and therefore the neighbouring C-13 and C-8 must be substituted by oxygen functions. (c) The signal at δ2.36 attributed to the C-12 methyl group was shifted to lower field by 0.65 ppm in comparison with that of cinncassiol D_4 [6–8] indicating the presence of an ether bond between C-12 and C-13. (d) The bond formation between C-13 and C-10 accounted for one isolated methine proton at δ2.61 attributable to the methine at C-10.

The final structure deduced for cinncassiol E is that shown by **1**. Cinncassiol E (**1**) is noteworthy because it is a novel diterpene with a new skeleton. The stereochemical evidence was not clear, but the stereochemistry of cinncassiol E was tentatively assumed to be as shown in **1** because of its probable biogenetic relationship with the cinncassiol D types of compounds.

EXPERIMENTAL

Isolation of cinncassiol E. The extraction and separation was described in the preceding paper [1], where cinncassiol E (35 mg) is referred to as compound X.

Cinncassiol E (**1**). FD-MS *m/z*: 421 $[M+Na]^+$, 399 $[M+1]^+$; EI-MS *m/z*: 380 $[M-H_2O]^+$, 352, 290, 194, 169, 149; ^{1}H NMR (100 MHz, pyridine-d_5): δ1.07, 1.14 (each 3H, *d*, J = 6 Hz, 18-Me), 1.73 (3H, *d*, J = 6 Hz, 1-Me), 1.76 (3H, *s*, 9-Me), 2.36 (3H, *s*, 12-Me), 2.13, 2.65 (each 1H, *d*, J = 13 Hz, 14-H_2), 2.61 (1H, *s*, 10-H); (CD_3OD): δ1.00 (6H, *d*, J = 6 Hz, 18-Me_2), 1.28 (3H, *d*, J = 7 Hz, 1-Me), 1.32 (3H, *s*, 9-Me), 1.54, 2.26 (each 1H, *d*, J = 13 Hz, 14-H_2), 1.60 (3H, *s*, 12-Me), 1.98 (1H, *s*, 10-H).

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Phytochemistry, Vol 24, No 8, pp 1850–1852, 1985
Printed in Great Britain

0031–9422/85 $3.00 + 0.00

KAURENIC ACID DERIVATIVES FROM *STEVIA EUPATORIA*

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(*Revised received* 4 *December* 1984)

Key Word Index—*Stevia eupatoria*; Compositae; Eupatoriae; kaurenes; 12β-ethoxy-*ent*-kaur-9(11),16-dien-19-oic acid, 12α-hydroxy-*ent*-kaur-16-en-19-oic acid

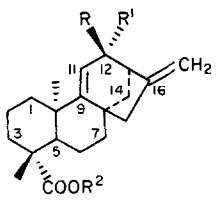
Abstract—Two kaurene type diterpenes were isolated from the aerial part of *Stevia eupatoria*. Their structures and stereochemistry were established by carbon and ^{1}H NMR, chemical transformation and correlation with known compounds.

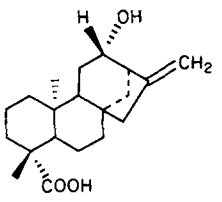
INTRODUCTION

Sweet diterpene glycosides isolated from *Stevia rebaudiana* [1, 2] and *S. paniculata* [3, 4] produce, by hydrolysis, several hydroxylated kaurenoic acid derivatives. Very few compounds of the latter type have been found in *Stevia* species which grow in Mexico. In this paper we describe the isolation and structure determination of 12β-ethoxy-*ent*-kaur-9(11),16-dien-19-oic acid (**1**) and the known 12α-hydroxy-*ent*-kaur-16-en-19-oic acid (**2**) from *S. eupatoria*.

