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Studies on the Constituents of Asclepiadaceae Plants. LXII.[1] The Structures of Two Glycosides, Cynafoside-A and -B, with a Novel Sugar Chain Containing a Pair of Optically Isomeric Sugars, D- and L-Cymaroses, from *Cynanchum africanum* R. BR.

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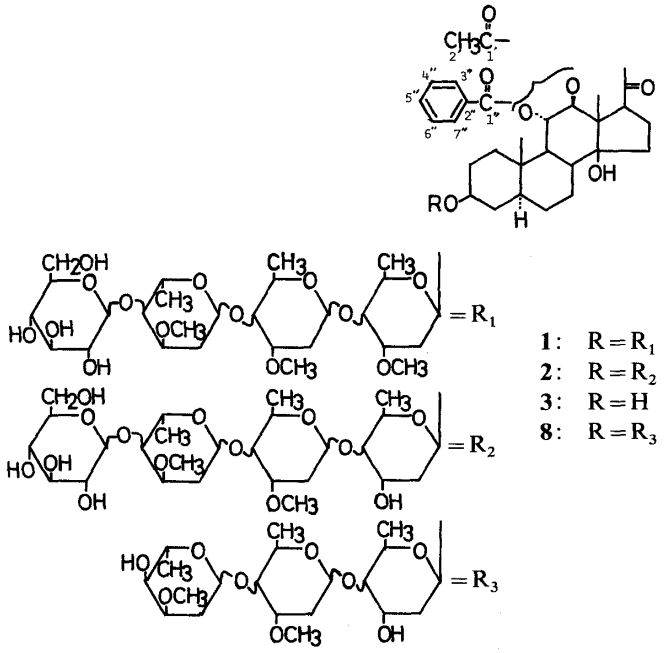
Two glycosides named cynafoside-A (**1**) and -B (**2**) were isolated from *Cynanchum africanum* R. BR. (Asclepiadaceae) which is toxic to stock in South Africa. Their structures were determined on the basis of spectral and chemical evidence, and are unusual in that they include both D- and L-cymaroses in the sugar chain.

Keywords——cynafoside-A; cynafoside-B; cynafogenin; optically isomeric sugars; D-cymarose; L-cymarose; *Cynanchum africanum*; Asclepiadaceae; cynanchosis

Cynanchum africanum R. BR. (Asclepiadaceae) is toxic to stock in South Africa owing to cynanchosis characterized by nervous and muscular intoxication.[2]

In this paper we wish to describe the structure determination of two new glycosides named cynafoside-A (**1**) and -B (**2**) (Chart 1), isolated from the dried leaf and stem of this plant. They showed positive Liebermann–Burchard and Keller–Kiliani[3] reactions, which



1: R = R_1
2: R = R_2
3: R = H
8: R = R_3

Chart 1

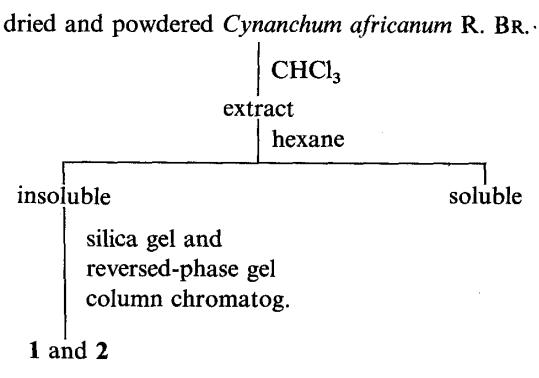


Chart 2. Extraction and Isolation of Glycosides

indicated the presence of steroidal glycosides with 2-deoxysugars. The extraction and separating procedures are shown in Chart 2.

On acidic hydrolysis of **1**, an aglycone (**3**) named cynafogenin was obtained. The molecular formula of **3** was established as $C_{30}H_{40}O_7$ by high-resolution electron impact mass spectrometry (HR-EI-MS). The presence of a mono-substituted phenyl group in **3** was suggested by the infrared (IR) absorptions at 1600, 1580, and 1490 cm^{-1}, the ultraviolet (UV) absorption at 230 nm, and five aromatic proton magnetic resonances at δ 7.49 (2H, t, J=7.9 Hz, 4″, 6″-CH), 7.62 (1H, tt, J=7.9, 1.2 Hz, 5″-CH), and 8.05 (2H, dd, J=7.9, 1.2 Hz, 3″, 7″-CH). A comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **3** (Table I) and condurangogenin A[4] indicated **3** to be a 3β,14β-dihydroxy-5α-pregnan-20-one derivative with two ester groups at C-11 and C-12 (Chart 1); this was supported by two proton resonances at δ 4.99 (1H, d, J=10.3 Hz, 12-CH) and 5.42 (1H, t, J=10.3 Hz, 11-CH) and IR absorptions at 1730, 1715, 1695 (C=O), and 1170 (C–O–C) cm^{-1}. Two ester groups were confirmed to be benzoate and acetate by the carbon signals at δ 166.6 (C-1″), 130.0 (C-2″, C-3″, and C-7″), 129.0 (C-4″ and C-6″), and 133.8 (C-5″) for the former and δ 170.2 (C-1′) and 21.2 (C-2′) for the latter, but the exact sites of linkage in **3** have not been determined yet.

