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Alkaloids from the seeds of *Strychnos wallichiana* Steud. ex DC. (*Strychnos cinnamomifolia* Thwaites var. *wightii* A. W. Hill)

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The seeds of a south Indian sample of *Strychnos wallichiana* Steud. ex DC., previously analysed by Short (1924) under the name *S. cinnamomifolia* Thwaites and thought to contain mostly brucine with a little strychnine, have now been shown to contain a new base 4-hydroxy-3-methoxystrychnine (I) as the major alkaloidal constituent. Smaller amounts of strychnine, 4-hydroxystrychnine, brucine, vomicine, 4-hydroxy-3-methoxy-*N*-methyl-*sec.*-pseudostrychnine (II), and novacine and possibly also α-colubrine are present.

In 1924 at the request of the then Director of the Royal Botanic Gardens, Kew, a sample of seeds of *Strychnos cinnamomifolia* Thwaites from Travancore, south India, was analysed in the Research Laboratories of the Pharmaceutical Society (Short, 1924; cf. Holland, 1924). Using a modification of the British Pharmacopoeia 1914 method of assay, Short determined 2·231% brucine and 0·342% strychnine, and qualitative tests indicated the presence of both these bases in the total alkaloid mixture.

Among the samples included in our screening program on Asian *Strychnos* species was one which came from the Kew Museum of Economic Botany and which comprised several seeds of *S. cinnamomifolia* belonging to the lot originally analysed by Short. Thin-layer chromatograms of the total alkaloid extract from this sample when sprayed with Dragendorff's reagent appeared to confirm that the alkaloids present were mainly brucine with some strychnine. However, gas-liquid chromatograms showed that the main peak belonged to an alkaloid having a retention time longer than that of brucine and that the strychnine peak was very small, while on spraying the thin-layer chromatograms with ferric chloride/perchloric acid or ceric sulphate/sulphuric acid the major part of the "brucine" spot gave a colour different from that of brucine and the "strychnine" spot gave a colour different from that of strychnine. Seeds from the lot analysed by Short are still in the Pharmaceutical Society's Museum at the University of Bradford (labelled *S. cinnamomifolia*, Greenish, 1924) and examination of the total alkaloid extract from a sample of them gave identical results on t.l.c. and g.l.c. A more detailed investigation was therefore undertaken in order to establish the identities of the alkaloids present.

METHODS AND RESULTS

The sample of seeds from the Kew Museum was received under the name *Strychnos cinnamomifolia* and was labelled J. F. Bourdillon 5/1/23 Travancore.

The ultraviolet spectra were recorded on a Unicam SP800 instrument and the infrared spectra on Unicam SP100 or SP200 instruments. The 100 MHz nmr spectra were run in deuterochloroform with TMS as internal standard on a Varian Associates HA100 instrument. The mass spectra were obtained with an A.E.I.902 high-resolution spectrometer having a direct-inlet system operating at a temperature of 205° and at 70 eV.

Thin-layer chromatography (t.l.c.). The plates were prepared with neutral silica gel G (Merck) and run in one of the following systems, depending on the mobility of the alkaloids being examined: (1) ethyl acetate–isopropanol–5·5% ammonia (45 : 35 : 20); (2) ethyl acetate–isopropanol–conc. ammonia (80 : 15 : 5); (3) ethyl acetate–isopropanol–conc. ammonia (100 : 2 : 1); and (4) n-butanol–0·1 N hydrochloric acid–7·4% aqueous potassium ferrocyanide (100 : 15 : 34).

The plates were sprayed with Dragendorff's reagent, but use was also made of 2% ceric sulphate in 2N sulphuric acid and 0·2 M ferric chloride in 35% perchloric acid. With the latter two sprays strychnine and *ar*-substituted strychnines and their derivatives with an N_a-carbonyl function give colours which differ according to the substitution pattern and which are largely independent of changes elsewhere in the molecule. Table 1 lists the colours obtained.

Preparative t.l.c. The plates, 20 × 20 or 20 × 40 cm, were made up with a 1-mm thick adsorbent layer comprising equal amounts of silica gel G and GF_{254} (Merck). When required, the plates were developed several times to ensure a better separation of the components in the mixtures being examined.

Gas-liquid chromatography (g.l.c.). The conditions were similar to those previously used in investigating *Strychnos* alkaloids (Bisset & Fouché, 1968; Bisset & Phillipson, 1971). A column temperature of 250° was used. A Becker 407 instrument was also used with a column temperature of 270° and a nitrogen flow-rate of 100 ml min^{-1}.

