Isolation and Partial Synthesis of 3-Methoxycarbonyl-7-formyl-1-benzoxepin-5(2*H*)-one,† the Ester of a Metabolite from Shake Cultures of the Fungus *Marasmiellus ramealis* (Bull. *ex* Fr.) Singer [1]

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The title compound was isolated from the esterified extract of *Marasmiellus ramealis* shake cultures and synthesized from the corresponding natural 3,7-bis(hydroxymethyl)benzoxepinone. In addition, several known metabolites, three new to *M. remealis*, were isolated from the culture fluid extract.

Examination of the surface cultures of the Tricholomataceae species *Marasmiellus ramealis* (Bull. *ex* Fr.) Singer, *tribus Collybieae*, previously called *Marasmius ramealis* (Bull. *ex* Fr.) Fr.,[2] resulted in the isolation of marasin (1),[3] 8-hydroxy-3-methylisocoumarin (2),[4] and 3,7-bis(hydroxymethyl)-1-benzoxepine-5(2*H*)-one (3).[5] In the course of our search for possible marasin precursors we grew the fungus in shake cultures for five days and the constituents of the culture fluid extract were analysed. The neutral fraction yielded marasin (1), the isocoumarin (2), and the dihydroisocoumarin (4). The latter compound, known as the fungal metabolite mellein,[6a] was new to *M. ramealis* cultures. Also new to this fungus were two constituents of the acid fraction: pyrrole-2-carboxylic acid, a known bacterial product,[7] and the fungal metabolite *p*-hydroxybenzoic acid;[6b] both compounds were isolated as the corresponding methyl esters.

Traces of polyacetylenes appeared in the least polar ester fraction and were followed by an aromatic compound of probable molecular formula $C_{14}H_{16}O_4$. None of these could be identified.

The most polar ester fraction yielded a crystalline metabolite (0.3 mg l^{-1} culture fluid). High-resolution mass spectrometry showed the molecular formula to be $C_{13}H_{10}O_5$. The signals in the ^1H n.m.r. spectrum accounted for a formyl proton, three aromatic protons in a 1,2,4-arrangement, a single olefinic proton, two equivalent protons on a carbon atom carrying oxygen, and three methyl ester protons.

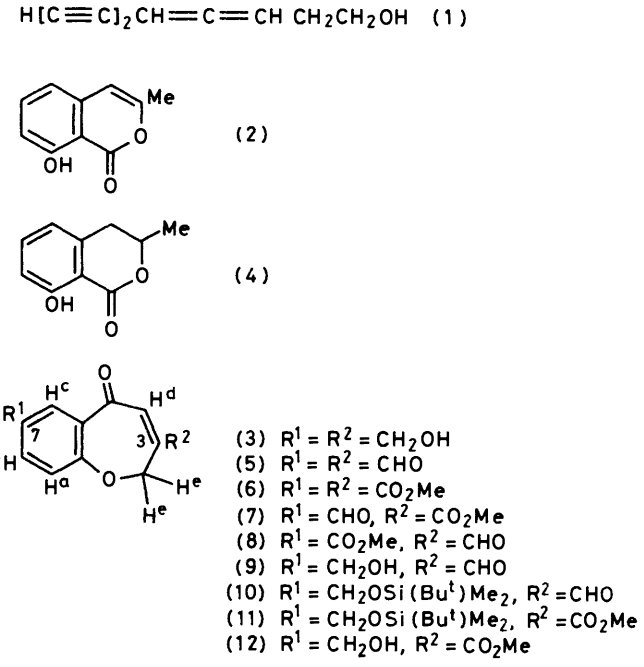
Comparison of the chemical shifts and patterns of the signals in the ^1H n.m.r. spectrum of the new metabolite with those in the spectra of the benzoxepinones (3), (5), and (6) [5] indicated that (a) the same ring system was present in all cases, (b) the differences arose through the substituents at positions 3 and 7 on the ring, (c) the substituents in the new benzoxepinone had to be the formyl and methoxycarbonyl groups, and (d) the ester was at the 3-position [compound (7)] rather than at C(7) [compound (8)].