TABLE I. ^{13}C-NMR Chemical Shifts of **3** and the Aglycone Moieties of **1**, **2**, and **8** (δ in ppm)

	3	**1**	**2**	**8**
C-1	39.7	38.0	37.9	38.0
C-2	32.8	30.3 (−2.5)	30.4 (−2.4)	30.4 (−2.4)
C-3	69.9	76.0 (+6.1)	76.0 (+6.1)	76.1 (+6.2)
C-4	39.7	35.4 (−4.3)	35.5 (−4.2)	35.4 (−4.3)
C-5	45.1	44.6	44.6	44.7
C-6	29.5[a)]	29.2[a)]	29.3[a)]	29.4[a)]
C-7	28.5[a)]	28.3[a)]	28.4[a)]	28.4[a)]
C-8	40.2	39.9	40.0	40.0
C-9	50.2	50.0	50.1	50.1
C-10	38.0	37.8	37.9	38.0
C-11	71.7	71.5	71.6	71.6
C-12	79.1	78.9	78.9	79.0
C-13	55.0	54.8	54.7	54.9
C-14	84.0	83.8	83.9	83.9
C-15	33.9	33.8	33.8	33.9
C-16	24.3	24.2	24.3	24.3
C-17	58.5	58.3	58.3	58.4
C-18	11.9	11.7	11.8	11.8
C-19	12.5	12.3	12.4	12.4
C-20	213.3	213.3	213.4	213.4
C-21	31.7	31.6	31.7	31.7
C-1′	170.4	170.2	170.3	170.3
C-2′	21.4	21.2	21.3	21.3
C-1″	166.8	166.6	166.7	166.7
C-2″	130.2	130.0	130.1	130.1
C-3″	130.2	130.0	130.1	130.1
C-4″	129.2	129.0	129.1	129.1
C-5″	133.9	133.8	133.9	133.9
C-6″	129.2	129.0	129.1	129.1
C-7″	130.2	130.0	130.1	130.1

Measured in C_5D_5N with TMS as an internal standard. *a—g)* in each column may be interchangeable (Tables I and II). ( ): glycosidation shifts.

D-Cymarose[5] (**4**); $[\alpha]_D^{16.5}$ +49.8° (c=0.57, H_2O), and glaucobiose[6] (4-*O*-β-D-glucopyranosyl-L-cymaropyranose) (**5**) were separated from the acidic hydrolysate of **1**. The ^{13}C-NMR spectra of methyl glycosides of **4** and **5** coincided with those of authentic samples[6,7] and the identity of **5** was confirmed by the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the methyl β-glycopyranoside of **5** (**5b**).

The 500 MHz ^{1}H-NMR spectrum of **1** showed three secondary methyl groups at δ 1.20, 1.21, and 1.25 (each 3H, d, J=6.4 Hz, 6-Me of cymarose) and three methoxyl groups at δ 3.38, 3.43, and 3.47 (each 3H, s, 3-OMe of cymarose). One α-linkage and two β-linkages of three cymaroses were revealed by the coupling constants of anomeric protons at δ 4.75 (1H, dd, J=10, 2 Hz), 4.81 (1H, dd, J=3, 1 Hz), and 4.83 (1H, dd J=10, 2 Hz). Partially relaxed Fourier-transform (PRFT) measurements[8] in the ^{13}C-NMR spectrum indicated that the terminal sugar of **1** was β-D-glucopyranose (Table II), and the next sugar was confirmed to be L-cymarose[9] (**6**) by the presence of **5** in the acidic hydrolysate of **1**. The carbon chemical shifts of the 4-*O*-β-D-glucopyranosyl-L-cymaropyranosyl moiety of **1** appeared to correspond to those of methyl α-glaucobioside[6] (**5a**) rather than those of methyl β-glaucobioside[6] (**5b**), and

TABLE II. ^{13}C-NMR Chemical Shifts of **4b**, **5a**, **5b**, **7b**, and the Sugar Moieties of **1**, **2**, and **8** (δ in ppm)

	1	**4b**	**5a**	**5b**	**2**	**7b**	**8**
	D-cym				digito		digito
C-1	95.9	99.4			95.9	99.6	96.0
C-2	37.1[b]	35.1			39.0	39.1	39.0
C-3	77.9[c]	78.5			68.5	68.3	68.5
C-4	83.2[d]	74.0			83.3	74.0	83.3
C-5	69.1[e]	71.0			67.4	70.3	67.5
C-6	18.5[f]	18.9			18.6[b]	18.8	18.6[b]
-OMe	58.7[g]	56.0				55.9	
	D-cym	57.8			D-cym		D-cym
C-1	100.2				99.6		99.7
C-2	36.9[b]				36.8		36.8
C-3	77.6[c]				77.6		77.7
C-4	82.1[d]				82.1		82.0
C-5	68.9[e]				69.2		69.3
C-6	18.4[f]				18.6[b]		18.6[b]
-OMe	58.4[g]				58.5		58.4
	L-cym				L-cym		L-cym
C-1	98.8		97.5	99.2	98.9		99.0
C-2	32.1		31.9	35.2	32.2		32.1
C-3	73.2		73.2	74.3	73.3		76.4
C-4	78.9		78.2	79.0	78.9		73.3
C-5	65.1		64.2	69.3	65.1		66.5
C-6	18.4[f]		18.3	18.9	18.5[b]		18.5[b]
-OMe	56.7		56.7	55.8	56.7		56.6
	glc		54.8	57.9	glc		
C-1	102.2*		101.7	101.8	102.2*		
C-2	75.1*		75.1	74.9	75.2*		
C-3	78.3*[,h]		78.4	78.4	78.3*[,c]		
C-4	71.7*		71.9	71.6	71.7*		
C-5	78.4*[,h]		78.4	78.2	78.5*[,c]		
C-6	62.9		62.8	62.7	62.9		

Measured in C_5D_5N with TMS as an internal standard. *a—h*) In each column may be interchangeable (Tables I and II). * The chemical shifts have the longest dipole–dipole relaxation times by PRFT measurements. D-cym, β-D-cymaropyranose; L-cym, α-L-cymaropyranose; digito, β-D-digitoxopyranose; glc, β-D-glucopyranose.

therefore the mode of linkage of **6** is α. The presence of a further two β-D-cymaropyranoses was suggested on the basis of the optical rotation of cymarose obtained by the acidic hydrolysis of **1**, and the ^{1}H- and ^{13}C-NMR spectra.

