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STEREBINS A, B, C AND D, BISNORDITERPENOIDS OF STEVIA REBAUDIANA LEAVES

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Abstract —— Structures of sterebins A, B, C and D, four new bisnorditerpenoids isolated from the leaves of Stevia rebaudiana, have been elucidated as shown in formulas 1, 2, 3, and 4, respectively, on the basis of spectral and chemical evidence.

The extracts of Stevia rebaudiana Bertoni (Compositae) and crude stevioside, the most abundant ent-kaurene glycoside constituent of this plant, have been mainly used in Japan as a sweetening agent, a taste modifier and a sugar substitute. Medicinal uses of this plant was originated in Paraguay in the form of aqueous decoctions of the leaves as a contraceptive agent and for the treatment of hyperglycemia.[1]

In the course of our search for active principles in plants used for antihyperglycemic purpose, we have taken up Stevia rebaudiana and isolated four novel bisnorditerpenoids which were designated as sterebins A, B, C and D. In this paper, we report the structure elucidation of these sterebins by means of spectroscopic data as well as the chemical reactions and transformation.

Sterebin A (1), m.p. 157-158°, $[\alpha]_D$ +39.6°, was analysed as $C_{18}H_{30}O_4$ supported by its EIMS (m/z: 292 (M^+-H_2O)) and ^{13}C NMR spectrum which showed signals for eighteen carbons (CH_3- x 5, $-CH_2$- x 3, >CH- x 2, >C< x 2, >CH-O x 2, →C-O x 1, -CH=CH- x 1 and >C=O x 1). The UV absorption at 228 nm (log ε 4.08) and the IR absorption bands at 3450 and 1675 cm^{-1} suggested the presence of an enone system and hydroxyl groups in the molecule, and the presence of the former functionality was further indicated from its ^1H NMR signals at δ 2.27 (3H s), 6.16 (1H d, J 16 Hz) and 6.75 (1H dd, J 16 and 10 Hz) assigned for a trans-α,β-unsaturated methyl ketone group. In addition to this, the ^1H NMR spectrum of sterebin A also showed signals for four tertiary methyls at δ 1.02, 1.05, 1.17 and 1.25 (3H s, each) and two mutually coupled carbinyl hydrogens at δ 3.38 (1H d, J 10 Hz) and 3.69 (1H t, J 10 Hz), the coupling constants of the latter two signals of which suggested the presence of 1,2-trans-diequatorial diol system in a cyclohexane ring in sterebin A. Supporting evidence for the presence of this diol system was disclosed from its ^{13}C NMR signals at δ 71.8 (d), 75.3 (s) and 85.3 (d) assigned to one tertiary hydroxyl bearing carbon and two secondary hydroxyl bearing carbons.

From the above data sterebin A looks a close relative to austroinulin (5),[2] an ent-labdane-type diterpenoid isolated from the same source and, therefore, a comparative study of the ^{13}C NMR data of sterebin A to those of austroinulin (5)[3] was made. As a result it was found that the carbon signal assignable to C-9 was displaced downfield by 4.7 ppm and the resonances due to C-8 and C-10 were shifted slightly upfield in the spectrum of sterebin A, while other carbon resonances (C-1 – C-7 and C-15 – C-18) were virtually identical in both the spectra. Further, the resonance positions of ^1H NMR signals at δ 0.85, 0.97 (3H s, each) and 1.15 (6H s) due to the four tertiary methyls (C-15 – C-18) of dihydrosterebin A (6) obtained from sterebin A by catalytic hydrogenation using 5% palladized charcoal were found superimposable with those of austroinulin (5). These observations confirmed the site for the trans-α,β-unsaturated methyl ketone group at C-9 very much like that of austroinulin (5), and indicated that both have the same relative configurations.