This assignment was confirmed by partial synthesis in which the aldehydoester (7) was prepared from the natural diol (3) by selective stepwise oxidation. The relative susceptibility of the allylic and benzylic hydroxy groups in the diol (3) to mild oxidation determined which of the two possible aldehydoesters, the desired compound (7) or its positional isomer (8), would be produced. Treatment of the diol (3) with a large excess of MnO_2 (8 : 1, w/w) was found [5] to yield the unstable dialdehyde (5) in 67% yield. Oxidation with a smaller amount of MnO_2 (4.5 : 1) under controlled conditions (monitoring of the reaction progress by t.l.c.) yielded the intermediate monoaldehyde in 73% yield.

Structure allocation to the oxidation product was achieved by comparing its n.m.r. spectrum with those of the diol (3) and the dialdehyde (5).[5] The three aromatic protons H^a, H^b, and H^c of the hydroxyaldehyde resonated at τ 2.94, 2.48, and 2.08 respectively; their chemical shifts were almost identical with those of the aromatic protons in the diol (3) (τ 2.96, 2.47, and 2.09), but at appreciably higher field than those of the aromatic protons in the dialdehyde (5) (τ 2.75, 1.29, and 1.43). Thus, the hydroxymethyl group had to be present on the benzene ring as represented in structure (9). This was also favoured by the chemical-shift differences observed for the olefinic proton H^d. It resonated in the hydroxy aldehyde (9) at τ 3.07 which is much closer to the chemical shift of the olefinic proton in the dialdehyde (5) (τ 2.92) than in the diol (3) (τ 3.53). The chemical-shift differences for the allylic proton H^e in the three compounds were, on the other hand, less distinctive and of no value in the structure allocation. H^e resonated in the hydroxy aldehyde (9) at τ 5.1, in the diol (3) at τ 5.3, and in the dialdehyde (5) at τ 4.94.

No distinction was possible in the original signal assignments [5] between the chemical shifts of the benzylic and allylic protons for the diol (3) and between the two aldehyde proton signals in the dialdehyde (5). There is now a strong indication, based on the spectrum of the hydroxy aldehyde (9), that (a) in the diol (3) the singlet at τ 5.35 belongs to the benzylic CH_2OH group and that at τ 5.65 to the allylic group and (b) the singlet at τ 0.21 in the dialdehyde (5) belongs to the oxepine formyl and that at −0.01 to the aromatic formyl group.

With the selective oxidation of the allylic alcohol group



(3) $R^1 = R^2 = CH_2OH$
(5) $R^1 = R^2 = CHO$
(6) $R^1 = R^2 = CO_2Me$
(7) $R^1 = CHO$, $R^2 = CO_2Me$
(8) $R^1 = CO_2Me$, $R^2 = CHO$
(9) $R^1 = CH_2OH$, $R^2 = CHO$
(10) $R^1 = CH_2OSi(Bu^t)Me_2$, $R^2 = CHO$
(11) $R^1 = CH_2OSi(Bu^t)Me_2$, $R^2 = CO_2Me$
(12) $R^1 = CH_2OH$, $R^2 = CO_2Me$

† Systematic name: methyl 7-formyl-2,5-dihydro-5-oxo-1-benzoxepine-3-carboxylate.

accomplished, the following route was chosen for the partial synthesis of the aldehydoester (7).

Treatment of the hydroxy aldehyde (9) with $Me_2(Bu^t)SiCl$ and imidazole [8] in methylene dichloride gave the silyl ether (10) in 80% yield. As expected, its mass spectrum showed only a very weak molecular ion (1%), and the major fragments occurred at *m/z* 275, 259, and 201 [loss of Bu^t, (Bu^t + CH_4), and $Me_2(Bu^t)SiO$, respectively]. The n.m.r. spectrum showed a signal pattern similar to that of the parent compound (9), with the exception that the broad hydroxy-proton singlet (τ 7.48) was replaced by two singlets (τ 9.82 and 8.97) due to the methyl and t-butyl protons, respectively.

