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ALKALOIDS OF SRI LANKAN *STRYCHNOS NUX-VOMICA*

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Key Word Index—*Strychnos nux-vomica*; *S. wallichiana*; Loganiaceae; indole alkaloids.

Abstract—Twenty-two identified alkaloids have been isolated from the root bark and leaves of a Sri Lankan *Strychnos* species supplied as *S. nux-vomica*. The following bases have not previously been obtained from this species: 10-hydroxystrychnine, 3,12-dihydroxystrychnine, 12-hydroxy-11-methoxystrychnine, 3,12-dihydroxy-11-methoxystrychnine, 12-hydroxystrychnine *N*-oxide, 12-hydroxy-11-methoxystrychnine *N*-oxide, 19,20-dihydroisostrychnine I, 16α,17β-dihydro-17α-hydroxyisostrychnine I (protostrychnine), *O*-methylmacusine B, 16-epi-*O*-methylmacusine B, and nor-melinonine B. The occurrence of a high proportion of 12-hydroxy- and 12-hydroxy-11-methoxy-substituted bases, together with morphological and anatomical evidence, suggests that the plant material may have originated from a hybrid between *S. nux-vomica* and *S. wallichiana*. Data on the tertiary alkaloid composition of different plant parts are presented and compared with corresponding data for Indonesian material. The quaternary alkaloids from the root bark of the Sri Lankan plant exhibited strong muscle-relaxant activity.

INTRODUCTION

Strychnos nux-vomica L. is a smallish tree, growing mainly in the Indian subcontinent, Sri Lanka, and Indo-China, which has globose fruits containing several greyish coloured flattened seeds embedded in a fleshy pulp. These seeds have long been known in pharmacy as the major source of strychnine and brucine, and consequently their alkaloids have been much investigated [1]. Closely related to *S. nux-vomica* is the huge jungle liane *S. wallichiana* Steud. ex DC., which has a somewhat similar distribution range and larger fruits containing up to 15 slightly larger fawn coloured seeds. In supplies of *S. nux-vomica* seeds from Sri Lanka and southern India, seeds of *S. wallichiana* have sometimes been encountered as a substitute; however, these latter have been shown to contain much 12-hydroxy-11-methoxystrychnine and 12-hydroxystrychnine in addition to strychnine and brucine [1]. The observation that material originating from a tree growing in Gampaha, Sri Lanka, and supplied as *S. nux-vomica* had a large proportion of 12-hydroxy-11-methoxy- and 12-hydroxy-substituted bases, which are not normally present in this species, prompted a closer examination of the alkaloid composition of the material; the results of this study are set out below.

RESULTS AND DISCUSSION

Table 1 lists the identified alkaloids isolated from the root bark and leaves. Most of them belong to series of bases which have previously been encountered in *S. nux-vomica* and other species, but several of the compounds are new and their structure elucidation is discussed briefly in the following paragraphs.

β-Carboline-type bases

O-Methyl- and 16-epi-O-methyl-macusine B (**1b, 1c**). Of the quaternary bases obtained it has been possible to determine the structures of only two. Their TLC properties and the colour produced with the $FeCl_3/HClO_4$ spray reagent were almost identical with those of nor-macusine B, which is also present in the plant. Their mass spectra, with major peaks at *m/z* 156, 168, 169, and 182, indicated the presence of a β-carboline skeleton [2]. They both had the same MW, the $M^{+\cdot}$ *m/z* 323 being accompanied by a stronger $M^{+\cdot} - 1$ peak with the formula $C_{21}H_{27}N_2O$. A $M^{+\cdot} - 15$ peak at *m/z* 308 corresponded to the removal of a methyl group, while the peak at *m/z* 277 could be attributed to the ion formed after loss of the side-chain at C-16. The likely structures suggested by these observations are *O*-methylmacusine B (**1b**) and its 16-epimer (**1c**). A semi-synthetic derivative of *O*-methylmacusine B prepared by refluxing macusine B with methanol in the presence of calcium carbonate gave R_f values similar to those of **1b** in three different solvent systems and thereby confirmed the identity. **1b** has been isolated from *S. usambarensis* Gilg and is considered to be an artefact [3].

The other quaternary base differed from **1b** only in its TLC behaviour; the UV and mass spectra were the same. The compound is evidently an isomer and examination of Dreiding models showed that the only site at which isomers are possible is C-16. The structure assigned to the compound is therefore 16-epi-*O*-methylmacusine B (**1c**).

