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DIHYDROBENZOFURANS FROM *HETEROBASIDION ANNOSUM*

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Dedicated to the memory of Tony Swain.
"Omne tulit punctum qui miscuit utile dulci"
Ars Poetica 343

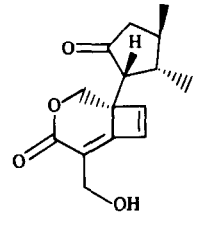
Key Word Index—*Heterobasidion annosum*; *Fomes annosus*; fomannoxin; fomannosin; fomajorins; dihydrobenzofuran.

Abstract—Fomannosin, fomannoxin and four additional new dihydrobenzofurans and a benzofuran were isolated from the culture broth of a single isolate D_3 of *Heterobasidion annosum*. The structures for the compounds were established by chemical and spectroscopic methods and confirmed by synthesis or by transformation from fomannoxin.

INTRODUCTION

Fomannosin (**1**) [1, 2], (S)-fomannoxin (**2**) [3] and the isocoumarins fomajorin S and D [4, 5] have been implicated in the phytopathogenic activity of the economically important wood rot fungus, *Heterobasidion annosum* (Fr.) Bref. [*Fomes annosus* (Fr.) Cooke]. Sitka spruce (*Picea sitchensis*) offers little resistance to invasion by *H. annosum* and decay to a height of three meters has been recorded. In the course of our continuing search for biologically active metabolites, seven compounds were identified in a single strain (D_3), four new closely related dihydrobenzofurans (**3–5**) as well as a benzofuran (**6**).

In this paper we report on their structures which were established by chemical and spectroscopic methods, and confirmed by synthesis or by transformation from fomannoxin (**2**). The separation and purification procedure for these isolates are presented in detail in the Experimental. The compounds are described herein according to their polarity (Fig. 1) and the isolates from the diethyl ether extract are tentatively named compounds A–F.



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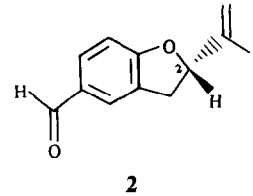

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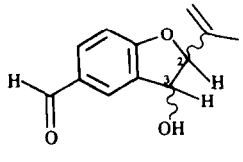

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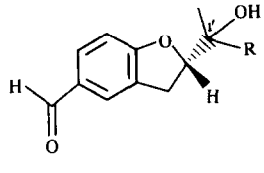
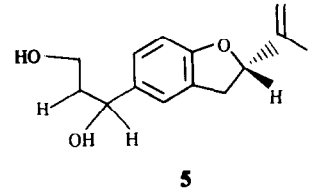


4a R = Me
4b R = CH_2OH



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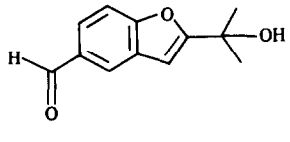

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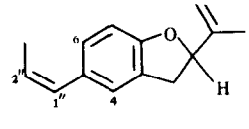

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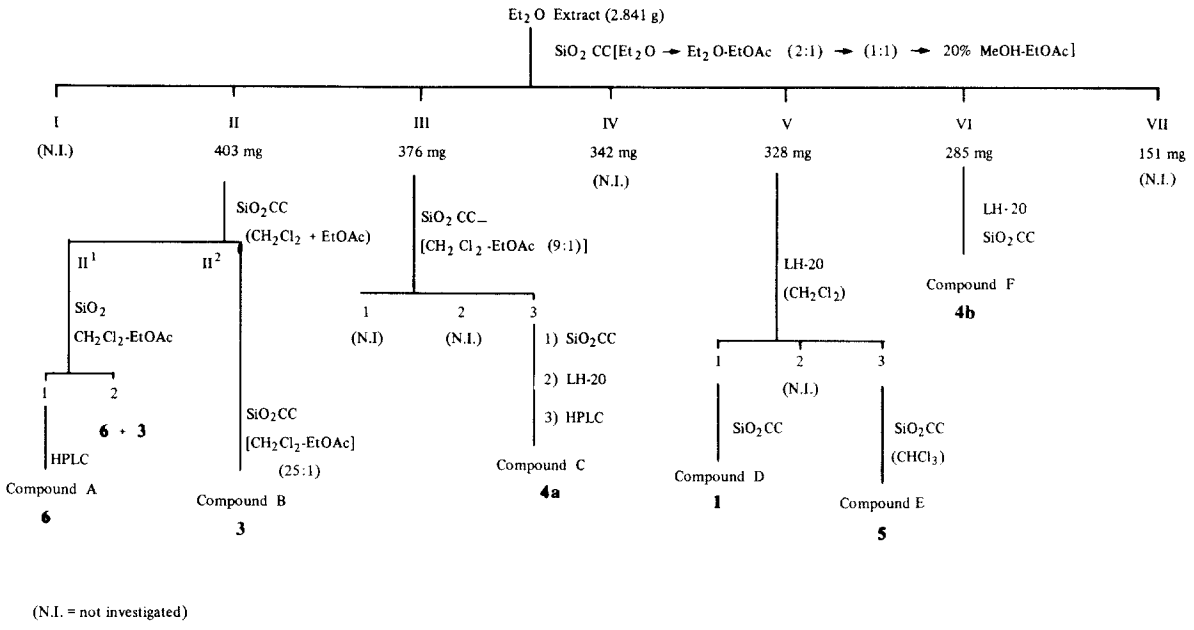

