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ALKALOIDS OF *UNCARIA LONGIFLORA**

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Abstract—Six isomers of the pentacyclic oxindole alkaloid mitraphylline and five of their *N*-oxides have been identified as being present in the leaves of *Uncaria longiflora* (Poir.) Merr. Isomitraphylline and mitraphylline *N*-oxides have been prepared and characterized.

INTRODUCTION

ACCORDING to Ridsdale,[1] who is currently undertaking a taxonomic revision of the genus *Uncaria*, *U. longiflora* (Poir.) Merr. is a complex species probably comprising of at least three major entities which cannot be readily delimited. One of these entities includes *U. pteropoda* Miq. and another, *U. longiflora* (Poir.) Merr. *sensu stricto* together with *U. havilandiana* S. Moore. The third entity corresponds to the range of variation which Miquel[2] described as *U. callophylla* Korth. var. *oligoneura* Korth. ex Miq.; *U. pachyphylla* Merr., *U. laevifolia* Elm. and *U. trinervis* Havil. comprise insufficiently known entities within *U. longiflora sensu lato*.

Although the name *U. longiflora* does not appear in the chemical literature, *U. pteropoda* Miq., now considered to be a subspecies of *U. longiflora*, is present. The results of screening indicate that the alkaloid content is extremely variable, e.g. the leaves and stems of *U. longifolia* Merr. (in error for *U. longiflora* Merr.) are reported to give only weakly positive alkaloid tests whereas the leaves of *U. pteropoda* Miq. screened at the same time were negative, although the roots, stems and seeds were positive.[3] On other occasions significant quantities of alkaloid have been reported to be present in the leaves, stems and roots.[4,5] The alkaloids isopteropodine and pteropodine have been isolated from stem, bark and root of *U. pteropoda* Miq.[6,7] Alkaloids from leaf material have not been characterized and the only samples from the *U. longiflora* complex previously investigated for their alkaloid content have been from Malaysia. This communication describes the identification of five alkaloids, including two new *N*-oxides, from a small sample of *U. longiflora* (Poir.) Merr. leaves obtained from Pulau Laut, Borneo, and the tentative identification of a further six alkaloids.

* Part II in a series "Alkaloids from *Uncaria* species."

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RESULTS AND DISCUSSION

During our screening of *Uncaria* species for the presence of alkaloids, a small sample of *U. longiflora* leaves yielded crystals of mitraphylline (UV, MS, R_f and R_t values identical with those of the authentic alkaloid). TLC of the mother-liquor indicated the presence of other alkaloids which were separated by preparative TLC. Five additional alkaloids were tentatively identified by TLC and GLC as isomers of mitraphylline, namely isomitraphylline, isopteropodine, pteropodine, uncarine F and speciophylline. The identification of speciophylline was confirmed (UV, MS, R_f and R_t values identical with those of the authentic alkaloid). TLC of more polar fractions indicated that the *N*-oxides of isopteropodine, pteropodine and speciophylline, which have recently been isolated from *U. bernaysii* F.v.M.,[8] were also present. Two further spots with low R_f values were noted and both gave a pink colour with the ferric chloride–perchloric acid reagent, indicating that they were closed E ring pentacyclic oxindole alkaloids of the mitraphylline-type. As mitraphylline and isomitraphylline were present in the plant extract it seemed reasonable to suppose that their *N*-oxides might also be present. The *N*-oxides of isomitraphylline and mitraphylline were therefore prepared and shown to correspond on TLC with the two unidentified spots in the extract. The natural compounds were separated by preparative TLC, yielding small amounts of two compounds which were identified as isomitraphylline *N*-oxide and mitraphylline *N*-oxide by TLC. Reduction of the two natural compounds with sulphurous acid yielded only the corresponding tertiary bases which were identified by TLC. The MS of natural mitraphylline, isomitraphylline and speciophylline *N*-oxides were consistent with those of the prepared compounds.

