Biologically Active Clerodane-type Diterpene Alcohol and its Glycosides from the Root-stalks of Ferns

Hamid Latif Siddiqui, Kiyotaka Munesada and Takayuki Suga *
Department of Chemistry, Faculty of Science, Hiroshima University, Kagamiyama, Higashi-Hiroshima 724, Japan

A diterpene alcohol showing a growth inhibition of lettuce was isolated from the root-stalks of *Dicranopteris pedata* and *Gleichenia japonica*. Its structure was determined to be (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol **1**, an aglycone of the two glycosides previously isolated from *G. japonica*; the chirality at both C-6 and C-13 was assigned as *S* by application of the β-D-glucosylation-shift rule and by measurement of ^{13}C NMR chemical shifts, respectively. Further, two glycosides showing growth inhibition were also isolated from *D. pedata* and they were characterised as the 13-*O*-β-L-fucopyranosyl-(1 ⟶ 2)-α-L-rhamnopyranoside **3** and 13-*O*-α-L-fucopyranosyl-(1 ⟶ 2)-[α-L-rhamnopyranosyl-(1 ⟶ 3)]-α-L-rhamnopyranoside **4** of the diterpene alcohol; the above assignment of the chirality at C-13 was established by measurement of nuclear Overhauser enhancement for the trisaccharide. In addition, a glycoside showing growth acceleration was also isolated and its structure was determined to be (6*S*,13*S*)-13-*O*-β-L-fucopyranosyl-6-*O*-α-L-rhamnopyranosylcleroda-3,14-diene-6,13-diol **2**.

Gleichenia japonica Spreng ('Urajiro' in Japanese) and *Dicranopteris pedata* Nakaike ('Koshida' in Japanese) are ferns belonging to the same family (Gleicheniaceae), which grow as a large community containing no other species of plants. Previously, we found that two clerodane-type and three labdane-type diterpene glycosides are present in the root-stalks of *G. japonica* and that three of them inhibit the growth of *Lactuca sativa* (lettuce).[1,2] However, the absolute configurations of the two clerodane-type diterpene glycosides have not been determined yet. We searched for allelopathic substances present in the root-stalks of *D. pedata* and have now isolated a clerodane-type diterpene alcohol and two corresponding glycosides showing growth inhibition of lettuce, together with a related diterpene glycoside. In addition, the absolute configurations of the two clerodane-type diterpene glycosides previously isolated from *G. japonica*[1] have also been determined.

Results and Discussion

Isolation of Compounds and their Biological Activities.—Fresh root-stalks of *D. pedata* were extracted with distilled water, followed by a mixed solvent of water and methanol (1:1, v/v) to give a water-soluble fraction. To remove less polar material, this fraction was then treated with chloroform. The chloroform-insoluble fraction was suspended in methanol to give a methanol-soluble fraction. The latter fraction was then subjected to reversed-phase (C_{18}) column chromatography, followed by silica gel column chromatography to give the four compounds **1**, **2**, **3** and **4**. The four compounds were each finally purified by reversed-phase HPLC. Compound **1** was also isolated from *G. japonica*, in addition to two clerodane-type diterpene glycosides **5** and **6**, previously reported.[1] The chromatographic, physical and spectral properties of samples of compound **1** isolated from the two ferns were completely identical. Table 1 shows the effects of the six compounds **1**–**6** on the elongation of the stem and root of lettuce. Compound **1** inhibited root and stem elongation at 100 ppm and its activity was at the same level as that observed for compound **5**. Further, two compounds, **3** and **4**, inhibited the elongation at 1000 ppm. On the other hand, two compounds, **2** and **6**, accelerated the elongation at 100 ppm.