(N.I. = not investigated)

Fig. 1. Separation of diethyl ether extract.

RESULTS AND DISCUSSION

The three-month-old cultures when harvested gave (2*S*)-(+)-fomannoxin (**2**) as the major constituent of the *n*-hexane extract and the identification was substantiated by formation of a 4-bromophenylhydrazone.

The diethyl ether soluble extract of the culture medium was chromatographed on a silica gel column using a gradient elution with diethyl ether, ethyl acetate and methanol to give seven fractions (Fig. 1). Fraction II of the diethyl ether extract contained two compounds. The less polar compound A analysed for $C_{12}H_{12}O_3$ (M^+ 204.0693). Its IR spectrum had a carbonyl absorption at 1684 cm^{-1} and a signal at δ 10.14 in its ^1H NMR spectrum were indicative of an aldehyde group. A signal at δ 1.70 (6H, *s*) adjacent to a hydroxyl group pointed to two identical methyl groups. The signals δ 5.75 (1H, *d*, J = 8.42 Hz), δ 7.84 (1H, *dd*, J = 8.42, 1.0 Hz), δ 8.08 (1H, *d*, J = 1.0 Hz) are typical of 1,2,4-trisubstituted benzene and resemble the pattern obtained for fomannoxin (**2**) [3]. The singlet at δ 6.70 (1H) was assigned to C-3 of a benzofuran skeleton. This assignment was confirmed by the ^{13}C NMR which showed one double bond other than the aromatic carbons at δ 111.93 (*d*), δ 158.15 (*s*). These observations indicated that compound A is 5-formyl-2-(isopropyl-1′-ol) benzofuran (**6**) and is a new natural product.

The more polar compound B in fraction II analysed for $C_{12}H_{12}O_3$ (M^+, m/z 204). An absorption signal in the IR spectrum at 1685 cm^{-1} and a singlet at δ 9.96 in the ^1H NMR were assigned to an aldehyde function. The signals for the aromatic protons and for an isopropyl group resembled those in the spectrum (^1H NMR) of fomannoxin (**2**). The characteristic signals for the methylene protons of **2** were absent. However a similarity of the H-2 and H-3 signals to those observed for toxol (5-acetyl-3-hydroxy-2-isopropenyldihydrobenzofuran) [6] suggested that the additional oxygen is accomodated as a hydroxyl at C-3. Compound B is assigned as 5-formyl-3-hydroxy-2-isopropenyldihydrobenzofuran (**3**). The ^{13}C NMR spectrum ($CDCl_3$) and the EIMS supported the proposed structure (**3**). The relative configuration at position 2 and 3 is tentatively assigned *cis* (J = 4.0 Hz).

