Pergamon

PII: S0031-9422(96)00779-0

Phytochemistry, Vol. 44, No. 8, pp. 1519–1522, 1997

0031-9422/97 $17.00+0.00

BISABOLANE SESQUITERPENOIDS FROM *SANTALUM AUSTROCALEDONICUM*

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(*Received in revised form* 30 *September* 1996)

Key Word Index—*Santalum austrocaledonicum*; Santalaceae; bisabolane-type sesquiterpenoids; lanceol; 6,13-dihydroxybisabola-2,10-diene; 7,13-dihydroxybisabola-2,10-diene; one- and two-dimensional NMR.

Abstract—Two new sesquiterpenoids, 6,13-dihydroxybisabola-2,10-diene and 7,13-dihydroxybisabola-2,10-diene, were isolated, together with (*E*)-anceol, from the heartwood of *Santalum austrocaledonicum* var. *austrocaledonicum*. The compounds were characterized by one- and two-dimensional NMR. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The heartwood of *Santalum austrocaledonicum* Viell. var. *austrocaledonicum* is grown in New Caledonia, especially on the Loyauté Islands [1], and has been exploited for a long time as the raw material of a precious essential oil.

In a previous paper [2], we reported on the isolation and structure determination of santalane derivatives from S. *insulare* var. *marchionense*. We now describe the isolation of two new bisabolane sesquiterpene alcohols from *S. austrocaledonicum* harvested on Maré Island. Their structures were established as 6,13-dihydroxybisabola-2,10-diene (**1**) and 7,13-dihydroxybisabola-2,10-diene (**2**) by means of one- (1D) and two-dimensional (2D) NMR. We report here also the occurence of (*E*)-lanceol (**3**) which was obtained along with the two new sesquiterpenoids.

RESULTS AND DISCUSSION

The high boiling fraction obtained by distillation of the dichloromethane extract of *S. austrocaledonicum* var. *austrocaledonicum* heartwood was chromatographed over a silica-gel column. The separation gave (*E*)-lanceol (**3**) and two new dihydroxylated sesquiterpenes: 6,13-dihydroxybisabola-2,10-diene (**1**) and 7,13-dihydroxybisabola-2,10-diene (**2**).

Compound **1** showed strong IR absorptions for hydroxyl groups (3374 cm^{-1}) and double bonds (1636 and 878 cm^{-1}). Its ^{13}C NMR spectrum contained 15 carbon atom signals with four at low field (δ 118.4, 138.4, 133.9 and 134.6) which were assigned to olefinic centres indicating the presence of two carbon–carbon double bonds. The signals appearing at δ 61.3 and 72.3 were characteristic of two oxygen-bearing carbon atoms of alcohol functions. The distortionless enhancement by polarization transfer (DEPT) [3] pulse sequence spectrum revealed the presence of three methyl groups, six methylene groups, three methine groups and three quaternary carbon atoms in the carbon skeleton of compound **1**.

The electron impact (EI)-MS spectrum of **1** showed no $[M]^+$, but a fragmention at *m/z* 220, probably resulting from the loss of one molecule of water, was observed. The mass spectral data were consistent with a molecular formula of $C_{15}H_{26}O_2$. All of these preliminary observations indicated that compound **1** was a monocyclic sesquiterpenoid.

In the ^1H NMR spectrum of compound **1**, three signals attributable to methyl groups were observed as a doublet at δ 0.88 ($^3J = 6.8$ Hz) and two singlets were observed at δ 1.62 and 1.72. The last two resonances were assigned to olefinic methyl groups. The proton spectrum also revealed two other ethylenic signals as a triplet at δ 5.24 ($^3J = 6.7$ Hz) and a singlet at δ 5.22. Two isochrone protons resonating as a singlet at δ 4.04 were assigned to a vinylic hydroxymethylene group.

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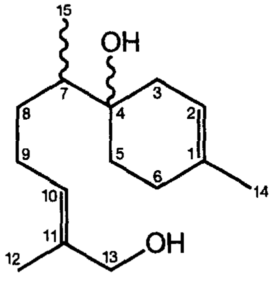

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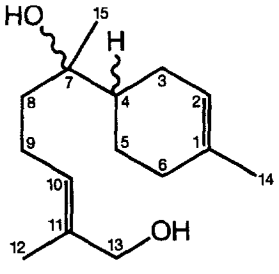

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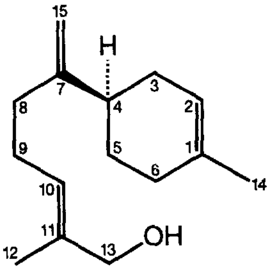

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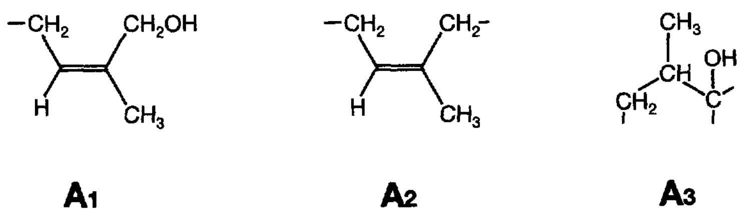

Fig. 1. Partial structures of compound 1.