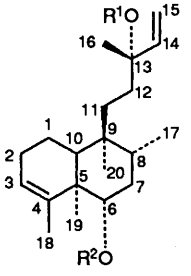
Structures of the Compounds.—High-resolution EI mass spectroscopy of compound **1** showed three characteristic fragment ions, at *m/z* 288.2453 ($C_{20}H_{32}O$, $M^+ - H_2O$), *m/z* 207.1578 ($C_{14}H_{23}O$) and *m/z* 71.0503 [MeC(OH)CH=CH_2]$^+$. The ^1H and ^{13}C NMR spectra of compound **1** coincided with those for the aglycone part of compound **5**, except for the carbon signal at δ_C 75.0 due to C-6. Compound **1**, on acetylation with Ac_2O and pyridine, afforded a monoacetate **7**. The IR spectrum of acetate **7** showed a band (3500 cm^{-1}) assignable to an hydroxy group, indicating the presence of a tertiary hydroxy group in compound **1**. These findings indicate that compound **1** is the aglycone of compound **5**. The enzymatic hydrolysis of glycoside **5** with β-glucosidase afforded compound **1** and D-glucopyranose. Upon comparison of the ^{13}C NMR spectra of compounds **1** and **5**, the C-6 carbon signal for compound **5** appeared at a lower field ($\Delta\delta$ + 10.7 ppm). By application of β-D-glucosylation-shift rule,[3,4] the chirality at C-6 of compounds **1** and **5** was determined to be *S*. Since the relative configuration of the aglycone part of compound **5** had already been elucidated by an NOE experiment,[1] the absolute configuration of compound **1** was thus determined, except for the chirality at C-13. Further, the chirality at C-13 was proposed to be *S* by the appearance of the C-16 Me carbon signal at δ_C 27.8 in the ^{13}C NMR spectrum compound of **1**.[5,6] Accordingly, the structure of compound **1** was determined to be (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol.

Compound **2** showed a molecular-ion peak at *m/z* 622 [M + Na + H]$^+$ in the secondary ionisation mass spectrum (SIMS). The molecular formula was determined to be $C_{32}H_{54}O_{10}$ by the high-resolution negative fast-atom bombardment mass spectrum [HR-(−)-FABMS]. The ^1H and ^{13}C NMR spectra of compound **2** coincided with those of compound **1**, except for the carbon signals at δ_C 86.3 and δ_C 80.2 due to C-6 and C-13, respectively, of the aglycone part and a series of signals due to two additional sugar moieties. The sugar moieties were found to be composed of one unit of β-L-fucopyranose (β-Fuc) and one unit of α-L-rhamnopyranose (α-Rha) by the similarity of their ^1H and ^{13}C NMR spectra with the literature data[7–9] and by interpretation of the 2D-COSY spectra. The position of the glycosidic linkage in compound **2** was determined by the NOE experiment. In the difference NOE spectrum of compound **2**, the

Table 1 Effects of compounds **1–6** on the elongation of the stems and roots of lettuce

Conc[a] (ppm)	Relative values (%)[a] compared with control (100%)											
	1		**2**		**3**		**4**		**5**		**6**	
	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root
1	88	81	119	110			101	107	98	83		
10	82	89	111	122			109	112	82	79	96	181
50					86	73						
100	81	88	127	131	90	77	111	109	77	85	142	123
1000					79	30	50	47			120	97

[a] Values are means of five duplications (s.d. is lower than 10% of the mean values).



	R^1	R^2
1	H	H
2		
3		H
4		H

	R^1	R^2
5	H	
6	H	
7	H	Ac
8		Ac

anomeric proton signals of α-Rha [δ_H 5.38 (br s)] and β-Fuc [δ 4.77 (d, J 7.8 Hz)] showed NOEs to the 6-H signal at δ_H 3.36 and the 16-Me signal at δ_H 1.57 (5), respectively. These findings indicate that the α-Rha links to the C-6, and β-Fuc to the C-13 of the aglycone part of compound **2**. Upon comparison of the ^{13}C NMR spectra of compounds **2** and **1**, the C-6 carbon signal for compound **2** was deshielded by $\Delta\delta$ + 11.3 ppm. According to the glycosylation-shift rule,[3,4] the chirality at C-6 of compound **2** was assigned to be *S*. Thus, the structure of compound **2** was determined to be (6*S*,13*S*)-13-*O*-β-L-fucopyranosyl-6-*O*-α-L-rhamnopyranosylcleroda-3,14-diene-6,13-diol.

