Pergamon

PII: S0031-9422(97)00017-4

Phytochemistry, Vol. 45, No. 3, pp. 579–584, 1997

0031-9422/97 $17.00+0.00

LUPANE TRITERPENOID GLYCOSYL ESTERS FROM LEAVES OF *ACANTHOPANAX DIVARICATUS*

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(*Received in revised form* 2 *December* 1996)

Key Word Index—*Acanthopanax divaricatus*; Araliaceae; triterpenoid saponin; lupane glycoside; chiisanoside; isochiisanoside; protochiisanoside; 22α-hydroxychiisanoside.

Abstract—Further investigation of the leaves of *Acanthopanax divaricatus* gave two analogues of chiisanoside, which is a lupane triterpenoid oligoglycosyl ester. The structures were established as 28-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 6)β-D-glucopyranosyl esters of 1β,11α-dihydroxy-3-oxo-lup-20(29)-en-28-oic acid and 1(*R*),11α,22α-trihydroxy-3,4-*seco*-lupa-4(23),20(29)-diene-3,28-dioic acid 3,11α-lactone based on chemical and spectroscopic evidence. In biosynthetic terms, one is the precursor of chiisanoside and the other is an oxygenated derivative chiisanoside.

INTRODUCTION

In our course of phytochemical studies on the genus *Acanthopanax*, we have isolated exclusively oleanane saponins from the leaves of *A. spinosus* Miq. [1, 2], *A. sieboldianus* Makino [3] and *A. nipponicus* Makino [4]. Oleanane saponins were also reported from the leaves of *A. senticosus* Harms [5, 6] and *A. hypoleucus* Makino [7]. However, only the leaves of *A. divaricatus* Seem. [8] and *A. chiisanensis* Nakai [9, 10] afforded lupane saponins. It is of chemotaxonomic interest that these two species gave no oleanane saponins. We have reinvestigated the leaves of *A. divaricatus* and isolated two novel lupane oligoglycosyl esters together with four known glycosides. This paper describes the isolation and structural determination of these compounds.

RESULTS AND DISCUSSION

The methanolic extract of the leaves of *A. divaricatus* gave two flavonol glycosides (**1** and **2**) and four triterpenoid saponins (**3**–**6**).

Based on the evidence of the NMR and FAB mass spectra, as well as on a comparison of the optical rotations with reported values, the flavonol glycosides **1** and **2** were shown to be identical with hyperin [11] and quercitrin [12], and the saponins **3** and **4** were identified as chiisanoside [9] and isochiisanoside [9], respectively. The assignments of the ^{13}C signals for chiisanoside and isochiisanoside [8, 10] were partly revised (Table 1) on the basis of 2D NMR experiments.

The new saponin, **5** (yield 0.39%), was named protochiisanoside. Its M_r was shown to be 956 by negative ion FAB mass spectroscopy, and its molecular formula was determined as $C_{48}H_{76}O_{19}$ by elemental analysis. The ^1H and ^{13}C signals of **5** were superimposable onto those of chiisanoside (**3**), except for the signals due to the ABC ring moiety. The ABC ring moiety of **5** showed ^1H NMR signals due to two tertiary methyls but lacked those of an isopropenyl group. In the ^{13}C NMR spectrum it contained the signal of a ketone carbonyl instead of an ester carbonyl. To avoid the confusion caused by the signals of the sugar moiety, **5** was treated with crude pectinase to give an aglycone (**5a**). The aglycone **5a** had a molecular formula $C_{30}H_{46}O_5$ and its ^1H NMR spectrum showed six tertiary methyls, a vinylidene group, an equatorial hydroxyl group substituted on C-11 (geminal proton at δ 3.89(*ddd*) and placed between methylene and methine groups ($J_{11,9}(=J_{ax,ax})=10.5$ Hz, $J_{11,12\alpha}(=J_{ax,ax})=10.5$ Hz, $J_{11,12\beta}(=J_{ax,eq})=5.4$ Hz) and another secondary hydroxyl group (δ 4.08, *dd*, $J_{1,2\beta}(=J_{ax,ax})=8.4$ Hz, $J_{1,2\alpha}(=J_{ax,eq})=1.8$ Hz) which must also be equatorial and placed between a tetrasubstituted sp^3 carbon atom and a methylene group (δ 2.21, *dd*, $J_{2\alpha,1}=1.8$ Hz, $J_{\text{geminal}}=13.9$ Hz and 3.03, *dd*, $J_{2\beta,1}=8.4$ Hz, $J_{\text{geminal}}=13.9$ Hz) adjacent to a fully substituted carbon. Its ^{13}C NMR spectrum

