59/THE ALKALOIDS OF *STRYCHNOS MEDEOLA* SAGOT EX PROGEL. STRUCTURE AND CONFIGURATION OF NORMACUSINE *B* BY SPECTRAL DATA. XXVI. ON THE ALKALOIDS OF *STRYCHNOS*.

GIOVANNI BATTISTA MARINI-BETTÒLO (*),
SILVIA ERAZO GIUFFRA, CORRADO GALEFFI
and ELISABETTA MIRANDA DELLE MONACHE

Laboratorio di chimica biologica, Istituto Superiore di Sanità, Roma.

(*) *Cattedra di chimica generale ed inorganica per scienze biologiche dell'Università*, Roma.

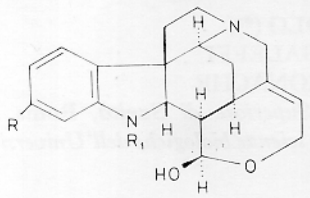
Summary. — 11-Methoxydiaboline ([I], figure 1), an alkaloid previously described by us, was isolated from the root bark of *Strychnos medeola*, while from the stem bark of the same plant normacusine *B* ([II], figure 1), already known by the name of deoxysarpagine, was obtained. This is the first time this alkaloid was found in a species of *Strychnos*, whereas it had been previously found in different plants of the *Apocynaceae* family. The structure and configuration of normacusine *B* was confirmed by MS as well as on the basis of ORD curve and NMR spectrum of the corresponding *O*-acetyl derivative ([III], figure 1).

Riassunto. — Dalla corteccia della radice di *Strychnos medeola* è stata isolata la 11-metossidiabolina ([I], figure 1), da noi precedentemente descritta, mentre dalla corteccia del fusto si è ottenuto un alcaloide, per la prima volta riscontrato nel genere *Strychnos*, la normacusina *B* ([II], figure 1), nota anche con il nome di desossisarpagina, la cui struttura e configurazione è stata determinata in base allo spettro di massa ed alla curva di dispersione ottica rotatoria ed allo spettro NMR del corrispondente *O*-acetato ([III], figure 1).

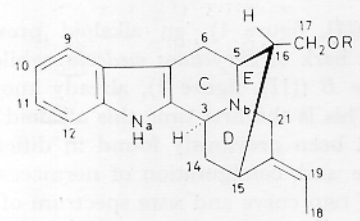
In the course of our systematic investigations of *Strychnos* alkaloids we have studied the alkaloids of *S. medeola*. This plant, which belongs to the section *Longiflorae*, is rather rare. No information on its uses in the preparation of curare or in popular medicine is available. The alkaloids were extracted from the pulverised plant material with dilute *Ac*OH and purified by CCD between $CHCl_3$ and phosphate buffer at pH=7. In both cases studied, that is of extraction of root and stem bark, one pure fraction consisted of a single alkaloid.

From the root bark it was obtained an alkaloid which crystallizes from *Et*O*Ac* and melts at 213–5 °C. From analytical data and MS (M^+ 382) the

raw formula $C_{22}H_{26}N_2O_4$ can be established. UV spectrum indicates an indolinic chromophore (λ_{max} in *Et*OH 295 nm, lg ε 3.81, 292 nm, lg ε 3.84 and 254 nm, lg ε 4.0). NMR spectrum (solvent $CDCl_3$, internal reference TMS), shows the presence of one methoxy group (δ 3.76 ppm) and of another methyl (2.35 ppm, s), probably due to N—CO—CH_3. MS and IR spectra are indistiguishable from those of 11-methoxy-diaboline ([I], figure 1), an alkaloid described by us the first time in [1].



		R	R_1
I	11-methoxy-diaboline	OCH_3	Ac
IV	diaboline	H	Ac
V	desacetyl-diaboline	H	H



		R
II	normacusine B	H
III	O-acetyl-normacusine B	Ac

Fig. 1.

Chromatographic properties and CCD behaviour are also identical with those of 11-methoxydiaboline. Melting point of our substance mixed with an authentic sample of the latter is undepressed.

It is the second time that this alkaloid is found in plants. This fact is rather interesting considering the possible role of diaboline ([IV], figure 1) and desacetyldiaboline ([V], figure 1) in the biogenesis of these alkaloids.

