1α-Hydroxyalliacolide, a Sesquiterpenoid Metabolite of *Marasmius alliaceus*. X-Ray Molecular Structure of 1α-Hydroxyalliacolide

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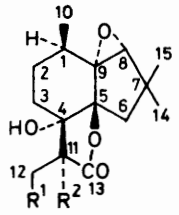
1α-Hydroxyalliacolide has been isolated from *Marasmius alliaceus* and its structure established by a combination of *X*-ray crystallographic and spectroscopic methods. The ^{13}C n.m.r. spectrum of alliacolide has been reassigned.

The Basidiomycete *Marasmius alliaceus* produces a novel series of metabolites possessing the sesquiterpenoid alliacane carbon skeleton and exemplified by alliacolide (**1**).[1-3] 11,12-Dehydroalliacolide (alliacol A) has cytotoxic properties.[3] In the course of biosynthetic studies,[4] we isolated a number of related fungal metabolites including 11- (**2**) and 12-hydroxyalliacolide (**3**).[5] In some of our more recent cultures the 12-hydroxyalliacolide fraction has contained a further metabolite, the structure of which forms the subject of this paper.



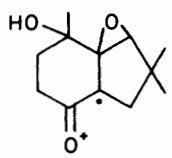
(**1**) $R^1 = R^2 = H$
(**2**) $R^1 = H$, $R^2 = OH$
(**3**) $R^1 = OH$, $R^2 = H$

The new metabolite, $C_{15}H_{22}O_5$, possessed i.r. absorptions at $\nu_{max.}$ 3 440, 3 300, and 1 750 cm^{-1} attributable to hydroxy and γ-lactone functionality. The ^1H and ^{13}C n.m.r. spectra (determined at 360 and 90.55 MHz respectively) (see Table 1) contained characteristic signals which were assigned to a secondary:tertiary epoxide, a secondary methyl group, three tertiary methyl groups, and an isolated methylene AB doublet. This suggested that the metabolite was a member of the alliacane series. The absence of $-\overset{|}{C}H(OH)$ signals in the ^1H n.m.r. spectrum indicated that the hydroxy groups were tertiary. The absence of one of the methyl-group doublets (δ_H 1.14) of alliacolide and the appearance of a new lower field methyl-group singlet at δ_H 1.38 suggested that the hydroxy group was at C-1 or C-11. Comparison of the ^{13}C n.m.r. spectrum with that of alliacolide (**1**) showed significant downfield shifts for the signals attributed to C-1, C-2 and, subsequently, *vide infra*, to that for C-10. This suggested that the additional hydroxy group was located at C-1. Confirmation came from the mass spectrum which showed significant fragments at *m/z* 209 and 191 corresponding to the ions (**4**) and (**5**). However, it was not possible to obtain unambiguous evidence for the stereochemistry of the hydroxy group. This was established by an *X*-ray crystallographic study (see Figure) which revealed that the new metabolite was 1α-hydroxyalliacolide (**6**).

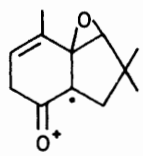
Table 1. N.m.r. data for compounds (**1**) and (**6**) ($CDCl_3$; δ values in p.p.m. from Me_4Si)

Carbon atom	Compound (**1**) δ_C	Compound (**6**) δ_C	δ_H
1	31.49	70.0	
2	25.30	34.87	1.7—1.86 (m)
3	28.20	29.40	
4	76.80	76.70	
5	92.54	92.03	
6	41.01	41.58	1.34, 2.05 (*J* 14 Hz)
7	38.50	38.37	
8	68.30	68.06	3.5 (s)
9	68.52	66.98	
10	17.33	22.90	1.38 (s)
11	45.12	45.94	2.71 (q, *J* 7.4 Hz)
12	7.5	7.2	1.18 (d, *J* 7.4 Hz)
13	175.95	174.98	
14	23.97*	23.80*	1.17 (s)
15	24.31*	24.21*	

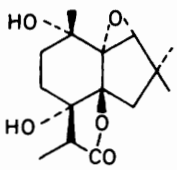
* Values in vertical columns may be interchanged.



