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STUDIES ON THE CONSTITUENTS OF ASCLEPIADACEAE PLANTS–LII[1]

THE STRUCTURES OF FIVE GLYCOSIDES GLAUCOSIDE-A, -B, -C, -D, AND -E FROM CHINESE DRUG "PAI-CH'IEN" *CYNANCHUM GLAUCESCENS* HAND-MAZZ

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Abstract—The glycosides of Chinese crude drug "Pai-ch'ien", dried root of *Cynanchum glaucescens* HAND-MAZZ (Asclepiadaceae) have been studied. Five new glycosides named glaucoside-A(4), -B(5), -C(6), -D(7), and -E(10) were isolated by silica gel column chromatography, and their structures were deduced by combination of analyses of their hydrolysates and spectroscopic methods using ^{1}H NMR, ^{13}C NMR, and FD-MS. Interestingly 5, 6, 7, and 10 were found to carry α-glycosyl linked L-cymaropyranoses in the terminal of their sugar chains while the other mode of linkages are β.

Chinese crude drug "Pai-ch'ien",[2] dried root of *Cynanchum glaucescens* HAND-MAZZ (Asclepiadaceae), has been used as an antitussive and expectorant in China. On the other hand "Pai-wei", dried root of *C. atratum* BGE (Asclepiadaceae), which is closely related to *C. glaucescens*, has been used as an antifebrile and diuretic. However, confusion exists not only in the therapy but also in the names between these two drugs but the many substitutes that are used.[2] The extracts of "Pai-ch'ien", and "Pai-wei", and their substitutes, which belong to the genus of *Cynanchum*, showed positive Liebermann-Burchard (L.B.) and Keller-Kiliani (K.K.) reactions as well as those of other asclepiadaceous plants, indicating the presence of steroidal glycosides with 2-deoxysugars. However, our preliminary studies on the constituents revealed that the aglycone moieties are sensitive to acid compared with those of other asclepiadaceous plants. From *C. grandifolium* HEMSL the steroidal compound, hirundigenin[3] was obtained. The previous communication[4] reported the structural elucidation of three new compounds named glaucogenin-A(1), -B(2), and -C mono-D-thevetoside (3) with a novel 13, 14:14, 15-diseco-pregnane-type skeleton and obtained from the hydrolysates of the crude glycosides of "Pai-ch'ien". We now report the full structures of five glycosides named glaucoside-A(4), -B(5), -C(6), -D(7), and -E(10), isolated from the same material. The hexane–benzene (1:1) and benzene soluble fractions of the crude glycosides, which contained a variety of glycosides, were subjected to silica gel column chromatography with various solvent systems to give five glycosides 4, 5, 6, 7 and 10 (yield: 0.008, 0.017, 0.010, 0.023 and 0.013% from the dried crude drug, respectively), each of which was an amorphous white powder and gave positive L.B. and K.K. reactions.

Glaucoside-A(4) gave 1 and oleandrose on hydrolysis, which were identified with authentic samples by TLC. The anomeric proton signal at δ4.55 (1H, dd, J = 10, 2 Hz) in the ^{1}H NMR is in good agreement with the ^{13}C NMR chemical shifts in pentadeuteropyridine (C_5D_5N) for the sugar moiety and those reported for methyl β-D-oleandropyranoside (11)[5] (Table 1) and established the β mode of linkage for the sugar.

Since the glycosidation shifts[6] were observed at C-2 (−2.5 ppm), C-3 (+8.7), and C-4 (−2.6) in the ^{13}C NMR for the aglycone moiety of 4, the sugar is linked with the C-3 OH group of 1. Moreover, the equivalent values for both the β-carbons supported the relative stereochemistry of the glycol moiety (2α, 3β) in the aglycone as reported by Tanaka *et al.*[7] This glycosidation shifts pattern was observed in the aglycone moieties of 5, 6 and 7 (Table 1), so that the sugar positions are linked at the C-3 OH group of 1. The occurrence of L-cymarose[8], D-oleandrose,[9] and D-digitoxose[10] has been confirmed in the hydrolysates of the crude glycosides of this drug; therefore the structure of 4 was established as glaucogenin-A 3-O-β-D-oleandropyranoside.

Glaucoside-B (5) liberated 1 and cymarose on hydrolysis. The ^{1}H NMR spectrum of 5 showed the signals due to three moles of cymarose: three secondary Mes at δ1.23, 1.24 and 1.27; three methoxyl Mes at δ3.40, 3.46 and 3.50. The anomeric proton signals were overlapped at δ4.75 integrating as 3H; the lanthanide induced shift experiments by addition of Eu(dpm)$_3$ revealed two sets of double doublet (J = 10, 2 Hz) and one broad doublet (J = 3 Hz), suggesting the presence of two β-linkages and one α-linkage in the sugar moiety of 5.