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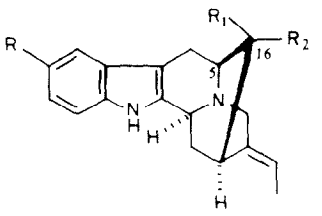
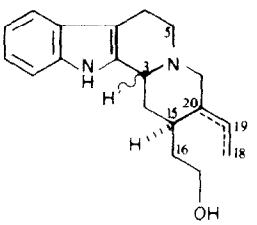


Ia R = R_1 = H, R_2 = CH_2OH
Ib R = R_1 = H, R_2 = CH_2OMe, N_b^+-Me
Ic R = R_2 = H, R_1 = CH_2OMe, N_b^+-Me



2a $\Delta^{19,20}$, 3α-H
2b $\Delta^{18,19}$, 3α-H, 20β-H
2c $\Delta^{18,19}$, 3α-H

Table 1. Alkaloids isolated from the root bark and leaves of Sri Lankan *Strychnos nux-vomica*

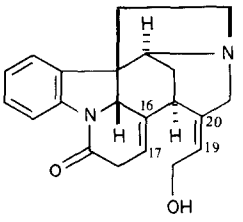
Alkaloid	Root bark	Leaves
Nor-macusine B (**1a**)	+*	+
O-Methylmacusine B (**1b**)	+	−
16-Epi-*O*-methylmacusine B (**1c**)	+	−
Nor-melinonine B (**2c**)	+	−
Isostrychnine I (**3a**)	+	+
19,20-Dihydroisostrychnine I (**3b**)	−	+
16α, 17β-Dihydro-17α-hydroxyisostrychnine I (protostrychnine) (**3c**)	+	−
Strychnine (**4a**)	+	+
10-Hydroxystrychnine (**4b**)	+	−
12-Hydroxystrychnine (**4c**)	+	+
10-Methoxystrychnine (β-colubrine) (**4d**)	+	−
12-Hydroxy-11-methoxystrychnine (**4e**)	+	+
10, 11-Dimethoxystrychnine (brucine) (**4f**)	+	+
Strychnine N_b-oxide	−	+
12-Hydroxystrychnine N_b-oxide	−	+
12-Hydroxy-11-methoxystrychnine N_b-oxide	−	+
10, 11-Dimethoxystrychnine N_b-oxide	−	+
3-Hydroxystrychnine (**4g**)	−	+
3, 12-Dihydroxystrychnine (**4h**)	−	+
3, 12-Dihydroxy-11-methoxystrychnine (**4i**)	−	+
3-Hydroxy-10, 11-dimethoxystrychnine (**4j**)†	−	+
12-Hydroxy-*N*-methyl-*sec*.-pseudostrychnine (vomicine) (**5a**)	−	+

* + = Present; − = not found.
†Isolated partly as the 3-ethyl ether.

Nor-melinonine B (**2c**). The mass spectrum of this base, with M^+ 296, indicated that it is a tetramethylene-tetrahydro-β-carboline, but as the base peaks are at *m/z* 223 and 225 it is clear that the skeletal structure differs from that of nor-macusine B (**1a**) and similar compounds which also occur in *S. nux-vomica* [1]. Acetic anhydride–pyridine acetylation at room temperature yielded a monoacetyl derivative which still had the *m/z* 223 and 225 peaks, showing that the hydroxyl function is not in that part of the molecule which gives rise to the ions represented by these peaks; most probably, the loss is of groups attached to ring D.

An attractive hypothesis is that the compound could differ from nor-macusine B as a result of cleavage between C-5 and C-16, and such a compound would have the correct MW. Of the possible candidates, geissoschizol (**2a**), which is related to geissoschizine, an indirect biogenetic precursor of strychnine and congeners [4], is eliminated as its mass

spectrum lacks the strong *m/z* 223 and 225 peaks. On the other hand, the general appearance of the mass spectrum of the quaternary base melinonine B, which results from thermal demethylation and is therefore that of the corresponding tertiary base nor-melinonine B [5], is strikingly similar to that of the present compound. The two very intense peaks at *m/z* 223 and 225 are present in the melinonine B spectrum. This evidence does not establish the stereochemistry, but the compound is not corynantheol (**2b**), which is isomeric with nor-melinonine B, since the TLC properties are different. This leaves nor-melinonine B itself as the most likely identity, the more so as melinonine B is known to occur in another *Strychnos* species, *S. melinoniana* Baill. [6]. Vamvacas *et al.* [7] assigned the 3β-H, 15α-H, 20β-H (pseudo) or 3β-H, 15α-H, 20α-H (epiallo) configuration to melinonine B after comparing dihydromelinonine B chloride with N_b-metho-dihydrocorynantheol chloride. The possibility of the present compound having the 3α-H, 15α-H, 20α-H (allo) or 3α-H, 15α-H, 20β-H (normal) configuration is eliminated by the TLC behaviour of the compound, for its lower HR_f values as compared with those for corynantheol point to a probable 3β-H, 15α-H and 20β-H or 20α-H (pseudo or epiallo) configuration [8]. The conclusion is that the present compound is almost certainly nor-melinonine B (**2c**), the residual doubt being due to the lack of evidence for the configuration at C-20.



