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Studies on the Constituents of Asclepiadaceae Plants. LIII.[1] The Structures of Glaucogenin-A, -B, and -C Mono-D-thevetoside from the Chinese Drug "Pai-ch'ien," *Cynanchum glaucescens* HAND-MAZZ

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The aglycone portion of the glycosides of the Chinese crude drug "Pai-ch'ien" was investigated. Three new compounds named glaucogenin-A (**1**), -B (**3**), and -C mono-D-thevetoside (**8**) were isolated and their structures were characterized on the basis of spectroscopic and chemical evidence, and that of **8** was determined by X-ray crystallography. They were found to possess an unprecedented 13,14: 14,15-disecopregnane-type skeleton.

Keywords——glaucogenin-A, -B, and-C mono-D-thevetoside; 13,14: 14,15-disecopregnane; "Pai-ch'ien"; *Cynanchum glaucescens*; Asclepiadaceae; X-ray crystallography

The Chinese crude drug "Pai-ch'ien"[2] 芫花叶白前, dried root of *Cynanchum glaucescens* HAND-MAZZ (Asclepiadaceae), has been used as an antitussive and expectorant in China. The previous paper[1] reported the structural characterization of five glycosides glaucoside-A, -B, -C, -D, and -E isolated from this drug, and the previous communication[3] described three new compounds, glaucogenin-A (**1**), -B (**3**), and -C mono-D-thevetoside (**8**), obtained from the hydrolysates of crude glycosides of this drug. We wish to describe herein the isolation and structural elucidation of **1**, **3**, and **8** in detail. As previously reported, when the crude glycosides were subjected to acid hydrolysis under the mild conditions usually applied to glycosides of asclepiadaceous plants,[4] the aglycone moieties were found to be susceptible to acid as judged by thin-layer chromatography (TLC). In order to obtain the genuine aglycones, the crude glycosides were hydrolyzed under even milder conditions, and the hydrolysates were separated by silica gel column chromatography to yield **1**, **3**, and **8** as crystals. Glaucogenin-A corresponded to a molecular formula of $C_{21}H_{28}O_6$ on the bases of its elemental analysis and electron impact mass spectrum (EI-MS, M^+, m/z: 376). The infrared (IR) absorptions at 3600, 3400, and 1730 cm^{-1} of **1** suggested the presence of hydroxyl and ester groups. Acetylation of **1** with acetic anhydride (Ac_2O) and pyridine (C_5H_5N) provided a diacetate (**2**), whose IR spectrum exhibited no hydroxyl absorption. The proton nuclear magnetic resonance spectrum (^{1}H-NMR) of **2** in deuterochloroform ($CDCl_3$) displayed two acetoxy-methine signals at δ 4.76 (1H, ddd, J=10, 10, 6 Hz) and 5.11 (1H, ddd, J=12, 10, 5 Hz), so that **1** bears two secondary hydroxyl groups. The ^{1}H-NMR specrum showed signals due to an angular methyl at δ 0.97 (3H, s) and an olefinic proton at δ 5.50 (1H, d, J=4.5 Hz), indicating that **1** is a Δ^5-steroid possessing an ordinary ring A and ring B. Moreover, the presence of an additional trisubstituted double bond was suggested by the deshielded signal at δ 6.27 (1H, d, J=2 Hz), which may be due to an enol ether function because of the absence of ultraviolet absorption corresponding to conjugated dienes or enones in **1**. The IR absorptions at 1710 and 1655 cm^{-1} in **1** may be attributed to this function. In the ^{13}C-NMR spectrum of **1** (Table I) in pentadeuteropyridine (C_5D_5N), the signals at δ 114.3 (s), 118.5 (s), 120.0 (d), 140.9 (s), and 143.8 (d) revealed the presence of two trisubstituted double bonds and a ketal function, which was supported by the fact that **1** gave a tetrahydro derivative (**7**) on catalytic hydrogenation, and the only ^{13}C-NMR signal of **7** in the low-field region was at δ 116.7 (s). The remaining ^{1}H-NMR signals of **1** were as follows: a tertiary methyl at δ 1.54 (3H, s); two protons at δ 1.08 (1H, t, J=12 Hz) and 3.46 (1H, dd, J=8, 2 Hz); two hydroxy-methine protons at δ 3.36 and 3.70 (each 1H, m); three

protons adjacent to oxygen at δ 3.88 (1H, dd, J=10, 9 Hz), 4.20 (1H, dd, J=9, 7 Hz), and 5.35 (1H, ddd, J=10, 8, 7 Hz). These physical constants and IR, ^{1}H-NMR, and MS data for **1** are in good agreement with those of vincetogenin[5] except for the specific rotation. Although Reichstein and co-workers reported the isolation of vincetogenin together with hirundigenin (**10**)[5] from *Vincetoxicum hirundinaria* Medikus, which is closely related to *C. glaucescens* Hand-Mazz, the structure has not been determined yet. Treatment of **1** with $LiAlH_4$ in THF gave a tetrol (**5**), whose ^{1}H-NMR showed an up-field shift of the signal at δ 4.52 (1H, ddd, J=10, 8, 6 Hz) which had been observed at δ 5.35 (1H, ddd, J=10, 8, 7 Hz) in **1**. In the ^{13}C-NMR, a hydroxyl methyl signal appeared at δ 7.15 (t) instead of the carbonyl carbon signal at δ 175.4 (s) of **1**, and thus the presence of a lactone ring in **1** was revealed. The tetrol (**5**) was acetylated to yield a tetraacetate (**6**), which showed ^{1}H-NMR signals due to an acetoxy-methylene group at δ 3.92 (1H, dd, J=11.5, 7 Hz) and 4.22 (1H, dd, J=11.5, 4 Hz); this led to the positioning of the acetoxy-methylene group next to a methine carbon. Therefore **1** was deduced to possess an unusual pregnane-type skeleton with two secondary hydroxyl groups, an enol ether function, a trisubstituted double bond, a ketal function, and a lactone ring.

