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A new alkaloid of *Strychnos*. The occurrence of 11-methoxy-diaboline in *Strychnos romeu-belenii* Krukoff and Barneby

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81. G. B. MARINI BETTÒLO, Elisabetta MIRANDA DELLE MONACHE, Silvia ERAZO GIUFFRA and Corrado GALEFFI. — **A new alkaloid of *Strychnos*. The occurrence of 11-methoxy-diaboline in *Strychnos romeu-belenii* Krukoff and Barneby.** (*)

Riassunto. — Dalla corteccia del fusto di *S. romeu-belenii* Krukoff e Barneby, una Loganiacea Sudamericana, è stato isolato un nuovo alcaloide indolico: la 11-metossi-diabolina (I). La sua struttura, proposta sulla base del confronto degli spettri IR, RMN, DOR e di massa con quelli della diabolina è stata confermata per conversione attraverso il N-desacetilderivato (III) (11-metossi-Wieland-Gumlich aldeide) in α-colubrina(II) (11-metossi-stricnina). Per acetilazione fornisce l'isomero in C-17 della condensammina(IV) denominato isocondensammina (V).

Inoltre la 11-metossi-Wieland-Gumlich aldeide (III) per riscaldamento con acido acetico ed acetato sodico dimerizza in 11,11'-dimetossi-caracurina V (VI).

Summary. — From the stem bark of *Strychnos romeu-belenii* Krukoff and Barneby, a Loganiacea of South America, a new indolinic alkaloid has been isolated to which the structure of 11-methoxy--diaboline (I) has been assigned on the basis of UV, IR, NMR ORD and MS data.

The structure was confirmed by converting (I) into α-colubrine (II) (11-methoxy-strychnine)through the N-deacetyl-derivative (III) (11-methoxy-Wieland-Gumlich aldehyde).

(I) by acetylation gives the isomer C-17 of condensamine (IV) for which we propose the name isocondensamine (V).

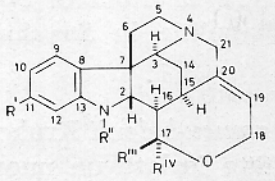
In addition, 11-methoxy-Wieland-Gumlich aldehyde (III) yields on heating with acetic acid and sodium acetate the dimeric 11,11'--dimethoxy-caracurine V (VI).

In the course of our investigations on the alkaloids of various *Strychnos* species ([1]), we report the isolation of a new tertiary alkaloid, the 11-methoxy-diaboline (I) fig. 1 from the stem bark of *S. romeu--belenii* Krukoff and Barneby of Brasil ([2]).

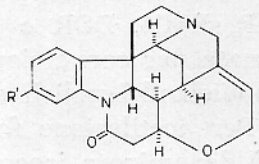
(*) Roma. — Istituto Superiore di Sanità. - Laboratorio di Chimica Biologica.

([1]) G. B. MARINI-BETTÒLO, E. MIRANDA DELLE MONACHE, C. GALEFFI, M. A. CIASCA RENDINA and A. VILLAR DEL FRESNO, Ann. Chimica, *60*, 444 (1970).

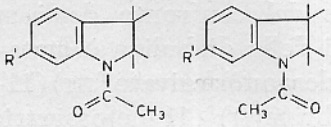
([2]) B. A. KRUKOFF, Memoirs of the New York Botanical garden, *20*, 22 (1969).



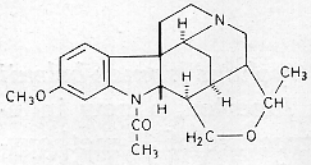
	R'	R''	R'''	R''''	
I	OCH_3	$COCH_3$	OH	H	11-methoxy-diaboline
III	OCH_3	H	OH	H	11-methoxy-Wieland-Gumlich aldehyde
IV	OCH_3	$COCH_3$	$OCOCH_3$	H	condensamine
V	OCH_3	$COCH_3$	H	$OCOCH_3$	isocondensamine
X	H	$COCH_3$	OH	H	diaboline
XI	H	H	OH	H	Wieland-Gumlich aldehyde
XIV	H	$COCH_3$	$OCOCH_3$	H	acetyl-diaboline A (henningsamine)
XIII	H	$COCH_3$	H	$OCOCH_3$	acetyl-diaboline B (jobertine)