The third compound, C (fraction III) mp 83–86° had a molecular formula $C_{12}H_{14}O_3$ which was established by elemental analysis and EIMS [M^+] *m/z* 206. Spectroscopic data (IR, UV, ^1H NMR, ^{13}C NMR) indicate that compound C is 5-formyl-2-(isopropyl-1′-ol)-2,3-dihydrobenzofuran (**4a**). A reference to **4a** has been made in a previous publication [7]. The identification was substantiated by the following. Reduction of **4a** with sodium borohydride afforded a 5-carbinol derivative [mp 114–116°, ^1H NMR; δ 4.58 (*s*, CH_2OH); ^{13}C NMR: δ 65.31 (CH_2OH)]. The absolute configuration at C-2 is assigned as S by comparison with the product of Markovnikov hydration [$Hg(OAc)_2/NaBH_4$] of the exocyclic double bond of (*S*)-2. The transformed product and **4a** were identical (mp, IR, UV, ^1H NMR).

The two component mixture (compounds D and E) in fraction V gave D, an unstable oil, as the major constituent which was hydrogenated to afford the known dihydrofomannosin mp 102°, $[\alpha]_D^{21}$ +347.5° ($CHCl_3$; *c* 0.21), [1, 2] which was identical (UV, EIMS, ^1H NMR) with an authentic sample.

The minor component E appeared from its spectroscopic data to be a fomannoxin-type compound. The EIMS registered a molecular ion M^+ *m/z* 234 corresponding to $C_{14}H_{18}O_3$ and had a base peak *m/z* 184. The UV spectrum was similar to that of **2**. The ^1H NMR spectrum had signals corresponding to 2-isopropenyl-2,3-dihydrobenzofuran but lacked the –CHO group so characteristic of fomannoxin (**2**). The additional signals registered a methyl (δ 1.13) as a doublet (J = 6.2 Hz) and two methine protons [δ 4.55 (*d*, J = 4.8 Hz) and δ 3.97 (dq, J = 6.2, 4.8 Hz)] adjacent to hydroxyl groups. The presence of a propanediol moiety

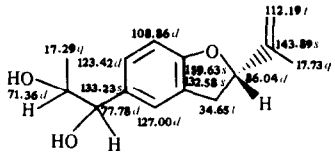

Fig. 2. ^{13}C NMR chemical shift values $\delta(CDCl_3)$ for **5**.

(MeCHOH·CHOH) at C-5 of the dihydrobenzofuran structure was established from the signals in its ^{13}C NMR spectrum (Fig. 2). These spectroscopic results indicate that the minor compound *E* is 5-(1″,2″-dihydroxypropyl)-2-isopropenyl-2,3-dihydrobenzofuran (**5**). To confirm this assignment a synthesis was undertaken.

A retrosynthetic analysis of **5** suggested that the diol might be formed stereospecifically from osmium tetroxide treatment on 2-isopropenyl-5-(*n*-propenyl)-2,3-dihydrobenzofuran (**7**) which in turn could be obtained by a Wittig reaction on fomannoxin (**2**). The synthesis of (±)-fomannoxin was achieved in good yield by heteroannulation of sodium phenate by isoprene dibromide with subsequent formylation [8]. The reaction of ethyltriphenylphosphonium bromide with (±)-fomannoxin gave a 61% yield of **7** which was separated from **2** by CC over silica gel. The ^1H NMR spectrum of the Wittig product of **7** had all the characteristic signals of (±)-fomannoxin with the exception of the –CHO group. The aromatic protons at C-4 and C-6 resonate 0.6 ppm upfield from those seen in the spectrum of **2**, due to the electron donating ability of the double bond. A double doublet at δ 1.88 was assigned to the –Me of the propenyl chain. The olefinic protons at δ 5.66 and 6.35 were assigned to H-2″ and H-1″ respectively with $J_{H-1'',H-2''} = 11.5$ Hz indicative of *cis*-coupling. Some *E*-isomer (*ca* < 5%) was observed to be present from the ^1H NMR spectrum. The geminal protons of the isopropenyl bond at δ 4.91 and 5.09 had a *J*-value of 1.29 Hz. The peaks of the ^{13}C NMR spectrum of **7** were assigned using established chemical shift data and by comparison with values recorded for **2**. The olefinic carbons of the propenyl group resonate at δ 125.31 (C-2″) and 129.69 (C-1″) and the methyl gives a signal at 14.74. The dihydrobenzofuran (**7**) was not stable at room temperature. Direct hydroxylation of **7** with osmium tetroxide in pyridine afforded a mixture of two diastereoisomers. Two distinct doublets at δ 1.03 and 1.11 ($J = 6.6$ Hz) in the ^1H NMR spectrum of the oxidation product indicate that the Me-3″ is present in two different environments. Similarly, two doublets at δ 4.27 ($J = 7.7$ Hz) and 4.54 ($J = 4.4$ Hz) were assigned to H-1″. The signals for the isopropenyl moiety do not reflect the presence of a mixture. It is concluded that the product is a mixture of *erythro* and *threo* diols in the ratio 11:9. A comparison of the ^1H NMR spectrum of **5** with that for the diol mixture pointed to an *erythro* configuration ($J = 4.6$ Hz) for the natural product 5-(1″, 2″-dihydroxypropyl)-2-(isopropenyl)-2, 3-dihydrobenzofuran. This tentative suggestion for the configuration assignment in **5** is based on results observed in a study of the preparation of 1-*p*-methoxyphenyl-1,2-propandiol from an oxidation of 4-methoxy-β-methylstyrene [9].

