Phytochemistry, 1972, Vol 11, pp 381 to 384 Pergamon Press Printed in England

SHORT COMMUNICATION

THE OCCURRENCE OF STRYCHNINE AND BRUCINE IN AN AMERICAN SPECIES OF *STRYCHNOS**

G B MARINI-BETTÒLO, M A CIASCA and C GALEFFI
Laboratori di Chimica Biologica, Istituto Superiore di Sanità, Rome, Italy

N G BISSET
Department of Pharmacy, Chelsea College of Science, University of London, Manresa Road, London SW3

and

B A KRUKOFF
c/o New York Botanical Garden, New York

(*Received* 23 *June* 1971)

Abstract—The isolation of strychnine and brucine from seeds of *Strychnos panamensis* Seem from Guatemala, Central America, is reported The alkaloids, 0 1% of each, were found only in those seeds which were mature and capable of germination, immature and overmature seeds were not found to contain either of the two bases This is the first time that strychnine and brucine have been identified with certainty in an American species of *Strychnos* Together with the results of previous work, *S panamensis* has now been shown to contain alkaloids of the strychnine, diaboline, and bis-quaternary types

INTRODUCTION

STRYCHNINE (I) was found together with brucine (II) for the first time in the seeds of the Asian *Strychnos nux-vomica* L. (Loganiaceae)[1] and later also in those of *S. ignatii* Berg.[2] Since then, strychnine and brucine have been shown to be present in other Asian *Strychnos* species, e.g. the seeds and bark of *S gauthierana* Pierre ex Dop (*S pierriana* A. W. Hill)[3,4] and the seeds of *S cinnamomifolia* Thwaites [5]

Strychnine has been found in only one African species, viz in the root bark and branch bark of *S. icaja* Baill [6–9] which seemingly may contain up to about 6% of it, the highest percentage so far known to occur naturally Recently, 4-hydroxystrychnine (III) has also

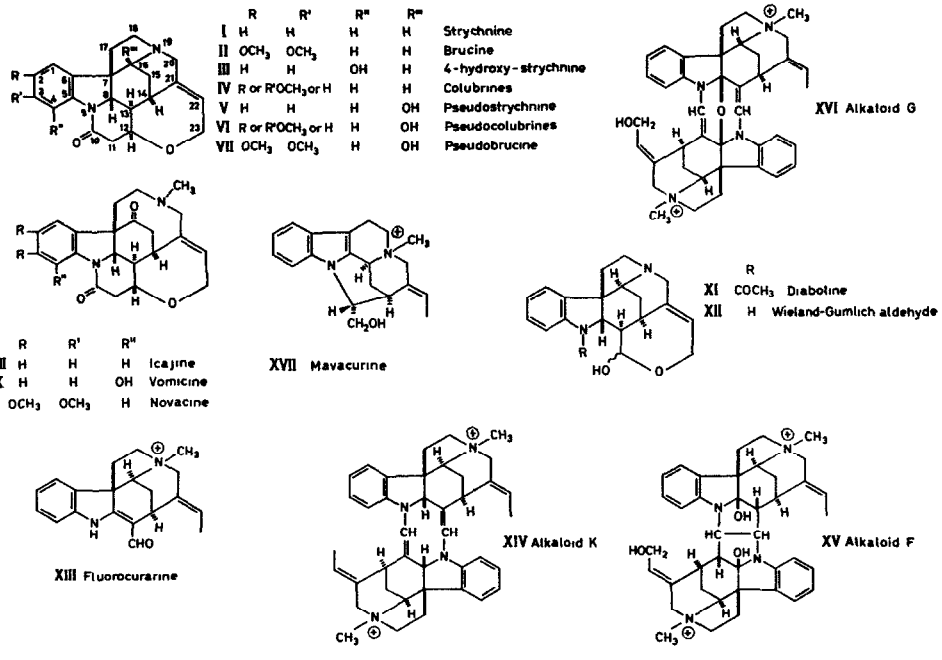
* Part XXII of the series "*Strychnos* alkaloids". For Part XXI see G B MARINI-BETTÒLO, E MIRANDA DELLE MONACHE, C GALEFFI, M A CIASCA RENDINA and A VILLAR DEL FRESNO, *Annali Chim* **60,** 444 (1970) A list of Parts I–XX is to be found in F DELLE MONACHE, E. CORIO, C. ROSSI CARTONI and G B MARINI-BETTÒLO, *Lloydia* **33,** 279 (1970)

