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DULCOSIDES A AND B, NEW DITERPENE GLYCOSIDES FROM *STEVIA REBAUDIANA*

MASARU KOBAYASHI, SHUJI HORIKAWA, ISOLDE H. DEGRANDI, JUNKO UENO, and HIROSHI MITSUHASHI
Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

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Key Word Index—*Stevia rebaudiana*; Compositae; new diterpene glycoside; natural sweet substance; dulcosides A and B; structural determination.

Abstract—New diterpene glycosides, dulcosides A and B were isolated from *Stevia rebaudiana* Bertoni and their structures were established as 19-*O*-β-glucopyranosyl-13-*O*-[α-rhamnopyranosyl (1-2)]-β-glucopyranosyl-steviol and 19-*O*-β-glucopyranosyl-13-*O*-[α-rhamnopyranosyl (1-2)-β-glucopyranosyl (1-3)]-β- glucopyranosylsteviol respectively. They showed moderate sweetness, *ca* 30 times more than that of sucrose.

INTRODUCTION

The South American shrub *Stevia rebaudiana* has received much attention as a new source of natural sweetening agents and its crude glycoside mixture is now used as a food additive in Japan. In addition to the main glycoside stevioside (**1**) [1], several other diterpene glycosides occur in *S. rebaudiana* and their relative abundance varies considerably according to the strain and locality of the plant [2]. We found 4 main glycosides and among them, rebaudioside A (**2**) [3], the monoglucosyl derivative of **1**), was present in amounts almost equal to those of **1**. The present paper deals with the structural determination of the other two diterpene glycosides, dulcosides A and B, which had moderate sweetness.

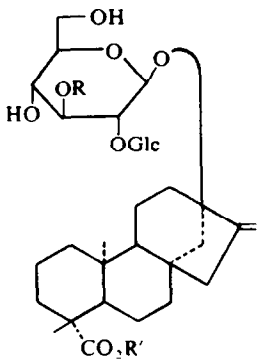
RESULTS AND DISCUSSION

Chromatography of the crude glycoside mixture from which a bulk of **1** was removed, gave compounds **3**, **1**, **4** and **2** in order of their elution. Compounds **1** and **2** were confirmed as stevioside and rebaudioside A from their physical constants and derivatives. Compounds **3** and **4** were new diterpene glycosides and designated dulcosides A (**3**) and B (**4**) after their sweet taste.

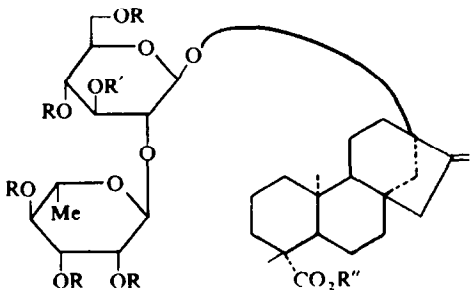
Dulcoside A, $C_{38}H_{60}O_{17}.2H_2O$, showed IR bands at 895 (terminal methylene) and 1730 cm^{-1} (ester). Its PMR spectrum(C_5D_5N) showed signals for two tertiary Me (δ 1.25 and 1.32) and one secondary Me (1.71, *d*, J = 6 Hz). The resolution at lower field was poor and only one vinylic (5.72, *s*) and an anomeric proton due to a β-glucosyl ester linkage 5.99, *d*, J = 7 Hz) were detected. Acid hydrolysis gave the rearranged aglycone isosteviol (**5**) [1b] as well as glucose and rhamnose in a 2:1 ratio. Although enzymatic hydrolysis of the compound using snail gut enzyme gave only the carboxylic acid **8**, the aglycone steviol (**7**) [1b] was obtained by periodate oxidation followed by hydrolysis. Alkaline hydrolysis gave (**8**) and 1,6-anhydroglucopyranose (**10**) which was identified by comparison with an authentic specimen. A similar result has been obtained for hydrolysis of (**1**) and (**2**) [1, 3]. The PMR (CD_3OD) of (**8**) showed signals for two tertiary Me (δ 1 and 1.2), one secondary Me (1.23, *d*, J = 6 Hz; in C_5D_5N, at 1.73) and two anomeric protons due to α- and β- glycosidic linkages at 5.32 (*d*, J = 1.5 Hz) and 4.56 (*d*, J = 7 Hz). On acid hydrolysis, (**8**) gave (**5**), and glucose and rhamnose in a 1:1 ratio. Methylation of **8** by Hakomori's method [4] gave the permethylate **9**, PMR ($CDCl_3$), δ 5.35 (1H, *d*, J = 1.5 Hz) and 4.48 (1H, *d*, J = 7 Hz). On methanolysis **9** gave isosteviol Me ester (**6**) as well as Me-3,4,6-tri-*O*-Me glucopyranoside and Me-2,3,4-tri-*O*-Me rhamnopyranoside indicating that the disaccharide residue at C-13 of steviol is 2-*O*-rhamnosylglucose (neohesperidose).