In the ^{13}C NMR of 5, the five sugar carbon signals distinguishable from the others (Table 2) by partially relaxed Fourier transform (PRFT) measurements[11] evidently correspond to those for methyl α-L-cymaropyranoside (13) rather than those for methyl β-D-cymaropyranoside (12); therefore the mode of linkage for the terminal cymarose is α. Consequently the structure of 5 as glaucogenin-A 3-O-α-L-cymaropyranosyl-(1→4)-β-L-cymaropyranosyl-(1→4)-β-L-cymaropyranoside. Since the signals ascribable to the terminal sugar in 5 are also found in 6, 7 and 10, they carry α-linked L-cymaropyranoses as the terminal sugar.

Glaucoside-C (6) gave 1, digitoxose, and cymarose on hydrolysis. The presence of two moles of cymarose and one mole of digitoxose in 6 was inferred from its ^{13}C NMR (Table 2) and ^{1}H NMR, namely three secondary Me and two methoxyl Me signals. The only available information for the sequence was given by its field desorption mass spectrum (FD-MS) in 6; the prominent

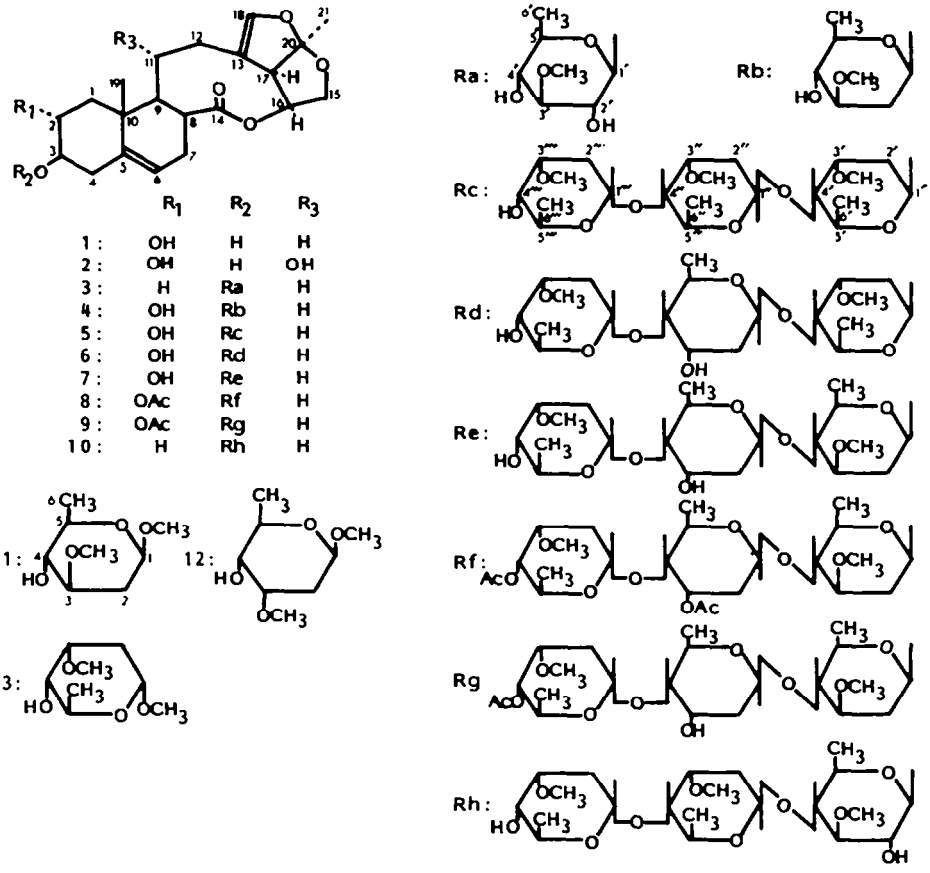

Fig. 1.

peaks at *m/z*: 650, 520, and 376 besides that of molecular ion peak are attributable to those derived by initial loss of the terminal cymarose, followed by digitoxose, and finally the cymarose linked to the aglycone, respectively in the manner as reported by Shulten *et al.*[12] (Fig. 2).

Glaucoside-D (**7**) showed essentially the same fragmentation pattern as **6** in its FD-MS, and showed similar ^{1}H NMR signals due to the sugars, indicating close analogy to **6**. On hydrolysis, **7** gave oleandrose besides cymarose and digitoxose. It was therefore suggested that the difference between **6** and **7** is only the sugar linked with the aglycone, namely cymarose in **6** and oleandrose in **7**. The question whether the terminal cymarose was linked to the C-3 or C-4 OH group of the digitoxose in **6** and **7** was resolved by the consideration of the ^{1}H NMR spectra of the tri- (**8**) and diacetate (**9**), which were obtained in approximately equal amounts by acetylation of **7**. In the ^{1}H NMR of **8** three low-field shifted acetoxymethine proton signals appeared at δ4.60 (1H, dd, J = 9.4, 3 Hz), 5.10 (1H, ddd, J = 12, 10, 5 Hz), and 5.35 (1H, m), which are assignable to 4‴-CH, 2-CH, and 3″-CH, respectively, while in **9** the signal due to 3″-CH at δ4.10 (1H, m) remained unchanged clarifying the presence of a 1→4 glycosyl linkage between the terminal cymarose and the digitoxose in **7**. The ^{13}C NMR of **6** and **7** (Table 2) indicated that the structure of the sugar chains are the same except for the sugar attached to the aglycones. In consequence, the structures of **6** and **7** were proposed as glaucogenin-A 3-O-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-L-cymaropyranoside and glaucogenin-A 3-O-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-oleandropyranoside, respectively. The low reactivity of the C-3″ OH group to acetylation compared with the others in **7** is presumably due to the steric hindrance caused by the α-glycosyl linked terminal cymarose as depicted in Fig. 2.

