

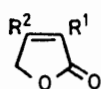
Metabolites of The Higher Fungi. Part 18.¹ 3-Butyl-4-methylfuran-2(5*H*)-one and 3-Butyl-4-methylenefuran-2(5*H*)-one. New γ -Butyrolactones from the Fungus *Hypoxylon serpens* (Persoon ex Fries) Kickx

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Culture solutions of the fungus *Hypoxylon serpens* contain a mixture of two isomeric γ -butyrolactones. These have been identified as 3-butyl-4-methylfuran-2(5*H*)-one (1a) and 3-butyl-4-methylenefuran-2(5*H*)-one (2). The synthesis of (1a) and the isomeric 4-butyl-3-methylfuran-2(5*H*)-one (1b) is described. At room temperature lactone (2) isomerises slowly to yield lactone (1a). This is the first report of the occurrence of a 4-*exo*-methylene γ -lactone as a natural product.

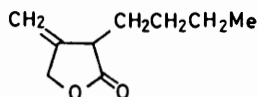
THE genus *Hypoxylon* is large containing over 120 species and varieties and during a chemotaxonomic survey of the metabolites produced in culture by the various members of this genus we have isolated two new lactones (1a) and (2) from the culture medium of a strain of the fungus *H. serpens*.†

The fungus grows readily on a solution of malt extract and produces a mixture of (1a) and (2) which can be isolated from the medium by solvent extraction. The components of the mixture yield brown stains with iodine on silica gel and can be separated by chromatography. The yield of the mixture is quite high (0.8 g l⁻¹) and the ratio (1a) : (2) is *ca.* 7 : 1.



(1a) R¹ = CH₂CH₂CH₂Me, R² = Me

(1b) R¹ = Me, R² = CH₂CH₂CH₂Me



(2)

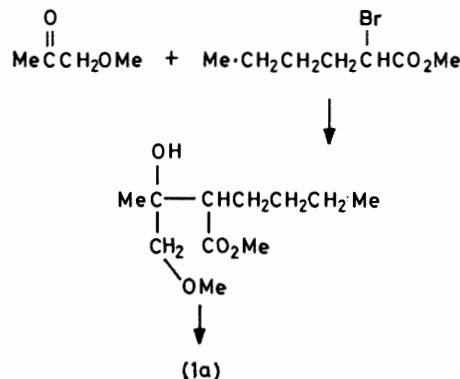
Lactone (1a), C₉H₁₄O₂, is an oil, b.p. 88 °C at 0.6 mmHg, which dissolves slowly in hot 2*N*-NaOH and is recovered unchanged on acidification of the solution. This property, together with an i.r. carbonyl absorption at 1750 cm⁻¹ proved the presence of a lactone structure. Attempts to prepare a crystalline derivative with a variety of amines were unsuccessful. The compound was not reduced by hydrogen at room temperature in the presence of either Pd-charcoal or Adams catalyst but gave a positive Legal test for unsaturated γ -lactones.

The ¹H n.m.r. spectrum gave the general structure. The presence of a butyl grouping is confirmed by absorptions at δ 0.89 (3 H, t, CH₃CH₂), 1.36 (4 H, m, CH₂CH₂), and 2.22 (2 H, t, =C-CH₂CH₂). Additional absorptions at δ 2.00 (3 H, s, CH₃-C=) and 4.58 (2 H, s, CH₂O) account for the remaining protons in the molecule and lead to the assignment of only two possible structures, (1a) or (1b).

Ozonolysis of (1a) gave an oil which with 2,4-dinitrophenylhydrazine yielded a mixture of *Z*- and *E*-isomers of a bis-derivative. The *Z* compound is yellow and

exhibits a chelated carbonyl absorption at 1694 cm⁻¹; and the *E* compound is orange and shows an unchelated carbonyl absorption at 1734 cm⁻¹. The formation of (*Z*)- and (*E*)-phenylhydrazone isomers is well known in the 2-oxo-acid series.^{2,3}

