Phytochemistry, Vol. 27, No. 10, pp. 3187 3196, 1988.
Printed in Great Britain.

0031 9422/88 $3.00 + 0.00

TERPENOIDS FROM *AGERATINA SALTILLENSIS*

NIANBAI FANG*, SANGGONG YU†, TOM J. MABRY, KHALIL A. ABBOUD and STANLEY H. SIMONSEN

Department of Botany, University of Texas at Austin, Austin, Texas 78713, U.S.A.; Department of Chemistry, University of Texas at Austin, Austin, Texas 78713, U.S.A.

(*Received in revised form* 21 *October* 1987)

Key Word Index—*Ageratina saltillensis*; Compositae; Eupatorieae; diterpenoids; sesquiterpene lactones; sesquiterpenes.

Abstract—Twenty-two terpenoids were isolated from *Ageratina saltillensis* including eighteen new compounds, namely, 16-hydroxy-3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 3α,4β,16-trihydroxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 3α,4β-dihydroxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 3α-methoxy-4β-hydroxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 3-oxo-4β-hydroxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, 2β-hydroxy-3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide. 2β-hydroxy-5β,10β-*cis*-17α,20α-cleroda-3,13(14)-diene-15,16-olide, (13*Z*)-2β-hydroxy-5β,10β-*cis*-17α,20β-cleroda-3,13(14)-diene-15-oic acid, (13*Z*)-2-oxo-5β,10β-*cis*-17α,20β-cleroda-3,13(14)-diene-15-oic acid, 3,4β-epoxy-5β-10β-*cis*-17α,20α-cleroda-15-oic acid, 16-hydroxy-3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-15-ol, 8β-5′-[5″-hydroxytigloyloxy]-tigloyloxy-3-dehydro-4β,15-dihydrozaluzanin C, 8β-5′-[5″-hydroxytigloyloxy]-tigloyloxy-4β,15-dihydrozaluzanin C, 4′-desoxy-3-desacetoxy-3β-hydroxyprovincialin, 5′-[5″-hydroxytigloyloxy]-5′-hydroxyheliangine, 3β-acetoxyliacylindrolide, 6-eudesmene-4α-ol and 6,15α-epoxy-1β,4β-dihydroxyeudesmane. The stereochemistry of one of the four known compounds, 3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, has been revised by X-ray analysis. The stereochemistry of four compounds were established by NOE experiments.

INTRODUCTION

Ageratina (tribe Eupatorieae, Compositae) produces a wide variety of terpenoids including sesquiterpene lactones of the germacrolides, guaianolides and heliangolides types [1–7]. Some species of *Ageratina* contain sesquiterpenes but no sesquiterpene lactones [8] and a few species contain diterpenes [2, 4, 9, 10]. Here we report 22 terpenoids from *Ageratina saltillensis* (B. L. Robo) King & Robinson, of which 13 are diterpenes, seven are sesquiterpene lactones and two are sesquiterpenes. However, the dichloromethane extract showed no evidence of chromene and thymol derivatives, which are common in other species of *Ageratina*. We report here detailed data for 18 compounds which are new. 3,4β-Epoxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide, the major component of plants in this population, was previously reported with an incorrect stereochemistry [5, 11], which has now been revised on the basis of X-ray analysis. The three known compounds are 20-hydroxygeranylnerol, 4′-desoxy-3-desacetoxy-3α-hydroxyprovincialin and liacylindrolide.

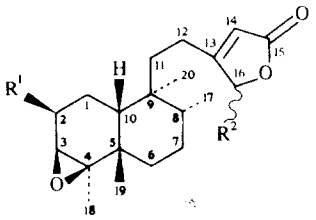
RESULTS AND DISCUSSION

Diterpenes (1–13)

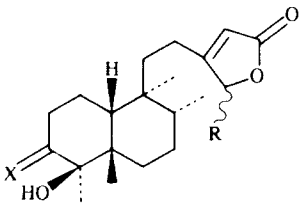
^{1}H NMR signals for the methyl groups (Table 1) and carbon resonances in the ^{13}C NMR spectra of each compound (Table 2) suggested that **1–13** are diterpenes. Moreover, comparison of the ^{1}H NMR, ^{13}C NMR and mass spectra data of **1** with data for a known compound [5, 11] indicated that **1** (mp 91°), $C_{20}H_{30}O_3$, had been reported earlier with an incorrect stereochemistry. In 1971 Anthonsen and Bergland obtained **1** from *Solidago shortii* and assigned the same configuration as that of the elongatolides, such as hardwickiic acid, which has a 3,4α-epoxide in the 5α,10β-*trans*-fused series [11]. In 1986 the same diterpene was isolated from *Ageratina cronquistii* [5] and on the basis of the stereochemistry of known clerodane diterpenes as well as the results of NOE experiments, the configuration was assigned as a 3,4α-epoxide in the 5β,10β-*cis*-fused series [5]. In the present study **1** was isolated as the major constitutent of *A. saltillensis* and X-ray analysis indicated that the structure and configuration of **1** is indeed 3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide. An ORTEP drawing of **1** with the atom labelling scheme is shown in Fig. 1. The atomic positional parameters with their equivalent isotropic thermal parameters, the anisotropic thermal parameters, all bond lengths and angles, and the torsion angles are available from the Cambridge Files. Observed and calculated structure factors are available upon request from the fourth author. The main objective in determining the crystal structure of **1** was to deduce the absolute configuration as well as the relative configurations at C-3, C-4, C-5, C-8, C-9 and C-10. Although the absolute configuration could not be determined, the stereo drawing of the molecule provides the relative configurations at the previously mentioned carbon atoms. Atoms O-1, C-19 and C-11 can all be seen occupying the proper axial positions relative to the ring. These atoms, therefore, are labelled β-substituents; C-17

*Hubei College of Chinese Traditional Medicine, Wuhan, China.

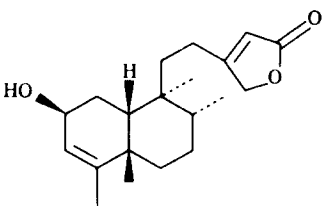
†Wuhan Institute of Medical Sciences, Wuhan, China.



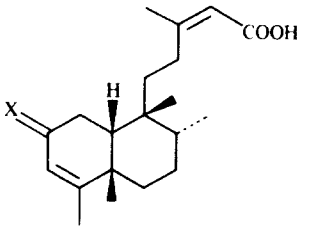
1 R^1 = H, R^2 = H
2 R^1 = H, R^2 = OH
7 R^1 = OH, R^2 = H



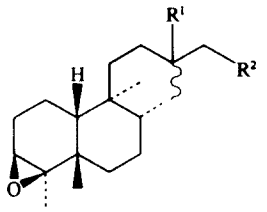
3 X = H,cOH, R = OH
4 X = H,cOH, R = H
5 X = H,cOMe, R = H
6 X = O, R = H



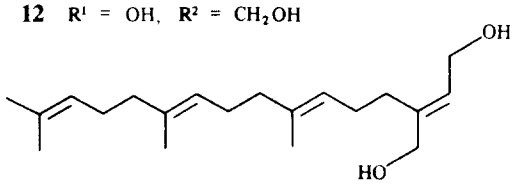
8



9 X = H,βOH
10 X = O



11 R^1 = H, R^2 = COOH
12 R^1 = OH, R^2 = CH_2OH



13

is in the equatorial position and so is an α-methyl group. The H-atom bonded to C-10 is labelled β because it occupies an axial position relative to the ring. The B-ring adopts the twisted conformation while the C-ring is planar as evidenced from by the respective endocyclic torsion angles. Carbon atoms 2, 3, 4 and 5 in the A-ring approach planarity because of the epoxy-ring. Since the coupling between the signal at δ1.62 (H-6a) and that at δ1.21 was exhibited in the 2D-COSY of **1**, the three-proton singlet at δ1.21 could be assigned to Me-19, not to Me-18 (see ref. [5], p. 421: 2D-COSY Plot of **1**). After correcting the assignment of Me-18 (δ1.15) and Me-19 (δ1.21), the NOE results [5] were consistent with the result of the X-ray analysis of **1**, namely, 3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide.

The mass spectrum of **2** exhibited a molecular ion peak at *m/z* 334 (7.3%) in accord with a $C_{20}H_{30}O_4$ formula, that is, one more oxygen than in **1**. The IR spectrum of **2** was similar to that of **1** except for a hydroxyl absorption at 3330 cm^{-1}. A hydroxyl group at C-16 clearly followed from the ^1H NMR signal for H-16: a two-proton signal at δ4.73 for **1** corresponded to the one-proton signal at δ5.95 for **2**, but the stereochemistry at C-16 could not be determined on the basis of these data. This assignment of a hydroxyl group at C-16 is strongly supported by the ^{13}C NMR spectrum of **2**, most notably by the signal at δ99.3 (*d*) for C-16. The ^1H and ^{13}C NMR data for **2** (Tables 1 and 2) indicated that except for the C-16 hydroxyl group compound **2** had the same structure as **1**. Therefore, compound **2** is 16-hydroxy-3,4-β-epoxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide.