Glaucoside-E (**10**) gave cymarose and **3** on hydrolysis. The ^{13}C NMR displayed signals due to two moles of cymarose, in good agreement with those observed for **5**, and the signals based on thevetose being affected by glycosidation shifts appeared as C-4 (+6.7 ppm), C-3 (−2.3) and C-5 (−1.2). Therefore the structure of **10** was deduced as glaucogenin-C 3-O-α-L-cymaropyranosyl-(1→4)-β-L-cymaropyranosyl-(1→4)-β-D-thevetopyranoside.

It is noteworthy that the cymarose which composes the glycosides of this drug belongs to L-series, which is found only rarely in nature.[8] Furthermore the four glycosides **5**, **6**, **7** and **10** bear α-glycosyl linked L-cymaropyranoses in the terminal of their sugar chains while the other mode of linkages are β.

Shoji *et al.* pointed out that the sugar sequences of cardiac and pregnane-type glycosides of asclepiadaceous plants obtained up to present have the 2,6-dideoxysugar, 6-deoxysugar, and glucose attached to the aglycone in that order.[13] However, in the case of **10**, the sugar linked with the aglycone is not a 2,6-dideoxysugar but a 6-deoxysugar. Thus, the glycosides in this drug were found to consist of the highly oxygenated aglycone with a novel skeleton and unique sugar chains, which cast a new concept on the biogenesis of asclepiadaceous pregnane-type glycosides. Further investigation on the glycosides of this drug is currently under way.

Table 1. ^{13}C NMR chemical shifts for 3, 4 and 11 and those for the aglycone moities of 5, 6, 7 and 10

	4	11	5	6	7	3	10
C- 1	44.7		44.5	44.6	44.6	36.6	36.6
C- 2	69.9(-2.5)		69.9	69.9	69.7	30.0	30.0
C- 3	85.4(+8.7)		85.5	85.4	85.2	78.1	78.2
C- 4	37.5(-2.6)		37.6	37.5	37.6	39.0	39.0
C- 5	139.8		139.7	139.7	139.7	140.7	140.1
C- 6	120.7		120.7	120.7	120.7	120.4	120.4
C- 7	30.0		30.0	30.0	30.0	30.0	30.0
C- 8	53.0		52.9	53.2	53.0	53.3	53.2
C- 9	40.2		40.2	40.2	40.2	40.7	40.7
C-10	39.5		39.4	39.4	39.4	38.7	38.7
C-11	23.9		23.0	23.8	23.8	23.9	23.9
C-12	28.4		28.4	28.4	28.4	28.4	28.4
C-13	118.4		118.4	118.4	118.4	118.4	118.4
C-14	175.2		175.2	175.3	175.1	175.4	175.3
C-15	67.7		67.7	67.6	67.7	67.7	67.7
C-16	75.5		75.5	75.5	75.5	75.5	75.5
C-17	56.4		56.1	56.1	56.1	56.2	56.2
C-18	143.8		143.8	143.8	143.8	143.8	143.8
C-19	19.0		18.9	18.9	18.9	18.6	18.6
C-20	114.2		114.2	114.3	114.1	114.3	114.2
C-21	24.8		24.8	24.8	24.7	24.8	24.8
C- 1'	99.3	C-1 101.0				102.4	
C- 2'	37.2	C-2 36.6				75.0	
C- 3'	81.3	C-3 81.3				88.0	
C- 4'	75.9	C-4 76.2				75.9	
C- 5'	73.0	C-5 72.6				72.6	
C- 6'	18.9	C-6 18.4				17.9	
-OMe	57.0	-OMe 56.0, 56.9				60.8	

(in C_5D_5N)

Table 2. ^{13}C NMR chemical shifts for the sugar carbons of 5, 6, 7 and 10 and those for 12 and 13

