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STRUCTURE OF THE STEROIDAL ALKALOID MULDAMINE AND ITS DEACETYL DERIVATIVE

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Key Word Index—*Veratrum californicum*; Liliaceae; steroidal alkaloid; muldamine; (22*S*,25*S*)-22,26-epiminocholest-5-ene-3β,16α-diol 16-acetate; deacetylmuldamine; teinemine; isoteinemine.

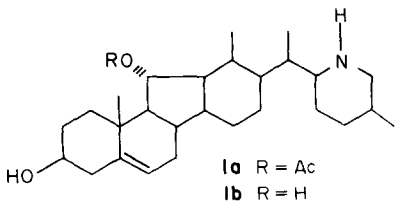
Abstract—Spectral techniques have established the structure of muldamine from *Veratrum californicum* as (22*S*, 25*S*)-22,26-epiminocholest-5-ene-3β,16α-diol 16-acetate, instead of the C-nor-D-homo steroid structure previously suggested. Its deacetyl derivative seems to be identical with teinemine, the configuration of which at C-22 is therefore *S* and not *R* as previously reported.

INTRODUCTION

The isolation of muldamine, one of the three principal benzene extractable alkaloids of the plant *Veratrum californicum* was reported in 1968[1]. Structural investigation of muldamine by spectroscopic techniques allowed provisional formulation[2] of the structure as an 11α-acetoxy-3β-hydroxy-Δ^5 derivative of the veratramine skeleton (**1a**). A reinvestigation of the structure of muldamine and its deacetyl derivative has shown that the structure proposed earlier[2] is in error and that muldamine should be represented as (22S,25S)-22,26-epiminocholest-5-ene-3β,16α-diol 16-acetate (**2**).

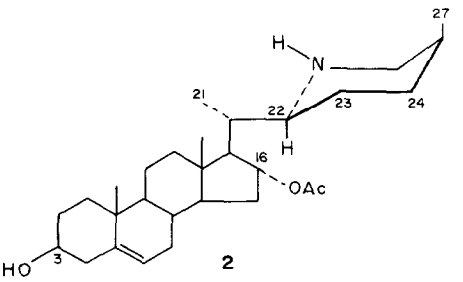
RESULTS

Muldamine was deacetylated to yield, after recrystallization from aqueous ethanol, deacetylmuldamine, mp 205–207°, $[\alpha]_D$ −38.7° ($CHCl_3$). Examination of high resolution ^1H and ^{13}C NMR spectral data were inconsistent with structure **1b** for deacetylmuldamine. In particular, the ^1H NMR spectrum of deacetylmuldamine contained two doublets due to C-methyl groups instead of the three required by **1b**, and the ^{13}C NMR spectrum contained signals due to three



1a R = Ac
1b R = H

quaternary carbon atoms rather than the two present in structure **1b**. In addition to the jervanine and veratranine alkaloids, several other alkaloid types have been isolated from *Veratrum* species[3], including the 22,26-epiminocholestane alkaloids, a subgroup



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which appeared to be a good candidate for the correct structures of muldamine and its deacetyl derivative.

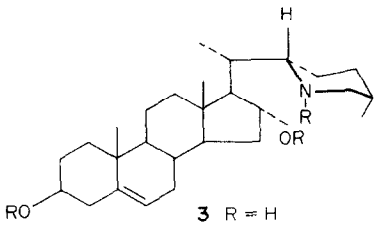
Perusal of ^{13}C NMR data for a wide variety of 22,26-epiminocholestanes[4] indicated excellent agreement between the observed values (Table 1) for deacetylmuldamine and the A, B and part of the C ring of dihydro-25-isoverazine A and the D, F and part of the C ring of dihydro-25-isosolafloridine B. Specifically, the chemical shifts for C-1 through C-12, and C-19, differed by no greater than δ0.4 for deacetylmuldamine in comparison to dihydro-25-isoverazine A and the resonances for C-11 through C-27 (excepting C-19) by no greater than δ 0.4 for deacetylmuldamine in comparison to dihydro-25-isosolafloridine B. In fact, C-13 through C-27 (excepting C-19) agreed within δ0.2 for the latter two compounds. These correlations indicated that the non A-ring hydroxy group of deacetylmuldamine was at C-16 and was α rather than β since similar compounds bearing a 16β-hydroxy substituent show a resonance at δ71[4] while 22,26-epiminocholestanes containing a 16α-hydroxy group show a signal at δ75[4], regardless of the configuration at C-22. The resonance positions shown by C-23 and C-27 indicated[4] that the C-27 methyl group of deacetylmuldamine is axial rather than equatorial. On the basis of the ^{13}C, ^1H NMR and mass spectra (see Experimental), the structure of deacetylmuldamine is established as