The aldehyde (10) was converted into the methyl ester (11) (53%) as described for the conversion of the dialdehyde (5) into the diester (6).[5] The ester (11) showed a weak molecular ion (2%) in the mass spectrum and the major fragments occurred at *m/z* 305, 289, and 231, thus revealing a fragmentation pattern analogous to that of the silyl ether (10). In the n.m.r. spectrum, the formyl-proton singlet (τ 0.13) of the aldehyde (10) was replaced by a signal due to the methyl ester (τ 6.18); the rest of the signal pattern remained unchanged.

Treatment of the silyl ether (11) with AcOH–water [8] at 20 °C gave the hydroxy ester (12) in 71% yield. In its mass spectrum the molecular ion constituted the base peak and prominent fragments appeared due to loss of OMe, CO_2Me, and (CO_2Me + CO). In the n.m.r. spectrum, the $Me_2(Bu^t)Si$-group singlets of the ether (11) were replaced by the broad singlet (τ 6.57) of the hydroxy proton.

The hydroxy ester (12) was oxidized with MnO_2 to the aldehydoester (7) (50%); this was found to be identical in all respects with the natural product.

Experimental

For general techniques see reference 9. Light petroleum refers to that fraction boiling in the range 30—40 °C.

Growth of Marasmiellus ramealis (*Bull.* ex *Fr.*) *Singer and Isolation of the Metabolites.*—The fungus was grown as a shake culture on a medium consisting of glucose (40 g), casein hydrolysate (1 g), KH_2PO_4 (1 g), KCl (0.5 g), $MgSO_4.7H_2O$ (0.5 g), $FeSO_4$ (0.01 g), and yeast extract (2 g) (per litre of water) for 5 d. The culture fluid (157.5 l; 210 flasks) was decanted and continuously extracted with diethyl ether (48 h). The extract was concentrated to *ca.* 200 ml and separated into a neutral and acidic fraction with saturated aqueous $NaHCO_3$.

Acidic fraction. The concentrated acidic fraction was esterified [MeOH–H_2SO_4 (24 : 1)] and the mixture of methyl esters was chromatographed on a SiO_2 column (300 g) with stepwise elution [light petroleum–Et_2O (4 : 1), to Et_2O–MeOH (19 : 1)], and 100-ml fractions were collected.

Fractions 4—8 were combined, concentrated, and the residue was separated by p.l.c. [CH_2Cl_2–light petroleum (1 : 3); continuous elution; 3.25 h] into two bands. The less polar band had $\lambda_{max.}$ (Et_2O) 337, 315, 296, 279, 251, and 240 nm, and the more polar one had $\lambda_{max.}$ (Et_2O) 345, 324.5, 306, 288infl., 244, and 231 nm; $\nu_{max.}$ ($CHCl_3$) 3 310, 1 730, and 840 cm^{-1}. Further attempts at purification failed.

The combined fractions 9—13 were further separated by repeated p.l.c. (CH_2Cl_2 and Et_2O–light petroleum mixtures; both continuous elution) into three fractions. The least polar fraction gave a metabolite (10 mg) which could not be identified, R_F (Et_2O) 0.64 (Found: M^+, 248.1047. $C_{14}H_{16}O_4$ requires *M*, 248.1046); *m/z* 248 (M^+, 60%), 233 (88), 217 (76), 216 (100), 203 (24), 202 (9), 201 (17), and 189 (40). The band of medium polarity yielded as the major component a white solid which, on crystallization (light petroleum), gave needles of methyl pyrrole-2-carboxylate (14 mg), m.p. and mixed m.p. 70—73 °C (lit.,[10a] 73 °C).

