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Studies on the Constituents of the Asclepiadaceae Plants. XXVIII.[1] Components of *Asclepias syriaca* L.

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It has been shown in the preceding papers[1,3,4] that the plants of Asclepiadaceae family contain a series of polyhydroxypregnane derivative. Tschesche, *et al.*,[5] and Reichstein, *et al.*,[6] demonstrated that the pregnane derivatives are precursors of cardenolides in *Digitalis spp.*

In an extension of our studies on this series, the present experiment was undertaken in order to find further pregnane compounds from *Asclepias syriaca* L., widely distributed in North America (Japanese name, "Otowata". Asclepiadaceae), and which had been proved to contain cardenolides.[7] Concerning the components of this plant, uzarigenin (1), syriogenin (2), and their glycosides have been isolated.[7]

According to the procedure described in the preceding papers[1,8] the aerial portion was extracted with chloroform, and the extract was precipitated several times with hexane. The methanol-soluble part, prepared from the precipitate, showed positive Keller-kiliani, Liebermann-Burchard, and Kedde reactions, suggesting the presence of cardiac glycosides containing 2-deoxysugar.

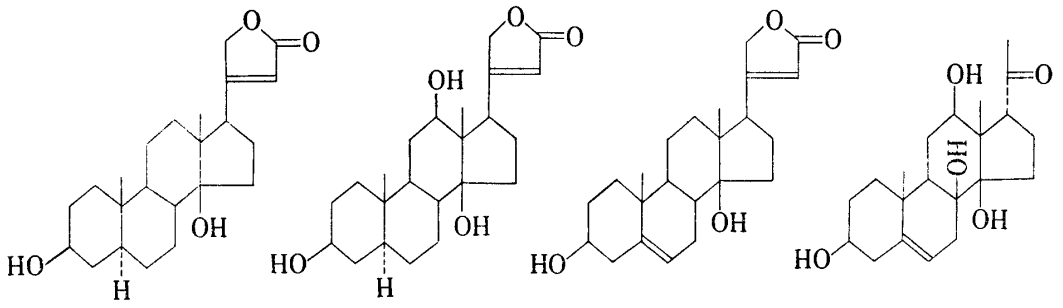
On hydrolysis of the crude glycoside by refluxing in 0.05 N sulfuric acid in 50% methanol,[9] followed by extraction with ether and chloroform, the ether layer furnished a Keller-Kiliani-negative aglycone mixture, and the extract was purified on alumina column developed with benzene, methylene chloride, and methanol. Five products, I, II, III, IV, and V, were obtained in crystalline state.

The chemical structures of crystal I, mp 253—264°, and crystal II, mp 105—112°, have been left unknown because of the small amount of the material available. Crystal III, mp 219—232°, was confirmed as uzarigenin[10] (1) by mixed fusion and comparison of thin-layer chromatogram and infrared (IR) spectra with those of authentic specimens. From the melting point and the mass spectrum data,[11] crystal IV, mp 237—241° was assumed to be xysmalogenin[12,13] (3), and identified by TLC in several systems with an authentic specimen kindly provided by Prof. Tschesche.

1) Part XXVII: H. Sawada, K. Hayashi, Y. Shimizu, and H. Mitsuhashi, *Phytochemistry*, accepted.
2) Location: *Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido.*
3) H. Mitsuhashi, Y. Shimizu, E. Yamada, I. Takemori, and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **10**, 808 (1962).
4) Y. Shimizu and H. Mitsuhashi, *Tetrahedron*, **24**, 4143 (1968).
5) R. Tschesche and U.G. Lilienweiss, *Z. Naturforsche*, **19b**, 265 (1964).
6) J.V. Euw and T. Reichstein, *Helv. Chim. Acta*, **47**, 711 (1964).
7) *a*) S. Bauer, L. Masler, O. Bauerova, and D. Sikl, *Experientia*, **17**, 15 (1961); *b*) *Idem*, *Collection Czech. Chem. Commun.*, **27**, 872 (1962); *c*) *Idem*, *ibid.*, **27**, 895 (1962).
8) H. Mitsuhashi and Y. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **8**, 313 (1960).
9) The condition usually applied to the hydrolysis of cardiac glycosides containing 2-deoxysugar; R.E. Winkler and T. Reichstein, *Helv. Chim. Acta*, **37**, 737 (1954).
10) S. Rangaswami and T. Reichstein, *Helv. Chim. Acta*, **32**, 939 (1949).
11) R. Reichstein, W. Stocklin, and T. Reichstein, *Helv. Chim. Acta*, **50**, 2139 (1967).
12) J. Poloria, A. Kuritskes, H. Jager, and T. Reichstein, *Helv. Chim. Acta*, **42**, 1437 (1959).
13) R. Tschesche, U. Freytag, and G. Snatzke, *Chem. Ber*, **92**. 3053 (1959).