Compound **3** showed a molecular ion peak at m/z 622 (M + Na + H)$^+$ and two fragment ion peaks, at m/z 293 (6-deoxyhexose-*O*-6-deoxyhexose)$^+$ and 147 (6-deoxyhexose)$^+$ in the SIMS. The molecular formula was determined to be $C_{32}H_{54}O_{10}$ by the HR-(−)-FABMS. The ^1H and ^{13}C NMR spectra of compound **3** coincided with those of **1**, except for the carbon signal at δ_C 79.8 due to the C-13 carbon signal of the aglycone part and a series of signals due to a sugar moiety.

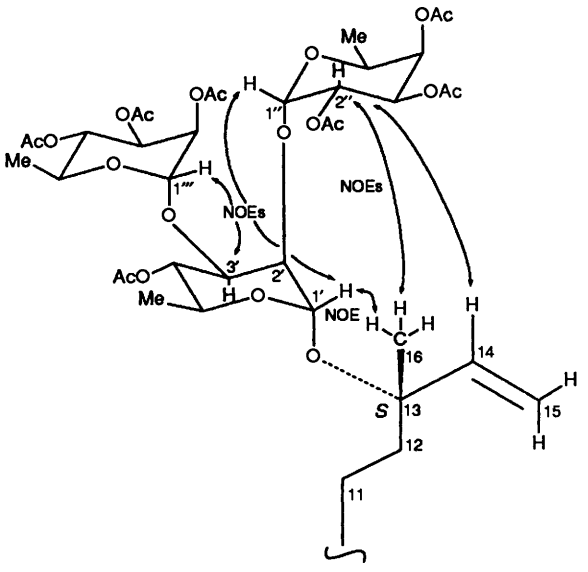


Fig. 1 Partial structure and NOEs of the octaacetate **8**

Further, the ^{13}C NMR spectrum for the sugar moiety of compound **3** was similar to that of compound **2**, except for the carbon signal at δ_C 83.0 due to C-2′ of α-Rha. The interglycosidic linkages were determined on the basis of the difference NOE spectrum. The proton signal at δ_H 5.65 (br s) due to 1′-H of α-Rha showed an NOE to the signal at δ_H 1.49 (s, 16-H_3) while the signal at δ_H 5.02 (d, *J* 7.8 Hz) due to 1″-H of β-Fuc showed an NOE to the signal at δ_H 4.39 due to 2′-H of α-Rha. From these findings, the sugar sequence in compound **3** was characterised to be β-L-fucopyranosyl-(1 ⟶ 2)-α-L-rhamnopyranoside. Thus, the structure of compound **3** was determined to be (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol 13-*O*-β-L-fucopyranosyl-(1 ⟶ 2)-α-L-rhamnopyranoside.

Compound **4** showed a molecular ion peak at *m/z* 768 $(M + Na + H)^+$ and a characteristic fragment ion peak at *m/z* 439 [6-deoxyhexose-*O*-6-deoxyhexose-*O*-6-deoxyhexose]$^+$ in the SIMS. The molecular formula was determined to be $C_{38}H_{64}O_{14}$ by the HR-(−)-FABMS. The ^1H and ^{13}C NMR spectra were identical with those of compound **3**, except for carbon signals due to a sugar moiety. The sugar moiety was found to be composed of two units of α-Rha and one unit of α-Fuc in view of the ^1H, ^{13}C and 2D-NMR spectra.[7–9] The interglycosidic linkages of the three sugars and the chirality at C-13 of the aglycone part were established on the basis of the difference NOE spectrum of the octaacetate **8** of **4** as depicted in the partial structure (Fig. 1). Two proton signals at δ_H 4.36 (br s, 2′-H) and δ_H 4.51 (dd, *J* 2.7, 8.7 Hz, 3′-H) of an α-Rha unit showed NOEs to the signals at δ_H 5.05 (s, 1″-H) of an α-Fuc unit and δ_H 5.26 (br s, 1‴-H) of another α-Rha unit, respectively. This characterised the sugar sequence as 13-*O*-α-L-fucopyranosyl-(1 ⟶ 2)-[α-L-rhamnopyranosyl-(1 ⟶ 3)]-α-L-rhamnopyranoside. Further, the proton signal at δ_H 1.28 due to 16-H_3 of the aglycone part showed NOEs to the signals due to 1′-H [δ_H 5.32 (br s)] of an α-Rha and 2″-H [δ_H 5.03 (d, *J* 7.1 Hz)] of an α-Fuc. The 2″-H signal showed an NOE to the signal at δ_H 5.66 (dd, *J* 8.3, 10.1 Hz) due to 14-H of the aglycone part. Considering the molecular structural model, these NOE data were best fitted with an *S*-chirality at C-13 of compound **4**. This also supported the chirality of this carbon in compound **1** described above. Thus, the structure of compound **4** was determined to be the 13-*O*-α-L-fucopyranosyl-(1 ⟶ 2)-[α-L-rhamnopyranosyl-(1 ⟶ 3)]-α-L-rhamnopyranoside of compound **1**.