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Table 1. ^{13}C Signals of compounds **3**, **3a**, **4**, **5**, **5a**, **5b**, **6**, **6a**, **6b** and **7** in C_5D_5N

C	**3**	**3a**	**4**	**5**	**5b***	**5a***	**7*†**	**6**	**6a**	**6b**
1	70.3	70.9	87.5	79.1	166.6	78.7	78.6	70.6	70.5	70.5
2	38.7	38.8	38.9	43.7	123.4	43.0	43.0	38.8	38.8	38.8
3	172.9	172.9	175.4	216.0	204.5	216.2	216.4	173.1	173.0	172.9
4	147.7	147.7	79.1	47.0	45.2	47.4	47.3	147.7	147.7	147.7
5	49.6	49.6	56.2	50.6	53.4	50.9	50.8	49.6	49.5	49.5
6	25.1	25.1	18.7	19.8	19.2	19.5	19.5	25.2	25.2	25.1
7	32.3	32.4	35.4	33.7	34.9	33.8	33.6	32.4	32.5	32.4
8	41.7	41.6	42.8	42.4	42.8	42.4	42.4[a]	41.7	41.6	41.6
9	44.0	44.0	48.9	56.2	49.6	55.5	55.2	44.1	44.0	44.1
10	44.1	44.1	46.9	44.3	43.0	44.0	43.9[a]	44.3	44.1	44.1
11	75.2	75.3	67.7	68.6	69.9	69.4	69.4	75.0	75.3	75.2
12	33.4	33.5	36.9	36.5	38.1	36.7	36.3	33.6	33.7	33.5
13	35.2	35.3	37.5	36.9	37.6	37.0	36.7	35.1	35.0	34.8
14	42.1	42.2	42.8	42.6	41.2	42.5	42.6[a]	42.2	42.2	42.0
15	29.5	29.6	30.3	30.0	30.1	29.8	27.4	29.0	29.1	28.7
16	32.1	32.6	32.2	32.1	32.8	32.1	35.3	26.7	27.0	26.7
17	56.7	56.3	57.0	56.8	56.6	56.3	42.9	62.9	62.5	60.7
18	48.6	49.5	49.5	49.3	49.2	48.6	47.6[b]	44.1	44.2	45.4
19	47.5	47.3	47.2	47.2	47.4	46.7	47.7b	47.8	48.0	47.3
20	150.4	150.5	150.5	150.2	150.7	149.7	150.2	150.6	151.0	‡
21	30.7	31.0	30.8	30.8	31.2	30.5	29.7	41.8	41.9	39.0
22	36.7	37.3	36.7	36.8	37.4	37.0	39.7	75.4	75.6	78.2
23	113.8	113.8	25.0	28.7	28.5	28.8	28.7	113.9	113.8	113.8
24	23.4	23.5	32.8	19.9	21.7	19.6	19.6	23.5	23.5	23.5
25	19.0	18.9	19.2	13.9	20.2	13.6	13.5	19.2	19.0	19.0
26	17.9	17.8	17.9	17.2	17.8	17.3	17.0	18.0	17.8	17.7
27	13.9	13.7	15.1	14.5	14.6	14.6	14.3	13.8	13.7	13.6
28	175.0	178.0	174.9	174.8	178.8	180.5	18.0	175.0	178.9	176.7
29	110.6	110.5	110.2	110.2	110.0	110.5	110.1	111.1	110.9	111.7
30	18.8	18.9	19.5	19.4	19.5	19.5	19.3	18.8	18.8	18.5
									CH_3CO	20.9
									$CH_3\underline{CO}$	170.1
28-*O*-Glc-1	95.3		95.3	95.2				95.5		
2	73.9[a]		73.9[a]	73.9[a]				73.9[a]		
(inner) 3	78.4		78.2	78.2				78.4		
4	70.9		70.9	70.9				70.8		
5	77.1		77.1	77.0				77.1		
6	69.4		69.4	69.4				69.4		
Glc-1	105.1		105.0	105.0				105.1		
2	75.2		75.3	75.2				75.2		
(outer) 3	76.4		76.4	76.4				76.4		
4	78.7		78.7	78.5				78.7		
5	78.0		78.0	77.9				78.0		
6	61.3		61.3	61.2				61.3		
Rha-1	102.7		102.7	102.6				102.7		
2	72.5[b]		72.5[b]	72.4[b]				72.5[b]		
3	72.7[b]		72.7[b]	72.6[b]				72.7[b]		
4	74.0[a]		74.1[a]	74.0[a]				74.1[a]		
5	70.5		70.3	70.2				70.3		
6	18.4		18.5	18.4				18.5		

* Measured in $CDCl_3$.

