[Chem. Pharm. Bull. 19(1) 202—205 (1971)]

UDC 547.92.02 : 581.192 : 582.675.1

Components of Adonis Plants

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(Received June 13, 1970)

It has already been shown that the roots of *Adonis amurensis* Regel et Radde (Japanese name "Fukujuso") (Ranunculaceae) and *Adonis vernalis* L. contain several cardiac glycosides.[2–6] We have reinvestigated these roots and isolated[7–9] adonilide (I), fukujusone (II),

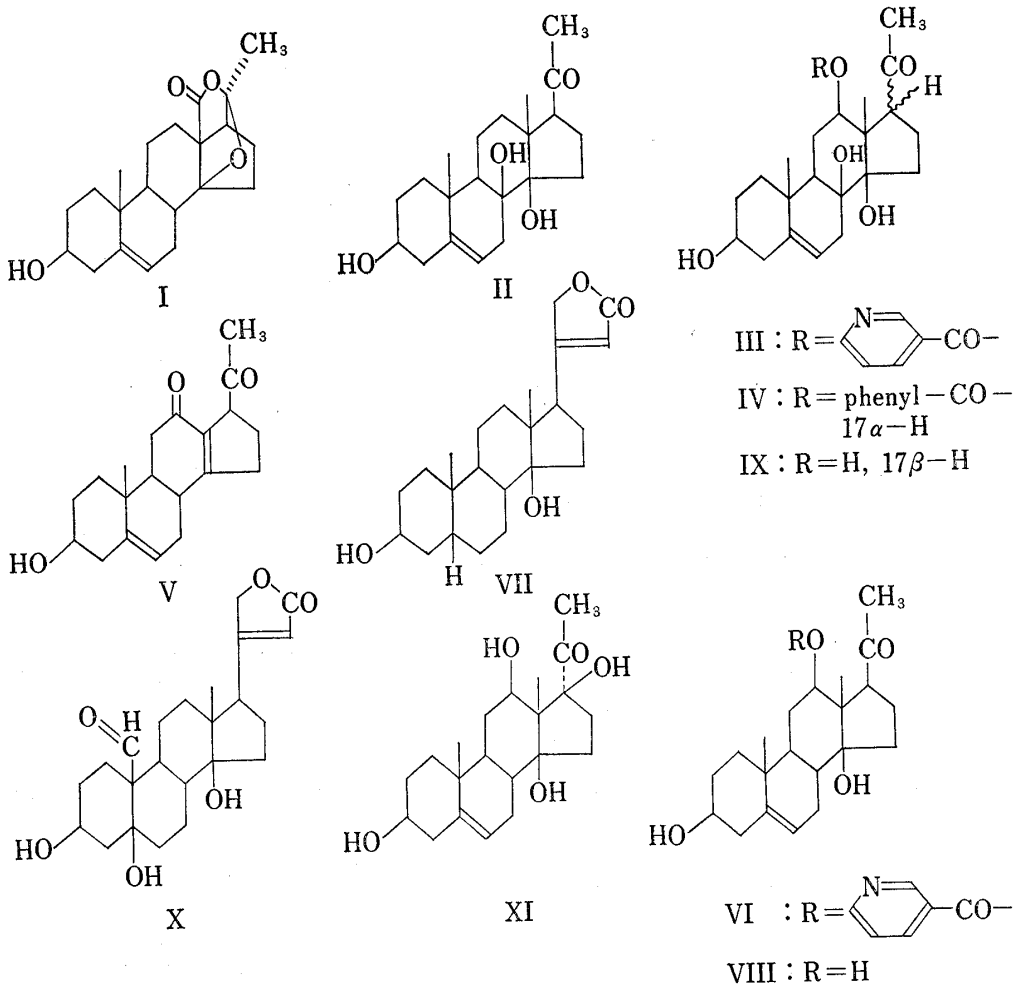

Chart 1

1) Location: *Kita-12-jo, Nishi-6-chome, Sapporo, 060, Japan*
2) R. Jaretzky, *Arch. Pharm.*, **273**, 334 (1935).
3) T. Reichstein and H. Rosenmund, *Pharm. Acta Helv.*, **15**, 150 (1940).
4) H. Rosenmund and T. Reichstein, *Pharm. Acta Helv.*, **17**, 176 (1942).
5) A. Katz and T. Reichstein, *Pharm. Acta Helv.*, **22**, 437 (1947).
6) F. Santavey and T. Reichstein, *Pharm. Acta Helv.*, **23**, 153 (1948).
7) Y. Shimizu, Y. Sato, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **15**, 2005 (1967).
8) Y. Shimizu, Y. Sato, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 2391 (1969).
9) Y. Shimizu, Y. Sato, and H. Mitsuhashi, *Experientia*, **25**, 1129 (1969).