The solid obtained from the most polar band was crystallized from chloroform to give needles of methyl *p*-hydroxybenzoate (206 mg), m.p. 126—127 °C (lit.,[10b] 127—129 °C).

Fractions 14—16 yielded a solid which was crystallized from acetone to give needles of *methyl 7-formyl-2,5-dihydro-5-oxo-1-benzoxepine-3-carboxylate* (7) (48 mg), m.p. 121—123 °C; R_F (Et_2O) 0.42 [Found: M^+, 246.0516. $C_{13}H_{10}O_5$ requires *M*, 246.0504; $(M - MeOH)^+$, 214.0259. $C_{12}H_6O_4$ requires *m/z*, 214.0252; $(M - CO_2Me)^+$, 187.0443. $C_{11}H_7O_3$ requires *m/z*, 187.0491; $(M - C_3H_3O_3)^+$, 159.0446. $C_{10}H_7O_2$ requires *m/z*, 159.0446]; $\lambda_{max.}$ ($CHCl_3$) 340 (ε 2 100), 270infl. (19 100), and 253.5 nm (25 600); $\nu_{max.}$ ($CHCl_3$) 2 820 (CHO), 1 720 (ester CO), 1 660 (aldehyde CO), 1 640, 1 605, 1 570, and 840 cm^{-1}; τ [$(CD_3)_2CO$] 6.14 (3 H, s, CO_2CH_3), 4.85 (2 H, s, H^e_2), 2.84 (1 H, s, H^d), 2.74 (1 H, d, *J* 8 Hz, H^a), 1.94 (1 H, dd, *J* 8 and 2 Hz, H^b), 1.54 (1 H, d, *J* 2 Hz, H^c), and −0.03 (1 H, s, CHO); *m/z* 246 (M^+, 100%), 215 (17), 214 (62), 203 (33), 187 (37), 186 (68), and 159 (55).

Neutral fraction. This was chromatographed on a SiO_2 column (300 g) with stepwise elution [light petroleum–Et_2O (3 : 1) to Et_2O–MeOH (19 : 1)] and 100-ml fractions were collected. Fractions 1—8 were concentrated and the solid residue was recrystallized (Et_2O–light petroleum) to give needles of 8-hydroxy-3-methylisocoumarin (2) (150 mg), m.p. 96—100 °C (lit.,[4] 99—100 °C). Fractions 9—10 gave, on further chromatography, 3,4-dihydro-8-hydroxy-3-methylisocoumarin (4) [4] (10 mg) as the major component. P.l.c. of the residue from fractions 13—16 gave marasin (1) [3] (118 mg).

2,5-Dihydro-7-hydroxymethyl-5-oxo-1-benzoxepine-3-carbaldehyde (9).—3,7-Bis(hydroxymethyl)-1-benzoxepine-5-(2*H*)-one (3) (110 mg, 0.5 mmol) and MnO_2 (500 mg) were stirred in CH_2Cl_2 (15 ml) for 1 h. The mixture was filtered (Celite), the MnO_2 residue was washed with warm EtOAc (50 ml), and the filtrate was concentrated. P.l.c. (EtOAc) of the residue gave three bands; the middle band contained the required *monoaldehyde* (9) (73 mg, 67%), b.p. 60—64 °C at 0.25 mmHg; R_F (Et_2O) 0.28 (Found: M^+, 218.0574. $C_{12}H_{10}O_4$ requires *M*, 218.0569); $\lambda_{max.}$ (EtOH) 332 (ε 1 400), 257.5 (7 200), 219 (11 900), and 202 nm (12 400); $\nu_{max.}$ ($CHCl_3$) 3 600 (free OH), 3 450br (bonded OH), 2 880 (CHO), 1 695 (aldehyde CO), 1 655 (ketone CO), 1 635, 1 610 (C=CCO and benzenoid system), 1 490, and 840 cm^{-1}; τ ($CDCl_3$) 7.48 (1 H, brs, disappears on D_2O addition, CH_2OH), 5.25 (2 H, s, CH_2OH), 5.1 (2 H, s, H^e_2), 3.07 (1 H, s, H^d), 2.94 (1 H, d, *J* 8.5 Hz, H^a), 2.48 (1 H, dd, *J* 8.5 and 2 Hz, H^b), 2.08 (1 H, d, *J* 2 Hz, H^c), and 0.3 (1 H, s, CHO); *m/z* 218 (M^+, 100%), 201 (4), 190 (21), 189 (34), 161 (49), 151 (23), 149 (19), 105 (23), and 77 (28).

