Secondary Mould Metabolites. Part 29.[1] Isolation and Structure Elucidation of Candicansol, 3-*epi*-Illudol and 1-*O*-Acetyl-3-*epi*-illudol, Novel Sesquiterpenoids from *Clitocybe candicans*, and Absolute Configuration of 3-*epi*-Illudol

Alberto Arnone, Rosanna Cardillo, Vincenza Di Modugno, and Gianluca Nasini
Dipartimento di Chimica del Politecnico, Centro del C.N.R. per le Sostanze Organiche Naturali, Politecnico di Milano, Piazza L. da Vinci 32, I 20133 Milano, Italy

The structural elucidation of candicansol (**1a**), 3-*epi*-illudol (**2a**), and 1-*O*-acetyl-3-*epi*-illudol (**3**), novel illudalane and protoilludane sesquiterpenoids isolated from cultures of *Clitocybe candicans* is based on a study of their ^1H and ^{13}C n.m.r. spectral data. The absolute configuration of 3-*epi*-illudol is also reported.

Protoilludanes (*e.g.* illudol), illudanes (*e.g.* illudin-M and -S), illudalanes (*e.g.* illudalic acid), and marasmanes (*e.g.* marasmic acid) belong to a series of sesquiterpenoid metabolites produced by Basidiomycetes. These compounds, which usually show antibacterial activity, are produced by the fungi to eliminate their bacterial predators. They may be formed from a common protoilludyl cation[2] which, in turn, arises by cyclization of farnesylpyrophosphate *via* a humulene intermediate.

In the course of a programme aimed to identify new bioactive metabolites from Basidiomycetes, we reported the isolation of several $\Delta^{2,3}$-protoilludene sesquiterpenoid aryl esters from pure cultures of *Armillaria mellea*,[3–5] *A. novae zelandiae*,[6] and *Clitocybe elegans*;[5] the structure of sulcatine, a Δ^5-norprotoilludene-7,8-diol produced by a strain of *Laurilia sulcata*, was also elucidated.[7]

In the present study, a culture of *Clitocybe candicans*, a non-toxigenic fungus whose fruit-body is very rare,[8] was investigated. Extraction of the pure culture grown in a liquid medium (malt–peptone–glucose) with ethyl acetate, followed by flash chromatography on silica gel, led to the isolation of three main metabolites, designated candicansol (**1a**), 3-*epi*-illudol (**2a**), and 1-*O*-acetyl-3-*epi*-illudol (**3**). In this paper we present full evidence for the structure elucidation of these metabolites based mainly on data obtained by ^1H and ^{13}C n.m.r spectroscopy.

Candicansol (**1a**) was isolated as a pale-yellow solid, m.p. 56–58 °C, $[\alpha]_D$ +30° (*c* 1 in MeOH), and had an analysis consistent with its formulation as $C_{15}H_{22}O_3$ (M^+, 250). The i.r. spectrum (KBr) showed a hydroxy band at 3 400 cm^{-1} while the u.v. spectrum [$\lambda_{max.}$(EtOH) 224, 240, and 300 sh nm (ε 8 300, 9 600, and 700)] indicated the presence of an extended conjugated system.[9]

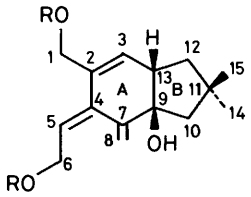
The ^{13}C n.m.r. spectrum of (**1a**) exhibited 6 sp^2- and 9 sp^3-hybridized carbon atoms. The sp^2 resonances indicated the presence of one methylene (δ 110.89), two methine (δ 131.08 and 127.86), and three quaternary (δ 149.26, 136.72, and 135.81) carbons while the sp^3 resonances were assigned to two methylene and one quaternary carbons bearing oxygen (δ 62.60, 60.30, and 84.63), two methyl (δ 33.25 and 32.77), two methylene (δ 52.61 and 47.28), one methine (δ 53.32), and one quaternary (δ 38.59) carbons.

Chemical-shift criteria and extensive use of low-power specific ^{13}C-{^1H} decoupling experiments, in conjunction with long-range ^{13}C-^1H (COLOC) and one-bond ^{13}C-^1H shift correlated 2D n.m.r spectra which enabled us to correlate ^1H and ^{13}C resonances, permitted their assignment (Table 1).

Addition of D_2O to the sample caused the resonances at δ_H 3.85, 3.85, and 3.70 in the ^1H n.m.r spectrum of (**1a**) to disappear, thus identifying three hydroxy groups.

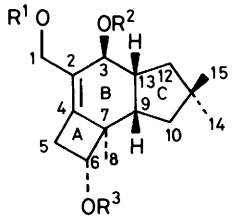
Acetylation of (**1a**) with pyridine–Ac_2O gave the hydroxy diacetate (**1b**) with the methylene protons assigned to 1-H_2 and 6-H_2 resonating downfield of *ca.* 0.5 p.p.m. with respect to (**1a**). These data indicate that the molecule contains two primary alcohol groups and that the remaining OH must be located at the quaternary carbon at δ_C 84.63.