Compound F, present in fraction VI of the diethyl ether extract was identified as (+)-5-formyl-2-(1′,2′-dihydroxyisopropyl-2,3-dihydrobenzofuran (**4b**) and has been detected (GC-MS) previously in spruce seedlings [9] which were grown in a nutrient solution containing (*S*)-fomannoxin. The dihydrobenzofuran (**4b**) has a molecular ion M^+ 222 and was assigned a molecular formula $C_{12}H_{14}O_4$. The IR spectrum has hydroxyl (μ 3380 cm^{-1}) and carbonyl (ν CO 1678 cm^{-1}) absorption signals. The presence of the 1,2-dihydroxyisopropyl group at C-2 is supported by signals in the ^1H NMR (δ 9.88) and ^{13}C NMR (δ 190.8). The presence of the aldehyde group was confirmed by its reduction with sodium borohydride to afford a triol (M^+ *m/z* 224).

The structure assigned to **4b** was supported by a comparison of the spectral data with that of a mixture of diastereomeric diols obtained on osmylation of (±)-fomannoxin. The diol mixture crystallized in needles ($CHCl_3$) mp 80–86°. The absolute stereochemistry for **4b** was not assigned due to a limited supply of the natural product. A minor component present in fraction VI had an identical R_f (0.16) with **4b**. Further study on this fraction is in progress.

Biological testing of **4b** shows it to possess to fungitoxic activity. The rate of production of the diol **4b** was monitored in a culture broth (strain 611) growing in Raulins medium and it was shown to peak shortly after S-(+)-fomannoxin reached its maximum (8 weeks). This observation is an indication that **2** is catabolized and detoxified by hydroxylation of the isopropenyl side chain. This finding is in agreement with earlier work on the catabolism and detoxification of rotenone [10].

The ethyl acetate extract was subdivided by solubility in chloroform. Chromatography of the latter fraction afforded fomannosin (**1**) and the dihydrobenzofuran (**4b**).

EXPERIMENTAL

Mps: uncorr. ^1H NMR (270 MHz and 60 MHz) and ^{13}C NMR (67.8 MHz) spectral determinations were taken in $CDCl_3$ unless otherwise stated at 25° using TMS as int. standard. EIMS were taken on an AEI MS 30 instrument and CIMS and HRMS were recorded on a modified MS-9 spectrometer. Merck Kieselgel 60 (70–230 mesh), Woelm dry silica TSC 04526 and Sephadex LH-20 were used as stationary phases for column chromatography.

Cultures. The stains of *Heterobasidion annosum* (Fr.) Bref were D_3 and 611 obtained from the Department of Botany, University College, Dublin. Cultures were maintained on malt agar slopes and plates. Stocks of mycelial plugs were retained in sterile H_2O.

Isolation and purification of metabolites. The strain D_3 of *Heterobasidion annosum* (Fr.) Bref was grown on a modified Raulins medium (9 l) contained in 40 × 1 l Roux flasks (250 ml of broth per flask) fitted with Morton closures and autoclaved at 120° for 20 min. The flasks were each inoculated with 4 × 10 mm plugs of mycelium from an agar plate of the fungus and the surface cultures were incubated at 21° in the dark. After three months growth the mycelia were harvested by filtration and the culture broth (9 l) was extracted 2× with Merck *n*-hexane, 2 × Et_2O, and 3× with EtOAc. Each extract was dried ($MgSO_4$) and evapd to give oily residues A (230 mg); B (2.841 g); C (1.559 g) respectively. The mycelium was homogenized with MeOH and the solvent evapd and partitioned × 3 in $CHCl_3$–MeOH–H_2O (13:7:4). The organic phase was collected and evapd and gave a residue (4.544 g).