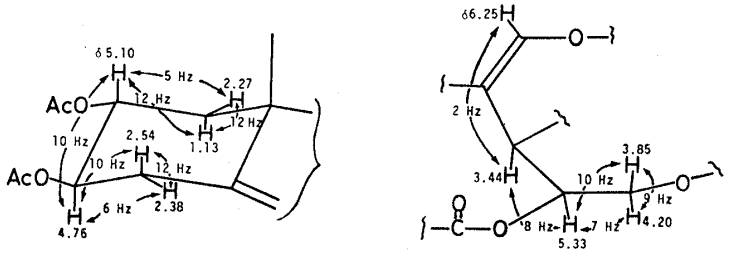


Fig. 1

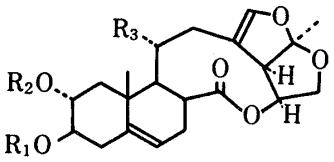
Glaucogenin-B (**3**) has the molecular formula $C_{21}H_{28}O_7$, and afforded a triacetate (**4**) on acetylation. The ^{13}C-NMR suggested the presence of another secondary hydroxyl group which is replaced by a methylene group in **1**. Proton spin decoupling experiments were carried out with **4** to provide the two partial structures shown in Fig. 1, considered to be common to **1** and **3**.

The aglycone moiety of glaucogenin-C mono-D-thevetoside (**8**) is similar to those of **1** and **3** in terms of spectroscopic properties, and liberated D-thevetose ($[\alpha]_D$ +30°) on hydrolysis under strongly acidic conditions, while an attempt to obtain the aglycone was unsuccessful. On acetylation, **8** gave a diacetate (**9**), whose IR showed no hydroxyl absorption. The ^{1}H-NMR showed two acetoxy-methine proton signals at δ 4.84 (1H, t, J=10 Hz) and 4.98 (1H, dd, J=10, 8 Hz) assignable to 4′-CH and 2′-CH of the thevetose, respectively, indicating that the aglycone moiety carries a single hydroxyl group with which the sugar linked. Consideration of the ^{13}C-NMR data for **8** (Table I) led to the location of the secondary hydroxyl group at C-3, though its configuration was unknown. The β-linkage of the sugar was deduced from the anomeric proton signal at δ 4.35 with a coupling constant of 8 Hz in the ^{1}H-NMR of **8**. Since no further refinement of the structure of these three compounds **1**, **3**, and **8** could be achieved, an X-ray crystallographic analysis was performed by using crystals of **9**, obtained from methanol (MeOH), to determine the structure and relative stereochemistry unequivocally. Crystals of **9** [$C_{32}H_{44}O_{11}$] are colorless prismatic needles; orthorhombic, a=19.270 (**6**), b=23.071 (**8**), c=7.155 (**3**) Å; space group $P2_12_12_1$, Z=4, D_x=1.26 g/cm^3. A crystal with dimensions 0.4×0.3×0.3 mm was used for measurements. The intensity data of 3180 independent reflections with $2\theta \leqq 130°$ were measured using Cu-$K\alpha$ radiation (λ=1.5481 Å) monochromated by means of graphite plates, with mixed $2\theta/\omega$ and ω scans on a Rigaku AFC-6[6] automated four-circle diffractometer. A total of 2087 reflections having intensities above the 2σ ($\pm$) level were collected and used for the structural determination. The crystal structure was refined by

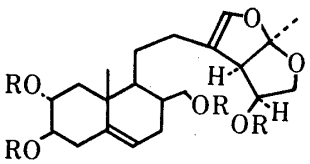
Table I. ^{13}C-NMR Chemical Shifts for **1**, **3**, **5**, **7**, and **8**

	1	**3**	**5**	**7**	**8**
C- 1	45.5	45.3	45.9	42.9	36.6
C- 2	72.4	73.2	72.5	72.6	30.0
C- 3	76.7	76.6	76.8	76.3	78.1
C- 4	40.1	40.1	40.7	36.7	39.0
C- 5	140.9	141.6	140.8	45.6	140.7
C- 6	120.0	126.9	121.6	27.2	120.4
C- 7	30.1	23.6	28.0	28.3	30.0
C- 8	53.2	51.4	49.0	53.2	53.3
C- 9	40.4	50.3	38.2	44.5	40.7
C-10	40.4	40.1	40.4	38.7	38.7
C-11	23.9	67.8	28.4	23.1	23.9
C-12	28.2	30.2	30.6	23.5	28.4
C-13	118.5	118.6	118.7	43.5	118.4
C-14	175.4	174.9	71.5	175.3	175.4
C-15	67.8	67.9	64.9	72.4	67.7
C-16	75.5	75.8	74.2	73.4	75.5
C-17	56.2	56.4	55.4	51.6	56.2
C-18	143.8	144.0	141.3	70.8	143.8
C-19	19.2	19.0	19.8	12.9	18.6
C-20	114.3	114.5	115.3	116.7	114.3
C-21	24.8	24.8	25.2	24.9	24.8
C- 1′					102.4
C- 2′					75.0
C- 3′					88.0
C- 4′					75.9
C- 5′					72.6
C- 6′					17.9
-OMe					60.8

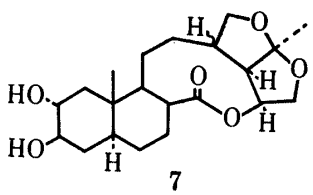
(in C_5D_5N)



