over anhydrous Na_2SO_4. Upon evaporation of solvent a crystalline product was obtained. Recrystallization from MeOH gave I (6 mg) as colorless prisms. mp 128—129°. Mixed mp on admixture with the authentic sample showed no depression.

Equlibration of Epimeric 16-Bromo-17-ketones (V and VI)——To a solution of each epimer (12 mg) in THF (1 ml)–EtOH (2 ml) was added 1% ethanolic KOH (1 ml), and the resulting solution was allowed to stand at room temperature for 40 min. The solution was diluted with ether, washed with H_2O dried over anhydrous Na_2SO_4. On usual work-up a crystalline-product was obtained. The optical rotation was measured on each sample. From 16α-bromo-17-ketone (VI): $[\alpha]_D^{16}$ −55.1° (c=0.18); from 16β-bromo-17-ketone (V): $[\alpha]_D^{16}$ −56.8° (c=0.26).

Polarography——Polarographic reductions were run by Yanagimoto Model PA-102 polarograph equipped with a capillary of the following characteristics: m=6.58 mg/sec, t=4.9 sec at a mercury height of 64.5 cm. An electrolysis solution was prepared by weighing the sample into a 10 ml volumetric falsk, dissolving it in iso-PrOH (*ca.* 5 ml) and adding the acetate buffer (pH 6.0) (2 ml). The solution was then made up to 10 ml with additional iso-PrOH. The sample solution thus prepared was deaerated by bubbling N_2 gas and then polarographed at 25±0.2°. Half-wave potential was expressed in volt *vs.* the saturated calomel electrode.

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UDC 581.19 : 582.572.2

Studies on Constituents of *Fritillaria camtschatcensis* Ker-Gawler

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Solanidine, solanthrene, and another alkaloid were isolated from the acid hydrolyzate of the methanol extract of the bulbs of *Fritillaria camtschatcensis*. A new oily substance was also isolated and a tentative structure was proposed for it.

Several plants of *Fritillaria* species are known to contain alkaloids with a cevanine skeleton.[2] It was expected that *F. camtschatcensis* might contain some steroidal alkaloids.

Dried and powdered bulbs of *F. camtschatcensis* collected in Teshio district, Hokkaido, were extracted with methanol, and the extract was hydrolyzed with 5% hydrochloric acid at 60° for 3.5 hr. A large amount of precipitate was formed during the hydrolysis, which was filtered off and extracted with chloroform. From the chloroform extract three alkaloids were isolated by means of silica gel chromatography. From the acid aqueous filtrate, an oily substance was obtained by extraction with chloroform and distillation of the extract under reduced pressure. The separation procedure is illustrated in Fig. 1. The oily substance was named compound I and the three alkaloids were named compounds II, III, and IV, in the order of chromatographic elution.

Compound III has mp 208—210° and much the same mass spectrum as that of solanidine.[3] Comparisons of compound III with the authentic sample of solanidine by means of thin-layer

1) Location: *Kita-12-jo, Nishi-5-chome, Sapporo; a)* Present address: *Tsumura-Juntendo Research Laboratory, Komae, Kitatama-gun, Tokyo.*

2) S. Ito, M. Kato, K. Shibata, and T. Nozoe, *Chem. Pharm. Bull.* (Tokyo), **11**, 1337 (1962); H. Morimoto and S. Kimata, *ibid.*, **8**, 302 (1960); M. Fukuda, *Nippon Kagaku Zasshi*, **69**, 167 (1948); **50**, 74 (1929); T.T. Chu and J.Y. Loh, *Hua Hsueh Hsueh Pao*, **21**, 241 (1955) [*C.A.*, **51**, 445 (1957)].

3) H. Budzikiewicz, *Tetrahedron*, **20**, 2267 (1964).

