Extraction and isolation. The plant material was air dried in the shade at room temp. and finally powdered. This material (260 g) was extracted at room temp. with Et_2O–MeOH (1:1) (1.5l. × 2) for 24 hr. The combined extracts were concd *in vacuo* and the residue defatted with MeOH. The residue (3.5 g) was chromatographed by CC on silica gel. The column was packed in petrol–Et_2O (1:1) and on elution with the same solvent gave fr 1 while fr 2 was eluted with petrol–Et_2O (1:3) and Et_2O.

Fr 1 on crystallization with petrol–Et_2O afforded colourless crystals (25 mg) of santonin (**4**) [6]. Fr 2 afforded a semisolid which on prep. TLC in Et_2O–petrol (3:2) gave a band at R_f 0.45 which on further prep. TLC in CH_2Cl_2–C_6H_6–Et_2O (3:3:1) with two developments gave three bands (2/1–2/3). Band 2/1 on HPLC (RP 8, MeOH–H_2O, 7:3, flow rate *ca* 3 ml/min, 100 bar) afforded 9 mg of **1** as a colourless oil (R_t 5.9 min) and 7 mg of **2** as a colourless crystals (R_t 7.1 min). Band 2/2 afforded **3** as colourless crystals, mp 176–177° [3] and band 2/3 was gallicin (**5**), mp 115° (114–116° [5]).

Compound **1**. Colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (γ-lactone), 1610, 1460, 1385, 1130, 1100; MS *m/z* (rel. int.): 250.1579 $[M]^+$ (18), 235 $[M-Me]^+$ (17), 232 $[M-H_2O]^+$ (8), 217 $[235-CO]^+$ (6), 61 (100), 55 (74); ^1H NMR ($CDCl_3$): δ5.04 (*dd*, *J* = 10, 2 Hz, H-5), 4.40 (*dd*, *J* = 10, 9 Hz, H-6), 2.83 (*ddd*, *J* = 16, 12, 4.5 Hz, H-2), 2.60 (*dd*, *J* = 12, 4.5 Hz, H-2'), 1.94 (*d*, *J* = 2 Hz, 4-Me), 1.21 (*d*, *J* = 7 Hz, 13-Me) and 1.03 (*d*, *J* = 7.5 Hz, 14-Me).

Compound **2**. Colourless crystals, mp 132° (128–130° [5]), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765 (γ-lactone), 1610, 1460, 1390, 1140, 1100; MS *m/z* (rel. int.): 248 $[M]^+$ (7), 230 $[M-H_2O]^+$ (5), 220 $[M-CO]^+$ 81 (100), 61 (65); ^1H NMR ($CDCl_3$): δ5.81 and 5.67 (2*s*, H-14), 5.03 (*dd*, *J* = 10, 2 Hz, H-5), 4.36 (*dd*, *J* = 10, 9 Hz, H-6), 1.77 (*d*, *J* = 2 Hz, 4-Me) and 1.24 (*d*, *J* = 7 Hz, 11-Me).

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TWO CALEINES FROM *CALEA ZACATECHICHI**

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Key Word Index—*Calea zacatechichi*; Compositae; Asteraceae; sesquiterpene lactones; caleines.

Abstract—Caleine E and F, two new sesquiterpene lactones, were isolated from *Calea zacatechichi*. Their structures and stereochemistry were determined by spectroscopic means and chemical derivatization of caleine E.

INTRODUCTION

Calea zacatechichi Schldl. is a wild shrub which grows in southern Mexican fields and has been used in folk medicine for stomach disease and in magic treatments as a dream inducer [1, 2]. Previous chemical work on *Calea zacatechichi* revealed the presence of caleines A (**3**), B (**4**), C and D, cromenes, flavones and acetylenic compounds [1, 3, 4].

The present investigation led to the isolation and identification of the known compounds 5 hydroxy-7,4'-dimethoxyflavone [5], acetylerioflorine [6], zexbrevine [7] and two new sesquiterpene lactones that we name caleine E (**1**) and F (**2**).

RESULTS AND DISCUSSION

Two different collections of *C. zacatechichi* were investigated for sesquiterpene lactones and flavonoids. The first collection (November 1979) afforded acetyleriof-

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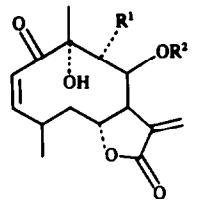
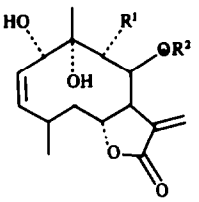


1 R^1 = H; R^2 = metacrilate
2 R^1 = H; R^2 = tiglate
3 R^1 = OAc; R^2 = angelate
4 R^1 = Oangelate; R^2 = Ac
6 R^1 = H; R^2 = isovalerate



5 R^1 = H; R^2 = metacrilate

lorine, zexbrevine and a new compound named caleine E (1).

