Pergamon

Tetrahedron Letters 40 (1999) 133–136

TETRAHEDRON LETTERS

Walleminol and Walleminone, Novel Caryophyllenes from the Toxigenic Fungus *Wallemia sebi*

Michael Frank[a], Emily Kingston[b], John C. Jeffery[b], Maurice O. Moss[a], Martin Murray[b], Thomas J. Simpson[b]* and Andrew Sutherland[b]

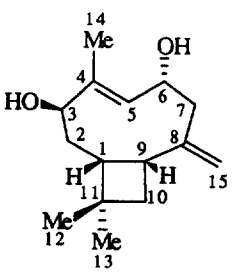
[a]School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH, UK

[b]School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK

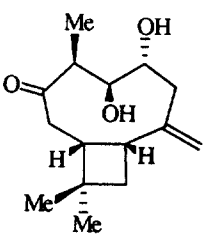
Received 11 September 1998; accepted 19 October 1998

Abstract. The structures and relative stereochemistries of walleminol (**1**) and walleminone (**2**), novel *cis*-fused *iso*-caryophyllenes from the toxigenic fungus, *Wallemia sebi*, have been established by detailed high-field 1D and 2D NMR and X-ray crystallographic studies.

Wallemia sebi is a common xerophyllic fungus found in grain, salt- and sugar-preserved foodstuffs, and house dust. An asexual fungus, it has been suggested to have affinities with the tremellinaceous basidiomycetes on the strength of an ultrastructural feature of its crosswall.[1] Studies[2] on a toxigenic strain of *Wallemia sebi* led to the isolation of two apparently related metabolites, designated walleminol A and walleminol B. We now report NMR and X-ray crystallographic studies which allow structures (**1**) and (**2**) to be assigned to these compounds which we now call walleminol and walleminone respectively.



(**1**)



(**2**)

Walleminol (**1**), isolated as colourless crystals, mp 128–130° C, has the molecular formula $C_{15}H_{24}O_2$. The absorption at 3414 cm^{-1} in the IR and the formation of a *bis*-trimethyl-silyl ether (M^+, 380) indicated the presence of two hydroxyl groups in the

0040-4039/99/$ - see front matter
PII: S0040-4039(98)02248-5

molecule. The NMR spectra (both ^{1}H and ^{13}C) contained more signals than could be explained by the molecular formula and displayed line broadening at ambient temperature which increased on warming. Preliminary X-ray crystallographic studies, due to extensive twinning of the crystals and disorder, did not define the structure but suggested the presence of fused nine and four membered rings, consistent with the sesquiterpene caryophyllene structure. The second product obtained from the cultures, walleminone (**2**), is an oil which is also obtained from walleminol on heating or prolonged standing in air. HRGCMS indicated the molecular formula $C_{15}H_{24}O_3$ and the IR and ^{13}C NMR spectra indicated the presence of a ketone function (1720 cm^{-1} and 223.8 ppm) in a medium-sized ring. In contrast to walleminol, walleminone gave well resolved ^{1}H and ^{13}C NMR spectra (Table 1) and these allowed the caryophyllene diol structure (**2**) with an unusual *cis* ring junction to be assigned. Most of the connectivities were determined from the ^{1}H-^{1}H COSY and ^{1}H-^{13}C correlation spectra (inverse mode, direct bonded PHSQC[3] and long-range HMBC[4]). The relative stereochemistry was obtained from full analysis of the proton coupling constants and nOe data.

Table 1. NMR characterisation of Walleminone **2**[a]

