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GERMACRANOLIDES, A GUAIANOLIDE WITH A β-LACTONE RING AND FURTHER CONSTITUENTS FROM *GRAZIELIA* SPECIES*

FERDINAND BOHLMANN,† CHRISTA ZDERO,† ROBERT M. KING‡ and HAROLD ROBINSON‡

† Institute for Organic Chemistry, Technical University Berlin, Strasse des 17. Juni 135, D-1000 Berlin 12, West Germany;
‡ Smithsonian Institution, Washington, DC 20560, U.S.A.

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Key Word Index—*Grazielia intermedia*; *G. dimorpholepsis*; *G. serrata*; Compositae; Eupatorieae; sesquiterpene lactones; germacranolides; guaianolides; melampolides; β-lactones; diterpenes.

Abstract—An investigation of three *Grazielia* species afforded, in addition to known compounds, several new sesquiterpene lactones, eight germacranolides, two melampolides and one guaianolide as well as three diterpenes, two geranylgeraniol derivatives and a labda-diene. Three of the sesquiterpene lactones had an additional β-lactone ring, one an acid function, and two others unusual ester functions. The structures were elucidated by spectroscopic methods and some chemical transformations. The chemotaxonomic situation is discussed briefly.

INTRODUCTION

The South American genus *Grazielia* (= *Dimorpholepis*) belongs to the *Disynaphia* group of the tribe Eupatorieae (Compositae) [1]. Prior to this study nothing was known on the chemistry of this genus and very little was known of the chemistry of the other genera placed in this group. A *Campovassouria* species was known to contain *ent*-kaurene derivatives [2], while from a *Symphiopappus* species kolavane derivatives had been isolated [3]. We have investigated three *Grazielia* species. Two of them afforded several new sesquiterpene lactones, which in part had very unusual structures.

RESULTS AND DISCUSSION

The aerial parts of *G. intermedia* (DC) K. et R. contain tridecapentaynene, germacrene D, bicyclogermacrene, lupeyl acetate, *ent*-kaurenic acid, the 15α-tiglinoyloxy derivative **16** [4] and spathulenol. The most polar fractions contained a very complex mixture of sesquiterpene lactones, which could only be separated with difficulty. Finally, eight compounds were isolated, the lactones **1**, **6**–**9**, **11**, **12** and **15**. The structure of **1** follows from the ^{1}H NMR data (Table 1). The sequence 5-H to 9-H is given by the spin decoupling data, while the stereochemistry of the double bonds is established by comparison of the chemical shifts of 1-, 5-, 14- and 15-H with those of closely related lactones [5, 6]. The ^{1}H NMR spectrum of **6** (Table 1) clearly indicates the presence of a 1,10-*cis* double bond with an aldehyde group at C-10 (δ = 6.62 *ddd* and 9.46 *d*). The presence of an angelate residue as well as the sequence 5-H to 9-H again follows from the chemical shifts and spin decoupling. The stereochemistry at these centres can be deduced from the coupling constants and by comparison of the data with those of similar lactones [7]. The ^{1}H NMR data of **7** (Table 1) shows that it is a 9β-hydroxy derivative of **6**. The stereochemistry follows from the coupling $J_{8,9}$, the downfield shift of 2α-H caused by the deshielding effect of the 9-hydroxy group and the missing W-coupling with 14-H. Models showed that a conformation with the substituents at C-4 and C-10 both above the plane the most likely. Furthermore the ^{1}H NMR data are very similar to those of the corresponding sesquiterpene lactones [8]. The lactone **8** is related to ovatifolin [9] and its desacetyl derivative [10]. Consequently the observed couplings are the same. The presence of an 8β-angeloyloxy derivative follows from the downfield shift of 8-H. The structure of the acid lactone **9** also follows from the ^{1}H NMR data (Table 1). The 1,10-*trans*-configuration can be deduced from the chemical shifts of 2- and 9-H, which are both deshielded by the carboxyl group i.e. an effect that requires the substituents at C-4 and C-10 to be above in plane. Addition of diazomethane gives one pyrazoline only, the ester **10**, the structure of which is supported by the ^{1}H NMR data (Table 1). The β-addition can be deduced by the downfield shift of the 6-H signal. **9** we have named grazielia acid. Its ^{1}H NMR data are different from those of germanin B [11], which is probably a 1,10-*cis* germacranolide (H-1: δ = 6.8 ppm).

The unusual structure of the dilactone **11** is established by the ^{1}H NMR data (Table 2) of the natural compound its tetrahydro derivative **13**, obtained by sodium borohydride reduction, and the pyrazoline **14**, formed by addition of diazomethane. Furthermore, the IR band at 1825 cm^{-1} is in agreement only with the presence of a β-lactone, especially as this band is shifted to 1835 cm^{-1} in the reduced compound **13**, which obviously has no strain. Therefore a strained γ-lactone, which could be the reason for the unusual IR band, is unlikely. Furthermore, since a γ-lactone in **15** (see below) seems to be impossible, the presence of β-lactones in **11** and **12** is supported on biogenetical grounds. Both reactions, the reduction as

*Part 318 in the series "Naturally Occurring Terpene Derivatives". For Part 317 see Bohlmann, F., Ahmed, M., Robinson, H. and King, R. M. (1981) *Phytochemistry* **20**, 1157.

