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ANGUSTINE AND RELATED ALKALOIDS FROM SPECIES OF *MITRAGYNA*, *NAUCLEA*, *UNCARIA*, AND *STRYCHNOS*

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Key Word Index—*Mitragyna; Nauclea; Uncaria;* Naucleeae; Rubiaceae; *Strychnos;* Loganiaceae; angustine; angustoline; angustidine; chemotaxonomy; indole alkaloids.

Abstract—Angustine and related alkaloids have been identified in extracts from species of *Mitragyna* Korth., *Nauclea* L., *Uncaria* Schreb. (Rubiaceae), and from African and Asian species of *Strychnos* L. (Loganiaceae). The chemotaxonomic significance of the occurrence of these alkaloids is discussed.

INTRODUCTION

THREE new pyridino-indolo-quinolizidinone alkaloids, angustine (**1a**), angustoline (**1b**), and angustidine (**1c**), have recently been isolated from *Strychnos angustiflora* Benth.[1] This communication describes the wider occurrence of these three alkaloids, not only in species of *Mitragyna* Korth., *Nauclea* L. and *Uncaria* Schreb. (Rubiaceae), but also in further African and Asian species of *Strychnos* L. (Loganiaceae).

RESULTS

The spectral properties of the yellow alkaloids Pa 6, isolated from *Mitragyna javanica* Koord. et Valeton,[2] and Gu 5, from *M. parvifolia* (Roxb.) Korth.,[3] are the same as those of angustine and co-chromatography with the authentic alkaloid has confirmed their identity. Examination of the extracts from monthly collections of Burmese *M. parvifolia* leaves shows that while angustine is present during the first half of the year there appear to be only traces of it present during the second half.

Angustine, obtained from the leaves of *Nauclea coadunata* Roxb. ex J. E. Smith [*Sarcocephalus coadunata* (Roxb. ex J. E. Smith) Druce], has been identified by its spectral properties and by co-chromatography with the authentic base.

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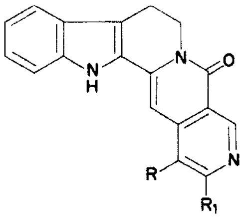
[1] AU, T. Y., CHEUNG, H. T. and STERNHELL, S. (1973) *J. Chem. Soc. Perkin I*, 13.

[2] SHELLARD, E. J., BECKETT, A. H., TANTIVATANA, P., PHILLIPSON, J. D. and LEE, C. M. (1967) *Planta Med.* **15**, 245.

[3] SHELLARD, E. J., PHILLIPSON, J. D. and GUPTA, D. (1969) *Planta Med.* **17**, 51.

Angustine and angustoline from the leaves of *Uncaria rhynchophylla* (Miq.) Miq. ex Havil. and *U. homomalla* Miq. have been identified by comparison of their TLC behaviour and of their UV and MS with those of the authentic alkaloids, while the presence of angustidine has been indicated by TLC. Angustine, identified by TLC and by its UV and MS, has also been isolated from the flowers of *U. bernaysii* F. v. M. and from the leaves, stems, and flowers of *U. guianensis* (Aubl.) Gmel.

An alkaloid isolated from *Strychnos camptoneura* Gilg et Busse has been identified as angustine by its TLC and spectral properties. TLC indicates that angustine-type alkaloids are present in a number of African and Asian *Strychnos* species (see Table 1 and Experimental).



(1a) $R = -CH{=}CH_2$; $R_1 = H$
(1b) $R = -CH(OH)Me$; $R_1 = H$
(1c) $R = H$; $R_1 = -Me$

DISCUSSION

The genera *Mitragyna*, *Nauclea* and *Uncaria* all belong to the tribe Naucleeae of the Rubiaceae, so that the discovery of alkaloids common to the three genera is not unexpected.

In the genus *Mitragyna* the occurrence of angustine appears to be restricted, since, in spite of the detailed investigations which have been carried out on the alkaloids of the 10 recognized species, it has been detected in only two of them: *M. javanica* and *M. parvifolia*.

Nauclea and its immediate allies (*Adina* Salisb., *Sarcocephalus* Afzel., *Neonauclea* Merr.) are in a state of taxonomic and nomenclatural confusion. Although angustine has been isolated from one *Nauclea* (or *Sarcocephalus*) species, further work is required to determine in what other species of this group of genera it is to be found.