The prepared *N*-oxides of isomitraphylline and mitraphylline were characterized by their R_f and R_t values, together with their UV, NMR and MS. TLC clearly indicated that the resulting compounds were more polar than the corresponding tertiary bases although the UV spectra and R_t values remained identical with those of the tertiary bases. The MS had molecular ions at *m/e* 384, showing the presence of one additional oxygen and this oxygen could easily be removed on reduction with sulphurous acid, to give the corresponding tertiary bases. The NMR spectra of isomitraphylline *N*-oxide and mitraphylline *N*-oxide were almost identical with those of the corresponding tertiary bases, with one important exception: a clear one-proton double doublet (*ortho* and *meta* coupling) at δ 8·14 in the spectrum of isomitraphylline *N*-oxide indicated that the oxygen at N-4 was adjacent to the C-9 proton as in the *N*-oxides of isorhynchophylline,[9] isopteropodine and uncarine F.[8] This signal is absent from the NMR spectrum of mitraphylline *N*-oxide, showing that the oxygen at N-4 is on the opposite side of the molecule to the C-9 proton. Support for these views was obtained from mass spectrometry. The known pentacyclic oxindole alkaloid *N*-oxides with their N-4 oxygen *anti* to the oxindole amide carbonyl have a molecular ion with a higher percentage relative abundance (isorhynchophylline *N*-oxide 24%, isopteropodine *N*-oxide 100%, uncarine F *N*-oxide 45%) than the corresponding isomers which have the N-4 oxygen *syn* to the amide carbonyl (rhynchophylline *N*-oxide 16%, pteropodine *N*-oxide 50%, speciophylline *N*-oxide 3·5%).[8,9] The prepared *N*-oxides of isomitraphylline and mitraphylline behave similarly, the % relative abundance of the molecular ion of isomitraphylline *N*-oxide being 38% and that of mitraphylline *N*-oxide being 13%. The NMR and mass spectral results show that the oxygen at N-4 in isomitraphylline *N*-oxide and mitraphylline *N*-oxide has the 4-*R* absolute configuration and that these compounds can be represented

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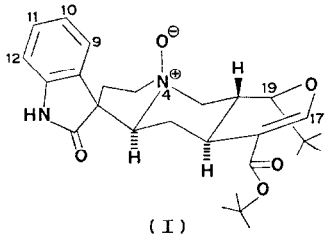
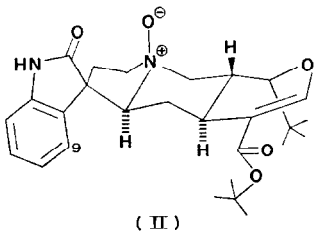
as I and II, respectively. With the discovery of the occurrence of isomitraphylline and mitraphylline *N*-oxides, eight pentacyclic oxindole alkaloid *N*-oxides are now known to occur naturally.



(I)



(II)

EXPERIMENTAL

The plant material was supplied by the Rijksherbarium, Leiden, and was labelled: Verhoef 101, Pulau Laut, Borneo, collected 24 December 1928.

The 100 MHz NMR spectra were determined in $CDCl_3$ using TMS as internal reference; the MS were determined on an AEI MS 902 high resolution mass spectrometer at 70 eV with inlet temp. between 210 and 240°. The TLC systems used were silica gel G/GF_{254} (Merck) (2:1) with (A) $CHCl_3$–Me_2CO (5:4); (B) EtOAc–*iso*PrOH–conc. NH_4OH (100:2:1); (C) EtOAc–*iso*PrOH–conc. NH_4OH (60:35:5); (D) MeOH–Et_2NH (96:8). The hR_fs are given in Table 1.

TABLE 1. THE hR_fS OF *Uncaria longiflora* ALKALOIDS

Compound	(A)	(B)	Compound	(C)	(D)
Isopteropodine	73	61	Isopteropodine *N*-oxide	37	76
Pteropodine	71	57	Pteropodine *N*-oxide	8	76
Uncarine F	67	41	Speciophylline *N*-oxide	3	50
Speciophylline	36	13	Isomitraphylline *N*-oxide	27	70
Isomitraphylline	68	50	Mitraphylline *N*-oxide	3	33
Mitraphylline	52	26			

The alkaloids were detected by using Dragendorff's reagent and 0·2 M $FeCl_3$ in 35% $HClO_4$ followed by heating at 90° for 1 hr. Isopteropodine, pteropodine and their *N*-oxides gave green spots which turned pink; the other alkaloids and their *N*-oxides gave pink spots. The GLC was carried out using an 0·5 m column packed with 5% SE 52 on Varoport 30 and a column temp. of 230°. The R_t of isopteropodine, pteropodine, uncarine F and speciophylline was 9·7 min and of isomitraphylline and mitraphylline 11 min.

Extraction and separation of alkaloids. The dried leaf powder (5 g) was moistened with 10% NH_4OH, macerated with EtOAc, filtered, extracted with 2% H_2SO_4, made alkaline with NH_4OH and taken into $CHCl_3$ which was washed, dried and concentrated to dryness; yield, 67 mg (1·3%). The residue yielded mitraphylline as colourless needles (7 mg) from $CHCl_3$–MeOH. TLC of the mother-liquor in systems A and B indicated the absence of heteroyohimbine alkaloids and the presence of isomitraphylline, mitraphylline, pteropodine, uncarine F, speciophylline and 'base-line' alkaloid and in systems C and D the presence of *N*-oxides of isomitraphylline, mitraphylline, isopteropodine, pteropodine and speciophylline. GLC indicated the presence of mitraphylline-type and pteropodine-type alkaloids. Mitraphylline (5·4 mg) and speciophylline (3·4 mg) were isolated after preparative TLC using system A. Isomitraphylline *N*-oxide (0·7 mg) was obtained by preparative TLC using system C.