Considering the structures of the diterpene alcohols and their glycosides in the root-stalks of *D. pedata* and *G. japonica*, the three glycosides **2**, **3** and **4** containing a 13-*O*-glycosidic linkage were found to be specifically present in the former plant. On the other hand, a series of labdane-type diterpene glycosides previously isolated from the latter[2] were not present in the former. Further, it is suggested that the growth-inhibition properties of the clerodane-type diterpene alcohol **1** are suppressed upon glycosylation at C-13, from consideration of the structures of the six compounds **1–6** and their effects on the growth of lettuce (Table 1).

Experimental

Optical rotations were measured in MeOH solution with a JASCO DIP-360 digital polarimeter. Values are now given in units of 10^{-1} deg cm^2 g^{-1}. SIMS spectra were obtained by detecting positive ions with a Hitachi M-80B double-focussing spectrometer equipped with an M-8086 Xenon beam-generating system. Analytical conditions were as follows: matrix, mixture of glycerol (2 mm^3) and 0.1% $MeCO_2Na$ in MeOH (1 mm^3); accelerating voltage, 3 keV. Assignment of mass number was achieved by comparing the spectra with the mass spectrum of CsI. High-resolution EI mass spectrum was measured at 70 eV and with an ion-source temperature of 200 °C. High-resolution FAB mass spectra were obtained by detecting negative ions with a JEOL JMS-SX 102A mass spectrometer. Analytical conditions were as follows: matrix, glycerol; accelerating voltage, 10 keV; calibration standard, PEG-600 and PEG-1000. ^1H, ^{13}C and 2D-NMR spectra were recorded on JEOL GSX-270 and GSX-500 spectrometers, with $SiMe_4$ as internal standard. *J*-values are given in Hz. GLC-MS analyses were performed on a Shimadzu QP-1000 instrument under the following GLC conditions: column, 0.21 mm × 50 m glass capillary column (WCOT) coated with OV-17; injector temp. 250 °C; column temp. 2 °C min^{-1} from 100 to 250 °C; carrier gas He; split ratio 60:1. GLC was performed on a Shimadzu GC-14A equipped with flame ionisation detector (FID) under the following conditions: 2.6 mm (i.d.) × 3 m glass column packed with 2% OV-17 on Chromosorb W-AWDMCS (80–100 mesh); column temp. 150 °C and injection temp. 250 °C. Analytical TLC was carried out on Merck 60 GF_{254} silica gel plates (thickness 0.25 mm).

Plant Material.—The root-stalks of *D. pedata* and *G. japonica* were collected in the Hiroshima Prefecture.

Extraction and Isolation.—Fresh root-stalks of *D. pedata* were washed with water and then air-dried. The dried root-stalks (3.05 kg) were cut into small pieces and immersed in distilled water (7 dm^3) for four days at room temp. After filtration, the root-stalks were then immersed in water–methanol (1:1 v/v; 5 dm^3) under the same conditions as above. The water and water–methanol extracts were combined, concentrated under reduced pressure and lyophilised to give a dark brown cake (86.6 g). To remove less polar substances, the cake was suspended in chloroform (400 cm^3) and the suspension was treated in an ultrasonic bath (37 kHz) for 1 h. After filtration, the insoluble material was suspended in methanol (500 cm^3) and then ultrasonicated as described above. The suspension was filtered and the filtrate, after removal of solvent under reduced pressure, gave a methanol-soluble fraction (67.7 g). This fraction was then subjected to reversed-phase column chromatography with the solvent systems water–methanol (2:1, 1:1, 2:3 and 1:4; 100 cm^3 each). The fractions eluted with water–methanol (2:3 and 1:4) were combined, and monitored with flash column chromatography on silica gel with the mixed solvent system chloroform (+1% water) in methanol (7.5:2.5) as eluent, to yield the four compounds **1–4**. The four compounds were finally purified by HPLC with a reversed-phase column [TOSOH ODS-C_{18},

300 × 7.5 mm; water–methanol (1:4)] to afford compounds **1** (18 mg), **2** (8 mg), **3** (8 mg) and **4** (50 mg).

