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OXINDOLE ALKALOIDS FROM *UNCARIA MACROPHYLLA**

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Key Word Index—*Uncaria macrophylla*; Rubiaceae; oxindole alkaloids; corynoxine; corynoxine B; isorhynchophylline; rhynchophylline.

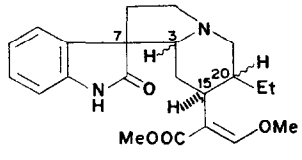
Abstract—Four isomeric oxindole alkaloids, isorhynchophylline, rhynchophylline, corynoxine and corynoxine B, have been isolated from the leaves of *Uncaria macrophylla* Wall. Corynoxine B has not previously been reported as a natural product.

INTRODUCTION

SEVERAL *Uncaria* species are known to produce heteroyohimbine, oxindole and roxburghine-type alkaloids,[1–4] but *U. macrophylla* does not appear to have been investigated for its alkaloid content.

RESULTS

During the process of screening *Uncaria* species for the presence of alkaloids, four small samples of *U. macrophylla* leaves collected in Assam, Indo-China and two locations in China were shown to contain oxindole alkaloids. TLC, including colour reactions with specific spray reagents and GLC indicated that the same four major alkaloids were present in all extracts and these were separated by preparative TLC. The alkaloids were identified by comparison of their UV and MS R_f and R_t values with reference alkaloids, as the four tetracyclic oxindole alkaloids isorhynchophylline (I, *normal*, C-7 *A* configuration), rhynchophylline (I, *normal*, C-7 *B* configuration), corynoxine (I, *allo*, C-7 *A* configuration) and corynoxine B (I, *allo*, C-7 B configuration). The corynoxine and corynoxine B used for



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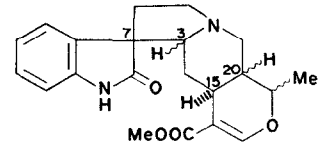

(II)

* Part III in the series. "Alkaloids from *Uncaria* species". For Part II see PHILLIPSON, J. D. and HEMINGWAY, S. R. (1973) *Phytochemistry* **12**, 2791.

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[1] SAXTON, J. E. (1965) in R. H. F. Manske, *The Alkaloids*, Vol. VIII, 59 and (1968) Vol. X, 521, Academic Press, New York.

[2] MERLINI, L., MONDELLI, R., NASINI, G. and HESSE, M. (1970) *Tetrahedron* **26**, 2255.

[3] MERLINI, L., NASINI, G. and HADDOCK, R. E. (1972) *Phytochemistry* **11**, 1525.

[4] MERLINI, L., NASINI, G. and PHILLIPSON, J. D. (1972) *Tetrahedron* **28**, 5971.

comparisons were not natural products but had been prepared from corynantheidine by treatment with *t*-butyl hypochlorite followed by methanolysis [5]

DISCUSSION

Isorhynchophylline and rhynchophylline occur in species of *Uncaria* and in species of the closely related genus *Mitragyna*.[1] Corynoxine has been reported from the bark of *Pseudocinchona africana* A. Chev.[6] but its C-7 isomer does not appear to have been isolated as a natural product. Four diastereoisomers of the corynoxine-type are theoretically possible, namely, *allo A* and *B* and *epiallo A* and *B*.[7] Four pteropodine isomers and their C-19 Me β analogues, the four rauniticine-oxindole alkaloids, are known to exist for the corresponding *allo/epiallo* pentacyclic oxindole alkaloids (II) [8,9]

Corynoxine (I, *allo*, *A*) has 7*S*, 3*S*, 4*R*, 15*S* and 20*S* absolute configuration;[10] isocorynoxine, stated to be the 7*R* isomer, has been prepared by acetic acid isomerization of corynoxine. However, since corynoxine corresponds to isorhynchophylline in 7*S*, 3*S* and 4*R* configurations and isocorynoxine corresponds to rhynchophylline at the same three centres, it was decided to retain the name corynoxine for the natural product, but to name the prepared isomer, corynoxine B.[7] When corynoxine was refluxed with acetic acid it yielded 20% corynoxine and 80% corynoxine B, and since corynoxine is known to be *allo* A,[11] corynoxine B must be *allo B*, *epiallo A* or *epiallo B*. As the preferred conformations of *allo B* (III) and *epiallo A* (IV) possess the N-4 lone pair *syn* to the lactam carbonyl it was decided that these two isomers would predominate in acid because of stabilization of the conjugate base by formation of an intramolecular hydrogen bond between the protonated lone pair and the lactam carbonyl. Pyridine isomerization of corynoxine yielded 80% corynoxine and 20% corynoxine B. As pyridine would be expected to stabilize *epiallo B* at the expense of *allo A* and since the same isomer appeared to be produced in pyridine and in acetic acid, it was considered that corynoxine B had the *allo B* configuration (III).[7]

Corynoxine and corynoxine B isolated from *U. macrophylla* correspond chromatographically to the samples of prepared corynoxines and on isomerization they were shown to be interconvertible with corynoxine B predominating in acid conditions and corynoxine in pyridine. This is consistent with *allo A* configuration for corynoxine and *allo B* configuration for corynoxine B.