R_1 R_2 R_3
1 : H H H
2 : Ac Ac H
3 : H H OH
4 : Ac Ac OAc



5 : R=H
6 : R=Ac



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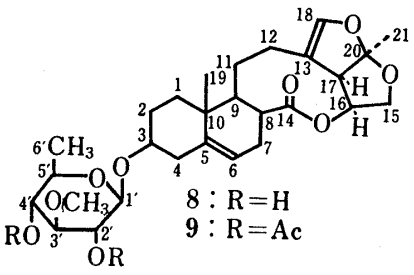

8 : R=H
9 : R=Ac

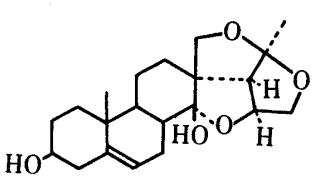

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Chart 1

TABLE II. Fractional Coordinates and Thermal Parameters

	X	Y	Z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
O(1)	−0.1707(5)	0.2929(4)	0.0991(15)	0.0028	0.0035	0.0296	−0.0013	−0.0012	0.0021
O(2)	−0.1711(5)	0.3294(4)	−0.2030(14)	0.0030	0.0036	0.0196	−0.0009	−0.0017	−0.0004
O(3)	0.0064(4)	0.3620(4)	−0.1219(12)	0.0024	0.0023	0.0177	−0.0005	0.0004	−0.0003
O(4)	0.0273(4)	0.4565(4)	−0.0672(14)	0.0029	0.0026	0.0241	−0.0002	−0.0010	0.0004
O(5)	0.3815(4)	0.3481(4)	0.5560(15)	0.0024	0.0023	0.0374	0.0000	−0.0035	0.0017
O(6)	0.4186(4)	0.4148(4)	0.7771(15)	0.0026	0.0027	0.0289	−0.0004	−0.0012	0.0006
O(7)	0.6221(5)	0.3810(4)	0.6676(16)	0.0027	0.0032	0.0354	0.0000	−0.0018	0.0014
O(8)	0.5125(4)	0.5662(3)	0.3817(14)	0.0032	0.0015	0.0238	−0.0001	−0.0008	0.0006
O(9)	0.5520(8)	0.2622(4)	0.4262(19)	0.0108	0.0020	0.0399	0.0003	−0.0041	0.0005
O(10)	0.5836(5)	0.4877(4)	0.8470(14)	0.0038	0.0024	0.0231	−0.0007	−0.0034	0.0005
O(11)	0.6166(5)	0.4584(5)	1.1278(15)	0.0042	0.0049	0.0243	−0.0013	−0.0035	0.0028
C(1)	0.0481(6)	0.4073(6)	0.0715(17)	0.0029	0.0025	0.0116	−0.0009	0.0020	−0.0003
C(2)	−0.0671(7)	0.3741(6)	−0.1514(22)	0.0026	0.0030	0.0263	−0.0006	−0.0015	0.0025
C(3)	−0.0998(7)	0.3215(11)	−0.2442(21)	0.0024	0.0045	0.0282	−0.0013	0.0010	−0.0038
C(4)	−0.1744(6)	0.3451(6)	−0.0125(21)	0.0023	0.0031	0.0236	−0.0008	−0.0009	0.0014
C(5)	−0.1063(5)	0.3808(7)	0.0271(18)	0.0012	0.0042	0.0148	0.0001	0.0000	0.0023
C(6)	−0.2447(7)	0.3767(8)	0.0226(26)	0.0019	0.0048	0.0352	−0.0001	−0.0013	−0.0036
C(7)	−0.1136(7)	0.2983(7)	0.2180(21)	0.0026	0.0033	0.0212	−0.0006	0.0001	0.0013
C(8)	−0.0762(6)	0.3455(6)	0.1926(19)	0.0020	0.0032	0.0178	−0.0005	0.0020	−0.0004
C(9)	−0.0127(6)	0.3650(6)	0.2962(19)	0.0018	0.0030	0.0176	−0.0008	0.0006	−0.0003
C(10)	0.0541(6)	0.3279(6)	0.2521(19)	0.0018	0.0026	0.0203	−0.0002	−0.0012	0.0017
C(11)	0.1181(6)	0.3685(6)	0.2068(18)	3.5452	0.0000	0.0000	0.0000	0.0000	0.0000
C(12)	0.1865(6)	0.3394(5)	0.2554(18)	3.6031	0.0000	0.0000	0.0000	0.0000	0.0000
C(13)	0.1893(7)	0.3414(6)	0.4828(21)	4.4294	0.0000	0.0000	0.0000	0.0000	0.0000
C(14)	0.2623(7)	0.3243(6)	0.5604(21)	4.5017	0.0000	0.0000	0.0000	0.0000	0.0000
C(15)	0.3161(7)	0.3681(6)	0.4868(20)	4.3579	0.0000	0.0000	0.0000	0.0000	0.0000
C(16)	0.3173(8)	0.3639(6)	0.2629(23)	5.5790	0.0000	0.0000	0.0000	0.0000	0.0000
C(17)	0.2476(7)	0.3762(6)	0.1858(20)	4.3494	0.0000	0.0000	0.0000	0.0000	0.0000
C(18)	0.2394(7)	0.4178(6)	0.0560(22)	4.9021	0.0000	0.0000	0.0000	0.0000	0.0000
C(19)	0.1713(8)	0.4344(6)	−0.0389(23)	5.4677	0.0000	0.0000	0.0000	0.0000	0.0000
C(20)	0.1196(6)	0.3852(5)	−0.0092(19)	3.7740	0.0000	0.0000	0.0000	0.0000	0.0000
C(21)	0.1931(7)	0.2784(6)	0.1828(28)	0.0030	0.0023	0.0467	−0.0007	0.0031	−0.0032
C(22)	0.4276(6)	0.3959(5)	0.5918(20)	0.0027	0.0017	0.0200	−0.0005	−0.0011	0.0014
C(23)	0.4609(7)	0.4624(6)	0.8205(19)	0.0028	0.0024	0.0177	−0.0010	−0.0009	−0.0006
C(24)	0.5373(6)	0.4378(5)	0.8228(19)	0.0027	0.0020	0.0194	0.0010	−0.0001	0.0009
C(25)	0.5564(6)	0.4111(6)	0.6389(19)	0.0016	0.0026	0.0201	−0.0005	−0.0013	0.0015
C(26)	0.5016(7)	0.3673(6)	0.5779(21)	0.0029	0.0023	0.0250	−0.0006	0.0010	−0.0003
C(27)	0.4404(8)	0.4849(7)	1.0082(26)	0.0037	0.0033	0.0316	−0.0007	−0.0035	−0.0015
C(28)	0.5207(8)	0.3025(7)	0.3054(25)	0.0044	0.0023	0.0379	−0.0002	0.0036	0.0028
C(29)	0.5321(12)	0.2997(7)	0.1122(27)	0.0101	0.0026	0.0288	−0.0006	0.0011	−0.0016
C(30)	0.6667(8)	0.3769(10)	0.5192(28)	0.0028	0.0080	0.0323	0.0004	0.0009	−0.0005
C(31)	0.6212(7)	0.4890(6)	1.0065(21)	0.0037	0.0029	0.0163	0.0002	−0.0005	−0.0004
C(32)	0.6757(8)	0.5391(6)	0.9870(23)	0.0037	0.0024	0.0305	−0.0012	−0.0001	−0.0012