Table 1. ^{13}C NMR chemical shifts* for muldamine (2) and deacetylmuldamine (3)†

Carbon	2	3	Carbon	2	3
1	37.4	37.3	15	34.7	34.5
2	31.6	31.6	16	79.9	75.3
3	71.4	71.3	17	58.1	62.8
4	42.3	42.3	18	13.0	13.6
5	141.1	140.8	19	19.4	19.4
6	121.0	121.4	20	39.3	38.2
7	31.7	31.6	21	13.0	15.9
8	31.2	31.6	22	59.3	61.4
9	50.0	50.0	23	25.2	22.4
10	36.4	36.5	24	30.8	30.3
11	20.8	20.7	25	27.4	26.9
12	39.8	39.8	26	52.6	51.5
13	43.2	44.2	27	16.6	16.6
14	54.0	53.9			

*Spectra recorded in $CDCl_3$; values in ppm downfield from TMS.

†Resonances were assigned with the aid of multiplicity separation and SFORD techniques.



3 R = H

4 R = Ac

(22*S*,25*S*)-22,26-epiminocholest-5-ene-3β,16α-diol (**3**). An X-ray crystallographic determination of **3** has confirmed this structure [Wong, R. Y., unpublished].

This structure is identical to one proposed by Kaneko *et al.*[5] for isoteinemine. However, the physical constants (mp, $[\alpha]_D$ and ^{1}H NMR spectrum) obtained for **3** agreed with those reported[5] for the compound named teinemine. In particular, the ^{1}H NMR resonance of the C-27 methyl doublet at δ0.97 in **3**, which is similar to that of related alkaloids containing an axial C-27 methyl group such as hapepunine[6], anrakorinine[6] and (22*S*,25*S*)-22,26-epimino-5α-cholestane-3β,16α-diol[7], reflects the axial orientation of the C-27 methyl group. Alternatively, the signal for the C-27 methyl doublet at δ0.83 in the alkaloid named isoteinemine [5] suggests an equatorial orientation for this group, similar to resonances reported for equatorial C-27 methyl groups in solaphyllidine[8], solaverbascine[9] and (22*R*,25*S*) - 22,26 - epimino - 5α - cholestane - 3β,16α - diol[7]. Thus, we conclude that deacetylmuldamine and teinemine are the same compound and may be represented as **3**.

The position of the *O*-acetyl group in muldamine was determined by inspection of ^{13}C NMR data since the signal due to C-16 in **3** at δ75.3 shifted downfield to δ79.9 in the acetyl derivative **2** while the C-3 resonance was essentially identical for the two alkaloids. Muldamine is therefore (22*S*,25*S*)-22,26-epiminocholest-5-ene-3β,16α-diol 16-acetate (**2**).

Acetylation of **2** yielded *O*,*N*-diacetylmuldamine (**4**)[2] which upon crystallization from aqueous ethanol gave crystals, mp 147.0–147.5°, in good agreement with the value of 147–148.5° reported[5] for *O*,*O*,*N*-triacetylteinemine. ^{1}H NMR data for **4** in deuterochloroform was nearly identical to that published[5] for *O*,*O*,*N*-triacetylteinemine. Doubling of several of the signals in the ^{13}C NMR spectrum of **4** was observed, a finding similar to that reported earlier for ^{1}H NMR resonances for the acetyl derivatives of other *Veratrum* alkaloids[10]. Presumably the additional NMR signals result from the presence of more than one conformer, either due to F ring inversion or restricted rotation about the bond between C-20 and C-22[10].

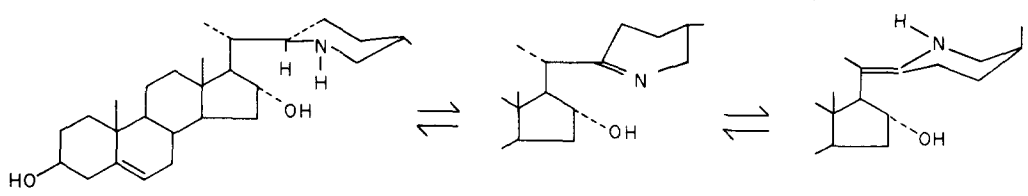
^{13}C NMR data of deacetylmuldamine are consistent with the piperidine ring positioned as shown in **3** with the amino group intramolecularly hydrogen bonded to the C-16 hydroxy group while the downfield shift of C-23 and C-24 in the ^{13}C NMR spectrum of muldamine, similar to that observed for solasodine relative to tomatidine[11], suggests a structure for muldamine, as in **2**, where the amino group is oriented away from the C-16 acetyl group, a moiety which does not permit hydrogen bonding. The δ2.9 upfield shift of the C-21 resonance of **2** relative to **3** suggests a strong γ-interaction between this carbon atom and the amino group and provides further evidence for a change in position of the F ring relative to the C-20, C-22 bond upon acetylation of the C-16 hydroxy group.

