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GUAIANOLIDES, HELIANGOLIDES AND OTHER CONSTITUENTS FROM *STEVIA ALPINA*

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Key Word Index—*Stevia alpina*; Compositae; Eupatorieae; guaianolides; heliangolides; sesquiterpene lactones; X-ray analysis.

Abstract— The aerial parts of *Stevia alpina* afforded in addition to estafietin, dehydroleucodin, achillin, 2-oxo-8-deoxyligustrin and two known heliangolides two new guaianolides, the two epimeric 10(14)-epoxyestafietins, and the new heliangolide 3-acetylpulverolide. The stereochemistry of the new epoxyestafietins was confirmed by X-ray analysis of one of the epimers.

INTRODUCTION

Representatives of the large New World genus *Stevia* (Compositae, tribe Eupatorieae, subtribe Ageratinae [1]) are being studied in several laboratories [2]. In the present article we describe our work on *Stevia alpina* Griseb, a species of central and northwestern Argentina [3]. The aerial parts yielded the known guaianolides estafietin (**1**) [4–11], dehydroleucodin (**2**) [2, 12–18], achillin (**3**) [19–28], 2-oxo-8-deoxyligustrin (**4**) [11] and the known heliangolides **7** and **8** [29] as well as common triterpenes and plant sterols. New sesquiterpene lactones from *S. alpina* were the two epimeric epoxyestafietins **5** and **6** and the heliangolide **9a**.

