

STRUCTURES OF ELAEODENDROSIDES M, N, O, P, Q, R AND S.

A SERIES OF CARDIAC GLYCOSIDES ISOLATED FROM ELAEODENDRON

GLAUCUM

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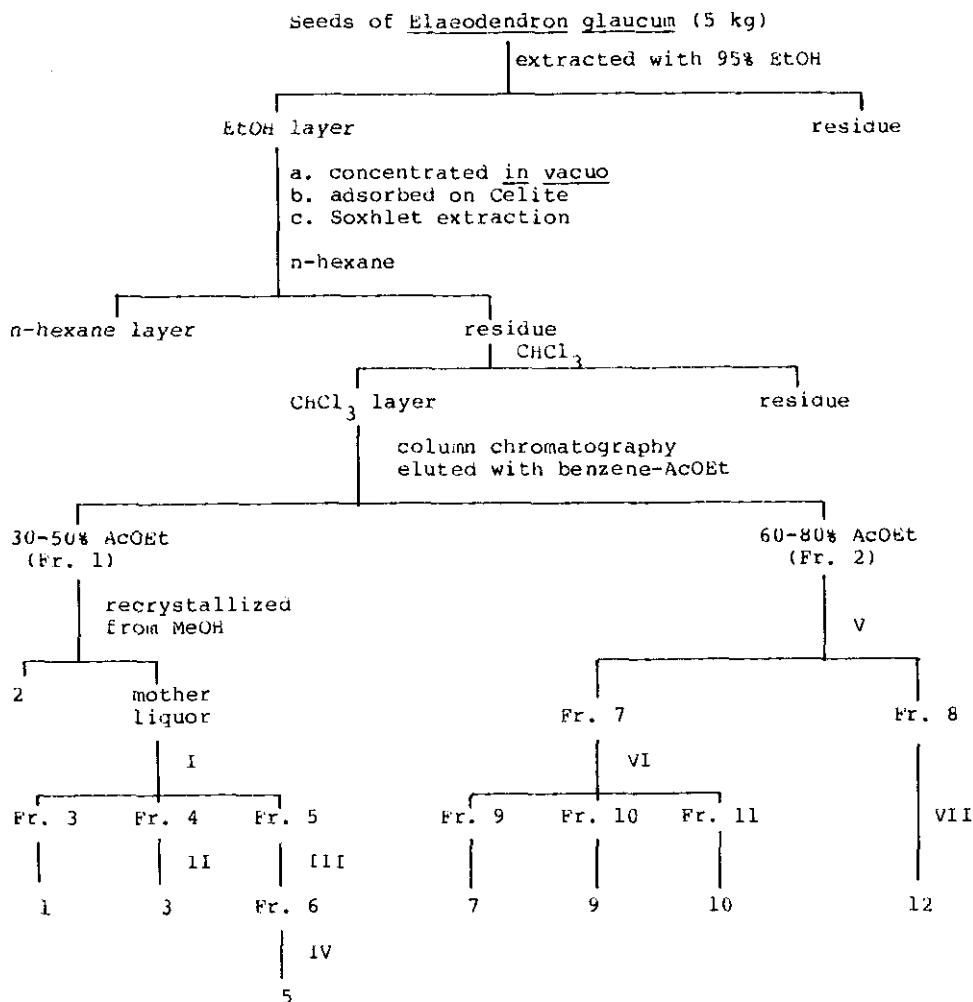
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Abstract-----Seven cardiac glycosides having an unusual sugar linkage, elaeodendrosides M, N, O, P, Q, R and S, were isolated from seeds of Elaeodendron glaucum.

These were unequivocally characterized on the basis of physical data and chemical correlation with related compounds previously isolated. These new compounds were tested for inhibition of Na⁺, K⁺-adenosine triphosphatase from guinea pig heart, and the structure-activity relationship has been discussed.

