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ALKALOIDS OF PERUVIAN *UNCARIA TOMENTOSA*

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Key Word Index—*Uncaria tomentosa*; Rubiaceae; alkaloid distribution; seasonal variation.

Abstract—Sixteen plants of *Uncaria tomentosa* were investigated for their alkaloid content and the alkaloid distribution in various parts of the plant was examined. Two chemical types were identified. Seventeen alkaloids were detected and a seasonal variation in the alkaloid content was observed. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Uncaria tomentosa (Willd.) DC. is said to be widely used in traditional medicine (known as 'uña de gato', cat's claw) by native people of the Peruvian rain forest. Numerous applications have been reported, including abscesses, arthritis, asthma, cancer, chemotherapy side-effects, contraception, disease prevention, fevers, gastric ulcers, haemorrhages, inflammations, menstrual irregularity, recovery from child-birth, rheumatism, skin impurities, urinary tract inflammation, weakness and wounds [1, 2]. It is unlikely that a single substance or at least a group of substances could be responsible for these diverse activities. However, some of them might be due to alkaloids. Previous workers have reported the presence of either pentacyclic or tetracyclic oxindole alkaloids and their indole precursors [3–8]. Traditionally, a tea is prepared from the bark of the root or the stalk, but, in some cases, the leaves are also used for treatments. As part of our continuing interest in Peruvian medicinal plants, we now present a comprehensive report on the alkaloid distribution in various parts of 16 individual *U. tomentosa* plants and the seasonal variation of the alkaloid content in the leaves of this species.

RESULTS AND DISCUSSION

Leaves and root samples of 13 plants of *U. tomentosa* from Peru were investigated for their alkaloid content and the results are summarized in Table 1. Two chemically different groups were present. Plants 1–6 contained pentacyclic oxindole and indole alkaloids. Plants 14–16 were cultivated in a greenhouse and displayed the same pentacyclic alkaloid pattern. In young leaves uncarine F (**4**) was the predominant oxindole alkaloid among pteropodine (**1**), isopteropodine (**2**) and speciophylline (**3**), while in mature leaves, **3** was more abundant. This is surprising considering that, in the isolated form, **3** and **4** are the thermodynamically more unstable isomers of this D/E *cis*-fused group of alkaloids which undergo isomerization very readily [9]; this does not necessarily reflect the situation in the plant. We assume that they are synthesized in the young leaves from the stereochemically-corresponding indole alkaloid, akuammigine **11**, which has not been identified before in *U. tomentosa*. Plants 4 and 14 were especially rich in **11**. The 3-epimer, tetrahydroalstonine **12**, was found only in small amounts. In general, the young leaves have the highest alkaloid concentration of all parts of the plant. Moving down the plant, the D/E *trans*-fused oxindole alkaloid, mitraphylline **5**, occurred in some of the older leaves and in the green bark of the twigs and, in addition, isomitraphylline **6** was present in the stem bark (Table 2). As the corresponding D/E *trans*-fused indole, ajmalicine, was not present in any of the samples, it would appear that **5** and **6** are produced from the D/E *cis*-fused oxindoles. It has been shown that this transformation is possible in *Mitragyna parvifolia* by feeding [^{14}C]pteropodine into the stem bark which was partly converted into [^{14}C]mitraphylline [10]. The roots contained all six pentacyclic oxindole alkaloids in proportions which reflect an acidic medium, preferring **1**, **3** and **5**.

Plant 6 was exceptional in that it produced in its leaves only the mitraphyllines **5** and **6**, which were absent in the leaves of the other plants examined. The expected precursor indole, ajmalicine, was not detected but instead the 3-epimer isoajmalicine **13** was found especially in the young leaves. Therefore, epimerization must take place in the course of the transformation to the oxindoles. This biosynthetic pathway has also been reported for several *Mitragyna* species [11]. Consequently, the root contained unusually high concentrations of **5** and **6**.

