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ABSOLUTE CONFIGURATION OF LONGIPINENYL EPOXYANGELATES FROM THREE *STEVIA* SPECIES

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Key Word Index—*Stevia salicifolia*; *S. mandonii*; *S. boliviensis*; Compositae; stereochemistry; absolute configuration; epoxyangelate esters; longipinene derivatives.

Abstract—The complete stereostructures of two longipinene derivatives from three *Stevia* species, which contain chiral epoxyangelate moieties are assigned as (4*R*,5*S*,7*R*,9*R*,10*R*,11*R*,2′*R*,3′*R*)- and (4*R*,5*S*,7*R*,9*R*,10*R*,11*R*,2′*S*,3′*S*)-9-angeloyloxy-7-(2′,3′-epoxy-2′-methylbutyryloxy)-1-oxolongipin-2-ene. These results follow from the preparation of the substances by incorporation of both enantiomers of epoxyangelic acid into 9-angeloyloxy-7-hydroxy-1-oxolongipin-2-ene, followed by comparison of the ^{1}H NMR data of the prepared esters with those of the natural substances. Minimum energy structures for both compounds using molecular mechanics calculations are also reported.

INTRODUCTION

The genus *Stevia* is widely distributed through the American continent from southwestern United States to northern Argentina. Chemical studies on *Stevia* have revealed that guaiane [1, 2], germacrane [3, 4] and longipinane derivatives [5, 6] are the most representative constituents of this taxon. Furthermore, in the last few years several *ent*-kaurene glycosides have attracted the attention of chemists and industrialists due to their economic significance as sweeteners [7–9]. Therefore, full and detailed chemical studies of each species of this genus is desirable.

In the present work, we describe the complete stereochemical elucidation of two longipinene derivatives (**1** and **2**) which contain chiral epoxyangelate moieties at the C-7 position. In previous studies, compound **1** was isolated from *Stevia boliviensis* [10], *S. mandonii* [10] and *S. salicifolia* [11], and compound **2** was obtained from *S. salicifolia* [11]. However, the complete stereochemistry of both substances, particularly the configuration of the epoxyangelate ester side chain, was not determined at that time due to the lack of knowledge of the absolute configuration of the two possible epoxyangelic acids. In this work, the stereochemical assignment is achieved by preparation of both substances, involving the use of both enantiomers of epoxyangelic acid [12], followed by comparison of the ^{1}H NMR data of the prepared compounds with those of the natural substances.

RESULTS AND DISCUSSION

Recently, we described the preparation and absolute configuration of (2*R*,3*R*)-(+)- and (2*S*,3*S*)-(−)-2,3-epoxy-2-methylbutanoic acids (*epoxyangelic acids*) by Sharpless asymmetric epoxidation of angelic acid [12]. On the other hand, we reported the preparation of monoester **3** almost a decade ago [13]. Incorporation of (2*R*,3*R*)-(+)- or (2*S*,3*S*)-(−)-epoxyangelic acid to monoester **3** was carried out in CH_2Cl_2 solutions using dicyclohexylcarbodiimide and 4-*N*,*N*-dimethylaminopyridine as the catalysts, giving diesters **1** and **2**, respectively.

Several chemical shift differences can be observed when comparing the ^{1}H NMR spectrum of **1** with that of **2**, thus permitting a clear distinction

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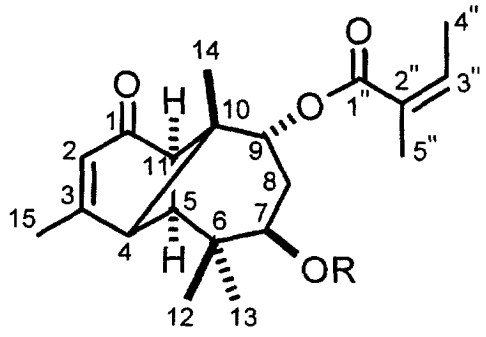
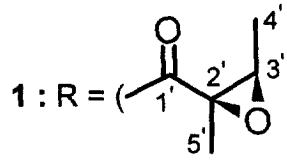
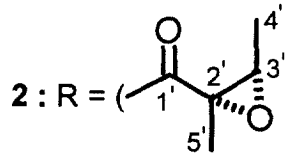






