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BISABOLENE DERIVATIVES FROM *STEVIA SALICIFOLIA**

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Key Word Index—*Stevia salicifolia*, Compositae, Eupatorieae, bisabolene-type sesquiterpenes, 1-hydroxy bisabol-15-oic acid, 1-acetoxy-bisabol-15-oic acid

Abstract—The aerial parts of *Stevia salicifolia* afforded two new bisabolene type sesquiterpenes The structures were elucidated by spectroscopic, chemical transformations and correlation with 15-acetoxy bisabol-1-one

INTRODUCTION

In continuation of our phytochemical survey of the genus *Stevia* [1–5], we have now reinvestigated *Stevia salicifolia* Cav var *typica* Rob, and in addition to the previously isolated diterpene stevinsol [6] we have found two new sesquiterpene acids whose structures were elucidated by chemical and spectroscopic methods

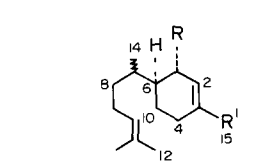
RESULTS AND DISCUSSION

The two new sesquiterpene acids were separated only after esterification with diazomethane with loss of large amounts of material

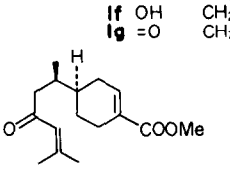
The less polar methyl ester **1a** was an unstable colourless oil, $C_{18}H_{28}O_4$, $[M^+]$ at *m/z* 308, $[\alpha]_D = +95°$, which was a bisabolene type sesquiterpene since its ^{13}C NMR spectrum (Table 1) displayed similar chemical shifts to known bisabolene derivatives [7, 8] The UV and IR spectra showed the presence of an α,β-unsaturated methyl ester grouping (1720 cm^{-1} and $\lambda_{max}^{MeOH} = 217$ nm) [9] The ^1H NMR spectrum showed a sharp three proton singlet at δ2 1 indicating the presence of an acetate group This assumption was supported by the mass spectrum peak at *m/z* 248 $[M-AcOH]^+$ (12 5) and IR absorption at 1732 cm^{-1} A vinylic proton at δ5 07 coupled to two vinylic methyls at δ1 57 and 1 65 was also observed establishing the presence of a terminal trisubstituted double bond The signal at δ5 40 was assigned to H-1, since it was shifted upfield to δ4 11 after hydrolysis

The low field signal at 6 67 was assigned to the vinylic proton (H-2) β to the carbomethoxy group which is coupled with H-1 since its irradiation converted the multiplet at δ5 40 to a triplet of doublets (J = 9, 1 2 Hz), indicating homoallylic coupling between H-1 and H-4 protons Further decoupling at δ2 30, the frequency of H-4, converted the multiplet at δ5 40 to a doublet of doublets (J = 9, 1 2 Hz) and collapsed the resonance at δ6 67 to a doublet (J = 1 2 Hz) The relative stereochemistry at C-1 and C-6 was deduced from the large coupling constant observed between H-1 and H-6 (J = 9 Hz), which agrees with a *trans*-diaxial relationship Since the side chain is probably β as in other bisabolene derivatives isolated from plants of the same genus [7, 10], the acetoxy group must be α and the corresponding H-1 proton, β axial The proposed structure **1a** was supported by chemical correlation with 15-acetoxy bisabol-1-one (**1g**) a sesquiterpene isolated from *S ovata* [11] Reduction of **1a** with lithium aluminium hydride gave a mixture of the diol **1e** and its dihydro derivative Selective acetylation of **1e** with acetic anhydride–pyridine afforded the monoacetate **1f** which was oxidized with Jones reagent to yield the ketone **1g**, whose IR and ^1H NMR spectra were identical with those previously published [11]

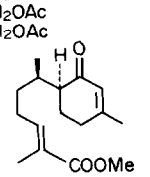
The more polar methyl ester **1b** was a colourless oil, $C_{16}H_{26}O_3$, $[M]^+$ at *m/z* 266, $[\alpha]_D = +41\,3°$ Its ^{13}C NMR (Table 1) and ^1H NMR (Table 2) spectra were very similar to those of the methyl ester **1a** Oxidation of **1b** with Jones reagent produced the keto-ester **1c**, whose spectroscopic data were different from those of dehydrojuvabione (**2**) [9] and compound **3** [12] Since dehydrojuvabione (**2**) possesses significant juvenile hormone activity [9], a biological activity study of compound



