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Note

Strychnos **alkaloids**

XXVII*. Separation and characterisation of isostrychnine in *Strychnos nux vomica* L. seeds

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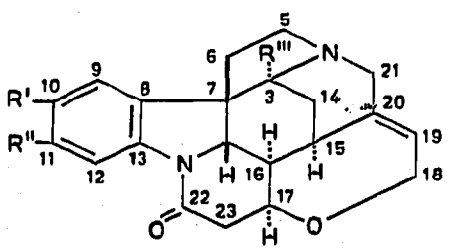
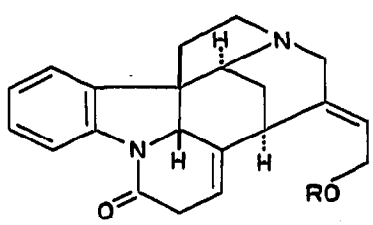
In a previous paper[1], we described the separation, from *Strychnos vomica* L. seed extract and from mother liquors obtained from crystallisation of strychnine sulphate, of nine known alkaloids (strychnine, α- and β-colubrines, brucine, pseudostrychnine, pseudobrucine, icajine, vomicine and novacine) and of four new alkaloids. The separation was obtained by counter-current distribution (CCD) using chloroform as the stationary phase and an aqueous buffered mobile phase. The pH of the buffer was varied discontinuously from neutral to acidic in order to extract the alkaloids with decreasing $K_r \cdot K_b$ values. (K_r is the partition coefficient, K_b is the dissociation constant).

Two of the new alkaloids were identified as 3-hydroxy-α-colubrine (I) and 3-hydroxy-β-colubrine (II) (or, according to the new IUPAC nomenclature, 16-hydroxy-α-colubrine and 16-hydroxy-β-colubrine), and their synthesis from α-colubrine and β-colubrine confirmed both of these structures[2].

The third alkaloid has now been identified as isostrychnine (III). This substance was first obtained synthetically by Leuchs and Schulte[3] in 1942 by treating strychnine (IV) with alkali. Recently, Heimberger and Scott[4] have reported the finding of isostrychnine in the roots of young seedlings of *S. nux vomica*. Isostrychnine from *S. nux*





		R'	R''	R'''
I	3-hydroxy-α-colubrine	H	OCH_3	OH
II	3-hydroxy-β-colubrine	OCH_3	H	OH
IV	strychnine	H	H	H

		R
III	isostrychnine	H
V	O-acetyl-isostrychnine	Ac

* Part XXVI: *Gazz. Chim. Ital.*, 103 (1973) 591.

vomica seeds, reported in our previous paper[1] as X_2, was distributed in a Craig Model Post CCD apparatus (200 stages, volume of both upper and lower phase 10 ml). Chloroform was used as the lower phase and phosphate buffer (pH 6.58) as the upper mobile phase.

The distribution curve obtained is shown in Fig. 1. The product $K_r \cdot K_b$ ($8.0 \cdot 10^{-9}$) was calculated from the pH value, the number of transfers ($n = 200$) and the position of the maximum of the curve ($r = 46$). K_r is $9.4 \cdot 10^{-2}$ as pK_b is 7.07 (ref. 5).