In addition, the ^1H n.m.r spectrum of (**1a**) (Table 2) presented absorptions for an isolated methylene group (10-H_2), a C(12)H_2-C(13)H fragment, two tertiary methyl groups (14- and 15-H_3), and four olefinic protons. Two of the latter correlate with the methylene carbon at δ_C 110.89 and were assigned as those of the *exo* double bond C(7)=C(8)H_2 while those



(**1**)

a ; R = H
b ; R = Ac



(**2a**) $R^1 = R^2 = R^3 = H$
(**2b**) $R^1 = R^2 = R^3 = Ac$
(**2c**) $R^1 - R^2 = CMe_2, R^3 = H$
(**2d**) $R^1 = COBu^t, R^2 = R^3 = H$
(**2e**) $R^1 = COBu^t, R^2 = R^3 = p\text{-}O_2NC_6H_4CO$
(**3**) $R^1 = Ac, R^2 = R^3 = H$
(**4**) $R^1 = R^2 = R^3 = H, 3\alpha\text{-}OH$

Table 1. ^{13}C N.m.r. data for candicansol (**1a**) in [$^2H_6$] acetone

Carbon	δ_C^a(p.p.m.)	^1J(CH)/Hz	$^{>1}J$(CH)/Hz
1	62.60 Td	140.5	6 (3-H)
2	135.81 Sbrddt		6 (5-H), 6 (13-H), 5 (1-H_2)
3	131.08 Ddtt	157	6 (13-H), 5 (1-H_2), 7 (12-H_2)
4	136.72 Sm		
5	127.86 Dbrt	154.5	5 (6-H_2)
6	60.32 Tbrs	141.5	
7	149.26 Sbrddd		10 (5-H), 2.5 (8-Ha), 2.5 (10-Hα)
8	110.89 Tbrs	157.5	
9	84.63 Sm		
10	52.61 Tm	128	
11	38.59 Sm		*ca.* 4 (14-H_3),[b] *ca.* 4 (15-H_3)[b]
12	47.28 Tm	129.5	
13	53.32 Dm	132	
14	32.77 Qm	124.5	5 (10-Hα),[b] 5 (12-Hβ)[b]
15	33.25 Qm	124.5	5 (10-Hα),[b] 5 (12-Hβ)[b]

[a] Capital letters refer to the pattern resulting from directly bonded (C,H) couplings [^1J(CH)] and small letters to that from (C,H) couplings over more than one-bound [$^{>1}J$(CH)]. S or s = singlet, D or d = doublet, T or t = triplet, Q = quartet, m = multiplet, and br = broad. [b] Determined by selective low-power decoupling experiments.

Table 2. ^1H N.m.r. chemical shifts (δ_H/p.p.m.) and ^1H-^1H coupling constants (J/Hz) for compounds (**1a**) and (**1b**) in [$^2H_6$] acetone

Proton	(**1a**)	(**1b**)	J	(**1a**)	(**1b**)
1a	4.25	4.81	1a, 1b	*b*	12.7
1b	4.23	4.67	1a, 3	*b*	1.0
3	5.66	5.78	1a, 13	*b*	1.7
5	5.84	5.74	1b, 3	*b*	1.0
6a	4.38	4.84	1b, 13	*b*	1.4
6b	4.29	4.80	3,5	0.8	0.8
8a	5.47	5.56	3,6a	0.5	0.5
8b	4.83	4.95	3,6b	0.9	0.8
10α	1.79	1.77	3,8b	0.3	0.3
10β	1.44	1.51	3,13	3.0	3.0
12α	1.48	1.51	5,6a	7.8	7.7
12β	2.21	2.22	5,6b	4.8	5.5
13	2.67	2.72	5,13	0.9	0.9
14	0.97	0.96	6a, 6b	13.7	13.4
15	1.17	1.18	6a, 13	0.7	0.7
1-OR	3.85	2.02[a]	6b, 13	1.0	1.0
6-OR	3.85	2.01[a]	8a, 8b	2.2	2.0
9-OH	3.70	3.85	10α, 10β	13.8	14.0
			10α, 9-OH	b	0.9
			10β, 13	1.5	1.4
			12α, 12β	12.8	12.7
			12α, 13	1.9	2.1
			12β, 13	8.3	8.4
			12β, 14	0.6	0.6

[a] Assignments may be interchanged. [b] Not assigned.

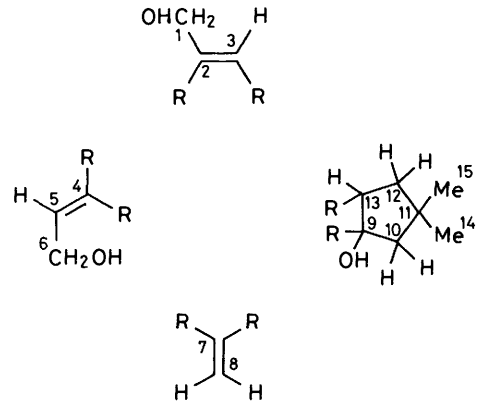


Figure 1.