Extraction. The ground seeds (23·4 g) were moistened with 5% potassium hydroxide solution (30 ml) and then shaken with chloroform–ether (1 : 2) (200 ml) for

Table 1. *Colours given by* Strychnos *alkaloids spotted on silica-gel t.l.c. plates after spraying with ceric sulphate/sulphuric acid and ferric chloride/perchloric acid.*

	$Ce_2(SO_4)_3/H_2SO_4$		$FeCl_3/HClO_4$	
Alkaloid	Colour directly after spraying	Colour after 5 min at about 90°	Colour after warming with hot air	Colour after 30 min at about 90°
Strychnine	—	Pale violet	Violet	Red
4-Hydroxystrychnine	V. pale violet	Reddish purple	Grey	Grey
2-Methoxystrychnine (β-Colubrine)	Brown	Violet	Grey	Greyish yellow
3-Methoxystrychnine (α-Colubrine)	Pale violet	Violet	Pale violet	Greyish pink
4-Hydroxy-3-methoxystrychnine	Pale yellow	Yellow	Yellowish green	Chocolate-brown
2,3-Dimethoxystrychnine (Brucine)	Brown-orange	Orange-yellow	Brownish red	Yellow
Diaboline	—	Orange	Light brown	Orange-red
Spermostrychnine	—	Purple-orange	Reddish purple	Greyish red
Strychnospermine	—	Orange-brown	Grey	Pink

40 min. The organic phase was removed and shaken out with N sulphuric acid (50, 50, and 30 ml). After basifying the combined acid extracts with conc. ammonia solution, the alkaloids were taken into chloroform (50, 50, and 30 ml). The combined chloroform extracts were washed with water (15 ml) and dried over anhydrous sodium sulphate. Removal of the solvent gave a residue of 0·750 g. The marc was stood over a second portion of chloroform–ether (1 : 2) (200 ml) for 2 days and then subjected to the same extraction procedure; this gave a further 0·036 g alkaloidal residue. Total yield 0·786 g = 3·36%.

Fractionation. T.l.c. of the crude alkaloids in system 1 indicated the presence of one major spot (R_F 0·84) and two minor ones (R_F 0·80 and 0·87). Repeated crystallization in methanol failed to remove the minor components. The crystals (375 mg) were therefore subjected to preparative t.l.c. in system 2 (run 3 times). The separation was still not complete, but the main band was divided into a front one-third (zone 1) and a rear two-thirds (zone 2) which were scraped off and eluted separately.

Zone 1. The residue from this zone (41 mg) was crystallized in methanol. T.l.c. in system 1 indicated the presence of at least three components. Two of these had the same R_F values and the same colours after spraying as 4-hydroxystrychnine and the new alkaloid 4-hydroxy-3-methoxystrychnine (see below, zone 2). Between the spots for these two components was a third one with an R_F value corresponding to strychnine and/or the colubrines, which in system 1 do not separate well, and with a colour, after spraying, different from those of the pure alkaloids. That this spot was probably a mixture was supported by the g.l.c. which showed a small peak at R_{Stry}^{270} 1·00 corresponding to strychnine and a somewhat larger peak at R_{Stry}^{270} 1·63 corresponding probably to a mixture of 4-hydroxystrychnine and α-colubrine. The mass spectrum of the crystals confirmed the presence of four alkaloids with molecular-ion peaks at *m/e* 380 (4-hydroxy-3-methoxystrychnine), 364 (a colubrine), 350 (4-hydroxystrychnine), and 334 (strychnine).

Zone 2. The residue from this zone was crystallized twice in methanol to give colourless elongated prisms (122 mg) of the new alkaloid *4-hydroxy-3-methoxystrychnine*, m.p. 234–236° (decomp.); R_{Bru}^{270} 1·14; $[\alpha]_D$ −18° (*c* 0·22, in chloroform); found M^+ 380·1725, required for $C_{22}H_{24}N_2O_4$ M^+ 380·1736; λ_{EtOH}^{max} 231, 270, and 301 nm, λ_{EtOH}^{min} 252·5 nm, no change on adding sodium hydroxide solution; ν_{max} (Nujol) 2700–2600 (H-bonding), 1660, 1635 (H-bonded amide C=O), 1603 (phenyl C=C stretching), 1580, 1255 (aromatic OMe), 1110, 1095, and 1045 cm^{-1}; δ 3·78 (3–H; s; aromatic OMe), 5·82 (1–H; ill-defined t; C–22 H), 6·50 (1–H; d, J_o = 9 Hz; C–1 H), 6·66 (1–H; d, J_o = 9 Hz; C–2 H), and 11·84 (1–H; s; disappearing on deuteration; H-bonded phenolic OH); mass spectrum peaks at *m/e* 380 (M^+; 100%), 351 (4), 350 (4), 323 (2), 279 (4), 267 (2), 244 (2), 243 (3), 242 (2), 190 (8), 189 (7), and 176 (9).

