PRIMATES, 30(1): 51–63, January 1989

Observations on the Illness and Consumption of a Possibly Medicinal Plant *Vernonia amygdalina* (DEL.), by a Wild Chimpanzee in the Mahale Mountains National Park, Tanzania

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ABSTRACT. Detailed observations on the consumption of *Vernonia amygdalina* (DEL.), a naturally occurring plant of known ethnomedicinal value, by an adult female chimpanzee (*Pan troglodytes schweinfurthii*) of M-group in the Mahale Mountains, Tanzania were made. Chewing the pith of several shoots, she sucked out and swallowed the astringent, bitter tasting juice; spitting out the fibrous remains. The female was followed during this period for 11 hr, over two consecutive days, and was recognized to be in irregular health at the time of consumption, exhibiting signs of lethargy, lack of appetite, and irregularity of bodily excretions. The low frequency and lack of seasonality in the usage of this plant suggest that it is sought after for reasons other than as a food source. These factors suggest that for chimpanzees, the consumption of this plant is primarily medicinal. The symptoms displayed by the female are the same as those experienced by people throughout tropical Africa who utilize this plant as a medicinal treatment for them. Interactions between the female and others suggest that they too were aware of her condition and coordinated their activities with the female and her infant.

Key Words: Chimpanzees; *Vernonia amygdalina*; Medicine.

INTRODUCTION

Observations on the consumption of certain plants containing medicinal properties have been reported for mammals (JANZEN, 1978), and in particular among primates (HAMILTON et al., 1978: *Papio ursinus*; PHILLIPS-CONROY, 1986: *Papio hamadryas*, *P. h.*×*P. anubis* hybrid; WRANGHAM & NISHIDA, 1983, TAKASAKI & HUNT, 1987: *Pan troglodytes schweinfurthii*). PHILLIPS-CONROY (1986) suggested that the leaves and nutritious, tasty berries of *Balânites aegyptiaca* (L.) DEL., were eaten by *P. hamadryas* and *P. h.*×*P. anubis* hybrid as a prophylactic agent against schistosomiasis. This plant was found to be a regular part of their diet along parts of the Awash river, Ethiopia, where schistosomiasis was most prevalent. For WRANGHAM and NISHIDA (1983), it was the peculiar feeding habits of *Aspilia* spp. at Gombe Stream and Mahale Mountains National Parks, Tanzania, which first drew their attention to the possible use of medicinal plants by chimpanzees. They argued that *Aspilia* spp. were not eaten for their caloric value, as the leaves of all of these species were swallowed slowly and without chewing. Subsequent chemical analysis (RODRIGUEZ et al., 1985) and another report on a different species eaten, (*Lippia plicata* BAKER, of known medicinal use) in a similar manner at Mahale (TAKASAKI & HUNT, 1987), strengthened their hypothesis.

PHILLIPS-CONROY (1986) points out the difficulty in distinguishing the relative nutritional and medicinal value of many primate plant foods with known active secondary compounds. The above reports cited medicinal use of the plants utilized, but provide little or no information about the physical state of the user before and after consumption. An ideal example of plant consumption primarily for medicinal value, would be one in which utilization is distinguishable from the daily dietary habits of the population and in which consumption is identifiable with sickness in the individuals concerned. This paper reports on yet another plant species of known ethnomedicinal value possibly utilized for its medicinal properties by chimpanzees at Mahale. Detailed observations on the change in health and behavior of one individual, before and after its consumption are presented. Based on these observations and other available material, the acquisition and possible use of medicinal plants in chimpanzees are discussed.

METHOD AND MATERIALS

The M-group of chimpanzees in Mahale Mountains National Park, Tanzania was studied by M.A.H. for two five-month periods (August–December) in 1985 and 1987. Adult males and females were selected as study subjects and focal animal sampling was conducted (ALTMANN, 1974) following individuals for as long as possible. All members of the group were individually identified (HIRAIWA-HASEGAWA et al., 1984). All plant species utilized by them, were identifiable by the local Kitongwe/Kiswahili vernacular and Latin names (NISHIDA & UEHARA, 1981, 1983). During November and December 1987, M.S., accompanied M.A.H. into the field, assisting in plant identification, tracking, and observation of the study

Fig. 1. *CH* and her 2.5-year-old male infant *CP*.

subjects. M.S. is trained by his grandfather in the traditional Tongwe use of local medicinal plants.

The adult female chimpanzee, *CH*, was born in K-group circa 1958 and transferred into M-group where she has remained since 1981 (NISHIDA, 1979; HIRAIWA-HASEGAWA & HASEGAWA, 1988). *CH* has one surviving dependent 2.5-year-old male infant, *CP* (Fig. 1).

The Plant Consumed

Vernonia amygdalina DEL. (Compositae), a shrub or tree reaching up to 8 m, occurs naturally throughout tropical Africa. It is also frequently planted around villages and sold at markets in West Africa for its many uses (DALZIEL, 1937; BURKILL 1985). Of direct relevance to this paper is the widespread medicinal use of *V. amygdalina* throughout tropical Africa against parasites and gastrointestinal disorders in people and their livestock. Table 1 gives a detailed description of some of these ethnomedicinal uses. One other species, *V. colorata* (WILLD.) DRAKE, very similar in appearance, is known by the same vernacular names and apparently is not distinguished from *V. amygdalina* by the people in tropical Africa where both are found (DALZIEL, 1937). The medicinal use of both species are the same (IRVINE, 1961; BURKILL, 1985).

In English, this plant is called 'bitter leaf' because of the astringent, bitter taste of its leaves, root, bark, and stems (WATT & BREYER-BRANDWIJK, 1962; BURKILL, 1985). A bitter principle,

Table 1. Some ethnomedicinal uses of *Vernonia amygdalina* in Africa.*

Disorder/illness	Parts utilized	Countries/comments
Parasites:		
Schistosomiasis	root, bark	Zimbabwe, Mozambique: mixed with *Vigna sinensis*
Malaria (fever)	root, stem-bark, leaves	E. Africa, Angola, Guinea, Nigeria, Ethiopia: a quinine substitute
Antihelmentic	root, leaves	E. Africa: treatment in children for trematodes used as a suppository
	root, seeds	Nigeria: enteritis, worms
	leaves	W. Africa: crushed in water and given to horses for worms, and there also reports of cattle grazing freely on it, especially in the evening
	leaves	Nigeria: for nursing infants, passed through mother's milk
Amoeba	root-bark	S. Africa: cold infusion used as substitute for ipecacuanha (ipecac) a source of emetine
Epidermal affections	leaves	Nigeria: treatment for ringworm, schistosome, and dermititis
Intestinal upsets:		
Constipation	leaf, sap	Nigeria, Tanzania, Ethiopia
Diarrhea	stem, root-bark	W. Africa
Unspecified	stem, root-bark, leaves	Angola, Nigeria, E. Africa
Miscellaneous:		
Scurvy	leaves	Sierra Leone, Nigeria, W. Cameroons; leaves sold in markets
"Heart weakness"	root	W. Africa: substance known as vernonin, a cardiotonic glycoside comparable to digitalin, a heart stimulant
Lack of appetite	leaf	W. Africa; leaves soaked and squeezed several times in cold water and boiled for use in soup
Coughing	leaf	Ghana, Nigeria, Tanzania
Rheumatism	stem, root-bark	Nigeria

*Sources taken from DALZIEL, 1937; WATT & BREYER-BRANDWIJK, 1962; KOKWARO, 1976; BURKILL, 1985.

vernonine, has been demonstrated and found to be 100% fatal when injected subcutaneously in mice at 10 g/kg body weight. It has also been found to have a hypotensive effect on dogs (IRVINE, 1961). M.A.H. conducted field experiments on the infusion's toxicity, using *Barbus semifer* minnows (3 g dried leaves/100 ml water) in a series of diluted test solutions. It was not possible to use shoots for the field bioassay because of problems with mold when drying the specimens. However, the chemical composition of the active substance found in the leaf is considered to be the same as that found in the pith. The solution was found to be fatal at 20% (% infusion of 500 ml water) within 6 hr.

NISHIDA and UEHARA (1981, 1983) included *V. amygdalina* in the natural diet of the chimpanzees of Mahale in both K- and M-groups. According to their 59-month survey between 1973 and 1981, the leaves, pith, and bark were utilized. There is no apparent seasonality in its use, as it was recorded year round. The relative frequency of utilization during this period is not given, but they cite 21 cases for which the feeding bout time was recorded (NISHIDA & UEHARA, 1983). UEHARA (unpubl. data), during 29 months of this survey (K-group only), recorded its use 26 times (15/21 cases given above) by 12 individuals. It was the pith that was utilized in all but two of these cases. In one of these cases the user, a male, was apparently recovering from an influenza-like sickness (see UEHARA & NYUNDO, 1983; UEHARA, unpubl. data). The four individuals, *CH*, *WD*, *GW*, and *WL* (all presently in M-group) were observed by UEHARA to have eaten this plant at least two or more times; *CH* was observed to do so seven times (UEHARA, unpubl. data). During M.A.H.'s ten-month study its consumption by chimpanzees in M-group was observed only once as described below. This plant is found in the woodland zone of the M-group's home range. According to WRANGHAM (1975), at Gombe, the pith of *V. colorata* is eaten. No indication however is given about the frequency of its use or the physical state of individuals observed consuming it.

OBSERVATIONS

On November 21–22, 1987, *CH* was observed for a total of 11 hr 8 min. The observations are split up into three periods for comparison of the change in *CH*'s activity patterns over time, and between *CH* and other adult females. The periods are defined as follows: day one (12:41–17:55; 267 min), day two/morning (9:07–12:49; 222 min), and day two/afternoon (13:06–16:05; 179 min). With the exception of an initial 28 min ad lib. observation between 12:41–13:22 on day one, all were made by focal animal sampling.

Figure 2 shows *CH*'s travel route during these two days of observation. The following is a condensed version of these observations.

DAY ONE (clear)

CH and *CP* are first seen at 12:41 (Fig. 2), in a mixed group with nine others (*LJ*, *KZ*, *JI*, *WD*, *MG*, *MA*, *WL*, *AS*, and *SF*). At 12:54 *CH* splits off from the group with the two other adult females and their three offspring, heading south away from the river, in the Hwasi valley, into the forest. At 13:05 we find *CH* resting in a tree and *CP* nearby. *WD*, her 8-month-old female infant *MG*, and adolescent son *MA* are with them. We follow *WD* as she and her offspring move off to forage at 13:22. At 13:56 we return following *WD* to *CH*, who is now sleeping in a day bed in the same tree as before (*CH* becomes the focal animal at this time).

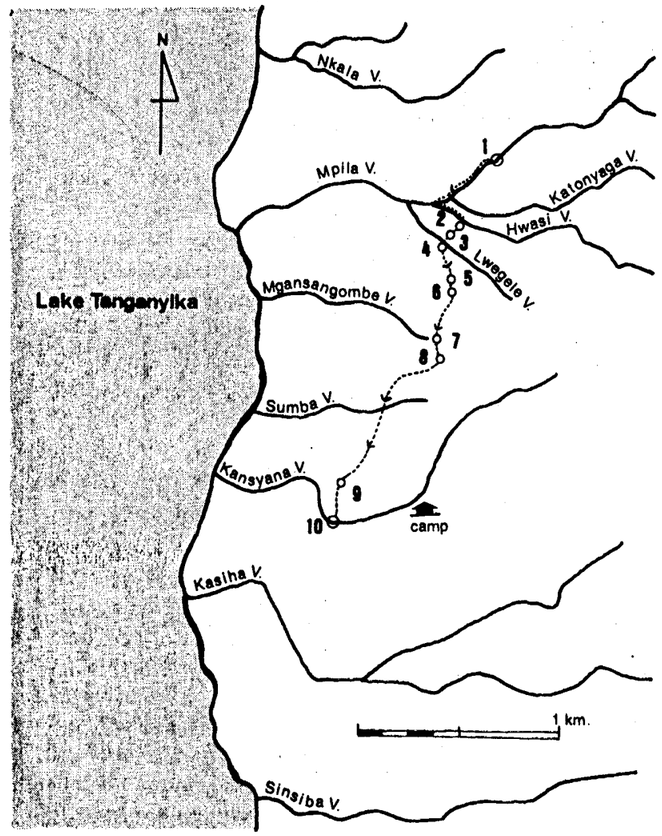

Fig. 2. Travel route of *CH* between November 21 and 22, 1987. 1: Nov. 21, 12:41; 2: 13:05; 3: 14:13; 4: 16:53–17:17; 5: 17:39–; 6: Nov. 22, 9:07; 7: 11:03–11:31; 8: 13:06; 9: 13:51; 10: 16:05.

At 14:02, *CH* climbs down and the group moves on south. While *WD*, *MG*, *MA*, and *CP* move ahead in the trees and on the ground foraging, *CH* slowly follows behind. *WD*, *MA*, and *CP* stop and feed on *P. purpureum*. At 14:13 *CH* goes directly to and sits down in front of a 2 m high shrub of *V. amygdalina* (Fig. 2, No. 3). She pulls down several young branches, approximately 2.5 cm in diameter at the base and 1 cm at the tip, to her lap and peels off the outer bark, leaves intact on the bark, exposing approximately 25 cm of pith from the tip down. This distal portion of the shoot is flexible and succulent (Fig. 3).

CH bites this portion off into several 5–7 cm portions, each time chewing them for several seconds. While doing so she makes a conspicuous sucking sound as she extracts and swallows the juice, spitting out the remaining fibers. Continuing in this manner, she processes several (not counted) shoot tips until 14:25.20. She pauses and begins moving her tongue around the inside of her mouth and teeth, opening and closing her lips slightly as if cleaning the inside. She makes no recognizable facial expression but her mouth seems to be affected by the substance she is eating. At 14:27.15 she shifts position and pulls down a dried stalk of *Pennisetum purpureum* SCHUMACK, and feeds on the larvae, eggs, and worker ants (*Crematogaster* sp.)

Fig. 3. Branch tips of *Vernonia amygdalina*.

found inside. At 14:30.54 she begins again to feed on *V. amygdalina* and until 14:35.07 she extracts the juice from four 15–25 cm long shoot tips.

During this time *CP* and *WD* sit nearby. *WD* is feeding on the tender pith of shoots of *P. purpureum*, and shows no interest in *V. amygdalina* although she is sitting on top of the bent over shrub to get at the grass. *CP* frequently shows interest in what *CH* eats by begging for the contents of her mouth. *CP* picks up pieces of the plant *CH* drops from her mouth and the discarded leaves and bark, putting them in his mouth for a few seconds but quickly discards them. *CP* continues to chew on the pith of small shoots of *P. purpureum* with *WD*. *CP* latter co-feeds with *CH* when she feeds on larvae, eggs, and worker ants (*Crematogaster* sp.).

After a brief rest, *CH* gets up and moves. The others follow behind. They occasionally forage on *Aframomum* sp. *CH* frequently stops to rest. At 15:01 *CH* and *WD* climb up a large tree. *WD* begins to feed on *Saba florida* (BENTH.) BULLOCK fruit and *CH* makes another day bed, resting until 15:30 when *WD* climbs down and sits on the ground below. *CH* leaves her bed and climbs part way down the tree. She stops and builds another day bed. *WD*, after a few minutes hesitation climbs back up into the tree and makes another bed next to *CH* at 15:33.

At 16:49 *CH* climbs out of her bed and urinates off the side. Viewed in bright light, her urine is unusually dark colored. *CH* slowly follows behind *WD*, *MA*, and *CP*. At 16:53 they cross the river in the Lwegele valley and *CH* takes a drink of water. They all forage on *Aframomum* sp. until 17:17 (Fig. 2, No. 4). *CH* slowly follows behind them, frequently stopping to rest. When *CH* reaches (17:29) the others, *WD* and *MA* have already begun to make their night beds. *CP* is waiting on the ground. *CH* attempts to defecate but appears to

Fig. 4. *CH* lying on a fallen tree to defecate.

be in pain when doing so. The discharge is slow but not firm. She climbs up and lays sideways on a fallen log and continues to defecate in small amounts (Fig. 4). At 17:36 *CH* climbs up into the closest tree attempting to defecate twice more, resting every meter or so. At 17:39 she begins to build a night bed and settles down at 17:44 (Fig. 2, No. 5). Observations are stopped at 17:55.

DAY TWO/MORNING (clear)

At 9:07 *CH* and *CP* are first seen, together with nine others in a mixed group (*DE*, *JI*, *WD*, *MG*, *MA*, *WL*, *AS*, *PT*, and *GW*) at a spot approximately 60 m from where they were left the night before (Fig. 2, No. 6). At 9:15 *CH* moves away and lays down at the edge of the group, leaving *CP* to play with two adolescents *AS* and *MA*, and a young adult female *PT*. At 9:59 *CP* follows *PT*, and *CH* slowly follows behind. Soon the whole group moves south-west.

At 10:00 *CH* splits off from the group leaving *CP* among them. She appears to be deficating normaly and urine color is back to normal. Until 10:38 *CH* remains separated from the group. *CH* frequently stops to rest or lie down. At 10:42 *CH* is seen for the first time today to eat. *CP* is now moving with her. *CH* briefly feeds on the fruits of *Garcinia huillensis* OLIV. and *Ficus urceolaris* WELW. ex HIERU. *CH*'s appetite appears to be coming back, although she frequently rests. Between 11:03–11:31 she forages on *P. angolensis* (WELW.) WARB. and *Ficus capensis* THUNB. (Fig. 2, No. 7). At this time *CH* and *CP* approach *DE*, *WD*, *MG*, *MA*, *GW*, and *PT*. They all lie down and rest for the next 72 min until 12:49 when they all suddenly stand up and move south.

DAY TWO/AFTERNOON (clear)

At 13:06 the group is relocated (Fig. 2, No. 8). *CH*, *CP*, and *GW* leave the group and continue south-west. Pausing only occasionally for the next 38 min, they cross the Sumba valley and climb down into the lower Kansyana valley (Fig. 2, No. 9). At 13:51 they begin to forage in the Kansyana valley along the river bed on *P. purpureum*. *Aframomum* sp., *F.*

ureolaris, *S. florida*, and *Crematogaster* sp. *CH* feeds steadily until the observations are terminated at 16:05.

DISCUSSION

CHANGES IN *CH*'S ACTIVITY PATTERNS

During these observations it appeared that *CH* was ill and that she gradually recovered. On day one, she appeared to be suffering from internal discomfort, lethargy, and a loss of appetite. This continued into the morning of day two. Later on in the morning, visible signs of a change were apparent. By that afternoon *CH* showed noticeable improvements in appetite and stamina.

In order to better evaluate the changes in *CH*'s behavior at these different stages, her activity was broken down into five basic patterns; lie in day bed or on ground, forage, travel, move-intermittent rest, and socialize. The results are given in Table 2. These results support the above conclusions.

On day one it was possible to directly compare her activity patterns with that of others in the same group. From 13:56 until 17:36 when *WD* began to build a bed for the night the two remained in close proximity. *CH* spent 51% of her time laying down and 28% foraging. The trend in *WD*'s behavior was reversed. She spent 54% of her time foraging and 35% resting in a day bed or on the ground.

For further comparison, activity patterns from focal observations of three adult females during similar time periods of the day in the same season, were calculated and given in Table 3. The variation that exists among individuals can partly be attributed to factors such as weather and group size; which indirectly affected day ranging patterns, feeding schedules, and opportunities for social contact (HUFFMAN, unpubl. data). These results further establish *CH*'s untypical behavior and change in health, suggesting marked recovery of appetite and strength sometime in the second day. The difference in the amount of time spent lying down and foraging between *CH* and these females is apparent.

INTERACTIONS BETWEEN *CH* AND OTHER CHIMPANZEES IN RELATION TO HER SICKNESS

Observations of interactions between *CH* and *CP* and a few other chimpanzees during this period, suggest that they were aware of her irregular health.

Table 2. Change in *CH* activity pattern during illness, with a comparison to that of *WD* for day one.*

Observation period		Lie in day bed or on ground	Forage[1]	Travel[2]	Move, intermittent rest[3]	Socialize	Time (min)
Day One:							
	CH	(115) 51%	(63) 28%	(0) 0%	(41) 18%	(5) 2%	224
(afternoon)	*WD*	(77) 35%	(119) 54%	(18) 8%	(0) 0%	(5) 2%	219
Day Two:							
(morning)	*CH*	(138) 62%	(36) 16%	(0) 0%	(41) 19%	(3) 3%	222
(afternoon)	*CH*	(0) 0%	(141) 79%	(39) 21%	(0) 0%	(0) 0%	179

**CH*: 625 focal min; *WD*: 219 ad lib. min. 1) Feeding and moving from one food source to another, but not stopping to rest for more than 5 min without feeding; 2) moving quickly, taking few rests, and rarely pausing to sit and rest. Movement between locations of other activities (i.e., sleep, eating, socializing, etc.); 3) moving slowly, pausing to sit and rest for periods frequently equally as long as time spent walking; not intermixed with other activities.

Table 3. Activity patterns of three adult females, observed during similar time periods as observations made on *CH*.

Observation period and individual	Lie in day bed or on ground	Forage[1]	Travel[2]	Move, intermittent rest[3]	Socialize	Time (min)
Morning:						
WD, Nov. 16 (9:51–12:51)	(0) 0%	(114) 63%	(0) 0%	(20) 11%	(46) 25%	180
GW, Oct. 26 (9:53–12:16)	(55) 38%	(44) 31%	(19) 13%	(25) 17%	(0) 0%	143
PU, Nov. 17 (9:30–12:38)	(14) 8%	(102) 56%	(0) 0%	(6) 3%	(61) 33%	183
Afternoon:						
WD, Nov. 16 (12:59–17:45)	(3) 1%	(117) 41%	(24) 8%	(49) 17%	(93) 33%	286
GW, Sept. 24 (13:00–17:17)	(0) 0%	(37) 22%	(4) 2%	(0) 0%	(126) 76%	167
PU, Nov. 17 (12:44–17:26)	(73) 27%	(177) 64%	(17) 6%	(8) 3%	(0) 0%	275

Watendele (*WD*); 466 focal min, *Gweklo* (*GW*); 310 focal min, *Pulin* (*PU*); 458 focal min. 1) Feeding and moving from one food source to another, but not stopping to rest for more than 5 min without feeding; 2) moving quickly, taking few rests, and rarely pausing to sit and rest. Movement between locations of other activities, (i.e., sleep, eating, socializing, etc.); 3) moving slowly, pausing to sit and rest for periods frequently equally as long as time spent walking; not intermixed with other activities.

On day one, *WD* remained with *CH* after they split off from the group. *WD* and her offspring left *CH* and *CP* alone for 34 min, but returned, as if expecting *CH* to still be there. For the rest of that day *WD* maintained a pace that allowed *CP* to move with them while *CH* followed slowly behind. In this way *WD* and *MA* acted as protectors of *CP*, as *CH* was not responding adequately to *CP* by letting him wander off on his own. *WD* could have easily left *CH* and joined other chimpanzees on several occasions. In one instance (15:30) the coordination of activities was obvious when *WD*, after initially climbing out of the tree, climbed back up into it after *CH* had refused to follow her down to the ground. *WD* build a day bed next to *CH* and they remained there for 76 min. *CH* was also partially responsible for maintaining this proximity by taking advantage of the time *WD* foraged in the same tree to build a bed and sleep (15:01).

On day two *GW* watched over *CP*, intervening when *CP* was abused by her older playmates, while *CH* slept. The group members allowed *CP* to move with them while *CH* stayed away to rest. At 13:06 *GW* moved away with *CH* when she separated from the group and watched over *CP* while *CH* feed on her own. During this period she made no attempts to forcefully draw *CP* away. Instead, she followed behind the two or stayed close ahead, following *CP* whenever he strayed too far from *CH*.

The consumption of *Vernonia amygdalina*, a plant of known medicinal properties (GITHENS, 1949; WATT & BREYER-BRANDWIJK, 1962; BURKILL, 1985), by a wild female chimpanzee was observed. Documented in detail for the first time, irregular health of the user at the time of consumption was confirmed.

From our experience in eating many of the food items of the M-group chimpanzees utilized during the research periods, we concluded that this plant was an exception to their (and our) normal taste preferences (HUFFMAN, unpubl. data). KALMUS (1970) notes the similarities in human and chimpanzee taste preferences, especially their aversion to strongly bitter tasting substances. We expected that the very bitter, astringent qualities of this plant should discourage chimpanzees from eating it. On the contrary, the adult female consumed in a liberal amount, the source of this bitterness in a highly concentrated form. The quickness with which *CH* began preparation, and consumption, as well as the time spent in the activity (1,006 sec.) shows that *CH* approached the plant with the intention of eating it. On the other hand, *WD* (known to have eaten this plant on several occasions), showing no apparent irregularities in

health at the time, paid no attention to this plant while foraging next to *CH*. The relatively low frequency with which this plant is consumed, despite an apparent lack of seasonality (NISHIDA & UEHARA, 1983), and year round availability, suggests that it is not sought after as a food source. These factors strongly suggest to us therefore that in chimpanzees, the consumption of *V. amygdalina* is primarily medicinal.

At this point information is limited and any further conclusions can only be speculative. However, suggestive evidence from a variety of sources and interesting similarities in the ethnomedicinal use of *V. amygdalina* and that by *CH*, have been recognized and raise important questions about the acquisition and use of medicinal plants by chimpanzees.

The strong bitter taste of *V. amygdalina* is an interesting factor in the use of this plant by chimpanzees. Among people of many cultures there appears to be an association made between bitterness and medicinal activity (GITHENS, 1949). Humans put up with the unpleasant bitter taste of many medicines, or disguise them, in order to benefit from the expected effects.

The Tongwe drink a cold water infusion of *V. amygdalina* extracted from 1–2 crushed leaves in 300–400 ml water for the relief of intestinal colic. It can take as long as 24 hr for the patient to feel better (M. SEIFU, pers. obs.; O. KABAJO, pers. comm.). It is not known whether *CH* had consumed *V. amygdalina* or other possibly medicinal plants on that day before we began to observe her. However, during our observations *CH* did not eat anything else different from her associates. The illness may have subsided in its own time, but it should be noted that approximately 23 hr had elapsed between observed consumption of this plant and *CH*'s remarkably obvious return to normal activity.

The continuation of the use of a given medicinal plant should be based on the experience of whether it produces satisfactory affects or not. It was noted above that the use of this plant has been recorded for several individuals, most of them more than once (UEHARA, unpubl. data). However, this does not explain how such plants are originally selected. Random experimentation does not seem likely, given the high degree of conservatism found in chimpanzee feeding habits (NISHIDA et al., 1983). A growing body of literature suggests that animals modify their diet on criteria other than just nutritional or caloric value, and that these modifications may partly be in response to specific hungers and long-delay learning (RICHTER, 1943; ROZIN & KALAT, 1971; ROZIN, 1977; REVUSKY, 1984). Such a mechanism would be beneficial to animals living in environments with a high risk exposure to disease (PHILLIPS-CONROY, 1986).

The correspondence recognized between disease and the initial selection of specific plants may in fact be partly a result of such physiologically based mechanisms. According to REWELL's (1969) description of intestinal infections in chimpanzees, it seems possible that *CH* may have been suffering from some such sickness. Avoiding any specific diagnosis however, the symptoms observed in *CH* (i.e., lethargy, loss of appetite, irregularities of bowel habit, and urine color) were comparable to those expected to be exhibited by humans suffering from some of the illnesses described in Table 1.

Of primary concern to us, are the observable social parameters of this phenomena. Not all individuals may be as equally sensitive to such cravings, thus the benefits of observational learning and cultural transmission, which allow many individuals through the experience of a few, to acquire new information (NISHIDA, 1987) should be of major importance. It has frequently been observed that chimpanzees show interest and concern for the injuries or sickness of others (see GOODALL, 1986). UEHARA and NYUNDO (1983) have reported a case of temporary adoption, in K-group, for at least six days by *GW* and *WL* of *CH*'s previous

infant *KB*, when *CH* was ill with a influenza-like sickness that had spread through the group. During our observations, the responses of a few individuals to *CH*'s 'untypical' behavior suggested that they were aware of her condition and coordinated their activities with *CH* and *CP*.

Given this interest and awareness, the use of medicinal plants may be acquired by others, through *observation and association* of its use in the *context of sickness*. Thus far, we have two good examples of observation and imitation of the use of this plant. One case described here of *CP* observing *CH*, and another recorded by NISHIDA (unpubl. data) in January 1986, of a 4-year-old female *AB* observing her 18-year-old brother *KZ*. *AB* too only tasted it for a few seconds. If this plant is used primarily in conjunction with sickness, this association should become all the more obvious to those individuals showing interest and concern. TAKAHATA et al. (1986) have demonstrated in chimpanzees how rapidly new feeding habits are acquired when the suitability of an item is noticed. Unlike other primates (e.g., ITANI, 1958: CAMBEFORT, 1981) adult chimpanzees are capable of initiating such new habits into the group (TAKAHATA et al., 1986).

In this respect, transmission of the medicinal use of plants in chimpanzees may differ from that of other non-human primates. Thus far, reports of the medicinal use of plants in other primates appears to be characterized by widespread use throughout the group as a part of their regular diet (HAMILTON et al., 1978; PHILLIPS-CONROY, 1986). However, further observations are necessary to confirm this.

CH has been observed to eat all three species of plants thus far suggested to be of medicinal value at Mahale (TAKASAKI & HUNT, 1987; UEHARA & NYUNDO, pers. comm.). This suggests that chimpanzees may be using a diverse variety of plants for medicinal purposes.

The above discussion raises many important questions about the acquisition of plant use by chimpanzees for possible medicinal use, and its possible distinction from that of other primates. Further detailed observations of individuals using these plants must be made before these hypothesis can be tested. It will be of importance to evaluate the physical state of the users. This is not an easy task, because the symptoms of illness may not be apparent by just a few minutes or even hours of observation. Given that chimpanzees appear to be using a variety of plants for this purpose, the illnesses themselves may vary in degree of severeness from a minor headache to severe cramps. The identification and laboratory analysis of constituents from *suspected* plant species utilized by chimpanzees, followed up by careful observation may prove to be an effective means of collecting information in the future.

Acknowledgements. M.A.H. wishes to give sincere thanks to the Tanzanian National Scientific Research Council and Serengeti Wildlife Research Institute for permission to conduct research at the Mahale Mountains National Park in cooperation with the Mahale Mountains Wildlife Research Centre. Special thanks go to Mr. E. T. MASSAWE, acting director of the research center and his staff and their families for their cooperation and friendship during both research periods. Our appreciation goes to Drs. T. NISHIDA and S. UEHARA, for generously making available their unpublished data and for commenting on an earlier draft of the manuscript. Dr. H. TAKASAKI provided invaluable suggestions on bioassay procedures and Dr. A. ROSITER, Kyoto University, identified the minnows used in the field experiments. Also we wish to thank Drs. H. HAYAKI, K. TAKASAKI, T. TSUKAHARA, R. NYUNDO, M., and H. BUNENGWA, for their companionship and humor in the field and for their valuable discussion at all stages of the project. M.A.H. also wishes to express deep appreciation for Dr. J. ITANI's endless encouragement and support, and also to the other members of the Laboratory of Human Evolution Studies for their advice and suggestions.

This study was financed by the Ministry of Education, Science, and Culture, Japan (Grant-in-Aid for Overseas Scientific Research Nos. 60041020 and 62041021 to Dr. T. NISHIDA).

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—Received *April 9, 1988*; Accepted *June 24, 1988*

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African Study Monographs, 12(4): 201–210, December 1991

BIOLOGICAL ACTIVITIES OF PLANT EXTRACTS FROM TROPICAL AFRICA

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ABSTRACT An investigation of plants in tropical Africa was conducted to search for new naturally bioactive substances. A total of 62 plant species were obtained from Cameroon, Zaire, and Tanzania. The extracts were tested for insecticidal, herbicidal, and fungicidal activities. Insecticidal activity was found in 23 extracts. Of the 51 extracts from Cameroon and Tanzania, herbicidal activity was detected in seven, and fungicidal activity in seven. *Polyascias fulva, Crassocephalum manii, Vernonia amygdalina, Vernonia vogetti, Poga oleosa, Gnidia glauca, Trema guinensis* and *Combretum bracteatum* were evaluated to be highly promising candidates for further investigation of their active constituents on the basis of their potency and/or broad spectrum of biological activities.

Key Words: Tropical African plants; Insecticidal activity; Herbicidal activity; Fungicidal activity; Medicinal plants.

INTRODUCTION

The tropical forest is one of the most interesting places in the world from the standpoint of bioactive natural product research. It is rich in plant species which are adaptive in a coevolutionary environment with regard not only to climatic factors but also to complex biological interactions. Tropical plants are surrounded by various competitors and consumers, and may be endowed with peculiar strategies for growth and reproduction, perhaps much more so than plants in other environments. A chemical defense system may be one possible strategy. It is well known that secondary plant metabolites (e.g. alkaloids, terpenoids, flavonoids, etc.) play significant roles in the biosphere as defensive substances that deter mammals and insects (Harborne, 1977). Phytoncides or allelopathic substances act as deterrents against microorganisms or other plant species (Harborne, 1977). The plants with medicinal properties are frequently used by local inhabitants (Lewis & Elvin-Lewis, 1977; Watt & Breyer-Brandwijk, 1962). Thus, the plants in tropical

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forests are valuable sources of naturally occurring bioactive substances. Although bioactive constituents of tropical plants have been extensively investigated (Hostettman & Marston, 1990; Watt & Breyer-Brandwijk, 1962), research has by no means been exhausted. Recent advances in biochemical and physiochemical methodology make it possible to investigate biologically and physiologically active plant constituents for new modes of activity.

Since 1983, we have conducted investigations to search for useful plants in the tropical rain forest of Cameroon (Ohigashi et al., 1987). Several new plant constituents with biological and physiological significance have been isolated from those plants (Koshimizu et al., 1992; Murakami et al., 1991a, 1991b; Ohigashi et al., 1989, 1991).

