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55. Hiroshi Morimoto and Seiichi Kimata : Studies on the Components of *Fritillaria Thunbergii* MIQ. I. Isolation of Peimine and its new Glycoside.

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The Chinese drug Pei-mu (貝母) is composed of dried bulbs of *Fritillaria roylei* (Liliaceae) and is said to be used in Chinese medicine as antipyretic, antitussive, expectorant, sedative, lactagog, diuretic, and so forth.[1,2] In recent years, with the finding that the pharmacological effect of Pei-mu resembles those of the alkaloids of Veratrum,[3] studies have been begun on the basic components of Pei-mu and plants belonging to *Fritillaria*, and as a result they were found to be steroidal alkaloids like the alkaloids of Veratrum. Chou and Chen[4] isolated peimine and peiminine from Pei-mu produced in China and pointed out that both have strong physiological activities. Thereafter, Chou and Chu,[5,6] Chi, Kao, and Chang,[2,7] and Wu[8] clarified the constants shown in Table I.

Relationship between peimine and peiminine was clarified by Chou and Chen from the fact that the former was oxidized by the Beckmann method to the latter, which in turn reverted to the former by reduction with ethanol and metallic sodium. Peimine has two acylable hydroxyl groups, but one of them is converted into a keto group in peiminine. The nitrogen in both alkaloids is tertiary and every attempt to denitrogenate resulted in failure. From these properties and the molecular formulae, Wu[8] assumed the compounds to have the skeleton (A) of dihydrosolanidine but the assumption lacked chemical support. Recently, Chu, Hwang, and Loh[9,10] isolated from the dehydrogenation products of peimine 2,5-lutidine (B) as a basic product and hydrocarbons, $C_{22}H_{20}$ (or $C_{20}H_{18}$) (C) and $C_{18}H_{14}$ (D), as neutral products. Of the hydrocarbons, (C) was found to be identical with the benzofluorene derivative obtained by dehydrogenation of jervine by Jacobs and Pelletier,[11] and (D) with 10-methyl-11*H*-benzo[*a*]fluorene synthesized by Gross and Lankelma.[12] At the same time, extensive experiments were conducted on the dehydrogenation of imperialine, an alkaloid of *F. imperialis*, and from the products, (B), (D), and veranthridine (E) were isolated.[13,14] The last compound is a base and played an important rôle in the elucidation of the structure of cevine which is an alkaloid of Veratrum having a modified steroid skeleton (F). From the result, imperialine was found to have (F), and, therefore, peimine and peiminine were also presumed to have the same skeleton.

The foregoing is the outline of the studies hitherto made on peimine and peiminine.

In the meantime the present authors also undertook investigation on the components of Bai-mo (貝母) (*Fritillaria Thunbergii* MIQ.) produced in Japan and found that it con-

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1) K. K. Chen, A. L. Chen, T. Q. Chou : J. Am. Pharm. Assoc., **22**, 639(1933).
2) Y. F. Chi, Y. S. Kao, K. J. Chang : J. Am. Chem. Soc., **58**, 1306(1936).
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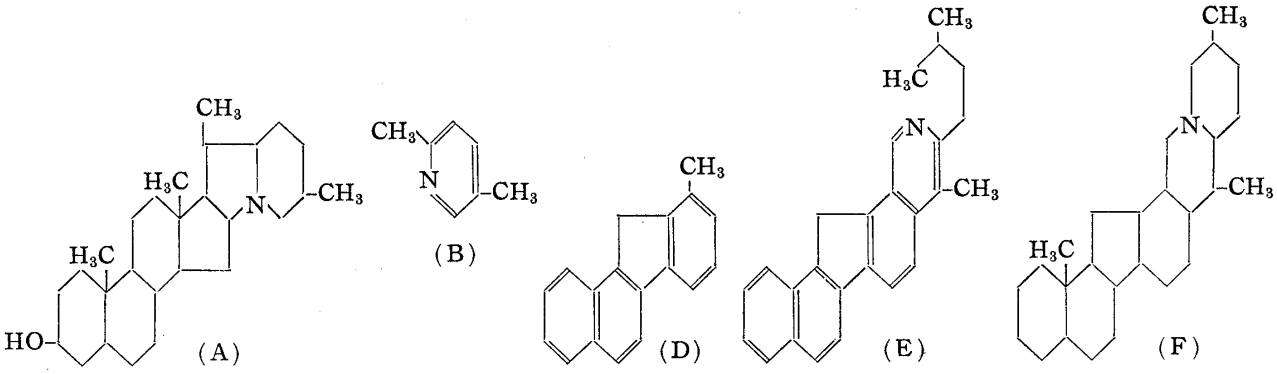


tains peimine as the main alkaloid and part of it in the form of glycoside. The results are presented herein.

