[Chem. Pharm. Bull.
17(11)2366—2370(1969)]

UDC 547.92.02

Conformational Analysis of Epimeric 16-Bromo-13α-estrones[1]

Toshio Nambara, Toshihiro Kudo, Hiroshi Hosoda,
Kikuko Motojima, and Shujiro Goya

Pharmaceutical Institute, Tohoku University[2]

(Received May 13, 1969)

It is well documented in the literatures that an alteration of the steroidal skeleton often exerts a significant influence on the reactivity of the remote site of molecule. These phenomena are interpreted in terms of the long-range conformational effect.[3] As a series of studies on the stereochemistry of 13α-steroids,[4] an interest in these respects prompted us to explore

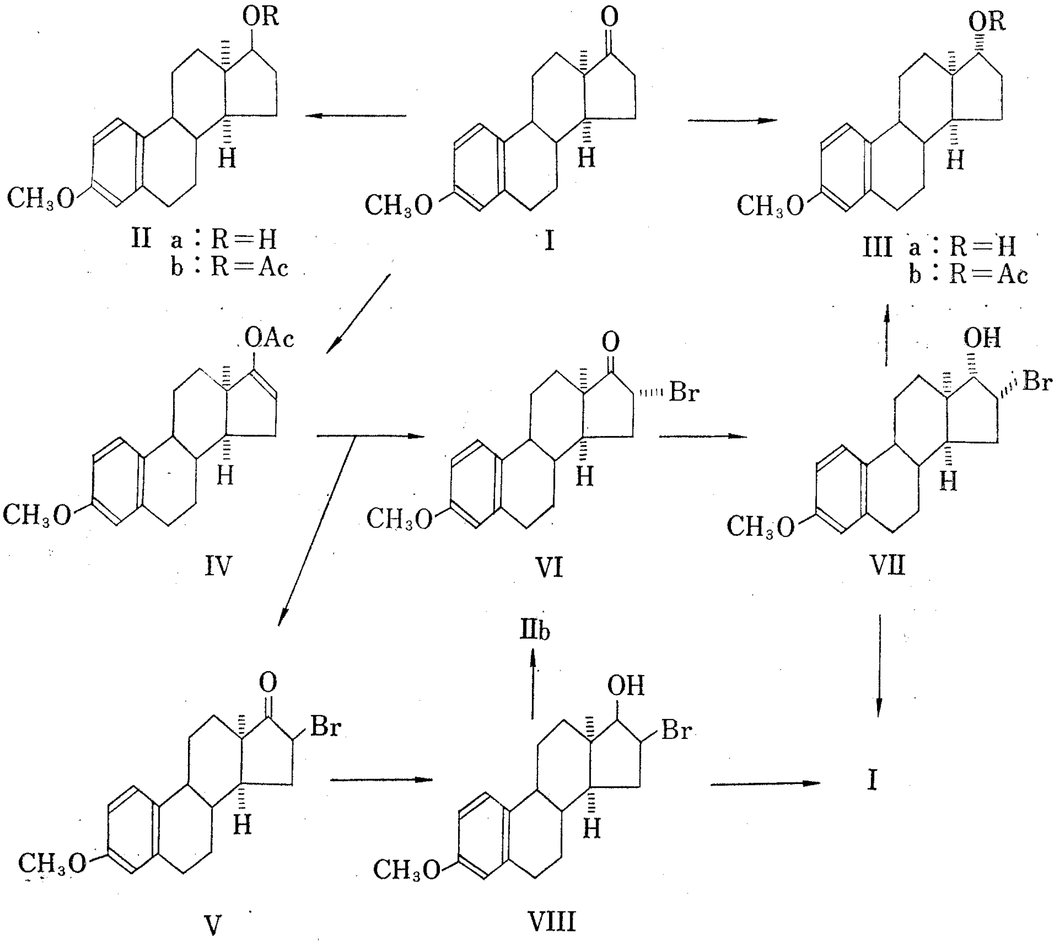

Chart 1

---

1) This paper constitutes Part XXXI of the series entitled "Analytical Chemical Studies on Steroids"; Part XXX: T. Nambara, H. Hosoda, and M. Usui, *Chem. Pharm. Bull.* (Tokyo), **17**, 1687 (1969).

2) Location: *Aobayama, Sendai.*

3) D.H.R. Barton, F. McCapra, P.J. May, and F. Thudium, *J. Chem. Soc.*, **1960**, 1297 and references quoted therein.