The ^1H NMR data for **3** ($C_{20}H_{32}O_5$) suggested that it was identical to **2** except for the 3, 4-epoxide being opened to a dihydroxyl system: δ3.00 (*d*, 5.9, H-3) for **2** (epoxide) and δ 3.56 (*br s*, H-3) for **3** (dihydroxyl system). This conclusion was supported by the ^{13}C NMR data. The signals at δ62.9 (C-3) and 66.5 (C-4) for **2** were replaced by signals at δ76.0 (C-3) and 77.1 (C-4) for **3**. If epoxide **2** is the biogenetic precursor for **3** the 3-hydroxyl group should be α since opening of the epoxide ring should give an anti-relationship of the two hydroxy groups. The ^1H NMR data for **3** indicated the same 16-hydroxy-butenolide side chain at C-9 as in **2**. Thus, we assign **3** as 3α,4β-16-trihydroxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide.

The structure of **4** could easily be deduced from the molecular formula $C_{20}H_{32}O_4$ ($[M]^+$ at *m/z* 336) and the ^1H and ^{13}C NMR spectra (Tables 1 and 2), especially when these data were compared with the same data for **1** and **3**. These comparisons indicated that **4** and **1** have the same butenolide side chain at C-9 and **4** and **3** have the same skeletal moiety. Thus, the structure of **4** is 3α, 4β-dihydroxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide.

Table 1. ^{1}H NMR data for diterpenes **1–12*** ($CDCl_3$, δ-scale in ppm, 360 MHz for **1–6** and **10–12**, 500 MHz for **7–9**)

H	1†	2	3‡	4	5	6	7	8	9	10	11	12
1	1.40 m	1.40						1.76	1.48	2.60 *dd*	1.40 *m*	1.40
1	1.29 *m*	1.29						2.02	2.12 *ddd*	2.29	1.13*m*	1.13
2	1.99 *m*	1.99	2.24	2.24			4.12 *m*	4.14 *m*	4.30		1.99 *m*	1.95
2	1.65 *m*	1.65									1.65 *m*	1.63
3	2.98 *d*	3.00	3.56 *br s*	3.54	3.00		3.21 *d*	5.49 *dd*	5.20 *br s*	5.70	2.99 *d*	2.97
6a	1.62 *m*	1.62						1.78	1.67	1.82	1.62	1.60
6b	1.38 *m*	1.38						1.54	1.51	1.52	1.38	1.30
7a	1.39 *m*	1.39						1.40	1.42	1.42	1.39	1.30
7b	1.44 *m*	1.44						1.45	1.38	1.38	1.44	1.30
8	1.51 *m*	1.51						1.45	1.47	1.58	1.51	1.45
10	1.40 *m*	1.40					1.40	1.50	1.35 *t*	1.92 *dd*	1.40 *m*	1.40
11a	2.01 *ddd*	2.15					2.03	2.03	1.52	1.52	1.96	1.68
11b	1.39 *m*	1.39						1.38	1.36	1.38	1.36	1.30
12a	2.46 *br ddd*	2.51	2.56	2.52	2.52		2.46	2.46	2.82 *ddd*	2.67	1.72 *br td*	1.75
12b	2.23 *br ddd*	2.33		2.27	2.27		2.22	2.31	1.95 *ddd*	2.05	1.65 *m*	1.60
13											1.87 *m*	
14a	5.79 *t*	5.74 *br s*	5.80	5.80 *t*	5.82	5.81	5.82	5.81	5.63 *br s*	5.60	2.39 *dd*	2.00 *m*
14b											2.12 *dd*	1.90 *m*
15												3.81 *m*
16a, b	4.71 *d*	5.95 *br d*	5.96	4.73 *d*	4.73	4.73	4.72	4.72	1.87 *d*	1.85	0.96 *d*	1.13
17	0.79 *d*	0.76	0.80	0.78	079	0.84	0.81	0.84	0.80 *d*	0.84	0.78 *d*	0.76
18	1.15 *s*	1.12	1.10	1.10	1.10	0.99	1.21	1.66 *d*	1.58 *d*	1.87 *d*	1.16 *s*	1.16
19	1.21 *s*	1.18	1.22	1.22	1.21	1.05	1.24	1.17	1.02	1.09	1.24	1.24
20	0.90 *s*	0.87	0.94	0.93	0.94	0.87	0.92	1.00	0.70	0.77	0.85	0.84
3-OMe					3.27 *s*							
4-OH						9.75 *s*						

J(Hz): Compounds **1** and **4–8**: 8,17 = 6.6; 11a, 12a = 4; 11a, 12b = 13; 12a, 12b, = 15.5; 14,16 = 1.2; compounds 1,2,11 and 12: 2, 3 = 5.9; compounds **3** and **4**: 8, 17 = 6.6; 11a, 11b = 13; 11a, 12a = 4; 11a, 12b = 13; 12a, 12b = 15.5; 16, OH = 6.6; Compound **7**: 2, 3 = 5.9; compound **8**: 2, 3 = 4.9; 3, 15 = 0.9; compound **9**: 1, 1 = 12; 1, 10 = 2; 1, 2 = 2.1; 1, 2 = 9; 1, 10 = 14; 3, 18 = 1; 8, 17 = 6.2; 11a, 12a = 5; 11a, 12b = 5, 11b, 12a = 5; 11b, 12b = 2; 12a, 12b = 12; 14, 16 = 1; compound **10**: 1, 10 = 3.1; 1, 1 = 17.5; 1, 10 = 14; 3, 18 = 1; 8, 17 = 5.9; 11a, 12a = 5.1; 11a, 12b = 3.9; 12a, 12b = 11.8; 14, 16 = 1; compound **11**: 11b, 12a = 4; 12a, 12b = 11; 13, 14a = 5.6; 13, 14b = 8.7; 13, 16 = 6.6; 14a, 14b = 15; compound **12**: 11b, 12a = 5; 12a, 12b = 13.

*Coupling patterns are not repeated if identical with the proceeding column.

†Used as a model compound.

‡$CDCl_3$ + 5% MeOH-d_4 as solvent.

Four other diterpenes (**5–8**) also contained an ethylbutenolide side chain at C-9 on the basis of ^{1}H and ^{13}C NMR spectra (Tables 1 and 2). For compound **5** (mass spectrum: *m/z* 350 for $C_{21}H_{34}O_4$), the presence of a methoxyl group was evident from the ^{1}H NMR (a three-proton singlet at δ3.27) and ^{13}C NMR spectra (singlet at δ57.3). Both **4** and **5** exhibited similar mass spectral fragmentation patterns (see Experimental); thus, they should have similar structures. The structural difference between **4** and **5** appears to be the presence of a hydroxyl group at C-3 for **4** and methoxyl group at the same position in **5**. This conclusion was supported by comparison of the ^{1}H and ^{13}C NMR spectra of **4** and **5** (Tables 1 and 2). Therefore, compound **5** is 3α-methoxy-4β-hydroxy-5β, 10β-*cis*-17α, 20α-cleroda-13(14)-en-15, 16-olide.

The IR spectrum of **6** indicated the presence of a hydrogen-bonded hydroxyl group ($\nu_{max}^{CHCl_3}$ 3200–3600 cm^{-1}, *br*). Moreover, from the comparison of the IR spectrum of **6** with that of **1**, it was evident that one more carbonyl group (1716 cm^{-1}, *s*) was present in **6**. The mass spectrum of **6** was similar to that of **1** (see Experimental), thus indicating the presence of a bicyclic clerodane skeleton with a butenolide side chain at C-9. The ^{1}H NMR spectrum of **6** exhibited three methyl singlets and one methyl doublet and showed no low field signals other than those for H-14, H-16a, b and a hydroxyl proton (which was strongly deshielded by hydrogen bonding). The carbonyl and hydroxyl groups are assigned to C-3 and C-4, respectively, and compound **6** is, therefore, 3-oxo-4β-hydroxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15,16-olide.

