Studies on the Constituents of *Atractylodes lancea*

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From the polar portion of the methanolic extract of the fresh rhizomes of *Atractylodes lancea* DC., nine new sesquiterpene glycosides [two guaiane-type glycosides, atractylosides A and B (1 and 2), and seven eudesmane-type glycosides, atractylosides C, D, E, F, G, H and I (3—9)] were obtained and their structures were characterized by enzymatic hydrolysis, spectroscopic means and X-ray analysis.

Keywords *Atractylodes lancea*; Compositae; sesquiterpene glycoside; guaiane type; eudesmane type; atractyloside; X-ray analysis

The rhizomes (Japanese name: Jutsu) of *Atractylodes* plants (Compositae) are an important crude drug used for regulation of body water (Kusuizai) and as a stomachic. Crude drugs derived from plants of this genus include Atractylodis Lanceae Rhizoma (*Atractylodes lancea* DC. or its varieties: 蒼朮 Changzhu) and Atractylodis Rhizoma (*A. japonica* KOIDZUMI or *A. ovata* DC.: 白朮 Baizhu). With regard to the ingredients of these crude drugs, the essential oils such as atractylon, hinesol and β-eudesmol are known, but the constituents in the polar portion of the methanolic extract of *A. lancea* have not yet been surveyed. We have now obtained nine new sesquiterpene glycosides together with syringin and tryptophan. This paper deals with the structural characterization of these new sesquiterpene glycosides of guaiane type, atractylosides A (**1**) and B (**2**), and of eudesmane type, atractylosides C, D, E, F, G, H and I (**3**—**9**).

Guaiane-Type Glycosides Atractyloside A (**1**), colorless needles, mp 227—230 °C, $[\alpha]_D$ +20.0°, showed a cluster ion peak $[M+H]^+$, at *m/z* 449 in the positive fast atom bombardment mass spectrometry (FAB-MS) and absorption bands due to a five-membered ring ketone at 1740 cm^{-1}. The carbon-13 nuclear magnetic resonance (^{13}C-NMR; Table I) exhibited fifteen carbon signals due to three methyls, four methylenes, three methines, four oxygenated carbons (s × 3 and t × 1) and one carbonyl group along with signals due to the β-D-glucopyranosyl residue (C-1′—C-6′, δ 96.5, 73.6, 76.5, 70.1, 75.8, 61.1). The ^{1}H–^{1}H shift-correlated spectrum (COSY) and ^{1}H–^{13}C COSY of **1** suggested the presence of a sequence of a ■–CH_2–CH_2–CH(■)–CH_2–CH(■)–CH(■)–CH_2–■ in the molecule and enabled us to assign the respective ^{1}H signals. This result also implied that **1** should be a sesquiterpene glycoside of guaiane type. The ring juncture was supposed to be *trans* from the coupling constant (J=11 Hz) between H-1 and H-5. On enzymatic hydrolysis **1** provided an aglycone (**1a**) and D-glucose. In the ^{13}C-NMR spectrum of **1**, signals due to two methyl groups at δ 23.2 and 23.5 were shifted by −4.2 and −4.7 ppm and a signal due to the carbon bearing a tertiary hydroxyl group at δ 80.5 was shifted by +7.8 ppm by comparison with those of **1a**, suggesting that the glucosyl moiety should be linked to the *tert*-hydroxyl group.[1] Oxidative ring-fission of **1** with *m*-chloroperbenzoic acid yielded a product (**1b**), whose proton nuclear magnetic resonance (^{1}H-NMR) and ^{13}C-NMR spectra showed a methyl ketone signal at δ 2.28 (3H, s), and signals due to a carbonyl and a carboxyl groups at δ 210.1 and 176.0, suggesting the occurrence of a 3-keto-4-hydroxyl partial structure in **1**. Therefore, the structure of **1** was deduced to be 1,5-*trans*-4,10,11,14-tetrahydroxyguaian-3-one 11-*O*-β-D-glucopyranoside. In order to verify this putative structure and determine the configurations, a single crystal of **1** was subjected to the X-ray analysis. Crystal data: $C_{21}H_{36}O_{10}$; orthorhombic $P2_12_12_1$; a=17.433 (4), b=19.447 (4), c=6.347 (1) Å; V=2151.7 (7) Å^3; Z=4. All unique diffraction maxima with $2\theta<120°$ were collected on a four-circle diffractometer using graphite-monochromated CuK_α (λ=

TABLE I. ^{13}C-NMR Data for **1**, **1a** and **2** (in Pyridine-d_5)

	1	1a	2
C-1	42.1	43.6	44.0
C-2	34.8	35.9	37.9
C-3	221.4	220.6	79.7
C-4	74.2	74.3	75.8
C-5	45.4	46.5	58.5
C-6	28.5	28.1	31.1
C-7	47.3	51.0	45.9
C-8	21.7	28.7	24.4
C-9	34.3	30.1	31.9
C-10	81.5	81.0	81.3
C-11	80.5	72.7	80.7
C-12	23.2	27.4	24.1
C-13	23.5	28.2	25.0
C-14	68.4	70.1	68.6
C-15	17.6	19.1	16.0
Glc-1′	96.5		98.6
Glc-2′	73.6		75.3
Glc-3′	76.5		78.8
Glc-4′	70.1		71.8
Glc-5′	75.8		78.1
Glc-6′	61.1		62.8

TABLE II. Atomic Coordinates ($\times 10^4$ or 10^3) and Equivalent Isotropic Temperature Factors ($Å^2 \times 10^2$) with Their e.s.d. Values in Parentheses for **1**