As a part of this long-term research, a third, more extensive field survey in Cameroon was carried out in 1988. This survey was conducted at two sites near Nkoélon and Nkanbé. Nkoélon, located in the rain forest of southwestern Cameroon, is close to Mvini village, a site at which previous surveys in 1983 and 1985 were conducted. Kaji indicated that the vegetation of this area is quite similar to that of the Mvini area (Kaji, 1990). At the Nkoélon area, random collection of plants (27 species) was undertaken, because the survey of useful plants in this area had already been completed (Ohigashi et al., 1987). On the other hand, the vegetation of the Nkambé area, located at the northwestern margin of the rain forest, is quite different from that at Nkoélon and can be characterized as either Afromontane or Afro-submontane (Letouzey, 1980). Medicinal plants in this area have been extensively surveyed by Jato (Jato, 1988). Furthermore, a preliminary survey in 1985 suggested that medicinal plants in the Nkambé area exhibited several biological activities at high rates (Ohigashi et al., 1987). Hence, further collection of medicinal plants (20 species) in this area was made.

Additional plant species were supplied from other African countries. One group of medicinal plants (11 species) were collected around Kinshasa, Zaire at the southern margin of the Central African rain forest by Muanza, and another group of plants (five species) containing four possible medicinal plants used by wild chimpanzees (Huffman & Sefu, 1989; Nishida, 1990; Takasaki & Hunt, 1987; Wrangham & Nishida, 1983) were collected from an area of savanna woodland in the Mahale Mountains National Park, Tanzania, by Nishida, Huffman and Takasaki.

This report describes insecticidal, herbicidal and fungicidal activities of these plant extracts, which were tested by our standard bioassay systems (Ohigashi et al., 1987).

MATERIALS AND METHODS

I. Preparation of Plant Extracts

All dried plant materials (usually 100 g dry weight) were immersed in methanol for 10–20 days at room temperature and then the extract solution was concentrated *in vacuo*. These methanol extracts were then submitted to the following bioassays,

and the activities in each assay were ranked by the standards as shown below.

II. Insecticidal Tests

Insecticidal activity was tested not only for the tobacco cutworm and the northern house mosquito, as conducted previously (Ohigashi et al., 1987), but also for the housefly and the pyrethroid-resistant diamondback moth.

1. Larval-Growth Inhibitory Activity against the Tobacco Cutworm (*Spodoptera litura*)

An emulsified aqueous solution (2 ml) of a test compound was mixed with a dose of artificial diet (13 g) and placed in a plastic cup (100 mm i.d., 35 mm depth) with ten of the 4th instar larvae. The test was usually conducted at a concentration of 4,000 ppm. In some cases, a concentration of 2,000 ppm was used because of limited sample availability. The mortality rate of the larvae and the feeding inhibitory rate to a control experiment were measured after six days. Inhibitory activity against larval growth measured by the mortality rate was evaluated using the following four ranks: +3, 100% mortality; +2, 90%–99% mortality; +1, 50–89% mortality; and − (inactive), less than 49% mortality. Feeding inhibition measured by the feeding speed was evaluated in four ranks: +3, more than 90% inhibition; +2, 50–89% inhibition; +1, 10–49% inhibition; and − (inactive), less than 9% inhibition.

2. Larval-Growth Inhibitory Activity against the Northern House Mosquito (*Culex pipiense pallense*)

Twenty 4th instar larvae were released in an emulsified aqueous solution (100 ml) of a test compound. The test was usually conducted at a concentration of 20 ppm. Some extracts, limited by availability, were tested at a reduced concentration (10 ppm) as in the case of the tobacco cutworm assay. After 24 hrs, the inhibitory activity of larval growth measured by the mortality rate was evaluated by the following four ranks: +3, 100% mortality; +2, 90–99% mortality; +1, 10–89% mortality; and − (inactive), less than 9% mortality. Thereafter, the surviving larvae were further reared until adult emergence. Inhibition of adult emergence compared to a control experiment was evaluated in four ranks: +3, more than 90% inhibition; +2, 80–89% inhibition; +1, 10–79% inhibition; and − (inactive), less than 9% inhibition.

3. Larval-Growth Inhibitory Activity against the Diamondback Moth (*Plutella xylostella*)

Leaf disks (2 cm in diameter) were punched out from a cabbage leaf. Both surfaces of the disks were treated with 20 μl of a 5,000 ppm solution of the methanol extract. Three treated disks and three untreated disks were alternately placed per petri-dish (10 cm in diameter) in which ten 3rd instar larvae were released. Inhibitory activity against larval growth measured by the mortality rate after two days was evaluated in four ranks: +3, 100% mortality; +2, 90–99% mortality; +1, 60–89% mortality; and − (inactive), less than 59% mortality. Feeding

inhibition was measured after five days by the percentage of leaf area consumed and evaluated in four ranks: +3, no feeding; +2, less than 10% consumed; +1, 11–80% consumed; and − (inactive), more than 81% consumed.

4. Killing Activity against the Adult Housefly (*Musca domestica*)

Thirty milligrams of the plant extracts in 1 ml of acetone was applied to bait consisting of 2 g of powder milk and 1 g of sugar placed on an aluminum tray. After air-drying, the bait and a cup of water (5 ml) were placed in a cage into which 50 housefly pupae were put. After two days, the adults emerged, and their mortality rate was determined after another seven days. Killing activity against the adult housefly was evaluated in four ranks: +3, more than 90% mortality; +2, 50–89% mortality; +1, 30–49% mortality; and − (inactive), less than 29% mortality.

III. Herbicidal Tests

Herbicidal activity was tested basically by the same methods previously reported (Ohigashi et al., 1987). A sanitary cotton sheet (50 mm × 50 mm) containing 0.1 ml of an emulsified solution (Tween 80-acetone, 1:10) of a test compound and 10 ml of water was placed into a glass tube (70 mm i. d., 100 mm depth). Normally the test was started at a concentration of 1,000 ppm. Seeds of test plants [three seeds of cucumber (*Cucumis sativus*), seven seeds of barnyard grass (*Echinochloa utilis*), 30 seeds of mustard (*Brassica junea*) or carrot (*Daucus carota* var. *sativa*)] were placed on the cotton. They were incubated at 27–28°C under a white fluorescent light (about 3,000 lux) for ten days. The rates of germination and the growth of the aerial part and/or the root were compared to control experiments. When growth inhibition was detected at 1,000 ppm, tests at further diluted concentrations were conducted. Activity was evaluated in four ranks: +3, more than 50% inhibition at 250 ppm; +2, more than 50% inhibition at 500 ppm; +1, more than 50% inhibition at 1,000 ppm; and − (inactive), less than 49% inhibition at 1,000 ppm.

IV. Fungicidal Tests

Fungicidal tests were conducted against ten species of phytopathogenic fungi by spraying emulsified extract solutions (500 ppm) on the host plants. The method used (Ohigashi et al., 1987) was basically the same in all of the tests, except for the host plant species and incubation conditions. Preventive effects on diseases by the fungi, *Pyricularia oryzae, Rhizoctonia solani, Cercospora arachidicola, Botrytis cineria, Phytophtora infestans* and *Venturia inaequalis*, were investigated. A typical run for *P. oryzae*: The emulsified aqueous solution of the extract (500 ppm) was sprayed onto the foliage of a host plant, *Oryza sativa*. After air-drying, spores of *P. oryzae* dispersed in water were sprayed onto the plant treated with the extract. The plant thus treated was incubated in a dark humidified room (99% humidity) for four days at 23°C. The preventive effect of the extract on the fungus disease was observed. Host plants and incubation conditions for the other fungi species were as follows: *C. arachidicola* on *Arachis hypogoea* for four days at 23°C

in a dark humidified room; *B. cineria* on *Cucumis sativus* for four days at 23°C in a dark humidified room; *P. infestans* on *Lycopersicum esculentum* for seven days at 23°C in a dark humidified room; *V. inaequalis* on *Malus domestica* for 18–20 days at 15°C in a greenhouse. In the case of *R. solani*, the spores precultured in a rice hull medium were inoculated on the base of the host plant, *Oryza sativa*, which was pretreated with an emulsified test solution of 500 ppm. The plant thus treated was incubated for seven days at 25–27°C in a humidified greenhouse (99% humidity).

The fungi, *Erysiphe graminis, Puccinia recondita, Pseudoperonospora cubensis* and *Plasmopara viticola* were used to identify the curative effects of extracts. The following is a typical test run: The spores of *E. graminis* were sprayed onto the foliage of the host plant, *Hordeum vulgare*. After preincubation in a humidified greenhouse (99% humidity) at 23–25°C for 18 hrs (preincubation), the emulsified aqueous solution of the extract (500 ppm) was sprayed onto a fungus infected plant. The plant thus treated was incubated at 23°C under continuous light (10,000 Lux) for ten days. The curative effect of the extract on the fungus disease was investigated. The host plants and incubation conditions for the other fungus were as follows: *P. recondita* on *Triticum aestivum*, preincubated for 18 hrs at 23–25°C in a humidified (99%) greenhouse and incubated for 12 days at 23°C under continuous light (10,000 lux); likewise, *P. cubensis* on *Cucumis sativus*, for 18 hrs at 23–25°C in a humidified (99%) greenhouse and additional three days at 23°C in the dark, followed by a further ten days at 28°C in a greenhouse; *P. viticola* on *V. vinifera*, for 18 hrs at 23–25°C in a humidified (99%) greenhouse and additional three days at 23°C in the dark, followed by further ten days at 28°C in a greenhouse.

The fungicidal activity was evaluated in six ranks: +5, 100% inhibition of the fungus disease; +4, 90–99% inhibition of the disease; +3, 70–90% inhibition of the disease; +2, 50–69% inhibition of the disease; +1, 20–49% inhibition of the disease; and − (inactive), less than 20% inhibition of the disease.

RESULTS AND DISCUSSION

All the plants from Zaire and Tanzania were identified. On the other hand, species identification of plants from Cameroon has been completed for 25 plants, and the genus was identified for eight plants. Further efforts to identify the plants are currently under way.

Table 1 shows the results of the standard bioassays for insecticidal, herbicidal and fungicidal activities of 48 plants, whose genera are identified. Unidentified plants were not listed except *APA, BOKKABOKUL* and *UNBAKFOLL* (vernacular name used at Nkoélon), which showed significant biological activities.

Insecticidal activities against four species of test insects were examined for all of the plant extracts (62 species). Against the tobacco cutworm, one of the standard test insects for insecticidal activity, 11 extracts were found to slightly inhibit larval growth, and 13 extracts inhibited larval feeding. Such effect on *Vernonia vogelli* was the most striking. Seven extracts exhibited inhibitory activity against larval

Table 1. Biological activities of the plant extracts.[a]

Plant		Biological Activity								
		Insect[d]								
		SL		CP		MD	PX			
Family Species (Part)[b]	Place[c]	M	D	M	E	M	D	Herb[e]	Fung[f]	Remarks[g]
Anacardiaceae										
Mangifera indica (B)	Zai	−	−	−	−	−	−	NT	NT	Med
Annonaceae										
Annona senegalensis (B)	Zai	−	−	−	−	−	−	NT	NT	Med
Apocynaceae										
Rauwolfia vomitoria (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Araliaceae										
Polyascias fulva (B)	NW-Cam	−*	+1*	−*	+2*	+3	−	−	+3PC	Med
Combretaceae										
Combretum racemosum (B)	Zai	+1	+2	−	−	−	−	NT	NT	Med
Combretum bracteatum (L)	SW-Cam	−	−	−	−	−	−	+3BJ, +3EU +3CS	−	
Terminalia sp. (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Compositae										
Aegeratum sp. (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Aspilia mossambicensis (L)	Tan	−	−	+1	+2	−	−	+1BJ	−	Med-PM
Crassocephalum manii (B)	NW-Cam	−	−	−	−	−	−	−	+5PC	Med
Vernonia amygdalina (L)	Tan	+1	+2	+1	−	−	−	−	+4PC, +2RS	Med, Med-PM
Vernonia guineensis (L)	NW-Cam	−	−	−	−	−	−	−	−	Med
Vernonia vogelli (L)	SW-Cam	+1*	+3*	−*	−*	−	−	−	−	
Ebenaceae										
Diospyros sp. (L)	SW-Cam	−*	+2*	−*	−*	−	−	−	−	
Euphorbiaceae										
Alchornia cordifolia (L)	Zai	−	+1	−	−	−	−	NT	NT	Med
Bridelia ferruginea (B)	Zai	−	−	−	−	−	−	NT	NT	Med
Euphorbia hirta (B)	Zai	−	−	−	−	−	−	NT	NT	Med
Hymenocardia acida (B)	Zai	−	−	−	+1	−	−	NT	NT	Med
Manniophyton fulvum (B)	Zai	−	−	−	−	−	−	NT	NT	Med
Flacourtiaceae										
Casearia sp. (L)	SW-Cam	+1*	+2*	−*	−*	−	−	−	−	
Irvingiaceae										
Desbordesia sp. (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Labiatae										
Ocimum gratissimum (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Ocimum sp. (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Leguminosae										
Afzelia bipindensis (L)	SW-Cam	−*	−*	−*	−*	−	−	+1BJ, +1EU +1CS, +1DC	−	
Crotalaria coolata (B)	NW-Cam	−	−	−	−	−	−	+1BJ	−	
Cynometra sp. (L)	SW-Cam	−*	+1*	−*	−*	−	−	−	−	
Daniella olivery (L)	NW-Cam	−*	−*	−*	−*	−	−	−	−	Med
Dialium pachyphyllum (L)	SW-Cam	−*	−*	−*	−*	−	−	−	+3RS	
Distemonanthus benthamianus (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Liliaceae										
Allium sativum (L)	NW-Cam	−	−	−	−	−	−	−	−	Med

(Table 1. cont.)

Malvaceae										
Sida rhombifolia (L)	Zai	+1	−	−	−	−	−	NT	NT	Med
Moraceae										
Ficus exasperata (L)	Tan	−	−	+1	−	−	−	−	−	Med-PM
Myrtaceae										
Psidium guajava (L)	Zai	−	−	−	−	−	−	NT	NT	Med
Olacaceae										
Olax subscorpioidea (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Strombosiopsis tetrandra (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Rhizophoraceae										
Poga oleosa (L)	SW-Cam	+1*	+1*	+1*	+2*	−	−	−	−	
Rosaceae										
Pygeum africanum (B)	NW-Cam	−	−	−	−	−	−	+2DC	+3PC	Med
Rubiaceae										
Mussaendra arcuata (L)	Tan	−	−	+1	−	−	−	−	−	Med
Nauclea latifolia (L)	NW-Cam	+1*	+1*	+1*	−*	−	−	−	−	Med
Nauclea sp. (L)	NW-Cam	+1*	−*	−*	−*	−	−	−	−	Med
Rothmannia withfieldii (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Tricalysia grossweileri (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Sapotaceae										
Zanha africana (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Thymeraceae										
Gnidia glauca (L)	NW-Cam	+1*	+2*	+1*	−*	−	+1	−	+3VI	
Verbenaceae										
Lippia plicata (L)	Tan	−	−	−	−	−	−	−	−	Med-PM
Vitex madiensis (RB)	Zai	−	+1	−	−	−	−	NT	NT	Med
Ulmaceae										
Trema guinensis (B)	NW-Cam	+1*	+2*	+1*	−*	−	−	−	−	Med
Trema orientalis (B)	NW-Cam	−	−	+2	−	−	−	−	−	Med
APA (L)	SW-Cam	−*	−*	−*	−*	+1	+2	+2Cu +2BJ	+2RS	
BOKKABOKUL (L)	SW-Cam	−*	−*	−*	+1*	−	+1	−	−	
UNBAFOLL (L)	SW-Cam	+1*	−*	−*	−*	−	−	+2BJ +2EU	−	

a) Each activity is expressed by the signature indicated in the Materials and Methods. NT: not tested.
b) Part extracted. B; bark; L: leaf; RB: root-bark.
c) Place collected. Zai: Zaire; NW-Cam: northwest Cameroon (Nkambé); SW-Cam: southwest Cameroon (Nkoélon); Tan: Tanzania.
d) Insect: insecticidal activity. SL: *Spodoptera litura*; CP: *Culex pipiense pallense*; MD: *Musca domestica*; PX: *Plutella xylostella*; M: mortality; D: feeding-deterrent activity; E: emergence inhibitory activity; *: test at 2000 ppm for SL and 10 ppm for CP.
e) Herb: herbicidal activity. BJ: *Brassica junea*; CS: *Cucumis sativus*; EU: *Echinochloa utilis*; DC: *Daucus carota*.
f) Fung: fungicidal activity. PC: *Pseudoperonospora cubensis*; RS: *Rhizoctonia solani*; VI: *Venturia inaequalis*; PV: *Plasmopara viticola*.
g) Med: local medicinal plant; Med-PM: possible medicinal plant used by wild chimpanzees.

growth of the mosquito, and six against adult emergence. Two extracts possessed killing activity of the adult housefly. The activity of *Polyascias fulva*, a medicinal plant found at the margin of the rain forest of Cameroon, was quite high. Deterrent activity against larval feeding of the diamondback moth, a pest to several cultivated vegetables, was found in three extracts. Thus, 23 out of the 62 extracts showed some kind of insecticidal activity and the activity-exhibiting rate (AER; 37%) was much higher than that (11%) in previous testing (Ohigashi et al., 1987). This may largely be due to the fact that the number of plants collected at the margin of the rain forests and savanna woodland was larger than that in the previous survey (Ohigashi et al., 1987). Then, it may be suggested that insect populations, particularly pest, are densest in such areas where human activities are high, hence the plants in these areas are endowed with defense systems against such insect attacks. Actually, 15 of the 23 extracts found to be active in the insecticidal tests were those from the plants collected at the margin of the forest or in the savanna woodland. Moreover, it may be proposed that some insecticidally active substances also possess significant value of physiological activity to humans. Among above 15 species, 11 species were locally used as medicinal plants (Jato, 1988) (Table 1).

Herbicidal activities, tested in cucumber, barnyard grass and carrot seedlings were found in seven out of 51 plant extracts. The AER was 13.7%. This rate was quite low compared with that (38%) of the previous study (Ohigashi et al., 1987). In this case, the fact that a fewer number of plants were collected from the rain forest may be again pointed out. These results may reflect that competition between plants is much more severe in the forest than the margin or outside of the forest, and plant growth inhibitory factors in the forest are much abundant. Among the herbicidally active plants, *Combretum bracteatum*, collected at Nkoélon, was most remarkable because of both the potency and the chemical characteristic of water solubility of the active constituent(s).

Fungicidal tests using 51 extracts against ten phytopathogens showed significant activity in seven extracts. Similarly to herbicidal activities, the AER (17.6%) in fungicidal tests was lower than that (31%) of the previous study (Ohigashi et al., 1987), suggesting severe competition between plant and microorganism in the rain forest. Of the 7 active extracts, those of *Crassocephalum manii* and *V. amygdalina* were indicated to contain invaluable active substances.

As a result of the biological activity tests, a total of 31 out of 62 species exhibited some kind of biological activity in the assays performed. The active constituents of *P. fulva, C. manii, V. amygdalina, V. vogetti, P. oleosa, Gnidia glauca, Trema guinensis* and *Combretum bracteatum* were evaluated to be important candidates for further study on the basis of the potency of each activity and/or the broad spectrum of activities displayed. It is hoped that these results will provide significant information for many areas of research in tropical Africa.

AKNOWELEDGMENTS This study was supported in part by a Grant-in-Aid for Scientific Research (International Scientific Research Program-Field Research) in 1988 and 1989 from the Ministry of Education, Science and Culture of Japan, and by a Grant-in-Aid

(Biocosmos program) from the Ministry of Agriculture, Forestry and Fisheries (BCP91–I-A-2). We are indebted to the MESRES and IMPM of the Republic of Cameroon, and to the Serengetti Wildlife Research Institute of Tanzania for permission to carry out the field research. We thank Dr. N. Hallé of the Muséum National d'Histoire Naturelle for identification of some of the plants.

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———Received *May 23, 1991*

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Short Communication

Bitter Principle and a Related Steroid Glucoside from *Vernonia amygdalina*, a Possible Medicinal Plant for Wild Chimpanzees

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Received December 14, 1990

Vernonia amygdaliana (Compositae) is a shrub widely distributed over savanna woodland in tropical Africa. Recently, the possible medicinal use of the tree by a wild chimpanzee has been reported by Huffman and Seifu.[1] They observed that an apparently sick adult female chewed the shoot of the shrub to extract the bitter juice, this shrub not being a regular food for chimpanzees of the study area in Tanzania. After this feeding habit, she seemed to return to normal activity, and hence, they concluded the consumption of the shoot to possibly be of medicinal value. The shrub is a well-known medicinal plant in tropical Africa,[2] and is also used as a tonic food in west Africa. This information further suggested that *V. amygdalina* contains a wide variety of physiologically or biologically active compounds. In fact, its anti-tumor agents[3] and insect anti-feedants[4] have already been reported. These compounds, however, may be only part of the constituents of physiological or biological significance. Therefore, a study of the bitter principles, which may carry significant physiological activity,[5] was conducted.

The water-soluble part of a methanol extract from the dry leaves (800 g) was successively separated on Amberlite XAD-2 (methanol–water), silicagel 60H (chloroform–methanol) and YMC-gel (ODS; methanol–water) to give a highly bitter fraction. This was then purified by preparative HPLC on μBondasphere C_{18} (acetonitrile–water) to yield a bitter principle, named vernonioside A_1 (**1**, 21.2 mg) and a related non-bitter compound, vernonioside B_1 (**2**, 217 mg).

Vernonioside B_1 (**2**), $[\alpha]_D^{25.0}$ +31.9° (c=0.94, MeOH), $C_{35}H_{52}O_{10}$ (FABMS m/z: 633, MH^+), showed UV absorption maxima in methanol due to a conjugated diene at 235 (ε, 13000), 243 (ε, 15000) and 250 nm (ε, 10000), and an IR absorption band (KBr) at 1775 cm^{-1} for γ-lactone. The presence of glucose was clarified by hydrolyzing with TFA and subsequent GLC analysis of the TMS-hydrolysates on OV-1. The ^{13}C-NMR (62.5 MHz, d_5-pyridine) signals at 102.29 (O–CH–O), 78.65, 77.07 (or 78.50), 75.34, 71.74 (CH–O, each) and 62.88 (CH_2–O) ppm due to glucose along with ^{1}H-NMR (250 MHz, d_5-pyridine) signal at 5.02 ppm (d, J=7.6 Hz) for the anomeric proton confirmed the glucose to be linked to an aglycone as a β-pyranoside.

The presence of two tertiary methyls (^{1}H-NMR: 0.75 and 0.87 ppm) and three secondary methyls (^{1}H-NMR: 1.25, 1.29 and 1.32 ppm) together with a lactone carboxyl group was suggestive of the aglycone being a C_{29} stigmastane-type steroid. The ^{1}H–^{1}H and ^{13}C–^{1}H COSY NMR spectra indicated that two of the secondary methyls at 1.25 and

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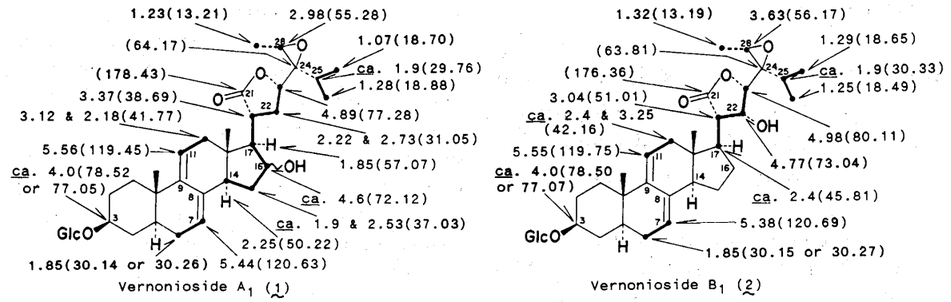

Fig. 1. Structures of Vernonioside A_1 and B_1, and Their Significant NMR Data.

The values express chemical shifts (δ ppm) for ^{1}H with those for ^{13}C in parentheses. ●—● with a bold line or bold dotted line shows the sequences confirmed by ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectra. A clear distinction between the protons at $C_{(7)}$ and $C_{(11)}$ was proved by long-range coupling between the protons at $C_{(7)}$ and $C_{(14)}$ in **2b**.

The $^{13}C_{(3)}$ signal in each compound was not clearly assigned even by the ^{1}H-^{13}C COSY spectrum because of overlapping of the ^{1}H signal at $C_{(3)}$ with those of the glucose. Hence, the signals at 78.52 ppm in **1** and 78.50 ppm in **2** may be interchangeable with those at 77.05 and 77.07 ppm, respectively.

1.29 ppm were due to an isopropyl ($C_{(25)}$–$C_{(26)}$–$C_{(27)}$), and the remaining methyl at 1.32 ppm, which was coupled to a proton at 3.63 ppm, was placed on $C_{(28)}$ at 56.17 ppm forming an epoxide with $C_{(24)}$ at 63.81 ppm. Hence, the lactone carboxyl group should be formed at $C_{(21)}$. Further NMR studies clearly confirmed the partial sequences shown in Fig. 1. A 7,9(11) diene was strongly suggested by the triple absorption maxima in the UV spectrum, which are known as a fingerprint for the diene in some triterpenoids and steroids.[6]

Enzymatic hydrolysis of vernonioside B_1 with β-glucosidase gave the real aglycone (**2b**), $C_{29}H_{42}O_5$ (HR-EIMS m/z: 470.3028, M^+; calcd., 470.3033), whose ^{13}C-NMR (d_5-pyridine) spectrum showed an upfield shift of a carbon signal at 78.50 (or 77.07 ppm) in **2** to 70.28 ppm. Biosynthetic considerations, as well as the multiplicity of the proton signal at *ca.* 3.8 ppm, attached to the carbon at 70.28 ppm, suggested the glucoside linkage to be completed with the hydroxyl at $C_{(3)}$ of the aglycone.

To confirm the structure of **2**, an X-ray diffraction analysis of **2b**, mp 205–210°C from ethyl acetate, was attempted. The crystalline data are as follows: space group, $P2_1$; z=2, a=17.082 (1), b=12.236 (1), c=6.230 (1) Å; β=92.81 (1)°, Dx=1.20 g cm^{-3}. All unique

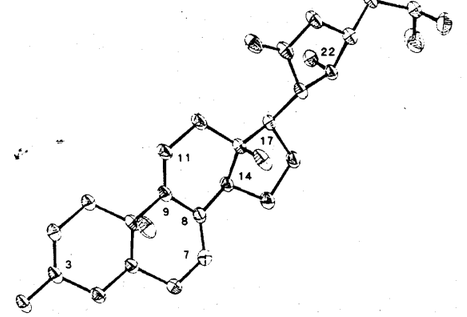

Fig. 2. Computer-generated Perspective Drawing of **2b** by X-ray Analysis.

intensity data were collected by a Rigaku AFC-5UD Jour-circle diffractmeter with graphite-monochromated Cu-Kα radiation. Analysis of 2179 independent reflections by both MULTAN 78 and the Rigaku program system gave the computer-generated perspective drawing shown in Fig. 2 with an R factor of 0.050, confirming the structure of vernonioside B_1 to be **2**.

The molecular formula of vernonioside A_1 (**1**), $[\alpha]_D^{24.0}$ +35.2° (c=0.23, MeOH), was determined as $C_{35}H_{52}O_{10}$ by its FABMS data

(m/z: 633, MH^+) and HR-EIMS data of the real aglycone (**1b**; m/z: 470.3014, M^+; calcd. for $C_{29}H_{42}O_5$, 470.3033) derived by enzymatic hydrolysis. The NMR data of **1** indicated that a β-glucopyranosyl group, a diene (UV λ_{max} (MeOH) nm (ε): 236 (12000), 243 (14000), 251 (9000), a γ-lactone (IR ν_{max} (KBr) cm^{-1}: 1760) and a trisubstituted epoxide were present in the same positions as those in **2**. Further NMR studies estimated the partial sequences shown in Fig. 1, confirming the hydroxyl at $C_{(16)}$. Thus, the structure of vernonioside A_1 was determined to be **1**. The stereochemistry of **1**, except for $C_{(16)}$, is presumed to be biosynthetically identical to **2**.

The minimum amount of vernonioside A_1 for a bitter taste was determined as 7 μg in a tentative sensory test with a paper disc (1 cm^2, 0.2 mm thickness),[5] and the activity was almost equal to that of quinine sulfate. Vernonioside B_1, on the other hand, did not show any bitter taste even at 200 μg. Hence, hydroxylation at $C_{(16)}$ may be significant for the bitter taste.

The structures of **1** and **2** are quite new, especially in the oxidation manner of the side chains of the aglycones. Further occurrence of the related bitter principles has been suggested, and the present data should provide significant information for their structural determination. Furthermore, it will be of interest to examine the physiological activity of each of these new steroid glucosides, and of the aglycones as well.

Acknowledgments. This study was supported by a Grant-in-Aid for General Scientific Research from the Ministry of Education, Science and Culture of Japan. We are indebted to Dr. Mitsuru Hirota at Shinshu University for taking the NMR data.

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野性チンパンジーの薬用植物と生理活性成分

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Possible Medicinal Plants Used by Wild Chimpanzees, and Their Physiologically Active Compounds

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As a new approach to discovering physiologically active natural compounds, possible medicinal plants used by wild chimpanzees have been studied. Physiological and biological activity tests of three such plants, *Vernonia amygdalina*, *Aspilia mossambicensis*, and *Ficus exasperata*, indicated that they contain a variety of active compounds. Particularly, the activities of *V. amygdalina* were remarkable, and hence further chemical studies were conducted. In the search for compounds of biological and physiological significance, in particular the bitter principles were investigated. Four known sesquiterpene lactones, vernodalin (1), vernolide (2), hydroxyvernolide (3) and vernodalol (4) were identified, and their anti-tumor and anti-bacterial activities were found. Furthermore, three bitter steroid glucosides and a related non-bitter glucoside, vernonioside A_1 (5), A_2 (6), A_3 (7) and B_1 (8) were isolated as new compounds. The significance of the use of *V. amygdalina* by chimpanzees for the control of parasite related disease and avenues for future research on this topic are discussed.

