Molecules **2003**, *8*, 670-677

molecules

ISSN 1420-3049
http://www.mdpi.org/

Sesquiterpenes from *Cymbopogon proximus*

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Received: 20 March 2003; in revised form: 23 July 2003 / Accepted: 26 July 2003 / Published: 15 August 2003

Abstract: In addition to four previously reported compounds: proximadiol (**1**), 5α-hydroxy-β-eudesmol (**2**), 1β-hydroxy-β-eudesmol (**4**) and 1β-hydroxy-α-eudesmol (**5**), two new sesquiterpenes, 5α-hydroperoxy-β-eudesmol (**3**) and 7α,11-dihydroxy-cadin-10(14)-ene (**6**) were isolated from the unsaponifiable fraction of the petroleum ether extract of *Cymbopogon proximus* STAPF. Isolation of compounds **2, 4** and **5** from the genus *Cymbopogon* is reported for the first time**.** The structure elucidation of these compounds was based primarily on 1D and 2D-NMR analyses.

Keywords: *Cymbopogon proximus*; Gramineae; proximadiol; sesquiterpenes; NMR; MS.

Introduction

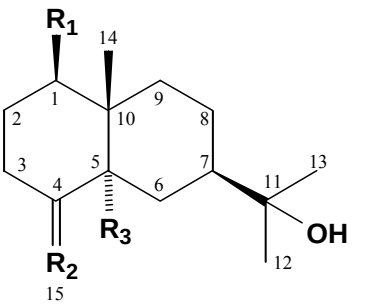
Cymbopogon proximus STAPF. (Gramineae) is a weed known as Halfabar that grows in the Egyptian desert. It is highly reputed in Egyptian folk medicine as an effective renal antispasmodic and diuretic agent [1,2]. Preliminary work on *Cymbopogon proximus* indicated that the unsaponifiable fraction of the petroleum ether extract of this plant possesses potent and unique antispasmodic properties, as it produces relaxation of the smooth muscle fibers without abolishing the propulsive movement of the tissue [3-6]. Bioactivity-assisted fractionation of the hexane extract lead to isolation of an active principle, proximadiol (0.02% yield) [5,6] and its bicyclic sesquiterpene diol chemical structure was confirmed by the spectral data [7-9]. In addition, two sesquiterpenes, elemol and

β-eudesmol, were also isolated from the unsaponifiable fraction of the fatty matter of the plant [10]. More thorough investigation of the unsaponifiable fraction revealed the presence of other sesquiterpenes besides the isolated ones, thus it was found desirable to pursue further study of the components of this plant.

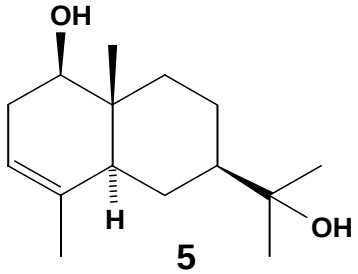
Results and Discussion

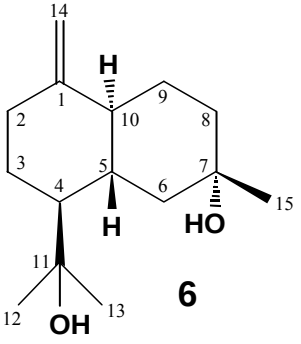
The unsaponifiable fraction of the petroleum ether extract of *Cymbopogon proximus* afforded six sesquiterpenes. In addition to the previously isolated proximadiol (**1**), two (**3** and **6**) were new and three (**2, 4,** and **5**) are newly reported in the genus (Scheme 1). The identification of these compounds was accomplished by examination of their spectral data (^{1}H-, ^{13}C-NMR, COSY, HMQC, HMBC and EIMS) and supported by comparison with published data of related compounds [11- 23].