	5	6	7	10	13	12
C-1'	97.8	97.8	98.2[e]	102.2		
C-2'	36.9	37.0	37.4	74.6		
C-3'	77.6[a]	77.8	78.9	85.7(-2.3)		
C-4'	81.9[a]	82.7	82.5	82.6(+6.7)		
C-5'	69.1	69.3[d]	71.7	71.4(-1.2)		
C-6'	18.5[b]	18.1[d]	18.4	17.8		
-OMe	58.4[c]	58.9	57.4[e]	60.4[f]		
C-1"	100.3	100.3	99.1[e]	98.6[f]		
C-2"	36.9	38.4	38.3	37.0		
C-3"	77.6	68.8	69.0	77.8		
C-4"	82.7[a]	80.0	80.6	82.2		
C-5"	69.1	67.6[d]	67.7	69.4		
C-6"	18.5	18.3[d]	18.4	18.6		
-OMe	58.9[c]			58.7[f]		
C-1'''	98.8*	98.4	98.2	98.8[f]	C-1 97.6	99.4
C-2'''	32.0*	32.2	32.1	31.9	C-2 31.9	35.1
C-3'''	76.3*	76.5	76.5	76.2	C-3 76.5	78.5
C-4'''	73.1*	72.7	72.7	73.1	C-4 73.2	74.0
C-5'''	66.2*	67.0	66.7	66.1	C-5 65.2	71.0
C-6'''	18.1[b]	18.5[d]	18.4	18.6	C-6 18.9	18.9
-OMe	56.4	56.8	56.7	56.4	-OMe 56.7, 54.7	57.8, 56.0

(in C_5D_5N)

a-f)Assignments may be interchanged, and the shifts with asteriks (*) have longest dipole-dipole relaxation time in the sugar carbons by PRFT measurements.

EXPERIMENTAL

Mps were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter at room temp. IR spectra were recorded on a JASCO A-102 spectrometer. ^1H NMR spectra were run on a JEOL FX-200 (200 MHz) in $CDCl_3$ solution and ^{13}C NMR spectra on a JEOL FX-100 (25 MHz) or a JEOL FX-200 (50 MHz) spectrometer in C_5D_5N soln with TMS as standard. Electron impact mass spectrometry (EI-MS) were determined with a JEOL JMS-D-300 mass spectrometer and field desorption (FD)-MS with a JEOL JMS-01SG-2. TLC was performed on Merck precoated plates, Kieselgel 60 F_{254}, and silica gel column chromatography on Wakogel C-200 or C-300.

Extraction from "Pai-ch'ien". The commercially available "Pai-ch'ien" (5.1 kg) was dried overnight at 45–50°, and powdered. This material (4.9 kg) was extracted exhaustively with $CHCl_3$ and concentrated to give a 240 g of dark-brown tar. This was dissolved in a small amount of $CHCl_3$ and gradually added hexane with stirring to remove oily substances.

The hexane soluble portion was decanted. The hexane insoluble portion (180 g) obtained by repeating it several times was then dissolved in MeOH and the insoluble portion was filtered

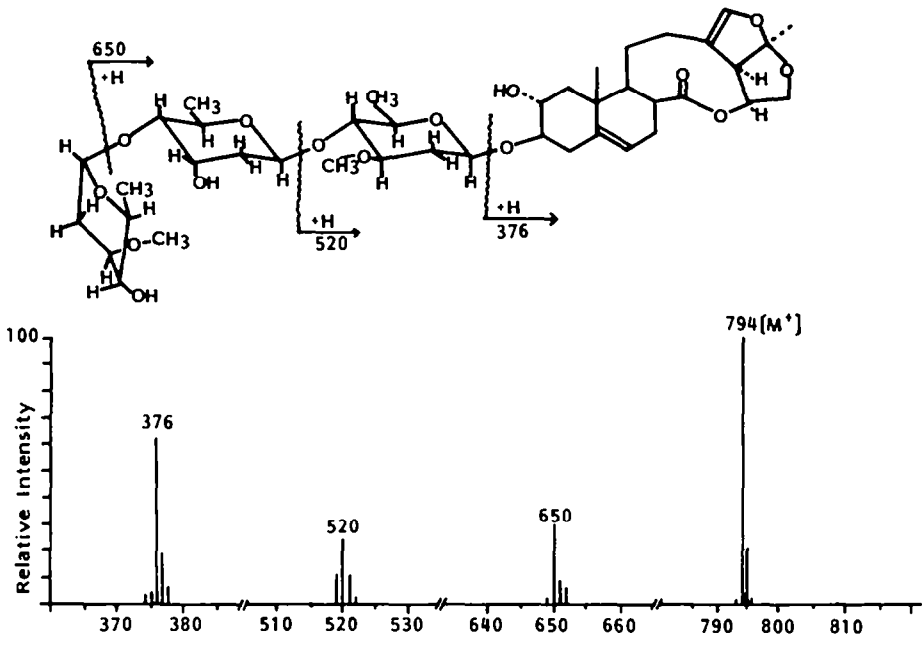

Fig. 2. FD Mass spectrum of 7.

off. The filtrate was evaporated to yield 165 g of crude glycosides as a dark-brown solid. This solid was further dissolved in hexane–benzene (1:1) and then in benzene. Each soluble portion, which contained several glycosides, was combined and concentrated to afford a dark-brown tar (110 g).