3a
3b $\Delta^{19,20}$ saturated
3c $\Delta^{16,17}$ replaced by H and OH

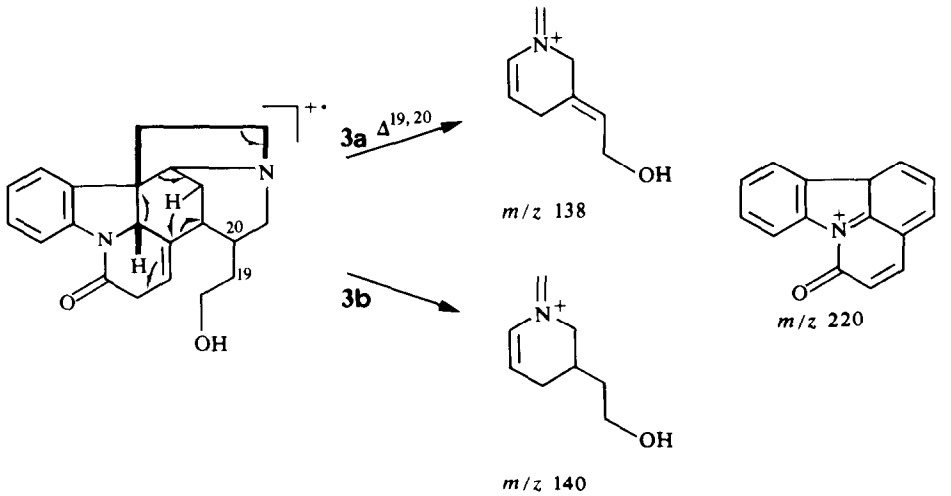
Isostrychnine-type bases

19,20-*Dihydroistrychnine I* (**3b**). The UV, IR, and mass spectral data for the alkaloid, which like isostrychnine I was present in one of the more polar fractions, revealed the occurrence of an unsubstituted N_a-acyl-dihydroindole chromophore, with the carbonyl as part of a lactam function. The presence of a hydroxyl group and the greater polarity of the substance as compared with members of the 'normal' series of strychnine bases suggested that the alkaloid might be related to isostrychnine I (**3a**). The mass spectrum with $M^{+\cdot}$ 336 and two peaks at $M^{+\cdot} - 31$ and $M^{+\cdot} - 45$, corresponding to loss of $-CH_2OH$ and $-CH_2CH_2OH$, respectively, indicated the possibility of a dihydroisostrychnine I structure with the 19,20- rather than 16,17-double bond saturated. Consistent with this is a further peak at *m/z* 140, which appears to be equivalent to the *m/z* 138 peak observed in the mass spectrum of isostrychnine I. Probably, the fragmentation is similar to that of akuammicine derivatives [9] and is initiated by migration of the 16,17-double bond into conjugation with the lactam carbonyl, followed by the usual retro-Diels–Alder process.

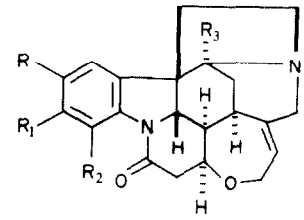
Accurate mass measurement of the *m/z* 220 peak common to the spectra of the dihydroisostrychnine I, isostrychnine I, and protostrychnine, is in agreement with the formula $C_{15}H_{10}NO$ and the structure shown may be proposed for this fragment.

The NMR spectrum supports the proposed structure, 19,20-dihydroisostrychnine I (**3b**). There is only one signal for an olefinic hydrogen, a multiplet at δ 5.8 which is slightly upfield from a similar signal in the spectrum of isostrychnine I. The signal for the two H-18 in the spectrum of **3b** is a triplet ($J = 7$ Hz) situated at δ 3.71, whereas in **3a**, where the hydrogen is adjacent to the 19,20-double bond, the two H-18 are seen as a doublet ($J = 6$ Hz) further downfield at δ 4.25.

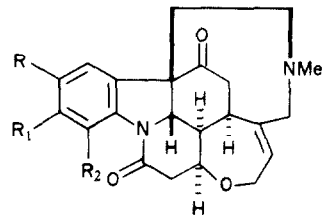
16α,17β-*Dihydro*-17α-*hydroxyisostrychnine I* (*protostrychnine*) (**3c**). The isolation of this alkaloid has been described elsewhere [10]. Protostrychnine appears to be structurally similar to the prestrychnine of Heimberger and Scott [11], which takes part in the



later stages of the biosynthesis of strychnine, the only difference between the two being that the former has a lactam ring not present in the latter. Occurrence of protostrychnine in the roots, the known biosynthetic site for alkaloids in *S. nux-vomica*, has obvious biosynthetic implications. The compound can be regarded as an immediate precursor of strychnine.



