Flavofungin: A Mixture of 13,15,17,19,21,23,25,27-Octahydroxy-31-isopropyl-14-methyl- and 13,15,17,19,21,23,25,27-Octahydroxy-14-methyl-31-s-butyl-hentriaconta-2,4,6,8,10,28-hexaen-31-olide

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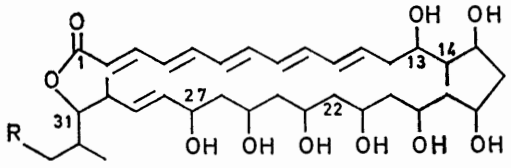
Flavofungin, the antifungal antibiotic from *Streptomyces flavofungini*, has been shown to be a mixture (*ca.* 10:1) of 13,15,17,19,21,23,25,27-octahydroxy-31-isopropyl-14-methyl- (1a) and 13,15,17,19,21,23,25,27-octahydroxy-14-methyl-31-s-butyl- (1b) hentriaconta-2,4,6,8,10,23-hexaen-31-olide. Its relationship to mycoticin is discussed.

In a preliminary communication [1] we reported that flavofungin, a macrolide antifungal antibiotic from *Streptomyces flavofungini*,[2] consists of a mixture of two compounds with structures (1a) and (1b) in a ratio of about 10 : 1. Mycoticin from *S. ruber* has been shown by Wasserman *et al.*[3] to consist of an approximately equimolar mixture of two compounds also with the same gross structures (1a) and (1b). Its biosynthesis from acetate and propionate has been studied.[4] We now give details of our work on flavofungin, and of the direct comparison of flavofungin with mycoticin.

On treatment with acetic anhydride in pyridine flavofungin yielded an octa-acetyl derivative. Mild alkaline hydrolysis regenerated flavofungin. The mass spectrum of the octa-acetate showed a molecular ion (M_1^+) for (1a) octa-acetate at *m/e* 986, and another (M_2^+) for (1b) octa-acetate at *m/e* 1000; $M_1^+ : M_2^+ \approx 9 : 1$. Successive loss of eight acetic acid units (60 a.m.u.)

[1] R. Bognár, B. O. Brown, W. J. S. Lockley, S. Makleit, T. P. Toube, B. C. L. Weedon, and K. Zsupán, *Tetrahedron Letters*, 1970, 471.
[2] J. Uri, and I. Békési, *Nature*, 1958, **181**, 988.
[3] H. H. Wasserman, J. E. Van Verth, D. J. McCaustland, I. J. Borowitz, and B. Kamber, *J. Amer. Chem. Soc.*, 1967, **89**, 1535.
[4] H. H. Wasserman, P. A. Zoretic, and P. S. Mariano, *Chem. Comm.*, 1970, 1634.

from each molecular ion were clearly visible. High resolution measurements on the peaks at *m/e* 926, 866, 806, and 686 (see Experimental section) (loss of 1, 2, 3, and 5 units of acetic acid) indicated a molecular formula of $C_{52}H_{74}O_{18}$ for M_1 (and therefore $C_{53}H_{76}O_{18}$ for M_2).



(1a) R = H
(1b) R = Me

Treatment of flavofungin with trimethylchlorosilane and hexamethyldisilazane produced the octatrimethylsilyl ether. In the mass spectrum the two expected molecular ions were found at *m/e* 1226 (M_1^+) and 1240 (M_2^+). Loss of up to six units of trimethylsilanol (90 a.m.u.) was observed; further losses did not give rise to ions significantly more abundant than those of other fragment ions.

These studies established the molecular formulae of (1a) and (1b) as $C_{36}H_{58}O_{10}$ and $C_{37}H_{60}O_{10}$, respectively, and indicated the presence in both molecules of eight reactive hydroxy-groups.

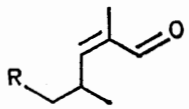
Hydrogenation of flavofungin over Adams catalyst, palladised charcoal, or Raney nickel yielded perhydroflavofungin. Its octa-acetate and octatrimethylsilyl ether showed increments of 12 a.m.u. relative to the unreduced materials on each of the peaks in the high mass region of the mass spectrum. Flavofungin thus contains six reducible carbon–carbon double bonds. The main compound obtained by hydrogenation of octa-acetylflavofungin was identical with that produced by acetylation of perhydroflavofungin.

In the u.v. spectrum, flavofungin exhibited maxima at 364 and 254 nm. Reduction with lithium aluminium hydride yielded material with $\lambda_{max.}$ 349, 331, and 318 nm, together with smaller amounts of a by-product with absorptions at 318, 304, and 290 nm. This behaviour suggested a pentaene chromophore conjugated to a carbonyl group, which on reduction gave a pentaene chromophore, not further conjugated, as well as some tetraene. Borohydride failed to produce an analogous effect, indicating that flavofungin contained a polyene ester (or lactone) function, which together with the eight hydroxy-groups accounts for all oxygen atoms.

Perhydroflavofungin was more readily hydrolysed than the parent compound. The product was identified as a nonahydroxyhexatriacontanoic acid by mass spectral studies on the derived nona-acetate and methyl ester. Since no carbon atoms are lost during the hydrolysis of the main constituent of flavofungin, the latter must be a lactone rather than an ester. Furthermore, since the carbonyl group is conjugated with the polyene system, flavofungin must be a polyene macrolide.

Fusion of perhydroflavofungin with potassium hydroxide gave tridecane-1,13-dioic acid as the only product with large molecular weight (in some experiments a small amount of undecane-1,11-dioic acid, the expected by-product,[5] was also obtained). Alkali fusion of flavofungin under the same conditions yielded no acids of high molecular weight. These results confirmed [5] that the portion of the molecule containing the chromophore is in an unbranched chain of thirteen carbon atoms, and that the first two oxygen functions are at C(1) and C(13).

The position of the sixth carbon–carbon double bond, not present in the chromophore but indicated by hydrogenation, was revealed by ozonolysis of octa-acetylflavofungin. This gave, among other products, 2,4-dimethylpent-2-en-1-al (2a), which was identified by comparison of its 2,4-dinitrophenylhydrazone with that of an authentic synthetic specimen. Presumably fission of the double bond gave initially an aldehyde with a β-oxygen



(2a) R = H
(2b) R = Me

substituent which was readily eliminated to form the observed α-methyl-αβ-unsaturated aldehyde. This α-methyl aldehyde cannot be derived by fission of the ω-double bond in the chromophore since it has been shown that the chain is unbranched between C(1) and C(13). The same degradation product (2a) was also obtained by oxidation of flavofungin in pyridine with permanganate. In the mass spectra of the 2,4-dinitrophenylhydrazone of both samples of (2a), there were also peaks due to *ca.* 8% of the homologue (2b). The spectrum contained a peak for the loss (43 a.m.u.) of the isopropyl group from the molecular ion of (2a), but no peak for a corresponding loss from the molecular ion of (2b) was observed. It was concluded that the extra carbon atom in the higher homologue of the antibiotic is located as shown in (2b).

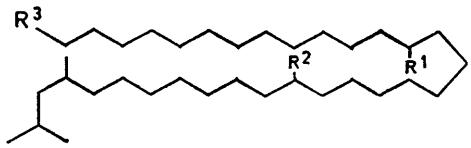
Neither flavofungin nor its perhydro-derivative was cleaved with periodate. Accordingly, neither is a 1,2-diol.

The carbon skeleton of flavofungin was established by reduction to the parent hydrocarbon by a route similar to that employed by Cope [6] in the elucidation of the structure of fungichromin. Perhydroflavofungin was converted into a polyol by reduction with lithium aluminium hydride. Treatment of the product with hydriodic acid and phosphorus produced a crude iodo-compound which was reduced with lithium aluminium hydride. This gave a hydrocarbon mixture, which was hydrogenated over Adams catalyst to reduce all unsaturated materials. Preparative g.l.c. gave a single hydrocarbon as the major product. Its mass spectrum exhibited peaks at *m/e* (*M* − 2), 323, 211, 210, and 85, indicating [6]

[5] R. A. Dytham and B. C. L. Weedon, *Tetrahedron*, 1960, **9**, 246.

