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ALKALOIDS FROM THE LEAVES OF *STRYCHNOS WALLICHIANA*

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Abstract—The leaves of *Strychnos wallichiana* Steud ex DC from Bangladesh contain icajine and novacine as their major alkaloids Smaller amounts of strychnine, brucine, pseudostrychnine, pseudobrucine, *N*-methyl-*sec*-pseudo-β-colubrine, 14-hydroxyicajine, strychnine *N*-oxide, and brucine *N*-oxide are also present The new bases 14-hydroxynovacine and icajine *N*-oxide have been isolated

INTRODUCTION

CERTAIN recent studies have shown that the alkaloid mixture in some leaf samples of *Strychnos nux-vomica* L contains a large proportion of *N*-methyl-*sec*-pseudo bases [1] We have examined the alkaloids present in the leaves of the related species *S wallichiana* Steud ex DC in order to determine whether this preponderance of *N*-methyl-*sec*-pseudo bases also occurs in other Asian species

RESULTS AND DISCUSSION

Of the *ca* 1% partially purified alkaloid mixture isolated, about three-quarters has been separated into identified components (Table 1) Among the less common alkaloids obtained are *N*-cyano-*sec*-pseudostrychnine (**2h**) and *N*-cyano-*sec*-pseudobrucine (**2i**), which are discussed elsewhere,[2] *N*-methyl-*sec*-pseudo-β-colubrine (**2c**), recently obtained from the fruit pericarp of *S nux-vomica*,[3] 14-hydroxyicajine (**2f**), previously found in the leaves of the African *S icaja* Baill ,[4] 14-hydroxynovacine (**2g**), the 2,3-dimethoxy analogue of the previous base, and a compound which appears to be the *N*-oxide of icajine (**2a**) These last two alkaloids are new and we first establish their structures before going on to discuss the results of our analysis

The spectral properties of **2g** (see Experimental) closely resemble those of novacine (**2e**) and they show that the base is a 2,3-dimethoxy-substituted N_a-acydihydroindole In the MS the molecular ion peak is at *m/e* 440, suggesting the presence of one more oxygen atom

* The work reported here is taken from the Ph D thesis submitted to the University of London (1972) Present address of A K C Natural Drugs Research Institute B C S I R Laboratories, Chittagong, Bangladesh

[1] ŠEFČOVIČ, P, DUBRAVKOVA, L and TORTO, F G (1968) *Planta Med* **16,** 143, MAIER, W and GROGER, D (1968) *Pharm Zentralhalle* **107,** 883, CHATTERJEE, A and BASA, S C (1967) *J Ind Chem Soc* **44,** 663, BISSET, N G and PHILLIPSON J D unpublished work

[2] BISSET N G CHOUDHURY A K and WALKER, M D (1974) *Phytochemistry* **13,** 255

[3] BISSET, N G and CHOUDHURY, A K (1974) *Phytochemistry* **13,** 265

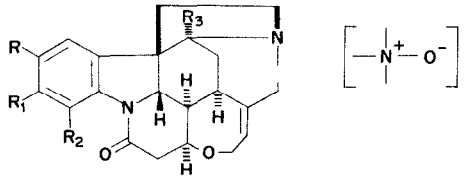
[4] BISSET, N G, DAS, B C and PARELLO, J (1974) *Tetrahedron* in press

TABLE 1 ALKALOIDS ISOLATED FROM THE LEAVES OF *Strychnos wallichiana*

Alkaloid	% Obtained	Alkaloid	% Obtained
Strychnine (1a)	0 009*	*N*-Methyl-*sec*-pseudo-β-colubrine (2c)	0 0003
Brucine (1d)	0 005*	Novacine (2e)	0 126†
Strychnine *N*-oxide	0 003*	14-Hydroxyicajine (2f)	0 018
Brucine *N*-oxide	0 002*	14-Hydroxynovacine (2g)	0 007
Pseudostrychnine (1e)	0 021	*N*-Cyano-*sec*-pseudostrychnine (2h)	0 011
Pseudobrucine (1f)	0 003	*N*-Cyano-*sec*-pseudobrucine (2i)	0 0003
Icajine (2a)	0 321†	Icajine *N*-oxide	0 0006

