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A New Sesquiterpene from *Nicotiana umbratica* Burbidge

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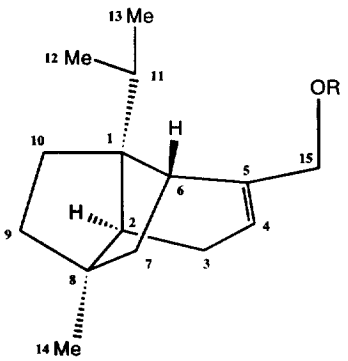
Abstract: A new sesquiterpene, (1*S*,2*S*,6*R*,8*R*)-1-isopropyl-5-(hydroxymethyl)-8-methyltricyclo[4.4.0.$0^{2,8}$]dec-4-ene (**1**), was isolated and identified from the leaf surface lipids of *Nicotiana umbratica* Burbidge. The structure of compound **1**, including the absolute stereochemistry, was established on the basis of its spectral data and X-ray diffraction analysis of 15-*p*-bromobenzoate (**1a**). © 1997 Elsevier Science Ltd.

INTRODUCTION

We have investigated the leaf surface lipids in various *Nicotiana* species as part of our reseach on tobacco aroma precursors and aromatic. The constituents in the leaf surface lipids are recognized to be important as precursors of tobacco aroma, especially in the case of oriental tobacco.[1-3] *N. umbratica* Burbidge (Solanaceae) is a herbaceous annual plant growing in the northwest of Western Australia (Pilbara district), which was known to have the characteristic aroma among these species. Previous study [4] relative to *N. umbratica* Burbidge showed that the leaf surface lipids contain several varieties of glycolipids, such as 6-*O*-acetyl-2,3,4-tri-*O*-acyl-α-D-glucopyranose and 2,3,4-tri-*O*-acyl-α-D-glucopyranosyl-3-*O*-acetyl-β-D-fructofuranoside. However, the above glycolipids don't contribute to the characteristic aroma. In our further studies on the aromatic constituents of *N. umbratica* Burbidge, we isolated a new sesquiterpene having an oily citrus-like aroma. In this paper, we wish to report the isolation and structural elucidation of (1*S*,2*S*,6*R*,8*R*)-1-isopropyl-5-(hydroxymethyl)-8-methyltricyclo [4.4.0.$0^{2,8}$]dec-4-ene (**1**).



1 R=H

1a R=*p*-Br-Benz

RESULTS AND DISCUSSION

The less polar compound **1** was isolated as a colorless oil from the leaf surface lipids ($CHCl_3$ extract). The compound **1** showed as reddish purple (single spot, *Rf*=0.37) on the silica gel TLC analysis with vanillin-sulfuric acid reagent. The high resolution (HR) FABMS spectrum showed the molecular ion peak at *m/z* 221.18878 $[M+H]^+$, corresponding to the molecular formular $C_{15}H_{24}O$, required four degrees of unsaturation. The IR spectrum indicated absorption bands at 3331 (OH) and 1671 (C=C) cm^{-1}. All the hydrogen and carbon atoms in the molecular were assigned by a combination of ^{1}H-NMR, ^{13}C-NMR, DEPT, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, HMBC, and NOESY experiments (in benzene-d_6). The ^{1}H-NMR spectrum (Table 1) displayed the presence of one isopropyl group (δ 0.80, 0.87, 2.03), one tertiary methyl (δ 1.02), one methylene conjugated to hydroxy group (δ 3.76, 3.79) and one olefinic proton (δ 5.25), respectively. These inferences were supported by the characteristic carbon resonances in the ^{13}C-NMR and DEPT spectrum (Table 1). Thus we supposed that the compound **1** was a tricyclic sesquiterpene including a primary alcohol.

Table 1. ^{1}H and ^{13}C-NMR Chemical Shifts and Assignments of Compound **1** (in benzene-d_6)*

^{13}C no.	δ (ppm)	^{1}H no.	δ (ppm)	Multiplicity (Hz)
C-1	52.7 (*s*)			
C-2	49.0 (*d*)	H-2	1.19	1H, *br.dd*, *J*=5.6, 1.3
C-3	24.5 (*t*)	H-3	1.95-2.08	2H, *m*
C-4	118.0 (*d*)	H-4	5.25	1H, *br.s*
C-5	147.2 (*s*)			
C-6	44.0 (*d*)	H-6	1.95	1H, *br.dd*, *J*=7.6, 1.3
C-7	48.2 (*t*)	H-7α	1.32	1H, *br.d*, *J*=11.7
		H-7β	1.56	1H, *ddd*, *J*=11.7, 7.6, 1.3
C-8	47.5 (*s*)			
C-9	40.0 (*t*)	H-9	1.20-1.25	2H, *m*
C-10	25.2 (*t*)	H-10α	1.62	1H, *ddd*, *J*=12.6, 7.1, 6.6
		H-10β	1.12	1H, *ddd*, *J*=12.6, 8.2, 7.6
C-11	26.8 (*d*)	H-11	2.03	1H, *hept.*, *J*=6.9
C-12	18.7 (*q*)	H-12	0.80	3H, *d*, *J*=6.9
C-13	18.9 (*q*)	H-13	0.87	3H, *d*, *J*=6.9
C-14	18.8 (*q*)	H-14	1.02	3H, *s*
C-15	65.8 (*t*)	H-15a	3.76	1H, *ABq*, *J*=13.4
		H-15b	3.79	1H, *ABq*, *J*=13.4

