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SOME SESQUI- AND DITERPENES FROM THE TRIBE EUPATORIEAE

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Key Word Index—*Trichogonia salviaefolia*; *Liatris spicata*; *Brickellia vernicosa*; Compositae; sesquiterpenes; guaianolide, germacranolides, dehydronerolidol derivatives; diterpenes; clerodane derivative; *ent*-kaurenic acid derivative.

Abstract—The aerial parts of *Trichogonia salviaefolia* afforded a new *ent*-kaurenic acid derivative and a germacranolide which, most likely, is the precursor of the trichosalviolides. The aerial parts of *Liatris spicata* gave 1α-hydroxybacchotricuneatin A and a guaianolide, which may be the precursor of spicatin. From *Brickellia vernicosa* some further dehydronerolidol derivatives were isolated.

INTRODUCTION

From *Trichogonia salviaefolia* several sesquiterpene lactones were isolated which belong to a new class [1]. A reinvestigation of the aerial parts gave in addition to the trichosalviolide isolated previously the germacranolide **1**.

RESULTS AND DISCUSSION

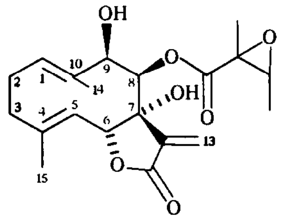
The structure of **1** followed from the ^{1}H NMR spectrum (Table 1). The presence of a 7-hydroxy group was indicated by the absence of allylic couplings with H-13. Many signals were close to those of the corresponding trichosalviolide [1]. However, from the typical signals of H-1, H-5, H-14 and H-15 the presence of a germacra-1(10),4-dien-6,12-olide could be deduced. Spin decoupling allowed the assignment of all signals, especially if deuteriobenzene was added. Thus irradiation of the H-1 signal (δ4.98) sharpened the broadened signal at δ1.49 and changed the multiplets at δ2.18 and 2.06. Irradiation at δ1.74 sharpened the H-5 signal. These results indicate the presence of two olefinic methyls which, together with the molecular formula, required the presence of a germacranolide. The chemical shift and the couplings of H-8 and H-9 only agree with the proposed configuration and the presence of an epoxy-2-methylbutyrate followed from the characteristic signals (2.79 *q*, 1.07 *d* and 1.33 *s*). The position of oxygen functions and the stereochemistry clearly followed from the ^{1}H NMR spectrum. The α-orientation of the 7-hydroxy group caused a downfield shift of H-9 if the data were compared with the shift of H-9 in 9β-hydroxy costunolide [2]. The lactone **1** most likely is the precursor of the trichosalviolides [1]. Furthermore the *ent*-kaurenic acid derivative **5** was isolated. Its structure followed from the molecular formula and from the ^{1}H NMR spectrum (see Experimental) which was nearly identical with that of similar esters like the corresponding 15α-angeloyloxy derivative [3]. As in similar cases the 9β-

Table 1. ^{1}H NMR spectral data of **1** and **2** (400 MHz, $CDCl_3$, TMS as internal standard)

	1 ($CDCl_3$–C_6D_6)	**2**
H-1	4.98 *br d**	3.13 *dd*
H-2	2.18 *m* 2.06 *m*	4.67 *br d*
H-3	2.25 *m* 2.01 *m*	5.75 *br s*
H-5	4.97 *br d**	2.62 *br dd*
H-6	4.95 *d**	4.59 *dd*
H-7	—	3.18 *dddd*
H-8	5.70 *d*	5.57 *ddd*
H-9α	4.83 *d*	2.72 *dd*
H-9β	—	2.86 *dd*
H-13	6.24 *s*	6.27 *d*
H-13′	5.81 *s*	5.52 *d*
H-14	1.49 *br s*	5.11 *br s*
H-14′		5.08 *br s*
H-15	1.74 *d*	1.97 *br s*
$H\text{-}3_1$	2.79 *q*	7.11 *q*
$H\text{-}4_1$	1.07 *d*	1.94 *d*
$H\text{-}5_1$	1.33 *s*	4.90 *d*
$H\text{-}5_{1'}$		4.83 *d*
$H\text{-}3_2$	—	6.89 *q*
$H\text{-}4_2$	—	1.88 *d*
$H\text{-}5_2$	—	4.30 *s*

*$CDCl_3$: H-1 = 5.17 *br dd*, H-5 = 5.13 *br d*, H-6 = 5.08 *d*.