Caleine E (1) $C_{19}H_{24}O_6$ mp 150–151° $[\alpha]_D = +47.4°$, shows IR absorption indicative for hydroxyl (3460), γ-lactone (1760), ester (1720), α,β-unsaturated ketone (1690) and terminal methylene (1630 cm^{-1}). Carefully comparison of the ^1H NMR spectra of caleine E (1) and neurolenin A (6) whose structure was established by X-ray crystallography [8], shows that they only differ by the ester at C-8, which in 1 is a metacrylate showing two one-proton broad singlets at δ6.0 and 5.0 and one three-proton singlet at 1.85. Further evidence is provided from the characteristic mass spectral peaks at *m/z* 262 $[M^+, C_4H_6O_2]$ and 69 $[C_4H_5O]^+$.

Reduction of 1 with sodium borohydride in the presence of $CeCl_3$ [10] gave the diol (5) whose ^1H NMR spectrum shows a broad singlet at δ4.3 for H-7 (Δδ = 1.65 ppm) and a multiplet at 3.65 for H-4 (Δδ = 0.57 ppm). The only explanation for these downfield shifts is that H-4 and H-7 in 5 are close to the newly formed alcohol at C-1 and must therefore be α-oriented.

The second collection of *C. zacatechichi* from the same locality gave the known 5-hydroxy-7,4'-dimethoxyflavone and a mixture (HPLC analyses) of caleine E (1) and another new compound named caleine F (2).

The purification of caleine F (2) was achieved by the addition of 1-propanethiol to the mixture of caleine E and F in a borate buffer solution (pH 9.2) [11]. The mixture of adducts was separated by preparative TLC and pure samples of 1 and 2 were obtained by heating ethyl acetate solutions of adducts over silica gel. HPLC analyses confirmed that no further transformations were induced during the above treatments.

The ^1H NMR spectrum of caleine F (2) showed similar signals as those for caleine E (1) and neurolenine A (6) (Table 1), except that a tiglate ester at C-8 is present. The mass spectrum of 2 confirmed the presence of the tiglate.

EXPERIMENTAL

Aerial parts (1.42 kg) of *Calea zacatechichi* Schldl. (collected on Nov. 1979, *ca* 30 km of Telixtlahuaca, Oaxaca, México, specimens were deposited at the Herbarium of the Instituto de Biología, UNAM, voucher: MEXU 324095), were extracted with $CHCl_3$ affording 21 g of residue. The extract was fractionated over tonsil [12] using hexane, $CHCl_3$ and ethyl acetate. The $CHCl_3$ extract (9.7 g) was chromatographed on silica gel using hexane–EtOAc as the developing solvent mixture.

Elution with hexane–EtOAc (6:4) gave 1.5 g of residue which were rechromatographed on silica gel with the same solvent mixture affording 50 mg of white crystals, mp 202–204° uncorr. ($CHCl_3$–isopropylether), identified as acetylerioflorine by comparison with an authentic sample [6] (mp, mmp, IR, ^1H NMR, MS, co-TLC). The fractions eluted with benzene–EtOAc (7:3) afforded 335 mg of caleine E (1) as white crystals, mp 150–151° uncorr. (Me_2O–isopropylether) $[\alpha]_D = +47.4°$ ($CHCl_3$); IR ν_{max} cm^{-1}: 3460, 1760, 1710, 1690, and 1630. UV (MeOH) λ_{max} 214 nm (ε = 13660). MS *m/z* (rel. int.): 348 $[M]^+$ (0.2). 330 (1.8), 244 (0.6), 67 (100). $C_{19}H_{24}O_6$ requires M^+ at *m/z* 348.

The fractions eluted with EtOAc yielded 50 mg of white crystals, mp 215–217° uncorr. ($CHCl_3$–isopropyl ether) identified