RESULTS AND DISCUSSION

The less polar fraction of *S. eupatoria*, afforded the ethoxy diterpenic acid (**1**) $C_{22}H_{32}O_3$, $[M]^+$ at *m/z* 344. The IR spectrum revealed the presence of a hydroxyl





2

	R	R¹	R²
1	OEt	H	H
1a	OEt	H	Me
1b	OH	H	H
1c	O	O	H

group of a carboxylic acid (3300–2700 cm^{-1}), a carbonyl (1699 cm^{-1}) and a terminal methylene group (1650, 980 and 885 cm^{-1}). The ^1H NMR spectrum (Table 1), showed signals for two tertiary methyl groups, an exocyclic methylene and a vinylic proton of a trisubstituted double bond.

Treatment of **1** with diazomethane gave the methyl ester (**1a**). Its ^1H NMR spectrum exhibited the signal of a methyl of a carbomethoxy group confirming the presence of a carboxylic acid.

Compound **1** losses C_2H_4 upon treatment with acid yielding **1b**, $C_{20}H_{28}O_3$, $[M]^+$ at *m/z* 316, which exhibited in the IR spectrum a strong hydroxyl absorption band at 3440 cm^{-1}. The ^1H NMR spectrum of **1b** did not show signals for the ethoxy group and a new signal was observed, which disappeared upon D_2O addition, which was attributable to a hydroxy group.

Oxidation of the hydroxy acid **1b** with Jones reagent gave an α,β-unsaturated ketone (**1c**), $C_{20}H_{26}O_3$, IR 1650 cm^{-1}; λ_{max}^{EtOH} 250 nm, log ε3.91. The ^1H NMR spectrum (Table 1) showed a remarkable paramagnetic effect of the carbonyl group on the chemical shifts of the terminal methylene protons at δ5.04 (1H, *br s*) and 5.29 (1H, *br s*) and for the vinylic proton at 5.28 (1H, *br s*).

A comparison of the ^1H NMR spectrum with data recorded for other known kaurene type diterpenes [5, 6] and the chemical shifts for **1b** and **1c** suggests that the hydroxy group and the trisubstituted double bond are located at C-12 and C-9, respectively.

A selective catalytic hydrogenation of the terminal methylene of **1c** gave **1d**, whose ^1H NMR spectrum showed signals for one new methyl group at δ0.9 (3H, *d*).

The ^{13}C NMR spectrum (Table 2) of the 12β-ethoxy-*ent*-kaur-9(11),16-dien-19-oic acid (**1**) confirms its structural identity. Resonance assignments were made with the aid of off-resonance decoupled (SFORD) spectra, based on comparison of general chemical shift arguments and reported values of related structures as well as those of tri- and tetracyclic diterpenoids [7–9].

The more polar fractions afforded the 12α-hydroxy-*ent*-kaur-16-en-19-oic acid (**2**), $C_{20}H_{30}O_3$, $[M]^+$ at *m/z* 318. The IR spectrum exhibited absorptions attributable to hydroxyl (3425 cm^{-1}), carbonyl (1699 cm^{-1}) and terminal methylene (1648 and 850 cm^{-1}) groups. The ^1H NMR spectrum (Table 2) showed signals for the two tertiary methyl groups, a carbinolic proton, a terminal methylene and a trisubstituted double bond.

The position of the hydroxyl group located at C-12 was deduced by comparison of chemical shifts of the ^1H NMR spectra of **2**, and with data published in the synthesis of grandiflorine acid [10]. The synthesis of **1b**, **1c** and compounds from the series **2** had been previously reported. The methyl ester of **1b** and the ketone **1c** had also been described as isolated from *Vellozia caput* [11], from *Ambrosia hispida* [12] and from *Viguiera excelsa* [5], respectively.

EXPERIMENTAL

Stevia eupatoria Will, was collected in September (1979), 5 km south of Ixlahuaca, State of Mexico. A voucher is on deposit at the Herbarium of the Instituto de Biología (UNAM), México.