4a R = R_1 = R_2 = R_3 = H
4b R = OH, R_1 = R_2 = R_3 = H
4c R = R_1 = R_3 = H, R_2 = OH
4d R = OMe, R_1 = R_2 = R_3 = H
4e R = R_3 = H, R_1 = OMe, R_2 = OH
4f R = R_1 = OMe, R_2 = R = H
4g R = R_1 = R_2 = H, R_3 = OH
4h R = R_1 = H, R_2 = R_3 = OH
4i R = H, R_1 = OMe, R_2 = R_3 = OH
4j R = R_1 = OMe, R_2 = H, R_3 = OH



5a R = R_1 = H, R_2 = OH
5b R = R_1 = OMe, R_2 = H

The other alkaloids isolated (Table 1) are members of series already known to be present in *S. nux-vomica*. They include a small quantity of 10-hydroxystrychnine (**4b**), a phenolic base of the 'normal' series now obtained for the first time from a natural source. Its *O*-methylated derivative, β-colubrine (**4d**), however, is a long-known minor alkaloid of the plant.

Alkaloids belonging to the pseudo, *N*-methyl-*sec.*-pseudo, and N_b-oxide series were, as expected, present only in the above-ground parts of the plant (cf. [1]). Of these, 3,12-dihydroxystrychnine (**4h**), 3,12-dihydroxy-11-methoxystrychnine (**4i**), and the N_b-oxides of 12-hydroxystrychnine (**4c**) and 12-hydroxy-11-methoxystrychnine (**4e**) were encountered for the first time. Vomicine (12-hydroxy-*N*-methyl-*sec.*-pseudostrychnine) (**5a**) appeared to be the only member of the *N*-methyl-*sec.*-pseudo series occurring in the leaves of the plant.

Identity of the Sri Lankan Strychnos *material investigated*

A large amount of 12-hydroxystrychnine (**4b**) was found in the material, which is unusual for *S. nux-vomica*, and much 12-hydroxy-11-methoxystrychnine (**4e**), not previously known in *S. nux-vomica*, was also isolated (cf. [1]). These findings raised the question of the identity of the plant being investigated. On comparison with authentic *S. nux-vomica* and also with authentic *S. wallichiana*, since Sri Lankan material of this species is known to contain 12-hydroxy-11-methoxystrychnine (cf. [1]), it was concluded that morphologically the Gampaha material more nearly resembled *S. nux-vomica* than *S. wallichiana*. This conclusion was based largely on the habit as a tree rather than climber and on the leaf, fruit, and seed characters. Nevertheless, stomatal counts of the leaves (see Experimental) had a mean intermediate between those for the leaves of the authentic *S. nux-vomica* and *S. wallichiana*. A second collection of Sri Lankan *Strychnos*, from Dambulla, about 100 km north-east of Gampaha, and also supplied as *S. nux-vomica*, gave a similar stomatal count and it likewise contained 12-hydroxy-11-methoxy-substituted bases.

These findings clearly differentiate the Sri Lankan plant from *S. nux-vomica* growing elsewhere in tropical Asia. It could be a hybrid between *S. nux-vomica* and *S. wallichiana*. If this is the case, it is evident that morphologically *S. nux-vomica* is the dominant parent. On the other hand, the contribution of the two putative parents to its alkaloid composition is more even.

Seasonal variation in alkaloid content

The tertiary alkaloid content of different parts of *S. nux-vomica* was also investigated on the basis of 12 consecutive monthly collections of material from Gampaha (Sri Lanka) and Bogor (Indonesia). The findings are set out in Table 2. Although the leaves of the Sri Lankan material tended to be richer in alkaloid than the Indonesian material, both had a substantially lower alkaloid content in the period October–February/March than in the remaining part of the year. The present work confirmed that the wood of the plant is poor in alkaloid compared with the bark; the figures for the root bark are considerably higher than those previously reported (cf. [1]).

There was no significant variation in the tertiary alkaloid composition of the Sri Lankan leaves throughout the year, and members of the 'normal' series (**4a–4f**) were predominant. In the underground parts, strychnine was the major alkaloid and brucine, the most highly substituted of the four main bases present, was the least abundant. In the aerial parts 12-hydroxy-11-methoxystrychnine dominated and, except in the trunk bark, brucine was again the least abundant. Interestingly, 12-hydroxy-11-methoxystrychnine was present in the trunk bark in far larger amounts than in any of the other plant parts examined.

The Indonesian *S. nux-vomica* presented a very different picture. No 12-hydroxy-11-methoxystrychnine was found in any of the plant parts studied and 12-hydroxystrychnine occurred only in traces in the mixture of leaf alkaloids. In the leaves, during the

Table 2. Content and composition of the tertiary alkaloid mixtures occurring in different parts of Sri Lankan (Gampaha) and Indonesian (Bogor) *Strychnos nux-vomica*