The ^{1}H and ^{13}C NMR spectral data for **7** (mass spectrum: *m/z* 334 for $C_{20}H_{30}O_4$) suggested that **7** had a skeleton similar to that of **1**. The IR spectrum of **7** indicated the presence of one hydroxyl group ($\nu_{max}^{CHCl_3}$ 3470 cm^{-1}); this was supported in the ^{1}H NMR spectrum by a signal at δ4.12 (*br s*). The hydroxyl could be assigned to C-2 from the ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2), which showed clearly that **7** and 2α,16-dihydroxy-3α,4α-epoxykolavenic-15-acid lactone [12] are epimers. The relative stereochemistry of **7** was determined by NOE experiments. Thus, in $CDCl_3$ clear effects were obtained between Me-20 (δ0.92), Me-17 (δ0.81), H-12b (δ2.22) and H-11b (δ1.58) and between Me-18 (δ1.21) and H-3 (δ3.21). These effects required the same stereo-

Table 2. ^{13}C NMR data for diterpenes **1**–**12** (90 MHz, $CDCl_3$, δ-scale in ppm)

C	1*	2	3†	4	5	6	7	8	9	10	11	12
1	20.2 *t*	19.8 *t*	18.0	17.9 *t*	18.3	26.5	29.4 *t*	34.8	36.5 *t*	35.5	19.8	19.9
2	30.4 *t*	30.2 *t*	28.9	28.9 *t*	28.9	35.9	63.0 *d*	64.7	69.3 *d*		30.5	30.5
3	62.5 *d*	62.9 *d*	76.0	76.0 *t*	85.3	207.3	63.6 *d*	123.8	124.4 *d*	125.4	62.7	62.8
4	65.8 *s*	66.5 *s*	77.1	76.8 *s*	‡	60.8	68.1 *s*	147.4	147.2 *s*	173.2	66.1	66.2
5	38.1 *s*	37.8 *s*	42.4	42.4 *s*	42.7	49.0	37.8 *s*	39.4	38.7 *s*	39.8	37.9	38.0
6	25.2 *t*	24.8 *t*	28.8	28.7 *t*	28.5	25.6	26.9 *t*	29.7	28.7 *t*	26.9	24.9	25.0
7	27.3 *t*	27.0 *t*	27.8	27.7 *t*	27.8	28.9	30.0 *t*	27.2	27.4 *t*	27.2	27.2	27.2
8	36.9 *d*	36.7 *d*	36.7	36.7 *d*	36.6	31.1	34.8 *d*	37.3	36.0 *d*	36.0	36.9	37.1
9	38.6 *s*	38.3 *s*	39.0	38.9 *s*	39.0	36.8	37.9 *s*	38.3	38.7 *s*	39.0	38.2	38.1
10	41.8 *d*	41.5 *d*	42.5	42.2 *d*	42.6	45.9	36.6 *d*	40.7	45.4 *d*	45.6	41.4	41.3
11	33.5 *t*	32.7 *t*	33.0	33.7 *t*	33.8	30.2	33.3 *t*	31.8	27.7 *t*	34.6	31.1	26.8
12	23.5 *t*	22.3 *t*	22.7	23.6 *t*	23.5	23.6	23.2 *t*	23.2	36.5 *t*	35.9	30.6	36.3
13	171.3 *s*	171.0 *s*	171.3	171.9 *s*	171.3	171.0	171.3 *s*	171.2	163.7 *s*	160.4	32.6	73.9
14	115.1 *d*	116.6 *d*	116.7	114.7 *d*	115.0	115.2	114.0 *d*	115.0	115.7 *d*	116.2	41.3	41.2
15	174.2 *s*	171.6 *s*	171.7	174.2 *s*	174.2	174.0	174.0 *s*	173.9	170.8 *s*	168.2	177.6	59.4
16	73.1 *t*	99.3 *d*	99.3	73.1 *t*	73.1	73.3	73.0 *t*	73.0	18.2 *q*	18.8	19.8	29.5
17	15.5 *q*	15.3 *q*	15.7	15.7 *q*	15.8	15.4	15.3 *q*	15.3	15.8 *q*	15.5	15.4	15.5
18	29.3 *q*	29.9 *q*	28.9	28.7 *q*	23.8	14.3	28.8 *q*	26.9	25.6 *q*	25.3	29.4	29.2
19	19.0 *q*	18.8 *q*	21.0	21.0 *q*	21.0	19.6	18.4 *q*	19.1	19.9 *q*	18.2	18.9	18.9
20	21.9 *q*	21.7 *q*	21.7	21.9 *q*	22.3	23.6	21.7 *q*	23.2	17.6 *q*	17.5	21.7	21.8
3-OMe					57.3							

*Used as a model compound.
†$CDCl_3$ + 5% MeOH-d_4 as solvent.
‡Signal for C-2 was not observed using a 7 mg sample.

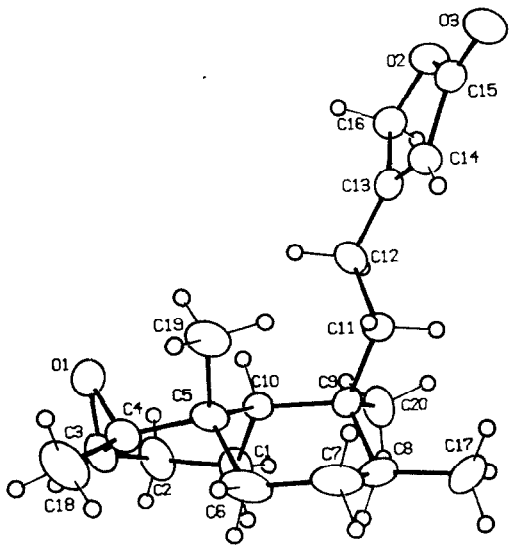


Fig. 1.

chemistry for **7** as for **1**. Moreover, NOE experiments suggested that the C-2 hydroxy group is β since irradiation of the signal at δ3.21 (H-3α) produced an NOE effect on the signals for H-2α (δ4.12) and CH_3-18α (δ1.21). The structure of compound **7** must be 2β-hydroxy-3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-13(14)-en-15, 16-olide.

The ^1H NMR spectrum of **8** differed from the one observed for **7**. Instead of a doublet at δ3.21 (H-3) and a singlet at δ1.21 (Me-18) as in **7**, two signals appeared at δ5.49 (*dd*) and at δ1.66 (*d*) for **8**. These signals were assigned to an H-3 vinylic proton and to a Me-18 vinylic methyl group, respectively. The ^{13}C NMR spectrum of **8** supported a 3,4-double bond with signals at δ123.8 and 147.4. The structure of **8** could be deduced from the ^1H and ^{13}C NMR data (Tables 1 and 2), which indicated that **8** and 2α,16-dihydroxykolavenic acid lactone [12] are epimers. The significant difference between the ^1H NMR spectrum of **8** and the spectrum for 2α,16-dihydroxykolavenic acid lactone [12] was for the signals for Me-19, Me-20, H-2 and H-3. NOE experiments were conducted to resolve the stereochemistry of **8**. Irradiation of the signal at δ1.00 (Me-20) markedly enhanced the signal at δ4.14 (H-2) and also enhanced the signal for Me-17 (δ0.84). This evidence indicated that the C-2 hydroxyl group has a β-orientation. Thus, the structure of compound **8** is 2β-hydroxy-5β,10β-*cis*-17α,20α-cleroda-3,13(14)-diene-15,16-olide.

That the skeleton of **9** is the same as that of **8** followed from comparison of their ^1H and ^{13}C NMR spectra. The ^1H NMR spectral data also indicated the difference between **9** and **8**. For compound **9** no signals appeared around δ5.80 and 4.71, which clearly showed that the butenolide side chain at C-9 in compounds **1**–**8** is not present in compound **9**. Moreover, in addition to four methyl groups at C-4, 5, 8 and 9, one more vinylic methyl group was evident (Table 1). In the 2D-COSY, two tertiary methyl groups on unsaturated carbons (at δ1.58 and 1.87 ppm) exhibited long range allylic coupling to olefinic protons at δ5.20 and 5.36 respectively. Both ^1H and ^{13}C NMR resonances agreed closely with published data –C(Me)=CH–COOH to be the terminal portion of the C-9 side chain in **9**. Moreover, the chemical shifts of the vinyl methyl and vinyl proton allow assignment of the C-9 side chain of **9** as part of a *Z*-olefin [13–16]. The

configuration of the skeletal part of **9** was assigned by NOE experiments. Irradiation of the signal at δ0.70 (Me-20) enchanced the signals at δ1.02 (Me-19) and 1.35 (H-10). Reciprocally, irradiation at δ1.02 established an NOE between Me-19 and CH_3-20 and also between CH_3-19 and H-10. These results suggested a *cis*-fused A/B-ring system with C-8α and C-9β methyl groups. Thus, compound **9** is assigned as (13*Z*)-2β-hydroxy-5β,10β-*cis*-17α,20β-cleroda-3,13(14)-diene-15-oic acid. By comparison of the data for the 5β,10β-*cis*-clerodanes **1**–**9**, it is evident that the ^{1}H NMR chemical shifts of the methyl groups at C-9 are different for different orientations in these compounds: for β 0.70 ppm and for α more than 0.87 ppm.

