Phytochemistry, Vol. 24, No. 3, pp. 495–499, 1985
Printed in Great Britain

0031–9422/85 $3.00 + 0.00

GERMACRANOLIDES FROM *PERYMENIUM KLATTIANUM* AND *PERYMENIOPSIS OVALIFOLIA*

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(*Received* 2 *April* 1984)

Key Word Index—*Perymenium klattianum*; *Perymeniopsis ovalifolia*; *Oyedaea*; Compositae; sesquiterpene lactones; germacranolides; chemical transformations.

Abstract—The aerial parts of *Perymenium klattianum* afforded, in addition to kaurane and beyerane derivatives, three germacranolides, two of them isolated for the first time. The structures were elucidated by spectroscopic methods and by some chemical transformations, which in part gave unusual products. *Perymeniopsis ovalifolia* gave known compounds only.

INTRODUCTION

The Central American genus *Perymenium* (Compositae, tribe Heliantheae) is placed by Stuessy in the subtribe Verbesininae [1] and by Robinson in the subtribe Ecliptinae [2]. So far, only two species have been investigated chemically [3, 4]. We have now studied the constituents of *P. klattianum* Fay and *Perymeniopsis ovalifolia* (A. Gray) H. Rob. The results will be discussed in this paper.

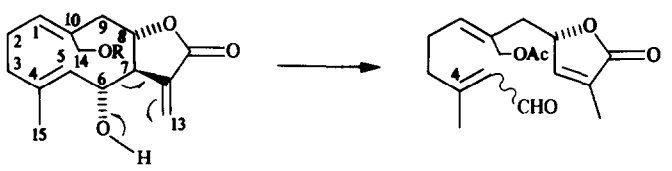
RESULTS AND DISCUSSION

The aerial parts of *Perymenium klattianum* afforded germacrene D, bicyclogermacrene, caryophyllene, squalene, *ent*-kaurenal, 9,11-dehydro-*ent*-kaurenal, *ent*-kaurenic acid, its 9,11-dehydro derivative, beyerenic acid [5], its 9,11-dehydro derivative [6], 12-hydroxy-9,11-dehydro-*ent*-kaurenic acid [7], lupeyl acetate, 14-acetoxydesacetyl-laurenobiolide (**2**), the corresponding 14-hydroxy derivative **1** and 14-acetoxy-8α-hydroxy-costunolide (**11**). The germacranolide **2** has been isolated previously in minute amounts from a *Schistostephium* species [8] but here it is the main constituent. We therefore have studied again the spectral properties of this germacranolide. The ^{1}H NMR spectrum at room temperature only showed highly broadened signals which even at 140° were still broad. At −40° the signals showed that a mixture of three conformations was present. The same was true for the ^{13}C NMR spectrum at this temperature (Table 1). Though not all signals could be assigned with certainty, the groups of signals and the ^{1}H NMR spectrum agreed nicely with the presence of the following conformations in **2**: I, methyls at C-4 and C-10 both below the plane; II, both methyls above the plane; and III, C-4 methyl above and C-10 below the plane (*ca* 10:9:6) (Table 2). The situation is slightly different in the case of laurenobiolide [9]. In order to get a fixed conformation, we transformed **2** by Cope rearrangement to **6**. However, in addition to **6**, the *E,Z*-isomeric aldehydes **3** and **4** were also obtained. In a second experiment, a sample, previously dissolved in deuteriochloroform and thus containing traces of acid, was heated in a sealed tube at 170°. In addition to **3**, **4** and **6**, also **7** and the cadinenolides **8** and **9** were obtained. The structure of **6** clearly followed from the ^{1}H NMR spectrum (Table 3). All signals were assigned by spin decoupling and the stereochemistry was established in deuteriobenzene by NOE difference spectroscopy. Clear NOEs were observed between H-5 and H-15, between H-5 and H-7, between H-7 and H-9α, and between H-6β, H-3′, H-8 and H-14′. An ω-coupling between H-14 and H-9α further established the proposed configuration. The structures of **3** and **4**, which could not be separated, also followed from the ^{1}H NMR spectral data (Table 3). Spin decoupling allowed the assignment of all signals. As the isomers were present in different concentrations (*E*-isomer being the major), the signals could be clearly matched to the isomers. **3** and **4** were obviously formed by an electro-cyclic reaction. The structure of **7** could also be deduced from the ^{1}H NMR