Plant part	Gampaha material % total alkaloid Oct. 1973–June 1974, July 1973–Sept. 1973	Principal alkaloids	Bogor material % total alkaloid Oct. 1973–Sept. 1974	Principal alkaloids
Leaves	2.5–4.5		0.3–5.0	
	Oct.–Feb. 2.5–3.1	**4e**(55)* **4a** (25), **4c** (10), **4f** (10)	Oct.–March 0.4–1.1	**5a** (50), **4f** (35), **5b** (10), **4a** (5)
	March–Sept. 3.3–4.5		April–Sept. 1.2–5.0	**4f** (55), **4a** (45)§
Twig bark	2.7–6.0	**4e** (55), **4a** (20), **4c** (10), **4f** (10)†	—	—
Twig wood	0.6–1.4	**4e** (60), **4a** (15), **4c** (15), **4f** (10)	—	—
Trunk bark	6.1–8.2	**4e** (65), **4f** (20), **4c** (10), **4a** (5)	2.0–5.0	**4f** (80), **4a** (10), **4j** (10)‖
Root bark	12.1–18.0	**4a** (40), **4e** (30), **4c** (15), **4f** (15)‡	11.3–17.3	**4a** (60), **4f** (40)¶
Root wood	0.8–1.7	**4a** (40), **4c** (30), **4e** (20), **4f** (10)	0.8–1.8	**4f** (60), **4a** (40)**

*Approximate percentage of the principal alkaloids.

†A small amount of alkaloids belonging to the 3-hydroxy (pseudo) series (cf. **4g**, etc.) was present.

‡The occurrence of some nor-macusine B (**1a**) was noted.

§Traces of *N*-methyl-*sec.*-pseudo bases (cf. **5**) were seen.

‖Nor-macusine B (**1a**), isostrychnine I (**3a**), and protostrychnine (**3c**) were present in traces.

¶Faint spots corresponding to the colubrines (cf. **4d**) and 3-hydroxybrucine (**4j**) were observed.

The chromatograms showed traces of the colubrines (cf. **4d) and the 3-hydroxy derivatives of strychnine and brucine (**4h**, **4j**).

period October–March bases of the *N*-methyl-*sec.*-pseudo series (**5a**, **5b**), in particular vomicine, predominated over strychnine and brucine; during the rest of the year brucine and strychnine were the principal alkaloids. Throughout the year, strychnine was the major alkaloid of the root bark and brucine was the chief base in the root wood, stem bark, and leaves.

Root bark of the Sri Lankan *S. nux-vomica* yielded 1.8% quaternary alkaloid extract. Screen-grip tests carried out on mice with this extract revealed, for the first time, that alkaloids present in this species have strong muscle-relaxant activity. The lowest dose bringing about a reversible muscle-relaxant effect was in the range 5–15 mg/kg and the lethal dose was in the range 15–25 mg/kg. No convulsions were observed. The quaternary alkaloids responsible for the activity have yet to be identified.

EXPERIMENTAL

Plant materials were collected between July 1973 and June 1974 in Gampaha and Dambulla, Sri Lanka, and between October 1973 and September 1974 in the Hortus Botanicus Bogoriensis, Bogor, Indonesia. Voucher specimens are kept in the Department of Pharmacy, Chelsea College, London.

^{1}H NMR spectra were recorded in $CDCl_3$ at 90 MHz. The ^{13}C NMR spectrum of protostrychnine was recorded in $CHCl_3$ at 25.15 MHz in the Fourier transform mode. High-resolution MS were determined at 70 eV and 185–230° using a direct inlet system.

The general techniques used in isolating and examining the alkaloids have been described in previous publications [10, 12, 13]. For TLC spraying 0.2 M $FeCl_3$ in 35% aq. $HClO_4$ was used, followed by heating at *ca* 90° [14]. Known alkaloids were identified by means of their mp, colour reactions, TLC properties and by comparison of their UV, IR, NMR, and MS with those of authentic samples. Only the data on the new alkaloids are listed below.

Alkaloid composition of monthly collections. In the visual assessment of the alkaloidal extracts from the monthly collections, a standard mixture of strychnine (1 mg), 12-hydroxy-11-methoxystrychnine (1.9 mg), 10,11-dimethoxystrychnine (1.6 mg), and 12-hydroxystrychnine (1.4 mg) was dissolved in 1 ml $CHCl_3$. 5, 10, and 15 μl of this soln and 10 and 15 μl of the standard solns of the monthly alkaloidal extracts, containing *ca* 4 mg in 1 ml $CHCl_3$, were spotted on 20 × 20 cm precoated Si gel plates (Camag) by means of calibrated Micropet capillary tubes. The plates were developed once to a distance of 18 cm in freshly prepared EtOAc–*iso*-PrOH–conc. NH_4OH (16:3:1), sprayed with $FeCl_3$–$HClO_4$ reagent, heated to 90° for 30 mins, and the relative sizes and intensities of the spots assessed visually.

Stomatal counts. For the leaf stomatal counts, 4 × 4 mm pieces were cut from the laminae near the midrib, mounted in 50% chloral hydrate soln with the lower epidermis uppermost, and cleared by gentle warming. The stomatal density was determined using a microscope equipped with a 40 × objective and a 6 × Huyghens grid eyepiece. Three mounts from three different parts of each of three leaves were prepared and three readings were taken from each mount. The following mean stomatal counts (stomata/mm^2) were obtained: Gampaha *Strychnos* 226, Dambulla *Strychnos* 212, *S. nux-vomica* 361 and 366 (lit. [15] 276–404), and *S. wallichiana* 178 (lit. [15] 164–194).

