Arjungenin, Arjunglucoside I, and Arjunglucoside II. A New Triterpene and New Triterpene Glucosides from *Terminalia arjuna*[1)]

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Arjungenin, a new triterpene isolated from *Terminalia arjuna*, is shown to be 2α,3β,19α,23-tetrahydroxyolean-12-en-28-oic acid (**1**). The structures of two new triterpene glucosides, arjunglucoside I and arjunglucoside II, isolated from the same plant, were determined to be β-D-glucopyranosyl 2α,3β,19α,23-tetrahydroxyolean-12-en-28-oate (**2**) and β-D-glucopyranosyl arjunolate (**3**), respectively.

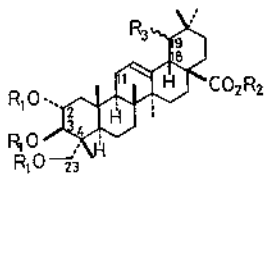
It has been reported that *Terminalia arjuna* (Combretaceae) contains ellagic acid, D(+)-mannitol, (+)-leucocyanidin, (+)-leucodelphinidin, β-sitosterol, oleanolic acid, arjunic acid, arjunolic acid, arjunetin, and a saponin ($C_{42}H_{68}O_{15}\cdot 2H_2O$).[2,3)] The methanol extract of the bark of the plant was recently examined, and a new sapogenin which we named arjungenin was isolated. In the present communication, we wish to report the structure determination leading to the structure **1** for arjungenin. An isolation of two new saponins named arjunglucoside I (**2**) and arjunglucoside II (**3**) from the same plant is also described.

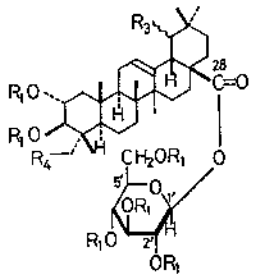
The molecular formula of $C_{30}H_{48}O_6$ was given for arjungenin (**1**), mp 293—294 °C (dec), $[\alpha]_D$ +29° (EtOH), based on its elemental analysis and mass spectral data. Arjungenin was treated with diazomethane to give a methyl ester (**4**), mp 162—165 °C, which on acetylation with acetic anhydride in pyridine afforded an ester triacetate (**5**), mp 137—138 °C. Treatment of **5** with acetic anhydride in the presence of perchloric acid gave an ester tetraacetate (**6**), an amorphous solid, which shows no IR absorption due to a hydroxyl group. Therefore, the nature of all six oxygen atoms involved in arjungenin is characterized. Arjungenin is a tetrahydroxy carboxylic acid.

In the mass spectra of arjungenin (**1**), **4**, **5**, and of **6**, characteristic peaks due to a retro-Diels-Alder-type cleavage of the olean-12-ene skeleton[4,5)] appear at *m/e* 264, *m/e* 278, *m/e* 278, and *m/e* 320, respectively, showing the presence of a hydroxyl group at the D/E ring of arjungenin. The ester triacetate (**5**) was heated under reflux with phosphoryl chloride in pyridine to give a mixture of dienes (**7** and **8**), which was then treated with dry hydrogen chloride in chloroform to afford a single product (**8**), mp 115—118 °C (UV and PMR spectral data: *cf.* Experimental). This product (**8**) was found to be identical with the diene (**8**) derived from methyl tri-*O*-acetylarjunolate (**9**)[6)] according to the procedures described by King *et al.*[3)] The Collins oxidation of **5** gave a ketone (**10**), an amorphous solid, whose ORD curve (a=+102) was almost identical with that (a=+102) of a 19-keto derivative (**11**)[2a)] prepared from arjunic acid (**12**).[2a)] Arjungenin is thus suggested to be 2α,3β,19ξ,23-tetrahydroxyolean-12-en-28-oic acid.

The structure of tomentosic acid has been determined to be 2α,3β,19β(equatorial), 23-tetrahydroxyolean-12-en-28-oic acid (**13**).[7)] As the physical constants of arjungenin and its methyl ester are not identical with those[7)] of **13** and its methyl ester, respectively, the hydroxyl group at C-19 of arjungenin (**1**) is considered to be in an α(axial) configuration. This received support from the following evidence.

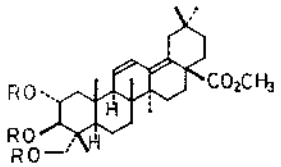
Reduction of a ketone (**14**) with sodium borohydride has been reported to yield a 19α(axial)-ol (**15**; methyl arjunate) almost quantitatively.[2a)] On reduction with sodium borohydride, the ketone (**10**) gave arjungenin ester triacetate (**5**) as a sole product. The PMR spectrum



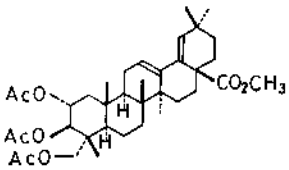


	R_1	R_2	R_3
1	H	H	α-OH
4	H	CH_3	α-OH
5	Ac	CH_3	α-OH
6	Ac	CH_3	α-OAc
9	Ac	CH_3	H
13	H	H	β-OH
24	H	H	H
27	CH_3	H	H

	R_1	R_3	R_4
2	H	α-OH	OH
3	H	H	OH
22	Ac	α-OH	OAc
25	Ac	H	OAc
26	CH_3	H	OCH_3
28	H	α-OH	H
29	Ac	α-OH	H



8 R=Ac
30 R=H



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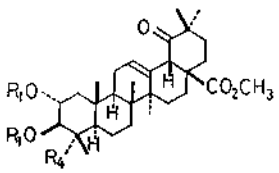
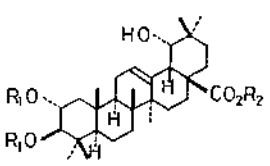
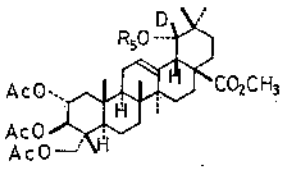


