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Studies on the Constituents of Asclepiadaceae Plants. XXVI.[1] Isolation of a New Glycoside from *Dreges volubilia* (L.) Benth.

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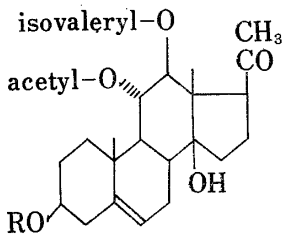
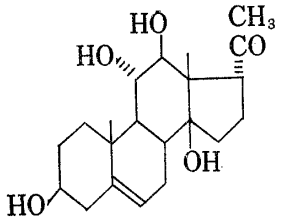
In 1965 and 1966, Reichstein and his co-workers studied the components of the seed of *Dregea volubilis* (L.) Benth. (Asclepiadaceae family) and confirmed the structure of drevogenin A, B, D, and P.[3] In the present paper we report the isolation of a glycoside, dregoside A from the stem of this plant.

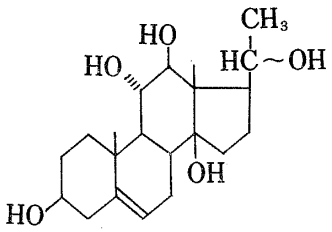
A methanolic extract of pulverized and dried stem of this plant obtained from Thailand was treated with hexane to precipitate a glycoside mixture showing positive Keller-Kiliani reaction,[4] which was submitted to mild acid hydrolysis and extracted with ether and chloroform. The aqueous layer gave a sugar mixture containing cymarose detected on PPC.[5] The ether layer was submitted to alumina column chromatography and silica gel TLC.T hese treatments yielded crystal-I, -II, and -III melting at 188—189.5°, 149.5—151°, and 219—224°, respectively.



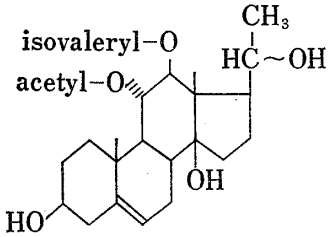
1. drevogenin A, (R=H)
2. dregoside A, (R=cymarosyl)



3. isodrevogenin P



4. drevogenin D



5. drebbysogenin G

Chart 1

Crystal-I forms prisms (from acetone/isopropyl ether), mp 188—189.5°, $[\alpha]_D^{17}$ +51.1° (c =1.01, MeOH), for which the molecular formula of $C_{28}H_{42}O_7$ was proposed from its elemental analysis. The infrared (IR) spectrum of crystal-I was very similar to that of drevogenin A

1) Part XXV: H. Mitsuhashi and H. Mizuta, *Yakugaku Zasshi*, **89**, 1352 (1969).
2) Location: *Kita-12-jo, Nishi-6-chome, Sapporo.*
3) *a*) H.H. Sauer, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **48**, 859 (1965); *b*) *Idem, ibid.*, **49**, 1632 (1966); *c*) *Idem, ibid.*, **49**, 1655 (1966).
4) T. Reichstein, J. von Euw, and T. Reichstein, *Helv. Chim. Acta*, **31**, 888 (1948).
5) Abbreviations used: PPC=paper partition chromatography, TLC=thin-layer chromatography.

(**1**) and the mass spectrum of crystal-I was identifiable with Reichstein's data.[3] Alkali hydrolysis of crystal-I gave a neutral compound melting at 186.5—198°, ORD negative Cotton effect, and the mass spectrum of this crystal coincided with that of isodrevogenin P (**3**).[3] The acidic portion gave acetic acid and isovaleric acid detected on PPC. Crystal-I was assigned to drevogenin A (**1**), whose nuclear magnetic resonance (NMR) spectrum is well explanable.[6]

Crystal-II $C_{35}H_{54}O_{10}$, mp 149—151.5°, $[\alpha]_D^{17}$ +43.9° (c=0.663, MeOH) exhibits positive Keller–Kiliani reaction and therefore crystal-II is a glycoside containing 2,6-dideoxysugar. Its mass spectrum showed the fragment ions of 2,6-dideoxysugar (Chart 2).[7]