1. はじめに

最近，野性霊長類の採食行動を通して，薬用的摂取と推察できる種々の植物が報告されつつある（Huffman and Seifu, 1989; Phillips - Conroy, 1986; Takasaki and Hunt, 1987; Wrangham and Nishida, 1983)。例えば，Wranghamと西田(1983)は，タンザニア国の西部タンガニーカ湖に沿った地域での調査において，チンパンジーが奇妙な食べ方で口にする植物について報告している。すなわち，キク科 *Aspilia* 属の三種，*A. mossambicensis*，*A. pluriseta* 及び *A. rudis* である。彼らの報告によれば，チンパンジーがこれら3種の植物を食べる際の特徴として，一枚一枚ゆっくりと，噛むことなく舌を使ってころがすよう

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に押さえつけ食べることである。面白いことに，ゴンベ地区での Wrangham の観察によれば（西田によるマハレ地区での *A. mossambicensis* の例は少し異なっているようである），この採食行動が早朝に限られているとのことである。この特徴的な食べ方は，通常の採食行動とは著しく異なり，栄養補給とは違った目的，例えば朝方の体調を整えるための刺激剤とも言うべき生理的効果を狙ったものと推定された。西田らはその後，*Aspilia* 属植物がアフリカで腹痛，腸内寄生虫の治療などに使われている事実も考慮し，Lodoriguez (1985) 等とその生理活性成分について共同研究を行い，*A. mossabicensis* 及び *A. pluriseta* 中に殺線虫，抗細菌作用等多様な活性を示す含硫ポリアセチレン化合物，thiaruburine - A の存在を見出した。このことより，タンザニアでのチンパンジーによる *Aspila* 属植物の採食は寄生虫駆除を目的とした薬用的利用と考えられるに至った（Wrangham and Goodall, 1989）。

一方，西田のグループの Huffman と Seifu (1989) は，同じタンザニアマハレ地区のチンパンジーが，キク科植物，*Vernonia amygdalina* を薬用的に利用しているとのさらに状況証拠に富んだ観察結果を報告している。日中の殆どを横臥して過ごし，また食欲不振，糞便異常など明らかに病気と判定できる雌チンパンジーが，日頃積極的には口にしない *V. amygdalina* の茎をしがみ，強い苦味を呈する樹液を飲んでいることを発見した。この行動は観察日に数回に渡ってくり返され，翌日の午後にはそのチンパンジーは平常の活動を取り戻したと報告している。*Aspilia* 属植物は健康な個体が摂取することから，予防薬的，あるいは健康の維持を目的としたものと考えられるに対して，*V. amygdalina* の例は，明らかに病気であったチンパンジーが平常の活動を取り戻したことから，治療効果を狙った採食であったと理解できる。

哺乳動物が栄養補給以外に薬用的目的に植物を利用していることはすでに Janzen (1978) によって示唆されていたが，最近，チンパンジーを含む多くの霊長類でそれらしき行動が数多く観察され，動物行動生態学的見地より注目されるに至っている。しかしながら，これまではその生態学的観察に留まっており，当該植物に含まれる薬理的，あるいは生理的活性成分にまで言及した例は，先に示した *Aspilia* の研究例を見るに過ぎない。

本研究グループの西田，Huffman 及び高崎等はこれまで，タンザニア国マハレ地区でのチンパンジーの行動生態学的調査より，彼らが非栄養的に摂取していると推定できる植物を種々確認し，さらに，地域住民によるそれら植物の利用法をも合わせて調査し，チンパンジーが薬用的に利用していると強く示唆される植物を数種選択してきた。このような状況下で，小清水，大東を初めとする天然物化学研究グループが参画し，これら植物の化学分析，特に生理活性成分に関する研究を開始している。このような研究は，チンパンジーの行動生態に化学的メスを入れるばかりでなく，生理活性天然物の分野に新しい拠点を築き，さらに民俗薬学的知見を深めることになると考えられる。ここでは，特に *V. amygdalina* の例を中心に，生理活性天然物化学の視点から，その生理的活性と活性に関与する成分についての研究を紹介し，併せて今後の展望について述べてみたい。

2. 方　法

植物材料

この研究で用いた植物は，いずれもタンザニア，マハレ地区で西田，Huffman，高崎により採取・乾燥されたものである。*V. amygdalina* については，一部，小清水，大東によりカメルーンで採取されたものを用いた。

植物成分の抽出及び精製

通常乾燥植物体はメタノールにより抽出され，抽出液は減圧下濃縮後，低温下（4℃）で保存され，生理活性試験等必要に応じて用いられた。*V. amygdalina* の大量処理における抽出法，並びに各種成分の精製については結果と考察の部に述べてある。得られた成分の化学的同定は，質量分析，紫外，赤外及び核磁気共鳴スペクトル，及びX線結晶解析により行った。

生理活性試験

表－1に示す生理活性が種々の *in vitro* 法により試験された。個々の方法については割愛する。本報告で陽性を示した活性については，結果と考察の項で一部触れるが，詳細な試験法については文献（Koshimizu et al., 1991）を参照していただきたい。

V. amygdalina の主要活性成分の分析

Huffman により供与された種子よりビニールハウス内で発芽生育させた *V. amygdalina* の葉を分析試料として用いた。新鮮葉2gを，それぞれ20mlのアセトン，及びメタノールで別個に3日間室温で浸漬抽出する。アセトン抽出液については，全液を5mlまで減圧下濃縮し，濃縮物の2／5量を，小量のアセトン溶液として市販のコスモシール140C_{18}－OPN（Nakarai Tesque 製）に吸着させる。この吸着ゲルを市販の C_{18} Sep-pak（Waters Co. 製）フィルター上にのせ，水中0，90，100％アセトニトリル液（各10ml）で順次溶出する。求めるセスキテルペンラクトン類は90％アセトニトリル溶出部に回収される。この溶出液を減圧下濃縮後，アセトニトリルにて全容2mlに調整し，メンブランフィルターで再度ロ過し，ロ液1μlを結果と考察の項で記した条件下 HPLC カラムに注入分析する。一方，メタノール抽出液については，濃縮後同様の操作で C_{18} Sep-pak 処理を施す。ただし，溶出溶媒として，水中メタノール濃度が0，90，100％の溶媒を用いる。目的とするステロイド配糖体類は90％メタノール溶出区に回収され，この区分の1μlを同様に HPLC 分析に供する。

3. 結果及び考察

V. amygdalina の抽出物の *in vitro* 系での生理活性

はじめにも述べたように，Huffman 等（1989）は *V. amygdalina* が明らかに病気と判定できるチンパンジーにより採食され，やがてその病状が確実に回復したことを報告している。一方，本植物は熱帯アフリカで広く利用されている民間薬用植物であり（Burkill, 1985; Dalziel, 1937; Kokwaro, 1976; Watt and Breyer-Brandwijik, 1962），その薬理的効果として，整腸，下痢止め，抗マラリアや駆虫効果などが知られている。また，西アフリカ・カメルーンでは，独特の苦味を有するこの葉が伝統的民族料理（葉はビッターリーフと，またその料理はンドレと呼ばれる）として野菜代わりに好んで食され，疲労回復，食欲増進に卓越した効果があるとされている。チンパンジー，さらにはヒトによる薬用的利用と共に，食品的利用もまた本植物の成分分析を試みる動機となった。すなわち，伝統的民族料理として定着してきたその背景には，その環境下で住む人々の健康を維持する上で重要な生理的効果を発揮する成分が備わっており，その効果が伝承されてきたものとも推察できる。このように，*V. amygdalina* には多彩な生理活性が期待できるものとして，まず，その抽出物の生理的，薬理的活性を *in vitro* 試験法で検討してみた。

脂溶性から水溶性に至る広範な物質をできるだけ漏れなく得る方法として，メタノールを溶媒に選び，*V. amygdalina* 乾燥葉を抽出した。次いで，このメタノール抽出物について，表1に示す15種

表1　試験した生理活性

抗腫瘍
抗発癌プロモショーン
免疫賦活及び抑制
細胞膜形態変化及びその抑制
細胞膜関連酵素の障害
表皮成長因子（EGF）の活性化
細胞分化
トリプシン活性阻害
抗エストロジェン
抗炎症
鎮痛
抗菌
苦味

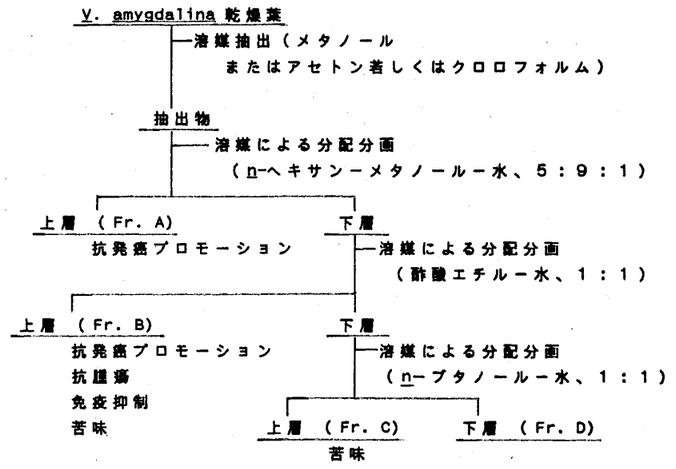

図1　*V. amygdalina* 葉抽出物の部分精製と生理活性の分布

の活性について，種々の *in uitro* 法で検討した。特に興味ある活性として，マウス白血病細胞P-388及びL-1210に対する強力な抗腫瘍活性，EBウイルス活性化法による抗発癌プロモーション抑制活性（Ohigashi et al., 1986），免疫抑制活性及びグラム陽性菌に対する抗菌活性が確認できた。

そこで，さらにそれぞれの活性物質の化学的性質を推察するために，粗メタノール抽出物を溶媒分画により部分精製し，上記4種の生理活性の動態を調べた。精製法及び精製による活性の分布状態は図1に示すようで，明らかにそれぞれの活性が何らかの化学因子により支配されていることが判明した。

一方，すでに述べたように，本植物には強い苦味が存在する。古くより苦味は多彩な生理活性を反映するものとして，天然生理活性物質の一次的スクリーニングに用いられてきた（Shiba, 1976）。苦味と生理活性との関連を伺わせる代表例として，抗マラリア剤として著名なキニーネ，鎮痛剤モルヒネなどのアルカロイド類，ウリ科植物の抗腫瘍性キュカビタシン類，抗結核菌作用を持つホップのフムロン，ルプロンなどのテルペン類を上げることができる。そこで，限られた試料から生理活性物質を広く探索する一手法として，苦味に着目することが有益であろうと推察し，苦味活性の動態をも追跡してみた。図1に部分精製における苦味活性の挙動が上記抗腫瘍，抗発癌プロモーション，免疫抑制及び抗菌活性と共に示してある。苦味活性は中極性部（Fr. B），及び極性部（Fr. C）に認められ，化学的性質の異なった広範な物質が関与していることが示唆された。興味あることに，苦味はメタノールでも抽出されるが，溶媒をより脂溶性に富むクロロフォルムやアセトンに代えるとその抽出効率が一段と増すことがわかった。さらに，この抽出法の違いによる苦味の差は，中極性部（Fr. B）の抗腫瘍，免疫抑制，抗菌活性の強弱と対応し，苦味成分がこれらの活性をも担っているのではないかと示唆された。

そこで，生理活性的に最も多様性のある中極性部（Fr. B）にまず着目し，含まれる苦味成分の究明を行った。

中極性部（Fr. B）の苦味成分

乾葉（100g）のアセトン抽出物を，ロ紙法試験による苦味活性を指標に順次精製し，苦味成分，化合物1−3，をそれぞれ500mg，29mg，14mg精製単離した。これらは，化学的，物理化学的諸性質よりそれぞれセスキテルペンラクトン類，

vernodalin (1)，vernolide (2)，hydroxyvernolide (3)と同定できた。それぞれの化学構造を図2に示した。いずれも既知成分で，表2に示すように各種 *Vernonia* 属植物より単離されている。一方，乾燥葉をメタノールで抽出すると，主成分，vernodalin (1)，は殆ど得られず，代わりに化合物4（vernodalol）が得られてくる。この事実は，メタノールを抽出溶媒に用いると，抽出過程で化合物1に存在する5員環ラクトンがメタノール分解を受けることを物語っている。それぞれの苦味活性（最小苦味発現量，MAB）は表3に示すようで，化合物1－3のそれらは強いが，4では弱いとの結果を得た。これは，化合物4に存在するテルペン部位の遊離水酸基が，ラクトンやエステルを形成することで苦味活性が高まることを示している。抽出溶媒としてメタノールを用いると，苦味活性が極端に低下する事実は，vernodalin(1)からvernodalol(4)への変換で説明できる。

先に述べた予備試験で，苦味と抗腫瘍及び抗菌活性の対応性が示唆されていたので，主成分として得られる vernodalin (1)及び vernodalol (4)を用いて両者の活性を検討してみた。表3にはその結果も示してある。抗腫瘍活性は，抗癌性物質の一次スクリーニング法として汎用されるマウス白血病培養細胞，P-388及びL-1210細胞で検定した。それぞれの細胞の3日後の増殖を，試験物質を含まない対照区に比し50%阻害する濃度（IC_{50}）で評価した vernodalin (1)の活性は0.086μg/ml（P-388）及び0.15μg/ml（L-1210）と強く，このIC_{50}値は強力な抗腫瘍剤，5-fluorouracilのそれに匹敵するものであった。一方，vernodalol (2)の活性は0.32μg/ml（P-388）及び0.61μg/ml（L-1210）と，1に比し約1／4の値を示した。種々のバクテリアを用いて検定した抗菌活性では，化合物1，2はグラム陽性菌に活性が認められ，その代表的菌種，*Bacillus subtilis*，及び *Micrococcus lutea* に対する最小生育阻止量（MIA）はそれぞれ表3に示すようであった。このように，抗菌試験においても，化合物1が2に比し10−50倍強い活性を持つことが示された。

Kupchan（1969）等は表2に示すように，抗腫瘍天然物質の開発過程で，ヒト由来KB細胞（口の類表皮癌由来）に対する増殖阻害（抗腫瘍活性）物質として vernodalin (1)及び vernolide(2)を単離している。さらに，これらセスキテルペンラクトン類が強力な抗腫瘍活性を示す化学的要因として，α，β−不飽和5員環ラクトンの存在を指摘している（Kupchan et al., 1970）。すなわち，このα，

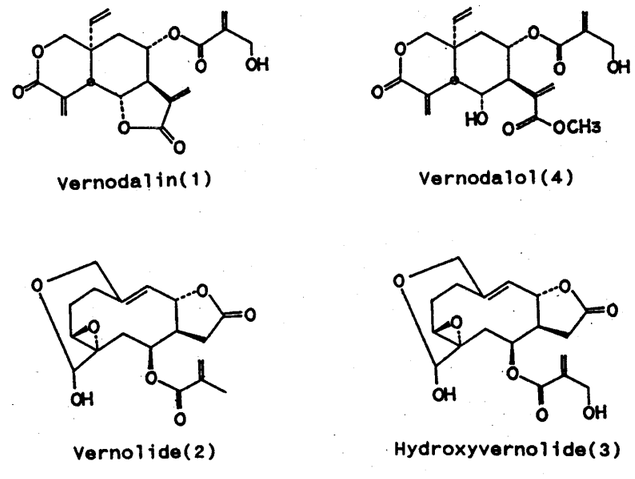

図2　*V. amygdalina* より得られ苦味性セスキテルペン類

表2 化合物1－4の *Vernonia* 属植物における分布とその生理活性

化合物	植物	生理活性
Vernodalin(1)	V. amygdalina[a]	KB細胞に対する毒性[a]
		アワヨトウ幼虫の摂食阻害[b]
	V. glabra[c]	
	V. neocorymbosa[d]	
Vernolide(2)	V. amygdalina[a]	KB細胞に対する毒性[a]
	V. colorata[e]	抗アメーバー赤痢[e]、抗蟯虫[e]
	V. neocorymbosa[d]	
	V. pectoralis[f]	
Hydroxyvernolide(3)	V. colorata[e]	抗アメーバー赤痢[e]、抗蟯虫[e]
	V. pectoralis[f]	
Vernodalol(4)	V. amygdalina[b]	アワヨトウ幼虫の摂食阻害[b]
	V. anthelmintica[g]	苦味[g]
	V. glaba[c]	

[a] Kupchan, S.M. et al., 1969. [b] Ganjian, I. et al., 1983.
[c] Jakupovic, J. et al., 1985. [d] Bohlmann, F. et al., 1983.
[e] Gasquet, M., et al., 1985. [f] Mompon, B. and Toubiana, R., 1976.
[g] Asaka, Y. et al., 1977.

表3 化合物1－4の生理活性

化合物	苦味活性	抗腫瘍活性 (IC_{50})		抗菌活性 (MIA)	
	(MAB)	P-388	L-1210	B.S	M.L
Vernodalin(1)	0.8 μg	0.086 μg/ml	0.15 μg/ml	5 μg	5 μg
Vernolide(2)	1.2 μg	NT	NT	NT	NT
Hydroxyvernolide(3)	1.0 μg	NT	NT	NT	NT
Vernodalol(4)	70.0 μg	0.32 μg/ml	0.61 μg/ml	50 μg	250 μg

苦味活性：最小苦味発現量(MAB)、抗腫瘍活性：50%増殖阻害濃度(IC_{50})、
抗菌活性：最小生育阻止量(MIA)、B.S: Bacillus subtilis、
M.L: Micrococcus lutea

β-不飽和5員環ラクトンが生体分子の求核中心と容易に反応することにより活性が発現するのであろうと推察している。抗腫瘍活性ばかりでなく，抗菌や苦味活性においても基本的には同じ機構で活性が発現されているものと推察できる。

これまで，生体分子との反応性に富んだα, β-不飽和5員環ラクトンを持つ多くのセスキテルペン類が見出され，また多様な生物・生理活性が報告されている。化合物1－4に限っても，表2に示すように，vernodalol (1)にはアワヨトウ幼虫に

対する摂食阻害活性が報告されている（Ganjian et al., 1983)。また興味あることに，vernolide(2)や hydroxyvernolide(3)には抗アメーバ赤痢や，抗蟯虫活性が報告されている（Gasquet et al., 1985)。後に改めて紹介するが，この二種の活性はチンパンジーの行動生態を物質レベルで解析する上で，格好の資料を提供してくれているものと考えられる。

中極性部（Fr. B）には免疫抑制活性も認められた。この活性にもまた一連のセスキテルペンラクトン類が関わっているのではないかと考えられ，現在本活性を検討中である。いずれにしても，本植物が熱帯アフリカで広く薬用植物として利用されているその化学的根拠の一つとして，vernodalin (1)を初めとするセスキテルペンラクトンの存在があげられよう。

極性部（Fr. C）の苦味成分

極性部（Fr. C）に存在する苦味成分は，メタノールで効率よく抽出される。また，この区分の苦味は化学的に互いによく似た挙動を示す数種の化合物からなっていることが判った。図3に示す方法に従って乾燥葉800gから得られるFr. C 区分を順次精製し，これまで，苦味成分（vernonioside A 群と命名）として5種（A_1－A_5）を，また非苦味性関連物質（vernonioside B 群）2種（B_1, B_2）を単離してきた。これら化合物の内，現在 vernonioside A_1－A_3 及び B_1 の4種の構造が図4に示すように確定できている（Ohigashi et al, 1991；Jisaka et al., 1991)。いずれも，炭素29個のスチグマスタン型ステロイドのグルコース配糖体で，化学構造的には，ステロイド側鎖部の酸化状態において極めて新規性のある化合物群である。

最小苦味発現量で評価した苦味活性は vernonioside A_2（5 μg），A_3（5 μg），A_1（7 μg）の順で，B_1 は200 μg でも苦味を示さなかった。このことから，ステロイド部の16位炭素についた酸素官能基が苦味活性に大きく寄与していることが判る。

これら vernonioside 類の生理活性についてはこれからの課題であるが，その構造から判断して，ステロイドに関連した種々の生理活性にまず注目し，各種誘導体を含め研究を展開中である。また，ステロイド側鎖部の酸化状態の類似性から，digitalin（図5）などの強心配糖体様活性にも注目している。西アフリカで，疲労回復や活力増進のため本植物が食べられている事実は，あるいはこれら vernonioside 類，若しくは関連化合物の存在に

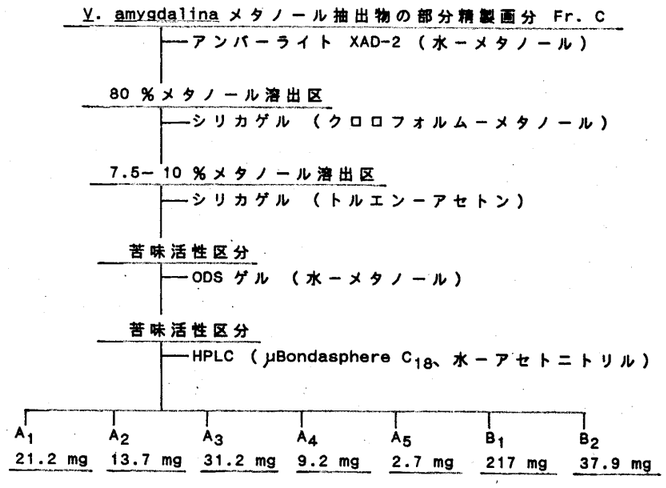

図3 Vernonioside 類の精製単離法

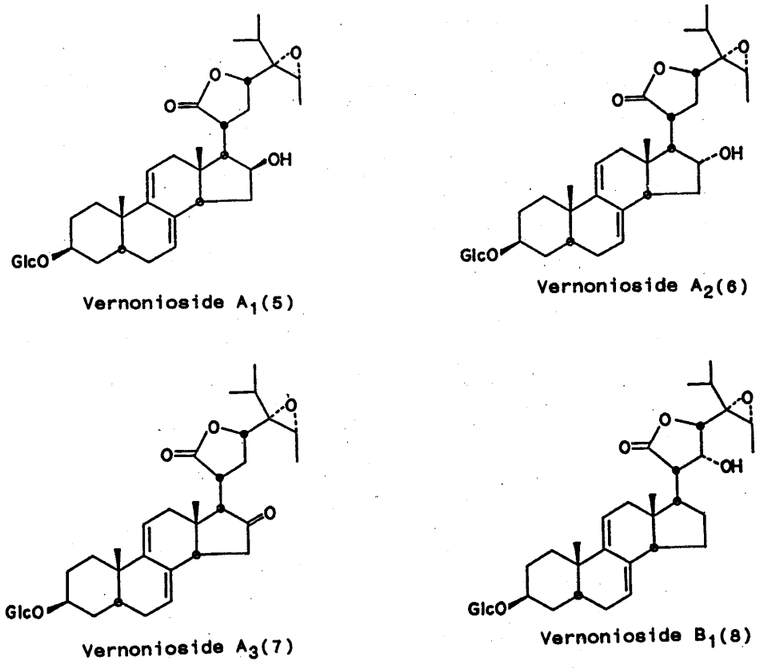

図4 Vernonioside A_1－A_3 及び B_1 の構造

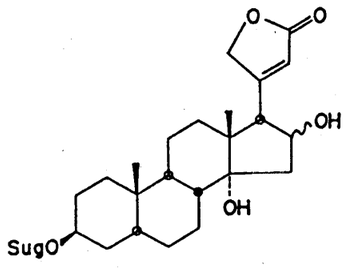

図5 Digitalin の構造

基づくものなのかもしれない。

その他のチンパンジー薬用植物抽出物の生理活性

西田（Wrangham and Nishida, 1983；西田, 1990）及び高崎（1987）等は，*V. amygdalina* 以外にチンパンジーが非栄養的に摂取していると推察できる植物を見出している。その中で，これまで *Aspilia mossambicensis*（キク科），*Ficus exasperata*（クワ科）を選び，*V. amygdalina* で行ったと同様の生理活性試験を試みた。*A. mossambicensis* は冒頭にも述べたように，チンパンジーが奇妙な食べ方をすることから注目された植物であるが，*F. exasperata* もまた同じように，早朝に一枚一枚ゆっくりと食べられることが観察されている。表－4に示すように，*A. mossambicensis* では極性部（Fr. C）に抗腫瘍活性及び免疫抑制活性が認められた。免疫抑制活性は中極性部（Fr. B）にも見出されている。さらに，非極性（Fr. A）及び中極性部（Fr. B）にタンパク分解酵素の一種であるトリプシンの活性を阻害する作用が認められた。一方，*F. exasperata* では，抗腫瘍活性と共に，顕著なトリプシン活生阻害作用が見出された。しかしながら，これらの活性は，*V. amygdalina* で認められたほど劇的なものではなかった。*A. mossabicensis* や *F. exasperata* は，*V. amygdalina* と違って，一見健康なチンパンジーによって摂取される。従って，両植物の利用は，治療よりむしろ予防的意味を含む平素の体調をコントロールするための用法と理解される。それぞれの生理活性がそれほど顕著でないことは，このことを反映しているものと考えられる。チンパンジーは予防薬的植物と治療薬的植物を巧みに使い分けているのかもしれない。

表4 *A. mossambicensis* 及び *E. exasperata* の生理活性

分画	*Aspilia mossambicensis* 抗腫瘍[a] P-388	L-1210	TRP[b] 阻害	免疫[c] 抑制 TD	NTD
Fr. A	−	−	+	NT	NT
Fr. B	−	−	−	++	++
Fr. C	+	+	+	++	++
Fr. D	−	−	−	−	−

分画	*Ficus exasperata* 抗腫瘍[a] P-388	L-1210	TRP[b] 阻害	免疫[c] 抑制 TD	NTD
Fr. A	−	−	++	−	−
Fr. B	+	+	++	−	−
Fr. C	+	+	−	−	−
Fr. D	+	+	−	−	−

[a]試験濃度：500 μg/ml、+: 60%以上の増殖阻害、−: 60%未満の増殖阻害
[b]トリプシン活性阻害活性、++: 50-100 μg/mlで50%以上の阻害、+: 100-1 mg/mlで50%以上の阻害、−: 1 mg/mlで50%未満の阻害
[c]TD: T細胞依存性抗体産生能、NTD: T細胞非依存性抗体産生能、試験濃度：50 μg/ml、++: 20%以下の溶血班形成細胞産生、−: 90%以上の溶血班形成細胞産生

さらに西田（1990）によれば，チンパンジーが噛まずにゆっくりと食べることにも重要な意味がありそうだとのことである。すなわち，活性成分は口腔粘膜を通して直接血液循環系に取り込まれ，作用器官へすばやく送られる可能性があると指摘している。チンパンジーは植物種をより分けるばかりでなく，処方せんまで会得しているのであろうか。

病気の治療を目的に利用していると考えられる植物のみならず，予防的効果が期待される植物にも，今後充分目を向ける必要がありそうである。

チンパンジーによる *V.amygdalina* の採食と薬用的意味

話は再び *V. amygdalina* に戻るが，Gasquet（1985）等は *V. colorata* より vernolide (2) 及び hydroxyvernolide (3) を抗蟯虫，抗アメーバー赤痢物質として単離している。*V. amygdalina* の民間での駆虫薬的用法は広く知られたところで，西田，Huffman らの調査地近郊でも実際に使用されている（Huffman, 1990）。一方，Huffman 等の1987年の調査によると，*V. amygdalina* の樹液を飲み，疾病状態から解放されたチンパンジーの最も可能性のある病気として，寄生虫病があげられている（Huffman et al., 1991）。

本研究において，我々は vernolide (2) 及び hydroxyvernolide (3) の存在を明らかにした。しかしながら，用いた植物葉中でのこれら二種の化合物の存在量は低かった。実際にはチンパンジーが摂取していた茎部または樹液中での両化合物の量が問題となろうが，それ以上に，2や3と構造的に類似した主成分，Vernodalin (1)，の活性の有無が注目される。vernodalin (1) の種々の生理活性は，我々がこれまで試した活性に限っても，vernolide (2) や hydroxyvernolide (3) のそれと同等，若しくは

上回っていた。恐らく，vernodalin (1)にも vernolide (2) や hydroxyvernolide (3) に匹敵する抗アメーバ，抗蟯虫活性が存在するであろう。また，これら二種に限らず，広く原生動物，回虫・条虫など寄生虫に活性を示すものと期待し，上記 Gasquet 博士等フランス・マルセイユ大のグループを初め，ロンドン大学の Wright 博士，国立予防衛生研の川中博士等との共同研究が始まっている。詳報は別の機会にゆずるが，川中博士による日本住血吸虫に対する試験で，vernodalin (1) は期待通りの成績を収めつつある。さらに，vernonioside 類の主成分，B_1 にも注目すべき活性が認められつつある。

一方，Huffman はチンパンジーの薬用的植物利用をさらに明快に解くために，調査対象グループの感染寄生虫の種の同定や，その季節的変動，さらには，各時期における非栄養的摂取植物種の克明な調査を始めつつある。その際，一方では，本報告で紹介した *V. amygdalina* の各成分の植物部位における存在量や，季節毎のその含量変化も重要な知見となろう。そこで，これら化合物の簡便な定量法が必要と考え，その方法に関する基礎的実験を試みた。

V. amygdalina の苦味成分及び関連化合物の簡便分析法

すでに述べたように，*V. amygdalina* の苦味成分は化学的性質より大きく二種のグループに分けられる。すなわち，比較的脂溶性に富んだセスキテルペン類（化合物１－４）と，水溶性の vernonioside 類である。これらをほぼ同じ操作で前処理でき，しかも複雑な操作を加えることなく分析できる手段として，化学実験室で日常的に用いられている HPLC 法での可能性を検討した。実際の実験条件の確立には，種々取捨選択がなされたが，ここでは現在最適と判断できる方法について以下紹介する。

図６はそれぞれのグループの化合物を HPLC 法で分析した例である。HPLC 装置は Waters 600

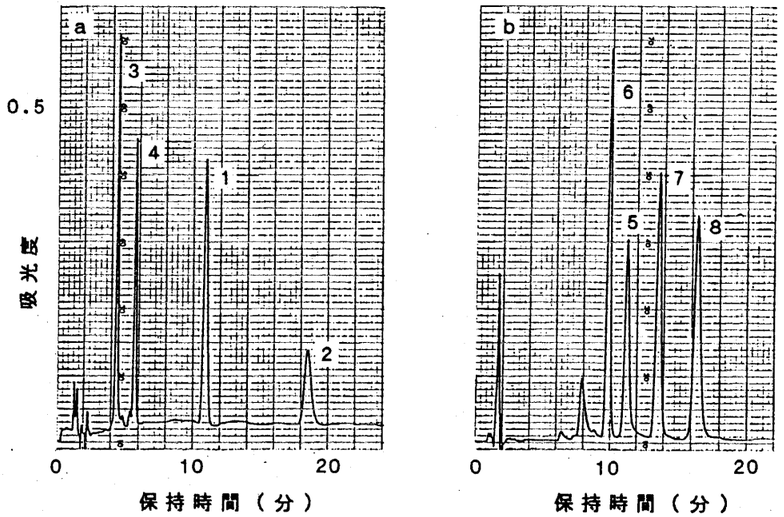

図６　化合物１－８の HPLC クロマトグラム
カラム：YMC AQ 301，溶出溶媒：化合物１－４（本図ａ）は25％アセトニトリル，化合物５－８（本図ｂ）は35％アセトニトリル，流速：１ml／分

（この型に限ることはない）を利用し，分析カラムとして市販の逆相型 ODS カラム（YMC AQ301, 4.6×100 mm）を用い，254若しくは220nmの紫外線による吸光度で検出した。セスキテルペン類は，水中25％アセトニトリル（容量％）の条件下で20分以内で良好に分離溶出された（図６a）。一方，vernonioside 類は，水中35％のアセトニトリルで望ましいピークの分離が認められた（図６b）。この実験データーより，基本的には HPLC 法で分析可能と示唆されたので，実際に，我々の研究室で種子より発芽生育させた *V. amygdalina* 新鮮葉について分析してみた。操作の詳細は方法の部に示してあるが，概略は次のようである。セスキテルペン類と vernonioside 類は溶媒により抽出効率が異なることから，新鮮葉（２g）を二組用意し，その一方はメタノールで，他方はアセトンでそれぞれ抽出した。次いで，両抽出物をそれぞれ市販の Sep-pak フィルターでろ過し，得られたロ液を濃縮後 HPLC で分析した。結果が図７に示してある。セスキテルペン類の分析では，主成分，vernodalin (1)，が殆ど独立したピークとして認められた（図７a）。予想されたように，vernodalin のメタノール分解物，vernodalol (4)のピークは認められなかった。微量成分，vernolide(2)や hydroxyvernolide (3)の確認は，この実験スケールではできなかった。一方，vernonioside 類（図７b）では，A群（５，６，７）のピークには，未同定物質のピークが数個接近し，やや複雑な様相を示したが，主成分 B_1 (8)は明瞭なピークとして認められた。ピークが明瞭であれば，それぞれの吸光度より定量が可能である。因みに，本実験で用いた試料では，vernodalin (1)及び vernonioside B_1 は１gの新鮮葉中にそれぞれ 1.8mg，及び 0.8mg 存在していたと算出された。Vernolide や vernonioside A 群等微量成分の分析にはさらに部分精製が要求され，また分離能のよいカラムの検討が必要であろうが，少なくとも現時点で，我々が注目している主要成分（vernodalin，vernonioside B_1）については，新鮮植物試料１gで充分定量分析が行えることがわかった。目下，この方法により，主要成分の植物部位における存在量やその季節的動向を明らかにし，チンパンジーの本植物摂取の意義を解析すべく研究を計画中である。

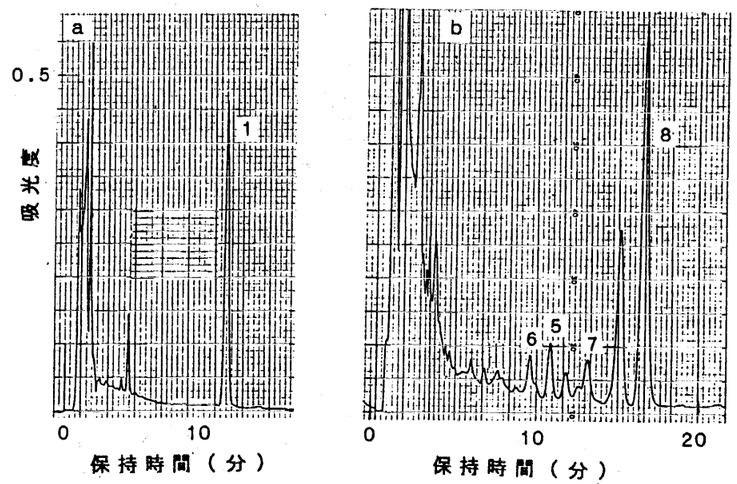

図７　*V. amygdalina* 新鮮葉抽出物の HPLC 分析
分析条件は図６のそれと対応する

4. おわりに

ここまで，*V. amygdalina* を主要題材として，生理活性天然物的視野よりチンパンジーの薬用的利用植物の意義について述べてきた。また，本稿では触れなかったが，*V. amygdalina* 中の抗発癌プロモーション抑制物質についても明らかにしてきた（Koshimizu et al., 1991）。結論として，動物行動生態学分野から供された植物材料には多様な生理活性物質が存在することが示唆されてきている。チンパンジーを初めとする野生霊長類の採食行動を通して有用天然物質を開発する試みは，生理活性天然物に対する新しいアプローチであると考えている。今後，動物生態学者の詳細な調査により，薬用をも含む霊長類の非栄養的摂取植物の解析が進み，それぞれの摂取意義が提起されることを願っている。さらに，このような研究は動物行動生態学分野にも新しい知見を与えるものと確信し，互いの協力の下，両分野の研究がさらに発展することを念じている。

謝 辞

本研究は文部省科学研究費－国際学術研究－の援助により行われた。現地調査に当たって，御協力いただいたタンザニア国，及びカメルーン国の関係政府機関に紙面を借りて厚く御礼申し上げます。

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Biosci. Biotech. Biochem., 56 (5), 845—846, 1992

Short Communication

Antischistosomal Activities of Sesquiterpene Lactones and Steroid Glucosides from *Vernonia amygdalina*, Possibly Used by Wild Chimpanzees against Parasite-related Diseases

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Received January 16, 1992

Vernonia amygdalina is a tropical African plant possibly used by wild chimpanzees for medicinal purposes.[1] We have previously reported new bitter steroid glucosides, vernonioside A_1—A_3, and a related vernonioside B_1 from this plant.[2,3] As another class of bitter constituents, four known cytotoxic sesquiterpene lactones were also isolated.[4] The most possible use of this plant by chimpanzees was suggested to be a medicine against parasite-related diseases; this idea is based on both the symptoms of the chimpanzee (loss of appetite, lethargy, constipation, dark colored urine, and apparent abdominal discomfort) (M. A. Huffman, personal communication) and local use of the plant by humans.[5] Schistosomiasis is one of the most common parasitosis throughout Africa. Here, we examined antischistosomal activities of the sesquiterpene lactones (vernodalin, 1; vernolide, 2; hydroxyvernolide, 3; vernodalol, 4), and the steroid glucosides (vernonioside A_1, 5; A_2, 6; A_3, 7; B_1, 8) including their aglycones (**7a**, **8a**, and **8b**) (Fig. 1) using *Schistosoma japonicum*.

Adult pairs of schistosomes (*S. japonicum*) were obtained aseptically from mice infected with cercariae for 8 weeks.[6] Antischistosomal activities were measured *in vitro* by the inhibition against movement (IM) and egg-laying (EL) of the schistosomes in triplicate experiments by the method previously reported.[6] The results are shown in Table I.

All of the sesquiterpene lactones (**1**—**4**) inhibited movement and egg-laying of schistosomes at 200 ppm, but not at 2 ppm, at the concentration of which a known antischistosomal drug, praziquantel[7] showed the activites (data not shown). At 20 ppm, only vernodalin (**1**) inhibited the movement and egg-laying. Vernonioside B_1 (**8**) also inhibited movement and egg-laying of the schistosomes at 200 ppm, but not at 20 ppm. Vernonioside A_1 (**5**) and A_3 (**7**) slightly inhibited egg-laying at 200 ppm. The aglycones **8a** and **8b**, however, clearly inhibited egg-laying at 20 ppm. The effects of **8b**, a secondary aglycone obtained by acid-hydrolysis of **8**,[3] was more potent than those of **8** and its real aglycone, **8a**.

