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Structure and absolute configuration of new acetylenic compounds isolated from cultures of *Clitocybe catinus*

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Abstract

Investigations of the extracts of a culture of *Clitocybe catinus* gave rise to the isolation of new acetylenic diols **1**–**3**. Their structure was determined on the basis of ^{1}H- and ^{13}C-NMR evidence and the absolute configuration elucidated by means of the modified Mosher's method.

Keywords: *Clitocybe catinus*; Basidiomycetes; Acetylene diols; Structural elucidation; Absolute configuration

1. Introduction

In the course of a screening program for biologically active metabolites from Basidiomycetes, we have studied many strains of *Clitocybe spp*. and reported the isolation of large number of protoilludane sesquiterpenoids from *C. elegans*, *C. candicans* and *C. illudens* (Nasini & Vajna de Pava, 1997), clavilactones from *C. clavipes* (Arnone, Cardillo, Meille, Nasini & Tolazzi, 1994) allenes from C. eucalyptorum (Arnone, Cardillo, Nasini & Vajna de Pava, 1993). Further investigation of the *Clitocybe* genus led to the isolation from a strain of *Clitocybe catinus* (Fr.) Quél. of three new acetylenes **1**–**3**; in the present paper, we describe their isolation, structural determination and assignment of the absolute configuration.

2. Results and discussion

The ethyl acetate extracts of the mycelial cultures of the fungus were chromatographed on silica gel and purified on preparative TLC (see Section 3) to afford compounds **1**–**3**; the IR spectra of all compounds showed absorptions at 3310–3360 and at 2220 cm^{-1} indicating the presence of hydroxyl groups and acetylenic functions, respectively.

The ^{1}H spectrum of compound **1** (see Section 3) revealed the presence of two sequences such as –C(1)H_2OH and C(6)H_3–C(5)H_2–C(4)HOH, the structures of which were readily determined by the observed geminal and vicinal coupling constants. The ^{13}C-NMR spectrum of **1** confirmed the ^{1}H-NMR data showing four signals attributable to one methyl, two methylene and one methine carbons; in addition, it presented two quaternary carbons at 86.60 and 83.03 whose chemical-shift values suggested that they are part of a –C(2)≡C(3)– group. Consistent with the latter assignment was the formation of the *cis* alkene derivative **4** ($J_{2,3}$ = 11.2 Hz) by reduction of **1** with hydrogen over Lindlar palladium.

At this point, it was only sufficient to link C-2 and C-3 with C-1 and C-4 to obtain the gross structure of **1**. The absolute configuration was then determined by applying the modified Mosher's method (Ohtani, Kusumi, Ishitsuku & Kakisawa, 1989) to the (S)- and (R)-MTPA (α-methoxy-α-trifluoromethyl)phenylacetic acid diesters **5** and **6** obtained by reacting **4** with the corresponding (−) and (+) MTPA acids. In fact, the $\Delta\delta$ values ($\delta_S - \delta_R$) depicted in Fig. 1 permitted us to assign as S, the chirality at C-4 because the protons on the left hand side of the MTPA plane shown in Fig. 2

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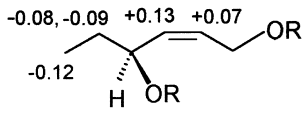
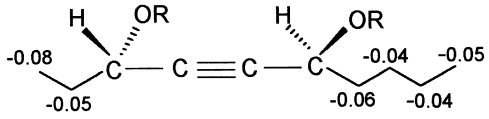


5 R = S (-) MTPA

6 R = R (+) MTPA



7 R = S (-) MTPA

8 R = R (+) MTPA

Fig. 1. Differences of the proton chemical shifts ($\Delta\delta = \delta_S - \delta_R$) of MTPA Mosher ester derivatives of compounds **4** and **2**.

had all negative numbers as a consequence of the shielding effect exerted by the phenyl ring of the esterifying acid.