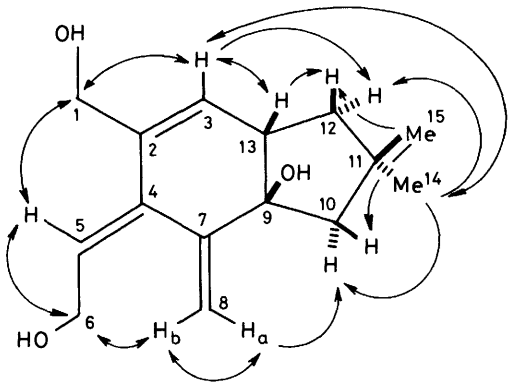


Figure 2. The n.O.e. connectivity pattern observed for candicansol (**1a**)

resonating at δ_H 5.66 and 5.84 which presented allylic and vicinal couplings to 1-H_2 and 6-H_2, respectively, pointed to the presence of the –C(3)H=C(2)CH_2OH and >C(4)=C(5)CH_2OH entities. From these and the following results the part structures depicted in Figure 1 were deduced.

The methylene protons resonating at δ_H 1.79 and 2.21 (10-Hβ and 12-Hβ) presented a 5 Hz coupling to both the tertiary methyl carbons C-14 and C-15 whose protons were coupled to the quaternary carbon resonance at δ_C 38.59 [C-11; ^2J(CH) ~ 4Hz]. These findings indicate that the two CH_2 groups are adjacent to C-11 and that the two Me groups are geminally disposed at C-11. Moreover, in the hydroxy diacetate (**1b**) 10-Hα and 10-Hβ presented W-type long-range coupling of 0.9 and 1.4 Hz with 9-OH and 13-H, respectively, thus suggesting the presence of the cyclopentane B in the molecule.

The (C,H) coupling constants of 5—7 Hz presented by C-3 with 1-H_2, 12-H_2, and 13-H, and those of 6 and 10 Hz presented by C-2 and C-7 with 5-H defined the mode of linkage between C-3 and C-13, C-2 and C-4, and C-4 and C-7 while the cross-peaks observed between C-9 and 8-H_2 in a COLOC experiment performed on compound (**1a**) defined that between C-7 and C-9.

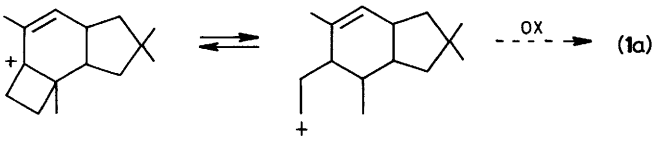
The n.O.e.s carried out on candicansol (see Figure 2 and Experimental section) not only are in agreement with the proposed structure but also allow assignment of stereochemistry to the double bonds as indicated in (**1a**) while the n.O.e. observed between 9-OH and 13-H (3%) in compound (**1b**) establishes the *cis* A, B ring junction.

Reduction of candicansol (**1a**) with 10% Pd on $BaSO_4$ was

Table 3. ^1H and ^{13}C N.m.r. data for compounds (**2a**), (**2b**) and (**3**) in[$^2H_6$]acetone

Proton	(**2a**) δ_H(p.p.m.)	(**2b**) δ_H(p.p.m.)	(**3**) δ_H(p.p.m.)	J(H, H)/Hz	(**2a**[b])	(**2b**)	(**3**)	Carbon	(**2a**) δ_C(p.p.m.)	(**2a**) ^1J(C, H)/Hz	(**3**) δ_C(p.p.m.)
1a	4.17	4.58	4.74	1a, 1b	12.0	12.6	12.5	1	59.25 T	142.5	60.84 T
1b	4.15	4.49	4.57	1a, 3	c	1.1	1.1	2	132.89 S		129.71 S
3	4.08	5.30	4.00	1a, 5α	1.6	1.7	1.8	3	74.65 D	142	73.31 D
5α	2.62	2.93	2.65	1a, 5β	c	0.9	1.0	4	137.31 S		141.27 S
5β	3.00	3.17	3.03	1b, 3	c	0.9	1.0	5	36.81 DD	139,133	37.43 DD
6	3.88	4.61	3.89	1b, 5α	1.6	1.5	1.6	6	75.92 D	150	76.07 D
8	0.98	0.99	1.00	1b, 5β	c	0.6	0.7	7	51.74 S		52.22 S
9	2.28	2.56	2.27	3, 5α	3.9	4.0	3.8	8	14.23 Q	126	14.26 Q
10α	1.36	1.33	1.38	3, 5β	1.9	2.0	2.0	9	45.93 D	130	46.09 D
10β	1.39	1.53	1.38	3, 13	8.6	9.2	8.8	10	42.08 T	127	42.24 T
12α	1.18	1.12	1.19	5α, 5	14.8	15.6	15.1	11	39.85 S		40.05 S
12β	1.78	1.59	1.77	5α, 6	7.3	7.6	7.5	12	47.32 T	127	47.55 T
13	2.23	2.44	2.20	5β, 6	7.2	7.4	7.0	13	50.90 D	ca.128	50.78 D
14	1.08	1.06	1.07	9, 10α	10.3	10.8	10.8	14	29.92 Q	124	29.92 Q
15	0.96	0.97	0.96	9, 10β	7.8	7.5	7.7	15	27.44 Q	124	27.47 Q
1-OR	4.00	1.96[a]	1.97	9, 13	11.7	12.2	11.7	OAc			171.13 S
3-OR	4.47	2.05[a]	3.90[a]	10α, 10β	12.5	12.7	12.5				20.94 Q
6-OR	4.66	2.03[a]	4.25[a]	10α, 15	0.8	0.8	0.8				
				10β, 12β	1.7	2.1	1.9				
				12α, 12	12.5	12.5	12.6				
				12α, 13	9.5	10.3	9.6				
				12α, 15	0.8	0.8	0.8				
				12α, 13	7.2	7.3	7.3				

[a] Assignments within each column may be interchanged. [b] Additional coupling constants: $^3J_{1.1\text{-OH}} = 5.5$, $^3J_{3.3\text{-OH}} = 5.7$, and $^3J_{6.6\text{-OH}} = 5.5$ Hz. [c] Not determined.