In order to confirm the nature of each glycosidic linkage, compound **8** was treated with weak acid and the monoglucoside formed was methylated to give **13**. Although the PMR ($CDCl_3$) revealed that the exocyclic double bond had migrated to the *endo* position as judged from the presence of only one vinylic proton at δ 5.08 (broad *s*), the β-glucosidic linkage in (**13**) was evident from the anomeric proton signal at 4.48 (*d*, J = 7 Hz). From these results, it was concluded that the structure of dulcoside A is 19-*O*-β-glucopyranosyl-13-*O*-[α-rhamnopyranosyl (1–2)]-β-glucopyranosylsteviol (**3**) which carries a neohesperidosyl instead of a sophorosyl group in (**1**). The sweetness of (**3**) was *ca* 30 times that of sucrose [5].

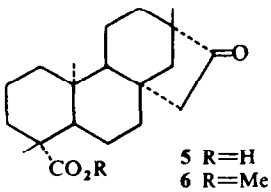
Dulcoside B, $C_{44}H_{70}O_{22}.3H_2O$, had a similar sweetness to Dulcoside A. On acetylation it gave the peracetate (**14**) and after alkaline hydrolysis, it gave the carboxylic acid (**15**) and 1,6-anhydroglucopyranose. The IR bands at 890 and 1725 cm^{-1} and the PMR signals (C_5D_5N) for two tertiary Me (δ 1.25 and 1.33), one secondary Me (1.66, *d*, J = 6 Hz), terminal methylene (5.13 and 5.69), and one β-glucosyl ester-anomeric proton (5.94, *d*, J = 7 Hz) suggest that the compound is also a derivative of steviol-β-glucosyl ester. In CD_3OD, the PMR signals of all 4 anomeric protons were observed at δ 4.47, 4.55, and 5.28 (each 1H, *d*, J = 7 Hz) for β- and at 5.37 (1H, broad *s*) for α-glycosidic linkages. Acid hydrolysis gave (**5**), glucose, and a smaller amount of rhamnose



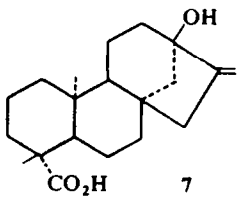
1 R=H R′=Glc
2 R=R′=Glc
19 R=Glc R′=H



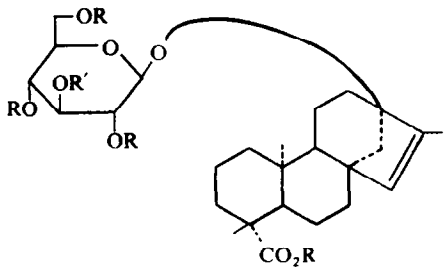
3 R=R′=H R″=Glc
4 R=H R′=R″=Glc
8 R=R′=R″=H
9 R=R′=R″=Me
14 R=Ac R′=R″=Glc(OAc)$_4$
15 R=H R′=Glc R″=H
16 R=Me R′=Glc(OMe)$_4$ R″=Me