The marc was re-extracted by maceration with methanol and the filtered extract was then concentrated to dryness and extracted with 5% HOAc. After basification with NH_4OH and washing with $CHCl_3$, the pH was adjusted to 2·0 by the addition of conc. HCl. The addition of aq. picric acid resulted in a slight precipitate which was separated by centrifugation. Mitraphylline *N*-oxide (1 mg) and speciophylline *N*-oxide (0 4 mg) were separated by preparative TLC of the picrate using system D.

Identification of mitraphylline and speciophylline. The UV (EtOH), MS, R_ts, R_fs in systems A and B, and colour with $FeCl_3/HClO_4$, were identical with those of the reference alkaloids.

Identification of isopteropodine, pteropodine, uncarine F *and isomitraphylline.* The R_ts, R_fs in systems A and B, and colour with $FeCl_3/HClO_4$, were identical with those of the reference alkaloids.

Identification of the natural N-*oxides*. The *N*-oxides of isomitraphylline, mitraphylline, isopteropodine, pteropodine and speciophylline had R_fs in systems C and D and colours with $FeCl_3/HClO_4$, identical with those of the *N*-oxides prepared from the corresponding tertiary alkaloids.[8] The natural *N*-oxides of isomitraphylline, mitraphylline and speciophylline[8] had UV identical to and MS qualitatively similar to those of the corresponding prepared *N*-oxides. On reduction with 5% H_2SO_3 they yielded single spots on TLC having R_fs and colours with $FeCl_3/HClO_4$, identical with those of the corresponding tertiary alkaloids.

Preparation of isomitraphylline and mitraphylline N-*oxides*. The *N*-oxides were prepared as previously described.[8]

Isomitraphylline N-*oxide*. Isomitraphylline (30·2 mg) yielded isomitraphylline *N*-oxide (12·1 mg, 39%). The UV spectrum (EtOH) and R_t were identical with those of isomitraphylline; the R_fs were as indicated above. NMR, δ 1·14 (3 H, *d*, *J* 6·5 Hz, C-19 Me), *ca.* 4 4 (1 H, *m*, C-19 H), 3·58 (3 H, *s*, MeO), 6·88 (1 H, *dd*, *J* 1·5 and 8·0 Hz, C-12 H), 7·02 (1 H, two overlapping *dd*, *J* 1·5 and 8 0 Hz, C-11 H), 7·20 (1 H, two overlapping *dd*, *J* 1·5 and 8·0 Hz, C-10 H), 7 45 (1 H, *s*, C-17 H), 8 14 (1 H, *dd*, *J* 1 5 and 8·0 Hz, C-9 H). MS, *m/e* 384 (M^+, 38%), 368 (M^+-16, 53%), 367 (M^+-17, 14%), 223 (100%), 208 (20%), 159 (30%), 146 (17%), 145 (17%), 144 (23%), 130 (40%), 69 (> 100%). Reduction of 1 mg with 5% H_2SO_3 yielded a single product identified as isomitraphylline by its R_fs and colour reaction with $FeCl_3/HClO_4$.

Mitraphylline N-*oxide*. Mitraphylline (30·7 mg) yielded mitraphylline *N*-oxide (14·3 mg, 45%). The UV spectrum (EtOH) and R_t were identical with those of mitraphylline, the R_fs were as indicated above. NMR, δ 1·09 (3 H, *d*, *J* 6 5 Hz, C-19 Me), *ca* 4·38 (1 H, *m*, C-19 H), 3·58 (3 H, *s*, Me O), 6·86–7·33 (4 H, *m*, C-9 H, C-10 H, C-11 H, C-12 H), 7·38 (1 H, *s*, C-17 H). MS, *m/e* 384 (M^+, 13%), 368 (M^+-16, 60%), 366 (M^+-18, 10%), 223 (100%), 208 (11%), 159 (35%), 146 (14%), 145 (8%), 144 (18%), 130 (32%), 69 (100%). Reduction of 1 mg with 5% H_2SO_3 yielded a single product identified as mitraphylline by its R_fs and colour reaction with $FeCl_3/HClO_4$.

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