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Alkaloids of Peruvian *Uncaria guianensis* (*Rubiaceae*)

By

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With 1 Figure.

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Summary

LAUS G. & KEPLINGER K. 2003. Alkaloids of Peruvian *Uncaria guianensis* (*Rubiaceae*). – Phyton (Horn, Austria) 43 (1): 1–8, 1 figure. – English with German summary.

Fourteen individual plants of *Uncaria guianensis* (AUBL.) GMEL. (*Rubiaceae-Coptosapelteae*) were investigated for their alkaloid content, and the alkaloid distribution in leaves and roots of the plant was examined by HPLC and TLC. Seven alkaloids were identified as mitraphylline, isomitraphylline, corynoxeine, isocorynoxeine, rhynchophylline, isorhynchophylline, and dihydrocorynantheine. All alkaloids have a *trans*-substitution pattern at C(15) and C(20). The total alkaloid content of *U. guianensis* was found to be considerably lower than in *U. tomentosa* (WILLD.)DC., the second South American species of this genus.

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Zusammenfassung

LAUS G. & KEPLINGER K. 2003. Alkaloide von peruanischer *Uncaria guianensis* (*Rubiaceae*). – Phyton (Horn, Austria) 43 (1): 1–8, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Vierzehn individuelle Pflanzen der Art *Uncaria guianensis* (AUBL.) GMEL. (*Rubiaceae-Coptosapelteae*) wurden auf ihren Alkaloidgehalt untersucht. Die Alkaloidverteilung in Blättern und Wurzeln wurde mit HPLC und DC untersucht. Sieben Alkaloide wurden identifiziert: Mitraphyllin, Isomitraphyllin, Corynoxein, Isocorynoxein, Rhynchophyllin, Isorhynchophyllin und Dihydrocorynanthein. Alle Alkaloide weisen ein *trans*-Substitutionsmuster an C(15) und C(20) auf. Der Gesamtalkaloidgehalt von *Uncaria guianensis* war wesentlich niedriger als bei *U. tomentosa* (WILLD.)DC., der zweiten südamerikanischen Art dieser Gattung.

Introduction

The species *Uncaria guianensis* (AUBL.) GMEL. (*Rubiaceae-Coptosapelteae*) is said to be widely used in traditional medicine (known as 'uña de gato', cat's claw, like many other plants with curved thorns) by native people of the Amazonian rain forest (CABIESES 1994: 43-52). The use of a decoction of stalks and leaves for the treatment of intestinal affections in Suriname was reported (RAYMOND-HAMET 1952). Due to their high tannin content the dried and powdered leaves are reportedly used for the healing of wounds, and an extract is used to treat dysentery (OSTENDORF 1962). Previous workers have found rhynchophylline and isorhynchophylline as the major alkaloids in the leaves of *U. guianensis* from Bolivia, Brazil, Suriname, and Venezuela, whereas in one sample from Guyana mitraphylline and isomitraphylline prevailed (HEMINGWAY & PHILLIPSON 1974, PHILLIPSON & al. 1978). In contrast, root samples from French Guiana were reported to contain mitraphylline, pteropodine, and speciophylline (LAVAULT & al. 1983). From Peruvian sources so far only the isolation of mitraphylline from the leaves has been described (ALVAREZ & al. 1988). Non-alkaloidal constituents of Peruvian *U. guianensis* were identified in the bark, i.e. the flavonoid aglycones kaempferol and dihydrokaempferol (ALVAREZ & al. 1988), and quinovic acid glycosides (YEPEZ & al. 1991). As part of our continuing interest in Peruvian medicinal plants (LAUS & al. 1997, KEPLINGER & al. 1999), we now present a comprehensive report on the alkaloid distribution in leaves and roots of fourteen individual *U. guianensis* plants.

Material and Methods

Plant material: The samples 1–3 (April 1997) and 4–6 (June 1999) were collected at an experimental plantation in Kivinaki, Province of Chanchamayo, Department of Junin, Region of Andres Avelino Caceres, Peru. Samples 7–14 were harvested along the road to Shaoashipango (June 1999), approximately 70 km south-east from Kivinaki. These habitats are situated at 600–800 m above sea level with a dry

and hot climate. The plants were fully grown. Voucher specimens are deposited at the Institute of Botany, University of Graz, Austria (GZU).