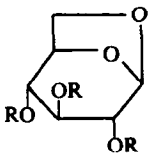
5 R=H
6 R=Me



7



12 R=R′=H
13 R=R′=Me
17 R=H R′=Glc
18 R=Me R′=Glc(OMe)$_4$



10 R=H
11 R=Ac

whilst periodate oxidation followed by hydrolysis, yielded the aglycone steviol (**7**). The carboxylic acid (**15**), which was also obtained by snail-enzymatic hydrolysis, gave (**5**), and glucose and rhamnose in a 2:1 ratio on acid hydrolysis. Methylation of (**15**) gave the permethylate (**16**) which on methanolysis afforded **6**, Me-2,3,4,6-tetra-*O*-Me glucopyranoside, Me-2,3,4-tri-*O*-Me rhamnopyranoside and Me-4,6-di-*O*-Me glucopyranoside, thus indicating the presence of a glucose moiety having terminal glucose and rhamnose at 2- and 3-*O*- or 3- and 2-*O*-. Mild acid hydrolysis of **15** gave the rhamnose-free bioside (**17**) whose permethylate (**18**) showed by PMR ($CDCl_3$), anomeric proton signals at δ 4.53 and 4.75 (each 1H, *d*, J = 7 Hz) corresponding to two β-glucosidic linkages. On methanolysis, **18** gave **6**, Me-2,3,4,6-tetra-*O*-Me glucopyranoside and Me-2.4.6-tri-*O*-Me glucopyranoside indicating the presence of a β-glucosyl (1-3)-β-glucosyl group in **17** and from these results it was concluded that the structure of dulcoside B was 19-*O*-β-glucopyranosyl-13-*O*-[α-rhamnopyranosyl (1-2)-β-glucopyranosyl (1-3)]-β-glucopyranosylsteviol (**4**).

Dulcosides A and B correspond to stevioside (**1**) and rebaudioside A (**2**) with their rhamnosyl groups replaced by glucosyl groups. However, the sweetness of **3** and **4** is less intense and is *ca* one tenth that of **1** or **2**. Up to the present, osladin [6] and dihydrochalcone derivatives [7] are the only known sweet substances having a neohesperidosyl group but their sweetness is greater than that of either **1** or **2**.

In their structural study of **2**, Kohda *et al.* also reported the co-occurrence of rebaudioside B in *S. rebaudiana* and that it was the carboxylic acid **19**, the alkaline hydrolysis product of **2** [3]. In the present study, however, we could not find the compound corresponding to **19**. We have confirmed the structure of **2** but, from our results, the alkaline hydrolysis of **2** gave **19**, mp 217–218°, $[\alpha]_D$ – 23.1°, significantly different from those reported

for rebaudioside B (mp 193–195°, $[\alpha]_D$ – 45.4°). The R_f of **19** on TLC was identical to that of dulcoside A, mp 193–195°.

EXPERIMENTAL

All mps were uncorr. PMR were recorded at 100 MHz with TMS as int. stand. IR were recorded in Nujol mull unless otherwise specified. GLC was carried out on a glass column (2 m × 3 mm i.d.) packed with 5% diethyleneglycol succinate at a column temp. of 140° for per Me sugars, 155° for monohydroxyper Me sugars, and 170° for Me-4,6-di-*O*-Me glucopyranoside. The following solvent systems were used for TLC, PC and column chromatography: (a) $CHCl_3$–MeOH–H_2O (6.5:3:1, lower layer); (b) *n*-BuOH–Py–H_2O (6:4:3); (*c*) MeOH–$CHCl_3$ (3:100); (d) MeOH–$CHCl_3$ (1:20).

