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SESQUITERPENE LACTONES FROM *BRACHYLAENA* SPECIES*

FERDINAND BOHLMANN and CHRISTA ZDERO

Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, West Germany

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Key Word Index—*Brachylaena transvaalensis*; *B. rotundata*; Compositae; sesquiterpene lactones; germacranolides; guaianolides; eudesmanolides; chemotaxonomy.

Abstract—The investigation of two *Brachylaena* species afforded in addition to known sesquiterpene lactones and other constituents five new lactones, a germacranolide, a guaianolide and three eudesmanolides. The structures were elucidated by spectroscopic methods. The chemotaxonomy of this complex genus is discussed.

INTRODUCTION

The Tarchonantheae have been excluded from the tribe Inuleae (Compositae) following detailed taxonomic studies [1–4]. However, no clear decision was made where to place this group. So far, little is known on the chemistry of the three genera *Tarchonanthus*, *Brachylaena* and *Synchodendron* belonging to this subtribe. The essential oil of *Brachylaena hutchinsii* afforded several sesquiterpenes, mainly cadinene and copaene derivatives [5, 6], while from *Tarchonanthus* species an acetylenic thiophene and also some simple sesquiterpenes were reported [7]. We have now investigated two *Brachylaena* species, which afforded several sesquiterpene lactones, whose occurrence may be useful for the placement of this genus.

RESULTS AND DISCUSSION

The aerial parts of *Brachylaena transvaalensis* Hutch. ex Phill. et Schweick, afforded the acetylenic compounds **1** and **2**, germacrene D, lupeyl acetate, linolic and linolenic acid, the germacranolides **6a** [8], **6b** [8], onopordopicrin (**7**) [9], salonitenolide (**9**) [10] and two further lactones, the epoxy derivative of **6b** (**8a**) as well as a dihydro derivative of zaluzanin C, the guaianolide **14a**. The structure of **8a**, which on acetylation afforded the acetate **8b**, followed from the molecular formula and the ^{1}H NMR data (Table 1). Though some signals were overlapping multiplets, comparison with the spectra of **6b** and **7** clearly showed that a germacranolide with oxygen functions at C-8 and C-15 was present. The chemical shifts of H-15 observed after acetylation established the position of the free hydroxyl group, while the characteristic doublets at δ 3.10 and 2.81 as well as the singlet at 1.58 indicated the presence of an epoxy isobutyrate which must be α-orientated at C-8 as the coupling $J_{7,8}$ was 9 Hz as in the spectra of **6a**, **6b**, **7** and **9**. The presence of a 6,12-*trans* lactone followed from the couplings $J_{5,6}$ and $J_{6,7}$. The ^{1}H NMR data (Table 2) of **14a** and of the acetate **14b**, obtained by mild acetylation, as well as the molecular formula showed that a dihydro derivative of a trimethylene sesquiterpene lactone with a free hydroxyl group must be present. The ^{1}H NMR data were similar to those of zaluzanin C. However, the H-13 methylene

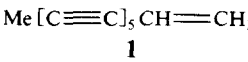
Table 1. ^{1}H NMR spectral data of compounds **8a** and **8b** (400 MHz, $CDCl_3$, TMS as internal standard)

	8a	**8b**
H-1	4.98*br dd*	4.97*m*
H-2	1.97*m*	2.0*m*
H-2′	2.22*m*	2.22*m*
H-3	2.56*ddd*	2.52*m*
H-3′	2.22*m*	2.22*m*
H-5	4.78*br d*	4.85*m*
H-6	5.04*br dd*	
H-7	3.02*dddd*	3.02*m*
H-8	5.12*br dd*	5.09*br dd*
H-9	2.53*br d*	2.52*m*
H-9′	2.41*dd*	2.42*dd*
H-13	6.39*d*	6.40*d*
H-13′	6.10*d*	6.10*d*
H-14	1.48*br s*	1.58*br s*
H-15	4.28*br d*	4.62*d*
H-15′	4.09*br d*	4.57*d*
OCOR	3.10*d*	3.09*d*
	2.81*d*	2.81*d*
	1.58*s*	1.58*s*
OAc	—	2.10*s*

J(Hz): 1,2 ~ 4; 1,2′ = 11; 2,3 = 2,3′ ~ 3; 3,3′ = 12; 5,6 = 6,7 = 10; 7,8 = 9; 7,13 = 3.5; 7,13′ = 3; 8,9′ = 10; 9,9′ = 13; $3'_1$, $3'_2$ = 6.