The material plant was cultivated at the Takeda Mutobe Agricultural Garden and, after being collected in May of 1957, sliced finely and dried at 55~60° for 16 hours. The material thus produced was treated as shown in the chart and described in the experimental part. The extraction of the components and chromatographic purification of the crude alkaloid were conducted under simultaneous investigation of their compositions by paper partition chromatography (Table II). As a result, it was found that the methanol extract contains, besides the main alkaloid (Rf 0.84), a considerable amount of another alkaloid (Rf 0.67) and choline (Rf 0.29), and that they can be separated from each other because majority of the alkaloids were extracted by chloroform (1,1), while choline remained in aqueous solution (1,2,2). The substance obtained from the two chloroform solutions (1,1 and 1,2,1) was purified by repeated chromatographies on Florisil and

Table I.

	Peimine ($C_{27}H_{45}O_3N$)		Peiminine ($C_{27}H_{43}O_3N$)
	Literature	Author's	Literature
m.p.(°C)*	223~224	223~224	212~213
$[\alpha]_D$	−19.2°(EtOH)	−19.4°(EtOH) −20°($CHCl_3$)	−62.5°(EtOH)
HCl salt	295 (decomp.)	291~294 (decomp.) $[\alpha]_D^{18}$ −18.5°(H_2O)	292 (decomp.)
HBr salt	288 (〃) 293.5~294 (〃)		295 (〃)
MeI salt		205~210 (decomp.) $[\alpha]_D^{18}$ −14°(H_2O)	269 (〃)
HSCN salt	262 (〃)		175
$HClO_4$ salt		273 (decomp.)	
HNO_2 salt		145 (〃)	

* All m.p.s are uncorrected.

Table II. Rf Value of Each Fraction in Paper Partition Chromatography

Base / Color / Fraction	Peimine Orange yellow	Peiminoside Orange yellow	Choline Violet red
MeOH-extract	0.84	0.67	0.29
$CHCl_3$ soln. (1,1)	0.84	0.67	
Aqueous soln. (1,2)	0.84	0.67	0.29
$CHCl_3$ soln. (1,2,1)	0.84	0.67	
Aqueous soln. (1,2,2)			0.29

Developing solvent : AcOH-BuOH-H_2O (1:4:5), ascending method.
Temperature : 15°
Toyo Roshi No. 131
Reagent : Dragendorff's reagent

