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ON THE ALKALOIDS OF STRYCHNOS—XXXI†

15-HYDROXYSTRYCHNINE, A NEW ALKALOID FROM *STRYCHNOS NUX VOMICA* L

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Abstract—The structure of a new alkaloid, the 15-hydroxystrychnine 12, isolated from seeds of *Strychnos nux vomica* L., has been established by analysis of spectroscopic data of 12 and of its O-acetyl derivative 13. The ^{13}C NMR spectrum of the O-acetyl derivative is in agreement with the proposed structure.

In a previous note[1] a new method was described for the separation of alkaloids by countercurrent distribution (ccd) at discontinuously decreasing pH, which made it possible to separate from the raw alkaloid mixture of *S. nux vomica* L. seeds nine known substances: strychnine 1, α- and β-colubrine 2 and 3, brucine 4, pseudostrychnine (= 3-hydroxystrychnine) 5, pseudobrucine (= 3-hydroxybrucine) 6, icajine 7, vomicine 8 and novacine 9, together with four new alkaloids. The structures of three of the latter were established as 3-hydroxy-α-colubrine 10, 3-hydroxy-β-colubrine 11[2] and isostrychnine.[3]

We report now the structure determination of the fourth alkaloid 12 (Fig. 1), which is also the most polar ($K_rK_b = 2 \times 10^{-9}$).

Compound 12, $C_{21}H_{22}N_2O_3$, m.p. 204–6° (crystals from EtOAc, $[\alpha]_D^{20} = -192.7$ ($c = 0.4$, $CHCl_3$), shows, in the IR spectrum ($CHCl_3$), bands of an OH group at 3460 cm^{-1} and of a δ-lactam ring at 1660 cm^{-1}. The UV spectrum (EtOH) is typical for an N-acyl-indoline (λ_{max}: 255, 280, 291 nm (log ϵ: 4.08, 3.60, 3.49)) and it is not modified by addition of NaOH. The mass spectrum of 12 shows peaks at *m/e* (%): 350 (M^+, 100), 333 (10), 178 (15), 144 (4), 143 (4), 130 (4); the last three peaks are characteristic of the sequence indoline–β–CH_2–CH_2–N_b.

In the ^1H NMR spectrum of 12 (Table 1) four signals quite typical and commune to strychnine structure 1 are present, i.e. at δ 8.07 (1H, d, J = 8 Hz, H–12), δ 5.88 (1 H, t, J = 6 Hz, H–19), δ 4.76 (1 H, ddd, J = 3, 3 and 8 Hz, H–17) and δ 4.15 (1 H, dd, J = 6 and 14 Hz, H–18_b).

From the above data it is possible to attribute to the alkaloid 12 the structure of a hydroxy derivative of 1.

In the mass spectrum of 12, the presence of the peak at *m/e* 178.080, ($C_{10}H_{12}NO_2^+$), corresponding to the right moiety of the molecule (Fig. 1), except for one hydrogen, instead of the corresponding peak at *m/e* 162 in 1 ($C_{10}H_{12}NO^+$), suggests that the OH group of 12 is in that fragment of the molecule. Moreover, the absence of new signals at relatively low field in the ^1H NMR spectrum of 13, the O-acetyl derivative of 12‡ (Table 1), suggests that the OH group in 12 is tertiary and probably allylic on account of the presence, in the mass spectrum of 12, of the ion at *m/e* 333 (10).

From the comparison of the ^1H NMR spectrum of 12 with that of 1[4] it is possible to assign unequivocally to the OH group the position 15 on the basis of the following considerations:

(i) The deshielding (0.49 ppm) for H–17 of 12, due to the 1–3 diaxial interaction with the OH group;

(ii) The loss of the coupling between H–15 and H–16 and between $H\text{–}14_{a,b}$ and H–15, observed in 1;

(iii) The loss of the homoallylic coupling between $H\text{–}18_a$ and H–15, present in 1.

In effect the ^1H NMR spectrum of 12 shows H–16 (δ 1.45) as a dd ($J_{2,16} = 10$ Hz and $J_{16,17} = 3$ Hz), H–19 (δ 5.88) as a dd reducible to a triplet (J = 6 Hz), whereas $H\text{–}14_b$ (δ 2.29) appears as a dd ($J_{gem} = 14$ Hz and $J_{3,14b} = 4$ Hz). Moveover the other signals reported in Table 1 for 12 and 1 are similar.

Further confirmation of the 15 position of the OH group was obtained by irradiation at δ 4.76 (H–17), which modified H–16 into a doublet ($J_{2,16} = 10$ Hz) and simultaneously the signals of H_a- and H_b-23 into a simple AB system. The irradiation at δ 1.45 (H–16) converted H–2 in a singlet and simplified H–17 into a dd ($J_{17,23a} = 8$ Hz and $J_{17,23b} = 3$ Hz).