The ^{1}H-NMR spectrum of compound **2** showed signals corresponding to 18 protons. The resonances at 3.63, 4.20 and 4.33, which were absent after the addition of D_2O to the sample were assigned to three hydroxy protons, while the analysis of the remaining protons indicated the presence of the C(1)H_2OH–C(2)H_2–C(3)HOH and C(10)H_3–C(9)H_2–C(8)H_2–C(7)H_2–C(6)HOH moieties. The ^{13}C-NMR spectrum showed 10 resonances; eight of them were attributable to one methyl, five methylene and two methine carbon atoms, while the two carbon atoms resonating at 86.63 and 85.01 (C-4 and -5) were indicative of a disubstituted acetylenic group. Thus, to complete the structure of **2**, we had only to connect C-4 and C-5 with C-3 and C-6.

Compound **3** presented ^{1}H- and ^{13}C-NMR spectra very similar to those exhibited by **2**, the only relevant difference being the presence in **3** of a methyl group in place of the CH_2OH unit. The absolute configuration of the metabolite was determined by applying the Mosher's method to the (S) and (R)-MTPA acetylenic derivatives **7** and **8** because of the difficulty to obtain the vinylic derivatives of **2** and **3** or to assign the NMR resonances of the corresponding fully reduced products. The negative values reported in Fig. 1 established that C-3 and C-6 possess the S configurations because all the protons disposed at the left hand side of the MTPA planes depicted in Fig. 2 underwent, as in derivative **5**, the shielding effect exerted by the phenyl rings. Compound **2** probably is an oxidation product of **3** and therefore, has the same absolute configuration.

Acetylenic functions in Basidiomycetes may derive from fatty acids by a series of dehydrogenations and β-oxidations. The most common chain lengths are C_9 and C_{10}, and an oxygenated function is present at one end of the chain. Genus *Clitocybe* produces some polyacetylenes active as inhibitors of fungi and of seed germination as *C. rhizophora* that produces an acetylenic C_9 triol and a related keto diol (Jones, Lowe & Lowe, 1964). C_{13} acetylenic antibacterial and antifungal compounds, containing an aromatic ring as peniophorin B were obtained from *Peniophora affinis* (Gerber, Shaw & Lechevalier, 1980) and as velutine from *Phanerochaete velutina* (Arnone, Nasini & Vajna de Pava, 1995).

Compounds **2** and **3** showed antibacterial activity (50 μg per disk) against *Bacillus subtilis* and *B. cereus*

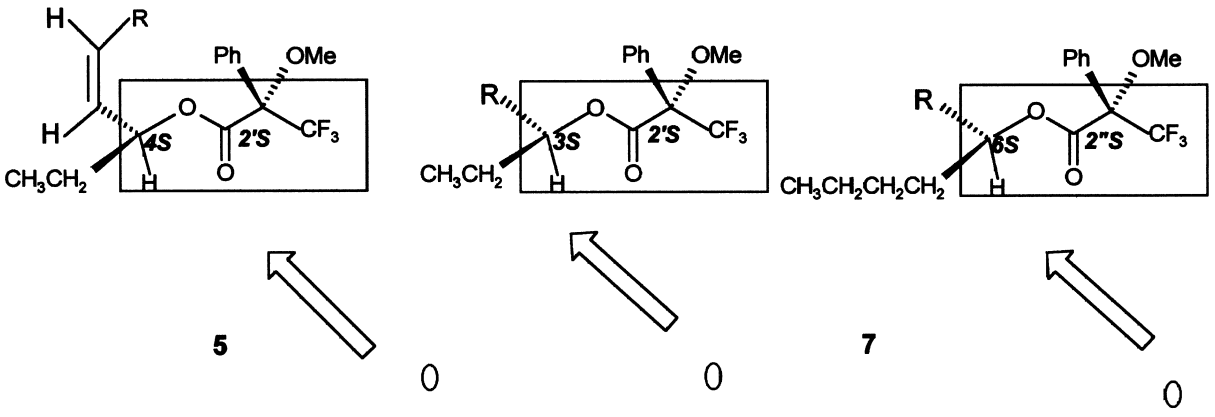
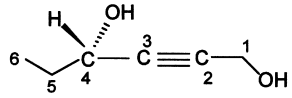
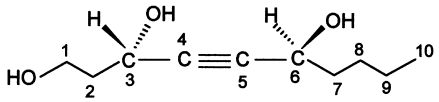


Fig. 2. MTPA planes for the (S)-MTPA esters used to assign the absolute configurations at C-4 for **1** and at C-3 and C-6 for **3**.