Residue A (230 mg) was re-extracted with *n*-hexane and the soluble fraction (216 mg) was chromatographed on a column of silica gel using *n*-hexane (200 ml) and CH_2Cl_2 (60 ml). The latter fraction contained (2*S*)-fomannoxin (175 mg) (**2**). The 4-bromophenylhydrazone of (2*S*)-fomannoxin crystallized as needles

(MeOH), mp 117–118°. (Found: C, 60.35; H, 4.86; N, 7.87; Br 22.67%. $C_{18}H_{18}N_2OBr$ requires: C, 60.35; H, 5.06; N, 7.87; Br 22.35%.) $[\alpha]_D^{20}$ +76.02 ($CHCl_3$ *c* 0.101). UV λ_{max}^{MeOH} nm(ε) 205 (37 000), 252 (14 600), 313 (28 300), 350 (34 300). ^1H NMR: δ 1.81 (3H, *d*, *J* = 1.0 Hz, Me), 3.08 (1H, *dd*, *J* = 16.0, 8.0 Hz, H_A-3), 3.48 (1H, *dd*, *J* = 16.0, 8.3 Hz, H_B-3), 4.98–5.14 (2H, *m*, $=CH_2$), 5.28 (1H, *dd*, *J* = 8.0, 8.3 Hz, H-2), 6.77–7.70 (9H, *m*, Ar–H + N=CH + NH).

Residue B (2.841 g) was chromatographed on silica gel (75 g) and eluted twice with Et_2O (18 × 50 ml), Et_2O–EtOAc (2:1→1:1), EtOAc–MeOH (4:1) and monitored by TLC [developer: CH_2Cl_2–EtOAc (5–0.2)] to give fractions I–VII (Fig. 1).

Fraction I was not further investigated.

Fraction II (403 mg) was chromatographed on silica gel (20 g) and eluted with CH_2Cl_2–EtOAc (25:1). The analysis (TLC) of subfraction 1 showed two bands (R_f 0.55 and 0.50) HPLC (normal phase) [eluent: $CHCl_3$–*n*-hexane 4:1, 1.5 ml/min] afforded the less polar fraction, the benzofuran (**6**) as an oil (7 mg). HRMS *m/z* 204.0693. $C_{12}H_{12}O_3$ requires *m/z* 204.0786. ^1H NMR: δ 1.70 (6H, *s*, 2 × Me), 3.82 (1H, *s*, OH), 6.82 (1H, *s*, H-3), 7.57 (1H, *d*, *J* = 8.6 Hz, H-7), 7.84 (1H, *dd*, *J* = 8.4, 1.6 Hz, H-6), 8.08 (1H, *d*, *J* = 1.6 Hz, H-4), 10.05 (1H, *s*, –CHO). ^{13}C NMR: δ 28.73 (2 × *q*), 69.35 (*s*), 100.93 (*d*) 111.93 (*d*), 126.2 (*d*), 125.8 (*d*), 128.9 (*s*), 132.26 (*s*), 158.15 (*s*), 165.17 (*s*), 191.82 (*d*). IR ν_{max} (KBr) 1684 cm^{-1}.

