Secondary Mould Metabolites. Part 31.[1] Isolation and Structure Elucidation of Illudins A and B, and Illudalenol, New Sesquiterpenoids from *Clitocybe illudens*

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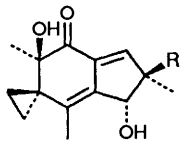
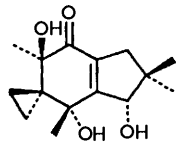
The structure and relative configuration of the three novel sesquiterpenes illudin A **4**, illudin B **5** and illudalenol **6**, which were isolated from cultures of *Clitocybe illudens* (= *Omphalotus olearius*), have been determined on the basis of ^{1}H NMR, ^{13}C NMR and 2D NMR (^{1}H–^{13}C HETCOR and ^{1}H–^{13}C COLOC) studies to be (2*S**,4*S**)-2,4-dihydroxyillud-5(9)-en-1-one, (2*R**,4*S**,6*S**,8*R**)-2,4,6,8-tetrahydroxyillud-5(9)-en-1-one and 4,12-dihydroxyilludala-2,5(9)-dien-1-one, respectively.

Clitocybe illudens, called Jack-O'-lantern mushroom because of its bioluminescent property, is commonly found in large clusters; when grown in liquid culture, it produces toxic sesquiterpenoids which possess antibacterial and antitumour activity.[2] The structure of the toxic metabolite illudin M **1** was first reported by McMorris and Anchel together with that of the inactive illudol;[3] two other compounds, illudin S **2** from *Lampteromyces japonicus*[4] and 4α-hydroxydihydroilludin M **3** from *C. illudens*,[5] have also been isolated. Interestingly the chemical and many of the morphological characteristics of *C. illudens* suggest that it does not belong to the genus *Clitocybe* (a genus that normally produces polyacetylenes but not protoilludane-related sesquiterpenoids) and it has been renamed *Omphalotus olearius*.[6] In contrast, in our previous work on sesquiterpenoids of Basidiomycete fungi, we have isolated, from cultures of *C. elegans*,[7] melledonals D and E, $\Delta^{2,3}$-protoilludene orsellinate esters and, from *C. candicans*,[8] candicansol, having the illudalane skeleton, and 3-*epi*-illudol.

A re-investigation of the fungus *C. illudens* (= *O. olearius*) was planned for two reasons: first to obtain a sample of illudol for comparison with our 3-*epi*-illudol[8] and second to isolate



1 R = Me
2 R = CH_2OH



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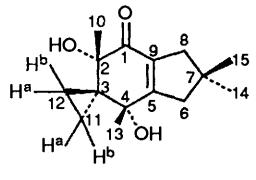

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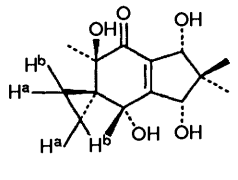

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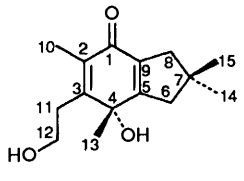

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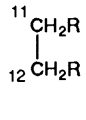
illudin M **1** for antitumour tests in view of its current interest as a possible chemotherapeutic agent.[9]

Our strain of *C. illudens* (CBS 164) was cultivated in agar cultures in a malt–peptone–glucose medium for one month. Under these conditions only illudin M **1**, illudalic acid, and illudoic acid[2] were isolated. A different metabolism was found when the fungus was grown in still cultures; extraction of the broth with EtOAc, after two months of growth, gave, besides illudin M and illudalic acid, a series of new metabolites, the illudine-type sesquiterpenoids **4** and **5**, for which we propose the names illudin A and illudin B, and the new illudalane derivative **6**. The present paper gives the full experimental evidence that led to the structural and stereochemical assignments of these new products.