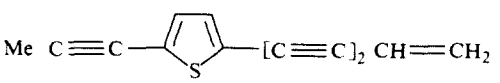
*Part 378 in the series 'Naturally Occurring Terpene Derivatives'. For Part 377, see Bohlmann, F., Singh, P., Jakupovic, J., Robinson, H. and King, R. M. (1982) *Phytochemistry* **20**, 707.

signals were replaced by a double quartet at δ 2.23 and a doublet at 1.22, indicating the presence of an 11,13-dihydrozaluzanin C. The stereochemistry at C-11 was deduced from the large coupling $J_{7,11}$ and the chemical shift of H-13. Spin decoupling supported the assignments of the signals. Irradiation of the downfield signal at 5.54 in the spectrum of the acetate collapsed the H-15 signals to doublets and also changed the H-2 signals in the expected way. Irradiation of the double doublet of the proton under the lactone oxygen (H-6) allowed the assignment of H-5 and H-7. All other signals were assigned in the usual way. The roots afforded **1–3**, lupeyl acetate, its Δ^{12}-isomer and 9-oxo-nerolidol (**4**), which was identical with a ketone obtained by oxidation of 9-hydroxy-nerolidol isolated from an *Artemisia* species [11]. Furthermore, a complex mixture of several sesquiterpene lactones was present, which could be separated only with difficulty. Finally 12 lactones were isolated, which included costunolide (**5**), dehydrocostuslactone (**10**) [12], dehydrozaluzanin C (**11**) [13], zaluzanin C (**12**) [13], dihydrodehydrocostuslactone (**13**) [14], 4β, 15-dihydrodehydrozaluzanin C (**15**) [15], tetrahydrodehydrozaluzanin C (**16**) [15], the furanoheliangolide **17** [16], tubiferin (**18**) [17] and three further eudesmanolides. ^{1}H NMR investigations of these lactones, which were available only in minute amounts, led to structures **19–21** (Table 3).



1

2



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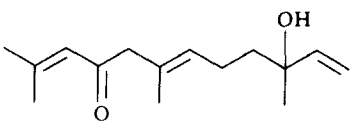
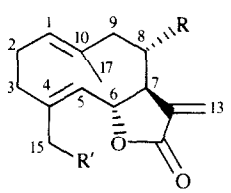
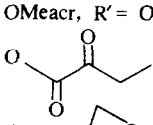
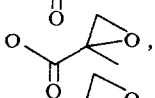


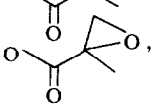
4

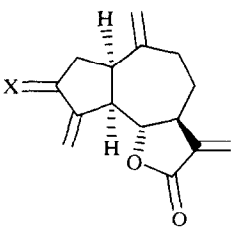
5 R = R′ = H
6a R = OiBu, R′ = OH
6b R = OMeacr, R′ = OH



7 R =  OH, R′ = OH

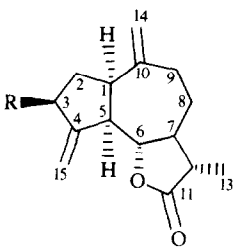
8a R =  R′ = OH

8b R =  R′ = OAc

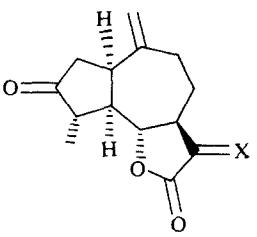


9 R = R′ = OH

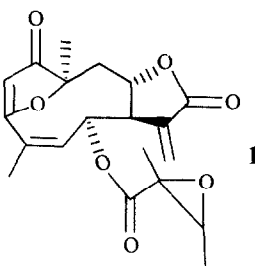
10 X = H_2
11 X = O
12 X = H, βOH



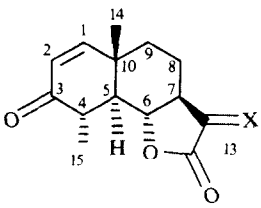
13 R = H
14a R = OH
14b R = OAc



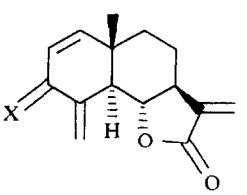
15 X = CH_2
16 X = H, α Me



17



18 X = CH_2
19 X = H, α Me



20 X = H, αOH
21 X = O

Table 2. ^{1}H NMR spectral data of compounds **14a** and **14b** (400 MHz, $CDCl_3$ TMS as internal standard)