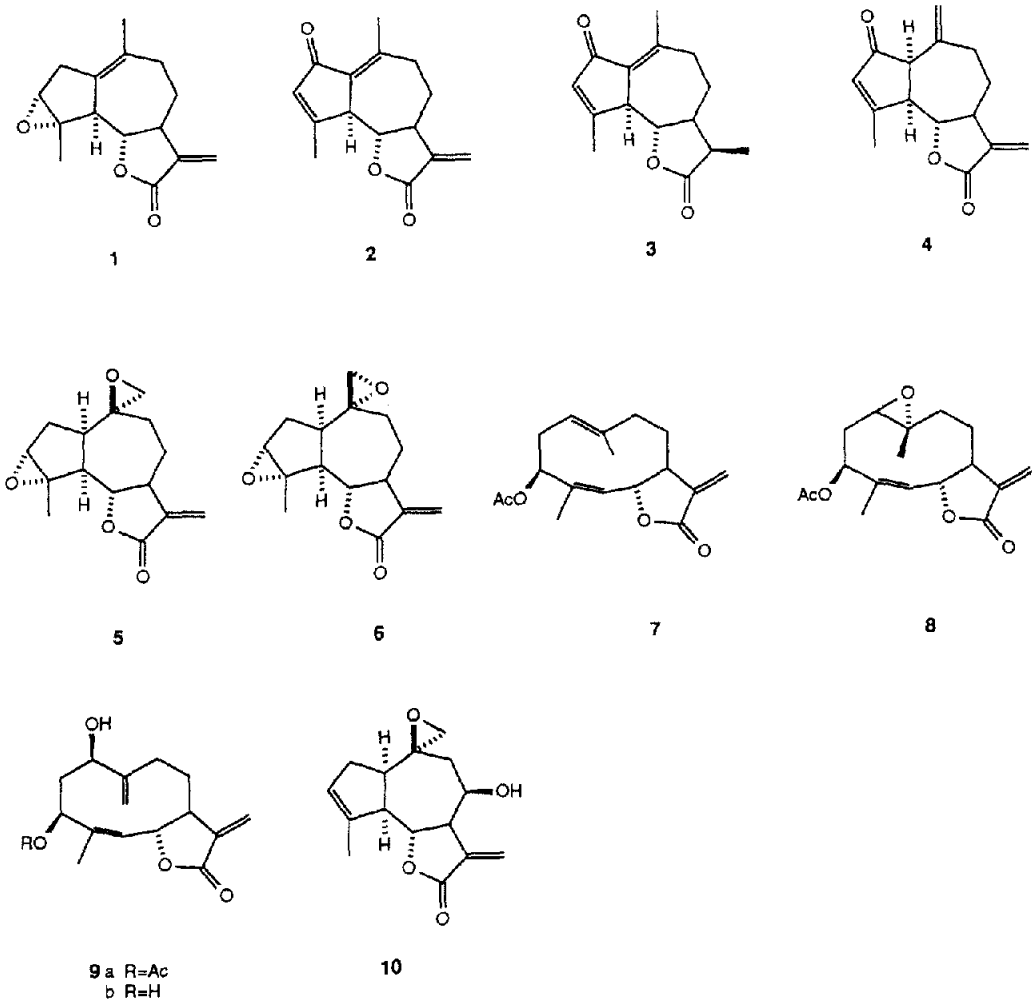
RESULTS AND DISCUSSION

Estafietin (**1**) was the main sesquiterpene lactone constituent of *S. alpina* followed by dehydroleucodin (**2**); the other lactones were isolated in very small amounts only. ^{1}H NMR spectra of two of these (Table 1) which were very difficult to separate and extensive decoupling which will not be described in detail indicated that they were the epimeric 10(14)-epoxides **5** and **6** derived from estafietin. The NMR spectra of the two differed significantly only in the chemical shift of H-7 (δ2.83 vs δ3.27). Furthermore oxidation of estafietin with *m*-chloroperbenzoic acid furnished predominantly the isomer exhibiting the H-7 signal at higher field. On this basis, and in the expectation that the conformation of a β-10(14)-epoxide would not differ significantly from that of bahia I (**10**) [30], the isomer exhibiting the H-7 signal at δ2.83 was assigned formula **5**.

To verify this conclusion an X-ray analysis of one of the very few available crystals of this isomer was undertaken. Crystal data are given in the Experimental section. Figure 1 is a stereoscopic view of the molecule which shows that the substance indeed possesses formula **5**. This formula also represents the absolute configuration as the absolute configuration of estafietin follows from its synthesis from (−)-santonin [31, 32]. A partial listing of torsion angles is given in Table 2; lists of bond lengths, bond angles, all torsion angles, atomic and final anisotropic thermal parameters are deposited at the Cambridge Crystallographic Centre. The torsion angles in Table 2 show that the conformation of **5** in the solid state is essentially identical with that of bahia I (**10**) [30], i.e. the seven-membered ring is a twist-chair with an approximate axis of symmetry passing through C-8. In this conformation the β-orientated oxygen of the 10(14)-epoxide exerts no significant effect on H-7 whereas the same conformation apparently also adopted by lactones of type **6** [33] results in deshielding of H-7 by the α-orientated epoxide oxygen. The congruence of **5** and **10** extends to the cyclopentane ($\Sigma\ |w| = 98$ vs 88°) and lactone ($\Sigma\ |w| = 70$ vs 61°) rings and, as expected, to the chirality of the lactone chromophore (−10.1 vs −11.5°).

Lactone **9a** was obviously related to its congeners **7** and **8** which had originally been found [29] in *Leucanthemopsis pulverulenta* (Compositae, tribe Anthemideae). That **9a** was also a heliangolide was evidenced by the values of $J_{7,13a}$ and $J_{7,13b}$ (2.5 and 2 Hz, respectively) and by the presence of allylic coupling and an NOE involving H-5 and H-15. Hence the unconjugated methylene group (broadened singlets at δ5.32 and 5.25) was attached to C-10. Spin decoupling established the sequence H-1 through H-3 and H-5 through H-9 with the coupling constants dictating the stereochemistry shown in the formula. Finally, the fact that the acetoxy group was attached to C-1 and not to C-3 became obvious on comparing the ^{1}H NMR spectrum of **9a** with that of pulverolide (**9b**), also a constituent of *L. pulverulenta* [34].

A brief survey of the phytochemical situation within *Stevia* has been presented recently [11]. Among the taxa containing sesquiterpene lactones guaianolides, either 6,12-*trans*- or 8,12-*cis*-fused but hardly ever occurring together, are most common (17 out of 21 taxa). Of these a few, including *S. alpina*, *boliviensis* [6], *pilosa* [28] and *yaconensis* [2, 11], elaborate only lactones normally associated with members of the Anthemideae. Whether



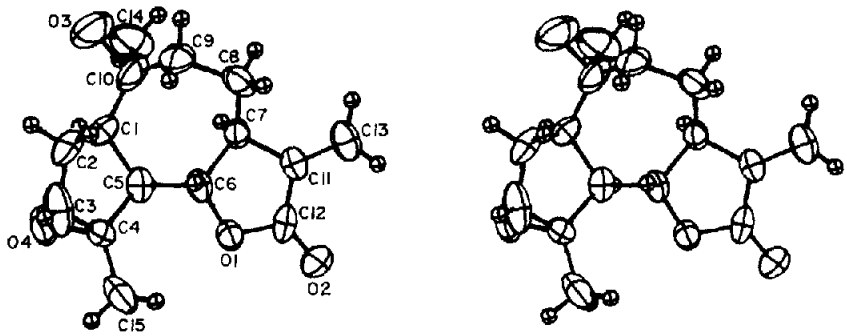


Fig. 1. Stereoscopic view of compound 5.

such findings point the way toward possible groupings within this large genus will require considerably more study.

