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Persicanidine A, a Novel Cerveratrum Alkaloid from the Bulbs of *Fritillaria persica*

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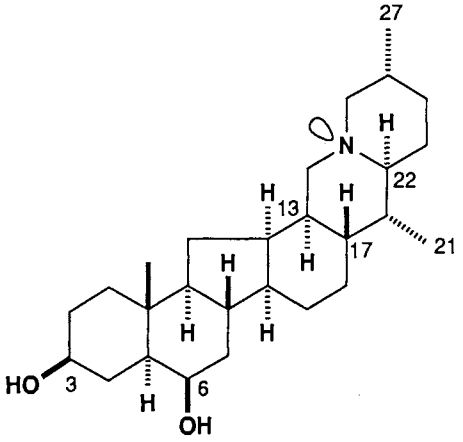
Persicanidine A, a novel cerveratrum alkaloid was isolated from the fresh bulbs of *Fritillaria persica* together with a known alkaloid, delavine. The structure was elucidated by spectroscopic data and X-ray crystallographic analysis. Persicanidine A is unique in structure having the D/E *trans* (H-13α/H-17β) and E/F *cis* (*cis*-quinolizidine) ring fusions. Persicanidine A and delavine were active as a cyclic AMP phosphodiesterase inhibitor.

The genus *Fritillaria* with about 100 species belongs to the subfamily Lilioideae in the Liliaceae and has a distribution in the temperate region of the Northern Hemisphere.[1] In traditional Chinese medicine, the bulbs of some *Fritillaria* plants are used as medicinal material [2] and a number of steroidal alkaloids have been isolated and identified.[3] *Fritillaria persica* is native to Cypress, southern Turkey and Iran, and one of the tallest species with large bulbs, sometimes reaching 36 inches in height, making the species distinct.[4] Our attention to the bioactive alkaloid in the bulbs has resulted in finding a novel cevanine alkaloid with the D/E *trans* (H-13α/H-17β) and E/F *cis* (*cis*-quinolizidine) ring fusions together with a known alkaloid, delavine. This communication mainly refers to the structural elucidation of the alkaloid based on spectroscopic data and X-ray crystallographic analysis.

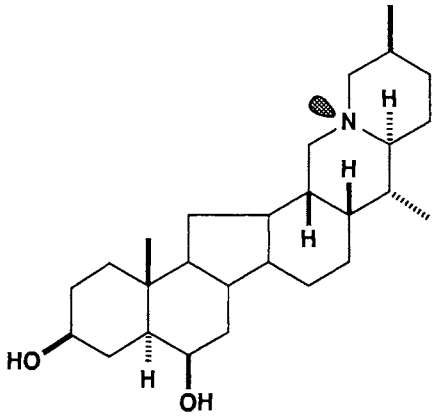
The commercially available fresh bulbs of *F. persica* (7.2 kg) were extracted with MeOH under reflux. The MeOH extract was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble phase was subjected to silica gel and ODS column chromatographies to yield persicanidine A (1.36 g) and delavine (690 mg).

Persicanidine A (**1**) was recrystallized from MeOH as colorless prisms, mp 208°C (dec.), $[\alpha]_D$ -7.8° ($CHCl_3$) and gave a positive color with Dragendorff reagent on TLC. The EI mass spectrum showed an accurate molecular ion peak at *m/z* 415.3507, confirming the molecular formula to be $C_{27}H_{45}NO_2$ (calcd: 415.3453) and a characteristic fragment ion peak of the cevanine type alkaloids without hydroxyl group at the C-20 position at *m/z* 111 (base peak).[5] The ^{13}C NMR spectrum ($CDCl_3$) showed a total of 27 carbons and the DEPT experiments allowed to assign the signals as CH_3 x 3, CH_2 x 11, CH x 12 and C x 1. The ^1H NMR spectrum ($CDCl_3$) showed signals for a tertiary methyl groups at δ 1.03 (s), two secondary methyl groups at δ 1.05 (d, J = 7.0 Hz) and 0.74 (d, J = 6.3 Hz), and two hydroxymethine groups at δ 3.85 (br s, $W_{1/2}$ = 7.0 Hz) and 3.66 (br m, $W_{1/2}$ = 23.0 Hz). The ^{13}C NMR spectrum of **1** resembled those of 5α-cevanine-3β,6β-diol alkaloids with resonaces due to the C-1 - C-10 (A and B rings), [3a] however, signals due to the C - F rings did not agree with

those of any other 20-deoxy-5α-cevanine alkaloids reported up to the present.[3a] Further, a diagnostic IR absorption at 2750 cm^{-1} due to the *trans*-quinolizdine group as observed in other cevanine alkaloids [3a,6] could not be detected in the IR spectrum of **1**. The above data suggested that **1** was a cevanine alkaloid with unusual ring fusions.