[6] A. C. Cope, R. K. Bly, E. P. Burrows, O. J. Ceder, E. Ciganek, B. T. Giles, R. F. Porter, and H. E. Johnson, *J. Amer. Chem. Soc.*, 1962, **84**, 2170.

its identity as a trimethyltritriacontane, (3a) or (3b). When the reaction sequence was repeated but with lithium aluminium deuteride in place of the hydride, an increment of 2 a.m.u. was observed in the peaks

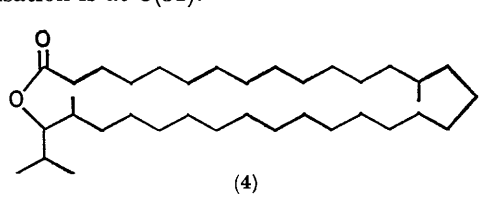


(3a) R^1 = Me, R^2 = H, R^3 = Me
(3b) R^1 = H, R^2 = Me, R^3 = Me
(3c) R^1 = Me, R^2 = H, R^3 = CHD_2
(3d) R^1 = Me, R^2 = H, R^3 = H

originally at *m/e* 504, 211, and 210. This established the deuteride hydrocarbon as (3c) and hence the unlabelled compound as 2,4,20-trimethyltritriacontane (3a). This assignment was further confirmed by hydrolysis of perhydroflavofungin, photochemical decarboxylation [7] of the resulting acid in the presence of lead tetra-acetate and iodine, and reduction to the hydrocarbon; in this case 2,4,20-trimethyldotriacontane (3d) was produced.

All the reductions of flavofungin to saturated hydrocarbons indicated the presence of four methyl groups in the macrolide. The high-field regions in the n.m.r. spectra of flavofungin and its derivatives were consistent with such a formulation. In the n.m.r. spectrum of the deca-acetate of the polyol (produced by reduction of perhydroflavofungin with lithium aluminium hydride) this feature was particularly clear: the methyl signals occurred as a system of three doublets (12H, each with *J* 7 Hz; 1 : 1 : 2). Another interesting feature of the same spectrum was a triplet at δ 4·05 p.p.m. (2H, *J* 6·5 Hz) attributable to the C(1) methylene group derived from the lactone carbonyl.

The position of lactonisation was shown by conversion of flavofungin into the saturated parent deoxylactone by a procedure which may prove of general use in the elucidation of the structures of other macrocyclic lactones. Perhydroflavofungin was converted into its octatosyl derivative, treatment of which with lithium bromide in dimethylformamide produced a mixture of unsaturated compounds. The least polar of these compounds exhibited conjugated heptaene absorption in the electronic spectrum. This ease of elimination lent support to the 1,3-relationship of the original hydroxy-groups. Hydrogenation of the mixture (first over Raney nickel and then over Adams catalyst) yielded the deoxylactone (4) whose mass spectrum showed a molecular ion at *m/e* 534. The only prominent fragment ions in the high mass region were observed at *m/e* 491 (*M* − C_3H_7) and 462 (*M* − C_4H_8O), and this establishes that lactonisation is at C(31).



(4)

On this evidence the only structures that can be written for the antibiotic are (1a) and (1b). These formulations require the 27-hydroxy-group to be allylic. All attempts to verify this directly by selective oxidation were unsuccessful. However, careful treatment of octa-acetylflavofungin in acetic acid with hydrogen and Adams catalyst produced hepta-acetylperhydroflavofungin (30%), and this provides strong support for the view that one of the acetoxy-groups in octa-acetylflavofungin is allylic. Under some conditions, hydrogenolysis (*ca.* 10%) was also observed with flavofungin.

No attempt to extend the double bond system in flavofungin by acid-catalysed dehydration succeeded. Experiments aimed at radical acetoxylation of octa-acetylflavofungin at the allylic 12-position also failed.

Apart from the proportions of the two constituents, our conclusions concerning the structures of flavofungin are the same as those of Wasserman *et al.*[3] for mycoticin. However for the latter no proof has yet been published for the position of the hydroxy-groups, or the presence of the allylic hydroxy-function. Their evidence for the structure of the hydrocarbon (3a), and hence for the presence of a 14-methyl group, rests on the implicit assumption that this methyl group is at the same position in (1a) and (1b). It does not preclude the possibility that this methyl group is at C(22) in (1a) [*i.e.* that the hydrocarbon is in fact (3b)].

We have carried out a direct comparison of flavofungin (m.p. 210°) with a sample of mycoticin (m.p. 220°) (kindly supplied by Professor H. H. Wasserman). No differences were observed in the following properties: i.r. (CsBr disc); u.v. (95% EtOH or dioxan); u.v. (95% EtOH) of the octa-acetates; mass spectra of the octa-acetates and octatrimethylsilyl ethers (except for the relative intensities of peaks due to the two homologues); n.m.r. ($CDCl_3$) spectra of the two antibiotics (except for the position of absorptions of the OH protons, δ 5·10 and 5·35 p.p.m., respectively) and of their octa-acetates; t.l.c. of the antibiotics, and their octa-acetate, perhydro-, and perhydro-octa-acetate derivatives; and hydrogenolysis. However the *X*-ray powder photographs of the two antibiotics showed significant differences, the pattern for flavofungin being more clearly defined than that of the less homogeneous mycoticin. Moreover measurements of specific rotations in dioxan solution gave some surprising results.

For mycoticin Wasserman *et al.*[3] reported $[\alpha]_D$ +64° (dioxan), but in our experience the specific rotation changes with time. Measurements 8 and 9 min after dissolution of a freshly purified sample of mycoticin in dioxan showed an increase in rotation which reached a sharp maximum at 9·5 min of $[\alpha]_D$ +81·7°. Subsequently this positive rotation decayed until after 15·5 min the rotation was zero. The solution then became laevorotatory, finally achieving a static $[\alpha]_D$ value of −18·2° after 33 min. Parallel measurements on flavofungin did not reveal the same behaviour, although the negative

[7] D. H. R. Barton and E. P.Serebryakov, *Proc. Chem. Soc.*, 1962, 309.

rotation increased slightly to give a final value after 20 min of $[\alpha]_D$ −36·5°. Solutions in dioxan of the two octa-acetates were both laevorotatory, but the rotations also varied with time. The variations with mycoticin octa-acetate were greater than those with the flavofungin derivative, but the latter was consistently the more laevorotatory, $[\alpha]_D$ *ca.* −66°. The c.d. curves of the two octa-acetates were similar in shape but quantitatively different (see Experimental section and Table).

Circular dichroism studies of octa-acetyl-mycoticin and -flavofungin in dioxan

Octa-acetylflavofungin		Octa-acetylmycoticin	
λ	Δε	λ	Δε
208	+2·34	210	+0·85
224	−2·34	224	−2·04
260	−5·19	260	−3·73
343	−2·51	345	−1·86
371	−2·18	362	−2·04
389	−2·51	380	−1·86

We conclude that the two constituents of flavofungin have the same gross structures as those of mycoticin, but no conclusion can yet be made concerning their absolute configurations. It is possible that the observed differences in the (final) optical properties of the two antibiotics and of their octa-acetates may simply reflect the different proportions of the two constituents, one of which has an additional chiral centre [C(32)]. The initial differences in the specific rotations of the two antibiotics must then be ascribed to some such phenomenon as micelle formation. However it cannot be excluded that the two antibiotics differ in absolute configuration at one or more asymmetric centre. The situation with regard to flavofungin and mycoticin is thus reminiscent of that between lagosin and fungichromin.[6]

EXPERIMENTAL

All operations involving flavofungin, mycoticin, and their completely acetylated derivatives were carried out in a nitrogen atmosphere. Solvents were redistilled before use. The antibiotics and their derivatives were stored under nitrogen at −50° in the dark. M.p.s were determined in evacuated capillary tubes and are uncorrected. Alumina for chromatography was graded according to Brockmann and Schodder [8] and was prepared from Merck neutral aluminium oxide. Light petroleum refers to that fraction b.p. 60—80° except where stated otherwise.