Air-dried root-stalks of *G. japonica* (4.36 kg) were treated in a similar manner to that used for *D. pedata*. Final purification by HPLC afforded compounds **1** (5 mg), **5** (16 mg) and **6** (73 mg).

Bioassay.—Bioassay of compounds **1–6** was performed on filter-papers contained in plastic petri dishes of radius 2.5 cm. Solutions, in EtOH, of different concentrations ranging from 1 to 1000 ppm were prepared. Each solution (0.5 cm^3) was placed on the filter-paper and the paper was allowed to dry in the air; this was followed by the addition of distilled water (0.5 cm^3). Ten lettuce seeds were placed in each petri dish and all the tested samples were maintained in the dark (25 °C). After 3 days, the lengths of the roots and stems of the germinated seeds were measured. The same procedure was applied for the control. The average value of a given parameter was measured in duplicate. Five such duplicates were used for statistical analysis. The results are given in Table 1.

(6S,13S)-*Cleroda*-3,13-*diene*-6,13-*diol* **1**.—Needles from hexane; $[\alpha]_D^{25}$ +17 ($CHCl_3$; *c* 0.525) [Found: $M^+ - H_2O$ (EI), 288.2453. $C_{20}H_{32}O$ requires *m/z*, 288.2452; 207.1578. $C_{14}H_{23}O$ requires *m/z*, 207.1748; 71.0503. C_4H_7O requires *m/z*, 71.0497]; δ_H(C_5D_5N; 500 MHz) 3.76 (ddd, *J* 3.8, 5.5 and 9.3, 6-H), 6.16 (dd, *J* 10.3 and 17.1, 14-H), 5.17 (dd, *J* 1.95 and 10.3, 15-H*Z*), 5.56 (dd, *J* 2.0 and 17.1, 15-H*E*), 1.49 (s, 16-H_3), 0.81 (d, *J* 6.4, 17-H_3), 2.19 (br s, 18-H_3), 0.76 (s, 19-H_3) and 1.27 (s, 20-H_3); δ_H(C_5D_5N; 125 MHz) 18.2 (C-1), 27.1 (C-2), 121.8 (C-3), 145.3 (C-4), 38.4 (C-5), 75.0 (C-6), 38.9 (C-7), 34.8 (C-8), 44.5 (C-9), 46.0 (C-10), 32.6 (C-11), 35.8 (C-12), 72.6 (C-13), 147.3 (C-14), 111.3 (C-15), 28.7 (C-16), 15.9 (C-17), 23.3 (C-18), 18.4 (C-19) and 16.0 (C-20); δ_C($CDCl_3$; 67.5 MHz) 73.4 (C-13), 145.1 (C-14), 111.8 (C-15) and 27.8 (C-16).

Acetylation of Compound **1**.—Compound **1** (5.8 mg) was treated with Ac_2O and pyridine (each 1 cm^3) for 2 h at 70 °C to give a monoacetate **7** (5.5 mg), R_f 0.69 [MeOH–$CHCl_3$ (1:24)]; ν_{max}($CHCl_3$)/cm^{-1} 3500 (OH), 1740 (C=O), 1370, 1220 and 1020; δ_H(C_5D_5N; 500 MHz) 4.85 (dd, *J* 4.6 and 11.5, 6-H), 6.14 (dd, *J* 10.5 and 17.4, 14-H), 5.16 (dd, *J* 1.8 and 10.5, 15-H*Z*), 5.55 (dd, *J* 1.8 and 17.4, 15-H*E*), 1.50 (s, 16-H_3), 0.72 (d, *J* 6.4, 17-H_3), 1.68 (s, 18-H_3), 0.69 (s, 19-H_3), 1.14 (s, 20-H_3) and 2.06 (3 H, s, OAc).