J (Hz): Compound **1**: 1, 2 = 11; 1, 2′ = 5; 5, 6 = 10; 5, 15 = 1; 8, 9 = 2.7; OCOR: 3′, 4′ = 5.5; compound **2**: 1, 2 = 6; 1, 5 = 8.5; 2, 3 = 3, 5 = 3, 15 ~ 1.5; 5, 6 = 10.5; 6, 7 = 8.5; 7, 8 = 7, 13 = 3.5; 7, 13′ = 3; 8, 9α = 8, 9β = 8; 9α, 9β = 13.5; $3_1, 4_1 = 3_2, 4_2 = 7$; $5_1, 5_1' = 12$.



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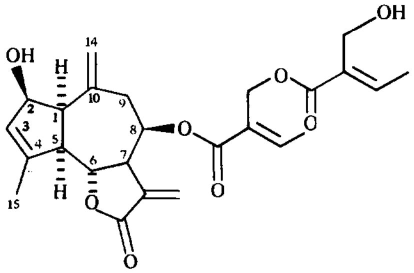

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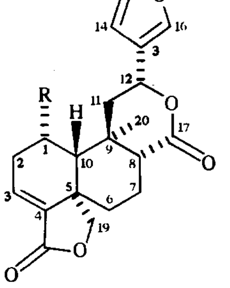
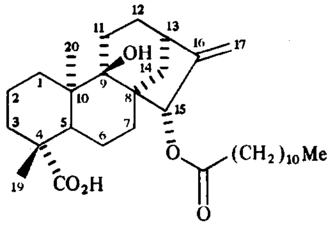


3 R = H
4 R = OH



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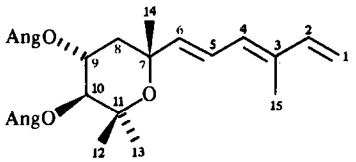

6 3E
7 3*E*,7-epi
8 3*Z*,7-epi

hydroxy group caused a considerable downfield shift of H-15 (5.94 *br s*). The nature of the ester side chain followed from the ^{1}H NMR signals and from the mass spectrum which showed loss of $C_{12}H_{24}O_2$. Elimination of the acid residue in 15-acyloxy kaurene derivatives is typical and has been observed in more than 20 cases.

Liatris spicata (L.) Willd. has been studied by several groups. In addition to widespread volatile compounds [4], flavones [5, 6] and euparin [5], the guaianolide spicatin were reported [7, 8]. A reinvestigation of the aerial parts gave the helianolides desacetyl 4′-desoxyprovincialin [9], its 9-epimer [8], 4′-desoxyprovincialin [8] and its 3-epimer [10], the *trans,trans*-germacranolide 3β-hydroxyliacylindrolide [10] as well as the guaianolide **2**, which obviously is the precursor of spicatin [8]. The ^{1}H NMR spectrum of lactone **2** (Table 1) was in part close to that of spicatin. The characteristic signals of the ester residue as well as the signals of H-5–H-8 and H-13 were nearly unchanged while the acetate singlet and the doublets of the epoxide protons were missing. Accordingly, the H-2 signal was shifted up field and additional low field broadened singlets at δ5.11 and 5.08 indicated a 10(14)-double bond, especially as the H-1 signal was shifted down field if compared with the chemical shift of H-1 in the spectrum of spicatin. We have named compound **2** prespicatin.

Furthermore bacchotricuneatin A (**3**) [11] and a further clerodane were isolated. The molecular formula was $C_{20}H_{22}O_6$ indicating the presence of an additional oxygen, which, following the IR band at 3600 cm^{-1}, obviously was a hydroxy group. The ^{1}H NMR spectrum (Table 2) was close to that of **3**. Careful spin decoupling showed that this group was at C-1 of bacchotricuneatin A (**3**). Irradiation of the lowfield double doublet at δ6.45 (H-3) caused a change of the ABXY-system (2.35 *m* and 2.28 *m*) which also was changed by irradiation at δ4.20. As the latter signal must be due to a proton under the hydroxy group these observations required a 1-hydroxy derivative. The couplings of H-1 indicated an axial hydroxy group while all the other couplings were nearly identical with those of **3**. Accordingly, the stereochemistry obviously was the same. Thus the second diterpene was

Table 2. ^{1}H NMR spectral data of **3** and **4** (400 MHz, $CDCl_3$, TMS as internal standard)