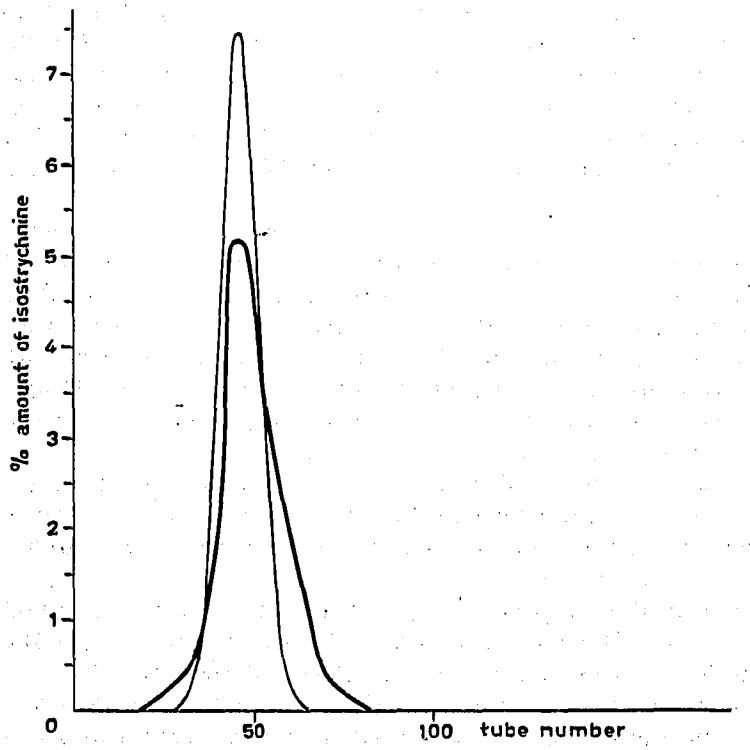


Fig. 1. Experimental (———) and theoretical (———) distribution curves of isostrychine between chloroform and buffer (pH 6.58) after 200 transfers.

The partition of a basic substance between a mobile aqueous and a stationary organic phase follows the equation[6]:

$$\log \frac{r}{n-r} = -\mathrm{pH} + \log \frac{K_b \cdot K_r}{K_w}$$

The theoretical distribution curve is also shown in Fig. 1 and was obtained by using the Laplace approximation, which permits the calculation of the percentage of substance, P_x, in every tube at a distance x from the maximum:

$$P_x = \frac{e^{-\frac{x^2}{2nms}}}{\sqrt{2\pi nms}}$$

where s and m are the solute fractions in the stationary and mobile phase, respectively.

The substance obtained after repeated extraction of the basified aqueous phase with chloroform was recrystallized from ethyl acetate as needles (m.p. 225–228°). The UV spectrum (ethanol) showed maxima at 294 nm (log $\varepsilon = 3.50$), 285 nm (log $\varepsilon = 3.60$) and 256 nm (log $\varepsilon = 4.05$), characteristic of an unsubstituted dihydroindole.

The IR spectrum of the compound dissolved in chloroform showed a broad band at 3450 cm^{-1} corresponding to the hydroxy stretching frequency; a further band at 1660 cm^{-1} could be attributed to the α-piperidone carbonyl stretching frequency.

In the mass spectrum, the molecular ion appeared at m/e 334 (86% of the base peak), which on the basis of elemental analysis corresponds to the empirical formula $C_{21}H_{22}N_2O_2$. The base peak at m/e 316, whose metastable peak m^* is at 300, corresponds to the elimination of a water molecule from an alcoholic function, which is assumed to be primary because of the presence of a peak at m/e 303 ($M^+ - 31$, 58%). The characteristic peaks of the indole fragmentation at m/e 144 (24%), 143 (11%) and 130 (18%) were also present.

Some nuclear magnetic resonance data (deuterochloroform; tetramethylsilane) of the substance in comparison with the isomeric strychnine are reported. The H-12 proton, for the deshielding of the α-piperidone carbonyl, resonates at 8.15 ppm, $J =$ 2 and 8 Hz (8.08 in strychnine). The olefinic proton H-19 is a perfect triplet at 5.60 ppm, $J = 6$ Hz, coupled with two protons of CH_2-19, which are a perfect doublet at 4.26 ppm. In strychnine, H-19 is a broadened triplet at 5.88, $J = 6$ Hz and CH_2-19 a double quartet at 4.09 ppm. Furthermore, an olefinic proton appears at 5.85 ppm (multiplet), which is absent in strychnine.

The substance was acetylated with pyridine in acetic anhydride and gave O-acetylisostrychnine (V), which was purified by CCD between chloroform and a buffer of pH 4.08 and was isolated as an oil identical to the synthetic specimen[7]. The calculated $K_a \cdot K_b$ product was $1.5 \cdot 10^{-11}$.

The above results suggest that the oxepinic ring present in strychnine is cleaved at the ethereal function. An open structure is thus obtained with the group $=CH-CH_2OH$ at one end and a double bond between C-16 and C-17 at the other.

In all other respects, the molecule is identical with strychnine. Further confirmation of this structure was obtained by direct comparison with an authentic specimen of isostrychnine prepared from strychnine[3] (mixed melting-point, IR and UV spectra). Isostrychnine is therefore the third new alkaloid obtained from *Strychnos nux vomica* extracts by CCD with the proposed technique using as the mobile phase a buffer with a discontinuously decreasing pH[1].

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