Table 1. ^{1}H NMR data of compounds **1** and **6**–**10** (270 MHz, TMS as internal standard)

	1		**6**		**7**	**8**	**9**	**10**
	($CDCl_3$)	(C_6D_6)	($CDCl_3$)	(C_6D_6)	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)	($CDCl_3$)*
1-H	5.10 *d* (*br*)	4.65 *dd* (*br*)	6.62 *ddd*	6.01 *ddd*	6.63 *ddd*	5.08 *d* (*br*)	5.90 *dd* (*br*)	5.70 *dd*
2-H	2.0–2.45 *m*	1.8–2.1 *m*	2.29 *dd* (*br*)	2.0–1.6 *m*	3.23 *ddd* (2α)	2.0–2.5 *m*	3.47 *dd* (*br*)	3.45 *dd* (*br*)
3-H			2.50 *m* 2.12 *dd* (*br*) 2.40 *ddd*		2.3–2.5 *m* 2.13 *m*		2.2–2.5 *m*	
5-H	4.73 *d* (*br*)	4.35	5.08*m*	4.69 *d* (*br*)	4.80 *d* (*br*)	4.84 *d* (*br*)	5.00 *d* (*br*)	4.97 *d* (*br*)
6-H	5.13 *dd*	5.07 *dd*		4.92 *dd*	5.11 *dd*	5.10 *dd*	5.11 *dd*	6.01 *dd*
7-H	3.02 *ddd* (*br*)	2.34 *ddd* (*br*)	2.50 *m*	2.21 *dddd*	2.65 *dddd*	2.95 *ddd* (*br*)	2.90 *ddd* (*br*)	2.48 *m*
8-H	5.98 *s* (*br*)	5.79 *d* (*br*)	6.49 *ddd*	6.49 *ddd*	6.33 *dd*	5.82 *d* (*br*)	5.79 *d* (*br*)	5.00 *d* (*br*)
9-H	4.36 *s* (*br*)	3.88 *d*	2.84 *dd* (*br*)	2.64 *dd* (*br*)	5.06*d* (*br*)	3.35 *dd*	3.57 *dd* (*br*)	3.56 *dd* (*br*)
9′-H			1.98 *m*	1.98 *m*		2.20 *d* (*br*)	2.22 *d* (*br*)	2.37 *d* (*br*)
13-H	6.35 *d*	6.34 *d*	6.25 *d*	6.13 *d*	6.26 *d*	6.32 *d*	6.32 *d*	2.27 *ddd*
13′-H	5.72 *d*	5.44 *d*	5.60 *d*	5.44 *d*	5.56 *d*	5.64 *d*	5.65 *d*	1.53 *ddd*
14-H	1.55 *s* (*br*)	1.48 *ddd*	9.46 *d*	9.13 *d*	9.36 *s*	4.25 *d* 3.82 *d*		
15-H	1.77 *d*	1.46 *d*	1.93 *s* (*br*)	1.59 *s* (*br*)	1.83 *d*	1.67 *d*	1.70 *s* (*br*)	1.91 *s* (*br*)
OCOR	6.17 *qq*	5.69 *qq*	6.10 *qq*	5.85 *qq*	6.18 *qq*	6.12 *qq*	6.05 *qq*	6.16 *qq*
	2.00 *dq*	1.91 *dq*	1.98 *dq*	1.88 *dq*	2.00 *dq*	1.98 *dq*	1.99 *dq*	2.05 *dq*
	1.89 *dq*	1.68 *dq*	1.85 *dq*	1.70 *dq*	1.88 *dq*	1.88 *dq*	1.77 *s* (*br*)	1.88 *dq*

*16-H 4.85 *ddd*, 4.67 *ddd*.

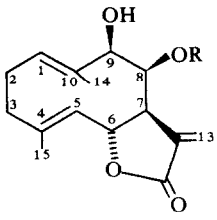
J (Hz): Compound **1**: 1,2 = 11; 1,2′ = 3.5; 1,15 = 2,15 = 5,15 ~ 1; 5,6 = 10; 6,7 = 9; 7,8 ~ 1; 7,13 = 3.5; 7,13′ = 3; 8,9 = 2.5; compound **6**: 1,2 = 10; 1,2′ = 7; 1,9 ~ 2; 2,3 ~ 2; 2,2′ = 13; 2′,3 = 6; 2′,3′ ~ 2; 3,3′ = 13; 5,6 = 10; 6,7 = 9; 7,8 = 2; 7,13 = 3.5; 7,13′ = 3; 8,9 = 7; 8,9′ = 10; 9,9′ = 14; 9′,14 = 1.5; compound **7**: 1,2 = 10; 1,2′ = 7; 1,9 ~ 2; 2,3 = 10; 3,3′ = 13; 5,6 = 10; 6,7 = 9; 7,8 = 2.5; 7,13 = 3.5; 7,13′ = 3; 8,9 = 5; compound **8**: 1,2 = 10; 5,6 = 10, 5,15 = 1; 6,7 = 9; 7,8 ~ 1; 7,13 = 3.5; 7,13′ = 3; 8,9 = 5; 8,9′ ~ 1.5; 9,9′ = 14; 14,14′ = 11; compound **9**: 1,2 = 12; 1,2′ = 3.5; 2,3 = 10; 2,2′ = 13; 2,3′ = 4; 5,6 = 10; 6,7 = 9; 7,8 ~ 1; 7,13 = 3.5; 7,13′ = 3; 8,9 = 6; 9,9′ = 14; compound **10**: 13,13′ = 13; 13,16 = 5; 13,16 = 10; 13′,16 = 10; 13′,16′ = 6; 16,16′ = 18; OAng: 3′,4′ = 7; 3′,5′ = 4′,5′ = 1.3.