	3	**4**
H-1	1.24 *m*	4.20 *br t*
H-1′	1.76 *br d*	—
H-2	2.46 *dddd*	2.35 *m*
H-2′	2.24 *dddd*	2.28 *m*
H-3	6.78 *dd*	6.45 *dd*
H-6β	1.34 *dddd*	1.17 *dddd*
H-6α	2.14 *m*	2.0–1.7 *m*
	1.96 *br dt*	
H-7	2.11 *br d*	
H-8	2.67 *dd*	2.52 *dd*
H-10	2.1 *m*	1.9 *m*
H-11	2.14 *dd*	2.06 *dd*
H-11′	1.94 *dd*	1.91 *dd*
H-12	5.40 *br dd*	5.27 *br dd*
H-14	6.43 *br d*	6.29 *br d*
H-15	7.47 *br s*	7.35 *br s*
H-16	7.44 *t*	7.29 *t*
H-19	4.30 *d*	4.57 *dd*
H-19′	3.94 *dd*	4.16 *d*
H-20	0.85 *s*	0.90 *s*
OH		1.70 *br s*

J (Hz): 1, 2 = 2; 2, 3 = 3.5; 2′, 3 = 6; 6α, 6β = 6β, 7α = 13; 6β, 7β = 4; 6β, 19 = 1.5; 7, 8 = 4.5; 7′, 8 = 11; 11, 11′ = 14; 11, 12 = 7.5; 11′, 12 = 10.5; 14, 15 = 15, 16 = 1.5; 19, 19′ = 8; (compound **3**: 1, 1′ = 14; 1, 2 ~ 3; 1, 2′ = 11; 1′, 2 ~ 3; 1′, 2′ ~ 4; 2, 2′ = 18; 2, 3 = 7.5; 2′, 3 = 2; 7, 8 = 4; 7′, 8 = 12).

Table 3. ^{1}H NMR spectral data of **6–8** (400 MHz, $CDCl_3$, TMS as internal standard)

	6*	**7†**	**8**
H-1c	5.05 *br d*	5.03 *br d*	5.17 *br dd*
H-1t	5.24 *br d*	5.21 *br d*	5.24 *br d*
H-2	6.41 *dd*	6.40 *dd*	6.92 *dd*
H-4	6.05 *br d*	6.02 *br d*	5.93 *br d*
H-5	6.76 *dd*	6.53 *dd*	6.65 *dd*
H-6	5.80 *br d*	5.78 *br d*	5.68 *br d*
H-9	5.22 *ddd*	5.38 *ddd*	5.37 *ddd*
H-10	5.10 *d*	5.12 *d*	5.11 *d*
H-12	1.35 *s*	1.47 *s*	1.46 *s*
H-13	1.25 *s*	1.36 *s*	1.37 *s*
H-14	1.18 *s*	1.28 *s*	1.28 *s*
H-15	1.96 *br s*	1.85 *br s*	1.87 *br s*
OAng	6.01 *qq*	6.06 *br q* (2H)	6.05 *br q* (2H)
	6.03 *qq*	1.95 *dq* (6H)	1.94 *dq* (6H)
	1.94 *dq*	1.86 *dq*	1.85 *dq*
	1.91 *dq*	1.79 *dq*	1.79 *dq*
	1.80 *dq* (6H)		

*In $CDCl_3$–C_6D_6: H-8 = 2.68 *dd* and 1.64 *dd*.

†In C_6D_6: H-8 = 2.70 *dd* and 1.51 *dd*.

J (Hz): 1c, 2 = 11; 1t, 2 = 17; 4, 5 = 11; 5, 6 = 15; 8, 8′ = 15; 8, 9 = 9, 10 = 10; 8′, 9 = 5; OAng: 3′, 4′ = 7; 3′, 5′ = 4′, 5′ = 1.5.

1α-hydroxybacchotricuneatin A (**4**). It is remarkable that the hydroxy group at C-1 led to small shift differences even of the signals of H-14–H-16 while the down field shift of the H-19 signals is expected and supported the proposed stereochemistry at C-1 and C-5.

The aerial parts of *Brickellia vernicosa* B. L. Robins. afforded as the main compound 3α-hydroxy-labd-7-en-15-oic acid [12] and the isomeric dehydronerolidol derivatives **6–8**. The separation was difficult but by repeated thin layer chromatography using silver nitrate coated silica gel all three isomers could be obtained pure. The structures easily could be deduced from the ^{1}H NMR spectra (Table 3) which were close to those of the known hydroxy angelates which were isolated from an *Ageratum* species [13]. Dehydronerolidol derivatives with angeloyloxy groups have been isolated from many *Brickellia* species and from some other genera of the tribe Eupatorieae.

