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BIOLOGICALLY ACTIVE CLERODANE-TYPE DITERPENE GLYCOSIDES FROM THE ROOT-STALKS OF *DICRANOPTERIS PEDATA*

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Key Word Index—*Dicranopteris pedata*; Gleicheniaceae; root-stalks; clerodane-type diterpene; glycosides; plant growth acceleration; plant growth inhibition.

Abstract—The molecular structure of the biologically active diterpene alcohol isolated previously from the root-stalks of *Dicranopteris pedata* and *Gleichenia japonica* was confirmed to be (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol by an X-ray crystallographic analysis, together with application of the octant rule to the Cotton effect observed in the CD spectrum of its 6-keto derivative. Further investigation of the root-stalks of *D. pedata* has resulted in the isolated two new glycosides, which were characterised as (6*S*,13*S*)-6-*O*-[β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]-13-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-fucopyranosyl]-cleroda-3,14-diene and (6*S*,13*S*)-6-*O*-[β-glucopyranosyl]-13-*O*-[β-fucopyranosyl-(1 → 2)-α-rhamnopyranosyl]-cleroda-3,14-diene. Of these two glycosides, the former glycoside accelerated the growth of the stems of lettuce and inhibited the growth of the roots. © 1997 Elsevier Science Ltd

INTRODUCTION

Previously, we isolated the biologically active diterpene alcohol from the root-stalks of *Dicranopteris pedata* Nakaike ('Koshida' in Japanese) and *Gleichenia japonica* Spreng ('Urajiro' in Japanese), which are ferns belonging to the same family (Gleicheniaceae), and its structure had been proposed to be (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol [1] by its ^{13}C NMR chemical shifts, by the observation of NOEs of its 13-*O*-glycoside and by application of the β-D-glucosylation-shift rule [2, 3] to the 6-*O*-glucoside. However, these experiments were insufficient to establish the full molecular structure of the compound; not only is application of the β-D-glucosylation-shift rule inadequate to determine a chirality at C-6 of such a sterically hindered compound possessing methyl group at the β-position, but also this diterpene alcohol has not been related to any compound having a well-defined structure to establish the skeletal structure. Therefore, further studies were required to elucidate completely the molecular structure of the biologically active diterpene alcohol. By the X-ray crystallographic study in combination with measurement of CD spectrum of its 6-keto derivative, the molecular structure of this deterpene alcohol has now been established completely as (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol (**1**). This identification supported the structure assigned previously to this compound [1].

The ferns grow as a large community containing no other species of plants. Previously, we also found, in addition to the presence of compound **1** [1], that five clerodane-type diterpene glycosides are present in the root-stalks of *D. pedata* [1, 4] and that two of these glycosides, together with three labdane-type diterpene glycosides, are also present in the root-stalks of *G. japonica* [5]. The clerodane-type diterpene alcohol and its five glycosides, and two of three labdane-type diterpene glycosides, are effective for the growth of lettuce [1, 4, 5]. Recently, four labdane-type diterpene glycosides were isolated from the fresh leaflets of *G. japonica* [6], together with two known glycosides [1, 4]. Our further investigation for allelopathic substances present in the root-stalks of *D. pedata* has now isolated two new glycosides of the clerodane-type diterpene alcohol. Of these two glycosides, the major one showed the characteristic behaviour for the elongation of lettuce, *Lactuca sativa* with acceleration of the growth of the stems but inhibition of the growth of the roots.

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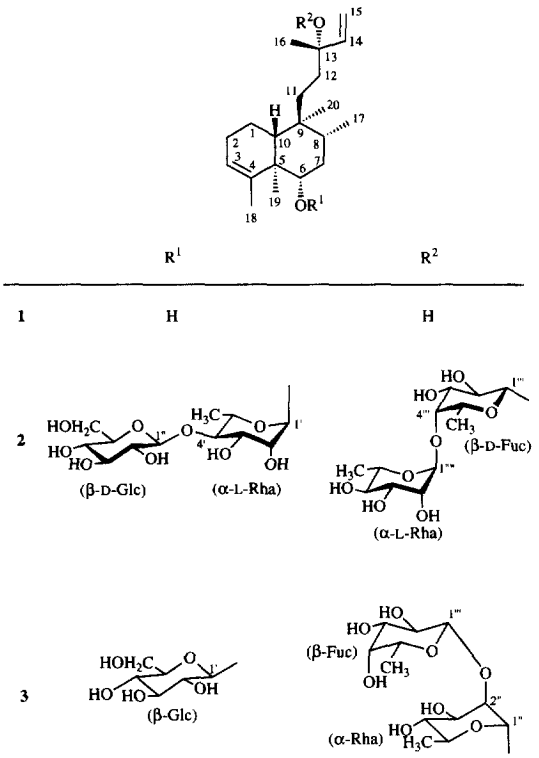