Separation of glycoside mixture. The aq. extract of *S. rebaudiana* from which *ca* 85% of **1** was removed by recrystallization from MeOH–EtOH, was used as the starting material. The material (20 g) was dissolved in dioxan (300 ml), mixed with Si gel (50 g), evapd to dryness *in vacuo* and placed on a column of dry Si gel (800 g). Elution using 10 l. of solvent a gave **3** (1.07 g), **1** (0.1 g), **4** (2.31 g) and **2** (0.3 g). R_f (Si gel HF 254-solvent a3 developments): **3**, 0.44; **1** 0.34; **4**, 0.29; **2**, 0.23. (a) *Dulcoside A* (**3**). mp 193–195° (MeOH); $[\alpha]_D$ – 46.7° (MeOH, *c* 0.45); IR: 3000–3600, 1730, 895 cm^{-1}; PMR (C_5D_5N): δ 1.25 and 1.32 (each 3H, *s*, tert-Me), 1.71 (3H, *d*, J = 6 Hz, sec-Me), 5.72 (1H, *s*, vinylic H), 5.99 (1H, *d*, J = 7 Hz, anomeric H). (Found: C, 55.54; H, 8.09. $C_{38}H_{60}O_{17}.2H_2O$ requires: C, 55.33; H, 7.82%). (b) *Stevioside* (**1**). mp 196–198° (MeOH) (lit. [1], 196–198°), identified with authentic sample. (*c*) *Dulcoside B* (**4**). mp 235–238 (H_2O); $[\alpha]_D$ – 28.7° (MeOH, *c* 3.55); IR: 3000–3600, 1725, 890 cm^{-1}; PMR (C_5D_5N): δ 1.25 and 1.33 (each 3H, *s*), 1.66 (3H, *d*, J = 6 Hz) 4.94, 5.02 and 5.94 (each 1H, *d*, J = 7 Hz, anomeric H), 5.13 and 5.69 (each 1H, *brs*, terminal methylene); (CD_3OD); δ 0.97 and 1.2 (each 3H, *s*), 1.21 (3H, *d*, J = 6 Hz), 4.47, 4.55 and 5.28 (each 1H, *d*, J = 7 Hz, anomeric H), 5.37 (1H, broad *s*, anomeric H), 5.16 (1H, *s*, vinylic H). (Found: C, 52.31; H, 7.55. $C_{44}H_{70}O_{22}.3H_2O$ requires: C, 52.58; H, 7.62%). (*d*) *Rebaudioside A* (**2**). mp 235–237° (MeOH) (lit. [3], mp 242–244°); $[\alpha]_D$ – 15.3° (MeOH, *c* 0.4) (lit. [3], –20.8°); IR: 3000–3600, 1730, 890 cm^{-1}; PMR (C_5D_5N): δ 1.23 and 1.30 (each 3H, *s*), 5.28 (1H, *d*, J = 7 Hz, anomeric H), 6.08 (1H, *d*, J = 7 Hz, anomeric H).

Dulcoside B peracetate (**14**). Compound **4** (102.6 mg) was acetylated (Ac_2O–Py, room temp.) to give 140 mg of **14**, mp 134–136°, $[\alpha]_D$ – 43.8° ($CHCl_3$, *c* 5.5); IR: 1730–1760, 900 cm^{-1}. (Found: C, 55.51; H, 6.45. $C_{70}H_{96}O_{35}$ requires: C, 56.14; H, 6.46%).