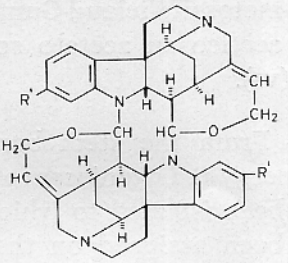
II R' = OCH_3 α-colubrine
XII R' = H strychnine



VIII IX



VII Strycnospermine



VI R' = OCH_3 11,11'dimethoxy-caracurine V
XV R' = H caracurine V

FIG. 1.

Whereas other examples of different Strychnos species contain very complex alkaloid mixtures ([3]), in *S. romeu-belenii* stem bark only one alkaloid is present in large quantity.

The latter, purified by countercurrent distribution between chloroform and phosphate buffer cristallizes from ethyl acetate, m.p. 214-6°.

The analytical data are in accordance with a raw formula $C_{22}H_{26}N_2O_4$ and MS indicates M^+ 382 (base peak).

The UV spectrum in ethanol, which shows two maxima at 295 (log ε 3.81) and 292 nm (log ε 3.84), a minimum at 273 (log ε 3.63) and a further maximum at 254 nm (log ε 4.01), is characteristic of an 11-methoxy,N-acyl-indoline (*).

([3]) C. GALEFFI, M. A. CIASCA RENDINA, E. MIRANDA DELLE MONACHE, A. VILLAR DEL FRESNO and G. B. MARINI-BETTÒLO, J. Chromatog, *45*, 407 (1969).

([4]) RAYMOND-HAMET, Ann. Pharm. franç., *8*, 482 (1950).

(*) α-colubrine (II) (11-methoxy-strychnine) shows maxima at 297 nm (log ε 3.77), 293 (3.75) and 255 (4.03) ([4]).

This structure is confirmed by a band at 1650 cm^{-1} in the IR spectrum, due to the stretching vibration of the amidic carbonyl without hydrogen bonding and by a band at 1600 cm^{-1} due to the aromatic system.

Furthermore the presence in the NMR spectrum (solvent $CDCl_3$, internal reference TMS) of a singlet corresponding to a methyl group at δ 2.35 ppm- i.e. to the same field as the N-acetyl of strychnospermine (VII) ([5]), suggests the presence of the same grouping.

To the limited rotation of this acetyl, as in strychnospermine, the complexity of the NMR signals, due to the two possible cisoid (VIII) and transoid (IX) conformations, should be attributed. In particular, whereas in both conformations the signals of the acetyl (δ 2.35 p.p.m.), of the methoxy group (δ 3.76 p.p.m.), and of the aromatic protons in C-9 (6.90 p.p.m., d, J 8.5 c.p.s.) and C-10 (6.63 p.p.m., q, 2.5 c.p.s. and 8.5 c.p.s.) are identical — as it happens in strychnospermine (ibidem) — the signal due to the proton in 12 is at 7.08 p.p.m. for the transoid conformation (IX), whereas is shifted to lower field, δ 7.57 p.p.m. (d, J 2.5 c.p.s.) for the cisoid conformation (VIII). This is due to the strong magnetic anisotropy of the neighbouring amidic carbonyl.

Furthermore, the identity of the aromatic substitution of the alkaloid (I) of *S. romeu-belenii* with that of α-colubrine (II) (cisoid structure) is confirmed by the identity of the corresponding signals (H-9 δ 7.00 p.p.m., d, J 8 c.p.s., H-10 6.60 p.p.m., q, J 2 and 8 p.p.m., H-12 7.74 p.p.m., d) ([6]).

