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THE GERMACRANOLIDES OF *VIGUIERA BUDDLEIAEFORMIS* STRUCTURES OF BUDLEIN-A AND -B‡

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Abstract—The structure of budlein-A, the main sesquiterpene lactone of *Viguiera buddleiaeformis* was established as the 8 angeloyl ester of 1 keto, 8-β, 14-dihydroxy germacra-2,4,11 (13)-trien-3, (10 β) oxido-6 α, 12-olide. Its structure and stereochemistry was determined by chemical and spectroscopic means. Budlein-B, found in the same plant as a minor constituent, is 8 α, 15-dihydroxygermacra-1 (10), 4, 11 (13)-trien-6 α, 12-olide.

In a previous paper we described two germacranolides, viguestenin and desacetylviguestenin, isolated from *Viguiera stenoloba* [1]. The present article deals with the isolation and structure determination of the 3(2H) furanone germacranolide budlein-A **1a**, a constituent of *V. buddleiaeformis*. Budlein-B was also isolated in low yield from the same plant; its structure and stereochemistry was found to be as depicted in **6a**.

Budlein-A (**1a**) $C_{20}H_{22}O_7$, mp 106–108°; $[\alpha]_D$ −82·3° MeOH, showed in the IR spectrum the characteristic bands of a 3(2H) furanone moiety [2,3] (1710 cm^{-1} keto group and 1590 cm^{-1} enolic double bond). The UV absorption (λ_{max} 266 nm, ε, 10000) indicated the conjugation of an additional double bond at C-4 with the 3(2H) furanone system, similar to that observed with calaxin and ciliarin [2] (zexbrevin which lacks the C-4 double bond absorbs at 259 nm) [3]. Budlein-A exhibited IR bands at 1760, 1650, 890 cm^{-1} and a pair of low field doublets in the PMR spectrum at 5·70 ppm (1H, *J* 2 Hz) and 6·30 ppm (1 H, *J* 2 Hz), characteristic of a γ-lactone conjugated with an exocyclic methylene group (UV absorption λ_{max} 215 nm, ε, 20800 both, lactone and ester chromophore). The presence of a OH group was indicated by an IR band at 3400 cm^{-1} and the formation of an acetate. In the MS, compound **1a** showed a M^+ at *m/e* 374 and other prominent peaks at *m/e* 274 (M^+-100), *m/e* 55 and a base peak at *m/e* 83 (Me–CH=C(Me)C≡O). These fragmentations are typical of the cleavage of esters of angelic, tiglic and senecioic acids. In the PMR spectrum of budlein-A a multiplet appeared at 6·08 ppm (1 H), a chemical shift typical of the vinyl proton of angelic acid. The vinyl hydrogen of tiglic acid appears at 7·0 ppm whereas the α-vinyl proton of senecioic acid resonates at higher field [4].

The PMR spectrum of budlein-A showed only signals for the C-10 Me group and the vinyl Me groups of the angelate ester moiety (Table 1). The typical signal for the C-4 Me group, common for lactones of this type [2], was not present; instead, the spectrum exhibited a broad singlet at 4·36 ppm (2 H) which was assigned to the $-CH_2-O-$ protons of an allylic primary alcohol. The vinyl hydrogen at C-5 appeared as a multiplet centered at 6·17 ppm (1 H, $J_{5,6}$ 4 and $J_{5,14}$ 1·5 Hz); the oxygen bridge of the dihydrofuranone system must lie between C-3 and C-10 of the germacranolide skeleton, since the C-10 Me group appeared as a singlet at 1·48 ppm (3H). Two overlapping complex signals centered at 5·33 and 5·23 ppm (1 H each) were ascribed to C-6 and C-8 protons. From the foregoing discussion we can assign structure **1a** for budlein-A, although at this point the alternative structure with the ester group attached to C-6 and the lactone closed to C-8 cannot be excluded.

The three previously described 3(2 H) furanone germacranolides, calaxin, ciliarin [2] and zexbrevin [3], contain a C-6 lactone closure, thus making structure **1a** more probable. This was confirmed in the following manner: in the PMR spectrum of compound **1a**, the signal at 5·33 ppm (1 H) was attributed to the C-6 allylic hydrogen of the lactone closure, since it was shifted upfield to 4·7 ppm (the typical position for the lactonic proton) in tetrahydrobudlein-A **2**. The multiplet centered at 5·23 ppm (1 H) in **1a** remained unchanged in compound **2** and was ascribed to the hydrogen at the carbon bearing the ester group. When the signal at 5·23 ppm was irradiated, the multiplet at 3·75 (proton at C-7) was simplified; at the same time the ABX system (C-9 methylene) collapsed to an AB system. This demonstrated that the signal at 5·23 corresponds to C-8 proton (ester base), the usual position for the ester group in lactones of this type [2,3,5]; therefore budlein-A is represented by **1a**.

