

# Sesquiterpenoid Constituents of the Liverwort *Frullania brotheri* STEPH.

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Two new sesquiterpene lactones, (+)- $\beta$ -frullanolide and (+)-brothenolide, have been isolated from the liverwort *Frullania brotheri* STEPH. One of them, (+)-brothenolide, has an allergenic property. The absolute structures of these lactones have been established by spectroscopic analysis and chemical transformation.

It is well-known that a number of sesquiterpene lactones have been isolated from the liverwort belonging to Frullaniaceae and that some of them have an allergenic property.<sup>1)</sup> In the course of our investigation on terpene constituents of the liverwort, we examined the constituents of *Frullania brotheri* STEPH. collected in Wakayama Prefecture around Mt. Ohto in June 1976 and isolated two new sesquiterpene lactones, (+)- $\beta$ -frullanolide (**1**) and (+)-brothenolide (**2**), one of which, **2**, had an allergenic property. This paper deals in detail with the structural determination of (+)- $\beta$ -frullanolide and (+)-brothenolide.<sup>2)</sup>

Column chromatographic separation on SiO<sub>2</sub> and AgNO<sub>3</sub>-SiO<sub>2</sub> of the ether extract of the fresh material gave lactones **1** and **2**. (+)- $\beta$ -Frullanolide (**1**), mp 165–167 °C, [ $\alpha$ ]<sub>D</sub> +178°, was obtained as colorless needles. The molecular formula C<sub>15</sub>H<sub>20</sub>O<sub>2</sub> of **1** was determined by the appearance of a molecular ion peak at 232.1453 in the high resolution mass spectrum (HR-MS). The IR spectrum (CHCl<sub>3</sub>) showed an  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone absorption bands at 1760 and 1670 cm<sup>-1</sup> and an exocyclic methylene absorption band at 910 cm<sup>-1</sup>. The <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>) indicated the presence of one tertiary methyl group ( $\delta$  0.92), four exocyclic methylene protons ( $\delta$  4.92, 5.09, 5.52, and 6.07) and one methine proton ( $\delta$  4.58) adjacent to oxygen. In the decoupling experiments of **1**, irradiation at  $\delta$  5.09 (14-H<sub>B</sub>) collapsed the proton of 5-H at  $\delta$  2.01 and the allylic methylene protons (3-H<sub>A,B</sub>) at  $\delta$  2.1–2.5 to sharp peaks, respectively. Also irradiation of 6-H at  $\delta$  4.56 collapsed a doublet of 5-H to a singlet and collapsed a multiplet of 7-H to sharp peaks. Furthermore, irradiation at  $\delta$  2.88 (7-H) converted a pair of doublets at  $\delta$  5.52 and 6.07 due to exocyclic methylene protons (13-H<sub>A,B</sub>) into a pair of singlets and doublet of doublets at  $\delta$  4.58 (6-H) into a doublet. These results indicated that  $\beta$ -frullanolide (**1**) had the partial structure **1a**. When the <sup>1</sup>H NMR spectrum of **1** was compared with those of eudesmanolides,<sup>3)</sup> it was similar to them. From these results, we assumed that lactone **1** had an eudesmanolide skeleton, as shown by structure **1**. This assumption was confirmed by a partial synthesis from costunolide (**3**)<sup>4)</sup> according to the following pathway (Chart 1).

Costunolide (**3**), isolated from costus root oil, was cyclized with thionyl chloride to give the cyclocostunolide mixture<sup>5)</sup> from which  $\beta$ -cyclocostunolide **4** was separated by column chromatography on 10% AgNO<sub>3</sub>-SiO<sub>2</sub>. This compound was identified as  $\beta$ -cyclocostunolide **4** with authentic spectra.<sup>5)</sup> Next, this compound was converted to **5** by ethanolysis with Na<sub>2</sub>CO<sub>3</sub> in ethanol. The IR spectrum showed hydroxyl and ester

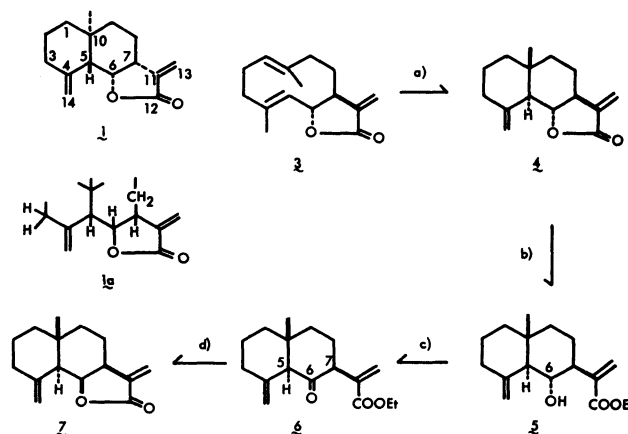


Chart 1. SOCl<sub>2</sub>/pyridine, b) Na<sub>2</sub>CO<sub>3</sub>/EtOH, c) Jones reagent, d) NaBH<sub>4</sub>/MeOH.