In addition to the melting point[7b] and the IR spectral data,[7c] the fact that the mass spectrum exhibited the molecular ion peak at *m/e* 390 and the peaks at *m/e* 372 ($M^+ - H_2O$), 354 ($M^+ - 2H_2O$), 336 ($M^+ - 3H_2O$), 262 ($C_{17}H_{24}O_2$),[14] 244 (262 $-H_2O$), 219 ($C_{15}H_{23}O$),[14] and 201 (219–H_2O) corresponding to a series of fragment ions arising from the cardenolide which contains three hydroxyl functions, suggested that crystal V, mp 263—266°, might be syriogenin[7b] (2). However, the comparison of TLC with the authentic syriogenin which had been kindly supplied by Dr. Masler, excluded this possibility. Hence, we asked Prof. Reichstein to compare the mass spectrum of this compound with that of syriogenin, and received his suggestion that this compound might be an isomer of syriogenin because all the main peaks correspond. However, no other evidence except the mass spectrum is presently available on this point, and therefore, further studies are required.

The weakly Keller–Kiliani–positive mixture of the chloroform extract, which had been obtained by hydrolysis of the crude glycosides, was chromatographed over alumina column. This treatment yielded a fraction whose main spot was similar to that of lineolon[4,15] (4) on TLC, but it could not be isolated in a pure state. On paper chromatography of the fraction, the main spot was identified with lineolon.



1 2 3 4

Chart 1

Experimental

Extraction from *Asclepias syriaca* L.——The aerial portion of the plant, cultivated and collected in our medicinal garden, was chipped, dried, and powdered. The powdered material (3.7 kg) was percolated with $CHCl_3$ at room temperature and, after evaporation of the solvent at below 60° *in vacuo*, deep green tar (140 g) was obtained. This tar was dissolved in $CHCl_3$ and reprecipitated several times with hexane to remove oily substances. A yellow precipitate (21.5 g), obtained by decantation of the hexane solution, was dissolved in MeOH, and the insoluble part was filtered off. The filtrate was evaporated to leave a crude glycoside mixture (12.0 g), which showed positive Keller–Kiliani reaction (deep blue), Liebermann–Burchard reaction (brown-reddish brown), and Kedde reaction (brown).

Acid Hydrolysis of the Crude Glycoside——The crude glycoside (10.0 g) was dissolved in 150 ml of MeOH and 50 ml of 0.2N H_2SO_4, and the mixture was refluxed for 35 min. After addition of 150 ml of H_2O, MeOH was evaporated *in vacuo* and the residue was extracted successively with ether and $CHCl_3$. Each organic layer was washed with H_2O, aqueous 5% $NaHCO_3$, and H_2O, and dried over Na_2SO_4. On evaporation of the solvent, ether layer gave an aglycone mixture (5.0 g) which showed negative Keller–Kiliani reaction, and $CHCl_3$ layer afforded a residue (1.75 g) showing weakly positive Keller–Kiliani reaction.

Column Chromatography of the Ether Extract——The ether extract (4.7 g) obtained from the acid hydrolysis of the crude glycoside, was submitted to chromatography over 200 g of alumina, giving results shown in Table I.

Crystal I: The oily fraction No. 26 was recrystallized from EtOH and crystal I was obtained (trace) mp 253—264°. Color test: Liebermann–Burchard reaction, bluish green–purple.

Crystal II: Recrystallization of the amorphous fraction No. 28 from MeOH gave a crystalline substance (trace), mp 105—112°. Color test: Liebermann Burchard reaction, yellowish brown–reddish brown.