Catalytic hydrogenation of **1a** was very complicated due to the presence of four double bonds and a hydrogenolizable allylic alcohol. After several attempts, there was obtained in a very low yield the tetrahydroderivative **2**. Compound **2** still contains the 3(2 H) furanone moiety (IR bands at 1710 and 1590 cm^{-1}) and the ester double bond (*m/e* 83). The saturation of the exocyclic methylene

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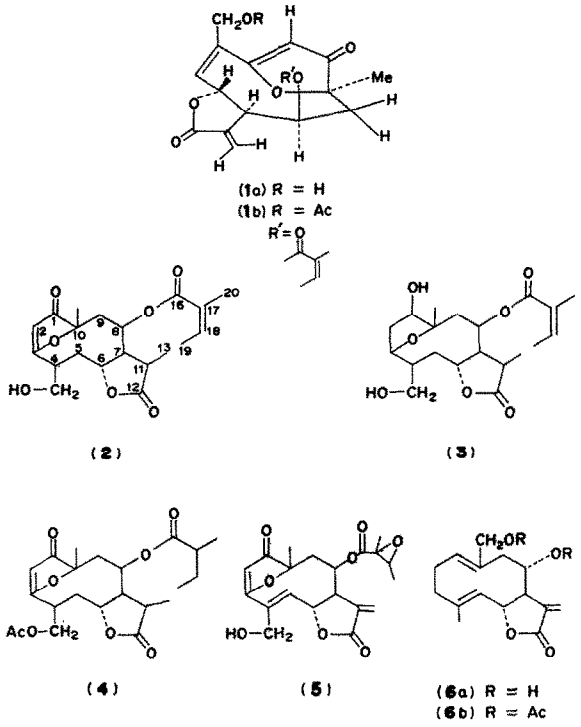

group was indicated by the absence of the low field vinyl proton doublets.

Sodium borohydride reduction of **1a** afforded octahydrobudlein-A **3** in which the carbonyl group at C-1 and the C-4 and C-11 double bonds were reduced. The ester double bond remained intact as indicated by the complex signal centered at 6·0 ppm (1 H). The C-6 hydrogen in **3** appeared as a multiplet centered at 4·5 ppm (1 H); the signal corresponding to the C-8 proton remained unchanged at 5·23 ppm.

Acetylation of budlein-A in the usual manner gave an unseparable mixture of several compounds. Budlein-A acetate **1b** was obtained when the acetylation was carried out under more basic conditions using triethylamine. The PMR spectrum of **1b** showed a singlet at 2·15 ppm (3 H) indicative of the acetoxy group; a singlet at 4·85 ppm (2 H) was assigned to the $-CH_2-O-$ protons at the base of the acetate. The MS showed a base peak at *m/e* 83 and other prominent peaks at *m/e* 356 (M^+-AcOH) and *m/e* 43 ($Me-C\equiv O^+$).

Catalytic hydrogenation of **1b** over palladium–charcoal gave a mixture of 3 compounds. Separation by TLC afforded in low yield hexahydrobudlein-A acetate **4**. In the vinyl proton region, **4** showed only the signal corresponding to the C-2 proton, thus indicating a complete saturation of the remaining double bonds.

Treatment of budlein-A with *m*-chloroperbenzoic acid afforded the epoxide **5**, in which only the ester double bond was epoxidized. In the PMR spectrum, budlein-A epoxide exhibited a doublet at 1·3 ppm (3 H, *J* 5 Hz), a singlet at 1·2 ppm (3 H) and a quartet centered at 3·1 ppm (1 H, *J* 4 and 5 Hz) attributed to the Me groups and the proton of the epoxide, respectively.

The stereochemistry of budlein-A at C-6 and C-8 was established from the dihedral angles (Table 1) calculated from the coupling constants. In the PMR spectra of compounds **1a**, **1b**, **2** and **3**, the signal corresponding to the C-7 proton has an abnormally low chemical shift (3·72 ppm), indicating that this hydrogen is close to the oxygen of the furan ring. If the usual assumption is made that the C-7 side chain is β-oriented, as in all sesquiterpene lactones of known stereochemistry, this proximity requires an α-oriented C-7 hydrogen, an α C-10 methyl group and a *cis* C-4 double bond (construction of a Dreiding model containing a *trans* C-4 double bond proved impossible). The calculated dihedral angle of 118° at the C_6–C_7 bond (the same angle was observed for zexbrevin) [3] indicated a β-configuration for the C-6 hydrogen or a *trans* lactone closure.