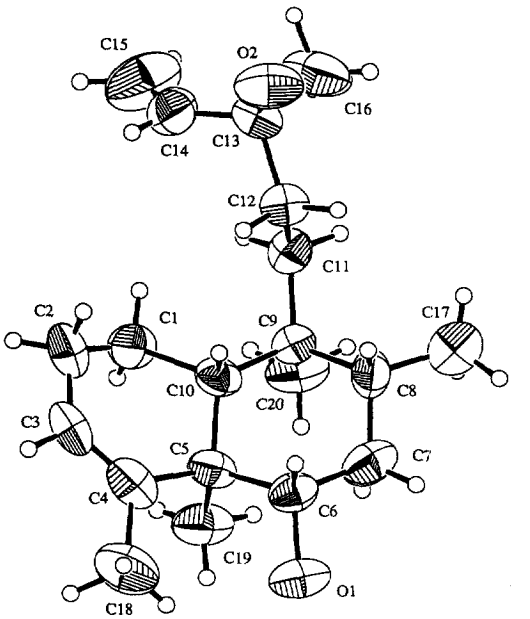

Fig 1. Perspective view of compound **1** with no OH hydrogen atoms and the numbering system used for X-ray molecular structure.

RESULTS AND DISCUSSION

In our previous experiments [1], attempts to recrystallize alcohol **1** from hexane had given precipitates as needle-like solids, but not crystals. Therefore, alcohol **1** was purified further by repeated preparative TLC and HPLC with a reversed-phase column (ODS) under the conditions as described in the Experimental, followed by recrystallization from hexane. Repeated recrystallization gave compound **1** as needles [mp 142.0–144.0°], which exhibited the same chromatographic and ^1H and ^{13}C NMR spectral properties and showed the same biological activity for the elongation of lettuce as reported previously for this compound [1]. Although the needles were obtained as twin crystals, the relative structure of compound **1** was confirmed by the X-ray analysis, as shown in Fig. 1. Further, the absolute configuration of compound **1** was clarified by measurement of the CD spectrum of its 6-keto derivative (**4**), which was prepared by oxidation of compound **1** with pyridinium-chlorochromate adsorbed on alumina (PCC/Al_2O_3) [7]. The CD curve of compound **4** exhibited a negative Cotton effect at 290 nm. Application of the octant rule [8] to this negative Cotton effect clearly indicated that the axial methyl group on C-5 locates in the bottom left rear octant. These results corroborated the validity of the structure possessing the *S*-chirality both at C-6 and C-13 assigned previously [1] to this diterpene alcohol. Consequently, the molecular structure of the biologically active diterpene alcohol (**1**) was concluded to be (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol.

The methanol-soluble fraction of fresh root-stalks of *D. pedata* was suspended in water, followed by successive extraction with hexane, ethyl acetate and *n*-butanol. The *n*-butanol extract was subjected to silica gel column chromatography and reversed-phase HPLC (ODS), followed by preparative TLC to give compounds **2** and **3**. Following the method described in the literature [1], compound **2** was used for the examination on the elongation of the stems and roots of lettuce and showed the characteristic activities; acceleration of stem elongation at 1 ppm, while inhibition of root elongation at 500 ppm, as illustrated in Fig. 2. Unfortunately compound **3** was obtained in small amounts insufficient to try such an examination.