Final proof of the structure was established by the synthesis of (1a) and its isomer (1b). The lactone (1a) was synthesised by condensing methyl 2-bromohexano-



ate with methoxyacetone in a Reformatsky reaction. Hydrolysis of the resulting hydroxy-ester, followed by demethylation of the methoxy-acid with HBr, resulted in spontaneous cyclisation to the required lactone. The isomeric lactone (1b) was similarly prepared from methyl 2-bromopropionate and 6-methoxyhexan-2-one. Both isomers have similar u.v. and i.r. spectra and the ¹H n.m.r. spectra show only small differences in the chemical shifts of the methyl and methylene protons. The C-2', C-3', C-4', and C-5' methylene protons of (1a) are all at a higher field than those of (1b) (0.1, 0.1, 0.22, and 0.07, respectively). The C-1' methyl protons of (1a) also appear at higher field (0.05) than those of (1b), but the C-5' methyl protons are at lower field (0.18) than in (1b). The best differentiation between the two isomers is obtained either by an examination of the differences in chemical shift produced by the addition of europium shift reagent or by comparing the mass spectra.

Addition of Eu(fod)₃ to solutions in chloroform of both natural and synthetic lactone (1a), causes the C-3' and C-4' methylene protons to move significantly further downfield than those of (1b). Conversely, the protons of the C-5' methyl group move further downfield in (1b)

† Isolated from stromata collected at Glyn Wood, Bangor, Gwynedd, and maintained under the collection No. 10(I)45 AJSW.

than in (1a). Movement of the C-5 methylene protons are little different for both isomers (Figures 1 and 2 and Table).

The mass spectrum of (1a) shows major ions at 154

The ion at m/e 112 in (1a) is presumably a rearrangement ion produced by loss of C_3H_6 from the side chain made possible by the close proximity of the side chain to the lactone carbonyl group.

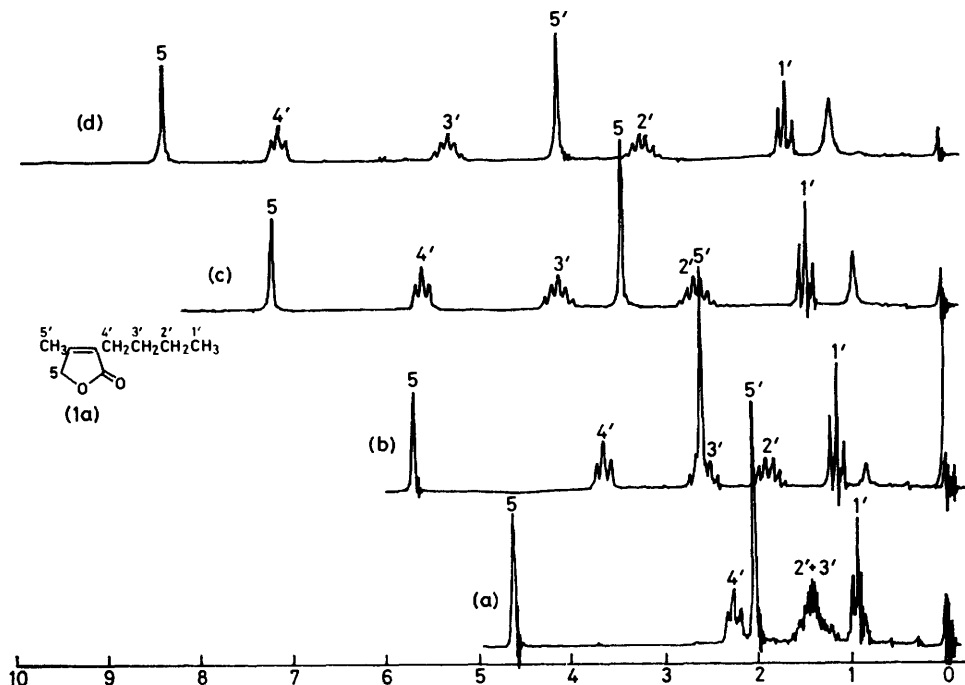


FIGURE 1 Effect of chemical shift reagent $Eu(fod)_3$ on natural and synthetic lactone (1a). $[Eu(fod)_3]/M$: (a) 0.00; (b) 0.60; (c) 1.30; (d) 1.50