* One bond carbon-proton connectivities were ascertained from the ^{13}C-^{1}H COSY experiment, while the carbon multiplicities were determined by the DEPT experiment.

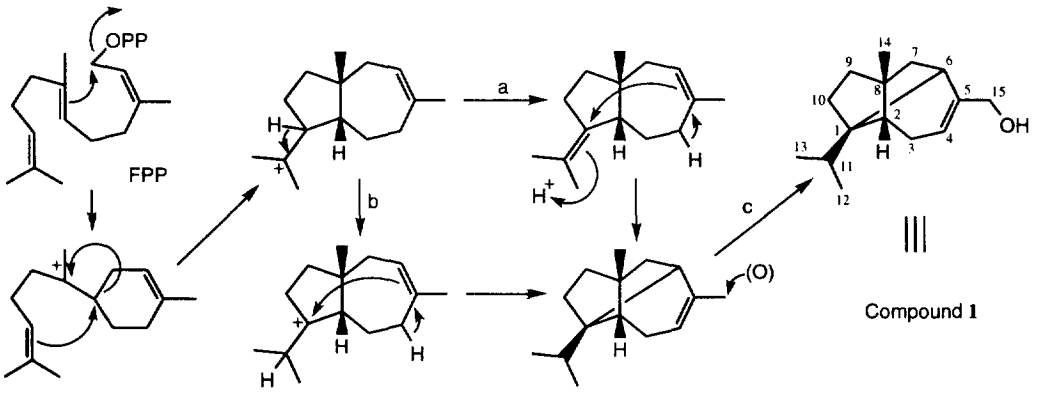
It was possible to trace all of the proton spin systems using the ^{1}H-^{1}H COSY experiment. Consequently, the ^{1}H-^{1}H COSY correlations defined four separate scalar coupled spin systems (H-2-H-3-H-4, H-6-H-7α-H-7β, H-9-H-10α-H-10β and CH_3-12-H-11-CH_3-13) and so on. In the HMBC [5] experiment (the delay time was optimized at 8.3 Hz), the correlations were observed from H-11, CH_3-12 and CH_3-13 to the quaternary C-1 resulted in the location of the isopropyl group adjacent to C-1. H-6 showed significant HMBC correlations to C-1, C-2 and C-10 while C-1 showed two-bond correlations from H-2, H-10α and H-10β, indicating that C-1 was contiguous to C-2, C-6 and C-10. H-2 exhibited a two-bond HMBC correlation to the quaternary C-8, while CH_3-14 exhibited the correlations to C-2, C-7, C-8 and C-9. From the stereochemical point of view, the relative configuration of compound **1** was investigated using the NOESY [6] experiment and the molecular model. In the

NOESY experiment (the mixing time of 1s was used), H-2 revealed the dipolar interaction with CH_3-13 and CH_3-14, while H-6 also revealed this with H-7β, H-10β and CH_3-12. The six membered ring was *cis*-fused to the other six membered ring (at C-1, C-6), which was corroborated on the basis of these NOESY relationship.

Absolute configuration

The absolute configuration was determined by X-ray crystallography by means of the comparison of the Bijvoet pair differences selected with anomalous difference between the observed and calculated structure factor amplitude. In the space group C2 the signs of the symmetry related indices for the Bijvoet pairs are divided into two groups, *i.e.* F(+) for (+++, -+-), and F(-) for (---, +-+). The structure factor amplitude was calculated to select reflections which have meaningful Bijvoet pair differences, and then five indices were assigned. The structure factor calculation was done by AUTOMR [7] with the anomalous dispersion terms of all atoms at the wave length of 1.54178Å. The crystallographically determined model structure was used for the calculation, and chiral centers of the model were taken as 1*S*, 2*S*, 6*R* and 8*R*. The diffraction intensities of each five indices were measured for four all symmetry related reflections again (Table 2). The observation conditions were similar to the data collection for the structure determination, except for the scan rate (4.0°/min. in ω) and scan width (1.01° + 0.200°tanθ) so as to measure more accurately. The mean structure factor amplitudes and their differences are presented in Table 3. In the all selected reflections, the signs of the observed Bijvoet differences coincide with those of the calculated ones of the (1*S*,2*S*,6*R*,8*R*) coordinate. Thus, it was concluded that the absolute structure is the (1*S*,2*S*,6*R*,8*R*) configuration. The crystal structure of the molecule is shown in a Ortep drawing (Fig. 1).