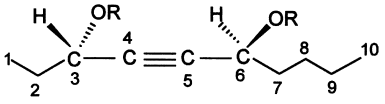
but not against *Escherichia coli*, *Saccharomyces cerevisiae*, or *Cladosporium cladosporioides*.



1



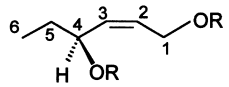
2



3 R = H

7 R = S (-) MTPA

8 R = R (+) MTPA



4 R = H

5 R = S (-) MTPA

6 R = R (+) MTPA

Block

3. Experimental

Flash CC was performed with Merck silica gel (0.04–0.06 mm) and TLC with Merck HF_{254} and RP-18 F_{254}. IR were determined on Perkin–Elmer 177 spectrophotometer; MS on a Finnigan-MAT-TSQ70 spectrometer; optical rotations on a JASCO-500 DIP-181 polarimeter. NMR spectra were recorded on a Bruker AC 250L spectrometer operating at 250.1 MHz for ^{1}H- and 62.9 MHz for ^{13}C-NMR; chemical shifts are in ppm (δ) from TMS as internal standard.

3.1. Cultivation of the fungus and isolation of compounds *1*–*3*

The strain *Clitocybe catinus* CBS 670.87 (Centraal Bureau vor Schimmelcultures) was inoculated in 10 Roux flasks containing MPGA (100 ml) (malt extract–peptone–glucose–agar 20:5:20:15 g/l) with a mycelium suspension. After 4 weeks growth at 24°C, the flasks were extracted with EtOAc containing 1% of MeOH. The combined extracts were dried (Na_2SO_4) and evaporated to give a crude extract 0.400 g. The mixture was chromatographed on a silica gel column using CH_2Cl_2–MeOH (gradient) as eluant, and purified on preparative TLC (PLC) with the same eluant to yield acetylenic compounds **1** (30 mg), **2** (70 mg) and **3** (90 mg) identified by means of ^{1}H- and ^{13}C-NMR analyses.

3.2. Compound *1*

Oil. $[\alpha]_D -5.2°$ (MeOH; *c* 0.1); CIMS *m/z* 97 [MH^+-18]; HRMS *m/z*: found 114.145 [M^+] ($C_6H_{10}O_2$ requires 114.144). IR ν_{max} cm^{-1}: 3320, 2220, 1015. ^{1}H-NMR (acetone-d_6) δ: 4.25 (1H, *dtt*, *J* = 5.6, 6.2 and 1.7 Hz, H-4), 4.19 (2H, *dd*, *J* = 6.0 and 1.7 Hz, H_2-1), 4.09 (1H, *d*, *J* = 5.6 Hz, OH-4), 4.01 (1H, *t*, *J* = 6.0 Hz, OH-1), 1.66 (1H, *ddq*, *J* = 13.5, 6.2 and 7.3 Hz, H-5a), 1.61 (1H, *ddq*, *J* = 13.5, 6.2 and 7.3 Hz, H-5b), 0.97 (3H, *t*, *J* = 7.3 Hz, H3-6). ^{13}C-NMR ($CDCl_3$) δ: 86.60 and 83.03 (*s*, C-2 and -3), 63.66 (*d*, C-4), 50.94 (*t*, C-1), 30.72 (*t*, C-5), 9.44 (*q*, C-6).