† Data taken from ref [13].

‡ Overlapped with solvent signal.

[a,b] Signals may be interchanged within each vertical column.

Multiplicities were established by DEPT experiments.

Table 2. ^{1}H NMR signals of compounds **3a**, **5a**, **5b**, **6a**, **6b** in C_5D_5N

H	3a	5a*	5b*	6a	6b
1	3.80 (*d*, 8.0)	4.08 (*dd*, 1.8, 8.4)	9.09 (*d*, 10.3)	3.80 (*d*, 8.1)	3.79 (*d*, 8.0)
2α	2.90 (*dd*, 8.0, 14.4)	2.21 (*dd*, 1.8, 13.9)	5.96 (*d*, 10.3)	2.90 (*dd*, 8.1, 14.6)	2.89 (*dd*, 8.0, 15.0)
2β	3.18 (*d*, 14.4)	3.03 (*dd*, 8.4, 13.9)	—	3.20 (*d*, 14.6)	3.18 (*d*, 15.0)
9	2.82 (*d*, 9.4)	1.62 (*d*, 10.5)	2.00 (*d*, 10.6)	2.87 (*d*, 9.0)	2.85 (*d*, 10.0)
11	4.69 (*ddd*, 9.4, 9.4, 9.4)	2.89 (*ddd*, 5.4, 10.5, 10.5)	4.27 (*ddd*, 5.1, 10.6, 10.6)	4.75 (*ddd*, 9.0, 9.0, 9.0)	4.73 (*ddd*, 10.0, 10.0, 10.0)
15β	1.83†	‡	1.75†	1.92†	1.85†
15α	1.21†	1.25†	1.25†	1.33†	1.28†
16α	1.60†	2.30†	1.55†	2.45 (*ddd*, 3.4, 13.0, 13.0)	1.92†
16β	2.64†	‡	2.60 (*ddd*, 3.0, 3.0, 11.5)	2.59†	2.45†
18	1.74 (*dd*, 10.8, 10.8)	1.67 (*dd*, 12.0 ,12.0)	1.78 (*dd*, 11.6, 11.6)	2.62 (*dd*, 11.2, 11.2)	2.28 (*dd*, 11.1, 11.1)
19	3.55 (*ddd*, 4.6, 10.8, 10.8)	3.00†	3.48 (*ddd*, 6.6, 11.6, 11.6)	3.70 (*ddd*, 5.0, 11.2, 11.2)	3.55 (*ddd*, 5.5, 11.1, 11.1)
21α	2.30†	‡	‡	2.75†	2.65 (*ddd*, 5.5, 15.0, 11.1)
21β	1.58†	‡	‡	1.85†	1.66 (*dd*, 15.0, 5.5)
22α	2.25†	‡	2.25†	—	—
22β	1.63†	‡	‡	4.87 (*d*, 5.0)	5.86 (*d*, 5.5)
23a	5.08 (*s*)	1.08 (*s*)	1.21 (*s*)	5.09 (*s*)	5.08 (*s*)
23b	5.19 (*s*)	—	—	5.20 (*s*, 1.6)	5.19 (*s*)
24	1.94 (*s*)	1.06 (*s*)	1.71 (*s*)	1.94 (*s*)	1.93 (*s*)
25	1.04 (*s*)	0.88 (*s*)	1.35 (*s*)	1.09 (*s*)	1.08 (*s*)
26	1.06 (*s*)	0.95 (*s*)	1.10 (*s*)	1.13 (*s*)	1.11 (*s*)
27	1.14 (*s*)	1.03 (*s*)	1.06 (*s*)	1.26 (*s*)	1.23 (*s*)
29a	4.71 (*s*)	4.65 (*s*)	4.62 (*s*)	4.78 (*s*)	4.75 (*s*)
29b	5.00 (*d*, 2.0)	4.78 (*d*, 1.5)	4.84 (*d*, 1.5)	5.13 (*d*, 1.6)	5.04 (*d*, 1.5)
30	1.76 (*s*)	1.70 (*s*)	1.70 (*s*)	2.04 (*s*)	1.83 (*s*)
					Ac 2.14 (*s*)

7* 4.08 (*dd*, 2.0, 8.5, H1), 2.21 (*dd*, 2.0, 13.4, H2α), 3.03 (*dd*, 8.4, 13.4, H2β), 1.61 (*d*, 10.7, H9), 3.89 (*ddd*, 10.7, 10.7, 5.3, H-11): data taken from ref [13].