The alkaloid obtained from stem bark shows a characteristic indolic spectrum (λ_{max} in *Et*OH 289 nm, lg ε 3.77, and 280 nm, lg ε 3.87, shoulder at 272 nm). The formula $C_{19}H_{22}N_2O$ can be assigned to the alkaloid on the basis of the elemental analysis and of the mass spectrum (M^+ 294 base peak); furthermore a partial β-carboline moiety can be established on the basis of the presence of the peaks m/e 169 and 168 (86 and 51% of the base peak respectively) (figure 2). The mass spectrum also suggests the presence of

(1) G. B. Marini-Bettòlo, E. Miranda Delle Monache, S. Erazo Giuffra, C. Galeffi, *Gazz. Chim. Ital.*, **101**, 971 (1971).

a primary alcoholic group ($M^+ - 31$, 34% of the base peak); the alkaloid gives an *O*-acetylderivative with Ac_2O and *Py* (M^+ 336, base peak and $M^+ - AcO$ 80%).

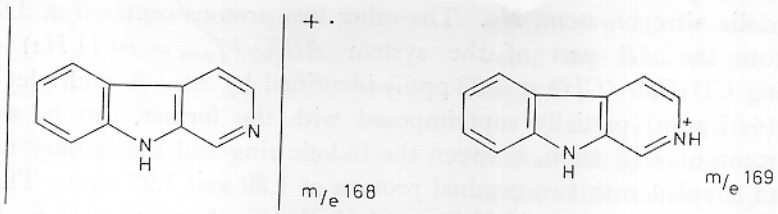


Fig. 2.

The NMR spectrum of the acetyl-derivative in $CDCl_3$ allows us to assign to the alkaloid the structure reported for normacusine B ([II], figure 1), with the exception of stereochemistry. Moreover, all the data reported in literature for this alkaloid and its acetylderivative ([2]), *i.e.* MS, UV spectra, rotatory power and m.p. are in accordance with those found by us.

Normacusine *B*, better known as deoxysarpagine, or tombozine or vellosiminol ([3]), was previously found in several species of *Apocinaceae* but never in a species of *Strychnos* (*Loganiaceae*).

Battersby and Yeowell ([4]) have obtained normacusine B by thermal cleavage of macusine *B* isolated from *S. toxifera* collected in Guyana and Bartlett and *alii*. demonstrated its structure by correlation with ajmaline ([5]). Because of the scarce solubility of the alkaloid in $CDCl_3$, the NMR spectrum was run with the *O*-acetyl-derivative ([III], figure 1). The information obtained from the spectrum of this substance, having a partial tetrahydro--β-carbonilic structure, complete the few data so far available on this molecule ([6]). The doublet corresponding to a methyl group at 1.54 ppm ($J=7$ Hz) is coupled with the only olefinic proton at 5.40 (multiplet); furthermore both methyl signals are splitted into a triplet ($J=1$ Hz) by homoallylic coupling, *cis* or *trans*, with two protons at 3.53 ppm. In fact by irradiating at 3.53 the

([2]) A. R. Battersby, D. A. Yeowell, *J. Chem. Soc.*, 4419 (1964).

([3]) M. F. Bartlett, R. Sklar, W. I. Taylor, E. Schlittler, R. L. S. Amai, Peter Beak, N. V. Bringi, E. Wenkert, *J. Am. Chem. Soc.*, **84**, 622 (1962). D. Stauffacher, *Helv. Chim. Acta*, **44**, 2006 (1961) and H. Rapoport, R. E. Moore, *J. Org. Chem.*, **27**, 2981 (1962).

([4]) A. R. Battersby, D. A. Yeowell, *Proc. Chem. Soc.*, 17 (1961).

([5]) M. F. Bartlett, E. Schlittler, R. Sklar, W. I. Taylor, R. L. S. Amai, E. Wenkert, *J. Am. Chem. Soc.*, **82**, 3792 (1960).

