[Chem. Pharm. Bull. 33(12)5231—5238(1985)]

Chemical and Chemotaxonomical Studies of Filices. LXI.[1] Chemical Studies on the Constituents of *Pronephrium triphyllum* HOLLT.

NOBUTOSHI TANAKA,[a] TAKAO MURAKAMI,*,[a] HIROSHI WADA,[a]
ALICIA B. GUTIERREZ,[b,2)] YASUHISA SAIKI[b]
and CHIU-MING CHEN[c]

Faculty of Pharmaceutical Sciences, Science University of Tokyo,[a] Funakawara-machi, Shinjuku-ku, Tokyo 162, Japan, Department of Pharmaceutical Sciences, Kobe Gakuin University,[b] Arise, Igawatani-machi, Nishi-ku, Kobe 673, Japan and Department of Chemistry, National Tsing Hua University,[c] Kuang Fu Road, Hsinchu, Taiwan, China

(Received April 18, 1985)

From the fronds of *Pronephrium triphyllum* HOLLT., three new glycosides, named triphyllins A, B and C, were isolated. Their structures were determined as (2*R*,4*S*)-5,7-di-β-D-glucosyloxy-6-hydroxymethyl-4′-methoxy-8-methylflavan-4-ol, (2*R*,4*S*)-5,7-di-β-D-glucosyloxy-4′-hydroxy-6-hydroxymethyl-8-methylflavan-4-ol and (2*S*)-7-β-D-glucosyloxy-5-hydroxy-4′-methoxy-6-methoxymethyl-8-methylflavanone, respectively, on the basis of spectral data and chemical correlations.

Keywords——*Pronephrium triphyllum*; Aspidiaceae; fern; flavan-4-ol; C-methylflavonoid; ^{13}C-NMR

In the previous paper, we reported the structure elucidation of a novel flavan-4-ol-type glycoside, eruberin B (III), and its derivative, eruberin A (IV), isolated from *Glaphyropteridopsis erubescens* (WALL.) CHING.[3] Further studies on the constituents of related ferns led to the isolation of three new analogous glycosides, named triphyllins A, B and C, from *Pronephrium triphyllum* HOLLT. (Japanese name: Kohmorishida, Aspidiaceae). In this paper, we describe the structure elucidation of these glycosides.

Triphyllin A (I), colorless needles, mp 252—253 °C, $[\alpha]_D^{20}$ +17° (c=1.0, MeOH), was formulated as $C_{30}H_{40}O_{16}$ on the basis of the elemental analysis and the field desorption mass spectrum (FD-MS). Compound I showed infrared (IR) [ν_{max}^{KBr} cm^{-1}: 3400, 1605, 1525, 1265, 1080] and ultraviolet (UV) [λ_{max}^{MeOH} nm (log ε): 227 (4.46), 276 (3.65), 283 (3.65)] spectra similar to those of eruberin B (III). The red coloration of I on treatment with 3% HCl also suggested the presence of a flavan-4-ol skeleton like that in III. On acid hydrolysis, I gave many anthocyanidine-type compounds, as expected, and D-glucose. The proton nuclear magnetic resonance (^{1}H-NMR) data of I are shown in Table I. Almost all the signals coincide with those of eruberin B (III, see also Table I) except that one of the aromatic methyl signals of III is replaced by the methylene signal at δ 6.06. In view of the molecular formula and the fact that I gave the undecamethyl ether (VII) on permethylation by Hakomori's method,[4] this methylene signal was assigned to a hydroxymethyl group bonded to the A-ring in place of a methyl group of eruberin B (III). To confirm the structure, the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of I was compared with that of III (Table II). The differences of their chemical shifts confirmed that the only structural difference between them is in the substituents of the A-ring, a methyl group in III and a hydroxymethyl group in I.

To determine the position of the hydroxymethyl group of I, the ^{1}H-NMR spectra of I

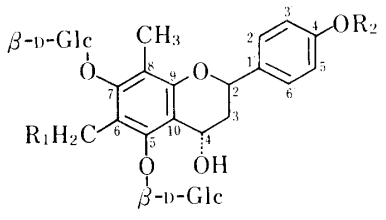
and III were also measured in dimethyl sulfoxide-d_6 (DMSO-d_6) solution. The signals of the methyl groups at C-6 and C-8 of III appeared at δ 2.28 and 2.10, respectively.[5] As their signals appeared at δ 3.13 and 2.65 in pyridine-d_5 solution, pyridine-induced chemical shifts[6] of 0.85 and 0.55 ppm were estimated for the methyl groups at C-6 and C-8, respectively. The values correspond to the number of neighboring glucosyl groups. On the other hand, the signal of the methyl group of I appeared at δ 2.17 in DMSO-d_6 solution. As it appeared at δ 2.63 in pyridine-d_5 solution, the value of the pyridine-induced chemical shift is 0.46 ppm, corresponding to that of the methyl group at C-8 of III. Therefore, the position of the hydroxymethyl group of I was determined to be at C-6.