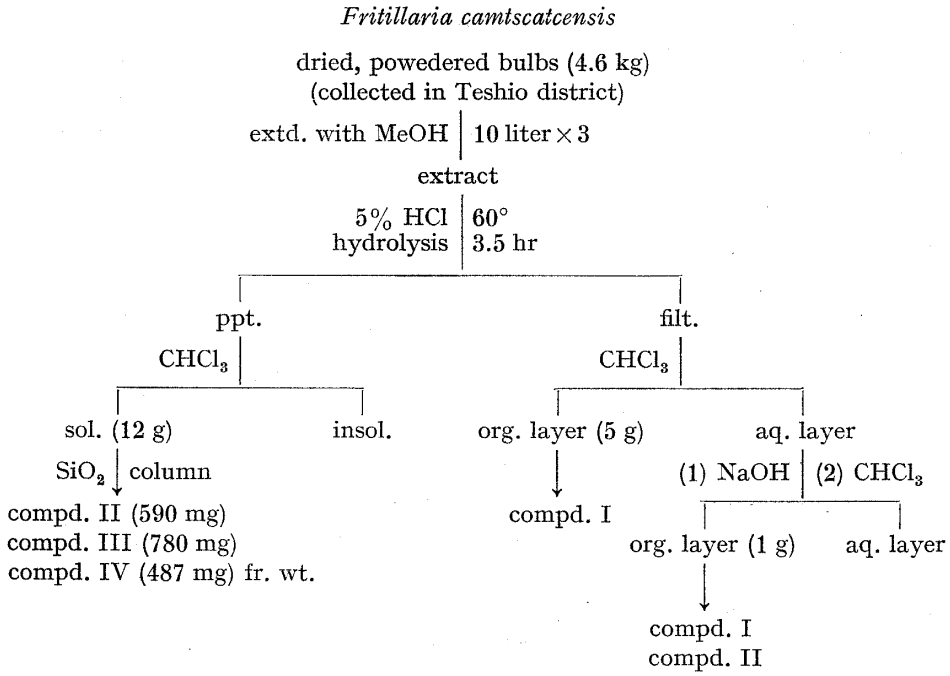

Fig. 1

chromatography, paper partition chromatography, infrared (IR), and mixed melting point were made and their identity was proved.

Compound II has mp 162—165° and a molecular formula of $C_{27}H_{41}N$, which corresponds to the anhydro derivative of solanidine. From these results and the main fragments in its mass spectrum, which are identical with that of solanidine, compound II is considered to be solanthrene,[4] probably an artefact formed during acid hydrolysis. Its ultraviolet (UV) spectrum indicates the position of the double bonds to be at 3- and 5-positions.[5]

Compound IV has mp 138—152° and colors pink with antimony trichloride similarly as compounds II and III. We have no further information on the substance because of scarcity of the material obtained.

So far as we know this is the first time that the alkaloids of solanidanine skeleton have been isolated from plants of *Fritillaria spp.* This is a quite interesting fact from the point of view that alkaloids of cevanine type might be synthesized through solanidine in plants and that *F. camtschatcensis* might lack the enzyme systems for this conversion. Absence of verticine, jervine, or veratramine was proved by comparison with their authentic samples on thin-layer chromatography.

Compound I has bp 105° (5 mmHg) and a molecular formula of $C_6H_6O_3$. It gives a diacetate and a mono-3,5-dinitrobenzoate. Thus, two hydroxyl groups and one carbonyl group (probably ketone) were assigned for the three oxygen atoms in the molecular formula on the basis of the IR spectra of the derivatives. Its nuclear magnetic resonance (MNR) spectrum shows two doublet peaks of AB-type, each of which corresponds to one proton, in vinyl proton region, a sharp two-proton singlet at δ=4.68, which can be assigned to a $-CH_2-(OH)$ group, and a broad two-proton singlet, which vanishes when treated with D_2O and was assigned to the hydroxylic protons. These results can be summarized in the following partial structure and the skeleton bracketed should contain a carbonyl and at least a double bond bearing two protons (Chart 1).

The degree of unsaturation for the skeleton is calculated to be four. Two unsaturation can be attributed to a carbonyl and a double bond. Three possibilities can be considered for

4) A. Soltys, *Ber.*, **66**, 762 (1933).
5) R.D. Haworth and J. Mckenna, *Chem. Ind.* (London), **1951**, 312.

the remaining two unsaturations: (i) Two double bonds, which means three double bonds as a whole on five carbon atoms, hence the presence of an allenic or acetylenic linkage, which however, could not be detected in the IR spectrum: (II) two rings which means a bicyclopentane system, that should be bicyclo [2.1.0] pentenone on the basis of the above mentioned requirements but which, is not possible from the IR spectrum; and (III) one double bond and one ring; there are four possible skeletons shown below (Chart 2).

