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STEREBINS E, F, G AND H, DITERPENOIDS OF *STEVIA REBAUDIANA* LEAVES

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Key Word Index—*Stevia rebaudiana*; Compositae; diterpenoid; labdane skeleton; sterebins E, F, G, H.

Abstract—New labdane-type diterpenoids, sterebins E, F, G and H, were isolated from *Stevia rebaudiana* leaves and their structures were elucidated on the basis of spectral and chemical studies.

INTRODUCTION

The leaves of *Stevia rebaudiana* Bertoni (Compositae) are well-known in Japan as a source of natural sweetener. In the traditional medicine of Paraguay, the water extract of this plant has been used as a contraceptive and for the treatment of diabetes mellitus [1]. During the course of our work to search for plant-origin hypoglycemic constituents, it was found that the ethyl acetate soluble fraction of the methanol extract of *Stevia rebaudiana* exhibited a promising activity. Systematic purification of this ethyl acetate soluble fraction led to the isolation of four labdane-type bisnorditerpenoids characterised as sterebins A, B, C and D [2]. Further fractionation of this active fraction has resulted in the isolation of four new labdane-type diterpenoids which we have now named sterebins E, F, G and H. This paper deals with the structure elucidation of these constituents.

RESULTS AND DISCUSSION

Sterebin E (**1**), $[\alpha]_D$ +29.9°, was indicated to have the molecular formula, $C_{20}H_{34}O_4$, on the basis of a mass ion peak at *m/z* 320 $[M-H_2O]^+$ and the analysis of its ^{13}C NMR spectrum (Table 1). In the ^1H NMR spectrum of sterebin E (**1**), signals assigned to four quaternary methyls at δ1.01, 1.03, 1.18 and 1.22 (3H each *s*), and two carbinyl hydrogens characteristic of a 1,2-*trans*-diequatorial diol system of a cyclohexane ring at δ3.45 (1H, *d*, *J* = 10 Hz) and 3.70 (1H, *t*, *J* = 10 Hz) corresponded well with those of sterebin A (**5**) [2]. Further, the signals due to the carbons of the decalin moiety (C-1–C-10) and four methyl groups attached to this moiety (C-17–C-20) were found virtually identical in the ^{13}C NMR spectra of sterebins E (**1**) and A (**5**). These observations indicated that sterebin E (**1**) has the same A- and B- rings as those of sterebin A (**5**).

The ^{13}C NMR spectrum exhibited the absence of a carbonyl group and the presence of a trisubstituted double bond (δ125.4, *d* and 134.8, *s*) and a disubstituted double bond (δ132.0 and 139.8, each *d*), which, along with its UV absorption maximum at 238 nm (log ε 3.87), suggested that these two double bonds are in conjugation. In addition to this, the ^1H NMR spectrum of sterebin E (**1**) showed signals for a vinylic methyl at δ1.83 (3H *s*) and three olefinic hydrogens at δ5.66 (1H, *t*, *J* = 7 Hz), 5.67 (1H, *dd*, *J* = 16 and 10 Hz) and 6.20 (1H, *d*, *J* = 16 Hz). Further, the one at δ5.66 was found to couple with an oxymethylene hydrogen signal at δ4.29 (2H, *d*, *J* = 7 Hz), which underwent a downfield shift to 4.70 by acetylation of sterebin E (**1**). These spectral data revealed the presence of a 5-hydroxy-3-methyl-1,3-pentadienyl group as a C-9 side chain of sterebin E (**1**).

The configurations of the two double bonds in the C-9 side chain were assigned to be 11*E* and 13*E*, respectively, from the large coupling constant between the C-11 and C-12 olefinic hydrogen signals (*J* = 16 Hz) and from the fact that the resonance position of the vinylic methyl carbon signal at δ12.9 matched well with that of (*E*)-3-methyl-1,3-pentadiene at 13.6 [3].