As is the case for eruberin B, once the glucosyl group at C-7 was hydrolyzed by crude hesperidinase, I formed an ether linkage between C-4 and C-2′ of the glucosyloxy group at C-5 to yield compound V.[7] The ^{1}H- and ^{13}C-NMR spectra of V confirmed it to be the 6-

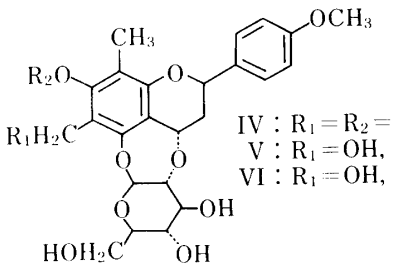
TABLE I. ^{1}H Chemical Shifts in Pyr.-d_5 (Multiplicity, Coupling Constant in Hz)

	Eruberin B (III)	Triphyrin A (I)	Triphyrin B (II)	Eruberin A (IV)	V
2-H	5.74 (br d, 12)	5.72 (br d, 12)	5.65 (br d, 12)	5.06 (br d, 12)	5.03 (br d, 12)
3-H_a	1.90 (br t, 12)	1.89 (br t, 12)	1.96 (br t, 12)	2.07 (br t, 12)	2.06 (br t, 12)
3-H_e	2.44 (br d, 12)	2.45 (br d, 12)	2.27 (br d, 12)	2.37 (br d, 12)	2.35 (br d, 12)
4-H	5.47 (br s)	5.52 (br s)	5.51 (br s)	5.30 (br s)	5.25 (br s)
6-CH_3	3.13 (s)	—	—	2.56 (s)	—
6-CH_2OH	—	6.06 (br s)	6.06 (br s)	—	5.45 (s)
8-CH_3	2.65 (s)	2.63 (s)	2.67 (s)	2.48 (s)	2.41 (s)
2′,6′-H	7.48 (d, 9)[a]	7.47 (d, 9)[a]	7.48 (d, 9)[a]	7.28 (d, 9)[a]	7.30 (d, 9)[a]
3′,5′-H	6.98 (d, 9)[a]	6.97 (d, 9)[a]	7.22 (d, 9)[a]	6.93 (d, 9)[a]	6.96 (d, 9)[a]
4′-OCH_3	3.68 (s)	3.69 (s)	—	3.68 (s)	3.69 (s)
Anomeric H	5.21 (d, 7)	5.33 (d, 7)	5.33 (d, 7)	5.59 (d, 8)	5.54 (d, 8)
	5.61 (d, 7)	5.48 (d, 7)	5.48 (d, 7)		

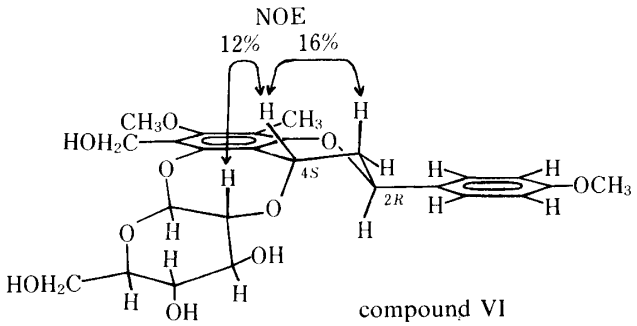
s, singlet; d, doublet; br s, broad singlet; br d, broad doublet; br t, broad triplet. *a*) Half of an $A_2'B_2'$ pattern.



I : R_1 = OH, R_2 = CH_3
II : R_1 = OH, R_2 = H
III : R_1 = H, R_2 = CH_3



IV : R_1 = R_2 = H
V : R_1 = OH, R_2 = H
VI : R_1 = OH, R_2 = CH_3



compound VI

Fig. 1

Table II. ^{13}C Chemical Shifts

	III DMSO-d_6	I DMSO-d_6	I Pyr.-d_5	II Pyr.-d_5	IV Pyr.-d_5	V Pyr.-d_5	IX Pyr.-d_5	VIII Pyr.-d_5
C-2	72.3	72.2	73.2	73.4	73.8	73.9	78.7	78.7
C-3	c)	c)	38.0	38.1	37.5	37.3	43.3	43.2
C-4	56.8	56.9	58.4	58.4	66.0	65.8	198.3	198.1
C-5	150.4[a)]	151.9[a)]	154.0[a)]	154.1[a)]	150.9[a)]	149.8[a)]	159.4[a)]	161.0[a)]
C-6	118.7[b)]	120.8[b)]	122.1[b)]	122.0[b)]	110.4[b)]	112.4[b)]	112.3[b)]	112.5[b)]
C-7	154.1[a)]	154.0[a)]	155.5[a)]	155.5[a)]	156.3[a)]	157.4[a)]	162.7[a)]	163.9[a)]
C-8	117.0[b)]	115.5[b)]	116.7[b)]	116.8[b)]	108.6[b)]	109.0[b)]	111.1[b)]	110.8[b)]
C-9	150.7[a)]	152.7[a)]	154.2[a)]	154.3[a)]	152.1[a)]	153.6[a)]	158.1[a)]	160.4[a)]
C-10	115.4[b)]	115.2[b)]	116.4[b)]	116.4[b)]	104.5[b)]	104.1[b)]	105.7[b)]	105.5[b)]
6-R	11.0	53.0	54.7	54.7	10.4	57.5	9.4	63.3
8-R	9.4	9.4	10.2	10.2	9.4	8.7	10.0	9.7
C-1′	133.4	133.2	134.4	132.8	133.7	133.4	131.5	131.2
C-2′,6′	127.3	127.3	127.9	128.1	128.0	128.1	128.2	128.2
C-3′,5′	113.7	113.8	114.3	116.2	114.2	114.2	114.5	114.5
C-4′	158.7	158.8	159.7	158.6	159.7	159.8	160.2	160.4
OCH_3	55.0	55.0	55.2	—	55.2	55.2	55.2	55.2
								58.1
G-1	104.3	104.0	105.9	105.9	102.2	101.7	105.8	106.4
	103.8	103.7	105.4	105.4				
G-2	73.9	73.9	75.6	75.7	79.4	79.3	75.7	75.7
	73.9	73.9	75.6	75.7				
G-3	75.9	76.1	78.2	78.3	76.0	75.9	78.4	78.3
	75.9	76.1	78.2	78.3				
G-4	69.9	70.0	71.6	71.6	71.5	71.2	71.5	71.6
	69.9	70.0	71.4	71.4				
G-5	76.8	77.0	78.7	78.7	76.6	76.3	78.7	78.7
	76.4	76.4	78.2	78.3				
G-6	61.1	60.9	62.4	62.4	62.8	62.4	62.6	62.8
	60.6	60.6	62.0	61.9				

a, b) Assignments with the same superscript for each compound may be interchanged. *c)* The signal was not identified, being overlapped by solvent signals.