A difference between the ^{1}H NMR spectra of **9** and **10** was observed for the signals for protons at C-2, 3 and 4. The signal for H-2 (δ4.30) for **9** was not present in the ^{1}H NMR spectrum of **10** and the two signals at δ5.70 (H-3) and 1.87 (Me-18) observed for **10** were replaced by signals at δ5.20 and 1.58 for **9**. These data are all compatible with an unsaturated moiety which involves C-3 and C-4 and a 2-oxoclerodane system. An isolated ABX system for H-1a,b and H-10 (Table 1) confirmed this conclusion [12, 14, 17]. Thus the structure of compound **10** must be (13*Z*)-2-oxo-5β,10β-*cis*-17α,20β-cleroda-3,13(14)-diene-15-oic acid, that is, the epimer of 2-oxokolavenic acid [14].

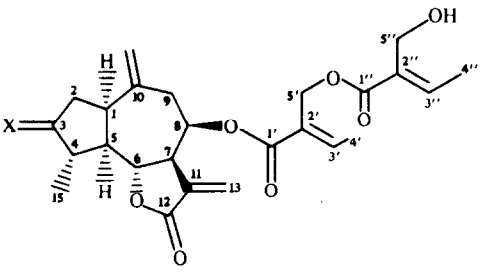
The mass spectrum of **11** exhibited a molecular ion at *m/z* 322 (12%) for $C_{20}H_{34}O_3$. The fragment at *m/z* 207 (85%) [M$-C_6H_{11}O_2$] provided evidence that **11** has a skeletal moiety of a 3,4-epoxy-clerodane. That the stereochemistry of **11** is the same as that of **1** was ascertained from the ^{1}H NMR chemical shifts of the methyl groups (Table 1). This led to an assignment in **11** of a 3,4β-epoxide and 17α,20α-methyl groups in the 5β,10β-*cis*-fused series. On the other hand, the formula $C_6H_{11}O_2$ for the C-9 side chain and no low field ^{13}C NMR signals other than one for a carbonyl group (δ177.6) required a saturated side chain containing a carboxyl group. The typical double doublets at δ2.39 and 2.12 and the methyl doublet at δ0.96 for –CH(Me)CH_2COOH suggested that the side chain at C-9 is –CH_2CH_2CH(Me)CH_2COOH [9, 18–20]. The stereochemistry at C-13, however, was not determined. Thus, the structure of compound **11** could be assigned as 3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-15-oic acid.

The ^{13}C NMR spectral data for **12** (Tables 1 and 2) showed that the butenolide part of **1** was replaced by a saturated side chain since all carbon signals for **12** appeared in the high field region. The molecular ion at *m/z* 324 and [M$-$side chain]$^+$ ion at *m/z* 207 suggested a formula of $C_6H_{13}O_2$ for the side chain at C-9. The ^{1}H NMR signal for H-15 (δ3.81) indicated a carbinol group (CH_2OH) at C-15 and the singlet at δ1.13 for Me-16 suggested a secondary hydroxyl group at C-13, conclusions which were confirmed by ^{13}C NMR signals at δ59.4 for C-15 and δ73.9 for C-13. Therefore, we assign this new natural product the structure 16-hydroxy-3,4β-epoxy-5β,10β-*cis*-17α,20α-cleroda-15-ol (**12**).

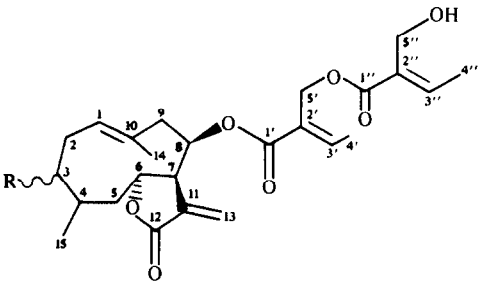
Spectral data (^{1}H and ^{13}C NMR) for compound **13** established that it was a known acyclic diterpene, 20-hydroxygeranylnerol [21,22].

Sesquiterpene lactones (**14**–**20**).

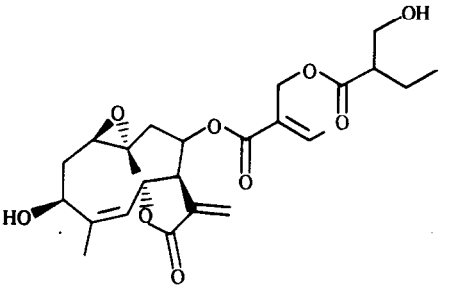
That compound **14** was a sesquiterpene lactone was indicated by the appearance of IR bands ($CHCl_3$) at



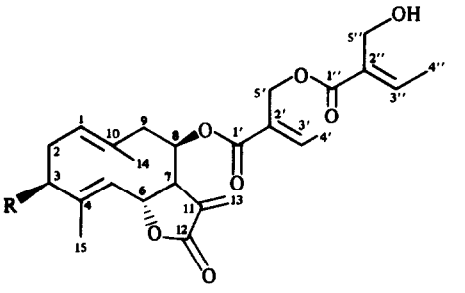
14 X = O
15 X = H,βOH



16 R = βOH
17 R = αOH



18



19 R = βAcO
20 R = H

1760 cm^{-1} and was further supported by the presence in the ^{1}H NMR spectrum (Table 3) of a characteristic pair of low field doublets at δ5.65 (1H, J = 3.0 Hz, H-13b) and 6.31 (1H, J = 3.4 Hz, H-13a). Moreover, in the ^{1}H NMR spectrum of **14**, two broadened singlets at δ4.97 and 4.82 suggested the presence of an exocyclic methylene function. Only one methyl doublet (δ1.28) was observed for

Table 3. ^{1}H NMR data for sesquiterpene lactones **14**–**16**, **18** and **19**. (360 MHz, $CDCl_3$, δ-scale in ppm)

H	14	15	16	18	19
1	3.08 *ddd*	2.75 *br ddd*	5.10 *m*	2.83 *dd*	4.86 *m*
2	2.60 *dd*	2.10 *ddd*	2.12 *m*	2.37 *ddd*	2.50 *m*
2	2.52 *dd*	1.70 *ddd*	2.73 *br dd*	1.75 *m*	2.28 *m*
3		3.65 *br ddd*	4.44 *dd*	4.43 *br dd*	5.17 *br dd*
4	2.37 *br dq*	1.85 *m*			
5	2.30 *ddd*	1.90 *m*	5.15 *br d*	5.24 *dd*	4.90 *m*
6	4.51 *t*	4.33 *t*	6.26 *br dd*	6.66 *dd*	5.06 *dd*
7	3.28 *dddd*	2.95 *dddd*	2.91 *br s*	2.85 *m*	2.89 *m*
8	5.74 *m*	5.54 *m*	5.24 *m*	5.17 *m*	5.78 *m*
9	2.72 *dd*	2.67 *br dd*	2.60 *m*	2.76 *dd*	2.78 *dd*
9	2.51 *dd*	2.29 *br dd*	2.36 *dd*	1.32 *dd*	2.33 *dd*
13a	6.31 *d*	6.12 *d*	6.29 *d*	6.33 *d*	6.20 *d*
13b	5.65 *d*	5.53 *d*	5.71 *d*	5.75 *d*	5.55 *d*
14a	4.82 *br s*	4.78 *br s*	1.67 *s*	1.42 *s*	1.44 *s*
14b	4.07 *br s*	5.02 *br s*			
15	1.28 *d*	1.13 *d*	1.73 *br s*	1.76 *br s*	1.72 *s*
3′	7.10 *q*	7.06 *q*	7.13 *q*	7.20 *q*	7.12 *q*
4′	1.99 *d*	1.88 *d*	1.89 *d*	1.93 *d*	1.94 *d*
5′a	4.89 *d*	4.79 *d*	4.82 *d*	4.84 *d*	4.93 *d*
5′b	4.85 *d*	4.77 *d*	4.80 *d*	4.80 *d*	4.82 *d*
3″	6.87 *q*	6.79 *q*	6.89 *q*	6.88 *q*	6.81 *q*
4″	1.96 *d*	1.83 *d*	1.86 *d*	1.87 *d*	1.85 *d*
5″a,b	4.31 *br s*	4.24 *br s*	4.31 *dd**	4.32 *dd*	4.26 *br s*
OAc					2.07 *s*

J(Hz): compounds **14**–**16**, **18** and **19**: 3′,4′ = 7.2; 5′a, 5′b = 12, 3″,4″ = 7.2; compound **14**: 1,2 = 1; 1,2 = 3; 1,5 = 9.0; 1,2 = 19.3, 4,5 = 10.5; 4,15 = 6.7; 5,6 = 9.1; 6,7 = 9.1; 7,8 = 2; 7,13a = 3.4, 13b = 2.9; 8,9 = 3, 8,9 = 4; 9,9 = 14.4; compound **15**: 1,2 = 6; 1,2 = 8; 1,5 = 10; 1,2 = 12, 2,3 = 9; 2,3 = 6; 3, 4 = 5; 4, 15 = 5.5; 6,7 = 9.3; 7, 8 = 2; 7, 13a = 3.3, 7, 13b = 2.3, 8, 9 = 4.2; 8,9 = 3.3; 9, 9 = 14; compound **16**: 2, 2 = 13.6; 2, 3 = 3.1; 2, 3 = 2.5; 5.6 = 10.4; 6, 7 = 2.2; 8, 9 = 3.5; 9, 9 = 14.8; compound **18**: 1, 2 = 4.9; 1,2 = 10.6; 2, 2 = 14.8, 2, 3 = 2; 5, 6 = 2.1; 6, 7 = 10.8; 7, 13a = 2.0; 7, 13b = 1.8; 8, 9 = 4.5; 8, 9 = 2.0; 9, 9 = 10.2. Compound **19**: 2, 3 = 10.4; 2, 3 = 6.1; 5, 6 = 9.8; 6, 7 = 9; 7,13a = 3.3; 7, 13b = 3.0;8, 9 = 4.2; 8, 9 = 2.3; 9, 9 = 14.3.