The mother-liquors from the original crystallization of the crude alkaloid mixture were subjected to preparative t.l.c. in system 2 (run 4 times) and four bands were removed.

Band 1. This band had an R_F value similar to that of brucine. The residue (83 mg) was shown by t.l.c. in system 1 to comprise at least two components. Renewed preparative t.l.c. in system 2 (run 4 times) afforded two main zones. The *upper zone* gave a residue (33 mg) which consisted chiefly of 4-hydroxy-3-methoxystrychnine (t.l.c.), while the *lower zone* gave material (27 mg) which was a mixture primarily of

brucine with some 4-hydroxy-3-methoxystrychnine and a little 4-hydroxystrychnine (t.l.c., g.l.c., and mass spectrum).

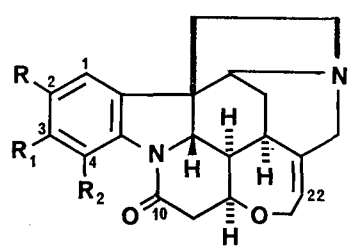
Band 2. The residue (119 mg) from this band, which had an R_F value similar to that of 4-hydroxy-3-methoxystrychnine, was found by t.l.c. to be mainly that alkaloid.

Band 3. This band had an R_F value similar to that of strychnine. Nevertheless, further investigation (t.l.c., g.l.c., and mass spectrum) of the residue (7 mg) showed it to consist mainly of an unidentified base (M^+ 318; R^{270}_{Stry} 0·64; R_F (system 1) 0·73, reddish brown with ferric chloride/perchloric acid) and small amounts of brucine and 4-hydroxy-3-methoxystrychnine.

Band 4. This band ran very near the solvent front. Check t.l.c. in system 4 after elution of the residue (48 mg) indicated that it contained at least three components. The material was therefore subjected to preparative t.l.c. in system 3 (run twice) and three zones were removed. The *most polar zone* gave material (2.1 mg) which proved to be identical with novacine (t.l.c., g.l.c., and mass spectrum). The residue (2·5 mg) from the *least polar zone* was shown by the same means to be vomicine. The *middle zone* gave a residue (14 mg) consisting of the new base *4-hydroxy-3-methoxy*-N-*methyl*-sec.-*pseudostrychnine*, R^{270}_{Bru} 2·36; $[\alpha]_D$+33° (*c* 0·24, in chloroform); λ^{max}_{EtOH} 232, 270, and 301 nm; ν_{max} (KCl disc) 2800–2400 (H-bonding), 1670 (C=O), 1635 (H-bonded amide C=O), 1604 (phenyl C=C stretching), 1580, 1255 (aromatic OMe), and 1115 cm^{-1}; δ 2·02 (3-H; s; *N*-Me), 3·79 (3-H; s; aromatic OMe), 5·95 (1-H; ill-defined t; C-22 H), 6·67 (1-H; d, J_o = 8·5 Hz; C-2 H), 7·21 (1-H; d, J_o = 8·5 Hz; C-1 H), 11·75 (1-H; s; disappearing on deuteration; H-bonded phenolic OH); mass spectrum peaks at *m/e* 410 (M^+; 100%), 395 (7), 394 (19), 352 (14), 351 (M^+ — 59; 45), 336 (14), 335 (14), 334 (8), 297 (3), 294 (5), 281 (13), 266 (7), 258 (6), 257 (7), 256 (7), 255 (9), 254 (6), 242 (11), 240 (8), 229 (10), 214 (11), 205 (12), 191 (17), 190 (11), 189 (12), and 176 (14).