	R	R'
1a	OAc	COOMe
1b	OH	COOMe
1c	=O	COOMe
1d	OH	COOH
1e	OH	CH_2OH
1f	OH	CH_2OAc
1g	=O	CH_2OAc



2 [9]



3 [12]

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Table 1 ^{13}C NMR spectra of compounds **1a** and **1b** 20 MHz, $CDCl_3$, TMS as int standard)

C	1a	1b
1	71 05 *d*	68 84 *d*
2	137 35 *d*	141 81 *d*
3	133 42 *s*	131 61 *s*
4	24 66 *t*	24 89 *t*
5	20 93 *t*	20 59 *t*
6	42 05 *d*	45 54 *d*
7	31 34 *d*	31 02 *d*
8	35 07 *t*	35 07 *t*
9	26 12 *t*	26 20 *t*
10	124 66 *d*	124 68 *d*
11	131 37 *s*	131 41 *s*
12	25 62 *q*	25 71 *q*
13	17 66 *q*	17 69 *q*
14	14 60 *q*	14 14 *q*
15	167 06 *s*	167 69 *s*
OMe	51 61 *q*	51 73 *q*
OAc	170 45 *s*	
	20 93 *q*	

The assignments of the carbon shifts are based on comparison with model compounds and off-resonance decouplings

Table 2 ^1H NMR data of compounds **1a–1e** (80 MHz, $CDCl_3$, TMS as int standard)

H	1a*	1b	1c	1d	1e
1	5 40 *m*	4 11 *m*		4 15 *m*	4 05 *m*
2	6 67 *d t*	6 77 *s* (*br*)	6 67 *s* (*br*)	6 90 *s* (*br*)	5 65 *s* (*br*)
4	2 30 *m*	2 30 *m*		2 30 *m*	
10	5 07 *t* (*br*)	5 07 *t* (*br*)	5 06 *t* (*br*)	5 08 *t* (*br*)	5 10 *t* (*br*)
12	1 65 *s* (*br*)	1 65 *s* (*br*)	1 66 *s* (*br*)	1 67 *s* (*br*)	1 68 *s* (*br*)
13	1 57 *s* (*br*)	1 57 *s* (*br*)	1 58 *s* (*br*)	1 58 *s* (*br*)	1 60 *s* (*br*)
14	0 80 *d*	0 80 *d*	0 82 *d*	0 82 *d*	0 81 *d*
15					4 00 *s* (*br*)
OMe	3 73 *s*	3 70 *s*	3 80 *s*		
OAc	2 10 *s*				

*Run at 90 MHz

J (Hz) 1,6 = 9, 9,10 = 7,14 = 7, 10,12 = 10,13 = 1 5, 1,2 = 1,4 = 1 2, 2,4 = 2 3

1c is in progress Finally, compound **1b** was identical with the hydrolysis product of **1a** And **1d** was obtained from **1a** by prolonged hydrolysis with potassium hydroxide in methanol

EXPERIMENTAL

Plant material S salicifolia was collected in the University campus at U N A M (Mexico, D F), in Sept, 1980 A voucher specimen is on deposit at the Herbarium of the Instituto de Biología (U N A M), México

Isolation of the products The aerial parts of the plant (2 5 kg) were extracted with petrol, giving 120 g crude syrup which was chromatographed on 1 5 kg of silica gel using $CHCl_3$–Me_2CO mixtures as eluants Fractions eluted with $CHCl_3$–Me_2CO (19 1) afforded 5 0 g stevinsol [6] and 3 0 g of a mixture of two acids which could not be separated A sample of this mixture (1 0 g) was methylated with excess CH_2N_2 in Et_2O and the mixture of methyl esters chromatographed on 30 g silica gel using petrol–Me_2CO as eluants, giving 400 mg **1a** and 500 mg **1b**

