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PREHISPANOLONE, A LABDANE DITERPENE FROM *LEONURUS HETEROPHYLLUS*

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Key Word Index—*Leonurus heterophyllus*; Labiatae; diterpenoids; prefuranic and furanic labdane derivatives; prehispanolone; hispanolone.

Abstract—A new labdane diterpene, prehispanolone, has been isolated from *Leonurus heterophyllus*. Its structure, 9α,13*R*;15,16-diepoxylabdan-14-en-7-one, was established by spectroscopic means as well as by examination of its derivatives.

INTRODUCTION

The whole plant of *Leonurus heterophyllus* Sweet, also known as 'YiMuCao' in Chinese, is a well-known herb in Chinese medicine for the treatment of gynaecological problems, including irregular menstruation, amenorrhea and postpartum haemorrhage as well as edema in chronic and acute nephritis [1].

Several alkaloids, including leonurine A and B, have been isolated from this plant [2–4] and many labdane diterpenoids have been isolated from related species in the same family over the last few years [5–10]. In our search for biologically active compounds, we examined the aerial parts of *Leonurus heterophyllus*. From this source a new labdane diterpene was isolated and named prehispanolone (**1**). It inhibited ^{3}H-platelet activating factor binding to rabbit platelet membranes with an IC_{50} of 4×10^{-6}M. Its structure was established by spectroscopic methods as well as from its rearranged and hydrogenated derivatives. It is of taxonomical interest to note that both *Leonurus sibiricus* and *Leonurus heterophyllus* contain labdanic diterpenoids whereas other *Leonurus* species contain only clerodanic diterpenoids [8].

RESULTS AND DISCUSSION

Prehispanolone (**1**) has a molecular formula $C_{20}H_{30}O_3$, as indicated by EI and high resolution mass spectra. Its IR spectrum showed ketone (1715 cm^{-1}) and enol–ether (3100, 1615 cm^{-1}) absorptions but did not show hydroxyl bands. Its ^{1}H NMR spectrum was consistent with a β,β-disubstituted dihydrofuran partial structure (at δ5.13 and 6.42, 1H each, *d*, $J=2.5$ Hz, H-14 and H-15; and an AB system at δ4.02 and 4.41, 1H each, *d*, $J=10.4$ Hz, 2H-16) and also with three tertiary methyl groups (at δ0.86, 6H, *s*, Me-18 and Me-19; δ1.11, 3H, *s*, Me-20) and a secondary methyl group (at δ0.99, 3H, *d*, $J=6.5$ Hz, Me-17). The fragments at *m/z* 82 and 96 in the mass spectrum of prehispanolone (**1**) were also indicative of the presence of a β,β-disubstituted dihydrofuran ring in the molecule. In its ^{1}H NMR spectrum (Table 1) the H-8 methine proton signal was a simple quartet (at δ2.69, 1H, *q*, $J=6.5$ Hz) and in the ^{1}H–^{1}H COSY spectrum the H-8 proton was coupled with the C-17 methyl group, so the methine carbon atom (C-8) must have two fully substituted carbon atoms attached to it. These data suggested that the ketone group should be at the C-7 position.

The configuration of the C-17 methyl group on C-8 must be equatorial as reflected by the coupling constant of the doublet ($J=6.5$ Hz), because an axial methyl group should have a larger value $J=8$ Hz [6]. This conclusion was also supported by the ^{1}H–^{1}H NOESY spectrum which showed that the H-8 was an axial proton coupled with the C-20 methyl group.

The 13*R*-configuration assigned to prehispanolone (**1**) was supported by ^{1}H–^{1}H NOESY spectrum which showed that the C-17 methyl group was coupled with the H-16 protons, but not coupled with H-14, this behaviour established the configuration of the C-13 centre of prehispanolone as *R* [5], which is thus 9α,13*R*;15,16-diepoxylabdan-14-en-7-one. The ^{13}C NMR spectrum (Table 2) confirmed all of the above assignments.

The structure of prehispanolone (**1**) was further confirmed by the ready conversion of **1** into hispanolone (**2**) by mild acid treatment. Hispanolone (**2**) from **1** also has a molecular formula of $C_{20}H_{30}O_3$ and its ^{1}H NMR spectrum was very similar to that of **1**. The difference was only a β-monosubstituted furan ring (at δ6.27, 7.36 and 7.23, 1H each, H-14, H-15 and H-16, respectively) in **2** instead of the β,β-disubstituted dihydrofuran of **1**. Its ^{13}C NMR spectrum also supported this conclusion. Hispanolone is a known compound. The ^{1}H NMR, ^{13}C NMR and mass spectra, as well as the [α] and mp of **2** derived from **1** were identical to those previously reported [5, 6] for natural hispanolone.