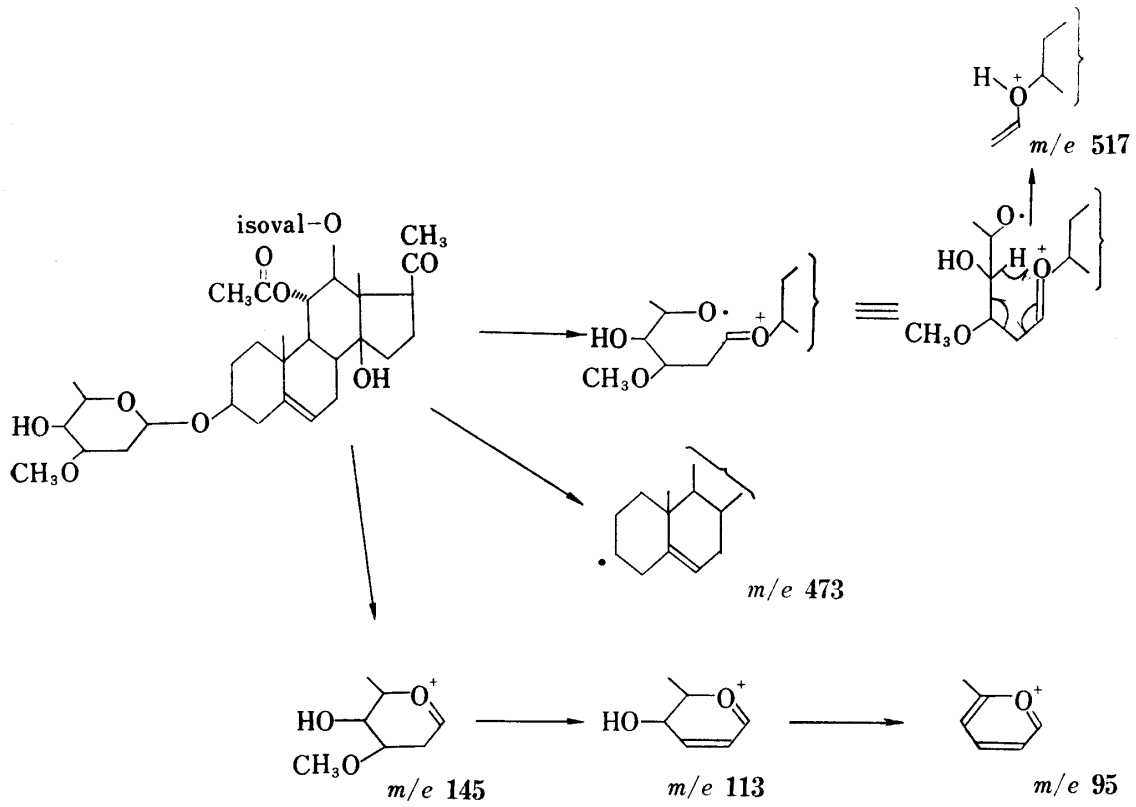


Chart 2

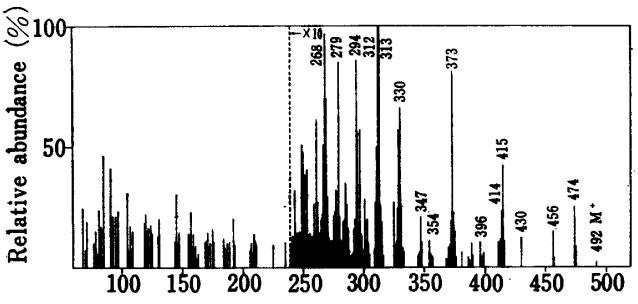


Fig. 1. Mass Spectrum of Crystal-III

The NMR spectrum of crystal-II was very similar to that of crystal-I except for the presence of a signal at τ 6.58 (3H, singlet, $-OCH_3$) and 8.73 (3H, doublet, J=7 cps, $-\overset{|}{C}H-CH_3$). A mild acid hydrolysis of this compound gave crystal-I (identified by mixed mp showing no depression) and cymarose detected by PPC. These facts let to the conclusion that crystal-II is a cymaroside of crystal-I [drevogenin A (**1**)], for which the name dregoside A (**2**) is proposed.