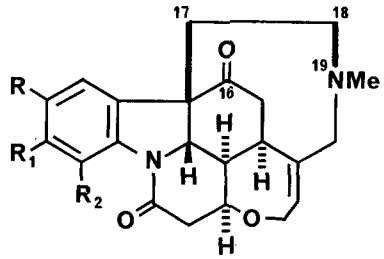
DISCUSSION

4-Hydroxy-3-methoxystrychnine (I). The spectroscopic properties of the new base are very similar to those of strychnine and brucine and it may reasonably be assumed in deriving the structure that it contains the same, or a closely related, ring system. High-resolution mass measurement indicates the molecular formula $C_{22}H_{24}N_2O_4$. Infrared absorption at 1255 cm^{-1} and a 3-proton singlet at δ 3·78 in the nmr spectrum reveal the presence of an aromatic methoxyl group. The infrared band at 1635 cm^{-1} may be assigned to a hydrogen-bonded carbonyl function, while the 1-proton signal at δ 11·84, disappearing on deuteration, points to the occurrence of a hydrogen-bonded phenolic hydroxyl group; presumably it is hydrogen-bonded to the carbonyl. The nmr spectrum also shows that there are two adjacent aromatic protons (AB quartet at δ 6·50 and 6·66, J_o = 9 Hz) and they may be assigned to C-1 and C-2 (cf. henningsoline (III); Biemann, Grossert & others, 1965). This requires that the methoxyl group be attached at C-3 and that the phenolic hydroxyl group be at C-4 where it can hydrogen-bond with the C-10 carbonyl (*cf.* 4-hydroxystrychnine; Sandberg, Roos & others, 1968). Further evidence in favour of the suggested structure is the ill-defined 1-proton triplet at δ 5·82, which evidently belongs to the ethylenic C-22 proton. Moreover, the appearance of the mass spectrum closely resembles that of strychnine (Biemann, 1962) in that it shows an intense molecular-ion peak with little accompanying fragmentation except for the "indole" peaks, which here are at *m/e* 176,



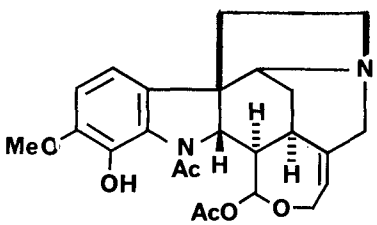
I

Strychnine
$R = R_1 = R_2 = H$
4-Hydroxystrychnine
$R = R_1 = H$, $R_2 = OH$
2-Methoxystrychnine (β-Colubrine)
$R = OMe$, $R_1 = R_2 = H$
3-Methoxystrychnine (α-Colubrine)
$R = H$, $R_1 = OMe$, $R_2 = H$
4-Hydroxy-3-methoxystrychnine
$R = H$, $R_1 = OMe$, $R_2 = OH$
2,3-Dimethoxystrychnine (Brucine)
$R = R_1 = OMe$, $R_2 = H$

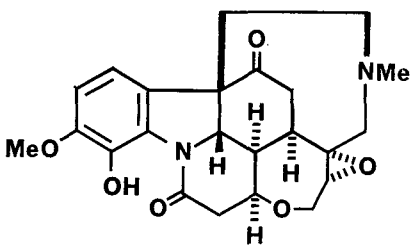


II

N-Methyl-*sec.*-pseudostrychnine (Icajine)
$R = R_1 = R_2 = H$
4-Hydroxy-*N*-methyl-*sec.*-pseudostrychnine (Vomicine)
$R = R_1 = H$, $R_2 = OH$
4-Hydroxy-3-methoxy-*N*-methyl-*sec.*-pseudostrychnine
$R = H$, $R_1 = OMe$, $R_2 = OH$
2,3-Dimethoxy-*N*-methyl-*sec.*-pseudostrychnine (Novacine)
$R = R_1 = OMe$, $R_2 = H$



III



IV

189, and 190 and correspond to the presence of methoxyl and hydroxyl substituents in the aromatic ring. The nature of the second new alkaloid isolated also supports the proposed structure.

*4-Hydroxy-3-methoxy-*N*-methyl-*sec.*-pseudostrychnine* (II). The spectroscopic properties of this compound are very similar to those of vomicine and icajine. The molecular-ion peak in the mass spectrum at *m/e* 410 is in agreement with a molecular formula of $C_{23}H_{26}N_2O_5$. That the substitution pattern in the aromatic ring is the same as in the previous alkaloid is clear from the "indole" peaks in the mass spectrum at *m/e* 176, 189, and 190 and also from the 3-proton singlet at δ 3·79 in the nmr spectrum, corresponding to an aromatic methoxyl group, and the 1-proton singlet at δ 11·75, which disappears on deuteration and which presumably belongs to a hydrogen-bonded phenolic hydroxyl function. In the IR spectrum there is a band at 1635 cm^{-1}, due to the C-10 hydrogen-bonded carbonyl function. Moreover, the nmr spectrum again indicates that the C-1 and C-2 protons are unsubstituted (AB quartet at δ 7·21 and 6·67, $J_o = 8·5$ Hz). The infrared band at 1670 cm^{-1} shows that a second carbonyl function is present and its location at C-16 may be inferred from the deshielding of the C-1 proton [downfield shift from δ 6·50 in (I) to δ 7·21 in (II)] and the presence of a small peak in the mass spectrum at *m/e* 256, belonging to an ion probably containing both carbonyl groups (Bisset, 1968). The 3-proton signal at δ 2·02, for a *N*-methyl group, and the important peak at *m/e* 351 (= $M^+ - 59$), representing loss