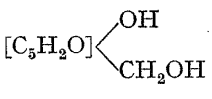

Chart 1

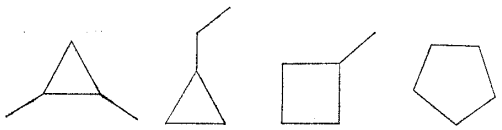

Chart 2

Though we have no positive reasons to reject a three- or four-membered ring, a five-membered ring was prefered simply on the basis of much more frequent occurrence of the latter than the former in nature. Thus, cyclopentadienone skeleton was assigned tentatively to compound I.

Since the coupling constant (J=4 Hz) for the two vinylic protons requires that they must be on adjacent carbon atoms, the three structures, A, B, and C, are probable (Chart 3).

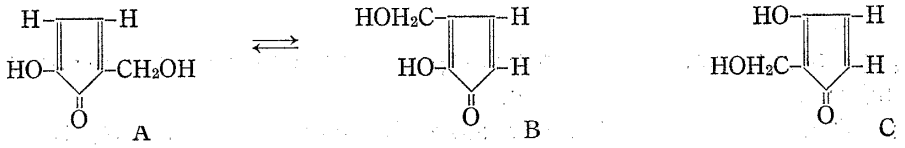

Chart 3

The structure C can be eliminated because the mono-3,5-dinitrobenzoate has a chelated OH-band at 3050 cm^{-1}. The structures A and B are tautomeric and it is not significant to distinguish them. We, therefore, would like to propose the tautomeric structure A or B for compound I.

The structures proposed have the same skeleton as that of methylreductic acid formed by thermolysis of calotropis glycosides.[6] Since compound I was isolated from the acid hydrolyzate, it can be an artefact. Hence, it would be interesting to examine the origin of compound I by isolating the alkaloids at a glycoside level.

Experimenta

Separation Procedure——Dried and powdered bulbs (4.6 kg) of *F. camtschatcensis* were extracted three times (with 10 liter each) of MeOH. MeOH was evaporated under reduced pressure and the residue was heated with 5% HCl at 60° for 3.5 hr. During this hydrolysis a large amount of precipitate was formed and collected by filtration. The precipitate was extracted with $CHCl_3$. On evaporation of the filtered and separated $CHCl_3$ solution, brown-black solid residue (12 g) was obtained, one-half of the fraction (6 g) was chromatographed on a column of silica gel (120 g). Elution was effected with $CHCl_3$ containing 0—5% EtOH, and the fractions containing compound II (390 mg), compound III (295 mg), and compound IV (244 mg) were collected (thin-layer chromatography check). Pure crystals of these compounds were obtained by preparative thin-layer chromatography (on silica plate; solvent $CHCl_3$:EtOH=9:1) and recrystallized from $CHCl_3$–MeOH.

The filtrate of the acid hydrolyzate was extracted with $CHCl_3$, and an oily substance was obtained from the organic layer. Pure compound was yielded by preparative thin-layer chromatography (on silica plate; solvent, $CHCl_3$) and distillation.

On basification of the acid aqueous layer and its extraction with $CHCl_3$ an oily substance (1 g) was obtained from the $CHCl_3$ layer and chromatographed to give a small amount of compounds I and III.

Properties of Compound I——bp 105° (5 mmHg); UV λ_{max}^{EtOH} mμ (log ε): 226 (3.46), 282 (4.04). IR ν_{max}^{neat} cm^{-1}: 3400, 1670, 1580. NMR (in $CDCl_3$) δ: 3.30 (2H, broad singlet), 4.68 (2H, singlet), 6.50 (1H, doublet,

6) G. Hesse and F. Reicheneder, *Ann.*, **526**, 252 (1936).

J=4 Hz), 7.25 (1H, doublet, J=4 Hz). Mass spectrum: M^+=126. *Anal.* Calcd. for $C_6H_6O_3$: C, 57.14; H, 4.80. Found: C, 56.40, H, 5.01.