12-O-nicotinoylisolineolon (or lineolon) (III), 12-O-benzoylisolineolon (IV)[10] and fukujusonorone (V), together with already known cardiac glycosides (Chart 1).

Further investigation was attempted in order to determine the components of the upper ground portion of *Adonis amurensis* and *A. vernalis* L.

i) Adonis amurensis

The flowering plants gathered at Nishino, a suburb of Sapporo City, on May 10, 1966, were percolated with 70% ethanol and purified as shown in Chart 2.

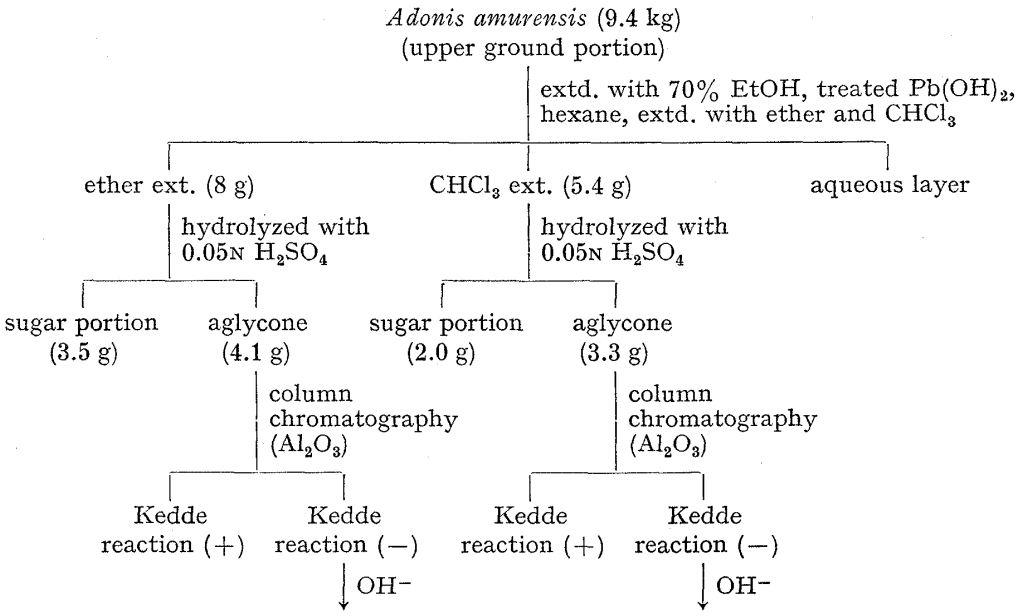

Chart 2. Extraction and Separation

The crude glycoside so obtained was hydrolyzed with 0.05N sulfuric acid showed positive Dragendorff reaction.

Both sugar fractions displayed a strong Keller Kiliani reaction. Chromatography of the aglycone, obtained by hydrolysis of the ether fraction, over alumina yielded three crystalline substances. One of them was nicotinoylisoramanone (VI), and one of the remainning two was digitoxigenin (VII), and this is the first example of the isolation of VII from *Adonis* Plants. The other was confirmed as isoramanone (digipurprogenin-II) (VIII), but this substance seems to be contained in plants in free and not ester form, because we never treated aglycone fraction with alkali.

The fractions negative to Kedde reaction obtained from alumina chromatography, were combined and hydrolyzed with alkali. The alkaline hydrolysate was treated by preparative thin-layer chromatography (PTLC) and two crystalline substances, lineolon (IX) and pergularin[11] (XI), were obtained.

The crude glycoside obtained from the chloroform extract was hydrolyzed with 0.05N sulfuric acid and strophanthidin (X), mp 136—138°, was obtained from the aglycone mixture. X had previously been isolated from the root of this plant by Reichstein and Santavey[6] and designated as cymarin (strophanthidin-cymarose). For the isolation of other compounds, the mother liquor left after isolation of strophanthidin (X) was combined and submitted to chromatography over alumina, and nicotinoylisoramanone (VI) was obtained. The sugar parts, which had been obtained by hydrolysis of the glycosides of both ether and chloroform

10) H. Mitsuhashi and H. Mizuta, *Yakugaku Zasshi*, **89**, 1352 (1969).
11) H. Mitsuhashi, T. Nomura, and M. Hirano, *Chem. Pharm. Bull.* (Tokyo), **14**, 717 (1966).

fraction, were examined. The aqueous layer, after extraction of aglycone, displayed a strong Keller–Kiliani reaction. Paper partition chromatography (PPC) sugars syrup showed the presence of D-cymarose, D-sarmentose and L-oleandrose.

ii) *Adonis vernalis* L.