([6]) L. D. Antonaccio, N. A. Pereira, B. Gilbert, H. Vorbrueggen, H. Budzikiewicz, J. M. Wilson, L. J. Durham, C. Djerassi, *J. Am. Chem. Soc.*, **84**, 2161 (1962).

signal of the methyl group is transformed in a simple doublet (J=7 Hz) whereas by irradiating at 5.40 ppm, the signal of the same methyl is transformed in a triplet (J=1 Hz). The two allylic protons at 3.53 ppm, that is at relatively low fields, must be bonded to a carbon atom near the tertiary non-indolic nitrogen atom, N_b. The other two protons centered at 3.95 ppm constitute the *AB* part of the system *ABX* ($J_{gem} = \sim 11$ Hz) of the grouping CH_2O*Ac* (CH_3 at 1.98 ppm) identified by MS. A multiplet at low fields (4.02 ppm) partially superimposed with the former, can be assigned to a proton on C–3 atom, between the indolic ring and the tertiary nitrogen N_b, and coupled with two geminal protons at 1.80 and 1.95 ppm. The other geminal protons ($J_{gem} = 15$ Hz) on the other carbon atom C–6, bonded to the indolic ring, are at 2.60 and 3.05 ppm and are further coupled ($J = 2$ and $J = 6$ Hz respectively), with another proton (H–5) at 2.70 ppm in α to N_b.

In the same range partially superimposed two other protons are present. The first constitutes the *X* part of the *ABX* system of the group CH—CH_2O*Ac*, and the other represents the allylic hydrogen of the last non aromatic carbon atom. This carbon atom and the other tertiary C–5 are the joint points of the fifth ring *E* and are bonded to the methyne group of CH—CH_2—O*Ac*.

The four protons of the aromatic moiety of the molecule are between 7.0 and 7.5 ppm, whereas the broadened signal at 8.05 ppm (which disappears with D_2O) belongs to the hydrogen of the indolic nitrogen N_a.

In order to establish the absolute configuration of the indolic alkaloids, the chirality of the center at C–3 near the only chromophore was previously correlated with the ORD curves in the yohimbane-quebrachamine alkaloids ([7]). In these alkaloids the ring *C* can be either in the hemi-chair or in the hemi-boat form, the ring *D* in the boat, chair or twist form, without affecting remarkably the atom dispositive near the chromophore itself.

In the case of our alkaloid, the combination of the conformation *C*/*D* *cis*(*C* is hemi-chair) with that of *D* in boat form does not allow the same disposition of the atom 3,4 (= N_b), 5,6 and 14 with respect to the chromophore as in *C*/*D* trans or *C*/*D* *cis* conformation (with C–2 axial with respect to the ring *E*) of the alkaloids of the yohimbane-quebrachamine series. Nevertheless in both cases the atoms with respect to the plane of the indolic ring, are on the same side with the identical absolute configuration of the C–3 center which, in any case, determines the sign of the Cotton effect of the ORD curves.

Analogously the α-configuration can thus be attributed to the H–3 of the *O*-acetyl-normacusine B ([III], figure 1) according to the positive

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([7]) W. Klyne, R. J. Swan, N. J. Dastoor, A. A. Gorman, H. Schmid, *Helv. Chim. Acta*, **50**, 115 (1967).

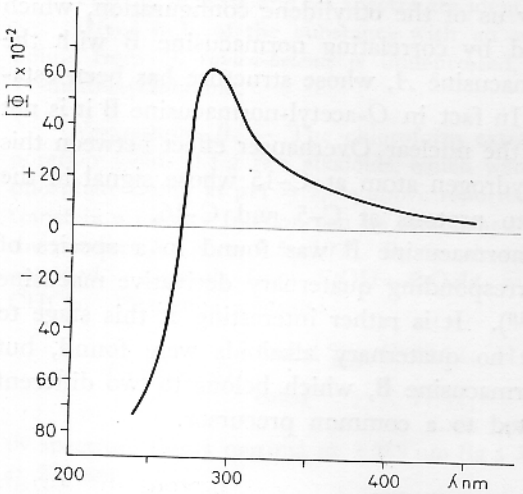
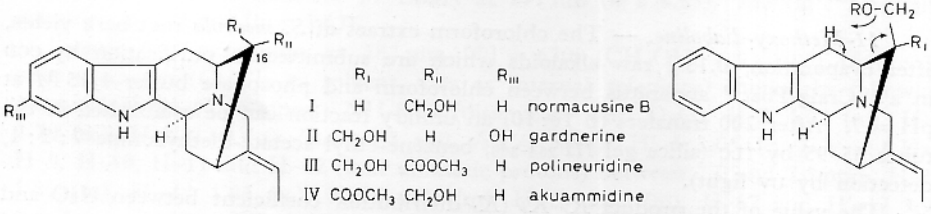


Fig. 3.