Position	δ_C	δ_H (J/Hz)	HMBC	nOe
1	50.1	2.3 (dddd, 12.8, 8.2, 3.3, 1.7)	H-9, H-2a, H-2b, H_3-12, H_3-13	H-2a, H-2b, H-9, H-10a, H-10b
2a	42.5	2.8 (dd, 12.8, 10.3)		H-2b, H-1
2b		2.1 (dd, 12.8, 2.0)		H-2a, H-1, H-15b
3	223.8			
4	45.4	3.4 (qd, 7.0, 2.6)		H_3-14, H-5, H-15a
5	80.7	3.2 (dd, 10.0[b], 2.6)	H-7a, H-7b, H-14	H-4, H-7a, H_3-14
6	71.4	3.3 (ddd, 11.1, 9.5[b], 2.2)	H-7a, H-7b	H-7a
7a	44.3	2.6 (dd, 14.4, 2.2)		H-7b, H-6, H-5
7b		2.4 (dd, 14.4, 11.1)		H-7a, H-15a
8	146.8		H-9, H-7a, H-7b, H-10b	
9	37.2	3.15 (ddd, 11.1, 8.2, 8.1)	H_2-15, H-2a, H-2b, H-11b	H-1, H-10a
10a	35.2	1.7 (ddd, 11.2, 8.1, 3.3)	H_3-12, H_3-13	H-10b, H-1, H-9
10b		2.0 (dd, 11.2, 11.1)		H-10a, H_3-13, H-15b
11	33.9		H-10a, H-10b, H_3-12, H_3-13	
12	29.4	1.3 (s)	H_3-13, H-10b	H-1
13	24.8	0.9 (s)	H-10b, H_3-12	H-10b
14	14.7	1.2 (d, 7.0)	H-4	H-4, H-5
15a	114.9	5.2 (d, 1.7)	H-7a, H-7b	H-15b, H-4, H-7b
15b		5.1 (d, 1.7)		H-15a, H-2b, H-10b

[a] Chemical shifts were measured at 125 MHz for ^{13}C and 500 MHz for ^{1}H in $CDCl_3$ solution.
[b] Coupling to hydroxyl protons.

Since walleminone is obtained as a single compound from walleminol in high yield, it appeared that the complications in the NMR spectra of walleminol must be due to the presence of stable conformers which have been shown to exist for caryophyllenes and

iso-caryophyllenes.[5,6] At +60° C the ^{1}H NMR spectrum of walleminol became very broad, particularly in the region between δ 3.5 and 5.2 where the signals due to hydrogens of oxygen-bearing methine and alkene groups occur. At 0° C, the signals became much sharper than at room temperature, and a ^{1}H-^{1}H COSY spectrum at this temperature, together with room temperature ^{1}H-^{13}C inverse correlation spectra (PHSQC and HMBC) allowed assignments of all the signals (^{1}H and ^{13}C) of both conformers, establishing the structure (**1**) for walleminol.[7] The conformers arise from mobility of the endocyclic double bond part of the molecule, rotation about the 3-4 and 5-6 bonds placing the olefinic methyl groups on opposite sides of the ring (Figure 1). Coupling constant and nOe data are in full agreement with these conformers.

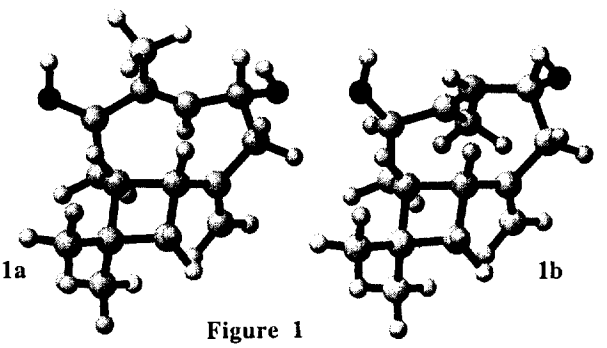
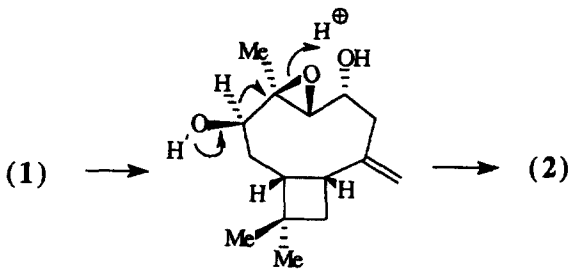


Figure 1

In the light of the NMR results, the X-ray structure determination was repeated. The crystal used was of a different space group (P3/2) from that originally studied. The measurements clearly established the *E*-configuration of the double bond in the nine-membered ring, but the extensive disorder observed can be understood if it is assumed that both conformers are present in the solid state. For example, considerable electron density is found near the 5-proton on the endocyclic double bond, which is close to the position occupied by the methyl group in the minor conformer. Final assignments of the two conformers were obtained from solutions in deuteriomethanol, in which walleminol is more soluble than deuteriochloroform, particularly at lower temperatures. Thus nOe spectra at -30° C clearly showed that the major conformer is **1a** with the methyl group on the same side as the ring junction protons, while in the minor conformer **1b** this methyl is on the opposite side of the ring and shows nOe effects with the two exocyclic methylene protons. The *cis* ring junction in (**1**) and (**2**) is unusual, but has been observed for plant[8] and fungal[9] caryophyllenes. The interconversion of walleminol to walleminone probably occurs *via* epoxidation of the *E*-alkene in walleminol, followed by rearrangement (Scheme 1). Such processes have been previously established for caryophyllene oxide.[10]