Table 1. ^{1}H NMR spectral data of **2** ($CDCl_3$, 400 MHz, TMS as internal standard, −40°)

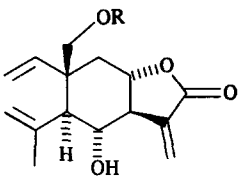
	Conformation		
	I	II	III
H-1	5.45 *br dd*	5.35 *m*	5.22 *br dd*
H-5	5.29 *br d*	4.77 *br d*	4.93 *br d*
H-6	4.02 *br dd*	4.24 *br dd*	4.30 *br dd*
H-7	3 17 *br d*	2.88 *m*	2.88 *m*
H-8	3.92 *br ddd*	3.96 *br dd*	4.52 *m*
H-13	6.41 *br s*	6.38 *br s*	6.41 *br s*
H-13′	6.20 *br s*	6.06 *br s*	6.25 *br s*
H-14	4.50 *br s*	4.59 *br d*	4.68 *br d*
H-14′		4.39 *br d*	4 45 *br d*
H-15	1.58 *br s*	1.75 *br s*	1.49 *br s*
OAc	2.07 *s*	2 13 *s*	2.10 *s*

J (Hz): I: 1, 2 = 8; 5, 6 = 5.5; 6, 7 = 10.5; 7, 8 = 8, 9β = 4; 8, 9α = 11; II: 5, 6 = 6, 7 = 10; 8, 9α = 10; 8, 9β = 6; 14, 14′ = 12; III: 5, 6 = 6, 7 = 10; 14, 14′ = 12

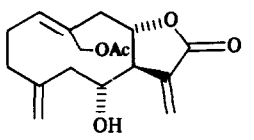


1 R = H
2 R = Ac

3 4E
4 4Z



5 R = H
6 R = Ac



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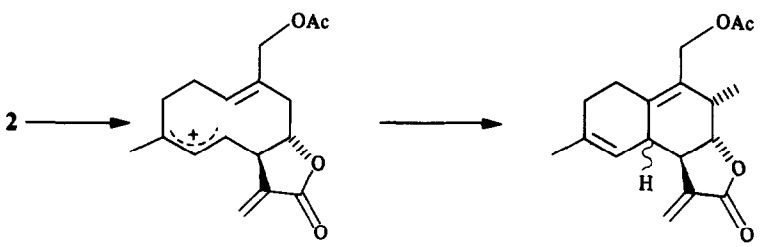
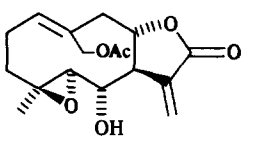


2a

8 6αH
9 6βH



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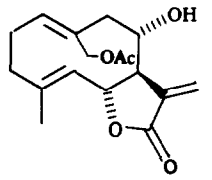


11

spectrum (Table 3), which showed sharp signals even at room temperature. All signals could be assigned by spin decoupling. Obviously **7** was formed by proton-catalysed isomerization of the Δ^4-double bond of **2**. It may be of interest that in the mass spectra of **1**, **2** and **6** the base peak (*m/z* 84) may be formed via **6** and **12** in a similar way. The structures of **8** and **9**, which could not be separated, followed from the ^{1}H NMR spectrum (Table 3) and spin decoupling. As again the two isomers were present in different concentrations, most of the signals could be assigned. The main isomer was **9**, its stereochemistry being easily deduced from the coupling $J_{6,7}$. As both isomers showed an 11 Hz coupling for $J_{7,8}$ the presence of *trans*-lactones was obvious. **8** and **9** also were most likely formed by acid catalysis. If **2** was first transformed to the corresponding allyl cation **2a**, an electrophilic attack of the 1(10)-double bond followed by loss of the H-1 proton would give **8** and **9**. Inspection of a model showed that no preferred attack should be expected, thus explaining the formation of both isomers. Further proof of the structure of **2** was the formation of the epoxide **10** by peracid oxidation of **2**. Surprisingly, only one isomer was obtained, which was identical to a lactone isolated previously [8].