In the preceding papers we reported the isolation and characterization of cardiac glycosides having an unusual sugar linkage, elaeodendrosides A-L and elaeodendrogenin from seeds of Elaeodendron glaucum Pers. (Celastraceae) by X-ray crystallography and chemical means.¹⁻⁶ The present paper describes the structures of elaeodendrosides M-S which have been isolated by the procedure similar to that previously reported (Chart 1, Fig. 1).^{1,4}

Elaeodendroside M (1), mp 277-279°C, was separated as colorless prisms. Ms spectral data provided the molecular formula C₂₉H₃₄O₁₀. The complete structure was determined as 1 by direct comparison with the authentic sample obtained by oxidation from elaeodendroside A (2).⁴ The uv spectrum (λ_{max} 284 nm) of 1 suggested the presence of the diosphenol structure. When adsorbed on



I: column chromatography; benzene-AcOEt (1:1), II: hplc (TSKgel ODS-80TM); MeOH-H₂O (5:3), III: hplc (TSKgel ODS-80TM); MeCN-H₂O (1:1), IV: prep. tlc; CHCl₃-acetone (3:1), V: column chromatography; benzene-AcOEt (1:3), VI: hplc (TSKgel ODS-80TM); MeCN-H₂O (2:3), VII: prep. tlc; CHCl₃-acetone (4:1).

Chart 1. Isolation of Elaeodendrosides

silica gel in methanol-acetone for a week, 2 yielded 1 together with ketol rearrangement products.⁴ This result implied that 1 might be an artifact formed from 2.

Elaeodendroside N (3), mp >300°C, was isolated as colorless amorphous substance. Field desorption (FD) ms spectral data provided the molecular formula C₂₉H₃₆O₁₀. In the ¹H nmr spectrum, 3 exhibited the signals of 18- and 19-methyl groups at 0.69 and 1.42 ppm together with the carbinyl proton at 3.85 ppm, indicating the presence of the 128-hydroxy-11-oxo moiety (Table 1).⁷