Protoilludane type Illudalane type

Scheme.

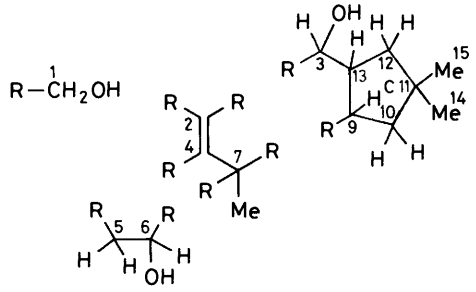


Figure 3.

attempted but only degraded products, which were hard to purify, were obtained.

From a biogenetic point of view, candicansol belongs to the rare illudalane family of sesquiterpenoids and derives from oxidation of the illudalane-type cation (see Scheme); it shows antibacterial activity against *Bacillus cereus* and *B. subtilis* but not against *Escherichia coli* (50 μg/disc). The only components, so far known[2] of this class are illudalic, illudoic and illudacetalic acids, and illudinine; these metabolites have all been isolated from a liquid culture of *Clitocybe illudens*(=*Omphalotus olearius*).[2]

The second metabolite (**2a**) was obtained as a glassy solid, m.p. 42—44 °C, $[\alpha]_D$ − 106° (*c* 1 in EtOH), and had an analysis consistent with its formulation as $C_{15}H_{24}O_3$. The electron impact mass spectrum lacked the molecular ion peak (M^+ at *m*/*z* 252), but a fragment was found at *m*/*z* 234 ($M^+ - H_2O$) due to the ready loss of water. In addition, strong peaks were observed at *m*/*z* 216 ($M^+ - 2H_2O$), 188, and 173 (base peak). Chemical ionization mass spectroscopy gave a distinct peak at *m*/*z* 253 ($M^+ + 1$). The u.v. spectrum displayed no absorbtion above 200 nm and the i.r. spectrum showed strong hydroxy absorption in the range 3 200—3 400 cm^{-1}.

The ^{13}C n.m.r. spectrum of (**2a**) (Table 3) contained 15 resonances attributable to: a tetra substituted carbon-carbon double bond, to one methylene carbon and two methine carbons bearing oxygen (δ 59.25, 74.65, and 75.92), to three methyl carbons, three methylene carbons, two methine carbons, and two quaternary carbons.

The ^1H n.m.r. spectrum of (**2a**) (Table 3) confirmed and extended these findings through the appearance of one primary and two secondary hydroxy protons coupled to the methylene protons at δ_H 4.17 and 4.15 (1-H_2) and to the methine protons at δ_H 4.08 and 3.88 (3-H and 6-H), and of three tertiary methyl groups at δ_H 1.08, 0.98, and 0.96.

The formation of the triacetate (**2b**) confirmed the presence of the three hydroxy groups. The above results, as corroborated by extensive use of ^1H-{^1H} decouplings on (**2b**), permitted the part structures shown in Figure 3 to be constructed.

The presence of the –C(1)H_2OH and –C(5)H_2–C(6)HOH groupings and of the sequence shown below readily followed

$$\begin{array}{c} C(12)H_2 \\ | \\ -C(3)HOH-C(13)H-C(9)H-C(10)H_2 \end{array}$$

from an analysis of the appropriate ^1H n.m.r multiplets. Furthermore, the W-type long-range coupling of 2.1 Hz between 10-Hβ and 12-Hβ and those of 0.8 Hz between 15-H_3 and both 10-Hα and 12-Hα are strong indicators that the molecule contains a cyclopentane ring C and that the C-15 methyl group (δ_H 0.96) is located at the quaternary carbon C-11. The presence of the additional C-14 methyl group (δ_H 1.08) at C-11 followed from the n.O.e.s observed between 14-H_3 and the

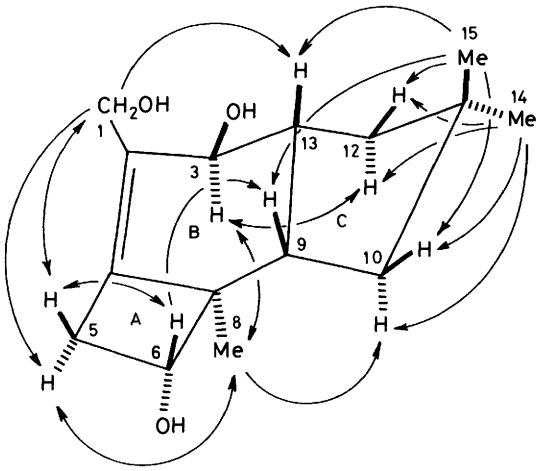


Figure 4. The n.O.e. connectivity pattern and the preferred conformation of 3-*epi*-illudol (**2a**)

10- and 12-methylene protons (2—3%). The remaining C-8 methyl group (δ_H 0.98) which exhibited a three-bond coupling of 5 Hz with the olefinic carbon at δ_C 137.31 was allocated at C-7.