[1] J PELLETIER and J B CAVENTOU, *Ann. Chim Phys* **8,** 323 (1818)
[2] J PELLETIER and J B CAVENTOU, *Ann Chim Phys* **10,** 142 (1819)
[3] H G BOIT and L PAUL, *Naturwissenschaften* **47,** 136 (1960)
[4] H T CHANG, YU I TUNG and C C LOU, *Acta Pharm Sinica* **10,** 365 (1963)
[5] A W HILL, *Bull Misc Inf R Bot Gdns Kew* 195 (1917)
[6] F SANDBERG, K ROOS, K J RYRBERG and K KRISTIANSON, *Acta Pharm Suecica* **6,** 103 (1969), *Tetrahedron Letters* 6217 (1968)
[7] A DENOËL, *J Pharm Belg* **5,** 59 (1950)
[8] F JAMINET, *Lejeunia* **15,** 23 (1951)
[9] A DENOËL, F JAMINET, G DETILLEUX, M VAN SUMSEN and L MERVEILLE, *Contribution a l'étude chimique des Strychnos du Congo Belge*, pp 105, 137, 159, Ministère des Colonies, Direction de l'Agriculture, Bruxelles (1953)

been found in the root bark of *S icaja*[6] There is some evidence that brucine may be present in the branch bark of *S cocculoides* Bak and in the root bark of *S spinosa* Lam (*S lokua* A Rich)[9]

Seeds of *S lucida* R Br from Australia have been shown to contain strychnine and brucine[10,11]

Strychnine is always accompanied by other, generally minor, alkaloids of the strychnine series [e g 4-hydroxystrychnine (III), the colubrines (IV), brucine (II)], of the pseudo series [e g pseudostrychnine (V), the pseudocolubrines (VI), pseudobrucine (VII)], and of the *N*-methyl-*sec*-pseudo series [e g icajine (VIII), vomicine (IX), novacine (X)]



Related to strychnine is diaboline (XI) (and deacetyldiaboline (XII) = Wieland-Gumlich aldehyde), which was obtained for the first time from the bark of *S diaboli* Sandw.[12] from Guyana, South America, then from the fruits of *S ignatii* Berg.[13] of Malayan origin, and more recently from the bark of *S henningsii* Gilg[14] from South Africa.

Diaboline (XI) has also been isolated from several *Strychnos* species of Central and South America *S. chlorantha* Prog , *S rondeletioides* Spruce, *S panamensis* Seem , *S jobertiana* Baill , *S solerederi* Gilg, *S castelnaeana* Wedd ,[15] as the major component of the tertiary bases from the stem and root barks of these plants

[10] F A L ANET, G K HUGHES and E RITCHIE, *Austral J Chem* **6,** 58 (1953)
[11] F H SHAW and I S DE LA LANDE, *Austral J Exptl Biol Med Sci* **26,** 199 (1948)
[12] H KING, *J Chem Soc* 955 (1949)
[13] C G CASINOVI, G B MARINI-BETTOLO and N G BISSET, *Nature, Lond* **193,** 1178 (1962)
[14] J S GROSSERT, J M HUGO, M E VON KLEMPERER and F L WARREN, *J Chem Soc* 2812 (1965)
[15] G B MARINI-BETTÒLO, *Il Farmaco, Sci Edn* **25,** 150 (1970)

The only quaternary base known so far from Asian *Strychnos* species is the monomeric C-mavacurine (XVII), isolated from the root bark of *S nux-vomica* cultivated in Guatemala from Indochinese seed [16] This compound has also been found to occur in several South American species *S macrophylla* B Rodr , *S. divaricans* Ducke, *S toxifera* Rob Schomb , *S. amazonica* Kruk , *S froesii* Ducke, *S mitscherlichii* Rich Schomb var. *amapensis* Kruk et Barneby, *S. subcordata* Spruce, *S melinoniana* Baill., *S parvifolia* DC , generally in the stem bark [15]