4) *a*) T. Nambara, H. Hosoda, and S. Goya, *Chem. Pharm. Bull.* (Tokyo), **16**, 1266(1968); *b*) T. Nambara, H. Hosoda, M. Usui, and J. Fishman, *Chem. Pharm. Bull.* (Tokyo), **16**, 1802 (1968); *c*) T. Nambara, H. Hosoda, and M. Usui, *Chem. Pharm. Bull.* (Tokyo), **17**, 375 (1969); *d*) T. Nambara, H. Hosoda, and M. Usui, *Chem. Pharm. Bull.* (Tokyo), **17**, 947 (1969).

the distant effect due to aromatic ring A on the conformation of ring D. The present paper deals with the preparation of two epimeric 16-bromo-17-ketones and the conformation of ring D on the basis of their physical constants.

An initial project was directed to the reduction of 13α-estrone 3-methyl ether (I) with sodium borohydride. Chromatographic separation of the reduction products gave the 17β-hydroxyl derivative (IIa) and its epimer (IIIa) in a ratio of 10 to 7. The configurations at C-17 of these compounds were deduced from nuclear magnetic resonance (NMR) spectral data. It has recently been demonstrated that the use of pyridine instead of chloroform results in the solvent shift and the extent of deshielding effect on methyl group vicinally situated to the hydroxylic function depends on the magnitude of the dihedral angle between these two groups.5) Therefore configuration of a 17-hydroxylic compound with the shift value of 0.22 ppm should be α and that of the epimer with 0.01 ppm should be defined as β. In addition the splitting pattern of C-17 proton and chromatographic behavior of the 17-ols also support this assignment. It is to be noted that the predominant reduction product of 13α-estrone is the 17β-ol in contrast to that of 13α-androstane,4a,6) presumably because the lack of C-10 methyl group would favor the formation of the 17β-hydroxyl derivative.

Treatment of I with isopropenyl acetate in the presence of anhydrous *p*-toluenesulfonic acid as catalyst gave the enol acetate (IV) in satisfactory yield. Subsequent bromination with an equimolar amount of reagent under non-enolizing conditions furnished the 16α-bromo-17-ketone (VI) accompanied with the 16β-bromo compound (V), whose separation was efficiently achieved by preparative thin-layer chromatography (TLC). The structures of these two epimers were established by the standard method of Fieser and Ettorre.7) Reduction of V with lithium aluminum hydride at −17° for a short period8) provided solely the 16β,17β-bromohydrin (VIII) in satisfactory yield. This *cis*-bromohydrin structure was rationalized by the formation of the 17-ketone when refluxed with methanolic potassium hydroxide. On the other hand reductive dehalogenation of VIII with hydrogen followed by acetylation gave 3-methoxy-13α-estra-1,3,5(10)-trien-17β-ol acetate (IIb), which proved to be identical with the authentic sample. Likewise, on metal hydride reduction VI was transformed into the *cis*-16α,17α-bromohydrin (VII), which was similarly confirmed by leading to the 17-ketone with alkali and to the 17α-hydroxylic compound by catalytic hydrogenation. It is of interest that metal hydride reduction of the two 16-bromo-17-ketones provides the *cis*-bromohydrins as sole product, respectively, probably due to the characteristic ring fusion of 13α,14α-steroid. Treatment of each 16-bromo-17-ketone with potassium hydroxide resulted in a mixture of two epimers. The optical rotation of equilibrated mixture was measured and in consequence the composition was found to be 25% 16β and 75% 16α.

Table I. Infrared Spectral and Polarographic Data

Substance	IR $\nu_{\max}^{\mathrm{CCl_4}}$ cm^{-1}	$\Delta\nu$	PG $E_{1/2}$ (*vs.* SCE) V
17-Ketone (I)	1741		
16β-Bromo-17-ketone (V)	1751	+10	−0.20
16α-Bromo-17-ketone (VI)	1753	+12	−0.20

---

5) *a*) P.V. Demarco, E. Farkas, D. Doddrell, B.L. Mylari, and E. Wenkert, *J. Am. Chem. Soc.*, **90**, 5480 (1968); *b*) T. Nambara, H. Hosoda, and M. Usui, *Chem. Pharm. Bull.* (Tokyo), **17**, 1687 (1969).

6) L.J. Chinn, *J. Org. Chem.*, **30**, 4165 (1965).

7) L.F. Fieser and R. Ettorre, *J. Am. Chem. Soc.*, **75**, 1700 (1953).

8) Otherwise the reaction proceeded in an abnormal manner in that bromine was lost and the products obtained were the 17-hydroxyl derivatives.9)

9) T. Nambara, H. Hosoda, and T. Shibata, *Chem. Pharm. Bull.* (Tokyo), in press.