(**4**)



(**5**)



(**6**)

In order fully to assign the n.m.r. spectra, both a two-dimensional one-bond ^{13}C–^1H n.m.r. shift correlation and, by using longer delay times, a second ^{13}C–^1H n.m.r. shift correlation to show geminal (^1H–C–^{13}C) and vicinal (^1H–C–C–^{13}C) connectivities, were obtained. This led to the assignments shown in Table 1. In the course of this work we became aware of a possible inconsistency with our original assignment of the methyl group carbon resonances for C-10, C-14, and C-15 in

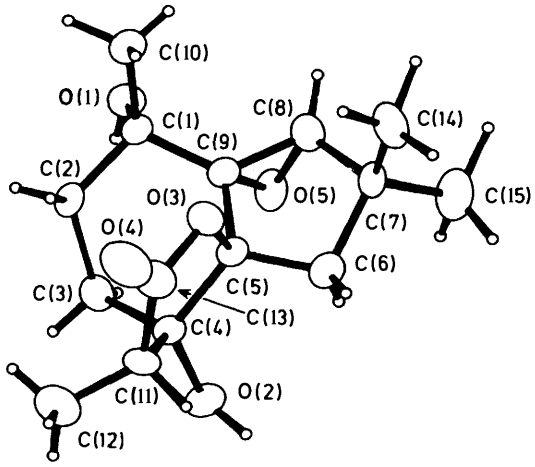


Figure. *X*-Ray molecular structure of 1α-hydroxyalliacolide (**6**)

alliacolide (**1**). Hence we also obtained two-dimensional ^{13}C–^{1}H chemical-shift correlations for alliacolide which led us to reassign the signal at δ_C 17.33 p.p.m. to C-10 whilst the signals at δ_C 23.97 and 24.31 p.p.m. were attributed to C-14 and C-15 (see Table 1). Since these signals are common to the other metabolites described previously,[5] the assignments should also be interchanged for these compounds. Although these resonances figured in our biosynthetic work, the difference between the coupling constants (35.1 and 36.6 Hz) only corresponds to one data point in the original Fourier transform ^{13}C n.m.r. spectrum and hence lies within the experimental error for that determination. The discrepancy was not revealed at the time of our original biosynthetic experiment. We have now redetermined the coupling pattern at 90.55 MHz using the original material. The enrichment was sufficient to enable us to carry out a two-dimensional ^{13}C–^{13}C COSY experiment to establish some of the ^{13}C–^{13}C connectivities. This showed that the signals at δ_C 17.33 and 31.49 p.p.m. (C-10 and C-1) were coupled (J 34.7 ± 0.3 Hz) and that those at δ_C 24.31 and 38.5 p.p.m. (C-14/15 and C-7) were also coupled (J 36.7 ± 0.3 Hz). The coupling constants were measured using a normal one-dimensional ^{13}C plot which also served to show which of the two signals at δ *ca.* 24 p.p.m. was coupled. This correction does not, however, change the conclusions concerning the labelling pattern of the alliacolide biosynthesized from [1,2-$^{13}C_2$]acetate. It does, however, reveal the power of two-dimensional ^{13}C–^{13}C n.m.r. experiments in determining the connectivity patterns in compounds biosynthetically enriched from multiply labelled precursors, particularly when coupling constants are of a similar order of magnitude.

Experimental

^{1}H and ^{13}C N.m.r. spectra were obtained on a Bruker WM 360 spectrometer. The accuracy of the ^{13}C coupling constants is estimated to be 0.3 Hz. The programs for the two-dimensional experiments including the COSY-90° experiment used standard Bruker software. Samples were *ca.* 15 mg in deuteriochloroform (0.5 ml). Light petroleum refers to the fraction boiling in the range 60—80 °C.

