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CAMTSCHATCANIDINE, AN ALKALOID FROM *FRITILLARIA CAMTSCHATCENSIS*

KÔ KANEKO, MIKAKO TANAKA, UTAKO NAKAOKA, YÔKO TANAKA, NAOTOSHI YOSHIDA and HIROSHI MITSUHASHI

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

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Key Word Index—*Fritillaria camtschatcensis*; Liliaceae; bulb; solanidanine alkaloids; 18-nor-17β-methyl-22,26-epiminocholestane.

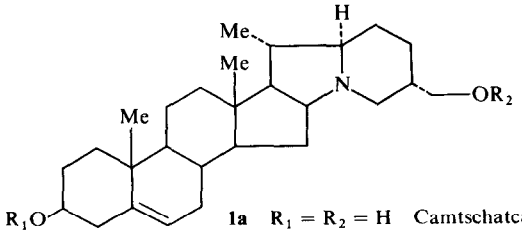
Abstract—From the hydrolysed glycoalkaloid fraction from bulbs of mature *Fritillaria camtschatcensis* in addition to already reported alkaloids a new solanidanine alkaloid, 22*R*,25*S*-solanid-5-ene-3β,27-diol (camtschatcanidine), was isolated and its structure elucidated by spectral analysis and its conversion to solanidine. Also veralkamine was identified from the same plant.

INTRODUCTION

Solanidine (**3**) was isolated from the bulb [1] and the aerial part [2, 3] of mature *Fritillaria camtschatcensis* as the main alkaloid as well as the *N*-methyl-22,26-epiminocholestene derivatives, hapepunine and anrakorinine, in addition to tomatidenol and solasodine, as minor alkaloids [2, 3]. In continuation of our work on the alkaloids of this plant, a new alkaloid, camtschatcanidine (**1a**) has been isolated from the bulb as well as the known 18-nor-17β-methyl-22,26-epiminocholestane alkaloid, veralkamine (**2**) originally isolated from *Veratrum album* var. *lobelianum* [4, 5]. This paper described both the isolation of these alkaloids and the structure elucidation of **1a**.

RESULTS AND DISCUSSION

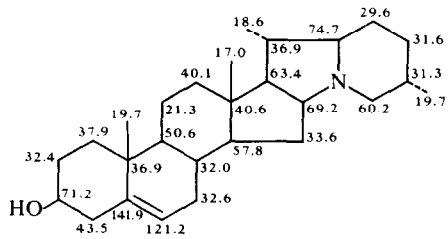
Employing the extraction and separation procedure previously reported [2, 3], 7.03 g of alkamines were obtained after hydrolysis of the glycosidic fraction isolated from 3.65 kg of bulbs of *F. camtschatcensis*. This crude alkaloid was fractionated by column chromatography on alumina and afforded five components, 1.75 g of solanidine (**3**), 1.4 mg of hapepunine, 44 mg of camtschatcanidine (**1a**), 3 mg of veralkamine (**2**), and 3.4 mg of a dihydroxylated spirosolane alkaloid.



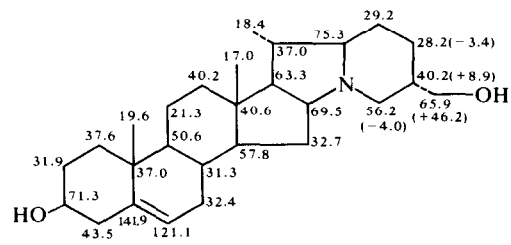
1a $R_1 = R_2 = H$ Camtschatcanidine
1b $R_1 = R_2 = Ac$
1c $R_1 = H$, $R_2 = Ms$
1d $R_1 = Ac$, $R_2 = Ms$

The mass spectrum of **1a** revealed the M^+ at *m/e* 413, the base peak at *m/e* 166 and a diagnostic peak at *m/e* 220. These three peaks correspond to the typical fragment ions of solanidanine alkaloids hydroxylated at ring E or F. This is also supported by the ^1H NMR spectrum which contains signals for one secondary Me group at δ 1.04, two tertiary Me groups at δ 0.96 and 1.05, one hydroxymethyl group at δ 3.82 (downfield shift to δ 3.92 on acetylation), a secondary OH group at δ 3.44 (downfield shift to δ 4.60 on acetylation), and an olefinic proton at δ 5.44.