Silica gel for t.l.c. was Merck Kieselgel H. U.v. and visible light absorption spectra were recorded on a Unicam SP 800 instrument and i.r. spectra were determined on Perkin-Elmer 257 or 225 spectrometers. N.m.r. spectra were recorded at 60 or 100 MHz with Varian A60 or HA 100 instruments. Mass spectra were determined on an A.E.I. MS 902 mass spectrometer. Selected peaks only are quoted (nominal mass units) and high resolution measurements where these were made.

Isolation of Flavofungin.—Streptomyces flavofungin nsp. was cultured in a medium containing asparagine (10^{-2} mol l^{-1}), glycerine (10^{-2} mol l^{-1}), biotin (0·01%), potassium dihydrogen phosphate (0·1%), and magnesium sulphate (0·1%).

After addition to the fermentation fluid of 1—3% of chloroform, the mycelium was filtered off. The still moist mycelium was stirred repeatedly with warm ethyl acetate. The extracts were separated and cooled, and the solid which separated was collected. Crystallisation from 90% aqueous ethanol or 50% aqueous methanol gave flavofungin as yellow needles, m.p. *ca.* 210° (decomp.), $[\alpha]_D$ −85—90° (*c* 0·2 in pyridine), −47—51° (*c* 0·15 in methanol, $\lambda_{max.}$ (methanol) 363 nm ($\varepsilon_1^{1\%}{}_{cm}$ 870); $\nu_{max.}$ (KBr) 3400, 2945, 1710, 1680, 1620, 1232, 1122, 1020, and 840 cm^{-1}, δ([$^2H_6$]Me_2SO, 100 MHz) 7·35—6·95(m), 6·50—6·70(m), 5·45—5·30(m), 4·85—3·55(m), 3·17(m), 3·12(m), 2·50—2·10(m), and 1·50—0·50(m) p.p.m.

Hydrolysis of Flavofungin.—Flavofungin (2·2 g) and sodium hydroxide (*ca.* 7 mol) in methanol (20 ml) were heated under nitrogen on a water-bath for 5 h. The reddish yellow solution was evaporated to dryness and the residue was dissolved in water (50 ml). The solution was extracted with ethyl acetate and the aqueous layer was then acidified with 10% sulphuric acid to give an amorphous yellow precipitate (1·45 g) which decomposed at 115—118°, $\lambda_{max.}$ (EtOH) 343 nm, $[\alpha]_D$ +1·7° (MeOH), +5·5° (pyridine). Catalytic hydrogenation of the product (1·43 g) gave a crystalline compound (0·78 g), m.p. 115°, identical with the product obtained by hydrolysis of perhydroflavofungin.

Octa-acetylflavofungin.—Acetic anhydride (1·3 ml) in dry pyridine (3 ml) was added slowly to flavofungin (100 mg) in pyridine (10 ml) at room temperature. The solution was kept for 2 days, after which it was diluted with water (50 ml) and acidified with 4N-hydrochloric acid. Ether extraction, evaporation, and crystallisation of the residue from ether gave *octa-acetylflavofungin* (75 mg), m.p. 151°, $\lambda_{max.}$ 364 and 254 nm ($\varepsilon_1^{1\%}{}_{cm}$ 740 and 53), $\nu_{max.}$ 1735, 1625, 1605, 1580, and 1240 cm^{-1}, δ ($CDCl_3$, 100 MHz) 7·7—5·5 (m, 12H), 5·5—4·3 (m, 9H), 2·1 (m, 24H), and 0·95 p.p.m. (broad, 12H); *m/e* 1000 ($M_2^{+\cdot}$, octa-acetylflavofungin B, 0·06%), 986 ($M_1^{+\cdot}$, octa-acetyl flavofunoin A, 0·35), 940 (M_2 − 60, 0·21), 926·497 (M_1 − 60, 2·5. $C_{50}H_{70}O_{16}$ requires *M*, 926·466), 9·8 (M_1 − 18 − 60, 0·19), 898 (M_2 − 60 − 42, 0·07), 884 (M_1 − 60 − 42, 0·29), 880 [M_2 − (2 × 60), 0·20], 866·422 [M_1 − (2 × 60), 1·9. $C_{48}H_{66}O_{14}$ requires *M*, 866·445], 848 [M_1 − 18 − (2 × 60), 0·40], 824 [M_1 − 42 − (2 × 60), 0·35], 806·413 [M_1 − (3 × 60), 2·1. $C_{46}H_{62}O_{12}$ requires *M*, 806·424], 746 [M_1 − (4 × 60), 2·4], 686·382 [M_1 − (5 × 60), 2·0. $C_{42}H_{54}O_8$ requires *M*, 686·382], 626 [M_1 − (6 × 60), 1·4], 566 [M_1 − (7 × 60), 1·1], 506 [M_1 − (8 × 60), 1·0], 60 (29), and 43 (100), o.r.d. (dioxan) $[\phi]_{385}$ −9120, $[\phi]_{336}$ +15,200, $[\phi]_{295}$ +15,200, $[\phi]_{264}$ +3040, $[\phi]_{248}$ +19,490, $[\phi]_{222}$ +21,300, and $[\phi]_{214}$ +24,350, c.d. (*c* 5·88 in dioxan) $(\Delta\varepsilon)_{389}$ −2·51, $(\Delta\varepsilon)_{371}$ −2·18, $(\Delta\varepsilon)_{343}$ −2·51, $(\Delta\varepsilon)_{250}$ −5·19, $(\Delta\varepsilon)_{224}$ −2·34, $(\Delta\varepsilon)_{208}$ +2·34 {lit.,[9] m.p. 141—142°, [α] −184° (pyridine)}.

Hydrolysis of Octa-acetylflavofungin.—Octa-acetylflavofungin (5 mg) was dissolved in ethanol (95%, 3 ml) and anhydrous potassium carbonate (130 mg) was added. The solution was stirred under nitrogen at room temperature for 1 day. Removal of the excess of carbonate by filtration, followed by evaporation, gave crude flavofungin which was purified by t.l.c. (Silica gel H; 15% methanolic dichloromethane). The product had the same u.v. and i.r. spectra as flavofungin and was inseparable from it on t.l.c.