The glycosidation shifts[10] of the aglycone carbon signals observed at C-2 (−2.5 ppm), C-3 (+6.1 ppm), and C-4 (−4.3 ppm) (Table I), suggested that the sugar moiety was linked to the C-3 hydroxyl group of **3**. Consequently, the structure of **1** was established to be cynafogenin 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside (Chart 1).

The aglycone of **2** was indicated to be **3** by comparisons of the ^{1}H- and ^{13}C-NMR (Table I) spectra of **2** with those of **1**.The acidic hydrolysis of **2** gave **3**, **4** $[\alpha]_D^{17}$ +54.1° (c=0.58, H_2O), D-digitoxose[11] (**7**) $[\alpha]_D^{17}$ +41.2° (c=0.34, H_2O), and **5**. Both **4** and **5** were identified from the ^{13}C-NMR spectra[6,7] of their methyl glycosides and **7** from the ^{1}H-NMR spectrum of its methyl β-glycopyranoside (**7b**).

The terminal sugar of **2** was indicated to be β-D-glucopyranose by PRFT measurements[8] in the ^{13}C-NMR spectrum (Table II). The enzymatic hydrolysis of **2** with β-glucosidase afforded desglucosyl-**2** (**8**). The structure of the sugar moiety of **8** was deduced to be as shown in Chart 1 from its ^{13}C-NMR spectra with PRFT measurements at seven pulse intervals (200, 180, 170, 160, 150, 140, and 120 ms) between 180° and 90° pulses (Table II). Methine signals at C-1, C-4, and C-5 of the terminal α-L-cymaropyranose were recovered at 200 ms, those at C-1, C-4, and C-5 of β-D-cymaropyranose at 170, 170, and 150 ms, respectively, and those at C-1, C-4, and C-5 of β-D-digitoxopyranose at 150, 140, and 150 ms, respectively.

In the 500 MHz ^{1}H-NMR spectrum of **8** all the protons of sugars were assigned by means of proton decoupling experiments, in which 1-CH, 3-CH, and 5-CH of each of the three sugars were irradiated. Owing to the acetylation of **8**, 4-CH of the terminal α-L-cymaropyranose was shifted from δ 3.27 to δ 4.43 and 3-CH of β-D-digitoxopyranose from δ 4.21 to δ 5.35; therefore β-D-cymaropyranose is linked at the C-4 hydroxyl group of β-D-digitoxopyranose. Thus, the structure of **2** was deduced to be cynafogenin 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-cymaropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-digitoxopyranoside (Chart 1).

Cynafosides are the second examples of compounds in which a pair of optically isomeric sugars, D- and L-cymaroses, exists in the sugar sequence of a glycoside. The first examples were wilfosides,[12] obtained from *Cynanchum wilfordi* HEMSLEY. The sugar chains of both cynafosides and wilfosides are similar, that is, those of **1** and **2** correspond to the loss of α-L-diginopyranose[13] from those of wilfoside C1G and wilfoside C2G, respectively.

The toxicity of these compounds to animals was not tested because of the small amounts of the glycosides obtained.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ with a JASCO DIP-4 digital polarimeter at room temperature. UV spectra were obtained in ethanol with a Shimadzu UV-220 spectrometer, and absorption maxima are given in nm. IR spectra were recorded in $CHCl_3$ on a JASCO A-102 spectrometer. ^{1}H-NMR spectra were run on JEOL GX-500 (500 MHz) and FX-200 (200 MHz) spectrometers in $CDCl_3$ or C_5D_5N, and ^{13}C-NMR spectra on JEOL FX-200 (50 MHz) and FX-90Q (22.5 MHz) machines in C_5D_5N with tetramethylsilane as an internal standard. Electron impact mass spectrometry (EI-MS) was carried out with a JEOL LMS-D-300 mass spectrometer. Thin layer chromatography (TLC) was performed on Merck precoated plates (Kiesel gel 60 F_{254}) with the following solvent systems: Rf_1 MeOH–$CHCl_3$ (5:95 (v/v)), Rf_2 MeOH–$CHCl_3$ (15:85), Rf_3 H_2O–MeOH–$CHCl_3$ (1:3:12, lower layer), Rf_4 acetone–hexane (1:1), and Rf_5 hexane–ethyl acetate (1:1). Column chromatography was carried out on Wakogel C-200 (200 mesh), Wakogel C-100 (100 mesh), or Lobar column Lichroprep RP-8 (reversed phase).