Enzymatic Hydrolysis of Compound **5**.—Compound **5** (10 mg) was dissolved in NaH_2PO_4–Na_2HPO_4 buffer (0.2 mol dm^{-3}; pH 6.0) (18 cm^3) and β-glucosidase (10 mg) was added. The mixture was incubated for 24 h at 30 °C. The reaction mixture was then poured into ice-cold water and extracted with chloroform (3 × 7 cm^3) to give **1** (1.0 mg). The aqueous phase was subjected to chromatography on a Dowex WGR column with distilled water as the solvent and the eluate was lyophilised, followed by acetylation with Ac_2O/pyridine. The acetate thus obtained was characterised as a mixture (α-:β-anomer, 1:2; on GLC and ^1H NMR spectroscopy) of the α and β anomers of pentaacetyl-D-glucopyranose by comparing its specific rotation {$[\alpha]_D^{25}$ +42.9 ± 4.3 (*c* 0.4, $CHCl_3$); lit.,[10] $[\alpha]_D^{25}$ +39.4} and other spectral data with those of an authentic sample.

(6S,13S)-13-O-β-L-*Fucopyranosyl*-6-O-α-L-*rhamnopyranosyl*-*cleroda*-3,14-*diene*-6,13-*diol* **2**.—$[\alpha]_D^{25}$ −50.3 (*c* 0.64, MeOH) [Found: M^- − H (FAB), *m/z* 597.3629. $C_{32}H_{53}O_{10}$ requires M − H, 597.3636]; (SIMS) *m/z* 622 (M + Na + H)$^+$; δ_H(C_5D_5N; 270 MHz) 3.36 (dd, *J* 4.4 and 11.0, 6-H), 6.24 (dd, *J* 10.7 and 17.6, 14-H), 5.26 (d, *J* 11.2, 15-H*Z*), 5.41 (d, *J* 17.1, 15-H*E*), 1.57 (s, 16-H_3), 0.70 (d, *J* 6.3, 17-H_3), 1.76 (s, 18-H_3), 1.14 (s, 19-H_3), 1.04 (s, 20-H_3), 4.77 (d, *J* 7.8, 1′-H), 4.32 (dd, *J* 7.8 and 8.3, 2′-H), 4.06 (dd, *J* 8.3 and 3.4, 3′-H), 4.02 (d, *J* 3.4, 4′-H), 3.72 (q, *J* 6.4, 5′-H), 1.51 (d, *J* 6.4, 6′-H), 5.38 (br s, 1″-H), 4.51 (br s, 2″-H), 4.45 (d, *J* 8.2, 3″-H), 4.30 (t, *J* 8.2, 4″-H), 4.28 (m, 5″-H) and 1.66 (d, *J* 4.9, 6″-H); δ_C(C_5D_5N; 67.5 MHz) 18.1 (C-1), 26.8 (C-2), 121.9 (C-3), 143.4 (C-4), 38.1 (C-5), 86.3 (C-6), 35.2 (C-7), 34.2 (C-8), 44.1 (C-9), 45.7 (C-10), 32.0 (C-11), 35.3 (C-12), 80.2 (C-13), 145.0 (C-14), 114.9 (C-15), 22.9 (C-16), 15.9 (C-17), 23.2 (C-18), 18.2 (C-19), 16.0 (C-20), 100.0 (C-1′), 72.8 (C-2′), 75.4 (C-3′), 75.4 (C-4′), 71.6 (C-5′), 17.5 (C-6′), 103.8 (C-1″), 74,6 (C-2″), 74.9 (C-3″), 73.9 (C-4″), 70.0 (C-5″) and 18.3 (C-6″).

