Coprinolone, an Oxygen-bridged Protoilludane from the Fungus *Coprinus psychromorbidus*: Structure Determination by Chemical and N.M.R. Studies aided by Biosynthetic Incorporation of [1,2-$^{13}C_2$]Acetate

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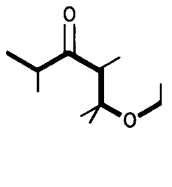
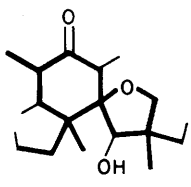
The structure of a sesquiterpene ketol, coprinolone, from the W2 isolate of the fungus *Coprinus psychromorbidus*, has been deduced as (**1a**) by chemical transformations and from ^1H and ^{13}C n.m.r. data; confirmation was obtained from the labelling pattern of the compound from cultures supplemented with [1,2-$^{13}C_2$]acetate.

During an investigation[1] of hydrogen cyanide production by the W2 isolate of a low-temperature basidiomycete (ATCC 26501), now classified as *Coprinus psychromorbidus*,[2,3] a new metabolite which we name coprinolone was found in Et_2O or EtOAc extracts of the culture filtrates and the mycelia. Chromatography over silicic acid–Celite (4 : 1) and crystallization from light petroleum gave coprinolone, m.p. 105—107 °C; $[\alpha]_D^{23}$ −95° (*c* 1.03, $CHCl_3$); i.r. (KBr) 3518 and 1703 cm^{-1}, having the constitution $C_{15}H_{22}O_3$ by microanalysis and precise mass measurement. The ^1H n.m.r. spectra in C_6D_6 or CD_2Cl_2 revealed one secondary and two tertiary methyl groups as well as a >CHOH and a $-CH_2O-$ group. The ^{13}C n.m.r. spectra confirmed and extended these findings through the appearance of signals for carbonyl carbon (δ_c 212.1), quaternary, methine, and methylene carbons bearing oxygen (93.3, 91.6, 75.4), three methyl (10.0, 13.7, 24.8), three methylene (20.7, 27.7, 30.6), three methine (43.7, 51.1, 51.9), and two quaternary carbons (41.8, 47.3). The absence of olefinic unsaturation showed coprinolone to be tetracyclic.

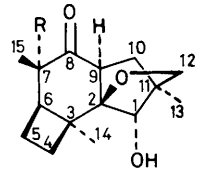
Treatment of coprinolone with *N*-bromosuccinimide in refluxing CCl_4 furnished a monobromo derivative, m.p. 145—148 °C (decomp.); $[\alpha]_D^{24}$ +127° (*c* 1.02, $CHCl_3$); i.r. (KBr) 3562 and 1713 cm^{-1}, with three tertiary methyl signals in the ^1H n.m.r. spectrum and a methine signal replaced by a new quaternary signal in the ^{13}C spectrum. These data indicate that the secondary methyl group in coprinolone is adjacent to the carbonyl group.



(**A**)

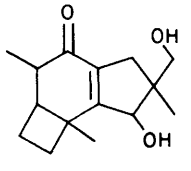


(**B**)

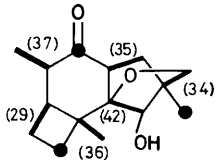


(**1**)

a ; R = H
b ; R = Br



(**2**)



(**3**)

Coprinolone was cleanly converted to a more polar compound when treated with 0.17 M KOH in MeOH at room temperature. Crystallization from EtOAc–hexane yielded a keto-diol, m.p. 144—146 °C, $[\alpha]_D^{25}$ −27° (*c* 0.81, $CHCl_3$); i.r. (KBr) 3410 and 1652 cm^{-1}; $\lambda_{max.}$ (EtOH) 252 nm (ε 8490). The ^{13}C spectrum in $CDCl_3$ contained signals for a carbonyl carbon (δ_c 200.0), two fully substituted olefinic centres (135.6, 162.4), methine and methylene carbons bearing oxygen (81.0, 69.8), as well as three methyl (10.5, 17.4, 25.2), three methylene (19.5, 30.9, 37.0), two methine (39.9, 47.9), and two quaternary carbons (40.5, 47.0). Base-catalysed opening of an oxide ring to yield an α,β-unsaturated ketone having a primary alcohol group accounts for these features and together with the bromination results leads to part structure (A) for coprinolone.

A series of n.m.r. experiments confirmed part structure (A) and led to its extension to (B). A heteronuclear correlation experiment revealed the specific proton absorptions associated with each protonated carbon. Those carbons which are one and two bonds from each of the three methyl groups were identified through a long-range heteronuclear correlation experiment utilizing the XCORFE sequence.[4] Additional information on the carbon–carbon connectivities was obtained from one- and two-dimensional double quantum coherence (INADEQUATE) spectra,[5] as summarized in structure (B), but the two remaining linkages could not be unequivocally established.

High resolution mass measurements showed that the base peak at M^+ −28 was due to the loss of ethylene. By analogy with results for the sterpuranes[6] and some protoilludenes,[7] this is strong evidence for the presence of a cyclobutane ring. Inclusion of this feature leads to the protoilludane structure (**1a**) for coprinolone; structures (**1b**) and (**2**) follow for the bromo derivative and the keto-diol.

To confirm these deductions, the fungus was grown on medium (750 ml) to which [1,2-$^{13}C_2$]acetate (0.06 M, 25 ml) was added on days 8 and 10. Extraction of the cultures on day 13, chromatography, and crystallization gave coprinolone (63 mg). From the relative intensities of the ^{13}C–^{13}C satellites exhibited by the paired carbons incorporated as intact acetate units the ^{13}C enrichment was found to be 4.0%. The labelling pattern is shown in (**3**) with J_{CC} values for intact acetate units given in parentheses. A similar labelling pattern was found for illudins S and M, metabolites of another basidiomycete believed to be formed through a pathway involving a protoilludyl intermediate.[8] Illudin S and the recently identified[9] protoilludane, 15-hydroxy-5′-*O*-methylmelledonal, are also oxygenated at C-1 and the β-methyl of the *gem*-dimethyl group. A 75 MHz ^{13}C 2D COSY experiment provided further confirmatory evidence for direct bondings of C-4 with C-3 and C-5, C-7 with C-6 and C-8, C-9 with C-2 and C-8, and C-11 with C-1, C-10 and C-13 in (**1a**).

Because of the difficulty of identifying *C. psychromorbidus* owing to its failure to produce fruiting bodies in culture, the isolation and characterization of (**1a**) from the W2 isolate may have taxonomic significance.

The ^1H–^1H coupling and nuclear Overhauser enhancement data leading to the relative stereochemistry shown in (**1a**) will be discussed in a full paper together with the results for a series of derivatives.

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