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SHORT REPORTS

GUAIANOLIDES FROM *STEVIA SANGUINEA*

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Key Word Index—*Stevia sanguinea*, Eupatorieae, Compositae, guaianolides, sesquiterpene lactones.

Abstract—The aerial parts of *Stevia sanguinea* afforded four new guaianolides and the flavone hispidulin.

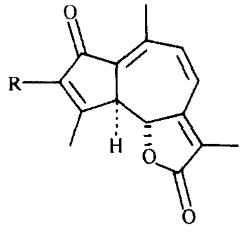
INTRODUCTION

Recent reports on the chemistry of the large New World genus *Stevia* (tribe Eupatorieae, subtribe Ageratinae [1]) have been summarized [2] In the present article, we continue our study of Argentine *Stevia* species [3] and describe our work on *Stevia sanguinea* Hieron., a taxon of northwestern Argentina [4]. The aerial parts yielded the new guaianolides **1a**–**4** as well as the flavone hispidulin.

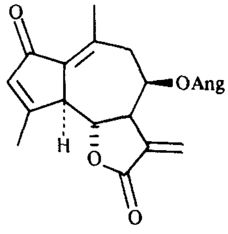
RESULTS AND DISCUSSION

Because of the limited quantity (70 g) of plant material, only small amounts of the various lactones were available for study. The mass spectrum of the least polar compound **1a** indicated the presence of chlorine and the molecular formula $C_{15}H_{13}ClO_3$, while the ^{1}H NMR spectrum (Table 1) showed that our substance was the chlorine analogue of a diosphenolic guaianolide **1b** from an Indiana collection of *Helianthus microcephalus* [5]. Other 2-chloro-1-oxo-1(10),3-guaiadienolides, though lacking the extended conjugation of **1a**, have been reported from *Trichogonia gardneri* [6] and *Lasiolaena santosii* [7].

Mass and ^{1}H NMR spectra, the latter accompanied by extensive decoupling which will not be discussed in detail, showed that a second lactone from *S. sanguinea* had the structure and stereochemistry depicted in **2**, a formulation with which the ^{13}C NMR spectrum (Table 2) was fully in accord. Analogues of **2** differing only in the nature of the ester group attached to C-8 have been reported from *Trichogonia santosii* [8], *Helianthus glaucophyllus* and *H microcephalus* [5].



1a R = Cl
1b R = OH



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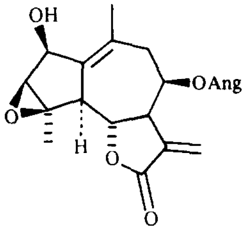

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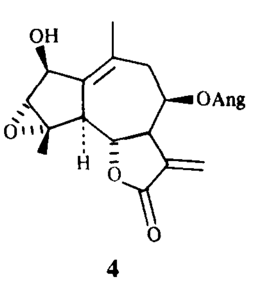

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Table 1 ^{1}H NMR spectra of compounds **1**–**4** ($CDCl_3$, 270 MHz)

H	**1**	**2**	**3***	**3**†	**3**+TAI	**4**‡	**4**+TAI‡
2	–	–	4.70 *br s*	4.57 *br s*	5.90	4.70 *br s*	5.90
3	–	6.23 *br s*	3.58 *dd* (2, 1)	3.43 *dd*	3.76	3.58 *dd*	3.76
5	3.46 *br d* (11.5)	3.54 *br d* (10)	3.25 *br d* (10)	3.08 *br d*	3.34	3.53 *br d* (10)	3.53
6	4.84 *dq* (11.5, 2)	4.08 *dt* (10)	4.02 *t* (10)	3.90 *t*	4.08	4.11 *t* (10)	4.10
7		3.16 *dddd* (10, 3.5, 3, 2)	3.03 *dddd* (10, 3, 3, 2)	2.70 *dddd*	3.07	3.16 *dddd* (10, 3, 3, 2)	3.18
8	6.64 *d* (11.5)	5.77 *dt* (5.5, 2)	5.67 *dt* (6, 2)	5.51 *dt*	5.68	5.81 *dt* (6, 2)	5.80
9a	6.29 *d* (11.5)	2.89 *dd* (15, 6)	2.66 *dd* (15, 6)	2.51 *dd*	2.71	2.87 *dd* (15, 6)	3.01
9b	–	2.73 *br d* (15)	2.47 *br d* (15)	2.22 *br d*	2.53	obsc	obsc
13a	2.03 *d*§ (2)	6.26 *d* (3.5)	6.22 *d* (3)	6.14 *d*	6.22	6.26 *d* (3)	6.23
13b		5.56 *d* (3)	5.49 *d* (3)	5.36 *d*	5.50	5.52 *d* (3)	5.51
14§	2.37 *d*(1)	2.35 *br s*	1.84 *br s*	1.78 *br s*	1.70	1.84 *br s*	1.70
15§	2.53 *d*(1)	2.35 *br s*	1.69 *s*	1.64 *s*	1.70	1.69 *s*	1.70
3′	–	6.11 *dq* (7, 1.5)	6.13 *dq*	6.00 *dq*	6.13	6.13 *dq*	6.13
4′§	–	1.91 *dq* (7, 1.5)	1.94 *dq*	1.90 *dq*	1.94	1.94 *dq*	1.94
5′§	–	1.77 *q*(1.5)	1.81 *q*	1.76 *q*	1.80	1.81 *q*	1.80