Although the alkaloid composition of many species of *Strychnos* from South America has been investigated,[15,17] so far only tertiary alkaloids belonging to the diaboline group or quaternary alkaloids[18] have been found, and never alkaloids of all three groups strychnine type, diaboline type, and quaternary type. Strychnine, although closely related structurally to diaboline and thus to the bis-quaternary alkaloids, has therefore been considered characteristic of Asian, African, and Australian *Strychnos* species

We now report on the examination of the seed of *S panamensis* Seem , a species growing from Mexico to Colombia and previously investigated by us [19] The samples, which were collected in Guatemala, were classified as overmature, mature and capable of germination, and immature. Only the mature seeds capable of germination gave an alkaloidal extract, from which we have isolated strychnine (I) and brucine (II)

This isolation of strychnine and brucine from the seeds of *S panamensis* lends some support to an early claim by Graham y Ponz[20] that strychnine and brucine are present in the seeds of *S. tabascana* Sprague et Sandw , a point previously discussed at some length by one of us.[21] This species is closely related to *S panamensis* and the seeds are used by the Mexicans on account of their high toxicity to kill dogs and other animals

The alkaloids were extracted from the mature seeds of *S panamensis* by treatment with acetic acid and then fractionated by counter-current distribution according to the method of Galeffi *et al* [22] Two alkaloids were finally isolated in a pure form and identified through their physico-chemical and chromatographic behaviour as strychnine (I) and brucine (II), respectively The yield was about 0·1 % of each alkaloid

This is the first time that strychnine and brucine have definitely been found in an American species of *Strychnos* and the assumption that strychnine is characteristic of non-American species is no longer correct. Tertiary and quaternary alkaloids were obtained previously from the bark of *S panamensis* [19] While among the tertiary alkaloids diaboline (XI) was characterized, the quaternary bases were found to comprise fluorocurarine (XIII) and alkaloids K (XIV), F (XV), and G (XVI) Hence, this is also the first example of a *Strychnos* species in which strychnine, diaboline, and bis-quaternary alkaloids have been found together, thus supporting a common biogenetic origin for all *Strychnos* alkaloids However, in this connection it must be borne in mind that Schlatter *et al* [23] have shown that

[16] A Guggisberg, M Hesse, H Schmid and P Karrer, *Helv Chim Acta* **49**, 1 (1966)
[17] G B Marini-Bettòlo, *Bull Soc Pharm Nancy* No 80, 5 (1969)
[18] A Pimenta, in *Curare and Curare-like Agents* (edited by D Bovet, F Bovet-Nitti and G B Marini-Bettòlo), p 149, Elsevier, Amsterdam (1959)
[19] R Pellicciari, F delle Monache, N Lozano Reyes, C G Casinovi and G B Marini-Bettòlo *Ann Ist Super Sanita* **2**, 411 (1966)
[20] J Graham y Ponz, *La Farmacia (Mexico)* **3**, 277 (1894)
[21] B A Krukoff, *Mem N Y Bot Gdn* **12 (2)**, 29, 31 (1965)
[22] C Galeffi, M A Ciasca Rendina, E Miranda delle Monache, A Villar del Fresno and G B Marini-Bettòlo, *J Chromatog* **45**, 407 (1969)
[23] Ch Schlatter, E E Waldner, H Schmid, W Maier and D Groger, *Helv Chim. Acta* **52**, 776 (1969)

deacetyldiaboline (Wieland-Gumlich aldehyde) (XII), a theoretically possible common precursor of the strychnine and bis-quaternary alkaloids, is not a precursor in the biogenesis of strychnine in *S nux-vomica*

It is interesting to observe that recently Angenot *et al*[24] have isolated quaternary alkaloids from *S usambarensis* Gilg—a species of tropical Africa, the root bark of which is used in Rwanda for the elaboration of an arrow poison which exhibits curarizing properties. This finding shows that in African *Strychnos* species as well all three types of alkaloids occur