Extraction and Isolation of Glycosides——The dried leaf and stem of *Cynanchum africanum* R. BR. (5.5 kg), obtained from South Africa, were pulverized and extracted with $CHCl_3$ at room temperature. A dark green tar

(290.03 g) obtained by concentration of the extract was dissolved in $CHCl_3$ (300 ml) and hexane (1800 ml) was poured into the solution. This mixture was decanted and hexane (1800 ml) was added again. The insoluble portion corresponded to a crude glycoside (79.89 g), which showed positive Liebermann–Burchard and Keller–Kiliani reactions. The crude glycoside was subjected to column chromatography on silica gel using solvents of increasing polarity from $CHCl_3$ to MeOH–$CHCl_3$ (1 : 1 (v/v)), to separate fraction A (19.72 g), fraction B (12.17 g), and fraction C (20.04 g). Fraction A included pigments for the most part, with very little glycoside. Fraction B was rechromatographed on silica gel with MeOH–$CHCl_3$ (7 : 93) to separate fraction B1 (2.47 g : a crude fraction containing **1**), fraction B2 (3.08 g : a crude fraction containing **2**), and fraction B3 (1.58 g). Rechromatography of fraction B1 on silica gel with hexane-acetone (2 : 5) and MeOH–$CHCl_3$ (5 : 95) and further on reversed-phase gel with H_2O–MeOH (15: 85 and 20 : 80) afforded **1** (109.3 mg, yield: 0.0020%). Rechromatography of fraction B2 on silica gel with hexane–acetone (1 : 3 and 1 : 2) and MeOH–$CHCl_3$ (6 : 94 and 7 : 93), and further on reversed-phase gel with H_2O–MeOH (15 : 85) gave **2** (207.6 mg : 0.0038%). *Rf* values : **1** (Rf_2 0.56) and **2** (Rf_2 0.51).

Cynafoside-A (1)——An amorphous powder, mp 142—144 °C, $[\alpha]_D^{17}$ +14.8 ° (c = 1.00, $CHCl_3$). *Anal.* Calcd for $C_{57}H_{86}O_{21} \cdot 4/3H_2O$: C, 60.59; H, 7.79. Found: C, 60.59; H, 7.92. UV $\lambda_{max}^{ethanol}$ nm (log ε): 230 (3.63), 275 (2.46), 283 (2.39). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1730, 1710, 1695, 1600, 1580, 1490, 1160. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.95 (3H, s, 19-Me), 1.15 (3H, s, 18-Me), 1.20, 1.21, 1.25 (each 3H, d, J = 6.4 Hz, 6-Me of cymarose), 1.65 (3H, s, 2′-Me), 2.07 (3H, s, 21-Me), 3.20, 3.23 (each 1H, dd, J = 9.5, 2.8 Hz, 4-CH of cymarose), 3.38, 3.43, 3.47 (each 3H, s, 3-OMe of cymarose), 3.69, 3.72 (each 1H, ddd, J = 3, 3, 3 Hz, 3-CH of cymarose), 3.83 (1H, dq, J = 9.5, 6.4 Hz, 5-CH of cymarose), 4.38 (1H, d, J = 7.8 Hz, 1-CH of β-D-glucopyranose), 4.75 (1H, dd, J = 10, 2 Hz, 1-CH of β-D-cymaropyranose), 4.81 (1H, dd, J = 3, 1 Hz, 1-CH of α-L-cymaropyranose), 4.83 (1H, dd, J = 10, 2 Hz, 1-CH of β-D-cymaropyranose), 4.99 (1H, d, J = 9.8 Hz, 12-CH), 5.41 (1H, t, J = 9.8 Hz, 11-CH), 7.49 (2H, t, J = 7.8 Hz, 4″, 6″-CH), 7.62 (1H, tt, J = 7.8, 1.0 Hz, 5″-CH), 8.05 (2H, dd, J = 7.8, 1.0 Hz, 3″, 7″-CH). ^{13}C-NMR (22.5 MHz, C_5D_5N): see Tables I and II.

Acidic Hydrolysis of 1——A solution of **1** (65.1 mg) in MeOH (15 ml) was allowed to react with 0.2 N H_2SO_4 (5 ml) at 60 °C for 15 min, then H_2O (15 ml) was added and the mixture was concentrated to 20 ml. The solution was kept at 60 °C for a further 30 min, and extracted with ether (20 ml). The ether layer was washed with satd. $NaHCO_3$ (5 ml × 3) and satd. NaCl (5 ml × 3), and the solvent was evaporated off to give a syrup, which was chromatographed on silica gel with acetone–hexane (1 : 3) to afford **3** (19.7 mg) as an amorphous powder. The aqueous layer was neutralized with satd. $Ba(OH)_2$. The precipitates were filtered off and the filtrate was evaporated to give a mixture of **4** and **5**, which were identified by TLC comparison with authentic samples. *Rf* values: **4** (Rf_3 0.59, Rf_4 0.42) and **5** (Rf_3 0.16). The sugar mixture was chromatographed on silica gel with H_2O–MeOH–$CHCl_3$ (1 : 3 : 12 (v/v), lower layer) to separate **4** (7.5 mg) and **5** (11.8 mg). **4**: a syrup, $[\alpha]_D^{16.5}$ +49.8 ° (c = 0.57, H_2O). **5**: a syrup, $[\alpha]_D^{17}$ −67.1 ° (c = 0.96, H_2O).