EXPERIMENTAL

The air dried plant material was extracted at 20° with $MeOH$–Et_2O–petrol, 1:1:1, for 20 hr and the extracts obtained were treated with MeOH to remove saturated hydrocarbons and then separated by column chromatography as reported previously [14]. The extract of the aerial parts (150 g) of *Trichogonia salviaefolia* (voucher RMK 8183) gave three CC fractions (SiO_2): 1 (Et_2O–petrol, 1:3), 2 (Et_2O) and (Et_2O–MeOH, 9:1). TLC (SiO_2, PF 254) of fraction 1 (Et_2O–petrol, 1:1) gave 35 mg **5** (R_f 0.40), TLC of fraction 2 (Et_2O–petrol, 2:1) the lactones isolated previously [1] while fraction 3 gave after repeated TLC (MeOH–Et_2O, 1:9) 5 mg **1** (R_f 0.70).

The extract of the aerial parts of *Liatris spicata* (230 g, voucher RMK 9319) gave a polar CC fraction (SiO_2, Et_2O–MeOH, 9:1 and 3:1). HPLC (RP 8, MeOH–H_2O, 3:2, flow rate 3 ml/min, *ca* 100 bar) afforded 4 mg **4** (R_t = 4.5 min), 4 mg 3β-hydroxyliacylindrolide (R_t 7 min), 3 mg bacchotricuneatin A (**3**) (R_t 7.5 min), 1.5 mg **2** (R_t 10.0 min), 5 mg 4′-desoxydesacetyl provincialin (R_t 11.8 min), 3-epi-4′-desoxydesacetyl provincialin (R_t 13.0 min), 4′-desoxyprovincialin (R_t 14.8 min) and 3-epi-4′-desoxyprovincialin (R_t 20.0 min).

The extract of the aerial parts (100 g) of *Brickellia vernicosa* (voucher 84/1645, collected near Cerocahui, Mexico) gave two CC fractions (SiO_2): 1 (Et_2O–petrol, 1:3 and 1:1) and 2 (Et_2O and Et_2O–MeOH, 9:1). TLC (SiO_2, PF 254, Et_2O–petrol, 1:20, two developments) of fraction 1 gave a less polar band containing 3 mg **6** (R_f 0.58) and a second with **7** and **8** (R_f 0.50). TLC (SiO_2, $AgNO_3$ coated, Et_2O–petrol, 1:9) affording 1 mg **7** and 0.5 mg **8**. Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material. The structure of **3** was elucidated by intensive ^{1}H NMR spectroscopy including spin decoupling and comparison with the data in the literature.

7α,9β-Dihydroxy-8β-[3,4-epoxy-2-methyl butyryloxy]-costunolide (**1**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1760 (γ-lactone), 1740 (CO_2R); MS *m/z* (rel. int.): 378 $[M]^+$ (1), 262 $[M - RCO_2H]^+$ (7), 244 $[262 - H_2O]^+$ (21), 83 (81), 55 (100).

Prespicatin (**2**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1765 (γ-lactone), 1720 ($C{=}CCO_2R$); MS *m/z* (rel. int.): 458.194 $[M]^+$ (1) (calc. for $C_{25}H_{30}O_8$: 458.194), 440 $[M - H_2O]^+$ (11), 244 $[M - RCO_2H]^+$ (10), 226 $[244 - H_2O]^+$ (64), 99 $[RCO]^+$ (100), 81 $[99 - CO]^+$ (46), 53 $[81 - CO]^+$ (58).

1α-Hydroxybacchotricuneatin A (**4**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620 (OH), 1770 (γ-lactone), 1620 (C=C); MS *m/z* (rel. int.): 358.142 $[\mathrm{M}]^+$ (18) (calc. for $C_{20}H_{22}O_6$: 358.142), 328 $[\mathrm{M}-CH_2O]^+$ (100), 247 $[328-C_5H_5O]^+$ (6), 95 $[C_5H_3O_2]^+$ (30).