Atom	x	y	z	B_{eq}
O3	5595 (1)	4065 (1)	4095 (5)	3.89 (6)
O4	4778 (1)	2849 (1)	5593 (4)	2.64 (5)
O10	7031 (1)	1799 (1)	1793 (4)	2.81 (5)
O11	5079 (1)	503 (1)	6949 (3)	1.97 (4)
O14	8607 (1)	2059 (1)	1612 (4)	2.76 (5)
C1	6848 (1)	2620 (1)	4598 (5)	1.84 (6)
C2	6627 (2)	3264 (1)	3319 (5)	2.49 (6)
C3	5892 (2)	3508 (1)	4337 (5)	2.23 (6)
C4	5584 (2)	2943 (1)	5811 (5)	2.01 (6)
C5	6056 (1)	2307 (1)	5150 (5)	1.84 (6)
C6	6052 (2)	1710 (1)	6724 (5)	1.96 (6)
C7	6286 (1)	1024 (1)	5694 (5)	2.07 (6)
C8	7170 (2)	924 (2)	5456 (6)	2.94 (7)
C9	7668 (2)	1568 (2)	5179 (5)	2.47 (7)
C10	7408 (2)	2115 (1)	3578 (5)	2.05 (6)
C11	5922 (1)	400 (1)	6843 (5)	2.15 (6)
C12	6100 (2)	−275 (1)	5720 (7)	3.26 (8)
C13	6127 (2)	349 (2)	9167 (6)	3.10 (7)
C14	8099 (2)	2514 (2)	2730 (6)	2.60 (7)
C15	5718 (2)	3167 (2)	8077 (5)	2.56 (7)
O1′	4701 (1)	1096 (1)	3999 (3)	2.17 (4)
O2′	3731 (1)	−242 (1)	7005 (4)	2.53 (4)
O3′	2514 (1)	312 (1)	4681 (3)	2.46 (4)
O4′	2951 (1)	1024 (1)	782 (4)	3.07 (5)
O6′	4203 (1)	2321 (1)	1902 (4)	2.90 (5)
C1′	4640 (2)	464 (1)	5121 (5)	1.92 (6)
C2′	3811 (2)	384 (1)	5864 (5)	1.84 (6)
C3′	3291 (1)	362 (1)	3931 (5)	1.92 (6)
C4′	3410 (2)	1014 (1)	2646 (5)	1.89 (6)
C5′	4265 (2)	1074 (1)	2076 (5)	2.01 (6)
C6′	4465 (2)	1714 (1)	862 (5)	2.46 (6)
HC1	711 (2)	279 (2)	606 (5)	2.6 (0.7)
H1C2	653 (2)	307 (2)	176 (7)	4.7 (0.9)
H2C2	707 (2)	367 (2)	332 (6)	4.2 (0.8)
HC5	584 (2)	209 (2)	363 (6)	3.0 (0.7)
H1C6	552 (2)	165 (1)	726 (5)	1.5 (0.6)
H2C6	636 (2)	181 (1)	800 (5)	2.2 (0.6)
HC7	610 (2)	104 (2)	413 (6)	2.9 (0.7)
H1C8	727 (2)	58 (2)	415 (6)	3.5 (0.7)
H2C8	738 (2)	65 (2)	664 (6)	4.0 (0.9)
H1C9	773 (2)	184 (2)	652 (7)	4.6 (0.9)
H2C9	821 (2)	138 (2)	473 (6)	3.7 (0.8)
H1C12	667 (2)	−42 (2)	580 (6)	3.5 (0.8)
H2C12	577 (2)	−69 (2)	639 (6)	2.9 (0.7)
H3C12	600 (3)	−31 (2)	408 (8)	6.6 (1.2)
H1C13	670 (2)	22 (2)	941 (7)	5.3 (1.0)
H2C13	600 (2)	79 (2)	992 (7)	4.5 (0.9)
H3C13	591 (2)	−12 (2)	971 (7)	4.0 (0.8)
H1C14	794 (2)	293 (2)	174 (6)	3.7 (0.8)
H2C14	836 (2)	272 (2)	390 (6)	2.5 (0.7)
H1C15	625 (2)	331 (2)	843 (6)	3.0 (0.7)
H2C15	540 (2)	363 (2)	837 (7)	4.6 (0.9)
H3C15	558 (2)	277 (2)	901 (7)	4.7 (0.9)
HC1′	481 (2)	4 (2)	403 (5)	2.9 (0.7)
HC2′	366 (2)	79 (1)	695 (5)	2.0 (0.6)
HC3′	343 (2)	−7 (1)	303 (5)	2.0 (0.6)
HC4′	324 (2)	142 (2)	358 (5)	2.2 (0.6)
HC5′	442 (2)	61 (2)	124 (5)	2.4 (0.6)
H1C6′	505 (2)	176 (2)	83 (6)	4.3 (0.9)
H2C6′	422 (2)	165 (2)	−57 (7)	3.9 (0.8)
HO4	462 (3)	265 (2)	433 (9)	7.7 (1.3)
HO10	752 (3)	154 (2)	91 (8)	7.2 (1.2)
HO14	887 (4)	228 (3)	55 (12)	13.0 (2.1)

Coordinates ($\times 10^3$).

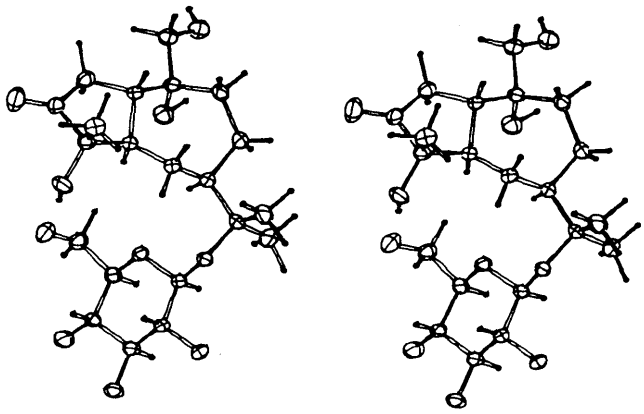
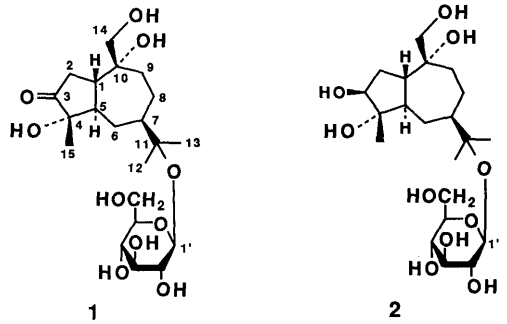


Fig. 1. ORTEP Drawing of **1**



1.5418 Å) radiation and the $2\theta-\omega$ scan technique. After correcting for Lorentz and polarization effects, 1816 independent reflections were observed, $I \geq 2.3\sigma(I)$. The structure was solved by the direct method with the MULTAN 82[2] series of programs. A block-diagonal least-squares refinement with anisotropic nonhydrogen atoms and isotropic hydrogen atoms lowered the R value to 0.036. The final atomic coordinates are listed in Table II and the ORTEP drawing is shown in Fig. 1. Consequently, the structure including the absolute configuration was concluded to be as shown in the formula.