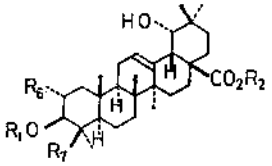
	R_1	R_4
10	Ac	CH_2OAc
11	Ac	CH_3
14	H	CH_3



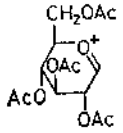
	R_1	R_2
12	H	H
15	H	CH_3
20	Ac	CH_3



16 R_5=H
17 R_5=Ac



	R_1	R_2	R_6	R_7
18	H	H	H	CH_2
19	H	H	OH	CH_2OH
21	Ac	CH_3	OAc	CH_2OAc



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of **5** shows a broad signal ($W_{1/2}$=6 Hz) at δ 3.10 due to a proton on C-18 (β, axial) and a multiplet at δ 3.35 due to a proton on C-19 (Table 1). When deuterium oxide was added, this multiplet changed into a doublet ($J_{18\beta,19\beta}$=4 Hz) suggesting an equatorial (β) nature for the proton on C-19. The presence of the same coupling constant ($J_{18\beta,19\beta}$=4 Hz) was observed in the spectrum of arjungenin ester tetraacetate (**6**) (Table 1). The ketone (**10**) was reduced with sodium borodeuteride (in dioxane, room temperature) to give a 19β-deuterated product (**16**). In the PMR spectrum of **16**, the signal at δ 3.10 due to a proton on C-18 appears as a broad singlet ($W_{1/2}$=3 Hz), while the multiplet at δ 3.35 is absent. When treated with acetic anhydride in the presence of perchloric acid, **16** yielded a 19β-deuterated tetraacetate (**17**). The signal due to a proton on C-18 of **17** is shifted and appears at δ 3.30 The evidence described above confirms the assignment for signals due to the protons on C-18 and C-19, and leads to an α(axial)-configuration for the hydroxyl group at C-19. The structure including absolute configuration of arjungenin should be represented by 2α,3β,19α,23-tetrahydroxyolean-12-en-28-oic acid (**1**).

Siaresinolic acid (**18**),[8,9] arjunic acid (**12**),[2a] and sericic acid (**19**)[10] are triterpene acids which have the same structure moiety (19α-hydroxyolean-12-en-28-oic acid structure) around the C/D/E rings as that of arjungenin (**1**). The assignments hitherto reported for the PMR signals due to the protons on C-18β and C-19β of methyl di-*O*-acetylarjunate (**20**) and of methyl tri-*O*-acetylsericate (**21**) seem to be erroneous. In the PMR spectrum of **20**, a triplet at δ 3.35 and a broad signal at δ 3.10 were described to be due to a proton on C-19β and a hydroxyl group on C-19α, respectively.[2a] However, we found that the latter signal (δ 3.10) remained unchanged after addition of deuterium oxide. The signal at δ 3.10 must be due to the proton on C-18β in accordance with our spectral data for arjungenin ester triacetate (**5**) (*cf.* Table 1). Bombardelli *et al.* reported that in the PMR spectrum of **21** a broad doublet at δ 3.16 (J=3 Hz) and a doublet at δ 3.34 (J=3 Hz) are due to the protons on C-19β and C-18β, respectively.[10] These assignments are considered to be reversed based on the observation described for **5** (*cf.*

Table 1. PMR spectral data (in δ values)

Compounds	**5**[a]	**6**[a]	**22**[b]	**25**[b]
t-CH_3	0.69s	0.68s	0.70s	0.75s
	0.91s	0.82s	0.92s	0.92s
	0.99s	0.90s	0.96s	0.92s
	0.99s	1.06s	0.99s	0.92s
	1.10s	1.06s	1.10s	1.10s
	1.25s	1.27s	1.25s	1.12s
-OCOCH_3	1.99s	1.99s	2.00s	1.99s
	2.01s	2.01s	2.02s	2.01s
	2.09s	2.08s	2.04s	2.03s
		2.10s	2.04s	2.03s
			2.04s	2.03s
			2.08s	2.06s
			2.11s	2.08s
$-CO_2CH_3$	3.62s	3.65s	—	—
$C_{(18\beta)}$-H	3.10[c]	3.35br.d	3.08[c]	2.85[c]
		J=4		
$C_{(19\beta)}$-H	3.35m[d]	4.92d	3.34[e]	—[e]
		J=4		
$-\underset{\underset{H}{|}}{C}_{(23)}$-OAc	3.58[f]	3.58[f]	3.61[f]	3.59[f]
	3.82[g]	3.82[g]	3.83[g]	3.85[g]
$H_{(5')}$-H	—	—	3.80m	3.80m
$-\underset{\underset{H}{|}}{C}_{(6')}$-OAc	—	—	4.06[h]	4.06[h]
			4.28[h]	4.28[h]
$C_{(3\alpha)}$-H, $C_{(2\beta)}$-H	*ca.* 5.10[j]	*ca.* 5.10[j]	—[k]	—[k]
$C_{(2')}$-H	—	—		
$C_{(3')}$-H	—	—		
$C_{(4')}$-H	—	—		
$C_{(12)}$-H	5.45t	5.50t	5.44[e]	5.32[e]
$C_{(1')}$-H	—	—	5.60d	5.58d
			J=8	J=8

a) Determined in $CDCl_3$ at 60 MHz. Coupling constants are expressed in Hz. s: singlet; d: doublet; br. d: broad doublet; t: triplet; m: multiplet. b) Determined in $CDCl_3$ at 100 MHz. c) Broad signal. d) On addition of D_2O, this multiplet changes into a doublet (J=4 Hz). e) Unresolved signal. f) A-part of AB-type quartet (J_{AB}=12 Hz). g) B-part of AB-type quartet. h) A-part of ABX-spin system (J_{AB}=12 Hz; J_{AX}=2 Hz). i) B-part of ABX-spin system (J_{AB}=12 Hz; J_{BX}=4 Hz). j) These signals are overlapped. k) These signals are overlapped and appear at δ 4.96—δ 5.30.