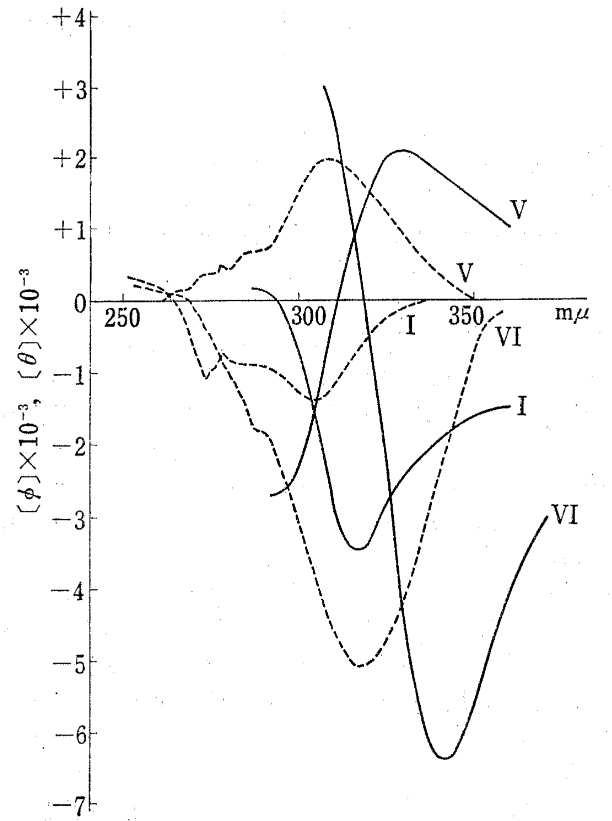

Fig. 1. Optical Rotatory Dispersion (——) and Circular Dichroism Curves (------) of I, V and VI in Methanol (at 11°)

The infrared spectral and polarographic data of the two epimeric 16-bromo-17-ketones and their parent ketone are listed in Table I. The shift values of infrared absorption spectra and the magnitude of half-wave potentials[10] reveal that the two C-16 positions in 13α-estrone are equivalent and bisectional. As to the NMR spectra the 16α-proton resonates at 4.45 ppm as quartet ($J=2.6$, 8.5 cps) while the 16β-proton at 4.12 ppm as triplet ($J=9.5$ cps). There can be seen no substantial difference in splitting pattern and coupling constants from the corresponding 13α-androstanes. The results of rotatory dispersion and circular dichroism show that the axial haloketone rule is also valid in the fused α-halocyclopentanones. As illustrated in Fig. 1, the 16β-bromo-17-ketone exhibits positive Cotton effect whereas its epimer and the parent ketone show negative sign.

Based upon these results it is concluded that conformation of ring D in 13α-estrone would practically be identical with that of 13α-androstane and the influence of angular distortion due to the aromatic ring on the remote site of molecule would be of a subtle nature.

Experimental[11]

Reduction of 3-Methoxy-13α-estra-1,3,5(10)-trien-17-one (I)——To a stirred solution of I (150 mg) in MeOH (10 ml) was added portionwise $NaBH_4$ (80 mg) at 0°, and the resulting solution was allowed to stand at 0° for 10 hr. After decomposition of excess $NaBH_4$ with AcOH (2 drops) the reaction mixture was diluted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent a crystalline residue obtained was submitted to preparative TLC using hexane–AcOEt (3:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.33) and recrystallization of the eluate from MeOH gave 3-methoxy-13α-estra-1,3,5(10)-trien-17α-ol (IIIa) (35 mg) as colorless needles. mp 134—136°. $[\alpha]_D^{16}$ +9.5° ($c=0.11$). *Anal.* Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.27; H, 8.96. NMR (5% solution in $CDCl_3$) δ: 0.96 (3H, s, 18-CH_3), 3.77 (3H, s, CH_3O–), 4.22 (1H, t, $J=7.9$ cps, 17β-H), 6.6—7.2 (3H, aromatic ring); (5% solution in pyridine) δ: 1.18 (3H, s, 18-CH_3). Acetate (IIIb): Usual acetylation of IIIa with Ac_2O and pyridine followed by recrystallization from MeOH gave IIIb as colorless needles. mp 66—67°. $[\alpha]_D^{18}$ +4.2° ($c=0.36$). *Anal.* Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.46; H, 8.32. NMR (5% solution in CCl_4) δ: 0.98 (3H, s, 18-CH_3), 1.98 (3H, s, CH_3COO–), 3.71 (3H, s, CH_3O–), 5.13 (1H, t, $J=7.9$ cps, 17β-H), 6.5—7.2 (3H, aromatic ring). Elution of the adsorbent corresponding to the spot (*Rf* 0.40) and recrystallization of the eluate from MeOH gave 3-methoxy-13α-estra-1,3,5(10)-trien-17β-ol (IIa) (50 mg) as colorless needles. mp 77—78°. $[\alpha]_D^{16}$ +95.8° ($c=0.13$). *Anal.* Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.15; H, 8.90. NMR (5% solution in $CDCl_3$) δ: 0.97 (3H, s, 18-CH_3), 3.77