Atractyloside B (**2**), a white powder, $[\alpha]_D$ −7.8° showed a $[M+Na]^+$ at m/z 473 in the positive FAB-MS. The ^1H- and ^{13}C-NMR spectra (Table I) of **2** were similar to those of **1**, except that a signal at δ 221.4 (s) due to the carbonyl group at C-3 in the ^{13}C-NMR spectrum of **1** had disappeared and a new signal at 79.7 (d) assignable to carbon bearing a *sec*-hydroxyl group appeared in that of **2**. Reduction of **1** with $NaBH_4$ afforded a product identical with **2**, and thus the structure of **2** was concluded to be 1,5-*trans*-3,4,10,11,14-pentahydroxyguaiane 11-*O*-β-D-glucopyranoside. The configuration at C_3-OH was concluded to be β from the observation of the nuclear Overhauser effects (NOE) between H_3-15 and H_2-6, H-1, H-2, and between H-3 and H-5, H-2α in the ^1H-NMR spectrum of the aglycone (**2a**) obtained by enzymatic hydrolysis of **2**.

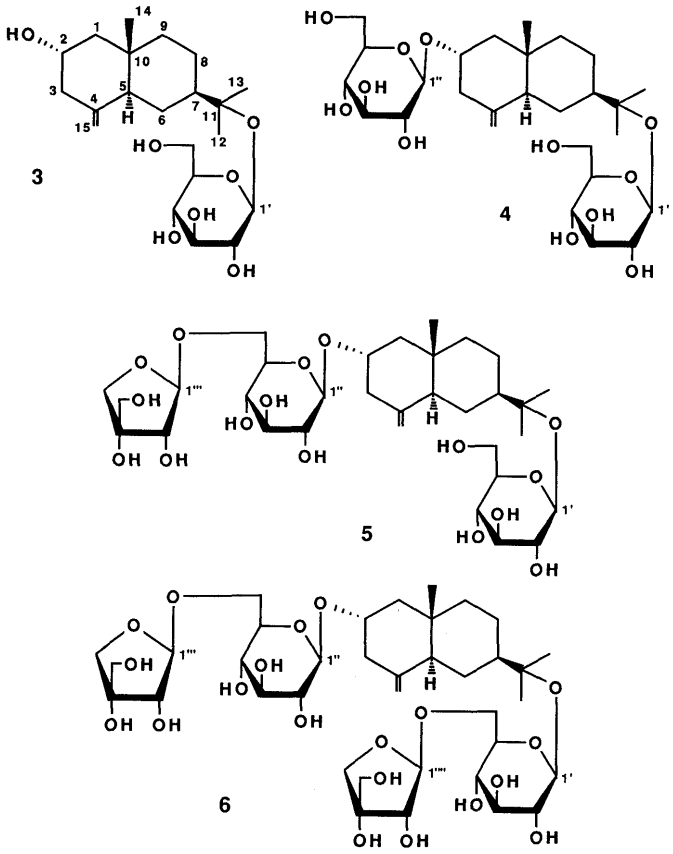
Eudesmane-Type Glycosides Atractylosides C (**3**), a white powder, $[\alpha]_D$ +12.0° (MeOH), D (**4**), a white powder, $[\alpha]_D$ −15.0° (MeOH), E (**5**), a white powder, $[\alpha]_D$ −39.4° (MeOH) and F (**6**), a white powder, $[\alpha]_D$ −99.8° (MeOH) showed the respective cluster ion peaks due to the molecular at m/z 399 $[M-H]^-$, 561 $[M-H]^-$, 717 $[M+Na]^+$ and 825 $[M-H]^-$ in the positive or negative FAB-MS. On

TABLE III. ^{13}C-NMR Data for **3**, **3a**, **4**, **5**, **6**, **7**, **7a**, **8** and **9** (in Pyridine-d_5)

	3	3a	4	5	6	7	7a	8	9
C-1	52.1	52.2	48.1	47.9	48.1	49.3	49.8	46.8	54.5
C-2	67.3	67.3	75.3	75.6	75.8	73.4	73.5	84.4	194.8
C-3	47.6	49.7	47.7	45.1	45.4	79.6	79.7	78.9	151.0(s)
C-4	150.2	150.6	150.6	148.5	148.7	152.3	152.6	150.3	146.9(s)
C-5	49.8	50.0	49.7	49.4	49.6	48.6	48.5	48.6	48.5
C-6	22.2	22.5	22.2	22.0	22.1	22.2	28.5	22.1	22.1
C-7	48.8	49.7	48.7	48.5	48.7	48.5	49.4	48.0	47.5
C-8	24.8	25.3	24.9	24.8	25.0	25.4	25.6	25.4	24.7
C-9	41.4	41.5	41.3	41.0	41.2	41.1	41.2	40.7	40.0
C-10	35.5	35.5	35.5	35.1	35.2	35.5	35.4	35.1	37.0
C-11	79.5	71.4	79.4	79.3	79.5	79.5	71.4	80.4	79.5
C-12	24.1	27.7	24.0	23.6	23.9	24.1	27.1	24.0	23.5
C-13	25.2	28.1	24.8	24.5	24.9	24.8	27.7	24.8	25.4
C-14	17.7	17.6	17.4	17.2	17.4	17.8	17.8	17.4	15.2
C-15	107.4	107.4	108.1	108.1	108.3	104.8	104.8	106.3	16.9
Glc-1′	98.7		98.7	98.4	98.7	98.7		98.7	98.7
Glc-2′	75.4		75.3[a]	75.0[a]	75.3[a]	75.7		74.7[a]	75.4[a]
Glc-3′	79.0		78.7[b]	78.4[b]	78.6[b]	78.9		78.0[b]	78.9[b]
Glc-4′	72.0		71.7[c]	71.7[c]	71.9[c]	72.0		72.0[c]	71.9[c]
Glc-5′	78.1		78.0[d]	77.7	77.9	78.6		77.8	78.4[d]
Glc-6′	63.2		62.8[e]	62.9	69.0	63.1		63.1	63.1[e]
Glc-1″			103.2	103.4	103.3			104.3	105.7
Glc-2″			75.4[a]	75.2[a]	75.4[a]			75.2[a]	76.2[a]
Glc-3″			79.0[b]	78.7[b]	79.0[b]			78.3[b]	78.6[b]
Glc-4″			72.0[c]	71.8[c]	72.0[c]			71.8[c]	71.4[c]
Glc-5″			78.4[d]	77.0	77.9			77.0	78.1[d]
Glc-6″			63.2[e]	69.2	69.2			69.1	62.7[e]
Api-1‴				110.9	111.2			111.2	
Api-2‴				76.8	77.0[d]			76.7	
Api-3‴				80.3	80.5			79.4	
Api-4‴				74.8	75.1			75.2	
Api-5‴				65.5	65.8			65.8	
Api-1⁗					111.2				
Api-2⁗					76.2[d]				
Api-3⁗					80.5				
Api-4⁗					75.1				
Api-5⁗					65.8				

a—*e*) may be interchangeable in each column.