	14a	**14b**
H-1	2.94*br ddd*	2.90*br ddd*
H-2	2.54*ddd*	2.46*ddd*
H-2′	1.75*ddd*	1.79*ddd*
H-3	4.54*br dd*	5.54*dddd*
H-5	2.78*dddd*	2.80*dddd*
H-6	4.03*dd*	3.99*dd*
H-7	1.89*dddd*	1.91*dddd*
H-8	2.13*dddd*	2.12*dddd*
H-8′	1.34*m*	1.31*br dd*
H-9	2.34*ddd*	2.51*ddd*
H-9′	2.02*ddd*	2.03*ddd*
H-11	2.23*dq*	2.23*dq*
H-13	1.22*d*	1.22*d*
H-14	4.96*br s*	4.92*br s*
H-14′	4.93*br s*	4.90*br s*
H-15	5.39*dd*	5.41*dd*
H-15′	5.30*dd*	5.27*dd*
OAc	—	2.10*s*

J(Hz): 1,2 = 1,2′ = 1,5 ~ 9; 2,2′ = 14; 2,3 = 2,3′ = 7.5; 3,15 = 5,15 = 2; 5,6 = 6,7 = 10; 7,8 = 4; 7,8′ = 11; 7,11 = 11.5; 8,8′ = 13; 8,9 = 8,9′ ~ 4; 8′,9 = 4; 8′,9′ = 10; 11,13 = 7.

Table 3. ^{1}H NMR spectral data of compounds **19–21** (400 MHz, $CDCl_3$, TMS as internal standard)

	19	**20**	**21**
H-1	6.69*d*	5.62*dd*	6.81*d*
H-2	5.88*d*	5.56*dd*	6.03*d*
H-3	—	4.72*br*	—
H-4	2.57*dq*	—	—
H-5	1.96*dd*	2.60*br d*	3.03*ddd*
H-6	3.98*dd*	4.46*dd*	4.13*dd*
H-7	1.63*m*	2.58*ddddd*	2.63*ddddd*
H-8α	1.90*m*	2.18*dddd*	2.14*m*
H-8β	1.74*m*	1.64*dddd*	1.7*m*
H-9α	1.60*m*	1.56*m*	1.38*m*
H-9β		1.73*ddd*	1.73*m*
H-11	2.28*dq*	—	—
H-13	1.21*d*	6.10*d*	6.14*d*
H-13′		5.40*br s**	5.46*d*
H-14	1.15*s*	0.92*s*	1.05*s*
H-15	1.34*d*	5.40*br s**	6.28*dd*
H-15′		5.04*br t*	5.71*br s*

*$CHCl_3$–C_6D_6 H-13′ 5.24*d*, H-15 5.34*br d*.

J(Hz): compound **19**: 1,2 = 10; 4,5 = 12; 4,15 = 7; 5,6 = 11; 6,7 = 10; 7,11 = 12; 11,13 = 7; compound **20**: 1,2 = 10; 1,3 = 2,3 ~ 2; 3,15 = 1.5; 5,6 = 11; 6,7 = 10.5; 7,8α = 3; 7,8β = 12; 7,13 = 3.3; 7,13′ = 3; 7,8α = 3; 7,8β = 12; 8α,8β = 13; 8α,9α = 8α,9β ~ 3; 8β,9α = 11; 8β,9β ~ 3; 9α,9β = 13; compound **21**: 1,2 = 10; 5,6 = 6,7 = 11; 5,15 ~ 2; 7,8α ~ 3; 7,8β ~ 10; 7,13 = 3.5; 7,13′ = 3.

