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ALKALOIDS OF *STRYCHNOS RUBIGINOSA**

GIOVANNI BATTISTA MARINI-BETTOLO,† CORRADO GALEFFI,‡
MARCELLO NICOLETTI† and IRENE MESSANA†

† Cattedra di Chimica Generale ed Inorganica, Facoltà di Scienze, Università di Roma e Centro Chimica dei recettori e delle molecole biologicamente attive del C.N.R., Roma, Italy; ‡Laboratorio di Chimica del Farmaco, Istituto Superiore di Sanità, Roma, Italy

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Key Word Index—*Strychnos rubiginosa*; Loganiaceae; alkaloids; strychnorubigine; ^{13}C NMR.

Abstract—A new corynantheine-type alkaloid, strychnorubigine, was identified from root bark of *Strychnos rubiginosa* D.C. 11-Methoxydiaboline and normacusine B were also isolated and the ^{13}C NMR spectral data of their *O*-acetyl derivatives are reported.

INTRODUCTION

In a previous paper the presence of alkaloids in *Strychnos rubiginosa* D.C. was reported [1, 2] but subsequently a reexamination of the plant stated that the plant under study was *S. parvifolia* [3, 4]. From the present sample of root bark of *S. rubiginosa* D.C., Bahia (Brazil) (Harley 19142), supplied by B. A. Krukoff, three tertiary alkaloids **1–3** were isolated by counter-current distribution (CCD). Two of them, also present in the stem bark of the same plant, are already known, 11-methoxydiaboline (**1**), described by us for the first time in *S. romeu-belenii* [5] and normacusine B (**2**), also found by us in several South American *Strychnos* species [6] and also obtained from several plants of the family Apocynaceae. The third alkaloid is a new one and was named strychnorubigine (**3**).

RESULTS AND DISCUSSION

By CCD with discontinuously decreasing pH [7], 11-methoxydiaboline (**1**) ($K_1K_b = 6\times10^{-8}$), normacusine B (**2**) ($K_1K_b = 1.2\times10^{-8}$) and strychnorubigine (**3**) ($K_1K_b = 1.4\times10^{-9}$) were separated in that order. **1** and **2** were identified by direct comparison with authentic specimens. Alkaloid **3**, $C_{22}H_{28}N_2O_4$, mp 126–127°, M^+ *m/e* 384, showed a UV spectrum, λ_{max}^{EtOH} 227, 274, 284 and 293 nm (log ε 4.68, 3.91 *sh*, 3.84 *sh* and 3.84), typical of a 9-methoxy indolic alkaloid [8]. This structure could be expanded to that of a methoxytetrahydro-β-carboline on the basis of the MS peaks at *m/e* 214 (15%), 200 (41), 199 (37) and 186 (26). Corresponding peaks result from the A, B and C ring pattern of yohimbane-type alkaloids [9].

In the ^{1}H NMR spectrum ($CDCl_3$) of **3**, besides the H-12 (δ 6.46, 1H, *dd*, $J = 3$ and 8 Hz) and the aromatic OMe group (3.86, 3H, *s*), a propenilic group Me—CH=C (1.57, 3H, *d*, $J = 7$ Hz and 5.55, 1H, *q*, $J = 7$ Hz) and another methoxy group (3.55, 3H, *s*) were evident. The latter belongs to a carbomethoxy group (IR $\nu_{C=O}$ 1720 cm^{-1}), whereas the fourth oxygen of **3** belongs to an alcoholic function (IR ν_{OH} 3400 cm^{-1}), probably primary ($M^+ - 18$ at *m/e* 366 (80) and $M^+ - 31$ at *m/e* 353 (80)). In fact by acetylation with Ac_2O and Py, alkaloid **3** gave the O-acetyl derivative **4**, mp 96–97°, whose ^{1}H NMR spectrum ($CDCl_3$) showed, together with an additional acetyl group (1.95, 3H, *s*), the shift of two protons to lower fields (4.30, 2H, *m*). In the MS spectra of **3** and **4** the presence of the peak at *m/e* 281 (base peak) could be related to the loss of the fragment MeOOC—CH—CH_2OR (R = H for **3** and R = Ac for **4**) corresponding to the branching in the 15-position of the D-ring and to allylic position; by fission of the same bond and elimination of the γ-H to the carbonyl group, according to McLafferty rearrangement, the ($M^+ - 1$) ion gives rise to a peak at *m/e* 279. The corynantheine-type structure **3** could thus be attributed to strychnorubigine.

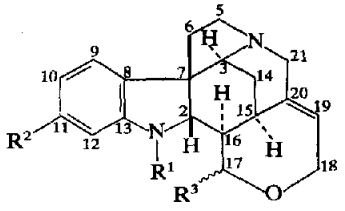
The absolute configuration H-3α was deduced from the positive Cotton effect of **4** [10] (first extremum at 296 nm, [Φ] = +5800), whereas the H-15α configuration could be suggested on the basis of biogenetic considerations of the C_{10} unit [11]. Therefore strychnorubigine may be considered the 9-methoxy derivative of isositzirikine (**5**), an alkaloid isolated from *Vinca rosea* Linn (Apocynaceae) [12]. A third natural representative of this group, found in *Vinca herbacea* W. K., is hervine (**6**), which is 11-methoxyisositzirikine [13].