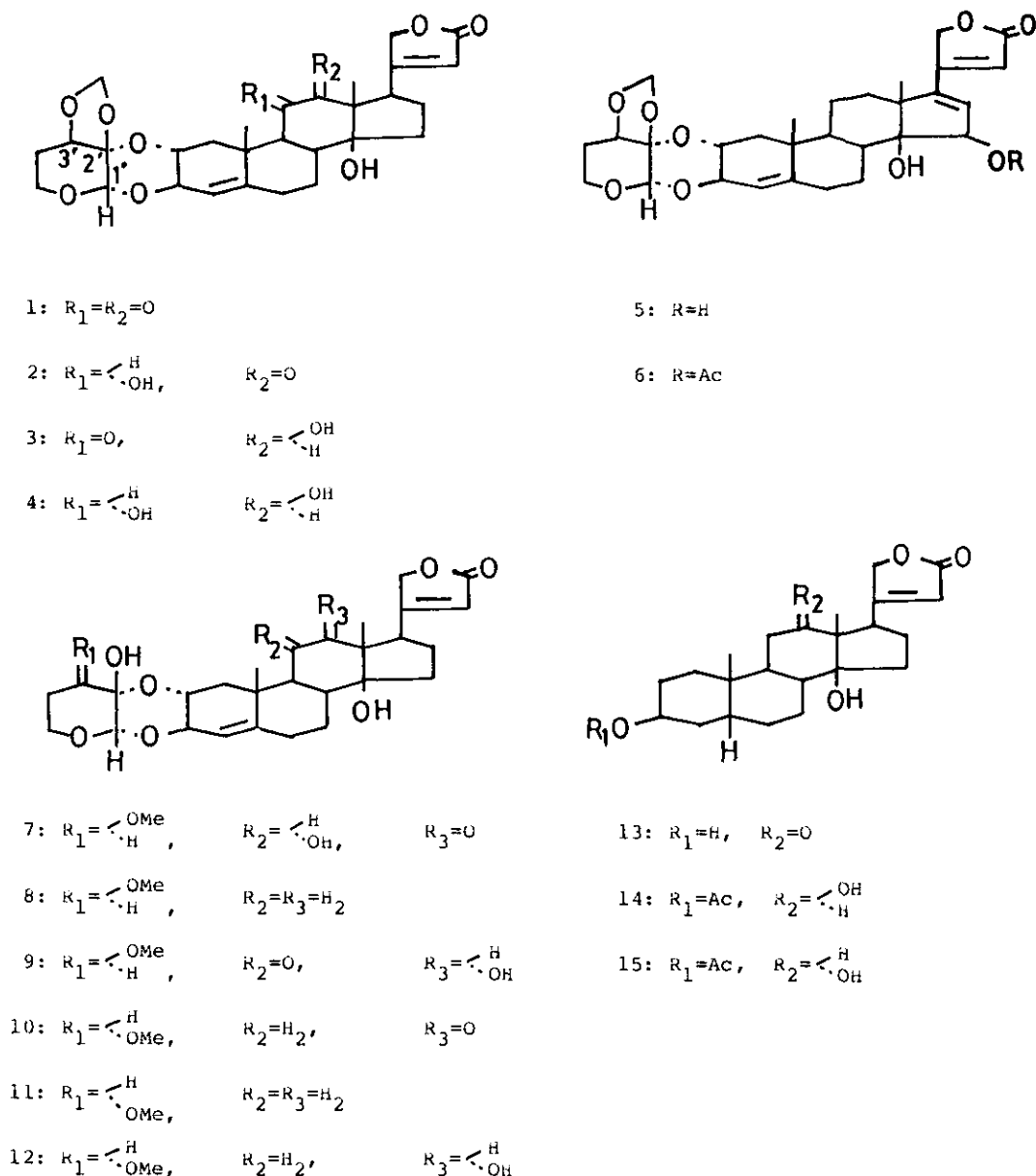


Fig.1. Structures of Elaeodendrosides and Related Compounds

The complete structure was elucidated by direct comparison with authentic sample obtained by oxidation from 11 α ,12 β -dihydroxyelaeodendroside D (4).⁴

Elaeodendroside O (5), mp 243-245°C, was obtained as colorless amorphous substance. Elemental analysis and ms spectral data permitted us to assign the molecular formula $C_{29}H_{36}O_9$ to 5. Compound 5 showed the maximum uv absorption at 265 nm and was quantitatively transformed into the cyclic phenylboronate,

indicating the existence of both 16-dehydro and cis-glycol structures.⁴ On usual acetylation 5 gave the acetate (6), whose ^1H - ^1H correlation nmr spectroscopy showed the coupling between 16-H and deshielded proton caused by acetylation. These data together with ^1H and ^{13}C nmr spectral data lent a support to assign the structure 5 to elaeodendroside O (Table 2).

Elaeodendroside P (7), mp 232-234°C, was isolated as colorless amorphous substance. The exact ms spectrum and elemental analysis data afforded the molecular formula $\text{C}_{29}\text{H}_{38}\text{O}_{10}$. Inspection of ^1H and ^{13}C nmr spectra permitted us to assign the structure of sugar moiety of 7 to that of elaeodendroside C (8).⁶ On the other hand nmr spectra of genin moiety of 7 were compatible with those of 2.⁴ On the basis of these data the structure 7 was assignable to elaeodendroside P.

Elaeodendroside Q (9), mp 252-253°C, was also isolated as colorless amorphous substance. When adsorbed on alumina in methanol-acetone overnight, 7 underwent ketol rearrangement to yield 9,⁴ which was identical with the isolated compound. In the ^1H nmr spectrum, 9 exhibited the carbinyl proton at 4.15 ppm, which supported the assigned structure.⁴

Elaeodendroside R (10), mp 231-233°C, was separated as colorless amorphous substance. Exact ms spectral data provided the molecular formula $\text{C}_{29}\text{H}_{38}\text{O}_9$. Inspection of ^1H and ^{13}C nmr spectra permitted us to assign the sugar moiety of 10 to that of elaeodendroside B (11).⁶ In the ^1H nmr spectrum, 10 exhibited the signals of 18- and 19-methyl groups at 1.08 and 1.20 ppm, indicating the presence of an oxo group at C-12, whose data was compatible with that of 12-oxoeleodendroside D.⁴ The ^{13}C nmr spectral data of 10 besides 12-oxo-digitoxigenin (13) also lent a support to assign the structure 10 to elaeodendroside R.⁸