1-*Acetoxy bisabol*-15 *oic acid methyl ester* (**1a**) Colourless oil, $[\alpha]_D$ +95 0° (EtOH, *c* 0 25), IR ν_{max}^{film} cm^{-1} 1735, 1720, 1650; UV λ_{max}^{MeOH} nm (ε) 217 (10358), EIMS (probe) 70 eV, *m/z* (rel int) 308 $[M]^+$ (1 0), 248 $[M-AcOH]^+$ (12 5), 181 $(M-42-C_6H_{13}]^+$ (27), 163 $[M-AcOH-C_6H_{13}]^+$ (59), 109 $[C_8H_{13}]^+$ (100), 82 $[C_6H_{10}]^+$ (42 5), 69 $[C_5H_9]^+$ (25)

1-*Hydroxy bisabol*-15-*oic acid methyl ester* (**1b**) Oil, $[\alpha]_D$ = +41 3° (EtOH, *c* 0 22), UV λ_{max}^{MeOH} nm (ε) 218 (8311), IR ν_{max}^{film} cm^{-1} 3410, 1715, 1650, 1250, EIMS (probe) 70 eV, *m/z* (rel int) 266 $[M]^+$ (14), 181 $[M-C_6H_{13}]^+$ (89), 109 $[C_8H_{13}]^+$ (100), 82 $[C_6H_{10}]^+$ (59), 69 $[C_5H_9]^+$ (61)

1-*Oxo bisabol*-15-*oic acid methyl ester* (**1c**) To a soln of **1b** (200 mg) in Me_2CO (10 ml), Jones reagent was added dropwise After 10 min 150 mg **1c** was obtained by usual work up Oil $[\alpha]_D^{25}$ −50 3° (EtOH, *c* 0 2227), UV λ_{max}^{MeOH} nm (ε) 235 (10,700), IR ν_{max}^{film} cm^{-1} 1725, 1680, 1250 EIMS (probe) 70 eV, *m/z* (rel int) 264 $[M]^+$ (10), 181 $[M-C_6H_{11}]^+$ (100), 179 $[M-C_6H_{13}]^+$ (42), 154 $[C_8H_{10}O_3]^+$ (23), 121 $[C_7H_5O_2]^+$ (23), 109 $[C_8H_{13}]^+$ (50), 95 $[C_7H_{11}]^+$ (18), 69 $[C_5H_9]^+$ (20)

1-*Hydroxy bisabol*-15-*oic acid* (**1d**) Compound **1a** (100 mg) was treated with 20% KOH–MeOH for 24 hr at room temp Usual work-up gave 45 mg **1d** oil $[\alpha]_D$ +40 2° (EtOH, *c* 0 142), UV λ_{max}^{MeOH} nm (ε) 220 (5077), IR ν_{max}^{film} cm^{-1} 3500–2500, 1700, 1650, EIMS (probe) 70 eV *m/z* (rel int) 252 $[M]^+$ (7 3), 234 $[M-H_2O]^+$ (4 9), 167 $[M-C_6H_{13}]^+$ (100), 149 $[M-H_2O-C_9H_{13}]^+$ (36 0), 109 $[C_8H_{13}]^+$ (91), 82 $[C_6H_{10}]^+$ (43), 69 $[C_5H_9]^+$ (31 7)

1-*Hydroxy bisabol*-15-*ol* (**1e**) $LiAlH_4$ was added to a cold soln of **1a** (200 mg) in THF Work-up as usual yielded 50 mg **1e** as a crystalline compound, mp 65–68° UV λ_{max}^{EtOH} nm (ε) 207 (11730), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3300, 1450, 1160

15-*Acetoxy bisabol*-1-*one* (**1g**) Compound **1e** (50 mg) was acetylated with Ac_2O–pyridine, the reaction being monitored by TLC After 10 min the reaction was worked up as usual to give 28 mg **1f** IR ν_{max}^{film} cm^{-1} 3400; ^1H NMR (60 MHz, $CDCl_3$) δ2 05 (3H, *s*, Ac), 4 50 (2H, *s* (*br*), H-15), 4 05 (1H, *d* (*br*), H-1) Oxidation of **1f** with Jones reagent as above, yielded after TLC 9 mg **1g** Colourless oil, IR and ^1H NMR spectra identical with those previously published [11]