The co-occurrence of the 3 different types of alkaloids (**1–3**) may suggest their biogenetic relationship. However, the hypothesis is partly confirmed by biosynthetic studies in *S. nux-vomica* L. [14], where a similar pattern of alkaloids (W. G. aldehyde (**7**) normacusine B (**2**) and geissoschizine (**8**)) is present.

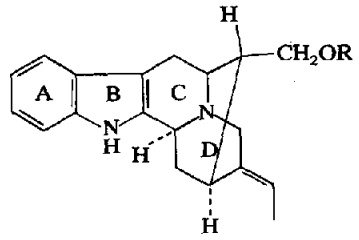
In Table 1, ^{13}C NMR spectral data of the *O*-acetyl derivatives of **1** and **2**, **9** and **10**, respectively, are reported.

The NMR analysis was carried out on compound **9**

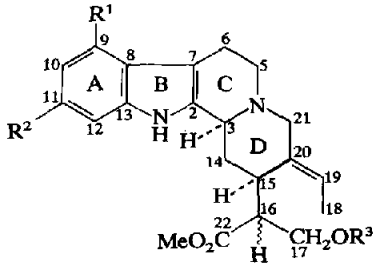
* Part XXXIII in the series "On the Alkaloids of *Strychnos*". For Part XXXII see Galeffi, C. and Marini-Bettolo, G. B. (1980) *Gazz. Chim. Ital.* (in press).



	R^1	R^2	R^3
1 11-Methoxydiaboline	Ac	OMe	OH
7 W. G. aldehyde	H	H	OH
9 Isocondensamine	Ac	OMe	OAc(α)
11 Henningsamine	Ac	H	OAc(β)
12 Diaboline	Ac	H	OH



2 Normacusine B, R = H
10 *O*-Acetylnormacusine B, R = Ac



	R^1	R^2	R^3	
3 Strychnorubigine	OMe	H	H	
4 *O*-Acetylstrychnorubigine	OMe	H	Ac	
5 Isositzirikine	H	H	H	
6 Hervine	H	OMe	H	
8 Geissoschizine	H	H	H	$\Delta^{16,17}$

(previously named isocondensamine [5]), having the α-configuration for the acetoxy group, in order to avoid the complicated pattern of signals due to the mixture of anomers in **1**. By comparison of the chemical shift values of **9** with those of henningsamine **11** (*N*-acetyldiaboline, β-OAc) [15], the differences for C(17) ($\Delta\delta = -8.5$ ppm), C(18) ($\Delta\delta = -2.5$ ppm) and C(15) ($\Delta\delta = -3.7$ ppm) suggested a boat conformation for **9** instead of a chair conformation for the oxepinic ring. However, two different conformations for the oxepinic ring were suggested for the two anomers of diaboline (**12**) [15]. The boat conformation in **9** places the acetoxy group in the preferred equatorial position.

Owing to the low solubility of alkaloid **2** in $CDCl_3$, the ^{13}C NMR spectrum of the corresponding acetyl derivative **10** (compound previously described by us [6]) was performed.

The combination of the conformation C/D *cis* (C in hemi-chair) with that of D in boat form shifts C(3) to higher fields than in a typical *cis*-quinolizidine structure [16]; however, the latter in the yohimbine series shows C(3) at a lower chemical shift value (54.4 ppm) than the *trans*-quinolizidine form (60.2 ppm). The other signals of **10** can be unambiguously assigned by comparison with corresponding ones in similar models.

EXPERIMENTAL

General. MS were recorded at 70 eV, ^1H NMR spectra at 90 MHz ($CDCl_3$ as solvent, TMS as internal reference).

Table 1. ^{13}C NMR Chemical Shifts*

	9	**10**
C(2)	64.7	134.9
C(3)	58.5	49.9
C(5)	51.2	54.5
C(6)	38.4	26.9
C(7)	57.6	103.9
C(8)	124.7	127.4
C(9)	121.4	117.9
C(10)	109.2	121.1
C(11)	159.3	119.1
C(12)	105.8	110.8
C(13)	139.7	136.3
C(14)	25.0	33.2
C(15)	29.5	27.6
C(16)	45.4	40.8
C(17)	93.6	66.1
C(18)	61.8	12.7
C(19)	122.7	116.9
C(20)	142.9	138.1
C(21)	53.1	55.9
NCOMe	169.2	
NCOMe	23.1	
OMe	55.3	
C=O	168.3	170.8
Me	20.9	20.9

* Values in ppm downfield from TMS; δ (TMS) = δ ($CDCl_3$) + 77.0 ppm.