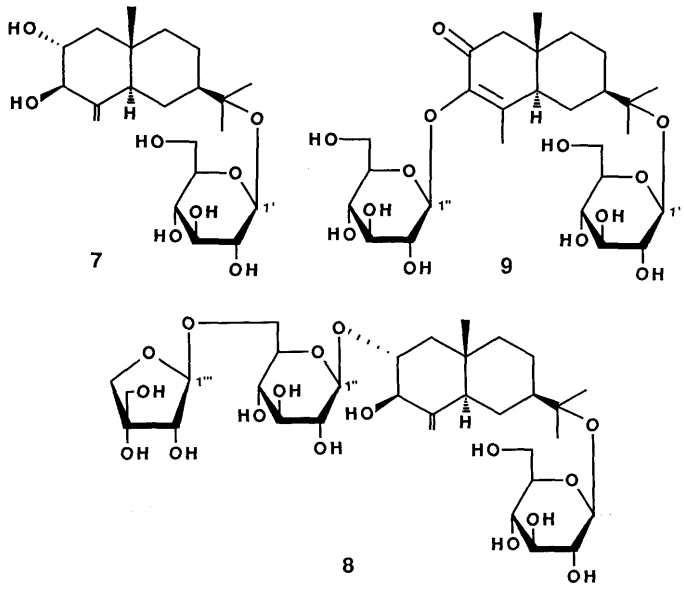
enzymatic hydrolysis, they all gave the same aglycone (**3a**), a white powder, $[\alpha]_D$ +31.7° (MeOH) (from **3**), whose ^{1}H-NMR spectrum was analogous to that of β-eudesmol, but it exhibited a signal (1H, dt, J=3, 12 Hz) due to a carbinol proton. Based on this coupling constant, it was concluded to be pterocarpol (2α-hydroxy-β-eudesmol).[3] This was also confirmed by the ^{1}H–^{1}H COSY and ^{13}C-NMR spectra (Table III). Liberated sugars were also detected by thin layer chromatography (TLC); glucose was obtained from **3** and **4**, and glucose and apiose from **5** and **6**. The sugar linkages were determined from the glycosylation shifts.[1] As listed in Table III, shifts at C-11 (+8.1 ppm) in **3**, and at C-2 (+8.0—+8.5 ppm) and C-11 (+7.9—+8.1 ppm) in **4**, **5** and **6** compared with those in **3a** were observed in the ^{13}C-NMR spectra. Moreover, the ^{13}C-NMR data indicated the presence of the β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl moiety in **5** and **6**. Therefore, the structures of **3** and **4** could be represented as 11-*O*-β-D-glucopyranosyl pterocarpol and 2,11-di-*O*-β-D-glucopyranosyl pterocarpol. Atractyloside F (**6**) was formulated as 2,11-di-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl pterocarpol. The electron impact mass spectrum (EI-MS) of the acetate of **5** showed peaks at *m/z* 766, 547, 331 and 259 due to $[M-\text{hexose}-OH]^+$, pentosyl hexosyl cation,



hexosyl cation and pentosyl cation, rspectively, in the EI-MS. Since the terminal hexosyl moiety attached to the *tert*-hydroxyl group is readily cleaved, the occurrence of the prominent peak at *m/z* 547 suggested that the terminal glucose is linked to the C-11-OH. Thus, the structure of **5** was tentatively deduced to be 2-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl pterocarpol 11-*O*-β-D-glucopyranoside.

Atractyloside G (**7**), a white powder, $[\alpha]_D$ +2.2° (MeOH) showed a cluster ion $[M-H]^-$ at *m/z* 415 in the negative FAB-MS. Enzymatic hydrolysis provided an aglycone (**7a**) and glucose. The ^{1}H-NMR signals of **7a** resembled those of pterocarpol (**3a**), except that a change in the signal pattern at H-2 (ddd, J=5, 9, 12 Hz, δ 4.11) and appearance of the oxygenated methine proton signal (1H, br d, J=9 Hz) at δ 4.35 were observed. The ^{1}H–^{1}H COSY of **7a** revealed a correlation of the above two protons, and thus **7a** was regarded as 3β-hydroxypterocarpol. The ^{13}C-NMR data (Table III) were also consistent with the above ^{1}H-NMR data. Furthermore, the ^{13}C-NMR data suggested the presence of a β-D-glucopyranosyl residue attached to the C-11-OH. Therefore, the structure of **7** was represented as 11-*O*-β-D-glucopyranosyl 3β-hydroxypterocarpol.

Atractyloside H (**8**), a white powder, $[\alpha]_D$ −33.2° (MeOH) exhibited a $[M+Na]^+$ at *m/z* 733, giving an aglycone identical with 3β-hydroxypterocarpol (**7a**) together with glucose and apiose. Analysis of the ^{13}C-NMR spectrum of **8** disclosed the presence of apiofuranosyl-(1→6)-β-D-glucopyranosyl and β-D-glucopyranosyl residues in the molecule. The appearance of a prominent peak at *m/z* 553 due to a $[M-\text{glc}-H_2O+Na]^+$ in the positive FAB-MS suggested that the glucosyl residue was linked to



the C-11-OH as in **5**. Therefore, the structure of **8** was tentatively expressed as shown in the formula.