absorption bands at 3460 and 1715 cm<sup>-1</sup>. In the <sup>1</sup>H NMR spectrum, the signals due to an ethyl group as the ester function at  $\delta$  1.30 and 4.23 were observed. The alcohol thus obtained was oxidized with Jones reagent to give keto ester **6**. The IR spectrum showed bands at 1718 cm<sup>-1</sup> due to carbonyl groups and at 910 cm<sup>-1</sup> due to an exocyclic methylene. In the <sup>1</sup>H NMR spectrum of **6**, the signals of 5-H and 7-H were observed at lower field (5-H:  $\delta$  3.08, s; 7-H: 3.67, t,  $J$ =10.0 Hz) than those of **5** (5-H:  $\delta$  1.90, d,  $J$ =11.0 Hz; 7-H: 2.60, dt,  $J$ =6.0 and 11.0 Hz). These results and the absence of a hydroxyl absorption in the IR and the signal of 6-H in the <sup>1</sup>H NMR of **6** indicated that hydroxyl group at C-6 was oxidized to keto group. Reduction of keto ester **6** with NaBH<sub>4</sub> in methanol gave a *cis* lactone **7**: [ $\alpha$ ]<sub>D</sub> -161.8°.

Compound **7** was identified in every respect with (+)- $\beta$ -frullanolide except for chiroptical property, CD (MeOH)  $\Delta\epsilon_{254}$ +1.5 for **7** vs.  $\Delta\epsilon_{254}$ -1.5 for **1** (Fig. 1). (+)- $\beta$ -Frullanolide is fully represented by structure **1**.

(+)-Brothenolide (**2**), mp 113–114 °C, [ $\alpha$ ]<sub>D</sub> +153.0°, CD (MeOH)  $\Delta\epsilon_{255}$ -1.6, was obtained as colorless needles. The molecular formula C<sub>15</sub>H<sub>20</sub>O<sub>2</sub> of **2** was determined by HR-MS together with the <sup>13</sup>C and <sup>1</sup>H NMR data. The IR spectrum (CHCl<sub>3</sub>) showed bands at 3040 cm<sup>-1</sup> due to a cyclopropane ring, and at 1760 and 1660 cm<sup>-1</sup> due to an  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone. The <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>) showed the signals of two tertiary methyl groups ( $\delta$  1.11, s and 1.36, s), one proton on the cyclopropane ring ( $\delta$  0.38, t,  $J$ =3.6 Hz), two olefinic protons ( $\delta$  5.52, d,  $J$ =0.6 and 6.09, d,  $J$ =0.6 Hz), one allylic methine ( $\delta$  2.72, m) and one methine proton ( $\delta$  4.76, dd,  $J$ =3.0 and 4.8 Hz) adjacent to oxygen. The <sup>13</sup>C NMR spectrum (CDCl<sub>3</sub>)

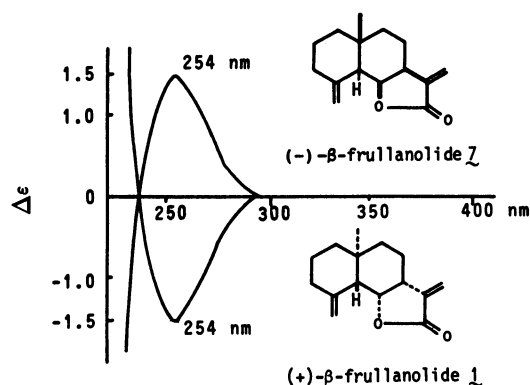


Fig. 1. CD spectra of (+)- and (-)-β-frullanolide.

also indicated the signals of  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone: C=O,  $\delta$  171.3; C-, 141.8; =CH<sub>2</sub>, 119.5 and -O-CH, 77.8.