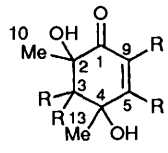
Illudin A **4** was isolated as a white solid, m.p. 82–84 °C; $[\alpha]_D$ −15° (*c* 0.1, $CHCl_3$), and analysed for $C_{15}H_{22}O_3$ (M^+, 250); chemical ionization mass spectroscopy gave a distinct peak at *m/z* 251 (MH^+) and a fragment was found at *m/z* 233 [(MH^+) − H_2O; base peak] due to the ready loss of water; in addition, strong peaks were observed at *m/z* 215 [(MH^+) − $2H_2O$] and 205. The IR spectrum ($CHCl_3$) exhibited absorptions at 3460 and 1670 cm^{-1}, due to hydroxy and unsaturated carbonyl groups, and the UV spectrum [λ_{max} 250 nm (ε 7700)] suggested the presence of a conjugated system.

The broad-band ^{1}H-decoupled ^{13}C NMR spectrum of compound **4** showed the presence of 15 carbon resonances (Table 1), the multiplicities of which were determined using the DEPT sequence.[10] Three of them (C-1, C-5 and C-9) were assigned to a fully substituted α,β-unsaturated ketonic moiety on the basis of their chemical-shift values[11] while the remaining signals were assigned to four methyl, four methylene and four quaternary (two of them, C-2 and C-4, oxygen-bearing) sp^3-hybridized carbon atoms. Moreover, the chemical-shift values and the magnitude[12] of the one-bond (C,H) couplings exhibited by C-11 and C-12 in the fully ^{1}H-coupled ^{13}C NMR spectrum of illudin A **4** indicated that they are part of a cyclopropane ring [δ_C 5.98, ^1J(CH) 162 Hz; δ_C 4.60, ^1J(CH) 163 Hz]. Accordingly, the ^{1}H NMR spectrum of illudin A **4** (Table 2) showed the presence of four tertiary methyl groups (10-, 13-, 14- and 15-H_3), an ABCD-like spin system attributable to the C(11)H_2–C(12)H_2 fragment **A**, two isolated methylene groups (6- and 8-H_2), and two aliphatic hydroxy groups which, through necessity, must be placed at C-2 and C-4).

The ^{1}H–^{13}C heteronuclear correlation (HETCOR) spec-



A



B

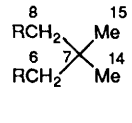


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Table 1 ^{13}C NMR data for compounds **4–6** in [$^2H_6$]acetone

	4			**5**			**6**		
Carbon	δ_C		^1J(CH) (Hz)	δ_C		^1J(CH) (Hz)	δ_C		^1J(CH) (Hz)
1	199.90 (199.17)[a]	S		202.13	S		184.48	S	
2	75.68 (75.36)	S		75.30	S		132.14	S	
3	36.10 (35.71)	S		33.89	S		157.47	S	
4	69.98 (69.69)	S		71.42	S		70.03	S	
5	167.34 (165.88)	S		160.71	S		164.81	S	
6	47.94 (47.32)	T	129	81.89	D	145	47.98	T	128
7	38.18 (37.64)	S		49.80	S		38.10	S	
8	45.10 (44.80)	T	131	78.82	D	147	45.37	T	130
9	133.07 (133.06)	S		135.27	S		134.11	S	
10	25.99 (25.79)	Q	128	27.22	Q	128	11.42	Q	127
11	5.98 (6.00)	T	162	3.93	T	162.5	33.05	T	127.5
12	4.60 (4.90)	T	163	7.15	T	162	61.30	T	143.5
13	25.10 (24.53)	Q	127	24.28	Q	127	26.90	Q	129
14	29.38[b] (28.88)	Q	125	16.12	Q	127	29.73[b]	Q	125
15	29.58[b] (29.23)	Q	125	25.25	Q	125	29.69[b]	Q	125

[a] Values in parentheses are chemical shifts in [$^2H_6$]benzene. [b] Assignments within each column may be interchanged.

