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SHORT COMMUNICATION

ISOLATION OF DIHYDROILLUDIN M FROM *CLITOCYBE ILLUDENS**

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Abstract—Dihydroilludin M (Ia) has been isolated from culture liquids of *Clitocybe illudens*. This metabolite differs from the dihydroilludin M obtained by borohydride reduction of illudin M, only in the configuration at C-7, one of three asymmetric centers in the molecule.

SIX RELATED sesquiterpenoids have been isolated so far from culture liquids of *Clitocybe illudens*.[1] We now report isolation of a seventh, dihydroilludin M (Ia).

Culture liquids of a homokaryotic strain of *Clitocybe illudens* yielded a fraction with an UV absorption maximum at 256 nm. The product obtained by further purification of this fraction on a column and then by preparative TLC could not be crystallized. The UV absorption spectrum of the compound was reminiscent of that of dihydroilludin M (Ia), a compound prepared[2] by borohydride reduction of illudin M (IIa). The IR and NMR spectra also were similar. However, the natural product did not crystallize when seeded with synthetic dihydroilludin M. It also had a slightly lower R_f on TLC. We therefore examined the possibility that these compounds might be stereoisomers.

Periodate oxidation of natural Ia yielded a keto aldehyde (III) identical with that obtained from synthetic Ia. Thus, synthetic and natural Ia have the same configuration at C-3.

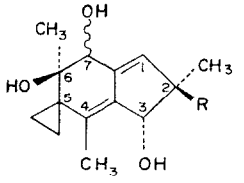
Comparison of the configuration at C-6 would require elimination of the asymmetric center at C-7. The most obvious method, MnO_2 oxidation to IIa, was ruled out because oxidation of Ia with MnO_2 does not yield IIa but gives the same keto aldehyde, III, as is obtained by periodate oxidation.[2] A second method was suggested by the finding[3] that hydrogenation of illudin M (IIa) with palladium-charcoal in ethyl acetate yields a product (IV) which can be oxidized with MnO_2 to the diketone (V). By analogy, we hoped to obtain a product (VI) from dihydroilludin M, which would yield the desired diketone on MnO_2 oxidation. Unexpectedly, hydrogenation of Ia yielded the ketol (IV) instead of a triol. The same product, identical in all respects with the ketol (IV) from illudin M, was obtained from both synthetic and natural dihydroilludin M. Thus, the natural and synthetic dihydroilludin M differ only in the configuration at C-7. This finding is in harmony with the likely assumption that biogenesis of illudin M and of dihydroilludin M involves the same enzyme systems. Borohydride reduction of the carbonyl at C-7 in illudin M results predominantly in a different configuration at this carbon atom than that produced biologically.

A corresponding illudin pair, illudin S (IIb)[4] and dihydroilludin S (Ib),[5] has been

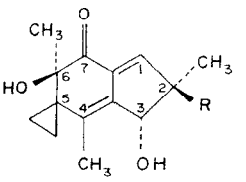
* Part VI of a series, "Metabolites of *Clitocybe illudens*". For Part V see Ref. 3.

[1] M. S. R. NAIR, H. TAKESHITA, T. C. MCMORRIS and M. ANCHEL, *J. Org. Chem.* **34**, 240 (1969).
[2] T. C. MCMORRIS and M. ANCHEL, *J. Am. Chem. Soc.* **87**, 1594 (1965).
[3] M. ANCHEL, T. C. MCMORRIS and P. SINGH, *Phytochem.* **9** (1970).
[4] M. TADA, Y. YAMADA, N. S. BHACCA, K. NAKANISHI and M. OHASHI, *Chem. Pharm. Bull.* **12**, 853 (1964).
[5] A. ICHICHARA, H. SHIRAHAMA and T. MATSUMOTO, *Tetrahedron Letters* 3965, (1969).