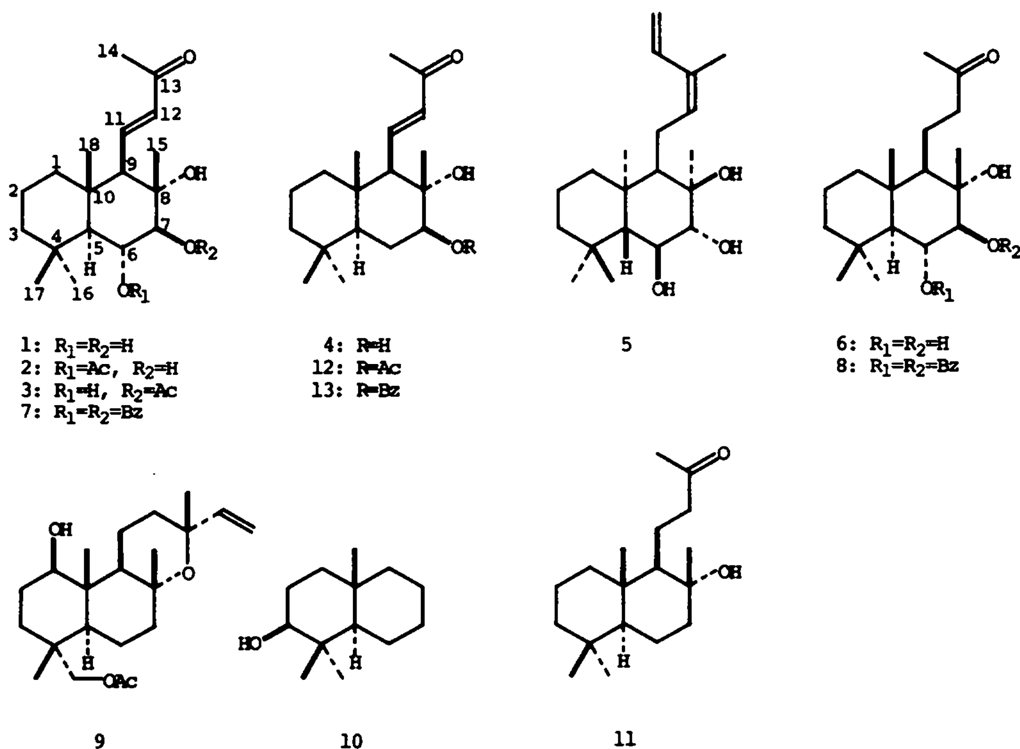
Benzoylation of sterebin A with benzoyl chloride in pyridine in the presence of p-dimethylaminopyridine afforded the dibenzoate (7) which was converted to the corresponding saturated-ketone (8) by hydrogenation over 5% palladized charcoal catalyst. The ketone (8) showed a typical exciton-split CD curve ($[\theta]_{238}$ -51800, $[\theta]_{223}$ +43800) associated with the 1,2-dibenzoate

system in the molecule which disclosed the absolute stereochemistry of C-6 and C-7 as R and S, respectively.[4]

Sterebin B (2), $[\alpha]_D$ +15.9°, was found to have molecular formula $C_{20}H_{32}O_5$ (m/z: 353 (M^++H)). In its ^1H NMR spectrum, it showed signals for five tertiary methyls at δ 0.94, 1.03, 1.15, 1.27 and 2.25 (3H s, each) and two olefinic hydrogens at δ 6.19 (1H d, J 16 Hz) and 6.82 (1H dd, J 16 and 10 Hz). These findings, along with the UV absorption maximum at 228 nm (log ε 4.02) and the IR absorption band at 1670 cm^{-1}, indicated the presence of the same conjugated enone group like sterebin A. The presence of an acetoxyl group in sterebin B was suggested from the IR absorption bands at 1735 and 1230 cm^{-1}, and this was confirmed from the ^1H and ^{13}C NMR signals at δ 2.09 (3H s), and 171.5 (s) and 21.8 (q), respectively. Sterebin B was hydrolysed with 1N potassium hydroxide to afford sterebin A, a fact indicating sterebin B to be a monoacetate of sterebin A. From a comparison of the carbinyl hydrogen signals in the ^1H NMR spectrum of sterebin B to those of sterebin A, it was found that the carbinyl hydrogen signal of sterebin B at δ 5.22 (1H dd, J 11 and 10 Hz) was shifted downfield by 1.53 ppm than that of sterebin A at δ 3.69 (1H t, J 10 Hz). Further, on going from sterebin A to sterebin B, the significant downfield shift of the ^{13}C NMR signal assignable to C-6 and the upfield shift of that due to C-7 was observed, while the rest of the ^{13}C NMR signals were virtually identical in both the spectra. The above fact logically placed the acetoxyl group of sterebin B at C-6.

Sterebin C (3), $[\alpha]_D$ +34.8°, had the same molecular formula $C_{20}H_{32}O_5$ (m/z: 334 (M^+-H_2O)) as sterebin B, and like sterebin B, it showed the UV absorption maximum at 228 nm (log ε 3.97) and the IR absorption bands at 3400, 1725, 1680 and 1230 cm^{-1} assigned for hydroxyls, enone and ester functionalities in the molecule. The ^1H NMR spectrum of sterebin C was found to match well with that of sterebin B, except the signals assigned to carbinyl hydrogens at C-6 and C-7. These signals in the case of sterebin C appeared at δ 3.85 (1H t, J 10 Hz) and 4.80 (1H d, J 10 Hz), whereas in sterebin B, they came at δ 5.22 (1H dd, J 11 and 10 Hz) and 3.51 (1H d, J 10 Hz), respectively. These facts clearly explained that sterebin C is the isomeric compound of sterebin B which bears the acetoxyl function at C-7 instead of C-6.