* In $CDCl_3$.

† δ-value was determined from CH-COSY and HH-COSY.

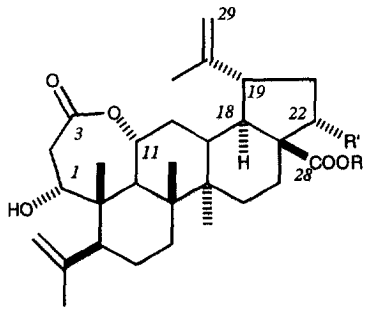
‡ Overlapped signal.

Multiplicity and *J*-value (Hz) in parentheses.

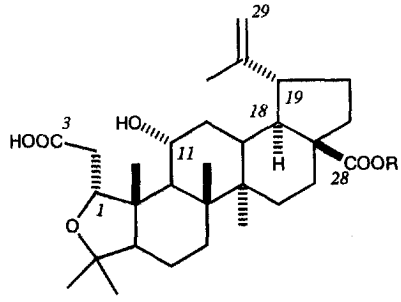
showed signals for a ketone carbonyl (δ 216.2), carboxyl (δ 180.5), two secondary oxygenated carbon (δ 78.7 and 69.4), and an isopropenyl (δ 149.7, 110.5, 19.5) group.

3,4-*seco*-Triterpenoids are derived biogenetically from the corresponding 3-ketones, therefore **5a** is the likely precursor of chiisanogenin (**3a**) and should be 1β,11α-dihydroxy-3-oxo-lup-20(29)-en-28-oic acid. Recently, Savona *et al.* [13] reported a triterpenoid with a closely related structure, 1β,11α-dihydroxy-lup-20(29)-en-3-one (**7**). The NMR data of **5a** are in complete agreement with the ABC ring moiety of **7** and the DE ring moiety of chiisanosgenin (**3a**) (Tables 1 and 2). Thus the structure of **5a** was established as 1β,11α-dihydroxy-3-oxo-lup-20(29)-en-28-oic acid. To confirm the whole structure of **5**, it was subjected to acid hydrolysis and selective cleavage of the ester glycoside. On acid hydrolysis, **5** afforded glucose (Glc) and rhamnose (Rha), as sugar components, and a different aglycone (**5b**) from the genuine aglycone (**5a**). The aglycone **5b** showed absorptions of hydroxyl, carboxyl and conjugated ketone groups in the IR spectrum, and signals due to an α,β-unsaturated ketone (δ 5.96, 9.09, *ABq*, J = 10.3 Hz) and only one secondary hydroxyl group (δ 4.27, *ddd*, J = 5.1, 10.6, 10.6 Hz) together with those due to six tertiary methyls and a vinylidene group in the ^{1}H NMR spectrum. This indicated that the α,β-unsaturated ketone of **5b** was formed by dehydration during the acid hydrolysis. Selective cleavage [14] of **5** gave an anomeric pair of methyl α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 6)-α,β-D-glucopyranosides (**8**) which was identified by direct comparison with an authentic sample [1]. The anomeric configuration of the ester-linked glucopyranosyl group was determined to be β from the $J_{1,2}$ value (8.2 Hz). All of the above findings confirmed the new saponin **5**, protochiisanoside, as the 28-*O*-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranosyl ester of 1β, 11α-dihydroxy-3-oxo-lup-20(29)-en-28-oic acid, a compound which is one of the precursors of **3**.