Scheme 1



	R_1	R_2	R_3
1	H	α-OH, CH_3	H
2	H	CH_2	OH
3	H	CH_2	OOH
4	OH	CH_2	H



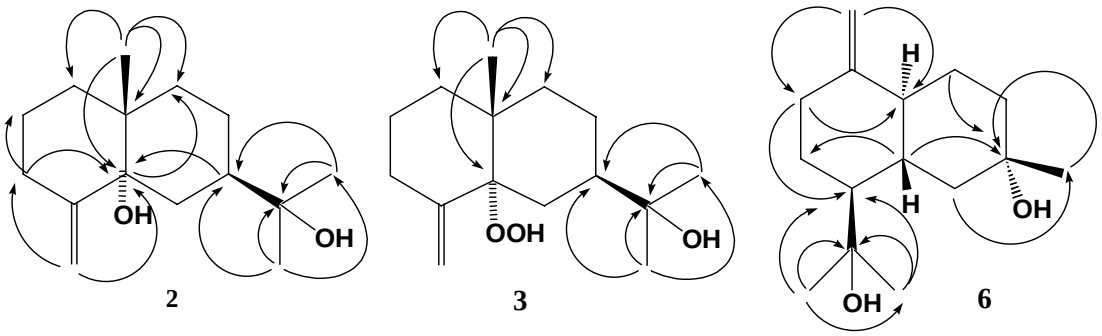


Compound **1** ($C_{15}H_{28}O_2$, EIMS *m/z* 240 $[M]^+$), was identified as proximadiol (**1**), by comparing its spectral data with those reported for the compound isolated from the same plant [7,8] and from the leaves of *Cryptomeria japonica* [9].

Compound **2** ($C_{15}H_{26}O_2$, EIMS *m/z* 238 $[M]^+$), was identified as 5α-hydroxy-β-eudesmol by comparing its spectral data (see Experimental and Table 1) with those of closely related compounds [11-13]. 5α-Hydroxy-β-eudesmol was previously isolated from the aerial parts of *Jasonia montana* [13]. A complete assignment of the ^{13}C-NMR data of **2** was accomplished using 2D NMR spectra (HMQC and HMBC, Figure 1) and is reported here for the first time (Table 1).

Compound **3** showed a molecular ion peak at *m/z* 254 $[M]^+$ in the EIMS, 16 mass units higher than that of **2** and corresponding to the molecular formula $C_{15}H_{26}O_3$. This finding suggested that **3** has an extra oxygen atom in its structure. The ^{1}H- and ^{13}C-NMR spectral data of **3** were found to be closely similar to those of **2**. The ^{13}C-NMR spectra of **3** showed signals for three methyl carbons (δ_c 28.0, 26.3 and 21.2), ascribed to C-12, C-13, and C-14, seven methylene carbons, a methine carbon (δ_c 43.1) and four quaternary carbons, two of which are oxygenated [δ_c 72.9 (C-11) and 87.2 (C-5)]. When compared with those in **2**, the downfield shifts of C-5 (+11.7 ppm) and C-15 (+ 4 ppm), and the upfield shifts of C-6 (-6.3 ppm) and C-4 (-3.3 ppm), suggested the placement of a hydroperoxy functional group at C-5. This assignment was further confirmed by the presence of HMBC correlation between the proton signals at δ_H 0.91 (CH_3-14) and C-1, C-5, C-9 and C-10 (Figure 1). Long-range correlations between the proton signals at δ_H 1.22 (H_3-12) and 1.25 (H_3-13) and C-7 and C-11 was also observed. The relative stereochemistry of the hydroperoxide group at C-5, was determined as α- by comparing the ^{1}H- and ^{13}C-NMR data of **3** with those reported for the closely related isomers 10-epi-5α-hydroperoxy-β-eudesmol and 10-epi-5β-hydroperoxy-β-eudesmol [14] (see Experimental and Table 1). From these data the structure of **3** was assumed to be 5α-hydroperoxy-β-eudesmol.

Figure 1. Significant HMBC correlations observed in compounds **2**, **3** and **6**



Compound **4** was assigned the molecular formula $C_{15}H_{26}O_2$ on the basis of EIMS data (*m/z* 238 $[M]^+$). The ^{1}H- and ^{13}C-NMR spectral data of **4** were very similar to those of selin-4(15)-en-1β,11-diol (i.e. 1β-hydroxy-β-eudesmol), previously isolated from the root wood of *Pterocarpus marsupium* [15]. This structure was confirmed by 2D NMR spectra (HMQC and HMBC).

Compound **5** had a molecular formula of $C_{15}H_{26}O_2$ (EIMS, *m/z* 238 $[M]^+$). By comparison of its ^{1}H- and ^{13}C-NMR spectra data with those previously reported, the structure of **5** was identified as 3-eudesmene-1β,11-diol (i.e. 1β-hydroxy-α-eudesmol) previously isolated from the leaves of *Cryptomeria japonica* [9].