Elaeodendroside S (12), mp 235-240°C, was also obtained as colorless amorphous substance. Upon oxidation with chromium trioxide 12 was led to 10, suggesting the presence of a hydroxyl group at the 12-position. The configurational assignment of the 12 α -hydroxyl group in 12 was supported by the chemical shift of C-18 in the ^{13}C nmr spectrum. The C-18 chemical shifts of digoxigenin 3-acetate (14) and 12-epidigoxigenin 3-acetate (15) were 9.0 and 17.1 ppm, respectively,⁸ while that of 12 was 17.3 ppm and compatible with the 12 α -hydroxylated structure. On the basis of these results, the structure 12 was assigned to elaeodendroside S.

Table 1. ^1H Nmr Chemical Shifts of Elaeodendrosides^a

	Elaeodendrosides									
	A(2)	M(1) ^b	N(3)	O(5)	C(8)	P(7)	Q(9)	B(11)	R(10)	S(12)
18,19-Me	1.10	1.02	0.69	1.35	0.90	1.12	1.09	0.89	1.08	0.86
21-CH ₂	1.40	1.51	1.42	1.18	1.14	1.40	1.18	1.12	1.20	1.12
	4.79	4.90	4.77	4.95	4.80	4.78	4.66	4.80	4.77	4.83
	4.90	(m)	4.90	(m)	4.97	4.90	5.17	4.97	4.89	4.94
	(dd, 18,2)		(dd, 18,2)		(dd, 18,2)	(dd, 19,2)	(dd, 19,2)	(dd, 18,2)	(dd, 19,2)	(dd, 19,2)
22-H	6.00	6.06	6.00	6.03	5.88	6.00	5.90	5.88	5.98	5.88
	(brs)	(brs)	(brs)	(brs)	(brs)	(brs)	(d,2)	(brs)	(brs)	(brs)
4-H	5.22	5.37	5.30	5.23	5.22	5.32	5.30	5.20	5.29	5.25
	(brs)	(brs)	(brs)	(brs)	(brs)	(brs)	(brs)	(brs)	(brs)	(brs)
2β-H	4.01		4.04	4.04	4.20	4.19	4.20	4.38	4.21	4.21
	(m)		(m)	(m)	(m)	(m)	(m)	(m)	(m)	(m)
3α-H	4.40		4.36	4.41	4.54	4.54	4.60	4.57	4.60	4.60
	(d,9)		(d,9)	(d,9)	(d,9)	(d,9)	(d,9)	(d,9)	(d,9)	(d,9)
1'β-H	4.67	4.70	4.67	4.68	4.67	4.68	4.70	4.55	4.58	4.58
3'-H	3.87			3.85	3.36	3.37	3.40	3.30	3.29	3.29
	(brs)			(brs)	(brs)	(brs)	(brs)	(dd, 11,5)	(dd, 11,5)	(dd, 11,5)
5'α-H	3.94		3.93	3.93				4.10	4.09	4.09
	(ddd, 12,6,3)		(ddd, 12,6,3)	(ddd, 12,6,3)	3.80	3.78	3.81	(m)	(ddd, 13,8,2)	(ddd, 13,8,2)
5'β-H	3.76		3.75	3.75				3.55	3.51	3.51
	(td, 12,3)		(td, 12,3)	(td, 12,3)				(m)	(td, 13,3)	(td, 13,3)
-OCH ₂ O-	5.13	5.14	5.17	5.15						
	5.21	5.20	5.23	5.20						
3'-OMe					3.42	3.42	3.45	3.47	3.47	3.47
others	2.50	3.36-	3.49	4.81		3.65	4.15			3.72
	(dd, 14,3)	4.30	(OH) ^c	(d,8+brs) ^d		(OH) ^c	(12β-H)			(brs)
	(1β-H)	(5H,m)	3.85	(15α-H)		4.47				(12β-H)
	4.45	2β-,	(2H,m) ^d	5.88		(dd, 12,4)				
	(d,12)	3'α-H,	(12α-,	(16-H)		(11β-H)				
	(11β-H)	5'-CH ₂	3'α-H)							

a) δ(ppm) in CDCl₃. J/Hz value in parentheses.

b) Measured at 100 MHz (JEOL JNM-FX-100).

c) The signal disappeared on treatment with D₂O.d) Treated with D₂O.