Identification of the framework of compound **1** was achieved by the concerted use of 2D heteronuclear (^1H–^{13}C,) chemical correlations [4, 5]. These experiments established the connections through bonds by observation of both direct (heteronuclear multiple quantum coherence (HMQC)) [6] and long-range (heteronuclear multiple bond connectivity (HMBC)) [7] correlation diagrams. The starting points of these connectivity networks were the two double bonds, the carbon and proton couplings (^2J or ^3J) of which were used to assign all atoms in their vicinity and thus to obtain structural fragments A_1 and A_2. The connectivities observed between the methyl proton shifts (0.88 ppm) and carbon atoms α and β to this group in the HMBC diagram led to a third partial structure A_3 (Fig. 1).

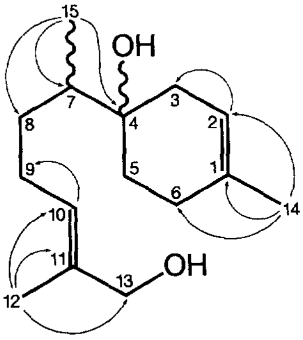

Fig. 2. Selected long-range proton–carbon (→) coupling pathways of compound **1** determined by HMBC and HMQC experiments.

These three partial fragments were expanded and then assembled on the basis of further examinations of intercoupling between each assigned carbon atom and proton detected in the HMBC and HMQC diagrams. The determining heteronuclear correlations observed for compound **1** are shown in Fig. 2.

The above information allowed, complete assignment of the proton and carbon NMR parameters for compound **1** (Table 1) and established its structure as 2-hepten-1-ol,2-methyl-6(4′-methyl-3′-cyclohexen-1-ol), called 6,13-dihydroxybisabol-2,10-diene. The ^{13}C NMR data are consistent with those of β-bisabolol [8], after taking into account the presence of a second hydroxyl group in **1**.

The IR spectrum of **2** displayed a large hydroxyl band (3382 cm^{-1}) and a characteristic double-bond band (1633 and 944 cm^{-1}). No molecular ion peak $[M]^+$ was detected in the EI-MS spectrum, which showed significant fragments at *m/z* 202 and 220 corresponding to $[M - 2H_2O]^+$ and $[M - H_2O]^+$, respectively, and suggesting the molecular formula $C_{15}H_{26}O_2$. The ^{13}C NMR spectrum of **2** confirmed the presence of 15 carbon atoms and exhibited four deshielded signals (δ 133.9, 134.5, 128.8 and 120.7) attributable to the sp^2 hybridized carbon atoms of two (C=C) double bonds. Two oxygen-bearing carbon atoms were shown by characteristic ^{13}C NMR chemical shifts at δ 74.6 and 61.51, respectively, which correspond to two hydroxylated carbon atoms. The DEPT experiment indicated the occurrence of three methyl groups, six methylene groups, three methine groups and three quaternary carbon atoms. Thus, **2** had to be a monocyclic sesquiterpenoid. In the ^1H NMR spectrum of **2**, the signals observed as singlets at δ 1.76, 1.62 and 1.10, respectively, were assigned as three methyl proton groups the carbon atoms of which were linked to quaternary carbon atoms, two of them (resonating at δ 1.76 and 1.62) were olefinic methyl

Table 1. ^1H and ^{13}C NMR chemical shifts for compounds **1** and **2** (HMBC and HMQC experiments; TMS)

	1			**2**		
C/H	δ_C	Group*	δ_H†‡	δ_C	Group*	δ_H†‡
1	133.91	C	–	133.92	C	–
2	118.41	CH	5.22 *br s*	120.70	CH	5.36
3	35.00	CH_2	1.85, 2.12	26.13	CH_2	2.1, 2.0
4	72.25	C	–	43.67	CH	1.53
5	30.38	CH_2	1.55, 1.65	24.07	CH_2	1.3, 1.2
6	27.01	CH_2	1.85	31.09	CH_2	2.0, 1.95
7	41.60	CH	1.41	74.58	C	–
8	31.24	CH_2	1.03	39.41	CH_2	1.49 *t* (8.2)
9	26.09	CH_2	1.95	21.94	CH_2	2.2, 2.1
10	128.36	CH	5.24 *t* (6.7)	128.75	CH	5.29 *t* (7.4)
11	134.57	C	–	134.48	C	–
12	21.32	CH_3	1.62 *s*	21.57	CH_3	1.76 *br s*
13	61.32	CH_2	4.04 *s*	61.51	CH_2	4.12 *d* (10), 4.08 *d* (10)
14	23.28	CH_3	1.72 *s*	23.55	CH_3	1.62 *br s*
15	13.74	CH_3	0.88 *d* (6.8)	23.86	CH_3	1.10 *s*

*Determined from DEPT spectra.