---

10) S. Goya, H. Hosoda, T. Kudo, C. Anzo, and T. Nambara, *Yakugaku Zasshi*, **89**, 336 (1969).

11) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were determined in $CHCl_3$ solution unless otherwise stated. Infrared spectra were obtained on carefully calibrated Hitachi Model 225 spectrophotometer. Optical rotatory dispersion and circular dichroism measurements were carried out on Nihon-Bunko Model ORD/UV-5 recorder. Nuclear magnetic resonance spectra were run on Hitachi Model H-60 spectrometer at 60 Mcps using $(CH_3)_4Si$ as an internal standard. For preparative TLC silica gel H (E. Merck AG) was used as an adsorbent.

(3H, s, CH_3O–), 6.6—7.2 (3H, aromatic ring); (5% solution in pyridine) δ: 0.98 (3H, s, 18-CH_3). Acetate (IIb): Usual acetylation of IIa with Ac_2O and pyridine followed by recrystallization from MeOH gave IIb as colorless prisms. mp 76°. $[\alpha]_D^{16}$ +93.3° (c=0.10). *Anal.* Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.76; H, 8.36. NMR (5% solution in CCl_4) δ: 1.03 (3H, s, 18-CH_3), 1.91 (3H, s, CH_3COO–), 4.30 (3H, s, CH_3O–), 4.83 (1H, m, 17α-H), 6.5—7.2 (3H, aromatic ring).

3-Methoxy-13α-estra-1,3,5(10),16-tetraen-17-ol Acetate (IV)——To a solution of I (1 g) in isopropenyl acetate (32 ml) was added anhydrous *p*-TsOH (220 mg) and refluxed for 25 hr. The resulting solution was concentrated to its half volume by slow distillation over a period of 2 hr. Additional isopropenyl acetate (16 ml) and anhydrous *p*-TsOH (110 mg) were added and concentrated again to its half volume during 7 hr. The reaction mixture was diluted with ether, washed with ice-cooled 5% $NaHCO_3$, and H_2O and dried over anhydrous Na_2SO_4. After usual work-up the residue was dissolved in hexane–benzene (2:1) and filtered through Al_2O_3 (25 g). After evaporation of solvent a crystalline product obtained was submitted to preparative TLC using hexane–AcOEt (4:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.74) and recrystallization of the eluate from MeOH gave IV (502 mg) as colorless leaflets. mp 87—88°. $[\alpha]_D^{16}$ −21.5° (c=0.09). *Anal.* Calcd. for $C_{21}H_{26}O_3$: C, 77.27; H, 8.03. Found: C, 77.17; H, 7.80.

Bromination of IV——To a stirred solution of IV (700 mg) in CCl_4 (35 ml) containing anhydrous K_2CO_3 (400 mg) was added a solution of the calculated amount of Br_2 dissolved in CCl_4 dropwise at 0°. The resulting solution was washed with 10% $NaHSO_3$, H_2O and dried over anhydrous Na_2SO_4. After usual work-up a crystalline product obtained was submitted to preparative TLC using hexane–AcOEt (3:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.63) and recrystallization of the eluate from MeOH gave 3-methoxy-16β-bromo-13α-estra-1,3,5(10)-trien-17-one (V) (108 mg) as colorless needles. mp 152—154°. $[\alpha]_D^{21}$ +51.2° (c=0.14). *Anal.* Calcd. for $C_{19}H_{23}O_2Br$: C, 62.13; H, 6.31. Found: C, 62.08; H, 6.24. NMR (5% solution in CCl_4) δ: 1.10 (3H, s, 18-CH_3), 3.72 (3H, s, CH_3O–), 4.45 (1H, q, J=2.6, 8.5 cps, 16α-H), 6.5—7.2 (3H, aromatic ring). Elution of the adsorbent corresponding to the spot (*Rf* 0.77) and recrystallization of the eluate from MeOH gave 3-methoxy-16α-bromo-13α-estra-1,3,5(10)-trien-17-one (VI) (312 mg) as colorless leaflets. mp 122—123°. $[\alpha]_D^{16}$ −92.1° (c=0.12). *Anal.* Calcd. for $C_{19}H_{23}O_2Br$: C, 62.13; H, 6.31. Found: C, 61.86; H, 6.26. NMR (5% solution in CCl_4) δ: 1.24 (3H, s, 18-CH_3), 3.72 (3H, s, CH_3O–), 4.12 (1H, t, J=9.5 cps, 16β-H), 6.5—7.2 (3H, aromatic ring).