Uncaria has recently been revised and its species divided among seven major groups.[4] The Indian and Chinese taxa comprising group 7 are rather heterogeneous, but they have been further arranged in two subgroups, one of which consists of *U. rhynchophylla* (Miq.) Miq. ex Havil., *U. sessilifructus* Roxb., and *U. laevigata* Wall. ex G. Don and the other of *U. scandens* (J. E. Smith) Hutch., *U. lancifolia* Hutch., and possibly *U. homomalla* Miq. Angustine-type bases have been identified in leaf samples of *U. rhynchophylla* and *U. homomalla*, species which are placed in different subgroups. The only other instances of the identification of angustine in the genus have been as a trace alkaloid in *U. bernaysii* and *U. guianensis;* in both these plants the angustine was accompanied by relatively large amounts of oxindole alkaloids. *U. bernaysii* occurs only in New Guinea and is the only member of group 4, while *U. guianensis* is included in group

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[4] RIDSDALE, C. E. (1972) *Numerical and Taxonomic Studies on the Genus* Uncaria *Schreb.*, Ph. D. Thesis, University of Bristol.

2 which comprises the Afro-American taxa. The fact that angustine has been detected in three of the seven groups of *Uncaria* may indicate that it occurs more widely in the genus. However, alkaloid-screening results suggest that angustine-type alkaloids are present as major alkaloids only in the species of group 7.[5]

Several yellow alkaloids were encountered during the screening of African *Strychnos* species for alkaloids and their presence or absence was used as one means of organizing the results.[6] Although the significance of these bases was not known, it was suggested that their occurrence implied the presence of a precursor common to the species in which they were found. The isolation of one of the yellow alkaloids from the African *S. camptoneura* and its identification as angustine is recorded in the Experimental; the base has now been obtained from an African and an Asian species.

During the screening of Asian *Strychnos* species for alkaloids,[7] angustine and other yellow alkaloids were detected in the extracts from some samples, including those of *S. angustiflora* the species from which they were first fully characterized.[1] These extracts and a selection of extracts from African species previously found to contain yellow alkaloid(s) have been co-chromatographed with authentic angustine, angustoline, and angustidine. The results are included in the Experimental and when combined with those previously reported,[6] give some indication of the distribution of these three alkaloids in African and Asian *Strychnos* (see Table 1).

TABLE 1. THE OCCURRENCE OF ANGUSTINE-TYPE ALKALOIDS IN AFRICAN AND ASIAN SPECIES OF *Strychnos*

Section	No. of species in section*	No. of species examined	No. of species with angustine-type alkaloids
1 Strychnos	12	10	1
2 Rouhamon	12	11	4
3 Breviflorae	12	12	4
4 Penicillatae	15	12	0
5 Aculeatae	1	1	0
6 Spinosae	4	4	0
7 Brevitubae	14	12	6
8 Lanigerae	29	26	8
9 Phaeotrichae	1	1	0
10 Densiflorae	8	8	0
11 Dolicanthae	9	8	6
12 Scyphostrychnos	1	1	1
Total	118	106	30

* The 71 known Central and South American species belong to sections 1, 2, and 3 only.[8]

There are *ca* 190 *Strychnos* species throughout the world,[8] and of the *ca* 120 occurring in Africa and Asia[8,9] the leaves of 106 have now been screened for alkaloids.[6,7] Angustine-type bases have been detected in 30 species, belonging to seven of the 12 sections into which the genus is divided.[8] Table 1 shows that these bases appear in a high proportion of the species belonging to the section Dolicanthae. This section has

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[5] PHILLIPSON, J. D. and HEMINGWAY, S. R. (1973) unpublished results.

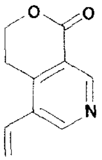
[6] BISSET, N. G. and PHILLIPSON, J. D. (1971) *Lloydia* **34**, 1.

[7] BISSET, N. G. and PHILLIPSON, J. D. (1973) unpublished results.

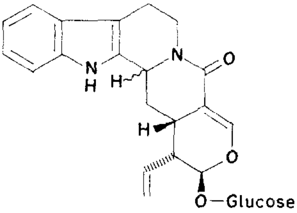
[8] LEEUWENBERG, A. J. M. (1969) *Meded. Landbouwhogesch. Wageningen* **69** (1), 1.

[9] BISSET, N. G., LEENHOUTS, P. W., LEEUWENBERG, A. J. M., PHILCOX, D., TIREL-ROUDET, C. and VIDAL, J. E. (1973) *Lloydia* **36**, 179.

affinity with the Lanigerae, Scyphostrychnos, and Brevitubae,[8] and through this last section with the Breviflorae and Rouhamon; all these sections have species which contain angustine-type bases. The five sections in which they have not been found (Table 1) comprise African and Asian species which mostly carry little or no alkaloid in their leaves. It is noteworthy that, apart from *S. angustiflora*, there is no evidence to suggest the presence of the yellow alkaloids in any other species, whether rich or poor in alkaloids, of the section Strychnos.