Octatrimethylsilylflavofungin.—Trimethylchlorosilane (0·1 ml) was added to a solution of flavofungin (10 mg) and

[8] H. Brockmann and H. Schodder, *Ber.*, 1941, **74**, 73.
[9] R. Bognár, I. Farkas, S. Makleit, M. Rakosi, J. Soltesz, L. Somogyi, and K. Zsupán, *Antibiotiki*, 1965, **10** (12), 1059.

hexamethyldisilazane (0·2 ml) in dry pyridine (1 ml). The mixture was shaken briefly and then kept at room temperature for 40 min. The solvent was removed under reduced pressure, and carbon tetrachloride (3 × 2 ml) was added to the residue and then evaporated. Chromatography of the residue (neutral alumina, grade II, benzene), collection of the main yellow band, and evaporation gave octatrimethylsilylflavofungin (17 mg, 90%), an extremely viscous yellow gum, $\lambda_{max.}$ (benzene) 382 and 364 nm, $\lambda_{max.}$ (light petroleum) 378, 360, and 344infl. nm, *m/e* (6 kV) 1240 ($M_2^{+\cdot}$, octatrimethylsilylflavofungin B, 0·005%), 1226 ($M_1^{+\cdot}$, octatrimethylsilylflavofungin A, 0·008) 1136 (M_1 — 90, 0·1), 1046 [M_1 — (2 × 90), 0·05], 956 [M_1 — (3 × 90), 0·05], 147 (100), 75 (100), 73 (100), and 44 (100), *m/e* (8 kV) 956 [M_1 — (3 × 90), 1·1], 866 [M_1 — (4 × 90), 1], 794 (0·23), 785 [M_1 — (3 × 147), 0·2], 776 [M_1 — (5 × 90), 0·9], 704 (794 — 90, 0·25), 695 (785 — 90, 0·5), 686 [M_1 — (6 × 90), 0·36], 605 (695 — 90, 1·2), 565 (655 — 90, 1·8), 515 (605 — 90 1·2), 475 (565 — 90, 2·3), 385 (475 — 90, 2), 217 (62), 211 (62), 191 (40), 147 (34), 143 (40), 117 (18), 103 (27), 75 (62), and 73 (100).

Perhydroflavofungin.—(*a*) *With Adams catalyst.* Flavofungin (500 mg) in methanol (50 ml) was added to a previously hydrogenated suspension of platinum oxide (Adams catalyst) (100 mg) in methanol (5 ml), and the mixture was shaken in an atmosphere of hydrogen until the uptake of gas had ceased (160 ml at 22° and 760 mmHg; uptake complete after 12 h). U.v. spectroscopy (MeOH) confirmed the absence of conjugated double bonds in the product. The catalyst was removed by filtration (Hyflo-supercel), and the filtrate was evaporated. The residue was chromatographed [silica gel (grade III) column (50 × 4 cm), ethanol–chloroform (1 : 5 v/v)]. Fractions of the eluate were collected, and their composition was analysed by t.l.c. [Kieselgel H, ethanol–chloroform (1 : 5, v/v)]. Appropriate fractions were combined and evaporated to give perhydroflavofungin (376 mg) (single spot on t.l.c.) as a viscous oil, which gradually solidified on storage at 5°, $\nu_{max.}$ (KBr disc) 3360, 2925, 2855, 1730, 1376, 1298, 1130, 1100, 1090, 981, 891, 841, and 825 cm^{-1}, δ([$^2H_6$]Me_2SO, 100 MHz) 0·6—0·9 (m), 1·0—1·9 (m), and 3·3—4·8 p.p.m. (m) (relative intensities *ca.* 3 : 11 : 5, respectively). This material was used without further treatment.

(*b*) *With palladium on carbon.* Palladium on carbon (10%; 2·5 g) was suspended in ethanol (50 ml). A solution of flavofungin (5·0 g) in ethanol (315 ml) and water (20 ml) was introduced and the mixture was shaken with hydrogen for 1 h. After filtration and evaporation, the residual gum was crystallized from aqueous ethanol to give needles of perhydroflavofungin (3·91 g), m.p. 149—150°, $[\alpha]_D$ −91° (pyridine).

(*c*) *With Raney nickel.* Flavofungin (9·3 g) was added to Raney nickel (11 g) in methanol (350 ml), and the mixture was shaken under hydrogen for 2 days. The solution was decanted from the catalyst and evaporated to give perhydroflavofungin (8·0 g), m.p. 151°, $[\alpha]_D$ −0·95° (methanol).

Perhydroflavofungin Acid.—Perhydroflavofungin (2·5 g) was heated with a solution of potassium hydroxide (1·8 g) in methanol (18 ml) on a water-bath for 5 h. The solution was evaporated, and the residue was dissolved in water (50 ml) and extracted with ethyl acetate (40 ml). The aqueous phase was acidified with 10% sulphuric acid to yield an amorphous precipitate, which was crystallised from methanol–water to give perhydroflavofungin acid as fine needles (1·8 g), m.p. 117—118°, $[\alpha]_D$ +3·8 (MeOH), 0° (pyridine), $\nu_{max.}$ (CsI disc) 3380, 2930, 2850, 1710, 1470, 1140, 1080, and 850 cm^{-1}.

Nona-acetylperhydroflavofungin Acid.—Perhydroflavofungin acid (38 mg) was dissolved in pyridine (1·5 ml) and acetic anhydride (0·5 ml) in pyridine (0·5 ml) was added slowly. The mixture was left for 2 days, after which water (0·5 ml) was added and the solution was stirred for 12 h. The solution was partitioned between ether and water, the aqueous phase being acidified to pH 3. The organic layer was separated, washed twice with water, dried ($MgSO_4$), and evaporated to yield the crude *nona-acetylperhydroflavofungin acid* (42 mg). A sample was purified by t.l.c. [silica gel H, 35% acetone in light petroleum (b.p. 40—60°)], $\nu_{max.}$ (CCl_4) 2950, 2920, 2850, 1740, 1430, 1370, 1260, 1020, and 945 cm^{-1}, *m/e* 1041 (*M* — 17, 1%), 998 (*M* — 60, 5), 938 [*M* — (2 × 60), 24], 878 [*M* — (3 × 60), 42], 818 [*M* — (4 × 60), 34], 758 [*M* — (5 × 60), 92], 698 [*M* — (6 × 60), 58], 657 (100), 638 [*M* — (7 × 60), 46], 578 [*M* — (8 × 60), 40], and 518 [*M* — (9 × 60), 30].

Nona-acetylperhydroflavofungin Acid Methyl Ester.—An ethereal solution of nona-acetylperhydroflavofungin acid (10 mg) was methylated by the addition of an excess of ethereal diazomethane. Removal of the solvent, and purification of the crude product by t.l.c. [silica gel H, 25% acetone in light petroleum (b.p. 40—60°)] gave *nona-acetylperhydroflavofungin acid methyl ester* (10 mg), $\nu_{max.}$ (CCl_4) 2920, 2850, 1735, 1430, 1365, 1250, 1120, 1020, and 945 cm^{-1}, *m/e* 1072 ($M^{+\cdot}$, 1%), 1040 (*M* — MeOH, 16), 1029 (*M* — C_3H_7, 6), 1012 (*M* — 60, 10), 952 [*M* — (2 × 60), 34], 928 (100), 892 [*M* — (3 × 60), 26], 832 [*M* — (4 × 60), 25], 772 [*M* — (5 × 60), 48], 712 [*M* — (6 × 60), 44], 652 [*M* — (7 × 60), 29], 592 [*M* — (8 × 60), 24], and 532 [*M* — (9 × 60), 18].

Perhydroflavofungin Acid Methyl Ester.—To methanolic perhydroflavofungin acid (1 mg), a solution of diazomethane was added until a permanent yellow colouration was achieved. The excess of diazomethane was removed by passing a stream of nitrogen through the solution and the solvent was then evaporated to leave perhydroflavofungin acid methyl ester, $\nu_{max.}$ (KBr), 3380, 2930, 2855, 1740, 1460, 1435, 1380, 1100, and 850 cm^{-1}. The methyl ester was acetylated as for perhydroflavofungin to yield nona-acetylperhydroflavofungin acid methyl ester, identical (i.r. and t.l.c.) with that obtained by methylation of nona-acetylperhydroflavofungin acid.

