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PROTOSTRYCHNINE, A NEW ALKALOID FROM *STRYCHNOS NUX-VOMICA*

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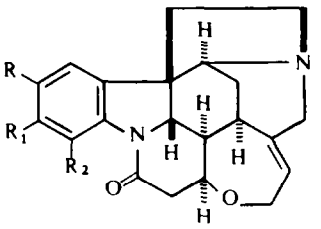
Key Word Index—*Strychnos nux-vomica*; Loganiaceae; indole alkaloids; protostrychnine; prestrychnine; biosynthesis.

Abstract—The tertiary bases from a sample of *Strychnos nux-vomica* contain, as well as the expected strychnine and brucine, an unusually high proportion of 4-hydroxy and 4-hydroxy-3-methoxy compounds. The biosynthetic implications of the isolation of a new alkaloid, 12β,13α-dihydro-12α-hydroxyisostrychnine, named protostrychnine, are discussed.

INTRODUCTION

The major tertiary alkaloids of *Strychnos nux-vomica* L. have usually been found to be strychnine (**1a**) and its 2,3-dimethoxy analogue brucine (**1c**) [1]. The biosynthesis of these two bases has been investigated most recently by Heimberger and Scott [2] who have demonstrated the existence of an aldol-acid (**2a**) as a precursor of strychnine.

Studies in our laboratories on material of Sri Lankan origin allowed isolation from the minor bases of a compound the structure of which represents one stage further in the biosynthesis of strychnine. This substance may thus be considered as the immediate precursor of strychnine and its isolation strongly supports the late stages of the biosynthetic pathway proposed by Heimberger and Scott [2].



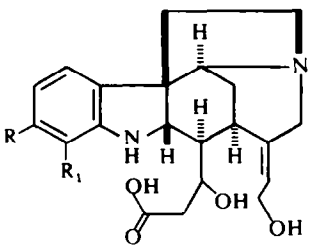
1a R = R_1 = R_2 = H
1b R = R_1 = H; R_2 = OH
1c R = R_1 = OMe; R_2 = H
1d R = H, R_1 = OMe; R_2 = OH

DISCUSSION

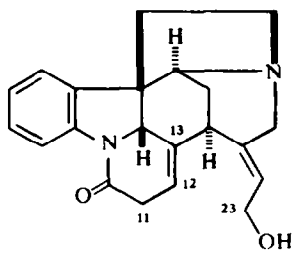
In the present work, the major tertiary alkaloids present in a root-bark extract of *S. nux-vomica* from Sri Lanka have been isolated and identified, in decreasing order of concentration, as: strychnine (**1a**), 4-hydroxy-3-methoxystrychnine (**1d**), 4-hydroxystrychnine (**1b**)*, brucine (**1c**), isostrychnine I (**3**) and normacusine B (**4**).

Among the minor bases is one, isolated in 0.1% yield, the MS of which has a M^+ corresponding with $C_{21}H_{24}N_2O_3$. The UV spectrum is almost superimposable on that of strychnine, while in the IR spectrum there are bands at 1660 and 1645 cm^{-1} due to a lactam carbonyl group and a broad band at 3350 cm^{-1} due to OH. The PMR spectrum is similar to that of isostrychnine I (**3**) but lacks the signal for the vinyl hydrogen on C-12. It shows, however, the presence of two OH groups by the occurrence of two 1H singlets at δ 2.05 and 1.98, both of which disappear on deuteration. In support of this, a diacetate is formed on acetylation. A doublet of doublets (J = 7 and 2 Hz) at δ 4.13 may be compared with a similar signal at δ 4.25 in the spectrum of isostrychine I and belongs to the methylene hydrogens of

* As much as one-third of the alkaloid mixture consisted of **1d**, not previously known as occurring in *S. nux-vomica*, and **1b**. This suggests that the plant from which the root-bark came, although morphologically indistinguishable from *S. nux-vomica*, could perhaps have been a hybrid between it and *S. wallichiana* Steud. ex DC. [3].



2a R = R_1 = H
2b R = OMe; R_1 = OH



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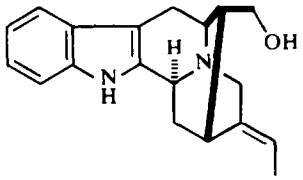

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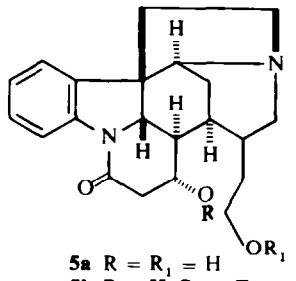