Cynafogenin (3)——An amorphous powder. Rf_1 0.53, Rf_4 0.58. mp 91—95 °C, $[\alpha]_D^{16}$ +76.5 ° (c = 0.92, $CHCl_3$). UV $\lambda_{max}^{ethanol}$ nm (log ε): 231 (4.23), 274 (3.12), 282 (3.05). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1730, 1715, 1695, 1600, 1580, 1490, 1170. EI-MS *m/z*: 512 (M^+), 494 ($M^+ - H_2O$), 105 ($C_6H_5CO^+$, base peak), 43 (CH_3CO^+). HR-EI-MS Calcd for $C_{30}H_{40}O_7$: 512.27738, Obsd: 512.27732. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.97 (3H, s, 18-Me), 1.16 (3H, s, 19-Me), 1.65 (3H, s, 2′-Me), 2.07 (3H, s, 21-Me), 3.56 (1H, tt, J = 11.0, 4.9 Hz, 3-CH), 4.99 (1H, d, J = 10.3 Hz, 12-CH), 5.42 (1H, t, J = 10.3 Hz, 11-CH), 7.49 (2H, t, J = 7.9 Hz, 4″, 6″-CH), 7.62 (1H, tt, J = 7.9, 1.2 Hz, 5″-CH), 8.05 (2H, dd, J = 7.9, 1.2 Hz, 3″, 7″-CH). ^{13}C-NMR (22.5 MHz, C_5D_5N): see Table I.

Methyl Glycosylations of 4 and 5——A solution of **4** (7.5 mg) in MeOH (2 ml) was allowed to react with 1% H_2SO_4–MeOH (2 ml) at room temperature for 20 min, then H_2O (2 ml) was added and the reaction mixture was neutralized with satd. $Ba(OH)_2$. The precipitates were filtered off and the filtrate was evaporated to give a mixture of methyl glycosides of **4**. Methyl α-cymarofuranoside (**4a**), methyl β-cymarofuranoside (**4b**), and methyl β-cymaropyranoside (**4c**) were identified by TLC comparison with authentic samples. *Rf* values: **4a** (Rf_5 0.19), **4b** (Rf_5 0.32), and **4c** (Rf_5 0.32). A mixture of **4a**, **4b**, and **4c**: ^{1}H-NMR (200 MHz, C_5D_5N) for **4a** δ: 1.43 (3H, d, J = 6.4 Hz, 6-Me), 3.30, 3.38 (each 3H, s, 1, 3-OMe), for **4b** δ: 1.51 (3H, d, J = 5.9 Hz, 6-Me), 3.32, 3.35 (each 3H, s, 1, 3-OMe), for **4c** δ: 1.52 (3H, d, J = 6 Hz, 6-Me), 3.42, 3.50 (each 3H, s, 1, 3-OMe). ^{13}C-NMR (22.5 MHz, C_5D_5N): see Table III. The ratio of **4a** : **4b** : **4c** was considered to be *ca.* 6 : 3 : 1 based on integration of the anomeric signals. A solution of **5** (11.8 gm) in MeOH (2 ml) was allowed to react in the same way as **4**, and a mixture of **5a** and **5b** was obtained as a syrup (7.4 mg). **5a**: Rf_3 0.30, **5b**: Rf_3 0.33. A mixture of **5a** and **5b**: ^{1}H-NMR (200 MHz, C_5D_5N) for **5a** δ: 1.41 (3H, d, J = 6.4 Hz, 6-Me), 3.31, 3.47 (each 3H, s, 1, 3-OMe), 5.01 (1H, d, J = 7.3 Hz, 1′-CH), for **5b** δ: 1.48 (3H, d, J = 6.4 Hz, 6-Me), 3.46, 3.56 (each 3H, s, 1, 3-OMe), 4.90 (1H, dd, J = 9, 2 Hz, 1-CH), 5.00 (1H, d, J = 7.8 Hz, 1′-CH). The ratio of **5a** : **5b** was considered to be *ca.* 1 : 2 based on integration of the methoxyl methyl signals. ^{13}C-NMR (22.5 MHz, C_5D_5N): see Table III. Chromatography of a mixture of **5a** and **5b** on silica gel with H_2O–MeOH–$CHCl_3$ (1 : 3 : 9 (v/v), lower layer) gave **5b** (3.6 mg), $[\alpha]_D^{16}$ −30.0 ° (c = 0.36, MeOH). ^{1}H-NMR (500 MHz, C_5D_5N) δ: 1.47 (3H, d, J = 6.3 Hz, 6-Me), 1.66 (1H, ddd, J = 13.4, 9.0, 2.4 Hz, 2-CH_{ax}), 2.28 (1H, ddd, J = 13.4, 4.4, 2.0 Hz, 2-CH_{eq}), 3.45, 3.55 (each 3H, s, 1, 3-OMe), 3.91 (1H, dd, J = 9.0, 2.9 Hz, 4-CH), 3.95 (1H, ddd, J = 8.8, 5.4, 2.5 Hz, 5′-CH), 3.98 (1H, dd, J = 8.3, 7.8 Hz, 2′-CH, 4.00 (1H, ddd, J = 4.4, 2.9, 2.4 Hz, 3-CH), 4.19 (1H, t, J = 8.8 Hz, 4′-CH), 4.21 (1H, t, J = 8.8 Hz, 3′-CH), 4.24 (1H, dq, J = 9.0, 6.4 Hz, 5-CH), 4.35 (1H, dd, J = 11.7, 5.4 Hz, 6′-CH), 4.52 (1H, dd, J = 11.7, 2.5 Hz, 6′-

TABLE III. ^{13}C-NMR Chemical Shifts of **4a**, **4b**, **4c**, **5a**, and **5b** Derived from **1** (δ in ppm)

	4a	**4b**	**4c**	**5a**	**5b**
C-1	105.7	106.3	99.5	97.7	99.4
C-2	38.8	39.7	35.2	32.0	35.2
C-3	88.8	89.7	78.6	73.4	74.5
C-4	81.1	82.3	74.1	78.7	79.2
C-5	67.5	68.3	71.1	64.4	69.5
C-6	20.1	21.0	19.0	18.3	18.9
-OMe	54.6	55.1	56.0	54.6	55.9
	56.8	56.6	57.8	56.8	58.0
C-1′				102.1	102.1
C-2′				75.2	75.2
C-3′				78.5	78.5
C-4′				71.9	71.9
C-5′				78.5	78.5
C-6′				62.9	62.9

Measured in C_5D_5N with TMS as an internal standard.