Octa-acetylperhydroflavofungin.—(*a*) Perhydroflavofungin (100 mg) was acetylated as for octa-acetylflavofungin, but for 3 days. Chromatography of the residue [silica gel H plates, 25% acetone in light petroleum (b.p. 40—60°)] yielded *octa-acetylperhydroflavofungin* as a viscous gum (100 mg), $\nu_{max.}$ (CCl_4) 2960, 2910, 1740, 1470, 1376, 1245, 1130, and 1030 cm^{-1}, δ(CCl_4, 100 MHz) 5·3—4·3 (m, 9H), 2·0 (m, 24H), 1·8(m), 1·35 (broad), and 0·85 p.p.m. (m, 12H), *m/e* 998 ($M^{+\cdot}$, 5%), 955(100), 938(65), 895(75), 875(100), 866(70), 836(100), 818(30), 796(20), 776(60), 758(40), 716(65), 698(60), 656(95), 638(40), 625(100), 607(10), 596(80), 578(25), 532(85), and 518(28).

(*b*) Octa-acetylflavofungin (5 mg) was dissolved in ethyl acetate (5 ml) and platinum oxide (Adams catalyst) (90 mg) was added. The suspension was hydrogenated at room temperature and 1 atm for 2 days. Filtration and evaporation yielded octa-acetylperhydroflavofungin (4·5 mg), identical with the compound prepared in (*a*).

Hydrolysis of Octa-acetylperhydroflavofungin.—Octa-acetylperhydroflavofungin (5 mg) was dissolved in methanol–

water (2 : 1) and anhydrous potassium carbonate (45 mg) was added. The solution was stirred overnight at room temperature. Evaporation of the solvent followed by preparative t.l.c. (silica gel H, 12% methanol in dichloromethane) yielded perhydroflavofungin (3 mg).

Octatrimethylsilylperhydroflavofungin.—To a solution of perhydroflavofungin (10 mg) in pyridine (1 ml) was added hexamethyldisilazane (0·2 ml) and trimethylchlorosilane (0·1 ml). The solution was kept at room temperature for 12 h, then diluted with water (70 ml) and acidified with 0·1N-hydrochloric acid (80 ml). Extraction with ether and evaporation yielded *octatrimethylsilylperhydroflavofungin* (12 mg) as a clear viscous gum, $\nu_{max.}$ ($CHCl_3$) 2910, 2843, 1725, 1375, and 830 cm^{-1}, *m/e* 1238 ($M^{+\cdot}$, 1%), 1148(4), 1058(20), 968(90), 878(100), and 788(65).

Reduction of Flavofungin.—(*a*) Lithium aluminium hydride (*ca.* 1 mg) was added to a cooled (−20°) solution of flavofungin (*ca.* 10 μg) in dry tetrahydrofuran, the progress of the reaction being followed by u.v. spectroscopy. The rapid appearance of conjugated pentaene ($\lambda_{max.}$ 349, 331, and 318 nm) and tetraene ($\lambda_{max.}$ 318, 304, and 290 nm) chromophores was observed. When the solution was left to warm to room temperature, the intensities of the conjugated tetraene bands soon exceeded those of the conjugated pentaene bands.

(*b*) Sodium borohydride (5 mg) was added to a solution of flavofungin (1 mg) in methanol (2 ml), and the mixture was kept at 0° for 48 h. No change in the u.v. spectrum ($\lambda_{max.}$ 363 nm) was observed during this period.

Alkali Fusion of Perhydroflavofungin.—(*a*) Perhydroflavofungin (35 mg) and powdered potassium hydroxide (450 mg) were intimately mixed and placed in a closed nickel crucible with inlet and outlet ports for nitrogen. The mixture was heated at 300° ± 5° (bath temperature) under a slow stream of nitrogen for 1 h, and then rapidly cooled to room temperature. The resulting dark mass was dissolved in water (15 ml) and the solution was adjusted to pH 3 by dropwise addition of 2N-hydrochloric acid with stirring. The mixture was continuously extracted with ether for 5 days, and the extracts were then evaporated. The residue was dried by repeatedly distilling pure benzene from it. Boron trifluoride–methanol (14% BF_3, 0·5 ml) was then added to the residue, and the mixture was heated under gentle reflux for 1 h and then cooled. The solution was poured into 5% aqueous potassium carbonate (5 ml) and well shaken. The mixture was extracted with chloroform (3 × 15 ml), and the extracts were washed with water, dried, and evaporated. Preparative g.l.c. (Aerograph A.90P, 20% SE-30 on Chromosorb W, 210°) gave the principal component, dimethyl tridecane-1,13-dioate (*ca.* 1 mg), as an almost colourless solid, *m/e* 272 ($M^{+\cdot}$, 1·2%), (*M* − 2, 0·9), 241·175 (*M* − 31, 25. Calc. for $C_{14}H_{25}O_3$: *M*, 241·180), 208 (*M* − 64, 6), 199 (*M* − 73, 30), 167 (*M* − 105, 11), 149 (*M* − 123, 7), 126 (14), 112 (30), 98 (100), 84 (50), 74 (80), 69 (30), 59 (26), 55 (56), and 41 (47).

The product did not separate from an authentic sample of dimethyl tridecanedioate on mixed g.l.c. (F11; silicone oil, 200°; diethylene glycol succinate, 195°).

(*b*) In another experiment, methylation of the ether extract with diazomethane followed by g.l.c. (2½% silicone gum rubber on Chromosorb W) gave the same compound, together with a smaller amount of dimethyl undecane-1,11-dioate (identified by mixed g.l.c. with an authentic specimen).

Alkali Fusion of Flavofungin.—Flavofungin (100 mg) and powdered potassium hydroxide (500 mg) were intimately mixed and treated as for perhydroflavofungin in (*a*). The resulting mass was dissolved in water (20 ml), and the solution was adjusted to pH 2 by dropwise addition with swirling of 2N-hydrochloric acid. The mixture was steam distilled until 50 ml of distillate had collected, and the residual solution was also retained. The two fractions were treated in the following ways. (i) The distillate was adjusted to pH 8 by dropwise addition of 1N-potassium hydroxide solution, evaporated under reduced pressure to low bulk (1 ml), and filtered under gravity through a column (5 × 0·5 cm) of acidic ion-exchange resin [Amberlite IR-120(H)]. The flask was washed with water (1 ml), and the washings were also filtered through the column. The volatile carboxylic acids in the filtrate were examined (as their ethylamine salts) by descending paper chromatography [Whatman No. 1 paper; eluant, n-butanol saturated with water; lower (vapour saturating) phase, 0·25N-ethylamine in n-butanol saturated with water; locating spray, 0·25% (w/v) Bromocresol Green in n-butanol–ethanol (1 : 1) according to the procedure of Lindqvist and Storgårds [10]. Comparison with authentic samples indicated the presence of much acetic or formic acid (or a mixture of both), some propionic acid, a little butyric or isobutyric acid (or both), and a trace of valeric acid (and/or a branched-chain isomer thereof). Higher acids were absent from the mixture.

(ii) The residue from steam distillation was continuously extracted with ether (50 ml) for 5 days. The extract was evaporated, and the residue was dried by azeotropic distillation of pure benzene. Boron trifluoride–methanol (14% BF_3, 0·8 ml) was then added, and the mixture was heated under gentle reflux for 1 h and then cooled. The resulting solution was poured into aqueous 5% potassium carbonate solution (10 ml) and well shaken. The mixture was extracted with chloroform (3 × 25 ml) and the extracts were washed with water, dried ($MgSO_4$), and evaporated. The composition of the residue was examined by g.l.c. (F11, diethylene glycol succinate, 195°), which showed the presence of at least 30 components of retention times 0—3 h, none of which could be regarded as a major constituent. Dimethyl tridecane-1,13-dioate was absent from the mixture (mixed g.l.c.). The products were not further investigated.