Compound **19** could be separated from **18** only by transforming the latter to the corresponding pyrazoline derivative. The ^{1}H NMR spectral data clearly showed the presence of one tertiary and two secondary methyls. Furthermore, the location of the conjugated keto group followed from the low-field doublets. A double doublet at 3.98 was obviously that of the hydrogen under the lactone oxygen (H-6). Irradiation of this signal allowed assignment of the signals of H-5 and H-7. Their irradiation established the signals of H-4, H-8 and H-11. As the H-4 signal, which was coupled with the methyl group, was at relatively low field, a neighbouring keto group was required, thus establishing the whole sequence from C-1 to C-9 and therefore revealing the presence of an eudesmanolide. The stereochemistry at C-4 to C-7 was deduced from the couplings observed. The large coupling $J_{4,5}$ and $J_{7,11}$ supported an α-orientation of the methyls at C-4 and C-11. The structure of **20** could also be deduced from the ^{1}H NMR data (Table 3). The position of the hydroxyl group followed from the splitting of the olefinic signals and from the result of decoupling experiments. Irradiation of the low-field broadened signal at 4.72 collapsed the olefinic signals to doublets and sharpened the signals of the exomethylene protons. As these signals were also coupled with a broadened doublet at 2.60, which was further coupled with the lactone proton, the assignments of H-1, H-2, H-3, H-5, H-6 and H-15 were possible. The remaining signals could be assigned by further decouplings. Inspection of a model supported the proposed α-orientation of the hydroxyl group at C-3, as an α-proton at C-3 should show a larger coupling $J_{2,3}$. We have named compound **20** brachylaenolide.

Having established the structures of **19** and **20**, the structure of **21** could be deduced from the ^{1}H NMR data by comparison with those of **18–20**. As expected, the signals of H-1, H-2, H-5 and H-15 were shifted downfield and one of the H-15 signals was a clear double doublet, caused by an allylic and a geminal coupling as could be established by spin decoupling. The structures were further established by oxidation of **20**, which gave a ketone identical with the natural compound.

The aerial parts of *Brachylaena rotundata* S. Moore also afforded the germacranolides **6a**, **6b** and **8a** as well as lupeyl acetate and its Δ^{12}-isomer.

The constituents isolated from the two species may support the results on pollen morphology [2–4], which showed relationships of the Tarchonantheae to either Mutisieae or Anthemideae. Placement in the latter tribe seems very unlikely as the acetylenes present in both *Brachylaena* and *Tarchonanthus* have never been detected in any member of the Anthemideae. However, a relationship to the Mutisieae is supported by the constituents found in the *Brachylaena* spp. In particular, the lactones isolated from genera belonging to the subtribe Gochnatiinae are in part similar to those of *Brachylaena* (*Actinoseris* [18], *Cnicothamnus* [19], *Dicoma* [20], *Gochnatia* [19, 21], *Moquinia* [22], *Pertya* [23] and *Wunderlichia* [18, 24]). A few lactones were also isolated from other subtribes of the Mutisieae. Acetylenes of the type **1–3** have also been isolated from the Mutisieae; but they are widespread in other plants. Also, sesquiterpene lac-

tones of the types described here have been isolated from members of other tribes. Clearly, more species have to be investigated to obtain a precise picture of the relationships of this group.

EXPERIMENTAL

The air-dried plant material, collected in Transvaal, was extracted with Et_2O–petrol (1:2) and the resulting extracts were separated first by column chromatography (Si gel) and further by repeated TLC (Si gel). Known compounds were identified by comparing their IR and ^1H NMR spectra with those of authentic material. The voucher specimens are deposited in the National Botanic Research Institute, Pretoria.