(2)



(3)

Angustine and angustoline are possibly derived from a tryptamine unit combined with a secologanin unit closely related to gentianine (**2**).[1] In some instances gentianine is an artefact formed during extraction procedures by the action of ammonia on glycosides such as sweroside,[10] and there is the possibility that angustine is also an artefact (*cf* the similar case: formation of cantleyine from ammonia and loganin).[11] Alternatively, angustine might arise possibly by reaction of vincoside- or isovincoside-lactam (**3**) with ammonia. Angustine-type alkaloids are present in *S. angustiflora* extracts worked up in the absence of ammonia.[1] Experiments comparing extracts prepared using either sodium carbonate or ammonia have also been carried out on samples of *S. camptoneura* and *S. soubrensis*[6] and a similar experiment on a sample of *M. parvifolia* is described in Experimental. Angustine is present in extracts prepared by both procedures, although more tends to be found in the extracts prepared with ammonia. In spite of these findings, angustine-type alkaloids need not be naturally-occurring, since nitrogen-containing substances in the plant material may perhaps take over the function of ammonia under basic extraction conditions. Whether they occur naturally or not, the presence of these alkaloids in plant extracts can be an indication of the presence of vincoside- and/or isovincoside-lactam and hence of the biogenetically important vincoside.[12] The lactams have in fact been obtained from *Adina* (Naucleeae–Rubiaceae)[13] and *Rhazya* (Apocynaceae), but again it is questionable whether they occur naturally.[14]

That angustine-type alkaloids have not been detected in some species and in some extracts of species which otherwise do contain them may perhaps be connected with seasonal variations in the extent of alkaloid biosynthesis; the findings with the monthly collections of *M. parvifolia* leaves are a pointer in this direction. However, because of the large number of samples examined, it is felt that the restricted distribution of these

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[10] Plouvier, V. and Favre-Bonvin, J. (1971) *Phytochemistry* **10**, 1697.

[11] Bisset, N. G. and Choudhury, A. K. (1974) *Phytochemistry* **13**, 265; Sévenet, T., Das, B. C., Parello, J. and Potier, P. (1970) *Bull. Soc. Chim. Fr.* 3120.

[12] Battersby, A. R. (1971) in *The Alkaloids* (Saxton, J. E., ed.) (Specialist Periodical Reports), Vol. 1, p. 31, The Chemical Society, London; Staunton, J. (1972) in *The Alkaloids* (Saxton, J. E., ed.) (Specialist Periodical Reports), Vol. 2, p. 1. The Chemical Society, London; Kompiš, I., Hesse, M. and Schmid, H. (1971) *Lloydia* **34**, 269.

[13] Blackstock, W. D., Brown, R. T. and Lee, G. K. (1971) *Chem. Commun.*, 910.

[14] Silva, K. T. D. de, Smith, G. N. and Warren, K. E. H. (1971) *Chem. Commun.*, 905.

compounds within the genera *Mitragyna, Uncaria,* and *Strychnos* may nevertheless be of taxonomic significance. The fact that they occur in the Naucleeae and in the Loganiaceae suggests that they may well be present in other indole-alkaloid producing genera of the Rubiaceae and also in genera of the Apocynaceae.

EXPERIMENTAL

The IR spectra were determined in Nujol, the 100-MHz NMR spectrum in DMSO-d_6 at 90°, and the MS with an AEI MS902 high-resolution spectrometer operating at 70 eV. TLC was carried out with plates spread with silica gel G/GF (Merck) (2 : 1) and run in the systems: (A) $CHCl_3$–Me_2CO (5 : 4); (B) EtOAc–*iso*PrOH–conc. NH_4OH (100 : 2 : 1); (C) EtOAc–*iso*PrOH–conc. NH_4OH (16 : 3 : 1); and (D) $CHCl_3$–EtOH (19 : 1). The hR_f values obtained were: Angustine, (A) 54; (B) 60; (C) 74; (D) 50; Angustidine, (A) 43; (B) 41; (C) 67; (D) 26; Angustoline, (A) 20; (B) 18; (C) 50; (D) 14. The alkaloids were detected by their intense yellow-white fluorescence in UV light (365 nm), by the yellow colour obtained on spraying with Dragendorff's reagent, and by the green–yellow colour obtained after spraying with 0·2 M $FeCl_3$ in 35% $HClO_4$ and heating at *ca* 90° for 15 min.