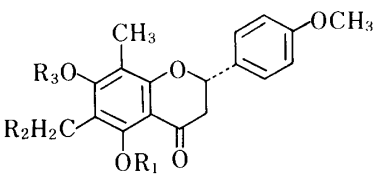
hydroxymethyl derivative of eruberin A (see Tables I and II). As the specific optical rotations of I (+17°) and V (+75°) were similar to those of eruberins B (+10°) and A (+88°), respectively, their absolute configurations seemed to be identical. To confirm their identity, the methyl ether of V (VI) was subjected to a proton nuclear Overhauser effect (NOE) experiment. Just as in the methyl ether of eruberin A,[3] 12% NOE was observed between the protons at C-4 and C-2′, indicating the absolute configurations at C-2 and C-4 to be *R* and *S*, respectively. Thus, the structure of I was established as (2*R*,4*S*)-5,7-di-β-D-glucosyloxy-6-hydroxymethyl-4′-methoxy-8-methylflavan-4-ol.

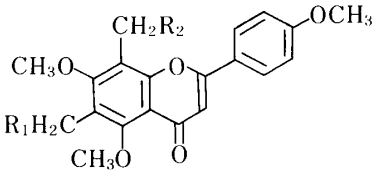
Triphyllin B (II), $C_{29}H_{38}O_{16}$, colorless needles, mp 210—215°C, $[\alpha]_D^{20}$ +18° (*c*=1.0, MeOH), showed IR (ν_{max}^{KBr} cm^{-1}: 3370, 1605, 1525, 1075) and UV [λ_{max}^{MeOH} nm (log ε): 227 (4.41), 277 (3.66), 283 (3.66)] spectra similar to those of triphyllin A (I). The ^1H- and ^{13}C-NMR data for II are shown in Tables I and II, respectively. Almost all the chemical shifts are in good agreement with those of I except at the B-ring. The differences of the spectral data indicate that II is the 4′-hydroxyl derivative of I. To confirm the structure, II was converted into I by treatment with diazomethane. Thus, II was determined to be 4′-demethyl derivative of I.

Triphyllin C (VIII), $C_{25}H_{30}O_{11}$, colorless needles, mp 188—190°C, $[\alpha]_D^{20}$ −2° (*c*=1.0, MeOH), gave a positive ferric chloride test. Compound VIII showed IR (ν_{max}^{KBr} cm^{-1}: 3350, 1645, 1595, 1515, 1260, 1080) and UV [λ_{max}^{MeOH} nm (log ε): 225 (4.55), 282 (4.20), 353 (3.70)]

absorptions characteristic of flavanone-type compounds. In the ^{1}H-NMR spectrum (in pyr.-d_5), VIII showed signals due to a 4′-methoxyphenyl group at δ 3.69 (3H, s), 6.99 (2H, d, J=8 Hz) and 7.48 (2H, d, J=8 Hz), and aromatic methyl group at 2.47 (3H, s), a methylene group at 4.89 (2H, br s), an aliphatic methoxy group at 3.53 (3H, s) and a glycosyl group at 5.60 (1H, d, J=7 Hz) and 3.8—4.6 (6H), together with ABX-type signals characteristic of a flavanone at 2.84 (1H, dd, J=17, 4 Hz), 3.16 (1H, dd, J=17, 12 Hz) and 5.35 (1H, dd, J=12, 4 Hz). These data indicated that the A-ring of VIII is substituted by a methyl group, a hydroxymethyl group and a glycosyloxy group, and one of the hydroxyl groups is methylated. Further, the bathochromic shifts of the UV absorption maxima on addition of $AlCl_3$ ($\lambda_{max}^{MeOH+AlCl_3}$ nm: 226, 311, 420) indicated the presence of a hydroxyl group at C-5.[8] In the light of these data, the ^{13}C-NMR spectrum of VIII was compared with that of the analogous glycoside, matteucinol 7-*O*-*β*-D-glucoside (IX), which had been isolated from *Matteuccia orientalis* (HOOK.) TREV. (Japanese name: Inugansoku, Aspidiaceae).[9] The differences of their chemical shifts (see Table II) indicated that one of the aromatic methyl groups at the A-ring in IX is substituted by a methoxymethyl group in VIII. On enzymatic hydrolysis using crude hesperidinase, VIII gave an aglycone (X), mp 127—128 °C, $[\alpha]_D^{20}$ −20° (c=0.2, MeOH), and D-glucose. The spectral data of X were consistent with the assigned structure, but the position of the methoxymethyl group, C-6 or C-8, remained ambiguous.