Table 2. ^{1}H NMR data of compounds **11**–**15** (270 MHz, $CDCl_3$)

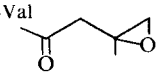
	11					
	($CDCl_3$)	(C_6D_6)	**12**	**13**	**14***	**15**
1-H	5.75 *d* (*br*)	4.69 *dd* (*br*)	5.76 *dd* (*br*)	2.34 *m*	5.77 *dd* (*br*)	—
2-H	2.88 *m*	2.60 *m*	2.88 *m*	1.6–1.9 *m*	2.88 *ddd*	3.52 *d* (*br*) 3.35 *d* (*br*)
3-H	2.45 *m*	1.75 *m*	2.42 *m*	2.34 *m* 2.14 *m*	2.47 *m*	5.65 *s* (*br*)
5-H	5.10 *d* (*br*)	4.48 *d* (*br*)	5.10 *d* (*br*)	5.32 *d* (*br*)	5.13 *dq*	3.70 *d* (*br*)
6-H	5.23 *dd*	5.04 *dd*	5.29 *dd*	4.91 *dd*	6.12 *dd*	4.26 *dd*
7-H	2.99 *ddd* (*br*)	1.99 *ddd* (*br*)	2.98 *ddd* (*br*)	2.0 *m*	2.62 *d* (*br*)	3.17 *ddd* (*br*)
8-H	6.06 *d* (*br*)	5.81 *d* (*br*)	6.05 *d* (*br*)	5.75 *s* (*br*)	5.30 *d* (*br*)	6.20 *d* (*br*)
9-H	5.12 *d* (*br*)	4.14 *d* (*br*)	5.07 *d* (*br*)	4.29 *dd*	4.84 *d* (*br*)	5.07 *s* (*br*)
13-H	6.45 *d*	6.37 *d*	6.43 *d*	1.38 *d*	2.37 *ddd*	6.37 *d*
13′-H	5.83 *d*	5.38 *d*	6.05 *d*		1.46 *ddd*	5.80 *d*
15-H	1.66 *d*	1.37 *d*	1.73 *s* (*br*)	1.93 *s* (*br*)	1.74 *d*	2.00 *dddd*
OCOR	6.14 *qq*	5.62 *qq*	3.06 *q*	6.24 *dq*	5.98 *qq*	6.15 *qq*
	1.97 *dq*	1.91 *dq*	1.27 *d*	2.03 *dq*	1.93 *dq*	1.93 *dq*
	1.91 *dq*	1.78 *dq*	1.58 *s*	1.94 *dq*	1.95 *dq*	1.80 *dq*
10-H	—	—	—	3.22 *ddd*	—	—
11-H	—	—	—	2.48 *dq*	—	—

*16-H 5.01 *ddd*; 16′-H 4.68 *ddd*.

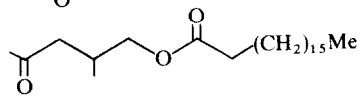
J (Hz): Compounds **11**/**12**: 1,2 = 12; 1,2′ = 3; 5,6 = 10; 5,15 = 1; 6,7 = 8.5; 7,8 ~ 0.5 7,13 = 3.5; 7,13′ = 3; 8,9 = 5.5; compound **13**: 1,10 = 5; 1′,10 = 3; 5,6 = 6,7 = 10; 8,9 = 1; 9,10 = 4; 11,13 = 12; compound **14**: 1,2 = 12; 1,2′ = 3; 2,3 = 10; 2,2′ = 13; 5,6 = 10; 6,7 = 9; 7,8 ~ 0.5; 8,9 = 5; 13,13′ = 13; 13,16 = 4; 13,16′ = 9; 13′,16 = 9; 13′,16 = 7.5; 16,16′ = 17.5; compound **15**: 2,2′ = 24; 2,3 = 2′,3 = 1.5; 2,5 = 2′,5 ~ 1; 2,9 = 2′,9 ~ 1.5; 2,15 = 2′,15 = 3,15 = 1.5; 5,6 = 10.5; 5,15 ~ 1; 6,7 = 9.5; 7,8 ~ 0.5; 7,13 = 3.5; 7,13′ = 3; 8,9 = 3; OAng: 3′,4′ = 7; 3′,5′ = 4′,5′ = 1.3; Epang: 3′,4′ = 5.

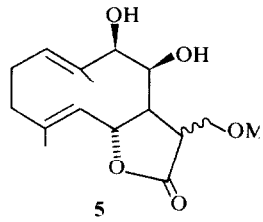


1 R = Ang
2 R = *i*-Val
3 R =



4 R =





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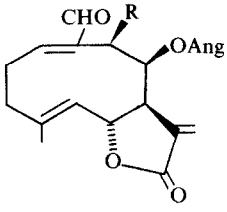
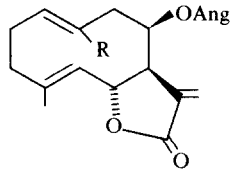
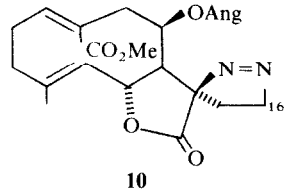