^{13}C NMR spectra were recorded at 100 MHz ($CDCl_3$ as solvent, TMS as internal reference). Proton-noise decoupled resonance spectra were performed for exact determination of ^{13}C chemical shifts, and single-frequency off-resonance decoupled spectra for differentiation of carbon species. ORD were recorded with a Cary 60 spectrophotometer. For CCD a Craig apparatus model Post (200 stages, 10:10 ml v/v) was utilized. TLC was carried out on Si gel F_{254} plates using EtOAc–C_6H_6–Et_2NH (5:4:1). Alkaloids were detected with UV (254 nm) and Dragendorff reagent.

Material. Root bark (150 g) and stem bark (120 g) of *S. rubiginosa* (Harley 19142) were collected in the State of Bahia (Brazil) and classified by B. A. Krukoff. A voucher specimen is kept in the Herbarium of the N.Y. Botanical Garden.

Extraction. Powdered bark was eluted overnight ×3 with 2% aq. HOAc. The percolated liquids were made alkaline with $NaHCO_3$ and extracted twice with $CHCl_3$. The residue after evapn of the pooled extracts amounted to 1 and 0.3% of root bark and the stem bark, respectively. The presence of quaternary alkaloids in the aq. phase was established by precipitation with aq. picric acid, after acidification to pH 2 with 6% HCl. The picrates were converted into chlorides by the usual method, but their amount was insufficient for successful subsequent separation and characterization.

Separation. Root bark extract was submitted to separation by CCD between $CHCl_3$ and Pi–citric acid buffer (mobile phase) with discontinuously decreasing pH. The separation was monitored by TLC; alkaloids were extracted with $CHCl_3$ from the aq. phase after alkalinization with $NaHCO_3$. The following alkaloids were eluted separately: at pH 7 alkaloid **1** ($K_rK_b = 6\times10^{-8}$) (78 mg); at pH 6 alkaloid **2** ($K_rK_b = 1.2\times10^{-8}$) (60 mg); and at pH 5.4 alkaloid **3** ($K_rK_b = 1.4\times10^{-9}$) (49 mg). Likewise but in smaller amounts, alkaloids **1** and **2** were also isolated from the extract of stem bark.

Alkaloid **1**. Crystals from EtOAc–cyclohexane, mp 212–214°. The IR and MS spectra as well as the rotation were identical to those of 11-methoxydiaboline [5].

Alkaloid **2**. Crystals from EtOH–EtOAc, mp 245–248°. The IR, ^1H NMR and MS spectra as well as the ORD curve were identical to those of normacusine B [6].

Alkaloid **3**, *strychnorubigine.* Crystals from EtOAc–*n*-hexane, mp 126–127° (decomp.). (Found: C, 68.98; H, 7.06; N, 7.08. $C_{22}H_{28}N_2O_4$ requires: C, 68.72; H, 7.34; N, 7.29%). UV λ_{max}^{EtOH} (nm): 227,274, 284, 293 (log ε 4.68, 3.91 sh, 3.84 sh, 3.84). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 and 1720. ^1H NMR: δ 7.1–7 (2H, *m*, H-10 and H-11), 6.46 (1H, *dd*, $J = 3$ and 8 Hz, H-12), 5.55 (1H, *q*, $J = 7$ Hz, H-19), 3.86 (3H, *s*, MeO arom), 3.55 (3H, *s*, COOMe), 1.57 (3H, *d*, $J = 7$ Hz, Me-CH). MS *m/e* (%): 384 (83, M^+), 383 (80), 366 (80), 354 (65), 353 (80), 281 (100), 279 (60), 214 (15), 200 (41), 199 (37), 186 (26), 170 (37).

O-*Acetyl derivative of* **3**. Purified by CCD between $CHCl_3$ and buffer at pH 3.6; $K_rK_b = 8.8\times10^{-12}$. Crystals from EtOAc–*n*-hexane, mp 96–97°. (Found: C, 67.90; H, 7.41; N, 6.25. $C_{24}H_{30}N_2O_5$ requires: C, 67.58; H, 7.09; N, 6.57%). ^1H NMR: δ 8.46 (1H, *br* signal, NH), 7.1–7 (2H, *m*, H-10 and H-11), 6.48 (1H, *dd*, $J = 3$ and 8 Hz, H-12), 5.5 (1H, *q*, $J = 7$ Hz, H-19), 4.3 (2H, *m*, H-17), 3.84 (3H, *s*, MeO), 3.5 (3H, *s*, COOMe), 1.95 (3H, *s*, CH_2OCOMe), 1.52 (3H, *d*, $J = 7$ Hz, Me-CH). MS *m/e* (%) 426 (90, M^+), 425 (75), 396 (56), 395 (30), 383 (8), 357 (26), 355 (23), 281 (100), 279 (61), 251 (90), 214 (8), 200 (51), 199 (57), 186 (30), 170 (63). ORD (MeOH): 296 nm (first extremum, Φ = +5800).

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