5a R = R_1 = H
5b R = H; R_1 = Tg
5c R = R_1 = Ac

the primary OH function at C-23. A signal at δ 3.92 is assigned to the methine hydrogen of a secondary OH group, placed at C-12 on the basis of the following considerations. If the location indicated is correct, dehydration should afford isostrychnine I or its Δ^{11}-isomer isostrychnine II and subsequent cyclisation should provide strychnine (**1a**). Indeed, after protection of the primary OH group as the tiglate (**5b**) [4, 5] and allowing the material to react with $POCl_3$ in pyridine, hydrolysis with 5% KOH in MeOH and treatment with 2N HCl in MeOH gave strychnine (**1a**) as one of the the reaction products. The only structure compatible with these findings is that of 12,13-dihydro-12-hydroxy-isostrychnine. The H-12 signal is a triplet (J = 9 Hz), which accords only with the presence of 12β- and 13α-hydrogens and a 12α-OH; supporting this is the cyclization to strychnine. The alkaloid is thus 12β,13α-dihydro-12α-hydroxy-isostrychnine (**5a**) and for it we propose the trivial name protostrychnine, in order to distinguish it from the prestrychnine of Heimberger and Scott [2, 6].

Root bark from *S. nux-vomica* cultivated in Java and exhibiting a more usual alkaloid composition, i.e. with strychnine and brucine as the principal bases [1], was also found to contain protostrychnine [7].

To determine whether protostrychnine might be an artefact generated from a strychnic acid derivative, such as prestrychnine (**2a**), by the action of the acid used in the isolation, some Sri Lankan root bark was extracted with alkaline EtOH, the solvent removed under reduced pressure and the basic solution extracted into $CHCl_3$. This normal isolation procedure gave 10.01% tertiary alkaloids, which included protostrychnine. The remaining aqueous solution was acidified with HCl, heated at 55° for 2 hr, cooled, neutralized with $NaHCO_3$ and extracted again with $CHCl_3$. This yielded only 0.04% additional alkaloids which included traces of strychnine (**1a**) and protostrychnine (**5a**) but also a slightly larger amount of 4-hydroxy-3-methoxystrychnine (**1d**). Evidently, the greater part of the protostrychnine existed as such in the plant material.

The isolation of protostrychnine has obvious biosynthetic implications for the *Strychnos* alkaloids. It could be regarded as an immediate precursor of strychnine, especially since it has been isolated from the root bark, the known site of alkaloid biosynthesis in *S. nux-vomica*, and its isolation furnishes additional evidence for the final stages of strychnine biosynthesis as envisaged by Heimberger and Scott [2, 6]. However, Heimberger's suggestion that true biosynthetic intermediates are so rapidly turned over as to be undetectable by isolation techniques [6] may mean that **5a** is rather the end product of a shunt pathway. In contrast with the findings of Heimberger [6], the fact that in the present work only very little extra strychnine was formed by the acid treatment implies that prestrychnine (**2a**), if present at all, must be in very low concentration. This could be because seedlings were used in the labelling experiments [6] whereas our material was from a well-developed tree. Nevertheless, because of the extra 4-hydroxy-3-methoxystrychnine (**1d**) obtained from the acid treatment, some *ar*-substituted non-cyclized compounds (e.g. **2b**) must also be present. It may well be that at the end of the biosynthetic pathway thare is a metabolic grid. On the one hand, oxidation of **2a** can lead to the formation of various *ar*-substituted prestrychnines which can then undergo lactamization and further cyclization; on the other hand, initial lactamization of **2a** can yield **5a** which can later be oxidized to *ar*-substituted protostrychnines and, by final ring closure, to the corresponding strychnines (e.g. **1a–d**).

EXPERIMENTAL

PMR spectra were recorded in $CDCl_3$ at 90 MHz. MS were determined at high resolution using a direct inlet system and operating at 70 eV. Root bark of *S. nux-vomica* was collected

in Gampaha, Sri Lanka, in 1973.

The dried ground root bark (79.6 g) was macerated with 1% aq. HOAc for 2 days, filtered, remacerated for a 4 further days and filtered again. The combined filtrates were acidified to pH 1 and treated with excess Mayer's reagent. The ppt. was collected by filtration, dissolved in Me_2CO–MeOH–H_2O (6:2:1) and passed through a column of Amberlite IRA 400 (Cl^- form). The eluate was neutralized with NH_4OH, evapd under red. pres. to remove Me_2CO and MeOH and then extracted with $CHCl_3$ (50 × 100 ml). The combined, dried $CHCl_3$ layers were evapd to dryness to yield the crude tertiary alkaloids (8.3 g, 10.4%).