The other NMR signals of the alkaloid are all broadened because of the limited rotation of the N-acetyl group and therefore the presence of the two conformomers (VIII and IX). Among these signals three, each corresponding to a proton, are worthwhile mentioning: a multiplet at 4.75 p.p.m. which can be attributed to the proton in 2, as in the other N-acetyl-indolinic alkaloids ([7]), a multiplet at 5.85 p.p.m. and a doublet at 5.25 p.p.m., J $\sim$ 2 c.p.s., shifted by acetylation to 6.14 p.p.m. . Both signals are common to diaboline (X) where they are attributed respectively to the olefinic hydrogen H-19, 5.85 p.p.m., m, and to H-17 of the O–C*H*OH–CH group, δ 5.28, d, J 2 c.p.s. (shifted by acetylation to 6.14 p.p.m.). In regard to the hydroxy group, the

([5]) F. A. ANET,, Can. J. Chem., *41*, 883 (1963).

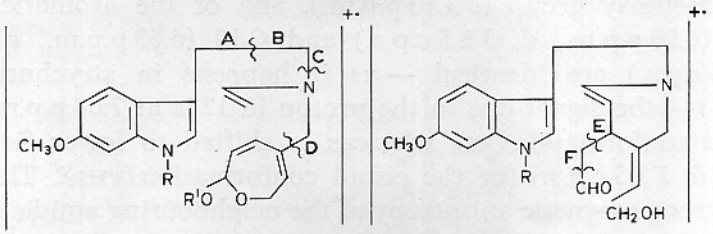
([6]) A. VILLAR DEL FRESNO, E. MIRANDA DELLE MONACHE, C. GALEFFI, M. A. CIASCA RENDINA and G. B. MARINI-BETTÒLO, Atti Accad. Naz. Lincei, Rend. Classe Sci. Fis. Mat. Nat. (8), *48*, 46 (1970).

([7]) N. G. BISSET, Alkaloids of some African species, Ph. D. Thesis, University of London, p. 46 (1968).

IR spectra of alkaloid (I) and of diaboline (X) ([8]) show perfect identity: a narrow band at 3650 cm^{-1} due to the free hydroxy group and large bands between 3400 and 3300 cm^{-1} and between 2700 and 2500 cm^{-1} due to the bonded hydroxy group. By the comparison of the mass spectra of the alkaloid (I) and of the diaboline (X) the identity of the non aromatic moiety of the two molecules is suggested, because the fragments attributed to the non aromatic moiety appear in both alkaloids with the same intensity (see especially peak B, table I)

TABLE I.

MS FRAGMENTATION OF 11-METHOXY-DIABOLINE, 11-METHOXY WIELAND--GUMLICH ALDEHYDE AND 11-METHOXY,O-ACETYL-DIABOLINE B



R	R'	
H	H	11-methoxy-Wieland-Gumlich aldehyde
$COCH_3$	H	11-methoxy-diaboline
$COCH_3$	$COCH_3$	11-methoxy,acetyl-diaboline B (isocondensamine)

MASS: % OF FRAGMENTS

	11-methoxy-diaboline		11-methoxy W.G. aldehyde		11-methoxy, O-acetyl-diaboline B	
	R=$COCH_3$; R'=H		R=R'=H		R=R'=$COCH_3$	
M^+	382	100	340	30	424	100
M^+-R'OH	364	7	322	4	364	22
F	353	64	311	11		
E	339	21	297	10		
M^+-R'COOH	336	21	294	2	336	22
M^+ -R					321	17
M^+ -R'OH	321	10			271	6
D	271	43	229	6	216	22
C	216	14	174	63	222	28
B	180	100	180	100	174	44
C-CH_2CO	174	95			162	15
B-R'OH	162	14	162	10	160	20
A-CH_2CO	160	39				

([8]) F. E. BADER, E. SCHLITTER and H. SCHWARZ, Helv. Chim. Acta, *36*, 1256 (1953).

whereas those containing indolic ring are increased by 30 mass units in (I) for the presence of a methoxy group ([9]). The direct comparison of the NMR spectrum of the alkaloid under consideration and that of diaboline as suggested by the previous analogies shows their perfect identity except for the aromatic zone, and the signal of the methoxy group. As in the spectrum of (I) so also in that of diaboline there is a superposition of signals of the two conformomers due to the limited rotation of the amidic acetyl group (in particular H-12 is at 7.92 p.p.m. in the cisoid conformation of the acetyl and 7.1 in the transoid form and covered by H-9, H-10 and H-11).