7-(t-Butyldimethylsilyloxymethyl)-2,5-dihydro-5-oxo-1-benzoxepine-3-carbaldehyde (10).—The formyl alcohol (9) (59 mg, 0.27 mmol), t-butyldimethylsilyl chloride (61 mg, 0.41 mmol), and imidazole (46 mg, 0.68 mmol) were stirred in CH_2Cl_2 (20 ml) at 20 °C for 8 h. The solution was filtered, and the filtrate was washed with HCl (0.1M; 50 ml) and brine, dried, and concentrated. The residue was subjected to p.l.c. [Et_2O–light petroleum (1 : 9); continuous elution; 2 h] and the material obtained from the major band was crystallized (light petroleum) to give yellow needles of the *silyloxy compound* (10) (72 mg, 80%), m.p. 59—61 °C; R_F (Et_2O) 0.68 {Found: $(M - Bu^t)^+$, 275.0736. $C_{14}H_{15}O_4Si$ requires *m/z*, 275.0733; $(M - Bu^t - CH_4)^+$, 259.0421. $C_{13}H_{11}O_4Si$ requires *m/z*, 259.0416; $[M - OSi(Bu^t)Me_2]^+$, 201.0575. $C_{12}H_9O_3$ requires *m/z*, 201.0598}; $\lambda_{max.}$ (EtOH) 332.5 (ε 1 800), 257.5

(9 800), 220 (18 800), and 201 nm (22 300); $\nu_{max.}$ (CCl_4) 2 720 (CHO), 1 705 (aldehyde CO), 1 660 (ketone CO), 1 640, 1 615 (C=CCO and benzenoid system), 1 255 ($SiMe_2$), 1 100, and 1 040 cm^{-1} (Si–O); τ (CCl_4) 9.82 (6 H, s, $SiMe_2$), 8.97 (9 H, s, Bu^t), 5.18 (2 H, s, CH_2OSi), 4.97 (2 H, s, H^c_2), 2.99 (1 H, s, H^d), 2.85 (1 H, d, *J* 8.5 Hz, H^a), 2.42 (1 H, dd, *J* 8.5 and 2 Hz, H^b), 2.04 (1 H, d, *J* 2 Hz, H^c), and 0.13 (1 H, s, CHO); *m/z* 332 (M^+, 1%), 275 (14), 260 (18), 259 (100), 201 (28), and 75 (28).