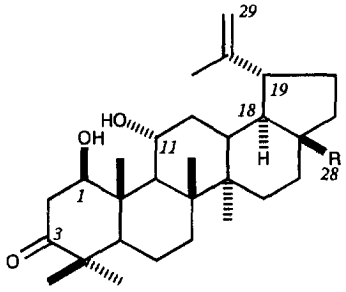
The second new saponin, **6** (yield 0.02%), named



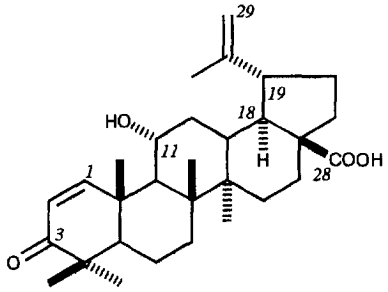
3 : R=-S, R'=-H
3a : R=-H, R'=-H
6 : R=-S, R'=-OH
6a : R=-H, R'=-OH
6b : R=-H, R'=-OAc



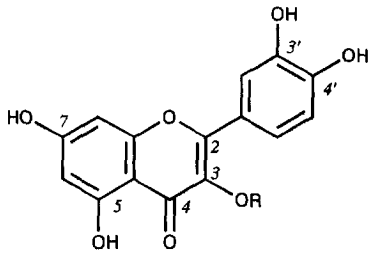
4 : R=-S
4a : R=-H



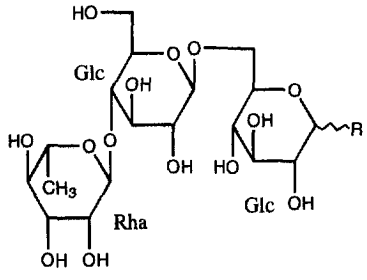
5 : R=-COO-S
5a : R=-COOH
7 : R=-CH_3



5b



1 : R=-β-D-Gal
2 : R=-α-L-Rha



S : R=-β
8 : R=-OCH_3

22α-hydroxychiisanoside, on FAB-mass spectrometry gave a $[M-H]^-$ ion at *m/z* 969, which is 16 mass units larger than that of **3** and was assigned the molecular formula $C_{48}H_{76}O_{20}$ by elemental analysis. Its ^{13}C NMR spectrum was similar to that of **3** except for the signals due to the DE ring moiety, suggesting that **6** was a monohydroxylated chiisanoside on its D or E ring. On enzymatic hydrolysis, **6** gave an aglycone (**6a**) whose $[M-H]^-$ ion appeared at *m/z* 499 which is larger than that of **3a** by 16 mass units. In the ^{13}C NMR spectrum of **6a** a new secondary hydroxyl carbon signal at δ 75.6, which correlated to δ 4.87 (*d*, $J = 5.0$ Hz) in the ^{13}C–^1H cosy spectrum. On acetylation with acetic anhydride in pyridine at room temperature, **6a** yielded a monoacetate **6b**, whereas chiisanogenin (**3a**) was not acetylated under the same conditions. Compound **6b** showed better resolution of signals and this facilitated the analysis of the position of the new hydroxyl group. Its acetylated hydroxymethylene proton at δ 5.86 (*d*, $J = 5.5$ Hz) was only coupled with the proton at δ 2.65 (*ddd*, $J_{21\alpha,22\beta} = 5.5$ Hz, $J_{21\alpha,19} = 11.1$ Hz, $J_{\text{geminal}} = 15.0$ Hz), which is ascribable to H-21α. The signal at δ 2.65 (H-21α) coupled with its geminal proton H-21β at δ 1.66 (*dd*, $J_{\text{geminal}} = 15.0$ Hz, $J_{21\beta,19} = 5.5$ Hz) and H-19, the characteristic ^1H-signal in lup-20(29)-en-28-oic acid type triterpenoids, at δ 3.55(*ddd*, $J_{19,21\beta} = 5.5$ Hz, $J_{19,18} = 11.1$ Hz, $J_{19,21\alpha} = 11.1$ Hz). Thus, it was deduced that the hydroxylation occurred on C-22 with α orientation. The signals due to H-18(*ax*) and H-16α(*ax*) of **6a** appeared abnormally deshielded by about 1 ppm compared to those of **3a**, which also demonstrated their 1,3-diaxial relationship with the

new hydroxyl group. Accordingly, the structure of **6a** was established as 1(*R*),11α,22α-trihydroxy-3,4-*seco*-lupa-4(23),20(29)-diene-3,28-dioic acid 3,11α-lactone. On acid hydrolysis and selective cleavage, **6** gave the same anomeric pair (**8**) and the same sugar component as above. The $J_{1,2}$ value (8.1 Hz) allowed the anomeric configuration of the ester linked glucopyranosyl group to be assigned as β. Consequently, the structure of **6** was formulated as the 28-*O*-α-L-rhamnopyranosyl (1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranosyl ester of 1(*R*),11α,22α-trihydroxy-3,4-*seco*-lupa-4(23),20(29)-diene-3,28-dioic acid 3,11α-lactone.