Band ii (R_f 0.5) afforded 5-formyl-3-hydroxy-2-isopropenyldihydrobenzofuran (**3**) as an oil. $[\alpha]_D^{20}$ +77.7° ($CHCl_3$; *c* 5.5). EIMS *m/z*: 204 (M^+ ($C_{12}H_{12}O_3$) 19%) 189(16), 148(100), 133(35) 105(27). IR ν_{max} (KBr) 3580 cm^{-1}, 1685 cm^{-1}. ^1H NMR; δ 1.75 (3H, *s*, Me), 2.3 (1H, *br d*, OH), 4.96, 5.01 (2H, *dd*, *J* = 3.9, 0.6 Hz, $=CH_2$), 5.10, 5.30 (2H, *dd*, *J* = 4.0 Hz, H-2, H-3), 7.00 (1H, *d*, 7.8 Hz, H-7), 7.81 (1H, *dd*, *J* = 8.4, 1.7 Hz, H-6), 7.94 (1H, *d*, *J* = 1.7 Hz, H-4), 9.82 (1H, *s*, CHO). ^{13}C NMR: δ 17.43 (*q*), 75.45 (*d*), 95.02 (*d*), 110.73 (*d*), 113.05 (*t*), 127.56 (*d*), 129.63 (*s*), 130.64 (*s*), 136.63 (*d*), 140.83 (*s*), 165.39 (*s*), 190.86 (*d*).

Fraction III (374 mg) was chromatographed on a silica gel column (eluent; CH_2Cl_2–EtOAc (10%–15%–50%)) and the major fraction (R_f 0.5) was rechromatographed on silica gel (eluent: CH_2Cl_2–EtOAc, 20:1) The relevant sub-fraction (R_f 0.5) was passed through a column of Sephadex LH-20 (eluent: *n*-hexane–CH_2Cl_2 1:4) and purified by HPLC (normal phase) [eluent $CHCl_3$–*n*-hexane 4:1, 1.5 ml/min], to afford an oil (10 mg), which on treatment with $CHCl_3$–*n*-hexane gave (*S*)-5-formyl-2-(isopropyl-1′-ol)-2,3-dihydrobenzofuran (**4a**) as needles, mp 83–85°. $[\alpha]_D^{21}$ +109.1° ($CHCl_3$; *c* 1.4). Found: C, 69.82; H, 6.80. $C_{12}H_{14}O_3$ requires C, 69.88; H, 6.84% IR ν_{max} (KBr) 3510 cm^{-1}, 2980 cm^{-1}, 1670 cm^{-1}. UV λ_{max}^{MeOH} nm (ε) 295 (15 907), 280 (14 316), 229 (14 316). ^1H NMR: δ 1.24, 1.37 (6H, 2 × s, Me), 1.99 (1H, *br s*, OH), 3.22 (2H, *d*, *J* = 9.1 Hz, H-3), 4.73 (1H, *t*, *J* = 9.1 Hz, H-2), 6.88 (1H, *d*, *J* = 8.1 Hz, H-7), 7.65 (1H, *dd*, *J* = 9.1, 1.5 Hz, H-6), 7.7 (1H, *d*, *J* = 1.1 Hz, H-4), 9.81 (1H, *s*, –CHO). ^{13}C NMR: δ 24.15, 26.77, 2 × *q* (2 × Me), 29.77 *t* (C-3), 71.69 *s* (C-1′), 90.78 *d* (C-2), 109.4 *d* (C-7), 126.0 *d* *(C-5), 128.8 *s* (C-4a), 130.67 (C-3), 133.0 *d** (C-4), 165.1 *s* (C-7a), 190. 7 *s* (CHO) (*interchangeable). The reduction product ($NaBH_4$), the 5-carbinol, crystallized as needles ($CHCl_3$–*n*–hexane) mp 114–116° (Found: C, 69.49; H, 7.6. $C_{12}H_{16}O_3$ requires C, 69.23; H, 7.69%). ^1H NMR δ: 1.21, 1.34 (6H, 2 × s, Me), 1.84 (2H, *br s*, 2 × OH), 3.13 (2H, *d*, *J* = 9.9 Hz, H-3), 4.58 (2H, *s*, $-CH_2OH$), 4.6 (1H, *t*, *J* = 9.9 Hz, H-2), 6.74, 7.09 (2H, 2 × *d*, *J* = 8.1 Hz, H-7, H-6), 7.2 (1H, *s*, H-4). ^{13}C NMR δ: 65.31 ($-CH_2OH$).

Fraction IV (362 mg) gave an unresolved mixture and was not further investigated.