In order to assign the structure of compound (**2a**) we have only to tie together the fragment containing the olefinic bond with the other ones. The fact that 5-H_2, and not 6-H and 9-H, presented long-range couplings with 1-H_2 and 3-H suggests that C-1, C-3, and C-5 are located at the olefinic carbons and that C-6 and C-9 are bonded to C-7. Moreover, the value of the direct (C,H) couplings of 139 and 133 Hz exhibited by C-5 is diagnostic of the presence of a cyclobutane ring. Thus, C-5 is linked to C-4 and C-3, as well as C-1, is bonded to C-2 to form the cyclohexene ring B.

These results indicate that compound (**2a**) possesses the same $\Delta^{2,4}$-protoilludene-1,3,6-triol skeleton as illudol (**4**), a metabolite of *Clitocybe illudens*, but with different stereochemistry since illudol has been reported[10] to have m.p. 130—132 °C and $[\alpha]_D$ − 116° (*c* 0.4 in EtOH). Additionally, it is possible to deduce that illudol (**4**) has the absolute configuration 3*R*, 6*R*, 7*R*, 9*S*, 13*R* on the basis of the *X*-ray analysis carried out on an illudol derivative[11] and comparison with a racemic sample of illudol obtained by stereospecific syntheses.[12,13] It was of interest therefore to correlate the two compounds (**2a**) and (**4**) by establishing the absolute configuration of the metabolite (**2a**).*

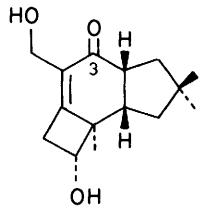
Configurational and Conformational Considerations in Connection with Compound (**2a**).—In the case of compound (**2a**) irradiation of the 15-methyl protons, assumed to be β, resulted in enhancement (see Experimental Section and Figure 4) of 9-H (5%), 13-H (5%), and of the geminal protons resonating at δ_H 1.39 (10-H; 2.5%) and 1.78 (12-H; 3.5%), this fact indicating that these protons are on the same β-side of the ring C and that the B, C junction is *cis*.

Furthermore, the similarity of the vicinal coupling constants between the *trans*-disposed 9-Hβ and 10-Hα, and 12-Hα and 13-Hβ (^3J 10.3 and 9.5 Hz; dihedral angles of *ca.* 150°) and those between the *cis*-disposed 9-Hβ and 10-Hβ, and 12-Hβ and 13-Hβ (^3J 7.8 and 7.2 Hz; dihedral angles of *ca.* 30°) are consistent with the cyclopentane ring C assuming preferentially the *exo*-envelope form shown in Figure 4. The presence of the above mentioned W-type long-range couplings between 10-Hβ and 12-Hβ, 10-Hα and 15-H_3β and 12-Hα and 15-H_3β are also in accord with the proposed geometry as well as the value of 11.7 Hz between 9-H and 13-H (dihedral angle of *ca.* 0°) which necessitates a near-eclipsed relationship of these protons. As a consequence, the cyclohexene ring B adopts a boat-like disposition in which 3-H and 8-H_3 are α-axially disposed as evidenced by the n.O.e.s between 3-H and 12-Hα (6%), 8-H_3 and 10-Hα (4.5%). Finally, the n.O.e.s between 8-H_3 and the methylene proton resonating at δ_H 2.62 (5-Hα; 3%) and between 6-H and the geminal methylene proton resonating at δ_H 3.00 (5-Hβ; 3%) require that the 6-OH is β-located.

The chirality of the 6-OH group in (**2a**) and thus the absolute configuration of (**2a**), *i.e.* 3*S*, 6*R*, 7*R*, 9*S*, 13*R*, were determined by the partial resolution method of Horeau[14] carried out on the acetonide derivative (**2c**). Furthermore, the absolute configuration at C-3 and C-6 was confirmed as 3*S* and 6*R* from the c.d. spectrum performed on the 1-*O*-pivaloyl-3,6-dinitrobenzoyl derivative (**2e**). In fact, according to the benzoate rule,[15] the c.d. curve exhibited a negative Cotton effect at 256 nm, thus indicating the left-handedness of the orientation of the benzoate groups. On the basis of the above evidence, compound (**2a**), which differs from illudol only in its chirality at C-3, is identified as 3-*epi*-illudol.

The metabolite (**3**) was identified as 1-*O*-acetyl-3-*epi*-illudol by comparison of the n.m.r. data (see Table 3) of (**2a**) and (**3**). The presence of an additional 1-*O*-acetyl group in (**3**) was evident from the three-proton singlet at δ_H 1.97 and the downfield shift of 0.4—0.6 p.p.m. observed for the 1-H_2 resonances. As expected, the metabolites (**2a**) and (**3**) afforded the same triacetate (**2b**), this fact proving the interrelationship between these compounds.