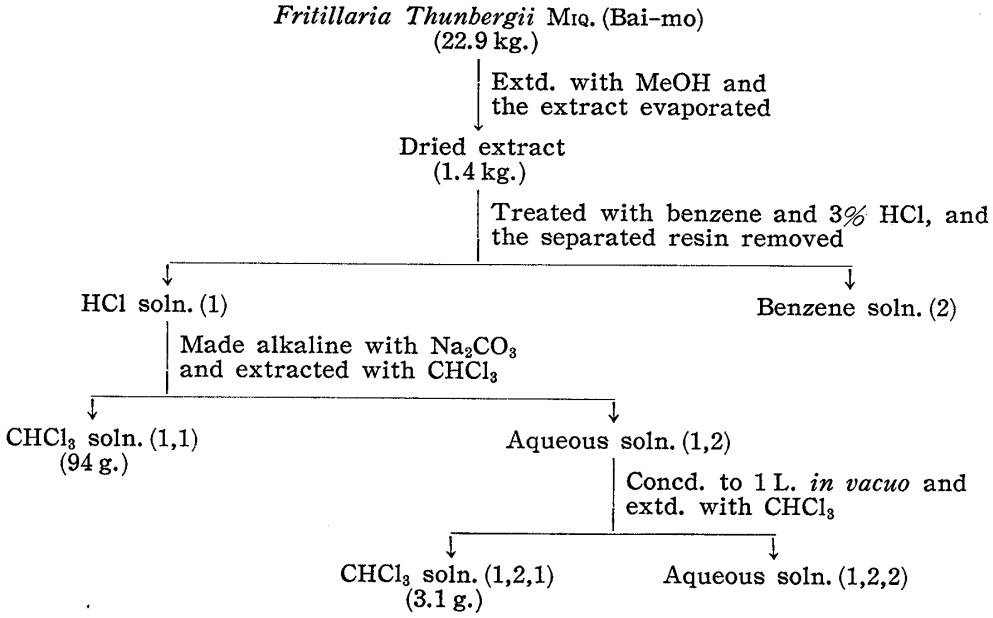


Chart 1. Isolation of Crude Alkaloid

the fraction showing Rf 0.84 was recrystallized from ethanol to colorless needles, m.p. 223~224°, $[\alpha]_D^{16}$ −19.4°(EtOH). From the analytical values and constants (Table I) of its various salts, the product was identified as peimine (I). The yield was 0.11% of the dried material.

The alkaloid with Rf 0.67, which is readily soluble in water, could not be crystallized but it was hydrolyzed by sulfuric acid into peimine and a sugar. The former was identified by mixed melting point determination, comparison of infrared spectrum, and by leading to its perchlorate, while the latter was led to its phenylosazone and the product was found to be identical with glucophenylosazone.

Since the alkaloid was found to be a glycoside of peimine, liberation of the sugar by hydrolysis with enzyme was attempted, and it was found that the alkaloid can be readily and completely hydrolyzed by β-glucosidase, giving peimine and D-glucose (Table III). As this is a new glycoside consisting of peimine and D-glucose, it was named peiminoside (II).

Table III. Rf Value of the Sugar produced by Enzymatic Hydrolysis

Sugar \ Solvent	AcOH-BuOH-H_2O (1:4:5 v/v)	BuOH-H_2O (1% NH_4OH w/v)	Phenol-H_2O (9:1 v/v)	Pyridine-H_2O-BuOH (25:40:45 v/v)
Sample	0.16	0.07	0.34	0.39
D-Glucose	0.16	0.07	0.34	0.39
D-Mannose	0.21	0.09	0.38	0.41
D-Fructose	0.18	0.08	0.47	0.35

Temperature : 19°
Toyo Roshi No. 131
Ascending method
Reagent : Benzidine reagent

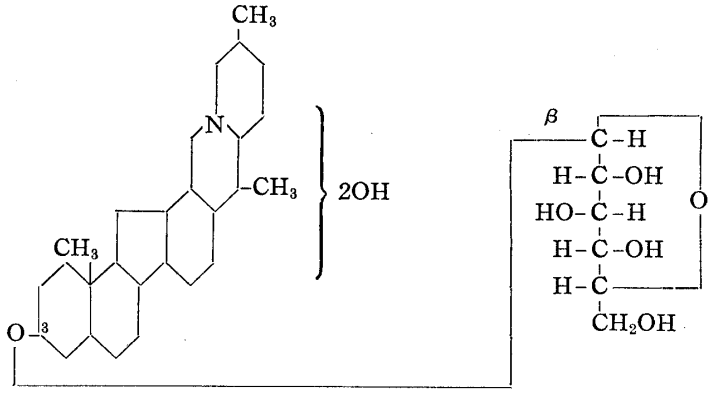
To clarify the structure of (II), it was led to pentamethylpeiminoside methiodide (III) by treating with methyl iodide and silver oxide in N,N-dimethylformamide. The product crystallized from a mixture of methanol and benzene to colorless fine needles, having double melting points (160° and 218~220°), $[\alpha]_D^{20}$ −20°(EtOH). From the analytical values and measurement of methoxyl groups, it was presumed that peiminoside contains one mole of D-glucose, and that four out of the five methoxyl groups in (III) are present in the sugar and the remaining one in the peimine part. The hydroxyl group still observed in the

infrared spectrum of (III) may be due to the comparatively inactive hydroxyl group in the peimine part.

The pentaacetylpeiminoside produced by treating (II) with acetic anhydride in pyridine could not be crystallized, but its perchlorate (IV), after purification by the addition of water to its methanol solution, was obtained as a colorless powder, m.p. ~195°, $[\alpha]_D^{20}$ −36° (EtOH).

Next, (III) was separated into the sugar and non-sugar parts by acid hydrolysis. The sugar part was found to contain only one kind of a sugar (V) by paper partition chromatography and it was led to its anilide (VI), m.p. 136~138°. As the anilide was presumed to be 2,3,4,6-tetra-O-methyl-D-glucose anilide from its melting point and analytical values, the methylated sugar (V) was synthesized from D-glucose by the known method[15] and the anilide of (V) was compared with the above anilide (VI), and they were found to be identical by mixed melting point determination and comparison of their infrared spectra and optical rotations.