Table 2 ^1H NMR chemical shifts (δ_H) and ^1H–^1H coupling constants (J/Hz) for compounds **4–6** in [$^2H_6$]acetone

Proton	**4**		**5**	**6**	J	**4**[d]	**5**	**6**
6α	2.64	(2.48)[a]		2.52	6α,6β	18.0		17.6
6β	2.47	(2.07)	4.52	2.64	6α,8α	1.9		1.9
8α	2.39[b]	(2.34)		2.39[b]	6α,8β	1.9		1.8
8β	2.29[b]	(2.30)	4.44	2.34[b]	6β,8α	2.0		1.9
10	1.29	(1.23)	1.50	1.85	6β,8β	1.6	1.4	1.9
11a	0.78	(1.00)	0.64	2.76	8α,8β	15.7		15.8
11b	0.81	(0.72)	0.69	2.76	10,11			0.6
12a	0.58	(0.77)	0.83	3.75	11a,11b	3.8	4.3	*e*
12b	0.51	(0.45)	0.45	3.75	11a,12a	9.6	9.6	*e*
13	1.40	(1.12)	1.24	1.37	11a,12b	6.1	5.8	*e*
14	1.12[c]	(0.94)	0.98	1.13	11b,12a	5.9	6.1	*e*
15	1.11[c]	(0.89)	1.14	1,11	11b,12b	9.7	9.5	*e*
2-OH	4.17	(4.04)[b]	3.87		12a,12b	4.2	3.9	*e*
4-OH	4.01	(2.94)[b]	3.76	4.77	6β,6-OH		6.1	
6-OH			4.77		8β,8-OH		5.5	
8-OH			4.00		12,12-OH			5.1
12-OH				4.33	13,4-OH	0.6		

[a] Values in parentheses are chemical shifts in [$^2H_6$]benzene. [b,c] Assignments within each column may be interchanged. [d] The J-value relative to the protons of the cyclopropane ring are in [$^2H_6$]benzene + 2 drops of [$^2H_6$]acetone. [e] Not assigned.

trum[13] of illudin A **4** identified the specific resonances associated with each protonated carbon while long-range ^1H–^{13}C heteronuclear correlation (COLOC) spectra[14] carried out in [$^2H_6$]acetone and [$^2H_6$]benzene (Figs. 1 and 2) were used to detect (C, H) couplings through two and three bonds. In [$^2H_6$]acetone the 14- and 15-methyl protons overlapped partly, thus not permitting us to assign individually their correlations with the adjacent carbons. By acquiring the COLOC spectrum in [$^2H_6$]benzene these protons could be resolved, as evidenced in Figure 2. The COLOC spectra showed that the methyl protons at C-10 couple with the quaternary C-1, C-2 and C-3 carbons and the methyl protons at C-13 couple with C-3, C-4 and C-5. These correlations, together with the above mentioned presence of a $>C(5)=C(9)-C(1)=O$ moiety in illudin A **4**, are consistent with fragment **B**. Similar correlations between both sets of methyl protons at C-14 and C-15 and C-6, C-7 and C-8 pointed to the presence of fragment **C**. At this point we had only to link C-11 and C-12 with C-3 to form the expected cyclopropane ring, and then C-5 with C-6, and C-8 with C-9, to obtain the gross structure **4** for illudin A.

The NOE difference spectra carried out on illudin A **4** (Table 3) agreed with the proposed structure and allowed determination of the relative configuration at C-2 and C-4. In fact, the NOEs observed between 10- and 13-H_3, and *vice versa*, (2 and 1.5%) indicated that these two methyl groups are *cis*-disposed.

Illudin B **5** was obtained as a glassy solid, m.p. 62–65 °C; $[\alpha]_D$ −9° (*c* 0.1, $CHCl_3$); elemental analysis and CI mass spectroscopy indicated the formula $C_{15}H_{22}O_5$ (MH^+, 283); the IR and UV spectra of compound **5** were similar to those exhibited by illudin A **4**, thus suggesting the presence of hydroxy and conjugated carbonyl groups.