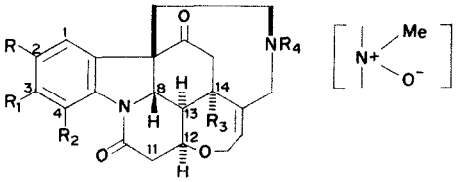
* + an additional 0 053% of mixed strychnine strychnine *N*-oxide + brucine/brucine *N*-oxide fractions
† + an additional 0 120% of mixed icajine + novacine fractions

than in **2e** which has its molecular ion peak at *m/e* 424, the broad IR absorption at 3400 cm^{-1} and the broad NMR signal at δ 3 5, which disappears on deuteration, show that the oxygen atom is present as an OH group The NMR spectrum also has a 1-hydrogen quartet at δ 4 85 (J 11-12 Hz, J' 4-5 Hz) Such a signal is observed in the spectrum of 14-hydroxyicajine (**2f**) and it is assigned to H-12,[4] and the downfield position results from deshielding by the OH group at C-14 (1,3-diaxial relationship) Similar considerations apply here The J values for the H-12 signal in the spectrum of **2g** indicate that the stereochemistry is the same as in **2e** and other *Strychnos* alkaloids The MS of the new base, with the 'indole' peaks at *m/e* 190 203 and 204, the presence of peaks at *m/e* (269), 270 and 285, and the absence of peaks at *m/e* 330 and 369 [4] confirms the suggested structure of 14-hydroxynovacine (**2g**)



(1)

(1a) R = R_1 = R_2 = R_3 = H
(1b) R = R_1 = H, R_2 = OH, R_3 = H
(1c) R = H, R_1 = OMe, R_2 = OH, R_3 = H
(1d) R = R_1 = OMe, R_2 = R_3 = H
(1e) R = R_1 = R_2 = H, R_3 = OH
(1f) R = R_1 = OMe, R_2 = H, R_3 = OH



(2)

(2a) R = R_1 = R_2 = R_3 = H, R_4 = Me
(2b) R = R_1 = H, R_2 = OH, R_3 = H, R_4 = Me
(2c) R = OMe, R_1 = R_2 = R_3 = H, R_4 = Me
(2d) R = H, R_1 = OMe, R_2 = OH, R_3 = H, R_4 = Me
(2e) R = R_1 = OMe, R_2 = R_3 = H, R_4 = Me
(2f) R = R_1 = R_2 = H, R_3 = OH, R_4 = Me
(2g) R = R_1 = OMe, R_2 = H, R_3 = OH, R_4 = Me
(2h) R = R_1 = R_2 = R_3 = H, R_4 = CN
(2i) R = R_1 = OMe, R_2 = R_3 = H, R_4 = CN

The spectral properties of the new *N*-oxide (see Experimental) point to similarity with icajine (**2a**) However, a peak in the MS at *m/e* 380 indicates the presence of an additional oxygen atom as compared with **2a** which has its molecular ion peak at *m/e* 364 The NMR signal for the *N*-methyl group is at δ 3 27, while in the spectrum of **2a** it is at δ 2 06, this considerable paramagnetic shift is plausibly explained as resulting from deshielding by an

[5] HOOTELE, C (1969) *Tetrahedron Letters* 2713, and references cited therein WERNER G and SCHICKLUSS R (1971) *Ann Chem* **746**, 65

oxide function on the same nitrogen atom *,[5] Reduction of the *N*-oxide with Zn–5N HCl gives a product having a R_f value in two systems similar to that of **2a** The compound is therefore formulated as icajine *N*-oxide Attempts to synthesize it were unsuccessful

Altogether, 14 alkaloids have been isolated and identified (Table 1) The *N*-methyl-*sec*-pseudo alkaloids icajine (**2a**) and novacine (**2e**) are the major components of the mixture and together they make up *ca* 80% of the alkaloids identified In contrast, strychnine (**1a**) and brucine (**1d**), together with the corresponding *N*-oxides and pseudo bases (**1e**) and (**1f**), amount to only *ca* 9% This predominance of the *N*-methyl-*sec*-pseudo bases over the normal and pseudo bases is thus in agreement with some of the previously reported analyses of the alkaloid mixtures in *S nux-vomica* leaves [1] Other analyses,[6] however, indicate that the normal bases strychnine and brucine may be the major components Alkaloids of the normal series are formed mainly in the roots[7] and it has been suggested[8] that as the alkaloids move up the plant to the leaves there is a gradual conversion via the *N*-oxides and bases of the pseudo series to alkaloids of the *N*-methyl-*sec*-pseudo series That this conversion process may be subject to seasonal influences could be at least a partial explanation for the different findings in the various analyses