1



2

The relative stereostructure of **1** was confirmed by X-ray crystal structure analysis as shown in Fig. 1.[7] The ring fusions are as follows: A/B *trans*, B/C *trans*, C/D *cis*, D/E *trans* (H-13α/H-17β) and E/F *cis* (*cis*-quinolizidine). The configurations were settled as 3-OH: β-equatorial, 6-OH: β-axial, 10-Me: β-axial, 20-Me: α-axial, 22-H: α, 25-Me: α-equatorial and a lone pair of the nitrogen: α. All the six membered rings, A, B, D, E and F are in the chair conformations.

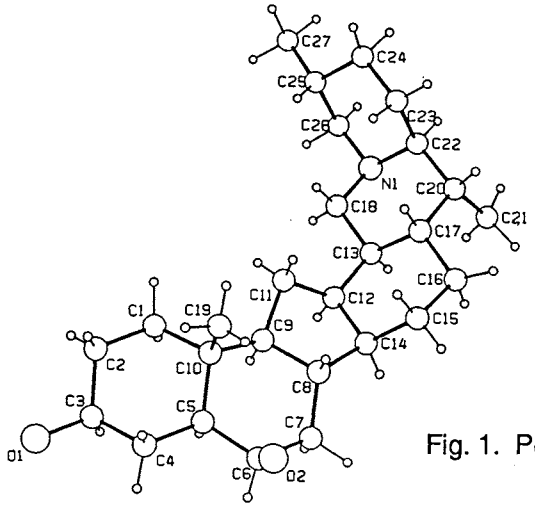


Fig. 1. Perspective drawing of persicanidine A.

3,6-*O*-Bis-*p*-bromobenzoate (**1a**) of **1** showed a negative first CD band centered at 251 nm (Δε -3.4) and a positive second CD band at 238 nm (Δε +2.2).[8] This indicated a counterclockwise orientation between the two chromophores (Fig. 2) [9] and that **1** had a usual steroidal absolute stereostructure. Thus, the structure of **1** was completely assigned. Confirmative assignments of the ^{13}C NMR signals were performed through the combined use of the ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra.[10] The ^{13}C chemical shift of the C-18 (a carbon attached

to a nitrogen atom) exhibited an unexpected upfield shift to appear at δ 51.8, compared with that of other cevanine alkaloids (δ 65 - 60), which must be caused by the 1,3-diaxial interactions between the C-22 - C-23 and C-25 - C-26 bonds, and the H-18 axial proton (Fig. 3).

Compound **2** was identified as delavine by the EI mass, IR, ^{1}H and ^{13}C NMR spectra.[11]

About 50 naturally occurring cevanine alkaloids have been reported up to now,[3a] however, persicanidine A (**1**) is the first cevanine alkaloid with the D/E *trans* (H-13α/H-17β) and E/F *cis* (*cis*-quinolizidine) ring fusions. It is interesting from the view point of biosynthesis of the steroidal alkaloids that a plant produces two cevanine alkaloids of different ring fusions.

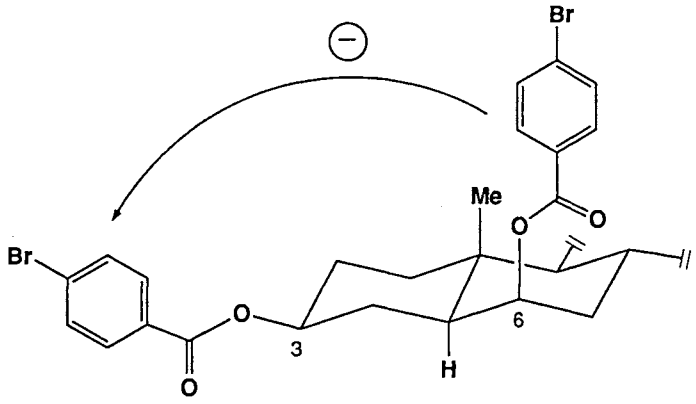


Fig. 2.

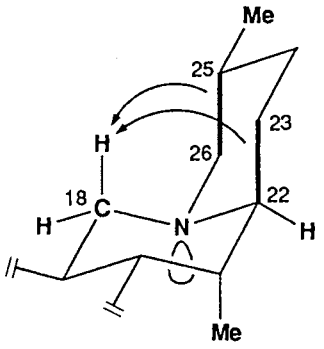


Fig. 3.