Table 1).

Arjunglucoside I (**2**) crystallized from aqueous methanol as colorless needles, mp 231 °C, $[\alpha]_D$ +17° (EtOH). Elemental analysis indicates the formula $C_{36}H_{58}O_{11}\cdot 2H_2O$. Acid-catalyzed methanolysis of **2** afforded arjungenin (**1**) and methyl D-glucoside. An alkaline hydrolysis of **2** yielded **1** and D-glucose. The IR spectrum of **2** shows an ester carbonyl absorption at 1730 cm^{-1} and no carbonyl absorption due to a free carboxylic acid. Therefore the presence of an ester glucoside linkage is suggested for **2**. Acetylation of **2** with acetic anhydride and pyridine gave a heptaacetate (**22**). The mass spectrum of **22** shows a peak at *m/e* 331 due to a fragment ion (**23**)[11] formed by cleavage of

the glucoside linkage; the appearance of a series of peaks at *m/e* 331, *m/e* 211, *m/e* 169, and *m/e* 109[11] suggests the presence of a tetra-*O*-acetylglucopyranosyl group for **22**. As the tetra-*O*-acetyl-D-glucopyranosyl group preferentially exists as a C1 form, the PMR spectrum of **22** ($J_{1',2'}$=8 Hz; Table 1) indicates the presence of a β-glucosyl linkage in the molecule.[12] The presence of an ester glucoside linkage in **22** received support from the appearance of signals due to a proton on C-1′ at δ 5.60 (Table 1). The structure of arjunglucoside I is thus shown to be β-D-glucopyranosyl 2α,3β,19α,-23-tetrahydroxyolean-12-en-28-oate (**2**).

The elemental analysis of arjunglucoside II (**3**; crystallized from aqueous methanol), mp 252 °C, $[\alpha]_D$ +37° (EtOH), fitted best for the molecular formula $C_{36}H_{58}O_{10}\cdot H_2O$.[13] On acid-catalyzed methanolysis, **3** yielded arjunolic acid (**24**) and methyl D-glucoside. An alkaline hydrolysis of **3** gave **24** and D-glucose. The IR spectrum of **3** shows the presence of an ester carbonyl group (1730 cm^{-1}) and the absence of a free carboxylic acid. Acetylation of **3** with acetic anhydride and pyridine gave a heptaacetate (**25**). When treated with methyl iodide in *N,N*-dimethylformamide in the presence of silver oxide, **3** gave a heptamethyl ether (**26**). On hydrolysis of **26**, arjunolic acid trimethyl ether (**27**) was obtained. Therefore, the presence of an ester glucoside linkage is suggested for **3**. The mass spectrum of **25** shows a series of peaks at *m/e* 331, *m/e* 211, *m/e* 169, and *m/e* 109[11] characteristic for the presence of a tetra-*O*-acetylglucopyranosyl group. The presence of a β-glucosyl linkage is suggested from the PMR spectrum of **25** ($J_{1',2'}$=8 Hz; Table 1). The structure of β-D-glucopyranosyl 2α,3β,23-trihydroxy-olean-12-en-28-oate (**3**; β-D-glucopyranosyl arjunolate) is thus given for arjunglucoside II.

Experimental

IR spectra were measured using a Hitachi EPI-G2 spectrometer. UV spectra were determined on a Hitachi EPS-3 spectrophotometer. ORD measurements were carried out on a JASCO Model J-20 spectrometer. Optical rotations were measured on a JASCO DIP-SL polarimeter. Mass spectra were taken on a Hitachi RMU-6-Tokugata mass spectrometer with a direct inlet system operating at 70 eV. PMR spectra were measured using a JEOL 4H-100 (100 MHz) or a Hitachi R-20 (60 MHz) spectrometer. Chemical shifts were expressed in δ downfield from TMS as an internal standard, and coupling constants in Hz. Thin layer chromatography (TLC) was carried out on Kieselgel G nach Stahl, Kieselgel GF_{254} nach Stahl, and Kieselgel 60 PF_{254} (E. Merck, Darmstadt). For column chromatography Wakogel C-200 (Wako Pure Chemical Ind.) was used. All mps were determined on a hot block and reported uncorrected.

Isolation. The bark (1.3 kg) of *Terminalia arjuna* were sliced and ground into powder and extracted with methanol (total 4 l) at room temperature. To the extract was added lead acetate (100 g) and a precipitated material then formed was filtered off. Water (*ca.* 12 l) was added to the filtrate to give a greenish-yellow precipitate which was separated by filtration. The mother liquor was concentrated under reduced pressure to give another crop of the greenish-yellow precipitate. The combined greenish-yellow material (material A; total 4 g) showed six spots on TLC and was subjected to separation on a column of silica gel (450 g). Eluted fractions were examined by TLC (silica gel).

