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SESQUITERPENE LACTONES FROM *BEDFORDIA ARBORESCENS*

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Key Word Index—*Bedfordia arborescens*; Compositae; sesquiterpene lactones; guaianolides.

Abstract—The aerial parts of *Bedfordia arborescens* afforded in addition to diterpenes and an eremophilanolide several further sesquiterpene lactones including a *seco*-guaianolide. The structures were elucidated by high-field ^{1}H NMR spectroscopy. The configuration of inuviscolide reported previously has been revised.

INTRODUCTION

The small genus *Bedfordia* with only three species belongs to a group of shrubby and arboreal species from Australia which are placed in the tribe Senecioneae [1]. The constituents of *B. salicina* have been investigated previously [2]. In addition to eremophilanes typical for the tribe Senecioneae, large amounts of sesquiterpene acids and some diterpenes were present. This combination is not very typical for Senecioneae. We have therefore studied a further species, *B. arboresens* Hockr. The results are discussed in this paper.

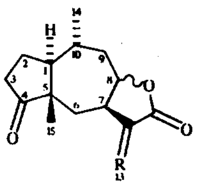
RESULTS AND DISCUSSION

The extract of the aerial parts of *B. arborescens* gave by careful separation β-farnesene, *ent*-kaurenic acid, the eremophilanolide **7** [3], the clerodane **8** [2] and the sesquiterpene lactones pseudoivalin [4], 2,3-dihydroaromaticin (**1**) [5] and compounds **2**–**6**.

The ^{1}H NMR spectrum of **2** (Table 1) was in part close to that of **1**. However, the signals of the exomethylene protons were replaced by a singlet at δ1.49 and also the couplings of H-8 were changed. As could be deduced from the molecular formula ($C_{15}H_{22}O_4$), an additional oxygen function was present, its nature following from the IR spectral band. All data therefore agreed with the presence of the hydroxy derivative **2**. NOE difference spectroscopy established this identification and allowed the assignment of the stereochemistry because so far all pseudoguaianolides have a 4β-methyl and a 7α-H. Saturation of H-15 gave a clear effect with H-6β (6%). Further effects were observed between H-13 and H-8, between H-14 and H-8 and between H-7 and H-8.

Lactone **3** was identical with a guaianolide isolated previously [6] but which was erroneously assigned as a 1α-H guaianolide. Reinvestigation of the configuration by NOE difference spectroscopy showed that the proton at C-1 was β-orientated. NOEs between H-15 and H-1 (5%), between H-1, H-8 (7%) and H-6β (4%) as well as between H-7 and H-5 (7%) established the configuration at all chiral centres if a normal lactone with 7α-H was present.

The ^{1}H NMR spectrum of compound **4** (Table 1) was close to that of **3**. However, the changed stereochemistry at C-8 followed from the downfield shift of the H-7 signal and the slightly changed couplings of H-8. NOE difference spectroscopy established this assumption by clear effects between H-8 and H-7 (5%). Furthermore, from the NOEs between H-15, H-1 (5%) and H-6β (5%) as well as between H-5 and H-7 (4%) the configuration of the remaining chiral centres could be deduced.



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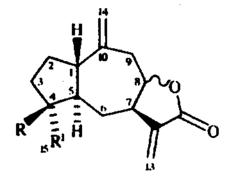
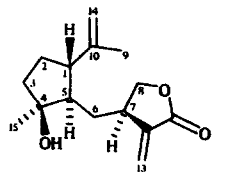
2 R = αMe,βOH; 8αH



	3	**4**	**5**
R	Me	Me	OH
R	OH	OH	Me
8-H	β	α	α



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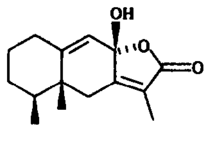

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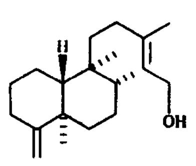

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The ^{1}H NMR spectrum of compound **5** (Table 1) was in part close to that of **4**. However, a different stereochemistry at C-4 was indicated by the downfield shift of the H-1 signal. The configurations of the chiral centres again were determined by NOE difference spectroscopy. Clear effects were observed between H-7, H-5 (6%) and H-8 (7%), and between H-15 and H-5 (4%) but there was no effect between H-15 and H-1.

The molecular formula of **6** ($C_{15}H_{22}O_3$) already indicated that this lactone had two more hydrogens. Spin

