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DITERPENE LACTONES AND OTHER CONSTITUENTS FROM *WEDELIA* AND *ASPILIA* SPECIES

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Key Word Index—*Wedelia regis*; *Aspilia floribunda*; Compositae; diterpenes; wederegiolide derivatives; sesquiterpenes; curcumene derivatives.

Abstract—From the aerial parts of *Wedelia regis* three diterpene lactones were isolated, which were obviously degradation products of *ent*-kaurenic acid. The roots afforded two derivatives of curcumene. *Aspilia floribunda* gave a further *ent*-kaurene derivative. The structures were elucidated by spectroscopic methods and chemical transformations.

INTRODUCTION

The genus *Aspilia* and the closely related genera *Wedelia* and *Steiractinia* are placed in the tribe Heliantheae, subtribe Ecliptinae. All contain *ent*-kaurene acid derivatives [1–5] and several species have eudesmanolides with a 10α-methyl group [6, 7]. We now have studied further *Wedelia* and *Aspilia* species and the results are discussed below.

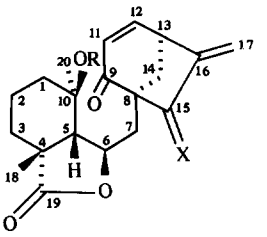
RESULTS AND DISCUSSION

The aerial parts of *Wedelia regis* H. Rob. afforded bicyclogermacrene, germacrene D, caryophyllene and spathulenol as well as three diterpenes. The main compound with molecular formula $C_{20}H_{26}O_6$ was obviously a γ-lactone as shown by the IR spectrum which had a characteristic band at 1770 cm^{-1}: the presence of a conjugated ketone and of hydroxyl was indicated by other IR bands (1660 and 3600 cm^{-1}). The MS fragmentation pattern showed that a diol was present and this accounted for all oxygens. Inspection of the ^1H NMR spectrum (Table 1) showed several signals which were close to those of a seco-*ent*-kaurane derivative [5], which, however, is only a keto lactone; the new compound showed some additional low field signals. The doublet at δ5.94 was coupled with the threefold doublet at δ7.44 which was further coupled with a broadened double doublet at 3.30 and by a w-coupling with threefold doublet at δ1.88. As the latter showed a geminal coupling we were most likely dealing with H-14. Further spin decoupling allowed the assignment of most signals. The presence of a 15-hydroxy group was indicated by the observed downfield shift of the methylene signals (H-17). The chemical shift of H-7α and the couplings of H-6 indicated that most likely a 6β,19-lactone was present. Manganese dioxide-oxidation afforded the ketone **5**, its ^1H NMR spectral data (Table 1) supported the proposed position of the hydroxy group as the H-17 signal was shifted downfield as well as the signals of H-13 and H-14β. A second, minor diterpene, obviously was an isomer. Most ^1H NMR signals (Table 1) were very similar. However, some clear differences indicated that the two lactones were most likely epimeric at C-15. In the spectrum of the minor isomer the H-14α signal was shifted downfield. Inspection of a model showed that this could be explained by a deshielding effect of a 15α-hydroxyl group. Similarly a downfield field shift of the H-7β signal could be observed in the spectrum of the main isomer. All data therefore agreed with the structures of **1** and **2** (main compound) for the lactones. As expected, the oxidation of **1** also led to the formation of the ketone **5**.

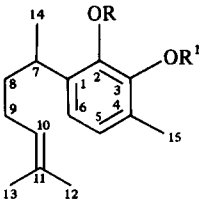
The third diterpene was the 15-*O*-acetate of **2**, and could be prepared by mild acetylation of **2** to the monoacetate **3**, which was identical with the natural product. Prolonged acetylation in the presence of Steglich-base [8] afforded a diester, which was the 10-acetoacetate as shown from the ^1H NMR spectrum (Table 1). Therefore the isomeric structure with a 6-hydroxy group and a 10,19-lactone as in wedelia-seco-kaurenolide [5] could be excluded. We have named the 15-deoxy derivative of **1** wederegiolide. The absolute configuration of this lactone followed from the observed Cotton-effect as most likely the octant-rule is valid in this case.

The roots afforded in addition to bicyclogermacrene and germacrene D a mixture of the two isomeric angelates **6** and **7**. All signals in the ^1H NMR spectrum of the mixture (Table 2) could be assigned as the two isomers were present in a slightly different concentration. Furthermore the usual shift differences between phenols and phenolic esters allowed the assignment of the relative position of the oxygen function in the curcumene derivatives. A free phenolic hydroxy group in **6** led to an upfield shift of the H-6 signal while in **7** the H-5 signal was at higher fields. Similarly the aromatic methyl signal was shifted downfield in the spectrum of **6** due to the deshielding effect of the neighbouring hydroxy group.