Table 1. Alkaloid distribution in different parts of Peruvian *Uncaria tomentosa* plants*

	Plant no.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	mg alkaloid g^{-1} plant material													
Young leaves														
Pteropodine **1**	3.3	5.2	3.0	0.82	2.9									0.59
Isopteropodine **2**	1.5	2.0	2.1	0.67	1.3									0.44
Speciophylline **3**	7.1	8.6	7.0	1.2	6.5									2.0
Uncarine F **4**	11.2	17.5	13.7	1.6	10.8									1.9
Mitraphylline **5**						2.0			0.03		0.06			
Isomitraphylline **6**						2.0			0.02		0.06			
Rhynchophylline **7**							9.4	9.2	10.7	12.9	10.0	6.5	10.9	
Isorhynchophylline **8**							17.1	14.8	20.8	24.2	21.0	14.1	18.1	
Corynoxeine **9**							0.21	0.27	0.44	0.45	0.24	0.22	0.39	
Isocorynoxeine **10**							0.32	0.29	0.17	0.28	0.45	0.31	0.33	
Akuammigine **11**	3.1	9.6	6.1	40.0	4.7									23.0
Tetrahydroalstonine **12**	0.30	0.62	1.5	0.85	0.32									
Isoajmalicine **13**						28.1								
Hirsutine **14**							0.93	2.3	1.7	2.3	1.8	1.6	1.8	
Dihydrocorynantheine **15**							1.7	4.8	1.6	3.1	1.9	1.3	4.4	
Hirsuteine **16**							0.08	0.21	0.11	0.12	0.13	0.08	0.14	
Corynantheine **17**								0.08					0.04	
Total	26.5	43.5	33.4	45.1	26.5	32.1	29.7	32.0	35.6	43.4	35.6	24.1	36.1	27.9
Mature leaves														
Pteropodine **1**	4.0	4.7	4.7	4.6	4.8									2.9
Isopteropodine **2**	1.7	3.0	2.0	3.1	0.9									2.0
Speciophylline **3**	4.9	10.8	5.7	7.4	5.1									5.0
Uncarine F **4**	1.1	2.8	1.4	1.9	1.1									2.2
Mitraphylline **5**						10.9			0.09		0.19			

Isomitraphylline **6**	0.09		0.11		0.25	8.7			0.09		0.19			
Rhynchophylline **7**							3.9	3.2	3.2	4.1	4.8	7.7	4.4	
Isorhynchophylline **8**							9.9	8.7	9.1	11.2	13.8	22.2	12.0	
Corynoxeine **9**							0.08	0.06	0.05	0.08	0.09	0.19	0.10	
Isocorynoxeine **10**							0.24	0.20	0.17	0.25	0.30	0.60	0.32	
Akuammigine **11**	0.05	0.39												9.2
Isoajmalicine **13**						0.31								
Hirsutine **14**							0.21	0.12		0.12		0.33	0.15	
Dihydrocorynantheine **15**							0.50	2.1	0.45	0.80	0.83	0.70	0.58	
Hirsuteine **16**								0.14						
Total	11.8	21.7	13.9	17.0	12.2	19.9	14.8	14.5	13.2	16.6	20.2	31.7	17.6	21.3
Roots														
Pteropodine **1**	2.9	3.9	3.9	5.6	2.3	3.0			0.35		0.61			2.7
Isopteropodine **2**	1.5	2.3	2.4	2.8	1.3	1.6			0.40		0.22			1.9
Speciophylline **3**	3.0	5.5	5.8	6.6	4.7	4.8			0.61		1.0			5.0
Uncarine F **4**	1.0	2.8	2.9	2.5	2.6	3.1			0.27		0.24			2.4
Mitraphylline **5**	1.9	2.7	1.7	2.3	2.9	4.7			1.5		1.2			5.0
Isomitraphylline **6**	1.0	1.5	0.8	1.1	1.9	3.1			1.1		0.92			3.3
Rhynchophylline **7**		0.19	0.28	0.17	0.18	0.11	6.8	8.7	6.0	5.0	1.9	5.9	10.3	
Isorhynchophylline **8**		0.64	0.94	0.58	0.23	0.38	16.0	19.4	15.9	13.2	10.3	15.4	21.6	
Corynoxeine **9**		0.01					0.32	0.28	0.30	0.26	0.21	0.31	0.51	
Isocorynoxeine **10**		0.01					0.59	0.51	0.55	0.49	0.40	0.57	0.95	
Akuammigine **11**		0.28	0.27	0.17	0.30	0.25								0.09
Hirsutine **14**			0.03				0.44	1.0	0.72	0.42	0.22	1.0	0.80	
Dihydrocorynantheine **15**		0.03	0.05	0.04	0.01		0.56	1.0	0.71	0.37	0.23	0.29	1.51	
Hirsuteine **16**				0.06	0.01			0.19	0.02		0.04			
Total	11.3	19.9	19.1	21.9	16.7	21.0	24.7	31.1	28.4	19.7	17.3	23.5	35.7	20.4