Part of the crude bases (6 g) was roughly fractionated on a column of Al_2O_3 (activity III) by elution with C_6H_6 then MeOH. The latter fraction (3.1 g) was further chromatographed on Al_2O_3 with C_6H_6 containing increasing concns of $CHCl_3$ as solvent. From the early fractions strychnine (**1a**) (*ca* 26%), 4-hydroxy-3-methoxystrychnine (**1d**) (*ca* 18%), 4-hydroxystrychnine (**1b**) (*ca* 9%), brucine (**1c**) (*ca* 9%), isostrychnine I (**3**) (*ca* 0.8%) and normacusine B (**4**) (*ca* 0.5%) were isolated, all identical in every respect with authentic specimens. Elution of the column with $CHCl_3$–MeOH (1:1) gave a yellow oil which on prep-TLC (Si gel layers, 1 mm thick, EtOAc–*iso*-PrOH–conc NH_4OH (16:3:1) as solvent, 8 developments) yielded an amorphous buff coloured solid (58.5 mg; 0.1%) of 12β-13α-dihydro-12α-hydroxyisostrychnine (**5a**), mp 201° (decomp): $[\alpha]_D^{20}$ = +5° (MeOH; *c*, 0.39); λ_{max}^{MeOH} 290, 280, 255 nm: ν_{max}^{Nujol} 3350, 1660, 1645, 1595 cm^{-1}; PMR δ8 (1H, *br d*, H-4), 7.35–7.1 (3H, *m*, H-3, H-2, H-1), 5.8 (1H, *t*, *J* = 7 Hz, H-22), 4.13 (2H, *dd*, *J* = 7 Hz, 2 Hz, H-23α, H-23β), 3.92 (1H, *t*, *J* = 9 Hz, H-12), 2.05 (1H, *s*, OH, disappears with D_2O), 1.98 (1H, *s*, OH, disappears with D_2O); MS *m/e* (rel. int.) 352 (40), 335 (39), 334 (73), 322 (27), 321 (100), 317 (3) (m* 352 → 334), 308 (9), 269 (7), 180 (29), 168 (12), 167 (13), 144 (37), 143 (21), 139 (62), 138 (29), 130 (42), 122 (41), 119 (34), 115 (13). Accurate mass measurements: Found: 352.1781, 334.1673, 321.1593 and 180.1015. $C_{21}H_{24}N_2O_3$, $C_{21}H_{22}N_2O_2$, $C_{20}H_{21}N_2O_2$ and $C_{10}H_{14}NO_2$ require: 352.1787, 334.1681, 321.1603 and 180.1025.

Acetylation. Treatment with Ac_2O and C_5H_5N in the usual way yielded 12α,23-diacetoxy-12β,13α-dihydro-isostrychnine (**5c**) as a pale cream solid, λ_{max}^{MeOH} 290, 280, 254 nm: ν_{max}^{Nujol} 3500, 1730, 1665, 1600 cm^{-1}; PMR δ8.05 (1H, *br d*, H-4), 7.40–7 (3H, *m*, H-3, H-2, H-1), 5.7 (1H, *t*, *J* = 7 Hz, H-22), 4.85 (1H, *t*, *J* = 9 Hz, H-12), 4.46 (2H, *dd J* = 7 Hz, 14 Hz), 2.03 (3H, *s*, acetate), 2.01 (3H, *s*, acetate); MS *m/e* (rel. int.) 436 (11), 393 (7), 376 (100), 364 (6), 350 (2), 333 (43). 324 (1) (m* 436 → 376), 317 (15), 303 (2), 295 (2) (m* 376 → 333), 275 (2), 267 (1) (m* 376 → 317), 264 (2), 263 (1), 262 (1), 261 (2), 260 (2), 234 (2), 220 (9), 210 (11), 196 (4), 183 (8), 181 (9), 180 (7), 179 (6), 178 (7) 158 (8), 144 (26), 143 (9), 130 (16), 122 (28), 120 (15), 119 (46), 115 (7). Accurate mass measurements: Found: 436.2008, 393.1793 and 376.1783. $C_{25}H_{28}N_2O_5$, $C_{23}H_{25}N_2O_4$ and $C_{23}H_{24}N_2O_3$ require: 436.1998, 393.1814 and 376.1787.

Synthesis of strychnine from **5a**. **5a** (18 mg) was dissolved in C_5H_5N and treated with tigloyl chloride in the normal way for 2 days. Usual work up gave a yellow oil which was redissolved in C_5H_5N, 3 drops of 10% $POCl_3$ in C_5H_5N added and the mixture left at room temp for 2.5 hr. Usual work up gave another yellow oil which was treated with 5% KOH in MeOH for 2 hr and then the soln was acidified with 2N HCl and allowed to stand for 1 hr at room temp. The crude product obtained by basification and extraction into $CHCl_3$ was subjected to prep-TLC as before. This allowed isolation of two main products—strychnine (**1a**) and isostrychnine I (**3**), both identical in every respect with authentic specimens.

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