Hydrogenation of **2** with Pd/C in EtOH yielded a dihydro compound **8** ( $m/z$  234). The <sup>1</sup>H NMR spectrum indicated the signal of a secondary methyl group at  $\delta$  1.06 instead of the protons of the exocyclic methylene in the spectrum of **2**. This compound was further reduced<sup>6)</sup> with PtO<sub>2</sub> in EtOH-AcOH to give a tetrahydro compound **9** ( $m/z$  236, C<sub>15</sub>H<sub>24</sub>O<sub>2</sub>). The IR spectrum indicated the absence of a cyclopropane ring absorption and the presence of *gem*-dimethyl absorption bands at 1380 and 1370 cm<sup>-1</sup>. In the <sup>1</sup>H NMR spectrum, a signal of a tertiary methyl group was observed in place of the protons on the cyclopropane ring. This fact showed that one of the two methyl groups in **2** should be attached to the cyclopropane ring. The presence of the partial structure **2a** was indicated by the above result, and the results of double irradiation experiments in the <sup>1</sup>H NMR spectrum of **2** with the aid of shift reagent. The signal of the proton at 7-H appeared as a multiplet which collapsed to a broad doublet of doublets ( $J=7.0$  and 10.2 Hz) on irradiation of 6-H. In addition, irradiation of the signal of 6-H converted the doublet of 5-H into a singlet. The fact that 7-H is coupled to the 8 $\alpha$ - and 8 $\beta$ -H's with  $J=7.0$  and 10.2 Hz indicated that it is axial. Thus the  $J_{8,7}$  value of 4.8 Hz shows that 6-H is equatorial. Therefore, the  $\gamma$ -lactone ring must be *cis*-fused at C-6 and C-7. The reduction of **9** with LAH afforded a diol **10**, the IR spectrum of which showed the presence of hydroxyl groups at 3500 and 3430 cm<sup>-1</sup>. The <sup>1</sup>H NMR spectrum showed the signals of methylene protons adjacent to oxygen as primary alcohol at  $\delta$  3.44 and 3.56. The diol **10** was acetylated with acetic anhydride and pyridine at room temperature to give monoacetate **11**, the IR spectrum of which showed bands at 3510 and 1720 cm<sup>-1</sup> due to the hydroxyl group and the carbonyl group, respectively. The <sup>1</sup>H NMR spectrum showed also the signals of the acetoxymethyl group at  $\delta$  2.06 and the -CH<sub>2</sub>-OAc group at  $\delta$  3.94 and 4.24. The monoacetate **11** was oxidized with Jones reagent to yield a corresponding keto compound **12**. The IR spectrum indicated the presence of a 6-membered ring ketone at 1715 cm<sup>-1</sup>. Furthermore, in the decoupling experiments of **2** using 360 MHz NMR, irradiation of 1-H<sub>A</sub> at  $\delta$  0.83 collapsed the doublet of doublets of 1-H<sub>B</sub> ( $\delta$  1.77)

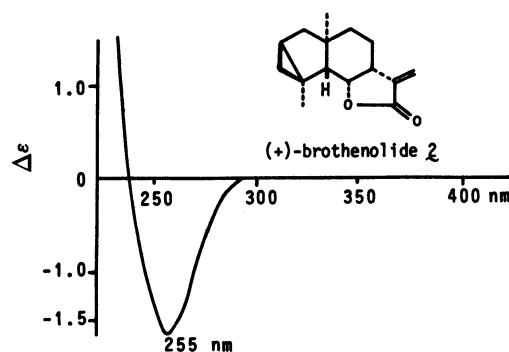


Fig. 2. CD spectrum of (+)-brothenolide.

TABLE 1. CHEMICAL SHIFT OF THE METHYL GROUPS (IN  $\delta$ )

|                           | 4-CH <sub>3</sub> | 4-CH <sub>2</sub> D | 10-CH <sub>3</sub> |
|---------------------------|-------------------|---------------------|--------------------|
| <b>11</b> -d <sub>2</sub> | 1.20              | 1.17                | 1.03               |
| <b>12</b> -d <sub>2</sub> | 1.34              | 1.07                | 0.91               |

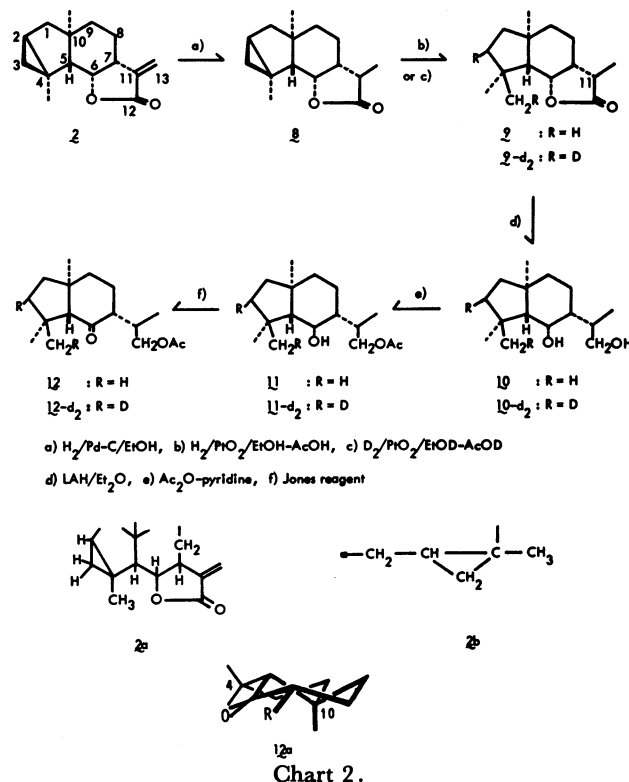


Chart 2.

to a doublet, and a multiplet of 2-H ( $\delta$  1.22) to a sharp peak. In addition, irradiation of 3-H<sub>A</sub> ( $\delta$  0.38) collapsed the doublet of doublets of 3-H<sub>B</sub> ( $\delta$  0.93) to a doublet, and 2-H ( $\delta$  1.22) to sharp peak. This result indicated that brothenolide (**2**) had a partial structure **2b**. All these data show that the new sesquiterpene lactone **2** is most favorably represented by formula **2**. The fact that the CD Cotton effects of lactones **1** and **2** have the same signs (Figs. 1 and 2) shows that they belong to the same chiroptical series.<sup>7)</sup>

The  $\beta$ -configuration of the cyclopropane ring was determined as follows. Reduction of dihydrobrothenolide **8** with deuterium instead of hydrogen (see above) in EtOD-AcOD, followed by LAH treatment and acetylation gave **11**-d<sub>2</sub> (the dideuterio derivative of **11**).