Isolation of each glycoside (**4**, **5**, **6**, **7**, and **10**). Part of the above less polar portion of the crude glycosides (40 g) was applied to a column of silica gel (400 g of Wakogel C-200), and the material was eluted with a solvent of increasing polarity from benzene–acetone (8:1) to acetone. Fraction 2 (3.48 g), benzene–acetone (5:1) eluted fraction, contained **4** and **5**. Rechromatography of this fraction on a column of silica gel (80 g of Wakogel C-300) with hexane–EtOAc (1:1), and further rechromatography with $CHCl_3$–acetone (7:1) gave **4** (92 mg) and **5** (198 mg).

Fraction 3 (8.0 g) was obtained by further elution with the same solvent system, which contained **6**, **7**, and **10**. The fraction was rechromatographed on a column of silica gel (160 g of Wakogel C-300) with 1.5% MeOH in $CHCl_3$ to separate five fractions (fraction A to E). Fraction C (2.24 g) containing **6** and **10** was further rechromatographed with hexane–EtOAc (1:2) to give **10** (163 mg), while rechromatography of this fraction with hexane–EtOAc–MeOH (50:50:1) yielded **6** (120 mg). Fraction D (1.07 g) containing **7** was rechromatographed with hexane–acetone (5:2), and then with hexane–EtOAc–MeOH (50:50:3) to furnish **7** (277 mg). Each of the five glycoside is an amorphous white powder. The *Rf* values of **4** and **5** on TLC are 0.50 and 0.75 ($CHCl_3$–acetone (3:1) as the solvent); **6**, **7** and **10** are 0.45, 0.40, and 0.50 (benzene–acetone (2:1) as the solvent), respectively.

Glaucoside-A (**4**). An amorphous powder, m.p. 112–117°, $[\alpha]_D$ + 7.17° (*c* = 1.20, $CHCl_3$). (Found: C, 64.41; H, 7.87. Calc for $C_{28}H_{40}O_9$: C, 64.59; H, 7.74%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450, 1730, 1710, 1655, 1440, 1380, 1310, 1160, 1100, 1070, 880. EI-MS *m/z*: 520 (M^+), 376, 330, 312, 145, 137, 114, 95, 87 (base peak), 43. ^1H NMR ($CDCl_3$) δ: 0.95 (3H, s, 19-CH_3), 1.01 (1H, t, *J* = 12 Hz, 1-CH_a), 1.35 (3H, d, *J* = 6 Hz, 6'-CH_3), 1.53 (3H, s, 21-CH_3), 3.41 (3H, s, 3'-OCH_3), 3.66 (1H, ddd, *J* = 12, 8, 4 Hz, 2-CH), 3.84 (1H, dd, *J* = 10, 9 Hz, 15-CH_β), 4.19 (1H, dd, *J* = 9, 7 Hz, 15-CH_a), 4.55 (1H, dd, *J* = 10, 2 Hz, 1'-CH), 5.30 (1H, ddd, *J* = 10, 8, 7 Hz, 16-CH), 5.42 (1H, d, *J* = 4.5 Hz, 6-CH), 6.27 (1H, d, *J* = 2 Hz, 18-CH). ^{13}C NMR: see Table 1.

Glaucoside-B (**5**). An amorphous powder, m.p. 115–120°, $[\alpha]_D$ −1.83° (*c* = 1.20, $CHCl_3$). (Found: C, 62.15; H, 8.11. Calc for $C_{42}H_{64}C_{15}$: C, 62.36; H, 7.98%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3450, 1730, 1710, 1655, 1450, 1380, 1310, 1160, 1090, 1050, 860. FD-MS *m/z*: 808 (M^+, base peak), 664 (M^+ − 144), 521, 376. ^1H NMR ($CDCl_3$) δ: 0.93 (3H, s, 19-CH_3), 1.01 (1H, t, *J* = 12 Hz, 1-CH_a), 1.23, 1.24, and 1.27 (each 3H, d, *J* = 6 Hz, 6'-, 6''-, and 6'''-CH_3), 1.53 (3H, s, 21-CH_3), 3.40, 3.46, and 3.50 (each 3H, s, 3'-, 3''-, and 3'''-OCH_3), 3.85 (1H, dd, *J* = 10, 9 Hz, 15-CH_β), 4.15 (1H, dd, *J* = 9, 7 Hz, 15-CH_a), 5.32 (1H, ddd, *J* = 10, 8, 7 Hz, 16-CH), 5.42 (1H, d, *J* = 4.5 Hz, 6-CH), 6.27 (1H, d, *J* = 2 Hz, 18-CH). ^{13}C NMR: see Table 1 and 2.