The compound **1** has an unique structure which is interesting from the biosynthetic point of view. Therefore we speculated about the possible biosynthetic pathway of this compound. The tricyclic sesquiterpene seems to arise from the cyclization of *cis*, *trans*-farnesyl pyrophosphate (FPP) via the five-seven membered ring system (carotane skeleton [8,9]), the deprotonation (a) or the prototropy (b) and the successive transannular cyclization. Finally, it appears that the formation of a primary alcohol occurs via the oxygenation (c) at the allylic methyl group (C-15). The formation of the compound **1** is shown in Scheme 1.



Scheme 1. Hypothetical Formation of Compound **1** from FPP.

Table 2. The Observed Amplitudes

hkl			+++	-+-	---	+-+
-3	-1	0	43.30(24)	44.82(24)	55.45(23)	55.17(23)
-1	-1	1	18.89(28)	18.10(29)	20.97(26)	22.85(25)
-1	1	-1	221.71(20)	227.80(34)	216.56(20)	207.70(20)
-3	1	1	125.49(21)	116.13(21)	114.31(21)	117.06(21)
-1	-1	2	120.34(21)	108.21(21)	113.43(21)	129.82(21)

Table 3. The Mean Amplitudes and Their Differences*

hkl			mean |Fo(+)|	mean |Fo(-)|	|Fc(+)|	|Fc(-)|	Δ|Fo|	Δ|Fc|
-3	-1	0	44.56	55.31	12.76	21.35	-10.75	-4.09
-1	-1	1	18.50	21.96	5.79	6.75	-3.46	-0.96
-1	1	-1	224.76	212.13	97.10	96.22	12.63	0.88
-3	1	1	120.81	115.69	47.35	46.53	5.12	0.82
-1	-1	2	114.28	121.63	56.78	57.50	-7.35	-0.72

* $|F(+)| = [|F(hkl)| + |F(-h-k-l)|] / 2$, $|F(-)| = [|F(-h-k-l)| + |F(h-kl)|] / 2$,
$\Delta|F| = |F(+)| - |F(-)|$

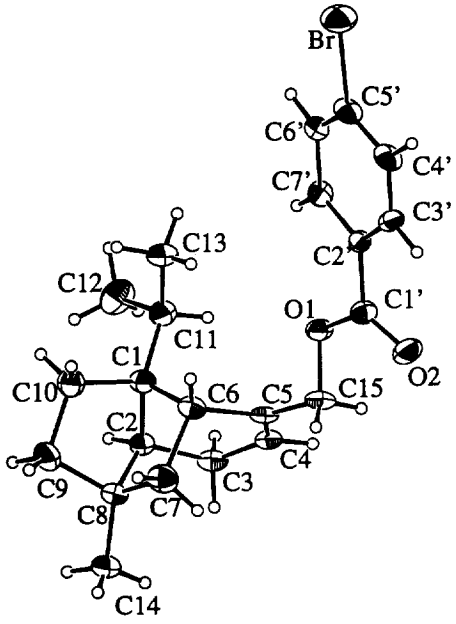


Fig. 1. Ortep drawing of 15-*p*-bromobenzoate (**1a**).

EXPERIMENTAL

General procedures. Melting points were uncorrected. IR spectra were recorded using a Nicolet system 800 FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter in $CHCl_3$. GCMS were measured using a Hewlett Packard 5971A. FABMS and HRFABMS were measured using a SHIMADZU KRATOS CONCEPT IIH mass spectrometer. ^1H, ^{13}C and 2D NMR were recorded using a Burker AM 500 spectrometer (^1H at 500.13 MHz, ^{13}C at 125.77 MHz) for compound **1** and a AC 300p spectrometer (^1H at 300.13 MHz, ^{13}C at 75.47 MHz) for compound **2**. For 2D NMR experiment, Bruker's standard program was used. Chemical shifts were expressed in δ (ppm) referring to solvent peaks : δ_H 7.15 and δ_C 128.0 for benzene-d_6. Thin layer chromatography (TLC) was carried out on silica gel $60F_{254}$ (Merck, thickness 0.2 mm) by using the solvent system hexane-ethyl acetate (4:1). The spots were developed by heating with vanillin-sulfuric acid reagent. Silica gel (Wakogel C300) was used for column chromatography. Preparative HPLC was performed using a silica gel column (YMC-pack A-014 SIL, Yamamura Chem. Co., 6 mm x 300 mm, flow rate : 1.5 ml/min., detector : reflective index). Preparative TLC was carried out on silica gel $60F_{254}$ (Merck, thickness 2 mm) by using the solvent system hexane-ethyl acetate (10:1).