14) G. Spiteller, *Z. Analyt. Chem.*, **197**, 1 (1963).
15) K.A. Jaeggi, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **46**, 694 (1963).

Table I. Chromatography of the Ether Extract

Fraction No.	Solvent	Eluted product (mg)	Note
1—12	benzene	205.1	
13—31	benzene–CH_2Cl_2	529.5	
(26)	benzene–CH_2Cl_2	16.4	crystal I
(28)	benzene–CH_2Cl_2	6.9	crystal II
30, 31	CH_2Cl_2	127.8	crystal III
32, 33	CH_2Cl_2	44.9	crystal IV
34, 35	CH_2Cl_2	67.7	crystal V
36—43	CH_2Cl_2–MeOH (20:1)	144.3	
44—46	CH_2Cl_2–MeOH (10:1)	155.9	
47—50	CH_2Cl_2–MeOH (10:3)	83.1	
51—56	CH_2Cl_2–MeOH (5:2)	113.4	
57—61	CH_2Cl_2–MeOH (1:1)	118.2	
62—65	MeOH	760.7	

each fraction: 300 ml

Crystal III: Repeated crystallization of fractions No. 30 and 31 from EtOH afforded needles (73.6 mg), mp 219—232°, $[\alpha]_D$ +15.5° (c=1.07, EtOH). IR ν_{max}^{Nujol} cm^{-1}: 3600, 3550 (OH), 1790, 1747 (C=O), 1617 (C=C). UV λ_{max}^{EtOH} mμ: 218. Mass Spectrum m/e: 356 (M^+-H_2O), 338 (M^+-$2H_2O$), 323 (M^+-$2H_2O$-CH_3), 312 (M^+-H_2O-CO_2), 246 ($C_{17}H_{24}O$), 203 ($C_{15}H_{23}$). The melting point of this compound was not depressed by admixture with an authentic specimen of uzarigenin.

Crystal IV: Combined fractions No. 32 and 33 were recrystallized from EtOH to rectangular plates (3.1 mg), mp 237—241°. UV λ_{max}^{MeOH} mμ: 218. Mass Spectrum m/e: 372 (M^+), 354 (M^+-H_2O, base peak), 339 (M^+-H_2O-CH_3), 336 (M^+-$2H_2O$), 328 (M^+-CO_2), 321 (M^+-$2H_2O$-CH_3), 310 (M^+-H_2O-CO_2), 306 (M^+-$2H_2O$-$2CH_3$), 298, 244 ($C_{17}H_{22}O$), 242, 229, 216, 201 ($C_{15}H_{21}$), 186, 105. Color test: Kedde reaction, reddish purple. The mass spectra of both compounds crystal IV and xysmalogenin were nearly identical, and the melting point of crystal IV was similar to that of xysmalogenin. This compound was identified with xysmalogenin by comparison of TLC (silica gel HF_{254} Merck, 5% MeOH/$CHCl_3$, AcOEt, ether, or 30% acetone/C_6H_6, detected by $SbCl_3$).

Crystal V: Combined fractions No. 34 and 35 were crystallized from acetone to needles (12.0 mg), mp 263—266°. IR ν_{max}^{direct} cm^{-1}: 3400 (OH), 1793, 1748 (C=O), 1619 (C=C). UV λ_{max}^{MeOH} mμ (log ε): 217.5 (4.17). Mass Spectrum m/e: 390 (M^+), 372 (M^+-H_2O), 354 (M^+-$2H_2O$), 339 (M^+-$2H_2O$-CH_3), 336 (M^+-$3H_2O$), 328 (M^+-H_2O-CO_2), 321 (M^+-$3H_2O$-CH_3), 310 (M^+-$2H_2O$-CO_2), 262 ($C_{17}H_{24}O_2$), 244 (262-H_2O), 229 (262-H_2O-CH_3), 219 ($C_{15}H_{25}O$), 211 (262-$2H_2O$-CH_3), 201 (219-H_2O), 186 (219-H_2O-CH_3). Comparison with authentic samples by TLC denied the possibility that crystal V might be syriogenin, periplogenin, mallogenin, sarmentogenin, or digoxigenin.