The fourth bisnorditerpenoid of this series designated as sterebin D (4), $[\alpha]_D$ +22.8°, had the molecular formula $C_{18}H_{30}O_3$ (m/z: 276 (M^+-H_2O)). The spectral data (UV and IR) spoke it to be a



congener of sterebins A, B and C. Unlike other sterebins, sterebin D showed only one carbinyl hydrogen signal at δ 3.58 (1H dd, J 11 and 5 Hz) whose coupling pattern indicated that it bears an equatorial-oriented hydroxyl group either at C-1, C-3 or C-7.

In order to clarify the position of the hydroxyl group in sterebin D, the ^{13}C NMR spectrum was analysed and it was found that the carbon signals assignable to C-10 and C-18 (δ 38.8 and 16.1) deviated to a great extent from those of jhanidiol 18-monoacetate (9) (δ 42.9 and 11.7),[5] indicating that sterebin D does not bear a C-1β-hydroxyl group. Furthermore, the presence of a C-3β-hydroxyl group in sterebin D was excluded from the fact that the resonance positions of the signals due to C-4, C-16 and C-17 (δ 33.3, 33.4 and 21.6) were quite different from those of the corresponding signals in trimethyl-trans-decanol (10) (δ 38.8, 27.4 and 15.2).[6] Hence the hydroxyl group was placed at C-7 which was verified from the evidence that the resonance values of the observed signals were well consistent with the calculated ones, obtained from the shieldings of bisnorlabdane derivative (11) as a reference[7] and the additive substituent parameters for the additional hydroxyl at C-7.[8]

The NOE experiments carried out in the ^{1}H NMR spectrum of sterebin D monoacetate (12) showed significant NOE for the H-12 signal by irradiation of H-9, indicating the spatially close arrangement of these two hydrogens. Further, NOE's observed between H-11 and H-15, and H-11 and H-18 confirmed that H-11 was close to H-15 and H-18. The CD spectrum of the monobenzoate (13) gave the split-type Cotton effects at 237 nm ([θ] +21300) and 217 nm ([θ] -12200). These signs of the Cotton effects indicated that the chromophores of the benzoyl and enone groups were twist in a clockwise manner.[4] Thus, sterebin D was found to be the same normal-labdane type bisnorditerpenoid as like other sterebins.

Only a few labdane-type bisnorditerpenoids were isolated so far,[7,9] and to the best of our knowledge, the series of sterebins are the first reported compounds which bear the highly-oxidized B-rings. Testing of the physiological activity of the sterebins is now in progress.

EXPERIMENTAL

Optical rotations were measured on a JASCO DIP-360 polarimeter and CD spectra on a JASCO A-3 instrument. IR spectra were recorded on a SHIMADZU IR-27G spectrometer, UV spectra on a SHIMADZU UV-202 spectrophotometer, ^{1}H and ^{13}C NMR spectra on a JEOL FX-100 spectrometer (TMS as internal standard). Low resolution EIMS were determined with a HITACHI M-52 spectrometer, and Field-Disorption MS with a JEOL-01-SG-2 spectrometer.

Isolation of sterebins A, B, C and D 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 (500 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. x 30 cm); solvent: CH_3CN-H_2O (35:65)) to yield sterebins A (1) (60 mg), B (2) (40 mg), C (3) (15 mg) and D (4) (20 mg).

sterebin A (1) as colorless needles from acetone, m.p. 157-158°, $[\alpha]_D$ +39.6° (c 0.63, MeOH); EIMS m/z: 292 (M^+-H_2O), 249, 127, 109; UV (MeOH) λ_{max} 228 nm (log ε 4.08); IR (KBr) ν_{max} 3450 (hydroxyl), 1675 (enone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.02, 1.05, 1.17, 1.25 (3H s, each), 2.01 (1H d, J 10 Hz), 2.27 (3H s), 3.38 (1H d, J 10 Hz), 3.69 (1H t, J 10 Hz), 6.16 (1H d, J 16 Hz), 6.75 (1H dd, J 16, 10 Hz); ^{13}C NMR (C_5D_5N) δ: 197.6 (s, C-13), 145.0 (d, C-11), 136.0 (d, C-12), 85.3 (d, C-7), 75.3 (s, C-8), 71.8 (d, C-6), 64.2 (d, C-9), 57.4 (d, C-5), 43.8 (t, C-3), 41.2 (t, C-1), 38.0 (s, C-10), 36.9 (q, C-16), 34.2 (s, C-4), 27.1 (q, C-14), 22.4 (q, C-17), 20.1 (q, C-15), 18.5 (t, C-2), 17.4 (q, C-18).