Identification of alkaloids: UV spectra were recorded during the chromatography using a Merck-Hitachi L-7450 diode-array detector and allowed classification of oxindole and indole alkaloids (PHILLIPSON & HEMINGWAY 1975). Authentic natural alkaloids **1–7**, isolated from *U. tomentosa* and identified by MS and NMR spectroscopy, were used as reference compounds for HPLC and TLC. In addition, the oxindole alkaloids in samples of *U. guianensis* showed the typical isomerization which has been described in detail (LAUS & al. 1996, LAUS 1998).

Analytical procedure: Air-dried samples were milled, divided into aliquots (30-50 mg), weighed and homogenized in $H_2O/CH_3CN/HCl$ conc (5 ml, 700:300:1). The extracts were neutralized with 0.1 M NaOH and diluted with 0.01M aqueous phosphate buffer pH7 to a final volume of 10 ml and filtered (0.45 µm) immediately prior to injection. The alkaloid content was determined by HPLC using CH_3CN/0.01 M phosphate buffer pH7 (38:62) as the eluent with a flow rate of 1.3 ml min^{-1} at 50°C. 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. Retention times were as follows: **1** 5.1, **2** 6.8, **3** 7.3, **4** 8.1, **5** 9.1, **6** 10.7, and **7** 20.1 min. An unidentified polar indole alkaloid eluted at 2.5 min. Crystallized mitraphylline and rhynchophylline were used for calibration for the pentacyclic and tetracyclic oxindoles, respectively. Commercial ajmalicine (Fluka) was used as a standard for the indole **7**, with a correction for the M_r.

Validation of assay procedure: The extraction yielded 97 to 107%, compared to a Soxhlet MeOH-extraction which was assumed to give 100% but changes the composition of the oxindole alkaloids due to isomerization. The calibration graphs were rectilinear from 0.05 up to 1 mg l^{-1} mitraphylline (6 data points; R^2=0.996; rel.s.d. 1.2%), rhynchophylline (6 data points; R^2=0.998; rel.s.d. 1.3%), and ajmalicine (6 data points; R^2=0.997; rel.s.d. 1.5%). Limit of quantitation was 0.05 mg l^{-1}, and limit of detection was 0.01 mg l^{-1}. System repeatability was 1.0% (n=6) at 0.5 mg l^{-1}. Accuracy was evaluated by standard addition to three samples at two concentration levels (150 and 200% of original concentration), and recoveries ranged from 96 to 103%.

Thin Layer Chromatography: Plant material was extracted with MeOH. Silica gel 60 F_{254} layers (thickness 0.2 mm) with concentrating zone (Merck) were developed using AcOEt/*iso*-PrOH/NH_3 conc (100:2:1), and spots were detected by spraying with either Ehrlich's reagent (1% in EtOH; HCl vapours) or $FeCl_3$ (0.2 M in 35% $HClO_4$; heating), as described earlier (PHILLIPSON & HEMINGWAY 1975).

Results and Discussion

HPLC analysis of extracts from leaves and roots showed the presence of, in order of elution, the oxindole isomer pairs mitraphylline **1** and isomitraphylline **2**, isocorynoxeine **3** and corynoxeine **4**, isorhynchophylline **5** and rhynchophylline **6**, and the indole alkaloid dihydrocorynantheine **7**. Their structures and stereochemical notation are given in Fig. 1. The numbering system is based on that customarily used for the hetero-yohimbinoid alkaloids. All compounds have a *trans*-substitution pattern at

C(15) and C(20). The results of the quantitative analyses are summarized in Tables 1 and 2.

Table 1. Alkaloid distribution in young leaves and root bark of Peruvian *Uncaria guianensis* plants [a]