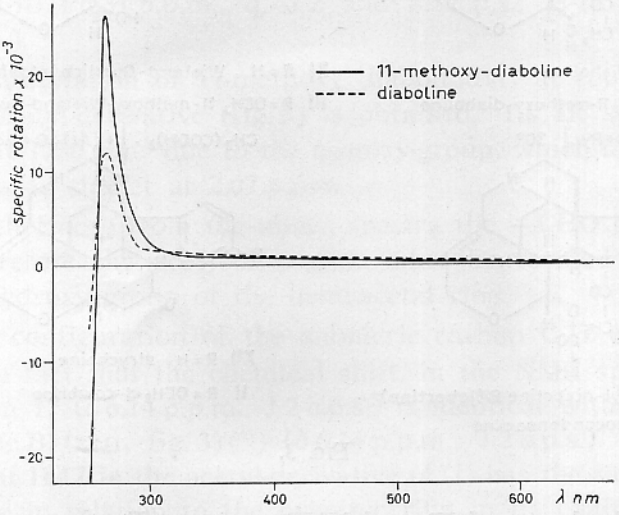


FIG. 2.

The identity of configuration at the nodal centres C-2 and C-7 and thus at C-3, C-15 and C-16 ([10]) of diaboline and alkaloid (I) was proved by the identity of ORD curves (fig. 2) which present a positive Cotton effect with the first extremum at 264 nm for both compounds with $[\alpha] + 12100$ for diaboline and 26300 for (I) (c $7 \cdot 10^{-3}$ in CH_3OH). Therefore the structure of 11-methoxy-diaboline can be attributed to alkaloid (I).

It is also possible to establish the presence of the hemiacetal ring (see tautomeric forms in fig. 3) and the absence of a —CHO

([9]) H. MÜLLER, M. HESSE, P. WASER, H. SCHMID and P. KARRER, Helv. Chim. Acta, *48*, 320 (1965); K. BIEMANN, J. S. GROSSERT, J. M. HUGO, J. OCCOLOWITZ and F. L. WAREN, J. Chem. Soc., 2814 (1965).

([10]) W. KLYNE, R. J. SWAN, B. W. BYCROFT and H. SCHMID, Helv. Chim. Acta, *49*, 833 (1966).