The antischistosomal activity of vernodalin (**1**), the most active compound *in vitro*, was then tested *in vivo*. As anticipated by the cytotoxicities against KB,[8] P-388, and L-1210 cells,[4] it was highly toxic, and was lethal at more than 5 mg (120 mg/kg) to the infected mouse with *S. japonicum* when orally administered. Oral administration of a non-lethal dose of **1** (2.5 mg) had no great effect on the parasite.

The quantitative analysis of **1** and **8** by HPLC (YMC AQ-301, 25% aqueous acetonitrile for **1** and 35% aqueous acetonitrile for **8**) showed that the leaves of *V. amygdalina* contained both **1** and **8** at high levels (2.81 mg and 0.61 mg per g of fresh leaves, respectively). The level of **8** in the piths of the stems (0.75 mg per g of fresh piths) was comparable to that in the leaves. Vernodalin (**1**), however, was detectd only at a small level in the piths (0.02 mg per g of fresh piths).

The sick chimpanzee was observed by Huffman[1] to ingest the piths of the young stems. Although it is not evident that the sick chimpanzee was infected by schistosomes, the above data may suggest that the sick chimpanzee can use steroid glucosides such as **8** in the piths for the remedy against common parasitosis such as schistosomiasis caused by African schistosomes, *S. haematobium* and *S. mansoni*, avoiding the ingestion of leaves or stem barks which contained the extremely toxic vernodalin (**1**) in high levels. Furthermore, active steroid glucosides like **8** may be metabolized into more active forms like **8a** and **8b** during digestion. An investigation of the *in vivo* activity of **8** as well as the structure-activity relationship of steroid glucosides is now in process.

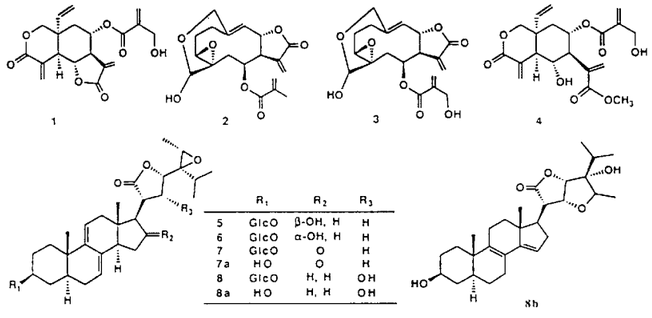

Fig. 1. Structures of Sesquiterpene Lactones, and Steroid Derivatives.

Table I. *In Vitro* Antischistosomal Activities

Compound	Well-1		Well-2		Well-3	
	200 ppm	20 ppm	200 ppm	20 ppm	200 ppm	20 ppm
	IM[a]: EL[b]	IM[a]: EL[b]	IM[a]: EL[b]	IM[a]: EL[b]	IM[a]: EL[b]	IM[a]: EL[b]
1	+: 57	++: 6	+: 0	++: 3	+: 3	++: 3
2	++: 0	+: 0	++: 0	+: 30	++: 0	+: 42
3	++: 0	−: 92	++: 0	−: 5	++: 101	−: 366
4	++: 0	−: 4	++: 4	−: 72	++: 0	−: 0
5	−: 47	NT[c]	−: 6	NT[c]	−: 15	NT[c]
6	−: 212	NT[c]	−: 772	NT[c]	−: 66	NT[c]
7	+: 0	−: 206	−: 48	−: 46	−: 166	−: 400
7a	−: 46	−: 286	−: 106	−: 153	−: 60	−: 60
8	+: 0	−: 0	+: 0	−: 1072	+: 0	−: 766
8a	+: 0	−: 16	+: 0	−: 66	+: 0	−: 33
8b	+: 0	+: 0	+: 0	−: 48	+: 0	−: 0
DMSO-1	−: 420		−: 1280		−: 760	
DMSO-2	−: 428		−: 466		−: 506	

To an adult pair of schistosomes in RPMI 1640 supplemented with 10% fetal calf serum (1 ml) in a well (16 mm in diameter, 17 mm in depth), each compound in DMSO (20 μl) was added. After incubation for 24 hr under 5% (CO_2 at 37 °C, movement of schistosomes and the number of the eggs laid were observed by the method previously reported.[6] The control wells contained DMSO (20 μl) without any compound.
[a] The inhibition of movement of schistosomes is shown as follows: ++, completely inhibited; +, incompletely inhibited; −, not inhibited.
[b] The number of eggs laid.
[c] NT, not tested.

Acknowledgments. This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan. We thank Mr. Daisuke Izutsu for his technical assistance.

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Tetrahedron Vol. 48, No. 4, pp. 625-632, 1992
Printed in Great Britain

0040-4020/92 $3.00+.00

Bitter Steroid Glucosides, Vernoniosides A1, A2, and A3, and Related B1 from a Possible Medicinal Plant, *Vernonia amygdalina*, used by Wild Chimpanzees.

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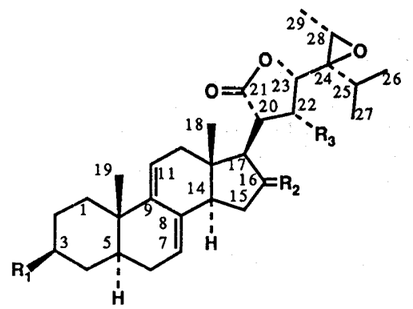
(*Received in Japan* 18 *October* 1991)

Key words: *Vernonia amygdalina*; Bitter compound; Medicinal plant; Chimpanzee; Vernonioside

Abstract: From Vernonia amygdalina, a possible medicinal plant used by wild chimpanzees, three bitter steroid glucosides, vernoniosides A1, A2, and A3 and a nonbitter vernonioside B1, were isolated. The oxygenation patterns of the aglycone parts were new, especially the pattern of the carboxyl group at C21. The oxygen functionalities at C16 were important for the bitter taste.

Some plants may be consumed for medicinal purposes by wild chimpanzees.[1,2] *Vernonia amygdalina* (Compositae), a tree growing throughout tropical Africa, is one of the most likely examples of such plants. At the Mahale Mountain in Tanzania, Huffman and Seifu [2] observed that shoots of this tree were chewed by an apparently sick adult female chimpanzee. *V. amygdalina* is used in tropical Africa as anthelmintic, antiscorbutic, and a quinine substitute, and it is eaten as a traditional tonic food in west Africa. These facts suggest that *V. amygdalina* contains a variety of physiologically or biologically active compounds. Some sesquiterpene lactones have been isolated as antitumor agents by Kupchan *et al.*[3] and as insect antifeedants by Ganjian *et al.*[4] To search for further physiologically and biologically active compounds, we have investigated the bitter compounds, in particular.[5] The structures of a bitter compound, vernonioside A1, and a nonbitter related vernonioside B1 were briefly reported,[6] without detailed physicochemical data or chemical characteristics. We recently isolated two new related bitter compounds, vernoniosides A2 and A3, and identified the configuration of the hydroxy group at C16 of vernonioside A1. Here, we describe in full the isolation, structures, and bitterness of these steroid glucosides.

The bitter *n*-butanol soluble part of a methanolic extract of the dried leaves of *V. amygdalina* contained a group of compounds that appeared as violet or blue spots on TLC with 0.5% vanillin in sulphuric acid-ethanol solution, and was separated by 4 steps of column chromatography and then purified by preparative HPLC on μBondasphere C_{18} to yield the bitter compounds vernonioside A1 (**1**), A2 (**2**), and A3 (**3**) and a related nonbitter compound, vernonioside B1 (**4**).



	R_1	R_2	R_3
1	OGlc	β-OH, H	H
1a	OH	β-OH, H	H
2	OGlc	α-OH, H	H
2a	OH	α-OH, H	H
3	OGlc	O	H
3a	OH	O	H
4	OGlc	H, H	OH
4a	OH	H, H	OH

Fig. 1. Structures of Vernoniosides and Their Aglycones.

The results of FABMS indicated that the molecular formulae of **1**, **2**, and **4** were $C_{35}H_{52}O_{10}$ and that the molucular formula of **3** was $C_{35}H_{50}O_{10}$. UV spectra of these compounds showed triple maxima at 235, 243, and 250 nm, indicating the presence of a conjugated diene chromophore. An IR absorption band due to a γ-lactone was observed with each compound. The following structural information was common to all of the compounds, as reported previously.[6] They were ß-glucopyranosides of the C_{29} stigmastane-type of steroids, and each contained an isopropyl group at C24, a trisubstituted epoxy ring system at C24-C28, and a γ-lactone cyclized between C21 and C23.

^1H-^1H and ^1H-^{13}C COSY NMR spectra of **4** confirmed the partial connectivities such as C23-C22-C20-C17, C6-C7, and C11-C12. The triple absorption maxima in the UV spectrum of **4** suggested the presence of a $\Delta^{7,9(11)}$ diene, as reported of some triterpenoids and steroids.[7] Hydrolysis of **4** with β-glucosidase gave the natural aglycone (**4a**), the spectral data of which corresponded with those of **4** except for ^{13}C NMR signals arising from C2, C3, and C4. The ^{13}C NMR signals of C2, C3, and C4 shifted from 30.15, 77.07 (or 78.50), and 34.54 ppm in **4** to 32.58, 70.28, and 38.78 ppm in **4a** (Table 1). From these data, the structure of vernonioside B1 seems to be **4** (Fig. 1).

The final structure of the steroid part of vernonioside B1 was found to be that of **4a** by X-ray diffraction analysis.[6] The perspective view of **4a** is shown in Fig. 2, where the $\Delta^{7,9(11)}$ diene is shown;

Table 1. ^{13}C NMR Assignments of **1**, **2**, **3**, **4**, **3a**, **4a**, and **4b**.

C	1	2	3	4	3a	4a	4b
1	35.01	34.92	34.83	35.01	35.11	35.30	35.75
2	30.14	30.08	30.06	30.15	32.49	32.58	32.63
3	77.05[a]	76.98[a]	76.93[a]	77.07[a]	70.14	70.28	70.28
4	34.52	34.48	34.40	34.54	38.69	38.78	39.32
5	39.30	39.16	39.11	39.25	39.57	39.73	41.31
6	30.26	30.19	30.06	30.27	30.20	30.39	22.38
7	120.63	120.99	122.72	120.68	122.91	120.85	36.07
8	136.13	135.87	133.41	136.53	133.47	136.59	X[b]
9	144.15	144.09	144.77	143.84	145.02	144.08	142.10
10	36.23	36.18	36.42	36.12	36.48	36.17	36.96
11	119.45	118.76	117.76	119.75	117.76	119.76	27.12
12	41.77	41.32	39.93	42.16	40.00	42.20	25.77
13	42.89	43.28	40.94	42.63	40.98	42.67	45.48
14	50.22	49.18	46.13	51.93	46.22	51.03	151.33
15	37.03	36.02	37.64	23.51	37.69	23.55	116.62
16	72.12	74.89	214.51	28.08	214.53	28.10	36.63
17	57.07	60.14	62.80	45.81	62.83	45.84	47.56
18	13.91	13.91	14.24	12.68	14.28	12.70	16.87
19	19.60	19.48	19.50	19.52	19.69	19.70	18.58
20	38.69	40.26	37.36	51.01	37.38	52.03	46.10
21	178.43	177.97	177.70	176.36	177.71	176.38	177.24
22	31.05	29.31	28.50	77.04	28.50	73.05	79.46
23	77.28	77.38	77.24	80.11	77.23	80.11	82.99
24	64.17	64.02	64.38	63.81	64.38	63.82	82.13
25	29.76	29.74	29.66	30.33	29.67	30.33	30.85
26	18.70	18.73	18.75	18.49	18.76	18.49	17.34
27	18.88	18.89	18.75	18.65	18.76	18.65	18.07
28	55.28	55.40	55.32	56.17	55.31	56.18	81.09
29	13.21	13.19	13.22	13.19	13.23	13.19	13.94
1'[c]	102.29	102.25	102.32	102.28			
2'[c]	75.36	75.30	75.37	75.34			
3'[c]	78.65	78.60	78.69	78.64			
4'[c]	71.74	71.71	71.80	71.74			
5'[c]	78.52[a]	78.46[a]	78.56[a]	78.50[a]			
6'[c]	62.89	62.86	62.93	62.88			

[a] The values of C3 and C5' may be reversed.
[b] This olefin carbon signal was not assigned exactly because it was superimposed by the signals arising from pyridine-d_5.
[c] The carbons of glucose were numbered as 1' to 6'.

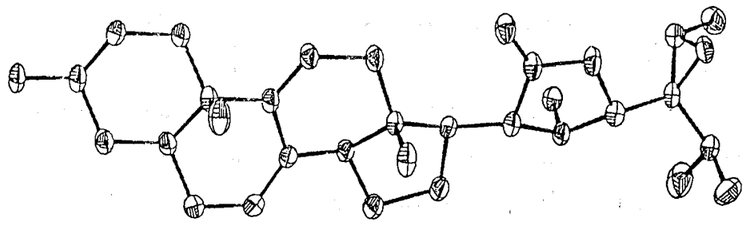


Fig. 2 Perspective View of **4a**.

Table 2. ^{1}H NMR Assignments of **1**,**2**,**3**, and **4**.

H	1	2	3	4
1α	1.30	1.26	1.29	1.28
1β	1.91	1.86	1.87	1.88
2α	2.18	2.16	2 15	ca. 2.2
2β	1.72	1.71	1.75	ca. 1.7
3	3.96	3.95	ca. 4.0	ca. 4.0
4α	2.07	2.02	2.04	2.05
4β	1.48	1.41	1.47	1.47
5	1.37	1.34	1.35	1.38
6α	1.83	1.82	ca. 1.8	ca. 1.8
6β	1.83	1.82	ca. 1.8	ca. 1.8
7	5.44, br s	5.40, br s	5.30, br s	5.38, br s
11	5.56, br d, *J*=6.3 Hz	5.50, br d, *J*=5.9 Hz	ca. 5.5, br d	5.55, br d, *J*=6.29 Hz
12α	2.19	2.34	ca. 2.3	2.37
12β	3.12, dd, *J*=17.8, 6.7 Hz	2.77, dd, *J*=17.4, 6.6 Hz	ca. 2.4	3.25, dd, *J*=18.0, 6.9 Hz
14	2.24	2.82	2.67	2.30
15α	2.53, dt, *J*=12.8, 6.9, 6.9 Hz	2.16	2.44, dd, *J*=18.0, 7.9 Hz	1.82
15β	1.91	2.16	2.17, dd, *J*=18.4, 12.7 Hz	ca. 1.5
16α	4.65, br s	-	-	1.65
16β	-	4.58	-	2.38
17	1.89	2.37	3.04	2.41
18	1.18, s	0.75, s	0.73, s	0.75, s
19	0.89, s	0.85, s	0.83, s	0.87, s
20	3.37, q, *J*=9.7 Hz	3.07, m	ca. 3.08	3.04, dd, *J*=9.6, 4.0 Hz
22α	2.28	2.32	2.32	-
22β	2.73, m	2.52, q, *J*=11.8 Hz	2.32	4.77, br s
23	4.89, dd, *J*=9.9, 5.9 Hz	4.93, dd, *J*=10.6, 5.5 Hz	4.90, t, *J*=8.1 Hz	4.98, d, *J*=2.3 Hz
25	1.89	1.86	1.92	2.01
26	1.07, d, *J*=7.2 Hz	1.08, d, *J*=7.2 Hz	1.04, d, *J*=7.2 Hz	1.25, d, *J*=7.4 Hz
27	1.28, d, *J*=7.1 Hz	1.28, d, *J*=7.0 Hz	1.26, d, *J*=6.7 Hz	1.29, d, *J*=7.6 Hz
28	2.98, q, *J*=5.3 Hz	3.01, q, *J*=5.6 Hz	3.17, q, *J*=5.6 Hz	3.63, q, *J*=5.5 Hz
29	1.23, d, *J*=5.5 Hz	1.19, d, *J*=5.5 Hz	1.24, d, *J*=5.4 Hz	1.32, d, *J*=6.1 Hz
1'	5.07, d, *J*=7.6 Hz	5.01, d, *J*=7.6 Hz	5.05, d, *J*=7.8 Hz	5.02, d, *J*=7.6 Hz
2'	4.07	4.06	4.08	4.06
3'	4.38	4.31	4.31	4.33
4'	4.30	4.27	4.27	4.30
5'	4.00	3.96	4.02	4.03
6'	4.38	4.40, br dd, *J*=11.9, 5.0 Hz	4.42	4.41, dd, *J*=11.9, 5.3 Hz
	4.60	4.57, br d, *J*=10.0 Hz	4.60	4.58, dd, *J*=11.8, 2.1 Hz

this allowed us to assign most of the ^{13}C (Table 1) and ^{1}H (Table 2) NMR signals of **4**, which was helpful for the structure elucidation of other vernoniosides.

Hydrolysis of **4** with HCl gave an aglycone (**4b**) with a molecular ion peak at m/z 470 in its EIMS, indicating that the molecular formula of **4b** was $C_{29}H_{42}O_5$. The UV spectrum of **4b** had a single maximum at 250 nm (ε 14000) arising from a diene chromophore. The ^{1}H NMR spectrum of **4b** showed only an olefinic proton signal at 5.45 ppm, which was coupled with methylene proton signals at 2.44 and 2.88 ppm. The methylene proton signals were coupled with a methine proton signal arising from 17-H (2.54 ppm) in the ^{1}H-^{1}H COSY NMR spectrum at 500 MHz. Thus, double migrations of double bonds from $\Delta^{7,9(11)}$ to $\Delta^{8,14}$ by proton addition and elimination reactions occurred during acid hydrolysis (Fig. 3). Furthermore, the ^{13}C NMR signals arising from C22, C24, and C28 shifted downfield from 73.05, 63.82, and 56.18 ppm in **4a** to 79.46, 82.13, and 81.09 ppm, respectively, in **4b** (Table 1). Additionally, the methine proton signal arising from 28-H shifted downfield from 3.64 ppm in **4a** to

4.31 ppm in **4b**. These shifts of the ^{13}C and ^{1}HNMR signals could be explained by the formation of a five-membered ether ring accompanied with opening of the epoxy ring as shown in Fig. 3.

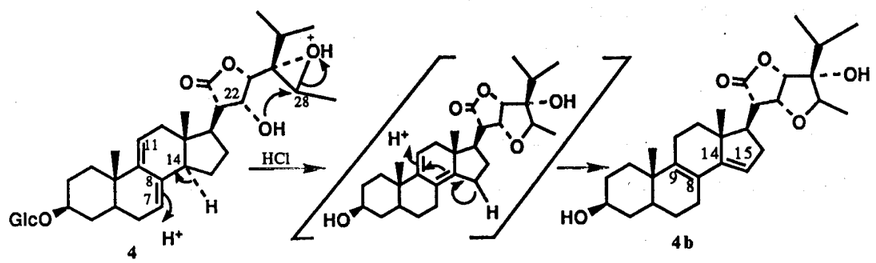


Fig. 3. Reaction of **4** Hydrolyzed with HCl to **4b**.

The structure of vernonioside A_1 was previously found to be that of **1** by comparison of ^{13}C and ^{1}H NMR data (Table 1 and 2, respectively) with those of **4**, except for the stereochemistry at C16.[6] Vernonioside A_2 (**2**) was found to be an isomer of **1** and **4** by its FABMS and by HR-EIMS of its natural aglycone **2a** obtained by enzymatic hydrolysis. ^{1}H-^{1}H and ^{1}H-^{13}C COSY NMR spectra of **2** clearly demonstrated the connectivity of C23-C22-C20-C17-C16-C15-C14, as for **1**. The C22 and C16 of **2** were of methylene (^{1}H NMR; 2.32 and 2.52 ppm) and hydroxymethine (^{1}H NMR; 4.58 ppm), respectively. Thus, **2** was identified as an epimer of **1** at C16.

Vernonioside A_3 had an IR absorption band at 1740 cm^{-1} and a ^{13}C NMR signal at 214.51 ppm (Table 1), indicating the presence of an additional carbonyl group other than that of C21. In the ^{1}H-^{1}H COSY NMR spectrum, both 15α-H and 15β-H appeared as double doublets (2.44 ppm, J=18.0, 7.9 Hz and 2.17 ppm, J=18.4, 12.7 Hz, respectively) coupled with a methine proton signal at 2.67 ppm arising from 14-H. Thus, **3** seemed to be a 16-oxo-derivative.

By $NaBH_4$ reduction of **3**, two products were obtained in the ratio of 5:1. The major product was identified spectroscopically as **1** and the minor one as **2**. Probably, hydride stereoselectively attacked the carbonyl carbon at C16 from the less hindered α-side to yield a β-hydroxy derivative as the major product. The results of $NaBH_4$ reduction of **3**, therefore, indicated not only that **3** is a 16-oxo-derivative, but also that **1** and **2** are 16β-hydroxy and 16α-hydroxy derivatives, respectively. Further evidence identifying the configurations at C16 of both **1** and **2** was their ^{1}H and ^{13}C NMR spectra. The ^{13}C NMR signals of C16 and C17 of **1** resonated at a higher field than those of **2**. Furthermore, the ^{1}H NMR signal due to 18-methyl resonated at a lower field and the 14- and 17-methine signals resonated at a higher field in **1** than **2**. These tendencies were in agreement with the data

reported on 2-alkyl hydroxycyclopentane and certain steroids.[8,9] On irradiation of 16-H, 2% of the NOE of the 18-methyl signal was observed in **2a**, but none was observed in **1a**. Thus, the configurations of the hydroxy group at C16 of **1** and **2** were β and α, respectively (Fig. 1).

The amounts needed for bitter taste of **1**, **2**, and **3** were 7, 5, and 5 μg, respectively (Table 3), which were almost equal to the amount needed of quinine sulphate. Vernonioside B_1 was not bitter even at 200 μg, suggesting that the presence of an oxygen atom at C16 may be important for the bitter taste. The glucosyl moiety was also important for bitter taste because aglycones **1a**, **2a**, and **3a** had small bitterness.

Table 3. Bitterness of Vernoniosides and Their Aglycones.

Compounds	Bitterness (μg)
1	7
1a	130
2	5
2a	150
3	5
3a	>200
4	>200
4a	>200
4b	>200

Acknowledgments

This study was supported by a Grant-in-Aid for General Scientific Research from the Ministry of Education, Science, and Culture, of Japan. We thank Dr. M. Nakagawa, Institute for Fundamental Research, Suntory,.Ltd. and Mr. H. Naoki, Suntory Institute for Bioorganic Research, for the ^{1}H-^{1}H COSY NMR at 500 MHz.

Experimental

General remarks

All melting points were measured on a Yanagimoto microapparatus and are uncorrected. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-200; IR, Shimadzu IR-435 (KBr); ORD, JASCO model J-5; ^{1}H and ^{13}C NMR, Brucker AC250 (250 MHz for ^{1}H, ref. TMS) and GE GN-500 (500 MHz for ^{1}H, ref. TMS); MS, JEOL JMS-DX300 (70 eV, 300 μA); HPLC was done with a μBondasphere C_{18} (ODS) column (Waters Associates Inc.). GC was done with an OV-1 column (FFS capillary column, 50 m x 0.24 mm i.d.). The bitterness was measured as the minimum amount for bitter taste by a filter paper method, in which a filter paper containing a known amount of test sample (1 cm^2, 0.2 mm, thick) was put on the tongue.[5]

Extraction of V. amygdalina *and isolation of* ***1, 2, 3, and 4***

Dried leaves of *V. amygdalina* (800 g), which had been collected and air-dried in 1987, were extracted with MeOH at room temperature for 7 days. The crude extract (94.9 g) was partitioned with *n*-hexane-MeOH-water (5:9:1) to yield a bitter lower layer. This portion was partitioned with EtOAc-water (1:1), and the bitter lower layer obtained (49.9 g) was further partitioned with water-saturated *n*-BuOH to yield a bitter *n*-BuOH-soluble part (17.6 g). The bitter part was chromatographed on Amberlite XAD-2 eluted stepwise with MeOH-water. The bitter eluate (80% MeOH) was rechromatographed on silica gel eluted stepwise with $CHCl_3$-MeOH to afford 7.5-10% MeOH eluates. The combined eluate was further

separated on silica gel 60H with toluene-acetone (2:3) under pressure to yield a bitter fraction (2.1 g). After being rechromatographed on ODS gel (MeOH-water/3:2), the bitter fraction was purified by HPLC on μBondasphere (acetonitrile-water/36:64, 8 ml/ min) to yield vernonioside A_1 (**1**, 21.2 mg, Rt 21.7 min), A_2 (**2**, 13.7 mg, Rt 19.0 min), A_3 (**3**, 31.2 mg, Rt 26.5 min) and B_1 (**4**, 217 mg, Rt 30.0 min). Vernonioside A_1 (**1**): Colorless needles, mp 244-246°C. $[\alpha]_D^{24}$ +35.2° (c 0.23, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1760. UV λmax (MeOH) nm (ε): 236 (12000), 243 (14000), 251 (9000). FABMS m/z: 633 (MH^+, $C_{35}H_{52}O_{10}$+H). Vernonioside A_2 (**2**): Colorless plates, mp 254-256°C. $[\alpha]_D^{22}$ +10.7° (c 0.30, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1760. UV λmax (MeOH) nm (ε): 235 (15000), 243 (17000), 250 (11000). FABMS m/z: 655 {($M+Na)^+$, $C_{35}H_{52}O_{10}$+Na}. Vernonioside A_3 (**3**): Colorless needles, mp 200-203°C. $[\alpha]_D^{24}$ -30.0° (c 0.25, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1775, 1740. UV λmax (MeOH) nm (ε): 235 (11000), 242 (12000), 250 (8000). FABMS m/z: 631 (MH^+, $C_{35}H_{50}O_{10}$+H). Vernonioside B_1 (**4**): Colorless needles, mp 208-211°C. $[\alpha]_D^{25}$ +31.9° (c 0.94, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1775. UV λmax: 235 nm (ε 13000), 243 nm (ε 15000), 250 nm (ε 10000). FABMS m/z: 633 (MH^+, $C_{35}H_{52}O_{10}$+H).

GLC analysis of sugar moiety of 4

Vernonioside B_1 (**4**) (100 μg) was hydrolyzed with 2N TFA (200 μl) at 125°C in a sealed tube for 1 hr. After the solvent was evaporated, the residue was dissolved in pyridine (20 μl), to which N,O-bis(trimethylsilyl)trifluoroacetamide (10 μl) was added. The mixture was left for 1 hr and the products were analyzed by GLC on OV-1 at 170°C. TMS-glucoses were observed at Rt 8.0 min and Rt 12.8 min.

Enzymatic hydrolysis

Vernonioside B_1 (10 mg) was dissolved in Triton X-100 (0.2 ml), and 50 mM sodium citrate-NaOH buffer (pH 5.0, 20 ml) and β-glucosidase (32 mg) were added. After incubation of the mixture at 35°C for 72 hr, the reaction products were extracted with *n*-BuOH (20 ml) three times. After chromatography of the *n*-BuOH-soluble part on silica gel and then ODS gel, the natural aglycone was recrystallized from EtOAc. **4a**: Colorless plates, mp 205-210°C. $[\alpha]_D^{25}$ +40.8° (c 1.03, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1760. UV λmax (MeOH) nm (ε): 236 (13000), 242 (14000), 250 (10000). ^{1}H NMR δ (pyridine-d5) ppm: 0.79 (3H, s), 1,00 (3H, s), 1.26 (3H, d, *J*=7.4 Hz), 1.30 (3H, d, *J*=7.4 Hz), 1.32 (3H, d, *J*=5.5 Hz), 1.45 (1H), 1.5 (1H), 1.51 (1H), 1.6 (1H), 1.62 (1H), 1.8 (2H), 1.9 (2H), 1.92 (2H), 1.99 (1H), 2.0 (1H), 2.1 (1H), 2.38 (1H), 2.39 (1H), 2.40 (1H), 2.44 (1H), 3.07 (1H, dd, *J*=9.9, 4.1Hz), 3.29 (1H, dd, *J*=17.9, 6.8 Hz), 3.64 (1H, q, *J*=5.6 Hz), 3.80 (1H, m), 4.79 (1H, br s), 5.00 (1H, d, *J*=2.4 Hz), 5.42 (1H, br s), 5.62 (1H, br d, *J*=6.2 Hz). HR-EIMS m/z: 470.3028 (M^+, calcd. for $C_{29}H_{42}O_5$, 470.3032). By the same procedure, three natural aglycones, **1a** (2.6 mg), **2a** (5.2 mg), and **3a** (2.6 mg), were obtained from 10 mg of each vernonioside and recrystallized from MeOH. **1a**: Colorless needles, mp 256-258°C. $[\alpha]_D^{28}$ +51.3° (c 0.08, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1760. UV λmax (MeOH) nm (ε): 236 (14000), 242 (1600), 251(11000). ^{1}H NMR δ (pyridine-d5) ppm: 1.02 (3H, s), 1.07 (3H, d, *J*=7.2 Hz), 1.21 (3H, s), 1.23 (3H, d, *J*=6.7 Hz), 1.29 (3H, d, *J*=7.1 Hz), 1.42 (1H), 1.55 (1H), 1.61 (1H), 1.79 (1H), 1.89(1H), 1.90(1H), 1.91 (1H), 1.93 (2H), 1.96 (1H), 2.02 (1H), 2.16 (1H), 2.22 (1H), 2.26 (1H), 2.27 (1H), 2.55 (1H, dt, *J*=12.6, 7.4, 7.4 Hz), 2.74 (1H, m), 2.99 (1H, q, *J*=5.5 Hz), 3.16 (1H, dd, *J*=17.6, 6.6 Hz), 3.39 (1H, m), 3.86 (1H, m), 4.65 (1H, m), 4.90 (1H, dd, *J*=10.2, 5.9 Hz), 5.48 (1H, br s), 5.63 (1H, br d, *J*=6.1 Hz). HR-EIMS m/z: 470.3014 (M^+, calcd. for $C_{29}H_{42}O_5$, 470.3032). **2a**: Colorless plates, mp 229-231°C. $[\alpha]_D^{19}$ +34.5° (c 0.26, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1760. UV λmax (MeOH) nm (ε): 235 (14000), 242 (17000), 251 (11000). ^{1}H NMR δ (pyridine-d5) ppm: 0.79 (3H, s), 0.98 (3H, s), 1.08 (3H, d, *J*=7.2 Hz), 1.19 (3H, d, *J*=5.5 Hz), 1.29 (3H, d, *J*=7.1 Hz), 1.38 (1H), 1.53 (1H), 1.60 (1H), 1.76 (1H), 1.87 (1H), 1.90 (2H), 1.96 (2H), 2.13 (1H), 2.17 (2H), 2.32 (1H), 2.39 (1H), 2.42 (1H), 2.54 (1H, m), 2.81 (1H, dd, *J*=17.3, 6.7 Hz), 2.86 (1H), 3.01 (1H, q, *J*=5.5 Hz), 3.07 (1H, dt, *J*=12.1, 7.6, 7.6 Hz), 3.84 (1H, m), 4.58 (1H, m), 4.93 (1H, dd, *J*=10.7, 5.5 Hz), 5.45 (1H, br s), 5.58 (1H, br d, *J*=5.6 Hz). HR-EIMS m/z: 470.3038 (M^+, calcd. for $C_{29}H_{42}O_5$, 470.3033). **3a**: Colorless needles, mp 228-230°C. $[\alpha]_D^{26}$ -12.5° (c 0.12, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1770, 1740. UV λmax (MeOH) nm (ε): 234 (11000), 242 (12000), 250 (8000). ^{1}H NMR δ (pyridine-d5) ppm: 0.76 (3H, s), 0.96 (3H, s), 1.04 (3H, d, *J*=7.2 Hz), 1.24 (3H, d, *J*=5.7 Hz), 1.26 (3H, d, *J*=7.3 Hz), 1.43 (1H), 1.50 (1H), 1.65 (1H), 1.80 (1H), 1.88 (2H), 1.89 (1H), 1.95

(1H), 2.00 (1H), 2.19 (1H, dd, *J*=18.2, 12.8 Hz), 2.20 (1H), 2.34 (2H), 2.40 (2H), 2.47 (1H), 2.70 (1H), 3.06 (2H), 3.17 (1H, q, *J*=5.6 Hz), 3.84 (1H, m), 4.89 (1H, t, *J*=8.1 Hz), 5.35 (1H, br s), 5.62 (1H, br d, *J*=5.4 Hz). HR-EIMS m/z: 468.2859 (M^+; calcd. for $C_{29}H_{40}O_5$, 468.2876).

Hydrolysis of 4 with HCl

To **4** (8.9 mg) in EtOH (0.2 ml), 2.0N HCl (5 ml) and toluene (5 ml) were added. After the solution was refluxed for 6 hr, the products in the upper layer were purified by preparative TLC (silica gel plate, toluene-MeOH/8:3) to yield an aglycone (**4b**, 3.8 mg). **4b**: Colorless needles, mp 259-261°C. $[\alpha]_D^{28}$ +13.3° (c 0.24, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1775. UV λmax (MeOH) nm (ε): 250 (16000). ^{1}H NMR δ (pyridine-d5) ppm: 1.02 (3H, s), 1.12 (3H, d, *J*=7.1 Hz), 1.16 (1H), 1.17 (3H, s), 1.25 (3H, d, *J*=6.6 Hz), 1.33 (3H, d, *J*=6.6 Hz), 1.37 (1H), 1.44 (2H), 1.54 (1H), 1.64 (1H), 1.72 (2H), 1.87 (1H), 2.07 (1H), 2.11 (1H), 2.12 (1H), 2.24 (3H), 2.38 (1H), 2.44 (1H), 2.54 (1H), 2.88 (1H), 2.98 (1H, br d, *J*=13.2 Hz), 3.11 (1H, dd, *J*=10.0, 6.5 Hz), 3.85 (1H, m), 4.31 (1H, q, *J*=6.4 Hz), 4.89 (1H, dd, *J*=6.5, 4.7 Hz), 4.96 (1H, d, *J*=4.7 Hz), 5.44 (1H, br s).