Scheme 1

References:

1. Moore R. T, *Antonie van Leuwenhook*, **1986**, *52*, 183-187.
2. Wood G. M.; Mann, P. J.; Lewis, D. F.; Reed, W. J.; Moss, M. O., *Food Additives and Contaminants*, **1990**, *7*, 67-69.
3. Bax, A.; Subramanian, S., *J. Magn. Reson.*, **1986**, *67*, 565-569.
4. Bax, A.; Summers, M. F., *J. Am. Chem. Soc.*, **1986**, *108*, 2093-2094.
5. Suginome, H.; Kondoh, T.; Gogonea, C.; Singh, V.; Goto, H.; Osawa, E., *J. Chem. Soc., Perkin Trans. 1*, **1995**, 69-81.
6. Barrero, A. F.; Molina, J.; Oltra, J. E.; Altarejos, J.; Barragán, A.; Lara, A.; Segura, M.; *Tetrahedron*, **1995**, *51*, 3813-3822.
7. Walleminol, major conformer δ_H (500 MHz, $CDCl_3$, 0°C) 0.90 (3H, s, H_3-13), 1.17 (3H, s, H_3-12), 1.58 (1H, ddd, J 10.7, 7.9, 3.4, H-10a), 1.60 (1H, d, J 15.3, H-2a), 1.73 (3H, s, H_3-14), 1.75 (1H, m, H-10b), 1.98 (1H, ddd, J 14.5, 11.9, 4.7, H-2b), 2.36 (1H, ddd, J 14.4, 6.4, 1.8, H-1), 2.36 (1H, dd, J 14.0, 6.4, H-7a), 2.52 (1H, d, J 14.0, H-7b), 2.86 (1H, ddd, J ca. 6, ca. 6, ca. 6 H-9), 4.61 (1H, d, J 4.0, H-3), 4.80 (1H, dd, J 6.7, 6.4, H-6), 4.99 (1H, s, H-15), 5.25 (1H, s, H-15), 5.25, 1H, d, J 5.8, H-5); δ_C (125 MHz, $CDCl_3$) 14.7 (C-12), 24.9 (C-14), 29.6 (C-13), 30.6 (C-10), 32.3 (C-11), 38.3 (C-2), 42.0 (C-9), 43.2 (C-1), 46.5 (C-7), 67.7 (C-6), 72.0 (C-3), 114.6 (C-15), 129.0 (c-5), 135.8 (C-4), and 146.3 (C-8); minor conformer δ_H (500 MHz, $CDCl_3$, 0°C) 0.91 (3H, s, H_3-13), 1.21 (3H, s, H_3-12), 1.68 (1H, ddd, J 14.6, 4.8, 2.0, H-2a), 1.75 (1H, m*, H-10a), 1.78 (1H, m*, H-10b), 1.78 (1H, m*, H-2b), 1.85 (1H, m*, H-14), 2.30 (1H, dd, J 14.3, 6.1, H-7a), 2.57 (1H, m*, H-1), 2.58 (1H, d, J 14.0, H-7b), 2.85 (1H, m*, H-9), 4.09 (1H, d, J 4.6, H-3), 4.67 (1H, dd, J 6.0, 6.0, H-6), 4.87 (1H, br, H-15), 5.05 (1H, d, J 1.2, H-15), 5.51 (1H, dq, J 4.0, 1.5, H-5): * signals obscured, multiplicities and couplings not determined: δ_C (125 MHz, $CDCl_3$) 19.1 (C-12), 25.4 (C-14), 29.6 (C-13), 30.9 (C-10), 32.4 (C-11), 38.9 (C-2), 42.5 (C-9), 43.0 (C-1), 48.3 (C-7), 70.3 (C-6), 74.4 (C-3), 115.0 (C-15), 121.4 (C-5), 139.5 (C-4), 146.2 (C-8).
8. Bohlmann, F.; Zdero, C., *Phytochemistry*, **1978**, *17*, 1135-1153.
9. Tsuboyama, S.; Sakurai, T.; Tsuboyama, K.; Doi, K.; *Bull. Chem. Soc. Japan*, **1986**, *59*, 1921-1924.
10. Tsui, W.-Y.; Brown, G., *J. Chem. Soc., Perkin Trans. 1*, **1996**, 2507-2509.