* *J*'s (Hz) 2,3 = 2; 2,14 = 1, 3,5 = 1, 5,14 = 1, 9b,14 ~ 1

† In $CDCl_3$-C_6D_6 2:1

‡ From mixture with compound **3**

§ Intensity three protons

The two remaining lactones, the second obtained only in admixture with the first, were stereoisomeric 2-hydroxy-3,4-epoxy-8-angeloxyguaia-1(10),11(13)-dien-6,12-olides based on the mass spectrum and the ^{13}C NMR spectrum (Table 2) of the pure isomer and on the ^{1}H NMR spectra (Table 1) which differed from each other only in the chemical shifts of the protons attached to the respective seven-membered rings. That both substances nevertheless possessed the same relative stereochemistry at C-5, C-6, C-7 and C-8, i.e. that H-5 and H-7 were α and H-6 and H-8 were β, could be deduced from the identical coupling constants involving the protons at these four centres. On the other hand, determination of the relative configurations at C-2, C-3 and C-4 presented difficulties since the signals of H-2, H-3 and H-15 in the spectra of the two isomers were superimposed, because the coupling constants involving H-2 and H-3 of isomeric 2-hydroxy-3,4-epoxyguaian-6,12-olides are essentially identical and because examination of the literature revealed considerable confusion in the assignment of stereochemistry to such compounds, especially when these also contain a 1(10)-double bond.

We base the tentative assignments shown in formulas **3** and **4** on the following information. (i) In the NOE difference spectrum of the pure isomer **3**, irradiation of H-2 produced a 10% enhancement of the H-3 signal, while irradiation of H-3 resulted in a 6.3% enhancement of the H-2 resonance. Consequently H-2 and H-3 of **3** were *cis*. In the NOE difference spectrum of the 2:1 mixture of **3** and **4**, these values were reduced by about one-third. Consequently H-2 and H-3 of the minor isomer were assumed to be *trans*. As expected, there was no difference in the degree of enhancement of the H-3 resonance on irradiation of H-15 since in either α- or β-orientation of the epoxide ring H-3 and H-15 are *cis*. (ii) Reaction of either isomer with trichloroacetylisocyanate (TAI) produced the same chemical shifts in the signals of H-2, H-3 and H-14, but did not significantly affect the remaining resonances. On this basis, we assume that the orientation of the hydroxyl group attached to C-2 is probably the same in both isomers. (iii) The H-5 resonance of the minor isomer was significantly downfield from the H-5 resonance of the isomer obtained in pure form. On this basis, we assume that the epoxide ring of the minor isomer is α-orientated, thus leading to expression **4** for it and to formula **3** for the isomer obtained in pure form. However, this leaves unexplained the chemical shift differences in the signals of H-6, H-7, H-8 and H-9 which are invariably at lower field in the spectrum of the presumed **4**. Conformational factors in the seven-membered rings of the two isomers cannot be invoked because the coupling constants are the same. Hence our assignments must remain tentative.

EXPERIMENTAL

Aerial parts (70 g) of *Stevia sanguinea* Hieron., collected in La Caldera, Ruta 68, km 1634, near Dique Campo Alegre, Salta

Table 2 ^{13}C NMR spectra of compounds **2** and **3** (67 89 MHz, $CDCl_3$)*

C	**2**	**3**
1	132 66 *s*	137 42 *s*[a]
2	195.08 *s*	72 47 *d*†
3	135 99 *d*†	67 20 *d*†
4	169 18*s*[a]	65 25 *s*
5	53 24 *d*†	52 88 *d*†
6	79 38 *d*†	76 10 *d*†
7	55 65 *d*†	56 70 *d*†
8	64.48 *d*†	65.31 *d*†
9	41.09 *d*†	38 59 *t*†
10	146 48 *s*	136 99 *s*[a]
11	134.65 *s*	134 87 *s*
12	168 15 *s*[a]	168 21 *s*
13	120 51 *t*†	119 98 *t*†
14	23 03 *q*†	23.59 *q*
15	19.69 *q*†	19 20 *q*
1′	166.85 *s*	166.81 *s*
2′	126.91 *s*	126.99 *s*
3′	139.81 *d*†	139 45 *s*
4′	20 40 *q*	20 42 *q*
5′	15 82 *q*	15 78 *q*