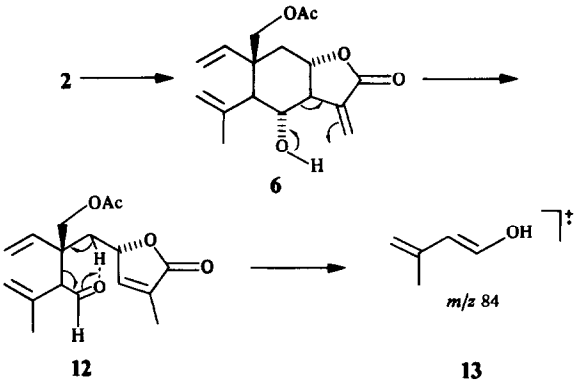
The structure of **1** followed from the molecular formula, the ^{1}H NMR spectrum (which was close to that of **2**), and the ^{1}H NMR spectrum (Table 3) of the Cope-rearrangement product **5**. In this case only traces of other compounds were obtained.

The structure of **11** clearly followed from the ^{1}H NMR spectrum (Table 3) and from NOE difference spectra which showed that in this case only one conformation, with both methyls above the plane, was present. Thus H-6 shows a strong NOE with H-8 and H-15, H-8 with H-6 and H-14', H-15 with H-2 and H-6, and H-7 with H-5 and H-9α. **11** is therefore an isomer of **2** with a 6,12-lactone

Table 2. ^{13}C NMR signals of 2* ($CDCl_3$, $CHCl_3$ as internal standard, −40°)

	Conformation		
	I	II	III
C-1	132.6	136.8	134.9
C-2	22.7	23.8	23.8
C-3	37.2	36.2	37.9
C-4	141.9	136.6	136.4
C-5	129.9	130.9	131.3
C-6	70.0	69.6	69.4
C-7	50.6	49.1	53.6
C-8	80.2	83.2	79.8
C-9	37.4	38.7	42.5
C-10	127.6	127.8	128.3
C-11	135.6	135.5	134.9
C-12	171.0	171.3	171.2
C-13	126.1	126.7	126.2
C-14	63.5	63.1	61.3
C-15	17.8	17.2	16.5
OAc	21.1	21.1	21.1
	170.2	170.2	170.5

*Some signals may be interchangeable; assignment following intensities (*ca* 10:9:6) and usual shift rules.



ring and the 8-epimer of ovalifolin [10], in which the H-8 signal differs characteristically. The spectral data were close to those of similar costunolide derivatives with 8α-oxygen functions.

From the aerial parts of *Perymeniopsis ovalifolia* (= *Oyedaea ovalifolia* A. Gray) only germacrene D, bicyclogermacrene, *ent*-kaurenic acid and its 15α-angeloyloxy and cinnamoyloxy derivatives were isolated. This allows no differentiation from those species which are still placed in the genus *Oyedaea*, where also *ent*-kaurene derivatives are common [11].

Table 3. ^1H NMR spectral data of 3–9 and 11 (400 MHz, $CDCl_3$, TMS as internal standard)