2,4-Dimethylpent-2-en-1-al (2a).—(*a*) Octa-acetylflavofungin (40·0 g) was dissolved in dichloromethane–methanol (2 : 9) and ozonised at −70°. After reductive decomposition, the mixture was diluted with water, shaken, and the dichloromethane layer was separated. After evaporation, a portion (16·33 g) of the residue (27·66 g) was dissolved in dry methanol (580 ml) and mixed with sodium methoxide [from sodium (1·45 g) in methanol (20 ml)]. The mixture was kept for 48 h at room temperature, adjusted to pH 6 with glacial acetic acid, and the solvent was removed under reduced pressure. The residue was dissolved in water (50 ml) and distilled into methanolic 2,4-dinitrophenylhydrazine hydrochloride, to give 2,4-dimethylpent-2-en-1-al 2,4-dinitrophenylhydrazone (identified by comparison with an authentic specimen), m.p. and mixed m.p. 167—171° (from ethanol), the n.m.r. was identical with that of the synthetic specimen from (*c*), *m/e* 306 [$M_2^{+\cdot}$ (2b), 6%], 292 [$M_1^{+\cdot}$ (2a), 70], 289 (M_2 − 17, 3), 275 (M_1 − 17, 47), 249

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(M_1 − 43, M_2 − 57, 42), 109 (41), 95 (66), 67 (48), 55 (59), and 41 (100). The m.p. was not depressed on admixture of the derivative with that from (*c*).

(*b*) Flavofungin (5·0 g) was dissolved in pyridine (100 ml) and a solution of potassium permanganate (10·0 g) in water (500 ml) was added to it dropwise with stirring. The solution was cooled, and the manganese dioxide removed by centrifugation. The combined aqueous pyridine solution (including the aqueous washings from the manganese dioxide) was distilled into a solution of 2,4-dinitrophenylhydrazine in methanolic hydrochloric acid to give the same 2,4-dinitrophenylhydrazone (70 mg), m.p. 171—171·5° (from ethyl alcohol) as that obtained from (*a*).

(*c*) Propionaldehyde (6·05 g) and isobutyraldehyde (15 g) were stirred for 3 h with ethanol (58 ml) containing potassium ethoxide [from potassium (4·057 g)], and then left overnight. After neutralisation with 5% sulphuric acid, the mixture was diluted with water and the aqueous phase was separated and extracted [ether (2 × 30 ml)]. The extracts were dried and evaporated. On distillation of the residue a fraction, b.p. 146—150°, was collected and left overnight with a trace of iodine and then distilled at atmospheric pressure. The fraction distilling between 120—160° was treated with a solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid to give 2,4-dimethylpent-2-en-1-al 2,4-dinitrophenylhydrazone (0·65 g), m.p. 171—172° (from 96% ethanol or benzene), δ($CDCl_3$) 9·14 (d, *J* 10 Hz, 1H), 8·34 (dd, *J* 10 Hz, 1H), 7·95 (d, *J* 10 Hz, 1H), 7·73 (s, 1H), 5·82 (d, *J* 10 Hz, 1H), 3·1—2·5 (m, 1H), 1·96 (d, *J* 1·2 Hz, 3H), 1·08 p.p.m. (d, *J* 7 Hz, 6H), *m/e* 292 ($M^{+\cdot}$, 89%), 275 (*M* − 17, 56), 249 (*M* − 43, 54), 109 (53), 95 (88), 67 (60), 55 (73), and 41 (100). The product did not separate from those obtained by (*a*) and (*b*) on mixed t.l.c.

2,4,20-*Trimethyltritriacontane* (3a).—A solution of perhydroflavofungin (200 mg) in dry tetrahydrofuran (2 ml) was added slowly to a cooled suspension of lithium aluminium hydride (1·2 g) in tetrahydrofuran (10 ml). The suspension was stirred under reflux for 2 days. The excess of reagent was destroyed by the cautious addition of ethyl acetate, and the mixture was acidified by the addition of 2N-hydrochloric acid (100 ml). The solution was extracted with n-butanol, the organic layer was washed with sodium hydrogen carbonate solution, twice with water, and then evaporated to yield crude perhydroflavofungin polyol (150 mg), $\nu_{max.}$ (KBr), 3330br, 2925, 2860, 1470, 1380, 1070, and 850 cm^{-1}.

Perhydroflavofungin polyol (20 mg) was acetylated with acetic anhydride-pyridine (1 ml, 1 : 1) as before, and usual work-up and t.l.c. (silica gel H, 25% acetone in light petroleum) yielded *deca-acetylperhydroflavofungin polyol* (18 mg) as a clear viscous gum, $\nu_{max.}$ (CCl_4) 2970, 2920, 2850, 1740, 1460, 1370, 1240, 1130, and 945 cm^{-1}, δ($CDCl_3$, 100 MHz) 5·00—4·50 (broad, 9H), 4·05 (t, *J* 7 Hz, 2H), 2·03 (m, 30H), 1·95—1·50 (broad), 1·35—1·20 (broad, 20H), 0·87 (d, *J* 7 Hz, 3H), 0·86 (d, *J* 6·6 Hz, 3H), and 0·84 p.p.m. (d, *J* 6·5 Hz, 6H), *m/e* 1044 (*M* − 42, 0·8%), 1026 (*M* − 60, 2·4), 966 [*M* − (2 × 60), 14], 906 [*M* − (3 × 60), 8], 846 [*M* − (4 × 60), 8], 786 [*M* − (5 × 60), 24], 726 [*M* − (6 × 60), 48], 666 [*M* − (7 × 60), 50], 606 [*M* − (8 × 60), 46], 546 [*M* − (9 × 60), 34], 486 [*M* − (10 × 60), 8], and 83(100).

A mixture of perhydroflavofungin polyol (180 mg), red phosphorus (150 mg), and hydriodic acid (64—68% w/w, 6 ml), was heated under reflux for 12 h. The solution was cooled, and then poured into chloroform. The organic layer was separated, washed twice with water, once with 1% sodium thiosulphate and again with water, dried ($MgSO_4$), and evaporated to give the crude iodo-compound as a clear oil (40 mg), $\nu_{max.}$ (CCl_4) 2950, 2920, 2860, 2850, 1460, 1375, 1365, and 1255 cm^{-1}. A solution of the iodo-compound (40 mg) in ether (15 ml) was heated under reflux for 12 h with lithium aluminium hydride (100 mg). Ethyl acetate was added, followed by 10% sodium potassium tartrate solution, and the resulting suspension was extracted with light petroleum (b.p. 40—60°). The extracts were dried ($MgSO_4$) and evaporated to yield the crude hydrocarbon as a clear oil (20 mg). Chromatography on alumina (Grade I) with light petroleum as eluant gave a slightly pure hydrocarbon (15 mg). The hydrocarbon (8 mg) was dissolved in light petroleum (10 ml) and platinum oxide (Adams catalyst) (25 mg) was added. The suspension was hydrogenated at room temperature and atmospheric pressure for 6 days. Filtration and evaporation of the solvent gave the saturated hydrocarbon (7 mg), $\nu_{max.}$ (CCl_4) 2950, 2920, 2860, 2850, 1460, 1375, and 1365 cm^{-1}. G.l.c. (20% SE.30 on chromosorb W, 317°) gave 2,4,20-trimethyltritriacontane (single symmetrical peak), $\nu_{max.}$ (CCl_4) 2960, 2930, 2880, 2860, 1470, 1380, and 1370 cm^{-1}, *m/e* 504 (*M* − 2, 1%), 491 (*M* − CH_3, 3), 463 (*M* − C_3H_7, 4), 449 (*M* − C_4H_9, 10), 421 (*M* − C_6H_{13}, 3), 323 (*M* − $C_{13}H_{27}$, 6), 322 (4), 211 (*M* − $C_{21}H_{43}$, 13), 210 (18), 85 (*M* − $C_{30}H_{61}$, 100).