Finally, confirmation of the structure of X, including the position of the methoxymethyl group, was achieved by chemical correlation. To obtain compounds related to X, hydroxylation and methoxylation of matteucinol dimethyl ether (XI)[10] were attempted. On treatment of XI with *N*-bromosuccinimide (NBS) in CCl_4, the aromatic methyl groups of XI were equally subject to bromination, though dehydrogenation of the heterocyclic ring took place at the same time. Thus, two pairs of hydroxylated (XIV and XV) and methoxylated (XVI and XVII) dehydromatteucinol dimethyl ethers, together with dehydromatteucinol dimethyl ether (XVIII), were obtained by hydrolysis or methanolysis of the products of the NBS reaction. Compounds XIV and XV were converted to compounds XVI and XVII, respectively, by methylation with methyl iodide and Ag_2O. The positions of the hydroxyl or methoxyl groups of these compounds were determined from the ^{1}H-NMR data. The hydroxyl or methoxyl groups introduced at the methyl groups of XI are considered to affect the chemical shifts of the neighboring protons by magnetic anisotropic effects rather than through-bond effects. Therefore, it was expected that the hydroxylation or methoxylation of the methyl group at C-6 would cause shifts for both of the A-ring methoxyl groups while those at C-8 would affect only one of them. In the cases of compounds XV and XVII, one of the A-ring methoxyl signals showed low-field shifts of 0.12 and 0.10 ppm, respectively, compared with that of compound XVIII (see Table III). On the other hand, both signals showed similar low-field shifts in the cases of compounds XIV and XVI. Therefore, the position of the





VIII: R_1=H, R_2=OCH_3, R_3=*β*-D-glucosyl
IX: R_1=R_2=H, R_3=*β*-D-glucosyl
X: R_1=R_3=H, R_2=OCH_3
XI: R_1=R_3=CH_3, R_2=H
XII: R_1=R_2=R_3=H
XIII: R_1=R_3=CH_3, R_2=OCH_3

XIV: R_1=OH, R_2=H
XV: R_1=H, R_2=OH
XVI: R_1=OCH_3, R_2=H
XVII: R_1=H, R_2=OCH_3
XVIII: R_1=R_2=H

Fig. 2.

TABLE III. ^{1}H Chemical Shifts in $CDCl_3$ (Multiplicity, Coupling Constant in Hz)

	XIV	XV	XVI	XVII	XVIII
3-H	6.61 (s)	6.57 (s)	6.60 (s)	6.59 (s)	6.60 (s)
5-OCH_3	3.97 (s)	3.88 (s)	3.93 (s)	3.89 (s)	3.88 (s)
6-CH_3	—	2.27 (s)	—	2.28 (s)	2.28 (s)
6-CH_2OR	4.79 (s)	—	4.54 (s)	—	—
7-OCH_3	3.90 (s)	3.91 (s)	3.89 (s)	3.89 (s)	3.79 (s)
8-CH_3	2.47 (s)	—	2.46 (s)	—	2.46 (s)
8-CH_2OR	—	5.02 (s)	—	4.76 (s)	—
2′,6′-H	7.82 (d, 9)[a]	7.81 (d, 9)[a]	7.83 (d, 9)[a]	7.83 (d, 9)[a]	7.83 (d, 9)[a]
3′,5′-H	7.00 (d, 9)[a]	6.99 (d, 9)[a]	7.00 (d, 9)[a]	7.00 (d, 9)[a]	7.00 (d, 9)[a]
4′-OCH_3	3.89 (s)	3.88 (s)	3.89 (s)	3.89 (s)	3.88 (s)
$-CH_2OCH_3$	—	—	3.43 (s)	3.51 (s)	—

s, singlet; d, doublet. *a*) Half of an $A_2'B_2'$ pattern.

hydroxymethyl or methoxymethyl group were unequivocally determined to be at C-6 for XIV and XVI, and at C-8 for XV and XVII.

The aglycone X was methylated to yield XIII by treatment with methyl iodide and potassium carbonate in acetone, and then dehydrogenated with chloranil. The product was identified as XVI. Thus, the position of the methoxymethyl group of X was concluded to be at C-6. On the basis of these results, the structure of triphyllin C (VIII) was determined to be 5,7-dihydroxy-4′-methoxy-6-methoxymethyl-8-methylflavanone 7-*O*-*β*-D-glucoside. The absolute configuration of VIII was established as (2*S*) by the circular dichroism Cotton effect, $[\theta]_{282}^{20}$ -41000° ($c=0.003$, MeOH), as compared with that of matteucinol 7-*O*-*β*-D-glucoside (IX), $[\theta]_{286}^{20}$ -44700° ($c=0.003$, MeOH).[11]

Experimental

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter. Circular dichroism (CD) spectra were recorded on a JASCO J-20 spectropolarimeter. Gas-liquid chromatography (GLC) was run on a Shimadzu GC-4BM-PF gas chromatograph with a flame ionization detector using a capillary column (30 m × 0.25 mm i.d., WCOT, SE-30, Wako Pure Chemical). The ^{1}H-NMR spectra were measured at 60 MHz with a Hitachi R600 spectrometer and at 100 MHz with a JEOL FX-100 spectrometer, using tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet). MS were taken at 70 eV on a Hitachi RMU-7M mass spectrometer with a direct inlet system. FD-MS were taken on a Hitachi M-80 spectrometer. UV spectra were recorded on a Hitachi 323 spectrometer and IR spectra on a Hitachi IR-215 spectrometer. ^{13}C-NMR spectra were measured at 25 MHz with a JEOL FX-100 spectrometer with ^{2}H internal lock, using tetramethylsilane as an internal standard. Activated charcoal (Wako Pure Chemical) and silica gel (above 100 mesh, Kanto Chemical) for column chromatography were used. Silica gel G (type 60, Merck) and Silica gel GF_{254} (type 60) were used for preparative layer chromatography (PLC).

