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ON THE ALKALOIDS OF STRYCHNOS—XXXVI†‡

THE ALKALOIDS OF *STRYCHNOS HIRSUTA* SPRUCE EX BENTHAM; TWO NEW β-CARBOLINIC ALKALOIDS, STRYCHNOHIRSUTINE AND TETRADEHYDROSTRYCHNOHIRSUTINE

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Abstract—The structures and configurations of two new β-carbolinic alkaloids, strychnohirsutine **1** and tetradehydrostrychnohirsutine **2**, isolated from stem and root bark of *Strychnos hirsuta*, were reported. ^{1}H NMR data of N,O-diacetylstrychnohirsutine **5** and ^{1}H and ^{13}C NMR data of O-acetyltetradehydrostrychnohirsutine **4** were listed. Alkaloid **1** was converted into **2** by catalytic dehydrogenation.

In a survey of American Strychnos in 1942 Krukoff and Monachino[1] mentioned *Strychnos hirsuta* Spruce ex Bentham as occurring in British Guiana. Later King[2] reported the curarizing activity of this species from the same country.

In the course of our research on Strychnos alkaloids we examined two samples of *Strychnos hirsuta* from North Brazil. Two new alkaloids, **1**, $C_{19}H_{24}N_2O_2$, m.p. 141–3°, and **2**, $C_{19}H_{20}N_2O_2$, m.p. 221–3°, were isolated from stem and root bark of a specimen (Prance *et al.* 22104). The latter alkaloid was also isolated from another sample of *S. hirsuta*, J. Murça Pires 2638.

Alkaloid **2**, M^+ at *m/z* 308.1537 (calculated 308.1524) showed a multimaximum UV spectrum identical to that of alstonidine **3**, β-carbolinic alkaloid isolated from *Alstonia constricta* F. Muell. (Apocynaceae).[3] Alkaloid **1**, instead, showed an indolic UV spectrum, which undergoes hypsochromic shift with 0.05 N alcoholic HCl. This behaviour, typical of a tetrahydro-β-carbolinic structure, is due to hindered homoconjugation between N_b and indolic chromophore.[4] Alkaloid **1** named strychnohirsutine, gave alkaloid **2** by catalytic dehydrogenation with hydrogen transfer.[5] The latter alkaloid is therefore tetradehydrostrychnohirsutine. In its ^{1}H NMR spectrum in $CDCl_3$ the signals of a methine group (δ 4.75, m), of a secondary methyl group (1.28, d, J = 7 Hz) and of a methylenic group (3.98 and 4.05) are quite distinct. The last signals are AB part of an ABX system, $HOCH_2$–CH, whose chemical shifts move downfield in O-acetyl derivative **4**, $C_{21}H_{22}N_2O_3$, m.p. 133–5°.

^{1}H NMR spectrum of the last compound was particularly helpful for the assignment of nearly all signals and for structure determination. H-5 and H-6 are at δ 8.35 and 7.75 (d, J = 6 Hz), respectively. The former gives two broad lines because spin-lattice relaxation is only partially effective to average spin–spin splitting of the coupling with the directly attached nitrogen.[6] H-9 signal is at 8.10 (dd, J = 2 and 8 Hz), whereas three aromatic proton signals are between 7.2 and 7.6. The above mentioned secondary methyl group (1.36, d, 7 Hz) is coupled with a hydrogen at 4.13 (double quartet), which is, in turn, coupled (J = 2 Hz) with a hydrogen at 2.18 (multiplet). The latter is further coupled with a proton at 4.52 (J = 9) and one at 4.58 (J = 6). The two protons (AB part of a ABX system, having geminal coupling, J = 11 Hz) belong to the above mentioned alcoholic function, acetylated in 4. The signal of the acetyl group is at δ 2.02. As a confirmation of the sequence Me–CH–CH–CH_2O–Ac, by irradiation at δ 1.36 the signal at 4.13 becomes a doublet (J = 2)–a double doublet at 4.22, J = 4 and 12 Hz is beneath it—whereas by irradiation at 2.18 the signals at 4.52 and 4.58 become a simple AB system (J_{gem} = 11 Hz). The low field quintet at δ 4.83 (4.75 in **2**) is coupled with two geminal protons (J_{gem} = 12 Hz) at 3.46 (J = 11) and 4.22 (J = 4) and a proton at 2.92 (J = 4). The last one, in turn, is coupled with two geminal hydrogens (J_{gem} = 16) at 3.17 (J = 4) and 3.64 (J = 12). In fact by irradiation at δ 2.92 the signal at 3.64 becomes a doublet (J = 16), the signal at 4.83 misses one J = 4 coupling, whereas by irradiation at 4.22 the quintet at 4.83 becomes a double doublet (J = 4 and 11 Hz) and the signal at 3.46 becomes a doublet (J = 11). Thus the sequence –CH_2–CH–CH–CH_2– turns out unambiguously. All the hydrogens of **4** are now identified, and moreover no carbon atom of the aliphatic moiety is quaternary. The skeleton of **4** is therefore pentacyclic and its third oxygen is ethereal because no hydroxy group band is present in its IR spectrum. On the basis of their chemical shift values the following assignments are practicable: the methine at δ 4.83 is to be bonded to N_a; the methylenic group, H_a 3.17 and H_b 3.64, is to be bonded to the β-carboline ring (in 3 position), and finally the other methylenic group, H_a 3.46 and H_b 4.22, and the methine