Elution with chloroform–methanol (47: 3) gave *arjunic acid*[2a] (**12**; 260 mg), mp >280 °C (dec), $[\alpha]_D$ +28° (*c* 3.1, EtOH), characterized as its methyl ester diacetate[2a] (**20**), mp 234—235 °C (crystallized from methanol), $[\alpha]_D$ +4° (*c* 1.6, EtOH); IR (Nujol) 3540, 1740, and 1640 cm^{-1}; PMR ($CDCl_3$) δ 0.69, δ 1.06, and δ 1.25 (each 3H, s; *t*-CH_3), δ 0.91 and δ 0.99 (each 6H, s; 2×*t*-CH_3), δ 1.98 and δ 2.05 (each 3H, s; 2×$OCOCH_3$), δ 3.62 (3H, s; CO_2CH_3), δ 3.10 (1H, m; $C_{(18\beta)}$–H), δ 3.35 (1H, d, J=4 Hz; $C_{(19\beta)}$–H), δ 4.75 (1H, d, J=11 Hz; $C_{(3\alpha)}$–H), δ 5.15 (1H, ddd, J=11, J=11, and J=4 Hz; $C_{(2\beta)}$–H), and δ 5.45 (1H, t; $C_{(12)}$–H); mass spectrum *m/e* 586 (M^+), *m/e* 568, *m/e* 527, *m/e* 278, *m/e* 260, *m/e* 245, *m/e* 219, and *m/e* 201 (base peak).

Found: C, 71.69; H, 9.54%. Calcd for $C_{35}H_{54}O_7$: C, 71.64; H, 9.28%.

Subsequent elution with chloroform–methanol (23: 2) yielded *arjunolic acid*[6,14] (**24**; 230 mg), mp 296—297 °C (dec.) (from aqueous methanol), $[\alpha]_D$ +61° (*c* 2.9, EtOH), characterized as its methyl ester,[6] mp 210—213 °C, $[\alpha]_D$ +61° (*c* 1.3, EtOH); IR (KBr) 3430, 1730, and 1640 cm^{-1}; PMR ($CDCl_3$) δ 0.73, δ 0.85, δ 1.03, and δ 1.15 (each 3H, s; *t*-CH_3), δ 0.95 (6H, s; 2×*t*-CH_3), δ 3.63 (3H s; CO_2CH_3), and δ 5.30 (1H, m; $C_{(12)}$–H); mass spectrum *m/e* 502 (M^+), *m/e* 484, *m/e* 466, *m/e* 262, *m/e* 249, *m/e* 203 (base peak), *m/e* 189, and *m/e* 133.

Found: C, 74.19; H, 10.12%. Calcd for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03%. This methyl ester was found to be identical (mp, $[\alpha]_D$, IR, PMR, and mass spectrum) with an authentic sample of methyl arjunolate.[14]

Successive elution with chloroform–methanol (9: 1) gave *arjungenin* (**1**; 20 mg), mp 293—294 °C (dec.)(from aqueous methanol), $[\alpha]_D$ +29° (*c* 2.6, EtOH), IR (KBr) 3400, 1690, and 1630 cm^{-1}; PMR (pyridine-d_5) δ 1.08 and δ 1.11 (each 6H, s; 2×*t*-CH_3), δ 1.19 and δ 2.09 (each 3H, s; *t*-CH_3), δ 3.59 (2H, s), δ 3.76 (1H, s), δ 4.15 (2H, m), and δ 5.52 (1H, s; $C_{(12)}$–H); mass spectrum *m/e* 504 (M^+), *m/e* 486, *m/e* 468, *m/e* 264, *m/e* 246, *m/e* 231, and *m/e* 201 (base peak).

Found: C, 71.41; H, 9.56%. Calcd for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59%.

Elution with chloroform–methanol (43: 7) yielded *arjunetin*[2b] (**28**; β-D-glucopyranosyl arjunate; 1.8 g), mp 232—234 °C (from aqueous methanol), $[\alpha]_D$ +15° (*c* 1.3, EtOH); IR (Nujol) 3370, 1730, and 1640 cm^{-1}; mass spectrum *m/e* 488, *m/e* 470, *m/e* 452, *m/e* 264, *m/e* 246, *m/e* 231, and *m/e* 201 (base peak) (no molecular ion peak was observed), characterized as its hexaacetate (**29**)[2b], mp 235—237 °C (from ethanol), $[\alpha]_D$ +1° (*c* 1.5, $CHCl_3$); IR (Nujol) 3510, 1760, 1730, and 1710 cm^{-1}; PMR ($CDCl_3$) δ 0.69, δ 0.90, δ 0.92, δ 0.95, δ 0.98, δ 1.06, and δ 1.25 (each 3H, s; *t*-CH_3), δ 1.99 (3H, s; $OCOCH_3$), δ 2.02 (9H, s; 3×$OCOCH_3$), δ 2.07 (6H, s; 2×$OCOCH_3$), δ 3.06 (1H, br. signal; $C_{(18\beta)}$–H), δ 3.34 (1H, br. signal; $C_{(19\beta)}$–H), δ 3.80 (1H, m; $C_{(5')}$–H), δ 4.06 (1H, as A part of ABX-system: $J_{6',6'}$=12 and $J_{5',6'}$=2 Hz; $C_{(6')}$–H), δ 4.28 (1H, as B part of ABX-system: $J_{6',6'}$=12 and $J_{5',6'}$=4 Hz; $C_{(6')}$–H), δ 4.74 (1H, d, $J_{2\beta,3\alpha}$=10 Hz; $C_{(3\alpha)}$–H), δ 5.44 (1H, m; $C_{(12)}$–H), and δ 5.60 (1H, d, $J_{1',2'}$=8 Hz; $C_{(1')}$–H); mass spectrum *m/e* 570, *m/e* 554, *m/e* 526, *m/e* 331, *m/e* 271, *m/e* 264, *m/e* 246, *m/e* 231, *m/e* 211, *m/e* 169, *m/e* 109, and *m/e* 43 (base peak) (no molecular ion peak was observed).

