REEXAMINATION OF THE STRUCTURE OF VERAMARINE

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The previously proposed α-orientation for the $C_{(16)}$-hydroxyl group of the alkaloid veramarine (*Ia*) has been revised; the ^1H and ^{13}C NMR data of *Ia* and the base-catalyzed solvolysis of its O-diacetate *Id* evidenced veramarine to be (25*S*)-cev-5-enine-3β,16β,20β-triol (*Ib*).

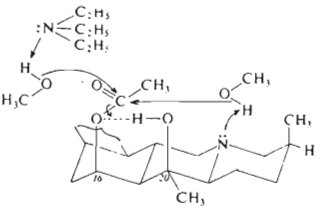
The amorphous base veramarine (*Ia*), isolated from the rhizome of *Veratrum album* subsp. *Lobelianum* SUESSENGUTH[1,2], is an alkamine of the cevanine type poor in the content of hydroxyl groups. Its structure was adduced from the physicochemical data of the base and its derivatives[2,3]. Another source of this alkaloid was found to be the rhizome of irradiated *V. grandiflorum* (MAX.) LOESSEN, from which veramarine (*Ib*) was isolated in a crystalline form. The identity of both alkaloids was confirmed by a direct comparison of its 3-acetate with that of the specimen.

To verify the structure *Ib* the NMR spectral data of the base were reexamined and the base-catalyzed solvolysis of the O-diacetate *Id* was investigated. The position of the multiplet (centered at δ 4·38, 8 Hz in the half-height width) of the hydrogen at $C_{(16)}$, which bears the hydroxyl group fits that for α-equatorial arrangement; on acetylation the position was downfield shifted to δ 5·35. Consequently, the $C_{(16)}$ hydroxyl have to be β-axially oriented.

Another proof for the $C_{(16)}$ β-hydroxyl orientation came from the ^{13}C NMR spectrum: comparison of the spectral data of *Ib* with those of veraflorizine (*II*), recently isolated from the same source as *Ib* (ref.[4,5]), showed a close pattern (Table I). An introduction of the β-axial hydroxyl group at $C_{(16)}$ caused significant shifts of $C_{(13)}$, $C_{(15)}$ and $C_{(16)}$ resonances in *Ib*. The observed Δδ values backed the configuration of the β-axial hydroxyl group at $C_{(16)}$: the upfield shift ($\Delta = -5$ ppm) of the $C_{(13)}$ signal because of the characteristic γ-effect, a downfield $C_{(15)}$ signal shift ($\Delta = 5$ ppm) because of the β-effect and a downfield $C_{(16)}$ signal shift ($\Delta = 45{\cdot}3$ ppm) because of the α-effect.

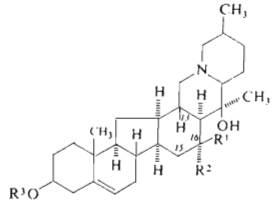
To support the configuration assignment at $C_{(16)}$ in *Ib*, compound *Id* was sub-

jected to a base-catalyzed solvolysis (ref.[6,7]). The methanolic solution of *Id*, allowed to stand at room temperature for 24 h, afforded *Ic* in a 68% yield. The reaction in trimethylamine buffer was significantly accelerated. The reactivity of the $C_{(16)}$ acetate in *Id* during the methanolysis entitled us to postulate a participation of the hydroxyl group at $C_{(20)}$ bearing a *cis*-1,3-diaxial relation to the ester group (Scheme 1).

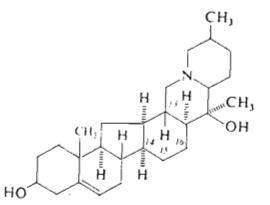


SCHEME 1

The X-ray analysis of veramarine monoacetate *Ic* confirmed the suggested structure as (25*S*)-cev-5-enine-3β,16β-20β-triol (ring junctions B/C *trans*, C/D *cis*, D/E *trans*, and E/F *trans*). The configuration at other chiral centers was settled to be $C_{(3)}$ hydroxyl β-equatorial, $C_{(10)}$ methyl β-axial, $C_{(20)}$ hydroxyl β-axial, $C_{(25)}$ methyl β-axial[8,9]. Thus, the previously proposed structure for veramarine was confirmed excepting the configuration of the $C_{(16)}$ hydroxyl group which was revised for β-axial orientation.



Ia, $R^1 = R^3 = H$, $R^2 = OH$
Ib, $R^1 = OH$, $R^2 = R^3 = H$
Ic, $R^1 = R^2 = H$, $R^3 = CH_3CO$
Id, $R^1 = R^3 = CH_3CO$, $R^2 = H$



II

Since veramarine is a $C_{(16)}$ oxygenated derivative of veraflorizine (*II*) it seems plausible to propose that *Ib* represents one of the intermediates in the postulated pathway of cevanine alkaloid biogenesis[4].

EXPERIMENTAL

Melting points were taken with a melting-point apparatus (Leitz, Wetzlar. FRG), the mass spectrum was recorded on a JMS D-300 Jeol, (Japan) spectrometer with a JMA 2000 Jeol, (Japan) Mass data analysis system. The ^1H and ^{13}C NMR spectra were measured with a JNM FX-100 Jeol spectrometer in deuteriochloroform (ppm downfield from tetramethylsilane $\delta = 0$), the IR spectrum of chloroform solution with a Hitachi grating infrared spectrometer, model 215, and optical rotation of chloroform solution with a Jasco DIP-4 (Japan) digital polarimeter.