* No entry means that the corresponding alkaloid was not detected (<0.01 mg g^{-1}).

Table 2. Alkaloid distribution in different parts of *Uncaria tomentosa* plants (Innsbruck, April 1994)*

	Young leaf	Mature leaf	Twig bark	Stem bark	Root
	mg alkaloid g^{-1} plant material				
Plant no. 15					
Pteropodine **1**	2.8	9.3	3.0	0.05	1.1
Isopteropodine **2**	1.9	4.1	0.63	0.06	0.57
Speciophylline **3**	12.2	9.6	1.6	0.08	2.1
Uncarine F **4**	47.9	2.4	0.63	0.03	0.91
Mitraphylline **5**			0.50	0.03	1.6
Isomitraphylline **6**		0.06		0.02	0.84
Akuammigine **11**					
Total	64.8	25.5	6.4	0.27	7.1
Plant no. 16					
Pteropodine **1**	3.1	5.2	1.7	0.32	1.5
Isopteropodine **2**	0.89	1.9	0.54	0.23	0.65
Speciophylline **3**	6.4	6.2	1.3	0.50	1.5
Uncarine F **4**	18.3	1.5	0.39	0.22	0.50
Mitraphylline **5**			0.55	0.30	1.6
Isomitraphylline **6**					0.72
Akuammigine **11**	1.7				
Total	30.4	14.8	4.5	1.6	6.5

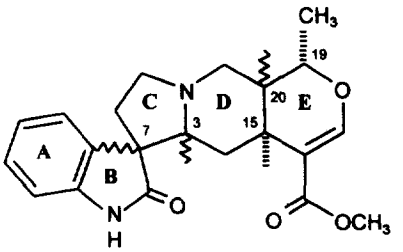
* No entry means that the corresponding alkaloid was not detected (<0.01 mg g^{-1}).

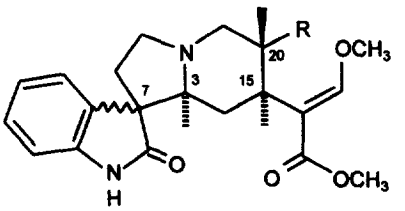
In contrast, in plants 7–13, the tetracyclic oxindole alkaloids, rhynchophylline **7** and isorhynchophylline **8** prevailed, along with their $\Delta^{18,19}$-congeners, corynoxeine **9** and isocorynoxeine **10**. The same four alkaloids occur together in other species of *Uncaria* [4]. In the leaves and roots, small amounts of the tetracyclic indole alkaloids, hirsutine **14**, dihydrocorynantheine **15** and traces of hirsuteine **16** were found; in two instances, corynantheine **17** was also detected. It is remarkable that no significant differences in the pro-

Table 3. Seasonal variation of alkaloids in leaves of *Uncaria tomentosa* (Innsbruck)*