Extraction and isolation of compound 1. The leaves of *Nicotiana umbratica* Burbidge were collected at our field of institute in May, 1991. The fresh leaves (2.4 kg) were treated with $CHCl_3$ for 10 sec and $CHCl_3$ layer was concentrated in vaccum with rotary evaporator. The concentrated extract (37.78 g) was applied to a column of silica gel (120 g), which was successively washed with $CHCl_3$, $CHCl_3$-acetone (1:1), acetone and MeOH to give 4 fractions. The $CHCl_3$ fraction (16.47 g) was chromatographed twice on silica gel column with the solvent system hexane-ethyl acetate, and the compound **1** was eluted with hexane-ethyl acetate (4:1). Further purification by HPLC on silica gel column with hexane-ethyl acetate (9:1) afforded compound **1** (88.1 mg).

(1S,2S,6R,8R)-1-isopropyl-5-(hydroxymethyl)-8-methyltricyclo[4.4.0.0^{2,8}]dec-4-ene(1). colorless oil, $[\alpha]_D^{23}$ +35.6° (*c*=0.94, $CHCl_3$). IR vmax (KBr) cm^{-1} : 3331, 2920, 2865, 1671, 1589, 1458, 1382, 1321, 1265, 1066, 834. GCMS *m/z* (rel. int.) : 220 ($[M]^+$, 8), 202 (8), 189 (13), 159 (15), 135 (14), 123 (100), 121 (24), 105 (21), 93 (19), 91 (52), 81 (33), 79 (26), 77 (25), 55 (23), 43 (31), 41 (58). HRFABMS *m/z* : 221.18878 $[M+H]^+$ (Calcd. for $C_{15}H_{25}O$, 221.19054).

p-Bromobenzoylation of compound 1. To a solution of compound **1** (30 mg, 0.136 mmol) in dry pyridine (1 ml), were added *p*-bromobenzoylchloride (75 mg, 0.342 mmol) in diethyl ether (1 ml). After stirring at room temperature for 3 days, the reaction mixture was diluted with diethyl ether (30 ml). This mixture was poured into water. After separation of organic layer, water layer was extracted with diethyl ether. The combined organic layer was washed with water and saturated $CuSO_4$ solution. This was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified with preparative TLC to afford 15-*p*-bromobenzoate (**1a**, 15 mg).

Compound 1a. mp 67.8-68.1°C (dec.), $[\alpha]_D^{23}$ +18.9° (*c*=0.35, $CHCl_3$). ^1H-NMR (benzene-d_6) : δ 0.74 (3H, *d*, *J*=6.9 Hz, H-12), 0.83 (3H, *d*, *J*=6.9 Hz, H-13), 0.98 (3H, *s*, H-14), 1.05 (1H, *ddd*, *J*=12.7, 8.8, 7.7 Hz, H-10β), 1.13 (1H, *br.s*, H-2), 1.15-1.25 (2H, *m*, H-9), 1.31 (1H, *br.d*, *J*=11.9 Hz, H-7α), 1.51 (1H, *ddd*,

J=11.9, 7.5, 1.2 Hz, H-7β), 1.57 (1H, *ddd*, *J*=12.7, 7.0, 6.7 Hz, H-10α), 1.90-2.00 (3H, *m*, H-3, 6), 2.02 (1H, *hept.*, *J*=6.9Hz, H-11), 4.59 (1H, *ABq*, *J*=12.3 Hz, H-15a), 4.63 (1H, *ABq*, *J*=12.3 Hz, H-15b), 5.30 (1H, *br.s*, H-4), 7.16, 7.83 (each 2H, *d*, *J*=8.4 Hz, benzoyl-H).

Crystal data for compound 1a. Colorless thin plate crystals of compound **1a**, recrystallized from EtOH, belong to the monoclinic space group C2. The diffraction data were measured on a MacScience four-circle diffractometer MXC-18 equipped with a device for graphite monochromated CuKα radiation (1.54178 Å). Crystal data : $C_{21}H_{27}O_2Br$, F.W.=391.35, *a*=21.393(5), *b*=7.823(2), *c*=14.007(2) Å, β=120.07(2)°, Z=4, Dc=1.281 g/cm^3, μ(CuKα)=28.25 cm^{-1}. A total of 1827 unique reflections were measured, and 1254 reflections (I>3.0σ(I)) were used for the structure determination and refinement. The structure was solved by SAPI91 [10] and DIRDIF92 [11]. The non-hydrogen atoms were refined anisotropically, while the included hydrogen atoms were not refined. The unweighted and weighted agreement factors were 0.062 and 0.072, respectively.

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