It is well known that Na⁺, K⁺-adenosine triphosphatase (Na⁺, K⁺-ATPase: EC 3.6.1.3) is an enzyme responsible for the active transport of Na⁺ and K⁺ across the cell membrane and is inhibited by cardiac steroids.^{9,10} The inhibitory activities of the newly isolated cardiac glycosides were tested with Na⁺, K⁺-ATPase from guinea pig heart. The molar concentrations exerting half-maximal inhibition (ID₅₀ value) are listed in Table 3.

Elaeodendroside P (7) having 11α-hydroxyl and 12-oxo groups showed approximately fifteen times higher activities than the isomeric 11, 12-ketols, elaeodendrosides Q (9) and N (3). These results are consistent with the previous findings¹⁰ that the presence of ring C substituent affected significantly the inhibitory activities.

Table 2. ^{13}C Nmr Chemical Shifts of Elaeodendrosides and Related Compounds^a

Elaeodendrosides and Related Compounds											
	A(2)	O(5)	C(8)	P(7)	Q(9)	B(11)	R(10)	S(12)	13 ^b	14 ^b	15 ^b
C- 1	43.3	41.2	41.6	43.9	41.4	41.6	41.0	41.2	29.4	30.5	30.5
2	71.1	71.3	67.9	67.5	67.0	67.5	67.1	67.3	27.9	25.0	25.1
3	70.7	70.8	70.8 ^c	70.7	70.7	70.9 ^c	70.7	70.7 ^c	66.5	70.2	70.5
4	119.6	117.5	117.7 ^c	120.8	121.0	117.7 ^c	119.6	118.4 ^c	33.7	30.5	30.5
5	146.7	147.2	146.4	145.7	144.2	146.4	145.2	146.5	36.1	36.9	37.0
6	32.2	31.9	31.9	32.2	31.9	31.9	31.3	31.7	26.2	26.3	26.3
7	29.2	29.0	29.3	29.4	28.3	29.2	29.3	29.6	21.9	21.5	21.1
8	41.4 ^c	41.7	41.6	41.4 ^c	39.8	41.6	41.0	41.8	41.4	41.4	42.0
9	53.8	51.0	50.7	54.0	58.9	50.6	47.6	44.5	33.3	32.6	29.5
10	42.2	40.6	41.0	42.4	41.9	40.8	40.7	40.4	35.7	35.0	35.0
11	74.4	20.1	21.4	74.5	212.3	21.4	37.3	29.6	37.4	30.2	29.7
12	213.0	38.6	39.5	213.0	82.6	39.4	211.3	75.0	211.4	75.0	76.6
13	63.4	51.8	50.0	63.4	55.7	50.1	64.4	53.6	64.0	55.6	52.0
14	84.9	84.6	84.2	85.1	82.6	84.2	85.2	84.4	86.5	85.5	85.5
15	33.1	75.9	33.1	33.1 ^d	34.2	33.1	32.8	34.8	33.1	33.3	34.8
16	27.1 ^d	136.7	27.2	27.1 ^d	28.3	27.2	27.0	29.2	26.9	27.4	28.9
17	40.5 ^c	143.2	51.2	40.5 ^c	45.6	51.2	40.4	45.8	39.9	45.7	45.6
18	17.2	17.4	16.1	17.2	18.9	16.0	16.6	17.3	16.5	9.0	17.1
19	20.3	19.8	20.0	20.3	21.1	19.8	19.3	19.7	23.3	23.6	23.6
20	174.0 ^e	159.1	174.4 ^d	174.0 ^e	174.3 ^c	174.3 ^d	174.6 ^c	177.2	174.5	175.6	175.7
21	73.7	71.8	73.7 ^c	73.7	75.0	73.6	73.8	73.9	73.7	73.3	73.8
22	118.8	113.7	118.8 ^c	118.8	117.5	118.7 ^c	118.5	117.2 ^c	118.6	117.6	117.7
23	173.8 ^e	174.3	175.8 ^d	173.8 ^e	173.1 ^c	175.6 ^d	174.2 ^c	174.5	173.5	174.4	174.6
1'	98.0	97.9	96.4	96.4	96.3	98.0	98.0	97.8			
2'	97.9	97.8	92.4	92.4	92.3	93.5	93.4	93.2			
3'	79.3	79.2	81.0	81.1	80.8	83.5	83.3	83.1			
4'	26.9 ^d	26.8	27.8	27.8 ^d	27.4	28.2	28.0	27.8			
5'	62.0	61.9	60.7	60.6	60.5	62.5	62.3	62.3			
MeO			58.2	58.1	57.9	57.9	57.8	57.7			
OCH ₂ O	94.6	94.7									