	Apr 94	Dec 94	Apr 95	May 95	Oct 95	Dec 95	Apr 96	May 96	Jul 96	Sep 96
Plant no. 16	mg alkaloid/g plant material									
Young leaves										
Pteropodine **1**	3.1	3.8	11.5	7.1	8.2	†	1.1	4.7	2.7	4.0
Isopteropodine **2**	0.89	2.3	4.2	2.2	2.6		1.3	1.8	1.7	1.4
Speciophylline **3**	6.4	13.4	14.0	13.7	9.4		8.5	6.5	7.4	8.3
Uncarine F **4**	18.3	7.4	64.4	44.6	2.2		25.9	29.6	39.8	15.2
Mitraphylline **5**					0.02					
Isomitraphylline **6**					0.10					
Akuammigine **11**	1.7	0.75	15.8	10.8			2.1	3.1	6.8	1.5
Tetrahydroalstonine **12**			4.1	3.5			0.15		1.2	0.1
Total	30.4	27.7	114.0	81.9	22.5		39.1	45.7	59.6	30.5
Mature leaves										
Pteropodine **1**	5.2	5.3	5.1	5.3	8.0	5.6	6.4	4.9	4.5	5.9
Isopteropodine **2**	1.8	3.3	3.4	2.4	0.38	3.2	3.8	1.7	3.8	3.3
Speciophylline **3**	6.2	12.7	12.0	15.2	8.9	9.6	9.4	11.5	10.8	11.0
Uncarine F **4**	1.5	2.4	3.6	4.4	1.7	2.3	2.6	3.7	3.3	2.6
Isomitraphylline **6**					0.28					
Akuammigine **11**				0.44				0.33	0.45	0.04
Total	14.7	23.7	24.1	27.7	19.3	20.7	22.2	22.1	22.9	22.8

* No entry means that the corresponding alkaloid was not detected (<0.01 mg g^{-1}).
† Young leaves not available.



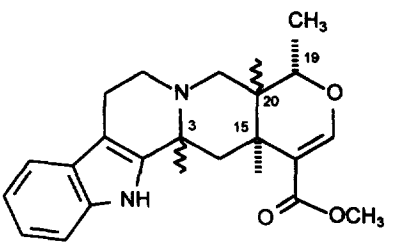


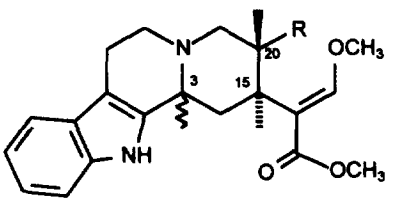
1-6

1	3*S*, 7*R*, 15*S*, 19*S*, 20*S*
2	3*S*, 7*S*, 15*S*, 19*S*, 20*S*
3	3*R*, 7*S*, 15*S*, 19*S*, 20*S*
4	3*R*, 7*R*, 15*S*, 19*S*, 20*S*
5	3*S*, 7*R*, 15*S*, 19*S*, 20*R*
6	3*S*, 7*S*, 15*S*, 19*S*, 20*R*

7-10

7	3*S*, 7*R*, 15*S*, 20*R* R=ethyl
8	3*S*, 7*S*, 15*S*, 20*R* R=ethyl
9	3*S*, 7*R*, 15*S*, 20*R* R=vinyl
10	3*S*, 7*S*, 15*S*, 20*R* R=vinyl





11-13

11	3*R*, 15*S*, 19*S*, 20*S*
12	3*S*, 15*S*, 19*S*, 20*S*
13	3*R*, 15*S*, 19*S*, 20*R*

14-17

14	3*R*, 15*S*, 20*R* R=ethyl
15	3*S*, 15*S*, 20*R* R=ethyl
16	3*R*, 15*S*, 20*R* R=vinyl
17	3*S*, 15*S*, 20*R* R=vinyl

portions of the main oxindole alkaloids between leaves and roots were found.

The seasonal variation in alkaloids was monitored in plant 16 (Table 3). Contents of uncarine F4 in the young leaves were high during spring and summer and decreased in autumn. However, in mature leaves no such variation could be observed. In a previous report, we described three plants of *U. tomentosa* which had shifted their alkaloid pattern in the course of years [8]. In the present study, plants 9 and 11 contained traces of pentacyclic alkaloids, in addition to the main tetracyclic alkaloids. This is probably an indication of a forthcoming shift. On the other hand, the small amounts of tetracyclic alkaloids in the roots of plants 2-6 could be remnants from a recent shift. It is not yet understood what influences cause a particular alkaloid pattern but it should be noted that plants from controlled environment contained only pentacyclic alkaloids.

In light of our results, it is not advisable to ascribe certain pharmacological properties to *U. tomentosa* in general. This species does not produce a constant, uniform pattern of constituents as far as the alkaloids are concerned; this factor has to be considered when plant extracts are screened for activity. Depending on whether a specific plant contains the tetracyclic or pentacyclic type of alkaloids, different activities should be expected. Therefore we propose to distinguish the two chemical modifications as *U. tomentosa* 'tetracyclic alkaloid-type' or 'pentacyclic alkaloid-type', respectively.