3-Methoxy-16α-bromo-13α-estra-1,3,5(10)-trien-17α-ol (VII)——To a solution of VI (250 mg) in anhydrous ether (30 ml) was added $LiAlH_4$ (220 mg) portionwise at −17° and allowed to stand for 5 min. The excess $LiAlH_4$ was decomposed with 10% H_2SO_4 and the reaction mixture was extracted with ether, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent a crystalline product obtained was submitted to preparative TLC using hexane–AcOEt (3:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.52) and recrystallization of the eluate from MeOH gave VII (96 mg) as colorless prisms. mp 128—129°. $[\alpha]_D^{18}$ +32.2° (c=0.25). *Anal.* Calcd. dor $C_{19}H_{25}O_2Br$: C, 61.79; H, 6.82. Found: C, 61.63; H, 6.69

Debromination of VII by Catalytic Hydrogenation——A solution of VII (40 mg) in MeOH (12 ml) was shaken with 5% Pd/$BaCO_3$ (150 mg) under a current of H_2 at room temperature for 48 hr. After removal of catalyst by filtration the filtrate was concentrated to give a crystalline product. Recrystallization from MeOH gave IIIa (18 mg) as colorless needles. mp 130—131°. Mixed mp on admixture with the authentic sample showed no depression and infrared (IR) spectra of two samples were entirely identical.

Transformation of VII into I with Potassium Hydroxide——A solution of VII (30 mg) in 5% methanolic KOH was refluxed for 3 hr. The resulting solution was diluted with AcOEt, washed with H_2O and dried over anhydrous Na_2SO_4. Upon evaporation of solvent a crystalline product was obtained. Recrystallization from MeOH gave I (12 mg) as colorless prisms. mp 128—129°. Mixed mp on admixture with the authentic sample showed no depression and IR spectra of two samples were entirely identical.

3-Methoxy-16β-bromo-13α-estra-1,3,5(10)-trien-17β-ol (VIII)——To a solution of V (68 mg) in anhydrous ether (10 ml) was added $LiAlH_4$ (52 mg) portionwise at −17° and allowed to stand for 5 min. The excess $LiAlH_4$ was decomposed with 10% H_2SO_4 and the reaction mixture was extracted with ether, washed with H_2O and dried over anhydrous Na_2SO_4. After evaporation of solvent a crystalline product was submitted to preparative TLC using hexane–AcOEt (3:1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.72) and recrystallization of the eluate from MeOH gave VIII (41 mg) as colorless needles. mp 118—120°. $[\alpha]_D^{16}$ +86.2° (c=0.12). *Anal.* Calcd. for $C_{19}H_{25}O_2Br$: C, 61.79; H, 6.82. Found: C, 61.85; H, 6.79.

Debromination of VIII by Catalytic Hydrogenation——A solution of VIII (25 mg) in MeOH (15 ml) was shaken with 5% Pd/$BaCO_3$ (90 mg) under a current of H_2 at room temperature for 48 hr. After removal of catalyst by filtration the filtrate was concentrated to give an oily product. Treatment of this crude product with Ac_2O (0.5 ml) and pyridine (1 ml) in the usual manner followed by recrystallization from MeOH gave IIb (10 mg) as colorless prisms. mp 76°. Mixed mp on admixture with the authentic sample showed no depression.

Transformation of VIII into I with Potassium Hydroxide——A solution of VIII (16 mg) in 5% methanolic KOH was refluxed for 3 hr. The resulting solution was diluted with AcOEt, washed with H_2O and dried 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.

Acknowledgement The authors are indebted to Hitachi, Ltd. for infrared spectral measurement and to all the staffs of central analytical laboratory of this Institute for elemental analyses and spectral measurements.

[Chem. Pharm. Bull. 17(11)2370—2373(1969)]

UDC 581.19 : 582.572.2

Studies on Constituents of *Fritillaria camtschatcensis* KER-GAWLER

HIROSHI MITSUHASHI, UKON NAGAI,[1] and TOHRU ENDO[1a)]

Faculty of Pharmaceutical Sciences, Hokkaido University[1]

(Received May 15, 1969)

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).