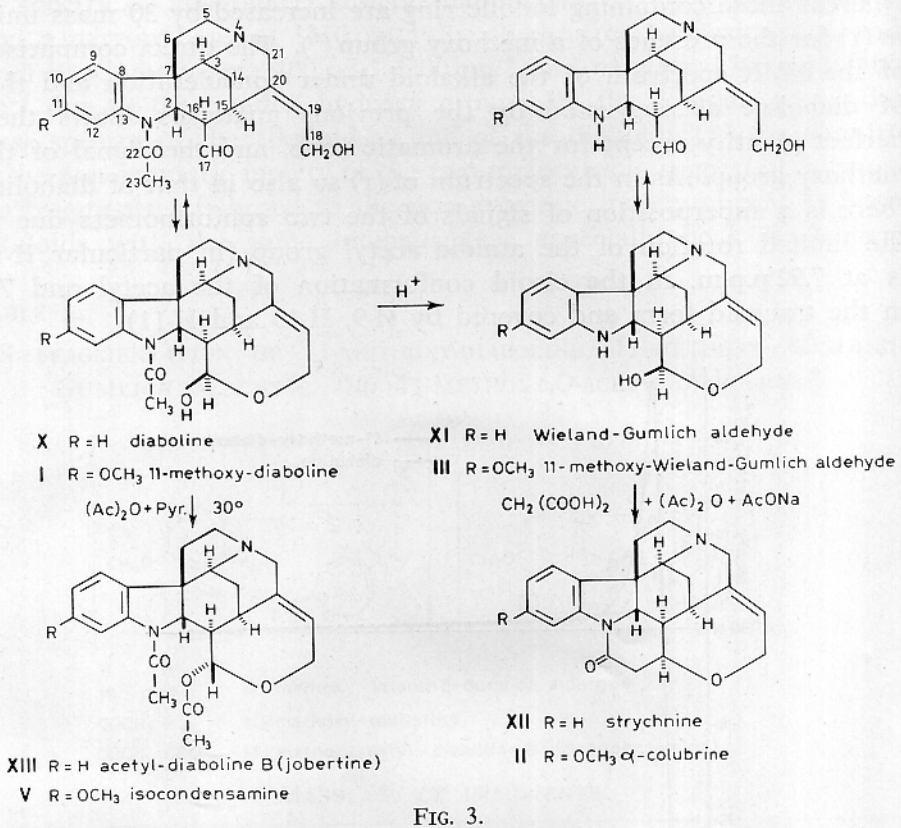


FIG. 3.

group, on the bases of IR and NMR spectra. Furthermore it can be inferred that a single isomer at H-17 is present because the area of the signal at 5.25 p.p.m. corresponds exactly to one proton.

That the configuration of the latter is α-axial as in diaboline is confirmed through the identity in both substances of the NMR signal of this proton ([11]).

The proposed structure of (I) was confirmed through the transformation of (I) into its N-deacetyl-derivative (III) and of this latter into α-colubrine (II, fig. 3).

The transformation was performed by heating (III) with malonic acid according to the conversion of Wieland-Gumlich aldehyde (XI) into strychnine (XII, fig. 3) ([12]).

The identity with α-colubrine was proved by direct comparison of Rf, UV, IR data and by a mixed melting point.

([11]) J. A. DEYRUP, H. SCHMID and P. KARRER, Helv. Chim. Acta, *45*, 2266 (1962)
([12]) F. A. L. ANET and R. ROBINSON, Chem. & Ind. (London), 245 (1953).

The N-deacetyl-derivative (III) obtained from (I) by acid hydrolysis (fig. 3) does not show the amidic carbonyl band in IR spectrum at 1650 cm^{-1}, whereas it shows two narrow bands at 3700 and 3450 cm^{-1} due to the groups NH and OH.

The mass spectrum is identical with that of W. G. aldehyde (XI) ([9]) except for the increase of 30 mass units in the indolic fragments of (III) for the presence of a methoxy group (table I).

In the NMR spectrum of the N-deacetyl derivative (III), the aromatic protons are sensibly shielded with respect to those of (I) because of the deacetylation of the nitrogen (H-9 δ 6.80 p.p.m., d, J 8.5 c.p.s.; H-10 δ 6.31 p.p.m., q, J 2 and 8.5 c.p.s.; H-12 δ 6.29 p.p.m., d, J 2 c. p. s.).

By acetylation of 11-methoxy diaboline (I) at room temperature a monoacetyl-derivative (fig. 3) is obtained. Its IR spectrum shows a band at 1750 cm^{-1} due to the acetoxy-group, which appears in NMR spectrum as singlet at 2.07 p.p.m. .

Furthermore from the above spectra the —CHO group is absent and therefore the acetyl-derivative must be formed by acetylation of the hydroxy group of the hemi-acetal ring.

The configuration of the anomeric carbon C-17 can be deduced from the fact that the chemical shift, in the NMR spectrum, of the proton in 17 (δ 6.14 p.p.m., J 2 c.p.s.) is identical with that of acetyl--diaboline B (XIII, fig. 3) ([11]) (δ 6.14 p.p.m., J 2 c.p.s.). This fact suggests that H-17 in the acetyl-derivative of (I) has the same β-equatorial structure in relation to the oxepinic ring in its chair conformation. For this acetyl-derivative we propose the name isocondensamine (V) as it is anomeric isomer of condensamine (IV) isolated from *S. holstii* Gilg.var.reticulata-condensata ([13]).

Condensamine has the same configuration at C-17 of the acetyl--diaboline A (H-17 α-axial), found in *S. henningsii* and *S. Chlorantha* ([14]) and named henningsamine (XIV).

The anomeric isomer (acetyl-diaboline B) was found in *S. jobertiana* ([15]) and named jobertine (XIII, fig. 3).