	3	4	5	6	7	8	9	11
H-1	5.51 *br t*	5.54 *br t*	5.78 *dd*	5.75 *dd*	5.77 *br dd*		—	5.16 *br dd*
H-2	2.36 *m*		5.19 *d* (*c*)	5.16 *d* (*c*)	2.50 *m*		*	2.34 *m*
H-2′			5.09 *d* (*t*)	5.07 *d* (*t*)	2.35 *m*		*	
H-3	2.28 *br t*	2.65 *br t*	5.27 *dq*	5.28 *br s*	2.50 *m*		*	2.40 *m*
H-3′			4.95 *br s*	4.90 *br s*	1.85 *m*		*	2.09 *m*
H-5	5.87 *br d*	5.91 *br d*	2.15 *d*	2.18 *d*	2.50 *br dd* 2.30 *br dd*	5.39 *br s*	5.50 *br s*	4.77 *br d*
H-6	9.99 *d*	9.81 *d*	4.20 *br dd*	4.14 *dd*	3.74 *br ddd*	3.41 *br s*	3.01 *br d*	4.64 *dd*
H-7	7.00 *qq*	6.99 *dq*	2.56 *dddd*	2.60 *dddd*	3.29 *dddd*	2.88 *dddd*	2.39 *dddd*	2.90 *dddd*
H-8	4.98 *m*		4.34 *ddd*	4.10 *ddd*	4.37 *ddd*	4.36 *ddd*		4.09 *br ddd*
H-9	2.44 *br dd*		2.56 *dd*	2.45 *dd*	3.01 *dd*	2.69 *br dd*	2.65 *dd*	2.82 *br d*
H-9′	2.37 *m*		1.73 *dd*	1.78 *dd*	2.08 *dd*	2.43 *br dd*		2.40 *dd*
H-13	1.90 *dd*		6.17 *d*	6.18 *d*	6.43 *d*	6.33 *d*	6.19 *d*	6.41 *d*
H-13′			5.96 *d*	5.97 *d*	5.74 *d*	5.51 *d*	5.73 *d*	6.30 *br s*
H-14	4.63 *br s*		3.68 *br s*	4.16 *d*	4.66 *d*	4.67 *br d*	4.64 *br s*	4.50 *br d*
H-14′				4.12 *d*	4.62 *d*	4.61 *br d*		4.27 *br d*
H-15	2.16 *d*	1.98 *d*	1.85 *br s*	1.84 *br s*	5.20 *br s*	1.61 *br s*	1.69 *br s*	1.60 *d*
H-15′					5.04 *br s*			
OAc	2.06 *s*		—	2.05 *s*	2.09 *s*	2.06 *s*		2.07 *s*

*Overlapped multiplets.

J (Hz): Compounds 3 and 4: 1, 2 = 3, 4 = 7; 5, 6 = 8; 5, 15 = 1.5; 7, 8 = 7, 13 = 8, 13 ~ 2; 8, 9 = 7; 9, 9′ = 15; compounds 5 and 6: 1, 2*c* = 10.5; 1, 2*t* = 17; 3, 3′ = 3, 15 = 1.5; 5, 6 = 6, 7 = 10; 7, 8 = 11.5; 7, 13 = 7, 13′ = 3; 8, 9 = 4; 8, 9′ = 9, 9′ = 12.5; 13, 13′ = 0.5; 14, 14′ = 12; compound 7: 1, 2 = 1, 2′ ~ 8.5; 5, 5′ = 14; 5, 6 = 11; 5′, 6 = 2; 6, 7 = 7, 13 = 7, 13′ = 3; 7, 8 = 7; 8, 9 = 5; 8, 9′ = 9, 9′ = 14, 14′ = 12.5; compounds 8 and 9: 7, 8 = 11; 7, 13 = 7, 13′ = 3; 8, 9 = 5; 8, 9′ = 12; 9, 9′ = 15.5 (compound 8: 6, 7 = 6; 14, 14′ = 12; compound 9: 6, 7 = 11); compound 11: 1, 2 = 1, 2′ ~ 8.5; 5, 6 = 9.5; 5, 15 = 1.2; 6, 7 = 7, 8 = 8; 7, 13 = 3.5; 8, 9 = 3; 8, 9′ = 11; 9, 9′ = 13.5; 13, 13′ = 1; 14, 14′ = 12.

From *Perymenium serratum* Blake (unpublished results) also only kudtdiol [12], 5-hydroxy-7,4'-dimethoxyflavanone and *ent*-kaurane derivatives, which seem to be characteristic of large parts of the subtribe, were isolated.

Perymenium is a genus of some 40 species, mostly confined to Mexico and Central America [13]. Only a few of these have been examined chemically, but the present data suggest relationships with a wide assortment of genera. For example, the major germacranolide **2** of *P. klattianum* also occurs as a minor constituent of *Schistostephium* (tribe Anthemideae). Nevertheless, Fay [13] relates *Perymenium* to *Melanthera* as do other workers (Turner, B. L., personal communication).

