

Short Communication

The separation of the minor alkaloids of *Strychnos nux vomica* L.

In the course of a systematic investigation of the tertiary alkaloids of *Strychnos*¹⁻⁴, we directed our attention to the alkaloids of *S. nux vomica* in order to establish a possible biogenetic relationship between the different alkaloids of the group and to find out whether diaboline, which we have found in many species as a common precursor of strychnine, is also present.

It is well known that *S. nux vomica* seeds contain strychnine and brucine as first shown by PELLETIER AND CAVENTOU⁵. These seeds are the principal source for the industrial extraction of the above-mentioned alkaloids. Later WARNAT⁶ detected in *S. nux vomica* the presence of α - and β -colubrine and of pseudostrychnine, and WIELAND AND OERTEL⁷ that of vomicine.

Novacine was found in 1952 by MARTIN *et al.*⁸ in the mother liquors of the preparation of strychnine.

BISSET⁹, in recent work on *Strychnos icaja*, isolated icajine, N-methyl-*sec.*-pseudostrychnine, and formulated the hypothesis that this alkaloid should be present in *S. nux vomica* as an intermediate of the biogenetic pathway of the tertiary alkaloids of *Strychnos*.

QUIRIN *et al.* have established the presence of strychnine, brucine, colubrine and vomicine as well as two unidentified alkaloids¹⁰ in the leaves of *S. nux vomica*.

In our investigations we have studied *S. nux vomica* seeds, root bark* and a concentrate of mother liquors from the industrial preparation of strychnine. The separation of the alkaloid mixture can be achieved by passing the raw alkaloids over a silica gel column using chloroform (containing 2 % of methanol) as eluent.

Three main fractions were obtained: the first containing pseudostrychnine, pseudobrucine; the second vomicine; and the third icajine and novacine.

Pseudostrychnine and pseudobrucine can be separated by chromatography on an Al₂O₃ (activity III) column using benzene-ethyl acetate (75:25) as eluent.

Icajine and novacine were separated on the same column using benzene-ethyl acetate (85:15) as eluent.

The five alkaloids reported above were isolated in a pure crystalline form and identified on the basis of their spectroscopical properties (U.V., IR, NMR), chemical analysis, colour reactions, m.p., chromatographic behaviour and by direct comparison with authentic specimens.

These findings show that in *S. nux vomica*, in addition to the known tertiary alkaloids, icajine** and pseudobrucine are present.

We showed thus that three types of tertiary alkaloids, *viz.* the strychnine type (I), the pseudostrychnine type (II) and the N-methyl-*sec.*-pseudostrychnine

* From Madras area supplied by the Central Drug Research Institute of Lucknow (India).

** N. G. BISSET has also found icajine in *S. nux vomica* seeds by gas chromatography (personal communication).

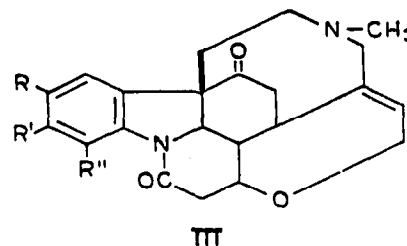
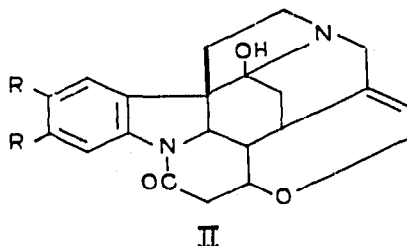
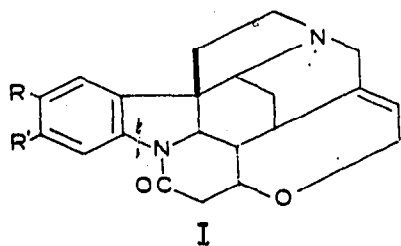


Fig. 1. Strychnine $R = R' = H$; Brucine $R = R' = OCH_3$; α -Colubrine $R = H$, $R' = OCH_3$; β -Colubrine $R = OCH_3$, $R' = H$.

Fig. 2. Pseudostrychnine $R = H$; Pseudobrucine $R = OCH_3$.

Fig. 3. Vomicine $R = R' = H$, $R'' = OH$; Icajine $R = R' = R'' = H$; Novacine $R = R' = OCH_3$, $R'' = H$.

type (III), are present in the seeds of this plant. In the root bark examined only pseudostrychnine and pseudobrucine were present. Strychnine, brucine and colubrine belong to type I, pseudostrychnine and pseudobrucine to type II, icajine (N-methyl-*sec.*-pseudostrychnine), novacine (N-methyl-*sec.*-pseudobrucine) and vomicine to type III.

Neither diaboline nor retuline were found in the samples examined. The finding of icajine and pseudobrucine in *S. nux vomica*, which were formerly obtained from *S. icaja* Baill⁹ and *S. gaultheriana*¹¹, respectively, shows that in *S. nux vomica* the complete biogenetic pathway of the alkaloids related to strychnine is present.

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- 1 C. G. CASINOVI, G. B. MARINI BETTÒLO AND N. G. BISSET, *Nature*, 193 (1962) 1178.
- 2 R. PELLICCIARI, F. DELLE MONACHE, E. CORIO AND G. B. MARINI BETTÒLO, *Ann. Ist. Super. Sanità*, 2 (1966) 411.
- 3 F. DELLE MONACHE, E. CORIO AND G. B. MARINI BETTÒLO, *Ann. Ist. Super. Sanità*, in press.
- 4 F. DELLE MONACHE, E. CORIO AND G. B. MARINI BETTÒLO, *Ann. Ist. Super. Sanità*, in press.
- 5 P. J. PELLETIER AND J. CAVENTOU, *Ann. Chim. Phys.*, 8 (1818) 323.
- 6 H. WARNAT, *Helv. Chim. Acta*, 14 (1931) 997.
- 7 H. WIELAND AND G. OERTEL, *Ann.*, 469 (1929) 193.
- 8 W. F. MARTIN, H. R. BENTLEY, J. A. HENRY AND F. S. SPRING, *J. Chem. Soc.*, (1952) 3603.
- 9 N. G. BISSET, *Compt. Rend.*, 261 (1965) 5237.
- 10 M. QUIRIN, J. LÉVY AND J. LEMEN, *Ann. Pharm. Franc.*, 23 (1965) 93.
- 11 H. G. BOIT AND L. PAUL, *Naturwiss.*, 47 (1960) 136.

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