Comparison of the ^1H and ^{13}C NMR spectra of compounds **4** and **5** (Tables 2 and 1) indicated that the gross structure of illudin B **5** differs from that of illudin A **4** in that one proton at C-6 and one at C-8 have been replaced by hydroxy groups. Specifically, the ^1H NMR spectrum of illudin B **5** showed the presence of two AM spin systems attributable to CHOH groupings, and the absence of the resonances which have been assigned in illudin A **4** to 6- and 8-H_2. Similarly, the ^{13}C NMR spectrum of illudin B **5** contained two resonances characteristic of oxygen-bearing methine carbon atoms (C-6 and C-8) whereas no such resonances were present in the spectrum of illudin A **4**. In addition, the values of 162.5 and 162 Hz observed for the ^1J(CH) of the carbons resonating at δ_C 3.93 and 7.15 (C-11 and C-12) confirmed the presence of a cyclopropane ring. The COLOC spectrum depicted in Fig. 3 showed the same

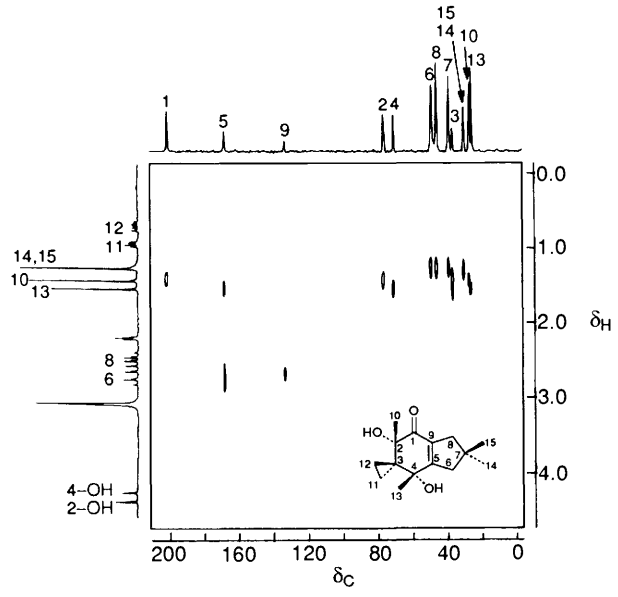


Fig. 1 COLOC spectrum of illudin A **4** in [$^2H_6$]acetone, optimized for the observation of long-range (C,H) couplings of *ca.* 8.3 Hz (D2 60 ms, D3 30 ms). A normal ^1H NMR spectrum is plotted along the vertical axis and the ^{13}C projection is on the horizontal axis.

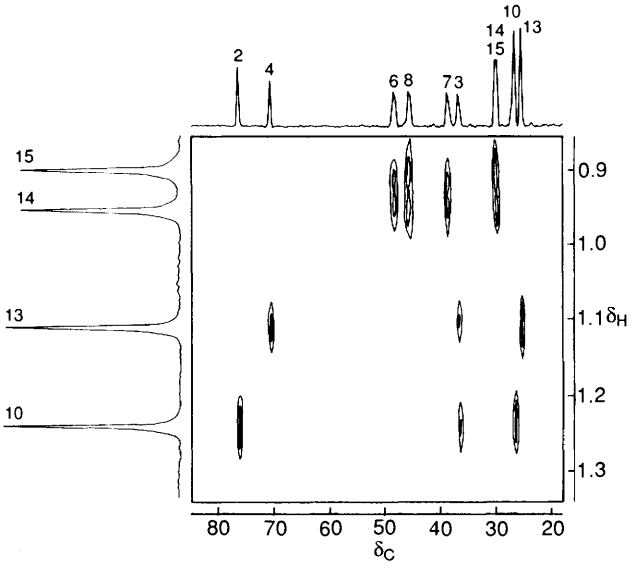


Fig. 2 Selected portion of the COLOC spectrum of illudin A **4** in [$^2H_6$]benzene, optimized for the observation of long-range (C,H) couplings of *ca.* 8.3 Hz (D2 60 ms, D3 30 ms). A normal ^1H NMR spectrum is plotted along the vertical axis and the ^{13}C projection is on the horizontal axis.