Table 1. PMR spectrum of budlein-A

Proton number	Chemical shift	Multiplicity	Coupling constants	Dihedral angles
H-2	5·65	*s*		
H-5	6·17	*t*, *d*	$J_{5,14} = 1{\cdot}5$	
H-6	5·33	*m*	$J_{6,7} = 4{\cdot}5$	47° or 118°
			$J_{5,6} = 4{\cdot}0$	50° or 120°
H-7	3·75	*m*	$J_{7,13} = 2{\cdot}8$	
			$J_{7,13} = 3{\cdot}0$	
H-8	5·23	*m*	$J_{8,9} = 5{\cdot}5$	42° or 125°
			$J_{8,9'} = 4{\cdot}0$	50° or 120°
			$J_{7,8} = 2{\cdot}0$	63° or 110°
H-9	2·51	*d*, *d*	$J_{9,9'} = 15$	
H-9′	2·32	*d*, *d*		
$14CH_2$-O	4·36	*d*, *t*		
$15CH_3$	1·48	*s*		
H-18	6·08	*q*, *q*	$J_{16,17} = 1{\cdot}5$	
			$J_{16,18} = 7$	
$19CH_3$	1·91	*m*		
$20CH_3$	1·78	*m*		

Spectra were determined in $CDCl_3$ at 100 MHz. Values are given in ppm (δ scale) relative to TMS as an internal standard. Signals are described as follows: *s* = singlet, *d* = doublet; *t* = triplet, *q* = quartet, *m* = multiplet. Coupling constants are given in Hz.

The configuration of the oxygen function at C-8 was found to be β-oriented, since the coupling constants between H_7-H_8, H_8-H_9 and H_8-$H_{9'}$ have small values. This is in agreement with the required dihedral angles (Table 1). Hence the structure and the stereochemistry for budlein-A must be as depicted in **1a**.

Budlein-B (**6a**) $C_{15}H_{20}O_4$, mp 162°; $[\alpha]_D$ + 3·1° MeOH, was isolated from the more polar fractions. It contains a γ-lactone conjugated with an exocyclic methylene group, as indicated by the IR bands (1765, 1665, 890 cm^{-1}) and the characteristic pair of low field doubles in the PMR spectrum at 5·60 ppm (1 H, *J* 1·5 Hz) and 6·30 ppm (1 H, *J* 2 Hz). The UV spectrum (λ_{max} 222 nm, ε, 8908) was in accord with the presence of an α, β-unsaturated γ-lactone. In the MS, compound **6a** showed a M^+ at *m/e* 264 and another prominent peak at *m/e* 246 (M^+-H_2O) and *m/e* 231 (M^+-18-15). The presence of two OH groups was indicated by the strong IR band at 3400 cm^{-1} and the formation of a diacetate. The PMR spectrum exhibited a doublet centered at 1·68 ppm (3 H, *J* 1 Hz), ascribed to a vinyl Me group. The six allylic protons appeared as a five proton multiplet centered at 2·28 ppm (C-2, 2 H; C-3, 2 H; C-9, 1 H); the complex signal at 2·82 ppm (2 H) was assigned to the C-7 and C-9 protons. In the low field region of the spectrum the vinyl protons at C-1 and C-5 appeared as complex signals at 5·15 ppm and 4·85 ppm, respectively. The expected signal for the vinyl Me group at C-10, found in the majority of the germacradienolides [5] was not present; instead, the spectrum exhibited a sharp symmetrical AB quartet at 3·9 ppm (2 H, *J* 1 and 5 Hz), assigned to the OH-methylene group. The broad quartet

at 5·0 ppm (1 H) was attributed to the C-6 lactonic proton.

Budlein-B, upon acetylation with pyridine-acetic anhydride, afforded budlein-B diacetate **6b** which was identical in all respects with zexbrevin D, previously isolated from *Zexmenia brevifolia* [6].

The stereochemistry at C-8 of budlein-B was established by the method developed by Horeau for determining the configuration of asymmetric centers containing a secondary OH group. Treatment of budlein-B with excess racemic α-phenylbutyric anhydride and work up in the usual manner afforded a laevorotatory optically active acid. Based on the Horeau procedure [7], the asymmetric center at C-8 must have the absolute configuration S (α-oriented OH group).

From all evidence presented above, we propose structure **6a** for budlein-B.

EXPERIMENTAL

Mp's are uncorrected. IR spectra were recorded in $CHCl_3$ and UV in 95% EtOH unless otherwise stated. Analyses were determined by Dr. Alfred Bernhardt, Max Planck Institute fuer Kohlenforschung, Muelheim, West Germany.