Cotton effect (first extremum at 287 nm, $[\Phi] + 6300$ in *Me*OH (figure 3). The configuration of the chiral centers 5 and 15 are consequentely determined being the joint points of the ring *E*.

Mass spectrometry allows to establish the relative positions of the substituents CH_2OH and H at C–16, which give rise to two series of alkaloids. In fact it has been observed that the mass spectra of the alkaloid under examination as well as those of the normacusine B series ($R' = H$, $R'' = CH_2OH$, [I], figure 4) also in inlet system different temperature always shows a slight peak corresponding to the elimination of water (2% of the base peak) whereas in the gardnerine series ($R' = CH_2OH$, $R'' = H$, [II], figure 4) it represents the 59% of the base peak ([8]). This difference in dehydratation behaviour already observed for the MS fragmentation of polineuridine ($R' = CH_2OH$, $R'' = COOCH_3$, [III], figure 4) and akuammidine ($R' = COOCH_3$ and $R'' = CH_2OH$, [IV], figure 4) ([6]) can be explained for the gardnerine and polineuridine series with the mechanism reported in figure 4.



	R_I	R_{II}	R_{III}	
I	H	CH_2OH	H	normacusine B
II	CH_2OH	H	OH	gardnerine
III	CH_2OH	$COOCH_3$	H	polineuridine
IV	$COOCH_3$	CH_2OH	H	akuammidine

Fig. 4.

In accordance with this mechanism in the mass spectrum of *O*-acetyl--derivative of the alkaloid under investigation the peak M^+—*Ac*OH is rather scarce (7% of the base peak) whereas the corresponding to M^+—*Ac*O (80% of the base peak) is very high.

([8]) S. Sakai, A. Kubo, J. Haginiwa, *Tetrahedron Letters*, **19**, 1485 (1969).

No evidence was obtained by us of the ethylidene configuration, which had been previously demonstrated by correlating normacusine B with the Wieland-Gumlich aldehyde and macusine *A*, whose structure has been established by X-ray diffraction ([9]). In fact in *O*-acetyl-normacusine B it is not possible to establish the figure of the nuclear Overhauser effect between this methyl group and the coplanar hydrogen atom at C–15 whose signal in the NMR spectrum is superimposed to protons at C–5 and C–16.

This is the first time that normacusine B was found in a species of *Strychnos*; previously only the corresponding quaternary derivative macusine B, was described in *S. toxifera* ([10]). It is rather interesting at this stage to consider here that in *S. medeola* no quaternary alkaloids were found, but only 11-methoxydiaboline and normacusine B, which belong to two different types of alkaloids, probably related to a common precursor.

EXPERIMENTAL

The plant material used in this research consisted of root bark (113 g) and stem bark (73 g) of *Strychnos medeola* (Nilo Silva 3408) collected by Nilo G. Silva on 1–12–1970 in the State of Para' (Brazil) in the basin of Rio Jari.

The material was identified and supplied to us by B. A. Krukoff. The voucher specimens are cited in this papers and deposited in the herbarium of the New York Botanical Garden.

Extraction. — Each sample was powdered and extracted by percolation with 2% *Ac*OH five times till negative reaction to the Dragendorff. The percolates were pooled and basified with $NaHCO_3$ and extracted four times with $CHCl_3$.

Quaternary alkaloids are absent because the aqueous phase, after acidification with *Ac*OH neither precipitate with Tanret reactive, nor yields, after acidification with hydrochloric acid, picrate with picric acid.

11-Methoxy-diaboline. — The chloroform extract of *S. medeola* root bark yields, after evaporation, 0.15 g raw alkaloids which are submitted to purification by CCD in a " Craig-Post " apparatus between chloroform and phosphate buffer 1/15 *M* at pH = 7. After 200 transfers (*v* 10:10) an unitary fraction can be established in the tubes 65–95 by TLC (silica gel $HF_{254-366}$, benzene-ethyl acetate-diethylamine 7 : 2 : 1, detection by UV light).

The value of the product $K_r \cdot K_b$ (K_r distribution coefficient between H_2O and $CHCl_3$; K_b dissociation constant), determinated according to the method described by one of us, is 6×10^{-8} ([11]). These fractions are pooled and the aqueous phase basified with $NaHCO_3$ and extracted with the chloroform phase. A residue of 43 mg is obtained after evaporation; crystals from *EtOAc*-cyclohexane melt at 213–5 °C.