Hydrolysis of **1a** Compound **1a** (50 mg) was hydrolysed with NaOH–MeOH at room temp After TLC 19 mg **1b** were obtained IR and ^1H NMR identical with natural product **1b**

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GUAIANE SESQUITERPENES FROM *MAGNOLIA WATSONII*

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Key Word Index—*Magnolia watsonii*, Magnoliaceae, sesquiterpenes, dehydrocostuslactone, watsonol A, watsonol B, 15-acetoxycostunolide, neolignans, obovatol, aporphine alkaloids

Abstract—The leaves and the trunk barks of *Magnolia watsonii* afforded two biosynthetic intermediates of dehydrocostuslactone (watsonol A and watsonol B) along with the neolignans, magnolol, hōnokiol and obovatol, and the aporphine alkaloids, liriodenine and asimilobine

In a recent chemotaxonomical investigation of the sesquiterpenes and the neolignans of magnoliaceous plants [1–6], it was found that the chloroform extracts of *Magnolia watsonii* Hook. fil contained two biosynthetic intermediates of dehydrocostuslactone (**11**), named watsonol A (**12**) and watsonol B (**13**). We now wish to report on the characterization of these new guaiane sesquiterpenes

The chloroform extracts of the fresh leaves and the trunk bark of *M watsonii* afforded three guaiane sesquiterpenes, the major one of which was identified as dehydrocostuslactone (**11**) [7, 8] The second sesquiterpene, watsonol A, mp 65–67°, $C_{15}H_{22}O_2$ (M^+ 234), was obtained as a crystalline substance Its IR spectrum ($CHCl_3$) showed bands assignable to a hydroxyl group (3430 cm^{-1}), and a double bond (1640 cm^{-1}) The ^1H NMR spectrum ($CDCl_3$) resembled that of dehydrocostuslactone (**11**), except for the presence of signals due to a hydroxymethylene group (δ4 06) in place of the γ-butyrolactone function of **11** Acetylation of watsonol A with acetic anhydride and pyridine afforded a diacetate, which showed two acetoxyl methyl signals at δ1 87 and 2 06 in its ^1H NMR spectrum In addition, the ^1H NMR spectrum exhibited signals typical of three terminal methylene double bonds (1H, *d*, J = 2 Hz, δ4 73, 1H, *s* (*br*), δ4 82, 1H, *s*, δ4 94, 1H, *s*, δ5 07, 2H, *s*, δ5 11) at C-15, C-14 and C-13, and a 1H as a triplet at δ3 27 (J = 9 Hz) for the proton attached to the carbon bearing the hydroxyl group at C-6 The latter signal shows the *trans*-diaxial disposition of the protons at C-5 (α), C-6 (β) and C-7 (α), as in dehydrocostuslactone (**11**)

On Jones oxidation of watsonol A, the oxidation product was obtained The structure of this compound was in agreement with dehydrocostulactone (**11**) (IR, MS and ^1H NMR) Therefore, the stereostructure of watsonol A is confirmed to be **12**

The third guaiane sesquiterpene, watsonol B, $C_{17}H_{24}O_3$ (M^+ 276), was obtained as an oil Its IR spectrum contained bands assignable to a hydroxyl group (3530 cm^{-1}), an acetoxyl group (1725 cm^{-1}), and a double bond (1640 cm^{-1}) The ^1H NMR spectrum was superimposable on that of watsonol A (**12**), except for the presence of a signal due to an acetoxyl methyl group

Watsonol B was acetylated with acetic anhydride and pyridine to give an acetate, which was identical with a diacetate of watsonol A Thus, the structure of watsonol B is elucidated as **13** Besides the three guaiane sesquiterpenes, the germacranolide sesquiterpenes, costunolide (**9**) and 15-acetoxycostunolide (**10**) [9], and the eudesman sesquiterpenes, α-eudesmol (**6**), β-eudesmol (**7**) and cryptomeridiol (**8**) [10] were isolated and characterized from the chloroform extracts of the fresh leaves and the trunk