3 : R = H

between the two diastereoisomers. For example, in $CDCl_3$ the epoxyangelate methyl groups Me-4′ and Me-5′ in **1** appear at δ 1.31 and 1.53, respectively ($\Delta\delta_{5'-4'}=0.22$), while in **2** they appear at δ 1.28 and 1.56 ($\Delta\delta_{5'-4'}=0.28$). In C_6D_6, these differences ($\Delta\delta_{5'-4'}$) have an opposite pattern since, as can be seen in Table 1, $\Delta\delta_{5'-4'}$ is larger (0.25) in **1** than in **2** (0.21). Concerning the signals of the protons geminal to oxygens at C-7 and C-9, in $CDCl_3$, they appear in **1** at δ 5.12 and 5.14, while in **2** they switch chemical shifts, appearing at δ 5.11 and 5.10, respectively. As previously mentioned, the stereochemistry of the epoxyangelate residues can be assigned by comparison of the ^{1}H NMR data of the synthetic compounds listed in Table 1 with those of the natural products [10, 11]. However, in order to accomplish a successful data comparison, the following detailed considerations must be taken into account. Literature data [11] given in C_6D_6 consistently differ from our results by *ca.* δ +0.055. This difference probably arises from distinct referencing procedures. In our case the remaining C_6D_5H signal was carefully centered at δ 7.15 [14]. On the other hand, the reported ^{1}H NMR data in $CDCl_3$ for natural **1** and **2** [10, 11] are in agreement with our results, excepting those for H-7 and H-9 of **2**, (**41** in reference [11]) which clearly are in error. This is evident if the reported data for this kind of hydrogens (H-7 and H-9) given in references [5, 6, 13] are taken into account. After these considerations, data comparison concludes that the stereochemistry of longipinene derivative **40** in reference [11] and **15** in reference [10] correspond to that of **1** (this work) and the stereochemistry of **41** in reference [11] corresponds to that of **2**

Table 1. ^{1}H NMR data of **1** and **2** (300 MHz).

H	**1** $CDCl_3$*	C_6D_6†	**2** $CDCl_3$*	C_6D_6†
2(*ddq*)	5.81	5.70	5.81	5.70
4(*d*)	2.66	1.98	2.66	1.99
5(*s*)	2.33	2.06	2.34	2.07
7(*dd*)	5.12	5.23	5.11	5.24
8α(*ddd*)	—‡	2.10	- ‡	2.15
8β(*ddd*)	2.28	1.81	2.25	1.92
9(*dd*)	5.14	5.05	5.10	5.03
11(*d*)	3.13	3.36	3.13	3.37
12(*s*)	1.08	0.67	1.09	0.68
13(*s*)	0.90	0.77	0.95	0.80
14(*s*)	1.01	0.91	1.01	0.90
15(*d*)	2.06	1.43	2.07	1.43
Epoxyang				
3′(*q*)	3.03	2.50	3.03	2.49
4′(*d*)	1.31	1.05	1.28	1.07
5′(*s*)	1.53	1.30	1.56	1.28
Ang¶				
3″(*qq*)	6.12	5.74	6.11	5.73
4″(*dq*)	2.02	2.05	2.00	2.06
5″(*dq*)	1.98	2.04	1.98	2.03

J (Hz): 2,4 = 2,11 = 2,15 = 1.5; 4,11 = 7.0; 7,8α = 1.5; 7,8β = 12.0; 8α,8β = 14.5; 8α,9 = 8β,9 = 3.5; 3′,4′ = 5.5; 3″,4″ = 7.0; 3″,5″ = 4″,5″ = 1.5.

* Chloroform signal centered at 7.27 ppm.

† Benzene signal centered at 7.15 ppm.

‡ Overlapped with the 4″ and 5″ signals.

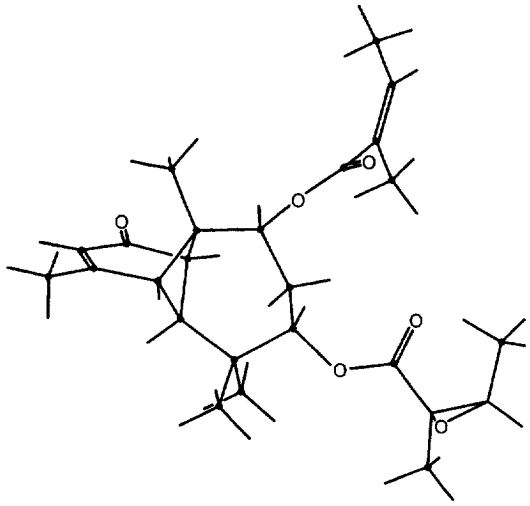
¶ Assigned according to [20].

Table 2. ^{13}C NMR data of **1** and **2** (75.4 MHz), internal standard TMS.