of the nitrogen bridge (C-17, C-18, N-19, and Me), demonstrates clearly that the base belongs to the *N*-methyl-*sec.*-pseudo series. An ill-defined 1-proton triplet at δ 5·95 indicates that the C-22 ethylenic proton is intact. All these data find close parallels in the spectra of the related compounds vomicine and icajine (Bisset, 1968).

The 4-hydroxy-3-methoxy substitution pattern is not new to *Strychnos* alkaloids and has been found previously in the base henningsoline (III), isolated from *S. henningsii* Gilg (Grossert, Hugo & others, 1965), and in 21,22-α-epoxy-4-hydroxy-3-methoxy-*N*-methyl-*sec.*-pseudostrychnine (IV), obtained from *S. icaja* Baill. (Bisset, 1968). The latter alkaloid is the epoxide of the new *N*-methyl-*sec.*-pseudo base whose isolation is now reported.

The findings in the present analysis go far to explain the results of the original assay by Short. The brucine was there determined by difference after oxidative destruction with nitric acid through formation of a quinone (Holmes, 1950) and it is evident that the 4-hydroxy-3-methoxystrychnine will also be readily destroyed under like conditions via a quinone of slightly different constitution. As colour reactions for brucine were positive, there would at the time be no reason to suppose that the alkaloid destroyed was not composed mostly of brucine. Similarly, although there appears to be very little strychnine in the seeds, the 4-hydroxystrychnine present would behave in the assay as if it were additional strychnine.

S. cinnamomifolia and its var. *wightii* belong to a group of large climbers which are characterized by long-tubed flowers and large many-seeded fruits and which are currently treated as comprising a single species—*S. wallichiana* Steud. ex DC. (Bisset & Philcox, 1971; Bisset, 1972). The plant occurs in Sri Lanka, south and north-east India, Bangla Desh, North Vietnam, south China, and the Andaman Islands. It includes such well-known forms as *S. colubrina* L., once the source of the best *lignum colubrinum* (snake-wood) (Bisset, 1972), and *S. gauthierana* Pierre ex Dop, a source of the Tonkinese drug *hoang nan*, at one time thought to be a remedy for leprosy and rabies (Bisset & Vidal, 1965).

During our screening program on Asian *Strychnos* species we have examined several samples of *S. wallichiana* and we have found that 4-hydroxy-3-methoxystrychnine occurs in materials from Sri Lanka and south India but not in those from Bangla Desh, Indo-China, and the Andaman Islands. It seems likely that the Sri Lanka *S. cinnamomifolia* which was analysed by Greenish (in Hill, 1917) and in which he found 2·07% brucine and a small percentage of strychnine also probably contained a notable percentage of 4-hydroxy-3-methoxystrychnine rather than just brucine.

For 4-hydroxy-3-methoxystrychnine the mean convulsive dose (CD50) and the mean lethal dose (LD50) in the mouse on subcutaneous injection have been determined by the method of Sandberg & Kristianson (1970) as about 9·0 and 9·8 mg kg^{-1}, respectively; these figures are not statistically different from each other. Tonic convulsions occur.

The introduction of 2- and/or 3-methoxy substituents into strychnine has the effect of increasing t.l.c. adsorption and g.l.c. retention (Phillipson & Bisset, 1970; Bisset, Dejestret, & Fouché, 1969) and of decreasing toxicity (Sandberg & Kristianson, 1970). In contrast, a 4-hydroxy substituent leads to a decrease in t.l.c. adsorption and an increase in g.l.c. retention, but to little change in toxicity. It is interesting to note therefore that the combination of 3-methoxy and 4-hydroxy substituents, as in the new alkaloid, brings about changes similar to those due to methoxy substitution alone.

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