Found: C, 64.07; H, 8.06%. Calcd for $C_{48}H_{70}O_{16}$: C, 63.85; H, 7.76%. Alkaline hydrolysis (4% KOH–EtOH, reflux, 5 h) of **28** gave arjunic acid (**12**) and D-glucose.

Subsequent elution with chloroform–methanol (21: 4) gave *arjunglucoside II* (**3**; β-D-glucopyranosyl arjunolate; 1 g), mp 252 °C (from aqueous methanol), $[\alpha]_D$ +37° (*c* 2.3, EtOH); IR (KBr) 3400, 1730, and 1630 cm^{-1}; mass spectrum *m/e* 488,

m/e 470, *m/e* 452, *m/e* 248 (base peak), *m/e* 233, *m/e* 203, *m/e* 189, and *m/e* 133 (no molecular ion peak was observed).

Found: C, 61.13; H, 8.83%. Calcd for $C_{36}H_{58}O_{10}\cdot 3H_2O$: C, 61.34; H, 9.15%.

Five among six constituents contained in material A were thus separated. The sixth constituent corresponding to the most polar spot on the TLC could be obtained from material B, rather than from material A, as described below. The mother liquor after separation of material A was treated with hydrogen sulfide to precipitate lead sulfide which was removed by filtration. The resulting filtrate gave, on evaporation of the solvent, a residue (material B; 3.2 g), containing the sixth constituent mentioned above as a main constituent (examined by TLC). Material B was subjected to separation on a column of silica gel (250 g). Elution with chloroform–methanol (83: 17) gave *arjunglucoside I* (**2**; 450 mg), mp 231 °C (from aqueous methanol), $[\alpha]_D$ +17° (*c* 2.8 EtOH); IR (KBr) 3410, 1730, and 1640 cm^{-1}; mass spectrum *m/e* 504, *m/e* 486, *m/e* 468, *m/e* 264, *m/e* 246, *m/e* 231, and *m/e* 201 (base peak) (no molecular ion peak was observed). Found: C, 63.16; H, 9.13%. Calcd for $C_{36}H_{58}O_{11}\cdot H_2O$: C, 63.14; H, 8.83%.

Methylation of Arjungenin (1). To arjungenin (**1**, 217 mg) in ether was added diazomethane in ether. The reaction mixture was treated as usual to give a residue (215 mg), which was crystallized from ether to yield arjungenin methyl ester (**4**), mp 162—165 °C; IR (KBr) 3430, 1720, and 1640 cm^{-1}; mass spectrum *m/e* 518 (M^+), *m/e* 500, *m/e* 482, *m/e* 278, *m/e* 260, *m/e* 245, *m/e* 219, and *m/e* 201 (base peak). Found: *m/e* 518.3503 (M^+). Calcd for $C_{31}H_{50}O_6$: *m/e* 518.3604.

Acetylation of Arjungenin Methyl Ester (4). Arjungenin methyl ester (**4**; 100 mg) dissolved in pyridine (1 ml) was treated with acetic anhydride (1 ml) overnight at room temperature. After the usual work-up, acetylated product (100 mg) was obtained and purified by column chromatography to give arjungenin methyl ester triacetate (**5**) mp 137—138 °C, $[\alpha]_D$ +8° (*c* 1.8, EtOH); IR (Nujol) 3520, 1740, and 1630 cm^{-1}, PMR (Table 1); mass spectrum *m/e* 644 (M^+), *m/e* 626, *m/e* 585, *m/e* 278, *m/e* 260, *m/e* 245, *m/e* 219, and *m/e* 201 (base peak). Found: C, 68.73; H, 8.89%. Calcd for $C_{37}H_{56}O_9$: C, 68.91; H, 8.75%.

Acetylation of Arjungenin Methyl Ester Triacetate (5). To arjungenin methyl ester triacetate (**5**; 23 mg) in acetic anhydride (2 ml) was added a drop of perchloric acid at −10 °C. The reaction mixture was kept at room temperature for 15 min, and poured into water. Extraction with ether and removal of the solvent gave a residue (22 mg), which was purified by preparative TLC to yield arjungenin methyl ester tetraacetate (**6**; 9 mg), an amorphous solid, IR (Nujol) 1730, and 1230 cm^{-1}, PMR (Table 1; signals due to four acetoxyl groups were observed); mass spectrum *m/e* 626 [(M−AcOH)$^+$], *m/e* 567, *m/e* 507, *m/e* 320, *m/e* 260, *m/e* 245, and *m/e* 201 (base peak)(no molecular ion peak was observed). Found: *m/e* 626.3820 [(M−AcOH)$^+$]. Calcd for ($C_{39}H_{58}O_{10}-C_2H_4O_2$): *m/e* 626.3815.

Dehydration of Arjungenin Methyl Ester Triacetate (5). Arjungenin methyl ester triacetate (**5**; 30 mg) in pyridine (0.5 ml) was treated with phosphoryl chloride (0.2 ml) under reflux for 6 h. The reaction mixture was treated in the usual manner to give a residue which was chromatographed to afford a mixture (24 mg) of dienes (**7** and **8**), an amorphous solid, UV (EtOH) λ_{max} 243, 252, and 261 nm, mass spectrum *m/e* 626 and *m/e* 201 (base peak). The fact that the product was a mixture of dienes (**7** and **8**) is suggested by the PMR spectrum. There appear signals at δ 5.40 (s; $C_{(19)}$–H) and δ 5.48 (m; $C_{(12)}$–H) due to olefinic protons of a 12,18-diene[28] system, besides those at δ 5.53 (d, J=11 Hz; $C_{(12)}$–H) and δ 6.43 (dd, J=11 and J=2 Hz; $C_{(11)}$–H) due to olefinic protons of the 11,13(18)-diene (**8**) (*vide infra*).