EXPERIMENTAL

General. For separation of mixtures Waters HPLC equipment (M 45 pump, U6 K injector and R-401 differential refractometer) was used. The column employed was an Alltech R Sil C-18 column (10 μm, 10 mm i.d. × 50 cm). R_fs were measured from the solvent peak.

Plant material. Aerial parts of *Stevia alpina* Griseb. were collected at the flowering stage on March 9, 1987 at km 45 of Road 307 to Tafí del Valle, Tucumán Province, Argentina. Voucher specimens (CANC 40) are deposited in the herbarium of the Instituto Miguel Lillo, S. M. de Tucumán.

Extraction of S. alpina. Flowers and leaves (430 g) were extracted with $CHCl_3$ (2 × 5 l) at room temp for 7 days to give

Table 1. ^{1}H NMR spectra of compounds **1**, and **5–9a** (270 MHz, $CDCl_3$)

H	1	1*	5	6	7	7*	8	9a
1	2.98 *ddd* (10.5, 8, 8)	2.77 *ddd* (10.5, 8, 8)	2.21 *ddd* (10.5, 8, 8)	~1.80	~5.20	~4.75	2.80 *dd* (10.5, 4)	4.06 *dd* (11, 4)
2a	2.07 *dd* (14, 8)	1.71 *dd* (14, 8)	2.10 *dd* (14, 8)	2.12 *dd* (12, 5.5)	~2.65	2.34 *ddd* (14.5, 10, 3)	2.60 *dt* (15.5, 4.5)	~2.40
2b	1.80 *ddd* (14, 10.5, 1)	1.24 *ddd* (13.5, 11, 1)	1.78 *ddd* (14, 11, 1.5)	1.76 *ddd* (12, 11, 1)	~2.30	1.80 *ddd* (14.5, 6.5, 4)	1.73 *ddd* (15, 10, 2)	~2.30
3	3.40 *br s*	2.91 *br s*	3.36 *br s*	3.37 *s*	5.23 *dd* (3.5, 2.5)	5.09 *dd* (4, 2.5)	5.30 *dd* (4.5, 2)	5.37 *dd* (5, 2.5)
5	2.31 *dd* (11, 8)	1.80 *dd* (10.5, 8)	2.42 *dd* (11, 8.5)	2.41 *dd* (11, 8)	5.16 *dq* (10.5, 1.5)	4.78 *dq* (11, 1)	5.28 *dq* (11, 1.5)	5.31 *dd* (10, 1.5)
6	4.07 *dd* (11, 9)	3.13 *dd* (11, 8.5)	4.10 *dd* (11, 9)	4.17 *dd* (11, 9)	5.29 *dd* (10, 2.5)	5.14 *dd* (11, 2.5)	5.65 *dd* (11, 2)	5.60 *dd* (10, 4)
7	2.87 *ddddd* (11.5, 8.5, 5, 3.5, 3)	2.14 *ddddd* (12, 9, 5, 3.5, 3)	2.82 *m*	3.23 *m*	~2.65	2.04 *dddd* (11, 2.5, 2, 2)	2.62 *dddd* (11, 2, 2, 2)	2.86 *ddddd* (11, 4, 3, 2.5, 2)
8a	~2.20	~1.45	~2.10	~2.25	1.96 *ddd* (13.5, 10.5, 3)	~1.40	~1.95	~2.30
8b	1.55 *m*	0.85 *m*	~1.60	~1.60			~1.85	~1.90
9a	~2.20	~1.60	~2.00	~2.00	2.40 *br d* (13)	~1.90	2.37 *ddd* (13.5, 5, 2)	2.67 *br ddd* (14, 8, 4)
9b	~2.20	~1.56	~1.60	~1.60	~2.30	~1.80	~1.20	~2.30
13a	6.21 *d* (3.5)	6.07 *d* (3.5)	6.23 *d* (3)	6.26 *d* (3.5)	6.30 *d* (2.5)	6.28 *d* (2)	6.34 *d* (2)	6.30 *d* (2.5)
13b	5.48 *d* (3)	4.84 *d* (3)	5.49 *d* (3)	5.53 *d* (3)	5.62 *d* (2.5)	5.07 *d* (2)	5.69 *d* (2)	5.64 *d* (2)
14a	4.95 *br s*	4.63 *d* (1)	2.64 *br d* (5)	2.63 *d* (5)	1.74 *br s*	1.46 *br s*	1.46 *br s*	5.32 *br s*
14b	4.88 *d* (1.5)	4.54 *d* (1.5)	2.60 *d* (5)	2.57 *d* (5)				5.25 *br s*
15†	1.62 *s*	1.61 *s*	1.63 *s*	1.62 *s*	1.80 *d* (1.5)	1.46 *br s*	1.91 *d* (1.5)	1.83 *d* (1.5)
OAc					2.15 *s*	1.71 *s*	2.12 *s*	2.05 *s*