The  $^1\text{H}$  NMR spectrum of **11** showed three tertiary methyl signals at  $\delta$  1.17, 1.20, and 1.03, one of which, at  $\delta$  1.17, appeared as a  $\text{CH}_2\text{D}$  signal in **11-d<sub>2</sub>**. Oxidation of **11-d<sub>2</sub>** with Jones reagent gave **12-d<sub>2</sub>**, the  $^1\text{H}$  NMR spectrum of which showed the signals of two tertiary methyl groups at  $\delta$  1.34 and 0.91, and a tertiary  $\text{CH}_2\text{D}$  group at  $\delta$  1.07. The chemical shifts of  $\text{CH}_3$  and  $\text{CH}_2\text{D}$  groups of **11-d<sub>2</sub>** and **12-d<sub>2</sub>** are shown in Table 1. One methyl peak at  $\delta$  1.20 in **11-d<sub>2</sub>** underwent a low field shift to  $\delta$  1.34 in **12-d<sub>2</sub>** whereas the other methyl peaks at  $\delta$  1.17 and 1.03 in **11-d<sub>2</sub>** shifted to  $\delta$  1.07 and 0.91 in **12-d<sub>2</sub>**. These shifts of methyl peaks were explained by the difference of anisotropic effects between  $6\alpha\text{-OH}$  and 6-keto function. The methyl signals at  $\delta$  1.17, 1.20, and 1.03 in **11-d<sub>2</sub>** were therefore assigned to  $4\beta\text{-Me}$ ,  $4\alpha\text{-Me}$ , and  $10\text{-Me}$  groups, respectively.

Molecular model **12a** clearly shows that  $4\text{-Me}$  group ( $\delta$  1.34) should have the  $\alpha$ -configuration since it is in-plane in relation to the carbonyl group. The  $\text{CH}_2\text{D}$  group in **11-d<sub>2</sub>** is thus  $\beta$ -oriented, and therefore, the cyclopropane methylene group is also  $\beta$ -oriented in brothenolide (**2**).

## Experimental

All melting points are uncorrected. IR spectra were measured with a Hitachi EPI-G2 spectrometer,  $^{13}\text{C}$  NMR spectra with a JEOL FX-100 (25.0 Hz) spectrometer,  $^1\text{H}$  NMR spectra with a JEOL FX-100 (100 MHz), a Nicolet NT-360 (360 MHz), or a Hitachi R-20B (60 MHz) spectrometer in deuteriochloroform solution containing tetramethylsilane as an internal standard, low resolution mass spectra with a Hitachi RMU-6 mass spectrometer and high resolution mass spectra with a JEOL-JMS 01SG-2, with direct inlet system operating at 70 eV. CD spectra were measured with a JASCO J-20 spectrometer. For column chromatography Kieselgel 60 (E. Merck, Darmstadt) was used. Thin-layer chromatography (TLC) was carried out on Kieselgel GF<sub>254</sub> (E. Merck, Darmstadt) in 0.25 mm thickness.

**Isolation.** The fresh material (100 g) of *Frullania brotheri* Steph. collected in Wakayama Prefecture around Mt. Ohto in June 1976 was extracted with ether at room temperature. The ether extract (1.8 g) was chromatographed on  $\text{SiO}_2$ . Elution with  $\text{CHCl}_3$  gave a lactone mixture that was subjected to further column chromatography on  $\text{SiO}_2$  impregnated with 10%  $\text{AgNO}_3$ . Elution with hexane-Et<sub>2</sub>O (20 : 1) yield a fraction of (+)-brothenolide. Next, elution with ether gave crude (+)- $\beta$ -frullanolide.

**(+)- $\beta$ -Frullanolide (1).** The crude material was recrystallized from hexane-EtOAc to yield colorless needles (**1**, 40 mg), mp 165–167 °C,  $[\alpha]_D^{25} +178.0^\circ$  ( $c$  1.17,  $\text{CHCl}_3$ ); CD,  $\Delta\epsilon_{254} -1.5$  ( $c$  0.012, MeOH); IR ( $\text{CHCl}_3$ ) 1760, 1670, and 910  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR  $\delta$  0.92 (3H, s,  $10\text{-CH}_3$ ), 4.58 (1H, dd,  $J=4.5$  and  $3.0$  Hz, 6-H), 4.92 (1H, br. s,  $14\text{-H}_A$ ), 5.09 (1H, br. s,  $14\text{-H}_B$ ), 5.52 (1H, d,  $J=0.6$  Hz,  $13\text{-H}_A$ ), and 6.07 (1H, d,  $J=0.6$  Hz,  $13\text{-H}_B$ );  $^{13}\text{C}$  NMR  $\delta$  18.3 (q), 23.0 (t), 24.6 (t), 34.7 (t), 37.5 (t), 38.8 (t), 41.4 (d), 43.0 (t), 50.9 (d), 77.8 (d), 109.7 (t), 119.0 (t), 141.7 (s), 145.7 (s) and 170.8 (s); MS  $m/z$  (%) 232 (93,  $\text{M}^+$ ), 217 [100,  $(\text{M}-\text{CH}_3)^+$ ], 176 (35), 171 (34), 91 (46), and 79 (37). Found:  $m/z$  232.1453. Calcd for  $\text{C}_{15}\text{H}_{20}\text{O}_3$ : M, 232.1445.