a) δ (ppm) in pyridine- d_5 . Assignments of the signals are based on CW off resonance, INEPT method or the report by Abe and Yamauchi.

b) from reference 8.

c-e) The signals with the same superscripts in each column may be interchanged.

Table 3. Inhibition of Guinea Pig Heart Na^+ , K^+ -ATPase by Elaeodendrosides

Compound	ID_{50}	Compound	ID_{50}
ouabain	157 ± 16.3^a (1.00) ^b	P (7)	214 ± 33.4 (0.73)
M (1)	39.9 ± 6.04 (3.93) ^c	Q (9)	3210 ± 295 (0.05)
N (3)	4420 ± 360 (0.04)	R (10)	714 ± 245 (0.22)
O (5)	731 ± 112 (0.21)	S (12)	2310 ± 368 (0.07)

a) mean $\pm$ S.E. ($\times 10^{-8}$ M, $n=3$).

b) Figures in parentheses express the relative potency.

c) from reference 10.

EXPERIMENTAL

Melting points were uncorrected. The following instruments were used.

Optical rotation; JASCO DIP-181 spectrometer, low ms spectra; Hitachi M-52 spectrometer, FD ms spectra; JEOL JMS-01SG-2 spectrometer, exact ms spectra

using chemical ionization (CI , CH_4); AEI MS-902 spectrometer, ^1H nmr spectra; JEOL JNM-GX-500 (500 MHz), ^{13}C nmr; JEOL JNM-GX-400 (100.4 MHz), high-performance liquid chromatography (hplc); JASCO TRI ROTAR equipped with a JASCO UVIDEC-100-II uv (240 nm) or Shimadzu SPD-M6A photodiode array uv (210-300 nm) detector, hplc column; TSKgel ODS-80TM (TOSOH), Develosil ODS-5 (Nomura Chemical) and Chemcosorb 5-ODS-H (Chemco) (5 μm ; 15 cm x 0.4 cm i.d.), hplc flow rate; 1 ml/min, preparative thin-layer chromatography (prep. tlc); silica gel HF₂₅₄ (E. Merck), column chromatography; silica gel (70-230 mesh) (E. Merck). Cycloextrin (CD) was kindly donated by Nihon Shokuhin Kako. Tetramethylsilane was used as an internal standard for nmr spectra. Abbreviations: s=singlet, d=doublet, t=triplet, dd=doublet of doublets, td=triplet of doublets, add=doublet of doublets of doublets, m=multiplet, br=broad.

Extraction of Steroidal Components

Seeds (5 kg) of Elaeodendron glaucum Pers. collected in India in March, 1975, were extracted with 95% EtOH (5 l) in a Soxhlet extractor. The ethanolic layer was concentrated in vacuo, and the residue was further extracted with n-hexane and then CHCl_3 in a Soxhlet extractor. The organic layer was concentrated in vacuo, and the residue (16 g) was chromatographed repeatedly as shown in Chart 1. The following cardiac glycosides were obtained.

Elaeodendroside M (1)(10 mg). mp 277-279°C, colorless prisms from acetone, $[\alpha]_{\text{D}}^{25} +66.7^\circ$ (c=0.15, CHCl_3 -MeOH (1:1)). ms m/z: 542 (M)⁺. $\text{uv } \lambda_{\text{max}}^{\text{MeOH}}$ nm: 217, 284.