Contrary to N-acetylindolinic alkaloids, 12 and 1 do not show any Cotton effect at 280 nm, which could be correlated to the absolute configuration of the chiral centres C–2 and C–7. However for these centres, as well as for the others, a configuration identical to that of 1 must be admitted. In fact the value of the rotary power of strychnine ($[\alpha]_D^{20} = -145$, $CHCl_3$) is in the same range of those of 12 and 13 ($[\alpha]_D^{20} = -151$ ($c = 0.9$, $CHCl_3$)).

The values of the chemical shifts of the ^{13}C NMR spectrum of 13 are reported in Table 2 and related to

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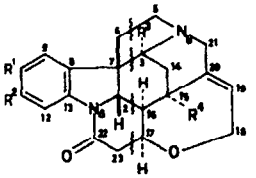
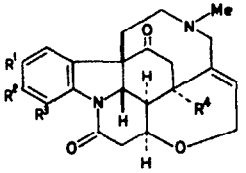
‡Compound 13 on mild alkaline conditions regenerated the alkaloid 12.

Wavy lines indicate the mass fragmentation

		R^1	R^2	R^3	R^4
1,	strychnine	H	H	H	H
2,	α-colubrine	H	OCH_3	H	H
3,	β-colubrine	OCH_3	H	H	H
4,	brucine	OCH_3	OCH_3	H	H
5,	pseudostrychnine	H	H	OH	H
6,	pseudobrucine	OCH_3	OCH_3	OH	H
10,	3-hydroxy-α-colubrine	H	OCH_3	OH	H
11,	3-hydroxy-β-colubrine	OCH_3	H	OH	H
12,	15-hydroxystrychnine	H	H	H	OH
13,	15-acetoxystrychnine	H	H	H	OAc





		R^1	R^2	R^3	R^4
7,	icajine	H	H	H	H
8,	vomicine	H	H	OH	H
9,	novacine	OCH_3	OCH_3	H	H
14,	15-hydroxyicajine	H	H	H	OH

Fig. 1.

Table 1. ^1H NMR spectra assignments[a]

Compound (solvent)	12 ($CDCl_3$)	12 (C_5D_5N)	1[b] ($CDCl_3$)	13 ($CDCl_3$)
H-2	3.89, d $J_{2,16}=10$	3.95	3.85, d $J_{2,16}=10$	4.05
H-3	ov.	ov.	3.92	4.12
H-5a	ov.	ov.	2.86	ov.
H-5b	3.20, m	3.11	3.18, ddd	ov.
H-6a and H-6b	1.8–2.0	1.75–1.9	1.87	1.85–2.0
H-9			7.14	
H-10	7.0–7.25	7.0–7.2	7.08	7.0–7.3
H-11			7.23	
H-12	8.07, d $J_{11,12}=8$	8.45	8.08, d $J_{11,12}=8$	8.08
H-14a	1.62, dd $J_{3,14a}=2$ $J_{14a,14b}=14$	1.54	1.43, ddd $J_{3,14a}=2$ $J_{14a,14b}=14$ $J_{14a,15}=2$	1.53
H-14b	2.29, dd $J_{3,14b}=4$	ov.	2.34, ddd $J_{3,14b}=4$ $J_{14b,15}=4$	2.18
H-15	—	—	3.13, dddd $J_{15,16}=3$ $J_{15,18a}=2$	—
H-16	1.45, dd $J_{16,17}=3$	1.50	1.25, ddd $J_{16,17}=3$	1.64
H-17	4.76, ddd $J_{17,23a}=8$ $J_{17,23b}=3$	4.89	4.27, ddd $J_{17,23a}=8$ $J_{17,23b}=3$	4.73
H-18a	ov.	4.05, m	4.05	4.16
H-18b	4.15, dd $J_{18a,18b}=14$ $J_{18b,19}=6$	4.10	4.13, dd $J_{18a,18b}=14$ $J_{18b,19}=7$	4.34
H-19	5.88, t $J_{18a,19}=6$	5.90	5.88, ddd $J_{18a,19}=6$ $J_{19,21b}=1$	5.97
H-21a	2.70, d $J_{21a,21b}=15$	2.58	2.71, d $J_{21a,21b}=15$	2.79
H-21b	ov.	3.78, d	3.69, dd	ov.
H-23a	3.06, dd $J_{23a,23b}=17$	3.32	3.10, dd $J_{23a,23b}=17$	3.18

Table 1. (*contd.*)

H-23b	2.73, dd	2.46	2.66, dd	2.70
Me				2.10

(a) chemical shifts as δ, coupling constants in Hz; d=doublet, dd=double doublet, ddd=double double doublet, dddd=double double double doublet, t=triplet, m=multiplet, ov.=signal overlapped.