Reduction of 3 with $NaBH_4$

To **3** (11.5 mg) in MeOH (2 ml), $NaBH_4$ (2 mg) was added. The mixture was left for 5 min while being stirred, and diluted acetic acid (about 20%, 1 ml) was added. After the solvent was evaporated, the products were extracted with *n*-BuOH. Purification of the *n*-BuOH extract by preparative HPLC on μBondasphere C_{18} (acetonitrile-water/35: 65) yielded vernonioside A_1 (**1**, 3.7 mg, R_t 32.0 min) and A_2 (**2**, 1 mg, R_t 28.4 min).

X-ray diffraction analysis of 4a

Crystal data of the single crystal used for X-ray analysis were as follows: monoclinic, space group $p2_1$, a=17.082(1), b=12.236(1), c=6.230(1) Å, β=92.81°(1), V=1301.0 $Å^3$, Z=2, Dx=1.202 g/cm^3. Intensity data of 2179 diffractions within 2Ø<130° were collected by a Rigaku AFC-5UD four-circle diffractometer with graphite-monochromated CuKα radiation (λ=1.54178 Å), and 2179 unique reflections with Fo>3σ (F) were used for the structure determination by a MULTAN 84 program system[10] (Rint=0.050). The perspective view is shown in Fig. 2.

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African Study Monographs, 14(4): 227–240, December 1993

FURTHER OBSERVATIONS ON THE USE OF THE MEDICINAL PLANT, *Vernonia amygdalina* (Del), BY A WILD CHIMPANZEE, ITS POSSIBLE EFFECT ON PARASITE LOAD, AND ITS PHYTOCHEMISTRY

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ABSTRACT This is the second detailed case study of the use of *V. amygdalina* (Del) by a wild chimpanzee suffering from gastrointestinal upset (flatulence and diarrhea). The female, who was followed for approximately 5 hours over a two-day period, recovered from her symptoms by the afternoon of the second day. Laboratory examination of two fecal samples, one collected approximately 1 hour and another 20.5 hours after ingestion of the plant's bitter pith, revealed a notable drop in the degree of parasitic infection by a *Ternidens* sp. Bioassay of the plant consumed by the female confirmed that the two most abundant and bioactive constituents, vernodalin and vernonioside B_1, were present. Vernonioside B_1 was found to occur at significant levels in both the leaves and pith, but the cytotoxic vernodalin was found only in the leaves. This suggests that vernonioside B_1 and its naturally occurring aglycones are likely to be the bioactive constituents ingested by chimpanzees. The estimated amount of vernonioside B_1 ingested by this female was found to be approximately equal to the amount contained in a traditional Tongwe medicinal preparation from a cold water extract of the leaves to treat similar gastrointestinal disorders in adult human patients. This report provides new evidence for the effectiveness of medicinal plant use in primates and strongly supports the current hypothesis regarding the use of *V. amygdalina* for the control of symptoms from parasitic and gastrointestinal illness by wild chimpanzees.

Key Words: *Pan troglodytes schweinfurthii*; Medicinal plants; *Vernonia amygdalina* Del.; Compositae; Parasite load; *Ternidens* sp.; Phytochemistry.

INTRODUCTION

In primates, and the chimpanzee in particular, various sources of evidence suggest that certain toxic plants are selected for their medicinal value (Huffman & Wrangham, in press).

The use of *Vernonia amygdalina* Del., by chimpanzees for its medicinal value

was first determined from the detailed observations of an ailing female's ingestion of this widely recognized African ethnomedical plant in the Mahale Mountains National Park, Tanzania (Huffman & Seifu, 1989). The symptoms displayed by this female were apparent lack of appetite, malaise, constipation and unusually dark colored urine. She meticulously removed the leaves and outer bark from several young shoots of *V. amygdalina* and chewed on the exposed piths, ingesting only the extremely bitter juice. Within 24 hours, she had regained her appetite and fully recovered from malaise and constipation (Huffman & Seifu, 1989).

At Mahale, there is a trend for greater chimpanzee use of *V. amygdalina* during the rainy season despite year round availability (Huffman et. al., 1990). During the rainy season, the number of chimpanzees with detectable parasitic infections also tends to increase, as does the number of different parasite species per individual (Huffman et al., 1990, in prep.).

Based on the female's symptoms and the ongoing investigation of parasitic infection in Mahale M group chimpanzees, it was hypothesized that the use of this plant by chimpanzees aid in the relieve gastrointestinal illness or parasite-related disease (Huffman et al., 1990, 1992).

Subsequent quantitative analysis and assays of the biological activity of *V. amygdalina* specimens (pith, bark, leaf, root) collected from Mahale have revealed the presence of two major classes of bioactive compounds in the plant: the sesquiterpene lactones and the steroid glucosides (Ohigashi et al., 1991a; Jisaka et al., 1992a, in press). The most abundant of these constituents, the sesquiterpene lactone vernodalin, and the steroid glucoside vernonioside B_1, have been shown to possess antibiotic, anti-parasitic, anti-amoebic, and anti-tumor properties (Asaka et al., 1977; Gasquet et al., 1985; Jisaka et al., 1992 a, b; Jisaka et al., 1993; Kupchan et al., 1969). Recently, *in vitro* anti-schistosomal (*Schistosoma japanicum*) activity tests have shown vernodalin, vernonioside B_1 and its naturally occurring aglycones to be the most bioactive constituents isolated from this species to date (Jisaka et al., 1992b). *In vivo* tests of vernodalin on schistosome-infected mice showed it to be lethal to the parasite at more than 5 mg. (120 mg./kg), but a lower dose (2.5 mg.) had no great effect on the parasite (Jisaka et al., 1992b). While similar *in vivo* tests on vernonioside B_1 are still being conducted, previous *in vitro* tests have failed to find any significant cytotoxicity in this compound, which suggests that vernonioside B_1's biological activity is of a different nature than that of vernodalin (Takaoka, pers. comm.).

Preliminary quantitative analyses of a specimen collected at Mahale showed that both vernodalin and vernonioside B_1 occurred in the leaves at significantly high levels (2.18 mg and 0.61 mg/g fresh leaves, respectively). However, while vernonioside B_1 occurred at comparably high levels in the pith (0.75 mg/g fresh pith) vernodalin occurred at levels low enough to be considered insignificant (0.02 mg/g fresh pith) (Jisaka et al., 1992b). It was then suggested that a key to understanding why chimpanzees select the pith rather than the leaves of *V. amygdalina* may lie in the *avoidance* of the highly toxic vernodalin in favor of the equally bioactive, less host-toxic vernonioside B_1 (Jisaka et al., 1992b).

To date, no direct evidence from fecal samples or plant material collected concurrently with observations of the use by chimpanzees of *V. amygdalina*, or any other

medicinal plant, have been available to test these hypotheses. In this paper, behavioral, parasitological, and phytochemical data collected during and subsequent to a recent observation at Mahale of another apparently ill adult female chimpanzee's use of *V. amygdalina* are presented. These data are discussed in relation to the above hypotheses concerning the possible use of the plant by chimpanzees for the control of parasite and/or other gastrointestinal illness.

MATERIAL AND METHODS

Observations were conducted on the M group of chimpanzees in the Mahale Mountains National Park, Tanzania, between October 1991 and January 1992.

Situated on the eastern shore of Lake Tanganyika, the study area's climate is influenced by weather from the lake and the mountainous terrain, which ranges from 772 m to 2,000 m above sea level. Chimpanzees are supported mainly by the semi-deciduous gallery forests between 780–1,300 m above sea level. The year is divided into two distinct seasons. The rainy season lasts from around mid-October to mid-May, during which rain falls an annual average of 1,800 mm (Nishida, 1990; Takasaki et al., 1990). For further description of the study group and site, see Nishida (1990).

In order to begin the systematic study of medicinal plant use in chimpanzees and to test hypotheses developing from this research, a multi-disciplinary research group called The C.H.I.M.P.P. Group (The Chemo-ethology of Hominoid Interactions with Medicinal Plants and Parasites) has been established and includes specialists in ethology, natural plant products, chemistry, parasitology, pharmacognosy, pharmacology, and traditional medicine (Huffman et al., 1992).

Focal-animal observations were conducted by M.A.H. with the assistance of M.S.K., following focal individuals for as long as possible, recording all social interactions, basic activity patterns, and feeding behavior. Visible cues to an individual's state of health were noted, specifically, stool type, urine color, sinus and respiratory congestion, etc.

During these observations, infrequently used chimpanzee "plant food items" observed to be ingested in a peculiar manner (e.g. for no apparent nutritional gain) or under unusual circumstances (e.g. associated with sickness) were collected. Most plant species utilized were identifiable by the local Kitongwe/Kiswahili vernacular and Latin names (Nishida & Uehara, 1983). Identification of some unknown species were made by G. Mwachala of the East African Herbarium, National Museums of Kenya.

The presence of possible phytochemical activity or ethnomedical use were investigated by cross-referencing species with the medicinal and poisonous plant literature of Africa (e.g. Watt & Breyer-Brandwijk, 1962; Kokwaro, 1976; Burkhill, 1985; Abbiw, 1990). When available, details of local Tongwe ethnomedical use was provided by M.S.K. and two other knowledgeable local informants, R. Nyundo and R. Kasakampe. Promising species were collected in bulk, dried thoroughly, and brought back to Kyoto University for a preliminary screening test of possible biological activity (Ohigashi et al., 1991b, 1993).

During focal-animal and *ad libitum* observations, fecal samples, when available, were collected immediately after discharge and stored individually in 5.0 ml Corning sterile cryogenic vials. At camp, the vials and contents were weighed and 1 gram fecal samples were fixed with a 10% formalin solution. The contents were thoroughly mixed in the vials before being sealed and stored in a cool dark room until transport to the laboratory, where they were examined by S.G. within 6 months after collection.

Each sample was microscopically checked for the presence of parasite eggs, and when present, species or genus level identification was made. Parasite loads were also measured using the McMaster's technique (expressed as eggs/g feces). Egg counts for each fecal sample were carried out three times and the parasite load for each sample was calculated as the mean value derived from those trials.

During the study, as part of an investigation on the chemical ecology and pharmacology of *V. amygdalina*, fresh samples from young shoots of this species (the part utilized most frequently by chimpanzees) were collected by M.A.H. every two weeks from three specific trees. On each sample day, one young shoot from each tree was collected and divided into three parts: young distal leaf, young branch (pith included), and older branch sections (pith included). Two, 2 gram samples of each part where placed separately into small glass specimen bottles, one containing methanol (MeOH) and the other acetone (Me_2CO), for preservation and later extraction and quantitative analysis of vernodalin and vernonioside B_1 in the laboratory. The samples were then stored in a cool dark place.

In this paper, plant materials collected during the first and last sample periods (October 27 and December 22) were used to assess possible seasonal differences in the relative abundance of the major bioactive compounds. These samples were used to represent the end of the dry season and mid-rainy season states of this species in the study area.

In the laboratory, quantitative analysis of the two most prevalent bioactive constituents, vernodalin and vernonioside B_1, were conducted by D.I. After Jisaka et al. (1992b), the Me_2CO extract was used for the analysis of vernodalin and the MeOH extract for that of vernonioside B_1. Each extract was concentrated to 10 ml, and 4 ml of this solution was poured onto Cosmosil 14OC_{18}-OPN gel (Nacalai Tesque, approximately 1g). After the excess solvent was removed *in vacuo*, the gel was transferred into a syringe (7 cm × 0.8 cm i.d.) equipped with a Sep-Pak C_{18} cartridge (Waters) at the outlet side. The components absorbed on the Cosmosil gel were successively eluted with 10 ml of water, 10 ml of 90% acentonitril (CH_3CN), and 100% CH_3CN under syringe pressure. The 90% CH_3CN eluate was concentrated, then redissolved with 1 ml of CH_3CN, and filtered with an H-13-5 filter (TOSOH) and again filled up to 2 ml with CH_3CN. Five μl of this solution was analyzed for vernodalin using HPLC [AQ-301, Column; ODS, 4.6 × 100 mm (YMC), CH_3CN-H_2O (25 : 75), 1 ml/min.] detected by the absorption intensity at UV_{220}. By the same procedure, a 90% aq. MeOH eluate was obtained from a sample of the MeOH extract and analyzed by HPLC [CH_3CN-H_2O (33 : 67)] detected for vernonioside B_1 by the absorption intensity at UV_{254} (Jisaka et al., 1992b). This procedure was repeated for a portion of shoot from the same plant ingested by the adult female reported here.

V. amygdalina seeds collected at Mahale were germinated and raised in the experimental green house of the Department of Agriculture, Kyoto University and in the home of K.K. Samples were obtained from these plants to determine average fresh weights of plant parts and for analytical comparison of the occurrence of vernodalin and vernonioside B_1 in fresh (mg/g sample f.W.) versus methanol and acetone extract states (μg/mg extract). There was no strong tendency for amounts of these compounds to vary according to state, and, therefore, for the following analyses, all measurements taken from extraction or fresh samples are expressed in the common unit mg/g fresh weight.

RESULTS

I. Behavioral Observations

On December 23–24, 1991, an adult female, FT (Fatuma: born circa 1963), was observed for a total of 5 hr. 4 min. The observations were split into two periods as follows: day one (14:37–18:15; 208 min.) and day two (09:32–11:47; 96 min.).

On day one at approximately 14:17, FT was observed by field assistant H. Bunengwa and researcher H. Yoshida to ingest the juice and some fibrous material from the piths of two shoots of *V. amygdalina* (Bunengwa & Yoshida pers. comm.). At approximately 14:33, Bunengwa made verbal contact with M.A.H. and M.S.K., and directed us to the plant used by FT. Two shoots were discarded, and there were distal young strips of bark and leaves left intact and the unchewed proximal portions of the shoot. These two shoots were each approximately 1 cm in diameter, 30 cm in length. A shoot of similar size and maturity from the same tree was collected for further study.

At 14:37, FT was located and focal observations begin. FT and her male infant PM (Pim: born 1988. 2) were traveling with a mixed sub-group of at least 10 members.

During these observations, gastrointestinal upset was evidenced by profuse flatulence and uncontrolled, yellow, liquid stools. FT's urine was clear in color. At 15:22, 1 hr. and 5 min. after ingesting *V. amygdalina*, a fecal sample was collected.

During this day's observations, FT spent 17% of her time resting, once in an elaborately made day-bed in a tree and the rest of the time on the ground. FT's infant PM frequently wandered out of view along with older playmates who solicited him to follow. At such times, FT, apparently waiting until the last possible moment, moved to maintain minimum visible contact. With frequent pauses to rest (36%) she slowly followed PM. When the pair reunited, FT groomed or was groomed by either the infant or his play-partners, which resulted in a few minutes more rest for FT. All of FT's grooming occurred in this context and accounted for 12% of her activity. The remaining 33% of her time was spent intermittently foraging on common food items: *Aframomum* sp. stalks, *Ficus exasperata* Vahl leaves, *Saba florida* (Benth.) Bullock fruits, and a small amount of clay from a termite mound. At 18:15 observations were discontinued.

On day two, members of the previous day's sub-group were located at 08:55 and at 09:32 FT and PM were located in a dense forest thicket with the sub-group, approximately 700 m south of where they were last sighted on the previous evening.

Of the total observation time (96 min.), FT spent 65% of it resting, and 10% grooming. Between rests she spent 23% of her time foraging on *Aframomum* sp. stalks, *Ficus* sp. leaves, and a small amount of clay from a termite mound.

FT's general condition appeared to have improved from the previous day and her stools, although small, were now solid. Fecal samples were collected at 10:55. Focal observations were discontinued at 11:47 when it became impossible to follow her through rough terrain.

At 13:03, 22 hrs. 46 min. after she was first observed to ingest *V. amygdalina*, she was relocated and identified as the capturer of an adult red colobus monkey. She maintained possession and consumed much of the carcass, despite attempts by two adult males, BA and AJ, to take it from her.

II. Parasitology

According to the analyses of four fecal samples collected from FT between October 29 and December 24, she was found to be harboring two intestinal nematode species, *Trichuris trichiura*, a *Ternidens* sp., and a protozoan commensulate *Troglodytella abrassarti*. Inspection of the two samples collected on December 23 and 24 (20 hr. 38 min. apart), after FT's ingestion of *V. amygdalina* pith, revealed a drop in the *Ternidens* sp. egg count from 130 to 15 eggs/g feces.

Table 1 compares this data for FT with changes in the level of *Ternidens* sp. infection over time with those of 7 other individuals sampled during this study period in which feces containing *Ternidens* sp. eggs occurred on more than one day sampled.

The decrease in egg count observed for FT following ingestion of the pith of *V. amygdalina* was not seen in other cases where multiple samples of the same individual (not observed to ingest this plant) were taken one to several days apart. A decrease was detected in only two (adult males NS, AJ) of the other 7 individuals, for which *Ternidens* sp. data was available (Table 1). For NS, a drop from 20 to 5 eggs/g feces was detected over a 10 day period. Such changes were not considered significant, as they were more likely due to the low level of infection and thus low density of eggs per sample. This is also considered to be the case in those samples in which no eggs were detected in individuals who had tested positive for *Ternidens* sp. at earlier or later trials.

On the other hand, for AJ, the apparently most ill and most seriously infected individual monitored during this study period, a total drop of 510 *Ternidens* sp. eggs/gram feces was detected over an 18 day period (Table 1). During focal observations AJ was not seen to use *V. amygdalina*; his steady recovery, however, may have been associated with extended feeding bouts on the young leaves of *Ficus exasperata* on several days during the period in which he appeared to be the sickest, and showed the highest levels of parasitic infection. The young leaves of this plant contain 5-methoxypsoralen, a well-known furanocoumarin. The young leaves have been estimated to posses effective nematocidal activity when consumed in

Table 1. Trends in level* of *Ternidens* sp. infection in wild chimpanzees of Mahale M Group at the onset of the 1991–92 rainy season.

name	age/sex class	date	infection level (eggs/gram feces)
AJ	adult male	Nov. 1	10
		Nov. 13	15
		Nov. 28	530
		Dec. 16	170
		Dec. 25	20
BA	adult male	Nov. 4	35
		Nov. 16	—
		Nov. 22	270
		Dec. 13	—
BO	old adult female	Oct. 29	<5
		Nov. 11	20
		Nov. 15	—
FT	adult female	Oct. 29	—
		Nov. 19	—
		Dec. 23	130
		Dec. 24	15
NS	adult male	Nov. 2	—
		Nov. 4	20
		Nov. 13	5
SL	adult female	Nov. 16	<5
		Nov. 18	—
		Jan. 6	45
TB	adult male	Nov. 4	—
		Nov. 7	5
		Nov. 16	—
		Jan. 6	33
WL	adult female	Oct. 28	10
		Nov. 27	—
		Dec. 2	140

* infection rate: eggs/g feces «McMasters technique»; eggs/g feces; —; not detected.

large quantities of 50–100 leaves (Rodriguez & Wrangham, 1993).

For other individuals sampled, it was more often the case for *Ternidens* sp. egg counts to increase over time. The average increase was 69.9 eggs/g feces (S.D.=84, range 5–236, n=7). This increase was highly variable between individuals, but the number of days between samples did not appear to affect the degree of measured increase (Table 1). These increases appear, rather, to reflect the frequently observed trend for an increase in parasite infection levels and the number of parasite species infections per individual during the rainy season (Huffman et al., 1990; Kawabata & Nishida, 1991; Huffman et al., in prep.).

III. Phytochemistry

Quantitative analyses revealed a difference in the relative distribution of ver-

Table 2. Comparison of the relative distribution and abundance of two bioactive compounds, vernonioside B_1 and vernodalin, extracted by plant part from *Vernonia amygdalina* specimens collected in the Mahale Mountains National Park.

sample	compound	young leaf	young stem mg/g fresh weigh	old stem	
FT	vernonioside B_1	0.51	0.38	0.08	
	vernodalin	0.47	0.00	0.00	
A, B, C	vernonioside B_1	1.19	0.69	0.61	mean value
		(0.82)	(0.32)	(0.21)	S.D
	vernodalin	1.19	0.20	0.11	
		(0.47)	(0.15)	(0.10)	

A sample of the shoot used by FT was collected on December 26, and shoot samples from trees A, B, and C were collected on October 27 and December 22, 1991.

nonioside B_1 and vernodalin among the test samples A, B, C, and the shoot from the plant ingested by FT (Table 2, 3). The reason for this is not known, but it can be speculated that this is due to either individual differences in plants sampled from different habitats or the longer lapse in time between collection and extraction of the FT sample as compared to that of A, B, C. However, in all samples, vernodalin occurred at relatively high levels in the young leaf but at very low levels in the young and old stem portions. Vernonioside B_1, on the other hand, occurred at equally high levels in the young leaf portion and at lower, yet still significant levels in the young and old stem portions.

Comparing the October 27 and December 22 sample sets (Table 3), there was a noticeable, general increase in the relative abundance of vernonioside B_1, particularly in the leaves. On the other hand, a relative decline in the abundance of vernodalin, especially in the young and old stems, was noted. Interestingly, vernodalin was completely absent from the stem portion of FT's sample, strongly sug-

Table 3. Comparison of the relative distribution and abundance, by plant part, of two bioactive compounds vernonioside B_1 and vernodalin, extracted from *Vernonia amygdalina* specimens collected on October 27 (I) and December 22 (II), 1991 in the Mahale Mountains National Park, Tanzania.

sample	young leaf	young stem mg/g fresh weight	old stem
vernonioside B_1			
I. A	0.47	0.37	0.36
B	0.59	0.60	0.39
C	0.58	0.53	0.57
II. A	1.76	0.66	0.64
B	2.54	1.32	0.93
C	1.18	0.66	0.75
vernodalin			
I. A	1.82	0.51	0.06
B	2.77	0.16	0.31
C	1.91	0.17	0.06
II. A	1.47	0.15	0.06
B	1.54	0.07	0.03
C	1.97	0.16	0.14

gesting vernonioside B_1 to be the most likely of the two compounds responsible for the observed effect on parasite load.

From a pharmacological standpoint, it would be valuable to compare the amount of active ingredients contained in a 'standard dose' taken by humans and that contained in the pith ingested by FT. Because neither the chimpanzees nor the Tongwe 'prepare' their medicine from methanol or acetone plant extracts, the rough estimation which follows is based on the amounts yielded by extraction with water.

One traditional Tongwe use of *V. amygdalina* in the treatment of parasitosis or gastrointestinal upset is the preparation of a cold water extract using 2–3 crushed leaves (approximately 10–15 g f.w.) in 300–400 ml of water. An analysis replicating this traditional method using fresh *V. amygdalina* leaves (3 trials) yielded 3.3–5.0 mg of vernonioside B_1. Based on the data in Table 2, the pith used by FT (60 cm, approximately 50–100 g f.w.; see methods) was estimated to have yielded roughly 3.8–7.6 mg vernonioside B_1 (cold water extraction yielded approximately 20% that of methanol extraction, Izutsu, 1993), or roughly an amount equal to that of the normal dosage prescribed to an adult Tongwe patient. In the future, a more detailed analysis will be necessary to make closer comparison.

DISCUSSION

Previous studies have discussed a variety of behavioral adaptations for combatting parasitosis and other unspecified diseases in primates. These behaviors include the alternation of sleeping sites and feeding sites (Freeland, 1980; Hausfater & Meade, 1982; McGrew et al., 1989) and the ingestion of certain plants, possibly for their pharmacological effect (Hamilton et al., 1978; Wrangham & Nishida, 1983; Phillips-Conroy, 1986; Takasaki & Hunt 1987; Huffman & Seifu, 1989; Huffman & Wrangham, in press).

The latter studies cite a variety of supporting evidence, such as ethnomedical and pharmacological information on bioactive compounds in a plant, its use restricted to certain habitats or seasons where risk of parasitic infection is higher than in other habitats or seasons, and observations of its use by ill individuals.

For example, Phillips-Conroy (1986) reported that *Papio* spp. in Ethiopia living in areas of high susceptibility to the contraction of schistosomiasis ingest the berries of *Balanites aegyptiaca* (L.) Delile, whereas the *Papio* spp. in non-risk areas do not. Based on these observations, she hypothesized that the baboons selected these berries for their medicinal purposes, and identified a steroidal saponin, diosgenin, as the likely agent responsible. Phillips-Conroy & Knopf (1986) tested this hypothesis by feeding diosgenin to schistosome-infected mice. However, instead of decreasing the infection, they found that the diosgenin-altered hormonal environment of the mice actually augmented their vulnerability to the disease, resulting in significantly increased levels of infection.

Another well-investigated example is that of 'leaf swallowing behavior' in chimpanzees which was first reported in detail by Wrangham (1975, 1977) and then by Wrangham and Nishida (1983) from the perspective of its possible non-nutritive

value. The rough surfaced leaves of *Aspilia mossambicensis* (Oliv.), *A. pluriseta* (O. Hoffm.) Wild, and *A. rudis* Oliv. & Hiern are usually selected one at a time and placed into the mouth, whereupon they are not chewed but swallowed whole. In addition to this peculiar method of ingestion, the presence of a powerful bioactive compound, the dithiane polyine thiarubrine A, in the leaves of *A. mossambicensis* and *A. pluriseta* was suggested to be of possibly anti-helmintic value to chimpanzees (Rodriguez et al., 1985; Wrangham & Goodall, 1989). However, Page et al. (1992) was able to confirm the presence of thiarubrine A only in the roots, not in the leaves, of these plants collected from both Mahale and Gombe. Page et al. (1992) succeeded, instead, in isolating two bioactive compounds, kaurenoic and grandiflorenic acid from the leaves of *A. mossambicensis* at Mahale. The anti-parasitic activity of these compounds are unknown.

Where previous studies have been unsuccessful, the present study provides three new types of key evidence, which are as follows: verification of the presence of a bioactive constituent(s) from a sample of the individual plant actually ingested, a clinically measured biological effect (decrease in parasite load) after ingestion, and a quantitative chemical comparison of the amount of active constituent(s) ingested by the study subject with a prescribed dose known to be effective in relieving symptoms in humans.

The observed decrease in the number of *Ternidens* eggs/g of FT's feces over the 20 hour period differed markedly from the other samples collected during this study. These results provide further support for the hypothesis that the consumption of *V. amygdalina* by chimpanzees aids in the relief of gastrointestinal or parasite-related disease (Huffman et al., 1990, 1992).

We do not rule out the possibility that factors other than the detected *Ternidens* sp. infection could have also affected FT's observed condition. However, infection by *Ternidens* can result in ulceration and cystic nodules on the walls of the large bowel where this parasite is found in the host (Beaver et al., 1984), resulting in overall gastrointestinal discomfort. FT's symptoms were consistent with this type of disease. These results do suggest that the ingestion of the bitter juices within the pith of *V. amygdalina*, which are known to contain active anti-parasitic properties *in vitro*, may also have some similar effect in chimpanzees.

Due to the unlikely event of complete eradication of parasites from the host, it is proposed that as a result of the ingestion of such plants, chimpanzees are in effect relieving the symptoms of illness by controlling the number of parasites carried, keeping parasitic infection and its effects on the health of the host to a minimum. Continued monitoring of the behavior and parasite levels of individuals with histories of *V. amygdalina* use over one season is one possible way of testing this hypothesis.

Although preliminary, these observations are in agreement with a previous report of another apparently ill adult female chimpanzee's use of *V. amygdalina* at Mahala (Huffman & Seifu, 1989). In both cases, a visible improvement in the condition of health (e.g. appetite, physical strength, urine and/or stool quality) was apparent within 20–24 hours after observed ingestion of the plant. This recovery time is similar to that recognized for local human inhabitants, the Tongwe, who use a cold water extract prepared from leaves of the same species for 'parasitosis'

or gastrointestinal upset (Huffman & Seifu, 1989).

While it was estimated that FT ingested an amount almost equal to that of the normal dosage prescribe to a Tongwe patient, the estimated figure may be slightly inflated because the above calculation is based on yield by extraction in water, not by chewing. Nonetheless, by this preliminary comparison, it becomes apparent that the amount of vernonioside B_1 ingested by FT is comparable to the effective dose traditionally prescribed for humans.

Investigations to determine the overall biological activity and possible synergistic effect of the primary aglycones of vernonioside B_1, found to occur in the pith, are now underway. Using a number of parasite species known to infect chimpanzees and humans as models, this information will provide a better understanding of the pith's pharmacological effect for chimpanzees, and will hopefully contribute to the ongoing search for readily available, natural plant-based antiparasitic agents useful to humans living in tropical regions of the world.

ACKNOWLEDGMENTS We wish to express our sincerest gratitude to the staff of the Tanzanian National Scientific Research Council, Tanzanian National Parks, Serengeti Wildlife Research Institute, the Mahale Mountains Wildlife Research Centre, and the National Museums of Kenya's East African Herbarium for their hearty support and generosity. Also we extend our deepest gratitude to H. Bunengwa, J. Itani, R. Kasakampe, G. Mwachala, T. Nishida, R. Nyundo, H. Ohigashi and H. Yoshida for their generous support, suggestions, and advise during various stages of the research and writing of the manuscript. We gratefully acknowledge the assistance of H. Takaoka and fellow staff at the Institute of Life Science of Snow Brand Milk Products Co. Ltd., for conducting the cytotoxicity tests and other assays on Vernonioside B_1. We thank H. Ohigashi and L. Turner for their valuable comments on the manuscript. Field research by M.A.H. in 1990–91 was supported by a grant under the Monbusho International Scientific Research Program to T. Nishida (#03041046) and in part during the preparation of this manuscript in 1992 by a Post-doctoral Fellowship from the Japan Society for the Promotion of Science. K. Koshimizu and D. Izutsu were supported in 1991–92 by a Grant-in-Aid for Monbusho International Scientific Research Program to K. Koshimizu (#03041048).

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————Accepted *October 29, 1993*

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International Journal of Primatology, Vol. 14, No. 2, 1993

Physiological Activities and the Active Constituents of Potentially Medicinal Plants Used by Wild Chimpanzees of the Mahale Mountains, Tanzania

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Received December 3, 1990; revised January 29, 1991

Potential medicinal plants for wild chimpanzees have been studied in order to discover their physiologically active compounds. Tests of the physiological activity of 3 plant species—Vernonia amygdalina, Aspilia mossambicensis, *and* Ficus exasperata—*indicate that they contain a variety of active compounds. From one species,* V. amygdalina, *an antitumor agent and 2 possible antitumor promoters are identified. Furthermore, steroid glucosides were isolated as the bitter substances. These structurally new compounds are expected to exhibit a number of significant physiological activities. The chemical investigation of possible medicinal plants used by chimpanzees should be helpful in recovering naturally occurring compounds of medicinal significance for human use.*

KEY WORDS: *Vernonia amygdalina; Pan troglodytes schweinfurthii*; medicinal plants; bitter substance; antitumor agent.

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0164-0291/93/0400-0345$07.00/0

INTRODUCTION

Recently, observations on the consumption of certain plants for possible medical purposes have been reported for primates. For example, leaves and berries of *Balanites aegyptica* are eaten by *Papio hamadryas* and *P. hamadryas* × *P. anubis* hybrids as a prophylactic agent against schistosomiasis (Phillips-Conroy, 1986). Wrangham and Nishida (1983) reported on the peculiar habits of feeding on *Aspilia* spp. (Compositae) by chimpanzees (*Pan troglodytes schweinfurthii*). At Gombe Stream and Mahale Mountains National Parks, Tanzania, they observed that the leaves of three species of *Aspilia* were swallowed slowly, without chewing. A similar chimpanzee feeding habit was reported for *Lippia plicata* (Verbenaceae) (Takasaki and Hunt, 1987). Subsequent chemical analysis of *Aspilia mosambicensis* revealed the presence of a bioactive compound, thiaburine A (Rodriguez *et al.*, 1985). It showed wide biocidal activity, including antimicrobial, nematocidal, and cytotoxic effects. These findings led researchers to conclude that the consumption of *Aspilia* spp. by chimpanzees may be of medicinal value, possibly as an anthelminthic (Wrangham and Goodall, 1989).

Another potentially medicinal plant, *Vernonia amygdalina* (Compositae), is a shrub which occurs widely throughout tropical Africa. Recently, Huffman and Seifu (1989) reported that an adult female chimpanzee consumed *V. amygdalina* in the Mahale Mountains National Park. She chewed the pith of several shoots of the plant, ingested the bitter juice, and spit out the fibrous remains. She appeared to be ill at the time. She spent more than 50% of her time lying in day beds or on the ground. This activity pattern was abnormal compared with that of a healthy individual traveling with her and with the usual patterns recorded for other adult females in the group during the same season. She also exhibited loss of appetite and irregularity of urination and defecation. However, within 23 hr after ingesting *Vernonia*, she recovered gradually from lethargy and loss of appetite. By afternoon of the second day her health had improved greatly, and she spent extensive time traveling and/or foraging.

Local people who use a cold water infusion of *V. amygdalina* for intestinal colic, report that it takes ≤24 hr before she or he feels better (Huffman and Seifu, 1989).

V. amygdalina is a well-known medicinal plant throughout tropical Africa. It is used to treat parasitic infections and gastrointestinal disorders (Watt and Breyer-Brandwijik, 1962; Dalziel, 1937; Kokwaro, 1976; Burkill, 1985). For example, it is an ingredient of "Ndole," a traditional cuisine of Cameroon that is believed to be very effective as a tonic food. Known as "bitter leaf," it has a strong unpleasant bitter taste.

In general, naturally occurring bitter substances are known to provide us with many valuable drugs, including quinine (antimalaria), morphine (analgesic), humulone and lupulone (antibacterial agents), and cucurbitacins (antitumor agents) (Shiba, 1976). The medicinal use of *V. amygdalina* by chimpanzees is suspected because of its low frequency of ingestion, its strongly bitter taste compared to other chimpanzee food items, the similarity of symptoms displayed by an apparently ill adult female that consumed the plant and exhibited symptoms like those that are displayed by humans who utilize the plant as a medicinal treatment, and its widely recognized medicinal use against parasites and gastrointestinal disorders by humans in tropical Africa (Huffman and Seifu, 1989).

The medicinal use of another plant, *Ficus exasperata* (Moraceae), by chimpanzees is suspected because of their peculiar feeding behavior with it, viz., they ingest its leaves slowly, singly, and without mastication (Nishida, 1990). This behavior is similar to that of chimpanzee feeding on *A. mossambicensis* (Wrangham and Nishida, 1983).