To determine the position of the hydroxymethyl group, the ^{13}C NMR spectrum of **1a** was compared with that of **3** [6]. Both showed a very similar pattern, thus assignment of many carbon atoms could be made. Substitution of **4** by OH at C-27 as in **1a** caused significant shifts at C-24 (−3.4 ppm), C-25 (+18.9 ppm), C-26 (−4.0 ppm) and C-27 (+46.2 ppm).



Solanidine (**3**)

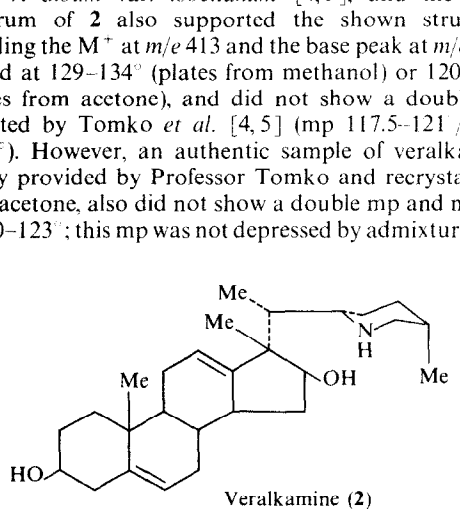


Camtschatcanidine (**1a**)

In order to establish the detailed stereochemistry of camtschatcanidine (**1a**), this 27-hydroxy-solanidine derivative has been converted into **3**. **1a** was treated with three equivalents of mesyl chloride in anhydrous pyridine affording, after high performance low pressure chromatography the monomesylate (**1c**) [MS *m/e*: 491 (M^+), 298, 244; ^{1}H-NMR (100 MHz, $CDCl_3$, TMS): δ 3.0 (3H, *s*, $-SO_2CH_3$), 4.06 (2H, *m*, $-CH_2OMs$)]. **1c** was transformed into the monoacetate (**1d**) which was reduced with lithium aluminium hydride in anhydrous ether leading to **3** (4.6 mg, 63.8% yield), identical in every respects with an authentic specimen of solanidine. Therefore, the structure of camtschatcanidine was established as (22*R*,25*S*)-solanid-5-ene-3β,27-diol (**1a**).

The ^{1}H-NMR spectrum of **2** exhibited six tertiary Me protons at δ 0.93, each three secondary Me protons at 0.81 and 0.96, two protons bearing an oxygen substituent at 3.50 (1H, *m*) and 4.05 (1H, *q*, J = 6 Hz), and two olefinic protons at 5.32.

The chemical shifts and coupling constants of these signals agree closely with those reported for veralkamine from *V. album* var. *lobelianum* [4, 5], and the mass spectrum of **2** also supported the shown structure, revealing the M^+ at *m/e* 413 and the base peak at *m/e* 98. **2** melted at 129–134° (plates from methanol) or 120–124° (plates from acetone), and did not show a double mp reported by Tomko *et al.* [4, 5] (mp 117.5–121°/167–169.5°). However, an authentic sample of veralkamine kindly provided by Professor Tomko and recrystallized from acetone, also did not show a double mp and melted at 120–123°; this mp was not depressed by admixture with **2**.



Veralkamine (**2**)

Five dioxygenated solanidanine alkaloids, rubijervine [7], isorubijervine [8, 9], epirubijervine [10], leptinidine [11, 12], and **1a** have been isolated from natural sources, up to present. From these, isorubijervine and epirubijervine were obtained from the genus *Veratrum* and suspected to be intermediates of the biogenesis of C-nor-D-homo steroidal alkaloids [10, 13]. It is interesting to note that *F. camtschatcersis* contains three steroidal alkaloids with a hydroxymethyl group, anrakorinine [2, 3], **1a** and possibly also one spirosolane alkaloid with an additional OH group at C-27, which was assumed from the ^{1}H NMR and mass spectra of this alkaloid and its acetate (cf. Experimental). However, cevanine alkaloids have not as yet been detected in this plant. According to our results, solanidine (**3**), solansodine [2, 3] and tomatidenol [2, 3] seem to be common alkaloids in *F. camtschatcensis* which are metabolized to **1a**, hapepunine, anrakorinine, and the 27-hydroxyspirosolane.