9β-Hydroxy-15-O-dodecanoyl grandifloric acid (**5**). Colourless oil; IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 3620 (OH), 3500–2700, 1700 (CO_2H), 1725 (CO_2R); MS *m/z* (rel. int.): 516 $[\mathrm{M}]^+$ (0.3), 316.204 $[\mathrm{M}-RCO_2H]^+$ (41), (Calc. for $C_{20}H_{28}O_3$: 316.204), 298 $[316-H_2O]^+$ (25), 270 $[316-HCO_2H]^+$ (32), 148 (100); ^{1}H NMR ($CDCl_3$): H-13 = 2.76 *m*, H-15 = 5.94 *br s*, H-17 = 5.16 and 5.09 *br s*, H-19 = 1.23 *s*, H-20 = 1.05 *s*; OCOR: 2.30 *t*, 1.23 *m*, 0.87 *t*; $[\alpha]_D^{22} = -47$ ($CHCl_3$; *c* 0.6).

9α,10β-Bisangeloyloxy-3E-agerafastin (**6**). Colourless oil; IR $\nu_{\max}^{\mathrm{CCl_4}}$ cm^{-1}: 1725, 1640 ($C{=}CCO_2R$), 3090, 1615, 995, 900 ($CH{=}CH_2$); UV $\lambda_{\max}^{\mathrm{Et_2O}}$ nm: 278, 268, 260; MS *m/z* (rel. int.): 416.256 $[\mathrm{M}]^+$ (3) (calc. for $C_{25}H_{36}O_5$: 416.256), 316 $[\mathrm{M}-RCO_2H]^+$ (7), 216 $[316-RCO_2H]^+$ (12), 201 $[216-\mathrm{Me}]^+$ (10), 136 $[C_{10}H_{16}]^+$ (12), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (56); $[\alpha]_D^{22} = -13$ ($CHCl_3$; *c* 0.3).

9α,10β-Bisangeloyloxy-7-epi-3E-agerafastin (**7**). Colourless oil; IR $\nu_{\max}^{\mathrm{CCl_4}}$ cm^{-1}: 1720, 1645 ($C{=}CCO_2R$), 3080, 1620, 990, 900 ($CH{=}CH_2$); MS *m/z* (rel. int.): 416.256 $[\mathrm{M}]^+$ (1) (calc. for $C_{25}H_{36}O_5$: 416.256), 316 $[\mathrm{M}-RCO_2H]^+$ (5), 216 $[316-RCO_2H]^+$ (16), 201 $(216-\mathrm{Me}]^+$ (12), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (59); $[\alpha]_D^{22} = +103$ ($CHCl_3$; *c* 0.1).

9α-10β-Bisangeloyloxy-7-epi-3Z-agerafastin (**8**). Colourless oil; IR $\nu_{\max}^{\mathrm{CCl_4}}$ cm^{-1}: 1720, 1645 ($C{=}CCO_2R$); MS *m/z* (rel. int.): 416.256 $[\mathrm{M}]^+$ (2) (calc. for $C_{25}H_{36}O_5$: 416.256), 316 $[\mathrm{M}-RCO_2R]^+$ (6), 216 $[316-RCO_2H]^+$ (14), 83 $[C_4H_7CO]^+$ (10), 55 $[83-CO]^+$ (55).

REFERENCES

1. Bohlmann, F., Zdero, C., Pickard, J., Robinson, H. and King, R. M. (1981) *Phytochemistry* **20**, 1323.
2. Kisiel, W. (1978) *Phytochemistry* **17**, 1054.
3. Bohlmann, F. and Le Van, N. (1977) *Phytochemistry* **16**, 579.
4. Karlsson, K., Wahlberg, I. and Enzell, C. R. (1973) *Acta Chem. Scand.* **27**, 1613.
5. Kagan, J. (1968) *Phytochemistry* **7**, 1205.
6. Wagner, H., Iyengar, M. A. and Herz, W. (1973) *Phytochemistry* **12**, 2063.
7. Herz, W., Paplawski, J. and Sharma, R. (1975) *J. Org. Chem.* **40**, 199.
8. Herz, W. and Sharma, R. P. (1976) *J. Org. Chem.* **41**, 1248.
9. Bohlmann, F. and Dutta, L. (1979) *Phytochemistry* **18**, 1228.
10. Bohlmann, F. and Dutta, L. (1979) *Phytochemistry* **18**, 847.
11. Wagner, H., Seitz, R., Lotter, H. and Herz, W. (1978) *J. Org. Chem.* **43**, 3339.
12. Bohlmann, F., Dutta, L., Robinson, H. and King, R. M. (1979) *Phytochemistry* **18**, 1889.
13. Bohlmann, F., Ludwig, G.-W., Jakupovic, J., King, R. M. and Robinson, H. (1983) *Phytochemistry* **22**, 983.
14. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1984) *Phytochemistry* **23**, 1978.