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4α-HYDROXYDIHYDROILLUDIN M—A NEW SESQUITERPENOID METABOLITE OF *CLITOCYBE ILLUDENS*

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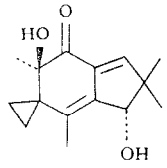
Key Word Index—*Clitocybe illudens*; basidomycete; illudin M; sesquiterpenoid.

Abstract—The structure of the new sesquiterpenoid 4α-hydroxydihydroilludin M has been established by a combination of spectral and X-ray studies.

The basidiomycete, *Clitocybe illudens* (*Omphalotus olearus*) produces a range of sesquiterpenoid metabolites typified by illudin M (**1**) and illudol (**2**) [1, 2]. In the course of biosynthetic studies [3, 4], we have isolated a new metabolite, $C_{15}H_{22}O_4$ from *C. illudens*. The IR spectrum possessed absorption characteristic of hydroxyl groups and an α,β-unsaturated ketone. The ^1H and ^{13}C NMR spectra revealed the presence of four tertiary methyl groups, three methylene groups, two of which formed part of a cyclopropane ring, one secondary and two tertiary alcohols, a fully substituted α,β-unsaturated ketone



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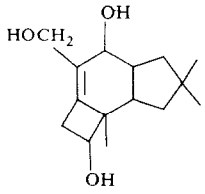

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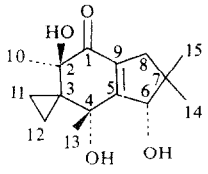

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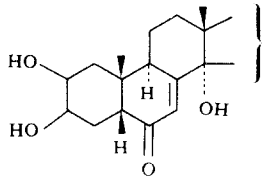

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and two further fully-substituted carbon atoms. The ^1H NMR signals of two of the methyl groups underwent a marked solvent shift between CD_3OD and deuteropyridine indicating the presence of adjacent hydroxyl groups. A pair of allylic proton signals showing geminal coupling (J = 15 Hz) and long range W coupling (1 and 1.5 Hz) to a secondary alcohol proton, were also clearly distinguishable. Bearing in mind the co-occurrence of the metabolite with the illudins, this data suggested the structure (**3**) without excluding a number of plausible alternatives or stereochemical variations. However because of the

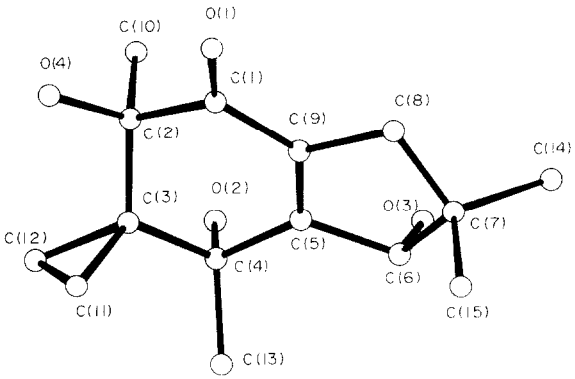

Fig. 1.

paucity of material, the structure and relative stereochemistry were established by direct X-ray methods. The circular dichroism curve showed a negative Cotton effect at 331 nm ($\Delta\epsilon = -0.86$) and a positive Cotton effect at 222 nm ($\Delta\epsilon = +7.75$). Using the helicity rule [5] the structure (Fig. 1) would predict a positive Cotton effect on the K band. The structure is in part enantiomeric to the ecdysone chromophore (**4**) [5] which has a positive effect at 340 nm and a negative effect at 240 nm. Although, because of the unquantifiable effect of the cyclopropane ring, this comparison is far from ideal, nevertheless it leads to an absolute configuration which is consistent with that of other illudin derivatives [6].

The structure of 4α-hydroxydihydroilludin M is of biogenetic interest since it represents, by dehydration a plausible immediate precursor of illudin M. It is less likely to be an artefact arising from illudin M by hydration since this would be expected to generate a hydroxyl group at C-8, i.e. β- to the carbonyl group of illudin M.