The ^{13}C-NMR spectrum of **6** ($C_{15}H_{26}O_2$, EIMS, *m/z* 238 $[M]^+$) (Table 1) showed signals characteristic of three methyl carbons, six methylene carbons, three methine carbons and three quaternary carbons, two of which are oxygenated [δ_c 74.2 (C-11) and 81.1 (C-7)] and the third one was assigned to C-1. This observation suggested the absence of an angular methyl group at C-10 (a signal for quaternary carbon was not seen around δ_c 37 ppm). The ^{1}H-NMR spectrum of **6** showed signals at

δ_H 1.18, 1.19 and 1.21 ppm (3H each), ascribable to three methyl groups attached to quaternary carbons bearing OH groups. The HMBC spectrum showed long-range correlations between the exomethylene protons signal at δ_H 4.69 (H_2-14) and the carbon signals at δ_c 37.8 (C-2) and 48.3 (C-10) (Figure 1). Similarly, the proton signal at δ_H 2.55m (H-2a) showed long-range correlations with C-14 (δ_c 106.5), C-4 (δ_c 47.4) and C-10 (δ_c 48.3). Significant correlations between C-4 and the methyl protons at δ_H 1.18 (H_3-12) and 1.19 (H_3-13), and between C-7 and the proton at δ_H 1.76 (H-5) were observed. A β- orientation was suggested for the isopropanol group at C-4, based on the downfield shift of C-4 (δ_c 47.4), compared to that of the *epi*-isomers (δ_c 41- 44) [19, 20]. In addition, comparison of the chemical shifts of the carbons of the isopropanol group with those of the other isolated compounds (**1** and **5**) further supported this suggestion**.** The relative stereochemistry of the methyl and hydroxyl groups at C-7, was inferred as β- and α-, respectively, based on the resonance of C-15 methyl group at δ_c 23.8, compared to that at δ_c 28 for α-methyl and β-hydroxyl groups [21, 22]. The stereo-orientation of H-5 was suggested as β-, based on the fact that the vicinal isopropanol group, which is β-oriented, must assume an equatorial position to avoid steric interaction with the axial H-10, in the *trans*-decalin skeleton. [19-23]. Therefore, compound **6** was identified as 7α,11-dihydroxy-cadin-10(14)-ene.

Table 1. ^{13}C-NMR spectral data of **2, 3, 4, 6**[a] and related compounds.

Position	**2**	**3**	10-epi-5β-hydro-peroxy-β-eudesmol [14]	10-epi-5α-hydro-peroxy-β-eudesmol [14]	**4**	**6**
1	34.9 *t*	34.4 *t*	33.5 *t*	35.8 *t*	79.3 *d*	153.8 *s*
2	21.9 *t*	21.5 *t*	20.1 *t*	23.5 *t*	31.4 *t*	37.8 *t*
3	31.7 *t*	32.0 *t*	32.4 *t*	34.2 *t*	34.2 *t*	25.6 *t*
4	152.1 *s*	148.8 *s*	148.7 *s*	148.8 *s*	148.9 *s*	47.4 *d*
5	75.5 *s*	87.2 *s*	87.0 *s*	89.5 *s*	47.5 *d*	52.5 *d*
6	31.0 *t*	24.7 *t*	23.4 *t*	28.0 *t*	24.4 *t*	40.6 *t*
7	43.5 *d*	43.2 *d*	40.7 *d*	43.7 *d*	48.9 *d*	81.1 *s*
8	22.3 *t*	22.5 *t*	22.7 *t*	22.4 *t*	22.1 *t*	27.2 *t*
9	34.2 *t*	34.3 *t*	35.2 *t*	35.4 *t*	36.9 *t*	27.5 *t*
10	37.8 *s*	38.7 *s*	38.1 *s*	38.9 *s*	40.1 *s*	48.3 *d*
11	72.8 *s*	72.9 *s*	74.1 *s*	72.9 *s*	72.8 *s*	74.2 *s*
12	26.8 *q*	26.3 *q*	28.7 *q*	26.5 *q*	27.0 *q*	26.9 *q*
13	27.8 *q*	28.0 *q*	29.9 *q*	27.6 *q*	27.2 *q*	27.1 *q*
14	19.9 *q*	21.2 *q*	22.8 *q*	21.3 *q*	10.2 *q*	106.5 *t*
15	107.6 *t*	111.6 *t*	111.1 *t*	108.0 *t*	106.8 *t*	23.8 *q*

[a] In $CDCl_3$ at 100 MHz.