Compound **2** showed a pseudomolecular-ion peak at m/z 929 $[M+Na]^+$ in the secondary ionisation mass spectrum (SIMS). The ^1H and ^{13}C NMR spectra coincided with those of compound **1**, except for the

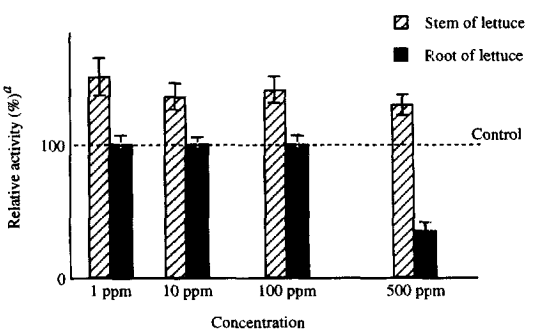

Fig 2. Effects of compound **2** on the elongation of the stems and roots of lettuce. [a]Relative activity (%) is illustrated to control (100%). Each activity is the mean of five duplications (s.d. is lower than 10% of the mean values).

carbon signals at δ_C 86.6 and δ_C 80.6 due to C-6 and C-13, respectively, of the aglycone part and a series of signals due to four additional sugar moieties. The ^1H and ^{13}C NMR spectra also suggested the sugar moieties to be composed of two units of α-rhamnopyranose, one unit of β-fucopyranose and one unit of β-glucopyranose by the similarity of their spectral data with the literature data [1, 9–11] and by the interpretation of the ^1H-^1H COSY and ^1H-^1H homonuclear Hartmann-Hahn (HOHAHA) spectra. Configurations and ratios of sugars in compound **2** were confirmed by GLC separation of sugar enantiomers as diastereoisomeric α-methylbenzylamino-1-deoxyalditols on an achiral fused-silica capillary column. Compound **2** was hydrolysed with hydrochloric acid to give a mixed sugar portion. Following the method described in the literature [12], the sugar portion was converted to the trimethylsilyl (TMS) ethers of 1-(L-α-methylbenzylamino)-1-deoxyalditols (MBA-alditols) and then subjected to GLC and co-GLC analyses with the TMS ethers of MBA-alditols derived from authentic enantiomeric sugars. On the basis of these GLC and co-GLC analyses, it was found that the sugar moiety of compound **2** was composed of L-rhamnose, D-fucose and D-glucose (2:1:1). The interglycosidic linkages of the four sugar moieties and the linking positions to the aglycone in compound **2** were determined by an HMBC experiment [13]. The anomeric proton [δ_H 5.23 (*br s*, H-1′)] of L-rhamnopyranose showed the HMBC cross peak with C-6 [δ_C 86.6] of the aglycone and the anomeric proton [δ_H 5.12 (*d*, J = 7.9 Hz, H-1″)] of D-glucopyranose exhibited the cross peak with C-4′ [δ_C 84.7] of the L-rhamnopyranose. These observations indicated the presence of the glycosidic linkage and the sugar sequence characterized as β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranoside, which links to the C-6 of the aglycone part. Further, it was observed that HMBC correlations of the anomeric proton [δ_H 4.66 (*d*, J = 7.3 Hz, H-1‴)] of D-fucopyranose to C-13 [δ_C 80.6] of the aglycone and the anomeric proton [δ_H 6.04 (*br s*, H-1⁗)] of another L-rhamnopyranose to C-4‴ [δ_C 78.3] of the D-fucopyranose. These observations indicated the presence of another glycosidic linkage and sugar sequence of α-L-rhamnopyranosyl-(1 → 4)-β-D-fucopyranoside, which links to the C-13 of the aglycone part. Thus, the structure of compound **2** was determined to be (6*S*,13*S*)-13-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-fucopyranosyl]-6-*O*-[β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]-cleroda-3,14-diene-6,13-diol.