Medicinal plants that are used by nonhuman primates should prove to be valuable targets in the search for naturally occurring compounds of biological or physiological significance. Although there are some difficulties in distinguishing the relative nutritional and medicinal value of many primate plant foods, the medicinal plants used by primates may be discovered through detailed analyses of the feeding habits and physical state of the consumer. Here, we wish to report on the value of several potentially medicinal plants from the standpoint of natural product chemistry, focusing primarily on the case of *V. amygdalina*.

METHODS

Plant Materials

Specimens of *Vernonia amygdalina, Aspilia mossambicensis*, and *Ficus exasperata* were collected from the Mahale Mountains National Park, Tanzania, by M.A.H. and T.N. *V. amygdalina* was collected near Nkanbe, in the northwestern part of Cameroon, by K.K. and H.O.

Chemical Analyses

Plant material (usually 100 g dry weight) was extracted with methanol for 10–20 days at room temperature, and the extracted solution was concentrated *in vacuo*. A hot water extract (for 30 min in boiling water) was

occasionally prepared. The methanol extract was separated by a solvent-partition procedure to yield nonpolar, medium polar, polar, and most polar fractions.

Column chromatography, and high-performance liquid chromatography (HPLC) were used to isolate physiologically active compounds. Gas liquid chromatography (GLC) was also used to analyze the compounds. Spectroscopic analyses (MS, IR, UV, ^{1}H- and ^{13}C-NMR) and X-ray diffraction analysis were employed to determine the chemical structures.

In Vitro Physiological Activities

Antitumor activities were assayed by measuring the growth inhibition of mouse leukemia P-388 and L-1210 cells and by cytotoxicity against KB cells from human tumor tissue (Doyle and Brandner, 1980). The inhibition against trypsin activity was assayed by measuring the effects of extracts on the hydrolysis of tosyl-L-arginine methyl ester by trypsin (Muramatsu *et al.*, 1965). Immunoresponse activities were examined by using a standard plaque-forming cell method against T cell-dependent (sheep red blood cell) and nondependent (lippopolysaccharides) antigens (Mishelle and Dutton, 1967). Antitumor promoting activity was assayed by the inhibition of Epstein–Barr virus (EBV) activation in Raji cells (Ohigashi *et al.*, 1986). Further physiological activities (such as bleb forming and anti-bleb forming, epidermal growth factor activation and its receptor-binding inhibition, adenosine deaminase inhibition, antiinflammation, analgesic, antiestrogen, and antimocrobial activities) were also tested, but no remarkable activities were detected.

RESULTS AND DISCUSSION

In vitro tests of physiological activity of the crude methanol extract showed antitumor activities with mouse leukemia P-388, L-1210, and human KB cells and inhibition of early antigen (EA) induction of EBV in Raji cells. Ohigashi *et al.* (1986) suggested that this response is correlated with antitumor promoting activity. Both T cell-dependent and nondependent immunosuppressive activities were also detected. Further separation by the solvent-partition procedure (Fig. 1) yielded four fractions, Fr-A (nonpolar), Fr-B (medium polar), Fr-C (polar), and Fr-D (most polar). As shown in Fig. 1, the antitumor activities were detected in Fr-B. Inhibitory activity of EBV-EA induction was detected in both Fr-A and Fr-B, and immunosuppressive activity was detected in Fr-B. Here, we first focused

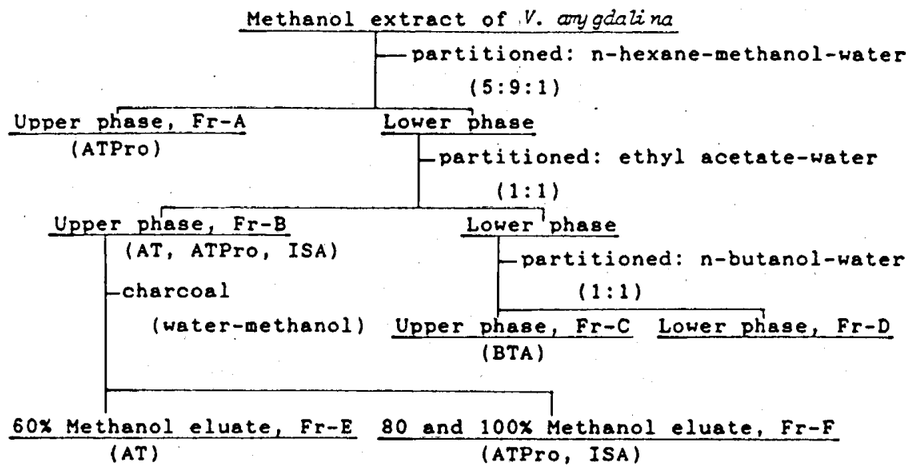


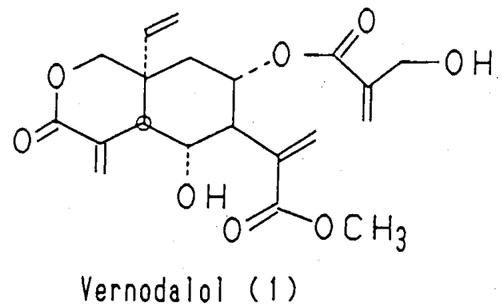
Fig. 1. Fractionation procedure and physiological activities of the fractions. ATPro, antitumor promoting activity; AT, antitumor activity; ISA, immunosuppressive activity; BTA, bitter taste activity.

on the constituents of Fr-B, in particular on the antitumor agent(s) and the inhibitors against EBV-EA induction.

Fr-B was chromatographically purified as shown in Fig. 1, giving two fractions, Fr-E and Fr-F, which contained an antitumor agent(s) and inhibitors of EBV-EA induction and immunosuppressor(s), respectively. Further chromatographical separation of Fr-E yielded an antitumor agent, which was identified as vernodalol (**1**; Fig. 2), an elemane-type sesquiterpene lactone (elemanolide). Vernodalol was first isolated as a bitter principle of *Vernonia anthelmintica*, a plant closely related to *V. amygdalina* (Asaka *et al.*, 1977). Ganjian *et al.* (1983) isolated it as an insect antifeedant from *V. amygdalina*. It was also synthetically derived from an antitumor agent, vernodalin, isolated from *V. amygdalina* (Kupchan *et al.*, 1969).

In the assays which we applied to the P-388 and L-1210 cells, vernodalol completely inhibited the growth of both cells at a concentration of 5 μg/ml. Although vernodalol was less active than the most active agent, 5-fluorouracil, it ranked as potent as a naturally occurring antitumor agent.

The *in vitro* antitumor activities were also detected in other fractions, and active constituents were thought to be vernodalol-related elemanolides as reported by Kupchan *et al.* (1969) and Rodriguez *et al.* (1976). However, vernodalol amounted to >2.7% of Fr-B by quantitative analysis using



Vernodalol (1)

Fig. 2. Structure of vernodalol.

HPLC. Thus, vernodalol might be a major component of the antitumor agents found in *V. amygdalina*.

The main inhibitors of EBV-EA induction in Fr-F were determined by GLC to be linoleic (2) and α-linolenic acid (3) (Fig. 3). Both acids inhibited EA induction by 40 ng/ml of a tumor promoter, TPA (Hecker, 1963), in the dose–response manner shown in Fig. 3. Tumor promoters are compounds that have no carcinogenic activity, but they act on cells that are initiated by carcinogenes to promote rapid tumor formation. Thus, the two-stage carcinogenesis by initiation–promotion has recently been accepted.

Application of inhibitors of promotion (antitumor promoters) is recognized as a new approach to the chemoprevention of cancer (Wattenberg, 1985; Ohigashi *et al.*, 1986). The inhibition of EBV-EA induction in Raji cells is now evaluated as a short-term method to find antitumor promoters (Ohigashi *et al.*, 1986; Tokuda *et al.*, 1986). The inhibitory activity of both acids (IC_{50} = 15 μg/ml) was higher than that of retinoic acid (IC_{50} = 25 μg/ml), a well-known potent antitumor promoter, suggesting that both acids are effective antitumor promoters.

The double bonds in the fatty acids should play an important role in this activity, because stearic acid, a saturated C_{18}-fatty acid, was inactive (Fig. 3). On the other hand, α-linolenic acid has been reported to be effective for the prevention of certain geriatric diseases, cancers, cerebral apoplexy, and allergic diseases, while linoleic acid is known to promote or to enhance them (Okuyama, 1990).

The relative ratio of α-linolenic acid-to-linoleic acid content was determined to be approximately 2.0, which is much higher than those of common edible oils from sesame, safflower, and soybean seeds (Potter, 1968). Although it is still uncertain whether the total amount of these unsaturated fatty acids is larger, and if the ratio (2.0) of α-linolenic-to-linoleic

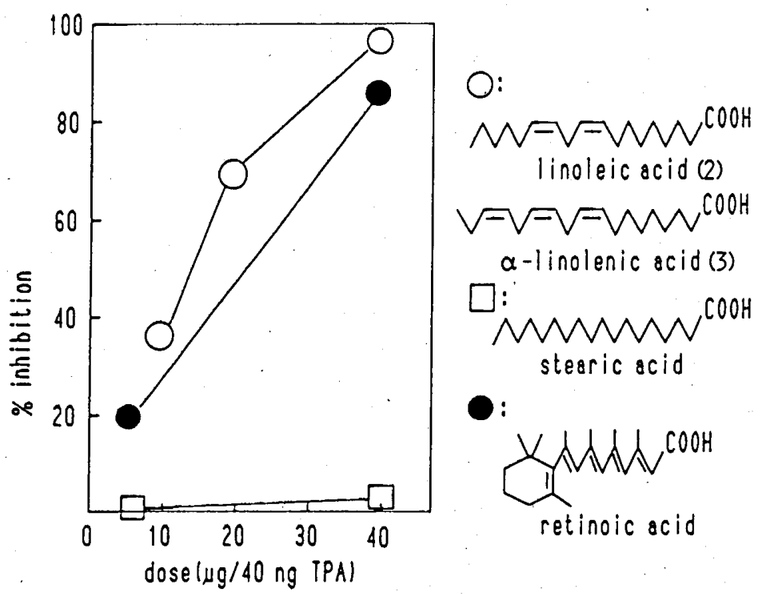


Fig. 3. Inhibitory activity of linoleic acid and α-linolenic acid against EBV-EA induction by TPA.

acid is higher in *V. amygdalina* than in other common vegetables, the fact that it is eaten by native people in Cameroon as a vegetable substitute may be significant from a physiological viewpoint.

The strong bitter taste was detected not in Fr-B-containing vernodalol but in the polar fraction, Fr-C. Purification monitored by a sensory test using the paper disk method afforded five bitter substances (BS-A–BS-E) and two related compounds (non-BS-F and G).

Figure 4 shows the purification scheme and yield of each compound together with the threshold value (BT) of the bitter taste. Among the isolated bitter substances, the structures of BS-A and BS-B were elucidated thus far as **5** and **6** by comparison of the spectral data with those for non-BS-F (**4**), whose structure was determined by X-ray diffraction analysis of its aglycon (Fig. 5). Thus BS-A and BS-B are new stigmastane-type steroid glucosides. Their bitter tastes were indicated by the oxygen-functional groups at C-16. The steroidal aglycons were structurally quite new, especially in the oxidation pattern of the side-chain part (C_{20}-C_{29}).

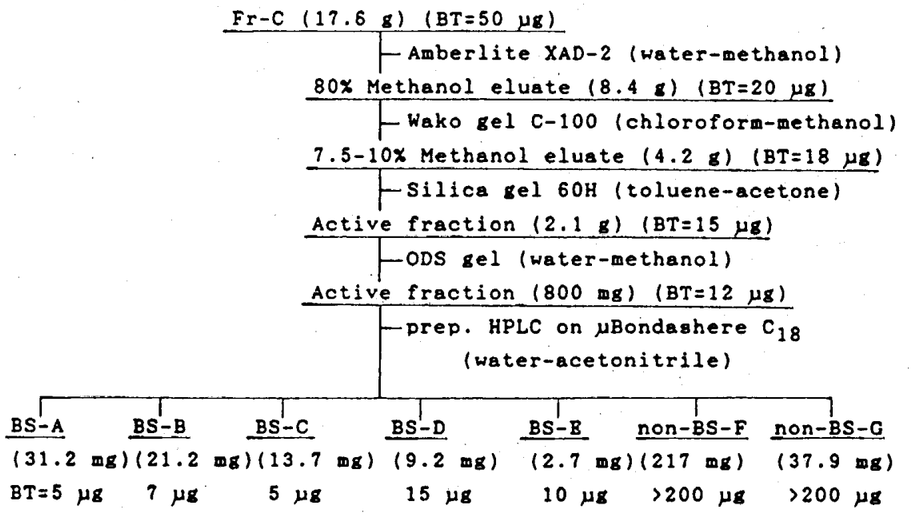


Fig. 4. Purification procedure and threshold value (BT) of the fractions.

Steroids and their metabolites, e.g., hormones and vitamins, are known to exhibit various physiological activities. Accordingly, it is important to learn what kinds of physiological activities might be exhibited by these new steroid glucosides and their aglycons.

Table I shows the physiological activities of the extracts of *A. mossambicensis* and *F. exasperata*. *A. mossambicensis* exhibited antitumor activities in the polar fraction and immunosuppressive activities in the medium polar and polar fractions. Inhibition against trypsin activity was also detected in the polar and nonpolar fractions.

F. exasperata showed antitumor activities in three fractions and remarkable inhibition against trypsin activity in two fractions.

The implications of chimpanzees foraging on plants for their medicinal value are intriguing. The potentially medicinal plants used by wild chimpanzees and analyzed here contain a wide variety of potent physiologically active compounds. While our chemical tests were not specifically directed at diseases carried by chimpanzees in the wild, their active biocidal properties lend further support to natural historical observations on the use of these plants for medicinal purposes by chimpanzees and humans.

For instance, Huffman and Seifu (1989) suggested that chimpanzees consumed *Vernonia amygdalina* in response to parasitic infection or possibly related gastrointestinal disorders. Gasquet *et al.* (1985) isolated vernolide,

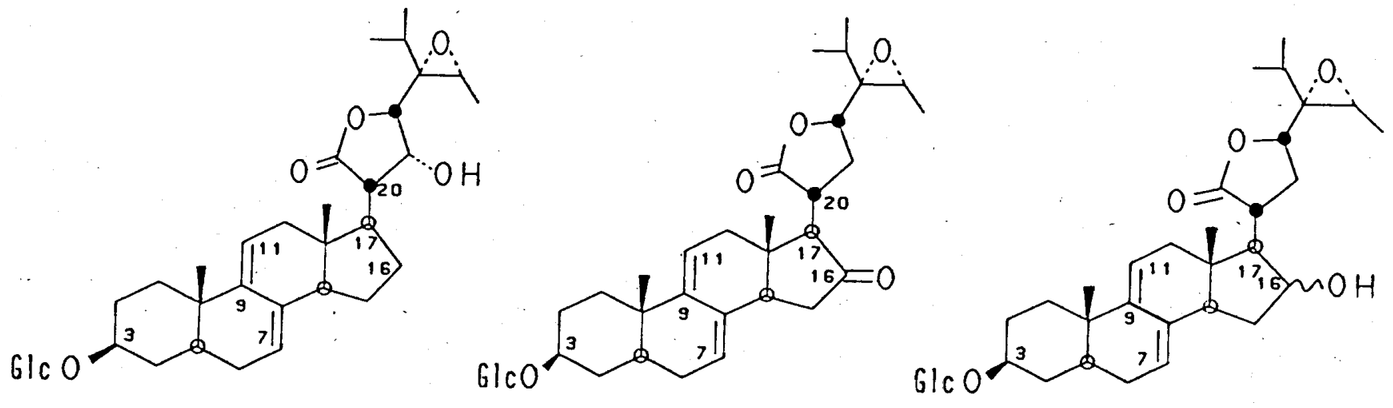


Fig. 5. Structures of bitter substances A and B (BS-A and BS-B) and a related compound (non-BS-F).

Table I. Physiological Activities of Two Potentially Medicinal Plants for Wild Chimpanzees

	Aspilia mossambicensis (Compositae)						*Ficus exasperata* (Moraceae)				
	AT[b]			ISP[d]			AT[b]			ISP[d]	
Fr[a]	P388	L1210	Trp[c]	T-dep	Non-T-dep	Fr[a]	P388	L1210	Trp[c]	T-dep	Non-T-dep
Fr-A	–	–	+	NT	NT	Fr-A	–	–	+	–	–
Fr-B	–	–	–	+	+	Fr-B	+	+	+	–	–
Fr-C	+	+	+	+	+	Fr-C	+	+	–	–	–
Fr-D	–	–	–	–	–	Fr-D	+	+	–	–	–

[a] Fr-A (non-polar), Fr-B (medium polar), Fr-C (polar), and Fr-D (most polar) were obtained in the same procedure as performed for *V. amygdalina*.

[b] Antitumor activities against P-388 and L-1210 cells. + or – indicates more or less than 60% inhibition of the cell growth at 500 μg/ml.

[c] Inhibition of trypsin activity. ++ or + indicates more than 50% inhibition at 50–100 μg/ml or at 100 μg – 1 mg/ml. – indicates less than 50% inhibition at 1 mg/ml.

[d] Immunosuppressive activity against T-cell dependent (T-dep) and nondependent (non-T-dep) antigens. ++ or – indicates less than 20% or more than 90% appearance of plaque-forming cells at 50 μg/ml. The ISP of Fr-A of *A. mossambicensis* was not tested, because of insolubility in the test solution.

a vernodalol-related sesquiterpenoid, from *Veronia colorata* as an antiparasitic compound. Occurrence of vernolide has also been known in *V. amygdalina* (Kupchan *et al.*, 1969). Currently Huffman is investigating the relationship between the use of *V. amygdalina* and parasitic infection, and we plan to combine these data with further biochemical tests of the actual effectiveness of *V. amygdalina* and other plants used by the Mahale chimpanzees on parasites that actually infest them.

The study of medicinal plant use by nonhuman primates opens a new area of primate studies, which promises to expand the complexity of primate behavior. Further, it may provide a novel means to find and to exploit naturally occurring compounds for human medicine.

ACKNOWLEDGMENTS

This study was partly supported by a Grant-in-Aid for Overseas Scientific Survey in 1988 and 1989, and a Grant-in-Aid for General Scientific Research in 1990, from the Ministry of Education, Science and Culture of Japan. M.A.H., T.N., and H.T. thank the Serengetti Wildlife Research Institute of Tanzania for permission to conduct research at the Mahale Mountains National Park in cooperation with the Mahale Mountains Wildlife Research Center. K.K. and H.O. thank the Ministry of Higher Education and Scientific Research and the Institute of Medical Science and Studies of Medicinal Plants for permission to conduct field research in Cameroon. The authors thank staff members at the Research Institute of Life Science of Snow Brand Milk Products Co., Ltd., for conducting some of the physiological activity tests. We are indebted to Mitsuru Hirota at Shinshu University for taking the NMR spectra at 250 MHz, to Keisuke Watanabe at Sumitomo Chemicals for measuring the FAB-MS, to Hiroshi Nozaki at Okayama University of Science for the X-ray diffraction analysis, and to Teruyoshi Takagaki and Mitsuo Jisaka for their technical assistance.

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原著論文
24

Biosci. Biotech. Biochem., 57 (5), 833–834, 1993

Note

Antitumoral and Antimicrobial Activities of Bitter Sesquiterpene Lactones of *Vernonia amygdalina*, a Possible Medicinal Plant Used by Wild Chimpanzees

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Received August 10, 1992

Vernonia amygdalina (Compositae), which is known for its very bitter taste, is a plant that has been reported for its possible medicinal use by wild chimpanzees.[1] In a previous study, we found two classes of bitter compounds in this plant: novel sigmastane-type steroid glucosides[2,3]; and four known sesquiterpene lactones, vernodalin (**1**), vernolide (**2**), hydroxyvernolide (**3**), and vernodalol (**4**).[4] Based on the presumption that a chimpanzee observed to ingest this plant was likely to be suffering from a parasite-related disease,[1] the *in vitro* antischistosomal activity of these bitter and related compounds has previously been investigated.[5] In addition, antitumoral and antibacterial activities were found in a partially purified fraction containing **1**–**3**. This report describes the *in vitro* antitumoral activity against mouse leukemia cells, P-388 and L-1210, and the antibacterial activity against Gram-positive bacteria, *Bacillus subtilis* and *Micrococcus lutea*.

Compounds **1**, **2**, and **3** were obtained (2.3 g, 52.5 and 70.1 mg, respectively) from the ethyl acetate-soluble part of a leaf extract (800 g).[4] The occurrence of **3** in *V. amygdalina* was first found in this study. Compound **4** was obtained as an artifact from **1** after extracting with methanol. Compounds **1**, **2**, and **3** tasted more bitter (bitterness: 0.8, 1.2, and 1.0 μg, respectively; see Table) than bitter steroids glucosides.[2,3]

Kupchan *et al.* have reported the antitumoral activity of **1** and **2**, using KB cells, and also pointed out the importance of the α-methylene-γ-lactone for this activity.[6,7] However, the antitumoral activity against such leukemia cells as P-388 and L-1210 have not been investigated for **1**–**4** and their derivatives, except for the *in vivo* activity of the homologous compounds, vernolepin (**5**) and vernomenin (**6**).[8] Therefore, the *in vitro* antitumoral activity against P-388 and L-1210 cells was tested for **1**–**4** and some of the derivatives (**5**–**10**) of **1** shown in Fig.

As shown in Table, vernodalin (**1**) and vernolide (**2**) exhibited potent activity (IC_{50} for P-388 and L-1210 cells: 0.11 and 0.17 μg/ml for **1** and 0.13 and 0.11 μg/ml for **2**, respectively), while the activity of hydroxyvernolide (**3**) and vernodalol (**4**) was weak. The lower activity of **3** compared with **2** could be explained by the loss of hydrophobicity in the acyl moiety. On the basis of the results for **1**, **5**, and **6**, the presence of the hydroxymethacryloyl moiety was not significant for the activity of the vernodalin-related compounds. However, it may still be possible that the activity of **1** can be increased if the acyl moiety is changed to a more hydrophobic group (methacryloyl group) as in the case of **2**. 4,15-Dihydrovernodalin (**7**) and 1,2,2′,3′-tetrahydrovernodalin (**8**) showed no significant change in activity compared to that of **1**. However, when the $\Delta^{11,13}$ double bond of **8** was saturated (compound **9**), the activity was much less. Moreover, **4** was less active than **1**, and **10** was inactive even at 50 μg/ml. Thus, the importance of the α-methylene-γ-lactone was also indicated for the antitumoral activity of **1** against P-388 and L-1210 cells.

The antibacterial activity of **1**–**10** against two Gram-positive (*Bacillus subtilis* and *Micrococcus lutea*) and two Gram-negative bacteria (*Escherichia coli* and *Agrobacterium tumefaciens*) (see Table) was measured by the pulp disc method. None of the compounds tested here exhibited activity against the two Gram-negative bacteria. However, compounds **1**, **2**, **3**, **5**, **6**, and **7** strongly inhibited the growth of *B. subtilis* and *M. lutea* at 5 μg/disk. The activity of the vernodalin derivatives decreased in the order of **8** > **9** = **4**, and **10** was inactive at 50 μg/disk. This structure–activity relationship correlates with that found for the antitumoral activity.

Interestingly, while the antitumoral and antibacterial activities of **10** were insignificant, its degree of bitterness was quite high (see Table). Only vernodalol (**4**) had low bitterness, suggesting that either α-methyl- or α-methylene-γ-lactone is important for the bitter taste of the vernodalin-related compounds.

Experimental

Preparation of the derivatives of 1. The structures of the derivatives of **1** prepared as next described were confirmed by IR, MS, and ^{1}H-NMR spectra (data not shown), although the configuration of the newly formed methyl groups is unknown.

Vernolepin (**5**) and vernomenin (**6**): Vernodalin (**1**, 50.7 mg) in dioxane (3.5 ml) was hydrolyzed with 5% KOH (0.7 ml) at room temperature for 1 h. The product was then refluxed in dry benzene (9 ml) containing

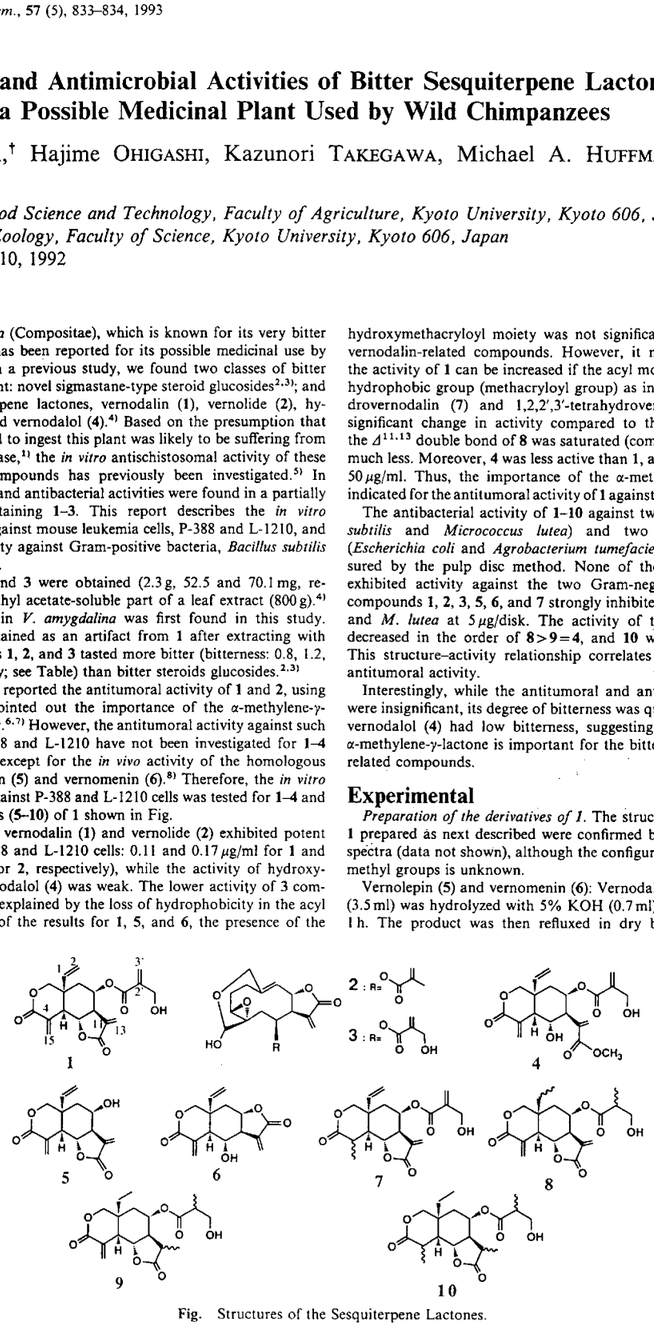

Fig. Structures of the Sesquiterpene Lactones.

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Table Biological Activity of the Sesquiterpene Lactones

Compound	Bitterness (μg)	Antitumoral activity IC_{50} (μg/ml)		Antimicrobial activity (μg/disk)	
		P-388	L-1210	*B. subtilis*	*M. lutea*
Vernodalin (**1**)	0.8	0.11	0.17	5	5
Vernolide (**2**)	1.2	0.13	0.11	5	5
Hydroxyvernolide (**3**)	1.0	0.92	1.25	5	5
Vernodalol (**4**)	70.0	0.32	0.61	50	50
Vernolepin (**5**)	1.2	0.12	0.29	5	5
Vernomenin (**6**)	1.0	0.17	0.32	5	5
4,15-Dihydrovernodalin (**7**)	0.8	0.07	0.15	5	5
1,2,2′,3′-Tetrahydrovernodalin (**8**)	2.5	0.14	0.26	10	10
1,2,11,13,2′,3′-Hexahydrovernodalin (**9**)	2.4	0.52	1.00	50	50
1,2,4,15,11,13,2′,3′-Octahydrovernodalin (**10**)	0.4	> 50	> 50	> 50	> 50

p-toluenesulfonic acid (8 mg) for 90 min to yield **5** and **6** (10.1 and 7.2 mg, respectively).[8] Dihydrovernodalin (**7**): Vernodalin (**1**, 57.7 mg) in MeOH (2 ml) was reduced with $NaBH_4$ (2 mg) at 0°C for 8 min to yield **7** (8.2 mg). 1,2,2′,3′-Tetrahydrovernodalin (**8**) and 1,2,11,13,2′,3′-hexahydrovernodalin (**9**): Vernodalin (**1**, 138 mg) in EaOAc (20 ml) was hydrogenated over 5% Pd–C (50 mg) to yield **8** and **9** (12.0 and 18.3 mg, respectively). 1,2,4,15,11,13,2′,3′-Octahydrovernodalin (**10**): Hexahydrovernodalin (**9**, 9.48 mg) in EtOH (1 ml) was reduced with $NaBH_4$ (1.13 mg) at 0°C for 10 min to yield **10** (4.89 mg).

Determination of bitterness. The minimum threshold level for bitterness was determined by tasting a filter paper (1 cm^2 × 0.2 mm thick) soaked with a known amount of the test compound.[4]

Determination of the in vitro antitumoral activity. P-388 or L-1210 mouse leukemia cells (1×10^4 cells) were incubated in RPMI 1640 medium supplemented with 10% fetal calf serum and a known amount of the test compound under 5% CO_2 at 37°C. After a 3-day incubation period, the number of cells was compared with that in the control medium without the test compound.[9] The concentration for a 50% inhibition (IC_{50}) of cell proliferation was determined.

Determination of the antibacterial activity. The bacteria were preincubated for 24 h in a shaken liquid medium (meat extract, 0.5%; peptone, 1%; NaCl, 0.5%). A portion of the precultivated medium (0.1 ml) was poured uniformly onto an agar medium (agar, 1.5%; extract, 0.5%; poptone, 1%; NaCl, 0.5%) in a Petri dish (8.5 cm diameter, 5 ml of medium). A paper disk (8 mm diameter × 0.5 mm thick) containing a known amount of the test compound was placed on the agar medium, which was then incubated for 24 h at 37°C. The minimum inhibiting dose per disk to form an antimicrobial zone was determined.

Acknowledgments. This study was supported by a Grant-in-Aid for General Scientific Research from the Ministry of Education, Science, and Culture of Japan. We thank members of the Institute of Life Science of Snow Brand Milk Products Co., Ltd. for the *in vitro* antitumoral activity tests.

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Phytochemistry, Vol. 34, No. 2, pp. 409–413, 1993
Printed in Great Britain

0031–9422/93 $6.00 + 0.00

Phytochemistry 34(2): 409-413.

STEROID GLUCOSIDES FROM *VERNONIA AMYGDALINA*, A POSSIBLE CHIMPANZEE MEDICINAL PLANT

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(*Received* 30 *November* 1992)

Key Word Index—*Vernonia amygdalina*; Compositae; medicinal plant; bitter compound; chimpanzee; steroid glucoside; vernonioside.

Abstract—Three new stigmastane-type steroid glucosides, vernonioside A_4, B_2 and B_3, as well as the aglycone of A_4, were isolated from *Vernonia amygdalina*, a possible medicinal plant used by wild chimpanzees. Vernonioside A_4 and its aglycone were bitter but others were not.

INTRODUCTION

Vernonia amygdalina, a shrub or tree growing throughout tropical Africa, is reported to be a possible medicinal plant used by wild chimpanzees [1]. As a part of an investigation of the bioactive constituents of this plant, we have previously reported three bitter steroid glucosides, vernoniosides A_1, A_2 and A_3, and a related nonbitter glucoside; vernonioside B_1 [2, 3]. In a subsequent investigation, we isolated another new bitter steroid glucoside, vernonioside A_4, and its aglycone, as well as two nonbitter related glucosides, vernonioside B_2 and B_3.

RESULTS AND DISCUSSION

A methanolic extract of the dried leaves was partitioned with *n*-hexane–methanol–water (5:9:1), and the lower layer (Fr-1) was further partitioned with ethyl acetate–water (1:1) to yield a bitter aqueous layer. When this aqueous layer was re-extracted with *n*-butanol, the bitterness was collected in the *n*-butanol-soluble part. This was chromatographed on silica gel with chloroform–methanol to yield a bitter fraction, which was further separated by ODS gel with methanol–water to yield 60 (Fr-2), 70 (Fr-3) and 80% (Fr-4) methanol eluates. Fr-2 contained a compound seen as a characteristic orange spot on an ODS TLC plate {R_f 0.47, methanol–water (18:7)} sprayed with vanillin–sulphuric acid and then heated. The compound corresponding to this spot was purified by chromatography on ODS and then by preparative HPLC (μBondasphere C_{18}) to yield vernonioside A_4 (**1**). Fr-3 also contained a compound showing an orange spot similar to **1** on an ODS TLC plate {R_f 0.38, methanol–water (3:1)}. This compound was purified by preparative HPLC (μBondasphere C_{18}) to give **1a**. Vernonioside B_3 {**3**, R_f 0.35, methanol–water (3:1) on an ODS TLC plate, yellow} was obtained from Fr-4 by preparative HPLC (μBondasphere C_{18}). On the other hand, the ethyl acetate-soluble part obtained from Fr-1 was separated on silica gel and then by preparative HPLC (μBondasphere C_{18}) to yield vernonioside B_2 (**2**), which could be seen as a blue spot on an ODS TLC plate {R_f 0.20, methanol–water (3:1)} sprayed with vanillin–sulphuric acid and then heated.