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

	2*	**3**	**4**	**5**	**6**
H-1	2.10 *m*	2.14 *br ddd*	2.05 *m*	3.39 *br ddd*	3.11 *br ddd*
H-5	—	1.67 *ddd*	1.40 *br dd*	1.74 *m*	1.74 *m*
H-6α	2.61 *dd*	2.29 *ddd*	2.07 *br dd*	1.55 *m*	1.38 *dd*
H-6β	1.28 *dd*	1.22 *m*	1.55 *ddd*		
H-7	2.33 *ddd*	2.67 *ddddd*	3.19 *ddddd*	3.04 *m*	3.18 *ddddt*
H-8	4.73 *ddd*	4.32 *ddd*	4.59 *ddd*	4.54 *ddd*	4.49 *dd* 3.91 *dd*
H-9α	1.91 *m*	2.58 *dd*	2.79 *dd*	2.67 *dd*	1.74 *br s*
H-9β	1.99 *m*	3.22 *br dd*	2.22 *dd*	2.49 *dd*	
H-13	1.49 *s*	6.23 *d*	6.31 *d*	6.28 *d*	6.25 *d*
H-13′		5.55 *d*	5.67 *d*	5.64 *d*	5.59 *d*
H-14	1.13 *d*	5.11 *br s*	4.99 *br s*	5.02 *br s*	4.92 *br s*
H-14′		4.99 *br s*	4.94 *br s*	4.91 *br s*	4.74 *br s*
H-15	0.97 *s*	1.22 *s*	1.21 *s*	1.32 *s*	1.40 *s*

*H-3 2.53 and 2.07 *m*, H-10 1.91 *m*.

J (Hz): compound **2**: 6α,6β = 15; 6α,7 = 3.5; 6β,7 = 10; 7,8 = 7; 8,9α = 6; 8,9β ~ 8; 10,14 = 7; compound **4**: 1,5 = 5,6β = 6β,7 = 12; 6α,6β = 14; 6α,7 = 4.5; 7,8 = 8.5; 7,13 = 2.7; 7,13′ = 2.5; 8,9α = 4; 8,9β = 9α,9β = 12; compound **5**: 1,2α = 9; 1,2β = 6; 1,5 = 10; 7,8 = 7; 7,13 = 2.3; 7,13′ = 2.0; 8,9α = 5; 8,9β = 11; 9α,9β = 13; compound **6**: 1,2α = 1,2β = 1,5 ~ 8; 5,6 = 6,7 ~ 7; 7,8 = 8; 7,8′ = 5.5; 7,13 = 3; 7,13′ = 2.5; 8,8′ = 8.5.

decoupling starting with the signal at δ3.18, which obviously was due to H-7, allowed the assignment of all signals. Furthermore, the presence of an 8,9-*seco*-guaianolide was indicated by the pair of doublets for H-8. All data therefore agreed with structure **6**, which we have named bedfordiolide. If the chemical shifts of H-1 in the spectra of compound **4** and **5** are compared with that of **6** it can be tentatively proposed that in **6** a 4β-hydroxyl group is present. As the lactone **6** surely is derived from **5** by a fragmentation pathway the stereochemistry at C-5 and C-7 is most likely identical in both compounds. The fragmentation pattern in the mass spectrum of **6** also supported the structure. After elimination of water (*m/z* 232), loss of $C_5H_5O_2$ (lactone moiety) and loss of $C_6H_7O_2$ (splitting of the 5,6-bond), prominent peaks at *m/z* 135 and 121 could be observed. Elimination of propylene from these fragments led to *m/z* 93 and 79.

The presence of eremophilanolides in *Bedfordia* indicates that this genus is clearly a member of the tribe Senecioneae. The isolation of guaianolides, pseudoguaianolides and sesquiterpene acids could be explained if the subarborescent Australian endemic, *Bedfordia*, is a relict, retaining an ancestral chemical pathway as it is certainly more primitive than the widespread herbaceous elements of the tribe such as *Senecio sensu stricto*.

EXPERIMENTAL

The air-dried aerial parts (1.3 kg, collected near Canberra, Australia, voucher 86-0062, deposited at the U.S. National Herbarium) were extracted with $MeOH–Et_2O$–petrol (1:1:1) and separated as reported previously [7]. The less polar CC fractions gave by prep. TLC (silica gel, PF 254) 5 mg β-farnesene, 10 mg *ent*-kaurenic acid and 20 mg **8**. The polar CC fractions (Et_2O and Et_2O–MeOH, 9:1) were further separated by prep. TLC (Et_2O–petrol, 3:1) affording 50 mg **1** (R_f 0.40), a mixture (R_f 0.50) which gave by repeated prep. TLC (Et_2O–petrol, 1:1, several developments) 2 mg **7** and 2 mg **6** and a further mixture (R_f 0.30) which gave by prep. TLC ($Et_2O–C_6H_6–CHCl_3$, 1:1:1) two bands (3/1 and 3/2). HPLC of 3/1 (RP 8, $MeOH–H_2O$, 13:7, *ca* 100 bar) gave 2 mg **2** (R_t 1.5 min), 2 mg **5** (R_t 2.0 min) and 4 mg **3** (R_t 2.5 min). HPLC of 3/2 (same conditions) gave 2 mg **4** (R_t 2.6 min) and 2 mg pseudoivalin. Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material.

*11β-Hydroxy-11,13-dihydro-8-*epi*-confertin* (**2**). Colourless gum; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3440 (OH), 1780 (γ-lactone), 1745 (C= O); MS *m/z* (rel. int.): 266.152 $[M]^+$ (44) (calc. for $C_{15}H_{22}O_4$: 266.152), 248 $[M-H_2O]^+$ (6), 230 (11), 207 (41), 189 (42), 161 (51), 121 (83), 97 (100).

