

ALKALOIDS AND IRIDOIDS FROM *STRYCHNOS NUX-VOMICA* FRUITS

N G BISSET and A K CHOUDHURY*

Pharmacognosy Research Laboratories, Department of Pharmacy, Chelsea College, University of London, Manresa Road, London SW3 6LX

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Key Word Index—*Strychnos nux-vomica*, Loganiaceae, indole alkaloids, 4-hydroxystrychnine, *N*-methyl-*sec*-pseudo- β -colubrine, iridoids, ketologanin, secologanin, cantleyine

Abstract—The alkaloid mixtures present in the fruit pericarp and fruit pulp of *Strychnos nux-vomica* L. are qualitatively very similar to that found in the seeds. In addition to alkaloids previously known to occur in this plant, small amounts of 4-hydroxystrychnine and the new base *N*-methyl-*sec*-pseudo- β -colubrine have been isolated. Cantleyine, which is a non-indolic base and an artefact, has also been obtained. The iridoid mixture in the fruit pulp is predominantly loganin with small amounts of related compounds, including the biogenetically important secologanin.

INTRODUCTION

STRYCHNINE (1a) and brucine (1e) have been reported to occur in the pericarp of *Strychnos nux-vomica* fruits, and a later TLC examination has been interpreted as suggesting that the colubrines (1c) and (1d), pseudostrychnine (1f), vomicine (2b), and strychnicine are also present^{1,2}. There is evidence that strychnine and brucine are present in the pulp of the fruits^{1,3}. We have carried out a more detailed analysis of the mixtures of alkaloids in these two materials in order to enable a comparison to be made with that found in the seeds. The iridoids of the fruit pulp have also been examined.

RESULTS AND DISCUSSION

Table 1 lists the alkaloids isolated and identified. Of these, three are new for *S. nux-vomica*: cantleyine (3), a non-indolic base which is an artefact (see below) and which has been obtained from an unidentified *Jasminum* species (Oleaceae) and from *Cantleya corniculata* (Becc.) Howard (Icacinaceae),⁴ 4-hydroxystrychnine (1b), previously isolated from the root bark of *S. icaia* Baill.,⁵ and *N*-methyl-*sec*-pseudo- β -colubrine (2c). This last base has not previously been obtained and its structure determination is therefore discussed first.

* 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 BOORSMA, W. G. (1902) *Meded. 's Lands Plantentuin (Batavia)* **52**, 11, (1902) *Bull. Inst. Bot. Buitenzorg* **14** (Pharmacologie I), 3.

² HIFNY SABER, A., ZAKI, A. Y. and ABD-EL-WAHAB, S. M. (1966) *Egypt Pharm. Bull.* **48**, 203.

³ FLUCKIGER, F. A. and HANBURY, D. (1874) *Pharmacographia*, 1st Edn, p. 384, Macmillan, London; DUNSTAN, W. R. and SHORT, F. W. (1883/84) *Pharm. J. [iii]* **14**, 1025.

⁴ HART, N. K., JOHNS, S. R. and LAMBERTON, J. A. (1969) *Australian J. Chem.* **22**, 1283; SEVENET, T., DAS, B. C., PARELLO, J. and POTIER, P. (1970) *Bull. Soc. Chim. Fr.* 3120.

⁵ SANDBERG, F., ROOS, K., RYRBERG, K. J. and KRISTIANSON, K. (1968) *Tetrahedron Letters* 6217, (1969) *Acta Pharm. Suec.* **6**, 103.

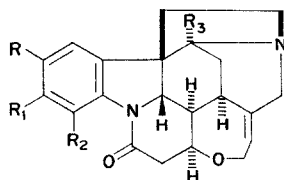
TABLE 1 ALKALOIDS FROM THE FRUIT PERICARP AND FRUIT PULP OF *Strachnos nux-tomica*

Alkaloid	Pericarp (%)	Pulp (%)	Alkaloid	Pericarp (%)	Pulp (%)
Strychnine (1a)	0.169*	0.040†	Brucine N-oxide	0.016*	0.009†
4-Hydroxystrychnine (1b)	0.0007	0.002	Icajine (2a)	0.006	0.002
β -Colubrine (1c)	0.004	—	Vomicine (2b)	0.091	0.013
Brucine (1e)	0.065*	0.030†	N-Methyl- <i>sec</i> -pseudo- β -colubrine (2c)	0.0007	
Pseudostrychnine (1f)	0.002	0.003	Novacine (2d)	0.020	0.018
Pseudobrucine (1g)	0.008	0.003	Cantleyne (3)	0.122	0.036
Strychnine N-oxide	0.005*	0.002†			