Glaucoside-C (**6**). An amorphous powder, m.p. 127–133°, $[\alpha]_D$ − 14.6° (*c* = 0.91, $CHCl_3$). (Found: C, 61.21; H, 7.88. Calc for $C_{41}H_{62}O_{14}\cdot 1/2H_2O$: C, 61.25; H, 7.90%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3450, 1730, 1710, 1655, 1310, 1160, 1050, 880. FD-MS *m/z*: 794 (M^+), 650 (M^+ − 144), 520 (650 − 130), 376 (520 − 144, base peak). ^1H NMR ($CDCl_3$) δ: 0.94 (3H, s, 19-CH_3), 1.01 (1H, t, *J* = 12 Hz, 1-CH_a), 1.22, 1.24, and 1.25 (each 3H, d, *J* = 6 Hz, 6'-, 6''-, and 6'''-CH_3), 1.53 (3H, s, 21-CH_3), 3.42, 3.46 (each 3H, s, 3'- and 3'''-OCH_3), 3.83 (1H, dd, *J* = 10, 9 Hz, 15-CH_β), 4.15 (1H, dd, *J* = 9, 7 Hz, 15-CH_a), 4.78 and 4.84 (each 1H, dd, *J* = 10, 2 Hz, 1'- and 1''-CH), 4.95 (1H, br. d, *J* = 3 Hz, 1'''-CH), 5.28 (1H, ddd, *J* = 10, 8, 7 Hz, 16-CH), 5.40 (1H, d, *J* = 4.5 Hz, 6-CH), 6.25 (1H, d, *J* = 2 Hz, 18-CH). ^{13}C NMR: see Table 1 and 2.

Glaucoside-D (**7**). An amorphous powder, m.p. 118–124°, $[\alpha]_D$ − 28.3° (*c* = 0.87, $CHCl_3$). (Found: C, 61.16; H, 7.71. Calc for $C_{41}H_{62}O_{15}\cdot 1/2H_2O$: C, 61.25; H, 7.90%), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1730, 1710, 1655, 1310, 1160, 1080, 1010. FD-MS *m/z*: 794 (M^+, base peak), 650 (M^+ − 144), 520 (650 − 130), 376 (520 − 144). ^1H NMR ($CDCl_3$) δ: 0.94 (3H, s, 19-CH_3), 1.06 (1H, t, *J* = 12 Hz, 1-CH_a), 1.25 and 1.32 (6H and 3H respectively, each d, *J* = 6 Hz, 6'-, 6''-, and 6'''-CH_3), 1.53 (3H, s, 21-CH_3), 3.42 (6H, s, 3'- and 3'''-OCH_3), 3.84 (1H, dd, *J* = 10, 9 Hz, 15-CH_β), 4.16 (1H, dd, *J* = 9, 7 Hz, 15-CH_a), 4.50, 5.01 (each 1H, dd, *J* = 10, 2 Hz, 1'-and 1''-CH), 4.92 (1H, br. d, *J* = 3 Hz, 1'''-CH), 5.31 (1H, ddd, *J* = 10, 8, 7 Hz, 16-CH), 5.42 (1H, d, *J* = 4.5 Hz, 6-CH), 6.27 (1H, d, *J* = 2 Hz, 18-CH). ^{13}C NMR: see Tables 1 and 2.

Acetylation of **7**. Compound **7** (41 mg) was acetylated with Ac_2O (1 ml) and C_5H_5N (2 ml) at room temp for 12 hr. An analysis on TLC with $CHCl_3$–acetone (5:1) showed the formation of approximately equal amounts of two products (*Rf*, 0.53 and 0.83). The usual work-up gave a white powder (53 mg), which was separated by silica gel column chromatography with $CHCl_3$–acetone (15:1) to yield **8** (18 mg) and **9** (22 mg) as an amorphous white powder in the order of elution.

Glaucoside-D triacetate (**8**). An amorphous powder, mp 104–109°, $[\alpha]_D$ − 32.7° (*c* = 1.10, $CHCl_3$). (Found: C, 60.85; H, 7.56.

Calc for $C_{47}H_{68}O_{18} \cdot 1/2H_2O$: C, 60.76; H, 7.38%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735, 1720, 1655, 1450, 1380, 1310, 1240, 1160. FD-MS *m/z*: 921 ($M^+ + H$), 920 (M^+), 734 ($M^+ - 186$), 562 (734 - 172, base peak). ^1H NMR ($CDCl_3$) δ: 1.00 (3H, s, 19-CH_3), 1.13 (1H, t, J = 12 Hz, 1-CH_a), 1.12 (3H, d, J = 6.4 Hz, 6‴-CH_3), 1.24, 1.27 (each 3H, d, J = 6 Hz, 6'- and 6''-CH_3), 1.53 (3H, s, 21-CH_3), 2.04, 2.10, and 2.14 (each 3H, s, -OC(=O)CH_3), 3.34, 3.40 (each 3H, s, 3'- and 3''-OCH_3), 3.62 (1H, ddd, J = 10, 10, 6 Hz, 3-CH), 3.71 (1H, m, 3‴-CH), 3.84 (1H, dd, J = 10, 9 Hz, 15-CH_β), 3.92 (1H, dq, J = 9, 6 Hz, 5'-or 5''-CH), 4.20 (1H, dd, J = 9, 7 Hz, 15-CH_α), 4.22 (1H, dq, J = 9, 6 Hz, 5''-CH), 4.48 (1H, dd, J = 9, 2 Hz, 1'-CH), 4.60 (1H, dd, J = 9.4, 3 Hz, 4''-CH), 4.84 (1H, br. d, J = 4 Hz, 1'''-CH), 4.92 (1H, dd, J = 9, 2 Hz, 1''-CH), 5.10 (1H, ddd, J = 12, 10, 5 Hz, 2-CH), 5.35 (2H, m, 3'- and 17-CH), 5.48 (1H, d, J = 4.5 Hz, 6-CH), 6.25 (1H, d, J = 2 Hz, 18-CH).