Isolation of budlein-A (**1a**) *and budlein-B* (**6a**). *Viguiera buddleiaeformis* (D.C.) Benth et Hook (10 kg) dried and ground was extracted 2× with EtOH and worked up in the usual manner [3], leaving an oily residue which was dissolved in hot C_6H_6 and chromatographed over Alcoa F-20 alumina. The fractions eluted with $CHCl_3$–Me_2CO (9:1) were combined and evaporated to dryness giving an amorphous residue; recrystallization from Me_2CO-isopropyl ether afforded pure budlein-A (3·65 g); mp 106–108°; $[\alpha]_D^{25}$ -82·3° (MeOH); λ_{max} 215, 266 nm; ε, 20800 and 10000; ν_{max} 3430, 1770, 1710, 1660, 1590 and 890 cm^{-1}. (Found: C, 64·03; H, 5·98; O, 29·99. $C_{20}H_{22}O_7$ requires: C, 64·15; H, 5·88; O, 29·94%). From the fractions eluted with $CHCl_3$–Me_2CO (4:1) there was obtained 400 mg of budlein-B; mp 162°; $[\alpha]_D^{25}$ + 3·1° (MeOH); λ_{max} 224 nm; ε, 7,200; ν_{max} 3350, 3450, 1765, 1665, 1150 and 1050 cm^{-1}. (Found: C, 68·30; H, 7·51; O, 24·32. $C_{15}H_{20}O_4$ requires: C, 68·28; H, 7·55; O, 24·27%).

Budlein-A acetate (**1b**). To a soln of budlein-A (175 mg) in CH_2Cl_2 (2·3 ml) was added Et_3N (0·58 ml) Ac_2O (0·23 ml) and C_5H_5N (0·015 ml). The mixture was agitated for 1·5 hr at room temp. Work up in the usual manner afforded a mixture of two compounds: **1b** was obtained by preparative-TLC, yield 130 mg; mp 114–116°; $[\alpha]_D^{25}$ −78·8° (MeOH); ν_{max} 1740 cm^{-1} (acetate); M^+ 412. (Found: C, 63·37; H, 5·88; O, 30·69. $C_{22}H_{24}O_8$ requires: C, 63·46; H, 5·76; O, 30·76%).

Tetrahydrobudlein-A (**2**). A soln of budlein-A (1 g) in MeOH (20 ml)) was hydrogenated for 5 hr with $Pd/CaCO_3$ (100 mg). The catalyst was filtered off and the soln evaporated to dryness. Purification by preparative TLC afforded 60 mg of tetrahydrobudlein-A; mp 43°; λ_{max} 220, 259 nm; ε, 7,000 and 10000; ν_{max} 3450, 1760, 1660 and 1590 cm^{-1}; M^+ *m/e* 378.

Octahydrobudlein-A (**3**). A soln of budlein-A (100 mg) in MeOH (8 ml) was mixed with a cold soln of $NaBH_4$ (68 mg) in MeOH (2 ml). The reaction mixture was kept at 0° for 1 hr. It was acidified and extracted with $CHCl_3$. The organic layer was washed, dried and evaporated to dryness. The crude product was recrystallized from AcOEt–hexane; yield 77 mg, mp 164–166°; $[\alpha]_D^{25}$ −168° (MeOH); ν_{max} 3430, 1760, 1720 and 1660 cm^{-1}. (Found: C, 62·53; H, 7·62; O, 29·86. $C_{20}H_{30}O_7$ requires; C, 62·82; H, 7·85; O, 29·32%).

Hexahydrobudlein-A acetate (**4**). A soln of **1b** (800 mg) in MeOH (20 ml) containing 300 mg of Pd/C −10% was hydrogenated for 12 hr at 4 kg/cm^2. The catalyst was filtered off and the soln evaporated to dryness; a mixture of 3 compounds was obtained. Separation by preparative TLC afforded 390 mg of pure hexahydrobudlein acetate; mp 75–78°; λ_{max} 258 nm; ε, 12,200. (Found: C, 62·60; H, 7·06; O, 30·40. $C_{22}H_{30}O_8$ requires: C, 62·55; H, 7·10; O, 30·33%).

Budlein-A epoxide (**5**). To a soln of **1a** (200 mg) in $CHCl_3$ (20 ml) was added recrystallized *m*-chloroperbenzoic acid (175 mg) and the soln was allowed to reflux for 48 hr. Work up in the usual manner yielded a mixture of several compounds. Separation by preparative TLC afforded 70 mg of **5** mp 66–69°; M^+ *m/e* 390.

Budlein-B diacetate (**6b**). A soln of **6a** (52 mg) in Ac_2O (0·5 ml) and C_5H_5N (0·5 ml) was 18 hr at room temp. Work up in the usual manner afforded 50 mg of budlein-B diacetate, mp 149–151°, identical in all respects with zexbrevin D. (Found: C, 65·38; H, 6·82; O, 27·47. $C_{19}H_{24}O_6$ requires: C, 65·50; H, 6·90; O, 27·60%).

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