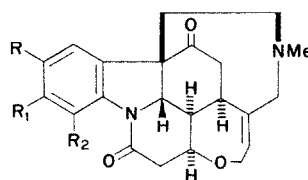
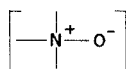
* + an additional 0.092% comprising mainly strychnine and brucine with a little strychnine N-oxide and brucine N-oxide

† + an additional 0.105% comprising strychnine and brucine and their N-oxides

The facts that **2c** came from the icajine/novacine fraction and that on TLC it runs between these two alkaloids suggests that it could be an *av*-monomethoxyicajine.⁶ Its MS has the molecular ion peak at m/e 394, consistent with the formula $C_{23}H_{26}N_2O_4$. 'Indole' peaks at m/e 160, 173 and 174 indicate the presence of an OMe substituent in the aromatic part of the indole moiety, while ions at m/e 240 and 335 ($M^+ - 59$) show that both C-10 and C-16 carbonyl functions are present, i.e. that the base belongs to the N-methyl-*sec*-pseudo series.⁷ The UV spectrum is very similar to that of β -colubrine (**1c**)⁸ and the new base is therefore formulated as N-methyl-*sec*-pseudo- β -colubrine (**2c**).



(1)



(2)

- (1a) R = R₁ = R₂ = R₃ = H
 (1b) R = R₁ = H, R₂ = OH, R₃ = H
 (1c) R = OMe, R₁ = R₂ = R₃ = H
 (1d) R = H, R₁ = OMe, R₂ = R₃ = H
 (1e) R = R₁ = OMe, R₂ = R₃ = H
 (1f) R = R₁ = R₂ = H, R₃ = OH
 (1g) R = R₁ = OMe, R₂ = H, R₃ = OH

- (2a) R = R₁ = R₂ = H
 (2b) R = R₁ = H, R₂ = OH
 (2c) R = OMe, R₁ = R₂ = H
 (2d) R = R₁ = OMe, R₂ = H

The fruit pericarp yielded 1.1% crude alkaloids, including *ca.* 0.19% strychnine, these figures are very much higher than the 0.24 and 0.016% previously reported.^{* 2} The compo-

* Some of the alkaloid identifications given in this reference (*loc. cit.* p. 209, Fig. V) are questionable. According to the TLC shown (in order of decreasing R_f s) in the system EtOAc: iPrOH: NH₄OH (9:7:4) while bases A, B and C may be identified as vomicine, strychnine, and brucine respectively, base D is unlikely to have been pseudostrychnine, which should run near the solvent front rather than behind brucine as indicated. On the other hand, the TLC suggests that the unidentified, somewhat more polar, bases F and G were probably strychnine and brucine N-oxides, however, these bases E and F were not observed to be present in the extract from the pericarp. The TLC also indicates that the highly polar base G was perhaps strychnine and/or brucine chloro-methobromide⁹ rather than strychnine¹ which was probably vomicine.¹⁰

¹ PHILLIPSON, J. D. and BISSET, N. G. (1970) *J. Chromatog.* **48**, 493.

² BISSET, N. G., DAS, B. C. and PARILLO, I. (1974) *Tetrahedron* in press.

³ RAYMOND-HAMPT, (1950) *Ann. Pharm. Fr.* **8**, 482. BOSTON, I. (1951) *J. Pharm. Belg.* [n.s.] **6**, 243.

⁴ PHILLIPSON, J. D. and BISSET, N. G. (1972) *Phytochemistry* **11**, 2547.

⁵ BISSET, N. G. and PHILLIPSON, J. D. (1973) *Phytochemistry* **12**, 2049.

sition of the alkaloid mixture (Table 1) is very much like that present in the seeds,¹¹ with strychnine (1a) and brucine (1e), bases of the normal series, as the dominant compounds

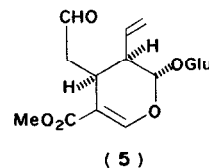
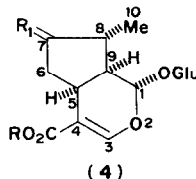
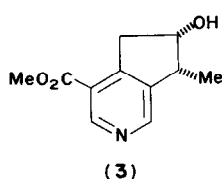
The fruit pulp gave 0.35% crude alkaloids and again the composition of the mixture (Table 1) closely resembles that from the seeds