(M^+) , 139 ($M - CH_3$), 125 ($M - CHO$), and 112 ($M - C_3H_6$), whereas in the same region of the spectrum

The isomeric lactone (2) is an optically active oil, $[\alpha]_D +77^\circ$, which isomerises slowly at room temperature

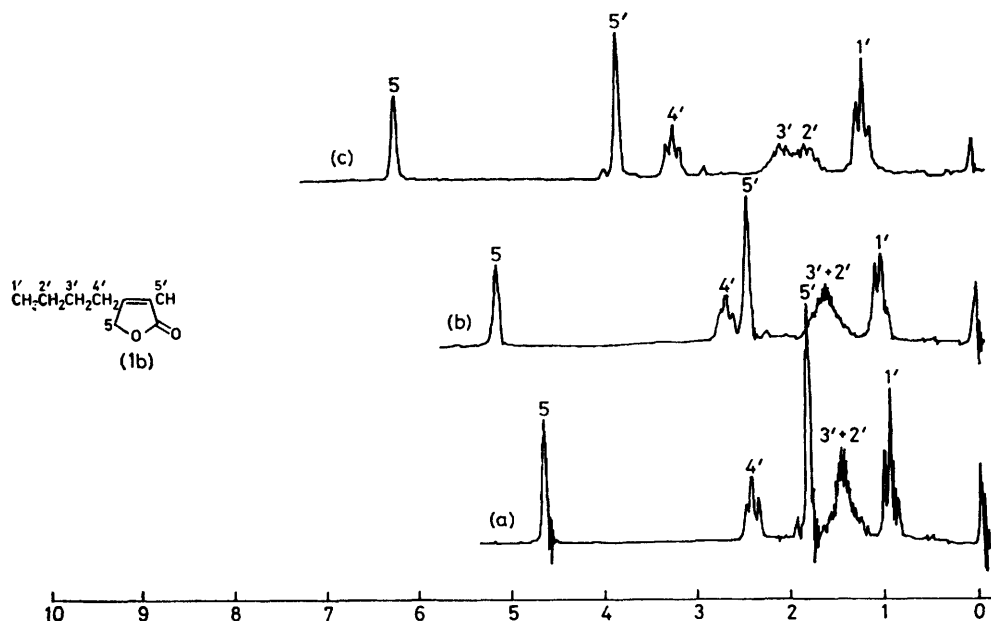


FIGURE 2 Effect of chemical shift reagent $Eu(fod)_3$ on synthetic lactone (1b). $[Eu(fod)_3]/M$: (a) 0.00; (b) 0.20; (c) 0.80

of (1b) only ions at 154 (M^+) and 125 ($M - CHO$) are observed. The remainder of the spectrum is the same for both isomers showing m/e 85, 83, 55, 43, 41, and 39.

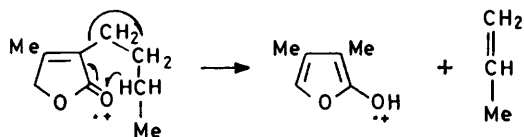
to yield (1a). There is no significant absorption in the u.v. spectrum above 200 nm and the i.r. carbonyl absorption occurs at 1782 cm^{-1} . This absorption is

typical of saturated γ -lactones.⁴ The mass spectrum is identical with that of (1a) but the n.m.r. spectrum lacks a

Effect of added $\text{Eu}(\text{fod})_3$ on n.m.r. spectra of lactones (a) and (b)

Protons	0.00 δ	[Eu(fod) ₃]/[lactone]					
		0.20		0.49		0.80	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	
1' (1a)	0.89	0.99	0.10	1.06	0.17	1.22	0.33
(1b)	0.94	0.99	0.05	1.02	0.08	1.13	0.19
2' (1a)	1.36	1.50	0.14	1.68	0.32	2.09	0.73
(1b)	1.46	1.55	0.09	1.63	0.17	1.74	0.28
3' (1a)	1.36	1.85	0.49	2.21	0.85	3.02	1.66
(1b)	1.46	1.55	0.09	1.63	0.17	2.00	0.54
4' (1a)	2.22	2.79	0.57	3.19	0.97	4.24	2.02
(1b)	2.42	2.59	0.17	2.78	0.36	3.14	0.72
5' (1a)	2.00	2.23	0.23	2.42	0.42	2.87	0.87
(1b)	1.82	2.27	0.45	2.75	0.95	3.69	1.87
5 (1a)	4.58	4.97	0.39	5.35	0.77	6.13	1.55
(1b)	4.65	4.98	0.33	5.33	0.68	6.08	1.43