EXPERIMENTAL

Material. Powdered mature tubers (3.65 kg) of *F. camtschatcensis* (L.) ker, harvested in the districts along the sea of Okhotsk, Hokkaido, at the end of June (after flowering), were extracted with hexane to remove fatty substances (10.7 g) and then with ammoniacal $CHCl_3$–MeOH affording 419 g of glycoside mixture.

Hydrolysis and separation of alkaloids. The hydrolysis of glycosides and separation of alkaloids are as previously described for similar studies on alkaloids from the aerial parts of this plant [2, 3], and 1.75 g of **4**, 1.4 mg of **5**, 44 mg of **1a**, 3 mg of **2**, and 3.4 mg of **3a** were isolated from 7.03 g of crude alkaloids. Mps are uncorrected.

Camtschatcanidine (**1a**). Mp 261–265° (needles, Me_2CO); $[\alpha]_D$ −19.4 (*c* 0.1, MeOH); (Calc. for $C_{27}H_{43}NO_2$: C, 78.40; H, 10.48; N, 3.39, found C, 78.30; H, 10.50; N, 3.38%); MS *m/e*: 413 (M^+), 220 ($C_{14}H_{20}NO$), 166 (base peak, $C_{10}H_{15}NO$); IR ν_{max}^{Nujol} cm^{-1}: 3400–3100 (OH), 1210 and 1150 (C-O); ^{1}H NMR (100 MHz, $CDCl_3$, TMS, chemical shift ppm): δ 0.96 (3H, *s*, 18-H), 1.04 (3H, *d*, J = 7 Hz, 21-H), 1.05 (3H, *s*, 19-H), 3.44 (1H, *m*, 3α-H), 3.80 (2H, *m*, 27-H), 5.44 (1H, *m*, 6-H); ^{13}C NMR (25 MHz, C_5D_5N, TMS, chemical shift in ppm, signal multiplicity obtained by off resonance decoupling expts): 37.6 (*t*, C-1); 31.9 (*t*, C-2); 71.3 (*d*, C-3); 43.5 (*t*, C-4); 141.9 (*s*, C-5); 121.1 (*d*, C-6); 32.4 (*t*, C-7); 31.3 (*d*, C-8); 50.6 (*d*, C-9); 37.0 (*s*, C-10); 21.3 (*t*, C-11); 40.2 (*t*, C-12); 40.6 (*s*, C-13); 57.8 (*d*, C-14); 32.7 (*t*, C-15); 69.5 (*d*, C-16); 63.3 (*d*, C-17); 17.0 (*q*, C-18); 19.6 (*q*, C-19); 37.0 (*d*, C-20); 18.4 (*q*, C-21); 75.3 (*d*, C-22); 29.2 (*t*, C-23); 28.2 (*t*, C-24); 40.2 (*d*, C-25); 56.2 (*t*, C-26); 65.9 (*t*, C-27).

Acetylation of **1a** *to O,O-diacetate* (**1b**). Acetylation of 4 mg of **1a** in 0.5 ml of pyridine with 0.3 ml of Ac_2O at room temp., overnight, followed by usual work up afforded 3 mg of **1b**, amorphous; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (C=O), 1250; MS *m/e*: 497 (M^+), 262, 208 (100); ^{1}H NMR (100 MHz, $CDCl_3$, TMS): δ 0.96 (3H, *s*, 18-H), 1.03 (3H, *s*, 19-H), 2.02 (3H, *s*, –OAc), 2.04 (3H, *s*, –OAc), 3.92 (2H, *m*, 27-H), 4.60 (1H, *m*, 3α-H), 5.36 (1H, *m*, 6-H).