The isolation of cantleyine is unexpected but not surprising. Sevenet *et al.*⁴ showed that it is formed when NH_4OH is used in the work-up, they were able to convert the iridoid loganin (4a) (see below) to cantleyine (3) in ca 5% yield. We find that when extracting 3 from the pericarp of *S. nux-vomica* fruits or making it from 4a,* use of Na_2CO_3 in the first basification and Na_2CO_3 or NH_4OH in the second one gave only little or no 3. Using NH_4OH in the first basification and Na_2CO_3 or NH_4OH in the second one afforded ca 5% of 3. The inference from these experiments is that in the formation of 3 from 4a introduction of the nitrogen (from the NH_4OH) into 4a probably takes place before hydrolysis, rather than first hydrolysis and then reaction with NH_4OH . Although the yield of 3 directly from 4a is very low, the amount of 4a present in *S. nux-vomica* fruit pulp and fruit pericarp is sufficient to account for the quantity of 3 isolated (Table 1).

TABLE 2. IRIDOIDS ISOLATED FROM THE FRUIT PULP OF *Strychnos nux-vomica*

Iridoid	% Obtained	Iridoid	% Obtained
Loganin (4a)	10.554	Ketologanin (4c)	0.046
Loganic acid (4b) ¹³	0.021	Secologanin (5)	0.005
Deoxyloganin (4d) ¹⁴	0.013		

Ca 3.4% loganin (4a) was obtained from the fruit pericarp. The fruit pulp, on the other hand, yielded ca 12.1% crude total iridoids, which after crystallization afforded ca 10.6% of 4a.¹² TLC of the mother-liquors showed that several other iridoids were present, and



(4a) R = Me, R₁ = OH, H

(4b) R = H, R₁ = OH, H

(4c) R = Me, R₁ = O

(4d) R = Me, R₁ = H, H

the identified ones obtained are listed in Table 2. Noteworthy is the overwhelming predominance of 4a. Ketologanin (4c)¹⁵ and secologanin (5)¹⁶ have not previously been found

* The plant material or aq. soln of loganin was basified with either Na_2CO_3 soln or NH_4OH soln. After extraction with an organic solvent the basic fraction was taken into acid which was then basified with either Na_2CO_3 soln or NH_4OH soln and worked up in the usual way.

¹¹ MARINI-BETTOLO, G. B., MONACHE, F., DELLE, G., ELABERT DE BROVETTO, A. and CORIO, E. (1968) *J. Assoc. Off. Anal. Chem.* **51**, 185, (1968) *J. Chromatog.* **32**, 178.

¹² DUNSTAN, W. R. and SHORT, F. W. (1983/84) *Pharm. J. [iii]* **14**, 1025, MERZ, K. W. and KREBS, K. G. (1937) *Arch. Pharm.* **275**, 217, MERZ, K. W. and LEHMANN, L. H. (1957) *Arch. Pharm.* **298**, 543.

¹³ JAMINET, F. (1951) *Lejeunia* **15**, 9, (1953) *J. Pharm. Belg. [n.s.]* **8**, 339-449.

¹⁴ BATTERSBY, A. R., BURNETT, A. R. and PARSONS, P. G. (1970) *J. Chem. Soc. Chem. Commun.* 826.

¹⁵ COSCIA, C. J., GUARNACCIA, R. and BOTTA, L. (1969) *Biochemistry* **8**, 5036.

¹⁶ BATTERSBY, A. R., BURNETT, A. R. and PARSONS, P. G. (1968) *J. Chem. Soc. Chem. Commun.* 1281.

in material of *S. nux-vomica*, but the isolation of secologanin is of special interest in view of its important rôle in the biosynthesis of indole alkaloids¹⁷

EXPERIMENTAL

Mp's are uncorrected. NMR spectra were determined in CDCl₃ soln at 60 MHz with TMS as internal standard ($\delta = 0.00$). MS were obtained with an A E I MS902 high-resolution instrument operating at 70 eV and inlet temperatures between 200° and 280° peak intensities are given as % of the largest peak above 100 m.u. which is taken as the base peak.