sterebin B (2) as colorless powder, $[\alpha]_D$ +15.9° (c 0.11, MeOH); FD-MS m/z: 353 (M^++H); EIMS m/z: 292 (M^+-AcOH), 274, 249, 127, 109, 43; UV (MeOH) λ_{max} 228 nm (log ε 4.02); IR (KBr) ν_{max} 3450 (hydroxyl), 1735, 1230 (acetoxyl), 1670 (enone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.94, 1.03, 1.15, 1.27 (3H s, each), 1.44 (1H d, J 11 Hz), 2.09, 2.25 (3H s, each), 3.51 (1H d, J 10 Hz), 5.22 (1H dd, J 11, 10 Hz), 6.19 (1H d, J 16 Hz), 6.82 (1H dd, J 16, 10 Hz); ^{13}C NMR ($CDCl_3$) δ: 197.9 (s, C-13), 171.5 (s, Ac), 142.7 (d, C-11), 135.8 (d, C-12), 82.8 (d, C-7), 75.3 (s, C-8), 72.8 (d, C-6), 62.6 (d, C-9), 56.4 (d, C-5), 43.3 (t, C-3), 40.8 (t, C-1), 38.2 (s, C-10), 35.9 (q, C-16), 33.5 (s, C-4), 27.7 (q, C-14), 22.1 (q, C-17), 21.8 (q, Ac), 19.7 (q, C-15), 17.8 (t, C-2), 17.1 (q, C-18).

sterebin C (3) as colorless powder, $[\alpha]_D$ +34.8° (c 0.07, MeOH); EIMS m/z: 334 (M^+-H_2O), 292, 249, 127, 109, 79, 42; UV (MeOH) λ_{max} 228 nm (log ε 3.97); IR (KBr) ν_{max} 3400 (hydroxyl), 1725, 1230 (acetoxyl), 1680 (enone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.04, 1.09, 1.17, 1.28, 2.11, 2.28 (3H s, each), 3.85 (1H t, J 10 Hz), 4.80 (1H d, J 10 Hz), 6.21 (1H d, J 16 Hz), 6.80 (1H dd, J 16, 10 Hz).

sterebin D (4) as colorless powder, $[\alpha]_D$ +22.8° (c 0.05, MeOH); EIMS m/z: 276 (M^+-H_2O), 251, 233, 109, 42; UV (MeOH) λ_{max} 228 nm (log ε 3.87); IR (KBr) ν_{max} 3450 (hydroxyl), 1675 (enone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 0.84, 0.91, 0.99, 1.24, 2.29 (3H s, each), 3.58 (1H dd, J 11, 5 Hz), 6.19 (1H d, J 16 Hz), 6.86 (1H dd, J 16, 10 Hz); ^{13}C NMR ($CDCl_3$) δ: 197.5 (s, C-13), 143.0 (d, C-11), 135.6 (d,

C-12), 79.6 (d, C-7), 76.0 (s, C-8), 64.0 (d, C-9), 53.6 (d, C-5), 41.5 (t, C-3), 40.6 (t, C-1), 38.8 (s, C-10), 33.4 (q, C-16), 33.3 (s, C-4), 27.8 (q, C-14), 27.7 (t, C-6), 21.6 (q, C-17), 18.6 (q, C-15), 18.3 (t, C-2), 16.1 (q, C-18).

Hydrogenation of sterebin A Sterebin A (1) (30 mg) in MeOH (10 ml) was stirred at room temperature with 5% Pd-C (10 mg) under H_2 for 15 hrs. The reaction product (30 mg) was purified by silica gel chromatography to yield dihydrosterebin A (6) (20 mg) as colorless powder, EIMS m/z: 312 (M^+) 294, 178, 42; ^1H NMR ($CDCl_3$) δ: 0.85, 0.97 (3H s, each), 1.15 (6H s), 2.12 (3H s), 3.38 (1H d, J 10 Hz), 3.62 (1H t, J 10 Hz); ^{13}C NMR ($CDCl_3$) δ: 210.8 (s, C-13), 85.4 (d, C-7), 76.6 (s, C-8), 71.6 (d, C-6), 57.6 (d, C-9), 57.3 (d, C-5), 45.7 (t, C-12), 43.4 (t, C-3), 39.9 (t, C-1), 39.3 (s, C-10), 36.2 (q, C-16), 33.8 (s, C-4), 30.0 (q, C-14), 22.0 (q, C-17), 19.4 (q, C-15), 18.3 (t, C-2, C-11), 16.5 (q, C-18).