3.3. Compound *2*

Oil; $\{\alpha\}_D -5.8°$ (MeOH, *c* 0.1); found, C, 64.7; H, 9.6%; $C_{10}H_{18}O_3$ requires C, 64.5; H, 9.7. IR ν_{max} cm^{-1} 3358, 2220, 1050. CIMS, *m/z* 169 [MH^+-18]. ^{1}H-NMR [acetone-d_6 ($CDCl_3$)] δ: 4.55 (4.68) (1H, *dt*, *J* = 2.0 and 6.5 Hz, H-3), 4.33, 4.20 and 3.63 (4.13, 3.58 and 3.30) (3H, *br* signals, 3 × OH), 4.32 (4.36) (1H, *dt*, *J* = 2.0 and 6.5 Hz, H-6), 3.77 (3.96) (1H, *ddd*, *J* = 10.5, 6.2 and 6.0 Hz, H-1a), 3.70 (3.85) (1H, *dt J* = 10.5 and 6.0 Hz, H-1b), 1.85 (1.98)(1H, *dddd*, *J* = 13.5, 6.5, 6.2 and 6.0 Hz, H-2a), 1.83 (1.96) (1H, *ddt*, *J* = 13.5, 6.5 and 6.0 Hz, H-2b), 1.66 (1.71) (1H, *ddt*, *J* = 13.0, 9.0 and 6.5 Hz, H-7a), 1.61 (1.67) (1H, *dddd*, *J* = 13.0, 7.8, 7.0 and 6.5 Hz, H-7b), 1.5–1.2 (1.5–1.2) (4H, *m*, H_2-8 and -9), 0.90 (0.91) (3H, *t*, *J* = 7.3 Hz, H_3-10). ^{13}C-NMR ($CDCl_3$) δ: 86.63 and 85.01 (*s*, C-4 and -5), 62.28 and 61.22 (*d*, C-3 and -6), 60.08 (*t*, C-1), 38.90 (*t*, C-2), 37.38, 27.38 and 22.38 (*t*, C-7, -8 and -9), 14.00 (*q*, C-10).

3.4. Compound *3*

Oil; $[\alpha]_D$-6.2 (MeOH, *c* 0.1); found, C, 70.7; H, 10.5%; $C_{10}H_{18}O_2$ requires C, 70.5; H, 10.7. IR ν_{max} cm^{-1} 3310, 2220, 1010; CIMS, *m/z* 153 [MH^+-18]. ^{1}H-NMR ($CDCl_3$): 4.36 (1H, *dt*, *J* = 1.8 and 6.7 Hz, H-6), 4.32 (1H, *dt*, *J* = 1.8 and 6.6 Hz, H-3), 3.25 (2H, *br* signal, 2 × OH), 1.71 and 1.67 (2H, *m*, H_2-7), 1.67 (2H, *m*, H2-2), 1.5-1.2 (4H, *m*, H_2-8 and -9), 1.00 (3H, *t*, *J* = 7.5 Hz, H_3-1), 0.91 (3H, *t*, *J* = 7.2 Hz H_3-10). ^{13}C-NMR ($CDCl_3$) δ: 86.07 and 85.66 (*s*, C-4 and -5), 63.55 and 62.28 (*d*, C-3 and -6), 37.73, 27.38 and 22.38 (*t*, C-7, -8 and -9), 30.73 (*t*, C-2), 14.00 (*q*, C-10), 9.53 (*q*, C-1).

3.5. Compound **4**

A mixture of compound **1** (50 mg) and Lindlar catalyst (5% palladium on calcium carbonate) in ethanol (5 ml) was stirred under hydrogen for 6 h, the catalyst was filtered off and the residue was purified by preparative TLC using CH_2Cl_2–methanol (15/1) as eluant to yield 30 mg of compound **4**. ^{1}H-NMR ($CDCl_3$) δ: 5.75 (1H, *dddd*, J = 11.2, 7.5, 5.7 and 1.2 Hz, H-2), 5.55 (1H, *dddd*, J = 11.2, 8.1, 1.3 and 1.2 Hz, H-3), 4.38 (1H, *dddd*, J = 8.1, 7.2, 6.2 and 1.2 Hz, H-4), 4.33 (1H, *ddd*, J = 13.0, 7.5 and 1.3 Hz, H-1a), 4.12 (1H, *ddd*, J = 13.0, 5.7 and 1.2 Hz, H-1b), 3.80 (2H, *br* signal, 2 × OH), 1.64 (1H, *ddq*, J = 13.8, 6.2 and 7.4 Hz, H-5a,), 1.53 (1H, *ddq*, J = 13.8, 7.2 and 7.4 Hz, H-5b), 0.91 (3H, *t*, J = 7.4 Hz, H3-6).

3.6. Compounds **5**, **6**, **7**, **8**

To two solutions of compounds **2** or **4**, respectively (10 mg) in CH_2Cl_2 (2 ml) containing DMAP (few crystals) and DCC (30 mg), (S)-(−) MTPA and (R)-(+)-MTPA were added, respectively. Each mixture was stirred at room temperature for 6 h, and the products (**5**, **6**, **7**, **8**) were purified by preparative TLC using hexane–EtOAc (2:1) as eluant.