A further compound (**5**), which may be an artefact generated during extraction, was isolated in very poor yield. It had an analysis consistent with its formulation as $C_{15}H_{22}O_3$ and differs in molecular weight by 2 mass units from 3-*epi*-illudol (**2a**).



(**5**)

This difference corresponds to oxidation of the 3-OH group present in (**2a**), as evidenced by the absence in the ^{1}H n.m.r. spectrum of (**5**) (see Experimental Section) of the resonance assigned to 3-H, and by the presence in the i.r. spectrum of (**5**) of a carbonyl absorption at 1 720 cm^{-1}. Photo-oxidation of 3-*epi*-illudol, as well as oxidation with Jones reagent, gave a compound identical with (**5**), thus confirming the proposed structure.

Experimental

M.p.s are uncorrected. U.v. spectra were measured for solutions in 95% EtOH on a JASCO Uvidec-510 spectrophotometer. I.r. spectra were recorded with a Perkin-Elmer 177 instrument. Flash chromatography was performed with Merck silica gel (0.04—0.063 mm), and t.l.c. with Merck HF_{254} silica gel. Mass spectra were taken on a VG-ZAB2 instrument at 70 eV. ^{1}H N.m.r. spectra were recorded on a Bruker CPX-300 (300.13 MHz) spectrometer and ^{13}C n.m.r. spectra on a Bruker AC 250L (62.9 MHz) instrument. Chemical shifts are in p.p.m. (δ) from $SiMe_4$ as internal standard. N.O.e. difference spectra were obtained by subtracting alternatively right-off resonance-free induction decays (FIDS) from right-on resonance-induced FIDS.

* Unfortunately no natural or synthetic sample of illudol (**4**) was available from M. Anchel,[10] T. Matsumoto,[12] or M. F. Semmelhack[13] for a direct comparison with (**2a**) and for more accurate n.m.r. analyses.

N.O.e. values reported in the text have only qualitative significance.

Isolation and Purification of Metabolites (**1a**), (**2a**), *and* (**3**).—A strain of *Clitocybe candicans* (CBS 168.67) received from Centraal Bureau voor Schimmel Cultures-Baarn, was maintained on MPGA (malt, peptone, glucose, agar 20:4:30:15 gl^{-1}) slants and sub-cultured in 50 stationary Erlenmeyer flasks (300 ml) containing a liquid medium MPG (50 ml) for 6 weeks at 24 °C; the culture filtrates separated from mycelium were extracted twice with EtOAc and the combined extracts were dried (Na_2SO_4) and evaporated to give a mixture (1.2 g) of crude metabolites. The mixture was chromatographed on a column of flash silica gel with hexane–EtOAc (2:1) as eluant, and purified further by preparative t.l.c. (p.l.c.) with CH_2Cl_2–MeOH (30:1 and/or 15:1), to yield the three metabolites in the following order of increasing R_F values: candicansol (**1a**) (120 mg), 3-*epi*-illudol (**2a**) (290 mg), and 1-*O*-acetyl-3-*epi*-illudol (**3**) 70 mg).

Candicansol (**1a**). This was obtained as a pale yellow solid (Found: C, 71.3; H, 8.6. $C_{15}H_{22}O_3$ requires C, 71.96; H, 8.86%); *m/z* (c.i.-isobutane) 251 ($M^+ + 1$), 233 [($M^+ + 1$) −18], and 215 [($M^+ + 1$) −36]; *m/z* (e.i.), 232, 215, 201, and 186; ^{13}C and ^1H n.m.r. data are reported in Tables 1 and 2, respectively.

Some connectivities established by n.O.e. difference experiments in [$^2H_6$]acetone + D_2O are as follows:

Proton irradiated	Proton affected(%)
1-H_2	3-H (9), 5-H (19.5).
3-H	1-H_2 (2), 12-Hα (2), 13-H (5.5), 14-H_3 (0.5).
5-H	1-H_2 (4.5), 6-H_2 (1.5).
6-H_2	5-H (8), 8-Hb (5).
8-Ha	8-Hb (28.5), 10-Hα (1).
8-Hb	8-Ha (31.5), 6-H_2 (1.5).
13-H	3-H (5), 12-Hβ (4).
14-H_3	3-H (2), 10-Hα (6), 12-Hα (3), 15-H_3 (1).
15-H_3	10-Hβ (4), 12-Hβ (5.5), 14-H_3 (1).

Biological Tests.—Antibacterial activity was tested with paper disks (6 mm diam.), soaked with candicansol (**1a**) (200, 100, and 50μg), which were placed on the surface of the nutrient agar, cooled at 45 °C, and poured into Petri dishes with a suspension of *Bacillus cereus* (ATCC 10702), *B. subtilis* (ATCC 6633), and *Escherichia coli* (ATCC 10536) as test micro-organisms.

3-*epi*-Illudol (**2a**). This was obtained as a glassy solid (Found: C, 71.2; H, 9.4. $C_{15}H_{24}O_3$ requires C, 71.39; H, 9.59%); *m/z* (c.i.-isobutane), 253 ($M^+ + 1$), 235 [($M^+ + 1$)−18], 217 [($M^+ + 1$) −36] (base peak), 199, 189, and 173; *m/z* (e.i.), 234 ($M^+ - 18$), 216 ($M^+ - 36$), 188, and 173 (base peak); ^1H and ^{13}C n.m.r. data are reported in Table 3.