Alkaline hydrolysis of **3** *and* **4**. (a) A soln of **3** (300 mg) in 10% aq. KOH (10 ml) was refluxed for 1 hr and neutralized with HOAc. Another 3 ml of HOAc was added, the mixture kept for 3 hr and the ppt. collected. The aq. layer was passed through 20 ml of Amberlite IR 120B column and evapd to dryness, giving 1,6-anhydroglucopyranose (**10**) as a single spot on TLC (solvent a). Its identity was confirmed by comparison with an authentic sample and TLC of the acetate derivative (**11**). The ppt. was recrystallized from MeOH to give 130 mg of **8**, mp 185–186.5°; $[\alpha]_D$ – 59.4° (MeOH, *c* 0.76); IR: 1715, 1635, 895 cm^{-1}; PMR (C_5D_5N): δ 1.20 and 1.33 (each 3H, *s*), 1.73 (3H, *d*, J = 6 Hz), 5.12 and 5.74 (each 1H, *s*, terminal methylene); (CD_3OD): δ 1 and 1.2 (each 3H, *s*), 1.23 (3H, *d*, J = 6 Hz), 4.56 (1H, *d*, J = 7 Hz, anomeric H), 5.32 (1H, *d* J = 1.5 Hz, anomeric H), 5.11 (1H, *s*, vinylic H). (Found: C, 59.95; H, 8.18. $C_{32}H_{50}O_{12}.H_2O$ requires: C, 59.60; H, 8.12). (b) A soln of **4** (200 mg) in 10% aq. KOH (10 ml) was refluxed for 1 hr, cooled, and neutralized with HOAc. After the addition of another 3 ml of HOAc, the mixture was concd to a small vol., diluted with H_2O and filtered. From the aq. layer, 1,6-anhydroglucopyranose was identified as in (a).

The filter cake was dried and triturated in MeOH. The MeOH extract was recrystallized from MeOH to give 100 mg of **15**, mp 209–210.5°; $[\alpha]_D$ – 52.9° (MeOH, *c* 0.87); IR: 3000–3500, 1690, 890 cm^{-1}; PMR (C_5D_5N): δ 1.23, and 1.35 (each 3H, *s*), 1.73 (3H, *d*, J = 6 Hz), 5.04 and 5.1 (each 1H, *d*, J = 7 Hz, anomeric H), 5.13 and 5.71 (each 1H, *s*, terminal methylene). (Found: C, 53.84; H, 7.90 $C_{38}H_{60}O_{17}.3H_2O$ requires: C, 54.14; H, 7.89%).

Enzymatic hydrolysis of **3** *and* **4**. (a) Pulverised snail digestive juice (50 mg) was mixed with 2 ml of acetate buffer (pH 5.5) and filtered. The filtrate was mixed with a soln of **3** (50 mg) in H_2O (20 ml). Several drops of toluene was added and the mixture was incubated at 25° for 14 days. The ppt., mp 184–187° was obtained in trace amounts and identified as **8** by IR and TLC (solvent a). (b) Dulcoside B (**4**) was treated by the same procedure and gave **15**, mp 205–208° as the only product.

Periodate oxidation of **3** *and* **4**. (a) A soln of **3** (300 mg) in dioxan–H_2O (1:1, 10 ml) was treated with 10% aq. $NaIO_4$ (10 ml) at room temp. for 18 hr. The solvent was evapd at 40° and the residue dissolved in 5% KOH in MeOH (5 ml) and refluxed for 2 hr. After neutralization with HOAc, most of the solvent was removed *in vacuo* and the residue diluted with H_2O (10 ml), extracted with Et_2O and worked up as usual. The Et_2O soluble products were dissolved in dioxan and submitted to preparative-TLC [$CHCl_3$–MeOH–H_2O (25:8:1)] on Si gel HF (254 and 366). The band corresponding with authentic steviol was removed and extracted with EtOAc giving a trace of **7**, mp 212–214.5° (MeOH) (lit. [1], 215°), IR: 3450, 1690, 1030, 880, 800, 760 cm^{-1}, identical with authentic **7**. (b) Dulcoside B (200 mg) was treated with 10% aq. $NaIO_4$ as in (a). The ppt. formed was removed by filtration and the filtrate evapd to dryness. It was dissolved in 5% KOH in MeOH (5 ml) and dioxan (2 ml) and refluxed for 2 hr. Usual work-up and preparative-TLC as in (a) gave a trace of **7**, mp 207–212°.