14,15-Dihydroprehispanolone (**3**) was a hydrogenated product of prehispanolone (**1**) and has a molecular for-

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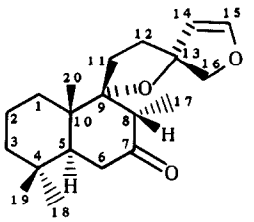
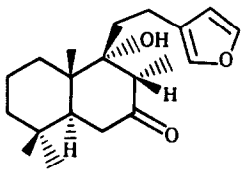


1



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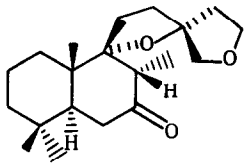

3

mula of $C_{20}H_{32}O_3$. Its ^{1}H NMR spectrum also was similar to that of **1**. The difference being consistent with the occurrence in **3** of a β,β-disubstituted tetrahydrofuran (at δ3.79 and 3.94, 1H each, *m*, 2H-15; δ3.75 and 3.58, 1H each, *d*, J = 8.6 Hz, 2H-16) instead of the β,β-disubstituted dihydrofuran ring of **1**. The ^{13}C NMR spectrum of 14,15-dihydroprehispanolone (Table 2) showed carbon resonances in complete agreement with structure **3** for this hydrogenated diterpene.

14,15-Dihydroprehispanolone (**3**) was a more stable compound than **1**. It cannot be transformed into 14,15-dihydrohispanolone under the same conditions where **1** was rearranged to hispanolone (**2**).

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on a 250 MHz spectrometer at 250 MHz and 62.9 MHz, respectively. $CDCl_3$ was used as solvent unless otherwise stated, with TMS as int. standard. IR spectra were recorded in $CHCl_3$. Mps: uncorr.

Commercial *Leonurus heterophyllus* Sweet, cultivated in Guangdong Province, China, was used. *Leonurus heterophyllus* used in this study was authenicated by Dr Paul But (Department of Biology, CUHK) and a sample is deposited in the Museum of the Chinese Medicinal Material Research Center, CUHK. Activity at the platelet activating factor (PAF) receptor on rabbit platelet membranes was determined by a ^{3}H-PAF radioreceptor assay [11].

Extraction and isolation of prehispanolone. Dried plant materials (1 kg) were extracted × 2 with Me_2CO (5 l) under reflux. The deep green extract was evapd to dryness under red. pres. at 30°. The residue (20 g) was chromatographed on a silica gel (Merck, 7734) column. Elution with hexane–EtOAc (4:1) gave crude prehispanolone (1 g). The crude product was treated with charcoal and purified by CC on a silica gel (Merck 9385) column with hexane–EtOAc (19:1) as eluting solvent, yielding pure prehispanolone (**1**) (250 mg).

Prehispanolone (**1**). A syrup, $[\alpha]_D^{22}$ −63.6° (C_6H_6; *c* 0.55); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3100, 2950, 2870, 1715, 1615, 1459, 1141, 1070, 1009, 973, 938, 860 and 727; ^{1}H NMR (250 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (62.9 MHz, $CDCl_3$): see Table 2: EIMS, *m/z* (rel. int.): 318 $[M]^+$ (8), 303 (1), 236 (59), 221 (6), 194 (16), 167 (17), 164 (13), 137 (21), 123 (77), 122 (40), 109 (51), 96 (22), 95 (60), 82 (68), 81 (100), 69 (38); high resolution MS: 318.2185, $C_{20}H_{30}O_3$ Calc. 318.2194.

Hispanolone (**2**) *from prehispanolone* **1**. Prehispanolone (**1**) (20 mg) was dissolved in 10 ml EtOAc and then 1 drop of 0.5% HCl soln was added. The soln was stirred at room temp. for 1 hr. The soln was dried over Na_2SO_4, filtered and the solvent removed. The residue was purified by silica gel (Merck 9385) column chromatography with hexane–EtOAc (9:1) as eluting solvent to give the hispanolone (**2**) (10 mg). Flake crystal from hexane–EtOAc; mp 145–146°; $[\alpha]_D^{22}$ −18.2° ($CHCl_3$; *c* 1.00); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3506, 3485, 2981, 2937, 2917, 2892, 1696, 1500, 1465, 875 and 757; ^{1}H NMR (250 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (62.8 MHz, $CDCl_3$): see Table 2; EIMS, *m/z* (rel. int.): 318 $[M]^+$ (6), 223 (5), 194 (38), 167 (5), 152 (14), 123 (56), 109 (100), 95 (50), 81 (76), 69 (26), 67 (22). EA: Found C, 75.28; H, 9.70. $C_{20}H_{30}O_3$ Calc. C, 75.42; H, 9.42%.