The isolation of 14-hydroxy-*N*-methyl-*sec*-pseudo bases is of some interest Alkaloids of this type have already been found in the leaves of the African *S icaja* [4] The 14-position in these and related alkaloids is equivalent to the 5-position in the iridoid loganin A label at this position in loganin is retained throughout the biosynthesis of indole alkaloids,[9] it is therefore possible that the 14-hydroxy group could be introduced at the start through biosynthesis from a 5-hydroxyloganin rather than loganin However, no 14-hydroxy bases of the normal and pseudo series are yet known, and although 5-hydroxy-iridoids have been isolated from several plants, they are not yet known to occur in *Strychnos* species [10]

Strychnos wallichiana occurs in Sri Lanka, south and north-east India, Bangladesh, North Vietnam, south China, and the Andaman Islands [11] Recently, Bisset and Phillipson have re-analysed seeds from south India [12] The main alkaloid is 4-hydroxy-3-methoxystrychnine (**1c**) and it is accompanied by a little strychnine, 4-hydroxystrychnine (**1b**), and brucine, small amounts of the corresponding *N*-methyl-*sec*-pseudo derivatives (**2d**), (**2a**), (**2b**), and (**2e**) are also present **1c** and **2d** have been found in the seeds and leaves of *S wallichiana* samples from Sri Lanka and south India, but they are not present in samples from Bangladesh, North Vietnam, and the Andaman Islands [13] The difference in the composition of the alkaloid mixtures in the samples from the two regions is distinct, but it seems not to be accompanied by any clear-cut morphological differences in the plants

* In the MS of the *N*-oxide the peak at *m/e* 321 (100%) corresponds to the loss of 59 m u from the molecular ion which has its peak at *m/e* 380 (8%) (m^*_{obs} 271, m^*_{calc} 271 05) This locates the oxygen atom on the nitrogen bridge Loss of the extra oxygen from the molecular ion gives a peak at *m/e* 364 (20%) It seems that the presence of the extra oxygen atom facilitates fragmentation of the nitrogen bridge (see Ref 4)

[6] QUIRIN, M, LEVY, J and LE MEN, J (1965) *Ann Pharm Fr* **23**, 93, BISSET, N G and PHILLIPSON, J D unpublished results

[7] SCHLATTER, C, WALDNER, E E, SCHMID, H, GROGER, D, STOLLE, K and MOTHES, K (1969) *Helv Chim Acta* **52**, 776, HEIMBERGER, S I and SCOTT, A I (1973) *J Chem Soc Chem Commun* 217

[8] BISSET, N G and PHILLIPSON, J D (1971) *J Pharm Pharmacol* **23**, Suppl 244S

[9] BATTERSBY, A R (1971) in *The Alkaloids* (SAXTON, J E, ed), Vol 1, p 31 (Specialist Periodical Reports), The Chemical Society, London

[10] PLOUVIER, V and FAVRE-BONVIN, J (1971) *Phytochemistry* **10**, 1697

[11] BISSET, N G and PHILCOX, D (1971) *Taxon* **20**, 537, BISSET, N G (1972) *Lloydia* **35**, 95

[12] BISSET, N G and PHILLIPSON, J D (1973) *J Pharm Pharmacol* **25**, 563

[13] BISSET, N G and PHILLIPSON, J D unpublished work

In contrast with other samples of *S. wallichiana* and samples of *S. nux-vomica* no 4-hydroxyalkaloids—4-hydroxystrychnine (**1b**) and vomicine (**2b**)—were isolated during the present analysis.

EXPERIMENTAL

*General*ities (see Ref. 3).

Source and identification of the plant material. *S. wallichiana* leaves were collected by A. K. C. with the help of the Forest Ranger, Rangi Chetra, Jury Range, Sylhet, Bangladesh, in September 1968. The material was identified by N. G. B. and Mr. D. Philcox at the herbarium of the Royal Botanic Gardens, Kew. Voucher specimens are kept in the Department of Pharmacy, Chelsea College.

Extraction of the alkaloids. 1955 g ground leaves were mixed with *ca.* 3200 ml of a 1:1 mixture of 50% conc. NH_4OH and 20% aq. Na_2CO_3. Extraction of the material in a Soxhlet with $CHCl_3$ followed by removal of the solvent gave 99 g residue which was redissolved in 500 ml $CHCl_3$ and shaken out with 3 × 700 ml 5% HCl. The combined acid extracts were basified with conc. NH_4OH and the alkaloids were taken into 8 × 1000 ml $CHCl_3$. Removal of the solvent gave 23·5 g (= 1·25%) crude bases. The alkaloids were further purified by dissolving them in 200 ml $CHCl_3$ and extracting them into 1 × 400, 1 × 300 and 6 × 200 ml 5% HCl. The combined acid extracts were basified with conc. NH_4OH and the alkaloids taken into 5 × 500 ml $CHCl_3$. The combined organic phases were dried over anhyd. Na_2SO_4 and taken to dryness. The yield of partially purified bases was 19·55 g (= 1%). Check TLC showed the presence of at least 10 alkaloids.