CD spectra have proved useful in enabling the exact differences in configuration to be established between the isomeric tetracyclic and pentacyclic oxindole alkaloids.[7,8,10–12] A negative CE in the 280-290 nm region and a positive CE in the 210 nm region are found for those alkaloids having a C-7 *A* configuration, whereas a positive CE in the 280–290 nm and a negative CE in the 210 nm region are present for those alkaloids with a C-7 *B* configuration. The pentacyclic oxindole alkaloids (II) show a negative CE at 252 nm for alkaloids with a C-3 *H* α configuration and a positive CE at the same wavelength for the

[5] BECKETT, A. H., DWUMA-BADU, D and HADDOCK, R. E. (1969) *Tetrahedron* **25**, 5961.
[6] CU, N. A, GOUTAREL, R. and JANOT, M. M. (1957) *Bull. Soc. Chim. Fr.* 1292.
[7] TRAGER, W. F., LEE, C. M., PHILLIPSON, J D, HADDOCK, R. E., DWUMA-BADU, D. and BECKETT, A. H. (1968) *Tetrahedron* **24**, 523
[8] BEECHAM, A. F., HART, N. K., JOHNS, S. R. and LAMBERTON, J. A. (1967) *Tetrahedron Letters* 991.
[9] SHAMMA, M., SHINE, R J., MORSINGH, F, KOMPIS, I., STICZAY, T., POISSON, J and POUSSET, J.-L. (1967) *J. Am. Chem. Soc.* **89**, 1739.
[10] POUSSET, J -L., POISSON, J., SHINE, R. J. and SHAMMA, M. (1967) *Bull. Soc. Chim Fr* 2766.
[11] POUSSET, J.-L., POISSON, J. and LEGRAND, M. (1966) *Tetrahedron Letters* 6283.
[12] POISSON, J. and POUSSET, J.-L. (1967) *Tetrahedron Letters* 1919.

C-3 *H* β isomers. Similar effects are noted for the tetracyclic oxindole alkaloids isorhynchophylline, rhynchophylline and corynoxine since their CD curves show negative CEs in the 255–265 nm region. This effect was noted in the CD spectrum of prepared corynoxine B which showed a positive CE at 255 nm indicating that it possesses a C-3 *H* β configuration, contrary to the evidence from the isomerizations.[7] The positive CE at 292 nm and the negative values below 220 nm in the CD spectrum of the natural corynoxine B are consistent with a C-7 *B* configuration. However, there is a slight negative CE at 267 nm which is in agreement with a C-3 *H* α configuration. Thus the isomerization data and the CD spectrum of the isolated corynoxine B show that it possesses 7*R*, 3*S*, 4*R*, 15*S* and 20*S* absolute configuration. It is difficult to explain why prepared corynoxine B showed a positive CE at 255 nm;[7] one possible explanation would be the presence of undetected *epiallo* A isomer together with corynoxine B. This seems unlikely as only two TLC spots were observed during the previous isomerization experiments.[7] Again, during the present work only two spots were observed for the isomerization products using several TLC systems, whereas TLC of similar isomerizations with the pteropodine isomers (II) readily showed the presence of four isomers. The CD spectrum of corynoxine from *U. macrophylla* is identical with the previously published spectrum of this alkaloid[7] showing that it possesses the same absolute configuration.

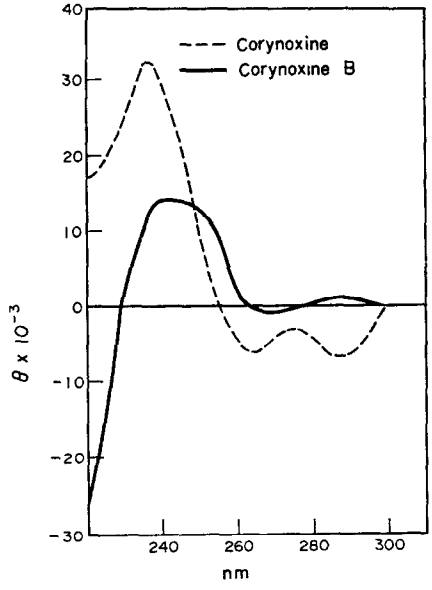
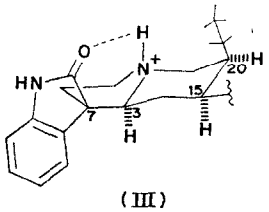


FIG. 1. CD CURVES OF CORYNOXINE AND CORYNOXINE B.