*in C_6D_6

†Intensity three protons.

Table 2. Selected torsion angles (deg) in **5** with standard deviation in parentheses

C(1)–C(2)–C(3)–C(4)	21.0	(1.2)
C(2)–C(3)–C(4)–C(5)	−2.6	(1.2)
C(3)–C(4)–C(5)–C(1)	−16.1	(1.0)
C(4)–C(5)–C(1)–C(2)	27.7	(0.9)
C(5)–C(1)–C(2)–C(3)	−30.4	(1.0)
C(5)–C(6)–C(7)–C(8)	95.4	(0.9)
C(6)–C(7)–C(8)–C(9)	−49.6	(1.1)
C(7)–C(8)–C(9)–C(10)	−31.6	(1.2)
C(8)–C(9)–C(10)–C(1)	86.1	(1.2)
C(9)–C(10)–C(1)–C(5)	−67.1	(1.2)
C(10)–C(1)–C(5)–C(6)	40.3	(1.2)
C(1)–C(5)–C(6)–C(7)	−61.2	(1.0)
C(6)–C(7)–C(11)–C(12)	16.8	(0.9)
C(7)–C(11)–C(12)–O(1)	−7.6	(1.0)
C(11)–C(12)–O(1)–C(6)	−7.0	(1.0)
C(12)–O(1)–C(6)–C(7)	18.0	(0.9)
O(1)–C(6)–C(7)–C(11)	−20.3	(0.8)
C(13)–C(11)–C(12)–O(2)	−10.1	(0.9)

Table 3. ^{13}C NMR spectra of compounds **1**, **2** and **4** (67.89 MHz)*

C	**1**(C_6D_6)	**2**($CDCl_3$)	**4**($CDCl_3$)
1	44.79 *d*	132.00 *s*	56.22 *d*
2	32.86 *t*†	195.64 *s*	206.21 *s*
3	62.24 *d*†	135.65 *d*	132.57 *d*†
4	64.96 *s*	169.38 *s*‡	177.54 s
5	50.71 *d*†	52.96 *d*†	53.23 *d*
6	79.42 *d*†	84.38 *d*†	83.32 *d*†
7	43.78 *d*†	53.08 *d*†	46.10 *d*†
8	28.85 *t*†	24.47 *t*†	31.17 *t*†
9	28.52 *t*†	37.25 *t*†	36.36 *t*†
10	146.55 *s*	151.68 *s*	144.07 *s*
11	140.43 *s*	138.64 *s*	138.47 *s*
12	168.34 *s*	168.97 *s*‡	169.29 *s*
13	118.37 *t*†	118.62 *t*†	121.04 *t*†
14	114.29 *t*†	21.75 *q*†	117.26 *t*†
15	18.59 *q*†	19.69 *q*†	19.81 *q*†

* Multiplicities established by DEPT pulse sequence.

† Assignments by single frequency heteronuclear decoupling.

‡ Assignments may be interchanged.

42 g (9.8% yield) of crude extract which was suspended in 380 ml of EtOH at 55°, diluted with H_2O (250 ml) and extracted successively with *n*-hexane (3 × 400 ml) and $CHCl_3$ (3 × 400 ml). Evaporation of the hexane fraction gave 26.4 g of residue a portion of which (4.0 g) was chromatographed over silica gel (125 g) using hexane–Et_2O (4:1), all fractions being monitored by TLC. Fractions showing a spot with the same R_f as β-amyrin were combined (0.56 g) and rechromatographed over silica gel (25 g, hexane–Et_2O 6:1) to give 205 mg of pentacyclic triterpenoids. A portion of this was processed by RP-HPLC (MeOH, 4 ml/min) to give, 0.6 mg of lupeol, 3.1 mg of pseudotaraxasterol, 3.7 mg of β-amyrin and 8.6 mg of germanicol, identified by comparison (^1H NMR spectrometry) with authentic samples.