Acknowledgments

The authors are grateful to Dr. Masao Hattori (Department of Cell-Resources Engineering, Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University, Toyama, Japan), and to the National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, Mississippi, USA), for the NMR and MS measurements.

Experimental

General

Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO FT/IR-230 IR spectrophotometer in KBr disks. 1D and 2D NMR spectra were obtained on Bruker Avance DRX 400 spectrophotometers (^{1}H-NMR at 400 MHz and ^{13}C NMR at 100 MHz) in $CDCl_3$ with TMS as an internal standard, and chemical shifts are reported in δ values. EIMS were recorded on a Shimadzu PQ-5000 instrument at 70 eV. Silica gel 60 (70-230 mesh, E. Merck), and neutral alumina (Sigma) were used for column chromatography and silica gel 60 H was employed for VLC. TLC was conducted on precoated Merck silica gel 60 F_{254} plates (0.25mm thickness), developed with either 4:1 toluene- acetone (solvent system A) or 1:1 toluene-ethyl acetate (solvent system B). Visualization was accomplished by spraying with *p*-anisaldehyde reagent followed by heating at 110ºC [24].

Plant material

The material was purchased at Harraz Herbal Drugstore in Cairo, Egypt in 2001 and was kindly identified by Dr. M. Gebali (Plant Taxonomy and Egyptian Flora Department, National Research Center, Giza, Egypt). A voucher specimen was deposited in the Herbarium, Faculty of Pharmacy, Cairo University.

Extraction and Isolation

The air-dried powdered *Cymbopogon proximus* herb (1.0 Kg) was successively extracted with petroleum ether (60-80ºC) and ethyl acetate in a Soxhlet apparatus, until complete extraction was effected. On removal of solvent, the petroleum ether extract left an oily residue (31.2 g). A part of this extract (30 g) was saponified using 10 % alcoholic potassium hydroxide [5] to give an unsaponifiable fraction (18.2 g). The unsaponifiable fraction (4.2 g) was dissolved in *n*-hexane (100mL) and VLC chromatographed over silica gel H (Merck, 45 g, 3.5 x 5 cm). Elution was carried out using n-hexane containing increasing amounts of chloroform and collecting 100 mL fractions. Similar fractions were pooled together based on TLC analysis using solvent system A. Fractions 4-5 (3.4 g, eluted with 5%

$CHCl_3$ in *n*-hexane) was rechromatographed on a column of alumina (140g, 4 x 26cm), eluted with *n*-hexane containing increasing amounts of EtOAc (up to 30%) and collecting 20 mL fractions. The eluted fractions were examined by TLC using solvent system B and similar fractions were pooled to give two main fractions, designated I and II, respectively.

Fraction I [from fractions 24-63 (1.2 g, 25% EtOAc / *n*-hexane)] showed a mixture of three main spots (R_f 0.21, 0.3, and 0.38, solvent system B). Fraction I was further chromatographed on a column of alumina eluting with 1-25 % EtOAc/hexane and collecting 250 fractions of 15 mL each. The eluted fractions were examined by TLC, using solvent system B. Three main sub-fractions, I-1 (fractions 97-98), I-2 (fractions 100-150) and I-3 (fractions 201-250) were obtained. Subfraction I-1 (54mg, 25% EtOAc/ *n*-hexane) on recrystallization afforded **1** (35mg). Subfraction I-2 (180mg, 25% EtOAc/hexane) was purified by VLC over silica gel 60H (2.5 x 18 cm, EtOAc/hexane, 75:100) to give compound **2** (14mg). Similarly, subfraction I-3 (180mg, 25% EtOAc/hexane) was purified by VLC eluted with EtOAc/toluene (80:140) to give **3** (6mg), **4** (34mg) **5** (18mg) and **6** (25mg) from fractions 11-14, 23-25, 26-28 and 36-41, respectively.

Fraction II [from fractions 68-96 (0.38 g, 25% EtOAc / *n*-hexane)] was re-chromatographed on a column of alumina to yield **1** (180mg) as a major compound.