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group at 4.13 are to be bonded to the ethereal oxygen. Two high field methynes are therefore bonded to one another: by double resonance it was impossible to ascertain the mutual coupling of their hydrogens (δ 2.92 and 2.18).

In confirmation of the above assignments and of the consistent structure **4** (see figure), H-15 shows long range coupling (CH–C–CH, J = 1 Hz) with H_b-17 (δ 4.22) and H-19. Both their signals narrow indeed by irradiation at 2.92 (H-15). On the basis of known bio-genetic considerations[7] of the indolic-isoprenic alkaloids the H-α configuration for C(15) of **4** was suggested. Same *H*-α configuration is to be for the chiral centre C(16) on account of the *cis* D/E ring junction (J_{15-16} = 4 Hz). Furthermore since in the CH–C–CH system effective coupling (J = 1–1.5 Hz) is confined to a planar zig-zag configuration  (hence the M or W or tail to tail expression[8]), H_b-17 (δ 4.22) and H-19 are to be α equatorial like H-15. The Me-18 group is therefore β-axial.

The configuration of the C-20 chiral centre could not be assigned on the basis of J_{19-20} value (J = 2 Hz, equatorial–equatorial or equatorial-axial relationship between H-19 and H-20). However the β-equatorial configuration in **4** for CH_2O–Ac could be assigned because, otherwise, a ring conformation having Me and CH_2O–Ac in trans diaxial relationship (and therefore H-19 and H-20 in *cis* diequatorial relationship) should be very improbable. The non-identity between J_{16-17b} (4 Hz) and J_{19-20} (2 Hz) could be explained by the strain of the pentacyclic system, which partially twists the chair conformation of E ring.

In the mass spectrum of tetradehydrostrychnohirsutine **2** peaks at *m/z* 249.1021 (Calc. for $C_{16}H_{13}N_2O^+$ 249.1027, 25%) and at *m/z* 205.0757 (Calc. for $C_{14}H_9N_2^+$ 205.0765, 100%) correspond—except for one hydrogen transfer—to fragmentations according to **a** and **b** dashes, respectively (see figure). In the mass spectrum of strychnohirsutine, **1** the molecular ion is base peak, whereas by retro Diels–Alder cleavage at C ring the peaks at *m/z* 283(78) and at *m/z* 180(94)—the latter with simultaneous loss of E ring-are generated.

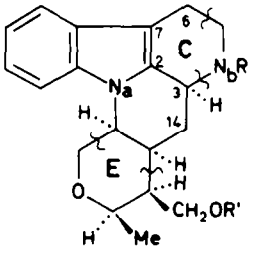
The IR spectrum of strychnohirsutine, **1** shows a band at 3500 cm^{-1} due to OH and NH groups, The band, of course, disappears in N,O-diacetylstrychnohirsutine, **5**. From ^1H NMR data of **4** and **5**, listed in Table 1, the identity of the D/E ring junction and of E ring conformation (J_{19-20} = 2 Hz, J_{16-17b} = 4 Hz) results.

The *cis* homoallilic coupling (J = 1 Hz) observed in **5** between one of the two hydrogens in 6 position and H-3 requires that they should be out of the plane which includes C(3), C(2), C(7) and C(6).[9]

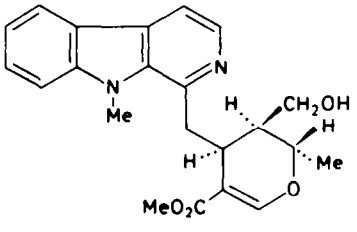
Assuming the validity of Klyne's rule to infer C(3) chiral centre configuration in yohimbane alkaloids[10] on the basis of ORD curves, the strong positive Cotton effect of **5** suggested the H-α configuration at C(3), as in the figure.