Extraction of the whole plant (6 kg) with MeOH yielded material (130 g) which was chromatographed on 600 g of silica gel. The elution with C_6H_6–EtOAc (9:1) gave **1** which was crystallized from hexane–Me_2CO; (0.01%) mp 170–172°; $[\alpha]_D^{20}$ + 63.7° (*c* 0.259; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200–2700 (OH), 1699 (CO_2H), 1650, 980, 885 (C=CH_2). MS *m/z* (rel. int.): 344 ($[M]^+$, $C_{22}H_{32}O_3$), 329 $[M-Me]^+$ (18.33), 229 $[M-C_6H_{11}O_2]^+$ (68.33), 157 $[M-C_{12}H_{11}O_2]^+$ (25.8), 141 $[M-C_{14}H_{19}O]^+$ (29.16), 107 $[M-C_{14}H_{21}O_3]^+$ (25), 93 $[M-C_{15}H_{23}O_3]^+$ (27 5). Calc. for $C_{22}H_{32}O_3$; C, 76.70; H, 9 36; O, 13.93. Found: C, 76.37; H, 9.57; O, 13.95%. Further column chromatography

Table 1. ^1H NMR data of compounds **1**–**1d** and **2** (100 and 60 MHz, $CDCl_3$, TMS as internal standard)

H	1	1a	1b	1c	1d	2
11	5 34 *d* (*br*)	5 32 *d* (*br*)	5.25 *d* (*br*)	5.82 *s* (*br*)	5.85 *s*	
12			3.82 *m*			3 84 *m*
13	2.90 *m*	2.90 *m*	2.71 *m*	3.42 *m*	2.82 *m*	2.68 *m*
17	4.98 *s* (*br*)	4.97 *s* (*br*)	4.82 *s* (*br*)	5.29 *s* (*br*)		4.77 *s* (*br*)
17′	4.92 *s* (*br*)	4.87 *s* (*br*)	4 94 *s* (*br*)	5.04 *s* (*br*)	0.91 *d*	4 84 *s* (*br*)
18	1.26 *s*	1.17 *s*	1.16 *s*	1.30 *s*	1.29 *s*	1 22 *s*
20	1.08 *s*	0.98 *s*	1.04 *s*	1.17 *s*	1.14 *s*	1 12 *s*
CO_2Me		3 65 *s*				
21	3.58 *m*	3.53 *m*				
22	1.24 *t*	1.20 *t*				

J (Hz); 11,12 = 4; 21, 22 = 8; 12, 13 = 2; 13, 14 = 3; 16, 17 = 6.

Table 2. ^{13}C NMR chemical shift values and assignments of carbons in compounds 1 and 2 (MHz, $CDCl_3$, TMS internal standard)

Carbon	1	2
1	38 25 (*t*)	40.68 (*t*)
2	18.44 (*t*)	19 05 (*t*)
3	29.20 (*t*)	38.09 (*t*)
4	43.39 (*s*)	43.51 (*s*)
5	44.87 (*d*)	56.86 (*d*)
6	20.16 (*t*)	21.86 (*t*)
7	40.77 (*t*)	33.13 (*t*)
8	43 44 (*s*)	43.66 (*s*)
9	159.87 (*s*)	56.12 (*d*)
10	39.03 (*s*)	38.59 (*s*)
11	116.07 (*d*)	41 23 (*t*)
12	80.25 (*d*)	71.78 (*d*)
13	46.42 (*d*)	51.32 (*d*)
14	47.38 (*t*)	26.17 (*t*)
15	44.75 (*t*)	49.23 (*t*)
16	153.29 (*s*)	152.67 (*s*)
17	107.98 (*t*)	104.76 (*t*)
18	23.32 (*q*)	28.44 (*q*)
19	178.46 (*s*)	180.70 (*s*)
20	15 73 (*q*)	14.5 (*s*)
21	64.15 (*t*)	
22	28.28 (*q*)	

elution with C_6H_6–EtOAc (6:1) gave the 12α-hydroxy-*ent*-kaur-16-en-19-oic acid (**2**) (0.013%), which was crystallized from hexane–Me_2CO, mp 240–243°; $[\alpha]_D^{20}$ −36.5° (*c* 0.200; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3425 (OH), 1699 (CO_2H), 1648, 850 (C=CH_2). MS *m/z* (rel. int.): 318 (M^+, $C_{20}H_{30}O_3$) 107 $[M-C_{12}H_{19}O_3]^+$ (100), 303 $[M-Me]^+$ (3.9), 300 $[M-H_2O]^+$ (25.7), 285 $[M-Me-H_2O]^+$ (26.7), 93 $[M-C_{13}H_{21}O_2]^+$ (53.4). Calc. for $C_{20}H_{30}O_3$· C, 75.43; H, 9 50; O, 15 07. Found: C, 75.50; H, 9 32; O, 14.56%.