Elaeodendroside N (3)(1 mg). mp >300°C, colorless amorphous substance from MeOH, $[\alpha]_{\text{D}}^{22} +104.3^\circ$ (c=0.12, CHCl_3). FD ms m/z: 545 (M+H)⁺.

Elaeodendroside O (5)(4 mg). mp 243-245°C, colorless amorphous substance from acetone. $[\alpha]_{\text{D}}^{16} +73.2^\circ$ (c=0.21, CHCl_3). Anal. Calcd for $\text{C}_{29}\text{H}_{36}\text{O}_9 \cdot 2\text{H}_2\text{O}$: C, 61.69; H, 7.14. Found: C, 61.32; H, 6.83. FD ms m/z: 528 (M)⁺. $\text{uv } \lambda_{\text{max}}^{\text{MeOH}}$ nm: 217, 265.

Elaeodendroside P (7)(10 mg). mp 232-234°C, colorless amorphous substance from MeOH-ether, $[\alpha]_{\text{D}}^{21} +47.0^\circ$ (c=0.34, CHCl_3). Anal. Calcd for $\text{C}_{29}\text{H}_{38}\text{O}_{10}$: C, 63.72; H, 7.01. Found: C, 63.29; H, 6.88. exact ms (CI) m/z: 547.2548 (M+H)⁺ (Calcd for $\text{C}_{29}\text{H}_{38}\text{O}_{10} + \text{H}$, 547.2543).

Elaeodendroside Q (9)(10 mg). mp 252-253°C, colorless amorphous substance from MeOH, $[\alpha]_{\text{D}}^{21} -6.8^\circ$ (c=0.57, CHCl_3). Anal. Calcd for $\text{C}_{29}\text{H}_{38}\text{O}_{10}$: C, 63.72; H,

7.01. Found: C, 63.62; H, 6.92. exact ms (CI) m/z: 547.2505 (M+H)⁺ (Calcd for C₂₉H₃₈O₁₀ + H, 547.2543).

Elaeodendroside R (10) (5 mg). mp 231-233°C, colorless amorphous substance from MeOH-ether, $[\alpha]_D^{21} +53.3^\circ$ (c=0.22, CHCl₃). exact ms (CI) m/z: 531.2585 (M+H)⁺ (Calcd for C₂₉H₃₈O₉ + H, 531.2594).

Elaeodendroside S (12) (7 mg). mp 235-240°C, colorless amorphous substance from MeOH-ether, $[\alpha]_D^{22} +63.8^\circ$ (c=0.80, CHCl₃). FD ms m/z: 533 (M+H)⁺.

Transformation of Elaeodendroside A (2) to Elaeodendroside M (1)

Elaeodendroside A (2) (20 mg) was treated with Jones reagent as described in the previous paper.⁴ Mixed mp of the oxidation product (15 mg) on admixture with 1 showed no depression.

Transformation of Elaeodendroside A (2) to Elaeodendroside N (3)

11 α ,12 β -Dihydroxyelaeodendroside D (4) (9 mg) obtained from 2⁴ was dissolved in AcOH (1 ml) and treated with 1.1 eq. of CrO₃ in AcOH-H₂O (1:1) (1.2 mg/ml) under ice-cooling for 1 h. The reaction mixture was extracted with AcOEt and washed successively with 5% NaHSO₃, 5% NaHCO₃ and H₂O. After drying over anhydrous Na₂SO₄, the organic layer was evaporated off in vacuo. The residue was subjected to prep. tlc using benzene-AcOEt (1:3) as a developing solvent. The zone corresponding to the spot of R_f 0.45 was scraped off and eluted with AcOEt. The dried eluate was recrystallized from MeOH to give colorless needles. mp >300°C. The chromatographic behaviors of the synthetic sample were entirely identical with those of 3. Hplc (Chemcosorb 5-ODS-H): MeCN-H₂O (2:3), t_R 5.36 min; MeCN-H₂O (2:5) containing 0.5% γ -CD, t_R 5.67 min, photodiode array detector.¹¹ Tlc [benzene-AcOEt (1:3)]: R_f 0.45.