Isolation Procedure——The air-dried fronds (800 g) of *Pronephrium triphyllum* HOLLT., collected in December at Amamiohshima-Island, Kagoshima prefecture, were extracted three times with 3 l of MeOH under reflux for 6 h. The combined extracts (9 l) and then 10 l of MeOH were passed over activated charcoal (100 g) packed in a column of 7 cm diameter to give fraction 1. The column was then eluted with 10 l of 30% $CHCl_3$ in MeOH to give fraction 2. Fraction 1 was concentrated to a syrup under reduced pressure. The syrup was mixed with silica gel (70 g) and applied to a silica gel column (120 g, 7 cm diameter). The column was eluted with $CHCl_3$ and MeOH to yield fractions containing triphyllins A and B. The mixture was rechromatographed on silica gel using a mixture of $CHCl_3$, MeOH and water (12 : 7 : 1) as an eluent to yield triphyllins A (I, 3.3 g) and B (II, 200 mg). Fraction 2 was concentrated to a syrup under reduced pressure and distributed between *n*-hexane (600 ml) and 98% MeOH (400 ml). The MeOH layer was evaporated under reduced pressure. The residue was chromatographed on silica gel using $CHCl_3$ and MeOH as eluents followed by PLC (solvent system, $CHCl_3$: MeOH = 10 : 1) to yield triphyllin C (VIII, 14 mg).

Triphyllin A (I)——Colorless needles from a mixture of EtOH and water, mp 252—253 °C, $[\alpha]_D^{20}$ $+17^\circ$ ($c=1.0$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1605, 1525, 1265, 1080. UV λ_{max}^{MeOH} nm (log ε): 227 (4.46), 276 (3.65), 283 (3.65). *Anal.*

Calcd for $C_{30}H_{40}O_{16}$: C, 54.87; H, 6.14. Found: C, 54.79; H, 6.11. FD-MS *m/z*: 695 (M+K), 679 (M+Na).

Triphyllin B (II)——Colorless needles from a mixture of MeOH and water, mp 210—215 °C, $[\alpha]_D^{20}$ +18° (*c*=1.0, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3370, 1605, 1525, 1075. UV λ_{max}^{MeOH} nm (log ε): 227 (4.41), 277 (3.66), 283 (3.66). *Anal.* Calcd for $C_{29}H_{38}O_{16}$: C, 54.20; H, 5.96. Found: C, 54.13; H, 6.12.

Triphyllin C (VIII)——Pale yellow fine needles from MeOH, mp 188—190 °C, $[\alpha]_D^{20}$ −2° (*c*=1.0, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350, 1645, 1595, 1515, 1260, 1080. UV λ_{max}^{MeOH} nm (log ε): 225 (4.55), 282 (4.20), 353 (3.70), $\lambda_{max}^{MeOH+AlCl_3}$ nm: 226, 311, 420. ^{1}H-NMR (100 MHz, in pyr.-d_5) δ: 2.47 (3H, s), 2.84 (1H, dd, *J*=17, 4 Hz), 3.16 (1H, dd, *J*=17, 12 Hz), 3.53 (3H, s), 3.69 (3H, s), 3.8—4.6 (6H), 4.98 (2H, br s), 5.35 (1H, dd, *J*=12, 4 Hz), 5.60 (1H, d, *J*=7 Hz), 6.99 (2H, d, *J*=8 Hz), 7.48 (2H, d, *J*=8 Hz). MS *m/e*: 474 ($M^+ - CH_3OH$), 313, 279, 134. *Anal.* Calcd for $C_{25}H_{30}O_{11}$: C, 59.28; H, 5.97. Found: C, 59.20; H, 6.01. CD: $[\theta]_{282}^{20}$ −41000° (*c*=0.003, MeOH).

Enzymatic Hydrolysis of Triphyllin A (I)——A solution of triphyllin A (100 mg) and crude hesperidinase (300 mg, Tanabe Pharm. Co.) in 0.05 M citrate buffer (pH 4.0, 100 ml) was stirred at 40 °C for 12 h. The reaction mixture was extracted with EtOAc. The extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on silica gel using 10% MeOH in $CHCl_3$ as an eluent to yield 32 mg of compound V.

Compound V——Colorless needles from MeOH, mp 130 °C (dec.), $[\alpha]_D^{20}$ +75° (*c*=1.0, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3370, 1620, 1525, 1260, 1155, 1060. UV λ_{max}^{MeOH} nm (log ε): 213 (4.64), 229 (4.39), 275 (3.42), 282 (3.39).

Methylation of Compound V——Compound V (26 mg) was dissolved in acetone (20 ml), and then CH_3I (6 ml) and anhydrous K_2CO_3 (2 g) were added. The mixture was stirred under reflux for 2 h and poured into ice-water. The products were extracted with EtOAc. The extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel using 7% MeOH in $CHCl_3$ as an eluent to yield 15 mg of compound VI.

Compound VI——Amorphous powder, $[\alpha]_D^{20}$ +95° (*c*=0.7, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3420, 1615, 1525, 1255, 1135, 1060. MS *m/z*: 490 (M^+), 328, 311. ^{1}H-NMR (100 MHz, in pyr.-d_5) δ: 2.05 (1H, br t, *J*=12 Hz), 2.24 (3H, s), 2.35 (1H, br d, *J*=12 Hz), 3.69 (3H, s), 3.88 (3H, s), 3.75—4.56 (6H), 4.99 (1H, br d, *J*=12 Hz), 5.07 (2H, br s), 5.24 (1H, br s), 5.57 (1H, d, *J*=8 Hz), 6.96 (2H, d, *J*=9 Hz), 7.30 (2H, d, *J*=9 Hz).