Sterebin F (**2**), $[\alpha]_D$ +40.3°, exhibited an identical mass ion peak at *m/z* 320 $[M-H_2O]^+$ to that of sterebin E (**1**), indicating the molecular formula to be $C_{20}H_{34}O_4$. Like sterebin E (**1**), sterebin F (**2**) showed the UV absorption maximum at 238 nm (log ε 4.03), exhibiting the presence of a conjugated diene system. The ^1H NMR spectrum of sterebin F (**2**) displayed signals due to four quaternary methyls (δ 1.01, 1.03, 1.18 and 1.20 (3H, each *s*)) and two carbinyl hydrogens of the decalin system at δ3.46 (1H, *d*, *J* = 10 Hz) and 3.71 (1H, *t*, *J* = 10 Hz). The chemical shifts and splitting patterns of these signals were essentially the same as those of sterebin E (**1**). On the other hand, the signals assigned to the C-12, C-14 and C-15 hydrogens of the C-9 side chain at δ6.54 (1H, *d*, *J* = 16 Hz), 5.58 (1H, *t*, *J* = 7 Hz), 4.14 and 4.37 (1H, each *dd*, *J* = 12 and 7 Hz) were appreciably shifted as compared with those of sterebin E (**1**), suggesting that sterebin F (**2**) is a geometrical isomer of sterebin E (**1**) at C-13. The shifts of the C-16 methyl and C-12 olefinic carbon signals from sterebin F (**2**) to sterebin E (**1**) ($\Delta\delta$ + 7.9 and −9.6 ppm, respectively) also substantiated the change of the configuration at C-13 of sterebin F (**2**). This observation established that sterebin F (**2**) has the 5-hydroxy-3-methyl-1*E*,3*Z*-pentadienyl side chain at C-9.

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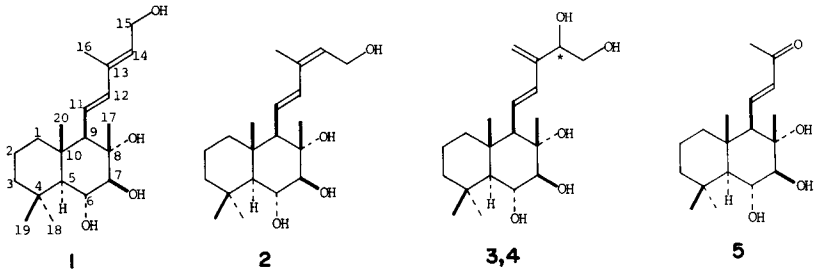

1 2 3,4 5

Sterebin G (**3**), $[\alpha]_D$ −5.9°, was found to have the molecular formula $C_{20}H_{34}O_5$ (*m/z*: 336 $[M-H_2O]^+$). Its UV and IR spectra (λ_{max}232 nm (log ε 3.74) and ν_{max}3400 cm^{-1}) indicated that it bears a 1,3-diene system and hydroxyls like the other two sterebins. The ^1H NMR signals assigned to the hydrogens of the A- and B-rings of sterebin G (**3**) were identical to those of sterebins E (**1**) and F (**2**). While the ^1H NMR signals appeared at δ5.21, 5.28 (1H, each *br s*), 5.82 (1H, *dd*, *J* = 16 and 10 Hz) and 6.15 (1H, *d*, *J* = 16 Hz) which pointed to the presence of a vinylidene and a *trans*-disubstituted double bond in the molecule. The presence of a 1,2-diol system in the C-9 side chain was indicated from the ^1H NMR spectrum which showed signals for oxymethylene and oxymethine hydrogens at δ3.62 (1H, *dd*, *J* = 11 and 7 Hz), 3.77 (1H, *dd*, *J* = 11 and 1.5 Hz) and 4.58 (1H, *dd*, *J* = 7 and 1.5 Hz). The chemical shift of the latter signal revealed that this moiety was next to the vinylidene group. Accordingly, it became clear that sterebin G (**3**) has the 4,5-dihydroxy-3-methylene-1-pentenyl group at C-9.

Table 1. ^{13}C NMR data of **1**, **2** and **5** (pyridine-d_5)

C	1*	2*	5†
1	41.5	41.4	41.2
2	18.8	18.7	18.5
3	44.2	44.1	43.8
4	34.4	34.4	34.2
5	57.8	57.8	57.4
6	72.0	72.1	71.8
7	85.6	85.6	85.3
8	75.5	75.5	75.3
9	65.2	65.4	64.2
10	38.3	38.2	38.0
11	132.0	132.2	145.0
12	139.8	130.2	136.0
13	134.8	133.5	197.6
14	125.4	128.2	
15	58.9	58.1	
16	12.9	20.8	27.1
17	20.3	20.3	20.1
18	37.0	37.0	36.9
19	22.6	22.6	22.4
20	17.7	17.7	17.4

*Run at 125 MHz.
†Run at 25 MHz.

Sterebin H (**4**), $[\alpha]_D$ −6.7°, had the molecular formula $C_{20}H_{34}O_5$ (*m/z*: 336 $[M-H_2O]^+$). It showed the same fragment ion peaks as well as UV and IR spectra as those of sterebin G (**3**), demonstrating that sterebin H (**4**) is a stereoisomer of sterebin G (**3**). Further, a detailed comparative study of the ^1H NMR spectra of sterebins H (**4**) and G (**3**) revealed that the difference was observed only in the signals due to the C-9 side chains, indicating that sterebin H (**4**) is an epimeric compound of sterebin G (**3**) at C-14.

