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LABDANE-TYPE DITERPENES FROM *STEVIA REBAUDIANA*

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Key Word Index—*Stevia rebaudiana*; Compositae; ^{13}C NMR; labdane-type diterpenes; jhanol; austroinulin; 6-*O*-acetyl-austroinulin.

Leaves of *Stevia rebaudiana* (Eupatoriae, Compositae), a wild herb of Paraguay, have attracted special attention as a new source of natural sweetener. Besides the major sweet principles, stevioside [1, 2] (kaurene-type diterpene glucoside), another major sweet glucoside, named rebaudioside-A [3] and several minor sweet glycosides, rebaudioside-C [4] (=dulcoside-B [5]), -D and -E [6] and dulcoside-A [5] were recently isolated from the leaves. Since a number of cytotoxic and anti-leukemic substances have been isolated from plants of Compositae, especially Eupatoriae, we have conducted a chemical investigation of constituents of the leaves, other than sweet glycosides, to substantiate the safety of the crude extract as a food-stuff.

Column chromatography of the ether-soluble fraction of the methanolic extract of the dried leaves afforded three crystalline compounds, **1**, **2** and **3**, along with β-amyrin acetate, esters of lupeol, and a mixture of sitosterol and stigmasterol.

The ^{1}H NMR spectrum, optical rotation and mp of **1** strongly suggested that this compound was identical with the labdane-type diterpene alcohol, jhanol previously isolated from *Eupatorium jhanii* [7]. The identification was confirmed by comparison of the ^{13}C NMR spectrum of **1** with that of jhanol acetate **4** reported by González *et al.* [7], and consideration of the acetylation shift of the carbon signals around 18-CH_2OH (Table 1) [8].

Table 1. ^{13}C NMR chemical shifts (25.15 MHz, in $CDCl_3$)

Carbon No.	1	4*	2	3
1	38.6	38.6	39.9	39.9
2	17.3	17.2	18.2	18.1
3	35.6	35.9	43.4	43.4
4	37.0	36.6	33.8	33.4
5	49.8	50.6	57.3	56.5
6	19.8	20.0	71.7	73.9
7	43.0	43.0	85.0	83.6
8	74.9	74.9	77.1	76.9
9	55.6	56.1	59.6	59.2
10	37.7	37.0	39.1	39.4
11	15.4	15.4	22.8	22.8
12	35.4	35.9	133.7	133.7
13	73.3	73.3	131.0	130.9
14	147.9	148.0	133.4	133.4
15	110.3	110.2	113.7	113.7
16	28.7	28.5	19.9	19.9
17	25.5	25.4	19.4	19.5
18	72.1	73.0	36.4	36.0
19	18.0	17.8	22.1	22.2
20	15.8	15.8	16.9	16.7

* Cited from ref. [7]. The value for C-16, δ 78.52 (may be a misprint) was revised.

Compound **2**, mp 74–77°, exhibited five methyl signals at δ 1.80, 1.19, 1.18, 1.00 and 0.88 (3H each, all *s*) in its ^{1}H NMR spectrum. The UV absorption maximum at 237 nm (log ε 4.06) in EtOH and ^{1}H NMR signals at δ 5.14 (1H, *d*(*br*), J = 10 Hz), 5.23 (1H, *d*(*br*), J = 18 Hz), 5.48 (1H, *t*, J = 7 Hz), 6.91 (1H, *dd*, J = 10 and 18 Hz) and 1.80 (*vide supra*, an allylic methyl) indicated the presence of a *Z*-type conjugated diene system, $-CH_2-CH=C(Me)-CH=CH_2$, which occasionally appears in labdane-type diterpenes [9]. The ^{13}C NMR signals of **2** at δ 133.7 (*d*), 133.4 (*d*), 131.0 (*s*) and 113.7 (*t*) also supported this partial structure [10]. The ^{13}C NMR signals of **2** at δ 71.7 (*d*), 85.0 (*d*) and 77.1 (*s*) suggested the presence of two secondary and one tertiary hydroxyl groups and ^{1}H NMR signals of this compound at δ 3.65 (1H, *t*, J = 10 Hz) and 3.42 (1H, *d*, J = 10 Hz) revealed a 1,2-*trans*-diequatorial-diol system, $>CH-CH(OH)-CH(OH)-C\leqslant$. These facts strongly suggested that the identity of **2** corresponded to austroinulin isolated from *Austroeupatorium inulaefolium*, whose structure was proposed by Bohlmann *et al.* [11], through the configuration of C-8 and the absolute stereo-structure seem to be unestablished. Although austroinulin was reported to be an oil, comparison of the optical rotation and the ^{1}H NMR spectrum of **2** with those reported by Bohlmann *et al.* [11] supported the identity, and the ^{13}C NMR signal assignments of **2** were consistent with this formulation (Table 1).