In summary, we have isolated four lupane triterpenoid oligoglycosyl esters, two of which were novel saponins, together with two known flavonol glycosides, from the leaves of *A. divaricatus*. No oleanane glycosides were found. It is notable from the chemotaxomomical point of view that there are two types of plants in the *Acanthopanax* spp.: one contains oleanane triterpenoid glycosides and the other lupane glycosides.

EXPERIMENTAL

Mps uncorr.; IR: KBr discs, unless otherwise stated; ^{1}H NMR: 400 MHz and ^{13}C NMR at 100 MHz; FID-GC: glass column (3 mm × 2.1 m) packed with 5% OV-17 at 155°. The solvents used for spectral determination were; C_5D_5N–TMS(NMR); MeOH($[\alpha]_D$), unless otherwise stated. CC: silica gel 60, RP-2, Sephadex LH-20 and Chromatorex ODS. The solvent systems for CC were all homogeneous.

Plant material. The plant was collected at Fujiyoshida city (Yamanashi Pref.), Japan, in November 1991 and botanically identified by one of the authors, Dr S. Isoda; the specimen has been deposited in the Herbarium of our school.

Extraction and separation. The dried leaves (630 g) were extracted with MeOH. After removal of the solvent by evapn, the extracts (147 g) were suspended in H_2O and partitioned with Et_2O (28.8 g). The H_2O layer was chromatographed on highly porous polymer resin (DIAION HP-20, Mitsubishi Chem. Ind. Co. Ltd, Tokyo, Japan) eluting with H_2O (70.1 g), MeOH (47.6 g) and Me_2CO (1.0 g), successively. The MeOH eluate was chromatographed on Sephadex LH-20 (MeOH) to give two frs, fr.I (43.1 g) and fr.II (4.5 g) in order of elution. Fr.II was repeatedly chromatographed on ODS CC (50% and 55% MeOH) to give **1** (2.45 g, 0.39%) and **2** (0.20 g, 0.03%). Fr.I was repeatedly chromatographed on silica gel CC ($CHCl_3$–MeOH–H_2O 30:10:1) and ODS CC (60% MeOH) to give four compounds, **3** (12.6 g; 2.0%), **4** (1.0 g, 0.16%), **5** (2.47 g, 0.39%) and **6** (0.12 g, 0.02%).

Hyperin. (**1**; yellow needles from MeOH, mp 234–235°) and *Quercitrin* (**2**; yellow needles from MeOH, mp 178–179°): mp, optical rotation, ^{1}H and ^{13}C NMR, and MS were identical with those in the lit. [11, 12].

Chiisanoside (**3**). A white powder $[\alpha]_D^{25}$ + 2.2° (*c* 1.10). FABMS (negative) *m/z*: 953 $[M-H]^-$, 483 $[M-(Glc\text{-}Glc\text{-}Rha)-H]^-$; IR ν_{max} cm^{-1} 3450 (OH), 1755, 1710 (COOR), 1640 (C = C); ^{1}H NMR δ: 1.03 (6H, *s*, H-27 and H-25), 1.12 (3H, *s*, H-26), 1.66 (3H, *s*, H-30), 1.71 (3H, *d*, *J* = 5.8 Hz, Rha H-6), 1.90 (3H, *s*, H-24), 2.71 (1H, *d*, *J* = 9.0 Hz, H-9), 3.09 (1H, *d*, *J* = 14.6 Hz, H-2), 3.37 (1H, *ddd*, *J* = 4.6, 10.7, 10.7 Hz, H-19), 3.73 (1H, *d*, *J* = 7.9 Hz, H-1), 4.52 (1H, *ddd*, *J* = 9.0, 9.0, 9.0 Hz, H-11), 4.62 (1H, *s*, H-29a), 4.88 (1H, *s*, H-29b), 4.96 (1H, *d*, *J* = 7.6 Hz, outer Glc H-1), 5.04 (1H, *s*, H-23), 5.16 (1H, *s*, H-23), 5.83 (1H, *s*, Rha H-1), 6.35 (1H, *d*, *J* = 8.2 Hz, inner Glc H-1).