The powdered crude drugs obtained from Paul Müggenburg (Hamburg) by kind arrangement of Prof. E. Stahl (Saarbrücken, Germany) was treated as shown in Chart 3, and the extract was treated as described in the preceding papers for examining ester-glycosides.[12–14] The aglycone mixture obtained by hydrolysis of the glycoside was chromatographed over silica gel column. Three crystalline substances, strophanthidin (X), adonilide (I), and unidentified substance of mp 151.3—153° were obtained. From the sugar fractions, L-oleandrose and D-cymarose were proved by PPC.

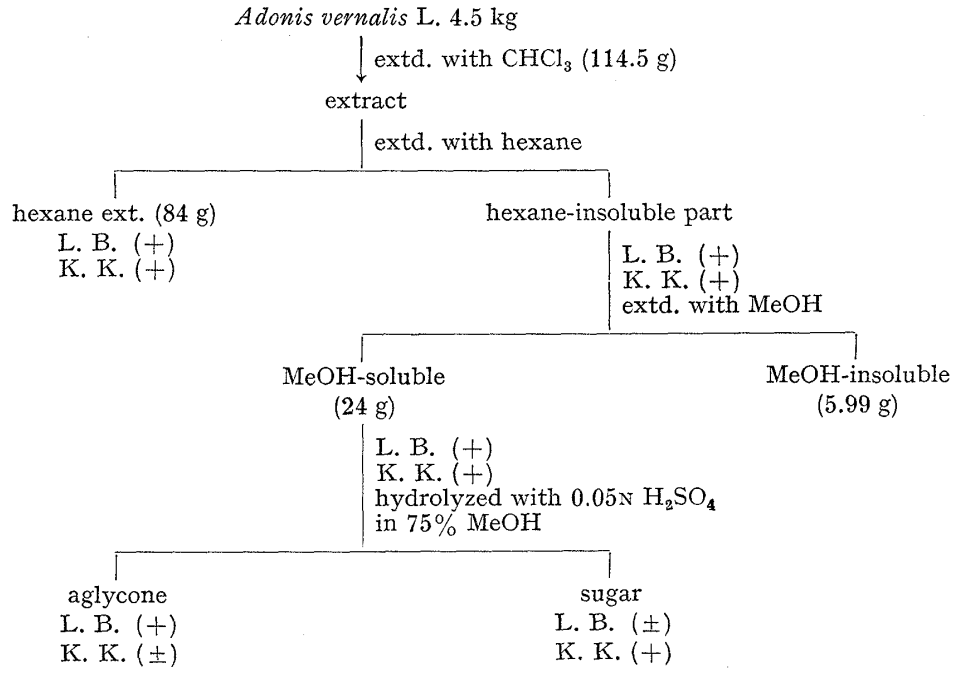

Chart 3

Experimental

Extraction of *A. amurensis*——The whole upper ground portion (9.4 kg) of *Adonis amurensis* REGEL et RADD was chipped and extracted three times with 70% EtOH, 50 liters of a dark green extract solution was shaken vigorously with freshly prepared Pb $(OH)_2$ for about 15 min, and then filtered. The filtrate was concentrated to 15 liters at 50—60° in a reduced pressure.

This concentrated residue was extracted successively with ether and $CHCl_3$. Upon evaporation of the solvent, 8 g of crude glycoside was obtained from the ether extract and 5.4 g from the $CHCl_3$ extract.

Hydrolysis of the Glycoside——A solution of 8 g of the crude glycosides from ether extract dissolved in 300 ml of MeOH was heated at 80° for 30 min with 300 ml of 0.1N H_2SO_4 on a water bath, 300 ml of H_2O was added, and MeOH was evaporated *in vacuo* at room temperature. The resulting mixture was extracted with CH_2Cl_2, which was washed with 5% $NaHCO_3$ solution and H_2O, and dried over Na_2SO_4. Evaporation of the solvent gave 4.1 g of a yellow powder which was considered to be the aglycone. Similarly as above, the crude glycoside from $CHCl_3$ extract gave 3.3 g of yellowish powder.