Alkaloids. TLC was carried out using neutral silica-gel G plates run in the following systems: CH₂Cl₂ or CHCl₃ containing 0–10% MeOH and EtOAc–PrOH–NH₄OH (9:7:4:16:3:1 or 100:2:1). The plates were sprayed with Dragendorff reagent. 1-mm thick preparative TLC plates (20 × 20 or 40 × 20 cm) were made from a 1:1 mixture of silica gel G and GF₂₅₄, they were run in an appropriate system and repeated development was carried out when necessary. GLC was performed as previously described¹⁸.

Iridoids. Routine TLC was done with neutral silica gel G plates in MeCOEt–MeOH–AcOH (3:1:1), MeCOEt–MeOH (4:1 or 7:3) or CH₂Cl₂–MeOH (17:3 or 4:1). The iridoids were detected by spraying with conc. H₂SO₄ or 1% KMnO₄ soln followed by heating to 110°. Preparative TLC plates (see above) were run in MeCOEt–MeOH (93:7:9:1:3:1 or 1:1), CH₂Cl₂–MeOH (97:3 or 94:6) or CHCl₃–MeOH (9:1 or 4:1). repeated development was carried out when necessary.

Source and identification of the plant material. The *S. nux-vomica* fruits were collected at Ambarnagar, Chittagong, Bangladesh, in September 1968 by Mr. Din Mohammad, botanist at the Natural Drugs Research Institute, Chittagong, who also identified the material. Voucher specimens are kept in the Department of Pharmacy, Chelsea College.

Extraction of the alkaloids from the fruit pericarp. 772 g ground pericarp was basified with 390 ml of a 1:1 mixture of 50% conc. NH₄OH and 20% aq. Na₂CO₃ soln. Extraction of the material in a Soxhlet with CHCl₃ followed by removal of the solvent gave 33 g residue which was treated with 2 × 100 and 1 × 50 ml 5% HCl. The combined acid extracts were washed with a little CHCl₃, then basified with conc. NH₄OH and the free bases taken into 3 × 250 and 1 × 200 ml CHCl₃, the combined organic phases were dried over anhyd. Na₂SO₄ and taken to dryness. The yield of crude bases was 8.5 g (= 1.1%). Check TLC showed the presence of at least 9 alkaloids.

Extraction of the alkaloids from the fruit pulp. 803 g wet pulp was basified with 300 ml of a 1:1 mixture of 50% conc. NH₄OH and 20% aq. Na₂CO₃ soln and then allowed to soak in 500 ml EtOH for 1 month, after filtration the pulp was re-extracted twice in the same way. Removal of the EtOH from the combined filtrates under reduced pressure gave a residue which was first refluxed with 3 × 200 ml petrol (b.p. 40–60°) and then extracted by warming with 3 × 120 ml CHCl₃–EtOH (5:1). The combined CHCl₃–EtOH extracts were taken to dryness and the residue treated with 3 × 100 ml CHCl₃. After concentrating the organic extracts to 100 ml, the alkaloids were taken into 2 × 200 and 1 × 100 ml 2% HCl. The combined acid extracts were basified with conc. NH₄OH and the free bases removed with 2 × 300 and 1 × 200 ml CHCl₃, the combined CHCl₃ extracts were dried over anhyd. Na₂SO₄ and taken to dryness. The yield of crude bases was 2.78 g (= 0.35%). Check TLC indicated the presence of 8–9 alkaloids.

Separation of the fruit-pericarp alkaloids. The crude bases were chromatographed over alumina (activity III) and eluted with C₆H₆ containing increasing proportions of CHCl₃, then with CHCl₃ alone and finally with CHCl₃ containing up to 50% MeOH. The fractions were grouped according to the results of check TLC. The smaller groups were further separated by preparative TLC, while the larger groups were first chromatographed over silica gel (eluants as for the alumina column) and then separated by preparative TLC.

Separation of the fruit-pulp alkaloids. The crude alkaloids were fractionated on silica gel (activity I) by elution with CHCl₃ containing from 4% up to 50% MeOH, after monitoring by TLC the fractions were combined into groups which were further separated individually by preparative TLC.