Compound I Diacetate——Compound I (100 mg) was reacted with a mixture of acetic anhydride and pyridine under reflux for 2 hr and the reaction mixture was worked up as usual. IR ν_{max}^{neat} cm^{-1}: 1755, 1680, 1580. NMR (in $CDCl_3$) δ: 2.12 (6H, singlet), 4.68 (2H, singlet), 6.50 (1H, doublet, J=4 Hz), 7.25 (1H, doublet, J=4 Hz). *Anal.* Calcd. for $C_{10}H_{10}O_5$: C, 57.14; H, 4.80. Found: C, 56.69; H, 4.80.

Compound I 3,5-Dinitrobenzoate——Compound I (100 mg) was reacted with 3,5-dinitrobenzoyl chloride (150 mg) in pyridine (2.0 ml) at room temperature overnight. mp 122—123.5°. IR ν_{max}^{Nujol} cm^{-1}: 3050, 1740, 1680, 1540, 1350. NMR (in $CDCl_3$) δ: 3.52 (2H, singlet), 6.75 (1H, doublet, J=4 Hz), 7.25 (1H, doublet, J=4 Hz), 9.10 (3H, multiplet), 9.60 (1H, singlet). Mass spectrum m/e: 320, 290, 195, 179, 165, 149, 125, 109. *Anal.* Calcd. for $C_{13}H_8O_8N_2$: C, 48.76; H, 2.52; N, 9.47. Found: C, 48.52; H, 2.21; N, 8.75.

Properties of Compound II——mp 162—165°. UV λ_{max}^{EtOH} mμ (ε) 228 (18600), 235 (19500), 243 (12400). Mass spectrum m/e: 379, 378, 364, 204, 150. *Anal.* Calcd. for $C_{27}H_{41}N$: C, 85.42; H, 10.89; N, 3.69. Found: C, 85.53; H, 10.80; N, 3.90.

Properties of Compound III——mp 208—210°. Mass spectrum m/e: 397, 396, 382, 204, 150.

Comparisons of Compound III with Authentic Solanidine——In the following "a" means authentic and "s" means the isolated compound III. Thin-layer chromatography (silica plate): Solvent A ($CHCl_3$:EtOH =10:1). *Rf* a=0.51, s=0.49; Solvent B (C_6H_6:C_6H_5N:AcOH=9:1:0.1), *Rf* a=0.60, s=0.57. Paper partition chromatography (descend., Toyo Roshi No. 50 sprayed with 1N tartaric acid): solvent ($CHCl_3$: cellosolve acetate:C_6H_5N=60:40:4), developing time 4.5 hr, distance moved, a=13.5 cm, s=13.5 cm. IR (Nujol): superposable. Mixed mp: a=207—211°, s=208—210°, mixt.=207—211°.

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UDC 547.963.3.07

Synthetic Nucleosides and Nucleotides. VII. A Direct Replacement of 6-Thiol Group of 6-Thioinosine and 6-Thioguanosine with Hydrazine Hydrate[1]

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As a conventional method for the synthesis of 6-substituted purine ribonucleosides have been employed the reaction of 6-halogeno or 6-alkylmercaptopurine ribonucleosides with several nucleophiles.[3] But, because the 6-thiol group of purine nucleosides is known to be less reactive than the 4-thiol group of pyrimidine nucleosides, direct replacement at 6-position of 6-mercapto-9-β-D-ribofuranosylpurine (6-thioinosine) (Ia) and 2-amino-6-mercapto-9-β-D-ribofuranosylpurine (6-thioguanosine) (Ib) with amino or substitute amino group has not been reported and failed when attempted.[4]

In previous paper of this series, the authors described the synthesis of 8-hydrazino derivatives of guanosine and xanthosine from the corresponding 8-bromo derivatives by treatment with 60% hydrazine hydrate in aqueous or methylcellosolve solution under mild condition.[5]

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1) Part VI of this series: M. Saneyoshi and F. Sawada, *Chem. Pharm. Bull.* (Tokyo), **17**, 181 (1969).
2) Location: *a*) *Tsukiji 5-chome, Chuo-ku, Tokyo*; *b*) *Numakage, Urawa, Saitama.*
3) J.A. Montgomery and H.J. Thomas, *Advan. Carbohyd. Chem.*, **17**, 301 (1962).
4) T. Naito, K. Ueno, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **12**, 951, (1964).
5) M. Saneyoshi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1616 (1968).