Some connectivities established by n.O.e. difference experiments in [$^2H_6$]acetone + D_2O are as follows:

Proton irradiated	Proton affected(%)
1-H_2	5-Hα (0.5), 5-Hβ (2.5), 9-H (−1.5), 13-H (2).
3-H	8-H_3 (1), 12-Hα (6).
5-Hα	5-Hβ (9), 8-H_3 (0.5).
5-Hβ	1-H_2 (1), 5-Hα (12), 6-H (2).
6-H	5-Hβ (3), 9-H (4.5).
8-H_3	3-H (5), 5-Hα (3), 10-Hα (4.5).
14-H_3	10-Hα (3), 10-Hβ (2), 12-Hα (2), 12-Hβ (2.5).
15-H_3	9-H (5), 10-Hβ (2.5), 12-Hβ (3.5), 13 (5).

1-*O*-*Acetyl*-3-epi-*illudol* (**3**) This was obtained as an oil, $[\alpha]_D$ −78.4° (*c* 0.2 in $CHCl_3$); $\nu_{max.}$(liq. film) 1 715 cm^{-1} (CO acetate); *m/z* 234 ($M^+ - 60$), 216, 202, 186, and 174; ^1H and ^{13}C n.m.r. data are reported in Table 3. Compound (**3**) gave upon acetylation a product identical with the above-described triacetyl derivative (**2b**).

Acetylation of Candicansol (**1a**).—Candicansol (**1a**) (50 mg) was dissolved in dry pyridine (0.5 ml) and treated with Ac_2O (1 ml) during 6 h at 0 °C. It was then extracted with CH_2Cl_2 and the extract washed with saturated aqueous $NaHCO_3$, water, saturated aqueous $KHSO_4$, and water and then finally dried (Na_2SO_4). Evaporation of the solvent gave the diacetate (**1b**), as an oil, $[\alpha]_D$ +6.9° (*c* 0.3 in MeOH); $\lambda_{max.}$(EtOH) 230 sh and 254 nm (ε 10 650 and 13 600); *m/z* 334 (M^+), 316 ($M^+ - 18$), 274 ($M^+ - 60$), 246, 228, and 214 (base peak); ^{13}C and ^1H n.m.r. data are reported in Tables 1 and 2.

Acetylation of 3-epi-*Illudol* (**2a**).—3-*epi*-Illudol (**2a**) (50 mg) was dissolved in dry pyridine (1 ml) and treated with Ac_2O (1.5 ml) overnight at 0 °C. Standard work-up followed by p.l.c. on silica gel in hexane–EtOAc (2:1) gave the triacetyl derivative (**2b**) (40 mg) as an oil; $[\alpha]_D$ +10.3° (*c* 0.1 in $CHCl_3$); *m/z* (c.i.-isobutane), 319 [($M^+ + 1$)−60], 259 [($M^+ + 1$)−120], 217 (base peak), 199, and 187. ^1H and ^{13}C N.m.r. data are reported in Table 3.

Acetonide Derivative of 3-epi-*Illudol* (**2a**).—A solution of (**2a**) (50 mg) in dry acetone (5 ml) was treated with a trace of sulphuric acid during 2 h at −20 °C. The acetone was evaporated and ice was added. The mixture was then extracted with CH_2Cl_2 and the extracts were washed with saturated aqueous $NaHCO_3$ and dried (Na_2SO_4). Evaporation of the solvent gave the acetonide (**2c**) (40 mg) as white crystals, m.p. 115—117 °C; *m/z* 292 (M^+), 234 ($M^+ - 58$), and 216; δ_H($CDCl_3$) 4.27 (2 H, br s, 1-H_2), 4.08 (1 H, m, 3-H), 3.94 (1 H, t, *J* 7.5 Hz, 6-H), 2.92 and 2.63 (2 × 1 H, m, 5-H_2), *ca.* 2.9 (1 H, 6-OH), 2.5—1.1 (6 H, m, 9- and 13-H, 10- and 12-H_2), 1.46 and 1.40 (2 × 3 H, s, MeCO), and 1.10, 1.03, and 0.96 (3 × 3 H, s, 8-, 14-, and 15-H_3).

Reaction of the Acetonide (**2c**) *with* (±)-2-*Phenylbutyric Anhydride.*—The acetonide (**2c**) (40 mg) and (±)-2-phenylbutyric anhydride (50 mg) were dissolved in dry pyridine (0.5 ml) and the solution was kept for 20 h at room temperature. (+)-2-Phenylbutyric acid with $[\alpha]_D$ + 15° (*c* 0.14 in pyridine) was obtained upon work-up of the reaction mixture according to the literature method.[14]