C-5 methyl absorption and shows two new absorptions at δ 5.09 (2 H, sextet, J 2 Hz) and 3.09 (1 H, m). The former is typical of exocyclic methylene groups⁵ and the latter can be ascribed to the new ring methine proton. The C-5 methyl absorption of (1a) appears when samples of (2) are kept at room temperature over three months; over this period isomerisation is still incomplete and the ¹H n.m.r. spectrum still shows the presence of unchanged (2). This isomerisation of (2) to yield (1a) proves that the exocyclic methylene group is attached to the ring at position 4. The ratio of isomers is probably (7 : 1)



correct since the yield of the mixture during incubation is low in the early stages of growth and increases markedly towards the end of the incubation period. *H. serpens* produces spores readily on solid media and the cultures develop the characteristic odour of the lactones as sporulation commences.

But-3-enolides are quite common as natural products, the ring system occurs in the cardenolides, and 4-hydroxybutenolides (tetronic acids) occur extensively in the fungi and lichens. Volatile butenolides bearing substituents at the 3- and 5-positions have been isolated from cultures of *Streptomyces griseus* and *S. odiferus*.⁶ Methylenebutanolides also occur naturally; 3-methylenebutanolide (tulipelin) is a powerful antifungicide occurring in tulip bulbs⁷ and in the dog-tooth violet *Erythronium americanum*,⁸ and protoanemonin is an example of the 5-methylenebutanolide system isolated from buttercups and many species of *Ranunculus*, *Anemone*, and *Clematis*.⁹ To our knowledge this is the first example of the occurrence of a 4-methylenebutanolide as a natural product.

EXPERIMENTAL

Isolation of 3-Butyl-4-methylfuran-2(5H)-one (1a) and 3-Butyl-4-methylenefuran-2(5H)-one (2).—*Hypoxylon serpens*

was cultured in Roux flasks (2 l) in a solution of malt extract (Boots, 40 g l⁻¹). After 8 weeks the solution was filtered from the colourless mycelium. The filtrate (10 l) was extracted three times with ether and the extract dried (Na_2SO_4) and evaporated to yield a pale yellow oil (8.3 g). The oil was dissolved in light petroleum (b.p. 60–80°)–ether–acetic acid (70 : 30 : 3) (25 ml) and the solution applied to a column of silica gel (3 × 60 cm). Development of the column with the same mixture of solvents gave two fractions. Fraction (i) yielded the *furanone* (2) as an oil (1.2 g) which was not purified further (Found: C, 69.9; H, 9.1. $\text{C}_9\text{H}_{14}\text{O}_2$ requires C, 70.1; H, 9.1%), $\delta(\text{CDCl}_3)$ 0.92 (3 H, t), 1.37 (4 H, m), 1.75 (2 H, m), 3.09 (1 H, m), 4.73 (2 H, q, J 2 Hz), and 5.09 (2 H, sextet J 2 Hz). Fraction (ii) gave a pale yellow oil (6.5 g) which was distilled to yield the *furanone* (1a) as an oil, b.p. 88° at 0.6 mmHg (Found: C, 70.0; H, 9.1. $\text{C}_9\text{H}_{14}\text{O}_2$ requires C, 70.1; H, 9.1%).