The anisotropic temperature factor is expressed in the form:
$\exp[-(h^2\beta_{11}+k^2\beta_{22}+l^2\beta_{33}+2hk\beta_{12}+2hl\beta_{13}+2kl\beta_{23})]$.
Standard deviation for the last digit ($\times 10^4$) is given in parentheses.

direct methods with the MULTAN program.[7] Lorentz and polarization corrections were applied, but absorption corrections were not made. The crystal structure was refined by using a block-diagonal least-squares method. The final R-value was 0.1186 without hydrogen atoms. The atomic parameters are listed in Table II.[8] An ORTEP drawing of a stereoscopic view of the molecular conformation with thermal ellipsoids is given in Fig. 2, bond lengths in Fig. 3, and bond angles in Fig. 4.

The absolute configuration of **8** was determined to be as depicted in Chart 1 on the basis that the thevetose belongs to the D-series.[9] The close analogy of the spectroscopic features and the apparent similarity among **1**, **3** and the aglycone moiety of **8** led to the structure of

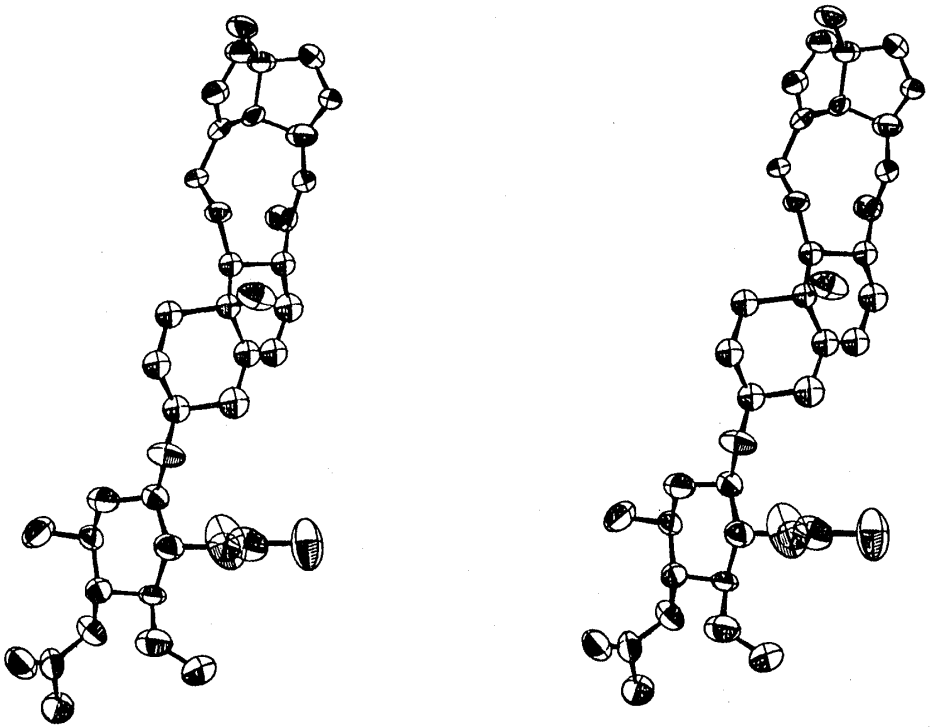

Fig. 2. Stereoscopic View of the Molecule of **9**

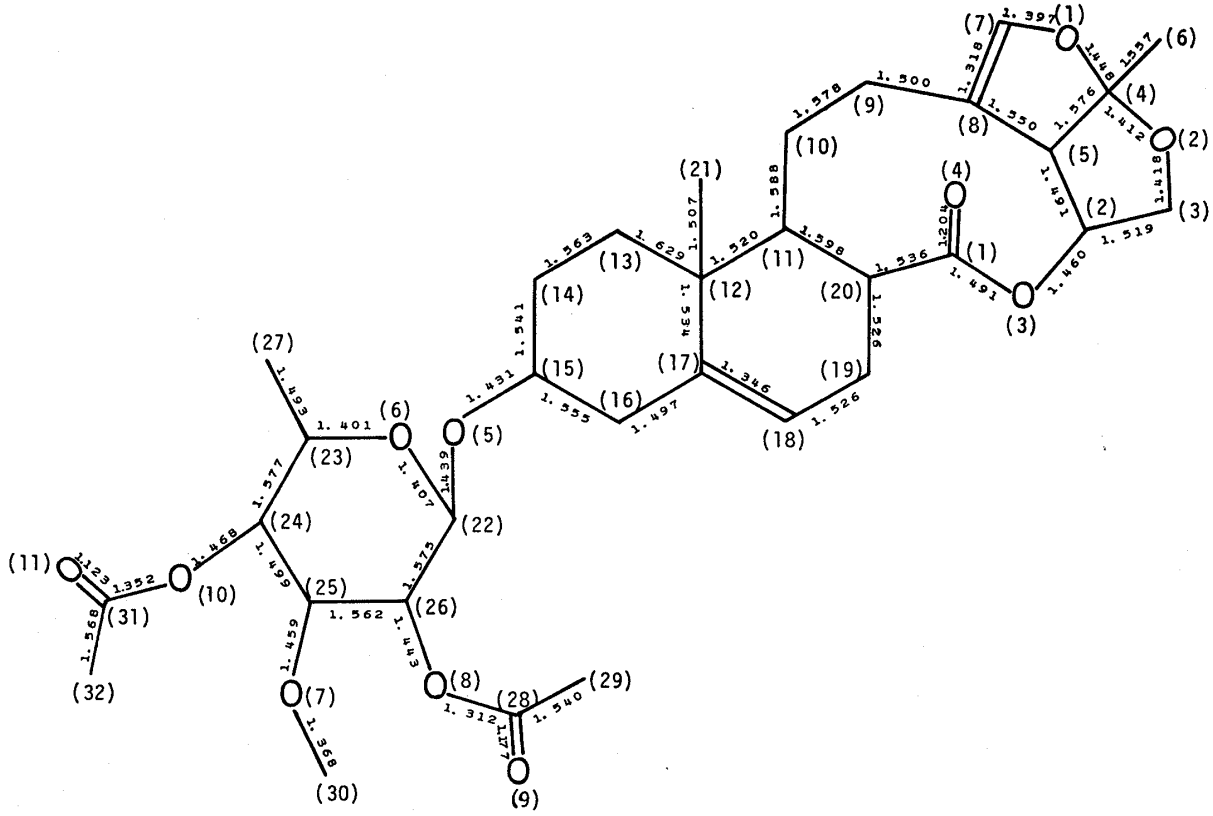

Fig. 3. Bond Lengths (Å) Together with Atomic Numbering in **9**

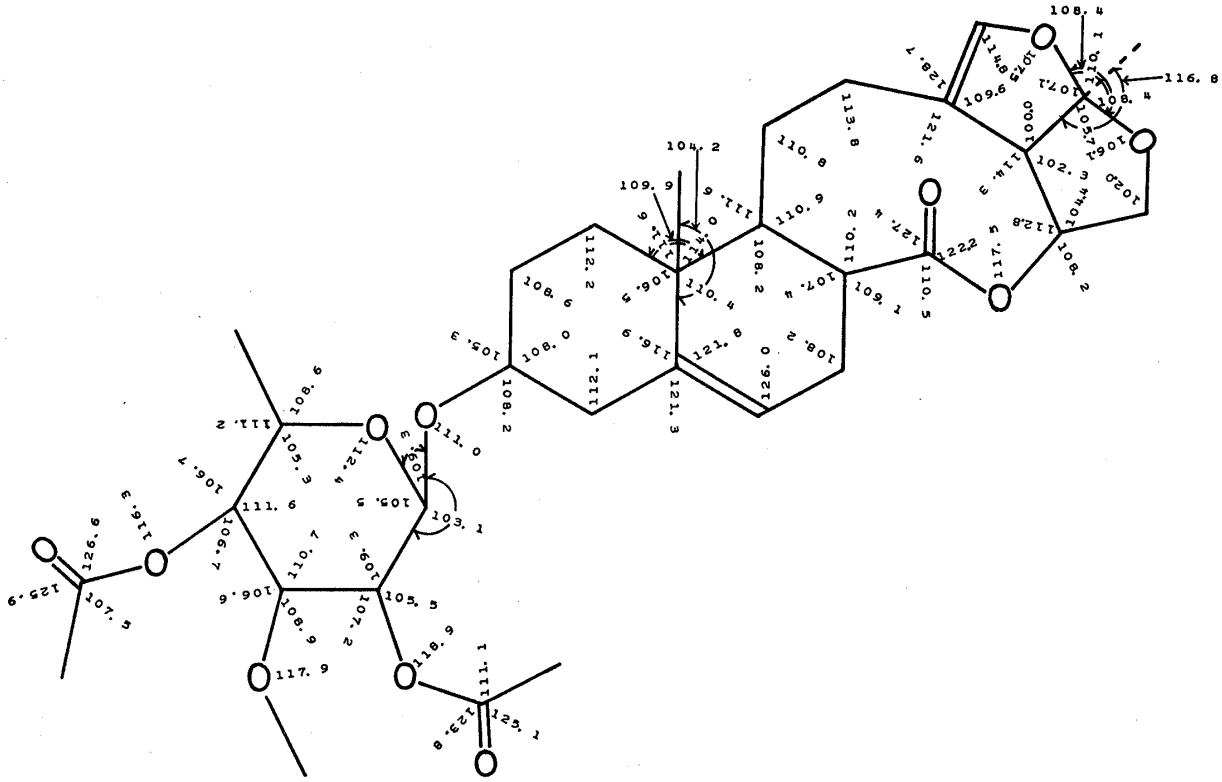

Fig. 4. Bond Angles(°) in **9**

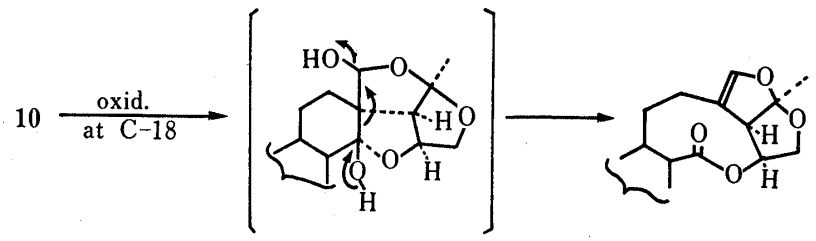

Fig. 5

glaucogenin-A (**1**) shown in Chart 1. Consideration of a Dreiding model of **3** indicated that only C-11$_\alpha$ was a suitable position for the remaining hydroxyl group, namely the rather large coupling constant (10 Hz) may be ascribable to coupling between 9-CH$_\alpha$ and 11-CH$_\beta$, with further coupling between 11-CH$_\beta$ and methylene protons at C-12. Thus, the structure of **3** was tentatively deduced to be as shown in Chart 1, with the remaining secondary hydroxyl group at C-11$_\alpha$.