Isolation of Metabolites.—*Marasmius alliaceus* was grown as described previously.[5] The crude extract from 9 litres of broth was separated into four fractions as described previously. The fourth fraction [containing (t.l.c.) two compounds of comparable R_F value but differing in their colours on visualization with methanolic sulphuric acid spray] was subjected to further column chromatography on silica (Merck 9385) in ethyl acetate–light petroleum. This gave 12-hydroxyalliacolide (**3**) (150 mg), identified by its i.r. and n.m.r. spectra. The product gave a red coloration on t.l.c. when sprayed with methanolic sulphuric acid. Further elution gave 1α-*hydroxyalliacolide* (**6**) (60 mg) which crystallized from ethyl acetate as needles, m.p. 164—166 °C; $[\alpha]_D^{23}$ +4.6° (*c* 1.0 in $CHCl_3$) (Found: C, 63.6; H, 7.5. $C_{15}H_{22}O_5$ requires C, 63.8; H, 7.8%); $\nu_{max.}$ 3 440, 3 300, and 1 750 cm^{-1}; *m/z* 282, 264, 246, 236, 221, 209, 191, and 178; ^{1}H and ^{13}C n.m.r. (see Table 1). The metabolite gave a blue coloration on t.l.c. when sprayed with methanolic sulphuric acid.

Table 2. Fractional atomic co-ordinates (× 10^4 for O and C), with estimated standard deviations in parentheses

	x	*y*	*z*
O(1)	5 889(2)	−129(1)	5 509(1)
O(2)	7 213(2)	−634(1)	9 029(1)
O(3)	5 961(2)	2 054(1)	8 154(1)
O(4)	4 067(3)	2 559(2)	9 138(1)
O(5)	8 342(2)	215(1)	6 653(1)
C(1)	5 592(3)	647(2)	6 306(2)
C(2)	4 472(3)	138(2)	7 056(2)
C(3)	5 259(3)	−550(2)	7 852(2)
C(4)	6 199(3)	134(2)	8 593(2)
C(5)	6 979(3)	1 095(2)	8 006(2)
C(6)	8 565(3)	1 451(2)	8 292(2)
C(7)	9 291(3)	1 981(2)	7 343(2)
C(8)	8 446(3)	1 411(2)	6 508(2)
C(9)	7 053(3)	906(2)	6 878(2)
C(10)	4 954(3)	1 665(2)	5 793(2)
C(11)	5 273(3)	762(2)	9 395(2)
C(12)	3 865(4)	226(3)	9 801(2)
C(13)	5 006(3)	1 874(2)	8 916(2)
C(14)	9 005(3)	3 232(2)	7 292(2)
C(15)	10 981(3)	1 740(3)	7 284(3)

Crystal Structure Determination.—$C_{15}H_{22}O_5$, M = 282.3, orthorhombic, space group $P2_12_12_1$, a = 8.881(1), b = 12.045 (1), c = 13.279(1) Å, V = 1 420.5 Å^3, Z = 4, D_c = 1.32 g cm^{-3}, monochromated Mo-K_α radiation, λ = 0.710 69 Å, μ = 0.92 cm^{-1}.

A crystal of approximate dimensions 0.4 × 0.3 × 0.3 mm was mounted on an Enraf-Nonius CAD4 diffractometer. Intensities for unique data with $2 < \theta < 25°$ were measured with an ω–2θ scan with a maximum scan time of 1 min. No correction was made for absorption. Out of 1 469 reflections measured, 1 282 with $|F^2|| > |\sigma(F^2)$ were used in the refinement where $\sigma(F^2) = [\sigma^2(I) + (0.04I)^2]^{\frac{1}{2}}/L_p$.

The structure was solved by direct methods using MULTAN and refined by full-matrix least-squares with anisotropic temperature factors. All hydrogen atoms were located on a difference map and were included in the refinement with isotropic temperature factors. Refinement converged at R = 0.036, R' = 0.047 with weighting scheme $w = 1/\sigma^2(F)$. All calculations were done on a PDP11/34 computer using the Enraf-Nonius SDP-plus program package.

Final non-hydrogen atom co-ordinates are shown in Table 2. Hydrogen-atom co-ordinates, temperature factors, bond lengths and angles, and torsion angles are deposited in Supplementary Publication No. SUP 56378 (6 pp.).* Structure factors are available from the editorial office on request.

* For details of the Supplementary Publications Scheme, see Instructions for Authors (1985), *J. Chem. Soc., Perkin Trans. 1*, 1985, issue 1.

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