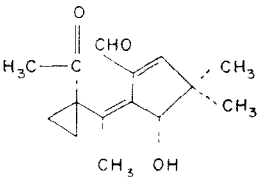
isolated from fruiting bodies of *Lampteromyces japonicus*. In this pair also, the configuration at C-3 and C-6 is the same. However, in contrast to the findings with dihydroilludin M, natural and synthetic dihydroilludin S apparently have the same configuration at C-7 as well.[5]



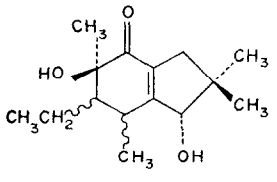
(Ia) R = CH_3
(Ib) R = CH_2OH



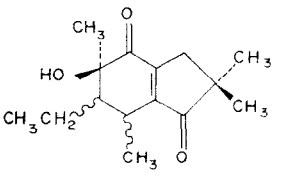
(IIa) R = CH_3
(IIb) R = CH_2OH



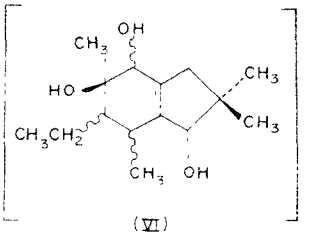
(III)



(IV)



(V)



(VI)

EXPERIMENTAL

Isolation of dihydroilludin M (Ia). The residue of an EtOAc extract of *Clitocybe illudens* culture liquids was fractionated by 50-tube countercurrent distribution between $CHCl_3$–H_2O. Tubes 10–25 showed an u.v. maximum at 256 nm. The gummy residue from these tubes, *ca.* 70 mg, was purified on a silica gel column (50 g) It was eluted with ligroin containing increasing amounts of EtOAc. The main fraction, *ca.* 30 mg with λ_{max} 256 nm was eluted with (3:1) ligroin–ethyl acetate and further purified by TLC. The fraction with R_f 0·55 on TLC with EtOAc–ligroin (1:1) was scraped off and eluted with EtOAc. The residue could not be crystallized, even on seeding with synthetic dihydroilludin M. λ_{max} 256 nm τ 3·98 (broad singlet, olefinic H); 4·17 (singlet, 20H); 5 25 (broad, 2 CH(OH); 8·68 (singlet OH); 8·14, 8 52, 8·73; 8 81 (singlets, 4-CH_3) and 8·84–9·52 (broad multiplet, about 4-cyclopropane H).

Oxidation of natural dihydroilludin M with sodium periodate. Production of the Ketoaldehyde (III). When this was done under the same conditions[2] as described for oxidation of synthetic Ia, the starting material was unchanged; even when the reaction time was increased from 1 hr to 24 hr the reaction was not complete. Oxidation in MeOH proved effective. About 7 mg of natural I and 20 mg of $NaIO_4$ in 4 ml of 50% MeOH stood at room temp. overnight. The solution was extracted with EtOAc. Evaporation of the solvent left a light yellow residue with λ_{max} 228 and 286 nm and R_f 0·68 in $CHCl_3$–MeOH (19·1), identical with that of the keto-aldehyde obtained from synthetic Ia.[2]

Hydrogenation of I. Production of IV. A solution of 100 mg synthetic Ia in 20 ml EtOAc was hydrogenated in the presence of 5% Pd/C until uptake of H_2 stopped (1 hr). The product, a light yellow semi-solid, was purified by preparative TLC using $CHCl_3$–MeOH (19.1). The main component, R_f 0·3 had λ_{max} 240 nm and an IR spectrum identical with that of the corresponding hydrogenation product (IV) from illudin M[3], which also had the same R_f in two different systems (EtOAc–ligroin (3:1), R_f 0·48, and $CHCl_3$–MeOH (19:1).

The results were exactly the same when natural Ia was hydrogenated under the same conditions.

The optical rotation for all three compounds was the same within the experimental error:

IV from synthetic Ia (0·99% in EtOH) $[\alpha]_D^{20} = -36$
IV from natural Ia (0·51% in EtOH) $[\alpha]_D^{20} = -35$
IV from IIa (1·18% in EtOH) $[\alpha]_D^{20} = -39$

The IR spectra of the corresponding hydrogenation products (IV) of illudin M, and of natural and synthetic dihydroilludin M were superimposable

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