6 R = H
7 R = OH



8 R = CH_2OH
9 R = CO_2H



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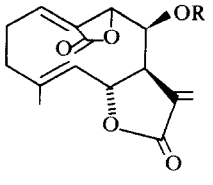
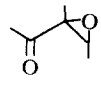
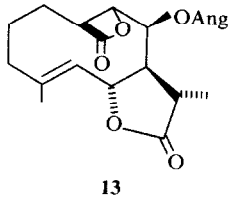


11 R = Ang
12 R =





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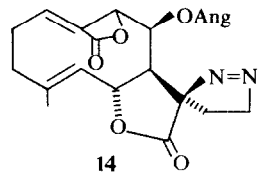

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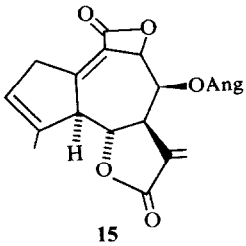

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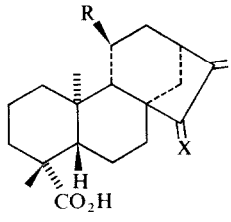
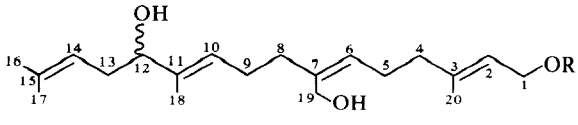
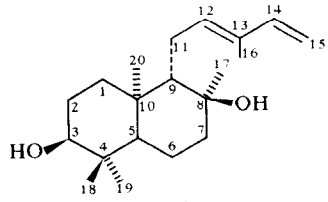


	16	**17**	**18**
R	H	OH	OH
X	α-OTigl	H	β-OH.H



19 R = H
20 R = Ac



21

well as the addition reaction, give one compound only, indicating a special steric situation. Inspections of models showed that the observed couplings and the stereospecific reactions can be explained best, if the β-lactone part is β-orientated. The configuration of **13** at C-10 and C-11 can be deduced from the observed couplings, while that of **14** follows from the downfield shift of 6-H caused by the deshielding effect of the azo group. The ^{1}H NMR data of **12** (Table 2) clearly shows that the ester residue of **11** is replaced by the corresponding epoxide of the angelate. All data are very similar to those of **11**. Although some differences in the chemical shifts are observed, the couplings are the same. The compound without a function at C-8 we have named grazuelolide. The structure of **15** was established by careful spin decoupling, which allowed the assignment of all signals and couplings. Irradiation at 2.00 (4-Me) causes a sharpening of the signals at 3.52, 3.35, 5.65 and 3.70, while irradiation of the double doublet at 4.24 (6-H) collapses the signal at 3.70 to a broadened singlet and the three-fold doublet at 3.14 to a broadened double doublet, indicating that these signals must be assigned to 5-H through 7-H. Irradiation of the 7-H signal sharpened the doublet at 6.20. The latter was coupled further with the broadened signal at 5.07, which on irradiation caused a sharpening of the broadened doublets at 3.52 and 3.35. Therefore the whole sequence from 2-H to 9-H is established. Inspection of a model showed that the configuration proposed for C-8 and C-9 would result in an angle of nearly 90° for the corresponding hydrogens. The presence of a β-lactone again followed from the IR band. The ^{13}C NMR signals (see Experimental) agree with the structure, though some signals cannot be assigned with certainty. Structure **15**, therefore, is another dilactone with a β-lactone ring. We have named the compound without an oxygen function at C-8 guaiagrazielolide to indicate the presence of an guanianolide related to grazielolide. Finally, two diterpenes **19** and **20** were isolated, **20** being the monoacetate of **19**, a dihydroxygeranylgeraniol which we have isolated from a *Lasiolaena* species [6]. The position of the acetate group follows from the ^{1}H NMR data. The triol gives a triacetate identical with the one obtained from the *Lasiolaena* compound.

The aerial parts of *G. dimorpholepsis* Baker contain tridecapentaynene, germacrene D, bicyclogermacrene, *ent*-kaurenic acid, the tiglate **16** and a further diterpene, which most probably is **21**. The ^{1}H NMR data (see Experimental) are very close to those of abienol and to those of a labdane derivative with an axial hydroxyl at C-3 and a 8(17) double bond. As the chemical shift of 17-H is the same as that of abienol the stereochemistry at C-8 probably is the same. Due to the axial hydroxyl group the 10-methyl signal, however, is shifted downfield. Though the absolute configuration was not determined an *ent*-labdane seems to be most likely as the optical rotation is opposite to that of abienol. The most polar fraction contained three sesquiterpene lactones, the germacranolides **2**–**4**. The ^{1}H NMR data of **2** are very similar to those of **1** (Table 3) and the nature of the ester residue follows from the typical NMR signals. The same is true for **3**. However, in this case the signals of the ester residue caused some confusion. The MS shows an acyl cation *m/e* 99 ($C_5H_7O_2$) indicating the presence of an epoxide of an unsaturated C-5 acid. The ^{1}H NMR spectrum in deuteriobenzene allows the assignment of the signals of the ester part, which can be established by spin decoupling. The broadened epoxide protons are coupled with the methyl group by a W-coupling, while the remaining methylene group, α to the carbonyl, displays two doublets. As far as we know this type of ester has not been reported from natural sources. The third lactone (**4**) also has an unusual ester residue, which again led to some

Table 3. ^{1}H NMR data of compounds **2**–**5** (270 MHz, $CDCl_3$)

