STRUCTURE OF THE ALKALOID VERACINTINE*

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Veracintine [20-(2-methyl-1-pyrrolin-5-yl)-5-pregnen-3β-ol], the constitution of which we have now confirmed by ^{13}C-NMR spectrometry, yielded when acetylated 21-acetamido-3β-acetoxy--20-methyl-21-(3-oxobutyl)-5-pregnene in addition to N,O-diacetyl derivative. The formation of the former was explained by the acetolysis of the isomerized heterocyclic moiety of the molecule to the corresponding enol *IIIb* and its rearrangement to a keto form *IIIa*.

The structural formula of veracintine (*I*) proposed on the basis of mass, infrared and ^{1}H-NMR spectral evidences of this alkaloid and its derivatives[1,2] was now confirmed by the ^{13}C-NMR spectrum. The acetylation of *I* with acetic acid anhydride in pyridine led, in addition to the already reported N,O-diacetyl derivative *II* (*cf.*[1,2]), to a compound of molecular formula $C_{30}H_{47}NO_4$ (*IIIa*). Its mass spectrum showed peaks at *m/e* 485 (M^+), 425, 223, 149 and 142 (base peak). The base peak was found by 18 mass units greater than that of N,O-diacetylveracintine. The formation of this compound is explained by an isomerization of the $C_{(25)}$=N double bond to $C_{(24)}$, analogously as with N,O-diacetyl derivative *II*, followed by an acetolysis resulting in the opening of the pyrroline ring. The enol *IIIb* thus formed underwent rearrangement to the more stable keto form *IIIa*.

The ^{1}H-NMR spectrum of *IIIa* (in p.p.m. at the δ scale) revealed 3 protons at 0·68 (s, $C_{(18)}$-methyl group), 3 protons at 1·00 (s, $C_{(19)}$-methyl group), 3 protons at 0·93 (d, J = 7 Hz, a methyl group at $C_{(20)}$) and 3 × 3 protons at 1·95, 2·00, 2·10 (singlets, 3 acetyl groups). The multiplet centred at 4·00 was ascribed to a proton at $C_{(21)}$, that at 4·6 to a proton at $C_{(3)}$ in the neighbourhood of an acetyl group and the AB doublet centred at 5·30 to a vinyl proton at $C_{(6)}$.

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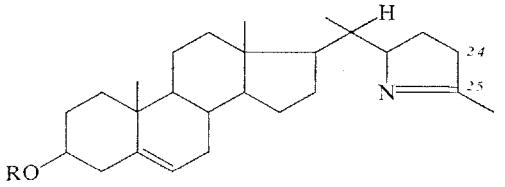
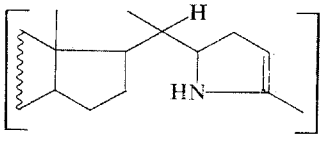
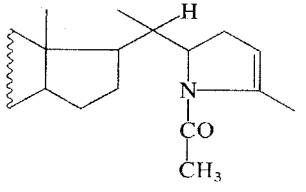
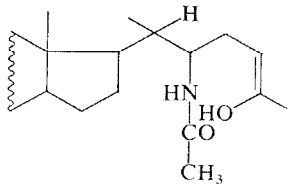