Atractyloside I (**9**), colorless needles, mp 156—158 °C, $[\alpha]_D$ +19.7°, showed cluster ion peaks due to $[M+H]^+$ and $[M+Na]^+$ at *m/z* 577 and 599, respectively, in the positive FAB-MS. The ^{1}H-NMR spectrum of **9** exhibited singlet signals due to methyl groups at δ 0.92, 1.24, 1.29 and 2.01, and signals of two anomeric protons of β-D-glucopyranosyl residues. The ^{13}C-NMR spectrum displayed signals ascribable to two glucopyranosyl residues and fifteen carbons, among which the signals at δ 194.8 (s), 154.3 (s) and 147.1 (s) suggested the presence of an α,β-unsaturated carbonyl group. Since signals due to the olefinic protons in this group were shifted toward lower field than usual, the presence of a hydroxyl group at the α-position on the α,β-unsaturated residue was suggested. Moreover, the molecular weight in the MS also supported the above evidence. The circular dichroism (CD) spectrum of **9** showed a positive Cotton effect having a maximum at 258 nm and a negative Cotton effect having a maximum at 323 nm.[4] The ^{1}H-NMR spectrum of the aglycone (**9a**) obtained by enzymatic hydrolysis of **9** displayed a vinyl methyl signal at δ 2.00 (d, J=2 Hz) ascribable to H_3-15. From the above accumulated evidence, the structure of **9** was determined as 2-keto-3-hydroxy-β-eudesmol 3,11-di-*O*-β-D-glucopyranoside.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. The ultraviolet (UV) and CD spectra were obtained with a Hitachi 556 type spectrometer and a JASCO J-500 spectrometer, respectively. The infrared (IR) spectra were recorded on a Hitachi 260-10 spectrometer. The EI-MS and FAB-MS were taken with JEOL JMS-01SG and JEOL JMS-DX-300 instruments, respectively. The ^{1}H- (400 MHz) and ^{13}C- (100.4 MHz) NMR spectra were recorded on a JEOL JNM-GX-400 spectrometer with tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). X-Ray intensities were collected with an Enraf–Nonius CAD4F-11 automatic diffractometer at room temperature. Column chromatography was performed with Kieselgel 60 (70—230 mesh, Merck), Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.) and MCI gel CHP 20P (75—150 μ, Mitsubishi Chemical Industries, Ltd.) and Bondapak C_{18} (Waters Associates). TLC was conducted on precoated Kieselgel 60 F_{254} plates (Merck, 0.2 mm thick).

Isolation of Atractylosides The fresh rhizomes (3.2 kg) of *A. lancea* DC. were extracted twice with 70% acetone. Concentration of the extracts under reduced pressure afforded a residue (170 g), which was partitioned between benzene and water. The aqueous layer (150 g) was chromatographed on an MCI gel CHP 20P column. Elution with water containing increasing amounts of methanol yielded nine fractions; fr. 1 (0.3 g), fr. 2 (3.3 g), fr. 3 (1.9 g), fr. 4 (1.1 g), fr. 5 (0.8 g), fr. 6 (0.7 g), fr. 7 (2.0 g), fr. 8 (1.0 g) and fr. 9 (1.6 g). Each fraction was further purified as shown in Chart 1 to give atractylosides A—I (**1**—**9**) together with the two known compounds, L-tryptophan, colorless plates, mp 237—239 °C, $[\alpha]_D^{28}$ −49.8° (c=0.25, pyridine), and syringin, colorless crystals, mp 184—186 °C, $[\alpha]_D^{27}$ −23.9° (c=0.50, MeOH). ^{1}H-NMR (methanol-d_4) δ: 3.22—3.78 (glc H_2-6), 3.85 (6H, s, OMe×2), 4.22 (2H, dd, J=2, 6 Hz, H_2-9), 4.88 (1H, d, J=7 Hz, glc H-1), 6.33 (1H, dt, J=6, 16 Hz, H-8), 6.55 (1H, d, J=16 Hz, H-7), 6.74 (2H, s, H-2,6).

Atractyloside A (1) Colorless needles, mp 227—230 °C, $[\alpha]_D^{27}$ +20.0° (c=0.52, 50% MeOH). CD ($c=3.35\times10^{-4}$, MeOH) [θ] (nm): $+5.77\times10^3$ (304), 0 (324), -0.209×10^3 (350). Positive FAB-MS *m/z*: 541 $[M+H+glycerol]^+$, 449 $[M+H]^+$, 431 $[M+H-H_2O]^+$, 253, 252, 235, 233. ^{1}H-NMR (pyridine-d_5) δ: 1.37, 1.40, 1.45 (each 3H, s, H_3-12,13,15), 1.45 (1H, td, J=11, 13 Hz, H-6), 1.74 (1H, td, J=7, 14 Hz, H-8), 2.13 (1H, dd, J=8, 13 Hz, H-9), 2.40 (1H, ddd, J=9, 10, 11 Hz, H-1), 2.46 (1H, ddd, J=4, 7, 14 Hz, H′-8), 2.53 (1H, dd, J=7, 14 Hz, H-9), 2.72 (1H, dd, J=9, 19 Hz, H-2), 2.77 (1H, t, J=11 Hz, H-5), 2.80 (1H, dd, J=5, 13 Hz, H′-6), 3.09 (1H, dd, J=9, 19 Hz, H′-2), 3.84 (1H, m, glc H-5), 3.86 (2H, br s, H_2-14), 3.94 (1H, dd, J=8, 9 Hz, glc H-2), 4.11 (1H, t, J=9 Hz, glc H-4), 4.24 (1H, t, J=9 Hz, glc H-3), 4.26 (1H, dd, J=5, 10 Hz, glc H-6), 4.44 (1H, dd, J=1, 10 Hz, glc H′-6), 4.97 (1H, d, J=8 Hz, glc H-1).

Enzymatic Hydrolysis of 1 A solution of **1** (20 mg) and crude naringinase (20 mg) in water was incubated at 38 °C for 8 h. After purification of the product by silica gel column chromatography with chloroform–methanol=7:1, **1** gave the aglycone (**1a**) (5.7 mg) and D-glucose, $[\alpha]_D^{20}$ +34.7° (c=0.51, H_2O). **1a**: a colorless powder, $[\alpha]_D^{23}$ +23.5° (c=0.57, MeOH). ^{1}H-NMR (pyridine-d_5) δ: 1.41, 1.42, 1.43 (each 3H, s, H_3-12,13,15), 1.61 (1H, td, J=11, 12 Hz, H-6), 1.91 (1H, td, J=9, 12 Hz, H-8), 2.08 (1H, m, H-7), 2.17 (1H, dd, J=9, 14 Hz, H-9), 2.44 (1H, td, J=9, 14 Hz, H-1), 2.51 (1H, ddd, J=5, 9, 12 Hz, H′-8), 2.60 (1H, dd, J=9, 14 Hz, H-9), 2.74 (1H, dd, J=9, 19 Hz, H-2), 2.77 (1H, m, H′-6), 2.82 (1H, dd, J=11, 12 Hz, H-5), 3.17 (1H, dd, J=9, 19 Hz, H′-2), 3.87 (2H, s, H_2-14).