EXPERIMENTAL

Plant material. Samples 1–13 were collected at an experimental plantation in Kivinaki, Province of Chanchamayo, Region of Andres Avelino Caceres, Peru. Roots were harvested in March 1994 and leaves in May 1994. At that time, plants were *ca* 2 years old. Plant 14 has grown since 1986 at the Botanical Garden of the University of Graz, Austria, from seeds obtained from the Botanical Garden near San Luis de Shoaro, Province of Chanchamayo. Plants 15 and 16 have grown since 1992 at the Botanical Garden of the University of Innsbruck, Austria, from root stocks obtained from Kivinaki. Unfortunately, plants 14 and 15 died during the study, making it impossible to gather more data on seasonal variation. Voucher

specimens are deposited at Immodal Pharmaka, GmbH.

Identification of alkaloids. Alkaloids (except **12**) were identified by UV, MS and NMR and behaviour on TLC. All data were in complete accordance with the lit. [12–14]. Compound **12** was identical with synthetic tetrahydroalstonine prepd from pteropodine by a published method [15].

Analytical procedure. Air-dried samples were milled, divided into portions (100–200 mg) and macerated ×5 for 30 min with MeOH–H_2O–conc. HCl (500:500:1). The combined extracts were filtered, neutralized with NaOH and dild with 0.01 M aq. Pi buffer (pH 7) to final vol. of 100 ml immediately prior to injn. Dilns were prepd if necessary to obtain concns within the calibrated working range. Alkaloid content was determined by HPLC using MeCN–0.01M Pi buffer pH7 (9:11) as eluent with a flow rate of 1.3 ml min^{-1} at 80°. LiChroCART 125 mm × 4 mm i.d. columns packed with LiChrospher 100 RP-18 (5 μm) were used (Merck). Detection was carried out at 247 nm. R_ts (min) were as follows **3** 2.0, **5** 2.3, **4** 2.6, **1** 2.9, **6** 3.2, **9** and **10** 3.5, **8** 4.1, **7** 4.3, **2** 4.4, **13** 4.5, **16** 4.7, **11** 5.1, **14** 5.8, **17** 6.3, **15** 7.8 and **12** 8.5. When tetracyclic alkaloids were found, samples were reanalyzed with a flow rate of 1.3 ml min^{-1} at 52° using MeCN–0.01M pi buffer pH7 (2:3) as eluent to give optimum resolution of these alkaloids. R_ts (min) were as follows: **3** 3.4, **4** and **5** 4.1, **1** 5.2, **6** 5.4, **10** 5.9, **9** 6.2, **8** 7.2, **7** 8.7 **13** 9.2, **16** 10.8, **11** 10.9, **14** 13.6, **17** 16.0 and **15** 19.5.

Validation of assay procedure. Extraction of leaves gave a 93.5% yield (s.d. 4.1) and roots yielded 92.4% (s.d. 3.7), compared with a Soxhlet MeOH-extraction which was assumed to give 100% (s.d.s 3.9 and 3.6%, respectively) but changing the composition of oxindole alkaloids due to isomerization. Pteropodine was used for calibration of oxindoles, applying correction factors for the M_r of tetracyclic alkaloids. Its purity was checked by HPLC (≥99%) and microanalysis (±0.3%, recrystallized from *t*-BuOMe). Ajmalicine (Fluka, ≥98%) was used as a standard for indoles. Calibration graphs were rectilinear from 0.005 up to 20 mg l^{-1} pteropodine (12 data points; $R^2 = 0.999$; s.d. 0.65 for six higher and 1.05 for six lower concns) and from 0.005 up to 1 mg l^{-1} ajmalicine (6 data points; $R^2 = 0.997$; s.d. 1.53). Accuracy was evaluated by standard addition to three samples at two concns (150 and 200% of original concn) and recoveries ranged from 99.3 to 100.8%. All determinations were carried out in duplicate. System repeatability was 1.03 ($n = 6$). Furthermore, we proved by analyses of fr. and dried halves of the same leaf that alkaloid composition remained unchanged during drying of samples.

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