Perymeniopsis ovalifolia is a monotypic element recently segregated from *Oyedaea* [14] on relatively trivial morphological grounds and it is said by its creator to have characters of both *Oyedaea* and *Perymenium* but stands closer to the latter. The present chemical data, as noted above, do not support separation from *Oyedaea*, nor does it stand especially apart from *Perymenium* where it is perhaps properly positioned (Turner, B. L., personal communication).

EXPERIMENTAL

The air-dried aerial parts of *P. klattianum* (600 g, voucher Turner 5330 TEX, *XAL*) was worked-up in the usual fashion [15]. CC fractions were as follows: 1 (petrol), 2 (Et_2O–petrol, 1:20 and 1:10), 3 (Et_2O–petrol, 1:3 and 1:1), 4 (Et_2O) and 5 (Et_2O–MeOH, 10:1). TLC ($AgNO_3$-coated SiO_2, petrol) of fraction 1 gave 100 mg germacrene D, 10 mg bicyclogermacrene, 20 mg caryophyllene and 30 mg squalene. TLC of fraction 2 (Et_2O–petrol, 1:10) gave 25 mg *ent*-kaurenal, 5 mg 9,11-dehydro-*ent*-kaurenal and 50 mg lupeyl acetate. TLC of fraction 3 (Et_2O–petrol, 1:3) afforded 300 mg *ent*-kaurenic acid, 450 mg 9,11-dehydro-*ent*-kaurenic acid, 100 mg beyeren-19-acid and 20 mg of its 9,11-dehydro derivative. TLC of fraction 4 (Et_2O) (after separation of 300 mg crystalline **2**) gave two bands (4/1 and 4/2). TLC of 4/1 (Et_2O–CH_2Cl_2, 1:9) afforded 15 mg **2** (R_f 0.18) and 15 mg 12-hydroxy-9,11-dehydro-*ent*-kaurenic acid. TLC of 4/2 (Et_2O–CH_2Cl_2, 1:2) gave 5 mg **11** (R_f 0.31) and 2 mg **1** (R_f 0.18). TLC of fraction 5 (Et_2O) gave 15 mg **2** (R_f 0.18) and 50 mg of a mixture (5/2), which on repeated TLC (Et_2O–CH_2Cl_2, 1:2) afforded 10 mg **11** (R_f 0.30) and 35 mg **1** (R_f 0.20). Known compounds were identified by comparing the 400 MHz ^1H NMR spectra with those of authentic material and by co-TLC in different solvent systems.

14-Hydroxydesacetyl-laurenobiolide (**1**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3420 (OH), 1760 (γ-lactone); MS *m/z* (rel. int.): 264.136 $[M]^+$ (4) (calc. for $C_{15}H_{20}O_4$: 264.136), 246 $[M-H_2O]^+$ (10), 228 $[246-H_2O]^+$ (12), 84 $[C_5H_8O$ (**13**)$]^+$ (100);

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 548 \quad 436\,nm}{+19 \quad +26 \quad +30 \quad +47} (CHCl_3;\ c\ 0.33).$$

10 mg **1** in 2 ml C_6H_6 was heated in a sealed tube for 2 hr at 170°. TLC (Et_2O–CH_2Cl_2, 1:2) gave 4 mg **1** (R_f 0.19), and 4 mg **5** (R_f 0.25), colourless oil; MS *m/z* (rel. int.): 264.136 $[M]^+$ (1), 246 $[M-H_2O]^+$ (3), 228 $[246-H_2O]^+$ (6), 84 $[13]^+$ (100)