Acid hydrolysis of **3**, **4**, **8** *and* **15**. (a) soln of **3** (50 mg) in N-HCl (5 ml) was refluxed for 3 hr. After cooling, most of the solvent was removed at 40° and the residue dried under N_2. The mixture was partitioned with $CHCl_3$ and H_2O, and the $CHCl_3$ layer evapd and recrystallized from MeOH to give 6 mg of **5**, mp 221.5–223° (lit. [1b], 231°), IR: 2500–3200, 1735, 1690 cm^{-1}, identical with an authentic sample. The aq. layer contained glucose and rhamnose, TLC (solvent a) and PC (solvent b), comparison with authentic samples. Compound **8** was treated in the same way giving **5**, glucose and rhamnose in a 1:1 ratio as estimated by PC and TLC. (b) Compounds **4** and **15** (each 50 mg) were treated in the same way as (a) giving **5**, mp 222–224°. The aq. layer contained glucose and a smaller amount of rhamnose (TLC and PC). From **15**, the ratio of glucose to rhamnose was *ca* 2:1 as estimated by PC and TLC.

Hakomori methylation of **8** *and* **15**. (a) A mixture of NaH (200 mg) and DMSO (5 ml) was stirred at 65° for 1 hr under N_2. A soln of **8** (100 mg) in DMSO (3 ml) was added and stirred at 65° for 20 min. To this was gradually added MeI (2 ml), the mixture stirred for 3 hr and then poured into H_2O. Extraction with $CHCl_3$ and usual work-up gave **9** as an oil, $[\alpha]_D$ – 31.8° (MeOH, *c* 2.99); PMR ($CDCl_3$): δ 0.84 and 1.18 (each 3H, *s*), 1.25 (3H, *d*, J = 6 Hz), 4.48 (1H, *d*, J = 7 Hz, anomeric H), 5.35 (1H, *d*, J = 1.5 Hz, anomeric H), 4.88 and 5.19 (each 1H, *s*, terminal methylene). (b) Compound **15** gave by the same procedure, the permethylate **16** as an oil, $[\alpha]_D$ – 24.3° (MeOH, *c* 3.77); PMR ($CDCl_3$): δ 0.84 and 1.17 (each 3H, *s*), 1.21 (3H, *d*, J = 6 Hz), 4.49 (2H, *d*, J = 7 Hz, two anomeric H), 5.23 (1H, *d*, J = 1.5 Hz, anomeric H), 4.86 and 5.2 (each 1H, *s*, terminal methylene); (C_5D_5N): δ 0.96 and 1.19 (each 3H, *s*), 1.5 (3H, *d*, J = 6 Hz), 4.87 and 4.93 (each 1H, *d*, J = 7 Hz, anomeric H), 5.74 (1H, *d* J = 1.5 Hz, anomeric H), 5.11 and 5.67 (each 1H, *s*, terminal methylene).

Partial acid hydrolysis and methylation of **8** *and* **15**. (a) A soln of **8** (300 mg) in NHCl (6 ml) and dioxan (6 ml) was heated at 80° for 2.5 hr. TLC (solvent a) of the mixture showed the formation of a less polar monoside (**12**) and rhamnose in trace amounts. After neutralization with 20% aq. NaOH, the mixture was concd to a small vol., diluted with H_2O and extracted with *n*-BuOH. The aq. layer contained rhamnose and a trace of glucose (TLC). The BuOH layer was washed with H_2O satd BuOH, satd aq. NaCl and evapd to dryness. Column chroma-

tography of the residue on Si gel (solvent a) afforded **12** as an oil, IR (film): 3000–3500, 1710 cm^{-1}. It was methylated (Hakomori) to the permethylate (**13**), PMR ($CDCl_3$): δ 0.83 and 1.17 (each 3H, *s*), 4.48 (1H, *d* J = 7 Hz, anomeric H), 5.08 (1H, *s*, vinylic H). (b) A soln of **15** (200 mg) in NHCl (6 ml) and dioxan (6 ml) was heated at 70° for 1.5 hr and worked up as in (a). Free rhamnose was confirmed in the aq. layer by TLC. The bioside thus obtained was methylated (Hakomori) to give chromatographically pure **18**, PMR ($CDCl_3$): δ 0.87 and 1.21 (each 3H, *s*), 4.53 and 4.75 (each 1H, *d*, J = 7 Hz, anomeric H), 5.13 (1H, *s*, vinylic H).