*Broadened singlet at 4.30 (90 MHz).

the skeletal part of **14**, and other resonances were coincident with the skeletal signals reported for 8β-4′-hydroxy-5′-[4″-hydroxytigloyloxy]-tigloyloxy-3-dehydro-4β,15-dihydrozaluzanin [23], which indicated that compound **14** is a guaianolide. The presence of a C_{10} diester side chain in **14** was evident from the ^{1}H NMR spectrum (Table 3). While the signals of H-3′(1H) and H-3″(1H) were split into quartets, those of H-4′(3H) and H-4″(3H) were doublets at δ1.99 and 1.96, respectively. Also the doublets at δ4.89 and 4.85, in conjunction with the broadened singlets at 4.31 (2H, H-5″a,b), suggested that the side chain of **14** was 5-hydroxytigloyloxy esterified with 5-hydroxytiglate [24, 25]. Thus, compound **14** is 8β-5′-[5″-hydroxytigloyloxy]-tigloyloxy-3-dehydro-4β,15-dihydrozaluzanin C.

The 8β-5′-[5″-hydroxytigloyloxy]-tigloyloxyl side chain of **15** could be deduced from the ^{1}H and ^{13}C NMR spectral data [24, 26]. In the mass spectrum of **15**, the fragments of the skeleton were nearly identical to those observed in the spectrum of **14**, except that the *m/z* values for each fragment from **15** were 2 units more than those from **14**. This observation was supported by the ^{13}C NMR spectral data (Table 4). A carbonyl signal at δ219.5 for **14** [23] was replaced by a signal at δ78.31 (*d*) for C-3 for **15**. The mass and IR spectral data, as well as correlation of the ^{1}H and ^{13}C NMR spectra of **15** (Tables 3 and 4) with known compounds, established that **15** is 8β-5′-[5″-hydroxytigloyloxy]-tigloyloxy-4β,15-dihydrozaluzanin C [23–29].

That compounds **16** and **17** possess the same C_{10} diester side chain at C-8 as do **14** and **15** followed from the typical ^{1}H NMR signals of a 5′-[5″-hydroxytigloyloxy]-tigloyloxyl group (Table 3). But, unlike **14** and **15**, compounds **16** and **17** are heliangolide sesquiterpene lactones, a skeletal type which can be deduced from the characteristic coupling constants $J_{7,13} \sim 2$ and other ^{1}H NMR signals, especially those for the two methyl groups. All ^{1}H NMR signals of these two compounds were nearly the same except for those for H-3, H-6 and Me-15, suggesting that **16** and **17** are isomers at C-3. While H-3α for **16** showed a double doublet at δ4.44 (J = 3.0 and 3.1 Hz) [24, 30, 31], this signal was shifted to δ4.61 (J = 12 and 5 Hz) in the spectrum of **17**. All spectral data showed that **17** was previously reported as 4′-desoxy-3-desacetoxyl-3α-hydroxyprovincialin from another member of the Eupatorieae, *Liatris cylindracea*

Table 4. ^{13}C NMR data for sesquiterpene lactones **15**–**18** (90 MHz, $CDCl_3$, δ-scale in ppm)

C	15	16	17	18
1	42.6 *d*	123.8	124.0	125.4 *d*
2	38.5 *t*	31.8	33.9	32.1 *t*
3	78.3 *d*	78.6	68.5	74.6 *d*
4	46.4 *d*	140.1	140.4	139.6 *s*
5	51.9 *d*	126.4	124.3	127.7 *d*
6	80.8 *d*	75.7	77.2	78.8 *d*
7	50.6 *d*	48.4	49.0	52.2 *d*
8	66.9 *d*	75.3	74.1	71.8 *d*
9	40.9 *t*	43.3	43.4	43.9 *t*
10	142.3 *s*	134.7	133.9	136.1 *s*
11	135.0 *s*	137.9	137.6	135.9 *s*
12	169.2 *s*	169.5	169.3	169.2 *s*
13	121.5 *t*	125.1	125.7	121.3 *t*
14	116.8 *t*	19.5	18.5	12.5 *q*
15	17.6 *q*	22.9	17.1	20.8 *q*
1′	165.4 *s*	165.4	165.3	165.0 *s*
2′	127.6 *s*	127.6	127.7	127.4 *s*
3′	145.7 *d*	145.8	146.1	146.1 *d*
4′	14.4 *q*	14.5	14.7	14.6 *q*
5′	56.4 *t*	56.3	56.8	56.4 *t*
1″	166.8 *s*	166.8	167.0	166.9 *s*
2″	131.6 *s*	131.6	131.8	131.5 *s*
3″	141.4 *d*	141.2	141.4	141.8 *d*
4″	14.0 *q*	14.0	14.1	14.1 *q*
5″	57.5 *t*	57.6	57.9	57.5 *t*

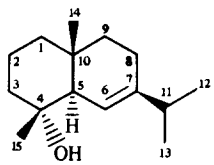
[24]. In the ^1H NMR spectrum of **16** the low field chemical shift (δ6.26 for H-6 in **16**) and coupling constants $J_{5,6}$ = 10.4 Hz and $J_{6,7}$ = 2.2 Hz are characteristic and showed the typical stereochemistry of 3β-hydroxy heliangolides [30, 31]. Thus, compound **16** was formulated as a new lactone, namely, 4′-desoxy-3-desacetoxyl-3β-hydroxyprovincialin; all spectra (^{13}C NMR, MS and IR) support this assignment. The ^{13}C NMR data of **17**, which had not been reported previously, are listed in Table 4.

The ^1H NMR spectrum of **18** showed the characteristic features of a 8β-5′-[5″-hydroxytigloyloxy]-tigloyloxyheliangolide sesquiterpene lactone. The 2D-COSY of **18** established that the multiplet signal at δ5.10 for H-1 of **16** [the X component of an ABX system (H-1, H-2α and 2β)] was replaced by a double doublet at δ2.83 (J = 4.9 and 10.6 Hz) for **18**. To account for the methyl singlet at δ1.42 (Me-14) in **18** an epoxy group is required between C-1 and C-10. Moreover, comparison of spectral data for the known leptocarpin [31], heliangine [32, 33] and epoxinobilin [34] with those for **18** revealed that all four had the same skeleton. The unusual low field ^1H NMR signals for H-6β at δ6.70 for all four compounds indicated the typical stereochemistry of heliangolides with 1β,10α-epoxy and 3β-hydroxyl groups. Thus, compound **18** is 5′-[5″-hydroxytigloyloxy]-5′-hydroxyheliangine.

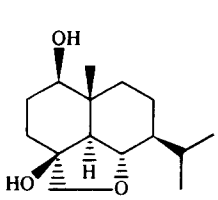
The characteristic coupling constants ($J_{7,13a}$ = 3.3, $J_{7,13b}$ = 3 Hz) and the chemical shift of two olefinic methyl groups in the ^1H NMR spectra of **19** and **20** indicated that these two compounds were not heliangolides but germacranolides. The presence of the same C-8 diester side chains in **19** and **20** as those found in **14**–**18** was apparent from the ^1H NMR spectra. Moreover, the ^1H and ^{13}C NMR spectra of **20** were identical to those of liacylindrolide, a compound previously isolated from *Liatris cylindracea* and *Neohintonia monantha* [24, 26]. The ^1H and ^{13}C NMR spectra of **19** showed the presence of an acetoxyl function (Table 3 and 4), and the chemical shift of the signal assigned to H-3 (δ5.17) suggested the attachment of the acetoxyl group to that position. The magnitude of $J_{2,3}$ (10.4 Hz) and $J_{2,3}$ (6.1 Hz) as well as the chemical shift of H-3 (δ5.17) showed that the acetoxy group should be β-oriented. Compound **19** is therefore 3β-acetoxyliacylindrolid.