In Table 2 ^{13}C NMR chemical shifts of O-acetyltetradehydrostrychnohirsutine, **4**, are reported. The aromatic signals were assigned by comparison with the spectral data of carbazole, naphthalene and isoquinoline.[11]

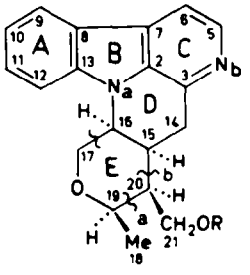
Strychnohirsutine and tetradehydrostrychnohirsutine may instead be considered related to alstonidine, **3**. The pentacyclic system of **1** and **2** is also present in talbotine **6**, isolated from another Apocynacea, *Pleiocarpa talbotii* Wernham,[12] but the biogenesis of the carbon system of ring E is different.



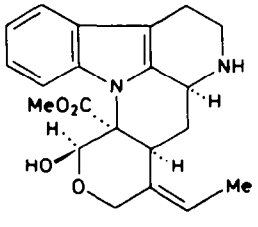
1 R = R' = H
5 R = R' = Ac



3 Alstonidine



2 R = H
4 R = Ac



6 Talbotine

Wavy lines indicate the mass fragmentation

Table 1. ^{1}H NMR assignments[a]

3				5.15	ddd	$J_{3-6}=1$; $J_{3-14a}=11$; $J_{3-14b}=5$
5	8.35	d[+]	$J_{5-6}=6$	3.03(2H)	m	
6	7.75	d		2.75(2H)	m	
9	8.10	dd	$J_{9-10}=8$; $J_{9-11}=2$	7.0–7.6		
10–12	7.2–7.6					
14a	3.17	dd	$J_{14a-15}=4$; $J_{gem}=16$	2.00	m	
14b	3.64	dd	$J_{14b-15}=12$	2.43	m	
15	2.92	m	$J_{15-16}=4$; $J_{15-17b}=1$; $J_{15-19}=1$	2.97	m	$J_{15-16}=5$
16	4.83	qu	$J_{16-17a}=11$; $J_{16-17b}=4$	4.60	qu	$J_{16-17a}=11$; $J_{16-17b}=4$
17a	3.46	dd	$J_{gem}=12$	4.09	dd	$J_{gem}=11$
17b	4.22	dd[b]		4.20	dd	
18(3H)	1.36	d	$J_{18-19}=7$	1.20	d	$J_{18-19}=7$
19	4.13	dq[b]	$J_{19-20}=2$	3.84	dq	$J_{19-20}=2$
20	2.18	m	$J_{20-21a}=9$; $J_{20-21b}=6$	1.97	m	
21a	4.52	dd	$J_{gem}=11$	4.42	dd	
21b	4.58	dd		4.49	dd	
MeCON				2.26	s	
MeCOO	2.02	s		2.12	s	

[a]Chemical shifts as δ, coupling constants in Hz, d = doublet, dd = double doublet, dq = double quartet, m = multiplet, qu = quintet, s = singlet.
[b]Broadened.

Table 2. Assignments of ^{13}C NMR spectrum of 4[a]

C(2)	140.4[b]	C(7)	126.2	C(11)	120.6	C(15)	32.9	C(19)	75.7
C(3)	140.6[b]	C(8)	121.5	C(12)	109.0	C(16)	47.1	C(20)	42.5
C(5)	136.0	C(9)	113.7	C(13)	140.4[b]	C(17)	70.4	C(21)	61.8
C(6)	129.2	C(10)	123.0	C(14)	28.6	C(18)	18.3	C=0	172.8
								Me	21.0

[a]In parts per million downfield from Me_4Si: $\delta(Me_4Si)=\delta(CDCl_3)+77.0$ ppm.
[b]These assignments may be reversed.

EXPERIMENTAL

A Craig Post apparatus (200 stages, 10:10 ml, upper and lower phase) was used for countercurrent distribution (CCD). The separation was monitored by tlc analysis on silica gel HF_{254} (solvent, benzene, AcOEt, $NHEt_2$ 7:2:1). Conventional mass spectra and exact mass measurements were obtained on an AEI 902 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with a Varian XL 100 (using $CDCl_3$ as solvent and TMS as internal standard) whereas ORD curve was recorded with a Cary 60 spectrophotometer.

Materials. Two samples of *Strychnos hirsuta* Spruce ex Bentham, i.e. J. Murça Pires 2638, root and stem bark, and Prance *et al.* 22104, leaves and root and stem bark, were collected in North Brazil and identified by Dr. B. A. Krukoff. A voucher specimen is kept in New York Botanical Garden.

Extraction. The dried material was powdered and eluted with 2% aqueous AcOH until negative Dragendorff reaction occurred. The liquid was made alkaline with $NaHCO_3$ and extracted twice with $CHCl_3$. The pooled extracts, after drying over Na_2SO_4 and evaporation, yielded a residue which amounted to 0.1% of the starting material for stem bark and to 0.3% for root bark, approximately. The residue from leaves was negative to Dragendorff reagent and not further examined. In all aqueous phases quaternary alkaloids were absent.