Isolation and identification of the alkaloids. (a) *Mitragyna* species. The yellow alkaloids Pa 6* isolated from *M. javanica*[2] and Gu 5 isolated from *M. parvifolia*[3] have hR_f values in TLC systems A–D and UV, IR, and MS identical with those of authentic augustine.

M. parvifolia (ex Maung Maung Gale, Burma, –/6–7/70). 60 g dried leaf extracted in the same way as for (b) yielded 50 mg (0·08 %) total alkaloid. 10 g of the same batch of leaves extracted by the same procedure, but substituting 5 % Na_2CO_3 for NH_4OH throughout, yielded 7 mg (0·07 %) total alkaloid. TLC (systems A–D) indicated that angustine was present in both extracts but was in higher concentration in the extract which had been prepared with NH_4OH.[15] Monthly collections of Burmese *M. parvifolia* leaves (from the same source as above) were extracted using NH_4OH. TLC (systems B–D) showed angustine to be present in the extracts from the collections dated December 1969 to July 1970 inclusive; thereafter, until January 1971, the date of the last collection examined, the angustine was only just detectable in the extracts.[15] With young plants of *M. parvifolia* (< 1 yr old), raised at Chelsea from Burmese seed, TLC (systems B–D) suggested that the younger leaves contained more of the yellow alkaloids, particularly angustine.[15]

(b) *Nauclea coadunata* (coll. Bamrung Tantisewie, Thailand, 1963). The dried powdered leaf (5·5 kg) was moistened with 10 % NH_4OH and macerated with EtOAc. The concentrated EtOAc extract was shaken with 2 % H_2SO_4 which was separated, washed with EtOAc, basified with NH_4OH, and extracted with $CHCl_3$. After being washed and dried, the $CHCl_3$ was removed to give an oily residue (3·2 g = 0·06 %). Chromatography of the residue over Al_2O_3 (Spence Type H) and elution with $CHCl_3$ yielded a major fraction as a green–black oil (0·90 g). The oil was suspended in Et_2O and extracted into $CHCl_3$ which was washed, dried, and concentrated to a brown semi-solid (0·49 g). This material yielded orange-yellow crystals from abs. EtOH–dry Et_2O; recrystallization from the same solvent mixture gave angustine (0·050 g = 0·001 %).

Identification of angustine. m.p. 283–284° (decomp.); λ_{max}^{EtOH} 222, 255, 292, 305, 380, and 400 nm (log ε 4·52, 4·20, 4·10, 4·10, 4·50, and 4·50); ν_{max} 3220 (NH), 1640 (amide C=O), 1600, 1575, 1145, 830, 815, and 740 cm^{-1}; δ† 9·18 (1 H, *s*; H–17), 8·73 (1 H, *s*; H–21), 7·55 (1 H, *dd*, *J* 8 Hz, *J'* 2 Hz; H–9), 7·40 (1 H, *dd*, *J* 8 Hz, *J'* 2 Hz; H–12), *ca* 7·20 (3 H, *m*; H–10, H–11, H–19), 7·18 (1 H, *s*; H–14), 5·92 (1 H, *dd*, *J* 11 Hz, *J'* 2 Hz; H–18), 4·35 (2 H, *t*, *J* 7 Hz; H–5), 3·18 (2 H, *t*, *J* 7 Hz; H–6); MS (245°) 313 (M^+; 100 %); TLC: hR_f values identical with those of angustine in systems A–D.

(c) *Uncaria* species. The total crude alkaloids were extracted by the method described under (b). The component alkaloids were separated by prep. TLC using system D and obtained as amorphous solids.

U. rhynchophylla (ex S. Sakai, coll. J. Haginawa, Chiba, Honshu, Japan, 4/7/63). 410 mg dried leaf gave 6·9 mg (1·68 %) total alkaloid which afforded 0·60 mg (0·15 %) angustine and 0·22 mg (0·05 %) angustoline. The angustine had UV spectrum and hR_f values on TLC in systems A–D identical with those of the authentic base; MS (240°) 313 (M^+; 100 %). The angustoline had UV spectrum and hR_f values on TLC in systems A–D identical with those of the authentic alkaloid; MS (240°) 331 (M^+; 100 %), 316 (M^+–Me; 68 %), 314 (M^+–OH; 41 %), and 313 (M^+–H_2O; 91 %). TLC in systems A–D of the total alkaloid extract indicated the presence of angustidine as well.