Compound **3** showed a pseudomolecular-ion peak at m/z 783 $[M+Na]^+$ in the SIMS. The ^1H and ^{13}C NMR spectra were identical with those of compound **1**, except for the carbon signals at δ_C 85.7 and δ_C 79.8 due to C-6 and C-13, respectively, of the aglycone part and a series of proton and carbon signals due to three additional sugar moieties. The ^1H and ^{13}C NMR spectra, and the interpretation of the ^1H-^1H COSY and ^1H-^1H HOHAHA spectra, suggested compound **3** to be a glycoside of compound **1** possessing the sugar moieties composed of α-rhamnopyranose, β-fucopyranose and β-glucopyranose. Such sugar compositions were also supported by the similarity of their spectral data with the literature data [1, 9–11]. Further, the ^1H and ^{13}C NMR spectra of compound **3** were identical with those of compound **2**, except for the carbon signals at δ_C 95.8, δ_C 83.1 and δ_C 72.9 due to C-1″, C-2″ and C-3″, respectively, in the rhamnopyranose moiety and then at δ_C 107.5 and δ_C 72.8 due to C-1‴ and C-4‴, respectively, in the fucopyranose moiety in addition to the anomeric proton signal at δ_H 5.01 (*d*, J = 8.3 Hz, H-1‴). The glycosidic linkage and the sugar sequence in compound **3** were determined by an HMBC experiment. The anomeric proton [δ_H 5.01 (*d*, J = 7.3 Hz, H-1′)] of glucopyranose showed the HMBC correlation to C-6 [δ_C 85.7] of the aglycone. This indicated the presence of 6-*O*-β-glucopyranoside as a partial structure. In addition, the anomeric proton [δ_H 5.01 (*d*, J = 8.3 Hz, H-1‴)] of fucopyranose showed the HMBC cross peak with C-2″ [δ_C 83.1] of rhamnopyranose and then the anomeric proton [δ_H 5.64 (*br s*, H-1″)] of the rhamnopyranose exhibited the correlation to C-13 [δ_C 79.8] of the aglycone. These observations were apparently indicative of the presence of 13-*O*-β-fucopyranosyl-(1 → 2)-α-rhamnopyranoside as another partial structure. Thus, the structure of compound **3** was determined to be (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol 6-*O*-β-glucopyranosyl-13-*O*-β-fucopyranosyl-(1 → 2)-α-rhamnopyranoside.

EXPERIMENTAL

General. Optical rotations were measured in MeOH soln with a JASCO DIP-360 digital polarimeter. The EI-MS was obtained with a Hitachi M-80B double focusing spectrometer at 70 eV and an ion-source temp. of 200°. SIMS spectra were obtained by detecting positive ions with an M-8086 Xenon beam-generating system. Analytical conditions were as follows: matrix, glycerol; accelerating voltage, 3 keV. Assignment of mass numbers was achieved by comparing the spectra with the MS of CsI. ^1H and ^{13}C NMR spectra were recorded on JEOL GSX-500 spectrometer, with TMS as int. standard. J-values are given in Hz. CD spectrum was recorded on a JASCO J-600 spectrometer at 25° in a quartz cell of 1.0 cm path length. UV spectrum was recorded on a Shimadzu UV-160A spectrophotometer. IR spectra were recorded on a JASCO FT/IR 5300 spectrometer. X-Ray data collection was performed on a Rigaku AFC7R diffractometer. GLC analyses were performed on a Shimadzu GC-14A equipped with flame ionisation detector (FID) under the following conditions: 0.25 mm × 25 m fused-silica capillary column (HiCap CBP1-M25-025); column temp. 250° and injection temp. 260°; carrier gas He; split ratio *ca* 100:1. Analytical and prep. TLC were carried out on Merck 60 GF_{254} silica gel plates with thickness 0.25

mm and 0.5 mm, respectively. HPLC was run on a JASCO 980 instrument equipped with a UV/VIS detector and a Wakosil-II 5C18 HG column (25 cm × 4.6 mm).