5α-Hydroxy-β-eudesmol (**2**):$C_{15}H_{26}O_2$; oily; EIMS, *m/z* 238 $[M]^+$, 220(M^+-H_2O), 205, 202, 187; IR λ_{max}: (3450, 1155, 1645, 895 cm^{-1}; ^{1}H-NMR: δ_H 4.82 (1H, br s, H-15a) 4.71 (1H, br s, H-15b), 2.61 (1H, *dd, J*=13.1,6.5 Hz, H-3a), 2.12 (1H, *dd, J*=13.1, 2.0 Hz, H-3b), 1.9 (1H, *m,* H-1a), 1.74 (1H, *dd, J*=13.3, 4.4 Hz, H-1b), 1.68 (1H, *m*, H-2a), 1.60 (1H, *m*, H-2b), 1.65 (2H, *m*, H-6), 1.63 (1H, *m*, H-8a), 1.62 (2H, *m*, H_2-9), 1.58 (1H, *m*, H-8b), 1.38 (1H, *dddd, J*=13.8,13.3, 4.4, 4.1 Hz, H-7), 1.23 (3H, s, Me-13), 1.21(3H, s, Me-12), 0.86 (3H, s, Me-14); ^{13}C-NMR, see Table 1.

5α-Hydroperoxy-β-eudesmol (**3**): $C_{15}H_{26}O_3$; oily; EIMS, *m/z* 254 $[M]^+$, 236 (M^+- H_2O), 221 (M^+-OOH), 203 (M^+-OOH -H_2O), 187 (M^+-2H_2O -CH_3), 162, 59 (hydroxyl isopropyl group); IR λ_{max}: 3610, 3400, 1155, 3365, 1640, 900 cm^{-1}; ^{1}H-NMR: δ_H 5.06 (1H, br s, H-15a) 4.79 (1H, br s, H-15b), 2.5 (1H, *m*, H-3a), 2.2 (1H, *m,* H-3b), 1.84 (1H, *m,* H-1a), 1.72 (1H, *m,* H-1b), 1.70 -1.55 (8H, *m*, H_2-2, H_2-6, H_2-8, H_2-9), 1.38 (1H, *m*, H-7), 1.25 (3H, s, Me-13), 1.22 (3H, s, Me-12), 0.91 (3H, s, Me-14); ^{13}C-NMR, see Table1.

1β-Hydroxy-β-eudesmol (**4**): $C_{15}H_{26}O_2$**;** m.p.156-8ºC; EIMS, *m/z* 238 $[M]^+$, 220 (M^+-H_2O), 207 ($C_{14}H_{23}O$, base peak); ^{1}H-NMR: δ_H 4.77 (1H, br s, H-15a) 4.52 (1H, br s, H-15b), 3.41(1H, *dd, J*=11.7, 4.6 Hz, H-1), 2.32 (1H, *dd, J*=13.6, 3.0 Hz, H-3a), 2.11 (1H, *dd, J*=13.8, 4.6 Hz, H-3b), 1.99 (1H, *m*, H-9a), 1.96 (1H, *m*, H-9b), 1.80 (1H, *m*, H-2a), 1.55 (1H, *m*, H-2b), 1.73 (1H, *m*, H-6a), 1.68 (1H, *m*, H-6b), 1.71 (1H, *m*, H-8a), 1.62 (1H, *m*, H-8b), 1.20 (1H, *m,* H-7), 1.16 (1H, *m,* H-5), 1.21 (6H, s, Me-13, Me-12), 0.68 (3H, s, Me-14); ^{13}C-NMR, see Table1.

7α, 11-Dihydroxy-cadin-10(14)-ene (**6**): $C_{15}H_{26}O_2$, oily; EIMS, *m/z* 238 $[M]^+$, 220 (M^+-H_2O), 205 (M^+-H_2O-CH_3), 202 (M^+- $2H_2O$), 187 (M^+-$2H_2O$ -CH_3); IR λ_{max}: 3450, 1155, 1645, 895 cm^{-1}; ^{1}H-NMR: δ_H 4.69 (2H, *s*, H_2-14), 2.55 (1H, *m*, H-2a), 2.06 (1H, *m,* H-2b), 2.18 (1H, *m*, H-10), 1.88 (1H, *m*, H-3a), 1.68 (1H, *m*, H-3b), 1.78 (1H, *m*, H-6a), 1.68 (1H, *m*, H-6b), 1.76 (1H, *m,* H-5), 1.75 (1H, *m,* H-4), 1.73 (1H, *m*, H-8a), 1.67 (1H, *m*, H-8b), 1.68 (1H, *m*, H-9a), 1.55 (1H, *m*, H-9b), 1.21 (3H, s, Me-15), 1.19 (3H, s, Me-13), 1.18 (3H, s, Me-12); ^{13}C-NMR, see Table 1.

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Sample Availability: Available from the authors