I, R = H



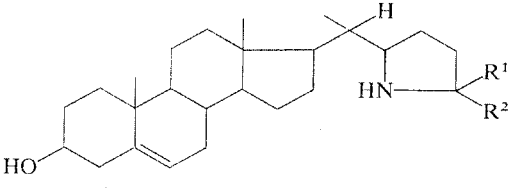


II, R = Ac

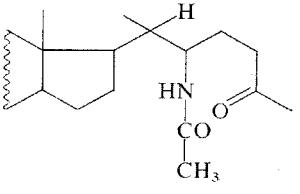


IIIb, R = Ac

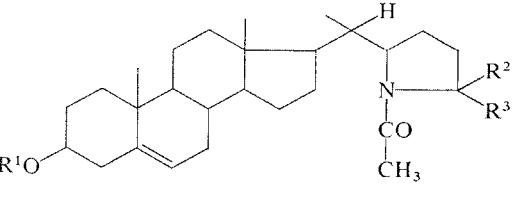
$NaBH_4$



Va, R^1 = H, R^2 = CH_3
Vb, R^1 = CH_3, R^2 = H



IIIa, R = Ac
IV, R = H



VI, R^1 = Ac, R^2 = H, R^3 = CH_3
VII, R^1 = H, R^2 = H, R^3 = CH_3

The infrared spectrum of this substance displayed absorption bands characteristic of an O-acetyl (1245 and 1730 cm^{-1}) and an N-acetyl (1675 cm^{-1}) group. A further band at 1715 cm^{-1} proved the presence of a newly formed carbonyl group. Basing upon arguments just presented a semisystematic name 21-acetamido-3β-acetoxy--20-methyl-21-(3-oxobutyl)-5-pregnene (*IIIa*) should be given to this derivative. The amide *IV* prepared from N,O-diacetyl ketone *IIIa* by heating in an aqueous--methanolic sodium hydrocarbonate solution displayed in its mass spectrum peaks at *m/e* 443 (M^+), 428 (M−15), 425 (M−18), 386 (M−57), 372 (M−71) and 142 (base peak). The absorption band of a hydroxyl group in the infrared spectrum at 1055 and 3360 cm^{-1} appeared instead of the O-acetyl vibration. The positions of signals of $C_{(18)}$- and $C_{(19)}$-methyl groups, a methyl group at $C_{(20)}$ and two acetyl groups in the ^{1}H-NMR spectrum remained virtually unchanged. The singlet of 3 protons at 2·00 (an O-acetyl group) disappeared and a multiplet at 3·5 (a proton at $C_{(3)}$ adjacent to a hydroxyl group) appeared.

Two stereoisomeric dihydro derivatives of R_F 0·22 (*Va*) and 0·14 (*Vb*) were obtained by sodium borohydride reduction of the azomethine double bond of veracintine. Compound *Va* was found to be identical with the dihydro derivative prepared by catalytical hydrogenation of veracintine in ethanol.

N,O-Diacetyldihydroveracintine (*VI*) prepared by acetylation of 25-dihydroveracintine (*Va*) displayed in the mass spectrum peaks at *m/e* 469 (M^+), 454 (M−15), 425, 84 and 126 (base peak); the latter was attributed to a species resulting from a bond cleavage between the steroid and heterocyclic moieties of the molecule. The infrared spectrum of substance *VI* showed absorption bands of an O-acetyl (1250 and 1725 cm^{-1}) and an N-acetyl (1620 cm^{-1}) group. The ^{1}H-NMR spectrum revealed signals of 3 protons at 0·67 (s, $C_{(18)}$-methyl group), 0·91 (d, J = 8 Hz, $C_{(21)}$-methyl group), 0·98 (s, $C_{(19)}$-methyl group), 1·25 (d, J = 8 Hz, $C_{(26)}$-methyl group), 2·01 and 2·05 (singlets, O-acetyl and N-acetyl groups), 3·95 (m, a proton at $C_{(22)}$), 4·5 (m, a proton at $C_{(3)}$ adjacent to an acetyl group). The AB doublet at 5·35 evidenced that the $C_{(5)}$ double bond was not reduced with sodium borohydride.

The hydrolysis of compound *VI* furnished substance *VII*. Its mass spectrum exhibited peaks at *m/e* 427, 412 (M−15), 409 (M−18), 84 and 126 (base peak). The infrared spectrum was characteristic of an N-acetyl group (1620 cm^{-1}), the ^{1}H-NMR spectrum indicated the presence of two methyl groups at 0·67 and 0·98 (singlets, $C_{(18)}$- and $C_{(19)}$-methyls), two methyl groups at 0·91 and 1·25 (doublets, J = 8 Hz each, $C_{(21)}$- and $C_{(26)}$-methyls) and one N-acetyl group at 2·05 (s). The multiplet associated with the proton adjacent to a hydroxyl group at $C_{(3)}$ was upfield shifted to 3·5; the position of another multiplet ascribed to the $C_{(22)}$-proton was the same as with the N,O-diacetyl derivative *VI*.

The ^{13}C-NMR spectrum of veracintine (Table I) was in favour of the structure proposed.

From the biological considerations and from papers published[3] so far we suppose that the methyl group at $C_{(20)}$ is like to have an *S* configuration, whilst the $C_{(17)}$-side chain of pregnane is β-oriented.

EXPERIMENTAL

The melting points were determined on a Kofler micro hot-stage, the optical rotations with a Bendix-Ericson 143 D apparatus; mass spectra were measured with an AEI-MS 902 spectrometer, ^{1}H-NMR spectra with a Tesla BS 487 B, ^{13}C-NMR spectra with a Bruker HX 90 E FT spectrometers both in deuteriochloroform, tetramethylsilane being the internal reference substance. The applied frequency in the former spectrum was 80 MHz, in the latter 22·63 MHz. The solvent system for TLC chromatography (alumina Woelm, neutral, dried at room temperature for 24 h) S_1 benzene–ethanol (98 : 2) and S_2 benzene–ethanol (9 : 1).