**Isolation of (+)-Brothenolide (2).** The crude fraction was recrystallized from hexane-EtOAc to give colorless needles (**2**, 270 mg), mp 113–114 °C,  $[\alpha]_D^{25} +153.0^\circ$  ( $c$  1.14,  $\text{CHCl}_3$ ); CD,  $\Delta\epsilon_{255} -1.6$  ( $c$  0.012, MeOH); IR ( $\text{CHCl}_3$ ) 3040, 1760,

1660  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (360 MHz)  $\delta$  0.38 (1H, t,  $J=3.6$  Hz,  $3\text{-H}_A$ ), 0.83 (1H, br. dd,  $J=3.6$  and  $12.5$  Hz,  $1\text{-H}_A$ ), 0.93 (1H, dd,  $J=3.6$  and  $8.7$  Hz,  $3\text{-H}_B$ ), 1.06 (1H, d,  $J=3.0$  Hz, 5-H), 1.11 (3H, s,  $10\text{-CH}_3$ ), 1.22 (1H, m, 2-H), 1.22 (1H, m,  $9\text{-H}_B$ ), 1.36 (3H, s,  $4\text{-CH}_3$ ), 1.47 (1H, m,  $8\text{-H}_A$ ), 1.60 (1H, ddd,  $J=3.0$ ,  $4.2$ , and  $12.6$  Hz,  $9\text{-H}_B$ ), 1.72 (1H, dd,  $J=7.5$  and  $12.5$  Hz,  $1\text{-H}_B$ ), 2.72 (1H, m, 7-H), 4.76 (1H, dd,  $J=3.0$  and  $4.8$  Hz, 6-H), 5.52 (1H, d,  $J=0.6$  Hz,  $13\text{-H}_A$ ), and 6.09 (1H, d,  $J=0.6$  Hz,  $13\text{-H}_B$ );  $^{13}\text{C}$  NMR  $\delta$  20.4 (q), 20.9 (q), 25.5 (t), 25.9 (d), 26.6 (s), 34.1 (t), 35.7 (t), 40.5 (d), 46.3 (t), 50.4 (s), 56.5 (d), 77.8 (d), 119.5 (t), 141.8 (s), and 171.3 (s); MS  $m/z$  (%) 232 (8,  $\text{M}^+$ ), 217 [100,  $(\text{M}-\text{CH}_3)^+$ ], 199 (39), 171 (44), 145 (31), 119 (45), 91 (41), and 79 (28). Found:  $m/z$  232.1472. Calcd for  $\text{C}_{15}\text{H}_{20}\text{O}_3$ : M, 232.1445.

**Isolation of Costunolide (3).** Costus root oil (3 g) was chromatographed on  $\text{SiO}_2$ , and eluted with  $\text{CHCl}_3$  to give costunolide (**3**, 270 mg), colorless needles, mp 104–105 °C (recrystallized from hexane),  $[\alpha]_D^{25} +127.0^\circ$  ( $c$  1.05,  $\text{CHCl}_3$ ). The IR, NMR, and mass spectra, and  $[\alpha]_D^{25}$  value of **3** were found to be identical with those of known (+)-costunolide.<sup>4)</sup>

**Cyclization of Costunolide (3).** A solution of costunolide (270 mg) in  $\text{CHCl}_3$  (50 ml) containing  $\text{SOCl}_2$  (0.1 ml) was kept at room temperature for 1 h. After removal of solvent under vacuum, the resulting residue was chromatographed on 10%  $\text{AgNO}_3\text{-SiO}_2$ . Elution with hexane-EtOAc (5 : 1) gave  $\beta$ -cyclocostunolide (160 mg), colorless needles, mp 67–68 °C (recrystallized from EtOH),  $[\alpha]_D^{25} +175.3^\circ$  ( $c$  0.93,  $\text{CHCl}_3$ ). The IR, NMR, and mass spectra, and  $[\alpha]_D^{25}$  value were identical with those of known (+)- $\beta$ -cyclocostunolide.<sup>5)</sup>