U. homomalla (*Poilane 9994*, South Vietnam: Ninh Thuân Prov., Ka Rom, 8/3/24). 302 mg dried leaf yielded 3·3 mg (1·09 %) total alkaloid, from which 0·30 mg (0·10 %) angustine, 0·56 mg (0·19 %) angustoline, and 0·40 mg (0·13 %) of a mixture of two yellow bases were obtained. The angustine and angustoline had hR_f values in the

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* The "correction" concerning the MW of Pa 6, given as a footnote in Ref. 2, is based on a misunderstanding and should be disregarded; the correct M^+ is 313.

† The slight differences between these chemical shifts and those previously reported for angustine[1] may perhaps be attributed to different operating temperatures.

[15] Houghton, P. J. and Shellard, E. J. (1973) unpublished results.

systems A–D and UV and MS identical with those of the authentic alkaloids. One of the components of the mixture had hR_f values in the systems A–D identical with those of angustidine.

U. bernaysii (*Ridsdale s.n.*, Territory of New Guinea: Lae, Markham River, –/–/68). 6·78 g dried flowers yielded 119 mg (1·75%) total alkaloid which gave 0·90 mg (0·013%) angustine, having hR_f values in systems A–D and UV and MS identical with those of the authentic alkaloid.

U. guianensis (*R. R. de Santos & R. Souza 1679*, Brazil: Mato Grosso, São Félix region, 6/6/68). TLC examination of the total alkaloid extracts from the leaves, stems, and flowers showed that essentially the same bases were present in each extract. 12·52 g combined plant material afforded 31·4 mg (0·25%) total alkaloid which gave 0·7 mg (0·005%) mainly angustine, identified by comparison of the hR_f values in the systems A–D and of the UV and MS with those of the authentic alkaloid.

(d) *Strychnos species*. (i) *African species*. *S. camptoneura* (*Leeuwenberg 5826*, Cameroun: *ca* 15 km east of Dimako, –/5/65). 2·2 kg powdered leaf was extracted as for (b), using CH_2Cl_2 instead of $CHCl_3$, and yielded 6·21 g (2·82%) total alkaloid. Crystallization from CH_2Cl_2 afforded 0·95 g of a mixture of antirhine and antirhine methobromide.[16] 0·45 g of the residue from the mother-liquors gave 0·1 g Et_2O-soluble material which yielded 17 mg (0·008%) angustine by prep. TLC using system A. The angustine had hR_f values in systems A–D and UV, IR, and MS identical with those of authentic angustine; accurate mass measurement found: 313·1209. Calc. for $C_{20}H_{15}N_3O$: 313·1215.

A yellow alkaloid having hR_f values identical with those of angustine has previously been reported to occur in several African species.[6] Re-examination by TLC (systems A or C, B, and D) using authentic reference alkaloids showed the presence of angustine, angustoline, and angustidine in the extracts of the following leaf samples: *S. odorata* A. Chev. (*Leeuwenberg 8040*; the three bases were also present in the unripe fruits), *S. samba* Duvign. (*Breteler 2051*), *S. scheffleri* Gilg (*Breteler, J. de Wilde, & Leeuwenberg 2444*), *S. trichoneura* Leeuwenberg (*Cours 2529*), and *S. xantha* Leeuwenberg (*Schmitz 7362*). Angustine and angustidine were indicated by similar means to be present in the extracts from *S. angolensis* Gilg (*Breteler, J. de Wilde, & Leeuwenberg 2376*) and angustine only in *S. floribunda* Gilg (*Leeuwenberg 4506*), *S. potatorum* L. f. (*Leippert 5968*), and *S. usambarensis* Gilg (*Leeuwenberg 4050*, *Leeuwenberg 3710*, and *Leeuwenberg & Voorhoeve 4935*).

(ii) *Asian species*. During the alkaloid screening of Asian species,[7] angustine, angustoline, and angustidine were indicated by TLC (systems A, B, and D) to be present in the extracts from the following leaf samples: *S. angustiflora* Benth. (*E. Cheng s.n.* and *Poilane 10802*), *S. borneensis* Leenh. (*Clemens 50260*), *S. minor* Dennst. (*N.G.F. 21692* and *Blake 15035*), *S. ovata* A. W. Hill (*F. Chow 70436* and *Lingnan Univ. 17194*), *S. umbellata* (Lour.) Merr. (*Lingnan Univ. 17470*), and *S. vanprukii* Craib (*Bourdillon 600* = *S. aenea* A. W. Hill and *L. Wray Jr. s.n.* = *S. quadrangularis* A. W. Hill; the three bases were also present in the stem bark, stem wood, root bark, and root wood of the Wray samples). Angustine only was found in *S. ledermannii* Gilg et Bened. (*B.S.I.P. 2698*).

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[16] BISSET, N. G. and PHILLIPSON, J. D. (1974) *Phytochemistry* **13**, in Press.