Sesquiterpenes (**21**, **22**)

The spectral data of compounds **21** and **22** showed the spectroscopic properties typical of the eudesmane and, moreover, showed the presence of the isopropyl groups, $CH(Me)_2$, (Tables 5 and 6). The structure of **21** ($C_{15}H_{26}O$) followed from the ^1H and ^{13}C NMR spectral data which was close to similar eudesmane derivatives [35–38]. The stereochemistry of **21** was assigned as 4α-hydroxy in the 5α,10β-*trans*-fused series on the basis of comparison of ^{13}C NMR data of **21** with those of similar compounds [37–41] which showed the C-14 methyl resonance at δ17.6 and C-15 methyl resonance at δ22.3. Thus, compound **21** is 6-eudesmene-4α-ol. The electron impact mass spectrum of compound **22** exhibited a molecular ion peak at *m/z* 254 (5.4%) corresponding to $C_{15}H_{26}O_3$, which implied three degrees of unsaturation. No sp^2 carbons were revealed by the ^{13}C NMR spectrum suggesting three rings for compound **22**. A broadened double doublet (1H, J = 11 and 4.1 Hz) at δ3.37 could be ascribed to Hα-1 [38, 41] and indicated a β-hydroxyl group at C-1. An AB system for H-15 and 15′ (δ3.17 and 3.60, 1H each, J = 9.1 Hz) permitted the assignment of a C-4 hydroxy group. Furthermore, an H-15 and 15′ AB system, in conjunction with the double doublet at δ3.73 (H-6, J = 9.4 and 11.6 Hz) and broadened doublet at 1.03 (H-5, J = 11.6 Hz) suggested the presence of 6,15α-epoxyl group in **22** [41–43]. Since the signals for H-5 and H-14, H-6 and H-15 overlap, respectively, four points (signals for H-5 and 14, for H-7, for H-1 and for H-6 and 15) were irradiated in NOE experiment. Careful inspection and comparison of four NOE spectra of **22** led to stereochemistry for **22** which was consistent with that assigned by coupling constants. All



21



22

Table 5. ^{1}H NMR data for sesquiterpenes **21** and **22** ($CDCl_3$, δ-scale in ppm, 360 MHz for **21** and 500 MHz for **22**)

H	21	22
1		3.37 *br dd*
2		1.98 *m*
2′		1.77 *m*
5		1.03 *d*
6	5.53 *br s*	3.73 *dd*
7		1.28 *m*
11	2.21 *br heptet*	
12	1.01 *d*	0.96 *d*
13		0.90 *d*
14	0.80 *s*	1.03 *s*
15	1.11 *s*	3.71 *d*
15′		3.60 *d*

J(Hz): **21**: 11, 12 = 11, 13 = 6.8; **22**: 1,2 = 11; 1,2′ = 4.1; 5, 6 = 11.6; 6,7 = 9.4; 11, 12 = 11, 13 = 6.9; 15, 15′ = 9.1.

Table 6. ^{13}C NMR data for sesquiterpenes **21** and **22** (90 MHz, $CDCl_3$, δ-scale in ppm)

C	21	22
1	40.7 *t*	80.5*
2	20.4 *t*	28.0†
3	43.2 *t*	39.7†
4	72.3 *s*	80.4†
5	54.4 *d*	57.6*
6	117.2 *d*	75.6*
7	143.8 *s*	51.2*
8	23.4 *t*	22.2†
9	39.5*t*	39.1†
10	33.8 *s*	33.2†
11	35.0 *d*	29.6*
12	21.8 *q*	18.5*
13	21.6 *q*	20.7*
14	17.6 *q*	12.8 *
15	22.3 *q*	76.5†

†Carbons are quaternary or methylene carbons.

*Carbons are methyl or methine carbons.

other spectral data supported the assignment of compound **22** as 6,15α-epoxy-1β,4β-dihydroxyeudesmane.

EXPERIMENTAL

Plant material. Ageratina saltillensis (B. L. Rob.) K & R was collected on November 31, 1985 from the N. L. 11.1 km of San Rafael (Hwy 57) to Galeana, NE of Ceno Potasy by Guy Nesom. A voucher specimen (Guy Nesom, No. 5283) is on deposit in the Plant Resources Center at the University of Texas at Austin, Austin, Texas.

General techniques. Dried leaves of *A. saltillensis* (563 g) were extracted with CH_2Cl_2 for 1 hr. The CH_2Cl_2 extract of the plant material was evapd at low temperature (~40°) until the extract was dry. The residue was dissolved in 100% MeOH and kept in a refrigerator overnight. After filtering to remove the ppt., H_2O was added to the filtrate until the soln reached approximately 80% aq. MeOH. This aq. extract was then partitioned against hexane (x4), and then the aq. soln was concd to a syrup at low temperature (~40°). The syrup was dried completely under a high vacuum system. The residue (10 g) was chromatographed over a silica gel column packed in hexane–EtOAc (19:1). Compounds were eluted with a hexane–EtOAc gradient, with increasing amounts of EtOAc. Compounds were purified by a combination of Sephadex LH-20 chromatography (cyclohexane–CH_2Cl_2–MeOH; 7:4:1), prep TLC (silica gel; hexane–EtOAc; 4:1, 3:2 or 1:3) and HPLC [using the following conditions: semi-prep. silica gel column (10 mm × 25 cm); RI detector; hexane–EtOAc (9:1, 7:3, 5:5, 3:7 or 1:9) as eluting solvent; flow rate 2.2 ml/min]. Compound **1** (1600 mg), **2** (32 mg), **3** (12 mg), **4** (27 mg), **5** (13 mg), **6** (9 mg), 7 (41 mg), **8** (13 mg), **9** (37 mg), **10** (7 mg), **11** (75 mg), 12 (21 mg), **13** (25 mg), **14** (5 mg), **15** (17 mg), **16** (219 mg), **17** (19 mg), **18** (8 mg), **19** (16 mg), **20** (6 mg), **21** (7 mg) and **22** (12 mg) were obtained. Mps: uncorr.

X-ray. Data were collected on a Syntex P 2_1 diffractometer with a graphite monochromator utilizing Mo-K_α radiation (λ = 0.71069 Å) and equipped with a Syntex LT−1 low temperature delivery system (163 K).* The crystal was orthorhombic, a = 7.625(2), b = 12.411(5), c = 18.494(9) Å, V = 1750.0(2) Å^3, space group $P2_12_12_1$, z = 4, Dx (g/cm^3, 163K) = 1.208, Dc (g/cm^3, 294K) = 1.199. Chemical formula, $C_{20}H_{30}O_3$, F.W. = 318.455, and $F(000)$ = 696 electrons. 5786 reflections were measured using ω-scan and symmetrically over 1° about $K_{\alpha 1,2}$ maximum and an offset of +1° in ω from $K_{\alpha 1,2}$ maximum at a scan rate of 3.0 deg./min (min) and 6.0°/min (max) and a 2θ range of 4.0 to 60.0°. 45 well centered reflections with $13.03° < 2\theta < 21.60°$ were used for least-squares refinement of the unit cell parameters. Crystal density was measured in a $ZnCl_2$ soln. Four reflections (200, 145, 303, 053) were remeasured every 196 reflections to monitor instrument and crystal stability. A total of 2383 reflections with intensity greater than 3σ were used in refining 248 variables. The refinement converged to a goodness of fit of 1.54 and a maximum shift/e.s.d. of 0.74, minimum and maximum peaks in ΔF map (eÅ^{-3}) were −0.14 and 0.23 respectively.

The structure was solved using direct methods (Multan-76) from which the locations of all non-hydrogen atoms were obtained. The structure was refined using full-matrix least squares method and SHELX-76.† Methyl hydrogen positions were calculated assuming an sp^3 geometry around the C-atom and their temperature factors were fixed at 0.05. All non-hydrogen atoms were treated anisotropically, hydrogen atoms isotropically. The refinement converged to R = 0.046 and Rw = 0.061 for 2383 reflections.

*Data reduction was carried out as described in ref. [44]. Crystal and instrument stability were monitored by remeasurement of 4 check reflections after every 196 reflections and the data were analysed as detailed in ref. [45].

†Relevant expressions are as follows:

$$\sum \omega(|F_o| - |F_c|)^2;\ \omega = 1/(\sigma(F_o))^2$$

$$R = \sum |(|F_o| - |F_c|)| / \sum |F_o|$$

$$R\omega = [\sum_\omega (|F_o| - |F_c|)^2 / \sum_\omega (|F_o|)^2]^{1/2}$$

$$S = [\sum_\omega (|F_o| - |F_c|)^2 / (m - n)]^{1/2}$$

16-*Hydroxy*-3,4β-*epoxy*-5β,10β-cis-17α,20α-*cleroda*-13(14)-*en*-15,16-*olide* (**2**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3330 (OH), 1750 (lactone), 1650 (C=C). MS (rel. int): 334 $[M]^+$ (7.3) ($C_{20}H_{30}O_4$), 319 $[M-Me]^+$ (42.5), 316 $[M-H_2O]^+$ (8.5), 305 $[M-29]^+$ (5.1), 287 $[305-H_2O]^+$ (12.7), 233 $M-C_4H_3O_2-H_2O]^+$ (23.5), 207 [M −side chain]$^+$ (54.1), 109 [side chain $-H_2O]^+$ (97), 43 (100).