14,15-*Dihydroprehispanolone* (**3**) *from prehispanolone* (**1**). Prehispanolone (20 mg) was dissolved in 10 ml EtOAc and then 10 mg 5% Pd/c was added as catalyst. The soln was stirred under H_2 at room temp. overnight. After filtration the solvent was

Table 1. ^{1}H NMR data of compounds **1–3** (δ values from internal TMS)

H	1	2	3
8	2.69 *q* (6.5)	2.74 *q* (6.5)	2.69 *q* (6.5)
14	5.13 *d* (2.5)	6.27	
15	6.42 *d* (2.5)	7.36	3.79 *m*
			3.94 *m*
16A	4.02 *d* (10.4)	7.23	3.75 *d* (8.6)
16B	4.41 *d* (10.4)		3.58 *d* (8.6)
Me-17	0.99 *d* (6.5)	1.12 *d* (6.5)	0.99 *d* (6.5)
Me-18	0.86 *s*	0.88 *s*	0.87 *s*
Me-19	0.86 *s*	0.90 *s*	0.87 *s*
Me-20	1.11 *s*	1.18 *s*	1.13 *s*

J (Hz) in parentheses.

Table 2. ^{13}C NMR data of compounds **1–3**

C	1	2	3
1	38.3 *t*	34.9 *t*	39.1 *t*
2	18.7 *t*	18.6 *t*	18.7 *t*
3	41.6 *t*	41.4 *t*	41.7 *t*
4	32.7 *s*	33.6 *s*	32.7 *s*
5	50.7 *d*	50.9 *d*	50.5 *d*
6	39.1 *t*	39.3 *t*	40.7 *t*
7	210.4 *s*	211.3 *s*	211.0 *s*
8	47.1 *d*	46.4 *d*	46.8 *d*
9	96.5 *s*	81.7 *s*	96.5 *s*
10	42.5 *s*	43.4 *s*	42.9 *s*
11	37.9 *t*	32.1 *t*	38.2 *t*
12	30.2 *t*	21.6 *t*	29.7 *t*
13	93.8 *s*	124.9 *s*	91.3 *s*
14	107.1 *d*	110.6 *d*	32.9 *t*
15	148.1 *d*	143.0 *d*	78.1 *t*
16	80.8 *t*	138.6 *d*	67.7 *t*
17	9.2 *q*	8.2 *q*	9.1 *q*
18	32.5 *q*	32.8 *q*	32.7 *q*
19	21.2 *q*	21.4 *q*	21.3 *q*
20	17.3 *q*	16.3 *q*	17.8 *q*

removed and the residue purified by CC on silica gel (Merck 9385) with hexane–EtOAc (9:1) as eluting solvent, to give 14,15-dihydroprehispanolone (**3**). A syrup; $[\alpha]_D^{22}$ −33.6 ($CHCl_3$; *c* 0.60); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 2978, 2871, 1710, 1465, 1101, 1058, 978, 929, 906, 755; ^1H NMR (250 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (62.9 MHz, $CDCl_3$): see Table 2; EIMS, *m/z* (rel. int.): 320 $[M]^+$ (4), 305 (4), 196 (73), 167 (6), 154 (37), 123 (20), 109 (22), 97 (10), 95 (12), 84 (7), 83 (100), 69 (22), 67 (17).

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A FURANOID DITERPENOID FROM *TINOSPORA MALABARICA*

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Key Word Index—*Tinospora malabarica*; Menispermaceae; tinosporicide.

Abstract—A new furanoid diterpenoid, tinosporicide, has been isolated from the fresh stems of *Tinospora malabarica*. Its structure was established on the basis of spectral studies.

INTRODUCTION

The plant family Menispermaceae has long served as a rich source of alkaloids. *Tinospora malabarica* (Miers) is cultivated throughout Pakistan and the aqueous extract of the plant is used in the indigenous system of medicine for the treatment of intermittent fever [1, 2], liver and eye ailments and it is also reputed as a tissue builder and emetic [3]. A number of chemical constituents have been reported from this plant [4–11]. In the present communication we report the isolation and structure determination of a new furanoid diterpenoid, tinosporicide (**1**), from the fresh stems of *T. malabarica*.

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RESULTS AND DISCUSSION

Tinosporicide (**1**) was isolated by column and thin-layer chromatography on silica gel (Experimental) as a crystalline compound, mp 260° (uncorr.) $[\alpha]_D^{23}$ (MeOH; *c* 1.02) +8.3°. Structure **1** has been proposed for tinosporicide on the basis of spectroscopic studies (UV, IR, MS, ^1H NMR, COSY-45, 2D *J*-resolved, NOE, broad-band ^{13}C NMR and DEPT experiments). Compound **1** is a diterpenoid of the clerodane series containing methyls at C-9 and C-5. It showed a UV spectrum characteristic for furanoid diterpenoids with λ_{max}^{MeOH} 212 nm [9–13]. The IR spectrum (KBr) displayed strong absorptions at 3470, 3450 (OH), 1758, 1717 (C=O) cm^{-1}, 1510, 880 (furan ring) cm^{-1}. The presence of a furan ring was also indicated by a positive Ehrlich colour test [14].