Treatment of the Mixture of Dienes (7 and 8) with Dry Hydrogen Chloride. Dry hydrogen chloride was passed through a solution of the mixture (24 mg) of dienes (**7** and **8**) in chloroform (3 ml) at room temperature for 1 h. After the usual work-up, a residue was obtained and purified by preparative TLC to give methyl 2α,3β,23-triacetoxyoleana-11,13(18)-dien-28-oate (**8**; 15 mg), mp 115—118 °C, $[\alpha]_D$ −120° (*c* 0.2, EtOH); UV (EtOH) λ_{max} 243.5 nm (log ε 4.41), 251.5 (4.47), 260.5 (4.27); IR (KBr) 1735 and 1630 cm^{-1}, PMR ($CDCl_3$) δ 0.80 (6H, s; 2×*t*-CH_3), δ 0.90, δ 0.94, δ 0.97, and δ 1.10 (each 3H, s; *t*-CH_3), δ 2.00, δ 2.01, and δ 2.10 (each 3H, s; $OCOCH_3$), δ 3.66 (3H, s; CO_2CH_3), δ 3.60 and δ 3.80 (each 1H, d, J=12 Hz; $C_{(23)}$–H_2), δ 5.15 (2H, m; $C_{(2\beta)}$–H and $C_{(3\alpha)}$–H), δ 5.53 (1H, d, J=11 Hz; $C_{(12)}$–H), and δ 6.43 (1H, dd, J=11 and J=2 Hz; $C_{(11)}$–H); mass spectrum *m/e* 626 (M^+), *m/e* 567, *m/e* 507, *m/e* 447, *m/e* 387, and *m/e* 187 (base peak). Found: C, 70.75; H, 8.81%. Calcd for $C_{37}H_{54}O_8$: C, 70.90; H, 8.68%.

Oxidation of Methyl Tri-O-acetylarjunolate (9) with Selenium Dioxide. This oxidation was effected according to the procedures described by King *et al.*,[3] which include alkaline hydrolysis at the final stage to give methyl 2α,3β,23-trihydroxyoleana-11,13(18)-dien-28-oate (**30**). Methyl tri-*O*-acetylarjunolate[6] (**9**; 51 mg) in acetic acid (2 ml) was heated with selenium dioxide (9 mg) under reflux for 17 h. After cooling, the reaction mixture was poured into water and extracted with ether. Removal of the solvent gave a residue (42 mg), which was purified by preparative TLC to afford 2α,3β,23-triacetoxyoleana-11,13(18)-dien-28-oate (**8**; 18 mg). This compound proved to be identical (mp, $[\alpha]_D$, IR, UV, PMR, TLC, and mass spectrum) with the diene (**8**) obtained from **5** by dehydration and by subsequent treatment with dry hydrogen chloride.

The Collins Oxidation of Arjungenin Methyl Ester Triacetate (5). To a solution prepared from chromium trioxide (260 mg), pyridine (0.5 ml), and dichloromethane (4 ml), was added a solution of arjungenin methyl ester triacetate (**5**; 220 mg) in dichloromethane (2 ml), and the whole was stirred at room temperature for 30 min. After the usual work-up a residue (200 mg) was obtained. The residue was purified by preparative TLC to yield methyl 2α,3β,23-triacetoxy-19-oxo-olean-12-en-28-oate (**10**, 150 mg), an amorphous solid; IR (Nujol) 1745 and 1710 (sh) cm^{-1}; PMR ($CDCl_3$) δ 0.74, δ 0.88, δ 0.98, and δ 1.19 (each 3H, s; *t*-CH_3), δ 1.09 (6H, s; 2×*t*-CH_3), δ 1.98, δ 2.00, and δ 2.07 (each 3H, s; $OCOCH_3$), δ 3.68 (3H, s; CO_2CH_3), δ 3.58 and δ 3.90 (each 1H, d, J=12 Hz; $C_{(23)}$–H_2), δ 5.10 (2H, m, $C_{(2\beta)}$–H and $C_{(3\alpha)}$–H), and δ 5.30 (1H, m; $C_{(12)}$–H); ORD (*c* 0.06, EtOH) $[\phi]_{315}$ +5300° and $[\phi]_{265}$ −4900° (a=+102); mass spectrum *m/e* 642 (M^+), *m/e* 582, *m/e* 522, *m/e* 462, *m/e* 276, and *m/e* 217 (base peak). Found: C, 69.11; H, 8.58%. Calcd for $C_{37}H_{54}O_9$: C, 69.13; H, 8.47%.

The Jones Oxidation of Methyl Di-O-acetylarjunate (20). To a solution of methyl di-*O*-acetylarjunate (**20**; 200 mg) in acetone (15 ml) was added the Jones reagent (2.2 ml), and the mixture was stirred at 0 °C for 20 min. After the usual treatment, a residue was crystallized from methanol to afford methyl 2α,3β-diacetoxy-19-oxo-olean-12-en-28-oate[28] (**11**; 120 mg), mp 200—201 °C; IR (KBr) 1720, 1440, 1360, and 1240 cm^{-1}; PMR ($CDCl_3$) δ 0.78, δ 1.00, and δ 1.20 (each 3H, s; *t*-CH_3), δ 0.91 and δ 1.10 (each 6H, s; 2×*t*-CH_3), δ 2.00 and δ 2.05 (each 3H, s; $OCOCH_3$), δ 3.70 (3H, s; CO_2CH_3), δ 4.77 (1H, d; J=11 Hz; $C_{(3\alpha)}$–H), δ 5.05 (1H, ddd; J=11, J=11, and J=4 Hz; $C_{(2\beta)}$–H), and δ 5.28 (1H, m; $C_{(12)}$–H); ORD (*c* 0.04, EtOH) $[\phi]_{315}$ +4900° and $[\phi]_{265}$ −5300° (a=+102); mass spectrum *m/e* 584 (M^+), *m/e* 525, *m/e* 464, *m/e* 405, *m/e* 276,

and *m/e* 217 (base peak). Found: C, 71.69; H, 9.23%. Calcd for $C_{35}H_{52}O_7$: C, 71.88; H, 8.96%.