Glaucoside-D diacetate (**9**). An amorphous powder, mp 92 - 95°, $[\alpha]_D$ - 48.9° (c = 0.56, $CHCl_3$). (Found: C, 61.55; H, 7.67. Calc for $C_{43}H_{66}O_{17}$: C, 61.49; H, 7.57%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1735, 1720, 1655, 1450, 1310, 1240, 1160. FD-MS *m/z*: 879 ($M^+ + H$), 878 (M^+), 692 ($M^+ - 186$), 562 (692 - 130, base peak). ^1H NMR ($CDCl_3$) δ: 1.00 (3H, s, 19-CH_3), 1.16 (3H, d, J = 6.4 Hz, 6‴-CH_3), 1.25, 1.29 (each 3H, d, J = 6 Hz, 6'- and 6''-CH_3), 1.53 (3H, s, 21-CH_3), 2.04, 2.13 (each 3H, s, -OC(=O)CH_3), 3.39 (6H, s, 3'- and 3''-OCH_3), 3.63 (1H, ddd, J = 10, 10, 6 Hz, 3-CH), 3.80 (1H, m, 3‴-CH), 3.84 (1H, dd, J = 10, 9 Hz, 15-CH_β), 4.10 (1H, m, 3''-CH), 4.20 (1H, dd, J = 9, 7 Hz, 15-CH_α), 4.57 (1H, dd, J = 9, 2 Hz, 1'-CH), 4.87 (1H, dd, J = 9, 3 Hz, 4''-CH), 4.97 (1H, br. d, J = 3 Hz, 1'''-CH), 5.06 (1H, dd, J = 9, 2 Hz, 1''-CH), 5.34 (1H, ddd, J = 10, 8, 7 Hz, 16-CH), 5.49 (1H, d, J = 4.5 Hz, 6-CH), 6.25 (1H, d, J = 2 Hz, 18-CH).

Glaucoside-E (**10**) An amorphous powder, mp 100–106°, $[\alpha]_D$ - 21.4° (c = 1.02, $CHCl_3$). (Found: C, 61.51; H, 8.21. Calc for $C_{42}H_{64}O_{15} \cdot 1/2H_2O$: C, 61.59; H, 8.12%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1730, 1710, 1655, 1460, 1380, 1310, 1160, 1110, 1080, 1050, 880, 830 FD-MS *m/z*: 808 (M^+, base peak), 664 ($M^+ - 144$), 520 (664–144), 360 (520–160). ^1H NMR ($CDCl_3$) δ: 0.92 (3H, s, 19-CH_3), 1.24 and 1.27 (3H and 6H respectively, each d, J = 6 Hz, 6'-, 6''-, and 6‴-CH_3), 1.54 (3H, s, 21-CH_3), 3.39, 3.49, and 3.62 each 3H, s, 3'-, 3''-, and 3‴-OCH_3), 3.89 (1H, dd, J = 10, 9 Hz, 15-CH_β), 4.19 (1H, dd, J = 9, 7 Hz, 15-CH_α), 4.35 (1H, d, J = 8 Hz, 1'-CH), 4.83 (1H, br. d, J = 4 Hz, 1'''-CH), 4.94 (1H, dd, J = 10, 2 Hz, 1''-CH), 5.33 (1H, ddd, J = 10, 8, 7 Hz, 16-CH), 5.48 (1H, d, J = 4.5 Hz, 6-CH), 6.25 (1H, d, J = 2 Hz, 18-CH). ^{13}C NMR: see Tables 1 and 2.

Isolation and identification of L-*cymarose,* D-*digitoxose, and* D-*oleandrose.* A portion of the less polar part (40 g) of the crude glycosides was dissolved in 600 ml MeOH and warmed to 50°. Then 200 ml 0.2 N H_2SO_4, which was prewarmed to 50°, was poured into the soln and kept at 50°. After 30 min the soln was neutralized with sat $Ba(OH)_2$ aq and the ppts filtered off.