In order to determine if the C-22 epimer of deacetylmuldamine could be formed as an artifact during the acidic hydrolysis of the crude mixture of *Veratrum* alkaloids[5], **2** was subjected to hydrolysis in methanolic hydrochloric acid. No evidence was obtained for the formation of the C-22 epimer of **3** under these conditions.

DISCUSSION

The biosynthesis of *Solanum* alkaloids has been postulated to proceed via the C-22 epimer (**5**) of deacetylmuldamine, which is presumably formed upon reduction of etioline (**6**)[12]. The occurrence of muldamine in *V. californicum*[1] and in *V. grandiflorum*[5] (teinemine) is not inconsistent with this proposal. Although an exhaustive extraction was not performed, the root and rhizome of *V. californicum* may contain up to 0.3–0.4% of muldamine [Keeler, R. F., unpublished]. In accordance with the relatively large amounts isolated[1, 5], **2**, which is of incorrect stereochemistry at C-22 to yield solanidine, could serve as a storage or end product of a particular pathway. *In vivo* oxidation of **3**, derived from **2** or as an intermediate in the formation of **2**, could yield **6** or its isomer **7** which upon reduction would produce the (22*R*,25*S*)-epiminocholestene, **5**. This compound has the proper C-22 stereochemistry to cyclize to solanidine, which then might undergo ring contraction to yield the jerveratrum alkaloids in a manner postulated by Kaneko *et al.* [13].

The alkaloid (mp 217°), isomeric to teinemine, which was isolated from *V. grandiflorum* in small amounts by Kaneko *et al.* [5] could be the 22*R*,25*S* compound (**5**). Our studies showing that acidic



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hydrolysis of muldamine *per se* does not appear to produce the C-22 epimer (**5**) of deacetylmuldamine indicates that **5** would not be produced as an artifact under the conditions used to hydrolyze[5] crude alkaloid glycoside. Further experiments to definitely establish the presence of **5** in *Veratrum* are warranted.

The revised structure of muldamine, lacking a rigid furanopiperidine system and a basic imino group accessible to the α-face of the steroid, is in accord with its reported lack of teratogenic activity in sheep[14] and low teratogenic activity in hamsters[15]. More recently, Nes *et al.*[16] have shown that muldamine inhibits growth and cholesterol induced sexual reproduction in *Phytophthora cactorum*, an observation which suggests a protective function for **2** in *V. californicum*.

EXPERIMENTAL

Mps were uncorr. ^{1}H and ^{13}C NMR spectra were measured at 100 and 25.03 MHz, respectively. The δ values are expressed in ppm downfield from TMS int. standard. EIMS (70 eV) and CIMS (100 eV) were measured on the direct probe inlet using *iso*-butane as reagent gas for the latter.

Deacetylmuldamine (**3**). Deacetylation of muldamine was conducted according to Keeler's procedure[2] yielding, after recrystallization from aq. EtOH, **3**, mp 205–207° (lit. [2] 201–203°), $[\alpha]_D^{27}$ −38.7° ($CHCl_3$; *c* 1.11). Kaneko *et al.* report[5] mp 204–209° and $[\alpha]_D^{20}$ −35.8° ($CHCl_3$) for teinemine. ^{1}H NMR ($CDCl_3$): δ0.71(3H, *s*, H-18), 0.97(3H, *d*, *J* = 5.5 Hz, H-27), 1.00(3H, *s*, H-19), 1.03(3H, *d*, *J* = 5.5 Hz, H-21), 3.47(1H, *m*, H-3), 4.04(1H, *m*, H-16), 5.34(1H, *m*, H-6). (C_5D_5N): 0.73(3H, *s*, H-18), 1.04(3H, *d*, H-21), 1.04(3H, *d*, H-27), 1.06(3H, *s*, H-19), 3.78(1H, *m*, H-3), 4.22(1H, *m*, H-16), 4.84(2H, *m*, 3 and 16-OH), 5.42(1H, *m*, H-6), 6.12(1H, *m*, N–H). EIMS, *m/z* (rel. int.): 415 $[M]^+$ (0.2), 150(2), 140(5), 99(7), 98 $[C_6H_{12}N]^+$ (100). CIMS, *m/z* (rel. int).: 416 $[M+H]^+$(28), 398 $[M+H-H_2O]^+$(18), 380 $[M+H-2H_2O]^+$(7), 140(6), 138$[C_9H_{14}O]^+$(3), 126 $[C_8H_{16}N]^+$(5), 99(7), 98(100).