Brachylaena transvaalensis *Hutch. ex Phill. et Schweick* (*voucher* 81/36). The roots (260 g) afforded 100 mg lupeyl acetate and its Δ^{12}-isomer (*ca* 2:1), 5 mg germacrene D, 0.2 mg **1**, 1 mg **2**, 1 mg **3**, 5 mg **4**, 5 mg **5**, 2 mg **10**, 2 mg **11**, 4 mg **12**, 5 mg **13**, 2 mg **15**, 5 mg **16**, 10 mg **17**, 4 mg **18**, 3 mg **19**, 2 mg **20** and 2 mg **21**. The aerial parts (300 g) gave 40 mg linolic and linolenic acid (1:1), 2 mg germacrene D, 5 mg lupeyl acetate, 0.1 mg **1**, 0.2 mg **2**, 2 mg **6a**, 2 mg **6b**, 10 mg **7**, 3 mg **8a**, 5 mg **9** and 2 mg **14a**. The lactones were separated by repeated TLC using first Et_2O–petrol mixtures and then mixtures of $CHCl_3$–C_6H_6–Et_2O.

Brachylaena rotundata (*voucher* 81/169). The aerial parts (50 g) afforded 50 mg lupeyl acetate and its Δ^{12}-isomer (2:1), 1 mg **6a**, 3 mg **6b** and 1 mg **8a**.

Salonitenolide-8-O-2,3-*epoxy isobutyrate* (**8a**). Colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1780 (lactone), 1740 (CO_2R); MS *m/z* (rel. int.): 246.125 $[M-RCO_2H]^+$ (3) ($C_{15}H_{18}O_3$), 228 $[246-H_2O]^+$ (9), 215 $[246-CH_2OH]^+$ (20), 119 (68), 91 (100). Compound **8a** (3 mg) was heated in 0.5 ml Ac_2O for 30 min at 70°. TLC (Et_2O–petrol, 3:1) afforded 2 mg **8b**, colourless gum (for ^1H NMR spectrum see Table 1).

11β,13-*Dihydrozaluzanin C* (**14a**). Colourless gum, which was purified as its acetate **14b** (heating in Ac_2O for 1 hr), colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1790 (lactone), 1745, 1250 (OAc); MS *m/z* (rel. int.): 248.141 $[M-ketene]^+$ (62) ($C_{15}H_{20}O_3$), 247 $[M-MeCO]^+$ (63), 230 $[M-AcOH]^+$ (27), 157 (70), 156 (67), 91 (78), 55 (100).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\ nm}{+11 \quad +14 \quad +17 \quad +26 \quad +41} \quad (CHCl_3;\ c0.23).$$

11β,13-*Dihydrotubiferin* (**19**). Separated from **18** by addition of CH_2N_2, which transformed **18** to the pyrazoline derivative. TLC (Et_2O–petrol, 3:1) afforded pure **19**. Colourless crystals, mp 136° (Et_2O–petrol), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1790 (lactone), 1685 (C=CCO); MS *m/z* (rel. int.): 248.141 $[M]^+$ (29) ($C_{15}H_{20}O_3$), 220 $[M-CO]^+$ (30), 205 $[220-Me]^+$ (11), 192 $[220-CO]^+$ (23), 173 (78), 69 (100).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\ nm}{+27 \quad +13 \quad 0 \quad -30 \quad -300} \quad (CHCl_3;\ c0.03).$$

Brachylaenolide (**20**). Colourless crystals, mp 138° (Et_2O–petrol), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1790 (lactone); MS *m/z* (rel. int.): 246.125 $[M]^+$ (54) ($C_{15}H_{18}O_3$), 231 $[M-Me]^+$ (18), 217 $[M-CHO]^+$ (28), 203 $[231-CO]^+$ (13), 173 (80), 91 (94), 55 (100).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\ nm}{+150 \quad +158 \quad +175 \quad +305 \quad +493} \quad (CHCl_3;\ c0.04).$$

Compound **20** (2 mg) in 2 ml Et_2O was stirred with 30 mg MnO_2. TLC (Et_2O–petrol, 3:1) afforded 1 mg **21**, identical with the natural compound.

Dehydrobrachylaenolide (**21**). Colourless crystals, mp 225° (*iso*-PrOH), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775 (lactone), 1680, 1620 (C=CCO); MS *m/z* (rel. int.): 244.110 $[M]^+$ (43), 229 $[M-Me]^+$ (11), 216 $[M-CO]^+$ (23), 215 $[M-CHO]^+$ (25), 201 $[229-CO]^+$ (15), 91 (100).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436 \quad 365\ nm}{+67 \quad +67 \quad +18 \quad -115 \quad +365} \quad (CHCl_3;\ c0.16).$$

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