Methanolysis of **9**, **16** *and* **18**. (a) A soln of **9** (90 mg) in 5% methanolic HCl (6 ml) was refluxed for 2 hr, cooled and most of the solvent was removed at 40° and the residue dried under N_2. Crystallization from MeOH gave **6**, mp 201–202° (lit. [1b], 201–202°); IR: 1740, 1710 cm^{-1}. GLC of the mother liquor showed the presence of Me-2,3,4-tri-*O*-Me rhamnopyranoside and Me-3,4,6-tri-*O*-Me glucopyranoside by comparison with authentic samples. Identification was also confirmed by TLC (solvent c). (b) Compound **16** (100 mg) was treated as in (a) and gave **6**, mp 201.5–202°. The mother liquor contained Me-2,3,4,6-tetra-*O*-Me glucopyranoside, Me-2,3,4-tri-*O*-Me rhamnopyranoside, and Me-4,6-di-*O*-Me glucopyranoside by comparison with authentic samples using GLC and TLC (solvent c, d). (c) Compounds **18** (50 mg) was treated as in (a) giving **6**, mp 200–201.5°. GLC of the mother liquor showed Me-2,3,4,6-tetra-*O*-Me glucopyranoside and Me-2,4,6-tri-*O*-Me glucopyranoside by comparison with authentic samples. Confirmation was also obtained by TLC (C_6H_6–Me_2CO, 1:1).

Alkaline hydrolysis of rebaudioside A. Alkaline hydrolysis of **2** using the procedure described for **3** and **4** gave the carboxylic acid **19**, mp 217–218°, $[\alpha]_D$ – 23.1° (MeOH, *c* 0.78): IR: 3050–3600, 1695, 895 cm^{-1}; PMR (C_5D_5N): δ 1.2 and 1.31 (each 3H, *s*), 5.3 and 5.5 (each 1H, *d*, J = 7 Hz, anomeric H), 5.03 and 5.65 (each 1H, *s*, terminal methylene). (Found: C, 54.04; H, 7.71. Calcd for $C_{38}H_{60}O_{18}.2H_2O$: C, 54.27; H, 7.67%). Methylation of **19** followed by methanolysis gave identical results as those reported in ref. [3].

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REFERENCES

1. (a) Wood, H. B., Allerton, R., Diehl, H. W. and Fletcher, H. G. (1955) *J. Org. Chem.* **20**, 875;
   (b) Mossetig, E. and Ness, W. R. (1955) *J. Org. Chem.* **20**, 884;
   (c) Wood, H. B. and Fletcher, H. G. (1956) *J. Am. Chem. Soc.* **78**, 207;
   (d) Vis, K. and Fletcher, H. G. (1956) *J. Am. Chem. Soc.* **78**, 4709;
   (e) Mossetig, E., Beglinger, U., Dolder, F., Lichiti, H., Quitt, P. and Waters, J. A. (1963) *J. Am. Chem. Soc.* **85**, 2305 and references cited therein.
2. Mitsuhashi, H. and Junko, U. (1975) unpublished.
3. Kohda, H., Kasai, R., Yamasaki, K., Murakami, K. and Tanaka, O. (1976) *Phytochemistry* **15**, 981.
4. Hakomori, S. (1964) *J. Biochem.* **55**, 205.
5. Kobayashi, M., Horikawa, S. and Mitsuhashi, H. (1977) unpublished.
6. Farnworth, N. R. (1973) *Cos. Perfum.* **88**, 27.
7. Horowitz, R. M. and Gentili, B. (1963) U.S. Patent 3,087,821.