([9]) A. T. McPhail, J. M. Robertson, G. A. Sim, A. R. Battersby, H. F. Hodson, D. A. Yeowell, *Proc. Chem. Soc.*, 223 (1961).

([10]) A. R. Battersby, R. Binks, H. F. Hodson, D. A. Yeowell, *J. Chem. Soc.*, 1848 (1960).

([11]) C. Galeffi, *J. Chromatog.*, in press.

The UV, IR, NMR and MS spectra are identical to those of 11-methoxydiaboline [1].

Mixed m.p. of the substance with an authentic specimen of 11-methoxy-diaboline from *S. romeu-belenii* is undepressed, thus confirming that the alkaloid is 11-methoxydiaboline.

Normacusine B. — The chloroform extract of *S. medeola* stem bark after evaporation yields 0.1 g raw alkaloids, which were distributed, between chloroform and phosphate buffer at pH = 7, as above reported for 11-methoxy-diaboline. After 200 transfers a unitary fraction can be established in tubes 30–50 by TLC. The value of the product $K_r \cdot K_b$ is 2.5×10^{-8}. By the above reported technique 24 mg alkaloid are obtained. Crystals from *Et*OH—*Et*O*Ac*, m.p. 200–220 °C, sparingly soluble in $CHCl_3$. Elemental analysis:

$C_{19}H_{22}N_2O$	found %:	C 77.13;	H 7.01;	N 8.89
	calcd :	77.52;	7.53;	9.52.

UV spectrum shows maxima at λ 289 nm (lg ε 3.77) and 280 (lg ε 3.87) and shoulder at 272 nm.

MS: M^+ 294 base peak, corresponding to $C_{19}H_{22}N_2O$, m/e 293 (M^+—1, 84% of the base peak), 263 (M^+—CH_2OH, 34%), 169 (86%) and 168 (51%) (figure 2).

O-Acetyl-normacusine B. — Normacusine B was acetylated overnight at room temperature with *Py* and Ac_2O. The product was purified by CCD between chloroform and potassium phosphate monobasic 1/15 *M*. After 180 transfers the acetylated alkaloid was located in tubes 7–19, and extracted as above reported; the value of the product $K_r \cdot K_b$ is 2×10^{-11}.

The substance after two crystallizations from *Et*O*Ac* melts sharply at 220–1 °C. $[\alpha]_D^{20} = +10.5 \pm 1.5$ (chloroform, $c = 0.3$). Elemental analysis:

$C_{21}H_{24}N_2O_2$	found %:	C 74.87;	H 6.97;	N 8.31
	calcd :	74.97;	7.19;	8.33.

UV spectrum shows maxima in *Et*OH at 291 nm (lg ε 3.55), 282 (lg ε 3.64) and shoulder at 276 nm (lg ε 3.63).

ORD: first extremum at 287 nm, $[\Phi]$ + 6300 (CH_3OH, c 9×10^{-3}) (figure 3). NMR spectrum ($CHCl_3$ as solvent, TMS as internal reference) shows the following chemical shifts (δ in ppm): NH 8.05 (disappears with D_2O), H–3 4.02 (m), H–5 2.70 (m), H–6 2.60 (q, $J_{gem} = 15$ and $J_{5-6} = 2$ Hz), H′–6 3.05 (q, $J_{5-6'} = 6$ Hz), H–9, H–10, H–11 and H–12 (four aromatic protons) between 7.0 and 7.5 ppm, H–14 1.80 (m), H′–14 1.95 (m), H–15 2.70 (m), H–16 2.70 (m), H–17 and H′–17 3.95 (q, $J_{gem} = 11$ Hz), CH_3—CO 1.98 (s), 3H–18 1.54 (doublet triplet, $J_{18-19} = 7$ and $J_{18-21} = 1$ Hz), H–19 5.40 (broadened quartet), 2H–21 3.53 (m, $J_{18-21} = 1$Hz).

MS: M^+ 336 (base peak), m/e 277 (M^+—CH_3COO).

We are indebted to dr. B. A. Krukoff, New York Botanical Garden, for supplying the plant material and for his suggestions and helpfull discussion.

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