2,4,20-*Trimethyldotriacontane* (3d).— Nona-acetylperhydroflavofungin acid (50 mg) was dissolved in redistilled benzene (20 ml) and lead tetra-acetate (180 mg) added. The suspension was irradiated under nitrogen (150 W tungsten-filament lamp), while iodine (190 mg) in benzene (5 ml) was added dropwise over 10 min. After 1·5 h photolysis, the mixture was poured into water. The organic layer was washed twice with sodium thiosulphate solution, twice with water, dried ($MgSO_4$), and evaporated to give a crude product. Purification by preparative t.l.c. (silica gel H; 20% acetone in light petroleum) gave the decarboxylated compound (40 mg), $\nu_{max.}$ (CCl_4), 2960, 2910, 2850, 1740, 1460, 1430, 1370, 1235, 1020, and 940 cm^{-1}. The compound (40 mg) was dissolved in hydriodic acid (6 ml, 64% w/w) and red phosphorus (100 mg) was added. The suspension was heated under reflux for 18 h and worked-up as in the preparation from perhydroflavofungin polyol to give the iodinated hydrocarbon (12 mg), $\nu_{max.}$ (CCl_4) 2960, 2940, 2860, 1465, and 1380 cm^{-1}. The product was reduced with lithium aluminium hydride, hydrogenated, and purified by g.l.c. as before to yield 2,4,20-trimethyldotriacontane, an oil (1·2 mg), $\nu_{max.}$ (CCl_4) 2960, 2930, 2880infl., 2860, 1470, 1380, and 1370 cm^{-1}, *m/e* 490 (*M* − 2, 28%), 477 (*M* − CH_3, 10), 463 (*M* − C_2H_5,10), 459 (*M* − C_3H_7, 15), 435 (*M* − C_4H_9, 35), 408 (10), 407 (*M* − C_6H_{13}, 10), 323 (*M* − $C_{12}H_{25}$, 25), 322 (20), 197 (*M* − $C_{21}H_{43}$, 45), 196 (80), and 85 (100).

[33,33-$^2H_2$]2,4,20-*Trimethyltritriacontane* (3c).—Perhydroflavofungin (200 mg) in tetrahydrofuran (2 ml) was added slowly to a cooled suspension of lithium aluminium deuteride (800 mg) in tetrahydrofuran (10 ml). The reduction was performed as described for the unlabelled compound (3a) (see before) to give the crude polyol (160 mg), $\nu_{max.}$ (KBr) 3400, 2920, 2850, 2200, 2100, 1460, 1400, 1300, 1090, 965, and 845 cm^{-1}. This was converted into the [33,33-$^2H_2$]-compound (3c) (1·1 mg) [as for the dotriacontane (3d)], $\nu_{max.}$ (CCl_4) 2960, 2930, 2860, 2320, 2200, 1465, 1370, and 1360 cm^{-1}, *m/e* 506 (*M* − 2, 14%), 493 (*M* − CH_3, 12), 479 (*M* − C_2H_5, 13), 465 (*M* − C_3H_7, 20), 451 (*M* − C_4H_9, 43), 423 (*M* − C_6H_{13}, 8), 323 (*M* − $C_{13}H_{25}D_2$, 17), 213 (*M* − $C_{21}H_{43}$, 17), 212(29), 85(89), and 57(100).

Conversion of Perhydroflavofungin into the Deoxylactone.—To perhydroflavofungin (200 mg) in dry pyridine (4 ml), toluene-*p*-sulphonyl chloride (1·8 g) in pyridine (5 ml) was added. The mixture was kept for 4 days at 5° and then poured into ether and washed well with water. The organic layer was separated, dried ($MgSO_4$), filtered, and evaporated. T.l.c. (Silica gel H, 30% acetone in light petroleum) yielded crude octatosylperhydroflavofungin (270 mg) as a clear gum, $\nu_{max.}$ (KBr) 3540, 3060, 3020, 2915, 2860, 1725, 1600, 1360, 1175, 890, and 550 cm^{-1}, $\lambda_{max.}$ (MeOH) 265 and 226 nm, $\delta(CDCl_3)$ 7·85, 7·68, 7·35, 7·25, 4·9—4·3, 2·45, 1·29, 0·89, and 0·80 p.p.m., containing two very similar compounds (*ca.* 1 : 1, t.l.c.) both of which were tosylates of perhydroflavofungin (i.r.).

The tosylated material (110 mg) was added to a saturated solution of anhydrous lithium bromide (5 g) in dimethylformamide (6 ml). The mixture was heated to 110° for 0·5 h, cooled, partitioned between light petroleum (b.p. 40—60°) and water, and the organic layer was washed well with water, dried ($MgSO_4$), and evaporated. Chromatography [silica gel column, gradient elution with benzene in light petroleum (b.p. 40—60°)] yielded a heptaene (0·3 mg), $\lambda_{max.}$ (light petroleum) 403, 380, and 360 nm, and a mixture of compounds (10 mg) with $\lambda_{max.}$ (hexane) 400, 375, 350, 330, 318, and 300 nm. This mixture was dissolved in dioxan (5 ml), Raney nickel (500 mg) in dioxan was added, and the mixture was stirred under hydrogen for 2 days. The suspension was filtered, Adams catalyst (300 mg) was added, and the mixture hydrogenated for 5 days. The suspension was filtered and the solvent was evaporated. T.l.c. (silica gel H, 7% chloroform in light petroleum) yielded the *deoxylactone* (2 mg), $\nu_{max.}$ (CCl_4), 2920, 2840, 1730, 1460, and 1380 cm^{-1}, $\lambda_{max.}$ end absorption only, *m/e* 534·538 ($M^{+\cdot}$, 29%. $C_{36}H_{70}O_2$ requires *M*, 534·538), 532 (*M* − 2, 28), 491·482 (*M* − C_3H_7, 60. $C_{33}H_{63}O_2$ requires *M*, 491·483), 462·479 (*M* − C_4H_8O, 50. $C_{32}H_{62}O$ requires *M*, 462·480), and 135(100).

Hydrogenolysis of Flavofungin.—Flavofungin (30 mg) was dissolved in ethanol (4 ml) and platinum oxide (200 mg) was added. The suspension was stirred under hydrogen at room temperature and atmospheric pressure for 4 days. Filtration and evaporation left a residue, the major component of which was perhydroflavofungin (mixed t.l.c., 15% methanol in dichloromethane). A second component was separated by t.l.c. (silica gel H, 12% methanol in dichloromethane) and acetylated with acetic anhydride (0·1 ml) in pyridine (1·0 ml) to give hepta-acetylperhydroflavofungin (2 mg), $\nu_{max.}$ (CCl_4) 2970, 2935, 2860, 1745, 1465, 1435, 1375, 1235, 1130, 1020, and 945 cm^{-1}. This compound was inseparable on t.l.c. from the corresponding hepta-acetylperhydromycoticin, prepared similarly from mycoticin.

Hydrogenolysis of Peracetylflavofungin.—Platinum oxide (200 mg) was added to peracetylflavofungin (30 mg) in glacial acetic acid (2 ml). The suspension was hydrogenated at room temperature and pressure for 2 days. After filtration and partitioning between ether and water, and usual work-up of the ether layer, t.l.c. (silica gel H, 27% acetone in petrol) gave *hepta-acetylperhydroflavofungin* (10 mg), $\nu_{max.}$ (CCl_4) 2970, 2935, 2860, 1745, 1465, 1435, 1375, 1235, 1130, 1020, and 945 cm^{-1}, $\delta(CCl_4)$ 0·75—1·0, 1·12—1·45, 1·9—2·1, and 4·5—5·2 p.p.m., *m/e* 940 ($M^{+\cdot}$, 5%), 897·519 (*M* − C_3H_7, 70. $C_{47}H_{77}O_{16}$ requires *M*, 897·521), 880·553 (*M* − 60, 20). $C_{48}H_{80}O_{14}$ requires *M*, 880·554), 820·532 [*M* − (2 × 60), 20. $C_{46}H_{76}O_{12}$ requires *M*, 820·533), 808·503 (*M* − HOAc − C_4H_8O, 30. $C_{44}H_{72}O_{13}$ requires *M*, 808·497), 760 [*M* − (3 × 60), 18], 700 [*M* − (4 × 60), 20], 640 [*M* − (5 × 60), 43], 580 [*M* − (6 × 60), 45], 520 [*M* − (7 × 60), 70], and 55(100).