Muldamine (**2**). A sample of **2** obtained by C_6H_6 extraction of *V. californicum* Durand[1] was used throughout this study. ^{1}H NMR ($CDCl_3$): δ0.73 (3H, *s*, H-18), 0.97 (3H, *d*, H-27), 1.00(3H, *s*, H-19), 1.04(3H, *d*, H-21), 2.07(3H, *s*, OAc), 3.48(1H, *m*, H-3), 4.93(1H, *m*, H-16), 5.32(1H, *m*, H-6). (C_5D_5N): 0.69(3H, *s*, H-18), 1.04(3H, *s*, H-19), 1.10(3H, *d*, *J* = 7.5 Hz, H-27), 1.16(3H, *d*, *J* = 7.0 Hz, H-21), 2.13(3H, *s*, OAc), 3.78(1H, *m*, H-3), 5.16(1H, *m*, H-16), 5.36(*m*, 1H, H-6), 6.09(1H, *m*, N–H). EIMS, *m/z* (rel. int.): 457$[M]^+$(0.4), 396$[M-H_2O-Ac]^+$(4), 150(10), 99(20), 98(100). CIMS, *m/z* (rel. int.): 459(14), 458$[M+H]^+$(37), 456$[M-H]^+$(7), 441(9), 440$[M+H-H_2O]^+$(23), 398(10), 382(5), 380$[M-OAc-H_2O]^+$(5), 301(5), 98(100).

O,N-*Diacetylmuldamine* (**4**). Acetylation of muldamine was performed according to Keeler's procedure[2] yielding the derivative which upon crystallization from aq. EtOH gave crystals, mp 147.0–147.5°, $[\alpha]_D^{27}$ −76.2° ($CHCl_3$; *c* 0.88). Kaneko *et al.* report[5] mp 147–148.5° for *O,O,N*-teinemine triacetate. ^{1}H NMR ($CDCl_3$): δ0.84 (3H, *s*, H-18), 0.86(3H, *d*, *J* = 6.8 Hz, H-21), 0.93(3H, *d*, *J* = 4.5 Hz, H-27), 1.00(3H, *s*, H-19), 2.01(3H, *s*, –OAc), 2.04(3H, *s*, –OAc), 2.08(3H, *s*, –NHAc), 4.57(1H, *m*, H-3), 5.17(1H, *m*, H-16), 5.36(1H, *m*, H-6). ^{13}C NMR($CDCl_3$): δ13.4(C-18), 14.5(C-21), 15.5, 15.8(C-27), 19.2(C-19), 20.4(C-11), 21.4(C-3COMe), 21.8(C-16 COMe), 22.2(NCOMe), 26.1(C-23), 27.7(C-2), 27.8(C-25), 30.5, 30.8(C-24), 31.5(C-8), 31.9(C-7), 33.3(C-15), 36.6(C-10), 36.9(C-1), 38.0(C-4), 39.3(C-20), 39.5(C-12), 43.7, 43.9(C-13), 50.0(C-9), 54.1, 54.3(C-14), 55.7(C-26), 56.8(C-17), 57.2(C-22), 73.8(C-3), 77.5(C-16), 122.1(C-6), 139.7, 139.8(C-5), 168.9, 169.0(C-16 CO), 170.4(C-3 CO), 171.2(NCO). EIMS, *m/z* (rel. int.): 497$[M-H-Ac]^+$(1), 141(24), 140$[C_8H_{14}NO]^+$(100), 98(64). CIMS, *m/z* (rel. int.): 542$[M+H]^+$(17), 483(10), 482$[M-OAc]^+$(19), 283(15), 182(8), 141(12), 140(100), 139(13), 117(10), 103(32), 101(13), 98(52).

Acidic hydrolysis of muldamine. Two ml 1 N HCl was added to 120 mg of **2** dissolved in 20 ml MeOH and the resulting soln heated under reflux for 6 hr. After evapn of the MeOH, the residue was redissolved in H_2O and the soln made basic with 50% aq NaOH. Extraction of the aq. soln with $Et_2O \times 3$ and overnight drying of the extracts over Na_2SO_4 gave, after evapn of the Et_2O, 100 mg of a mixture of alkaloids. This material (60 mg) was purified by prep. TLC on Si gel (MeOH–$CHCl_3$, 1:4) yielding 32 mg ($R_f \sim 0.2$) deacetylmuldamine (**3**), 5 mg ($R_f \sim 0.5$) starting material (**2**) and 2 mg ($R_f \sim 0.8$) of an unidentified alkaloid whose ^{1}H NMR spectrum did not agree with that reported[5] for the C-22 epimer of **3**.

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