Methyl ester of **1** The methylation of 200 mg of **1** with CH_2N_2 produced **1a** after crystallization from hexane–Me_2CO (190 mg); mp 85–87°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 1725 (methyl ester); 1650, 870 (C=CH_2). MS *m/z* (rel. int.): 358 ($[M]^+$, $C_{22}H_{34}O_3$) (90.1), 343 $[M-Me]^+$ (19.7), 229 $[M-C_7H_{13}O_2]^+$ (75 4), 155 $[M-C_{14}H_{19}O]^+$ (21 3), 107 $[M-C_{15}H_{23}O_3]^+$ (34.4), 93 $[M-C_{16}H_{25}O_3]^+$ (29.5). Calc. for $C_{23}H_{34}O_3$; C, 77.05; H, 9.56; O, 13.39 Found: C, 76.59; H, 9 45; O, 13 49%.

Hydrolysis of ether **1** The acid **1** (150 mg), dissolved in Me_2CO, was refluxed with 3 ml conc HCl for 30 min and monitored by TLC. The reaction mixture was diluted with H_2O, neutralized with saturated $NaHCO_3$ soln and extracted with $CHCl_3$. After usual work up the residue yielded **1b**, after hexane–Me_2CO crystallization, mp 175–180°; $[\alpha]^{20}$ +63.81°; IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1699 (CO_2H), 1650, 880 (C=CH_2). MS *m/z* (rel. int.): 316 ($[M]^+$, $C_{20}H_{28}O_3$) (26.2), 301 $[M-Me]^+$ (37.4), 298 $[M-H_2O]^+$ (27 8), 283 $[M-Me-H_2O]^+$ (29.5), 201 $[M-C_6H_{11}O_2]^+$ (39 3); 147 $[M-C_{10}H_{17}O_2]^+$ (21.58), 141 $[M-C_{12}H_{15}O]^+$ (22 58), 107 $[M-C_{12}H_{17}O_3]^+$ (38.7), 93 $[M-C_{13}H_{19}O_3]^+$ (45.1), 41 (100). Calc. for $C_{20}H_{28}O_3$; C, 75.91, H, 8.92; O, 15.17. Found: C, 74.71; H, 8.83; O, 13.70%.

Preparation of **1c**. A soln of **1b** (100 mg) in Me_2CO (50 ml) was oxidized with 0.4 ml of Jones reagent at −20°, the reaction being monitored by TLC After 30 min the reaction was worked up as usual to give 93 mg of **1c** from hexane–Me_2CO crystallization, mp 279–280°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3200–2800 (OH), 1699 (CO_2H), 1650 (C=O), 1600, 892 (C=CH_2). Calc. for $C_{20}H_{26}O_3$; C, 76.40; H, 8 34; O, 15.27 Found. C, 75 65; H, 8.33; O, 15.89%

Hydrogenation of **1c** A soln of 79.5 mg of **1c** in 15 ml of EtOAc was hydrogenated using 7 9 mg of PtO as catalyst. Filtration and evaporation gave 70 mg of **1d**, mp 244–246°; UV λ_{max}^{MeOH} nm: 217 (ε3800); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200–2800 (OH), 1690 (CO_2H), 1650 (C=O). Calc. for $C_{20}H_{28}O_3$; C, 75.91; H, 8.92; O, 15.17. Found: C, 75.23; H, 9.15; O, 15.82%.

Acknowledgement—We thank M. Rosales Hoz for many valuable discussions.

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