8-epi-*Inuviscolide* (**4**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1780 (γ-lactone); MS *m/z* (rel. int.): 248.141 $[M]^+$ (6.5) (calc. for $C_{15}H_{20}O_3$: 248.141), 230 $[M-H_2O]$; (23), 215 $[230-Me]^+$ (14), 190 (82), 159 (48), 145 (72), 119 (100).

*4,8-Bis-*epi*-inuviscolide* (**5**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1780 (γ-lactone); MS *m/z* (rel. int.): 248.141 $[M]^+$ (4) (calc. for $C_{15}H_{20}O_3$ 248.141), 230 $[M-H_2O]^+$ (40), 190 (43), 159 (48), 145 (51), 119 (100).

Bedfordiolide (**6**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3605 (OH), 1780 (γ-lactone); MS *m/z* (rel. int.): 250.157 $[M]^+$ (6) (calc. for $C_{15}H_{22}O_3$: 250.157), 235 $[M-Me]^+$ (22), 232 $[M-H_2O]^+$ (7), 217 $[235-H_2O]^+$ (10), 135 $[232-C_5H_5O_2]^+$ (67), 121 $[232-C_6H_7O_2]^+$ (100), 93 $[135-C_3H_6]^+$ (72), 79 $[121-C_3H_6]^+$ (56).

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GUAIANOLIDE FROM *SAUSSUREA LAPPA*

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Key Word Index—*Saussurea lappa*; Compositae; α-methylene-γ-lactone; biological activity; guaianolide; germacranolide; sesquiterpenoids; 12-methoxydihydrocostunolide.

Abstract—A new compound, 12-methoxydihydrodehydrocostuslactone, has been isolated from the essential oil of *Saussurea lappa*. The structure and stereochemistry were established by spectroscopic and chemical methods.

INTRODUCTION

The essential oil from costus roots (*Saussurea lappa* Clarke) is a rich source of two sesquiterpene lactones, dehydrocostuslactone (**1**) and costunolide (**2**) in addition to other minor components [1]. The biological activity of the oil is mainly attributed to the presence of these α-methylene-γ-lactones [2–4]. As a part of our continuing investigations of structure–biological activity relationships, we have isolated a new naturally occurring guaianolide, 12-methoxydihydrodehydrocostuslactone (**3**), from the essential oil.

RESULTS AND DISCUSSION

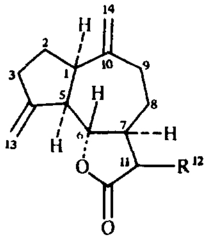
The new lactone (**3**), $C_{16}H_{22}O_3$ (HRMS $[M]^+$ 262.365), $[\alpha]_D^{20}+19°$, exhibited ^{1}H NMR data characteristic of dehydrocostuslactone (**1**) and dihydrodehydrocostuslactone (**4**) (Table 1). The presence of an additional singlet for three hydrogens at δ3.34 suggested that one of the oxygen atoms was present as $-OCH_3$. This was supported by a mass ion peak at m/z 231 $[262-31]^+$. Its IR spectrum showed bands at 1780, 1030 (γ-lactone), 3080, 1640 and 890 (exomethylene double bond) cm^{-1}. All the above data suggested that the new compound was the guaianolide 12-methoxydihydrodehydrocostuslactone (**3**).

Further proof of the structure was obtained by the synthesis of **3** (IR and NMR) from dehydrocostuslactone upon treatment with methanol under alkaline conditions. In addition to this, it also established the stereochemistry at all centres except that of the 12-methoxy group. This reaction of dehydrocostuslactone is reversible under basic conditions and hence it should yield the thermodynamically more stable epimer at C-11. In *trans*-fused butanolides like santonin and related compounds the more stable configuration of the C-11 methyl is the one in which 12-methyl group is *trans* to the hydrogen at C-7. On the basis of this agreement [5], if it holds good in guaianolides also, the 12-methoxy group in compound (**3**) should have the β-configuration.

12-Methoxydihydrocostunolide (**6**) isolated earlier [6] from the essential oil was suspected to be an artefact produced from costunolide during isolation since methanol was used as the solvent for chromatographic elution. However, the isolation of the same compound in this study proves beyond doubt that this compound is a natural product. The presence of compounds **3** and **6** in the oil suggests that the parent lactones **1** and **2** might be present in bound form in the plant and that they migrate to other parts, especially in roots, of the plant as their 12-methoxy derivatives where they are again regenerated to perform various biological functions like root initiation and alleliopathogenicity. It has already been shown that lactones **1** and **2** are inhibitors of germination [2–4].

EXPERIMENTAL

Isolation of compound **3**. The essential oil (10 g), obtained by solvent extraction (hexane) from the powdered roots of *S. lappa*, was allowed to stand at 0° for several days when a solid (6.0 g)



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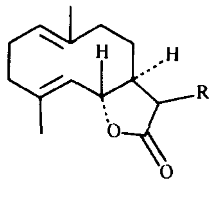
4 R = —Me



2 R = ═CH₂
5 R = —Me
6 R = —CH₂OMe