Attempts to Dehydrate Flavofungin.—(*a*) Flavofungin (2 mg) in dry, ethanol-free chloroform (2 ml) was treated with chloroform saturated with dry hydrogen chloride (2 drops), and stirred at room temperature. The reaction was followed by t.l.c. (Kieselgel H, 20% methanol in dichloromethane) and u.v. spectroscopy. After 48 h a broad absorption at *ca.* 320 nm had appeared, but t.l.c. detected no products less polar than flavofungin.

(*b*) Redistilled phosphoryl chloride (2 drops) was added to flavofungin (1 mg) in dry pyridine (1 ml) and the mixture was stirred at 20° for 36 h. The u.v. spectrum was unchanged [$\lambda_{max.}$ (pyridine) 369 nm], and t.l.c. (Kieselgel H, 30% ethanol in chloroform) revealed no products less polar than flavofungin. The mixture was then warmed at 60° for 24 h. This treatment resulted only in the gradual production of brown decomposition products, which remained at the origin on t.l.c. (Kieselgel H, 30% ethanol in chloroform).

(*c*) A 1% solution (w/v) of methanesulphonyl chloride in dry *NN*-dimethylformamide (2 ml) was added to flavofungin (2 mg) in the same solvent (1 ml). After stirring at 20° for 5 min, 2,4,6-trimethylbenzene (1 ml) was added, and the resulting solution was treated with sulphur dioxide in dry *NN*-dimethylformamide [2 drops of 5% (w/v)] and stirred at 20° for 1 h. The u.v. spectrum (chloroform) was unchanged. The mixture was warmed at 40° for 2 h and then evaporated almost to dryness under reduced pressure. The residue was taken up in chloroform (20 ml) and was washed with saturated sodium hydrogen carbonate solution, then with water, and evaporated, leaving a brown gum in very low yield. T.l.c. (Kieselgel H, 15% methanol in dichloromethane) showed the presence of flavofungin (mixed t.l.c.) and several more polar compounds. The latter were not further investigated.

Attempted Allylic Oxidation of Flavofungin.—(*a*) Fetizon's reagent (90 mg, freshly prepared) in methanol (10 ml) and flavofungin (5 mg) were heated under reflux under nitrogen for 30 min. The filtrate was examined by t.l.c. and u.v. spectrophotometry. No αβ-unsaturated ketone was detected.

(*b*) Flavofungin (2 mg) in ethanol (1 ml) was stirred with active manganese dioxide (40 mg) at room temperature for 2 weeks. No oxidation product was detected. The same result was obtained in dioxan and pyridine.

(*c*) Flavofungin (2 mg) was shaken with a suspension of nickel dioxide (15 mg) in dioxan (1 ml) under nitrogen for 24 h at room temperature. Only flavofungin was present in the mixture (t.l.c. and u.v.).

(*d*) Flavofungin (5 mg) was dissolved in pure dioxan (2 ml) and 2,3-dichloro-5,6-dicyanobenzoquinone was added. The reaction was performed with 1·4, 2·5, and 3·5 mol. equiv. of quinone and 15 mol. equiv. of flavofungin. No αβ-unsaturated ketone absorbtion was detected (u.v.) in any case.

Attempted Preparation of 12-Acetoxyflavofungin Octa-acetate.—Octa-acetylflavofungin (2 mg) in ethanol-free chloroform (2 ml) was added to a cooled (−20°), stirred solution of *N*-bromosuccinimide (2 mg) and acetic acid (0·05 ml) in ethanol-free chloroform (2 ml). The mixture was stirred at this temperature for 15 min and then at room temperature for a further 1 h. T.l.c. (Kieselgel H, ether) indicated the presence of octa-acetylflavofungin only.

Comparisons of Mycoticin and Flavofungin.—Peracetyl-, perhydro-, and peracetylperhydro-mycoticin were prepared by the methods employed for the corresponding flavofungin derivatives. The antibiotics were recrystallised under identical conditions.

T.l.c. The antibiotics and their derivatives did not separate from mixed chromatograms on silica gel H in the following systems: mycoticin and flavofungin, 15% methanol in dichloromethane, R_F 0·3; 45% ethanol in dichloromethane, R_F 0·4; 50% propan-2-ol in chloroform, R_F 0·4: perhydromycoticin and perhydroflavofungin, 10% methanol in dichloromethane, R_F 0·5; 25% ethanol in chloroform, R_F 0·7; 35% propan-2-ol in chloroform, R_F 0·4: octacetyl-flavofungin and -mycoticin, 30% acetone in light petroleum (b.p. 40—60°), R_F 0·5; ether, R_F 0·7; 30% ethyl acetate in chloroform, R_F 0·7; 55% ether in benzene, R_F 0·5: octa-acetylperhydro-flavofungin and -mycoticin, 30% acetone in light petroleum (b.p. 40—60°), R_F 0·6; 50% ether in benzene, R_F 0·4; 35% ethyl acetate in benzene, R_F 0·5; 30% ethyl acetate in chloroform, R_F 0·5.

M.p.s. Mycoticin, 220°; flavofungin, 210°; perhydromycoticin, 139°; perhydroflavofungin, 150°; octa-acetylmycoticin, 155—156°, and octa-acetylflavofungin, 151°.

I.r. No differences were detectable in the following derivatives: mycoticin and flavofungin (KBr disc); octa-acetyl-mycoticin and -flavofungin (CsI disc, CCl_4); and octa-acetylperhydro-mycoticin and -flavofungin (CCl_4).

U.v. Mycoticin, $\lambda_{max.}$ (methanol) 364; (dioxan) 382, 365, and 344 nm; flavofungin, $\lambda_{max.}$ (methanol) 364; (dioxan) 382, 365, and 344 nm; octa-acetylmycoticin, $\lambda_{max.}$ (methanol) 364 nm; octa-acetylflavofungin, $\lambda_{max.}$ (methanol) 364 nm.

N.m.r. The spectra of antibiotics in [$^2H_5$]pyridine were identical except for the position of the hydroxy-absorption (δ 5·35 for mycoticin and 5·10 p.p.m. for flavofungin). The spectra of octa-acetyl-mycoticin and -flavofungin in $CDCl_3$ were also identical.

Mass spectra. Octa-acetyl-mycoticin and -flavofungin, compared under identical operating conditions, indicated a ratio of mycoticin A : mycoticin B of 2 : 1 and flavofungin A : flavofungin B of 8 : 1 from measurements of the peaks at *m/e* 926 and 940 ($M_1 = 60$, $M_2 - 60$) but were otherwise identical. Pertrimethylsilyl-mycoticin and -flavofungin were also identical under the same conditions except for the varying quantity of the homologues present.

X-*Ray powder photographs.* The *X*-ray powder patterns produced by mycoticin and flavofungin showed distinct differences between the antibiotics, the pattern of mycoticin being more complicated than that of flavofungin.

We thank the Hungarian Academy of Sciences and the BIOGAL Pharmaceutical Factory (Debrecen) for financial support, and the S.R.C. for research studentships (to B. O. B. and W. J. S. L.). We are indebted to Dr. P. M. Scopes (Westfield College) for the o.r.d. and c.d. measurements, Dr. M. B. Hursthouse (Queen Mary College) for the *X*-ray powder photographs, Dr. É. R. Dávid (Debrecen) for microanalyses, and Dr. S. Szabó (Debrecen) for some of the light absorption data.

[2/274 *Received*, 7*th February*, 1972]