*Multiplicities established by DEPT pulse sequence

†Assignments by single frequency heteronuclear decoupling

[a] Assignments may be interchanged

Province, Argentina, in March 1986 and identified by Dr Luis Ariza Espinar (voucher on deposit in the Museo Botanico, Córdoba), were exhaustively extracted with $CHCl_3$ at room temp., yielding after removal of the solvent 8 3 g of crude extract. This was shaken with a mixture of MeOH (94 ml), hexane (395 ml) and H_2O (32 ml) The aq layer was sepd, washed with hexane, concd to smaller vol. *in vacuo* and extracted with $CHCl_3$ Evaporation of the $CHCl_3$ yielded 3 1 g of crude gum which was adsorbed on silica gel (10 g) and chromatographed over 150 g of the same adsorbent packed in hexane, 50 ml fractions being collected. The polarity of the eluent was increased by adding Me_2CO in the ratios 1 24 (fr 1–6), 2 23 (fr 7–12), 3 22 (fr. 3–18), 4:21 (fr 19–24), 1·4 (fr 25–30), 6 19 (fr 31–36), 7 18 (fr 37–42), 8 17 (fr 43–48), 9 16 (fr 49–54), 2 3 (fr. 55–60), 11.14 (fr. 61–66), 12 13 (fr. 67–72), 13 12 (fr 73–78) and Me_2CO (fr 79–84), all fractions being monitored by TLC. Fr 55–63 which exhibited similar behaviour on TLC (hexane–Me_2CO 3:2) were combined (137 mg) and rechromatographed over silica gel (10g, C_6H_6–Me_2CO 9.1, 5 ml fractions). Fr 17 gave 4 1 mg of **1a**, trituration of fr 19 with Et_2O resulted in crystallization of 10 mg of **2**.

Fr. 64–69 of the original chromatogram were combined (0.12 g) and rechromatographed over silica gel ($CHCl_3$) Fr. 12–17 contained 7.1 mg of a 2:1 mixture of **3** and **4** Fr 18–21 yielded 25.6 mg of **3**. Fr 81–83 of the original chromatogram were combined (72 mg) and were rechromatographed over Sephadex LH-20 with MeOH. This yielded 5 mg of hispidulin identified by comparison with an authentic sample Rechromatography of Fr 84–85 of the original column (53 mg) over silica gel (4 g, C_6H_6–EtOAc 1:1) gave in fr 14–16 3 5 mg of a crude sesquiterpene lactone mixture which was not identified further.

(5R,6R)-3-*Chloro*-2-*oxoguia*-1(10),3,7(11), 8-*tetraen*-6,12-*olide* (**1a**). Yellowish solid (no mp taken), IR ν^{KBr} cm^{-1} 1744, 1688, 1629, 1566, EIMS *m/z* (rel. int) 278 and 276 (14.6 and 50 9, M^+), 263 and 261 (0 7 and 2.5), 250 and 248 (1 4 and 2 7), 241 (7 0), 221 (35 3), 213 (100); ^{1}H NMR Table 1

(5S,6R,7R,8R)-8-*Angeloxy*-2-*oxoguaia*-1(10),3,11(13)-*trien*-6, 12-*olide* (**2**). Crystalline solid (no mp taken), IR ν^{KBr}cm^{-1} 1775, 1716, 1687, 1640, 1619, EIMS *m/z* (rel int) 342 (3.4, M^+), 243 (39 2), 83 (44.5), 55 (100); ^{1}H NMR and ^{13}C NMR Tables 1 and 2

[2S(?),3S(?)4R(?),5S,6S,7R,8R]-8-*Angeloxy*-3,4-*epoxy*-2-*hydroxyguaia*-1(10),11(13)-*dien*-6,12-*olide* (**3**) Solid (no mp taken), IR ν^{KBr}cm^{-1} 3400, 1771, 1713, EIMS *m/z* (rel int.) 360 (1 8, M^+), 260 (62.9), 242 (16.2), 83 (32 0), 55 (100), ^{1}H and ^{13}C NMR. Tables 1 and 2.

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