Compounds **1**, **3**, and **8** have an unprecedented skeleton, namely 15,20α: 18,20β-diepoxy-13,14: 14,15-disecopregna-5,13(18)-dien-14-oic acid 16-oxy-lactone. Although the occurrence of **10** in this material has not been confirmed yet, the biogenesis of this peculiar skeleton can be speculated to be as depicted in Fig. 5; after hydroxylation at C-18 of **10**, the nine-membered lactone ring is generated together with the enol ether function. This type of lactonization is known to occur as a chemical reaction of ryanodine[10] and diterpenes in *Cinnamomum cassia*.[11]

This crude drug, based on asclepiadaceous plants, thus provides an interesting insight into biogenetic oxygenation processes of pregnane-type steroids. Further investigation of the aglycones associated with the glycosides in this material is under way.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter at room temperature. IR spectra were recorded on a JASCO A-102 spectrometer. ^{1}H-NMR spectra were run on a JEOL FX-200 (200 MHz), and ^{13}C-NMR spectra on a JEOL FX-100 (25 MHz) or FX-200 (50 MHz) spectrometer in $CDCl_3$ or C_5D_5N solution with tetramethylsilane as a standard. EI-MS were determined with a JEOL JMS-D-300 mass spectrometer. TLC was performed on Merck precoated plates (Kieselgel 60 F_{254}), and column chromatography on Wakogel C-200 or C-300.

Isolation of 1, 3, and 8——A part of the hexane–benzene (1: 1) and benzene soluble portion of the crude glycosides (40 g) reported in the previous paper[1] was dissolved in 600 ml of MeOH and warmed to 50°C. Then 200 ml of 0.2 N H_2SO_4, which had been prewarmed to 50°C, was poured into the solution and the mixture was kept at around 50°C. After 30 min, the solution was neutralized with saturated aqueous $Ba(OH)_2$ and the precipitates were filtered off. The filtrate was concentrated and chromatographed on a column of silica gel (200 g of Wakogel C-300) with