EXPERIMENTAL

Isolation and characterization of 4α-hydroxydihydroilludin M. Clitocybe illudens (ATCC 11719) (13.5 l.) was grown as described previously [3] on a yeast–peptone medium for 6 weeks. The broth was extracted with EtOAc at pH 2 and the metabolites separated by CC on Si gel in

30% EtOAc–$CHCl_3$ and by PLC in MeOH–$CHCl_3$ (1:9) to afford illudin M (287 mg) identified by its ^1H NMR spectrum. 4α-Hydroxydihydroilludin M (3) (50 mg) crystallized from EtOAc–petrol as prisms, mp 196–198°, $[\alpha]_D$ +15.4 (c 0.2 in MeOH) (Found: C, 67.9; H, 8.6. $C_{15}H_{22}O_4$ requires C, 67.6; H, 8.3%.) IR ν_{max} cm^{-1}: 3510, 3420, 3300 *br*, 1680. δ^1H NMR (C_5D_5N) 1.0 (2H, *m*), 1.15, 1.20, 1.55 and 1.82 (each 3H, *s*), 2.4 and 2.52 (2H, AB, *dd*, 15 Hz, 1 and 1.5 Hz respectively), 4.9 (1H, *m*). $\delta(CD_3OD)$ 0.8 (2H, *m*), 1.0, 1.10, 1.23 and 1.42 (each 3H, *s*), 2.20 (2H, *m*) 4.55 (1H, *m*). $\delta^{13}C$ NMR 201.5 (*s*, C − 1), 75.5 (*s*, C − 2), 33.8 (*s*, C − 3), 71.1 (*s*, C − 4), 161.6 (*s*, C − 5), 84.2 (*d*, C − 6), 43.6 (*s*, C − 7), 42.1 (*t*, C − 8), 132.9 (*s*, C − 9), 24.9 (*q*, C − 10), 4.7 (*t*, C − 11), 7.0 (*t*, C − 12), 22.7 (*q*, C − 13), 19.8 (*q*, C − 14), 26.7 (*q*, C − 15).

X-ray structure determination. Crystal data $C_{15}H_{22}O_4$, M = 266.34, orthorhombic, a = 10.532(4), b = 10.386(3), c = 12.966(7) Å, U = 1421.6 Å^3, Z = 4, Dc = 1.24 g/cm^3, $F(000)$ = 576. Mo–Kα radiation, λ 0.71073 Å, μ = 0.96 cm^{-1}. Space group $P2_12_12_1$ from systematic absences of $h00$ for h odd, $0k0$ for k odd and $00l$ for l odd.

A crystal of *ca* 0.25 × 0.25 × 0.25 mm was used for data collection on an Enraf-Nonius CAD4 diffractometer. Cell dimensions were derived from the setting angles for 25 reflections. Intensities for unique reflections with $2 < \theta < 25°$ were measured by a $\theta/2\theta$ scan using monochromated Mo-Kα radiation and with a scan width of $\Delta\theta = (1.0 + 0.35 \tan\theta)°$. The scan speed for each reflection was determined by a rapid pre-scan at 5°/min and all reflections with $I < \sigma(I)$ were coded as unobserved. The remainder were re-scanned to give $I/\sigma(I)$ of 50, subject to a maximum scan time of 120 sec. Two standard reflections monitored every 30 min showed no significant variations in intensity. After correction for Lorentz and polarization effects but not for absorption, 973 reflections with $|F^2| > 3\sigma(F^2)$ were used in the structure refinement. The value of $\sigma(F^2)$ was calculated from:

$$\sigma(F^2) = \sqrt{[\sigma^2(I) + (0.06I)^2]}/Lp.$$

The positions of the carbon and oxygen atoms were found by direct methods using the MULTAN programme. These atoms were refined by full matrix least squares with anisotropic temperature factors. A low-angle difference map revealed the positions of all the hydrogen atoms except for that bonded to O(2). These were included at positions from the map with a common isotropic temperature of B = 6.0. Further refinement with the hydrogen atom parameters fixed converged at R = 0.077, R' = 0.090, where the weighting scheme was $w = 1/\sigma^2(F)$ and the maximum shift to error ratio was <0.01. A final difference map everywhere was < 0.3 electron/Å^3.

The structure solution and refinement was done on a PDP11/34 computer using the Enraf-Nonius Structure Determination Package. Final atom co-ordinates, lists of temperature factors, hydrogen atom positions and final structure factors are deposited with the Cambridge Crystallographic Centre.

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REFERENCES

1. McMorris, T. C. and Anchel, M. (1965) *J. Am. Chem. Soc.* **97**, 1594.
2. Ayer, W A. and Browne, L. M. (1981) *Tetrahedron* **37**, 2199.
3. Hanson, J. R., Marten, T. and Nyfeler, R. (1976) *J. Chem. Soc. Perkin Trans.* 1, 1212.
4. Bradshaw, A. P. W., Hanson, J. R. and Siverns, M. (1978) *J. Chem. Soc. Chem. Commun.* 303.
5. Scopes, P. M. (1975) *Fortschr. Chem. Org. Naturst.* **32**, 167.
6. Harada, N. and Nakanishi, K. (1970) *J. Chem. Soc. Chem. Commun.* 310.