**Ethanolysis of  $\beta$ -Cyclocostunolide (4).** 0.05 M (1 M=1 mol  $\text{dm}^{-3}$ )  $\text{Na}_2\text{CO}_3$  aqueous solution (0.2 ml) was added to a solution of  $\beta$ -cyclocostunolide (150 mg) in EtOH (10 ml), and the mixture was kept at room temperature for 2 d. The reaction mixture was diluted with  $\text{H}_2\text{O}$ , evaporated to remove EtOH under reduced pressure and extracted with ether. Ether layer was washed with  $\text{H}_2\text{O}$  and dried over  $\text{Na}_2\text{SO}_4$ . After removal of solvent under reduced pressure, the resulting residue was chromatographed on  $\text{SiO}_2$ . Elution with  $\text{CHCl}_3$  gave hydroxy ester **6**, colorless needles, mp 95–96 °C (recrystallized from hexane),  $[\alpha]_D^{25} +25.2^\circ$  ( $c$  0.92,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ) 3460 and 1715  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR  $\delta$  0.83 (3H, s,  $10\text{-CH}_3$ ), 1.30 (3H, t,  $J=8.0$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 1.90 (1H, d,  $J=11.0$  Hz, 5-H), 2.60 (1H, dt,  $J=6.0$  and  $11.0$  Hz, 7-H), 3.92 (1H, t,  $J=11.0$  Hz, 6-H), 4.23 (2H, q,  $J=8.0$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 4.68 (1H, br. s,  $14\text{-H}_A$ ), 4.96 (1H, br. s,  $14\text{-H}_B$ ), 5.69 (1H, br. s,  $13\text{-H}_A$ ), and 6.27 (1H, br. s,  $13\text{-H}_B$ ); MS  $m/e$  (%) 278 (trace,  $\text{M}^+$ ), 232 [53,  $(\text{M}-\text{EtOH})^+$ ], 217 (33), 123 (61), and 81 (100). Found:  $m/z$  278.1864. Calcd for  $\text{C}_{17}\text{H}_{24}\text{O}_3$ : 278.1869.

**Oxidation of 5 with Jones Reagent.** A few drops of Jones reagent were added slowly to a stirred solution of **5** (80 mg) in acetone (10 ml) at 0 °C, and the mixture was further stirred for 2 d at room temperature. The reaction mixture was treated in the usual way to give a residue which was chromatographed on  $\text{SiO}_2$ . Elution with  $\text{CHCl}_3$  gave keto compound **6**, colorless needles, mp 64 °C (recrystallized from hexane),  $[\alpha]_D^{25} +10.6^\circ$  ( $c$  1.0,  $\text{CHCl}_3$ ); IR ( $\text{CHCl}_3$ ) 1718, 1640, and 910  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR  $\delta$  0.83 (3H, s,  $10\text{-CH}_3$ ), 1.27 (3H, t,  $J=8.0$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 3.08 (1H, s, 5-H), 3.63 (1H, t,  $J=10.0$  Hz, 7-H), 4.18 (1H, q,  $J=8.0$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 4.98 (1H, br. s,  $14\text{-H}_A$ ), 5.54 (1H, br. s,  $13\text{-H}_A$ ), 5.88 (1H, br. s,  $14\text{-H}_B$ ), and 6.32 (1H, br. s,  $13\text{-H}_B$ ); MS  $m/z$  (%) 276 (55,  $\text{M}^+$ ), 230 [92,  $(\text{M}-\text{EtOH})^+$ ], 175 (89), 135 (100), and 107 (97). Found:  $m/z$  276.1714. Calcd for  $\text{C}_{17}\text{H}_{24}\text{O}_3$ : M, 276.1706.

**Reduction of 6 with  $\text{NaBH}_4$ .** After  $\text{NaBH}_4$  (4.4 mg) was added to an ice-cooled solution of **6** (26 mg) in MeOH (0.3 ml), the mixture was stirred at room temperature for 2 h. The

reaction mixture was diluted with H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The CH<sub>2</sub>Cl<sub>2</sub> layer was treated in the usual way to give a residue (20 mg) which was chromatographed on TLC (solvent system; hexane-EtOAc=10:1). Colorless needles (7, 8 mg) were obtained by recrystallization from hexane-EtOAc, mp 164–165 °C,  $[\alpha]_D^{25} -161.8^\circ$  (c 0.64, CHCl<sub>3</sub>); CD,  $\Delta\epsilon_{254} +1.5$  (c 0.012, MeOH). Found:  $m/z$  232.1449. Calcd for C<sub>15</sub>H<sub>20</sub>O<sub>2</sub>: M, 232.1445. The IR, NMR and mass spectra were found to be identical with those of (+)- $\beta$ -frullanolide (1).