3α,4β,16-*Trihydroxy*-5β,10β-cis-17α,20α-*cleroda*-13(14)-*en*-15,16-*olide* (**3**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460 (OH), 3250 (OH), 1750 (lactone), 1650 (C=C). MS (rel. int): 352 $[M]^+$ (3.7) ($C_{20}H_{32}O_5$), 334 $[M-H_2O]^+$ (2.5), 224 $[334-C_6H_6O_2]^+$ (9.1), 207 $[334-C_6H_7O_2\text{-}H_2O]^+$ (20.8), 189 $[207-H_2O]^+$ (40), 109 [side chain $-H_2O]^+$ (86.1), 95 $[109-CH_2]^+$ (97.4), 81 $[95-CH_2]^+$ (81), 43 (100).

3α,4β-*Dihydroxy*-5β,10β-cis-17α,20α-*cleroda*-13(14)-*en*-15,16-*olide* (**4**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460 (OH), 1780, 1745 (lactone), 1632 (C=C). MS (rel. int): 336 $[M]^+$ (7.9) ($C_{20}H_{32}O_4$), 319 $[M-15]^+$ (6.4), 226 [M − side chain + 1]$^+$ (100), 111 [side chain]$^+$ (79).

3α-*Methoxy*-4β-*hydroxy*-5β,10β-cis-17α,20α-*cleroda*-13(14)-*en*-15,16-*olide* (**5**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470 (OH), 1780, 1748 (lactone), 1636 (C=C). MS (rel. int): 350 $[M]^+$ (2.4) ($C_{21}H_{34}O_4$), 335 $[M-15]^+$ (2.7), 240 [M-side chain + 1]$^+$ (100), 111 [side chain]$^+$ (78.6).

3-*Oxo*-4β-*hydroxy*-5β,10β-cis-17α,20α-*cleroda*-13(14)-*en*-15,16-*olide* (**6**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200–3600 (*br* OH), 1780, 1748 (lactone), 1636 (C=C). MS (rel. int): 334 $[M]^+$ (2.2) ($C_{20}H_{30}O_4$), 319 $[M-Me]^+$ (31), 318 $[M-O]^+$ (35), 305 $[M-29]^+$ (21), 304 $[319-Me]^+$ (23), 289 $[318-29]^+$ (28), 235 $[318-C_4H_3O_2]^+$ (30), 208 $[318-C_6H_6O_2]^+$ (20), 111 [side chain]$^+$ (100), 43 (93).

2β-*Hydroxy*-3,4β-*epoxy*-5β,10β-cis-17α,20α-*cleroda*-13(14)-*en*-15,16-*olide* (**7**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470 (OH), 1780, 1748 (lactone), 1635 (C=C). MS (rel. int): 334 $[M]^+$ (1.5) ($C_{20}H_{30}O_4$), 319 $[M-Mc]^+$ (3.6), 316 $[M-H_2O]^+$ (2.2), 288 $[M-CO-H_2O]^+$ (4.8), 273 $[288-Me]^+$ (35.9), 223 [M − side chain]$^+$ (3.6), 205 $[223-H_2O]^+$ (14.7), 111 [side chain]$^+$ (100).

2β-*Hydroxy*-5β,10β-cis-17α,20α-*cleroda*-3,13(14)-*diene*-15,16-*olide* (**8**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 1780 (lactone), 1637 (C=C). MS (rel. int): 318 $[M]^+$ (2) ($C_{20}H_{30}O_3$), 300 $[M-H_2O]^+$ (44.5), 285 $[M-H_2O-Me]^+$ (24.6), 235 $[M-C_4H_3O_2]^+$ (76.8), 203 $[M-H_2O-C_5H_5O_2]^+$ (21.4), 189 $[M-H_2O-C_6H_7O_2]^+$ (39.2), 111 [side chain]$^+$ (100).

(13Z)-2β-*Hydroxy*-5β,10β-cis-17α,20β-*cleroda*-3,13(14)-*diene*-15-*oic acid* (**9**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400(OH), 3400–2600, 1690 (CO_2H), 1640 (C=C). MS (rel. int): 320 $[M]^+$ (1.2) ($C_{20}H_{32}O_3$), 302 $[M-H_2O]^+$ (31.5), 287 $[302-Me]^+$ (22.6), 269 $[287-H_2O]^+$ (16.3), 203 $[M-H_2O-C_5H_7O_2]^+$ (23.5), 189 $[M-H_2O-C_6H_9O_2]^+$ (100), 119 (80.1), 105 (80.5), 95 (82.4), 91 (68.6).

(13Z)-2-*Oxo*-5β,10β-cis-17α,20β-*cleroda*-3,13(14)-*diene*-15-*oic acid* (**10**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400–2600, 1690 (CO_2H), 1650 (C=CO). MS (rel. int): 318 $[M]^+$ (1.6) ($C_{20}H_{28}O_3$), 300 $[M-H_2O]^+$ (5.9), 285 $[M-H_2O-Me]^+$ (15.7), 259 $[M-C_2H_3O_2]^+$ (20.4), 243 $[259-O]^+$ (11), 205 $[M-C_6H_9O_2]^+$ (46.5), 189 $[205-O]^+$ (29.8), 121 (100), 109 (86.6), 95 (95.6), 83 (40.4).

3,4β-*Epoxy*-5β,10β-cis-17α,20α-*cleroda*-15-*oic acid* (**11**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450–2600, 1720 (CO_2H). MS (rel. int): 322 $[M]^+$ (11.7) ($C_{20}H_{34}O_3$), 307 $[M-Me]^+$ (11.9), 304 $[M-H_2O]^+$ (16.7), 293 $[M-29]^+$ (24.6), 237 $[M-C_4H_5O_2]^+$ (16.8), 223 $[M-C_5H_7O_2]^+$ (19), 207 [M − side chain]$^+$ (84.6), 109 (100).

16-*Hydroxy*-3,4β-*epoxy*-5β,10β-cis-17α,20α-*cleroda*-15-*ol* (**12**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH). MS (rel. int): 324 $[M]^+$ (2.6) ($C_{20}H_{36}O_3$), 309 $[M-Me]^+$ (13.6), 306 $[M-H_2O]^+$ (9.9), 291 $[309-H_2O]^+$ (8.9), 273 $[291-H_2O]^+$ (6.9), 221 $[M-C_5H_{11}O_2]^+$ (10.7) 207 [M − side chain]$^+$ (91.9), 43 (100).

8β-5′-[5″-*Hydroxytigloyloxy*]-*tigloyloxy*-3-*dehydro*-4β,15-*dihydro-zaluzanin C* (**14**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490 (OH), 1770 1700 (lactone, C=CCO_2R, C=O), 1650 (C=C). MS (rel. int): 458 $[M]^+$ (2.7) ($C_{25}H_{30}O_8$), 440 $[M-H_2O]^+$ (10), 361 $[M-C_5H_5O_2]^+$ (51), 343 $[M-C_5H_7O_3]^+$ (91), 263 $[M-C_{10}H_{11}O_4]^+$ (27), 245 $[M-C_{10}H_{13}O_5]^+$ (100), 244 $[M-C_{10}H_{14}O_5]^+$ (43), 99 $[MeCH=C(CH_2OH)CO]^+$ (94.8), 81 $[MeCH=C(CH_2OH)CO-H_2O]^+$ (84).

8β-5′-[5″-*Hydroxytigloyloxy*]-*tigloyloxy*-4β,15-*dihydrozaluzanin C* (**15**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490 (OH), 1763 (lactone), 1718 (C=CCO_2R), 1650 (C=C). MS (rel. int): 460 $[M]^+$ (1) ($C_{25}H_{32}O_8$), 442 $[M-H_2O]^+$ (4.4), 424 $[442-H_2O]^+$ (1), 363 $[M-C_5H_5O_2]^+$ (12.6), 345 $[M-C_5H_7O_3]^+$ (25.7), 265 $[M-C_{10}H_{11}O_4]^+$ (11.3), 247 $[M-C_{10}H_{13}O_5]^+$ (51.3), 246 $[M-C_{10}H_{14}O_5]^+$ (84.3), 229 $[247-H_2O]^+$ (100), 99 $[MeCH-C(CH_2OH)CO]^+$ (100), 81 $[MeCH=C(CH_2OH)CO-H_2O]^+$ (94.6).

4′-*Desoxy*-3-*desacetoxyl*-3β-*hydroxyprovincialin* (**16**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490 (OH), 1752 (lactone), 1710 (C=CCO_2R), 1650 (C=C). MS (rel. int): 460 $[M]^+$ (0.5) ($C_{25}H_{32}O_8$), 442 $[M-H_2O]^+$ (2), 362 $[M-C_5H_6O_2]^+$ (2), 344 $[M-C_5H_8O_3]^+$ (3.8), 264 $[M-C_{10}H_{12}O_4]^+$ (3), 246 $[M-C_{10}H_{14}O_5]^+$ (69), 228 $[246-H_2O]^+$ (96), 99 $[MeCH=C(CH_2OH)CO]^+$ (100), 81 $[MeCH=C(CH_2OH)CO-H_2O]^+$ (99.9).