From the above results peiminoside can be concluded to be a glucoside produced by the combination of peimine with pyranose-type D-glucose at C-1 of the latter. In general, natural steroids and modified steroids have a hydroxyl group at C-3 and their glycosides are formed by the combination of a sugar with this hydroxyl group. Phytochemically, it is reasonable to think that peimine also has a hydroxyl group at C-3 capable of taking part in the formation of a glycoside. Hence, if the fundamental skeleton (F) assigned to peimine is recognized, the structure of peiminoside can be presumed to be O-β-D-glucopyranosyl-(1→3)-peimine (G), ($C_{33}H_{55}O_8N$).



(G)

Compared with peimine, peiminoside was found to have strong blood pressure depressing activity, the details of which will be reported elsewhere.

Experimental

Extraction of the Crude Base—22.9 kg. of powdered dried bulbs of *Fritillaria Thunbergii* MIQ. (Bai-mo, 貝母) was extracted twice with 80 and 60 kg. of MeOH at 60° for 16 hr. with stirring, and the combined extracts were evaporated at a temperature below 50° under a reduced pressure, leaving 1.4 kg. of an extract. The extract was dissolved in 3 L. of benzene and the solution was extracted with 1.0, 0.5, 0.5, and 0.5 L. of 3% HCl. After removal of the separated resin, the acid extract (1) was made alkaline with Na_2CO_3 and the precipitated basic substance was extracted with 2.0, 1.0, and 1.0 L. of $CHCl_3$. The $CHCl_3$ extract (1,1) was washed with water, dried over anhyd. Na_2CO_3, and evaporated under a reduced pressure to leave 94 g. of a crude alkaloid.

The above aqueous solution (1,2) was concentrated to about 1 L. and then extracted with 0.3 and 0.2 L. of $CHCl_3$. The $CHCl_3$ solution (1,2,1) was dried on anhyd. Na_2CO_3 and evaporated under a reduced pressure to give a second crop (3.1 g.) of the crude alkaloid.

15) Org. Syntheses, **20**, 97(1940).

Paper chromatography (Table II) revealed that the $CHCl_3$ solutions (1,1 and 1,2,1) contain two alkaloids (Rf 0.84 and 0.67) and the aqueous solution (1,2,2) choline (Rf 0.29).

Isolation of Peimine (I)—The crude alkaloid was adsorbed on Florisil and eluted successively with benzene, benzene-MeOH (9:1), benzene-MeOH (2:1), and MeOH. It was found by paper chromatography that peimine (I) (Rf 0.84) was contained mainly in the eluates of benzene and benzene-MeOH (9:1), and peiminoside (II) (Rf 0.67) in the eluates of benzene-MeOH (2:1) and MeOH. The process was repeated to purify peimine and the resulting product was recrystallized from EtOH to colorless needles, m.p. 223~224°, $[\alpha]_D^{16}$ −19.4° (c=1, EtOH), $[\alpha]_D^{17}$ −20° (c=1, $CHCl_3$). The yield was 25 g. *Anal.* Calcd. for $C_{27}H_{45}O_3N$: C, 75.13; H, 10.51; N, 3.24. Found: C, 75.02; H, 10.17; N, 2.95. Hydrochloride: Colorless prisms, m.p. 291~294° (decomp.), $[\alpha]_D^{18}$ −18.5° (c=1, H_2O), after recrystallization from water. *Anal.* Calcd. for $C_{27}H_{45}O_3N \cdot HCl$: C, 69.27; H, 9,90; N, 2.99; mol. wt., 468. Found: C, 68.91; H, 9.95; N, 2.90; mol. wt. (Barger method), 450±10.

Perchlorate: A solution of 110 mg. of (I) in 3 cc. of 20% AcOH was warmed with 50 mg. of $NaClO_4$ for a while and concentrated under a reduced pressure. The separated product was recrystallized from water to colorless prisms, which colored from about 255° and decomposed at 273°, $[\alpha]_D^{23}$ −15° (c=0.05, H_2O). *Anal.* Calcd. for $C_{27}H_{45}O_3N \cdot HClO_4$: C, 60.94; H, 8.71; N, 2.63. Found: C, 60.63; H, 8.72; N, 2.62.