The FAB mass spectrum (*m/z* 649, MH^+) data of vernonioside A_4 (**1**) and the HREI mass spectral data (*m/z* 486.2969, calcd for $C_{29}H_{42}O_6$, 486.2981) of its natural aglycone (**1a**) obtained by enzymatic hydrolysis indicated that the molecular formula of **1** is $C_{35}H_{52}O_{11}$ (M_r 648). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **1** were very close to those of vernoniosides A_1 (**4**) and A_2 (**5**), and all the other related compounds A_3 (**6**), and B_1 (**7**) reported [3], suggesting that **1** is also a stigmastane-type steroid glucoside. The presence of a β-orientated β-glucosyl moiety at C-3 of the aglycone was indicated by comparison of the ^{13}C NMR data arising from the sugar part and from C-2, C-3 and C-4 (δ30.2, 76.9 and 34.5, respectively) of the aglycone with those of **4**–**7** [3]. The ^1H–^1H and ^1H–^{13}C COSY NMRs showed the connectivity of C-17-C-16-C-15-C-14. The chemical shift of H-14 (δ2.91) was largely shifted from that of vernonioside A_1 (**4**), but close to that of vernonioside A_2 (**5**) (Table 1). This shows that the 16-hydroxy group is α-orientated [4]. Further evidence for the configuration of the hydroxy group at C-16 was obtained by noting the close chemical shifts of H_3-18 (δ0.66) and H_2-15 (δ2.22) to those of

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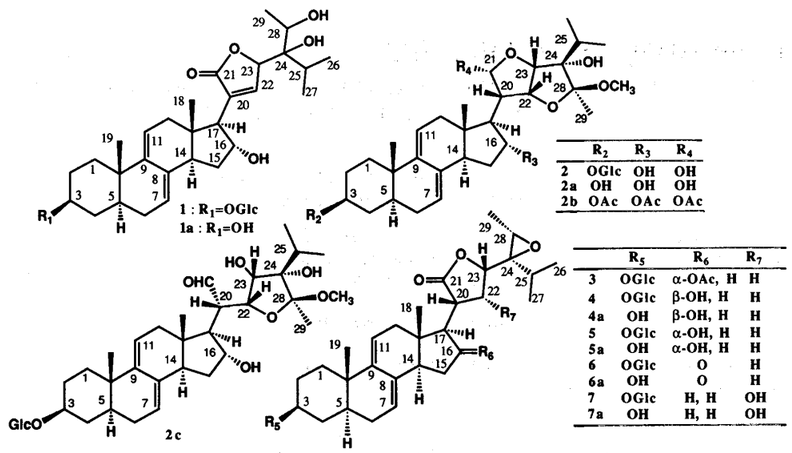

	R_2	R_3	R_4
2	OGlc	OH	OH
2a	OH	OH	OH
2b	OAc	OAc	OAc

	R_5	R_6	R_7
3	OGlc	α-OAc, H	H
4	OGlc	β-OH, H	H
4a	OH	β-OH, H	H
5	OGlc	α-OH, H	H
5a	OH	α-OH, H	H
6	OGlc	O	H
6a	OH	O	H
7	OGlc	H, H	OH
7a	OH	H, H	OH

vernonioside A_2 (**5**) (Table 1) [3, 4]. Further NMR data, along with the UV absorption maxima at 234, 241 and 251 nm clearly showed the presence of $\Delta^{7,9(11)}$ diene [5]. H-17 appeared as a broad doublet at a lower field (δ3.17, J = 6.9 Hz) than those of other vernonioside (**4**–**7**) previously reported [3]. Moreover, long range couplings between H-17 and H-22, and between H-17 and H-23, were shown by the ^1H–^1H COSY NMR spectrum. These data, together with both the ^{13}C NMR data of C-20, C-22 and C-23 (δ135.8, 151.5 and 83.6, respectively) and the IR absorption band at 1735 cm^{-1}, indicated the occurrence of an α,β-unsaturated-γ-lactone in **1** in place of a saturated γ-lactone in A_1–A_3 and B_1. The presence of the isopropyl group (C-25, C-26 and C-27) was confirmed by the ^1H–^1H and ^1H–^{13}C COSY NMR. A tertiary carbon signal at δ70.4 coupled to a methine proton at δ4.41 and a quaternary carbon signal at δ79.1 were, therefore, attributed to C-28 and C-24, respectively. The configurations at C-23, C-24 and C-28 of **1** were unresolved. The co-occurrence of the natural aglycone (**1a**) of **1** in Fr-3 was proved during the purification process of **1**–**3**.

The FAB mass spectral data of vernonioside B_2 (**2**) showed a peak at *m/z* 703 $[M+Na]^+$ which shifted to *m/z* 719 $[M+K]^+$ when KBr was added to the matrix. The HR-EI mass spectral data of its natural aglycone (**2a**) obtained by enzymatic hydrolysis indicated a peak at *m/z* 500.3118, which was considered to be an $[M-H_2O]^+$ ion (calcd for $C_{30}H_{44}O_6$, 500.3138). These data revealed that the molecular formula of **2** is $C_{36}H_{56}O_{12}$ (M_r 680). The UV spectrum indicated absorption maxima at 235, 242 and 251 nm, characteristic to the $\Delta^{7,9(11)}$-diene chromophore [5] which was further confirmed by the similarities of ^1H and ^{13}C NMR signals (Table 1) arising from the steroid-cyclic system of **2** to those of other vernonioside. The ^1H NMR data of H-14, H_2-15 and H_3-18 suggested that the oxygen substituent at C-16 was α-orientated as in the case of **1** and **5**. The IR and ^{13}C NMR spectra of **2**, however, indicated the absence of either α,β-unsaturated or saturated γ-lactone, commonly present in vernoniosides hitherto described. The ^1H–^1H and ^1H–^{13}C COSY NMR spectra clearly proved the connectivities of C-23-C-22-C-20-C-17-C-16-C-15-C-14 and C-20-C-21. The chemical shifts of C-21 (δ99.1) and H-21 (δ5.90) suggested that C-21 forms either a hemiacetal or an acetal. The ^1H NMR spectrum of the acetylated natural aglycone (**2b**) showed downfield shifts of the signals due to H-3, H-16 and H-21 (from δ3.84, 4.45 and 5.91 in **2a** to δ4.8, 5.74 and 6.58 in **2b**, respectively), confirming C-21 to form a hemiacetal. The presence of the isopropyl group (C-25, C-26 and C-27) was also shown by the ^1H–^1H COSY NMR spectrum of **2**. A quaternary carbon signal at δ113.1 together with singlet methyls at δ1.60 (H_3-29) and δ3.33 (OMe) proved the formation of a methyl ketal at C-28. The remaining quaternary carbon signal at δ82.0, therefore, was assigned to C-24. The NOESY spectrum recorded by a phase sensitive mode showed significant cross-peaks between H-20 and each proton at C-21, 22 and 23, H-22 and each proton at C-21 and C-23, H-23 and one of the methyl (δ1.29) of the isopropyl group, and H-22 and the methoxy group showing that all of the hydrogens at C-20–C-23, and the isopropyl and the methoxy groups are β-orientated. The β-glucosyl moiety at C-3 should also be β-orientated on the basis of ^1H and ^{13}C NMR data. Thus, the structure of **2** was deduced for vernonioside B_2. HPLC analysis showed that vernonioside B_2 exists as an equilibrium mixture of two compounds (**2**: minor compound, 4:1, R_t = 7.2 and 5.2 min, respectively) in 45% aqueous acetonitrile at 20°. The conversion of **2** to the minor compound was suppressed by preparation of the aqueous acetonitrile solution under cooling. The minor compound must be either the 21-formyl-23-hydroxy form (**2c**) or the anomer of **2**. The formation of methyl ketal at C-28 could have possibly occurred during the extraction step. It is very interesting that a steroid glucoside VE-1, which forms a hemiacetal at C-21, has been isolated from

Table 1. ^{1}H NMR assignments of compounds **1** and **2**

H	1	2	4*	5*
1α	1.23	1.21	1.30	1.26
1β	1.79	1.82	1.91	1.86
2α	2.14	2.12	2.18	2.16
2β	1.70	1.72, *br d* J=13.7 Hz	1.72	1.71
3α	3.93	3.97	3.96 *m*	3.95
4α	2.00	2.03	2.07	2.02
4β	1.40	1.43	1.48	1.41
5α	1.32	1.36	1.37	1.34
6α	1.82	1.85	1.83	1.82
6β	1.82	1.85	1.83	1.82
7	5.41	5.43, *br s*	5.44, *br s*	5.40, *br s*
11	5.45	5.48, *br d* J=6.2 Hz	5.56, *br d* J=6.3 Hz	5.50, *br d* J=5.9 Hz
12α	2.17	2.21	2.19	2.34
12β	2.59	2.54	3.12, *dd* J=17.8, 6.7 Hz	2.77, *dd* J=17.4, 6.6 Hz
14α	2.91	2.83, *m*	2.24	2.82
15α	2.22	2.21	2.53, *dt* J=12.8, 6.9, 6.9 Hz	2.16
15β	2.22	2.08	1.91	2.16
16α	—	—	4.65, *br s*	—
16β	5.17	4.55	—	4.58
17α	3.17, *br d* J=6.9 Hz	2.58	1.89	2.37
18	0.66, *s*	0.65, *s*	1.18, *s*	0.75, *s*
19	0.79, *s*	0.86, *s*	0.89, *s*	0.85, *s*
20	—	2.32, *m*	3.37, *m*	3.07, *m*
21	—	5.90, *br d* J=3.2 Hz	—	—
22	8.02, *s*	4.73, *dd* J=6.0, 6.0 Hz	2.28, *m*	2.32
			2.73, *m*	2.52, *q* J=11.8 Hz
23	5.75, *s*	4.86, *d* J=6.0 Hz	4.89, *dd* J=9.9, 5.9 Hz	4.93, *dd* J=10.6, 5.5 Hz
25	2.48, *heptet* J=7.2 Hz	2.39	1.89	1.86
26	1.25, *d* J=6.8 Hz	1.15, *d* J=6.6 Hz	1.07, *d* J=7.2 Hz	1.08, *d* J=7.2 Hz
27	1.27, *d* J=7.3 Hz	1.29, *d* J=6.7 Hz	1.28, *d* J=7.1 Hz	1.28, *d* J=7.0 Hz
28	4.41	—	2.98, *q* J=5.3 Hz	3.01, *q* J=5.6 Hz
29	1.59, *d* J=6.2 Hz	1.60, *s*	1.23, *d* J=5.5 Hz	1.19, *d* J=5.5 Hz
1'†	5.00, *d* J=7.2 Hz	5.03, *d* J=7.9 Hz	5.07, *d* J=7.6 Hz	5.01, *d* J=7.6 Hz
2'†	4.04	4.03	4.07	4.06
3'†	4.33	4.32	4.38	4.31
4'†	4.26	4.27	4.30	4.27
5'†	3.94	4.02	4.00	3.96
6'†	4.39	4.38	4.38	4.40, *br dd* J=11.9, 5.0 Hz
	4.59, *br d* J=11.2 Hz	4.6	4.60	4.57, *br d* J=10.00 Hz
OMe	—	3.33, *s*	—	—

*Data from ref. [3].
†The carbons of glucose.

Table 2. ^{13}C NMR assignments of compounds **1**, **1a**, **2**, **2a** and **3**

C	1	1a	2	2a	3	4*	5*
1	34.9	35.2	34.9	35.2	34.9	35.0	34.9
2	30.2	32.6	30.1	32.5	30.1	30.1	30.1
3	76.9	70.2	77.0	70.2	77.0	77.1	77.0
4	34.5	38.8	34.5	38.8	34.5	34.5	34.5
5	39.1	39.6	39.1	39.6	39.2	39.3	39.2
6	30.1	30.3	30.2	30.4	30.1	30.3	30.2
7	121.3	121.5	121.3	121.5	121.6	120.6	121.0
8	132.7	132.7	135.8	(135)‡	135.0	136.1	135.9
9	144.3	144.6	144.1	144.3	144.2	144.2	144.1
10	36.2	36.3	36.2	36.2	36.2	36.2	36.2
11	118.4	118.4	118.6	118.6	118.3	119.5	118.8
12	40.8	40.9	41.7	41.8	40.8	41.8	41.3
13	44.6	44.7	43.6	43.7	43.1	42.9	43.3
14	49.6	49.7	49.1	49.2	48.9	50.2	49.2
15	35.7	35.8	35.2	35.3	33.0	37.0	36.0
16	74.5	74.6	76.2	76.3	76.8	72.1	74.9
17	58.7	58.8	56.0	56.1	56.3	57.1	60.1
18	14.0	14.1	14.5	14.6	13.9	13.9	13.9
19	19.4	19.6	19.5	19.7	19.4	19.6	19.5
20	135.8	135.8	48.5	48.6	39.7	38.7	40.3
21	175.1	175.1	99.1	99.2	177.3	178.4	178.0
22	151.5	151.5	81.0	81.0	29.1	31.1	29.3
23	83.6	83.6	90.1	91.2	77.0	77.3	77.4
24	79.1	79.1	82.0	82.0	64.1	64.2	64.0
25	32.7	32.8	32.4	32.4	29.8	29.8	29.7
26	18.8	18.8	17.4	17.5	18.7	18.7	18.7
27	18.8	18.8	18.5	18.5	18.7	18.9	18.9
28	70.4	70.4	113.3	113.4	55.3	55.3	55.4
29	19.1	19.1	17.4	17.5	13.2	13.2	13.2
1'†	102.3	—	102.3	—	102.3	102.3	102.3
2'†	75.3	—	75.3	—	75.3	75.4	75.3
3'†	78.6	—	78.6	—	78.7	78.7	78.6
4'†	71.7	—	71.8	—	71.8	71.7	71.7
5'†	78.5	—	78.4	—	78.5	78.5	78.5
6'†	62.9	—	62.9	—	62.9	62.9	62.9
OMe	—	—	48.5	48.5	—	—	—
OCOMe	—	—	—	—	21.2	—	—
					170.7		

*Data from ref. [3].
†The carbons of glucose.
‡Obstructed by the signals of pyridine-d_5.

Vernonia extensa by Sakai *et al.* [6]. 16-Oxygenated stigmastane-type steroids and their glucosides may be rich in *Vernonia* species.

Vernonioside B_3 (**3**), $C_{37}H_{54}O_{11}$, was indicated to contain an acetyl group by the IR (1725 cm^{-1}), and ^{1}H NMR (δ2.04, 3H) spectra. The ^{13}C NMR spectrum corresponded well to that of **5** except for the chemical shifts of C-15, C-16 and C-17 as shown in Table 2. The upfield shifts of C-15 and C-17 and the downfield shift of C-16 of **3** from those of **5** indicated that the 16-hydroxyl should be acetylated. This was further supported by the ^{1}H NMR spectrum, where an acetylation shift of H-16 by 0.95 ppm was observed as compared with that of **5**.

The bitter activities (BA) of **1**–**3**, and the aglycones **1a** and **2a** were determined by the threshold amount using a filter paper tasting method [3]. The bitterness of **1** and **1a** (BA: 30 and 7 μg, respectively) were comparable to that of quinine sulphate (BA: 17 μg). Interestingly, the aglycone **1a** tasted more bitter than the glucoside **1**, while the aglycones **4a**, **5a** and **6a** (BA: 130, 150 and >200 μg, respectively) tasted less bitter than their glucosides **4**–**6** (BA: 5, 5 and 7 μg, respectively) as reported previously [3]. It may be possible that the glycol group at C-24 and C-28 in vernonioside A_4 (**1**) acts as a hydrophilic portion in place of a sugar moiety. Excess hydrophilicity could result in the loss of bitterness. Thus, the balance between hydrophilicity and hydrophobicity in a molecule must be important for the bitterness of this compound. Vernonioside B_2 (**2**) and its aglycone (**2a**) did not taste bitter (BA: >200 μg for both). Furthermore, vernonioside B_3 (**3**) (BA: >200 μg), the 16-acetate of **5**, lacked bitterness, suggesting that the free hydroxyl (**4** and **5**) at C-16 is important for bitterness.

EXPERIMENTAL

General remarks. All melting points were measured on a Yanagimoto microapparatus and are uncorr. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-200; IR, Shimadzu IR-435 (KBr); optical rotation, JASCO model J-5; ^{1}H and ^{13}C NMR, Brucker AC250 (250 MHz for ^{1}H, ref. TMS) and Varian VXR-200 (200 MHz for ^{1}H, ref. TMS); MS, JEOL JMS-DX300 (70 eV, 300 μA).

*Isolation of vernonioside A_4 (**1**), its aglycone (**1a**), B_2 (**2**) and B_3 (**3**).* The methanolic extract of the dried leaves of *V. amygdalina* (dry wt 1.0 kg) was partitioned with *n*-hexane–MeOH–H_2O (5:9:1) to yield a bitter lower layer (Fr-1), which was further partitioned with EtOAc–H_2O. The lower layer was extracted with *n*-BuOH to yield a bitter *n*-BuOH-soluble part. The *n*-BuOH-soluble part was chromatographed on silica gel eluted stepwise with $CHCl_3$–MeOH to give a 20% MeOH eluate. The eluate was further chromatographed on ODS gel with MeOH–H_2O to yield 60 (Fr-2), 70 (Fr-3) and 80% (Fr-4) MeOH eluates. Fr-2 was purified by prep. HPLC on μBondasphere C_{18} {19×150 mm, MeCN–H_2O (7:18), 8 ml min^{-1}} to yield vernonioside A_4 [3β-glucosyl-7,8,9,11,20,22-hexadehydro-16α,24ξ,28ξ-trihydroxy-5α-stigmastane-21,23-carbolactone (**1**), 59.6 mg, R_t 21.0 min]. Vernonioside A_4 (**1**): mp 242–243° (from MeOH); $[\alpha]_D^{24}$ −39.6° (MeOH; *c* 0.10); FABMS (NBA), *m/z*: 649 $[M+H]^+$, 671 $[M+Na]^+$; UV λ_{max}^{MeOH} nm (log ε): 229 (4.26)sh, 234 (4.28), 241 (4.28), 251 (4.11); IR ν_{max}^{KBr} cm^{-1}: 3350, 1735. The 70% MeOH eluate was purified by prep. HPLC on μBondasphere C_{18} (MeCN–H_2O, 7:13) to yield the naturally occurring aglycone (**1a**) of **1** (21.3 mg, R_t 25.1 min). The aglycone (**1a**) of vernonioside A_4 (**1**): $[\alpha]_D^{22}$ −36.8° (MeOH; *c* 0.57); UV λ_{max}^{MeOH} nm (log ε): 228 (4.11), 234 (4.18), 242 (4.18), 250 (3.95); IR ν_{max}^{KBr} cm^{-1}: 3350, 1730; FABMS (NBA) *m/z*: 487 $[M+H]^+$; HR-EIMS 70 eV, *m/z*: 486.2951 (M^+; calcd for $C_{29}H_{42}O_6$, 486.2982); ^{1}H NMR (250 MHz, pyridine-d_5): δ0.70 (3H, *s*, H-18), 0.92 (3H, *s*, H-19), 1.26 (3H, *d*, *J*=6.9 Hz, H-26), 1.28 (3H, *d*, *J*=6.8 Hz, H-27), 1.39 (H-1α), 1.49 (H-5), 1.58 (H-4β), 1.60 (3H, *d*, *J*=6.5 Hz, H-29),

1.75 (H-2β), 1.87 (2H, H-4α), 1.88 (3H, H-1β, H-6α, H-6β), 2.11 (H-2α), 2.25 (2H, H-15α, H-15β), 2.28 (H-12α), 2.50 (1H, *heptet*, J=7.0 Hz, H-25), 2.59 (1H, *br d*, J=16.5 Hz, H-12β), 2.95 (1H, *m*, H-14), 3.20 (1H, *br d*, J=7.2 Hz, H-17), 3.83 (1H, *m*, H-3), 4.42 (1H, *q*, J=6.3 Hz, H-28), 5.20 (1H, *m*, H-16), 5.46 (1H, *br s*, H-7), 5.53 (1H, *br d*, J=5.5 Hz, H-11), 5.77 (1H, *s*, H-23), 8.03 (1H, *s*, H-22). Vernonioside B_3 {(23*S*,24*R*,28*S*)-16β-acetoxy-3β-glucosyl-7,8,9,11-tetradehydro-24,28-epoxy-5α-stigma-stane-21,23-carbolactone (**3**), 28.4 mg} was isolated from the 80% MeOH eluate by prep. HPLC{μBondasphere C_{18}, 19 × 150 mm, MeCN–H_2O (2:3), 8 ml min^{-1}, R_t 28.5 min}. Vernonioside B_3 (**3**): $[\alpha]_D^{22}$ −7.6° ($CHCl_3$; *c* 0.39); UV λ_{max}^{MeOH} nm (log ε): 227 (4.00), 235 (4.15), 242 (4.20), 250 (4.04); IR ν_{max}^{KBr} cm^{-1}: 3350, 1760, 1725; FABMS (NBA) *m/z*: 697 $[M+Na]^+$, (NBA+KBr) *m/z*: 713 $[M+K]^+$; ^1H NMR (260 MHz, pyridine-d_5): δ0.70 (3H, *s*, H-18), 0.82 (3H, *s*, H-19), 1.07 (3H, *d*, J=7.2 Hz, H-26), 1.26 (3H, *d*, J=6.9 Hz, H-27), 1.26 (3H, *d*, J=5.5 Hz, H-29), 1.26 (H-1α), 1.36 (H-5), 1.39 (H-4β), 1.72 (H-2β), 1.81 (H-6α, H-6β), 1.87 (H-1β), 1.88 (H-15β), 1.89 (H-25), 2.00 (H-4α), 2.04 (3H, *s*, OCOMe), 2.1 (H-2α), 2.13 (H-15α), 2.23 (H-22α, H-22β), 2.28 (H-12α), 2.45 (1H, *dd*, J=6.6, 5.9 Hz, H-17), 2.56 (H-14), 2.61 (1H, *dd*, J=17.4, 6.3 Hz, H-12β), 3.07 (H-20), 3.13 (1H, *q*, J=5.5 Hz, H-28), 3.97 (H-3), 4.01 (H-5′), 4.06 (H-2′), 4.28 (H-4′), 4.30 (H-3′), 4.42 (H-6′), 4.60 (H-6′), 4.90 (1H, *t*, J=8.1 Hz, H-23), 5.03 (1H, *d*, J=7.7 Hz, H-1′), 5.48 (H-11), 5.32 (1H, *br s*, H-7), 5.53 (H-16). The EtOAc-soluble part of Fr-1 was chromatographed on silica gel and eluted stepwise with $CHCl_3$–MeOH to give 10–20% MeOH eluates. The combined eluate was successively rechromatographed on ODS gel with acetonitrile–water (7:15), silica gel with $CHCl_3$–MeOH (19:1), ODS gel with MeOH–water (13:7), and then purified by prep. HPLC on μBondasphere C_{18} (acetonitrile–water, 47:53, 8 ml min^{-1}) to yield vernonioside B_2 {(22*R*,23*S*,24*R*,28*S*)-3β-glucosyl-28-methoxy-7,8,9,11-tetradehydro-16α,21,24-trihydroxy-21,23:22,28-diepoxy-5α-stigmastane (**2**) 46 mg, R_t 14.0 min}. Vernonioside B_2 (**2**): mp 260–261° (from MeOH); $[\alpha]_D^{24}$ +65.3° (MeOH; *c* 0.453); FABMS (NBA), *m/z*: 703 $[M+Na]^+$, (NBA+KBr) *m/z*: 719 $[M+K]^+$; UV λ_{max}^{MeOH} nm (log ε): 235 (4.20), 242 (4.26), 251 (4.08); IR ν_{max}^{KBr} cm^{-1}: 3400.

Enzymatic hydrolysis of vernonioside A_4 and B_2. Vernonioside A_4 (**1**, 36.3 mg) or B_2 (**2**, 18.6 mg) was hydrolysed with β-glucosidase as previously reported [3] to yield the corresponding natural aglycones (**1a**, 12.1 mg, and **2a**, 8.03 mg, respectively). Compound **2a**: mp 207–209° (from MeOH); $[\alpha]_D^{25}$ +93.5° (MeOH; *c* 0.246); FABMS (NBA), *m/z*: 541 $[M+Na]^+$; HR-EIMS, *m/z*: 500.3118 ($[M-H_2O]^+$; calcd for $C_{30}H_{44}O_6$, 500.3128); UV λ_{max}^{MeOH} nm (log ε): 238 (3.95), 246 (4.00), 254 (3.85); IR ν_{max}^{KBr} cm^{-1}: 3400; ^1H NMR (250 MHz, pyridine-d_5): δ0.69 (3H, *s*, H-18), 0.99 (3H, *s*, H-19), 1.16 (3H, *d*, J=6.6 Hz, H-26), 1.30 (3H, *d*, J=6.8 Hz, H-27), 1.36 (H-1α), 1.51 (H-5α), 1.60 (H-4β), 1.61 (3H, *s*, H-29), 1.77 (H-2β), 1.92 (H-6α and H-6β), 1.94 (H-1β), 1.98 (H-4α), 2.09 (H-15β), 2.13 (H-2α), 2.25 (H-15α), 2.26 (H-12α), 2.35 (H-20β and H-25), 2.60 (H-12β), 2.64 (H-17α), 2.88 (1H, *m*, H-14α), 3.34 (3H, *s*, OMe), 3.84 (1H, *m*, H-3α), 4.54 (1H, *br s*, H-16β), 4.75 (1H, *t*, J=6.1 Hz, H-22), 4.87 (1H, *d*, J=6.0 Hz, H-23), 5.47 (1H, *br s*, H-7), 5.55 (1H, *br d*, J=5.2 Hz, H-11), 5.91 (1H, *br s*, H-21).

Acetylation of **2a**. The natural aglycone (**2a**, 6.28 mg) of **2** was acetylated with pyridine–Ac_2O to yield a triacetylated aglycone (**2b**, 2.86 mg). Compound **2b**: $[\alpha]_D^{22}$ +125° (MeOH; *c* 0.152); FABMS (NBA), *m/z*: 667 $[M+Na]^+$; UV λ_{max}^{MeOH} nm (log ε): 234 (4.15), 242 (4.20), 250 (4.04); IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1730, 1720; ^1H NMR (200 MHz, pyridine-d_5): δ0.80 (3H, *s*, H-18), 0.86 (3H, *s*, H-19), 1.06 (3H, *d*, J=7.0 Hz, H-26), 1.11 (3H, *d*, J=6.6 Hz, H-27), 1.64 (3H, *s*, H-29), 2.06, 2.10, 2.16 (OCOMe each), 3.29 (3H, *s*, OMe), 4.60 (1H, *t*, J=5.8 Hz, H-22), 4.71 (1H, *d*, J=5.8 Hz, H-23), 4.8 (1H, *m*, H-3), 5.4 (2H, *m*, H-7 and H-11), 5.74 (1H, *br t*, J=7.4 Hz, H-16), 6.58 (1H, *d*, J=6.8 Hz, H-21).

Acknowledgements—This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, of Japan. Financial support was also given by Shorai Kagaku Foundation. We thank Mr Daisuke Izutsu for his technical assistance.

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霊長類研究 Primate Res. **9**：179−187，1993

総 説

野生チンパンジー薬草利用研究: 成果と展望

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はじめに

霊長類の食物選択において，植物の二次化合物の存在は大きな影響を持っているとされている。そのため彼らの食用植物選択戦略に関する多くの研究は，サルがなぜ，そして，どのように毒物質を含むこの二次化合物に対抗しているのか，という課題に焦点を当ててきた（Glander, 1982）。ところが，著名な生態学者，ペンシルベニア大学・D.H.Janzen は，文献を５０年ほど遡って，世界各地からの野生動物の逸話を調べ，霊長類を含む多くの哺乳類はこの二次化合物を，痛み止め，解毒，寄生虫駆除など健康維持のために利用してるとの指摘をした（Janzen, 1978）。たしかに，病原体，寄生虫などは様々な病気を引き起こすことによって，宿主の行動や繁殖適応度に大きな影響を与える（Hart, 1990; Holmes and Zohar, 1990などを参照のこと）。したがって，進化の過程において，動物はこれらの悪影響を抑える必要性があったと考えられる。

１９７０年代後半から次第に，霊長類が，栄養補給以外に，いわば薬用的に植物を利用している実例が報告され始めた（Hamilton et al., 1978; Wrangham and Nishida, 1983; Phillips-Conroy, 1986）。

チンパンジーの採食行動と薬用植物

葉の呑み込み行動

野生チンパンジーの薬草利用研究は，１９８３年にR.W. Wranghamと西田利貞のアスピリア属（キク科）３種（*Aspilia mossambicensis*, *A. pluriseta*, *A. rudis*）の特異な採食行動の報告によって始まった（Wrangham and Nishida, 1983）。彼らは，タンザニアの西部，タンガニーカ湖に沿った地域にあるゴンベとマハレ両国立公園のチンパンジーが，栄養補給のためではなく，何らかの薬理的効果を目的として，これら植物の葉の一枚一枚を噛まずに飲み込むことを報告した。WranghamとGoodall（1989）によると，ゴンベのチンパンジーのアスピリア摂取には性差があるという。つまり，オトナ雌がオトナ雄の約三倍の割合で葉を食べる（アスピリアを摂取する日数／個体が５日以上個体追跡法によって観察された日数：２年間の記録より）。マハレチンパンジーのアスピリア摂取量における性差についてはまだ調べられていないが，アスピリアを摂取すると観察された雄と雌それぞれの個体数には統計的に有意な差は認められていない（Huffman et. al., 1990）。

アスピリア属植物は，アフリカのバントゥ民族の伝統的な生薬として広く使われている。例えば，根や葉は止血剤，外傷，強壮剤，痛み，寄生虫の駆除，腹痛，催乳剤などの薬的利用が記載されている（e.g. Kokwaro, 1976; Abbiw, 1990）。

カリフォルニア大学・アーバイン校のRodriguez ら（1985）は，*A. mossambicensis* がその生理活性成分分析によって，殺線虫や抗細菌

1993年3月22日受付，1993年5月19日受理

作用をもつチアルブリンAを含むことを見出し，Wranghamと西田の薬理的効果説を支持した。その後 WranghamとGoodall (1989) は，チンパンジーによるアスピリアの採食は寄生虫駆除を目的としたものであろうと報告している。

マハレとゴンベの両調査地において，一見健康そうなチンパンジー（同時に複数の個体の場合もある）がアスピリアを採食するのは一般的に観察されることから（Wrangham and Nishida, 1983），この植物は寄生虫感染に対する予防的な効果を持っている可能性がある。

ところが，ブリテイッシュ・コロンビア大学のPageとTowers は最近，タンザニアとケニアで採取したアスピリアを数回分析し，葉からは一度もチアルブリンAが確認できず，根のみに存在していると主張している（Page et al., 1992）。さらに，Pageら（1992）はマハレで採取したアスピリアの葉からジテルペンkaurenoic acidとgrandiflorenic acidを単離し，それらの生理活性分析から両者にモルモットの子宮を収縮させる作用があることを確かめた。Pageら（1992）はこの事実と，ゴンベで認められた採食量における性差とを関連づけて，チンパンジーのメスは自らの繁殖能力を調整するためにアスピリアを利用するのではないかという新しい仮説を提出した。さらに，チンパンジーのオトナ雄や，他の性的に未熟な個体に対しても，アスピリアの両化合物は抗細菌や抗肝臓毒作用を及ぼしているに違いないと指摘している（Page，私信）。

一方，TakasakiとHunt（1987）は，*Lippia plicata*（キク科）の葉がまた，アスピリアと同じように，噛まれることなしにチンパンジーにより摂取されていることを発見し，この植物が薬草として利用されている可能性を報告した。

１９９３年１月現在，日本や欧米の研究者によるアフリカ大型類人猿の調査によって，アスピリア（３種）やリビア（１種）以外に，これらと同じような様式で摂取される１５種もの植物が報告されている（表１）。東アフリカでは，ゴンベとマハレ以外に，キバレのチンパンジー（R.W. Wrangham），中央アフリカでは，ザイール・カフジービエガのヒガシローランドゴリラとチンパンジー（山極寿一ら），ザイール・ワンバのボノボ（黒田末寿ら）などの例が，さらに，西アフリカでは，コートジボアール・タイ森のチンパンジー（C. Boeschら），ボッソウのチンパンジー（松沢哲郎ら）の例があげられる(Huffman & Wrangham, 1993)。

ベルノニアの苦い髄の吸い込み行動

著者は，１９８７年にチンパンジーが薬として利用したと考えられる新たな植物 *Vernonia amygdalina* (Del.)の採食場面を観察した(Huffman & Seifu, 1989)。明らかに病気と判定できるオトナ雌のチンパンジーが，この植物の若い茎部の葉とその樹皮を取り除き，露出した髄をしがみ，滲み出た苦い樹液を飲んだのである。この雌は翌日の午後には平常の活動を取り戻した。

著者は，再び，１９９１年度に調査を行い，その期間中に，同じような場面に出会った。糞分析の結果，その個体は寄生虫感染症であったと判定された（Huffman and Wrangham, 1993; Huffman et al., in press)。

以前から，*V. amygdalina* はマハレMと旧K両集団のチンパンジーより，低頻度ではあるが，採食される植物として記録されている（Nishida and Uehara, 1983)。一見健康そうなチンパンジーが利用することもあることから（西田，上原，保坂，私信），上に示した観察が示唆する治療的な利用方法だけではなく，健康維持など，いわば予防的な利用方法もあると考えられる。しかし，野外では健康であるかどうかを判断することが難しく，より客観的な判定方法を求める必要がある。

マハレより約１５０ｋｍ北に生息しているゴンベのチンパンジーは，*V. amygdalina* に極めて近い種（形態的，化学的）である*V.colorata*を，同じような採食法で食べている（Wrangham, 1975)。ゴンベの観察では，採食前後のチンパンジーの詳細な健康状態は不明であるが，現地に住むトングウエ族を含めてアフリカの多くの人々は，この *V. amygdalina* を腹痛や寄生虫感染などの治療に薬として用いている。著者はさきの病気のチンパンジーの行動から読み取れる症状（食欲不振，倦

表１　チンパンジーやボノボ，ゴリラが葉をのみ込むと確認された食物とその民間薬用法，薬理効果，生物活性および化学的特性（1993年1月現在）

地域，植物種名［確認者ら］	*民間薬用法，薬理効果，生物活性，化学的特性など［参考文献］
(Pan troglodytes schweinfurthii)	
Mahale, Tanzania:	
Aspilia mossambicensis (Oliv.) [1]	駆虫，局所殺菌，解熱，腹痛軽症，子宮収縮，抗バクテリア，抗肝細胞毒 抗白せん催乳，生理痛軽症[2,3,4,5,6,7]
Lippia plicata Baker [8]	腹痛軽症，生理痛軽症，殺菌[2,8]
Commelina diffusa? Burm. f. [9]	洗眼，頭痛，過剰摂取による対家畜毒 [10,11]
Ficus exasperata Vahl [9]	疝痛，せき止め，矢毒，対家畜毒，腎臓痛，抗線虫，殺虫[2,10,12,13]
Trema orientalis (L) Blume [14]	駆虫，吐薬，抗黄疸，殺虫，アルカロイド解毒，高タンニン，アルカロイド[2,10,12,15]
Melastomastrum capitatum Vahl A. & R Fernandes [16]	腹痛 [16]
Gombe, Tanzania:	
A. pluriseta (O. Hoffm.) Wild [1]	抗皮膚感染症，局所殺菌，抗真菌，抗線虫，抗生，子宮収縮，抗肝細胞毒，抗バクテリア [3,17,4,5,6]
A. rudis Oliv. & Hiern [1]	**NA
Hibiscus aponeurus Sprague & Hutch [18]	NA
Kibale, Uganda:	
Aneilema aequinoctiale (P. Beau.) Loudon [20]	NA
Rubia cordifolia L. [21]	腹痛，rubatro，含生物活性アントラキノン，含環求ヘキサペプチト [7,16,21]
Kahuzi-Biega, Zaire:	
Commelina cecilae C.B. Clarke [22]	NA
Ipomoea involucrata P. Beauv. [22]	NA
Lagenaria abyssinica (Hook. f.) C. Jeffrey [22]	NA
(P.t.verus)	
Bossou, Guinea:	
Ficus mucuso Ficalho [23]	NA
Polycephalium capitatum (Baill.) [24]	止血剤 [25]
Tai forest, Ivory Coast:	
Manniophyton fulvum Mull. Arg. [26]	下痢止め [27]
Tristemna coronatum Benth. [26]	NA
Dichaetanthera africana (Hook. f.) Jacques-Felix [26] (= Sakersia africana)	NA
(P. paniscus)	
Wamba, Zaire:	
Manniophyton fulvum Mull. Arg. [28]	下痢止め [27]
(Gorilla gorilla graueri)	
Kahuzi-Biega National Park, Zaire:	
Commelina cecilae C.B. Clarke [22]	NA

*人間とチンパンジーの共通利用部分の薬理的効果についてのみ掲げた; **NA: 情報なし; 1. Wrangam and Nishida, 1983; 2. Ohigashi et al., 1991a; 3. Rodriguez et al., 1985; 4. Page et al., 1992; 5. Lwande et al., 1985; 6. Yang et al. 1986; 7. Kokwaro, 1987; 8.Takasaki and Hunt, 1987; 9. Newton and Nishida, 1990; 10. Abbiw, 1990; 11. Russo, 1992; 12. Terashima et al., 1991; 13. Rodriguez et al., 未発表; 14. 川中健二, 未発表; 初観察者; 15. Oates et al., 1977; 16. M.S. Kalunde, 私信; 17. Wrangham and Goodall, 1989; 18. E. Mpongo, Wrangham and Goodall, 1989に引用; 19. R.W. Wrangham, 未発表; 20. Wrangham and Goodall, 1989; 21. Itokawa et al., 1983; 22. 山極寿一, 私信; 23. 中村美穂, 私信; 初観察者; 24. 松沢哲郎, 1992; 25. Sugiyama and Koman, 1992; 26. C. Boesch, 準備中; 27. D. Muanza, 私信.; 28. 黒田末寿, 私信.