Table 1. ^1H NMR data* of sesquiterpene lactones 1, 2, 5 and 8

	Neurolenine A (6) [8]	Caleine E (1)	Caleine F (2)	5
1-H				4.3 *br s*
2-H	6.58 *d* (12)	6.45 *d* (12)	6.45 *d* (12)	5.25 *m*
3-H	5.88 *t* (12)	5.85 *t* (12)	5.85 *t* (12)	5.25 *m*
4-H	3.08 *m* (12, 6)	3.08 *m* (12, 6)	3.05 *m* (12, 6)	3.65
6-H	4.5 *dd* (12, 5)	4.5 *dd* (12, 5)	4.5 *dd* (12, 6)	4.65 *ddd* (12, 5, 1)
7-H	2.61 *br s*	2.65 *br s*	2.65 *br s*	4.3 *br s*
8-H	5.2 *ddd* (11, 6, 3)	5.3 *ddd* (10, 4, 2)	5.30 *ddd* (10, 4, 2)	5.5 *m*
13-H	6.27 *br s* (1)	6.23 *d* (2)	6.23 *d* (2)	6.2 *br s*
13-H'	5.73 *d* (1.5)	5.74 *d* (2)	5.74 *d* (2)	5.7 *br s*
14-H	1.44 *s*	1.42 *s*	1.42 *s*	1.3 *s*
15-H	1.13 *d* (7)	1.14 *d* (6)	1.14 *d* (6)	1.0 *d* (6)
	0.89 *d* (7)	6.0 *br s*	6.75 *m*	5.95 *br s*
	1.31 *d* (7)	5.0 *br s*	1.75 *m*	5.0 *br s*
	3.09 *m*	1.85 *s*		1.8 *s*

*At 80 MHz in $CDCl_3$ with TMS as internal standard. Chemical shifts are in ppm. Values in parentheses are coupling constants in Hz.

as zexbrevin by comparison with an authentic sample [7], (mp, mmp, IR, ^{1}H NMR, MS, co-TLC) and 200 mg of a mixture of caleine E (1) and caleine F (2).

A second collection of *C. zacatechichi* from the same locality (May 1982) gave, after chromatographic separation, 25 mg of yellow crystals, mp 170–172°, identified as 5-hydroxy-7,4'-dimethyl flavone, by comparison with an authentic sample [5] (mp, mmp, IR, ^{1}H NMR, MS, co-TLC) and 200 mg of a mixture of caleine E (1) and caleine F (2).

HPLC analysis. Solvent: EtOAc isopropyl ether–hexene (3:3:4) column: micropack Si10, detector: refraction index, flow rate: 230 ml/hr, retention time: caleine F (2) 9 min 90%, caleine E (1) 7.2 min 10%.

Isolation of caleine F (2). A sample of 60 mg of the above mixture was dissolved in 2 ml of THF to which 0.1 ml of propanethiol and 2 ml of buffer pH 9.2 (borate) were added. After 18 hr at room temp. the reaction mixture was diluted with 5 ml of buffer soln. Usual work up [11] gave 70 mg of a mixture of adducts. The mixture was separated by prep. TLC (Me_2CO–$CHCl_3$ 1:1) yielding 20 mg of the adduct of 1 and 18 mg of the adduct of 2. Each adduct was dissolved in 10 ml of EtOAc to which 30 g of silica gel were added. After refluxing the suspensions for 18 hr, 10 mg of 1 and 5 mg of 2 were obtained. Caleine F (2) shows mp 141–143°, IR ν_{max} cm^{-1}: 3470, 1765, 1715, 1695, 1645. MS *m/z* (rel. int.): 362 (0.7), 344 (0.3), 83 (100), 55.1 (39.8). $C_{20}H_{26}O_6$ requires M^+ at *m/z* 362.

Reduction of 1. A sample of 100 mg of caleine E (1) was dissolved in 2.5 ml soln of 0.4 M of $CeCl_3 \cdot 6H_2O$ and then treated with 11.4 mg of $NaBH_4$ at 0°. After 15 min it was quenched with a sat. soln. of NaCl. Usual work up [10] gave 22 mg of 5, IR ν_{max} cm^{-1}: 3460, 1760, 1710, 1660.

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KAURENE DERIVATIVES FROM *ALEPIDEA AMATYNSIA*

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Key Word Index—*Alepidea amatynsia*; Umbelliferae; diterpenes; kaurene derivatives.

Abstract—*Alepidea amatynsia* afforded several known diterpenes, *ent*-16-kauren-19-oic acid, its 9(11)-dehydro derivative, *ent*-16-kauren-12-on-19-oic acid, wedelia *seco*-kaurenolide and a further *seco*-diterpene. The structure of the latter was established by ^{1}H NMR spectroscopy.

INTRODUCTION

The small South African genus *Alepidea* (Umbelliferae, subfamily Saniculoideae, tribe Saniculeae) so far has not been studied chemically. Careful separation of the extracts of the roots and the aerial parts of *A. amatynsia* Eck. et Leyh. each yielded *ent*-16-kauren-19-oic acid, its 9(11)-dehydro derivative, the corresponding 12-keto derivative [1], wedelia *seco*-kaurenolide 1 [2] and a further diterpene, the 3β-acetoxy derivative 2. The structure of 2 followed from the molecular formula and from the ^{1}H NMR and ^{13}C NMR spectral data which were in part very similar to those of 1 [2] (Table 1). The presence of an acetoxy group at C-3 was deduced from the downfield shift of the C-2, C-3 and C-4 signals and the shielding effect at C-5, while the couplings of H-3 indicated a β-orientation of the oxygen function. An α-acetoxy group