3.7. Compound **5**

^{1}H-NMR ($CDCl_3$) δ: 7.6–7.3 (10 H, *m*, ArH), 5.80 (1H, *br ddd*, J = 10.2, 7.4, and 6.5 Hz, H-2), 5.65 (1H, *br ddd*, J = 9.5, 7.0 and 6.0 Hz, H-4), 5.62 (1H, *br dd*, J = 10.2 and 9.5 Hz, H-3), 5.10 (1H, *ddd*, J = 13.0, 7.4 and 1.3 Hz, H-1a), 4.95 (1H, *br dd*, J = 13.0 and 6.5 Hz, H-1b), 3.54 and 3.51 (6H, *q*, J = 1.2 Hz, 2 × OMe), 1.69 (1H, *ddq*, J = 13.9, 7.0 and 7.4 Hz, H-5a), 1.57 (1H, *ddq*, J = 13.9, 6.0 and 7.4 Hz, H-5b), 0.80 (3H, *t*, J = 7.4 Hz, H_3-6).

3.8. Compound **6**

^{1}H-NMR ($CDCl_3$) δ: 7.6–7.3 (10 H, *m*, ArH), 5.73 (1H, *dddd*, J = 11.0, 7.1, 6.7 and 1.0 Hz, H-2), 5.61 (1H, *dddd*, J = 9.5, 7.0 and 6.0 and 1.0 Hz, H-4), 5.49 (1H, *dddd*, J = 11.0, 9.5, 1.5 and 1.3 Hz, H-3), 5.07 (1H, *ddd*, J = 13.0, 7.1 and 1.5 Hz, H-1a), 4.97 (1H, *br ddd*, J = 13.0, 6.7 and 1.3 Hz, H-1b), 3.55 and 3.52 (6H, *q*, J = 1.2 Hz, 2 × OMe), 1.77 (1H, *ddq*, J = 14.0, 7.0 and 7.4 Hz, H-5a), 1.66 (1H, *ddq*, J = 14.0, 6.0 and 7.4 Hz, H-5b), 0.92 (3H, *t*, J = 7.4 Hz, H_3-6).

3.9. Compound **7**

^{1}H-NMR ($CDCl_3$) δ: 7.6–7.3 (10H, *m*, ArH), 5.58 (1H, *dt*, J = 1.7 and 6.7 Hz, H-6), 5.54 (1H, *dt*, J = 1.7 and 6.6 Hz, H-3), 3.58 (6H, *q*, J = 1.2 Hz, 2 × OMe), 1.83 (2H, *dq*, J = 6.6 and 7.4 Hz, H_2-2), 1.78 (2H, *ddd*, J = 7.2, 6.7 and 3.8 Hz, H_2-7), 1.38 (2H, *m*, H_2-8), 1.32 (2H, *m*, H_2-9), 0.95 (3H, *t*, J = 7.4 Hz, H_3-1), 0.86 (3H, *t*, J = 7.2 Hz, H_3-10).

3.10. Compound **8**

^{1}H-NMR ($CDCl_3$) : δ 7.6–7.3 (10H, *m*, ArH), 5.56 (1H, *dt*, J = 1.7 and 6.7 Hz, H-6), 5.52 (1H, *dt*, J = 1.7 and 6.6 Hz, H-3), 3.54 (6H, *q*, J = 1.2 Hz, 2 × OMe), 1.88 (2H, *dq*, J = 6.6 and 7.4 Hz, H_2-2), 1.84 (2H, *ddd*, J = 6.8, 6.7 and 4.5 Hz, H_2-7), 1.42 (2H, *m*, H_2-8), 1.36 (2H, *m*, H_2-9), 1.03 (3H, *t*, J = 7.4 Hz, H_3-1), 0.91 (3H, *t*, J = 7.2 Hz, H_3-10).

3.11. Biological tests

Antibacterial and antifungal activity were tested with paper disks (6 mm diameter), soaked with metabolites **2** and **3** (100 and 50 g dissolved in $CHCl_3$–MeOH, 2:1), and placed in a suitable culture medium on Petri dishes with *Escherichia coli* (ATCC 10586) *Bacillus subtilis* (ATCC, 6633), *Bacillus cereus* (ATCC 1072), *Saccharomyces cerevisiae* (NCYC 729), *Cladosporium cladosporioides* (IPV F 167) as test microorganisms.

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