correlations observed in illudin A **4** between the 10- and 13-methyl protons and the C-1, C-2, C-3, C-4 and C-5 carbons, and other correlations between the 14- and 15-methyl protons and the C-6, C-7 and C-8 carbons. All these findings, together with the NOE difference results shown in Table 3, permitted us to assign structure **5** as the stereostructure of illudin B. The NOEs observed between 10-H_3, assumed as being α, and 4-OH (0.5%), and between 13-H_3 and 2-OH (0.5%) suggested that 10- and 13-H_3, contrary to what happens in illudin A **4**, are *trans*-disposed while the NOEs observed between 10- and 14-H_3, and *vice-versa*, (0.5 and 0.5%) imply that these two methyl groups are on the same, α-side of the molecule. Finally, the fact that NOEs of 6% were observed between 15-H_3 (β) and 6- and 8-H, and NOEs of 1% between 14-H_3 (α) and the same

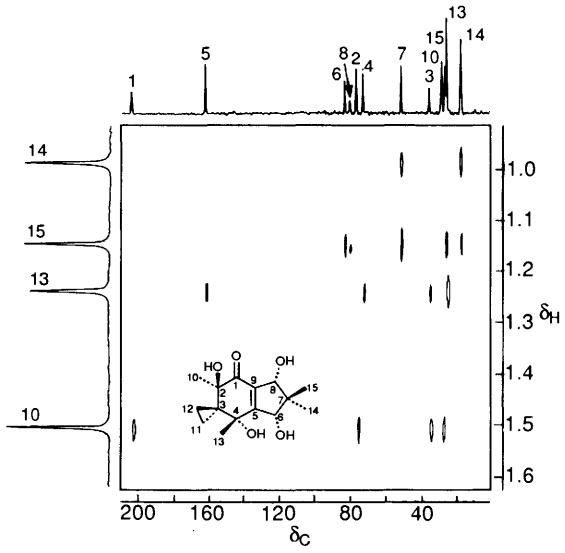
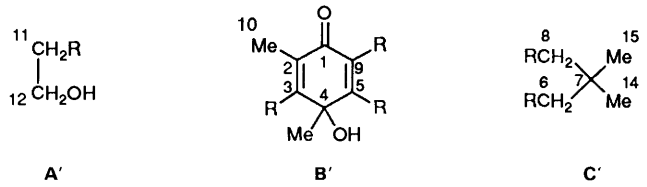


Fig. 3 Selected portion of the COLOC spectrum of illudin B **5** in [$^2H_6$]acetone, optimized for the observation of long-range (C,H) couplings of *ca.* 6.3 Hz (D2 80 ms, D3 40 ms). A normal ^1H NMR spectrum is plotted along the vertical axis and the ^{13}C projection is on the horizontal axis.

methine protons indicated that 6- and 8-H are β-disposed and hence that the geminal 6- and 8-OH groups are α-disposed.

The third metabolite, compound **6**, for which we propose the name illudalenol, belongs to the rare illudalane family. It was isolated as an oil, $[\alpha]_D$ + 6° (*c* 1.8, $CHCl_3$); the CI mass spectrum established the molecular formula of compound **6** as $C_{15}H_{22}O_3$ (MH^+, 251); the IR spectrum ($CHCl_3$) showed absorption bands at 3380 and 1630 cm^{-1} attributable to hydroxy and carbonyl groups, and the UV spectrum showed absorptions at 200, 245 and 290 nm (ε 6500, 8500 and 1900), thus indicating the presence, in illudalenol **6**, of a more conjugated chromophore than that in illudins A and B.