Crystal-III, mp 219—224°, shows IR bands at 1745 and 1250 cm^{-1} and NMR signals at τ 7.93 (3H, singlet), 8.60 (3H, singlet), 8.76 (3H, singlet) and 8.95 (3H, doublet, J=7.5 cps). These data suggest that crystal-III has a pregnane type skeleton and an acetoxyl moiety. Alkali hydrolysis

6) Details are given in the experimental section.
7) R. Tschesche, P. Welzel, and H.W. Fehlhaber, *Tetrahedron*, **21**, 1797 (1965).

a small amount of crystal-III gave a spot on PPC (chloroform/formamide system) and on silica gel TLC (10% methanol in chloroform system), which coincided with that of drevogenin D (**4**).[3] Crystal-III has a molecular weight of 492 according to its mass spectrum in (Fig. 1). Bhatnagar, *et al.* isolated drebyssogenin G (**5**), mp 212—227°, which is 11-O-acetyl-12-O-isovaleryl drevogenin D from *Dregea abyssinica* (Hochst.) K. Schum. (an African Asclepiadaceae plant).[8] Judging from the similarity of the mass spectra between crystal-III and (**5**), crystal-III seemed to be identical with (**5**) though direct comparison was not made.

Experimental

All melting points were measured on a Kofler block hot stage and are uncorrected. IR spectra were taken on Shimadzu IR type model and NMR data were obtained by a Hitachi model H-6013 and Varian A-60 in $CDCl_3$ (tetramethylsilane was used as internal standard). ORD curves were measured by JASCO ORD/UV-5.

Extract——Ground stem of *Dregea volubilis* (L.) Benth. (1.5 kg) obtained from Thailand, was dried, purverized, and percolated with MeOH at room temperature. Dark yellow tar (36 g) obtained by evaporation of MeOH *in vacuo* was dissolved in $CHCl_3$ (150 ml) and reprecipitated with hexane (1.5 l) to remove oily substances. Finally, 31 g of a yellow precipitate was obtained and this precipitate showed positive Keller–Kiliani reaction and positive Liebermann–Burchard reaction. The precipitate was dissolved in MeOH and insoluble powder was filtered off. The filtrate was evaporated under a reduced pressure to give a crude glycoside mixture (30 g), which showed positive Keller–Kiliani and Liebermann–Burchard reactions.

Acid Hydrolysis of Glycoside Mixture——The crude glycoside was refluxed with 0.05N H_2SO_4 in 50% MeOH on a water bath. MeOH was evaporated *in vacuo*, the residue was extracted successively with ether and $CHCl_3$. The both extracts were washed with H_2O, 5% $NaHCO_3$ aqueous solution, and H_2O, and dried over Na_2SO_4. After removal of the solvent, powdered substance was obtained from ether and $CHCl_3$ layers. The aqueous layer, obtained on hydrolysis of the glycoside, was neutralized with 5% $Ba(OH)_2$ aqueous solution and a small amount of $BaCO_3$ was added. H_2O was evaporated to dryness under reduced pressure and the residue was extracted with MeOH. The precipitated $BaSO_4$ was centrifuged off. Evaporation of MeOH gave a sugar portion as a syrup showing strong Keller–Kiliani reaction. Similar procedure as above was repeated three times. The reaction conditions and results are summarized in Table I.