Methyl 7-(t-*Butyldimethylsilyloxymethyl*)-2,5-*dihydro*-5-*oxo*-1-*benzoxepine*-3-*carboxylate* (11).—The silyloxy aldehyde (10) (117 mg, 0.35 mmol), MnO_2 (210 mg, 2.4 mmol), KCN (42 mg), and AcOH (0.4 ml) were stirred in MeOH (20 ml) for 12 h. The mixture was filtered (Celite) and the filtrate was diluted with Et_2O (80 ml); the mixture was then washed (brine), dried, and concentrated. P.l.c. [Et_2O–light petroleum (1 : 9); continuous elution; 2 h] of the residue gave the *ester* (11) (67 mg, 53%), b.p. 130—138 °C (block) at 0.2 mmHg; R_F (Et_2O) 0.61 {Found: $(M - Bu^t)^+$, 305.0854. $C_{15}H_{17}O_5Si$ requires *m/z*, 305.0863; $(M - Bu^t - CH_4)^+$, 289.0531. $C_{14}H_{13}O_5Si$ requires *m/z* 289.0530; $[M - OSi(Bu^t)Me_2]^+$, 231.0654. $C_{13}H_{11}O_4$ requires *m/z* 231.0651}; $\lambda_{max.}$ (EtOH) 345 (ε 1 400), 280 (4 500), 251.5 (6 400), 220 (12 600), and 203.5 nm (17 200); $\nu_{max.}$ (CCl_4) 1 730 (ester CO), 1 655 (ketone CO), 1 640, and 1 615 cm^{-1} (C=CCO and benzenoid system); τ (CCl_4) 9.94 (6 H, s, $SiMe_2$), 9.09 (9 H, s, Bu^t), 6.18 (3 H, s, CO_2CH_3), 5.35 (2 H, s, CH_2OSi), 5.11 (2 H, s, H^e_2), 2.98 (1 H, d, *J* 8 Hz, H^a), 2.85 (1 H, s, H^d), 2.54 (1 H, dd, *J* 8 and 2 Hz, H^b), and 2.19 (1 H, d, *J* 2 Hz, H^c); *m/z* 362 (M^+, 2%), 305 (40), 289 (100), and 231 (9).

Methyl 2,5-*Dihydro*-7-*hydroxymethyl*-5-*oxo*-1-*benzoxepine*-3-*carboxylate* (12).—A solution of the silyloxy ester (11) (306 mg, 0.87 mmol) in AcOH–water (2 : 1; 6 ml) was stirred at 20 °C for 3.5 h. The mixture was diluted with water (20 ml) and the aqueous solution was extracted with Et_2O. The extract was washed (brine), dried, filtered, and concentrated. The residue was subjected to p.l.c. (Et_2O; continuous elution; 2 h) and gave the *hydroxy ester* (12) (149 mg, 71%), R_F (Et_2O) 0.28; b.p. 175—185 °C (block) at 0.25 mmHg (Found: M^+, 248.0682. $C_{13}H_{12}O_5$ requires *M*, 248.0685); $\lambda_{max.}$ (Et_2O) 348 (ε 1 200), 279 (4 400), 250 (5 500), 220 (14 200), and 205 nm (17 100); $\lambda_{max.}$ ($CHCl_3$) 3 610 (OH), 1 725 (ester CO), 1 655 (ketone CO), 1 635, and 1 610 cm^{-1} (C=CCO and benzenoid system); τ ($CDCl_3$) 6.57 (1 H, br s, disappears on D_2O addition, OH), 6.15 (3 H, s, CO_2CH_3), 5.38 (2 H, s, CH_2OH), 5.05 (2 H, s, H^e_2), 2.98 (1 H, d, *J* 8 Hz, H^a), 2.83 (1 H, s, H^d), 2.52 (1 H, dd, 8 and 2 Hz, H^b), and 2.14 (1 H, d, *J* 2 Hz, H^c); *m/z* 248 (M^+, 100%), 217 (10), 216 (33), 189 (33), 188 (57), and 161 (38).

Methyl 7-*Formyl*-2,5-*dihydro*-5-*oxo*-1-*benzoxepine*-3-*carboxylate* (7).—The hydroxy ester (12) (145 mg, 0.58 mmol) and MnO_2 (1.5 g) were stirred in CH_2Cl_2 (15 ml) for 2 h. Filtration (Celite), concentration of the filtrate, and crystallization (Me_2CO) of the residual solid gave needles of the required aldehydoester (7) (72 mg, 50%), m.p. and mixed m.p. 121—123 °C; R_F (Et_2O) 0.42. The spectra were identical with those of the natural product.

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References

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