The aerial parts of *Aspilia floribunda* (Gardn.) Baker afforded, in addition to known compounds, a further ent-kaurenic acid derivative, the angelate **9**. The structure followed from the molecular formula, which could be calculated from the mass spectrum as both fragments [M $-H_2O$] and [M – angelic acid] were present, and from the ^1H NMR spectrum (see Experimental), which was close to

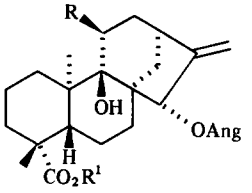


	1	2	3	4	5
X	αOH, H	βOH, H	βOAc, H	βOAc, H	=O
R	H	H	H	$COCH_2COMe$	H



6 R = Ang, R^1 = H

7 R = H, R^1 = Ang



8 R = R^1 = H

9 R = OH, R^1 = Me

Table 1. ^{1}H NMR spectral data of **1**–**5** (400 MHz, $CDCl_3$, TMS as internal standard)

	1	2	3	4	5
H-5	2.00 *d*	2.00 *d*	1.93 *d*	2.00 *d*	1.96 *d*
H-6	4.35 *dd*	3.89 *dd*	4.28 *dd*	4.55 *dd*	4.39 *dd*
H-7α	3.01 *d*	3.31 *d*	3.20 *d*	3.03 *d*	3.38 *d*
H-7β	1.74 *dd*	1.40 *dd*	1.46 *dd*	1.50 *m*	1.43 *dd*
H-11	5.84 *d*	5.94 *d*	6.01 *d*	6.01 *d*	5.91 *d*
H-12	7.37 *ddd*	7.44 *ddd*	7.42 *ddd*	7.35 *ddd*	7.43 *ddd*
H-13	3.45 *br dd*	3.30 *br dd*	3.35 *br dd*	3.32 *dd*	3.73 *br dd*
H-14α	2.17 *ddd*	1.88 *ddd*	2.04 *ddd*	2.03 *br d*	2.36 *ddd*
H-14β	2.36 *d*	2.33 *d*	2.39 *d*	2.50 *d*	2.97 *br d*
H-15	4.17 *br d*	4.42 *br s*	5.73 *br s*	5.71 *br s*	—
H-17	5.30 *br d*	5.22 *br d*	5.23 *br d*	5.22 *br d*	6.04 *br s*
H-17′		5.20 *br d*	5.15 *br d*	5.11 *br d*	5.49 *br s*
H-18	1.34 *s*	1.33 *s*	1.32 *s*	1.33 *s*	1.35 *s*
H-20	1.31 *s*	1.30 *s*	1.30 *s*	1.72 *s*	1.31 *s*
OH	3.33 *br s*	4.13 *br s*	3.80 *br s*		3.08 *s*
OAc	—	—	1.99 *s*	1.99 *s*	—
OCOR	—	—	—	2.54 *d*	—
				2.44 *d*	
				2.25 *s*	

J (Hz): 5, 6 = 10.5; 6, 7β = 11, 12 = 9.5; 7α, 7β = 16; 12, 13 = 7.5; 12, 14α = 1.5; 13, 14α = 4.5; 13, 17 ~ 1; 14α, 14β = 12.5; 15, 17 ~ 1; compound **4**: OCOR: $2_1'$, $2_2'$ = 16; compound **5**: 6, 7β = 10; 14α, 14β = 12; 12, 14α = 2; 13, 14α = 4.

Table 2. ^{1}H NMR spectral data of **6** and **7** (400 MHz, $CDCl_3$, TMS as internal standard)

	6	7
H-5	6.99 *d*	6.74 *d*
H-6	6.76 *d*	6.94 *d*
H-7	3.09 *dq*	2.78 *dq*
H-10	5.11 *tqq*	5.04 *tqq*
H-12	1.66 *br s*	1.53 *br s*
H-13	1.54 *br s*	1.50 *br s*
H-14	1.21 *d*	1.15 *d*
H-15	2.25 *s*	2.14 *s*
OAng	6.33 *br q*	
	2.10 *br s*	

J (Hz): 5, 6 = 8; 7, 8 = 7, 14 = 7.

that of the methyl ester of **8** [9]. The position of the additional hydroxy group could be deduced by spin decoupling. Irradiation of the H-13 signal allowed the assignment of H-12 and H-12′. As the broadened doublet of δ3.93 which obviously was the signal of hydrogen at the hydroxy group bearing carbon, collapsed to a singlet on irradiation of H-12 the position was settled. The β-orientation followed from the small coupling constant. The roots of the plant also contain **9** together with **8**, *ent*-kaurenic acid and stigmasterol.