Separation. The extract was submitted to CCD between $CHCl_3$ and phosphate–citric acid buffer (mobile phase). Alkaloids were extracted with $CHCl_3$ from the aqueous phase after alkalinization with $NaHCO_3$. From the basic extract of root and stem bark of *S. hirsuta* J. Murça Pires 2638 at pH 2.6 alkaloid **2**, $K_rK_b=6.3\times10^{-12}$,[13] was isolated (0.09 and 0.03% of the drug, respectively). From the basic extract of root and stem bark of *S. hirsuta* Prance *et al.* 22104 at pH 6 alkaloid **1**, $K_rK_b=1.5\times10^{-8}$ (0.10 and 0.02%, respectively) and at pH 2.6 alkaloid **2** (0.06 and 0.02%, respectively) were isolated.

Alkaloid 1; *strychnohirsutine.* Crystals from AcOEt and n-hexane, m.p. 141–3°, UV (EtOH), λ_{max}: 228, 276, 284, 293(sh) nm (log ϵ: 4.51, 3.84, 3.86, 3.75), in 0.05 N ethanolic HCl: 224, 274, 280, 290(sh) nm (log ϵ: 4.47, 3.80, 3.79, 3.65); IR ($CHCl_3$), ν_{max}: 3500 cm^{-1}, $[\alpha]_D^{20}=-6.3$ (c = 0.5, $CHCl_3$), M^+ at *m/z* 312 (Found: C, 73.34; H, 7.39; N, 8.80. Calc. for $C_{19}H_{24}N_2O_2$: C, 73.04; H, 7.74; N, 8.97%).

Alkaloid 2; *tetradehydrostrychnohirsutine.* Crystallizes with difficulty from ethyl ether in refrigerator, m.p. 221–3°, UV (EtOH), λ_{max}: 240, 254, 282, 291, 346, 360 nm (log ϵ: 4.68, 4.38, 3.99, 4.22, 3.77, 3.82), in 0.05 N ethanolic HCl: 252, 304, 385 nm (log ϵ: 4.50, 4.24, 3.68). The solutions of the alkaloid are fluorescent. M^+ at *m/z* 308.1537 (Found: C, 74.26; H, 6.39; N, 8.98. Calc. for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N, 9.09%).

Dehydrogenation of strychnohirsutine **1**. Palladium black (50 mg) was added to an aqueous solution (20 ml) of alkaloid **1** (100 mg) and of maleic acid (200 mg) and the suspension was refluxed 3 hr. Further palladium black (50 mg) and maleic acid (100 mg) were added and the suspension was refluxed 1 hr more. The catalizer was removed by filtration, the solution was made alkaline with $NaHCO_3$ and extracted with $CHCl_3$. The residue of the organic phase was purified by CCD between $CHCl_3$ and buffer at pH 2.6 and the obtained compound was identified as tetradehydrostrychnohirsutine, **2**, after crystallization from ethyl ether.

O-Acetyltetradehydrostrychnohirsutine **4**. Alkaloid **2** (200 mg) was acetylated with a mixture of pyridine and Ac_2O (4 ml, 1:1 v/v). After 2 days the reagents were evaporated and the residue was purified by CCD between CCl_4 and cyclohexane (3:2 v/v) and buffer at pH 3.6, $K_rK_b=4.5\times10^{-11}$, m.p. 133–5° (from cyclohexane),

MS, *m/z* (%): 350 (100), 307 (10, metastable peak at 269), 291 (24, metastable peak at 242), 249 (14), 205 (78). (Found: C, 71.49; H, 6.17; N, 7.75. Calc. for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 8.00%).

N,O-Diacetylstrychnohirsutine **5**. Alkaloid **1** (200 mg) was acetylated with a mixture of pyridine and Ac_2O (5 ml, 1:1 v/v). After 2 days the reagents were evaporated and the residue was purified by column chromatography (silica gel, solvent CH_2Cl_2, AcOEt 9:1 v/v). Crystals from benzene and n-hexane, m.p. 114–5°, UV (EtOH), λ_{max}: 225, 274, 280, 290 nm (log ϵ: 4.48, 3.81, 3.84, 3.70); MS, *m/z* (%): 396 (100), 353 (37), 325 (23), 282 (21), 180 (50); ORD (MeOH), [ϕ] (λ_{max}, nm): +6900 (302), first extremum. (Found: C, 69.75; H, 7.02; N, 7.06. Calc. for $C_{23}H_{28}N_2O_4$: C, 69.67; H, 7.12; N, 7.07%).

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