Table I. Reaction Condition for Hydrolysis of Crude Glycoside Mixture

Reaction No.	Weight (g)	Reflux time (min)	$CHCl_3$ (g)	Ether (g)	Sugar portion (g)
1	10	25	1.5	2.5	2
2	10	60	1.5	3	5
3	9	60	1	2.5	4.5

$CHCl_3$ portion showed positive Keller–Kiliani and Liebermann–Burchard reaction.
Ether portion showed positive Liebermann–Burchard and negative Keller–Kiliani reactions.
Sugar portion showed negative Liebermann–Burchard and positive Keller–Kiliani reactions.

Ether extract from each of the three reactions showed approximately similar thin-layer chromatogram. Therefore, they were combined. $CHCl_3$ and sugar portions were likewise combined.

Column Chromatography of Aglycone from Ether Layer——Etherlayer (8 g) was submitted to chromatography over 240 g of Al_2O_3, giving results shown in Table II.

Paper Chromatography of Sugar Portion of Glycoside Mixture——The syrupy sugar from the glycoside mixture was separated into three fractions by preparative TLC (silica gel HF_{254} Merck, 20×20 cm^2, 5% MeOH/$CHCl_3$) and the fraction with second mobility was extracted with AcOEt. The residue by evaporation of AcOEt was submitted to PPC ($CHCl_3$/$HCONH_2$, 6 hr, detected by $SbCl_3$). Presence of cymarose was confirmed by direct comparison with an authentic sample.

Crystal-I [Drevogenin A (1)]——Fraction No. 23—36 (Table II) was recrystallized from acetone/*i*-Pr_2O to 216 mg of plates, mp 188—189.5°, $[\alpha]_D^{17}$ +51.1°. *Anal.* Calcd. for $C_{28}H_{42}O_7$: C, 68.54; H, 8.63. Found: C, 68.19; H, 8.57. IR ν_{max}^{Nujol} cm^{-1}: 3450—3300, 1742, 1720. NMR τ 4.48 (1H, multiplet, C-6, vinylic), 4.68

8) *a*) von Ajay S. Bhatnagar, H. Kaufmann, S. Stöcklin, and T. Reichstein, *Helv. Chim. Acta*, **51**, 117 (1968); *b*) von Ajay S. Bhatnagar, W. Stöcklin, and T. Reichstein, *ibid.*, **51**, 133 (1968).

Table II. Alumina Chromatography of Ether Layer

Fract. No. (300 ml each)	Solvent	Weight (mg)	Fract. No. (300 ml each)	Solvent	Weight (mg)
1	benzene	61.4	38—39	CH_2Cl_2	336.3
2—7	benzene–CH_2Cl_2 (4:1)	10.7	40—41	CH_2Cl_2	228.5
8—10	benzene–CH_2Cl_2 (1:1)	14.1	42	CH_2Cl_2	236.6
11—12	benzene–CH_2Cl_2 (1:1)	trace	43—48	CH_2Cl_2	451.9
13—19	benzene–CH_2Cl_2 (1:1)	trace	49—53	CH_2Cl_2	129.5
20—22	CH_2Cl_2	12.7	54—74	CH_2Cl_2	101.8
23—36	CH_2Cl_2	823.0	75—90	CH_2Cl_2	132.9
37	CH_2Cl_2	1101.9	91—last	CH_2Cl_2	144.6

(1H, tirplet, $J=9$ cps, C-11β), 5.21 (1H, doublet, $J=9$ cps, C-12α), 6.49 (1H, multiplet, C-3α), 6.98 (1H, multiplet, C-17α) 7.83 (3H, singlet, C-21 $-COCH_3$), 8.04 (3H, singlet, $-OAc$), 8.90 (*ca.* 12H, multiplets, C-18, C-19, and isovaleric methyls). ORD ($c=0.199$, MeOH) trough $[\alpha]_{270}$ $-170°$, peak $[\alpha]_{308}$ $+850°$, a$=+50$.