Acid Hydrolysis of Triphyllin A (I)——Triphyllin A (I, 30 mg) was hydrolyzed with 3% HCl (5 ml) at 90 °C for 3 h. The reaction mixture was neutralized with 3% Na_2CO_3 solution and evaporated. The residue was chromatographed on silica gel using 30% MeOH in $CHCl_3$ as an eluent to yield 5 mg of D-glucose, $[\alpha]_D^{20}$ +41° (*c*=0.2, H_2O). Its trimethylsilyl ether was identical with an authentic sample on GLC, t_R 7.7 and 11.1 min (column temp., 180 °C).

Permethylation of Triphyllin A (I)——NaH (0.5 g) was added to dimethyl sulfoxide (10 ml) and the mixture was stirred at 70 °C for 1 h under N_2. A solution of tirphyllin A (I, 100 mg) in dimethyl sulfoxide (5 ml) was added, and the whole was stirred at room temperature for 1 h under N_2. Then, CH_3I (10 ml) was added, and the mixture was stirred at room temperature for 2 h under N_2, then poured into ice-water. The products were extracted with EtOAc. The extract was washed with water, dried over anhydrous Na_2SO_4, and evaporated. The residue was subjected to PLC (solvent system, $CHCl_3$: Et_2O = 3 : 1) to yield 40 mg of the undecamethyl ether (VII).

The Undecamethyl Ether VII——Colorless syrup, $[\alpha]_D^{20}$ −4° (*c*=0.7, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3010, 2940, 2840, 1605, 1520, 1455, 1385, 1315, 1135, 1095. ^{1}H-NMR (100 MHz, in $CDCl_3$) δ: 1.80 (1H, br t, *J*=12 Hz), 2.12 (3H, s), 2.21 (1H, br d, *J*=12 Hz), 3.35 (6H, s), 3.41 (3H, s), 3.44 (3H, s), 3.54 (3H, s), 3.56 (3H, s), 3.58 (3H, s), 3.63 (3H, s), 3.66 (3H, s), 3.69 (3H, s), 3.82 (3H, s), 3.0—3.9 (12H), 4.62 (2H, br s), 4.75 (1H, d, *J*=7 Hz), 4.88 (1H, d, *J*=7 Hz), 5.29 (1H, br d, *J*=12 Hz), 6.89 (2H, d, *J*=9 Hz), 7.34 (2H, d, *J*=9 Hz).

Conversion of Triphyllin B (II) into Triphyllin A (I)——An excess of diazomethane in ether was added to a solution of triphyllin B (II, 100 mg) in MeOH (5 ml), and the solution was allowed to stand for 6 h. The solvent was evaporated off and the residue was recrystallized from a mixture of EtOH and water to give triphyllin A (I, 60 mg). This product was found to be identical with an authentic sample on direct comparison (TLC, IR, ^{1}H-NMR, ^{13}C-NMR).

Enzymatic Hydrolysis of Triphyllin C (VIII)——A solution of triphyllin C (VIII, 15 mg) and crude hesperidinase (50 mg) in 0.05 M citrate buffer (pH 4.0, 20 ml) was stirred at 40 °C for 2 h. The reaction mixture was extracted with EtOAc. The extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel using $CHCl_3$ as an eluent to yield 6 mg of compound X. The water layer was neutralized with 5% $NaHCO_3$ solution and concentrated. The residue was chromatographed on silica gel using 20% MeOH in $CHCl_3$ as an eluent to yield 2 mg of D-glucose, $[\alpha]_D^{20}$ +32° (*c*=0.1, H_2O). Its trimethylsilyl ether was identical with an authentic sample on GLC; t_R 7.7 and 11.1 min (column temperature, 180 °C).

Compound X——Pale yellow needles from MeOH, mp 127—128 °C, $[\alpha]_D^{20}$ −20° (*c*=0.2, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3200, 1645, 1615, 1525, 1365, 1300, 1275, 1210, 1180, 1125. UV λ_{max}^{MeOH} nm (log ε): 227 (4.15), 294 (3.84), 341 (3.30). ^{1}H-NMR (60 MHz, in $CDCl_3$) δ: 2.00 (3H, s), 2.78 (1H, dd, *J*=18, 5 Hz), 3.08 (1H, dd, *J*=18, 10 Hz), 3.47 (3H, s), 3.82 (3H, s), 4.75 (2H, s), 5.38 (1H, dd, *J*=10, 5 Hz), 6.92 (2H, d, *J*=9 Hz), 7.38 (2H, d, *J*=9 Hz). MS *m/z*: 344 (M^+), 312, 178, 134.

Methylation of Compound X——Compound X (5 mg) was dissolved in acetone (3 ml), and then CH_3I (1 ml) and anhydrous K_2CO_3 (300 mg) were added. The mixture was stirred under reflux for 10 h. After cooling, the mixture was

filtered and the filtrate was evaporated. The residue was subjected to PLC (solvent system, $CHCl_3$: Et_2O = 7 : 1) to yield 2 mg of compound X dimethyl ether (XIII).

XIII——Pale yellow needles from MeOH, mp 95—97 °C, $[\alpha]_D^{20}$ +40° (c = 0.05, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1680, 1590, 1515, 1405, 1255, 1115. UV λ_{max}^{MeOH} nm (log ε): 229 (4.29), 273 (3.80), 328 (3.35). ^{1}H-NMR (60 MHz, in $CDCl_3$) δ: 2.15 (3H, s), 2.80 (1H, dd, J = 17, 4 Hz), 3.05 (1H, dd, J = 17, 11 Hz), 3.43 (3H, s), 3.84 (6H, s), 3.89 (3H, s), 4.44 (2H, br s), 5.36 (1H, dd, J = 11, 4 Hz), 6.92 (2H, d, J = 9 Hz), 7.36 (2H, d, J = 9 Hz). MS m/z: 372 (M^+), 341, 238, 207, 179, 134.