Ozonolysis, followed by the sodium borohydride reduction of the above four sterebins afforded the same tetraol which was also obtained by the same reactions of sterebin A (**5**). This observation demonstrated that sterebins E (**1**)–H (**4**) are the *normal* labdane-type diterpenoids.

EXPERIMENTAL

Isolation of sterebins E, F, G and H. The EtOAc soluble fraction (200 g) of the methanolic extract (1.5 kg) obtained from the *Stevia rebaudiana* leaves (4.5 kg) was chromatographed over silica gel (1.5 kg). The column was eluted with *n*-hexane–EtOAc mixtures in order of increasing polarity. Rechromatography of the EtOAc eluates (20 g) over silica gel (600 g) using CH_2Cl_2–MeOH as a solvent gave the fractions which were further separated by HPLC [column: TSK LS-410 ODS SIL (2.54 cm i.d. × 30 cm); solvent: CH_3CN–H_2O (4:6)] to yield sterebin E (**1**), F (**2**), G(**3**) and H (**4**) (70, 150, 10 and 10 mg, respectively).

Sterebin E (**1**), colourless powder, $[\alpha]_D$ +29.9° (*c* 0.17, MeOH), EIMS (direct inlet) 70 eV, *m/z*: 320 $[M-H_2O]^+$, 308, 277; UV λ_{max}^{MeOH} nm (log ε): 238 (3.87); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380; ^1H NMR (500 MHz, $CDCl_3$): δ1.01, 1.03, 1.18, 1.22, 1.83 (3H, each *s*), 1.89 (1H, *d*, *J* = 10 Hz), 3.45 (1H, *d*, *J* = 10 Hz), 3.70 (1H, *t*, *J* = 10 Hz), 4.29 (2H, *d*, *J* = 7 Hz), 5.66 (1H, *t*, *J* = 7 Hz), 5.67 (1H, *dd*, *J* = 16 and 10 Hz), 6.20 (1H, *d*, *J* = 16 Hz).

Sterebin F (**2**), colourless powder, $[\alpha]_D$ +40.3° (*c* 0.16, MeOH); EIMS (direct inlet) 70 eV, *m/z*: 320 $[M-H_2O]^+$, 308, 277; UV λ_{max}^{MeOH} nm (log ε): 238 (4.03); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300; ^1H NMR (500 MHz, $CDCl_3$): δ1.01, 1.03, 1.18, 1.20, 1.90 (3H, each *s*), 1.92 (1H, *d*, *J* = 10 Hz), 3.46 (1H, *d*, *J* = 10 Hz), 3.71 (1H, *t*, *J* = 10 Hz), 4.14 (1H, *dd*, *J* = 12 and 6 Hz), 4.37 (1H, *dd*, *J* = 12 and 8 Hz), 5.58 (1H, *t*, *J* = 7 Hz), 5.73 (1H, *dd*, *J* = 16 and 10 Hz), 6.54 (1H, *d*, *J* = 16 Hz).

Sterebin G (**3**), colourless powder, $[\alpha]_D$ −5.9° (*c* 0.50, MeOH), EIMS (direct inlet) 70 eV, *m/z*: 336 $[M-H_2O]^+$, 318, 300, 275, 109; UV λ_{max}^{MeOH} nm (log ε): 232 (3.74); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400; ^1H NMR (500 MHz, $CDCl_3$): δ1.00, 1.03, 1.17, 1.22 (3H, each *s*), 1.84 (1H, *d*, *J* = 10 Hz), 3.44 (1H, *d*, *J* = 10 Hz), 3.62 (1H, *dd*, *J* = 11 and 7 Hz), 3.73 (1H, *t*, *J* = 10 Hz), 3.77 (1H, *dd*, *J* = 11 and 1.5 Hz), 4.58 (1H, *dd*, *J* = 7 and 1.5 Hz), 5.21 (1H, *br s*), 5.28 (1H, *br s*), 5.82 (1H, *dd*, *J* = 16 and 10 Hz), 6.15 (1H, *d*, *J* = 16 Hz).

Sterebin H (**4**), colourless powder, $[\alpha]_D$ −6.7° (*c* 0.50, MeOH), EIMS (direct inlet) 70 eV, *m/z*: 336 $[M-H_2O]^+$, 218, 300, 275,

109; UV λ_{max}^{MeOH} nm (log ε): 232 (3.65); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300; ^1H NMR (500 MHz, $CDCl_3$): δ1.00, 1.03, 1.18, 1.21 (3H, each *s*), 1.86 (1H, *d*, *J* = 10 Hz), 3.44 (1H, *d*, *J* = 10 Hz), 3.57 (1H, *dd*, *J* = 12 and 7 Hz), 3.73 (1H, *t*, *J* = 10 Hz), 3.76 (1H, *dd*, *J* = 12 and 3 Hz), 4.61 (1H, *dd*, *J* = 7 and 3 Hz), 5.22 (1H, *br s*), 5.33 (1H, *br s*), 5.78 (1H, *dd*, *J* = 16 and 10 Hz), 6.17 (1H, *d*, *J* = 16 Hz).