**Hydrogenation of Brothenolide (2).** Lactone 2 (25 mg) and palladium charcoal (10 mg) in EtOH (2 ml) were stirred under hydrogen atmosphere at room temperature for 3 h. The catalyst was removed by filtration and the solvent was evaporated to give a residue (25 mg), which was purified by recrystallization from hexane (8, 18 mg). Colorless needles, mp 105–106 °C; IR (CHCl<sub>3</sub>) 3040 and 1770 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  0.32 (1H, t,  $J=3.6$  Hz, 3-H<sub>A</sub>), 1.06 (3H, d,  $J=7.0$  Hz, 11-CH<sub>3</sub>), 1.18 (3H, s, 10-CH<sub>3</sub>), 1.36 (3H, s, 4-CH<sub>3</sub>), and 4.53 (1H, dd,  $J=3.0$  and 4.8 Hz, 6-H); MS  $m/z$  (%) 234 (7, M<sup>+</sup>), 219 [100, (M-CH<sub>3</sub>)<sup>+</sup>], 161 (57), 145 (71), and 107 (45). Found:  $m/z$  234.1613. Calcd for C<sub>15</sub>H<sub>22</sub>O<sub>2</sub>: M, 234.1608.

**Hydrogenation of Dihydro Lactone (8).** Dihydro lactone 8 (20 mg) and platinum catalyst (prepared from platinum oxide; 10 mg) in EtOH (1.5 ml) and AcOH (0.3 ml) were stirred under hydrogen atmosphere at room temperature for 8 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give a residue (21 mg). Tetrahydro lactone 9 was obtained by recrystallization from hexane, (9, 15 mg), colorless needles, mp 138–140 °C; IR (CCl<sub>4</sub>) 1780, 1380, and 1370 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  1.04 (3H, s), 1.07 (3H, s), 1.17 (3H, s), 1.20 (3H, d,  $J=6.6$  Hz, 11-CH<sub>3</sub>), and 4.52 (1H, dd,  $J=3.0$  and 4.8 Hz, 6-H); MS  $m/z$  (%) 236 (1.5, M<sup>+</sup>), 221 [16, (M-CH<sub>3</sub>)<sup>+</sup>], 177 (43), 147 (62), 123 (40), 109 (100), and 107 (69). Found:  $m/z$  236.1762. Calcd for C<sub>15</sub>H<sub>24</sub>O<sub>2</sub>: M, 236.1751.

**Reduction of Tetrahydro Lactone (9) with Lithium Aluminium Hydride.** After a solution of LAH (10 mg) in dry Et<sub>2</sub>O (2 ml) was added to an ice-cooled solution of tetrahydro lactone 9 (12 mg) in dry ether (2 ml), the mixture was kept at room temperature for 3 h. The reaction mixture was treated in the usual way to give a residue (15 mg) which was chromatographed on SiO<sub>2</sub>. Elution with CHCl<sub>3</sub>-MeOH (15:1) gave a diol compound (10, 10 mg), colorless viscous oil, IR (film) 3500, 3430, and 1045 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  0.98 (3H, d,  $J=7.0$  Hz, 11-CH<sub>3</sub>), 1.02 (3H, s), 1.19 (6H, s), 3.44 (1H, dd,  $J=5.0$  and 12.0 Hz, H<sub>A</sub>CH-OH), 3.56 (1H, dd,  $J=6.5$  and 12.0 Hz, H<sub>B</sub>CH-OH), and 4.14 (1H, br. s, 6-H); MS  $m/z$  (%) 240 (trace, M<sup>+</sup>, C<sub>15</sub>H<sub>28</sub>O<sub>2</sub>), 222 [43, (M-H<sub>2</sub>O)<sup>+</sup>], 209 (100).

**Acetylation of Diol Compound (10).** A solution of diol 10 (10 mg) in dry pyridine (2 ml) and Ac<sub>2</sub>O (1 ml) was kept at room temperature for 3 h. The reaction mixture was treated in the usual way to give a residue (13 mg), which was purified by SiO<sub>2</sub> column chromatography using CHCl<sub>3</sub> to give monoacetate, (11, 8 mg), colorless viscous oil, IR (film) 3510, 1720, and 1370 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  1.03 (3H, s, 10-CH<sub>3</sub>), 1.06 (3H, d,  $J=7.0$  Hz, 11-CH<sub>3</sub>), 1.17 (3H, s, 4-CH<sub>3</sub>), 1.20 (3H, s, 4-CH<sub>3</sub>), 2.06 (3H, s, COCH<sub>3</sub>), 3.94 (1H, dd,  $J=7.5$  and 11.0 Hz, 12-H<sub>A</sub>), 4.18 (1H, br. s, 6-H), and 4.24 (1H, dd,  $J=2.1$  and 11.0 Hz, 12-H<sub>B</sub>); MS  $m/z$  (%) 282 (5, M<sup>+</sup>, C<sub>17</sub>H<sub>30</sub>O<sub>3</sub>), 142 (58), 141 (100), and 140 (42).