Ozonolysis of 3-Butyl-4-methylfuran-2(5H)-one (1a).—To a solution of pentane saturated with ozone at 0 °C was added the lactone (1 g) at 0°. After 5 min the pentane was removed *in vacuo* and the residual oil dissolved in methanol and hydrogenated over 5% Pd– CaCO_3 . The reduction was stopped after the absorption of 150 ml of hydrogen. The solution was filtered and the methanol removed *in vacuo*. Treatment of the residual oil with 2,4-dinitrophenylhydrazine in ethanol gave an immediate orange-yellow precipitate which was filtered off and chromatographed on silica gel with benzene–chloroform (70 : 30) as eluant. A number of minor yellow bands were eluted first and discarded. The main orange-yellow band was eluted with benzene–chloroform (1 : 1) to yield first a yellow and then an orange component. Evaporation and recrystallisation from acetic acid gave the yellow (*Z*)-2-oxopropyl 2-oxohexanoate bis-2,4-dinitrophenylhydrazone, m.p. 201°, ν_{max} 1695 cm^{-1} (Found: C, 46.2; H, 4.0; N, 19.9. $\text{C}_{21}\text{H}_{22}\text{O}_{10}\text{N}_4$ requires C, 46.2; H, 4.0; N, 20.5%), and the orange (*E*) derivative, m.p. 174°, ν_{max} 1745 cm^{-1} (Found: C, 46.1; H, 4.1; N, 19.1%).

Methyl 2-[1-Hydroxy-1-(methoxymethyl)ethyl]hexanoate.—A mixture of methoxyacetone (8.8 g), methyl 2-bromohexanoate (21.9 g), and ether (50 ml) was added in small portions to activated zinc wool (6.6 g). After addition of the first 20 ml, the reaction was initiated by warming, and then the remainder of the mixture added at a sufficient rate to maintain gentle reflux. The mixture was refluxed for 6 h and ice-cold sulphuric acid (2N) added to destroy the zinc complex. The ether layer was separated, washed twice with sodium hydrogen carbonate solution, then dried (Na_2SO_4) and evaporated. Distillation of the residual oil gave *methyl 2-[1-hydroxy-1-(methoxymethyl)ethyl]hexanoate* (9.6 g) as a colourless oil, b.p. 80–82° at 1.0 mmHg, (Found: C, 60.3; H, 10.0. $\text{C}_{11}\text{H}_{22}\text{O}_4$ requires C, 60.5; H, 10.1%).

3-Butyl-4-methylfuran-2(5H)-one (1a).—A mixture of the above ester (10 g), ethanol (20 ml), and 2N-sodium hydroxide (30 ml) was refluxed for 2 h. After cooling, the solution was extracted with ether, acidified with dilute sulphuric acid, and re-extracted twice with ether. Evaporation of the ether gave 2-[1-hydroxy-1-(methoxymethyl)ethyl]hexanoic acid (8.3 g) as an oil which was not purified further.

A mixture of the acid (8.3 g) and HBr–HAc (25 ml; 45% HBr) was refluxed for 2 h. The brown solution was evaporated *in vacuo* to remove the acetic acid and the

residual oil poured onto ice. The mixture was rendered alkaline with 2*N*-sodium carbonate and extracted with ether. Evaporation of the ether and distillation of the residue gave 3-butyl-4-methylfuran-2(5H)-one (1a) (4.3 g) as an oil, b.p. 88° at 0.6 mmHg (Found: C, 70.05; H, 9.3. C₉H₁₄O₂ requires C, 70.1; H, 9.1%). The i.r., ¹H n.m.r., and mass spectra were identical with those of the natural product.

Methyl 3-hydroxy-3-(methoxymethyl)-2-methylheptanoate was similarly prepared from methyl 2-bromopropionate (16.7 g) and 1-methoxyhexan-2-one (13 g) by the method described above and was obtained as an oil (9.6 g), b.p. 82° at 0.7 mmHg (Found: C, 60.2; H, 10.1. C₁₁H₂₂O₄ requires C, 60.5; H, 10.1%). Hydrolysis of the ester (6 g) gave 3-hydroxy-3-(methoxymethyl)-2-methylheptanoic acid (4.3 g) as a viscous oil which after demethylation and cyclisation yielded 3-methyl-4-butylfuran-2(5H)-one (2) (2.7 g) as an oil, b.p. 96–98 °C at 0.8 mmHg (Found: C, 70.3; H, 9.0. C₉H₁₄O₂ requires C, 70.10 H, 9.1%),

ν_{\max} . 1 746 cm⁻¹, δ (CDCl₃) 0.94 (3 H, t), 1.46 (4 H, m), 1.82 (3 H, s), 2.42 (2 H, t), and 4.65 (2 H, s).

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