N,O-Diacetylveracintine (*II*) and 21-acetamido-3β-acetoxy-20-methyl-21-(3-oxobutyl)-5-pregnene (*IIIa*)

A solution of veracintine (100 mg) in pyridine (3 ml) and acetic acid anhydride (2 ml) was heated under reflux condenser on a steam bath for 30 min. The reaction mixture was evaporated under diminished pressure and the residue (126 mg) chromatographed over alumina Reanal (5 g, neutral., activity grade II). The amorphous N,O-diacetylveracintine (*II*), R_F 0·73 (S_1), $[\alpha]_D^{25}$ −98° (*c* 1·0, chloroform) was obtained by elution with benzene. Mass spectrum *m/e* 467 (M^+), 407, 150, 124, 108, 82. Elution with benzene–ethanol (99 : 1) afforded 21-acetamido-3β-acetoxy-20-methyl-21-(3-oxobutyl)-5-pregnene (*IIIa*), R_F 0·37 (S_1), m.p. 165–168°C (acetone), $[\alpha]_D^{23}$ −28·8° (*c* 1·0, chloroform).

21-Acetamido-3β-hydroxy-20-methyl-21-(3-oxobutyl)-5-pregnene (*IV*)

An aqueous $NaHCO_3$ solution (26 mg, 3 ml) was added to a solution of substance *IIIa* (26 mg) in methanol (5 ml) and heated under reflux condenser on a steam bath for one hour. The solvent was distilled off *in vacuo*, the residue diluted with water and extracted with chloroform (5 . 10 ml). The chloroform extract was washed with water, filtered and evaporated. Yield 16 mg, R_F 0·22 (S_1), m.p. 254–255°C (methanol), $[\alpha]_D^{22}$ −80° (*c* 0·14, ethanol).

TABLE I

Chemical Shifts (in p.p.m. relative to TMS) in the ^{13}C-NMR Spectrum of Veracintine

C 1	37·7	C 8	31·8	C 15	24·1	C 22	74·6
2	31·8	9	50·6	16	28·4	23	26·6
3	71·0	10	36·4	17	53·6	24	39·2
4	42·2	11	21·3	18	12·6	25	174·3
5	140·9	12	39·7	19	19·2	26	19·2
6	120·8	13	42·2	20	41·2		
7	31·8	14	56·8	21	11·3		

Dihydroveracintine *Va, Vb*

Ethyl acetate (1 ml) and $NaBH_4$ (80 mg) were added to a methanolic solution of veracintine (120 mg, 10 ml). The solution was allowed to stand at room temperature overnight, concentrated to about 3 ml, diluted with water, acidified with 5% H_2SO_4, made alkaline with ammonia and extracted with chloroform. The chloroform extract was evaporated and the residue (122 mg) chromatographed over alumina (5·5 g Reanal, neutr., activity grade IV). Elution with benzene afforded *Va*, R_F 0·22 (S_2), m.p. 178–180°C (acetone), $[\alpha]_D^{23}$ −35·4 (*c* 0·93, ethanol), with benzene-methanol (199 : 1) substance *Vb*, R_F 0·14 (S_2), m.p. 165–168°C (acetone), $[\alpha]_D^{23}$ −40° (*c* 0·9, ethanol).

N,O-Diacetyldihydroveracintine *VI*

A solution of dihydro derivative *Va* (22 mg) in pyridine (1 ml) and acetic acid anhydride (1 ml) was reacted for 18 hours at room temperature, evaporated and the residue (25 mg) crystallized from acetone. R_F 0·63 (S_1), m.p. 184°C, $[\alpha]_D^{22}$ −58° (*c* 0·58, ethanol).

N-Acetyldihydroveracintine *VII*

An aqueous $NaHCO_3$ solution (27 mg, 3 ml) was added to a methanolic solution of substance *VI* (29 mg, 7 ml) and heated under reflux condenser on a steam bath for 1 h. The reaction mixture was diluted with water, extracted with chloroform and the organic layer evaporated. Yield 24 mg, R_F 0·3 (S_1), m.p. 257–259°C (acetone), $[\alpha]_D^{22}$ −51° (*c* 0·51, ethanol).

The ^{1}H-NMR, *mass and infrared spectra were recorded in the Department of Analytical Chemistry, Institute of Chemistry, the* ^{13}C-NMR *spectrum in the Institut de Chimie des Substances Naturelles.*

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