Dehydrogenation of XIII——XIII (2 mg) was dissolved in xylene (1 ml), and chloranil (10 mg) was added. The mixture was refluxed for 8 h and subjected to PLC (solvent system, $CHCl_3$: EtOAc = 2 : 1) to yield 0.3 mg of dehydrogenated product. It was identical with an authentic sample (XVI) synthesized from matteucinol dimethyl ether (XI) on direct comparison (IR, TLC, mixed fusion).

Isolation of Matteucinol and Matteucinol 7-*O*-*β*-D-Glucoside——Air-dried fronds (500 mg) of *Matteuccia orientalis* (HOOK.) TREV. (Japanese name: Inugansoku, Aspidiaceae) collected in September at Mt. Mitsuishi, Chiba prefecture, were extracted 3 times with 3 l of MeOH under reflux for 6 h. The combined extracts and then 10 l of MeOH were passed over activated charcoal (60 g) packed in a column of 6 cm diameter. The resulting solution was concentrated to a syrup under reduced pressure. The syrup was chromatographed on a silica gel column (120 g) using $CHCl_3$ (1200 ml, frac. 1), 10% MeOH in $CHCl_3$ (600 ml) and 20% MeOH in $CHCl_3$ (1200 ml, frac. 2) as eluents. Fraction 1 was rechromatographed on silica gel using $CHCl_3$ as an eluent, followed by PLC (solvent system, $CHCl_3$: Et_2O = 3 : 1) to yield 400 mg of matteucinol (XII). Fraction 2 was rechromatographed on silica gel using 10% MeOH in $CHCl_3$ as an eluent to yield a mixture of matteucinol 7-*O*-*β*-D-glucoside and desmethoxymatteucinol 7-*O*-*β*-D-glucoside. The mixture was subjected to high performance liquid chromatography on Hitachi gel 3019 (solvent, 80% MeOH) to yield 35 mg of matteucinol 7-*O*-*β*-D-glucoside (IX).

Matteucinol (XII)——Pale yellow needles from $CHCl_3$, mp 169—171 °C, $[\alpha]_D^{20}$ −22° (c = 1.0, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3300, 1630, 1610, 1520, 1260, 830. UV λ_{max}^{MeOH} nm (log ε): 226 (4.56), 297 (4.37), 347 (3.70). ^{1}H-NMR (100 MHz, in $CDCl_3$) δ: 2.06 (3H, s), 2.08 (3H, s), 2.78 (1H, dd, J = 17, 4 Hz), 3.07 (1H, dd, J = 17, 12 Hz), 3.83 (3H, s), 5.31 (1H, dd, J = 12, 4 Hz), 6.91 (2H, d, J = 8 Hz), 7.35 (2H, d, J = 8 Hz). MS m/z: 314 (M^+), 207, 206, 181, 180, 152, 134, 121, 119, 91.

Matteucinol 7-*O*-*β*-D-Glucoside——Pale yellow needles from MeOH, mp 164—165 °C, $[\alpha]_D^{20}$ +10° (c = 0.4, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3430, 1645, 1590, 1520, 1125, 1070. UV λ_{max}^{MeOH} nm (log ε): 223 (4.58), 281 (4.31), 362 (3.68); $\lambda_{max}^{MeOH+AlCl_3}$ nm: 225, 289, 313, 362. ^{1}H-NMR (100 MHz, in pyr.-d_5) δ: 2.53 (3H, s), 2.62 (3H, s), 2.90 (1H, dd, J = 17, 4 Hz), 3.21 (1H, dd, J = 17, 12 Hz), 3.89 (3H, s), 3.8—4.6 (6H), 5.37 (1H, dd, J = 12, 4 Hz), 7.00 (2H, d, J = 8 Hz), 7.49 (2H, d, J = 8 Hz). MS m/z: 476 (M^+), 314 (aglycone), 180, 152, 134. CD: $[\theta]_{285}^{20}$ −44700° (c = 0.003, MeOH).

Methylation of Matteucinol——Matteucinol (XII, 200 mg) was dissolved in acetone (20 ml), then CH_3I (10 ml) and anhydrous K_2CO_3 (3 g) were added. The mixture was stirred under reflux for 18 h and filtered. The filtrate was evaporated and the residue was chromatographed on silica gel using $CHCl_3$ as an eluent to yield 120 mg of matteucinol dimethyl ether (XI).

Matteucinol Dimethyl Ether (XI)——Yellow needles from MeOH, mp 113—114 °C, $[\alpha]_D^{20}$ +51° (c = 0.7, MeOH). IR ν_{max}^{KBr} cm^{-1}: 1685, 1615, 1590, 1520, 1345, 1250, 1160, 1115, 840, 830. UV λ_{max}^{MeOH} nm (log ε): 226 (4.46), 273 (4.06), 332 (3.55). ^{1}H-NMR (60 MHz, in $CDCl_3$) δ: 2.14 (3H, s), 2.16 (3H, s), 2.74 (1H, dd, J = 16, 5 Hz), 3.05 (1H, dd, J = 16, 10 Hz), 3.74 (3H, s), 3.81 (3H, s), 3.83 (3H, s), 5.36 (1H, dd, J = 10, 5 Hz), 6.91 (2H, d, J = 9 Hz), 7.39 (2H, d, J = 9 Hz). MS m/z: 342 (M^+), 208, 193, 165, 134.