Plant material, extraction and purification. Budding *V. grandiflorum* (MAX.) LOESSEN was cultivated in a 4-fold dilution of Hoagland solution in the dark for 10 days. The resulting etiolated plants were irradiated by a red fluorescent light (Mitsubishi FLR-40P Japan), maximum energy at 660 nm from the top of the plants for 2 days. Dilute ammonia was added to the dried and powdered rhizome, and the mixture was extracted with chloroform–ethanol. The extract (550 g), hydrolyzed with a 1M-HCl in methanol for 6 h afforded the crude mixture of alkaloids (116·1 g), which was separated into secondary and tertiary base fractions according to the Jacobs method[10]. The tertiary base fraction (68·1 g) was separated by column chromatography on alumina (Merck, 30-fold excess, standard III) by consecutive elution with benzene, 10% ether in benzene, chloro-

TABLE I

^{13}C NMR Assignments for veramarine (*Ib*) and veraflorizine (*II*)

Carbon[a]	*Ib*	*II*	Carbon[a]	*Ib*	*II*
1	38·2	38·2	15	30·8	25·2
2	31·5[1]	31·5	16	66·1	20·8
3	71·9	71·9	17	50·4	49·0
4	42·0	41·9	18	61·9[3]	61·9[2]
5	141·7	142·0	19	19·1	19·0
6	122·3	122·3	20	73·2	71·1
7	31·5[1]	31·3	21	19·9	20·4
8	38·7	38·7	22	70·0	70·4
9	5·46	54·3	23	19·2	18·7
10	37·0	37·0	24	28·8[1]	29·3[1]
11	29·2[1]	29·5[1]	25	27·6	27·8
12	41·5	41·7	26	17·3	17·4
13	32·7	37·6	27	62·2[3]	62·7[2]
14	43·7	44·7			

[a] Resonances with the same superscripts[1,2,3] may be interchanged.

form and 10% methanol in chloroform to give fractions containing solanidine, verazine, rubijervine, and veratramine. Each fraction was further purified by thin-layer chromatography affording shinonomenine (49 mg), veraflorizine (5·7 mg) and veramarine (*Ib*, 250 mg).

Veramarine, m.p. 119—122°C (acetone-water), $[\alpha]_D$ —112·7° (*c* 0·22, chloroform). For $C_{27}H_{43}NO_3$ calculated: 429·3241, found: 429·3228 M^+, further peaks in the mass spectrum, *m/z*: 112·1130 ($C_7H_{14}N$, base peak), and 111. IR spectrum, cm^{-1}: 3 600, 3 420, 2 775. ^1H NMR spectrum: 1·04 (s, 3 H, 19-H), 1·10 (d, 3 H, J = 7 Hz, 27-H), 1·19 (s, 3 H, 21-H), 3·52 (m, 1 H, 3α-H), 4·36 (m, 1 H, $W_{0.5}$ = 9 Hz, 16α-H), and 5·38 (m, 1 H, 6-H).

Methanolysis of veramarine: A solution of *Id* 20·5 mg in methanol (3 ml) and water (0·5 ml), left to stand at room temperature for 24 h was extracted with chloroform after being made alkaline and the chloroform extract was purified by thin-layer chromatography on silica gel in cyclohexane–ethyl acetate–methanol–diethylamine (2 : 2 : 1 : 0·1). Yield 12·7 mg of *Ic*, m.p. 253 to 255°C and 4·4 mg of *Id*. The melting point of *Ic* was not depressed on admixture with the specimen. A solution of *Id* 11 mg in chloroform (0·5 ml), triethylamine (0·5M, 3 ml), acetic acid (0·5M, 0·5 ml), and methanol (90%, 0·5 ml) was allowed to stand at room temperature. The rate of methanolysis was monitored by thin-layer chromatography every hour (solvent system cyclohexane–ethyl acetate–methanol 2 : 2 : 1); R_F values: *Ib* 0·40, *Ic* 0·60, *Id* 0·75. The reaction equilibrium was reached after 10 to 14 h the ration *Ic* : *Id* being 7 : 3.

Veramarine 3-*monoacetate* (*Ic*): m.p. 253—255°C. IR spectrum, cm^{-1}: 3 450, 2 775, 1 720, 1 260. ^1H NMR spectrum: 1·04 (s, 3 H, 19-H), 1·08 (d, 3 H, J = 7 Hz, 27-H), 1·18 (s, 3 H, 21-H), 2·02 (s, 3 H, —$OCOCH_3$), 4·34 (m, 1 H, $W_{0.5}$ = 8 Hz, 16α-H), 4·60 (m, 1 H, 3α-H), and 5·40 (m, 1 H, 6-H). The melting point of *Ic* was not depressed on admixture with authentic veramarine 3-monoacetate[2].

Veramarine O-*diacetate* (*Id*): m.p. 205—210°C. IR spectrum, cm^{-1}: 3 300, 2 775, 1 720, 1 250. ^1H NMR spectrum: 1·03 (s, 3 H, 19-H), 1·07) d, 3 H, J = 7 Hz, 27-H), 1·10 (s, 3 H, 21-H), 1·93 (s, 3 H, —$OCOCH_3$), 2·00 (s, —$OCOCH_3$), 4·52 (m, 1 H, 3α-H), 5·36 (m, 1 H, 6-H), and 5·50 (m, 1 H, $W_{0.5}$ = 8 Hz, 16α-H).

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