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THE STRUCTURE OF HAPEPUNINE FROM *FRITILLARIA CAMTSCHATCENSIS*; A NEW N-METHYL-22,26-EPIMINOCHOLESTENE

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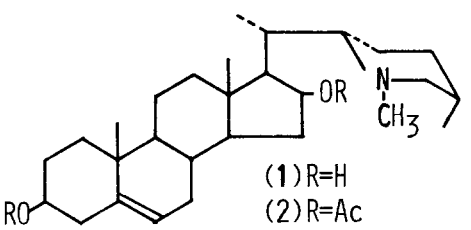
Isolation of solanidine from *Fritillaria camtschatcensis* has been reported by Mitsuhashi *et al.*[1] In addition to solanidine, a new alkaloid named hapepunine (1) after the Ainu name for the original plant, "Hapepui", was isolated from the mature plant of *Fritillaria camtschatcensis* Ker-Gawlr.

The basic fraction from the hydrolysate, which was obtained from the aerial part of the mature plant (after flowering), was crystallized from acetone, and solanidine was separated in about one-third weight of the basic fraction. The residue was separated by column chromatography on alumina to give the new alkaloid hapepunine (1), in addition to tomatidenol (3) and solasodine (4).



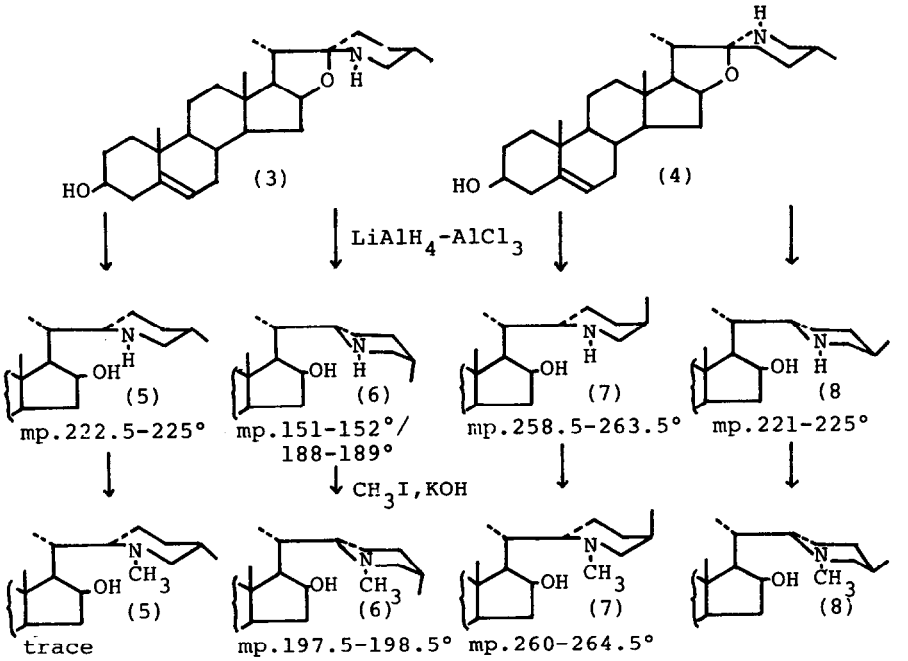
(1) R=H
(2) R=Ac

Hapepunine (1), mp 196.5-198.5°, $[\alpha]_D$ -72.57°, $C_{28}H_{47}NO_2$ (elementary analysis) afforded on acetylation in pyridine a diacetate (2), mp 207-212° $\nu_{max}^{CHCl_3}$ 1720 and 1680 cm^{-1}, δ 1.98 (3H, s), 2.00 (3H, s).

The nmr spectrum of (1) displayed two singlets (3H, each) at δ 0.96 and 1.02, indicative of C-18 and C-19 angular methyl groups of a normal steroidal ring system with Δ^5-double bond,[2] two doublets (3H, each, j = 6 Hz) at δ 1.03 and 1.09 corresponding to two secondary methyl groups at C-21 and C-27, and additional methyl signal at δ 2.30 (3H, s) for an N-methyl group, and a signal at δ 5.36 for a vinyl proton.

One of other protons, a multiplet centered at δ 3.52, is associated with α-hydrogen at C-3 [bearing with β-hydroxyl group] (this signal shifted downfield to δ 4.56 on acetylation), and the other a multiplet centered at δ 4.50 and showed 12 Hz in the half height width is associated with α-hydrogen at C-16 [bearing with β-hydroxyl group] (this signal shifted downfield to δ 5.24 on acetylation). The mass spectrum of (1) revealed ions at *m/e* 429 (M^+) and 112 (base peak). In the light of the nmr spectrum of (1), it is assumed that the base peak at *m/e* 112 is assigned to the N-methyl-piperidyl side chain moiety produced as a result of a bond fission between C-20 and C-22 of N-methyl-22,26-epiminocholestane, by comparison with the mass spectrum of teinemine,[3] which showed its base peak at *m/e* 98. From these properties, (1) was expected as N-methyl-22,26-epiminocholest-5-ene-3β,16β-diol and it remained to determine the configurations at C-22 and C-25 of (1).

In order to confirm the configurations at C-22 and C-25 of (1), tomatidenol (25S) and solasodine (25R) were reduced respectively with $LiAlH_4$ with $AlCl_3$ following the method of Sato[4] and Schreiber,[5] and four products were obtained and were proved to be isomeric in the configuration of C-22 and C-25 of dihydrospirosolane, namely, (22R,25S)-and (22S,25S)-dihydro-tomatidenol from tomatidenol and (22R,25R)- and (22S,25R)-dihydrosolasodine from solasodine, but the amount of(8) isolated prevented the further study. Each isomer was methylated with MeI in KOH, and three kinds of N-methyl



derivatives, (22R,25S) (5), (22S,25S) (6), and (22R,25R) (7) were obtained. Each isomer showed different Rf value on Silica gel tlc (DC-Fertigplatten, Kiesel gel 60 F_{254}, Merck); (5), 0.14; (6), 0.32; (7), 0.43; (1), 0.32, (cyclohexane:EtOAc:MeOH = 2:2:1). The Rf value and the physical constants of hapepunine (1) agreed completely with those of (6), and the melting point of (1) was not depressed by admixture with (6). From these results, hapepunine (1) was identified as N-methyl-(22S,25S)-22,26-epiminocholest-5-ene-3β,16β-diol.

Hapepunine (1) is the first compound which isolated from natural source as 16β-hydroxy -22,26-epiminocholestane derivative. In this plant, tomatidenol (3) accumulated in the aerial part at budding, then it gradually decreased during plant development. In progression of plant growth, the content of hapepunine (1) was constant, but N-methyl-trihydroxy-22,26-epimino-cholestene (the detail will be publishable elsewhere in the near future) gradually accumulated and its content reached about three times higher than

that of tomatidenol (3). It therefore appears that hapepunine (1) is synthesized from tomatidenol in the aerial part by biological degradation, not from the precursor of spirosolane biosynthesis.

REFERENCES

1. H. Mitsuhashi, U. Nagai, and T. Endo, Chem. Pharm. Bull. (Tokyo), 17, 2370 (1969)

2. R.F. Zurcher, Helv. Chim. Acta, 46, 2054 (1963).

3. K. Kaneko, M.W. Tanaka, E. Takahashi, and H. Mitsuhashi, Phytochemistry, 16, 1620 (1977).

4. Y. Sato and N. Ikekawa, J. Org. Chem., 26, 1945 (1961).

5. K. Schreiber and H. Ronsch, Tetrahedron, 21, 645 (1965).