By heating 11-methoxy-W.G.aldehyde (III) with acetic acid and sodium acetate in the absence of air, a dimerization occurs as with

([13]) J. Bosley, J. Pharm. Belg., *6*, 150 and 243 (1951).

([14]) J. S. Grossert, J. M. Hugo, M. E. von Klemperer and F. L. Warren, J. Chem. Soc., 2812 (1965).

([15]) F. Delle Monache, E. Corio and G. B. Marini-Bettòlo, Ann. Ist. Sup. Sanità, *3*, 564 (1967).

Wieland-Gumlich aldehyde (XI) and 11,11'-dimethoxy-caracurine (VI) is obtained.

The finding of a new derivative of diaboline in another South American *Strychnos* is in accordance with previous results of our investigations ([1]) and adds new data for the interpretation of the biogenetic relations between tertiary and quaternary alkaloids in *Strychnos* genus.

EXPERIMENTAL

The material used in the present work is constituted of the stem bark of *Strychnos romeu-belenii* Krukoff and Barneby, collected in Itacarè (Bahia) Brasil, identified and supplied by B. A. Krukoff ([2]).

1.1 kg bark is throughly powdered and percolated overnight with 2% acetic acid. The operation is repeated twice. The eluates are pooled, basified with sodium bicarbonate and extracted with chloroform. The chloroform extract after evaporation gives a 3.7 g residue which is purified by c.c.d. in a Craig apparatus (200 transfer, v 10: 10) between chloroform and phosphate buffer 1/15 M pH 6. The distribution is monitored by thin layer chromatography on Silica Gel $HF_{256+366}$ using, as solvent, benzene: ethyl acetate: diethylamine 7: 2: 1 (revealed with Dragendorff reagent or directly by UV light).

After 200 transfers an unitary abundant alkaloidal fraction is identified between tubes 130 and 174 whereas small quantities of minor alkaloids are found in tubes 56-110 and 1-30. The three fractions are separately basified with sodium bicarbonate and extracted with chloroform. The chloroform layer is stirred with water, dried over sodium sulphate and evaporated. The residue of the main fraction yields 2.3 g of alkaloid, whereas the minor fractions give 20 and 35 mg respectively, and were not examinated.

11-*methoxy-diaboline* (I). — The main fraction, practically unitary at the TLC, was crystallized from ethyl acetate and cyclohexane and then twice from pure ethyl acetate. Prisms, mp 214-6°, M^+ 382 (base peak), $[\alpha]_D^{20}$ + 20 c · 1 chloroform). Cotton effect: see fig. 2. Analysis:

	found%:	C 68.79;	H 6.75;	N 7.26;
for $C_{22}H_{26}N_2O_4$	calcd. :	69.09;	6.85;	7.33.

UV in ethanol: λ_{max} at 295 nm (log ε 3.81), 292 nm (log ε 3.84) and 254 nm (log ε 4.01), λ_{min} at 273 nm (log ε 3.63). IR in chloroform: bands

at 1650 cm^{-1} (amidic carbonyl without hydrogen bond), at 1600 cm^{-1} (aromatic ring), between 2700 and 2500, and 3400 and 3300 cm^{-1} due to hydrogen bonded hydroxy group, and at 3650 cm^{-1} due to the free hydroxy group.

Some features of the NMR spectrum (deuterochloroform as solvent, TMS as internal reference): δ 2.35 p.p.m. s (CH_3CON); δ 3.76 p.p.m., s (OCH_3); δ 4.75 p.p.m., m (H-2); δ 5.25 p.p.m., J 2 c.p.s., d (H-17); δ 5.85 p.p.m., m (H-19); δ 6.90 p.p.m., J 8.5 c.p.s., d (H-9); δ 6.63 p.p.m., J 2.5 and 8.5 c.p.s., q (H-10); H-12 is at 7.57 p.p.m., J 2.5 c.p.s. d, in the cisoid and 7.8 c.p.s. in the transoid conformation.

Mass spectrum fragmentation is reported in Table I.