Persicanidine A (**1**) and delavine (**2**) showed medium inhibitory activity on cyclic AMP phosphodiesterase (**1**: IC_{50} 24.7 x 10^{-5} M; **2**: 8.8 x 10^{-5} M).[12] Steroidal alkaloids isolated from other Liliaceae plants are now being assayed.

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References

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2) "Useful Plants of the World," ed by M. Hotta, Heibonsha Ltd., Publishers, Tokyo (1989), p. 468.
3) *a*) A. U. Rahman, "Handbook of Natural Products Data," Elsevier Science Publishers B. V., Amsterdam (1990), Vol. 1; *b*) I. W. Southon and J. Buckingham, "Dictionary of Alkaloids," Chapman and Hall Ltd., London, New York (1989).
4) J. E. Bryan, "Bulbs," Timber Press Inc., Portland, Oregon (1989), Vol. 1, p. 178.
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7) Crystal system: monoclinic. Crystal dimensions (mm): 0.500 x 0.400 x 0.200. No. reflections used for unit cell determination (2θ range): 25 (88.5 - 89.9°). Omega scan peak width at half-height: 0.18. Lattice parameters: a = 24.023 (8)Å, b = 8.251 (2)Å, c = 17.578 (5)Å, β = 133.86 (1)°, V = 2512 (1)Å^3. Space

group: C2 (#5). Z value: 4. D_{calcd}: 1.141 g/cm^3. F_{000}: 956. $\mu_{(CuK\alpha)}$: 5.16 cm^{-1}. Diffractometer: Rigaku AFC5R. Radiation: CuKα (λ = 1.54178 Å). Temp : 23 °C. Attenuators: Ni foil (factors: 3.5, 12.7, 44.9). Take-off angle: 6.0°. Detector aperture: 6.0 mm horizontal, 6.0 mm vertical. Crystal to detector distance: 25.8 cm. Scan type: ω-2θ. Scan rate: 32.0°/min (in omega), 2 rescans. Scan width: (1.63 + 0.30 tanθ)°. $2\theta_{max}$: 120.1°. No. of reflections measured: total: 2072, unique: 2016 (R_{int} = 0.052). Corrections: lorentz-polarization, absorption (*trans.* factors: 0.77 - 1.35), secondary extinction (coefficient: 0.32312E-05). Structure solution: direct methods. Refinement: full-matrix least-squares. Function minimized: Σ w(|Fo| - |Fc|)^2. Least-squares weights: $4Fo^2/\sigma^2(Fo^2)$. p-Factor: 0.09. Anomalous dispersion: all non-hydrogen atoms. No. observations (I > 3.00 σ (I)): 1836. No. variables: 414. Reflection/parameter ratio: 4.43. Residuals: R; R_w: 0.056; 0.077. Goodness of fit indicator: 1.66. Max shift/error in final cycle: 0.65. Maximum peak in final diff. map: 0.43 e$^-$/Å^3. Minimum peak in final diff. map: -0.21 e$^-$/Å^3.

8) Some spectral data of **1a**: EIMS *m/z* (%): 781 $[M]^+$ (12), 183 (29), 111 (100); IR ν_{max} (KBr) cm^{-1}: 2930, 2860 (CH), 1715 (C=O), 1590, 1485 (aromatic rings); UV λ_{max} (EtOH - dioxane, 9 : 1) nm (log ε): 245 (4.59); ^{1}H NMR ($CDCl_3$) δ: 7.87, 7.85, 7.60, 7.53 (each 2H, d, *J* = 8.5 Hz, *p*-bromobenzoyl moieties), 5.28 (1H, br s, $W_{1/2}$ = 8.3 Hz, H-6), 5.00 (1H, br m, $W_{1/2}$ = 25.2 Hz, H-3), 1.21 (3H, s, H-19), 1.18 (3H, d, *J* = 6.5 Hz, H-21), 0.90 (3H, d, *J* = 5.8 Hz, H-27).

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10) ^{13}C NMR data of **1** ($CDCl_3$) δ: 39.5, 31.4, 72.0, 34.8, 48.0, 73.3, 39.7, 39.1, 57.8, 35.6, 25.6, 40.2, 33.2, 40.8, 25.1, 27.8, 30.6, 51.8, 14.8, 38.5, 14.7, 63.6, 24.2, 35.0, 23.6, 62.9, 19.6 (C-1 - C-27).

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