the solvents of increasing polarity from $CHCl_3$–acetone (6: 1) to acetone to obtain four fractions (fractions 1 to 4). Fraction 1 (1.64 g) mainly contained a mixture of methyl glycosides. Fraction 2 (1.75 g), containing **8**, was rechromatographed with hexane-ethyl acetate (EtOAc) (1: 2) to give a fraction (450 mg) containing mainly **8**, and crystallization from acetone gave a pure sample of **8** (102 mg) as colorless fine needles. Fraction 3 (13.4 g), containing **1** and **3**, was rechromatographed on a column of silica gel (80 g of Wakogel C-300) with hexane–EtOAc (1: 2) to provide four fractions (fractions A to D). Fraction B, which contained **1**, was then rechromatographed with 0.7% MeOH in $CHCl_3$ to give a fraction (650 mg) containing mainly **1**. Crystallization from hexane–acetone afforded a pure sample of **1** (192 mg) as colorless needles. Fraction D (930 mg), containing **3**, was further rechromatographed with $CHCl_3$–acetone (5: 1) to give a fraction which contained mainly **3**. Crystallization from MeOH yielded a pure sample of **3** (30 mg) as colorless prisms.

Glaucogenin-A (**1**)——Colorless needles, mp 225—231°C, $[\alpha]_D$ +78.1° (c=1.07, MeOH). *Anal.* Calcd for $C_{21}H_{28}O_6$: C, 67.00; H, 7.50. Found: C, 67.20; H, 7.52. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3400, 1730, 1710, 1655, 1310, 880. EI-MS m/z: 376 (M^+), 358 (M^+-H_2O), 330, 312, 137 (base peak), 43. ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, s, 19-CH_3), 1.08 (1H, t, J=12 Hz, 1-CH_α), 1.54 (3H, s, 21-CH_3), 3.36 (1H, m, 3-CH), 3.46 (1H, dd, J=8, 2 Hz, 17-CH), 3.70 (1H, m, 2-CH), 3.88 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.20 (1H, dd, J=9, 7 Hz, 15-CH_α), 5.35 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.50 (1H, d, J=4.5 Hz, 6-CH), 6.27 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR: see Table I.