Reduction of the Ketone (10) with Sodium Borohydride. The ketone (**10**; 16 mg) in dioxane (1.5 ml) was treated with sodium borohydride (76 mg) under stirring at room temperature for 2 h. The reaction mixture was diluted with water, acidified with diluted sulfuric acid, and extracted with ether. Removal of the solvent gave a residue (11 mg), showing one spot (on TLC) corresponding to the 19α-ol (**5**). This residue was passed through a column of silica gel [elution with benzene–acetone (47: 3)] to afford arjungenin methyl ester triacetate (**5**; 8 mg) (identified by mp, mixed mp, $[\alpha]_D$, IR, PMR, and mass spectra).

Similar reduction of the ketone (**10**; 20 mg) in dioxane (1.2 ml) with sodium borodeuteride (102 mg) gave a residue (16 mg), which was purified by preparative TLC to yield 19β-deuterated arjungenin methyl ester triacetate (**16**; 13 mg). The IR spectrum of **16** is almost superimposable with that of **5**.

In the PMR spectrum ($CDCl_3$) of **16**, a broad singlet (1H, $W_{1/2}=3$ Hz; $C_{(18\beta)}$–H) appears at δ 3.10. The multiplet signal at δ 3.35 (1H; $C_{(19\beta)}$–H) observed for **5** is absent in the spectrum of **16**. The other PMR spectral data of **16** and **5** are identical. The mass spectrum of **16** shows the following peaks: *m/e* 645 (M^+), *m/e* 627, *m/e* 586, *m/e* 279, *m/e* 261, *m/e* 246, *m/e* 220, and *m/e* 202 (base peak).

Acetylation of the 19β-Deuterated Product (16). The 19β-deuterated product (**16**; 11 mg) was acetylated according to the procedures described for the acetylation of **5**. 19β-Deuterated arjungenin methyl ester tetraacetate (**17**; 6 mg) was obtained as an oil. The IR spectra of **17** and arjungenin methyl ester tetraacetate (**6**) are almost identical. The PMR spectrum ($CDCl_3$) of **17** shows a broad singlet (1H) at δ 3.30 due to $C_{(18\beta)}$–H; no signal due to $C_{(19\beta)}$–H was observed. The other PMR spectral data of **17** and **6** are the same. The mass spectrum of **17** shows the following peaks: *m/e* 627 [$(M-AcOH)^+$], *m/e* 568, *m/e* 508, *m/e* 321, *m/e* 261, *m/e* 246, and *m/e* 202 (base peak) (no molecular ion peak was observed).

Methanolysis of Arjunglucoside I (2). To a solution prepared from methanol (6 ml), water (2 ml) and sulfuric acid (0.3 ml), was added arjunglucoside I (**2**; 77 mg), and the resulting mixture was heated under reflux for 4 h. The reaction mixture was poured into water and extracted with ether. The ethereal layer was treated as usual to give a residue, which was crystallized from aqueous methanol to afford arjungenin (**1**; 45 mg) (identified by mp, mixed mp, $[\alpha]_D$, IR, PMR, TLC, and mass spectrometry). The aqueous layer was passed through a column of Dowex 1-X2 (OH^-) and a column of Dowex 50W-X8 (H^+), successively, and concentrated under reduced pressure to give a residue. The residue (methyl D-glucoside) was trimethylsilylated and subjected to examination by GLC [column: 2% Silicone OV 17 on Chromosorb WAW, 4(mm)×1.5(m), column temperature: 160 °C; detection: FID, detector temperature: 190 °C, carrier gas: N_2 45 ml/min; instrument: Shimadzu GC-4A PF] to identify the silylated product as methyl tetra-*O*-trimethylsilyl-D-glucoside (retention time: 8.6 min).

Alkaline Hydrolysis of Arjunglucoside I (2). To a solution prepared from methanol (5 ml), water (3 ml) and potassium hydroxide (0.3 g), was added arjunglucoside I (**2**; 170 mg). The resulting mixture was heated under reflux for 5 h, poured into water, acidified by addition of hydrochloric acid, and extracted with ether. From the ethereal layer arjungenin (**1**; 100 mg) (identified by mp, mixed mp, $[\alpha]_D$, IR, PMR, TLC, and mass spectrometry) was obtained. The aqueous layer was passed through a column of Dowex 1-X2 (OH^-) and then a column of Dowex 50W-X8 (H^+), and concentrated under reduced pressure to give a residue, which was identified to be D-glucose ($R_f=0.52$) [on Avicel plate (Funakoshi Pharmaceutical Co., Ltd.), developing with pyridine–ethyl acetate–acetic acid–water (5: 5: 1: 3), visualized by aniline hydrogen phthalate at 80 °C].