The $CHCl_3$ extract on evaporation at red. pres. furnished a residue (9.5 g), a 4.5 g portion of which was chromatographed (silica gel, 150 g) using $CHCl_3$ and increasing amounts of Et_2O (0–10%). Twenty fractions were collected. Frs 2–7 (combined wt 1.75 g) which showed a major spot on TLC were rechromatographed (silica gel, 70 g) using $CHCl_3$ (31 fractions). Frs 4–12 of the rechromatogram yielded 1.367 g of almost pure estafietin (**1**), mp 105° (heptane–EtOAc, 1:3), IR ν_{max}^{KBr} cm^{-1}: 3068, 3003, 2962, 2869, 1756, 1664, 1637, 1450, 1403, 1381, 1335, 1307, 1264, 1150, 1014, 988, 966, 944, 908, 817. As the ^1H NMR data for **1** in the literature [4–11] are not very complete and as ^{13}C NMR data for **1** are lacking, they are listed in Tables 1 and 3, respectively. Evaporation of the mother liquors from recrystallization of **1** followed by HPLC (MeOH–H_2O 2:1, 2.5 ml/min) gave 2.8 mg of additional crystalline **1** (R_t 22 min) and 3.0 mg of **7** as a gum whose ^1H NMR spectrum (Table 1) coincided with the less detailed 60 MHz spectrum reported in the literature [29].

Fractions 13–16 of the rechromatogram (62 mg) were purified by HPLC (MeOH–H_2O 2:1, 3.0 ml/min) to give 0.5 mg of **6** as a gum (R_t 10 min), 2 mg of crystalline achillin (**3**) (R_t 15.5 min) whose ^1H NMR spectrum coincided with that of authentic material and 23 mg of crystalline dehydroleucodin (**2**) (R_t 16.5 min) identical with authentic material [2] as well as 2 mg of additional somewhat impure **2**. The previously unreported ^{13}C NMR spectrum of **2** is listed in Table 3. Frs 17 and 18 (combined wt 9.2 mg) gave after RP-HPLC separation (MeOH–H_2O, 8:5; 3.0 ml/min) 1.5 mg of **5** as a solid (R_t 11.8 min), 1.5 mg of a 2:3 mixture of **5** and **6** (R_t 12.5 min) and 1.5 mg of solid **8** (R_t 15.5 min), (mp not taken because of scarcity of material) whose ^1H NMR spectrum (Table 1) coincided with the less detailed 60 MHz spectrum reported in the literature [29]. Frs 19–27 of the rechromatogram were undefined mixtures. Frs 28–31 (combined wt 15.6 mg) on HPLC (MeOH–H_2O, 8:5; 2.5 ml/min) furnished in one of the middle fractions 3.1 mg of **9a**. Frs 8–20 of the original chromatogram (combined wt 850 mg) after repeated purification by TLC and eventual HPLC (MeOH–H_2O, 5:3; 5 ml/min) gave 6.4 mg of **5** as a gum (R_t 13.5 min), 5.4 mg of impure **8**, 8.3 mg of crystalline **4** (R_t 20.5) whose ^1H NMR spectrum coincided with that reported in the literature [5] and whose ^{13}C NMR spectrum is listed in Table 3, 4.4 mg of crystalline **3** (R_t 26.5 min), 20.8 mg of crystalline **2** (R_t 29.5 min) and 9.0 mg of crystalline **1** (R_t 48 min).

Epoxidation of estafietin. To estafietin (230 mg) in 8 ml of CH_2Cl_2 and 2.5 ml of 0.5 M $NaHCO_3$ cooled in ice was added in small portions 2.3 g of 68% *m*-chloroperbenzoic acid. The mixture was stirred overnight, filtered and dild with 15 ml of $CHCl_3$. The organic layer was washed with 10% aq. $Na_2S_2O_3$ (3 × 4 ml), 1 M NaOH (3 × 4 ml) and H_2O (2 × 4 ml). Chromatography (silica gel, 10 g) of the residue (184 mg) after drying and evapn at red. pres. furnished 55 mg of unchanged estafietin and 63 mg of a 2:3 mixture of α- and β-epoxides a portion of which was separated by HPLC (MeOH–H_2O, 5:3; 1.5 ml/min) to give 3.0 mg of the β-epoxide (R_t 10.5 min) and 2.2 mg of α-epoxide (R_t 11.2 min).