Acetylation of 1——Compound **1** (10 mg) was acetylated with Ac_2O–C_5H_5N at room temperature, and **2** (8 mg) was obtained as colorless plates from acetone, mp 253—259°C, $[\alpha]_D$ ±0° (c=0.76, $CHCl_3$). *Anal.* Calcd for $C_{25}H_{32}O_8$: C, 65.20; H, 7.00. Found: C, 64.90; H, 6.86. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735, 1730, 1655, 1240, 1160, 1120, 1080, 1040. EI-MS m/z: 460 (M^+), 414, 400 (M^+—AcOH), 358, 354, 137, 43 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, s, 19-CH_3), 1.13 (1H, t, J=12 Hz, 1-CH_α), 1.53 (3H, s, 21-CH_3), 2.03 (6H, s, –OC(=O)CH_3), 3.43 (1H, dd, J=8, 2 Hz, 17-CH), 3.84 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.16 (1H, dd, J=9, 7 Hz, 15-CHα), 4.76 (1H, ddd, J=10, 10, 6 Hz, 3-CH), 5.11 (1H, ddd, J=12, 10, 5 Hz, 2-CH), 5.35 (1H, ddd, J=10, 8, 7 Hz, 17-CH), 5.50 (1H, d, J=4.5 Hz, 6-CH), 6.25 (1H, d, J=2 Hz, 18-CH).

$LiAlH_4$ Reduction of 1——A solution of 32 mg of **1** in 5 ml of THF was treated with excess $LiAlH_4$, and the mixture was stirred for 2 h at room temperature. Work-up in the usual manner gave a yellow oil, which was purified by silica gel column chromatography with 6% MeOH in $CHCl_3$ to yield **5** (15 mg) as a colorless oil, $[\alpha]_D$ −55° (c=1.2, MeOH). High resolution (HR)-EI-MS m/z: 380.2207 (M^+, Calcd for $C_{21}H_{32}O_6$: 380.2199). IR ν_{max}^{Nujol} cm^{-1}: 3300, 1655, 820. MS m/z: 380 (M^+), 362 (M^+-H_2O), 344 (M^+-2H_2O), 320, 137 (base peak), 121, 109, 43. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, s, 19-CH_3), 1.51 (3H, s, 21-CH_3), 3.03 (1H, dd, J=8, 2 Hz, 17-CH), 4.00 (1H, dd, J=10, 6 Hz, 15-CH_α), 4.52 (1H, ddd, J=10, 8, 6 Hz, 16-CH), 5.45 (1H, d, J=5 Hz, 6-CH), 6.27 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR: see Table I.

Acetylation of 5——Compound **5** (10 mg) gave, on acetylation as described above, **6** (11 mg) as a colorless oil, $[\alpha]_D$ −63° (c=1.1, $CHCl_3$). HR-EI-MS m/z: 548.2601 (M^+, Calcd for $C_{29}H_{40}O_{10}$: 548.2621). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735, 1655, 1440, 1370, 1240, 1040. EI-MS m/z: 548 (M^+), 488 (M^+—AcOH), 428 (M^+—2AcOH), 368 (M^+—3AcOH), 308 (M^+—4AcOH), 137, 43 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, s, 19-CH_3), 1.12 (1H, t, J=12 Hz, 1-CH_α), 1.54 (3H, s, 21-CH_3), 2.03, 2.04, 2.05, 2.06 (each 3H, s, –OC(=O)CH_3), 3.23 (1H, dd, J=8, 2 Hz, 17-CH), 3.67 (1H, dd, J=10, 8 Hz, 15-CH_β), 3.92 (1H, dd, J=11.5, 7 Hz, 14-CHa or –CHb), 4.09 (1H, dd, J=8, 6.5 Hz, 15-CH_α), 4.22 (1H, dd, J=11.5, 4 Hz, 14-CHa or –CHb), 4.75 (1H, ddd, J=12, 10, 6 Hz, 3-CH), 5.16 (1H, ddd, J=12, 10, 4.5 Hz, 2-CH), 5.29 (1H, ddd, J=10, 8, 6.5 Hz, 16-CH), 5.46 (1H, d, J=4.5 Hz, 6-CH), 6.25 (1H, d, J=2 Hz, 18-CH).

Hydrogenation of 1——A solution of 39 mg of **1** in 3 ml of 90% AcOH was added to a suspension of prereduced PtO_2 (20 mg) in 1 ml of AcOH, and the whole was stirred under hydrogen at room temperature for 1 h. TLC analysis with EtOAc showed the formation of one major (Rf, 0.20) and two minor products (Rf, 0.22 and 0.25). The catalyst was filtered off, the filtrate was concentrated, and the residue was separated

by silica gel column chromatography with hexane–EtOAc (1: 2) to isolate the major product as a white powder, which was crystallized from hexane–acetone to give **18 mg** of **7** as colorless needles, mp 217—220°C, $[\alpha]_D$ +14° (c=0.8, $CHCl_3$). HR-EI-MS m/z: 380.2191 (M^+ Calcd for $C_{21}H_{32}O_6$: 380.2199). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3350, 1730, 1460, 1380, 1160, 1040. EI–MS m/z: 380 (M^+), 365, 323, 254, 83 (base peak), 43. ^{1}H-NMR ($CDCl_3$) δ: 0.80 (3H, s, 19-CH_3), 0.98 (1H, t, J=12 Hz, 1-CH_α), 1.57 (3H, s, 21-CH_3), 2.05 (1H, dd, J=12, 5 Hz, 1-CH_β), 2.42 (1H, dd, J=12, 5 Hz, 4-CH_α), 3.44 (1H, m, 3-CH), 3.63 (1H, ddd, J=12, 10, 5 Hz, 2-CH), 3.95, 4.10 (each 1H, dd, J=10, 7 Hz, 15-CH_2), 5.77 (1H, ddd, J=9, 7, 7 Hz, 16-CH). ^{13}C-NMR: see Table I.