Hydrolysis of Aglycone——A solution of 350 mg of the noncrystalline aglycone fractions negative to the Kedde reaction, obtained by column chromatography over alumina, dissolved in 10 ml of 5% MeOH–KOH

12) H. Mitsuhashi and Y. Shimizu, *Steroids*, **2**, 1373 (1963).
13) K.A. Jaeggi, E.K. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **46**, 694 (1963).
14) H. Mitsuhashi and Y. Shimizu, *Tetrahedron*, **24**, 4143 (1968).

was kept overnight at room temperature. MeOH was evaporated and the residue was extracted with ether. Evaporation of ether gave 50 mg of a crystalline mass which was purified by preparative TLC using SiO_2-HF_{254}, and lineolon (IX) and pergnlarin (XI) were obtained. IX was recrystallized from acetone to prisms, mp 242—246°, which showed no depression on mixed fusion with authentic lineolon. XI was recrystallized from acetone–H_2O to prisms, mp 220—234°, and identified as pergularin,[11] which had previously been obtained from *Metaplexis japonica* MAKINO.

Extraction of *Adonis vernalis* L.——The upper ground portion of the plant was dried, powdered, and 4.5 kg of the powder was extracted with $CHCl_3$ until the extract solution became colorless. The extract was concentrated and 114.5 g of a residue was obtained. This residue was treated with hexane and the hexane-soluble part was discarded. The residue was treated with MeOH (1 liter) and the MeOH solution was evaporated below 60°, giving 24 g of a MeOH-soluble material. This residual substance (10 g) was dissolved in 450 ml of MeOH and 150 ml of 0.2N H_2SO_4, and the mixture was refluxed for 45 min. The solvent was evaporated *in vacuo* at room temperature and the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 5% $NaHCO_3$ solution and H_2O, dried over Na_2SO_4, and evaporation of the solvent gave 7.65 g of an aglycone. The aqueous layer was neutralized with saturated $Ba(OH)_2$ water and concentrated to a syrup under a reduced pressure. The syrupy sugar fraction (2.1 g) thus obtained on hydrolysis of the glycoside was submitted to paper chromatography for comparison with the authentic samples and D-cymarose and L-oleandrose were detected. Solvent system $CHCl_3$–$HCONH_2$, room temperature, 6—7 hr, descending, Toyo Roshi No. 51.

Chromatography of Aglycone——Column chromatography of 2.1 g of the aglycone over 200 g of SiO_2 gave results shown Table I.

TABLE I

Fract. No.	Solvent system	Eluted product (mg)	Note
1— 14	$CHCl_3$	407	
15— 29	1% MeOH–$CHCl_3$	129	crystal III
30— 39	1% MeOH–$CHCl_3$	282	
40— 42	1% MeOH–$CHCl_3$	86	crystal II
43— 52	1% MeOH–$CHCl_3$	270	
53— 66	3% MeOH–$CHCl_3$	364	
67— 75	5% MeOH–$CHCl_3$	196	
76— 82	5% MeOH–$CHCl_3$	230	crystal I
83— 89	7% MeOH–$CHCl_3$	47	
90—114	7% MeOH–$CHCl_3$	191	
115—121	15% MeOH–$CHCl_3$	91	

each fraction, 100 ml

Crystal I——Crystal I was recrystallized from MeOH to 58 mg of prisms mp 139—142°. It showed identical behaviour as strophanthidin (X) and a mixed fusion showed no depression of mp. The IR spectra also confirmed their identity.

Crystal II——Recrystallization from acetone gave 6 mg of plates, mp 151.5—153°. Liebermann-Burchard reaction (−), Keller-Kiliani reaction (−), Kedde (−). IR ν_{max}^{Nujol} cm^{-1}: 3440, 1730, 1720, 1620, 1275, 1240, 1180, 1100, 960. UV: end absorption. Mass Spectrum *m/e*: M^+ 196, 181, 178, 163, 154, 153, 150, 140, 135, 125, 109, 107, 95. From the results of high resolution mass spectrum (196.1033), the formula $C_{11}H_{16}O_3$ is proposed.

Crystal III——The substance was recrystallized from acetone ether and 5 mg of crystal mp 260°, was obtained. *Anal.* Calcd. for $C_{21}H_{28}O_4$: mol. wt., 344.4549. Found: mol. wt., 344.1988. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1780, 1285, 1190, 1150, 840. Mass Spectrum *m/e*: M^+ 344, 326, 311, 300, 293, 283, 267, 265, 257, 255, 239, 237, 223, 215, 197, 183, 171. Mixed fusion with the authentic specimen of adonilide (I) showed no depression of mp.

Acknowledgement The authors are much indebted to Prof. Dr. E. Stahl (Germany) and Paul Müggenburg (Hamburg) for collection of *Adonis vernalis* L. They express their gratitude to Miss Y. Imai for mass spectral measurement, and to Miss Y. Kishio and Miss H. Fujisawa for NMR spectral measurement.