Purification of (6S,13S)-*cleroda*-3,13-*diene*-6,13-*diol* (**1**). The diterpene alcohol previously isolated from the root-stalks of *D. pedata* [1] was used for further purification as follows. At first, this alcohol was subjected to reversed-phase CC (ODS) with the solvent systems of H_2O–MeOH (2:3 and 1:4; 100 ml each). Each eluate was then subjected to prep. TLC (0.5 mm thickness), respectively, with the mixed solvent system using EtOAc–hexane (1:4). The band at R_f 0.22 on each TLC was collected and the compound was eluted from the silica gel with $CHCl_3$. The eluate gathered was again subjected to reversed-phase CC (ODS) with the solvent system of H_2O–MeOH (1:4; 100 ml), followed by prep. TLC under the same condition described above. Finally, this compound was purified by duplicates of HPLC sepn on a reversed-phase column (ODS)[H_2O–MeOH (1:4)] and then by recrystallization from hexane to give compound **1** as colourless needles, R_f 0.22 [EtOAc–hexane (8:2)]; mp 142.0–144.0°; $[\alpha]_D^{25}$ −6.5° ($CHCl_3$; *c* 0.29). EIMS *m/z* (rel. int.): 306 $[M]^+$ (2), 288 $[M-H_2O]^+$ (40), 71 (100). IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 1651 (C = C). ^1H NMR (500 MHz, $CDCl_3$): δ 1.55 (2H, *m*, H_2-1), 2.03 (2H, *m*, H_2-2), 5.22 (1H, *br s*, H-3), 3.53 (1H, *dd*, *J* = 4.1, 11.5 Hz, H-6), 1.47 and 1.58 (each 1H, *m*, H_2-7), 1.57 (1H, *m*, H-8), 1.26 (1H, *m*, H-10), 1.26 and 1.38 (each 1H, *m*, H_2-11), 1.32 (2H, *m*, H_2-12), 5.87 (1H, *dd*, *J* = 10.1, 17.4 Hz, H-14), 5.07 and 5.20 (each 1H, *d*, *J* = 10.1 and 17.4 Hz, respectively, H_2-15), 1.26 (3H, *s*, H_3-16), 0.80 (3H, *d*, *J* = 6.4 Hz, H_3-17), 1.83 (3H, *br s*, H_3-18), 1.01 (3H, *s*, H_3-19), 0.71 (3H, *s*, H_3-20). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.7 (C-1), 26.6 (C-2), 122.3 (C-3), 143.7 (C-4), 44.0 (C-5), 75.6 (C-6), 37.8 (C-7), 34.4 (C-8), 38.0 (C-9), 45.4 (C-10), 31.8 (C-11), 35.0 (C-12), 73.4 (C-13), 145.1 (C-14), 112.0 (C-15), 27.9 (C-16), 15.5 (C-17), 22.4 (C-18), 14.9 (C-19), 18.0 (C-20). ^{13}C NMR (125 MHz, C_5D_5N): δ 145.3 (C-4), 44.5 (C-5), 75.0 (C-6), 38.9 (C-7), 72.6 (C-13), 147.3 (C-14), 111.3 (C-15), 28.7 (C-16). The behaviour on TLC and the ^1H and ^{13}C NMR spectra of compound **1** coincided completely with those of the diterpene alcohol reported previously [1], except for the $[\alpha]_D$ value. The difference might arise from a contamination by a small amount of impurity due to the imperfect purification. Thus, the $[\alpha]_D$ value of compound **1** should be corrected as described in this paper.

X-Ray crystal structure analysis. Intensity measurements were made with $2\theta \leqslant 110.2°$ by using graphite monochromated Cu-Kα radiation at 20° on a Rigaku AFC7R diffractometer. A total of 2471 independent reflections were collected, of which 1410 were considered to be observed [$I > 3\sigma(I)$]. The structure was solved by direct methods and expanded using Fourier techniques. The nonhydrogen atoms were refined anisotropically by full-matrix least-squares refinement. H atoms were included but not refined. The structure was finally refined to $R = 0.077$ ($R_w = 0.101$). Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, U.K.

Crystal data of compound **1**. $C_{20}H_{34}O_2$, M_r = 306.49, monoclinic, space group $P2_1$, *a* = 9.540 (5), *b* = 22.40 (1), *c* = 9.601 (5) Å, β = 112.03 (4)°, *U* = 1901 (1) Å^3, *Z* = 4, D_c = 1.07 g cm^{-3}.

Oxidation of compound **1**. Following the method described in the literature [7], PCC/Al_2O_3 reagent was prepd as follows; pyridine (19.8 g) was added to a soln of chromium trioxide (25.0 g) in 6 M HCl (45 ml) within 10 min at 40°. The mixt. was kept at 10° until a yellow-orange solid formed. Reheating to 40° gave a soln. Alumina (208 g) was then added to the soln with stirring at 40°. After evapn of H_2O, the orange solid was dried in vacuum for 2 hr at 100° to give PCC/Al_2O_3 reagent. To a soln of compound **1** (2.1 mg) in dry C_6H_6 (0.1 ml) was added PCC/Al_2O_3 (30 mg), followed by stirring for 2 hr at room temp. The reaction mixt., after filtration, was subjected to prep. TLC with a solvent mixt. of ETOAc–hexane (4:1) to give the ketone derivative (**4**) (0.75 mg), R_f 0.30 [EtOAc–hexane (4:1)]. EIMS *m/z* (rel. int.): 304 $[M]^+$ (100). UV λ_{max}^{MeOH} nm (log ε): 238 (2.76). CD $\Delta\varepsilon_{290}$ −1.25 (MeOH; *c* 0.006). IR ν_{max}^{neat} cm^{-1}: 3450 (OH), 1707 (C = O), 1670 (C = C). ^1H NMR (500 MHz, $CDCl_3$): δ 1.60 (2H, *m*, H_2-1), 2.02 (2H, *m*, H_2-2), 5.37 (1H, *br s*, H-3), 2.05 (1H, *m*, H-7), 2.80 (1H, *t*, *J* = 12.7 Hz, H-7), 2.00 (1H, *m*, H-8), 5.83 (1H, *dd*, *J* = 11.0, 17.4 Hz, H-14), 5.07 and 5.19 (each 1H, *d*, *J* = 11.0 and 17.4 Hz, respectively, H_2-15), 1.25 (3H, *s*, H_3-16), 0.88 (3H, *d*, *J* = 7.3 Hz, H_3-17), 1.81 (3H, *br s*, H_3-18), 1.41 (3H, *s*, H_3-19), 0.96 (3H, *s*, H_3-20).