**Oxidation of Acetate (11) with Jones Reagent.** A drop of Jones reagent was added to a solution of 11 (10 mg) in acetone (1 ml) at 0 °C, and the mixture was further stirred for 3 h at room temperature. The reaction mixture was treated in the usual way to give a residue (10 mg), which was chromatographed on TLC using CHCl<sub>3</sub> to afford keto acetate, (12, 6 mg), colorless viscous oil, IR (film) 1735 and 1715 cm<sup>-1</sup>; <sup>1</sup>H

NMR  $\delta$  0.91 (3H, s, 10-CH<sub>3</sub>), 0.98 (3H, d,  $J=7.0$  Hz, 11-CH<sub>3</sub>), 1.07 (3H, s, 4-CH<sub>3</sub>), 1.34 (3H, s, 4-CH<sub>3</sub>), 2.03 (3H, s, COCH<sub>3</sub>), 2.12 (1H, s, 5-H), 2.24 (1H, m, 7-H), 3.93 (1H, dd,  $J=7.1$  and 11.0 Hz, 12-H<sub>A</sub>), and 4.09 (1H, dd,  $J=2.1$  and 11.0 Hz, 12-H<sub>B</sub>); MS  $m/z$  (%) 280 (17, M<sup>+</sup>, C<sub>17</sub>H<sub>28</sub>O<sub>3</sub>), 265 [36, (M-CH<sub>3</sub>)<sup>+</sup>], and 220 [100, (M-60)<sup>+</sup>].

**Preparation of D-Labelled Compounds (9-d<sub>2</sub>), (11-d<sub>2</sub>), and (12-d<sub>2</sub>).**

Dihydro lactone 8 (10 mg) and platinum catalyst (prepared from platinum oxide; 5 mg) in EtOD (1.5 ml) and AcOD (0.3 ml) were stirred under deuterium atmosphere at room temperature for 8 h. The reaction mixture was filtered and the filtrate was evaporated *in vacuo* to give a residue which was chromatographed on TLC using CHCl<sub>3</sub> to give 9-d<sub>2</sub> (7 mg), MS  $m/z$  (%) 238 (1, M<sup>+</sup>, C<sub>15</sub>H<sub>22</sub>D<sub>2</sub>O<sub>2</sub>), and 110 (100); IR (CCl<sub>4</sub>) 1780, 1380, and 1370 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  1.04 (3H, s, 10-CH<sub>3</sub>), 1.08 (2H, s, 4-CH<sub>2</sub>D), 1.16 (3H, s, 4-CH<sub>3</sub>), 1.20 (3H,  $J=6.5$  Hz, 11-CH<sub>3</sub>), and 4.51 (1H, br. s, 6-H).

**Conversion of 9-d<sub>2</sub> into 11-d<sub>2</sub>.** A mixture of 9-d<sub>2</sub> (7 mg) and LAH (10 mg) in dry ether (3 ml) was stirred at room temperature for 3 h. The reaction mixture was treated in the usual way. The residue was treated with acetic anhydride (0.5 ml) and pyridine (1 ml) at room temperature for a day and then worked up in the usual way. The resulting residue was chromatographed on TLC using CHCl<sub>3</sub> to yield 11-d<sub>2</sub> (4 mg); MS  $m/z$  (%) 284 (54, M<sup>+</sup>, C<sub>17</sub>H<sub>28</sub>D<sub>2</sub>O<sub>3</sub>), 269 [31, (M-CH<sub>3</sub>)<sup>+</sup>] and 209 (100); IR (film) 3500, 1720 and 1380 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  1.03 (3H, s, 10-CH<sub>3</sub>), 1.06 (3H, d,  $J=7.0$  Hz, 11-CH<sub>3</sub>), 1.17 (2H, s, 4-CH<sub>2</sub>D), 1.20 (3H, s, 4-CH<sub>3</sub>), 2.06 (3H, s, COCH<sub>3</sub>), 3.94 (1H, dd,  $J=7.5$  and 11.0 Hz, 12-H<sub>A</sub>), 4.18 (1H, br. s, 6-H), and 4.24 (1H, dd,  $J=2.1$  and 11.0 Hz, 12-H<sub>B</sub>).

**Oxidation of 11-d<sub>2</sub> with Jones Reagent.** To a solution of 11-d<sub>2</sub> (4 mg) in acetone (1 ml) was added a drop of Jones reagent at 0 °C. The mixture was stirred for a day at room temperature, and then worked up in the usual way. The residue was chromatographed on TLC using CHCl<sub>3</sub> to afford 12-d<sub>2</sub> (2 mg), MS  $m/z$  (%) 282 (32, M<sup>+</sup>, C<sub>17</sub>H<sub>26</sub>D<sub>2</sub>O<sub>3</sub>), 267 [28, (M-CH<sub>3</sub>)<sup>+</sup>], and 222 [100, (M-60)<sup>+</sup>]; IR (film) 1735 and 1715 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  0.91 (3H, s, 10-CH<sub>3</sub>), 0.98 (3H, d,  $J=7.0$  Hz, 11-CH<sub>3</sub>), 1.07 (2H, s, 4-CH<sub>2</sub>D), 1.34 (3H, s, 4-CH<sub>3</sub>), 2.03 (3H, s, COCH<sub>3</sub>), 2.13 (1H, s, 5-H), 2.24 (1H, m, 7-H), 3.93 (1H, dd,  $J=7.1$  and 11.0 Hz, 12-H<sub>A</sub>), and 4.09 (1H, dd,  $J=2.1$  and 11.0 Hz, 12-H<sub>B</sub>).

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