Dehydrogenation and Hydroxylation of Matteucinol Dimethyl Ether (XI)——A mixture of matteucinol dimethyl ether (26 mg), NBS (27 mg) and benzoyl peroxide (0.5 mg) in CCl_4 (4 ml) was refluxed for 1.5 h and evaporated under reduced pressure. The residue was suspended in a mixture of dioxane (8 ml) and water (3 ml) and refluxed for 8 h. The reaction mixture was poured into ice-water and the products were extracted with ether. The extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated. The residue was subjected to PLC (solvent system, $CHCl_3$: EtOAc = 2 : 1) to yield XIV (5 mg), XV (5 mg) and XVIII (3 mg).

XIV——Colorless needles from a mixture of *n*-hexane and EtOAc, mp 184—185 °C. UV λ_{max}^{MeOH} nm (log ε): 266 (4.35), 322 (4.51). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1645, 1610, 1595, 1515, 1375, 1110, 835. MS m/z: 356 (M^+), 341, 310, 298.

XV——Colorless needles from a mixture of *n*-hexane and EtOAc, mp 222—223 °C. UV λ_{max}^{MeOH} nm (log ε): 266 (4.05), 322 (4.25). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1645, 1610, 1595, 1515, 1370, 1125, 835. MS m/z: 356 (M^+), 341, 323, 311, 292.

XVIII——Colorless needles from a mixture of *n*-hexane and EtOAc, mp 201—202 °C. UV λ_{max}^{MeOH} nm (log ε): 268 (3.94), 321 (4.12). IR ν_{max}^{KBr} cm^{-1}: 1650, 1610, 1595, 1515, 1375, 1275, 830. MS m/z: 340 (M^+), 325, 307.

Dehydrogenation and Methoxylation of Matteucinol Dimethyl Ether (XI)——A mixture of matteucinol dimethyl ether (51 mg), NBS (54 mg) and benzoyl peroxide (0.5 mg) in CCl_4 (8 mg) was refluxed for 1.2 h and evaporated. The residue was dissolved in MeOH (20 ml) and the solution was refluxed for 6 h, poured into water and extracted with EtOAc. The extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated. The residue was subjected to PLC (solvent system, *n*-hexane : EtOAc = 1 : 1) to yield XVI (11 mg), XVII (10 mg) and XVIII (6 mg).

XVI——Colorless needles from a mixture of *n*-hexane and EtOAc, mp 179—180 °C. UV λ_{max}^{MeOH} nm (log ε): 266 (3.92), 322 (4.07). IR ν_{max}^{KBr} cm^{-1}: 1650, 1610, 1595, 1515, 1470, 1370, 1270, 1010, 830. MS m/z: 370 (M^+), 355, 339,

325, 310.

XVII——Colorless needles from a mixture of *n*-hexane and EtOAc, mp 155—158 °C. UV λ_{max}^{MeOH} nm (log ε): 266 (3.79), 321 (4.05). IR ν_{max}^{KBr} cm^{-1}: 1650, 1615, 1595, 1515, 1370, 1275, 830. MS *m/z*: 370 (M^+), 355, 337, 325, 309, 295.

Methylation of XIV (or XV)——XIV (or XV) (3 mg) was dissolved in CH_3I (5 ml) and 300 mg of Ag_2O was added. The mixture was stirred under reflux for 5 h and filtered. The filtrate was evaporated under reduced pressure. The residue was crystallized from *n*-hexane to yield colorless needles of XVI (or XVII). The product was identical with an authentic sample on direct comparison (TLC, IR, mixed fusion).

References and Notes

1) Part LX: T. Kuraishi, H. Maehashi, T. Murakami, Y. Saiki and C.-M. Chen, *Yakugaku Zasshi*, **105**, 937 (1985).
2) Present address: *Universidad Nacional de Cordoba, Calle Obispo Trejo Y Sanabria 242, 5000 Cordoba, Argentina.*
3) N. Tanaka, T. Sada, T. Murakami, Y. Saiki and C.-M. Chen, *Chem. Pharm. Bull.*, **32**, 490 (1984).
4) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
5) Assignments may be reversed, though those given are preferred because 2.10 is nearer than 2.28 to the chemical shift (2.17) of the methyl group at C-8 of I. In the case of the reversed assignments, pyridine-induced chemical shifts of 1.03 and 0.37 ppm are estimated for the methyl groups at C-6 and C-8, respectively. These values also lead to the same conclusion.
6) P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari and E. Wenkert, *J. Am. Chem. Soc.*, **90**, 5480 (1968).
7) If the 7-hydroxyl group is present in a free form, the elimination of the 4-hydroxyl group occurs easily under such mild conditions as enzymatic hydrolysis (pH 4.0). Presumably, the formation of a *p*-quinone methide form of the A-ring facilitates this elimination. Subsequent ether linkage formation occurs in such a manner as to make an axial bond in order to minimize the steric interaction with the oxygen atom attached to C-5.
8) T. J. Mabry, K. R. Markham and M. B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, 1970, p. 171.
9) A. Hiraoka, *Biochem. Syst. Ecol.*, **6**, 171 (1978); K. Mohri, T. Takemoto and Y. Kondo, *Yakugaku Zasshi*, **102**, 310 (1982).
10) Matteucinol dimethyl ether (XI) was obtained from matteucinol (XII), which has been isolated from *M. orientalis* (HOOK.) TREV., by treatment with methyl iodide and potassium carbonate in acetone.
11) W. Gaffield, *Tetrahedron*, **26**, 4093 (1970).