	2	**3**	**4***	**5†**
1-H	5.08 *dd* (*br*)	4.61 *dd* (*br*)	5.08 *dd* (*br*)	5.08 *dd* (*br*)
2-H 3-H	2.2–2.45 *m*	1.7–2.1 *m*	2.2–2.5 *m*	2.2–2.5 *m*
5-H	4.69 *d* (*br*)	4.30 *d* (*br*)	4.67 *d* (*br*)	4.59 *d* (*br*)
6-H	5.07 *dd*	5.13 *dd*	5.05 *dd*	5.16 *dd*
7-H	2.98 *ddd* (*br*)	2.28 *ddd* (*br*)	2.98 *ddd* (*br*)	2.32 *m*
8-H	5.95 *d* (*br*)	5.78 *d* (*br*)	5.98 *d* (*br*)	4.33 *s* (*br*)
9-H	4.30 *d* (*br*)	3.76 *d* (*br*)	4.29 *d* (*br*)	4.09 *d* (*br*)
13-H	6.34 *d*	6.43 *d*	6.35 *d*	3.79 *dd*
13′-H	5.72 *d*	5.56 *d*	5.75 *d*	3.68 *dd*
14-H	1.55 *s* (*br*)	1.52 *s* (*br*)	1.54 *s* (*br*)	1.61 *s* (*br*)
15-H	1.79 *d*	1.57 *d*	1.77 *d*	1.74 *d*
OCOR	2.24 *d*	2.24 *d*		
	2.13 *m*	2.14 *m*		
	0.97 *d*	1.07 *s* (*br*)		
	0.96 *d*	2.33 *d* (*br*)		
		2.12 *d* (*br*)		

J (Hz): 1,2′ = 4; 5,6 = 10; 5,15 = 1.5; 6,7 = 9; 7,8 ~ 1; 7,13 = 3.5; 7,13′ = 3; 8,9 = 2.5; OCOR (**2**): 2′, 3′ = 3′, 4′ = 3′, 5′ = 6.5; OCOR (**3**): $2'_2$, $2'_2$ = 14; 4′, 5′ ~ 0.5; $5'_1$, $5'_2$ = 5.

*OCOR: 17-H 2.47 *dd* (*J* = 15, 6 Hz), 17′-H 2.23 *dd* (*J* = 15, 6 Hz), 18-H 2.35 *m*, 19-H 0.98 *d* (*J* = 6.5 Hz), 20-H 4.05 *dd* (*J* = 11, 5 Hz), 20′-H 3.91 *dd* (*J* = 11, 6 Hz), 22-H 2.30 *t* (*J* = 7.5 Hz), 23-H 1.62 *m*, 24-38-H 1.25 *m*, 39-H 0.88 *t* (*J* = 6.5 Hz).

†OMe 3.38 *s*.

confusion. The ^{1}H NMR spectrum (Table 3) if compared with those of **1** and **2**, showed additional signals, which must be due to a large ester residue. The MS showed a very small $M^{+\cdot}$ at *m/e* 630 and prominent peaks at *m/e* 267.269 ($C_{18}H_{35}O$), 101.060 ($C_5H_9O_2$) and 246.127 ($C_{15}H_{18}O_3$). While the latter ion is obviously $[M-RCO_2H]^+$, the first ion can be assigned to a stearoyl cation and the second should be the acyl cation of a C-5 hydroxy acid. In combination with the additional ^{1}H NMR signals these facts can be correlated only with the structure of the ester part in **4**. Reaction of **4** in methanol–water with potassium carbonate affords, in addition to methyl stearoate, methyl[4-stearoyloxy]-isovalerate and the lactone **5**, the product of the saponification and of the addition of methanol to the 11,13-double bond. The ^{1}H NMR data of the reactions products again supported the proposed structure of the natural compound. This is the first report of a sesquiterpene lactone with this ester residue. Only eupassofilin [12] has a somewhat similar ester group, a tiglate substituted with a β-hydroxy stearoyloxy residue. The roots of *G. dimorpholepsis* afforded tridecapentaynene, germacrene D, α-pinene, *ent*-kaurenic acid and the tiglate **16**.

The aerial parts of *G. serrata* (Spreng) K. et R. contain tridecapentaynene, germacrene D, bicyclogermacrene, *ent*-kaurenic acid, the tiglate **16**, 11β-hydroxy-15-oxo-*ent*-kaurenic acid (**17**) and the corresponding diol **18**. The last two compounds have been isolated from *Eupatorium album* [13]. The roots gave tridecapentaynene, α-pinene, bicyclogermacrene, germacrene D, *ent*-kaurenic acid, the tiglate **16** and the ketone **18**.

The overall picture of the constituents of *Grazielia* species investigated is not uniform, but the compounds isolated show relationships to the genera *Symphiopappus* and *Campovassouria*, as far as the diterpenes are concerned. These and the sesquiterpene lactones isolated indicate a relationship to the *Eupatorium* group too, though the special types isolated in this study are different from those of the genus *Eupatorium*. Obviously more species of the *Disynaphia* group need to be investigated if we are to have a clear picture of the chemotaxonomy of this group.

EXPERIMENTAL

The air dried plant material, collected in north eastern Brazil, was extracted with Et_2O–petrol (1:2) and the resulting extracts, after treatment with MeOH to remove long chain saturated hydrocarbons, were first separated by column chromatography (Si gel, act. grade II) and further by repeated TLC (Si gel) and, if necessary, by HPLC (reversed phase). Known compounds were identified by comparing their IR and ^{1}H NMR spectra with those of authentic samples.