Plant material. The root-stalks of *D. pedata* were collected in the suburbs of Hiroshima city in June 1993 and identified by the authors (S. O. and Y. H.). A voucher specimen is kept in the laboratory of Y. H. Seeds of lettuce, *Lactuca sativa*, were germinated on moist filter-papers under the light of 1500–2000 lux (25°). The 2-day-old seedlings of lettuce were used for the bioassay of the glycoside **2**.

Bioassay. Bioassay of compound **2** was performed on filter-papers contained in plastic petri dishes radius 4.5 cm. Solns, EtOH, of different concs ranging from 1 to 500 ppm were prepd. Each soln (1.8 ml) was placed on the filter-paper and the paper was allowed to dry in the air; this was followed by the addition of distilled H_2O (1.8 ml). The 2-day-old seedlings of lettuce were placed in each petri dish and all the tested samples were incubated under the light of 1500–2000 lux (25°). After 3 days, the lengths of the roots and stems of the seedlings 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 Fig. 2.

Extraction and isolation of glycosides. Fresh root-stalks of *D. pedata* were washed with distilled H_2O and then air-dried. The dried root-stalks (3.5 kg) were

cut into small pieces and immersed in distilled H_2O (7 l.) for 4 days at room temp. After filtration, the rootstalks were then immersed in H_2O–MeOH (1:1; 5 l.) under the same conditions as above, followed by immersion in MeOH (4.6 l.) to give the MeOH extract (22.0 g). The MeOH extract suspended in H_2O–MeOH (1:1, 300 ml) was extracted successively with hexane, EtOAc and *n*-BuOH. The *n*-BuOH extract (5.9 g) obtained was subjected to silica gel CC with the gradient solvent system using $CHCl_3$ and MeOH as eluent (with MeOH increasing form 1–100%) and the eluate was then chromatographed on a silica gel column with the mixed solvent system using EtOAc–*iso*-PrOH–H_2O (6:1:0.5) as eluent to yield two frs. containing compounds **2** and **3**, respectively. These frs. were subjected to reversed-phase CC (ODS) with the mixed solvent system using H_2O–MeOH (3:7) as eluent. Then, each eluate obtained was finally purified by prep. TLC with the mixed solvent system using EtOAc–*iso*-PrOH–H_2O (6:2:1) as eluent to afford compounds **2** (11 mg) and **3** (1.5 mg). Compounds **2** and **3** gave spots with R_f 0.30 and 0.40, respectively, on analytical TLC [EtOAc–*iso*-PrOH–H_2O (6:2:1)].