(b) spectral parameters reported by Carter et al.[4] (approximate figures).

those of 1.[5] The α effect (+46 ppm) on C-15, due to the introduction of the acetoxy group, as well as the β effect on C-14 (+3.8 ppm) and C-16 (+2.4 ppm) are quite evident. Also the shielding of C-17 (−3.3 ppm) due to the 1-3 diaxial interaction with the OH group in C-15 confirms the effect observed in the ^{1}H NMR spectroscopy.

In the series of strychnine (the most abundant alkaloid present in *S. nux vomica* L.) this is the first time that substitution in the 15 position has been found. We now recall that from another Strychnes species, *S. icajia* Baill,[6] the alkaloids of the so-called N-methyl-pseudostrychnine type were isolated, i.e. icajine 7 and its 15-hydroxy derivative 14. The modifications of the ^{1}H NMR spectra

Table 2. ^{13}C NMR spectra assignments[a]

Compound	13	1 [c]		13	1
C(2)	61.3[b]	59.9[b]	C(15)	77.4	31.4
C(3)	62.6[b]	59.8[b]	C(16)	50.4	48.0
C(5)	51.7	50.1	C(17)	74.0	77.3
C(6)	42.5	42.6	C(18)	64.0	64.3
C(7)	51.5	51.7	C(19)	131.6	126.8
C(8)	131.2	132.4	C(20)	137.7	140.2
C(9)	122.2	121.9	C(21)	54.0	52.4
C(10)	124.3	123.8	C(22)	168.6	168.0
C(11)	128.7	128.1	C(23)	42.5	42.2
C(12)	116.1	115.8	C=O	168.6	
C(13)	142.2	141.8	Me	21.3	
C(14)	30.5	26.7			

[a] In parts per million downfield from Me_4Si: $\delta(Me_4Si) = \delta(CDCl_3) + 77.0$ ppm.

[b] Within a given column these assignments may be interchanged.

[c] Assignements reported by Wenkert et al[5].

between these two substances are analogous to those observed between 1 and 12.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded with a Varian XL 100 (using $CDCl_3$ as solvent, if not differently reported and TMS as internal standard). Conventional mass spectra were obtained on an LKB 9000 S spectrometer and exact mass measurement on an LKB 2091 with data system. Tlc analysis was performed on silica gel HF_{254} (solvent, $CHCl_3$, t-BuOH, $NHEt_2$ 7:2:1) and the spots revealed using the Draghendorff reagent.

Material. The alkaloid 12 (indicated in the previous work[1] as X_1) was isolated from the mother liquor of strychnine sulphate obtained from Sandoz firm (Milan, Italy). The product was not an artefact; in fact the presence of this alkaloid in the *S. nux vomica* L. seeds was confirmed by chromatographic analysis.

Alkaloid 12:15-*hydroxystrychnine.* The alkaloid was purified by CCD between $CHCl_3$ and phosphate buffer at pH 6.4 ($K_aK_b = 2 \times 10^{-8}$); crystals from AcOEt, m.p. 204–6°; UV (EtOH), λ_{max}: 255, 280, 291 nm (log ε: 4.08, 3.60, 3.49); IR ($CHCl_3$), ν_{max}: 3460 and 1660 cm^{-1}; $[\alpha]_D^{20} = -192.7$ ($c = 0.4$, $CHCl_3$); MS, *m/e* (%): 350 (M^+, 100), 333 (10), 178 (15), 144 (4), 143 (4), 130 (4), (Found: 178.080. $C_{10}H_{12}NO_2^+$ requires: 178.087; Found: C, 71.90; H, 6.18; N, 7.91. Calc. for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 8.00%).

15-*Acetoxystrychnine* 13. Compound 12 (100 mg) was acetylated with a mixture of pyridine and Ac_2O (3 ml, 1:1 v/v) and the soln allowed to stand for 7 days, until the reaction was complete. The reagents were evaporated and the residue was purified by CCD between $CHCl_3$ and phosphate–citric acid buffer at pH 3.8 ($K_aK_b = 5.4 \times 10^{-10}$). Crystals from AcOEt and *n*-hexane (76 mg), m.p. 198–201°; $[\alpha]_D^{20} = -151$ ($c = 0.9$, $CHCl_3$); MS, *m/e* (%): 392 (M^+, 68), 349 (9), 334 (25), 333 (100, strong metastable peak at *m/e* 283), 144 (58), 130 (23). (Found: C, 70.55; H, 6.06; N, 7.10. Calc. for $C_{23}H_{24}N_2O_4$: C, 70.39; H, 6.16; N, 7.14%).

Saponification of 15-*acetoxystrychnine.* Compound 13 (20 mg) dissolved in MeOH (3 ml) was added to 5% $KHCO_3$ aq (5 ml). After 10 days the mixture was diluted with water and extracted with $CHCl_3$. After purification by CCD ($CHCl_3$ and buffer at pH 6.4) and crystallization from AcOEt, 12 (11 mg) was obtained; the product was confirmed by comparison of m.p., rotatory power and by tlc analysis.

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