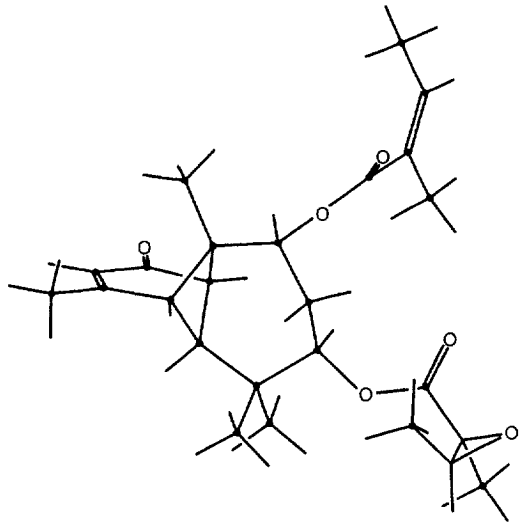
C	**1** $CDCl_3$	C_6D_6	**2** $CDCl_3$	C_6D_6
1	202.60	200.65	202.67	200.62
2	122.82	123.21	122.84	123.24
3	170.04	169.29	170.08	169.03
4	48.39	48.13	48.40	48.14
5	65.56	65.19	65.60	65.25
6	32.60	32.70	32.58	32.73
7	73.98*	74.15	74.13*	74.14
8	37.24	37.19	37.27	37.23
9	74.22*	74.52	74.31*	74.62
10	55.84	55.30	55.83	55.28
11	53.97	54.29	53.98	54.28
12	18.81	18.63	18.95	18.79
13	26.05	25.77	26.05	26.00
14	21.29	21.32	21.32	21.29
15	23.29	22.73	23.35	22.74
Epoxyang				
1′	168.95	168.31	168.90	168.27
2′	59.59	59.27	59.86	59.57
3′	59.82	59.57	59.75	59.34
4′	13.76	13.73	13.63	13.87
5′	19.17	19.32	19.20	19.35
Ang†				
1″	167.05	167.00	167.16	167.06
2″	127.74	127.00	127.79	127.49
3″	139.04	139.03	138.86	138.68
4″	15.84	16.04	15.84	15.99
5″	20.57	20.83	20.57	20.83

* May be interchanged.

† Assigned according to [20].



1



2

Fig. 1. Minimized structures of longipinene derivatives **1** (E_{MMX} = 58.4 Kcal/mol) and **2** (E_{MMX} = 58.3 Kcal/mol) obtained by molecular mechanics [15].

(this work). HETCOR experiments of both substances (**1** and **2**) together with literature values for longipinene diesters [5, 6, 13] allows the assignment of the ^{13}C NMR signals, which are listed in Table 2.

Once all the chiral centers of **1** and **2** are completely defined, the minimum energy conformations of **1** and **2** can be obtained by molecular mechanics calculations (MMX) [15]. The stereostructures are shown in Fig. 1. Finally, it is worth mentioning that diverse compounds containing epoxyangelate moieties possess relevant biological activities as tumor inhibitors [16], antibiotics [17] or anti-inflammatory agents [18] and that in one case, the biological activity has been specifically attributed to the presence of the epoxyangelate [18].

EXPERIMENTAL

General

^1H and ^{13}C NMR spectra (Tables 1 and 2) were measured at 300 and 75.4 MHz, respectively, from $CDCl_3$ and C_6D_6 solns with TMS as the int. standard, unless otherwise stated. CC was carried out on silica gel (230–400 mesh ASTM).

(4R,5S,7R,9Rr,10R,11R,2'R,3'R)-9-angeloyloxy-7-(2',3'-epoxy-2'-methylbutyryloxy)-1-oxo-longipin-2-ene (**1**)

A solution of **3** [13] (25 mg) in CH_2Cl_2 (2 ml) was treated with CH_2Cl_2 solns of dicyclohexylcarbodiimide (90 mg in 3 ml), *N,N*-dimethylaminopyridine (15 mg in 1 ml) and (+)-epoxyangelic acid (**4**) [12] (10 mg in 0.5 ml, *e. e.* = 80%) at room temp. for 48 h. The reaction mixture was filtered and chromatographed on silica gel using hexane-EtOAc (95:5) as eluent. The first frs yielded **1** (26 mg, 70%, *ds* = 90%), $[\alpha]_D^{20} = +58$ (*c* 0.45, $CHCl_3$). IR ν_{max}^{CHCl3} cm^{-1}: 3010, 1745, 1671, 620, 1422, 1260, 1145, 788. UV λ_{max}^{EtOH} nm (log ϵ): 216 (4.16), 249 (3.89). EIMS 70 eV, *m/z* (rel. int.): 430 M^+ (10), 331 (5), 303 (5), 232 (8), 214 (19), 173 (16), 83 (100), 55 (66), 43 (79).

(4R,5S,7R,9R,10R,11R,2'S,3'S)-9-angeloyloxy-7-(2',3'-epoxy-2'-methylbutyryloxy)-1-oxo-longipin-2-ene (**2**)

Obtained as described for **1** but using (−)-epoxyangelic acid (**5**) [12] (10 mg, *e. e.* = 56%) to give **2** (28 mg, 74%, *ds* = 80%), $[\alpha]_D^{20} = +35.2$ (*c* 0.40, $CHCl_3$). IR ν_{max}^{CHCl3} cm^{-1}: 3028, 1742, 1674, 1618, 1420, 1266, 1152, 783. UV λ_{max}^{EtOH} nm (log ϵ): 217 (4.21), 248 (3.91). EIMS 70 eV, *m/z* (rel. int.): 430 M^+ (2), 331 (5), 303 (7), 232 (6), 214 (12), 173 (9), 145 (8), 83 (100), 43 (65).

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