	Plant no.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	mg alkaloid g^{-1} plant material													
Young leaves														
Mitraphylline **1**	0.23	0.22	0.03	0.64	0.22	0.10	0.10	0.17	0.02	0.17	0.04	0.02	<0.01	0.01
Isomitraphylline **2**	0.15	0.18	0.04	0.66	0.17	0.52	0.12	0.22	0.04	0.15	0.04	0.02	<0.01	0.02
Isocorynoxeine **3**	0.12	0.01	0.06				0.02		0.04	0.05		0.02		0.01
Corynoxeine **4**	0.06	<0.01	0.03				0.04		0.02	0.01		<0.01		<0.01
Isorhynchophylline **5**	1.51	0.10	0.29	0.02	<0.01		0.74	<0.01	0.22	1.07	0.02	0.24	0.03	0.11
Rhynchophylline **6**	0.55	0.03	0.08	0.01	0.03	<0.01	0.40	<0.01	0.11	0.46	<0.01	0.10	0.01	0.06
Dihydrocorynantheine **7**							0.53			0.13		0.11		0.18
Total [b]	2.62	0.54	0.53	1.33	0.42	0.62	1.95	0.39	0.45	2.04	0.10	0.51	0.04	0.39
Root bark		c		c										
Mitraphylline **1**	0.65	0.83	2.98	0.31	0.06	0.04	0.38	0.41	0.80	0.34	0.68	0.40	0.89	0.54
Isomitraphylline **2**	0.45	0.55	0.93	0.18	0.03	0.01	0.17	0.17	0.41	0.05	0.27	0.14	0.21	0.18
Isocorynoxeine **3**	0.01	0.01	<0.01	0.02			<0.01		<0.01					
Corynoxeine **4**	<0.01	<0.01	0.02				<0.01		<0.01	<0.01				
Isorhynchophylline **5**	0.30		0.17	<0.01			0.22	<0.01	0.20	0.03	<0.01	<0.01	<0.01	
Rhynchophylline **6**	0.11		0.27	<0.01	<0.01		0.10	<0.01	0.11	0.13	0.01		0.02	0.02
Total [b]	1.52	1.39	4.37	0.51	0.09	0.05	0.87	0.58	1.52	0.55	0.96	0.54	1.12	0.74

[a] No entry means that the corresponding alkaloid was not detected (< 0.002 mg g^{-1}).
[b] Values < 0.01 mg g^{-1} not counted.
[c] Whole root.

In five young leaf samples the pentacyclic oxindole alkaloids mitraphylline **1** and isomitraphylline **2** were the major tertiary bases, whereas in six samples the tetracyclic oxindole alkaloids isorhynchophylline **5** and rhynchophylline **6** dominated. Two young leaves had a very low alkaloid content. Dihydrocorynantheine **7** was detected in only four of the leaves. In contrast, in all root samples mitraphylline **1** prevailed, and the concentration of the corynoxeine isomers **3** and **4** was very low (Table 1). The alkaloids pteropodine and speciophylline, which were reported as major constituents of the root (LAVAULT & al. 1983), could not be detected. Most of the alkaloids in the root were contained in the bark and phloem (97%), and the xylem was almost alkaloid-free (3%). In very fine root samples (samples 2 and 4), bark and wood could not be separated. These values are therefore not comparable.

Table 2. Alkaloid distribution in mature leaves and stem bark of Peruvian *Uncaria guianensis* plants [a]

	Plant no.		
	5	8	10
	mg alkaloid g^{-1} plant material		
Mature leaves			
Mitraphylline **1**	0.18	0.40	0.20
Isomitraphylline **2**	0.16	0.49	0.08
Isocorynoxeine **3**			0.06
Corynoxeine **4**			0.02
Isorhynchophylline **5**	<0.01		1.41
Rhynchophylline **6**	<0.01		0.52
Total [b]	0.34	0.89	2.29
Stem bark			
Mitraphylline **1**	0.01	0.05	0.02
Isomitraphylline **2**	<0.01	0.02	<0.01
Isorhynchophylline **5**			0.04
Rhynchophylline **6**			0.03
Total [b]	0.01	0.07	0.09

[a] No entry means that the corresponding alkaloid was not detected (<0.002 mg g^{-1}).
[b] Values <0.01 mg g^{-1} not counted.

Mature leaves of three plants were also examined. Their alkaloid composition was qualitatively and quantitatively similar to that of the young leaves. Three samples of stem bark showed a similar pattern but much lower alkaloid content than the root bark (Table 2).

There is evidence from diode-array data that the young leaves from Shaoashipango (samples 7–14) contained a yet unidentified polar indole alkaloid, only traces of which could be detected in the leaf samples from Kivinaki (samples 1–6). However, this alkaloid was present in root samples of the latter plants. More plant material is needed for isolation and identification of this compound.