Benzoylation of sterebin A Benzoyl chloride (150 mg) was added to a solution of sterebin A (1) (12 mg) and p-dimethylamonopyridine (5 mg) in dry pyridine (15 drops). The reaction mixture was stirred at room temperature for 24 hrs, poured into water, and extracted with EtOAc. The organic layer was washed with water, dried over Na_2SO_4 and concentrated under the reduced pressure. The residue was purified by silica gel chromatography to give dibenzoylsterebin A (7) (10 mg) as colorless powder, EIMS m/z: 518 (M^+), 274, 249, 231; ^1H NMR ($CDCl_3$) δ: 0.97 (6H s), 1.25, 1.43 (3H s, each), 1.69 (1H d, J 11 Hz), 2.27 (3H s), 2.28 (1H d, J 10 Hz), 5.33 (1H d, J 10 Hz), 5.85 (1H dd, J 11, 10 Hz), 6.28 (1H d, J 16 Hz), 6.85 (1H dd, J 16, 10 Hz).

Hydrogenation of dibenzoylsterebin A Dibenzoylsterebin A (7) (5 mg) was hydrogenated by the same manner as described above to afford the saturated ketone (8) (3 mg) as colorless powder, EIMS m/z: 502 (M^+-H_2O), 398, 380; CD (MeOH) $[\theta]_{238}$ -51800, $[\theta]_{223}$ +43800; ^1H NMR ($CDCl_3$) δ: 0.95, 1.06, 1.25, 1.35, 2.13 (3H s, each), 2.75 (2H m), 5.25 (1H d, J 10 Hz), 5.72 (1H dd, J 11, 10 Hz), 7.04-7.84 (10H m).

Alkaline hydrolysis of sterebin B Methanol-water (1:1) (1 ml) solution of sterebin B (2) (5 mg) was refluxed with 1N KOH (5 drops) for 24 hrs. The reaction mixture was diluted with water (3 ml) and extracted with EtOAc to afford sterebin A (1) (3 mg) as colorless needles from acetone, m.p. 154-155°, which was identified by mmp, $[\alpha]_D$ +38.3° (c 0.05, MeOH), and ^1H NMR ($CDCl_3$) δ: 1.03, 1.06, 1.17, 1.25 (3H s, each), 2.01 (1H d, J 10 Hz), 2.27 (3H s), 3.41 (1H d, J 10 Hz), 3.71 (1H t, J 10 Hz), 6.17 (1H d, J 16 Hz), 6.77 (1H dd, J 16, 10 Hz).

Acetylation of sterebin D To a solution of sterebin D (4) (5 mg) in pyridine (0.5 ml), acetic anhydride (1 ml) was added. The reaction mixture was kept at room temperature for 24 hrs, evaporated under reduced pressure and chromatographed over silica gel to give acetylsterebin D (12) as colorless powder, EIMS m/z: 294 (M^+-AcOH); ^1H NMR ($CDCl_3$) δ: 0.83, 0.90, 1.00, 1.25 (3H s, each), 1.98 (1H d, J 10 Hz), 2.10 (3H s), 2.26 (3H s), 4.76 (1H dd, J 12, 5 Hz), 6.18 (1H d, J 16 Hz), 6.79 (1H dd, J 16, 10 Hz).

Benzoylation of sterebin D Benzoylation of sterebin D (4) (5 mg) was performed as the same manner as mentioned above to yield monobenzoylsterebin D (13) (5 mg) as colorless powder, EIMS m/z: 398 (M^+); CD (MeOH) $[\theta]_{237}$ +21300, $[\theta]_{217}$ -12200; UV (MeOH) λ_{max} 229 nm (log ε 4.28); ^1H NMR ($CDCl_3$) δ: 0.84, 0.92, 1.05, 1.24 (3H s, each), 2.07 (1H d, J 10 Hz), 2.25 (3H s), 5.01 (1H dd, J 12, 5 Hz), 6.19 (1H d, J 16 Hz), 6.82 (1H dd, J 16, 10 Hz), 7.29-8.12 (5H m).

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