Screen-grip tests. The tests were carried out by Dr. L. Bohlin, Institute of Pharmacognosy, Uppsala University, Sweden, employing a method described elsewhere [16].

O-Methylmacusine B (**1b**). UV λ_{max}^{MeOH} nm: 230, 267, 271, 278, 281, 289; MS (230°) *m/z* (rel. int.) 323 ($M^{+\cdot}$, $C_{21}H_{27}N_2O$, 4), 322 (17), 308 (57), 294 (52), 293 (45), 279 (11), 277 (34), 276 (7), 263 (33), 185 (11), 184 (14), 183 (55), 182 (47), 180 (33), 169 (96), 168 (100), 167 (52), 156 (27), 154 (27), 144 (16), 143 (23), 130 (26), 122 (48); accurate mass measurement: found 322.2029, $C_{21}H_{26}N_2O$ requires 322.2045.

16-Epi-O-methylmacusine B (**1c**). UV λ_{max}^{MeOH} nm: 227, 269, 278, 280.5, 288.5; IR ν_{max}^{KBr} cm^{-1}: 3350, 2930, 2860, 1600, 1445, 1390, 1195, 1130, 1115, 1055, 938, 755; MS (185°) *m/z* (rel. int.) 323 ($M^{+\cdot}$, $C_{21}H_{27}N_2O$, 4), 322 (23), 308 (61), 294 (46), 293 (50), 279 (8), 277 (46), 276 (8), 263 (23), 185 (23), 183 (69), 182 (58), 180 (42), 169 (92), 168 (100), 167 (58), 156 (31), 154 (27), 144 (23), 143 (19), 130 (31), 122 (65).

Nor-melinonine B (**2c**). UV λ_{max}^{MeOH} nm; 231, 273, 279, 281, 289; MS (190°) *m/z* (rel. int.) 296 ($M^{+\cdot}$, $C_{19}H_{24}N_2O$, 65), 295 (74), 265 (13), 226 (18), 225 (100), 224 (25), 223 (100), 209 (7), 197 (18), 184 (22), 169 (30), 156 (24), 144 (13), 143 (13), 130 (10), 129 (12), 115 (12); accurate mass measurements: found 296.1886, 265.1718, 225.1387, 197.1081, $C_{19}H_{24}N_2O$, $C_{18}H_{21}N_2$, $C_{15}H_{17}N_2$, $C_{13}H_{13}N_2$ require 296.1889, 265.1705, 225.1392, 197.1079. Treatment of **2c** with Ac_2O–pyridine at room temp. yielded the mono-acetyl derivative: MS (185°) *m/z* (rel. int.) 338 (M^+, $C_{21}H_{26}N_2O_2$, 36), 337 (37), 279 (9), 257 (11), 239 (6), 225 (61), 224 (23), 223 (100), 209 (7), 208 (9), 197 (11), 196 (12), 184 (12), 169 (14), 156 (12), 144 (11), 143 (11), 130 (11), 129 (12), 111 (20).

19,20-Dihydroisostrychnine I (**3b**). $[\alpha]_D^{21}$ + 55° (MeOH; *c* 0.044) UV λ_{max}^{MeOH} nm: 254, 282, 292; IR ν_{max} cm^{-1}: 3400, 2950, 1660, 1640, 1595, 755; ^{1}H NMR data δ (from TMS) 8.12 (1H, *d*, *J* = 9 Hz, H-12), 7.2 (3H, *m*, H-9, H-10, H-11), 5.8 (1H, *m*, H-17), 3.71 (2H, *t*, *J* = 7 Hz, 2 × H-18), 1.99 (1H, *s*, disappearing with D_2O, OH-18); MS (200°) *m/z* (rel. int.) 366 ($M^{+\cdot}$, $C_{21}H_{24}N_2O_3$, 100), 318 ($M^+ - 18$, 5), 317 (14), 316 (29), 306 (9), 305 ($M^+ - 31$, 27), 303 (21), 292 (33), 291 ($M^{+\cdot} - 45$, 34), 279 (20), 264 (10), 247 (16), 236 (24), 220 (27), 180 (13), 167 (12), 144 (15), 143 (12), 140 (46), 130 (16), 115 (13); accurate mass measurements: found 336.1829, 305.1654, 291.1500, 220.0769, 140.1081, $C_{21}H_{24}N_2O_2$, $C_{20}H_{21}N_2O$, $C_{19}H_{19}N_2O$, $C_{15}H_{10}NO$, $C_8H_{14}NO$ require 336.1838, 305.1654, 291.1497, 220.0762, 140.1075.