11-*methoxy Wieland-Gumlich aldehyde* (III). — (N-deacetyl,11-methoxy-diaboline) 0.5 g of 11-methoxy-diaboline (I) are refluxed for 2h with 30 ml H_2SO_4 1N. The solution is basified with sodium bicarbonate and extracted with chloroform. The chloroform extract is purified by c.c.d. in the Craig apparatus between chloroform and phosphate buffer at pH 6. After 200 transfers the product (revealed at the TLC analysis with ceric sulphate) is collected in tubes between 160 and 190. This fraction is basified with sodium bicarbonate and extracted with chloroform. The chloroform extract is stirred with a small volume of water, dried over sodium sulphate and evaporated. The residue, crystallised twice from benzene, melts at 166-8°.

IR bands (in chloroform) at 3700 and 3450 cm^{-1} due to the NH and OH groups. UV (in ethanol): λ_{max} at 303 nm (log ε 3.70) and 264 nm (log ε 3.80). MS fragmentation data are reported in Table I.

Some features of the NMR spectrum: δ 6.80 p.p.m. J 8.5 c.p.s., d (H-9); δ 6.31 p.p.m. J 2 and 8.5 c.p.s., q (H-10); δ 6.29 p.p.m., d (H-12). Analysis:

	found%:	C 69.94;	H 7.01;	N 8.18;
for $C_{20}H_{24}N_2O_3$	calcd. :	70.56;	7.11;	8.23.

α-*colubrine* (II) *from* 11-*methoxy W. G. Aldehyde* (III). — 0.2 g 11-methoxy W. G. aldehyde (III) are refluxed with malonic acid, acetic anhydride and sodium acetate according to the method of Anet and Robinson ([12]). The product crystallizes from benzene-hexane, mp 181-3°. This substance has the same Rf, IR spectrum in chloroform, UV spectrum in ethanol and NMR spectrum as α-colubrine.

A mixed melting point of the substance with a pure sample of α-colubrine is undepressed.

11-*methoxy,O-acetyl-diaboline B* (V) (*Isocondensamine*). — 0.15 g 11-methoxy-diaboline (I) are acetylated with pyridine and acetic anhydride for 48 h at 30°. The reactives are eliminated under high vacuum at room temperature. The acetylderivative is purified from the starting product by c.c.d. with the Craig apparatus between chloroform and ethyl acetate (7:3) and phosphate buffer pH 6.5. After 900 transfers, a homogeneous fraction is obtained in tubes 75 to 125, which after basification is extracted with chloroform. The residue, after evaporation, is crystallized three times from heptane and melts at 112-5°, M^+ 424, $[\alpha]_D^{20}$ + 36 (c 0.4 chloroform), UV spectrum: λ_{max} 298, 292 and 252 nm. MS fragmentation data are reported in Table I.

Analysis:

	found% :	C 67.80;	H 6.79;	N 6.44;
for $C_{24}H_{28}N_2O_5$	calcd. :	67.90;	6.65;	6.60.

11,11'-*dimethoxy-caracurine* V (VI). — 0.1 g 11-methoxy W. G. aldehyde (III) is heated in absence of air with acetic acid and sodium acetate according to the method proposed [16] for the dimerization of W. G. aldehyde (XI) to caracurine V (XV).

The product was purified by c.c.d. between chloroform and phosphate buffer pH 6. The fraction obtained after 200 transfers between tubes 20 and 60 (violet colour with ceric sulphate, negative to Dragendorff reagent) was basified with sodium bicarbonate and extracted with chloroform. The chloroform extract, after washing with water and evaporation, gives a residue which does not crystallise and is purified by dissolution in chloroform and precipitation with hexane. UV spectrum in ethanol: λ_{max} at 303 and 280 nm. IR spectrum in chloroform: absence of OH and NH bands. Mass spectrum: M^+ 644.

The authors are indebted to Mr G. Conti for the NMR spectra, to Mr M. Martinangeli for the mass spectra and to Mr G. Del Guercio for the ORD spectra.

[16] K. BERNAUER, F. BERLAGE, W. von PHILIPSBORN, H. SCHMID and P. KARRER, Helv. Chim. Acta, *41*, 2293 (1958).