Oxidation of 1 with *m*-Chloroperbenzoic Acid *m*-Chloroperbenzoic acid (20 mg) was added to a solution of **1** (20 mg) in water (10 ml). The mixture was then stirred for 1 h at room temperature and evaporated under reduced pressure to give a residue, which was subjected to silica gel chromatography with $CHCl_3$–MeOH–H_2O=7:3:0.5 to afford **1b** (14 mg). **1b**: a white powder, $[\alpha]_D^{11}$ +59.1° (c=0.23, MeOH). Negative FAB-MS *m/z*: 537 $[M+glycerol-H_2O-H]^-$, 445 $[M-H_2O-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 1.24, 1.39 (each 3H, s, H_3-12,13), 1.25—1.40 (2H, m, H-6,8), 1.93—2.05 (3H, m, H-7, H_2-9), 2.28 (3H, s, H_3-15), 2.35 (1H, dd, J=7, 14 Hz, H′-8), 2.42 (1H, dd, J=10, 12 Hz, H-1), 2.59 (1H, br d, J=12 Hz, H-5), 3.05 (1H, t, J=10 Hz, H-2), 3.18 (1H, dd, J=3, 12 Hz, H′-6), 3.23 (1H, t, J=10 Hz, H′-2), 3.90 (1H, m, glc H-5), 3.92 (1H, dd, J=8, 9 Hz, glc H-2), 3.99, 4.04 (each 1H, d, J=12 Hz, H_2-14), 4.11 (1H, t, J=9 Hz, glc H-4), 4.19 (1H, t, J=9 Hz, glc H-3), 4.26 (1H, dd, J=6, 12 Hz, glc H-6), 4.51 (1H, dd, J=3, 12 Hz, glc H′-6), 4.98 (1H, d, J=8 Hz, glc H-1).

X-Ray Analysis of 1 Crystal data are as follows: $C_{21}H_{36}O_{10}$, M_r=448.51, orthorhombic, space group $P2_12_12_1$, a=17.433 (4), b=19.447 (4), c=6.347 (1) Å, V=2151.7 (7) Å^3, Z=4, D_c=1.384 g·cm^3, $F(000)$=968, T=298 K. Intensity data were recorded on an Enraf–Nonius CAD4F-11 four circle automatic diffractometer with graphite monochromated MoK_α radiation. One thousand eight hundred and sixteen independent reflections were observed on the basis of the criterion $F_o>2\sigma(F_o)$. The structures were solved by the direct method using MULTAN 82 and refined by the block-diagonal least-squares method. The final refinements with anisotropic temperature factors lowered the R value to 0.036.

Atractyloside B (2) A white powder, $[\alpha]_D^{27}$ −7.8° (c=1.00, MeOH). Positive FAB-MS *m/z*: 473 $[M+Na]^+$. ^{1}H-NMR (pyridine-d_5) δ: 1.38, 1.45, 1.52 (each 3H, s, H_3-12,13,15), 1.35—1.45 (2H, m, H-6,8), 1.95 (3H, m, H′-6, H-7,9), 2.18 (1H, m, H-1), 2.28 (1H, t-like, J=11 Hz, H-2), 2.65 (1H, t, J=11 Hz, H-5), 2.86 (3H, m, H′-2,8,9), 3.79, 3.86 (each 1H, d, J=10 Hz, H_2-14), 3.85 (1H, m, glc H-5), 3.95 (1H, dd, J=8, 9 Hz, glc H-2), 4.15 (1H, t, J=9 Hz, glc H-4), 4.23 (1H, t, J=9 Hz, glc H-3), 4.31 (1H, dd, J=3, 10 Hz, glc H-6), 4.48 (1H, br d, J=10 Hz, glc H′-6), 4.74 (1H, t, J=9 Hz, H-3), 5.00 (1H, d, J=8 Hz, glc H-1).