CH), 4.88 (1H, dd, J=9.0, 2.0 Hz, 1-CH), 4.97 (1H, d, J=7.8 Hz, 1′-CH).

Cynafoside-B (2)——An amorphous powder, mp 152—154 °C, $[\alpha]_D^{17}$ +8.8° (c=1.00, $CHCl_3$). *Anal.* Calcd for $C_{56}H_{84}O_{21}\cdot H_2O$: C, 60.52; H, 7.80, Found: C, 60.65; H, 8.02. UV $\lambda_{max}^{ethanol}$ nm (log ε): 230 (3.63), 275 (2.46), 283 (2.39). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430, 1730, 1710, 1695, 1600, 1580, 1490, 1160. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.94 (3H, s, 19-Me), 1.15 (3H, s, 18-Me), 1.20, 1.23, 1.26 (each 3H, d, J=6.4 Hz, 6-Me of cymarose), 1.64 (3H, s, 2′-Me), 2.07 (3H, s, 21-Me), 3.38, 3.50 (each 3H, s, 3-OMe of cymarose), 4.37 (1H, d, J=7.8 Hz, 1-CH of β-D-glucopyranose), 4.80 (1H, dd, J=3, 1 Hz, 1-CH of α-L-cymaropyranose), 4.81 (1H, dd, J=10, 2 Hz, 1-CH of β-D-cymaropyranose), 4.91 (1H, dd, J=9.8, 2.0 Hz, 1-CH of β-D-digitoxopyranose), 4.99 (1H, d, J=9.8 Hz, 12-CH), 5.41 (1H, t, J=9.8 Hz, 11-CH), 7.49 (2H, t, J=7.8 Hz, 4″, 6″-CH), 7.62 (1H, tt, J=7.8, 1.5 Hz, 5″-CH), 8.50 (2H, dd, J=7.8, 1.5 Hz, 3″, 7″-CH). ^{13}C-NMR (22.5 MHz, C_5D_5N): see Tables I and II.

Acidic Hydrolysis of 2——A solution of **2** (84.0 mg) was allowed to react in the same way as **1**, and products were separated to provide **3** (15.1 mg), **4** (6.8 mg), **5** (5.0 mg), and **7** (3.7 mg). *Rf* values: **3** (Rf_1 0.53, Rf_4 0.58), **4** (Rf_3 0.59, Rf_4 0.42), **5** (Rf_3 0.16), and **7** (Rf_3 0.42, Rf_4 0.04). **3**: $[\alpha]_D^{17}$ +80.1° (c=0.94, $CHCl_3$). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.97 (3H, s, 19-Me), 1.16 (3H, s, 18-Me), 1.65 (3H, s, 2′-Me), 2.07 (3H, s, 21-Me), 3.57 (1H, m, 3-CH), 5.00 (1H, d, J=10.3 Hz, 12-CH), 5.43 (1H, t, J=10.3 Hz, 11-CH), 7.49 (2H, t, J=7.5 Hz, 4″, 6″-CH), 7.63 (1H, tt, J=7.5, 1.5 Hz, 5″-CH), 8.05 (2H, dd, J=7.5, 1.5 Hz, 3″, 7″-CH). **4**: $[\alpha]_D^{17}$ +54.1° (c=0.58, H_2O). **5**: $[\alpha]_D^{17}$ −71.9° (c=0.47, H_2O). **7**: $[\alpha]_D^{17}$ +41.2° (c=0.34, H_2O). Methyl glycosylations of **4**, **5**, and **7** were carried out by the procedure described above and gave mixtures of the methyl glycosides. A mixture of **4a**, **4b**, and **4c** (6.0 mg): **4a** (Rf_5 0.19), **4b** (Rf_5 0.32), and **4c** (Rf_5 0.32). ^{13}C-NMR (22.5 MHz, C_5D_5N): see Table IV. A mixture of **5a** and **5b** (5.0 mg): **5a** (Rf_3 0.30) and **5b** (Rf_3 0.33). ^{13}C-NMR (22.5 MHz, C_5D_5N): see Table IV. Among the methyl glycosides of **7** (3.5 mg), **7b** is a main product. **7b**: Rf_3 0.54. ^{1}H-NMR (90 MHz, C_5D_5N) δ: 1.58 (3H, d, J=5.9 Hz, 6-Me), 1.97 (1H, ddd, J=13.2, 9.8, 2.9 Hz, 2-CH_{ax}), 2.40 (1H, ddd, J=13.2, 3.4, 2.0 Hz, 2-CH_{eq}), 3.53 (3H, s, 1-OMe), 3.60 (1H, dd, J=9.3, 2.9 Hz, 4-CH), 4.26 (1H, dq, J=9.3, 5.9 Hz, 5-CH), 4.42 (1H, ddd, J=3.4, 2.9, 2.9 Hz, 3-CH), 5.12 (1H, dd, J=9.8, 2.0 Hz, 1-CH).