A new compound, **3**, mp 173–174°, $C_{22}H_{36}O_4$, showed a UV absorption maximum at 237 nm (log ε 4.21) in EtOH, four quaternary methyl proton signals at δ 0.88, 0.97, 1.01, and 1.22 (3H each, all *s*) and proton signals due to the same conjugated diene system as that of **2** at δ 1.79 (3H, *s*), 5.11 (1H, *d*, J = 11 Hz), 5.19 (1H, *d*, J = 17 Hz), 5.47 (1H, *t*, J = 7 Hz) and 6.89 (1H, *dd*, J = 11 and 17 Hz). The presence of an acetoxyl function in **3** was demonstrated by IR absorption bands (in $CHCl_3$) at 1750 and 1260 cm^{-1}, a proton signal at δ 2.13 (3H, *s*), and ^{13}C NMR resonances at δ 171.0 and 21.8. Alkaline saponification of **3** afforded a crystalline compound which was identical with **2**, proving that **3** must be a monoacetate of **2**. On going from **2** to **3**, the carbon signal of C-6 was displaced downfield by 2.2 ppm and the signals due to

C-5 and C-7 were shifted slightly upfield, while other carbon resonances remained almost unshifted. This indicated that the acetoxyl group of **3** must be located at C-6 [8]. Analysis of proton signals of **3** at δ 3.47 (1H, *d*, *J* = 10 Hz) and 5.07 (1H, *dd*, *J* = 10 and 11 Hz) by a double irradiation experiment also supported this formulation.

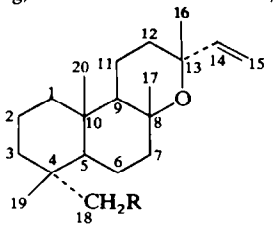
EXPERIMENTAL

Mps are uncorr. NMR spectra were taken in $CDCl_3$ at 25° using TMS as internal standard (^1H NMR at 100 MHz and FT-mode ^{13}C NMR at 25.15 MHz).

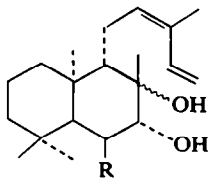
Extraction and isolation of compounds. The plant material was cultivated at Izu Experimental Station of Medicinal Plants, National Institute of Hygienic Sciences, Shizuoka Prefecture, Japan. Dried leaves of *Stevia rebaudiana* (3 kg) were exhaustively extracted with MeOH at room temp. The MeOH soln was concd to dryness *in vacuo*. After suspension of the residue in H_2O, the non-glycoside fraction was extracted with Et_2O. The Et_2O extract was concd *in vacuo* and the residue was chromatographed on a Si gel column. Elution with C_6H_6–EtOAc (6:1) afforded three crystalline compounds, **1**, **2** and **3**, along with a mixture of stigmasterol and sitosterol. The non-polar fraction was rechromatographed on Si gel using *n*-hexane–C_6H_6 (4:1) as an eluent and afforded three esters of lupeol and β-amyrin acetate.

Identification of compound **1** (*jhanol*). Compound **1** (190 mg) was isolated as needles, mp 137–139°, $[\alpha]_D^{18}$ +34° ($CHCl_3$, *c* 0.69) (lit. [7], mp 139–141°, $[\alpha]_D$ +27°). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 3080, 1638, 1120, 1095, 982, 920; ^1H NMR: δ0.75, 0.83, 1.27, 1.30 (each 3H, *s*), 3.10, 3.42 (each 1H, *d*, *J* = 12 Hz), 4.91 (1H, *dd*, *J* = 2 and 12 Hz), 5.12 (1H, *dd*, *J* = 2 and 18 Hz), 5.89 (1H, *dd*, *J* = 12 and 18 Hz). (Found: C, 78.62; H, 11.38. Calc. for $C_{20}H_{34}O_2$: C, 78.38; H, 11.18%).

Identification of compound **2** (*austroinulin*). Compound **2** (1.8 g) was isolated as needles, mp 74–77° (from MeOH), $[\alpha]_D^{25}$ +33.6° ($CHCl_3$, *c* 0.48) (lit. [11], oil, $[\alpha]_D^{24}$ +24°). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420; UV λ_{max}^{EtOH} nm (log ε): 237 (4.06). (Found: C, 72.65; H, 10.64. Calc. for $C_{20}H_{34}O_3 \cdot \frac{1}{2}H_2O$: C, 72.47; H, 10.64%).



1 R = OH
4 R = OAc



or its enantiomer

2 R = OH
3 R = OAc

6-O-Acetyl-austroinulin (**3**). Compound **3** (4.4 g) was isolated as needles, mp 173–174° (from EtOAc). $[\alpha]_D^{25}$ +36.8° ($CHCl_3$, *c* 0.46). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 1750, 1260. UV λ_{max}^{EtOH} nm (log ε): 237 (4.21). (Found: C, 72.20; H, 10.03. $C_{22}H_{36}O_4$ requires: C, 72.49; H, 9.96%).

Saponification of **3**. Compound **3** (250 mg) was refluxed in 1% KOH/MeOH soln (20 ml) for 1.5 hr. The reaction mixture was diluted with H_2O (50 ml) and extracted with Et_2O. From the Et_2O-extract, **2** (80 mg) was crystallized and identified by mmp, $[\alpha]_D$ (+34,2°) and ^{13}C NMR analysis.

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