16α,17β-Dihydro-17α-hydroxyisostrychnine I (protostrychnine) (**3c**). The physical data for this compound have been published elsewhere [10]. The ^{13}C NMR data are δ (from TMS) 171.4 (C-23), 140.7 (C-13), 137.2 (C-20), 134.3 (C-8), 128.6 (C-11), 126.5 (C-19), 124.9 (C-10), 122.1 (C-9), 115.9 (C-12), 68.0 (C-17), 66.6 (C-2), 62.0 (C-3), 57.1 (C-18), 56.8 (C-21), 53.9 (C-16), 53.6 (C-5), 51.2 (C-7), 45.7 (C-22 or C-6), 43.2 (C-6 or C-22), 28.3 (C-14), 27.7 (C-15).

10-Hydroxystrychnine (**4b**). UV λ_{max}^{MeOH} nm: 260, 299, 310 (sh); bathochromic shift on addition of NaOH soln to UV λ_{max}^{MeOH} 268 nm; ^{1}H NMR ($CDCl_3$ + CD_3OD): δ (from TMS) 7.87 (1H, *d*, *J* = 9 Hz, H-12), 6.70 (1H, *d*, *J* = 9 Hz; H-11), 6.66 (1H, *s*, H-9), 5.95 (1H, *m*, H-19); MS (210°) *m/z* (rel. int.) 350 (M^+, $C_{21}H_{22}N_2O_3$, 100), 163 (6), 162 (13), 161 (8), 160 (9), 159 (17), 146 (13), 134 (8), 120 (14), 107 (12); accurate mass measurement; found 350.1630, $C_{21}H_{22}N_2O_3$ requires 350.1630.

3,12-Dihydroxystrychnine (**4h**). $[\alpha]_D^{21}$ + 55° (MeOH; *c* 0.21) UV $\lambda_{max}^{CHCl_3}$ nm: 268, 280 (sh), 299; IR ν_{max}^{Nujol} cm^{-1}: 3600, 3450, 2700–2500, 1630, 1620, 1600, 1575, 1460, 1430, 1300, 1260, 1220, 1195, 1185, 1099, 1075, 1040, 985, 950, 919, 870, 820, 795, 760, 745, 730; ^{1}H NMR δ (from TMS) 11.70 (1H, *s*, disappearing with D_2O, OH-12), 7.40 (1H, *q*, *J* = 7.5 and 2 Hz, H-9), 7.01

(1H, *t*, $J = 7.5$ Hz, H-10), 6.80 (1H, *q*, $J = 7.5$ and 2 Hz, H-11), 5.9 (1H, *m*, H-19), 4.3 (1H, *m*, H-17), *ca* 4.1 (2H, *m*, 2 × H-18), 1.78 (1H, *s*, disappearing with D_2O, OH-3); MS (cf. [13]) (200°) *m/z* (rel. int.) 366 ($M^{+\cdot}$, $C_{21}H_{22}N_2O_4$, 100), 350 ($M^{+\cdot} - 16$, 39), 349 ($M^{+\cdot} - 17$, 22), 348 ($M^{+\cdot} - 18$, 27), 321 (7), 226 (7), 225 (8), 214 (8), 213 (7), 201 ($M^{+\cdot} - 165$, 76), 160 (65), 159 (100), 146 (50), *m** 126 (*m/z* 321 → 210).

3,12-*Dihydroxy*-11-*methoxystrychnine* (**4i**). $[\alpha]_D^{21} + 38°$ (MeOH; *c* 0.25) prisms (EtOAc); UV λ_{max}^{MeOH} nm: 240, 271; UV λ_{min}^{MeOH} 253 nm; IR ν_{max}^{Nujol} cm^{-1}: 3450, 2700–2500, 1660, 1630, 1595, 1570, 1335, 1298, 1250, 1210, 1190, 1150, 1130, 1095, 1070, 1045, 870, 760, 750; ^1H NMR δ (from TMS) 11.88 (1H, *s*, disappearing with D_2O, OH-12), 7.33 (1H, *d*, $J = 9$ Hz, H-9), 6.68 (1H, *d*, $J = 9$ Hz, H-10), 5.94 (1H, *br t*, H-19), 4.37 (1H, *m*, H-17), *ca* 4.1 (2H, *m*, 2 × H-18), 3.84 (3H, *s*, OMe-11), 3.75 (1H, *d*, $J = 9$ Hz, H-2), 3.3 (1H, *m*, H-15), 3.3 (1H, *m*, $J = 9$ Hz, H-22), 3.10 (1H, *d*, $J = 9$ Hz, H'-22), 2.28 (1H, *s*, disappearing with D_2O, OH-3), 1.38 (1H, *dt*, $J = 3$ and 11 Hz, H-16); MS (cf. [13]) (200°) *m/z* (rel. int.) 396 ($M^{+\cdot}$, $C_{22}H_{24}N_2O_5$, 28), 381 (23), 380 ($M^{+\cdot} - 16$, 96), 379 ($M^{+\cdot} - 17$, 33), 378 ($M^{+\cdot} - 18$, 100), 365 (6), 349 (11), 231 ($M^{+\cdot} - 165$, 5), 190 (11), 189 (30), 176 (13); accurate mass measurements: found 396.1696, 380.1758, 378.1577, 349.1546, 231.0907, $C_{22}H_{24}N_2O_5$, $C_{22}H_{24}N_2O_4$, $C_{22}H_{22}N_2O_4$, $C_{21}H_{21}N_2O_3$, $C_{13}H_{12}NO_3$ require 396.1686, 380.1737, 378.1580, 349.1552, 231.0896.