Ridsdale,[13] who has recently revised the genus *Uncaria*, has grouped *U. macrophylla* with the *Uncaria cordata-insignis-sclerophylla* complex. The species within this complex do not have a high oxindole alkaloid content but where oxindole alkaloids are present they are of the tetracyclic type (I).[14] However, Ridsdale has observed some morphological affinity between *U. macrophylla* and *U. bernaysii* F.v.M., a taxon distinct within the genus. A few

[13] RIDSDALE, C. E. (1972) Ph.D. Thesis, University of Bristol.
[14] PHILLIPSON, J. D. and HEMINGWAY, S. R. unpublished observations.

of the samples of *U. bernaysii* examined contained tetracyclic oxindole alkaloids,[14] as in *U. macrophylla*, although the majority contained the pentacyclic oxindole and heteroyohimbine-types with the *allo* and *epiallo* configurations.[15,16] In view of this it may be concluded that there is possibly taxonomic significance, not in the type of oxindoles present, but in the high oxindole alkaloid content of plants in these two taxa.



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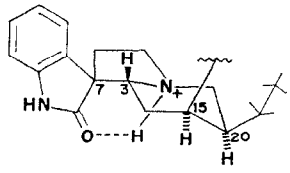

(IV)

EXPERIMENTAL

The leaf samples, supplied by the Rijksherbarium, Leiden, were from the following collections: (a) Masters s.n., Assam, Nov. 1844; (b) Poilane 20 048, Indo-China, 1932; (c) Tsang, 26 860, Kung P'ing Shan, Kwangtung, China, 1936; (d) Wang, 75 929, Cheli, Yunnan, China, 1936. The mass spectra were determined on an AEI MS 902 high resolution mass spectrometer at 70 eV at 220°. The TLC systems used were silica gel G/GF_{254} (Merck) (2:1) with (A) $CHCl_3$–EtOH (95:5) and (B) Et_2O–EtOAc (1:1). The alkaloids were detected with Dragendorff's reagent and with 0·2 M $FeCl_3$ in 35% $HClO_4$ as blue spots (tetracyclic oxindoles) on initial heating and as pink spots after heating at 90° for 1 hr. The hR_fs for systems (A) and (B) respectively are, corynoxine 66, 59; corynoxine B 37, 30; isorhynchophylline 57, 53, rhynchophylline 30, 12. GLC separations were obtained with an 0·5 m column of 5% SE 52 on Aeropak 30 at 230° using a nitrogen pressure of 36 kg/cm². The R_t are 10·6 min for isorhynchophylline–rhynchophylline and 9 8 min for corynoxine-corynoxine B.

Extraction and separation of alkaloids. The dried powdered leaf samples were moistened with 10% NH_4OH and macerated for 2 days with EtOAc. The filtered EtOAc extract was extracted with 2% H_2SO_4 which was made alkaline with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ was washed, dried and concentrated to dryness. The yields of crude alkaloid were as follows: (a) 0·547 g gave 2 3 mg (0 42%); (b) 9 589 g gave 13 4 mg (0 14%); (c) 1 353 g gave 5·0 mg (0·37%); (d) 1 440 g gave 5·3 mg (0·37%) TLC and GLC indicated that extracts of (a–d) were similar. Each extract was separated by preparative TLC using system (B) giving amorphous corynoxine 8·2 mg, corynoxine B 3 0 mg, isorhynchophylline 6 5 mg and rhynchophylline 5 9 mg

Identification of alkaloids The 4 isolated alkaloids had UV and MS which were consistent with those of reference alkaloids. The R_ts and R_fs (systems (A) and (B)) and colours with $FeCl_3/HClO_4$ were identical with those of reference alkaloids.

CD spectra were determined in MeOH; corynoxine (1 100 mg/5 ml) $[\theta]_{236}$ + 32 800, $[\theta]_{265}$ − 6340, $[\theta]_{288}$ − 6910; corynoxine B (0 460 mg/3 ml) $[\theta]_{240}$ + 14 900, $[\theta]_{267}$ − 660, $[\theta]_{292}$ + 1350 (Fig. 1).

Isomerizations of corynoxines. Corynoxine and corynoxine B (< 1 mg of each) were refluxed separately with 50% aq. HOAc for 24 hr at 123°. The extracted alkaloids (after making alkaline with NH_4OH and extracting into $CHCl_3$) were examined quantitatively by TLC using a Joyce–Loebel Chromoscan [16] Estimation of the alkaloid ratios showed that in each case the ratio of corynoxine to corynoxine B was 2:3. The two residues from HOAc isomerization were separately refluxed in pyridine for 14 hr at 126°. Similar extraction and quantitative TLC enabled the ratio of corynoxine to corynoxine B to be determined as 4 1 in each case. No spots were noted which could be attributed to *epiallo* alkaloids

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[15] BEECHAM, A. F., HART, N. K., JOHNS, S. R. and LAMBERTON, J. A (1968) *Australian J. Chem.* **21**, 491.
[16] PHILLIPSON, J. D. and HEMINGWAY, S R (1973) *Phytochemistry* **12**, 1481.