†Determined from the cross-sections of the HMQC diagram.

‡*J* (Hz) in parentheses.

groups. Two olefinic protons were observed in the ^1H NMR spectrum as a triplet at δ 5.29 ($^3J = 7.4$ Hz) and a multiplet at δ 5.36. As for **1**, the complete ^1H and ^{13}C chemical shift assignments of **2** (Table 1) and the establishment of its structure were achieved on the basis of various connectivities derived from 2D dimensional experiments HMQC and HMBC with inverse detection). Thus, **2** was determined as 2-heptene-1,6-diol-2-methyl-6-(4′-methyl-3′-cyclohexen-1-yl), and named 7,13-dihydroxybisabol-2,10-diene. Its ^{13}C and ^1H NMR parameters showed similar values to those of α-bisabolol [9], except for the chemical-shift difference due to replacement of a vinyl methyl by an hydroxymethylene group.

Close examination of the ^{13}C NMR data (including DEPT) of **3** allowed us to identify this constituent as (*E*)-lanceol, which also has a bisabolane skeleton. Good agreement was observed between the ^{13}C assignments for **3** and other related structure assignments such as these for β-bisabolene and β-bisabolenol [10, 11]. (*E*)-Lanceol has previously been found as a constituent of other *Santalum* species such as *S. lanceolatum* and *S. spicatum* [12, 13], but no dihydroxy derivatives have been reported in these species.

EXPERIMENTAL

General. Dried heartwood of *S. austrocaledonicum* var. *austrocaledonicum* (447 g) was finely rasped and then extracted with CH_2Cl_2 for 8 hr in a Soxhlet apparatus, yielding 54 g of extract. The crude extract was submitted to partial distillation under reduced pressure (vaccum 2 mmHg), providing 3.5 g of heavy fr. This fr. was chromatographed over a silica gel column using hexane to remove hydrocarbons, followed by OEtAc to give the polar fraction. This mainly comprised sesquiterpenoids, and was separated by CC over silica gel using a shallow gradient of $CHCl_3$–iPrOH from 99 : 1 to 98 : 2. Compound **3** (75 mg) was eluted first, followed by **1** (140 mg) and then **2** (40 mg). Repeated elutions were necessary to purify every compound. All 1D and 2D NMR spectra were recorded in $CDCl_3$ solns (^1H at 400.13 MHz; ^{13}C at 100.61 MHz; TMS as int. standard). Standard Bruker pulse sequences were used for 2D experiments. For other experimental details see references cited in [14].

6,13-*Dihydroxybisabola*-2,10-*diene* (**1**). $C_{15}H_{26}O_2$, oil, $[\alpha]_D^{25} = -84.6°$ ($CHCl_3$; *c* 1.2). IR ν_{max} cm^{-1}: 3374, 2919, 1636, 1436, 1377, 1067, 1011, 878. EI-MS 70 eV, *m/z* (rel. int.): 238 $[M]^+$ (0), 220 (3), 205 (2), 202 (1), 187 (2), 151 (8), 137 (21), 121 (14), 109 (36), 95 (33), 93 (47), 81 (34), 68 (49), 55 (47), 43 (100).

7,13-*Dihydroxybisabola*-2,10-*diene* (**2**). $C_{15}H_{26}O_2$, oil, $[\alpha]_D^{25} = -43.3°$ ($CHCl_3$; *c* 2.2). IR ν_{max} cm^{-1}: 3382, 2925, 1633, 1449, 1376, 1104, 1005, 944, 756. EI-MS 70 eV, *m/z* (rel. int.): 238 $[M]^+$ (0), 220 (1), 202 (1), 187 (1), 161 (1), 159 (1), 145 (3), 132 (9), 109 (7), 107 (13), 95 (17), 93 (14), 81 (11), 67 (15), 55 (16), 43 (100).

(E)-*Lanceol* (**3**). $C_{15}H_{24}O$, oil, $[\alpha]_D^{25} = -67.4°$ ($CHCl_3$; *c* 6). IR ν_{max} cm^{-1}: 3362, 2920, 1641, 1440, 1378, 1007, 889, 799. EI-MS 70 eV, *m/z* (rel. int.): 220 $[M]^+$ (0), 202 (4), 187 (6), 159 (18), 134 (20), 119 (25), 105 (23), 93 (63), 79 (50), 67 (46), 55 (33), 43 (100). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 133.8 (*s*) C-1, 120.7 (*d*) C-2, 31.4 (*t*) C-3, 39.8 (*d*) C-4, 28.3 (*t*) C-5, 30.8. (*t*) C-6, 153.9 (*s*) C-7, 35.0 (*t*) C-8, 26.4 (*t*) C-9, 128.1 (*d*) C-10, 134.6 (*s*) C-11, 21.3 (*q*) C-12, 61.5 (*t*) C-13, 23.5 (*q*) C-14, 107.5 (*t*) C-15.

Acknowledgements—The authors would like to thank Mr M. Point for providing plant material, and Dr P. Cosson for botanical information.

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