It seemed to be of interest to compare the two South American *Uncaria* species, *U. guianensis* and *U. tomentosa* (WILLD.)DC., both of which are used in traditional medicine. Inflorescences and fruits are markedly different but can only rarely be observed. Differences in the size and shape of the chromosomes were also noted (TEPPNER & al. 1984). One of the more apparent differences in the vegetative parts is the shape of the thorns, spirally twisted and sensitive in *U. guianensis*, as distinct from streight to sickle-shaped, very pungent, and not sensitive in *U. tomentosa* (TEPPNER & al. 1984, KEPLINGER & al. 1999). In the present work, the alkaloid content was found to be generally lower in *U. guianensis* than in *U. tomentosa*. On

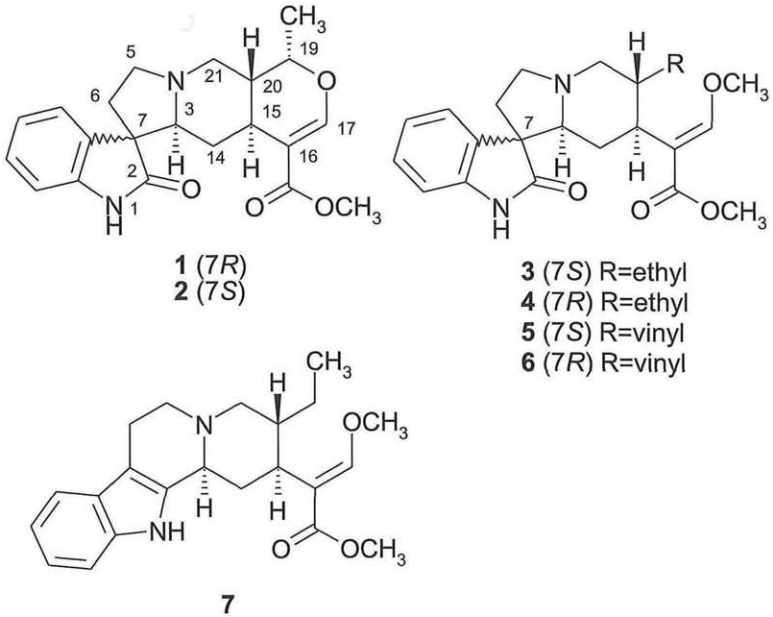

Fig. 1. Structures and stereochemical notations of the alkaloids in Peruvian *Uncaria guianensis*. – **1** = mitraphylline, **2** = isomitraphylline, **3** = isocorynoxeine, **4** = corynoxeine, **5** = isorhynchophylline, **6** = rhynchophylline, **7** = dihydrocorynantheine.

average, young leaves of *U. tomentosa* (14 samples) possessed a forty-fold higher alkaloid content than *U. guianensis* (14 samples), and the average of the three lowest *U. tomentosa* leaves still was ten times the average alkaloid content of the three highest *U. guianensis* leaves. Also, the alkaloid concentration in the roots of *U. guianensis* was twenty times lower than in the roots of *U. tomentosa*. The pronounced development of chemotypes in *U. tomentosa* (pentacyclic alkaloid-type and tetracyclic alkaloid-type) (LAUS & al. 1997) was not encountered in *U. guianensis*. None of the *U. guianensis* samples examined contained alkaloids with a *cis*-substitution pattern at C(15) and C(20) like *U. tomentosa* (LAUS & KEPLINGER 1994). No significant qualitative and quantitative variability between young and mature leaves of the same *U. guianensis* plant was observed which is also in contrast to *U. tomentosa* (LAUS & al. 1997). Thus, the opinion that *U. guianensis* and *U. tomentosa* are not closely related species (KEPLINGER & al. 1999, TEPPNER & al. 1984) is corroborated by our results. Furthermore, whereas the alkaloid pattern in the leaves of *U. guianensis* resembles that of *U. rhynchophylla* (MIQ.) HAVIL., the alkaloid content in the roots of these two species is totally different (LAUS & TEPPNER 1996, YAMANAKA & al. 1983).

In conclusion, the low alkaloid content in *U. guianensis* suggests that the reported traditional uses may be explained by other constituents rather than alkaloids, as has been suggested recently (AGUILAR & al. 2002, SANDOVAL & al. 2002), or that *U. guianensis* was possibly used as a poor substitute for *U. tomentosa*, or that these two similar species were simply confused.

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