12-*Hydroxystrychnine* N_b-*oxide* (cf. **4c**). UV λ_{max}^{MeOH} nm: 264, 295; MS (210°) *m/z* (rel. int.) 366 ($M^{+\cdot}$, $C_{21}H_{22}N_2O_4$, 42), 351 (40), 350 ($M^{+\cdot} - 16$, 100), 349 ($M^{+\cdot} - 17$, 38), 348 ($M^{+\cdot} - 18$, 60), 335 (14), 332 (17), 321 (16), 320 (13), 319 ($M^{+\cdot} - 47$, 52), 236 (14), 201 (13), 196 (11), 184 (13), 163 (17), 162 (20), 161 (14), 160 (28), 159 (48), 146 (33), 135 (26), 129 (20), 119 (31), 107 (32); accurate mass measurements: found 366.1571, 350.1637, 319.1438, $C_{21}H_{22}N_2O_4$, $C_{21}H_{22}N_2O_3$, $C_{20}H_{19}N_2O_2$ require 366.1579, 350.1630, 319.1446. Reduction of the compound with 5% sulphurous acid at room temp. overnight yielded the corresponding tertiary base **4c**, identified by TLC.

12-*Hydroxy*-11-*methoxystrychnine* N_b-*oxide* (cf. **4e**). $[\alpha]_D^{21} + 91°$ (MeOH; *c* 0.175) plates (MeOH); UV λ_{max}^{MeOH} nm: 237, 268; UV λ_{min}^{MeOH} 252 nm; MS (205°) *m/z* (rel. int.) 396 ($M^{+\cdot}$, $C_{22}H_{24}N_2O_5$, 40), 380 ($M^{+\cdot} - 16$, 100), 379 ($M^{+} - 17$, 32), 378 ($M^{+} - 18$; 50), 365 (10), 363 (7), 362 (8), 352 (7), 351 (9), 350 (18), 349 ($M^{+\cdot} - 47$, 61) 280 (12), 204 (9), 190 (17), 189 (18), 176 (19); accurate mass measurements: found 396.1678, 380.1747, 349.1566, $C_{22}H_{24}N_2O_5$, $C_{22}H_{24}N_2O_4$, $C_{21}H_{21}N_2O_3$ require 396.1685, 380.1736, 349.1552. Reduction of the compound with 5% sulphurous acid at room temp. overnight yielded the corresponding tertiary base, **4e**, identified by TLC.

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REFERENCES

1. Bisset, N. G. and Phillipson, J. D. (1976) *Lloydia* **39**, 263.
2. Antonaccio, L. D., Pereira, N. A., Gilbert, B., Vorbrueggen, H., Budzikiewicz, H., Wilson, J. M., Durham, I. J. and Djerassi, C. (1962) *J. Am. Chem. Soc.* **84**, 2161.
3. Angenot, L. (1975) *Planta Med.* **27**, 24.
4. Bisset N. G. (1980) in *Indole and Biogenetically Related Alkaloids* (Phillipson, J. D. and Zenk, M. H., eds.) p. 27. Academic Press, London.
5. Hesse, M. (1974) *Indolalkaloide—Progress in Mass Spectrometry*, Vol. 1, p. 141, Vol. 2, p. 59. Verlag Chemie, Weinheim.
6. Schlittler, E. and Hohl, J. (1952) *Helv. Chim. Acta* **35**, 29.
7. Vamvacas, C., von Philipsborn, W., Schlittler, E., Schmid, H. and Karrer, P. (1957) *Helv. Chim. Acta* **40**, 1793.
8. Phillipson, J. D. and Shellard, E. J. (1967) *J. Chromatogr.* **31**, 427.
9. Budzidiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry–Alkaloids* Vol. 1, pp. 46 and 133. Holden-Day, San Francisco.
10. Başer, K. H. C., Bisset, N. G. and Hylands, P. J. (1979) *Phytochemistry* **18**, 512.
11. Heimberger, S. I. and Scott, A. I. (1973) *J. Chem. Soc. Chem. Commun.* 217.
12. Bisset, N. G. and Choudhury, A. K. (1974) *Phytochemistry* **13**, 265.
13. Bisset, N. G., Das, B. C. and Parello, J. (1973) *Tetrahedron* **29**, 4137.
14. Bisset, N. G. and Phillipson, J. D. (1973) *J. Pharm. Pharmacol.* **25**, 563.
15. Tirel-Roudet, C. (1970) Thèse 3 ème Cycle, Faculté des Sciences, Université de Paris, pp. 177 and 179.
16. Sandberg, F. (1966) *Proc. Int. Symp. Med. Pl.*, Kandy, Dec. 1964 p. 187.