(6S, 13S)-13-O-[α-L-*Rhamnopyranosyl*-(1 → 4)-β-D-*fucopyranosyl*]-6-O-[β-D-*glucopyranosyl*-(1 → 4)-α-L-*rhamnopyranosyl*]*cleroda*-3,14-*diene*-6,13-*diol* (**2**). $[\alpha]_D^{25}$ −45° (MeOH; *c* 0.055). SIMS *m/z* 929 $[M+Na]^+$. IR ν_{max}^{neat} cm^{-1}: 3390 (OH) and 1650 (C = C). ^1H NMR (500 MHz, C_5D_5N including 10% CD_3OD): δ 1.40 and 1.80 (each 1H, *m*, H_2-1), 1.85 and 1.90 (each 1H, *m*, H_2-2), 5.12 (1H, *br s*, H-3), 3.34 (1H, *dd*, *J* = 4.6, 11.0 Hz, H-6), 1.58 and 2.07 (each 1H, *m*, H_2-7), 1.42 (1H, *m*, H-8), 1.19 (1H, *br d*, *J* = 11.9 Hz, H-10), 1.28 (2H, *m*, H_2-11), 1.50 (2H, *m*, H_2-12), 6.16 (1H, *dd*, *J* = 10.6, 17.9 Hz, H-14), 5.26 and 5.38 (each 1H, *d*, *J* = 11.0 and 17.7 Hz, respectively, H_2-15), 1.53 (3H, *s*, H_3-16), 0.74 (3H, *d*, *J* = 6.4 Hz, H_3-17), 1.74 (3H, *br s*, H_3-18), 1.05 (3H, *s*, H_3-19), 0.66 (3H, *s*, H_3-20), 5.23 (1H, *br s*, H-1′), 4.37 (1H, *m*, H-2′), 4.41 (1H, *dd*, *J* = 3.2, 9.6 Hz, H-3′), 4.27 (1H, *t*, *J* = 9.6 Hz, H-4′), 4.21 (1H, *m*, H-5′), 1.63 (3H, *d*, *J* = 6.4 Hz, H_3-6′), 5.12 (1H, *d*, *J* = 7.9 Hz, H-1″), 3.97 (1H, *m*, H-2″), 4.01 (1H, *m*, H-3″), 4.07 (1H, *m*, H-4″), 3.72 (1H, *m*, H-5″), 4.22 (1H, *dd*, *J* = 11.9, 4.6 Hz, H-6″), 4.33 (1H, *m*, H-6″), 4.66 (1H, *d*, *J* = 7.3 Hz, H-1‴), 4.12 (1H, *m*, H-2‴), 4.01 (1H, *dd*, *J* = 3.2, 9.6 Hz, H-3‴), 4.10 (1H, *m*, H-4‴), 3.73 (1H, *m*, H-5‴), 1.46 (3H, *d*, *J* = 6.4 Hz, H_3-6‴), 6.04 (1H, *br s*, H-1⁗), 4.70 (1H, *br d*, *J* = 3.7 Hz, H-2⁗), 4.35 (1H, *m*, H-3⁗), 4.10 (1H, *m*, H-4⁗), 4.32 (1H, *m*, H-5⁗), 1.57 (3H, *d*, *J* = 6.4 Hz, H_3-6⁗). ^{13}C NMR (125 MHz, C_5D_5N including 10% CD_3OD): δ 18.1 (C-1), 26.9 (C-2), 123.2 (C-3), 143.5 (C-4), 44.2 (C-5), 86.6 (C-6), 35.3 (C-7), 34.4 (C-8), 38.3 (C-9), 45.9 (C-10), 32.2 (C-11), 35.3 (C-12), 80.6 (C-13), 144.7 (C-14), 115.1 (C-15), 22.9 (C-16), 16.0 (C-17), 22.9 (C-18), 16.3 (C-19), 18.1 (C-20), 103.2 (C-1′), 72.3 (C-2′), 72.6 (C-3′), 84.7 (C-4′), 68.4 (C-5′), 18.1 (C-6′), 106.5 (C-1″), 76.3 (C-2″), 78.3 (C-3″), 71.5 (C-4″), 78.3 (C-5″), 62.7 (C-6″), 99.9 (C-1‴), 72.3 (C-2‴), 76.0 (C-3‴), 78.3 (C-4‴), 70.7 (C-5‴), 18.0 (C-6‴), 103.2 (C-1⁗), 72.3 (C-2⁗), 72.6 (C-3⁗), 73.8 (C-4⁗), 70.2 (C-5⁗), 18.4 (C-6⁗).