The broad-band ^1H-decoupled ^{13}C NMR spectrum of illudalenol **6** (Table 1), together with DEPT experiments, showed the presence of five resonances attributable to a fully substituted α,β,α′,β′-unsaturated ketonic moiety (C-1, C-2, C-3, C-9 and C-5), and ten signals attributable to four methyl, four methylene (one of them, C-12, oxygen-bearing), and two quaternary (one of them, C-4, oxygen-bearing) sp^3-hybridized carbon atoms. The ^1H NMR spectrum of illudalenol **6** (Table 2) confirmed and extended these findings through the appearance of four tertiary methyl groups (10-, 13-, 14- and 15-H_3), an AA′XX′Y spin system attributable to the C(11)H_2–C(12)H_2OH fragment **A′** ($^3J_{AX}$ + $J_{AX'}$ 14.5 and $^3J_{XY}$ = $^3J_{X'Y}$ = 5.1 Hz), an additional aliphatic hydroxy group which must be located at



A′ B′ C′

C-4, and two isolated methylene groups (6- and 8-H_2). The two- and three-bond correlations observed in the COLOC spectrum of illudalenol **6** depicted in Fig. 4 between the methyl protons at C-10 and the C-1, C-2 and C-3 carbons, and between the methyl protons at C-13 and C-3, C-4 and C-5 indicated the presence of fragment **B′**, while the correlations between the methyl protons at C-14 and C-15, and C-6, C-7 and C-8 are consistent with fragment **C′**. The fact that the methylene

Table 3 Selected connectivities established by NOE difference experiments in [$^2H_6$]acetone

Proton irradiated[a]	Proton affected (%) in compound 4	5	6
10	11a (1), 12a (3), 13 (2), 2-OH (3)	11a (2.5), 12a (1), 14 (0.5) 2-OH (1.5), 4-OH (0.5)	11 (2.5), 12 (2)
11			10 (2), 12 (6), 13 (1.5)
13	6α (1), 6β (3), 10 (1.5), 11b (1.5), 12b (4), 4-OH (3)	6 (5), 11b (5), 12b (8.5), 2-OH (0.5), 4-OH (1)	6α (1.5), 6β (3.5), 11 (3.5), 12 (1)
14	6α (4.5), 6β (4.5), 8α (4.5), 8β (4.5)	6 (1), 8 (1), 10 (0.5), 15 (1), 4-OH (0.5), 6-OH (1), 8-OH (0.5)	6α (3.5), 6β (3.5), 8α (3.5), 8β (3.5)
15		6 (6), 8 (6), 14 (1)	

[a] In compounds **4** and **6**, 14- and 15-methyl protons were irradiated together.

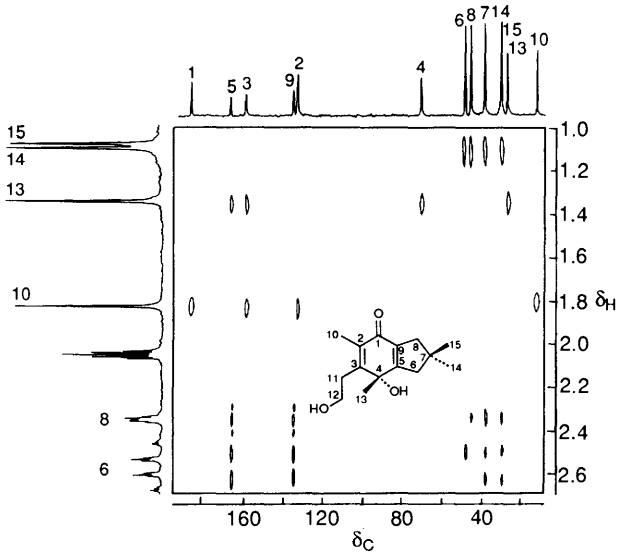
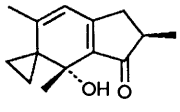


Fig. 4 COLOC spectrum of illudalenol **6** in [$^2H_6$]acetone + D_2O, optimized for the observation of long-range (C,H) couplings of *ca.* 8.3 Hz (D2 60 ms, D3 30 ms). A normal ^1H NMR spectrum is plotted along the vertical axis and the ^{13}C projection is on the horizontal axis.