The occurrence of ent-kaurene derivatives again showed the close relationship of the genera *Aspilia* and *Wedelia*, from which similar seco-*ent*-kauranes were isolated from *Wedelia* species [5]. Further *Aspilia* species [unpublished results] gave mainly *ent*-kaurenic acid derivatives, especially those with α- and β-orientated oxygen functions at C-15 which have also been observed in *Wedelia* species.

EXPERIMENTAL

The air dried plant material (collected in the province Bahia, Brazil) was worked-up as usual [10]. The extract of the roots of *Wedelia regis* (voucher RMK 8192) (125 g) gave CC fractions as follows: 1 (petrol) and 2 (Et_2O–petrol, 1:3 and 1:1). TLC of fraction 1 (silica gel, $AgNO_3$-coated, Et_2O–petrol, 1:10) gave 3 mg bicyclogermacrene and 8 mg germacrene D, while TLC of fraction 2 (Et_2O–petrol, 1:2) afforded a mixture of 1.8 mg **6** and 2.2 mg **7** (calc. from the ^{1}H NMR spectra) which could not be separated. The extract of the aerial parts (400 g) gave CC fractions as follows: 1 (petrol), 2 (Et_2O–petrol, 1:10) and 3 (Et_2O–petrol, 1:1, Et_2O and Et_2O–MeOH, 20:1). TLC of fraction 1 (silica gel, $AgNO_3$-coated, Et_2O–petrol, 1:20) gave 120 mg bicyclogermacrene and 130 mg germacrene D. TLC of fraction 2 (Et_2O–petrol, 1:10) gave 5 mg caryophyllene-1,10-epoxide and 10 mg spathulenol while TLC of fraction 3 (Et_2O–MeOH, 20:1) afforded 3 mg **1** (R_f 0.45), 5 mg **3** (R_f 0.65) and 20 mg **2** (R_f 0.40). The extract of the aerial parts (470 g) of *Aspilia floribunda* (voucher RMK 8574) afforded CC fractions (Et_2O–petrol, 1:1, and Et_2O), their TLC (Et_2O–petrol, 1:1) gave 25 mg **9** (R_f 0.37). The same compound (20 mg) was obtained from the extract of 40 g roots. Known compounds were identified by comparing their 400 MHz ^{1}H NMR spectra with those of authentic material.

15α-*Hydroxywederegiolide* (**1**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 3420 (OH), 1770 (γ-lactone), 1660 (C=CC=O); MS *m/z* (rel. int.): 346.178 $[M]^+$ (5) (calc. for $C_{20}H_{26}O_6$: 346.178), 328 $[M-H_2O]^+$ (12), 310 $[328-H_2O]^+$ (6), 300 $[328-CO]^+$ (8), 282 $[300-H_2O]^+$ (8), 267 $[282-Me]^+$ (4), 174 (31), 150 $[C_9H_{10}O_2$, McLafferty$]^+$ (38), 109 (100).

Compound **1** (3 mg) in 1 ml $CHCl_3$ was stirred for 4 hr with 30 mg MnO_2. TLC (Et_2O) afforded *ca* 1 mg **5**, colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1775 (γ-lactone), 1740 (C=O), 1670 (C=CC=O); MS *m/z* (rel. int.): 344 $[M]^+$ (1), 326 $[M-H_2O]^+$ (5), 298 $[326-CO]^+$ (3), 280 $[298-H_2O]^+$ (2), 109 (41), 84 (78), 73 (76), 61 (100).

15β-*Hydroxywederegiolide* (**2**). Colourless crystals, mp 170° (Et_2O–petrol); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1770 (γ-lactone), 1660 (C=CC=O); MS *m/z* (rel. int.): 346.178 $[M]^+$ (4) (calc. for $C_{20}H_{26}O_6$: 346.178), 328 $[346-H_2O]^+$ (18), 300 $[328-CO]^+$ (16), 282 $[300-H_2O]^+$ (10), 267 $[282-Me]^+$ (4), 150 $[C_9H_{10}O_2$, McLafferty$]^+$ (78), 109 (100), 94 (60). CD (MeCN): $\varepsilon_{333} = -3.2$; $\varepsilon_{267} = -5.9$.

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589 \quad 578 \quad 546 \quad 436\ \text{nm}}{-363 \quad -384 \quad -453 \quad -938} \quad (CHCl_3;\ c\ 0.17).$$

5 mg **2** were stirred in 1 ml CH_2Cl_2 1 hr with 50 mg MnO_2. TLC (Et_2O) afforded 3 mg **5**, identical with the ketone obtained from **1**. Acetylation of **2** (Ac_2O, 1 hr, room temperature) afforded **3**, identical with the natural compound (^{1}H NMR).