Table II. Chromatography of the Chloroform Extract

Fraction No.	Solvent	Eluted product (mg)	Note
1—13	CH_2Cl_2	79.3	
14—16	CH_2Cl_2–MeOH (99:1)	70.2	
17	CH_2Cl_2–MeOH (99:1)	54.7	amorphous
18—34	CH_2Cl_2–MeOH (99:1)	148.8	
35—37	MeOH	147.0	

each fraction: 50 ml

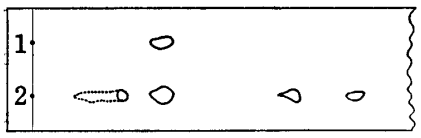


Fig. 1

system: $CHCl_3$/$HCONH_2$ 5 hr (R.T.)
1 Lineolon 2 Fr. No. 17

Column Chromatography of the $CHCl_3$ Extract——The $CHCl_3$ extract (1.65 g) obtained from the acid hydrolysis of crude glycoside, was chromatographed over 53 g alumina and the results are summarized in Table II. Several efforts to crystallize the eluted product were in vain.

Paper Chromatography of Fraction No. 17——Fraction No. 17 gave a main spot which was very similar to that of lineolon in TLC. A paper chromatographic examination result of fraction No. 17 is shown in Fig. 1.

Acknowledgement The authors are much indebted to Prof. T. Reichstein (Basel) for sending them the authentic samples of mallogenin and sarmentogenin and for helpful advice. They express their gratitude to Dr. L. Masler (Bratislava) and Prof. R. Tschesche (Bonn)for their kinds supply of the authentic samples of syriogenin and xysmalogenin. Thanks are also due to Mr. Yoshida for the collection of the plant material and to Miss Imai for mass spectra measurement.

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Optical Rotatory Dispersion of Nitrobenzene Derivatives. II.[1] N-*o*-Nitrobenzoyl L-α-Amino Acids

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In the previous work,[1] the optical rotatory dispersion (ORD) of *o*-nitrobenzoates of optically active secondary alcohols was studied. The relation between the sign of their Cotton effect and their absolute configuration was found and interpreted by assuming unsymmetrical twisting of the nitrobenzene chromophore induced by the neighbouring asymmetric center. In this paper, the ORD behaviour of similar derivatives of α-amino acids was examined.

Nine N-*o*-nitrobenzoyl L-α-amino acids were prepared and their ORD curves were measured in methanol. They showed Cotton effect centered at 330 nm, whose first extrema appeared near 370 nm and the second near 310 nm. Their molecular amplitudes with sign are presented in Table I together with those of the corresponding carboxylate ions and some methyl esters.

TABLE I. Molecular Amplitudes of N-*o*-Nitrobenzoyl L-α-Amino Acids and Their Methyl Esters

Compound No.	Parent amino acid	Free acid[a]	Carboxylate ion[b]	Methyl ester[c]
I	lysine[d]	−48.2	−34.5 (+14.3)	−33.9 (+13.7)
II	leucine	−46.2	+ 7.8 (+54.0)	
III	valine	−42.9	− 2.6 (+40.3)	−24.9 (+18.0)
IV	isoleucine	−41.6 −33.4[e]	− 2.4 (+39.2)	−28.2 (+13.4)
V	alanine	−36.1	− 1.6>[f]	
VI	serine	−35.6		
VII	tyrosine[d]	−12.5	+ 7.5<[f]	
VIII	phenylalanine	−11.3	+15.8 (+27.1)	− 0.9 (+10.4)
IX	tryptophan	− 0.5	+27.5 (+28.0)	

The values in parentheses mean the difference from those of the corresponding free acids.
a) measured in methanol *b*) measured in 4% $NaHCO_3$
c) measured in methanol *d*) bis (*o*-nitrobenzoyl) derivative
e) measured in chloroform
f) The molecular rotation values at the first extrema divided by 100 are presented since the second extrema were not determined.

1) Part I: U. Nagai and H. Iga, *Tetrahedron*, **26**, 725 (1970).
2) Location: *Kita-12, Nishi-6, Sapporo*; *a*) Present address: *Research Laboratory, Torii Seiyaku Co., Ltd., 3-14-3 Minamiyahata, Ichikawa.*