Grazielia intermedia (voucher RMK 8297). The aerial parts (1.5 kg) afforded 1 mg tridecapenta-3,5,7,9,11-yn-1-ene, 20 mg germacrene D, 5 mg bicyclogermacrene, 40 mg lupeyl acetate, 400 mg *ent*-kaurenic acid, 60 mg 15α-tiglinoyloxy-*ent*-kaurenic acid, and a mixture of sesquiterpene lactones, which could only be separated with considerable loss of material. Finally after TLC and HPLC 20 mg **1** (Et_2O–petrol, 3:1), 10 mg **6** and 6 mg **7** (HPLC, MeOH–H_2O, 7:3) and 3 mg **8**, 3 mg **9** (Et_2O–petrol, 3:1, HPLC, MeOH–H_2O, 13:7), 10 mg **11** and 2 mg **12** (Et_2O–C_6H_6–$CHCl_3$, 3:1:1), 10 mg **15** (Et_2O–petrol, 3:1, HPLC, MeOH–H_2O, 7:3). Furthermore 20 mg **19** (Et_2O–MeOH, 20:1) and 10 mg **20** (Et_2O) were obtained.

Grazielia dimorpholepsis (voucher RMK 8384). The roots (120 g) afforded 2 mg tridecapentaynene, 10 mg α-pinene, 3 mg germacrene D, 100 mg *ent*-kaurenic acid and 20 mg **16**, while the aerial parts (500 g) yielded 1 mg tridecapentaynene, 50 mg germacrene D, 3 mg bicyclogermacrene, 500 mg *ent*-kaurenic acid, 11 mg **2** (Et_2O–petrol, 9:1), 2 mg **3** (Et_2O–petrol, 9:1), 15 mg **4** (Et_2O–petrol, 9:1), 100 mg **16** and 3 mg **21** (Et_2O–petrol, 3:1).

Grazielia serrata (Spreng.) K. et R. (voucher RMK 8378). The roots (200 g) afforded 2 mg tridecapentaynene, 20 mg α-pinene, 2 mg bicyclogermacrene, 5 mg germacrene D, 150 mg *ent*-kaurenic acid, 50 mg **16** and 10 mg **17**, while the aerial parts (250 g) yielded 0.1 mg tridecapentaynene, 80 mg germacrene D, 5 mg bicyclogermacrene, 500 mg *ent*-kaurenic acid, 80 mg **16**, 60 mg **17** and 30 mg **18**.

8β-Angeloyloxy-9β-hydroxycostunolide (**1**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3630 (OH), 1780 (γ-lactone), 1730 (C=CCO_2R); CIMS (isobutane) *m/e* (rel. int.): 347 $[M+1]^+$ (100) ($C_{20}H_{26}O_5$), 329 $[M+1-H_2O]^+$ (35), 247 $[M+1-AngOH]^+$ (32), 229 $[247-H_2O]^+$ (34).

8β-Isovaleryloxy-9β-hydroxycostunolide (**2**). Colourless gum: IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (OH), 1775 (γ-lactone), 1750 (CO_2R); CIMS (isobutane) *m/e* (rel. int.) 349 $[M+1]^+$ (100), 331 $[M+1-H_2O]^+$ (43), 247 $[M+1-RCO_2H]^+$ (62), 229 $[247-H_2O]^+$ (47).

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589 \quad 578 \quad 546\,\text{nm}}{+14.2 \quad +15.0 \quad +17.8} \quad (c = 1.07,\ CHCl_3).$$

8β-[3,4-Epoxyisovaleryloxy]9β-hydroxycostunolide (**3**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3630 (OH), 1780 (γ-lactone), 1755 (CO_2R); MS *m/e* (rel. int.): 246.127 $[M-RCO_2H]^+$ (27) ($C_{15}H_{18}O_3$), 228 $[246-H_2O]^+$ (17), 99 $[RCO]^+$ (100), 71 $[99-CO]^+$ (56); CIMS (isobutane): 363 $[M+1]^+$ (38), 345 $[363-H_2O]^+$ (20), 247 $[M+1-RCO_2H]^+$ (100), 229 $[247-H_2O]^+$ (65), 99 $[RCO]^+$ (52).

8β-[4-Stearoyloxyisovaleroyloxy]-9β-hydroxycostunolide (**4**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (OH), 1775 (γ-lactone), 1740 (CO_2R); MS *m/e* (rel. int.): 630 $[M]^{+\cdot}$ (0.5), 267.269 $[C_{18}H_{35}O]^+$ (62), 246.127 $[M-RCO_2H]^+$ (52) ($C_{15}H_{18}O_3$), 228 $[246-H_2O]^+$ (39), 101 $[C_5H_9O_2]^+$ (100) ($C_5H_9O_2$); CIMS (isobutane): 631 $[M+1]^+$ (6), 247 $[M+1-RCO_2H]^+$ (100), 229 $[247-H_2O]^+$ (72), 101 $[C_5H_9O_2]^+$ (54).

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,\text{nm}}{+11.0 \quad +11.4 \quad +14.0 \quad +31.0} \quad (c = 1.2,\ CHCl_3).$$

To 10 mg **4** in 2 ml MeOH, 20 mg K_2CO_3 in 0.5 ml H_2O was added. After 1 hr dil. H_2SO_4 was added and the compound extracted with Et_2O. TLC afforded 3 mg methyl stearate, 2 mg methyl-4-stearoyloxyisovalerate, colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745 (CO_2R); ^{1}H NMR ($CDCl_3$): δ 2.43 *dd*, 2.20 *dd* (2-H), 2.32 *m* (3-H), 4.01 *dd*, 3.92 *dd* (4-H), 1.00 *d* (5-H), 2.30 *t*, 1.62 *m*, 1.25 *m*, 0.88 *t* ($OCO(CH_2)_{16}Me$); and 4 mg **5**, colourless gum, ^{1}H NMR see Table 3.