Glaucogenin-B (**3**)——Colorless prisms, mp 269—272.5°C, $[\alpha]_D$ +135° (c=0.23, MeOH). *Anal.* Calcd for $C_{21}H_{28}O_7$: C, 64.27; H, 7.19. Found: C, 64.22; H, 7.37. IR ν_{max}^{Nujol} cm^{-1}: 3500, 3350, 1730, 1710, 1655 1310, 880. EI–MS m/z: 392 (M^+), 374 (M^+-H_2O), 341, 328, 137 (base peak), 43. ^{1}H-NMR (C_5D_5N) δ: 1.01 (3H, s, 19-CH_3), 1.53 (3H, s, 21-CH_3), 2.50 (1H, dd, J=12, 4.5 Hz, 1-CH_β), 2.83 (1H, t, J=10 Hz, 4-CH_β), 3.60 (1H, dd, J=8, 2 Hz, 17-CH), 3.92 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.12 (1H, dd, J=9, 7 Hz, 15-CH_α), 5.44 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.82 (1H, br s, 6-CH), 6.26 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR: see Table I.

Acetylation of 3——Compound **3** (12 mg) gave, on acetylation as described above, **4** (9 mg) as colorless prisms from MeOH, mp 256—258°C, $[\alpha]_D$ +82.7° (c=0.37, $CHCl_3$). *Anal.* Calcd for $C_{27}H_{34}O_{10}$: C, 62.54; H, 6.61. Found: C, 62.36; H, 6.58. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735, 1730, 1655, 1380, 1310, 1240, 1080. EI-MS m/z: 518 (M^+), 458 (M^+—AcOH), 352, 137, 43 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, s, 19-CH_3), 1.13 (1H, J=12 Hz, 1-CH_α), 1.53 (3H, s, 21-CH_3), 2.03 (9H, s, –OC(=O)CH_3), 2.27 (1H, dd, J=12, 5 Hz, 1-CH_β), 2.38 (1H, dd, J=12, 6 Hz, 4-CH_α), 2.54 (1H, dd, J=12, 10 Hz, 4-CH_β), 3.44 (1H, dd, J=8, 2 Hz, 17-CH), 3.85 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.20 (1H, dd, J=9, 7 Hz, 15-CH_α), 4.76 (1H, ddd, J=10, 10, 6 Hz, 3-CH), 5.10 (1H, ddd, J=12, 10, 5 Hz, 2-CH), 5.33 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.48 (1H, br s, 6-CH), 5.70 (1H, dt, J=10, 2 Hz, 11-CH), 6.25 (1H, d, J=2 Hz, 18-CH).

Glaucogenin-C Mono-D-thevetoside (**8**)——Colorless fine needles, mp 187—190.5°C, $[\alpha]_D$ +27.4° (c=1.03, $CHCl_3$). *Anal.* Calcd for $C_{28}H_{40}O_9$: C, 64.59; H, 7.74. Found: C, 64.19; H, 7.87. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3500, 1730, 1710, 1655, 1310, 1070, 880. EI-MS m/z: 520 (M^+), 491, 360, 342, 314, 137 (base peak), 43. ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, s, 19-CH_3), 1.13 (3H, d, J=6 Hz, 6′-CH_3), 1.54 (3H, s, 21-CH_3), 3.46 (1H, dd, J=8, 2 Hz, 17-CH), 3.66 (3H, s, 3′-QCH_3), 3.85 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.16 (1H, dd, J=9, 7 Hz, 15-CH_α), 4.35 (1H, d, J=8 Hz, 1′-CH), 5.35 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.45 (1H, d, J=4.5 Hz, 6-CH), 6.26 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR: see Table I.

Acetylation of 8——Compound **8** (36 mg) gave, on acetylation as described above, **9** (25 mg) as colorless prisms from MeOH, mp 180—182°C, $[\alpha]_D$ +32.6° (c=1.13, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{44}O_{11}$: C, 63.56; H, 7.33. Found: C, 63.52; H, 7.40. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1730, 1710, 1655, 1440, 1380, 1240, 1160, 1030. EI-MS m/z: 604 (M^+), 297, 296, 245, 137, 84, 43 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, s, 19-CH_3), 1.21 (3H, d, J=6 Hz, 6′-CH_3), 1.53 (3H, s, 21-CH_3), 2.10, 2.17 (each 3H, s, –OC(=O)CH_3), 3.38 (3H, s, 3′-QCH_3), 3.84 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.20 (1H, dd, J=9, 7 Hz, 15-CH_α), 4.32 (1H, d, J=8 Hz, 1′-CH), 4.84 (1H, t, J=10 Hz, 4′-CH), 4.98 (1H, dd, J=10, 8 Hz, 2′-CH), 5.35 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 6.26 (1H, d, J=2 Hz, 18-CH).

Acidic hydrolysis of 8——Compound **8** (60 mg) was refluxed with 1 N H_2SO_4 in 50% MeOH (4 ml) for 6 h, then the solution was diluted with water (2 ml) and concentrated to 1/2 the initial volume. The solution was heated for a further 3 h, neutralized with 5% NaOH, and evaporated to dryness. The salt deposited on addition of MeOH was filtered off, then the filtrate was concentrated to give a dark-brown tar. This tar was subjected to silica gel column chromatography with 12% MeOH in $CHCl_3$ to yield a pure sample of D-thevetose (4 mg), which formed colorless needles after being dried, mp 116—119°C, $[\alpha]_D$ +30° (c=0.4, H_2O). This product was indistinguishable on TLC (Rf, 0.3 (solvent, $CHCl_3$–MeOH=3: 1)) from an authentic sample.

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References and Notes

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