(6S,13S)-*Cleroda*-3,14-*diene*-6,13-*diol* 13-O-β-L-*Fucopyranosyl*-(1 ⟶ 2)-α-L-*rhamnopyranoside* **3**.—$[\alpha]_D^{25}$ −28.6 (*c* 0.74, MeOH) [Found: M^- − H (FAB), *m/z* 597.3627. $C_{32}H_{53}O_{10}$ requires M − H, 597.3636]; (SIMS) 622 (M + Na + H)$^+$; δ_H(C_5D_5N; 500 MHz) 3.76 (ddd, *J* 3.8, 5.5 and 11.0, 6-H), 5.60 (d, *J* 5.5, 6-OH), 5.93 (dd, *J* 11.0 and 17.4, 14-H), 5.23 (d, *J* 10.7, 15-H*Z*), 1.49 (s, 16-H_3), 0.78 (d, *J* 6.4, 17-H_3), 2.18 (s, 18-H_3), 0.72 (s, 19-H_3), 1.26 (s, 20-H_3), 5.65 (br s, 1′-H), 4.39 (br s, 2′-H), 4.47 (dd, *J* 2.9 and 9.3, 3′-H), 4.08 (dd, *J* 9.3 and 9.3, 4′-H), 4.29 (dq, *J* 9.3 and 5.9, 5′-H), 1.56 (d, *J* 5.9, 6′-H), 5.02 (d, *J* 7.8, 1″-H), 4.38 (dd, *J* 7.8 and 8.2, 2″-H), 4.05 (dd, *J* 8.2 and 3.4, 3″-H), 3.99 (d, *J* 3.4, 4″-H), 3.77 (q, *J* 5.8, 5″-H), and 1.5 (d, *J* 5.8, 6″-H); δ_C(C_5D_5N; 67.5 MHz) 18.3 (C-1), 27.1 (C-2), 121.9 (C-3), 145.2 (C-4), 38.3 (C-5), 74.9 (C-6), 38.8 (C-7), 34.7 (C-8), 44.5 (C-9), 46.0 (C-10), 32.0 (C-11), 35.2 (C-12), 79.8 (C-13), 143.1 (C-14), 115.2 (C-15), 22.9 (C-16), 15.9 (C-17), 23.2 (C-18), 18.3 (C-19), 16.0 (C-20), 95.7 (C-1′), 83.0 (C-2′), 72.7 (C-3′), 74.9 (C-4′), 69.4 C-5′), 18.5 (C-6′), 107.4 (C-1″), 72.8 (C-2″), 74.5 (C-3″), 72.6 (C-4″), 71.6 (C-5″) and 17.3 (C-6″).

(6S,13S)-*Cleroda*-3,14-*diene*-6,13-*diol* 13-O-α-L-*Fucopyranosyl*-(1 ⟶ 2)-[α-L-*rhamnopyranosyl*-(1 ⟶ 3)]-α-L-*rhamnopyranoside* **4**.—$[\alpha]_D^{25}$ −48 (*c* 2.0, MeOH) [Found: M^- − H (FAB), *m/z* 743.4222. $C_{38}H_{63}O_{14}$ requires M − H, 743.4214]; (SIMS) *m/z* 768 (M + Na + H)$^+$, 293 and 147; δ_H(C_5D_5N; 500 MHz) 3.78 (ddd, *J* 3.8, 5.5 and 11.0, 6-H), 5.55 (d, *J* 5.5, 6-OH), 5.98 (dd, *J* 11.0 and 17.4, 14-H), 5.27 (d, *J* 11.0, 15-H*Z*), 5.32 (dd, *J* 2.3 and 17.4, 15-H*E*), 1.52 (s, 16-H_3), 0.89 (d, *J* 6.4, 17-H_3), 2.21 (s, 18-H_3), 0.82 (s, 19-H_3), 1.31 (s, 20-H_3), 5.58 (br s, 1′-H), 4.67 (br d, 2′-H), 4.69 (dd, *J* 2.5 and 9.2, 3′-H), 4.32 (t, *J* 9.2, 4′-H), 3.8 (dq, *J* 5.5 and 9.2, 5′-H), 1.47 (d, *J* 5.5, 6′-H), 5.30 (s, 1″-H), 4.36 (d, *J* 10.5, 2″-H), 4.32 (dd, *J* 4.0 and 10.5, 3″-H), 4.34 (d, *J* 4.0, 4″-H), 3.92 (q, *J* 6.4, 5″-H), 1.57 (d, *J* 6.4, 6″-H), 6.05 (br s, 1‴-H), 4.65 (br d, *J* 2.5, 2‴-H), 4.78 (dd, *J* 2.5 and 9.2, 3‴-H), 4.32 (t, *J* 9.2, 4‴-H), 4.57 (dq, *J* 6.4 and 9.2, 5‴-H) and 1.78 (d, *J* 6.4, 6‴-H); δ_C(C_5D_5N; 125 MHz) 18.2 (C-1), 27.4 (C-2), 123.3 (C-3), 145.2 (C-4), 38.4 (C-5), 75.1 (C-6), 38.9 (C-7), 34.9 (C-8), 44.5 (C-9), 46.0 (C-10), 32.1 (C-11), 35.4 (C-12), 79.6 (C-13), 142.9 (C-14), 115.1 (C-15), 23.8 (C-16), 15.9 (C-17), 23.6 (C-18), 18.2 (C-19), 16.0 (C-20), 95.8 (C-1′), 79.7 (C-2′), 77.9 (C-3′), 72.7 (C-4′), 70.2 (C-5′), 18.2 (C-6′), 105.8 (C-1″), 74.1 (C-2″), 75.1 (C-3″), 72.5 (C-4″), 71.5 (C-5″), 17.3 (C-6″), 103.8 (C-1‴), 72.5 (C-2‴), 72.2 (C-3‴), 72.7 (C-4‴), 70.2 (C-5‴) and 18.7 (C-6‴).