Enzymatic Hydrolysis of 2 with β-Glucosidase——A suspension (7 ml) of **2** (82.3 mg) in 0.3 M NaOAc buffer solution adjusted to pH 5.5 was treated with a suspension (10 ml) of snail (*Fruticola gainesil*) β-glucosidase (191.8 mg) at 37 °C for 89 h. The products were extracted with $CHCl_3$ (50 ml) and the solvent was evaporated off to give a syrup, which was chromatographed to afford desglucosyl-**2** (**8**, 12.0 mg).

Desglucosyl-2 (8)——An amorphous powder. Rf_1 0.59, Rf_4 0.55. mp 103—105 °C, $[\alpha]_D^{16}$ +17.2° (c=1.21, $CHCl_3$). *Anal.* Calcd for $C_{50}H_{74}O_{16}\cdot 3/4H_2O$: C, 63.57; H, 8.06, Found: C, 63.58; H, 8.36. UV $\lambda_{max}^{ethanol}$ nm (log ε): 231 (4.19), 274 (2.88), 282 (2.79). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1730, 1715, 1695, 1600, 1580, 1160. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.95 (3H, s, 19-Me), 1.15 (3H, s, 18-Me), 1.20, 1.23, 1.27 (each 3H, d, J=6.4 Hz, 6-Me of β-D-cymaropyranose,6-Me of β-D-digitoxopyranose, and 6-Me of α-L-cymaropyranose, respectively), 1.62 (1H, ddd, J=12, 9.5, 3 Hz, 2-CH_{ax} of β-D-cymaropyranose), 1.64 (3H, s, 2′-Me), 1.68 (1H, ddd, J=12, 9.5, 3 Hz, 2-CH_{ax} of β-D-digitoxopyranose), 1.74 (1H, ddd, J=12, 3.4, 3 Hz, 2-CH_{ax} of α-L-cymaropyranose), 2.05 (1H, ddd, J=12, 3, 1.8 Hz, 2-CH_{eq} of β-D-digitoxopyranose), 2.06 (3H, s, 21-Me), 2.11 (1H, ddd, J=12, 3, 1.8 Hz, 2-CH_{eq} of β-D-cymaropyranose), 2.28 (1H, ddd, J=12, 3, 1.5 Hz, 2-CH_{eq} of α-L-cymaropyranose), 3.19 (1H, dd, J=9.5, 2.8 Hz, 4-CH of β-D-digitoxopyranose), 3.22 (1H, dd, J=9.5, 2.8 Hz, 4-CH of β-D-cymaropyranose), 3.27 (1H, dd, J=9.5, 2.8 Hz, 4-CH of α-L-

TABLE IV. ^{13}C-NMR Chemical Shifts of **4a**, **4b**, **4c**, **5a** and **5b** Derived from **2** (δ in ppm)

	4a	**4b**	**4c**	**5a**	**5b**
C-1	105.7	106.3	99.5	97.7	99.4
C-2	38.7	39.7	35.1	32.0	35.3
C-3	88.8	89.7	78.6	73.4	74.6
C-4	81.0	82.3	74.0	78.7	79.3
C-5	67.5	68.3	71.1	64.4	69.5
C-6	20.1	21.0	19.0	18.3	18.9
-OMe	54.6	55.0	56.0	54.9	55.9
	56.8	56.6	57.7	56.8	58.0
C-1′				102.1	102.1
C-2′				75.3	75.3
C-3′				78.5	78.5
C-4′				71.9	71.9
C-5′				78.5	78.5
C-6′				62.9	62.9

Measured in C_5D_5N with TMS as an internal standard.

cymaropyranose), 3.38, 3.50 (each 3H, s, 3-OMe of cymarose), 3.59 (1H, ddd, J=3, 3, 2.8 Hz, 3-CH of α-L-cymaropyranose), 3.69 (1H, ddd, J=3, 3, 2.8 Hz, 3-CH of β-D-cymaropyranose), 3.77 (1H, dq, J=9.5, 6.4 Hz, 5-CH of β-D-digitoxopyranose), 3.92 (1H, dq, J=9.5, 6.4 Hz, 5-CH of β-D-cymaropyranose), 4.04 (1H, dq, J=9.5, 6.4 Hz, 5-CH of α-L-cymaropyranose), 4.21 (1H, ddd, J=3, 3, 2.8 Hz, 3-CH of β-D-digitoxopyranose), 4.79 (1H, dd, J=3.4, 1 Hz, 1-CH of α-L-cymaropyranose), 4.80 (1H, dd, J=9.5, 1.8 Hz, 1-CH of β-D-cymaropyranose), 4.91 (1H, dd, J=9.5, 1.8 Hz, 1-CH of β-D-digitoxopyranose), 4.99 (1H, d, J=10.4 Hz, 12-CH), 5.41 (1H, t, J=10.4 Hz, 11-CH), 7.49 (2H, t, J=7.6 Hz, 4″, 6″-CH), 7.61 (1H, tt, J=7.6, 1.5 Hz, 5″-CH), 8.05 (2H, dd, J=7.6, 1.5 Hz, 3″, 7″-CH). ^{13}C-NMR (22.5 MHz, C_5D_5N): see Tables I and II.

Acetylation of 8——**8** (3.0 mg) was dissolved in pyridine (1 ml), and acetic anhydride (0.8 ml) was added. The reaction mixture was kept at room temperature overnight, then H_2O (20 ml) was added, and the whole was extracted with $CHCl_3$ (10 ml). The $CHCl_3$ layer was washed with 2 N HCl (10 ml × 3), satd. $NaHCO_3$ (10 ml × 3), and satd. NaCl (10 ml × 3), and dried over anhydrous sodium sulfate. Then, the solvent was evaporated off to give acetyl-**8** (**9**, 1.5 mg).