Nitrite: A solution of 120 mg. of the hydrochloride of (I) in 3 cc. of water was left standing with a solution of 60 mg. of $NaNO_2$ in 1 cc. of water and colorless prisms separated out. The product was insoluble in $CHCl_3$, and colored from about 130° and decomposed at 145°. *Anal.* Calcd. for $C_{27}H_{45}O_3N \cdot HNO_2$: C, 67.75; H, 9.69; N, 5.85. Found: C, 67.25; H, 9.68; N, 5.15.

Methiodide: To a solution of 200 mg. of (I) in 1.5 cc. of Me_2CO 4 cc. of MeI was added and the mixture was evaporated after being heated on a water-bath for 2 hr. The residue was dissolved in small quantity of hot BuOH and a large quantity of Et_2O was added. The separated product was collected, washed quickly with Et_2O, and dried. The product was readily soluble in water, MeOH, and EtOH but insoluble in $CHCl_3$, benzene, and Me_2CO; m.p. 205~210° (decomp.), $[\alpha]_D^{18}$ −14.0° (c=1, H_2O). A sample of the product was dried at 110° for 8 hr. *in vacuo* and subjected to analysis. *Anal.* Calcd. for $C_{27}H_{45}O_3N \cdot CH_3I$: C, 58.63; H, 8.44; N, 2.44; I, 22.13. Found: C, 58.25, 58.46; H, 8.39, 8,35; N, 2.55; I, 20.56, 20.35.

Isolation of Peiminoside (II)—The peiminoside (II) (Rf 0.67) purified by chromatography on Florisil was a pale brown powder. It is readily soluble in water, MeOH, and EtOH, sparingly soluble in benzene, and hardly crystallizable. The yield was 18 g.

Hydrolysis of Peiminoside (II) (Formation of Peimine (I) and D-Glucose)—i) Hydrolysis with acid: A solution of 500 mg. of (II) dissolved in 10 cc. of *N* H_2SO_4 was refluxed for 5 hr. The reaction mixture was neutralized exactly with $NaHCO_3$ and then extracted with five 10-cc. portions of $CHCl_3$. The extract was washed with water, dried, and evaporated, and the residue (310 mg.) was recrystallized from EtOH to colorless needles, m.p. 223~224°, which showed no depression on admixture with an authentic sample of peimine (I), and its IR spectrum was also in complete agreement with that of the latter. A part of the product was converted to its perchlorate, which was identified by mixed m.p. determination with an authentic sample and by comparing its IR spectrum with that of the latter.

The above aqueous solution was evaporated to dryness and a solution of the residue in 4 cc. of water was heated with 0.4 g. of 50% AcOH and 0.4 g. of phenylhydrazine on a water-bath for 1 hr. when yellow needle-like crystals (140 mg.) separated out on cooling. The product crystallized from 80% EtOH in yellow needles, m.p. 196° (decomp.). It was identified by mixed m.p. determination with authentic glucophenylosazone and by comparison of IR spectra of the two. *Anal.* Calcd. for $C_{18}H_{23}O_4N_4$: C, 60.15; H, 6.45; N, 15.59. Found: C, 60.78; H, 6.47; N, 15.54.

ii) Hydrolysis with enzyme: The following three solutions were used as the test solution: (a) A solution of 5 mg. of (II) and 5 mg. of β-glucosidase in 0.5 cc. of 0.02*N* AcOH, (b) a solution of 5 mg. of (II) in 0.5 cc. of 0.02*N* AcOH, and (c) a solution of 5 mg. of β-glucosidase in 0.5 cc. of 0.02*N* AcOH. Each of the solutions was left to stand at 30° for 24 hr. and the reaction mixture was subjected to paper chromatography (Table III). As a result, D-glucose was detected only in (a).

Pentamethylpeiminoside Methiodide (III)—A solution of 4.4 g. of (II) in 200 cc. of N,N-dimethylformamide was shaken with 50 g. of Ag_2O and 100 cc. of MeI at 26° for 60 hr. and after addition of additional 50 g. of Ag_2O and 50 cc. of MeI the shaking was continued at 26° for 40 more hr. The resulting precipitate was filtered off and water was added to the filtrate until no more yellow precipitate separated out. Then KCN solution was added until the yellow precipitate disappeared and the solution was extracted with eight 100-cc. portions of $CHCl_3$. The $CHCl_3$ solution was washed with water, dried, and evaporated, and the brown viscous residue, after being crystallized by treatment with benzene, was recrystallized by adding benzene to its solution in MeOH to fine colorless needles having double m.p.s of 160° and 218~220°, $[\alpha]_D^{20}$ −20° (c=1.0, EtOH). Yield, 1.9 g. *Anal.* Calcd. for $C_{33}H_{50}O_3N(OCH_3)_5 \cdot CH_3I$: C, 58.12; H, 8.51; I, 15.75; $5CH_3O$, 19.26. Found: C, 57.96; H, 8.64; I,

15.28; CH_3O, 19.00.