Separation of the alkaloids. The crude alkaloids (19·55 g) were chromatographed over silica gel (activity I). *Ca.* 2 l. $CHCl_3$–MeOH (49:1) was passed through the column before the first alkaloid-positive fractions emerged. 50-ml fractions were then collected and after check TLC combined into 9 groups. The groups were mostly separated further by preparative TLC. However, the icajine–novacine fractions (group 3) were rechromatographed over alumina (activity III) and eluted with C_6H_6–EtOAc (17:3). Likewise, the strychnine–brucine fractions (group 7) were rechromatographed over alumina (activity III) eluted with C_6H_6, EtOAc (1:1), EtOAc, EtOAc–MeOH (17:3) and MeOH and then further purified by preparative TLC.

Group	Eluant		Fraction	Eluted (g)	Alkaloids obtained
1	$CHCl_3$–MeOH	49:1	1–2	0·26	**1c**, **1f**
2	$CHCl_3$–MeOH	49:1	3–6	1·80	**1c**, **2h**, **2i**, **2a**, **2c**, **2e**
3	$CHCl_3$–MeOH	49:1	7–30	9·01	**1c**, **2h**, **2a**, **2b**
4	$CHCl_3$–MeOH $CHCl_3$–MeOH	49:1 24:1	31–92 93–120	1·53	**2a**, **2b**
5	$CHCl_3$–MeOH $CHCl_3$–MeOH	24:1 9:1	121–220 221–250	1·53	**2a**, **2b**, **2f**, **2g**
6	$CHCl_3$–MeOH	9:1	251–275	0·36	**2f**, **2g**
7	$CHCl_3$–MeOH $CHCl_3$–MeOH	9:1 1:1	276–294 295–344	1·01	**2a** N-oxide, **1a**, **1d**
8	$CHCl_3$–MeOH MeOH	1:1	345–388 389–413	0·52	**1a**, **1d**
9	MeOH MeOH–AcOH	49:1	414–461 462–564	1·13	**1d**, **1a** N-oxide, **1d** N-oxide

Identification of the alkaloids. Known alkaloids were identified by means of their m.p., colour reactions and TLC properties and by comparison of their UV, IR and/or MS with those of authentic compounds available in our laboratory. 14-*Hydroxynovacine* (**2g**): fine needles from MeOH, dec. from 200°; on UV λ_{max}^{EtOH} 217 (log ε 4·64), 265 (4·39) and 301 (4·27) nm, λ_{min}^{EtOH} 242 (log ε 4·32) and 286 (3·99) nm; IR ν_{max}^{Nujol} 3200, 3400, 1655 and 1500 cm^{-1}; NMR δ 2·10 (3-H, s, NMe), 3·52 (1-H, broad s, disappearing on deuteration, C-14 OH), 3·90 (6-H, s, 2× *a*-OMe), 4·35 (1-H, *d*, *J ca.* 11 Hz, H-8), 4·86 (1-H, *q*, *J ca.* 11 Hz, *J ca.* 4·5 Hz, H-12), 6·00 (1-H, broadened *t*, H-22), 7·32 (1-H, s, H-1), 7·78 (1-H, s, H-4); MS *m/e* 440 (M$^+$, $C_{24}H_{28}N_2O_6$, 100%), 425 (8), 381 (3), 286 (14), 285 (13), 271 (5), 270 (7), 269 (7), 267 (5), 266 (6), 244 (5), 243 (8), 204 (6), 203 (5) and 190 (5). *Icajine N-oxide*: needles from MeOH; UV λ_{max}^{EtOH} 210 (log ε 4·66), 255 (4·32), 283 (3·91) and 289 (sh, 3·80) nm, λ_{min}^{EtOH} 237 (log ε 4·23) nm; IR ν_{max}^{Nujol} 1655 and 763 cm^{-1}; MS *m/e* 380 (M$^+$ $C_{22}H_{24}N_2O_4$, 8%), 364 (20), 363 (16), 362 (9), 322 (40), 321 (100), 320 (8), 214 (70), 213 (32), 212 (24), 210 (11), 209 (12), 199 (11), 198 (33), 197 (37), 196 (49), 183 (11), 168 (15), 162 (10), 144 (18), 143 (14), 131 (10) and 130 (28). *N-Cyano-sec-pseudostrychnine* (**2h**) and *N-cyano-sec-pseudobrucine* (**2i**) (see Ref. 2).

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