protons at C-6 and C-8 were both coupled to C-5 and C-9 in the COLOC spectrum and that it was observed NOE enhancements between 10-H_3 and 11-H_2, and *vice versa*, (2.5 and 2%) (Table 3) and an homoallylic coupling of 0.6 Hz between these protons defined the mode of linkage between C-5 and C-6, C-8 and C-9, and C-3 and C-11, leading to structure **6**. Sesquiterpenoids with a cyclopropane ring, such as illudins M and S, have attracted much interest for their biological activity as alkylating agents. Recently, ptaquiloside, a norsesquiterpenoid with the illudane skeleton, was isolated from the edible bracken fern *Pteridium aquilinum*.[15] This compound was converted under mild alkaline conditions into the dienone **7**, which is regarded as the active form of ptaquiloside. In fact, the cyclopropane ring of compound **7** is highly reactive as an electrophile and reacts with amino acids, nucleotides and nucleosides.[15]



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Ptaquiloside is a potent carcinogen, whereas illudins S and M show antitumour activity. Recent studies[16] have shown that illudin M **1** is strongly cytotoxic toward human adenocarcinoma cells (IC_{50}; 6.8 μg cm^{-3} at 24 h). In both the above cases, the driving force for the opening of the cyclopropane ring under nucleophilic attack, *i.e.* alkylation of the biological substrate, is the consequent aromatization of the six-membered ring.[17,18] The lack of such a possibility, at least under mild conditions, may explain the very low, if any, antitumour activity (IC_{50} > 10 000 μg cm^{-3} in the same system) of compounds **4** and **5**.

That the cyclopropane ring in illudin A **4** maintains some reactivity is shown by the isolation of illudalenol **6**. The hypothesis that this compound is an artifact of the isolation procedure is unlikely, because (i) the extraction was performed under mild conditions, (ii) the compound was always found in different fermentation batches, (iii) no corresponding ring-opened compound deriving from illudin M, certainly more easy to form, is present in the mixture.

Work is in progress to identify other minor metabolites from the same source.

Experimental

M.p.s were measured on a Kofler apparatus and are uncorrected. UV spectra were measured for solutions in 95% EtOH on a JASCO Uvidec-510 spectrophotometer. IR spectra were recorded with a Perkin-Elmer 177 instrument. TLC and PLC were performed with Merck HF_{254} silica gel. Optical rotations were measured on a JASCO DIP-181 polarimeter. Mass spectra were taken on a VG-ZAB2 instrument at 70 eV. NMR spectra were recorded on a Bruker AC 250L operating at 250.1 MHz for ^1H and 62.9 MHz for ^{13}C. Chemical shifts are in ppm (δ) from $SiMe_4$ as internal standard. NOE difference spectra were obtained by subtracting alternatively right-off resonance-free induction decays (FIDs) from right-on resonance-induced FIDs. NOE-values reported in the text have only qualitative significance. DEPT, HETCOR, and COLOC spectra were performed using the DEPT,[10] XHCORRDC,[13] and COLOC[14] pulse sequences of the AC 250L software. Owing to the complexity of the purification procedure, we report the R_f-values in hexane–EtOAc (1:1) and CH_2Cl_2–MeOH (15:1), respectively.

Isolation and Purification of Metabolites **4**–**6**.—A strain of *Clitocybe illudens* Sacc. [= *Omphalotus olearius* Singer (CBS 164.51)] received from Centraal Bureau voor Schimmel Cultures-Baarn, was maintained on MPGA (malt, peptone, glucose, agar; 20:4:30:15 g dm^{-3}) slants and sub-cultured in 80 stationary Erlenmeyer flasks (250 cm^3) containing a liquid medium MPG (50 cm^3) for 8 weeks at 24 °C; the culture filtrates separated from mycelium were extracted twice with EtOAc and the extracts were dried (Na_2SO_4) and evaporated to yield a mixture (1.6 g) of sesquiterpenoids. The mixture was chromatographed on a column of flash silica gel with hexane–

EtOAc (2:1) as eluent, and purified further by PLC with CH_2Cl_2–MeOH (30:1 and/or 15:1) to yield the major metabolites in the following order of decreasing R_f-value: illudin A **4** (10 mg), illudalic acid (120 mg), illudin M **1** (100 mg), illudoic acid (3 mg), illudalenol **6** (15 mg), and illudin B **5** (18 mg).