β-Epoxyestafietin ([1*R*,3*R*,4*S*,5*S*,6*S*,7*S*,10*S*]-3,4,10(14)-*diepoxyguai*-11(13)-*en*-6,12-*olide*) (**5**). Crystals, mp 159–161° ($CHCl_3$–EtOAc, 1:1); IR ν_{max} cm^{-1}: 1749, 1662, 1250, 1138, 1020, 990, 901, 816; EIMS *m/z* (rel. int.): 262 (10.9), 247 (60.7), 245 (10.3), 229 (12.4), 201 (43.0); ^1H NMR spectrum in Table 1.

α-Epoxyestafietin ([1*R*,3*R*,4*S*,5*S*,6*S*,7*S*,10*R*]-3,4,10(14)-*diepoxyguai*-11(13)-*en*-6,12-*olide*) (**6**). Crystals, mp 113–119°; IR ν_{max} cm^{-1}: 1762, 1661, 1263, 1141, 1020, 998, 891, 816; EIMS *m/z* (rel. int.): 262 (22.5) $[M]^+$, 260 (35.6), 247 (100), 244 (40.7), 229 (26.3), 201 (25.6); MS PCI *m/z* (rel. int.): 263 (100) $[M+1]^+$, 261 (28.1), 245 (22.9), 217 (3.8), 199 (2.8), 117 (9.4), 99 (6.6); ^1H NMR spectrum in Table 1.

3-*Acetylpulverolide* ([1*R*,3*S*,6*S*,7*S*]-3-*acetoxy*-1-*hydroxygermacra*-4*Z*,10(14),11(13)-*trien*-6,12-*olide*) (**9a**). Gum; EIMS *m/z* (rel. int.): 306 (0.5), 291 (1.4), 262 (8.0), 246 (10.0), 231 (9.2); PCIMS *m/z* (rel. int.): 307 (28.1), 289 (5.0), 279 (16.3), 263 (74.8), 247 (100), 229 (77.2), 201 (13.5); ^{1}H NMR spectrum in Table 1.

X-Ray analysis of **5**. Single crystals of **5** prepared by slow evapn of a $CHCl_3$–EtOAc (1:1) soln were orthorhombic, space group $P2_12_12_1$ with $a=8.130(6)$, $b=10.507(9)$, $c=15.817(10$ Å, $d_{calcd}=1.29$ g/cm^3 for $Z=4$ ($M_r=262.3$). The intensity data were measured on a CAD4 Enraf Nonius diffractometer. The limited amount of material available (*ca* 3 mg) resulted in only a few crystals of poor quality. The best measured $0.2\times0.2\times0.1$ mm^3 and was used for determination of the unit cell dimensions and for data collection. No absorption correction was necessary ($\mu=0.86$). A total of 1409 reflections were measured for $\theta\leq50°$ of which 945 were considered to be observed [$\geq 1\sigma$ (I)]. The main part of the structure was found using direct methods (MULTAN 78 [35]) with the remainder of the atoms located from successive least squares and difference Fourier techniques. The original assignments for O(3) and C(14) (see Fig. 1) were reversed and led to unreasonable thermal parameters. When the atom designations were changed to the present labelling scheme refinement proceeded smoothly, thus confirming the correctness of the latter assignments. In the final refinement anisotropic thermal parameters were used for non-hydrogen atoms. Methyl hydrogens were located from a difference Fourier map; the remaining hydrogen atom parameters were calculated assuming idealized geometry. Hydrogen atom contributions were included in the structure factor calculations, but their parameters were not refined. The final discrepancy indices were $R=7.9$ and $R_w=10.1$ for the 945 observed reflections. The final difference Fourier map was essentially featureless with no peaks greater than 0.18 eÅ^{-3}.