Reaction of 3-epi-*Illudol* (**2a**) *with Pivaloyl Chloride.*—3-*epi*-Illudol (**2a**) (100 mg), pivaloyl chloride (0.3 ml), and dry pyridine (1 ml) were left at —20 °C for 1 h. Standard work-up followed by p.l.c. on silica in hexane–EtOAc (2:1) afforded the pivaloate (**2d**) (80 mg) as an oil; $\nu_{max.}$(liq. film) 3 400 (OH) and 1 710 cm^{-1} (CO ester); δ_H($CDCl_3$) 4.77 and 4.53 (2 × 1 H, br d, *J* 12.4 Hz, 1-H_2), 3.97 (1 H, br ddd, *J* 8.8, 3.9, and 2.0 Hz, 3-H), 3.95 (1 H, dd, *J* 7.3 and 7.1 Hz, 6-H), 3.14 (1 H, br ddd, *J* 15.2, 7.1, and 2.0 Hz, 5-Hβ), *ca.* 2.7 (2 H, 3- and 6-OH), 2.68 (1 H, br ddd, *J* 15.2, 7.3 and 3.9 Hz, 5-Hα), 2.26 and 2.18 (2 × 1 H, m, 9- and 13-H), 1.83 (1 H, ddd, *J* 12.5, 7.3, and 2.0 Hz, 12-Hβ), 1.42 (1 H, ddd, *J* 12.5, 7.7, and 2.0 Hz, 10-Hβ), 1.33 (1 H, br dd, *J* 12.5 and 10.8 Hz, 10-Hα), 1.22 (1 H, br dd, *J* 12.5 and 9.7 Hz, 12-Hα), 1.19 (9 H, s, Bu^t), 1.09 and 1.01 (2 × 3 H, s, 8- and 14-H_3), and 0.95 (3 H, br s, 15-H_3).

Reaction of 1-O-*Pivaloyl*-3-epi-*illudol* (**2d**) *with* 3,6-p-*Dinitrobenzoyl Chloride.*—Compound (**2d**) (50 mg) dissolved in dry pyridine (1 ml) was treated with *p*-dinitrobenzoyl chloride (0.2 ml) at room temperature for 3 h. The resulting precipitate was filtered off, washed with Et_2O, and crystallized from EtOAc–hexane (1:4) to give compound (**2e**) as a white solid, m.p. 104—106 °C; c.d. (*c* 1.7 × 10^{-5} g ml^{-1} in iso-octane) 256 and 224 nm (Δε −6.0 and +2.9); $\lambda_{max.}$ 200 and 254 nm (ε 40 700 and 34 700);

$\nu_{max.}$(liq. film) 1 725 cm^{-1} (CO ester); *m/z* 606 (M^+); δ_H($CDCl_3$) 8.35 and 8.30 (2 × 4 H, m, ArH), 5.70 (1 H, m, 3-H), 4.99 (1 H, t, *J* 7.5 Hz, 6-H), 4.73 and 4.60 (2 × 1 H, br d, *J* 12.5 Hz, 1-H_2), 3.5—2.5 (4 H, m, 5-H_2, 9- and 13-H), 1.9—1.2 (4 H, m, 10- and 12-H_2), 1.13, 1.10 and 1.02 (3 × 3 H, s, 8-, 14-, and 15-H_3), and 1.10 (9 H, s, Bu^t).

Photo-oxidation of 3-epi-*Illudol* (**2a**).—3-*epi*-Illudol (**2a**) (150 mg) was dissolved in MeCN (100 ml) in Pyrex test tubes with benzophenone (100 mg) and the oxygen was removed with a N_2 stream. The tubes were then placed in a Rayonet reactor equipped with 350 nm lamps and irradiated for 1 h at room temperature. Evaporation of the solvent under reduced pressure followed by flash chromatography with hexane–EtOAc (3:1) gave compound (**5**) as an oil; $[\alpha]_D$ +23.2° (*c* 0.01 in MeOH); $\nu_{max.}$(Nujol) 3 400 (OH) and 1 720 cm^{-1} (CO band); *m/z* (c.i.-isobutane) 251 (M^+ +1) 233, 217, 203, and 189; δ_H ($CDCl_3$) 4.26 (1 H, ddd, *J* 12.7, 1.6, and 1.0 Hz, 1-Ha), 4.20 (1 H, ddd, *J* 12.7, 1.0, and 0.5 Hz, 1-Hb), 4.11 (1 H, dd, *J* 7.2 and 6.8 Hz, 6-Hβ), 3.30 (1 H, dddd, *J* 16.5, 6.8, 1.0, and 0.5 Hz, 5-Hβ), 299 (1 H, ddd, *J* 12.7, 10.5, and 7.4 Hz, 13- or 9-H) 2.93 (1 H, dddd, *J* 16.5, 7.2, 1.6, and 1.0 Hz, 5-Hα), 2.72 (1 H, dt, *J* 10.5 and 7.6 Hz, 9- or 13-H), 1.86 (1 H, dd, *J* 12.5 and 7.4 Hz, 12-Hβ), *ca.* 1.8 (2 H, 1- and 6-OH), 1.60 (2 H, d, *J* 7.6 Hz, 10-H), 1.48 (1 H, dd, *J* 12.7 and 12.5 Hz, 12-Hα), 1.22, 1.12, and 0.99 (3 × 3 H, s, 8-, 14-, and 15-H_3).

A small amount of the same compound (**5**) was also isolated (5 mg) during the chromatographic separation of the crude mixture of metabolites and by oxidation of 3-*epi*-illudol with Jones reagent.

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Received 10*th March* 1989; *Paper* 9/01071H