EXPERIMENTAL

Collection The samples of *Strychnos panamensis* seeds were from the collection *Armando Guillen 201*, Municipality of Chicacao, a few kilometres from the railway station Nahualate, Suchitepequez, Guatemala, altitude 500 ft They were identified by one of us (B A K)[21] The seeds were classified as overmature, mature and capable of germination, and immature

Extraction All three types of seeds were submitted to the extraction procedure for both tertiary and quaternary alkaloids, but only the sample indicated as capable of germinating gave a certain amount of extract We report here only the extraction of the mature seeds capable of germination

Seeds (1 kg) were ground and percolated with three lots of 5% HOAc for 24 hr each time The combined percolates were basified with $NaHCO_3$ and extracted twice with $CHCl_3$ The $CHCl_3$ extracts, after being washed with water and dried over anhydrous Na_2SO_4, were evaporated to dryness 0 22 G residue was obtained The alkaline aqueous phase was then brought to pH 2 with 10% aq HCl and a saturated aqueous solution of picric acid added An abundant precipitate formed, which was collected and washed with water The picrate was dissolved in the minimum amount of acetone and the solution was passed through a Dowex 2 × 4 column (Cl^- form) equilibrated with acetone–H_2O (9 1) The same mixture was used for the elution, which was monitored with ceric sulphate reagent After evaporation of the solvent, 5 5 g residue was obtained, comprising the chlorides of the alkaloids

Separation and identification of the alkaloids The chlorides were fractionated by counter-current distribution in a Craig apparatus between EtOAc and H_2O The separation was followed by means of TLC on silica-gel plates using benzene–EtOAc–$NHEt_2$ (7 2 1) After 4000 transfers (recycling), tubes 130–199 contained a homogeneous chloride-free fraction, the residue from which crystallized in EtOH The crystalline product was identified as strychnine from its m m p and IR and NMR spectra

A better separation of the alkaloids was obtained by the partition method at variable pH For this, the alkaloids were distributed between $CHCl_3$ and M/15 phosphate buffer After 400 transfers at pH 7, 500 at pH 6 5, 600 at pH 6, 300 at pH 5 5, and 600 at pH 5, two homogeneous fractions were obtained, in tubes 145–199 and 85–144, respectively The solutions were basified with $NaHCO_3$ and extracted with $CHCl_3$ After evaporation of the solvent, two alkaloids were obtained

The first one, from tubes 145–199, strychnine, crystallized in EtOH and had m p 268°, undepressed on admixture with the authentic base, λ_{max}^{EtOH} 254, 278 (shoulder), and 288 (shoulder) nm, $\nu_{max}^{CHCl_3}$ 1650 (C=O of α-piperidone ring) and 1610 and 1490 cm^{-1} (C=C phenyl), δ^{CDCl_3}* 5 57 (1 H, t, J = 7 Hz, C-22 proton), 7 0–7 3 (3 H, m, C-1, C-2, C-3 protons), 8 08 ppm (1 H, q, J = 8 Hz, J' = 2 Hz, C-4 proton), mass spectrum *m/e* 334 (M^+, base peak), 144, 143, and 130

The second alkaloid, from tubes 85–144, brucine, also crystallized from EtOH and had m p 178°, undepressed on admixture with the authentic base, λ_{max}^{EtOH} 263 and 301 nm, $\nu_{max}^{CHCl_3}$ 1650, 1610, and 1490 cm^{-1} (assignments as for strychnine), δ^{CDCl_3} 3 81 and 3 86 (2 × 3 H, 2 × s, 2 × OCH_3 group), 6 75 (1 H, s, C-1 proton), and 6 75 ppm (1 H, s, C-4 proton), mass spectrum *m/e* 394 (M^+, base peak), 204, 203, and 190

* Relative to TMS as internal standard

[24] L ANGENOT, A DENOËL and M GOFFART, *J Pharm Belg* **25**, 73 (1970)

Key Word Index—Strychnos panamensis, Loganaceae, strychnine, brucine, indole alkaloids