Acetylation of Arjunglucoside I (2). Arjunglucoside I (**2**; 38 mg) in pyridine was treated with acetic anhydride overnight at room temperature. The reaction mixture was treated as usual to give a residue, which was purified by column chromatography to afford arjunglucoside I heptaacetate (**22**; 30 mg), mp 147—149 °C, $[\alpha]_D$ −7° (*c* 1.4, EtOH); IR (KBr) 3520, 1740, and 1630 cm^{-1}; PMR (Table 1); mass spectrum *m/e* 331, *m/e* 271, *m/e* 264, *m/e* 246, *m/e* 231, *m/e* 211, *m/e* 201, *m/e* 169, *m/e* 109, and *m/e* 43 (base peak) (no molecular ion peak was observed). Found: C, 62.08; H, 7.59%. Calcd for $C_{50}H_{72}O_{18}$: C, 62.49; H, 7.55%.

Methanolysis of Arjunglucoside II (3). A mixture of arjunglucoside II (**3**; 130 mg), methanol (5 ml), water (1 ml), and sulfuric acid (0.2 ml), was heated under reflux for 4 h. Water was added to the reaction mixture and the resulting mixture was extracted with ether. From the ethereal layer arjunolic acid[6] (**24**; 85 mg) was obtained (identified by mp, $[\alpha]_D$, IR, TLC, and mass spectrometry). The aqueous layer was treated as described in the methanolysis of **2** to give a residue, which proved to be methyl D-glucoside (identified by its conversion to methyl tetra-*O*-trimethylsilyl-D-glucoside; detection by GLC).

Alkaline Hydrolysis of Arjunglucoside II (3). A mixture of arjunglucoside II (**3**; 100 mg), methanol (4 ml), water (2 ml), and potassium hydroxide (0.2 g), was heated under reflux for 4 h. The reaction mixture was poured into water, acidified by addition of hydrochloric acid, and extracted with ether. The ethereal layer gave, after the usual work-up, arjunolic acid[6] (**24**; 60 mg) (identified by mp, $[\alpha]_D$, IR, TLC, and mass spectrometry). The aqueous layer was treated as described in the alkaline hydrolysis of **2** to give a residue, which proved to be identical with D-glucose.

Acetylation of Arjunglucoside II (3). Arjunglucoside II (**3**; 40 mg) in pyridine was acetylated with acetic anhydride overnight at room temperature. After the usual work-up, a residue was obtained. The residue was chromatographed to yield arjunglucoside II heptaacetate (**25**; 46 mg), mp 136—140 °C, $[\alpha]_D$ +26° (*c* 2.0, $CHCl_3$); IR (Nujol) 1740 and 1630 cm^{-1}; PMR (Table 1), mass spectrum *m/e* 331, *m/e* 271, *m/e* 248, *m/e* 233, *m/e* 211, *m/e* 203, *m/e* 189, *m/e* 169, *m/e* 133, *m/e* 109, and *m/e* 43 (base peak) (no molecular ion peak was observed). Found: C, 62.33; H, 7.54%. Calcd for $C_{50}H_{72}O_{17}\cdot H_2O$: C, 62.35; H, 7.74%.

Methylation of Arjunglucoside II (3). To a solution of arjunglucoside II (**3**; 110 mg) in *N*,*N*-dimethylformamide (3 ml), were added methyl iodide (4 ml) and silver oxide (1.2 g), and the whole was stirred at 60 °C for 24 h. After cooling, the reaction mixture was filtered to remove insoluble material, which was washed several times with chloroform. The washings were combined with the filtrate. Evaporation of the solvents gave a residue, which was dissolved in chloroform. This chloroform solution was washed successively with water, aqueous sodium thiosulfate solution, and with brine, and dried over calcium chloride. Removal of the solvent gave a residue, which was chromatographed to give arjunglucoside II heptamethyl ether (**26**; 30 mg), an amorphous solid, IR (KBr) 1740 and 1635 cm^{-1}; PMR ($CDCl_3$) δ 0.68, δ 0.78, δ 0.98, and δ 1.16 (each 3H, s; *t*-CH_3), δ 0.94 (6H, s; 2×*t*-CH_3), δ 3.33, δ 3.38, δ 3.43, and δ 3.65 (each 3H, s; OCH_3), δ 3.54 (9H, s; 3×OCH_3), δ 5.32 (2H, m; $C_{(12)}$–H and $C_{(13)}$–H); mass spectrum *m/e* 748 (M^+), *m/e* 704, *m/e* 584, *m/e* 530, *m/e* 528, *m/e* 498, *m/e* 497, *m/e* 248, *m/e* 219, *m/e* 203, *m/e* 187 (base peak), and *m/e* 155. Found: C, 67.53; H, 9.79%. Calcd for $C_{43}H_{72}O_{10}\cdot H_2O$: C,

67.33; H, 9.72%.

Acid Hydrolysis of Arjunglucoside II Heptamethyl Ether (26). A mixture of the heptamethyl ether (**26**; 100 mg), methanol (8 ml), and hydrochloric acid (0.3 ml), was heated under reflux for 7 h. The reaction mixture was poured into water and extracted with ether. Evaporation of the solvent gave a residue, which was chromatographed to afford arjunolic acid trimethyl ether (**27**; 43 mg), mp 140—150 °C (dec); IR (Nujol) 1690 cm^{-1}; PMR ($CDCl_3$) δ 0.65, δ 0.72, and δ 1.12 (each 3H, s; t-CH_3), δ 0.94 (9H, s; $3\times t$-CH_3), δ 3.31, δ 3.42, and δ 3.52 (each 3H, s; OCH_3), δ 5.27 (1H, br. signal; $C_{(12)}$–H); mass spectrum *m/e* 530 (M^+), *m/e* 528, *m/e* 498, *m/e* 466, *m/e* 453, *m/e* 248 (base peak), *m/e* 233, *m/e* 203, *m/e* 189, and *m/e* 133. Found; *m/e* 530.3887. Calcd for $C_{33}H_{54}O_5$: *m/e* 530.3968.

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