Elaeodendroside A (2) was also obtained from the above prep. tlc (R_f 0.57).

Elaeodendroside O Acetate (6)

Elaeodendroside O (5) (2 mg) was dissolved in pyridine (2 ml) and treated with Ac₂O (2 ml) for 24 h. After evaporation of the solvent under an N₂ gas stream, the residue was subjected to prep. tlc using CHCl₃-acetone (3:1) as a developing solvent. The zone corresponding to the spot of R_f 0.55 was scraped off and eluted with AcOEt to give elaeodendroside O acetate (6) as colorless oily substance (1 mg). ¹H Nmr (CDCl₃) δ : 1.18 (3H, s, 19-Me), 1.35 (3H, s,

18-Me), 2.11 (3H, s, MeCO), 3.75 (1H, td, $J=12$, 3 Hz, 5' β -H), 3.85 (1H, m, 3' α -H), 3.93 (1H, add, $J=12$, 6, 3 Hz, 5' α -H), 4.03 (1H, m, 2 β -H), 4.41 (1H, d, $J=9$ Hz, 3 α -H), 4.68 (1H, s, 1' β -H), 4.93 (2H, dd, $J=18$, 2 Hz, 21-CH₂), 5.17, 5.22 (each 1H, s, -OCH₂O-), 5.22 (1H, brs, 4-H), 5.68 (1H, brs, 15 α -H), 5.83 (1H, brs, 16-H), 6.08 (1H, brs, 22-H). FD ms m/z : 570 (M)⁺.

Elaeodendroside O Phenylboronate

Elaeodendroside O (5) (< 1 mg) was dissolved in acetone (0.5 ml) and treated with phenylboronic acid (< 1 mg) at room temperature for 10 min. Evaporation of the solvent gave the phenylboronate. Tlc [benzene-AcOEt (1:3)]: R_f 0.90; 5, R_f 0.38. FD ms m/z : 614 (M+H)⁺.

Transformation of Elaeodendroside P (7) to Elaeodendroside Q (9)

Elaeodendroside P (7) (2 mg) dissolved in acetone-MeOH (1:1) (0.5 ml) was treated with aluminum oxide 90 (E. Merck) as described in the previous paper.⁴ The crude product was subjected to prep. tlc using benzene-AcOEt (1:2) as a developing solvent. The zone corresponding to the spot of ²R_f 0.27 was scraped off and eluted with AcOEt. Evaporation of the solvent gave elaeodendroside Q (9) (< 1 mg) as colorless amorphous substance. The chromatographic behaviors of the rearranged product were entirely identical with those of 9. Hplc (TSKgel ODS-80TM): MeCN-H₂O (5:8), t_R 6 min; MeOH-H₂O (4:3), t_R 9 min.

Transformation of Elaeodendroside S (12) to Elaeodendroside R (10)

Elaeodendroside S (12) (1 mg) dissolved in AcOH (0.1 ml) was treated with 1% CrO₃ in AcOH-H₂O (1:1) (0.1 ml) under ice-cooling for 45 min. After extraction with AcOEt, the organic layer was washed successively with 5% NaHSO₃, 5% NaHCO₃, H₂O and then dried over anhydrous Na₂SO₄. After evaporation of the solvent the crude product was subjected to prep. tlc using benzene-AcOEt (1:2). The zone corresponding to the spot of ²R_f 0.33 was scraped off and eluted with AcOEt. Evaporation of the solvent gave elaeodendroside R (10) (< 1 mg) as colorless amorphous substance. The chromatographic behaviors of the product were entirely identical with those of 10. Hplc (Develosil ODS-5): MeCN-H₂O (2:5) containing 0.5% γ -CD, t_R 5.83 min; MeOH-H₂O (1:1), t_R 7.85 min.

Biological Test using Na^+ , K^+ -ATPase

The inhibitory activities were tested with Na^+ , K^+ -ATPase from guinea pig heart according to the procedure described in the previous papers.^{9,10}

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