8β-Angeloyloxy-14-oxo-acanthospermolide (**6**). Colourless crystals, mp 84°; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3610 (OH), 2720, 1690 (C=CCHO), 1775 (γ-lactone), 1730, (C=C–CO_2R): MS *m/e* (rel. int.): 344.163 $[M]^{+\cdot}$ (0.5) ($C_{20}H_{24}O_5$), 244 $[M-AngOH]^+$ (6), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (50).

$$[\alpha]_{24^\circ}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\,\text{nm}}{-38.6 \quad -40.9 \quad -49.8 \quad -118.4 \quad -340.7} \quad (c = 0.43,\ CHCl_3).$$

8β-Angeloyloxy-9β-hydroxy-14-oxo-acanthospermolide (**7**). Colourless gum: IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3610 (OH), 2720, 1690, 1630 (C=CCHO), 1775 (γ-lactone), 1730, 1650 (C = CCO_2R); MS

m/e (rel. int.): 360.157 $[M]^{+\cdot}$ (0.2) ($C_{20}H_{24}O_6$), 342 $[M - H_2O]^+$ (0.3), 277 $[M - C_4H_7CO_2]^+$ (10), 260 $[M - AngOH]^+$ (9), 242 $[260 - H_2O]^+$ (7), 231 $[260 - CHO]^+$ (4), 83 $[C_4H_7CO]^+$ (100), 55 $[83 - CO]^+$ (95).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\,nm}{+19.0 \quad +19.4 \quad +21.4 \quad +33.5 \quad +160.0}$$

($c = 0.49$, $CHCl_3$).

14-O-*Desacetylovatifolin*-8-O-*angelate* (**8**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (OH), 1775 (γ-lactone), 1730, 1650 (C=CCO_2R); MS *m/e* (rel. int.): 346.178 $[M]^{+\cdot}$ (2) ($C_{20}H_{26}O_5$), 246 $[M - AngOH]^+$ (23), 83 $[C_4H_7CO]^+$ (100).

Grazielia acid (**9**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500–2600, 1690 (C=CCO_2R), 1780 (γ-lactone), 1730, 1650 (C=CCO_2R); MS *m/e* (rel. int.): 360.157 $[M]^{+\cdot}$ (1) ($C_{20}H_{24}O_6$), 342 $[M - H_2O]^+$ (1), 260 $[M - AngOH]^+$ (6), 242 $[260 - H_2O]^+$ (5), 83 $[C_4H_7CO]^+$ (100), 55 $[83 - CO]^+$ (55). To 3 mg **9** in 1 ml Et_2O excess of CH_2N_2 was added. After 15 min the soln was evapd and the residue was purified by TLC (Et_2O–petrol, 3:1); 2 mg **10** were obtained. Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1785 (γ-lactone), 1730, 1650 (C=CCO_2R); MS *m/e* (rel. int.): 416 $[M]^{+\cdot}$ (0.5), 388 $[M - N_2]^+$ (0.5), 316 $[M - AngOH]^+$ (1), 288 $[316 - N_2]^+$ (5), 256 $[288 - MeOH]^+$ (6), 228 $[256 - CO]^+$ (6), 83 $[C_4H_7CO]^+$ (100), 55 $[83 - CO]^+$ (86).

8β-*Angeloyloxy-grazielolide* (**11**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1825 (β-lactone), 1775 (γ-lactone), 1730, 1655 (C=CCO_2R); MS *m/e* (rel. int.): 258.089 $[M - AngOH]^+$ (2) ($C_{15}H_{14}O_4$), 214 $[258 - CO_2]^+$ (3), 83 $[C_4H_7CO]^+$ (100), 55 $[83 - CO]^+$ (62); CIMS (isobutane): 359 $[M + 1]^+$ (75) ($C_{20}H_{24}O_6$, 1), 259 $[M + 1 - AngOH]^+$ (7), 83 $[C_4H_7CO]^+$ (100).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{-74.5 \quad -77.3 \quad -86.8 \quad -131.8} \quad (c = 0.22,\ CHCl_3).$$

To 5 mg **11** in 1 ml MeOH, 10 mg $NaBH_4$ and after 5 min dil. H_2SO_4 were added. TLC (Et_2O–petrol, 3:1) afforded 3 mg **13**; colourless crystals, mp 167° (Et_2O–petrol); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1835 (β-lactone), 1790 (γ-lactone), 1725, 1650 (C=CCO_2R); MS *m/e* (rel. int.): 263 $[M - OAng]^+$ (1), 83 $[C_4H_7CO]^+$ (100); CIMS (isobutane): 363 $[M + 1]^+$ (52) ($C_{20}H_{28}O_6 + 1$), 319 $[M + 1 - CO_2]^+$ (10), 263 $[M + 1 - AngOH]^+$ (21), 219 $[263 - CO_2]^+$ (24), 83 $[C_4H_7CO]^+$ (100).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\,nm}{+28.6 \quad +31.1 \quad +35.4 \quad +71.4 \quad +137.5}$$

($c = 0.28$, $CHCl_3$).