Illudin A **4** R_f 0.6 and 0.5 (Found: C, 71.7; H, 8.7. $C_{15}H_{22}O_3$ requires C, 71.97; H, 8.86%); ^{13}C and ^1H NMR data are reported in Tables 1 and 2, respectively.

Illudin B **5**. This compound, isolated as an impure oil after PLC on silica gel, was obtained as a glassy solid by PLC on RP-18 plates developed with MeOH–water (2:1). R_f 0.15 and 0.3 (Found: C, 63.6; H, 7.7. $C_{15}H_{22}O_5$ requires C, 63.81; H, 7.85%); *m/z* (CI, isobutane) 283 (MH^+, 3%), 265 [(MH^+) − 18, 78], 247 [(MH^+) − 36, 100], 233 (75) and 219 (100); ^{13}C and ^1H NMR data are reported in Tables 1 and 2, respectively.

Illudalenol **6**. R_f 0.2 and 0.4 (Found: C, 71.7; H, 8.6. $C_{15}H_{22}O_3$ requires C, 71.97; H, 8.86%); *m/z* (CI, isobutane) 251 (MH^+, 100%), 233 [(MH^+) − 18, 68], 223 (12), 217 (15) and 205 (10); ^{13}C and ^1H NMR data are reported in Tables 1 and 2.

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References

1 Part 30. A. Arnone, G. Assante, G. Nasini and O. Vajna de Pava, *Phytochemistry*, 1990, **29**, 2499.
2 W. A. Ayer and L. M. Browne, *Tetrahedron*, 1981, **37**, 2199.
3 T. C. McMorris and M. Anchel, *J. Am. Chem. Soc.*, 1963, **85**, 831.
4 A. Achihara, H. Shirahama and T. Matsumoto, *Tetrahedron Lett.*, 1969, 3965.
5 A. P. W. Bradshaw, J. R. Hanson and P. B. Hitchcock, *Phytochemistry*, 1982, **21**, 942.
6 S. T. Carey, *Mycologia*, 1974, **66**, 951; R. B. Woodward and T. R. Hoye, *J. Am. Chem. Soc.*, 1977, **99**, 8007.
7 A. Arnone, R. Cardillo, V. Di Modugno and G. Nasini, *Gazz. Chim. Ital.*, 1988, **118**, 517.
8 A. Arnone, R. Cardillo, V. di Modugno and G. Nasini, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1995.
9 M. J. Kelner, T. C. McMorris, W. T. Beek, G. M. Zamora and R. Taetle, *Cancer Res.*, 1987, **47**, 3186.
10 D. M. Doddrell, D. T. Pegg and M. R. Bendall, *J. Magn. Reson.*, 1982, **48**, 323.
11 D. H. Marr and J. B. Stothers, *Can. J. Chem.*, 1965, **43**, 596.
12 C. S. Foote, *Tetrahedron Lett.*, 1963, 579.
13 A. Bax, *J. Magn. Reson.*, 1983, **53**. 517; V. Rutar, *J. Magn. Reson.*, 1984, **58**, 306; J. A. Wilde and P. H. Bolton, *J. Magn. Reson.*, 1984, **59**, 343.
14 H. Kessler, C. Griesinger, J. Zarbock and H. R. Loosli, *J. Magn. Reson.*, 1984, **57**, 331; H. Kessler, C. Griesinger and J. Lautz, *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 444.
15 M. Ojika, K. Wakamatsu, H. Niwa and K. Yamada, *Tetrahedron*, 1987, **43**, 5261.
16 Laboratori Farmitalia, Milano; unpublished results.
17 N. Morisaki, J. Furukawa, H. Kobayashi, S. Iwasaki, S. Nozoe and S. Okuda, *Chem. Pharm. Bull.*, 1987, **35**, 2678.
18 T. C. McMorris, M. J. Kelner, R. K. Chadha, J. S. Siegel, S. Moon and M. M. Moya, *Tetrahedron*, 1989, **45**, 5433.

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