The filtrate was concentrated, and chromatographed on a column of silica gel (200 g of Wakogel C-300) with a solvent of increasing polarity from $CHCl_3$–acetone (6:1) to acetone to fractionate roughly into four fractions (fraction 1–4). Fraction 1 (1.64 g) contained mainly a mixture of methyl glycosides of cymarose, oleandrose, and digitoxose. To fraction 1 (16.4 g) was added 500 ml 0.05 N H_2SO_4, and the total mixture as kept around 80° for 1 hr, then the soln was neutralized with sat $Ba(OH)_2$ aq and the ppts filtered off. The filtrate was concentrated, and chromatographed on a column of silica gel (80 g of Wakogel C-200) with hexane–EtOAc (1:2) to give a fraction (4.87 g), which contained L-cymarose and D-oleandrose. Further elution with the same solvent afforded a fraction containing D-digitoxose (880 mg), to which EtOAc was added to form crystalline ppts. Recrystallization from the same solvent yielded D-digitoxose (550 mg) as colorless prisms, m.p. 110 - 112°, $[\alpha]_D$ + 48° (c = 1.8, H_2O)[10]. Found: C, 48.43; H, 8.31. Calc for $C_6H_{12}O_4$: C, 48.64; H, 8.16%). The structure was confirmed by comparison of the ^1H NMR data for its four methyl glycosides; methyl α- and β-D-digitoxopyranosides, and methyl α- and β-D-digitoxofuranosides with those for reported.[14]

The fraction, which consisted of L-cymarose and D-oleandrose, was in turn rechromatographed on a column of silica gel (80 g of Wakogel C-300) with 1.5% MeOH in $CHCl_3$ to give two fractions; fraction A (2.63 g) and fraction B (1.09 g), which contained L-cymarose and D-oleandrose, respectively. Fraction A was further purified with hexane–acetone (2:1), then with 2% MeOH in benzene to form a crystalline solid (1.36 g) after drying, which was dissolved in acetone. A small amount of hexane was added to the soln to yield L-cymarose (150 mg) as colorless fine needles, m.p. 92–95°, $[\alpha]_D$ - 50° (c = 1.0, H_2O).[8] (Found: C, 51.60; H, 8.87. Calc for $C_7H_{14}O_4$: C, 51.84; H, 8.70%). A 5 ml of 11% HCl-MeOH soln was added to a soln of 400 mg L-cymarose in 5 ml MeOH, and stirred for 4 hr. The soln was then neutralized by addition of excess of Ag_2CO_3, the ppts were filtered off, and the filtrate was concentrated. The mixture of methyl glycosides was chromatographed on a column of silica gel (20 g of Wakogel C-300) with hexane–EtOAc (4:1) to give a mixture of methyl β-L-cymaropyranoside and β-L-cymarofuranoside (177 mg), then methyl α-L-cymaropyranoside (15 mg) (13), followed by methyl α-L-cymarofuranoside (140 mg) as a syrup in the order of elution.

The structure and $^4C_1$ conformation of **13** were confirmed by its ^1H NMR data ($CDCl_3$) δ: 1.28 (3H, d, J = 6.3 Hz, 6-CH_3), 1.66 (1H, ddd, J = 15.1, 4.6, 3.4 Hz, 2-CHβ), 1.81 (1H, ddd, J = 15.1, 3, 1.2 Hz, 2-CH_α), 3.20 (1H, dd, J = 9.5, 4 Hz, 4-CH), 3.35, 3.43 (each 3H, s, 1- or 3-OCH_3), 3.60 (1H, m, 3-CH), 3.86 (1H, dq, J = 9.5, 6.3 Hz, 5-CH), 4.64 (1H, br d, J = 4 Hz, 1-CH). The ^1H NMR data for the other three compounds were identical with those for reported.[8a,15] Fraction B (1.09 g) was purified by silica gel column chromatography with hexane–acetone (2:1) to give D-oleandrose as a colorless syrup, $[\alpha]_D$ - 11° (c = 1.1, H_2O).[9] Found: C, 50.89; H, 8.79. Calc for $C_7H_{14}O_4$: C, 51.85; H, 8.70%). The structure was confirmed by comparison of the ^1H NMR data for its methyl α- and β-D-oleandropyranosides with those for reported.[8a]

Acidic hydrolysis of each glycoside (**4**, **5**, **6**, **7**, *and* **10**). To a soln of 3 mg of each glycoside in 1 ml MeOH was added 2 ml of 0.1 N H_2SO_4 and kept at 60° for 30 min, then the soln was diluted with water (2 ml) and concentrated to 1/2 volume. The soln was again kept at 60° for a further 30 min, nuetralized with sat $Ba(OH)_2$, aq and the ppts filtered off. The filtrate was concentrated to give a yellow syrup, which was analyzed by TLC with three solvent systems: solvent A, $CHCl_3$–MeOH (9:1); solvent B, CH_2Cl_2–EtOH (9:1); and solvent C, benzene–acetone (5:3). The *Rf* values of **1**, **3**, cymarose, oleandrose, and digitoxose in the order of 0.51, 0.55, 0.47, 0.43 and 0.21 with solvent A; 0.53, 0.56, 0.42, 0.33 and 0.21 with solvent B, and 0.43, 0.49, 0.34, 0.31 and 0.17 with solvent C, respectively. In the hydrolysate of **4**, **1**, and oleandrose were identified by TLC with authentic samples with these solvent systems. Similarly, cymarose and **1**; cymarose, digitoxose, and **1**; cymarose, digitoxose, oleandrose, and **1**; and cymarose and **3** were identified in the hyrolysates of **5**, **6**, **7** and **10**, respectively.

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