5′-[5″-*Hydroxytigloyloxy*]-5′-*hydroxyhehiangine* (**18**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3740 (OH), 1750 (lactone), 1710 (C=CCO_2R), 1650 (C=C). MS (rel. int): 262 $[M-C_{10}H_{14}O_5]^+$ (6.3) (M^+: 476, $C_{25}H_{32}O_9$), 245 $[M-C_{10}H_{13}O_5-H_2O]^+$ (16.8), 244 $[262-H_2O]^+$ (13.9), 227 $[245-H_2O]^+$ (15.9), 99 $[MeCH=C(CH_2OH)CO]^+$ (100), 81 $[MeCH=C(CH_2OH)CO]^+$ (96.8).

3β-*Acetoxyliacylindrolid* (**19**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3510 (OH), 1768 (lactone), 1740 (OAc), 1715 (C=CCO_2R), 1650 (C=C). MS (rel. int): 442 $[M-AcOH]^+$ (3) (M^+:502, $C_{27}H_{34}O_9$), 344 $[442-C_5H_6O_2]^+$ (4), 246 $[M-AcOH-C_{10}H_{12}O_4]^+$ (70.8), 228 $[246-H_2O]^+$ (53.5), 99 $[MeCH=C(CH_2OH)CO]^+$ (84.5), 81 $[MeCH=C(CH_2OH)CO-H_2O]^+$ (100).

6-*Eudesmene*-4α-*ol* (**21**). MS (rel. int): 222 $[M]^+$ (2.5) ($C_{15}H_{26}O$), 207 $[M-Me]^+$ (14.3), 204 $[M-H_2O]^+$ (18.8), 161 $[M-H_2O-C_3H_7]^+$ (32.5), 151 (25.2), 121 (31.9), 109 (52.7), 43 $[C_3H_7]^+$ (100).

6,15α-*Epoxy*-1β,4β-*dihydroxyeudesmane* (**22**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200–3580 (OH). MS (rel. int): 254 $[M]^+$ (5.4) ($C_{15}H_{26}O_3$), 239 $[M-Me]^+$ (9.1), 236 $[M-H_2O]^+$ (5.9), 222 $[M-MeOH]^+$ (66.3), 209 (36.5), 206 (38.3), 180 (79.3), 43 $[C_3H_7]^+$ (100).

Acknowledgements—This work was supported by the National Science Foundation (Grant BSR-8402017), the National Institutes of Health (GM-35710), the Texas Advanced Technology Research Program and the Robert A. Welch Foundation (Grant F-130). K.A.A. and S.H.S. wish to thank the Robert A. Welch Foundation (Grant F-017) for support of the X-ray study. The authors thank Guy Nesom for collecting and identifying the plant material

REFERENCES

1. Romo, J., Rios, T. and Quijano, L. (1968) *Tetrahedron* **24**, 6087.
2. Quijano, L., Calderon, J. S., Gomez. G. F., Garduno, J. T. and Rios C, T. (1980) *Phytochemistry* **19**, 1975.
3. Bohlmann, F., Jakupovic, J. and Lonitz, M. (1977) *Chem. Ber.* **110**, 301.
4. Bohlmann, F., Banerjee, S., Wolfrum, C., Jakupovic, J., King, R. M. and Robinson, H. (1985) *Phytochemistry* **24**, 1309.
5. Gage, D. (1986) Ph.D. Dissertation. University of Texas at Austin, U.S.A.

6. Melek, F, R., Gage. D. A. and Mabry, T. J. (1985) *J. Nat. Prod.* **49**, 331.
7. Guerrero, C., Diaz, E., Martinez, M., Taboada, J., Plata, S. M. and Tellez, J. (1977) *Rev. Latinoam. Quim.* **8**, 123.
8. Bohlmann, F. and Gupta. R. K. (1981) *Phytochemistry* **20**, 1432.
9. Calderon, J. S., Quijano, L., Garduno, M., Gomez, F. and Rios, T. (1983) *Phytochemistry* **22**, 2617.
10. Gonzalez, A. G., Arteaga, J. M., Brelon, J. L. and Fraga, B. M. (1977) *Phytochemistry* **16**, 107.
11. Anthonsin, T. and Bergland, G. (1971) *Acta Chem. Scand.* **25**, 1924.
12. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 1657.
13. Anthonsen, T. and McCrindle, R. (1969) *Acta Chem. Scand.* **23**, 1068.
14. Hasan, C. M., Healey, T. M. and Waterman, G. (1982) *Phytochemistry* **21**, 1365.
15. Rossomando, P. C., Giordano, O. S., Espineira, J. and Joseph-Nathan, P. (1985) *Phytochemistry* **24**, 787.
16. Forster, P. G., Ghisalberti, M. L. and Jefferies, P. R. (1985) *Phytochemistry* **24**, 2991.
17. Bohlmann, F., Zdero, C., Gupta, R. K., King, R. M. and Robinson, H. (1980) *Phytochemistry* **19**, 2695.
18. Bohlmann, F., Zitzkowski, P., Suwita, A. and Fiedler, L. (1978) *Phytochemistry* **17**, 2101.
19. Bohlmann, F., Ahmed, M., Robinson, H. and King, R. M. (1981) *Phytochemistry* **20**, 1439.
20. Bohlmann, F., Jakupovic, J., Dhar, A. K., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 843.
21. Bohlmann, F., Dhar, A. K., Jakupovic, J., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 1077.
22. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 2021.
23. Pfeil, R. M., Gage, D. A., Lee, E. F., Miski, M., Mabry, T. J. and Powell, A. M. (1987) *Phytochemistry* **26**, 195.
24. Bohlmann, F. and Dutta, L. N. (1979) *Phytochemistry* **18**, 847.
25. Bohlmann, F. and Turner, B. L. (1985) *Phytochemistry* **24**, 1263.
26. Fang, N., de Luengo, D. and Mabry, T. J. (1986) *Phytochemistry* **25**, 2665.
27. Bohlmann, F. and Chen. Z. (1982) *Phytochemistry* **21** 2120.
28. Bohlmann, F., Singh, P., Borthakur, N. and Jakupovic, J. (1981) *Phytochemistry* **20**, 2379.
29. Halim, A. F., Zaghloul, A. M., Zdero, C. and Bohlmann, F. (1983) *Phytochemistry* **22**, 1510.
30. de Pascual-T, J., Gonzalez, M. S., Moreno Valle, M. A. and Bellido, I. S. (1983) *Phytochemistry* **22**, 1985.
31. Martinez J., R., Ayamante B., I. S., Nunez-Alarcon, J. A., and de Vivar, A. R. (1979) *Phytochemistry* **18**, 1527.
32. Morimota, H., Sanno, Y. and Oshio, H. (1966) *Tetrahedron* **22**, 3173.
33. Nishikawa, M., Kamiya, K., Takabatake, A. and Oshio, H. (1966) *Tetrahedron* **22**, 3601.
34. Holub, M. and Samek, Z. (1977) *Collect. Czech. Chem. Commun* **42**, 1053.
35. Bohlmann, F., Zdero, C., Berger, D., Suwita, A., Mahanta, P. and Jeffrey, C. (1979) *Phytochemistry* **18**, 79.
36. Bohlmann, F., Knoll, K., Zdero, C., Mahanta, P. K., Grenz, M., Suwita, A., Ehlers, D., Le Van, N., Abraham, W. R. and Natu, A. A. (1977) *Phytochemistry* **16**, 965.
37. Rose, A. F. and Sims, J. J. (1977) *Tetrahedron Letters* 2935.
38. Bohlmann, F., Grenz, M., Gupta, R. K., Dhar, A. K., Ahmed, M., King, R. M. and Robinson, H. (1980) *Phytochemistry* **19**, 2391.
39. Dalling, D. K., Grant, D. M. and Paul, E. G. (1973) *J. Am. Chem. Soc.* **95**, 3718.
40. Ayers, W. A., Browne, L. M., Fung, S. and Stothers, J. B. (1976) *Can. J. Chem.* **54**, 3272.
41. Bohlmann, F., Ates (Goren), N., King, R. M. and Robinson, H. (1983) *Phytochemistry* **22**, 1675.
42. Box, G. S., Bardouille, V. and Chan, W. R. (1977) *Phytochemistry* **16**, 987.
43. Bohlmann, F., Ahmed, M., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 1434.
44. Riley, P. E. and Davis, R. E. (1976) *Acta Crystal.* **332**, 381.
45. Henslee, W. H. and Davis R. E. (1975) *Acta Crystal.* **331**, 1511.