Pentaacetylpeiminoside Perchlorate (IV)—A solution of 1 g. of (II) in 6 cc. of pyridine was left to stand with 4 cc. of Ac_2O at 37° for 24 hr. The reaction mixture was diluted with 50 cc. of water, extracted with benzene, and the benzene solution was washed with water, dried, and evaporated, leaving 1.25 g. of a residue. All attempts to crystallize the residue were unsuccessful. Therefore, the residue (640 mg.) was dissolved in 20 cc. of 20% AcOH, the solution was warmed with 320 mg. of $NaClO_4$ on a water bath for 1 hr., and the mixture was evaporated under a reduced pressure. The residue was dissolved in a small amount of MeOH and water was added to precipitate a colorless powder, m.p. ~195°, $[\alpha]_D^{20}$ −36° (c=0.5, EtOH). Yield, 520 mg. *Anal.* Calcd. for $C_{33}H_{50}O_3N(CH_3COO)_5 \cdot HClO_4$: N, 1.55. Found: N, 1.49.

Hydrolysis of Pentamethylpeiminoside Methiodide (III) (Formation of 2,3,4,6-Tetra-O-methyl-D-glucose (V))—A solution of 1.23 g. of (III) in 45 cc. of a mixture of MeOH and conc. HCl (8:1) was refluxed for 4 hr. and, after addition of 57 cc. of water, concentrated to ca. 40 cc. under a reduced pressure. The concentrate, after being refluxed for 2.5 hr. again with 3.3 cc. of conc. HCl and treated with active carbon, was extracted with ten 15-cc. portions of $CHCl_3$. The $CHCl_3$ solution was washed with $NaHCO_3$ solution, dried over anhyd. Na_2SO_4, and evaporated to leave 370 mg. of a pale yellow viscous liquid (V). The liquid was dissolved in 5.6 cc. of MeOH and the solution was refluxed with 300 mg. of aniline and 5 mg. of NH_4Cl for 2 hr. After addition of 200 cc. of water, the mixture was extracted with fifteen 15-cc. portions of $CHCl_3$, the extract was dried over anhyd. Na_2SO_4, and evaporated to dryness, leaving 300 mg. of a residue. The residue was recrystallized from MeOH to colorless needles, m.p. 136~138°, $[\alpha]_D^{24}$ +237° (c=1.0, Me_2CO). The product showed no depression in m.p. on admixture with authentic 2,3,4,6-tetra-O-methyl-D-glucose aniline (VI) and its IR spectrum and $[\alpha]_D$ were in accord with those of the latter. *Anal.* Calcd. for $C_{16}H_{25}O_5N$: C, 61.72; H, 8.09; N, 4.50. Found: C, 62.24; H, 7.93; N, 4.35.

Synthesis of 2,3,4,6-Tetra-O-methyl-D-glucose Anilide (VI)—A solution of 600 mg. of 2,3,4,6-tetra-O-methyl-D-glucose (V), produced according to the method given in the literature,[15] dissolved in 9 cc. of MeOH was refluxed with 480 mg. of aniline and 10 mg. of NH_4Cl for 2 hr. The reaction mixture was diluted with 100 cc. of water, the resulting precipitate was sublimed at 130°/0.02 mm. Hg, and the sublimate was recrystallized from MeOH to colorless needles, m.p. 138°, $[\alpha]_D^{20}$ +239° (c=1.0, acetone). *Anal.* Calcd. for $C_{16}H_{25}O_5N$: C, 61.72; H, 8.09; N, 4.59. Found: C, 61.87; H, 8.13; N, 4.54.

The authors wish to express their gratefulness to the members in charge of elementary analysis and measurement of physical constants.

Summary

Peimine (I) and Peiminoside (II), a new glycoside ($C_{33}H_{55}O_8N$), were isolated from bulbs of *Fritillaria Thunbergii* Miq. (Bai-mo, 貝母) produced in Japan, and the latter was found to be a glycoside consisting of peimine and D-glucose.

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