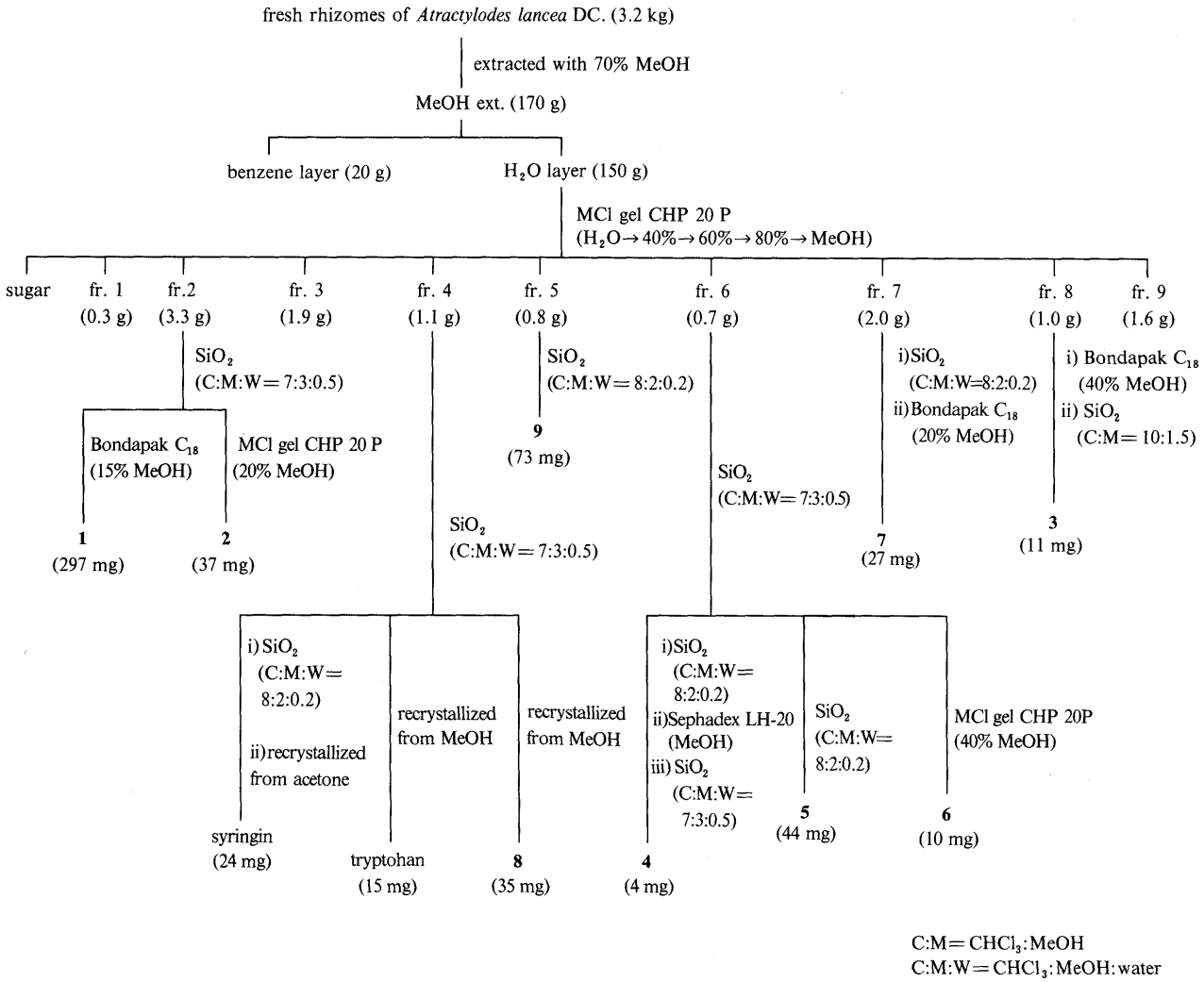


Chart 1

Enzymatic Hydrolysis of 2 A mixture of **2** (37 mg) and crude naringinase (20 mg) in water (20 ml) was incubated at 37 °C for 50 h and then evaporated under reduced pressure to give a residue, which was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O = 8 : 2 : 0.2 to provide the aglycone **2a** (3 mg). **2a**: a white powder. ^{1}H-NMR (pyridine-d_5) δ: 1.38, 1.41, 1.58 (each 3H, s, H_3-12,13,15), 1.37 (1H, m, H-6), 1.55 (1H, m, H-8), 1.82 (1H, t-like, J = 11 Hz, H-6), 1.95 (2H, m, H-7,9), 2.16 (1H, ddd, J = 3, 12, 13 Hz, H-1), 2.33 (1H, t, J = 12 Hz, H-2), 2.71 (1H, t, J = 10 Hz, H-5), 2.86 (3H, m, H-2,8,9), 3.86, 3.91 (each 1H, d, J = 11 Hz, H_2-14), 4.79 (1H, dd, J = 9, 10 Hz, H-3).

$NaBH_4$ Reduction of 1 **1** (20 mg) in MeOH (10 ml) was reduced with $NaBH_4$ (100 mg), with stirring for 16 h at room temperature. Usual work-up gave the products, which were separated by silica gel column chromatography to yield a product (18.7 mg) identical with **2** in terms of $[\alpha]_D$, ^{1}H- and ^{13}C-NMR spectra.

Atractyloside C (3) A white powder, $[\alpha]_D^{19}$ +12.0° (c = 0.95, MeOH). Negative FAB-MS m/z: 399 $[M-H]^-$. ^{1}H-NMR (methanol-d_4) δ: 0.71, 1.23, 1.25 (each 3H, s, H_3-14,12,13), 1.10—1.80 (10H, m), 1.95 (1H, dd, J = 3, 12 Hz, H-3), 3.10—3.40 (6H, m), 3.66 (1H, dd, J = 5, 12 Hz, glc H-6), 3.77 (1H, m, H-2), 3.80 (1H, dd, J = 3, 12 Hz, glc H′-6), 4.47 (1H, d, J = 8 Hz, glc H-1), 4.56, 4.80 (each 1H, br s, H_2-15).

Enzymatic Hydrolysis of 3 **3** (10 mg) was hydrolyzed with crude naringinase in the same way as described for **1** and **2** to afford the aglycone **3a** (1.2 mg), and glucose (on TLC). **3a**: a white powder, $[\alpha]_D^{16}$ +31.7° (c = 0.12, MeOH). ^{1}H-NMR (pyridine-d_5) δ: 0.82, 1.39, 1.40 (each 3H, s, H_3-14,12,13), 1.29, 1.32 (each 1H, m, H_2-9), 1.40—1.60 (4H, m, H-1,6,7,8), 1.82 (1H, d, J = 13 Hz, H′-8), 1.86 (1H, d, J = 13 Hz, H-5), 2.02 (1H, br d, J = 15 Hz, H-6), 2.10 (1H, dd, J = 2, 12 Hz, H-1), 2.36 (1H, t, J = 12 Hz, H-3), 2.96 (1H, dd, J = 3, 12 Hz, H-3), 4.10 (1H, dt, J = 3, 12 Hz, H-2), 4.67, 4.91 (each 1H, br s, H_2-15).

Atractyloside D (4) A white powder, $[\alpha]_D^{17}$ −15.0° (c = 0.95, MeOH). Negative FAB-MS m/z: 561 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.66, 1.44, 1.42 (each 3H, s, H_3-14,12,13), 1.10—1.50 (5H, m), 1.60 (1H, t, J = 12 Hz, H-8), 1.72 (1H, d, J = 12 Hz, H′-8), 1.82 (1H, d, J = 13 Hz, H-5), 1.94 (1H, d, J = 13 Hz, H-6), 2.15 (1H, br t, J = 5 Hz, H-1), 2.33 (1H, t, J = 12 Hz, H-3), 3.15 (1H, dd, J = 4, 12 Hz, H′-3), 4.52, 4.73 (each 1H, br s, H_2-15), 5.04, 5.05 (each 1H, d, J = 7 Hz, glc H-1 × 2). Enzymatic hydrolysis of **4** in the same way as described for **3** liberated the aglycone (identical with **3a**) and glucose.

Atractyloside E (5) A white powder, $[\alpha]_D^{30}$ −39.4° (c = 1.00, MeOH). Positive FAB-MS m/z: 717 $[M+Na]^+$, 537 $[M-glc-H_2O]$, 405 [m/z 537-api]. ^{1}H-NMR (pyridine-d_5) δ: 0.68, 1.39, 1.41 (each 3H, s, H_3-14,12,13), 1.10—1.40 (5H, m), 1.57 (1H, t, J = 11 Hz, H-8), 1.67 (1H, d, J = 11 Hz, H′-8), 1.80 (1H, d, J = 12 Hz, H-5), 1.90 (1H, d, J = 12 Hz, H-6), 2.09 (1H, d, J = 12 Hz, H-1), 2.32 (1H, t, J = 12 Hz, H-3), 3.14 (1H, dd, J = 3, 12 Hz, H′-3), 3.80—4.70 (m), 4.73, 4.90 (each 1H, br s, H_2-15), 4.96, 5.01 (each 1H, d, J = 8 Hz, glc H-1), 5.77 (1H, br s, api H-1). Atractyloside E (**5**) on enzymatic hydrolysis also liberated the aglycone (identical with **3a**) together with glucose and apiose. A mixture of **5** (5 mg), acetic anhydride (0.3 ml) and pyridine (0.5 ml) was kept standing at room temperature and evaporated under reduced pressure to give a residue, which was subjected to silica gel column chromatography (benzene–AcOEt = 2 : 1) to afford the acetate (3 mg) of **5**, a white powder. EI-MS m/z: 766 (M − hexosyl 4Ac − H_2O), 547 (pentosyl hexosyl 6Ac cation), 331 (hexosyl 4Ac cation), 259 (pentosyl 3Ac cation).