(6S,13S)-13-O-[β-*fucopyranosyl*-(1 → 2)-α-*rhamnopyranosyl*]-6-O-[β-*glucopyranosyl*]*cleroda*-3,14-*diene*-6,13-*diol* (**3**). SIMS *m/z* 783 $[M+Na]^+$. IR ν_{max}^{neat} cm^{-1}: 3370 (OH) and 1656 (C = C). ^1H NMR (500 MHz, C_5D_5N): δ 1.55 and 1.70 (each 1H, *m*, H_2-1), 2.05 (2H, *m*, H_2-2), 5.25 (1H, *br s*, H-3), 3.78 (1H, *dd*, *J* = 3.7, 11.9 Hz, H-6), 1.76 and 2.45 (each 1H, *m*, H_2-7), 1.60 (1H, *m*, H-8), 1.43 (1H, *br d*, *J* = 12.9 Hz, H-10), 1.25 (2H, *m*, H_2-11), 1.55 (2H, *m*, H_2-12), 5.95 (1H, *dd*, *J* = 11.0, 17.4 Hz, H-14), 5.24 (1H, *d*, *J* = 11.0 Hz, H-15), 5.26 (1H, *d*, *J* = 17.4 Hz, H-15), 1.51 (3H, *s*, H_3-16), 0.79 (3H, *d*, *J* = 6.4 Hz, H_3-17), 2.37 (3H, *br s*, H_3-18), 1.27 (3H, *s*, H_3-19), 0.68 (3H, *s*, H_3-20), 5.01 (1H, *d*, *J* = 7.3 Hz, H-1′), 4.03 (1H, *m*, H-2′), 4.19 (1H, *m*, H-3′), 4.00 (1H, *m*, H-4′), 3.95 (1H, *m*, H-5′), 4.34 (1H, *m*, H-6′), 4.51 (1H, *dd*, *J* = 11.0, 2.8 Hz, H-6′), 5.64 (1H, *br s*, H-1″), 4.35 (1H, *m*, H-2″), 4.45 (1H, *dd*, *J* = 9.2, 2.8 Hz, H-3″), 4.03 (1H, *m*, H-4″), 4.28 (1H, *dd*, *J* = 6.4, 9.2 Hz, H-5″), 1.56 (3H, *d*, *J* = 6.4 Hz, H_3-6″), 5.01 (1H, *d*, *J* = 8.3 Hz, H-1‴), 4.20 (1H, *m*, H-2‴), 4.00 (1H, *m*, H-3‴), 4.35 (1H, *m*, H-4‴), 3.80 (1H, *d*, *J* = 6.4 Hz, H-5‴), 1.53 (3H, *d*, *J* = 6.4 Hz, H_3-6‴). ^{13}C NMR (125 MHz, C_5D_5N): δ 18.2 (C-1), 27.2 (C-2), 122.2 (C-3), 145.2 (C-4), 44.7 (C-5), 85.7 (C-6), 35.8 (C-7), 34.6 (C-8), 38.3 (C-9), 46.3 (C-10), 32.2 (C-11), 35.3 (C-12), 79.8 (C-13), 143.2 (C-14), 115.2 (C-15), 23.0 (C-16), 16.0 (C-17), 24.0 (C-18), 16.9 (C-19), 18.3 (C-20), 104.5 (C-1′), 75.4 (C-2′), 79.3 (C-3′), 72.9 (C-4′), 78.3 (C-5′), 63.1 (C-6′), 95.8 (C-1″), 83.1 (C-2″), 72.9 (C-3″), 75.7 (C-4″), 69.4 (C-5″), 18.5 (C-6″), 107.5 (C-1‴), 72.0 (C-2‴), 74.7 (C-3‴), 72.8 (C-4‴), 71.7 (C-5‴), 17.3 (C-6‴).

Identification of enantiomeric sugars in compound **2**. Identification of enantiomeric sugars was carried out on GLC following the method described in literature [12]. Compound **2** (3 mg) was hydrolysed with a soln of 3% HCl/50% 1,4-dioxane (1 ml) at 80° for 3 hr. The reaction mixt. was washed with a small amount of $CHCl_3$ to remove a degradation product. The aq. layer was neutralized with aq. NaOH, followed by evapn of H_2O under vacuum to give a sugar portion as a syrup. A mixt. of a soln of the syrup in H_2O (0.1 ml) and a soln of L-(−)-α-methylbenzylamine (MBA) (14 mg) and $NaBH_3CN$ (0.4 mg) in EtOH (0.1 ml) was kept at 40° for 3 hr. Several drops of HOAc were added to the reaction mixt. The mixt. was evapd and further coevapd with MeOH (1 ml). The oily residue obtained was dried over silica gel in a vacuum desiccator overnight and then mixed with dry CH_3CN (0.1 ml) and *N*,*O*-bis-(trimethylsilyl)acetamide (0.025 ml). After standing for 15 min at room temp. in a stoppered tube, followed by addition of a small amount of hexane to extract the TMS ethers of MBA-alditols. The hexane extract was subjected to GLC and co-GLC analyses. The gas chromatogram exhibited the presence of three peaks, whose ratios were 2:1:1. Each peak was identified as the TMS ethers of MBA-alditols of L-rhamnose, D-fucose and D-glucose,

respectively, by co-GLC with the TMS ethers of MBA-alditols derived from authentic enantiomeric sugars.

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