Isochiisanoside (**4**). A white powder, $[\alpha]_D^{23}$ − 5.55° (*c* 0.35). FABMS (negative) *m/z*: 971 $[M-H]^-$, 501 $[M-(Glc\text{-}Glc\text{-}Rha)-H]^-$; IR ν_{max} cm^{-1} 3400 (OH), 2800–2600, 1730 (COOR), 1710 (COOH), 1640 (C=C); ^{1}H NMR δ: 1.20 (3H, *s*, H-27), 1.26 (6H, *s*, H-23 and H-26), 1.44 (3H, *s*, H-25), 1.54 (3H, *s*, H-24), 1.75 (3H, *s*, H-30), 1.76 (3H, *d*, *J* = 5.9 Hz, Rha H-6), 1.87 (1H, *dd*, *J* = 11.0, 11.0 Hz, H-18), 2.08 (1H, *d*, *J* = 11.0 Hz, H-9), 2.45 (1H, *ddd*, *J* = 12.5, 2.2, 2.2 Hz, H-12a), 2.73 (1H, *ddd*, *J* = 12.5, 2.2 2.2 Hz, H-16), 3.42 (1H, *ddd*, *J* = 11.0, 11.0, 4.8 Hz, H-19), 3.91 (1H, *dd*, *J* = 13.8, 2.2 Hz, H-2), 4.70 (1H, *s*, H-29a), 4.87 (1H, *s*, H-29b), 5.01 (1H, *d*, *J* = 7.8 Hz, outer Glc H-1), 5.12 (1H, *dd*, *J* = 11.4, 2.2 Hz, H-1), 5.90 (1H, *s*, Rha H-1), 6.39 (1H, *d*, *J* = 8.1 Hz, inner Glc H-1); ^{13}C NMR: Table 1.

Protochiisanoside (**5**). A white powder, $[\alpha]_D^{25}$ − 4.3° (*c* 1.01). FABMS (negative) *m/z*: 955 $[M-H]^-$, 485 $[M-(Glc\text{-}Glc\text{-}Rha)-H]^-$; IR ν_{max} cm^{-1} 3450 (OH), 1750 (COOR), 1705 (C=O), 1640 (C=C); $C_{48}H_{76}O_{19}\cdot 2H_2O$ requires: C 58.05, H 8.12, found: C 57.84, H 8.20; ^{1}H NMR δ: 1.15 (6H, *s*, H-25 and H-27), 1.17 (3H, *s*, H-23), 1.21 (3H, *s*, H-26), 1.24 (3H, *s*, H-24), 1.70 (3H, *s*, H-30), 1.75 (3H, *d*, *J* = 6.1 Hz, Rha H-6), 2.64 (1H, *d*, *J* = 13.2 Hz, H-2α), 3.30 (1H, *dd*, *J* = 8.8, 13.2 Hz, H-2β), 3.41 (1H, *ddd*, *J* = 4.6, 10.0, 10.0 Hz, H-19), 4.52 (1H, *d*, *J* = 8.8 Hz, H-1), 4.63 (1H, *s*, H-29a), 4.85 (1H, *s*, H-29b), 4.99 (1H, *d*, *J* = 7.9 Hz, outer Glc H-1), 5.91 (1H, *s*, Rha H-1), 6.39 (1H, *d*, *J* = 8.2 Hz, inner Glc H-1); ^{13}C NMR: Table 1.

22α-*Hydroxychiisanoside* (**6**). A white powder, $[\alpha]_D^{23}$ + 16.7° (*c* 0.25). FABMS (negative) *m/z*: 969 $[M-H]^-$, 499 $[M-(Glc\text{-}Glc\text{-}Rha)-H]^-$; IR ν_{max} cm^{-1} 3400 (OH), 1750, 1710 (COOR), 1640 (C=C); $C_{48}H_{76}O_{20}\cdot 2H_2O$ requires: C 57.24, H 7.81, found: C 57.63, H 7.79; ^{1}H NMR δ: 1.07 (3H, *s*, H-25), 1.19 (3H, *s*, H-26), 1.21 (3H, *s*, H-27), 1.74 (3H, *d*, *J* = 6.2 Hz, Rha H-6), 1.95 (3H, *s*, H-24), 1.96 (3H, *s*, H-30), 3.58 (1H, *ddd*, *J* = 4.9, 11.0, 11.0 Hz, H-19), 3.77 (1H, *d*, *J* = 12.6, H-1), 4.56 (1H, *ddd*, *J* = 9.0, 9.0, 9.0 Hz, H-11), 4.72 (1H, *s*, H-29a), 4.83 (1H, *d*, 4.1 Hz, H-22), 4.98 (1H, *d*, *J* = 7.7 Hz, outer Glc H-1), 5.05 (1H, *s*, H-29), 5.07 (1H, *s*, H-23a), 5.20 (1H, *s*, H-23b), 5.87 (1H, *s*, Rha H-1), 6.42 (1H, *d*, *J* = 8.1 Hz, inner Glc H-1); ^{13}C NMR: Table 1.