Identification of the alkaloids. Known alkaloids were identified by means of their mp, colour reactions and TLC properties and by comparison of the UV, IR and/or MS with those of authentic samples available in our laboratory: *N-Methyl-sec-pseudo-β-columbine* (2c) needles from Me₂CO, UV $\lambda_{\max}^{\text{EtOH}}$ 226 (log ϵ 4.38), 261 (3.95) and 300 (3.92) nm, $\lambda_{\min}^{\text{EtOH}}$ 244 (log ϵ 4.23) and 277 (3.74) nm, MS 394 (M⁺, C₂₃H₂₆N₂O₄, 100%), 337 (11), 336 (20), 335 (70), 334 (10), 320 (17), 318 (12), 281 (10), 276 (9), 266 (10), 242 (8), 241 (13), 240 (14), 239 (12), 226 (12), 214 (10), 213 (15), 212 (9), 198 (12), 197 (10), 186 (7), 184 (7), 175 (12), 174 (10), 173 (14), 160 (18), 58 (30) and 57 (27).

¹⁷ BATILSBY, A. R. (1971) in *The Alkaloids* (SAXTON, J. E., ed.) Vol. 1, p. 31 (Specialist Periodical Reports). The Chemical Society, London. STAUNTON, J. (1972) in *The Alkaloids* (SAXTON, J. E., ed.) Vol. 2, p. 1 (Specialist Periodical Reports), The Chemical Society, London. HEIMBERGER, S. I. and SCOTT, A. I. (1973) *J. Chem. Soc. Chem. Commun.* 215.

¹⁸ BISSET, N. G. and PHILLIPSON, J. D. (1971) *Lloydia* 34, 1.

Extraction of the fruit-pulp iridoids 600 g air-dried pulp was macerated with 1200 ml EtOH for 1 week and then filtered from the solvent, the process was repeated 3 × Concentration of the combined filtrates yielded 252 g extract, refluxing it with petrol (b p 40–60°) removed 9.4 g material The remaining extract was treated several times with CHCl₃–EtOH (4 : 1) This removed 79.7 g material which was refluxed with several lots of petrol (b p 40–60°) and repeatedly treated with CHCl₃ to leave 53 g final residue consisting mainly of crude loganin (4a) The ca 163 g of material insoluble in CHCl₃–EtOH (4 : 1) was dissolved in H₂O and thoroughly shaken and warmed with charcoal for some time After filtration, the charcoal was extracted several times with EtOH to give a further 19 g loganin The filtrate which still gave a positive loganin test was concentrated under reduced pressure and again treated with charcoal, from which an additional 12 g crude loganin was obtained All three lots of crude loganin were combined and recrystallized several times from EtOH to give ca 60 g pure loganin The residue from the combined mother-liquors of the loganin crystallizations was extracted with petrol (b p 40–60°) and CHCl₃ and check TLC of the final 12.5 g of residue showed the presence of several other iridoids besides loganin

Separation of the fruit-pulp iridoids The 12.5 g iridoids was chromatographed over silica gel, elution did not start until more than 1200 ml CH₂Cl₂–MeOH (24 : 1) had passed through the column and was continued with CH₂Cl₂–MeOH (24 : 1, 9 : 1, 4 : 1 and 1 : 1) More than 560 fractions of 25 ml were collected and ca 11.1 g iridoids recovered After check TLC the fractions were combined into 16 groups which were further separated by preparative TLC

Identification of the iridoids Loganin (4a) was identified as such from the m p and spectral (UV, IR, NMR and MS) properties of the free iridoid and its pentaacetate which were in agreement with those reported^{15, 19}

Loganic acid (4b) was identified by means of the m p and spectral (UV, IR and NMR) properties of the acid and its pentaacetate, which accorded well with published data¹⁵ Methylation of the acid with CH₃N₂ afforded 4a as the only product, identified by m p and UV and IR spectra

Deoxyloganin (4d) was identified from its UV and IR spectra and from the NMR spectrum of its tetraacetate The TLC behaviour and the UV spectrum of the tetraacetate were identical with those of an authentic sample²⁰

Ketologanin (4c) was identified by means of the m p and spectral (UV, IR, NMR and MS) properties of the free iridoid and its tetraacetate, which were identical with those reported in the literature¹⁵

Secologanin (5) was identified from the spectral (UV, IR, NMR and MS) properties of the free iridoid and its tetraacetate²¹ The TLC and spectral properties of the free iridoid were identical with those of an authentic sample

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¹⁹ BATTERSBY, A. R., HALL, E. S. and SOUTHGATE, R. (1969) *J. Chem. Soc. C*, 721

²⁰ INOUE, H., ARAI, T. and MIYOSHI, Y. (1964) *Chem. Pharm. Bull. Japan* **12**, 888

²¹ SOUZU, I. and MITSUHASHI, H. (1970) *Tetrahedron Letters* 191