Conversion of **1a** *to* **4**. Mesylation of 7.5 mg of **1a** in 3 ml of dry pyridine with 3 mol of MsCl at room temp., overnight, followed by usual work up. Half of the product was purified by high performance low pressure chromatography [Si gel HF_{254} (Type 60, Merck), cyclohexane–EtOAc–MeOH, 2:2:0.5, 7 kg/cm^2] and afforded 3.4 mg of camtschatcanidine monomesylate (**1c**) [MS *m/e*: 491 (M^+), 298, 244; ^{1}H NMR (100 MHz, $CDCl_3$, TMS): δ 3.0 (3H, *s*, $-SO_2CH_3$), 4.06 (2H, *m*, $-CH_2OMs$)]. The remaining half was combined with **1c**, then acetylated in the usual manner affording the monoacetate (**1d**). A soln of mixed acetate in 3 ml of dry Et_2O was reduced with 36 mg of $LiAlH_4$ in dry Et_2O, then refluxed for 1 hr. The remaining reductant was decomposed by cautious addition of aq. Et_2O, then extracted with Et_2O. The Et_2O extract purified by high performance low pressure chromatography, as described above, afforded 4.6 mg of **4** (63.8% yield) [MS *m/e*: 397 (M^+), 204, 150 (100); ^{1}H NMR (100 MHz, $CDCl_3$, TMS): δ 0.82 (3H, *d*, J = 6 Hz, 21 or 27-H), 0.84 (3H, *s*, 18-H), 0.92 (3H, *d*, J = 7 Hz, 21 or 27-H), 1.01 (3H, *s*, 19-H), 2.24 (2H, *m*, 26-H), 2.56 and 2.80 (1H each, *m*, 16α- or 22α-H), 3.48 (1H, *m*, 3α-H), 5.32 (1H, *m*, 6-H), mp 205–210°, 1.4 mg of **1c** (15.7%), and 1.5 mg of **1a** (20%). The mp of **4** was not depressed by admixture with natural product.

Veralkamine (**2**). Mp 120–123° (plates Me_2CO); 129–134° (plates MeOH); $[\alpha]_D$ 78.1 (*c* 0.32, $CHCl_3$); MS *m/e*: 413 (M^+), 125, 98 (100); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1040, 1005; ^{1}H NMR (100 MHz, $CDCl_3$, TMS): δ 0.81 and 0.96 (3H each, J = 7 Hz, 21 or 27-H), 0.93 (6H, *s*, 18 and 19-H), 2.80 (2H, *br s*, 26-H), 3.50 (1H, *m*, 3α-H), 4.04 (1H, *q*, J = 6 Hz, 16α-H), 3.32 (2H, *m*, 6 and 12-H). The mp of **2** from Me_2CO was reported 117.5–121°/167.5–169.5° [4], the authentic specimen [4] was recrystallized from Me_2CO, and melted at 120°/123°, without a double mp. The mp of **2** was not depressed by admixture with the authentic specimen.

Hydroxyspirosolane (**3a**). Mp 230.5–233°; M^+ 429.3235 (Calc. for $C_{27}H_{43}NO_3$: 429.3229); MS *m/e*: 429 (M^+), 414, 154,

130 (100), 129; ^{1}H NMR (100 MHz, C_5D_5N): δ 0.91 (3H, *s*, 18-H), 1.05 (3H, *s*, 19-H), 1.13 (3H, *d*, J = 7 Hz, 21-H), 3.74 (3H, *m*, 3α-H and 27-H), 4.64 (1H, *m*, 16-H), 5.36 (1H, *m*, 6-H).

Acetylation of **3a** *to triacetate* (**3b**). Acetylation of 2 mg of **3a** in 0.5 ml of pyridine with 0.3 ml of Ac_2O at room temp. overnight, followed by usual work-up, afforded 1.5 mg of *N,O,O*-triacetate (**3b**), mp 128–129°; MS *m/e*: 597 (M^+), 583 ($M^+ - CH_3$), 554 ($M^+ - Ac$), 537 ($M^+ - HOAc$), 522 ($537 - CH_3$), 494 ($537 - Ac$), 434 ($494 - HOAc$), 224 (100); ^{1}H NMR (100 MHz, $CDCl_3$, TMS): δ 0.92 (3H, *s*, 18-H), 1.04 (3H, *s*, 19-H), 1.08 (3H, *d*, J = 7 Hz, 21-H), 2.03 (3H, *s*, −OAc), 2.07 (3H, *s*, −OAc), 2.22 (3H, *s*, NAc), 4.04 (3H, *m*, 27-H and 16-H), 4.64 (1H, *m*, 3α-H), 5.38 (1H, *m*, 6-H).

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