15β-*Acetoxywederegiolide* (**3**). Colourless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3480 (OH), 1775 (γ-lactone), 1750 (OAc), 1675 (C=CC=O); MS *m/z* (rel. int.): 388.189 $[M]^+$ (1) (calc. for $C_{22}H_{28}O_6$: 388.189), 328 $[M-HOAc]^+$ (9), 310 $[328-H_2O]^+$ (4), 300 $[328-CO]^+$ (5), 295 $[310-Me]^+$ (4), 282 $[300-H_2O]^+$ (6), 109 (100). To 5 mg **3** in 0.1 ml Ac_2O 10 mg *p*-dimethyl aminopyridine [8] were added. After 12 hr at room temperature and TLC (Et_2O) 2 mg **4** was obtained, colourless oil, ^{1}H NMR see Table 1; MS *m/z* (rel. int.): 370 $[M-HO_2CCH_2COCH_3]^+$ (2), 328 $[370-$ketene$]^+$ (3), 310 $[370-HOAc]^+$ (3), 282 $[310-CO]^+$ (2), 109 (38), 84 (100).

2-*Angeloyloxy-3-hydroxy- and 2-hydroxy-3-angeloyloxy-α-curcumene* (**6** and **7**). Inseparable colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580 (OH), 1735 (PhOCOC=C); MS *m/z* (rel. int.): 316.204 $[M]^+$ (21) (calc. for $C_{20}H_{28}O_3$: 316.204), 149 (61), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (95).

Methyl-9β,11β-dihydroxy-15α-angeloyloxy-ent-kaurenoate (**9**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1730 (CO_2R); MS *m/z* (rel. int.): 428.256 $[M-H_2O]^+$ (2) (calc. for $C_{26}H_{36}O_5$: 428.256), 346.215 $[M-RCO_2H]^+$ (12) (calc. for $C_{21}H_{30}O_4$: 346.214), 328 $[346-H_2O]^+$ (36), 269 $[328-CO_2Me]^+$ (35), 161 (100), 83 $[C_4H_7CO]^+$ (54), 55 $[83-CO]^+$ (51); ^{1}H NMR (400 MHz, $CDCl_3$): δ2.18 (*br d*, H-3α), 1.09 (*ddd*, H-3β), 3.93 (*br d*, H-11α), 1.65 (*m*, H-12α), 2.12 (*m*, H-12β), 2.84 (*br s*, H-13), 6.20 (*br s*, H-15β), 5.28 (*br s*, H-17), 5.11 (*br s*, H-17′), 1.20 (*s*, H-18), 0.96 (*s*, H-20), 3.83 (*s*, OH), 3.65 (OMe), 6.09 (*qq*, H-3′), 2.00 (*dq*, H-4′), 1.88 (*dq*, H-5′); *J* (Hz): 2α, 3β = 3α, 3β = 13; 2β, 3β = 4; 11α, 12β = 2.5; 12β, 13 ~ 2; 3′, 4′ = 7; 3′, 5′ = 4′, 5′ = 1.5.

$$[\alpha]^{\lambda}_{24^\circ} = \frac{589 \quad 578 \quad 546 \quad 436\ \text{nm}}{-24 \quad -26 \quad -31 \quad -65} \quad (CHCl_3;\ c\ 0.25).$$

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REFERENCES

1. Schteingarst, C. D. and Pomilia, A. G. (1981) *Phytochemistry* **20**, 2589.
2. Bohlmann, F. and Le Van, N. (1977) *Phytochemistry* **16**, 579.

3. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 2329.
4. Bohlmann, F., Knoll, K.-H., Robinson, H. and King, R. M. (1980) *Phytochemistry* **19**, 971.
5. Bohlmann, F., Ziesche, J., King, R. M. and Robinson, H. (1981) *Phytochemistry* **20**, 751.
6. Bohlmann, F., Jakupovic, J., Ates(Gören), N., Schuster, A., Pickardt, J., King, R. M. and Robinson, H. (1983) *Liebigs Ann. Chem.* 962.
7. Bohlmann, F., Zdero, C., Jakupovic, J., Ates(Gören), N., King, R. M. and Robinson, H. (1983) *Liebigs Ann. Chem.* 1257.
8. Höfle, G. and Steglich, W. (1972) *Synthesis* 619.
9. Bohlmann, F. and Le Van, N. (1977) *Phytochemistry* **16**, 579.
10. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1984) *Phytochemistry* **23** (in press).