Acetylation of sterebin E (**1**). A mixture of sterebin E (**1**) (10 mg), Ac_2O (2 ml) and pyridine (1 ml) was kept at room temp. for 24 hr. The reaction mixture was freed from organic solvents *in vacuo* and purified by silica gel chromatography to yield sterebin E diacetate as amorphous powder (7 mg), EIMS (direct inlet) 70 eV, *m/z*: 422 $[M]^+$; UV λ_{max}^{MeOH} nm (log ε): 235 (3.94); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1725, 1240; ^1H NMR (100 MHz, $CDCl_3$): δ1.01, 1.02, 1.17, 1.24, 1.84, (3H, each *s*), 1.96 (1H, *d*, *J* = 10 Hz), 2.07, 2.18 (3H, each *s*), 3.83 (1H, *dd*, *J* = 8 and 10 Hz), 4.70 (2H, *d*, *J* = 7 Hz), 4.83 (1H, *d*, *J* = 10 Hz), 5.58 (1H, *t*, *J* = 7 Hz), 5.67 (1H, *dd*, *J* = 16 and 10 Hz), 6.20 (1H, *d*, *J* = 16 Hz).

Ozonolysis followed by $NaBH_4$ *reduction of sterebins E* (**1**)*–H* (**4**) *and A* (**5**). Sterebins E (**1**)–H (**4**) or A (**5**) (5 mg each) in MeOH (5 ml) was ozonized at −60° for 10 min and then the reaction mixture was reduced with $NaBH_4$ to give the residue (5 mg each) which was chromatographed over silica gel to yield the tetraol (3 mg each) as colourless powder, $[\alpha]_D$ +4.7° (*c* 0.02, MeOH), ^1H NMR (500 MHz, $CDCl_3$): δ0.86, 1.00, 1.16, 1.33 (3H, each *s*), 3.44 (1H, *d*, *J* = 10 Hz) 3.60 (1H, *t*, *J* = 11 Hz), 3.94 (1H, *dd*, *J* = 11 and 4 Hz), 3.95 (1H, *t*, *J* = 10 Hz).

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DITERPENEBUTENOLIDES IN *SOLIDAGO GIGANTEA*

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Key Word Index—*Solidago gigantea*; Asteraceae; diterpenebutenolides; 6-deoxy-solidagolactone IV-18,19-olide; 2β-*O*-β-D-glucopyranosyl-6-deoxy-solidagolactone IV-18,19-olide.

Abstract—6-Deoxy-solidagolactone IV-18,19-olide and 2β-*O*-βD-glucopyranosyl-6-deoxy-solidagolactone IV-18,19-olide, two new diterpenebutenolides of the *cis*-clerodane type, were isolated from *Solidago gigantea*. Lactones of this type were not detected in *S. virgaurea* and *S. canadensis*.

INTRODUCTION

2β-Hydroxy-6-deoxy-solidagolactone IV-18,19-olide (**1**, identical with L in [1]) was reported as a possible artifact of acid hydrolysis of the crude extract of *Solidago gigantea* Ait. [1]. Due to its butenolide ring, **1** is selectively detectable by spraying with Kedde reagent.

As we have shown that the compound is stable to heat and acid treatment, it must be a genuine constituent of *S. gigantea*. To establish if compounds of this type could serve as additional markers for the differentiation of the medicinally used *Solidago* species a TLC comparison of *S. virgaurea* L., *S. canadensis* L. and *S. gigantea* Ait. was performed in addition to a preparative scale investigation of *S. gigantea*.

RESULTS AND DISCUSSION

The crude chloroform–methanol extract of the freeze-dried aerial parts of diploid *S. gigantea* plants, collected at the full flowering stage, was purified and concentrated by column chromatography. In the resulting fractions 12 Kedde-positive spots were detected by TLC, one of them corresponding to **1**. Furthermore, two main components were isolated, and their structures determined by means of EIMS, FABMS, ^1H and ^{13}C NMR spectroscopy as 6-deoxy-solidagolactone IV-18,19-olide (**2**) and 2β-*O*-β-D-glucopyranosyl-6-deoxy-solidagolactone IV-18,19-olide (**3**).

On TLC screening of several different samples of *S. virgaurea* and *S. canadensis* of cytologically defined