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REFERENCES

1. King, R. M. and Robinson, H. (1987). *The Genera of the Eupatorieae (Asteraceae)*. Monographs in Systematic Botany, Missouri Botanical Garden.
2. Sosa, V. E., Oberti, J. C., Gil, R. R., Rúveda, E. A., Goedken, V. L., Gutiérrez, A. B. and Herz, W. (1989) *Phytochemistry*, (In press).
3. Ariza Espinar, L. and Cerana, M. M. (1986) *Bol. Acad. Nac. Cienc. Córdoba* **57**, 381.
4. Sanchez-Viesca, F. and Romo, J. (1963) *Tetrahedron* **19**, 1285.
5. Bohlmann, F., Borkowski, H. and Arndt, C. (1966) *Chem. Ber.* **99**, 2828.
6. Bohlmann, F., Dorner, W., King, R. M. and Robinson, H. (1979) *Phytochemistry* **18**, 673.
7. Uchio, Y., Tomosue, K., Nakayama, M., Yamamura, A. and Waki, T. (1981) *Phytochemistry* **20**, 2691.
8. Adenekov, S. M., Mukhometzhanov, M. N., Kagarlitskii, A. D. and Turmukhambelov, A. Zh. (1984) *Khim. Prir. Soedin.* **20**, 603.
9. Jakupovic, J., Chau-Thi, T. V., Warning, V. and Bohlmann, F. (1986) *Phytochemistry* **25**, 1663.
10. Zdero, C., Bohlmann, F. and Müller, M. (1987) *Phytochemistry* **26**, 2763.
11. Zdero, C., Bohlmann, F., King, R. M. and Robinson, H. (1988) *Phytochemistry* **27**, 2835.
12. Bohlmann, F. and Zdero, C. (1972) *Tetrahedron Letters* 621.
13. Bohlmann, F. and Zdero, C. (1974) *Chem. Ber.* **107**, 1044.
14. Bhadane, N. R., Kelsey, R. G. and Shafizadeh, F. (1975) *Phytochemistry*, **14**, 2561.
15. Bohlmann, F. and Zdero, C. (1979) *Phytochemistry* **18**, 336.
16. Bohlmann, F., Suwita, A., Robinson, H. and King, R. M. (1979) *Phytochemistry* **18**, 2055.
17. Bohlmann, F., Kramp, W., Gupta, R. K., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 2375.
18. Huneck, S., Zdero, C. and Bohlmann, F. (1986) *Phytochemistry* **25**, 883.
19. White, E. H. and Winter, R. E. K. (1963) *Tetrahedron Letters* 137.
20. Romo de Vivar, A. and Olmos, F. (1968) *Rev. Soc. Quim. Mex.* **12**, 212A.
21. Shafizadeh, F., Bhadane, N. R. and Kelsey, R. G. (1974) *Phytochemistry* **13**, 669.
22. Popova, A., Rybalko, K. S. and Evstratova, R. I. (1974) *Khim. Prir. Soedin.* **10**, 528.
23. Dominguez, X. A. and Cardenas, E. (1975) *Phytochemistry* **14**, 2511.
24. González, A. G., Bermejo, J. J., de la Rosa, A. and Massanet, G. W. (1976) *Anal. Quim.* **72**, 695.
25. Ohno, N., Gershenzon, J., Roane, C. and Mabry, T. J. (1980) *Phytochemistry* **19**, 103.
26. Tsankova, E. and Ognyanov, I. (1985) *Planta Med.* 10.
27. de la Fuente, R. J., Diaz, O. L., Morales, I., Oberti, J., Sosa, V. E. and Herz, W. (1986) *Planta Med.* 243.
28. Martinez, V. M., Muñoz-Zamora, A. and Joseph-Nathan, P. (1988) *J. Nat. Prod.* **51**, 221.
29. de Pascual-Teresa, J., González, M. S., Moreno-Valle, M. A. and Bellido, T. S. (1983) *Phytochemistry* **22**, 1985.
30. Herz, W., Govindan, S. and Blount, J. F. (1980) *J. Org. Chem.* **45**, 3163.
31. Edgar, M. T., Greene, A. E. and Crabbé, P. (1979) *J. Org. Chem.* **44**, 159.
32. Ando, M., Akahane, A., Yamaoka, H. and Takase, K. (1982) *J. Org. Chem.* **47**, 3909.
33. McPhail, A. T. and Sim, G. A. (1973) *Tetrahedron* **29**, 1751.
34. de Pascual-Teresa, J., Moreno-Valle, M. A., Gonzalez, M. S. and Bellido, I. S. (1984) *Phytochemistry* **23**, 1178 (1984).
35. Main, P. "Multan 78. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data", Department of Physics, University of York, York, England.