Acetylation of Compound **4**.—Compound **4** (10 mg) was acetylated in the same manner as described above to afford octaacetate **8** (9 mg) R_f 0.7 [MeOH–$CHCl_3$ (3:97)]; ν_{max}($CHCl_3$/cm^{-1} 1740 (CO), 1425, 1365, 1130 and 920; δ_H(C_6D_6; 500 MHz) 4.94 (dd, *J* 4.6 and 10.1, 6-H), 5.66 (dd, *J* 8.3 and 10.1, 14-H), 5.39 (d, *J* 8.3, 15-H*Z*), 5.61 (dd, *J* 10.1 and 2.0, 15-H*E*), 1.28 (s, 16-H_3), 0.75 (d, *J* 5.50, 17-H_3), 2.13 (s. 18–H_3), 0.71 (s, 19-H_3), 1.21 (s, 20-H_3), 5.32 (br s, 1′-H), 4.36 (br s, 2′-H), 4.51 (dd, *J* 2.7 and 8.7, 3′-H), 5.60 (dd, *J* 8.3 and 9.2, 4′-H) 4.68 (dq, *J* 6.4 and 9.2, 5′-H), 1.49 (d, *J* 6.4, 6′-H), 5.05 (s, 1″-H), 5.03 (d, *J* 7.1, 2″-H), 5.58 (d, *J* 7.1, 3″-H), 5.59 (d, *J* 2.0, 4″-H), 3.68 (q, *J* 6.4, 5″-H), 1.16 (d, *J* 6.4, 6″-H), 5.26 (br s, 1‴-H), 5.44 (dd, *J* 1.8

and 6.4, 2‴-H), 5.78 (dd, *J* 6.4 and 8.3, 3‴-H), 5.63 (dd, *J* 8.3 and 9.2, 4‴-H), 4.47 (dq, *J* 6.4 and 9.2, 5‴-H), 1.49 (d, *J* 6.4, 6‴-H) and 1.58, 1.62, 1.64, 1.66, 1.68, 1.70, 1.71 and 1.73 (each 3 H, s, OAc × 8).

(6S,13S)-*Cleroda*-3,14-*diene*-6,13-*diol* 6-O-β-*D*-*Glucopyranoside* **5**.—$[\alpha]_D^{25}$ −10.7 (*c* 0.24, MeOH) [Found: M^- − H (FAB), *m/z* 467.3001. $C_{26}H_{43}O_7$ requires M − H, 467.3006].

(6S,13S)-*Cleroda*-3,14-*diene*-6,13-*diol* 6-O-α-L-*Rhamnopyranosyl*-(1 ⟶ 2)-β-D-*glucopyranoside* **6**.—$[\alpha]_D^{25}$ −21.8 (*c* 0.07, MeOH) [Found: M^- − H (FAB), *m/z* 613.3596. $C_{32}H_{53}O_{11}$ requires M − H, 613.3585.

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