息，便秘気味，血尿等）に基づいて，彼らは寄生虫感染による症状をコントロールするために，この植物を利用したのではないかとの仮説を立て，これを実証するために，１９８９年から他分野の研究者と本格的な共同研究活動を開始した。

共同研究グループ，The C.H.I.M.P.P. Group (Chemo-ethology of Hominid Interactions with Medicinal Plants and Parasites)，の目的とするところは，動物行動学，植物化学，寄生虫学及び生薬学などの幅広い分野から，チンパンジーの薬用的植物利用とその認識についてさらに詳しく研究することである。

植物の薬効成分と生理活性

京都大学農学部の小清水弘一と大東肇は，チンパンジーが食用とする植物のうち，薬効成分を持つと考えられる植物を対象に，その生理活性成分についての分析を進めている。*V. amygdalina* からは，既知の生理活性成分が単離されている。たとえば，ＫＢ細胞に対する毒性や抗菌活性，抗赤痢アメーバ活性などを持つセスキテルペンラクトン類，vernodalin, vernolide, hydroxyvernolide などである（Gasquet et al., 1985; 大東ら, 1991ｂ; Jisaka et al. 1993)。また，新規な化合物として，スチグマスタン型ステロイドのグルコース配糖体(vernoioside A1-4; vernonioside B1-3)が単離構造決定された（Jisaka et al., 1992a; Jisaka et al. in press)。さらに，これまでに，vernodalinとvernonioside B1 などに抗住血吸虫作用があることが認められている（Jisaka et al., 1992b)。また，vernodalinにはマラリアやレシマニア原虫に対する薬用効果があることが示されている（P. Timmon-David; C.W. Wright, G.C. Kirby, D. Allen, D.C. Warhurst, J.D. Phillipson 私信)。

チンパンジーの薬的植物利用の可能性をさらに追求するために，著者がマハレチンパンジーの食物リスト（Nishida and Uehara, 1983）とアフリカの民族薬用植物リストを突き合わせた結果，人間が寄生虫症や胃腸炎に対して利用する植物種とチンパンジーの採食する種が重なる１２種の植物が新たにリストアップできた（表２)。

ここで注意すべきことは，両者の摂取する部分（たとえば，葉，樹皮，果実など）が同じ点である。なぜなら，種が同じであっても，摂取する部分が異なれば，その含まれている成分の有無や濃度も異なることがあり，それらの部分の誘起する作用が異なる可能性があるからだ。従って，民族薬用植物リストによる種だけから，チンパンジーの採食する植物の薬効性を判定することは危険である。

著者は，１９９１－９２年度，小清水のグループとの共同野外調査によって，これらチンパンジーの採食植物を（特に利用部分に注意を払って）採取した。それらの生理活性や活性成分について，現在詳細な分析が行われている。

表２　マハレM集団のチンパンジーの植物性食物*のうち，同じ植物部分がアフリカの民族薬用植物として寄生虫症や胃腸炎などに利用されることが新たに確認された種

植物種	**部分	民間薬用法，薬理効果，生物活性，化学的特性など［参考文献］
Annona senegalensis Pers.	Bk	下剤止め，赤痢，diterpenes, kaurenoic acid 関連化合物，抗癌，抗生物質 [1, 2]
Asystasia gangetica (L.) T. And.	Lf	駆虫 [3]
Cissus oliveri (Engl.) Gilg.	Lf	下剤 [3, 4]
Combretum molle G. Don	Lf	駆虫，triterpinoids [3, 5]
Croton sylvaticus Hochst. ex Krause	Lf	下剤 [3]
Erythrina abyssinica DC.	Lf, Bk	抗殺菌，マラリア，胃炎，抗原虫レシマニアの弱い活性，トリコモナス撲滅性の弱い活性，抗アメーバ赤痢の弱い活性，D-threopentaric acid, 3-Carbon 2-deoxy-α-lacton [3, 6, 7]
Hibiscus cannabinus L.	Lf	胃炎，赤痢，kaempferol [4, 8]
Lannea schimperi (A. Rich.) Engl.	Lf	下剤，抗アメーバ赤痢？[3]
Leea guineensis G. Don	Lf	腸炎 [1]
Parkia filicoidea Oliv.	Sd	弱い下剤，収斂剤，抗原虫レシマニアの弱い活性[4, 7]
Stephania abyssinica (Dillon & A. Rich.) Walp.	Lf	下剤，oxoaporphine alkaloid, phenanthrene alkaloids (6種) [1, 4]
Ximenia americana L.	Fr	駆虫 [3]

*Nishida and Uehara, 1983; **人間とチンパンジーの共通利用部分の薬理的効果についてのみ掲げた; Bk: 樹皮, Lf: 葉, Sd: 種子, Fr: 実 - 果実; 1. Githens, 1949; 2. Adesogan et al. 1976; 3. Kokwaro, 1987; 4. Watt and Breyer-Brandwijk, 1962; 5. D. Izutsu, Masters Thesis; 6. Kamat et al. 1981; 7. R. Elias, unpublished data; 8. Pakudina et al. 1976

国立予防衛生研・川中正憲，産業医科大学・嶋田雅尭，さらにはロンドン大学・J.D. Phillipson, C.W. Wrightとマーセイユ大学・G. Balansard, P. Timon-David, R. Eliasら寄生虫学及び生薬学の共同研究者達は，これらの抽出物について，マハレのチンパンジーが感染する可能性のある住血吸虫，原生動物，回虫，条虫などに対する薬用効果を検討している。現在までに，赤痢アメーバ，マラリア原虫，レイシマニア原虫に対する効果が一部の抽出に認められている（C.W. Wright, J.D. Phillipson; R. Elias, 私信)。

寄生虫感染症

１９８９年度から，著者はマハレM集団のチンパンジーについて，より包括的な健康状況の調査を行なうため，追跡個体の糞を定期的に採集している。京都大学霊長類研究所の後藤俊二氏がその試料を用いて，寄生虫感染度，感染症の同定などを検討してきた。その結果，糞線虫，鞭虫，吸虫，ランブル鞭虫などが検出された。１９８９－１９９０年度と１９９１－１９９２年度に検査されたチンパンジー（それぞれに４９個体と３５個体）糞試料から最も多く検出された寄生虫種は，糞線虫の*Ternidens* sp.（３９%，６９%）であった。それ以外の種の検出率は年によって多少異なったが，糞線虫の*Strongyloides fuelleborni*（２０%，２３%），鞭虫の*Trichuris trichiura*（１６%，２９%）の検出率は高かった（ハフマン，後藤，未発表資料)。

薬用植物摂取と寄生虫感染症

寄生虫感染の発症する個体は乾季より雨季に集中する傾向があり，さらに，雨季が進むにつれて感染する寄生虫の種数も上昇する傾向が認められた。(Huffman et. al., in prep)。１９９１－１９９２年度の検査試料では，病弱ぎみなオトナ雄と雌４個体において最も高い感染度数（糞１ｇ当たり１３０－５３０卵）を示した種は*Ternidens*であると同定された。*Ternidens*は，宿主の大腸に居ついて，大腸炎や潰瘍を起こす寄生虫(Brack, 1987)で，チンパンジーが雨季に示す際立った症状（下痢，便秘，食欲不振，疲れぎみ，など）は，*Ternidens*による直接的または間接的な影響による可能性が高いといってよいだろう。

１９９１年度の調査中に，再び，病気（下痢，食欲不振，疲れぎみ）と思われたオトナ雌が*V.amygdalina* の若い茎の髄に含まれている苦い樹液を飲み，翌日の午後には，平常の活動を取り戻したことが確認された。糞分析の結果，その個体は*Ternidens* による感染症であったことがわかり，また，その苦い樹液を摂取した１時間後と２０時間後の寄生虫感染度を比べてみると，その感染度が糞１ｇ当たり１３０卵から１５卵まで，明らかに減ったことが認められた（Huffman et al., in press)。

さらに，すでに記録されている植物採食資料を詳しく検討したところ，*V.amygdalina* は殆ど例外なく雨季に限って摂取されていることが明らかとなった（Huffman et. al., 1990)。アスピリアの採食と寄生虫感染についても，同じような傾向が認められている（Kawabata and Nishida, 1991)。このような事実は，チンパンジーの薬用植物摂取と寄生虫感染症との関わりを間接的に示しているものであり，病気の予防や治療に対する薬草利用をより一層把握するための有効な手がかりになると考えられる。

薬草利用行動の獲得

霊長類による薬用的植物利用行動の獲得は近年，多くの関心を集めている。表１で示されるように，現在まで，チンパンジーやボノボ，さらにはゴリラが呑み込むと確認された１９の植物種には，様々な民間薬的利用法や薬理効果が知られている。一方，これら植物は共通した物理的特徴を備えている。それは，*D. africana*を除けば，全ての種の葉の表面がザラザラしているということである（Huffman and Wrangham, 1993)。この特徴がアフリカの類人猿にとってどのような意味を持っているかは分からないが，呑み込み行動において何らかの重要な役割を持っているように思われ

る。彼らによって，そのザラザラした感覚が薬理成分の存在を知らせる信号として認知されているのか，それとも，実際に何らかの物理的な効果を果たしているのかなど，薬草利用の学習過程に関する今後の研究課題の一つとして残されている。

野生霊長類の薬草利用説を正しいとした上で，種や集団レベルでお互いに接触のないチンパンジーやボノボあるいはゴリラ同士が，類似した特徴を持つ同属の植物を同じように利用している事実を考えると，大型類人猿が共通の薬理効果認識機構を潜在的に持っていると考えられる（Huffman and Wrangham, 1993）。

最近，薬効成分をもつ植物の利用は，大型類人猿に限らず，他の霊長類においても報告されている。南米ではブラジルにおけるムルキ（K. Strier），中南米では，オマキザル（M. Baker）さらに日本では，ニホンザル（伊谷純一郎ら）についてその可能性が指摘されている（Baker, 投稿中; 伊谷, 1992; Strier, 1993）。このことは，今後，薬効成分を持つ植物利用に関する研究が，各種霊長類の文化的能力の研究，さらにその種間の比較研究など新しい課題へ発展する可能性を示している。

研究の今後の展望

以上の結果に基づいて，１９９１年度以後私たちは，マハレM集団を対象に，個体追跡法によって特定個体の行動を徹底的に追跡・記録し，その個体の糞と採食植物の採取を進めている。これらの試料についての多面的な分析は目下進行中であるが，個体の行動上に現われる異常症状の認定，ならびに糞分析による寄生虫感染症の確認とその感染度の判定によって，最終的には，チンパンジーの疾病への自主的対処の様態とその効能をより客観的に把握できると考えている。

本研究は，これまで体系的には調査されていない野生チンパンジーの薬草利用について，新しい調査法を設定し，解析を始めたものであり，新しい分野を開拓するものとして多彩な分野を統合させる試みでもある。この成果は，チンパンジーの行動と生態の研究において，日常の食生活における健康の維持，疾病ならびにそれへの対応についての認知とその進化といった点で，新たな問題提起につながると考えている。

また，この研究は，霊長類の病気，特に寄生虫感染症に対する霊長類各種の自主的対処についての研究でもある。この研究により，寄生虫感染症に対する個体の行動の変化や，その行動変化が他個体や集団全体に及ぼす影響なども知ることができるはずである。対象地域と種を広げることによって，霊長類の病理学的変異と，それへの対処についての文化的，非文化的行動といった視点の研究も加えることができるにちがいない。薬効成分を持つ植物の利用には，生理状態の変化と植物利用とを因果的に結び付ける高度な能力と経験を要すると考えられるので，この研究を通じて，霊長類の文化的能力の基盤である認知能力の種間の相違という新たな問題にも光が当てられるだろう。私達は，霊長類の薬的植物利用の研究を推進するために，霊長類学者，化学者さらには薬学者からなる国際的研究チーム（The C.H.I.M.P.P. GROUP）として，上記課題の長期的な研究を一層推進していくつもりである。

謝　辞

本稿をまとめるのにあたり，西田利貞教授，小清水弘一教授，大東肇助教授，後藤俊二氏，R.W. Wrangham 教授をはじめとする京都大学動物学教室，食品工学農業分析学研究室，霊長類研究所およびハーバード大学の方々に，さらに川中正憲博士，J.D. Phillipson教授，C.R. Wright博士，G.C. Kirby博士，G. Balansard教授，P. Timon-David教授，R. Elias教授らからさまざまな御協力と御援助をいただいた。また，川中健二教授，上原重男教授，M.S. Kalunde氏，山極寿一博士，中村美穂氏，松沢哲郎教授，C. Boesch博士，黒田末寿博士，R. Elias 博士の御助言と未発表資料を教えていただいた。以上の方々に厚くお礼を申し上げたい。本研究の一部は，文部省科学研究費補助金（海外学術調査，代表京都大学教授西田利貞，課題番号：No. 61043017, 62041021, 63043017, 03041046）の援助を受けた。また，本稿をまとめるのにあたり，日本学術振興会奨励研究の援助を受けた。

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(Summary)

An Investigation of the Use of Medicinal Plants by Wild Chimpanzees. Current Status and Future Prospects.

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It has been proposed that chimpanzees use a number of toxic plant species for their medicinal value. Based on behavior, plant pharmacology, and ethnomedical information, hypotheses concerning the medicinal use of some of these plants by chimpanzees include the following: control of parasites, treatment of gastrointestinal disorders, regulation of fertility, and possible anti-bacterial or anti-hepatotoxic activity. With regards to bitter pith chewing and whole leaf swallowing behaviors, 20 medicinal plant species have been observed to be used not only by chimpanzees, but also by bonobos and lowland gorillas at 7 sites (Mahale, Gombe, Kibale, Kahuzi-Biega, Wamba, Tai, Bossou) across Africa. A detailed description is given of the research program currently being carried out by the author and colleagues of the international research team, The C.H.I.M.P.P. Group, and in particular, of the ongoing multi-disciplinary research into the chimpanzee use of *Vernonia amygdalina* (Del.) in the Mahale Mountains National Park Tanzania. The hypothesis that this species has medicinal value for chimpanzees comes from detailed observations by the author of ailing individuals' use of the plant. Quantitative analysis and assays of the biological activity of *V. amygdalina* have revealed the presence of two major classes of bioactive compounds. The most abundant of these constituents, the sesquiterpene lactone vernodalin, and the steroid glucoside vernoioside B1 (and its aglycones) have been demonstrated to possess antibiotic, anti-tumor, anti-amoebic, anti-malarial, anti-leishmanial, and anti-schistosomal properties. At Mahale, the particular parts of an additional 12 plant species ingested by chimpanzees are recognized for their traditional use against parasite or gastrointestinal related diseases in humans. Their physiological activities are now being investigated in the laboratory.

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MÉTISSAGES EN SANTÉ ANIMALE
de Madagascar à Haïti

sous la direction de
Kakule KASONIA et Michel ANSAY

Édition botanique par Martine BAERTS et Jean LEHMANN

1994

PRESSES UNIVERSITAIRES DE NAMUR
CTA
ACCT

Les plantes médicinales utilisées par les chimpanzés sauvages

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Dans les montagnes à l'Ouest de la Tanzanie, dans le parc de Mahale, près du lac Tanganyika, des hommes observent depuis bientôt 30 ans, un groupe de chimpanzés hôtes habituels de ce parc de Mahale, considéré avec ses 1600 km² de végétation tropicale comme un dernier sanctuaire pour ces anthropoïdes qui nous ressemblent.

Les observateurs sont les gardiens du parc, experts en botanique et en médecine traditionnelle. Ils sont encadrés par une équipe de chercheurs japonais, le Japon ayant conclu, avec la Tanzanie, un projet de coopération sur l'étude des chimpanzés.
Cela fait bientôt 30 ans que ces chimpanzés sont suivis à la trace, les pisteurs les connaissent tous, ils en ont identifié 98, chacun reconnaissable par un caractère particulier. Les chimpanzés se déplacent habituellement en petits groupes de 20 à 30 individus, parfois 70 individus ont pu être observés dans la même journée.

Au départ, l'étude était essentiellement éthologique : les observateurs ont donc tout noté, ce qu'ils mangent, leur aspect physique, leur mode de vie, leurs attitudes, leurs comportements.
Dans leur nourriture, ils ont recensé 328 éléments différents, dont 198 espèces végétales (surtout feuilles et fruits), des insectes, des petits mammifères, des oiseaux, des œufs, des fourmis, des termites et plus étonnant la termitière aussi.

Avec un peu d'expérience, ils remarquent un changement dans les habitudes alimentaires de certains animaux : au lieu de mâcher les feuilles, comme ils le font d'habitude, ils paraissent choisir une espèce végétale particulière, à feuilles rugueuses.
Les feuilles sont mises entières, 1 à 1, dans la bouche; ils paraissent remuer, frotter cette feuille pendant 5 secondes environ dans leur bouche, sans la mâcher, pour enfin l'avaler. Ces feuilles se retrouveront, entières, non digérées dans les fèces.
Ils notent également le comportement particulier d'une femelle malade, elle traîne à l'arrière du groupe, puis s'éloigne avec son petit. Après s'être reposée elle part ramasser de jeunes pousses végétales, mais elle rejette les feuilles et la partie extérieure de la tige pour ne récupérer que la moelle centrale qu'elle mâche avec application avant d'en avaler le jus. Ce jus, nos patients observateurs l'ont goûté, il est plus amer que le sulfate de quinine. Vingt-quatre heures après, notre femelle paraît à nouveau en bonne santé et suit le reste du groupe.
Ces modifications comportementales ont suggéré deux hypothèses :

- *Les chimpanzés sont-ils capables de sélectionner les plantes pouvant améliorer leur état de santé ?*
- *Et, si tel est le cas, les plantes ainsi retenues permettront-elles de trouver de nouveaux médicaments utilisables en thérapeutique humaine ?*

Depuis une dizaine d'années, les chercheurs japonais ont essayé de vérifier ces hypothèses par une étude rationnelle. Pour cela :

- ils relèvent de façon systématique les plantes utilisées par les chimpanzés et réalisent leur identification botanique;
- ils étudient en parallèle l'aspect physique, le comportement de l'animal, de manière à établir une corrélation : état de santé – plantes utilisées;
- pour rester le plus rigoureux possible dans cette interprétation, ils procèdent également à des analyses coprologiques afin d'identifier les parasites dont les chimpanzés seraient porteurs;
- ils réalisent une étude cas par cas en suivant un schéma précis : analyse des fèces d'un animal identifié (les différents parasites et leur nombre sont notés), observation de son comportement (fatigue, somnolence) ou d'autres symptômes (constipation, signe de souffrance ou de maladie possible...), étude des plantes qu'il mange, de la manière dont il les mange;

- ils vérifient l'efficacité du supposé traitement en contrôlant à nouveau des fèces (numération des parasites), amélioration ou non des symptômes, modification du comportement ...
- enfin, ils étudient les variations d'utilisation des plantes :
  – d'un site à un autre (autres parcs en Tanzanie et en Ouganda),
  – entres différentes espèces de primates : chimpanzés, gorilles, singes,
  – en fonction des saisons et de la charge parasitaire;
- avec l'aide des tradipraticiens, ils étudient la pharmacopée traditionnelle afin de vérifier si ces plantes sont également utilisées en médecine traditionnelle, et pour quelles pathologies.

Cette première partie du travail, basée essentiellement sur des observations, sur le recueil d'échantillons (plantes, selles) et sur l'information, se réalise sur le terrain. Une collaboration étroite existe entre pisteurs, éthologues, botanistes et tradipraticiens.

Une autre étape consiste à isoler et identifier les principes actifs contenus dans ces plantes. Puis il faudra effectuer une recherche bibliographique concernant les molécules identifiées afin de retrouver une bioactivité déjà décrite : antiparasite, antibactérienne, antivirale, antifongique, antihépatotoxique, antitumorale ou régulatrice de la fertilité.
Pour les molécules nouvelles, des tests pharmacologiques reconnus, avec une méthodologie précise, devraient permettre de vérifier leur activité pharmacologique et leur toxicité éventuelles, avant d'envisager une possible utilisation en thérapeutique humaine.

Afin de mener à bien l'ensemble de ce très lourd programme, les chercheurs intéressés se sont regroupés en 1989 pour former le C.H.I.M.P.P. GROUP (Chemo-ethology of Hominoïd Interactions with Medicinal Plants and Parasites).
Avec comme principaux organisateurs : les Docteurs HUFFMAN, KOSHIMIZU et OHIGASHI de l'Université de Kyoto au Japon, c'est un groupe multinational (Japon, France et Angleterre) et pluridisciplinaire (zoologie, botanique, écologie, chimie, parasitologie, pharmacognosie, pharmacologie, éthologie).
Les premiers résultats de ce vaste programme ont été publiés par M.A. HUFFMAN et R.W. WRANGHAM.

Qu'ont-ils obtenu ?

Les primates étudiés sont des chimpanzés (*Pan troglodytes schweifn urthii*, P. t. *verus*), bonobos (*Pan panicus*) et des gorilles (Gorilla gorilla graueri). Ces animaux ont été observés sur 5 sites différents, en Tanzanie et en Ouganda .
Sur les 198 espèces végétales utilisées, 13 (4 familles, 7 genres) ont été répertoriées comme susceptibles d'être utilisées pour leurs propriétés médicinales :

- la moelle de 2 plantes est mâchée avec application,
- les feuilles de 11 autres plantes sont avalées entières.

Plantes dont la moelle amère est mâchée

L'étude botanique a permis son identification, il s'agit essentiellement de *Vernonia amygdalina* : elle est utilisée par les animaux malades, les feuilles et l'écorce sont éliminées alors que la moelle est mâchée pour en extraire le suc amer.
Une femelle malade, ayant perdu l'appétit, constipée, émettant des urines foncées, 24 h après cette ingestion retrouve l'appétit, un transit intestinal et un comportement normal. Une autre femelle diminue son parasitisme intestinal avec le même traitement. D'autres chimpanzés sucent de manière identique la moelle de *Vernonia colorata.*
Or, *V. amygdalina* et *V. colorata* sont des espèces très voisines, en médecine traditionnelle africaine, elles sont utilisées toutes deux pour les maux d'estomac, comme anthelminthique, fébrifuge et comme anti bilharzien.

D'autre part, l'analyse phytochimique de *V. amygdalina* collectée dans le parc de Mahale, donne 2 classes de composés bioactifs :

- des lactones sesquiterpéniques : vernodaline, vernolide, hydroxy-vernolide, vernodalol;
- et un nouveau groupe de glucosides stéroliques : les vernoniosides A1, A2, A3, et le vernonioside B1 (OHIGASHI *et col.*, 1991; JISAKA , *et col.* 1992).

Ces composés sont présents dans les feuilles, l'écorce, la racine et la moelle, mais le vernonioside B1 est présent en grande quantité dans la moelle.

De nombreux travaux antérieurs ont déjà démontré l'activité anthelminthique, amoébicide, antitumorale et antibiotique de lactones sesquiterpéniques.
Des expériences récentes, réalisées par les chercheurs du CHIMPP Group, sur les lactones sesquiterpéniques, mais également sur les glucosides stéroliques, indiquent d'autres activités parasiticides. JISAKA a trouvé, *in vitro,* une action antibilharsienne alors que des essais préliminaires de TIMON-DAVID, de KIRBY et ALLEN, semblent indiquer également une activité leishmanicide et antimalarique.
En résumé, si on considère :

- la faible fréquence d'ingestion de la moelle de *V. amygdalina*;
- le fait qu'elle soit utilisée lorsque l'animal paraît malade ou parasité (à Mahale, elle est utilisée en plus grande quantité pendant la saison des pluies, période pendant laquelle les infections parasitaires augmentent);
- que le goût amer est habituel dans la nourriture normale des chimpanzés;
- que la valeur nutritionnelle de la partie absorbée est inexistante par rapport à sa valeur pharmacologique (démontrée expérimentalement);
- que l'animal paraît guéri après cette absorption.

On peut en déduire logiquement, que les chimpanzés utilisent cette plante, non pas pour se nourrir, mais pour améliorer leur état de santé et plus particulièrement pour réduire leurs infections parasitaires.

Plantes dont les feuilles sont avalées entières

Ces plantes ont des feuilles épaisses à surface rugueuse. Elles sont avalées à une heure précise (à l'aube, environ 1 h après le lever du nid, l'animal restant à jeun). Les feuilles ne sont pas mâchées, seulement frottées avec la langue puis avalées entières. On les retrouve non digérées dans les selles.
L'étude botanique indique qu'il s'agit essentiellement *d'Aspilia mossambicencis, A. pluriseta* et *A. rudis.*
Dans le parc de Mahale, 8 autres plantes sont avalées de la même façon : *Lippia plicata, Commelina diffusa, Ficus exasperata, Trema orientalis, Aneilema aequinoctiale, Hibiscus aponeurus, Rubia cordifolia et Melastomastrum capitatum.*
Ce mode particulier d'absorption des feuilles a été retrouvé dans d'autres populations de chimpanzés, dans d'autres sites, avec l'utilisation d'autres espèces végétales présentes dans ces endroits.

L'observation des animaux a montré :

- qu'il existe un pic saisonnier dans la consommation des feuilles *d'Aspilia,* on note une augmentation à la saison des pluies alors que la charge parasitaire est la plus importante.
- qu'un mâle hyperparasité par divers nématodes (Strongles, Trichures, ternide) absorbe une grande quantité de feuilles de *Ficus exasperata.* Le nombre de parasites a diminué et son état de santé s'est amélioré.
- que les femelles chimpanzés mangent les feuilles *d'Aspilia pluriseta* et *d'Aspilia rudis* en plus grande quantité que les mâles.

En médecine traditionnelle, ces plantes sont utilisées pour éliminer les parasites intestinaux, pour soulager les maux de tête, faire tomber la fièvre paludique, soulager les troubles gastro-intestinaux, pour guérir les infections et plus spécifiquement pour *A. pluriseta et A. rudis* utilisées comme abortif ou inducteur du travail chez les parturientes.
L'analyse physicochimique des feuilles d'*Aspilia* a permis d'isoler une diathiane polyine et la thiarubrine A qui ont un pouvoir antibiotique, anthelminthique et antifongique (RODRIGUEZ *et col.*, 1985; TOWERS *et col*; WRANGHAM et GOODALL, 1989).
A. pluriseta et A. rudis renferment encore 2 diterpènes : l'acide kaurenoique et l'acide grandiflorenique activateurs des contractions utérines (PAGE *et col.*).
Des feuilles de *Ficus exasperata,* les chercheurs du CHIMPP group ont isolé un 5-methoxypsoralen et quelques furanocoumarines à effet nématocide (NEWTON et NISHIDA, 1990; HUFFMAN et GOTO).
Cette activité nématocide apparaît à la concentration de 13 µg/ml, par extrapolation la même activité nématocide serait obtenue par les chimpanzés en consommant 50 à 100 feuilles (les produits bioactifs sont plus abondants dans les jeunes feuilles).

Les chimpanzés utilisent-ils réellement ces plantes dans un but thérapeutique ?

Il est permis de le supposer si on considère que :

- La valeur nutritive de ces feuilles est nulle puisqu'elles sont retrouvées apparemment intactes dans les selles.
- L'état de santé des animaux s'est trouvé amélioré après leur utilisation.

Ces plantes sont utilisées en médecine traditionnelle. Des substances chimiques à activité pharmacologique spécifique ont été retrouvées.

Mais des questions restent posées :

- Les chimpanzés choisiraient-ils ces feuilles parce qu'elles sont rugueuses ? Le critère physique est-il essentiel pour leur choix ?
- Toutes les feuilles avalées entières (à part une espèce) sont rugueuses, hérissées de poils. On peut faire une analogie avec les carnivores domestiques (comme le chat) qui mangent de l'herbe, sans qu'il y ait pour cela une reconnaissance chimique significative.
- Pourtant, d'autres plantes à feuilles rugueuses n'intéressent pas les chimpanzés.
- Les critères chimiques, ou tout au moins les propriétés pharmacologiques, seraient reconnus par les chimpanzés capables d'effectuer une sélection entre les différentes espèces végétales.

On a essayé d'établir une corrélation entre le mode d'utilisation et le mode d'action de ces plantes.
Le fait que la feuille, d'une dureté particulière, soit avalée et non mâchée, éviterait à la thiarubine A d'être détruite par le suc gastrique permettant ainsi à la drogue d'atteindre sa cible. La feuille jouerait le rôle d'une capsule.
Mais les feuilles sont frottées, retournées dans la bouche avant d'être avalées, la drogue pourrait alors passer directement dans le sang, sans passer par l'estomac. Dans certaines espèces de composées, la drogue se situe dans les poils des feuilles. Il y aurait alors un passage per-lingual possible.

Ces questions ne sont pas élucidées, comme bien d'autres encore

- Dans quel but les chimpanzés mangent-ils les écorces de certains arbres, écorces indigestes, parfois toxiques, sans valeur nutritive, mais la médecine traditionnelle leur accorde un effet purgatif, tonique, émétique ou même antidote de certains poisons ?
- La géophagie observée a-t-elle pour but d'absorber les tannins ou d'autres toxines accumulées dans l'estomac ?

Des expériences sont en cours pour essayer de répondre à ces questions

- Il nous reste aussi à comprendre comment les chimpanzés ont appris à reconnaître les plantes médicinales ?
- Par l'éducation ? Les jeunes apprennent avec leur mère à identifier les plantes qui les soignent, les nourritures qui leur sont bénéfiques et les plantes toxiques.

- Nos observateurs japonais ont vu de jeunes chimpanzés, suivant l'exemple de leur mère, sucer la moelle de *V. amygdalina,* sans avaler le suc amer.
- Par un apprentissage personnel ? Une plante toxique tue rarement l'animal, mais le rend malade. Une expérience de ce genre lui suffira par la suite à l'éviter. De même il se souviendra des plantes bienfaisantes.

En 1992, le 1er Symposium sur l'utilisation des plantes par les animaux, s'est tenu à Chicago, à l'instigation de l'Association américaine médicinale pour le progrès de la science.
Plusieurs témoignages, semblables à ceux réalisés avec les chimpanzés, ont permis de consacrer une nouvelle discipline, la *Zoopharmacognosie.*
Cette nouvelle discipline, illustrée par cette étude sur l'utilisation des plantes médicinales par les chimpanzés n'en est encore qu'à son début.
Pourra-t-elle un jour déboucher sur l'identification de nouvelles molécules utilisables par la médecine humaine ?
On peut l'espérer, car déjà des brevets ont été déposés pour exploiter les propriétés de l'*Aspilia.*

En tant qu'ethnopharmacologues, nous savons tous qu'il faudra poursuivre ces études de la manière la plus rationnelle possible, avec une rigueur sans faille. Mais, arrivés à la fin de l'histoire, pourquoi ne pas rêver un peu. Imaginer un monde où tout nous a été donné dès le départ, et dont nous, pauvres humains, à force d'évolution, avons perdu le code. Alors pourquoi ne pas se retourner vers les animaux, qui eux savent encore … peut-être.

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