To 5 mg **11** in 1 ml Et_2O excess of CH_2N_2 in Et_2O was added. After 5 min the solution was evapd: colourless crystals (**14**), mp 168° (Et_2O), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1830 (β-lactone), 1785 (γ-lactone), 1740 (C=CCO_2R).

8β-[2,3-*Epoxy*-2-*methylbutyryloxy*]-*grazielolide* (**12**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1825 (β-lactone), 1770 (γ-lactone), 1740 (CO_2R); MS *m/e* (rel. int.): 258.089 $[M - RCO_2H]^+$ (9) ($C_{15}H_{14}O_4$), 214 $[258 - CO_2]^+$ (12), 81 (100).

8β-*Angeloyloxyguaiagrazielolide* (**15**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1835 (β-lactone), 1785 (γ-lactone), 1735 (C=CCO_2R); ^{13}C NMR ($CDCl_3$): δ C-1 to C-15, 37.0, 122.4, 133.9, 58.5, 74.0$^+$, 48.7, 78.4$^+$, 63.4, 126.7, 150.0, 133.3, 167.6, 126.1, 160.9, 16.2 ($^+$ may be interchangeable); MS *m/e* (rel. int.): 356.126 $[M]^{+\cdot}$ (3) ($C_{20}H_2O_6$), 83 $[C_4H_9CO]^+$ (100), 55 $[83 - CO]^+$ (92); CIMS (isobutane): 357 $[M + 1]^+$ (100), 257 $[M + 1 - AngOH]^+$ (6), 83 $[C_4H_7CO]^+$ (18).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{-66.7 \quad -73.1 \quad -85.8 \quad -171.7} \quad (c = 0.12,\ CHCl_3).$$

12,19-*Dihydroxygeranylgeraniol acetate* (**20**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1740, 1240 (OAc); MS *m/e* (rel. int.): 295.191 $[M - CH_2CH{=}CMe_2]^+$ (0.5) ($C_{17}H_{27}O_4$), 235 $[295 - AcOH]^+$ (10), 217 $[235 - H_2O]^+$ (12), 199 $[217 - H_2O]^+$ (6), 69 $[C_5H_9]^+$ (100); ^1H NMR ($CDCl_3$): δ 4.57 *br d* (1-H, J = 7 Hz), 5.32 *br t* (2-H, J = 7 Hz), 5.40 *br t* (6-H, 10-H), 399 *dd* (12-H, J = 7, 6 Hz), 5.10 *br t* (14-H, J = 7 Hz), 1.64 *br s* (16-, 17-H), 1.78 *br s* (18-H), 4.12 *br s* (19-H), 1.73 *br s* (20-H). 10 mg **20** were heated for 1 hr with 0.5 ml Ac_2O at 70°. After evaporation and TLC 9 mg **19** were obtained, identical with an authentic sample [6].

3β-*Hydroxy*-ent-*abienol* (**21**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3620 (OH), 1640, 940, 900 (C=C); MS *m/e* (rel. int.): 288.245 $[M - H_2O]^+$ (5) ($C_{20}H_{32}O$), 189 $[C_{14}H_{21}]^+$ (11), 81 $[C_6H_9]^+$ (100); CIMS (isobutane): 289 $[M + 1 - H_2O]^+$ (66), 271 $[289 - H_2O]^+$ (100).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{-27 \quad -27 \quad -36 \quad -51} \quad (c = 0.3,\ CHCl_3).$$

^1H NMR ($CDCl_3$): δ 3.42 *br dd* (3-H), 5.61 *br t* (12-H), 6.34 *dd* (14-H), 5.06 *br d* (15t-H), 4.91 *br s* (15c-H), 1.79 *br s* (16-H), 1.20 *s* (17-H), 0.88 (18-H), 0.85 *s* (19-H), 0.98 *s* (20-H) (J Hz: 2,3 = 3; 11,12 = 7; 14, 15*t* = 17; 14, 15*c* = 10).

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REFERENCES

1. King, R. M. and Robinson, H. (1972) *Phytologia* **23**, 305.
2. Bohlmann, F., Suwita, A., King, R. M. and Robinson, H. (1980) *Phytochemistry* **19**, 111.
3. Vichnewski, W., Murari, R. and Herz, W. (1979) *Phytochemistry* **18**, 129.
4. Bohlmann, F. and Zdero, C. (1979) *Phytochemistry* **18**, 492.
5. Herz, W., Murari, R. and Govindan, S. V. (1979) *Phytochemistry* **18**, 1337.
6. Bohlmann, F., Jakupovic, J., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, (in press).
7. Bohlmann, F., Jakupovic, J. Zdero, C., King, R. M. and Robinson, H. (1979) *Phytochemistry* **18**, 625.
8. Bohlmann, F., Ziesche, J., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 263.
9. Gnecco, S., Poyser, J. P., Silva, M., Sammes, P. G. and Tyler, T. W. (1973) *Phytochemistry* **12**, 2469.
10. Hoeneisen, M., Silva, M. and Bohlmann, F. (1980) *Phytochemistry* **19**, 2765.
11. Konovalova, O. A., Rhybalko, K. S. and Sheichenko, V. I. (1974) *Khim. Prir. Soedin* **10**, 578 (Engl. 591).
12. Herz, W. and Sharma, R. P. (1976) *J. Org. Chem.* **41**, 1015.
13. Herz, W. and Sharma, R. P. (1976 *J. Org. Chem.* **41**, 1021.