Enzymatic hydrolysis of **3**, **5** *and* **6**. A mixt. of **5** (120 mg) and crude pectinase (1 ml in 50% glycerol, Sigma

Chemical Co.) was incubated at 40° for 48 hr. The reaction mixt. was subjected to CC on RP-2 (eluting with H_2O and MeOH, successively) to give **5a** (15 mg) from the MeOH eluate. Compound **6** (117 mg) was hydrolyzed in the same way, yielding **6a** (33 mg). Compound **3** (150 mg) was hydrolyzed with crude hesperidinase (50 mg, Sigma Chemical Co.) to give **3a** (100 mg).

Chiisanogenin (**3a**). A white power, $[\alpha]_D^{26}+76.0°$ (*c* 0.90). EIMS *m/z*: 484 $[M]^+$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 1730 (COOR), 1705 (sh, COOH), 1650 (C=C); ^{13}C NMR: Table 1. ^1H NMR; Table 2.

Protochiisanogenin (**5a**). A white powder, $[\alpha]_D^{25}$ $+0.30°$ (*c* 0.50). FABMS (negative) *m/z*: 485 $[M-H]^-$; EIMS *m/z*: 468.3257 $[M-H_2O]^+$ $C_{30}H_{44}O_4$ requires: 468.3240; IR ν_{max} cm^{-1} 3450 (OH), 2800-2600, 1715 (cyclic ketone), 1705 (sh, COOH), 1650 (C=C); ^{13}C NMR; Table 1; ^1H NMR; Table 2.

22α-Hydroxychiisanogenin (**6a**). A white powder, $[\alpha]_D^{23}+79.8°$ (*c* 0.25). FABMS (negative) *m/z*: 499 $[M-H]^-$; EIMS *m/z*: 500.3089 $[M]^+$. $C_{30}H_{44}O_6$ requires: 500.3138; IR ν_{max} cm^{-1} 3400 (OH), 1750 (COOR), 1710 (COOH), 1640 (C=C); ^{13}C NMR; Table 1; ^1H NMR; Table 2.

Acid hydrolysis of **5**. A soln of **5** (100 mg) in 0.5 M HCl (50% dioxane) was heated at 70° for 3 hr. After cooling, the reaction mixt. was concd to half vol. and subjected to CC on RP-2 (eluting with H_2O and MeOH, successively) to give **5b** (35 mg) from MeOH eluate.

Dehydroprotochiisanogenin (**5b**). A white powder, $[\alpha]_D^{25}+0.14°$ (*c* 0.50). FABMS (negative) *m/z*: 467 $[M-H]^-$; EIMS *m/z*: 468 $[M]^+$; IR ν_{max} cm^{-1} 3450 (OH), 2800–2600, 1700 (COOH), 1720, 1670 (conjugated ketone), 1650 (sh, C=C); ^{13}C NMR: Table 1; ^1H NMR; Table 2.

Selective cleavage of ester glycoside linkage of **5** *and* **6**. A soln of **5** (71 mg) and LiI (70 mg) in 2,6-lutidine (3 ml) and dry MeOH (3 ml) was refluxed for 17 hr under a N_2 atm. After cooling, the reaction mixt. was diluted with 50% aq. MeOH (3 ml), deionized with Amberlite MB-3(H^+, OH^- form) and evapd to dryness. A suspension of the residue in H_2O was extracted with $CHCl_3$. The aq. layer afforded methyl α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl (1 → 6)-α,β-D-glucopyranosides (8 mg), identified by direct comparison with authentic sample [1]. By the same reaction, **6** (78 mg) afforded the same anomeric pair of methyl triglycosides (13 mg).

Acetylation of **3a** *and* **6a**. A mixt. of **6a** (19 mg), C_5H_5N (1 ml) and Ac_2O (1 ml) was left overnight at room temp. The reaction mixt. was subjected to CC on silica gel ($CHCl_3$–MeOH, 10:1) to give **6b** (15 mg). In the same way, **3a** (20 mg) gave **3a** (17 mg).

Acknowledgements—Thanks are due to the staff of the Analytical Centre of this school for measurements of spectra and elemental analysis.

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