14-Acetoxydesacetyl-laurenobiolide (**2**). Colourless crystals (Et_2O), mp 129°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1760 (γ-lactone), 1730, 1220 (OAc); MS *m/z* (rel. int.): 306.147 $[M]^+$ (6) (calc. for $C_{17}H_{22}O_5$: 306.147), 246 $[M-HOAc]^+$ (20), 228 $[246-H_2O]^+$ (28), 84 $[13]^+$ (100); $[\alpha]_D$ +76.6° ($CHCl_3$; *c* 0.36). 30 mg **2** in 3 ml C_6H_6 was heated 2 hr in a sealed tube at 170°, affording a 3:1 mixture of **2** and **6**. When 30 mg **2** was heated for 2 hr at 200°, TLC (Et_2O, 3 developments) gave 20 mg **2** (R_f 0.64), 4 mg **6** (R_f 0.67) and 2 mg **3** and **4** (*ca* 2:1) (R_f 0.48). The same reaction with a sample of **2** which had been dissolved in $CDCl_3$ and then evapd, was heated in C_6H_6 for 2 hr at 170°. TLC (Et_2O–CH_2Cl_2, 17:3) gave in addition to the compounds isolated above 1 mg **7** (R_f 0.10) and 1 mg of a mixture of **8** and **9** (*ca* 2:3) (R_f 0.59).

3 and **4**: Colourless oil; MS *m/z* (rel. int.): 306 $[M]^+$ (0.5), 264 $[M-ketene]^+$ (2), 246 $[M-HOAc]^+$ (8), 217 $[246-CHO]^+$ (7), 84 $[13]^+$ (100).

6: Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580, 3460 (OH), 1770 (γ-lactone), 1740, 1230 (OAc); MS *m/z* (rel. int.): 306 $[M]^+$ (0.5), 264 $[M-ketene]^+$ (2), 246 $[M-HOAc]^+$ (8), 217 $[M-CHO]^+$ (7), 84 $[13]^+$ (100);

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{-11 \quad -14 \quad -16 \quad -24} (CHCl_3;\ c\ 0.25).$$

7: Colourless oil; MS *m/z* (rel. int.): 306 $[M]^+$ (5), 246 $[M-HOAc]^+$ (16), 228 $[246-H_2O]^+$ (20), 55 (100).

8 and **9**: Colourless oil; MS *m/z* (rel. int.): 288 $[M]^+$ (5), 246 $[M-ketene]^+$ (12), 228 $[M-HOAc]^+$ (100), 213 $[228-Me]^+$ (14), 200 $[228-CO]^+$ (20), 132 $[M-C_5H_4O_2(RDA)-HOAc]^+$ (16).

20 mg **2** in 1 ml CH_2Cl_2 and 0.5 ml saturated $NaHCO_3$ soln were stirred for 30 min at room temp. with 20 mg *m*-chloroperbenzoic acid. Usual work-up gave a crude product, its ^1H NMR spectrum being identical to that of **10** and showing only traces of a second epoxide. TLC (Et_2O, 3 developments, R_f 0.25) gave pure **10**.

14-Acetoxy-8α-hydroxycostunolide (**11**). Colourless crystals (Et_2O), mp 114°; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1770 (γ-lactone), 1740, 1230 (OAc); MS *m/z* (rel. int.): 246.126 $[M-HOAc]^+$ (10) (calc. for $C_{15}H_{18}O_3$: 246.126), 228 $[246-H_2O]^+$ (18), 91 $[C_7H_7]^+$ (100);

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{+74 \quad +89 \quad +103 \quad +196} (CHCl_3;\ c\ 0.35).$$

Isolation of the constituents from Perymeniopsis ovalifolia (*voucher turner* 152 19*BTEX*). 560 g of aerial parts were worked up as usual [15]. CC fractions were as follows: 1 (petrol), 2 (Et_2O–petrol, 1.3 and 1:1) and 3 (Et_2O and Et_2O–MeOH, 9:1). TLC of fraction 1 gave 10 mg germacrene D and 2 mg bicyclogermacrene. TLC of fraction 2 (Et_2O–petrol, 1:3) afforded 200 mg *ent*-kaurenic acid, 50 mg 15α-angeloyloxy-*ent*-kaurenic acid and 100 mg 15α-cinnamoyloxy-*ent*-kaurenic acid.

Acknowledgements—We thank the Deutsche Forschungsgemeinschaft for financial support and Professor B. L. Turner, University of Texas at Austin, for the plant material.

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