Alkali Hydrolysis of Crystal-I——A solution of 239 mg of Crystal-I dissolved in 10 ml of 5% MeOH/KOH was refluxed for 2.5 hr. After adding 10 ml of H_2O, MeOH was evaporated in a reduced pressure. The residual aqueous layer was extracted with BuOH saturated with H_2O, washed successively with 2N HCl and H_2O, and dried over Na_2SO_4. Evaporation of the solvent left an oily substance and it was separated by preparative tlc (four plates of 20×20 cm^2, silicagel HF_{254} Merck, 10% MeOH/CH_2Cl_2 as a solvent). The main band was extracted with AcOEt. Evaporation of the solvent gave a crystalline mass, which was recrystallized from MeOH/ether to 42 mg of needles, mp 186.5—198°, *m/e*: 364 (M^+), 346, 328, 313, 310, 295, 285, 267, 249, 208 (as base peak), 190, *etc.*

Paper Chromatography of Acid Portion——The aqueous layer of above hydrolysate was acidified with 40% H_3PO_4 and extracted with ether by a continuous extractor. The ether layer was submitted to ppc with a solvent system of BuOH saturated with conc. NH_4OH, and detected by spraying slightly basic bromthymol blue aqueous solution. Paper, Toyo Roshi No. 51, 28°, 20 hr.

	Rf	
Acids from Crystal-I	0.23	0.76
Isovaleric acid		0.78
Acetic acid	0.22	

Crystal-II [Dregoside A (2)]——The mother liquor of recrystallization of (1) was submitted to preparative tlc (5% MeOH/$CHCl_3$) and 170 mg of needles was obtained, whose recrystallization from i-Pr_2O gave white needles, mp 149.5—151°. *Anal.* Calcd. for $C_{35}H_{54}O_{10}$: C, 66.22; H, 8.57. Found: C, 65.82; H, 8.60;. $[\alpha]_D^{17}$ +43.9° ($c=0.663$, MeOH), positive Keller–Kiliani reaction, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3400, 1745, 1700, 1250, 1085.

Acid Hydrolysis of Crystal-II——A solution of 37 mg of crystal-II dissolved in 3 ml of MeOH was refluxed with 3 ml of 0.1N H_2SO_4 on a water bath. MeOH was evapotated *in vacuo* after adding excess H_2O, extracted with $CHCl_3$ EtOH (3:2). The extract was washed successively with H_2O, 5% $NaHCO_3$ aqueous solution and H_2O, and dried over Na_2SO_4 (first washing was added to the aqueous layer). Evaporation of the solvent left an oily substance which showed two spots on silicagel tlc and separation by preparative tlc gave crytsal-I (4.8 mg) which was identified by mixed fusion, and the starting material (13.2 mg).

The aqueous layer was neutralized to pH 7 with 5% $Ba(OH)_2$ and evaporated *in vacùo* at 50° on a water bath. The residue was dissolved in MeOH and insoluble precipitate was centrifuged off. Removal of MeOH gave 8.5 mg of syrup, which was submitted to ppc on Toyo Roshi No. 51, developed with $CHCl_3$/$HCONH_2$, 28°, 6 hr, showed only one spot which was identified with cymarose.

Crystal-III——By the preparative tlc of the fraction No. 43—48 (350 mg) (Table II), 17 mg of white plates mp 219—224°, was obtained from hexane/i-Pr_2O, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500—3300, 1745, 1245. NMR τ: 4.42 (1H, multiplet, vinylic), 4.56 (1H, triplet, $J=11$ cps), 5.13 (1H, doublet, $J=11$ cps), 7.93 (3H, singlet, $-OAc$), 8.60 (3H, singlet), 8.76 (3H, singlet), and 8.95 (3H, doublet, $J=7.5$ cps). Mass spectrum is shown in Fig. 1.

Alkali Hydrolysis of Small Amount of Crystal-III——Crystal-III (about 1 mg) was dissolved in 0.2 ml of 5% MeOH/KOH and allowed to stand overnight at room temperature. The reaction mixture was examined to ppc (solvent system $CHCl_3$/$HCONH_2$) for 22 hr, $R_{drevogeninD}$ 1.00, and tlc (30% MeOH/$CHCl_3$, silicagel HF_{254} *Rf*$=0.36$ with the same mobility of drevogenin D.

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