Atractyloside F (6) A white powder, $[\alpha]_D^{19}$ −99.8° (c = 0.50, MeOH). Negative FAB-MS m/z: 825 $[M-H]^-$, 693. ^{1}H-NMR (pyridine-d_5) δ: 0.67, 1.39, 1.46 (each 3H, s, H_3-14,12,13), 1.10—1.40 (5H, m), 1.56 (1H, t, J = 12 Hz, H-8), 1.65 (1H, d, J = 12 Hz, H′-8), 1.75 (1H, d, J = 12 Hz, H-5), 1.88 (1H, d, J = 12 Hz, H-6), 2.08 (1H, d, J = 9 Hz, H-1), 2.34 (1H, t, J = 12 Hz, H-3), 3.15 (1H, t, J = 4, 12 Hz, H′-3), 3.90—4.75 (m), 4.59, 4.74 (each 1H, br s, H_2-15), 4.96, 4.98 (each 1H, d, J = 7 Hz, glc H-1 × 2), 5.76,

5.78 (each 1H, d, $J=2$ Hz, api H-1 × 2). On enzymatic hydrolysis **6** also afforded the aglycone (identical with **3a**) together with glucose and apiose.

Atractyloside G (7) A white powder, $[\alpha]_D^{19}$ +2.2° ($c=0.045$, MeOH). Negative FAB-MS m/z: 507 $[M+\text{glycerol}-H]^-$, 415 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.87, 1.44, 1.45 (each 3H, s, H_3-14,12,13), 1.20—1.70 (6H, m), 1.90 (2H, m, H-5,8), 2.05 (2H, m, H-1,6), 3.90—4.40 (7H, m, sugar), 4.50 (1H, dd, $J=2$, 11 Hz, glc H-6), 4.87, 5.80 (each 1H, br s, H-15), 5.06 (1H, d, $J=8$ Hz, glc H-1).

Enzymatic Hydrolysis of 7 A mixture of **7** (10 mg) and crude naringinase (10 mg) in water (10 ml) was incubated at 38 °C for 16 h. Usual work-up gave an aglycone (**7a**) (2 mg) together with glucose (on TLC). **7a**: a white powder, $[\alpha]_D^{16}$ +5.1 ($c=0.12$, MeOH). ^{1}H-NMR (pyridine-d_5) δ: 0.86, 1.40, 1.41 (each 3H, s, H_3-14,12,13), 1.27 (2H, m, H_2-9), 1.49 (1H, m, H-7), 1.53 (1H, m, H-6), 1.61 (1H, m, H-8), 1.68 (1H, t, $J=12$ Hz, H-1), 1.82 (1H, br d, $J=11$ Hz, H-8), 1.93 (1H, br d, $J=11$ Hz, H-5), 2.06 (1H, br d, $J=12$ Hz, H-6), 2.11 (1H, dd, $J=5$, 12 Hz, H-1), 4.11 (1H, ddd, $J=5$, 9, 12 Hz, H-2), 4.35 (1H, br d, $J=9$ Hz, H-3), 4.97, 5.87 (each 1H, br s, H_2-15).

Atractyloside H (8) A white powder, $[\alpha]_D^{27}$ −33.2° ($c=0.50$, MeOH). Positive FAB-MS m/z: 733 $[M+Na]^+$, 553 $[m/z\ 733-\text{glc}-H_2O+Na]^+$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.69, 1.42, 1.43 (each 3H, s, H_3-14,12,13), 1.12—1.63 (5H, m, H_2-9, H-6,7,8), 1.74 (1H, br d, $J=14$ Hz, H-1), 1.80 (1H, br d, $J=11$ Hz, H′-8), 1.95 (1H, br d, $J=12$ Hz, H-5), 2.03 (2H, m, H′-1,6), 3.90—4.80 (m, sugar), 4.80, 5.79 (each 1H, br s, H_2-15), 4.90, 5.04 (each 1H, d, $J=8$ Hz, glc H-1 × 2), 5.72 (1H, d, $J=2$ Hz, api H-1).

Enzymatic Hydrolysis of 8 **8** (10 mg) was hydrolyzed with crude naringinase as described above for **7** to give the same aglycone **7a** (2 mg) as for **7**, together with glucose and apiose.

Atractyloside I (9) Colorless needles, mp 156—158 °C, $[\alpha]_D^{27}$ +19.7° ($c=1.00$, MeOH). Positive FAB-MS m/z: 599 $[M+Na]^+$, 572 $[M+H]^+$, 415, 235. CD ($c=4.51\times10^{-4}$, MeOH) $[\theta]$ (nm): $+1.66\times10^3$ (258), 0 (288), -0.69×10^3 (323). ^{1}H-NMR (pyridine-d_5) δ: 0.90, 1.36, 1.45, 2.15 (each 3H, s, H_3-14,12,13,15), 1.10—1.40 (4H, m, H-6,8, H_2-9), 1.72 (2H, m, H′-6, H-7), 2.23 (1H, d, $J=16$ Hz, H-1), 2.40 (1H, d, $J=16$ Hz, H′-1), 2.48 (2H, m, H-5, H′-8), 3.90—4.55 (12H, m, sugar), 5.05, 5.36 (each 1H, d, $J=8$ Hz, glc H-1 × 2).

Enzymatic Hydrolysis of 9 A mixture of **3** (17 mg) and crude naringinase (24 mg) in water was incubated at 37 °C for 48 h to furnish an aglycone **9a** (6 mg) and glucose. **9a**: a white powder. ^{1}H-NMR (pyridine-d_5) δ: 0.89 (3H, s, H_3-14), 1.30—1.42 (3H, m, H-6, H_2-9), 1.33 (1H, m, H-8), 1.39, 1.41 (each 3H, s, H_3-12,13), 1.60 (1H, m, H-7), 1.76 (1H, br dd, $J=5$, 14 Hz, H′-6), 2.00 (3H, d, $J=2$ Hz, H_3-15), 2.29 (1H, d, $J=16$ Hz, H-1), 2.31 (1H, m, H′-8), 2.38 (1H, br d, $J=13$ Hz, H-5), 2.26 (1H, d, $J=16$ Hz, H′-1).

Acknowledgements The authors are grateful to Prof. O. Tanaka, Hiroshima University, for providing crude naringinase, and to Mr. S. Setoguchi, Yoshitomi Pharmaceutical Co., Ltd. for facilities for X-ray measurement.

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