Acetyl-8 (9)——^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.95 (3H, s, 19-Me), 1.15 (3H, s, 18-Me), 1.20, 1.20, 1.23 (each 3H, d, J=6.4 Hz, 6-Me of sugars), 1.65 (3H, s, 2′-CH), 2.07 (3H, s, 21-Me), 2.11, 2.12 (each 3H, s, -OAc), 3.19 (1H, dd, J=9.5, 2.8 Hz, 4-CH of β-D-digitoxopyranose), 3.23 (1H, dd, J=9.5, 2.8 Hz, 4-CH of β-D-cymaropyranose), 3.35, 3.52 (each 3H, s, 3-OMe of cymarose), 3.65 (1H, ddd, J=3, 3, 2.8 Hz, 3-CH of α-L-cymaropyranose), 3.69 (1H, ddd, J=3, 3, 2.8 Hz, 3-CH of β-D-cymaropyranose), 3.78 (1H, dq, J=9.5, 6.4 Hz, 5-CH of β-D-digitoxopyranose), 3.92 (1H, dq, J=9.5, 6.4 Hz, 5-CH of β-D-cymaropyranose), 4.36 (1H, dq, J=9.5, 6.4 Hz, 5-CH of α-L-cymaropyranose), 4.43 (1H, dd, J=9.5, 2.8 Hz, 4-CH of α-L-cymaropyranose), 4.80 (1H, dd, J=9.5, 1.8 Hz, 1-CH of β-D-cymaropyranose), 4.80 (1H, dd, J=3.4, 1 Hz, 1-CH of α-L-cymaropyranose), 4.92 (1H, dd, J=9.5, 1.8 Hz, 1-CH of β-D-digitoxopyranose), 4.99 (1H, d, J=10.4 Hz, 12-CH), 5.35 (1H, ddd, J=3, 3, 2.8 Hz, 3-CH of β-D-digitoxopyranose), 5.41 (1H, t, J=10.4 Hz, 11-CH), 7.49 (2H, t, J=7.6 Hz, 4″, 6″-CH), 7.62 (1H, tt, J=7.6, 1.5 Hz, 5″-CH), 8.05 (2H, dd, J=7.6, 1.5 Hz, 3″, 7″-CH).

References and Notes

1) Part LXI: Z. Zhang, Z. Jun, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.*, **33**, 4188 (1985).
2) J. M. Watt and M. G. Breyer-Brandwijk, "The Medicinal and Poisonous Plants of Southern and Eastern Africa," E. and S. Livingstone Ltd., Edinburgh, London, 1962, p. 129.
3) J. von Euw and T. Reichstein, *Helv. Chim. Acta*, **31**, 883 (1948).
4) K. Hayashi, K. Wada, H. Mitsuhashi, H. Bando, M. Takase, S. Terada, Y. Koide, T. Aiba, T. Narita, and D. Mizuno, *Chem. Pharm. Bull.*, **28**, 1954 (1980). Chemical shifts of C-3 and C-11 were corrected as follows: C-3 (δ 69.9) and C-11 (δ 71.8).
5) D. A. Prins, *Helv. Chim. Acta*, **29**, 378 (1946); O. Renkonen, O. Shindler, and T. Reichstein, *ibid.*, **46**, 1691 (1963).
6) T. Nakagawa, K. Hayashi, K. Wada, and H. Mitsuhashi, *Tetrahedron Lett.*, **1982**, 5431.

7) The ^{13}C-NMR spectra of methyl α- and β-cymarofuranosides have not been published, and were as follows. ^{13}C-NMR of methyl α-cymarofuranoside (25 MHz, C_5D_5N) δ: 20.0 (C-6), 38.7 (C-2), 54.6, 56.7 (1, 3-OMe), 67.4 (C-5), 81.0 (C-4), 88.7 (C-3), 105.6 (C-1). ^{13}C-NMR of methyl β-cymarofuranoside (25 MHz, C_5D_5N) δ: 21.0 (C-6), 39.6 (C-2), 55.1, 56.5 (1, 3-OMe), 68.2 (C-5), 82.2 (C-4), 89.6 (C-3), and 106.2 (C-1).
8) A. Allerhand and D. Doddrell, *J. Am. Chem. Soc.*, **93**, 2777 (1971); A. Neszely, K. Tori, and G. Lukacs, *J. Chem. Soc., Chem. Commun.*, **1977**, 613.
9) A. F. Krasso, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **46**, 1691 (1963); J. S. Brimacombe, R. Hanna, M. S. Saeed, and L. C. N. Tucker, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2583.
10) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175; S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, *J. Am. Chem. Soc.*, **100**, 3331 (1978).
11) H. Kiliani, *Arch. Pharm.*, **234**, 481 (1896); B. Iselin and T. Reichstein, *Helv. Chim. Acta*, **27**, 1203 (1944); O. Shindler and T. Reichstein, *ibid.*, **35**, 730 (1952).
12) S. Tsukamoto, K. Hayashi, and H. Mitsuhashi, *Tetrahedron*, **41**, 927 (1985); *idem, Chem. Pharm. Bull.*, **33**, 2294 (1985).
13) C. W. Shoppee and T. Reichstein, *Helv. Chim. Acta*, **23**, 975 (1940).