Fraction V (328 mg) was passed through Sephadex LH-20 and eluted with CH_2Cl_2, and the fraction R_f 0.46 afforded after purification on a silica gel column, fomannosin (**1**) (57 mg) which was characterized as the reduction product dihydrofomannosin mp 102° (identical IR, UV, MS and ^1H NMR as an authentic sample). A sub-fraction (R_f 0.35) was chromatographed on silica gel twice with $CHCl_3$ as eluent to afford an oil (**5**) (7 mg). EIMS *m/z* 234 $[M]^+$ ($C_{14}H_{18}O_3$). ^1H NMR: δ 1.13 (3H, *d*, *J* = 6.2 Hz, –Me-2″), 1.77 (3H, *s*, Me-1′), 3.03 (1H, *dd*, *J* = 15.7, 8.8 Hz, H_A-3), 3.34 (1H, *dd*, *J* = 15.7, 8.8 Hz, H_B-3), 3.97 (1H, *dq*, *J* = 6.2, 4.8 Hz, H-2″), 4.55 (1H, *d*, *J* = 4.8 Hz, H-1″), 4.91, 5.08 (2H, *br s*, $=CH_2$), 5.18 (1H, *t*, *J* = 8.8 Hz, H-2), 6.77 (1H, *d*, *J* = 8.1 Hz, H-7), 7.08 (1H, *br d*, H-6), 7.20 (1H, *br s*, H-4). ^{13}C NMR: δ 17.29 (*q*), 17.84 (*q*), 34.72 (*t*), 71.36 (*d*), 77.78 (*d*), 86.05 (*d*), 108.91 (*d*), 112.23 (*t*), 123.33 (*d*), 127.05 (*d*), 127.12 (*s*), 132.65 (*s*), 143.92 (*s*), 159.69 (*s*).

Synthesis of racemic 5-(1″,2″-*dihydroxypropyl*)-2-*isopropenyl*-2,3-*dihydrobenzofuran* (**5**). (±)-5(*n*-Propenyl)-2-isopropenyl-2,3-dihydrobenzofuran (**7**). A mixture of (±)-fomannoxin (**2**) [6] (76 mg) and 18-crown-6- (15 mg) in dry THF (5 ml) was added to a suspension of ethyltriphenylphosphonium bromide (1.79 g) and K_2CO_3 in dry THF (20 ml). The reaction was refluxed for 52 hr under N_2. The solvent was evapd and the residue was washed with Et_2O. Column chromatography on silica gel [eluent: *n*-hexane–EtOAc (9:1)] gave (*Z*)-5-(*n*-propenyl)-2-isopropenyl-2,3-dihydrobenzofuran (**7**) as the major product an oil (46 mg), (*E*)-(**7**) (2.3 mg) and unreacted fomannoxin. EIMS *m/z* 200 (M^+ 52%) *m/z* 185(100). ^1H NMR: δ 1.77 (3H, *s*, Me-2′), 1.88 (3H, *dd*, *J* = 7.1, 1.8 Hz, Me-3″), 3.04 (1H, *dd*, *J* = 15.6, 8.5 Hz, CH_{2A}-3), 3.34 (1H, *dd*, *J* = 15.6, 9.5 Hz, CH_{2B}-3), 4.91 (1H, *m*, *J* = 1.29 Hz, $=CH_{2A}$), 5.09 (1H, *m*, *J* = 0.82 Hz, $=CH_{2B}$), 5.17 (1H, *dd*, *J* = 9.0, 8.5 Hz, H-2), 5.66 (1H, *dq*, *J* = 11.5, 7.1 Hz, H-2″), 6.35 (1H, *dd*, *J* = 11.5, 1.9 Hz, H-1″), 6.77 (1H, *d*, *J* = 8.2 Hz, H-7), 7.08 (1H, *d*, *J* = 8.2 Hz, H-6), 7.12 (1H, *s*, H-4). ^{13}C NMR: δ 14.74 q (Me-3″), 17.29 q (Me-2′), 34.78 (*t*), 85.97 (*d*), 108.81 (*d*), 112.16 *t* (=CH_2), 124.64 (*d*)*, 125.31 *d* (C-2″), 126.6 (*s*), 128.97 (*d*)*, 129.69 *d* (C-1″), 130.35 (*s*), 144.05 (*s*), 158.42 (*s*). (* interchangeable).

5-(1″,2″-*Dihydroxypropyl*)-2-*isopropenyl*-2,3-*dihydrobenzofuran* (**5**). Osmium tetroxide (0.5 g) was added to a solution of (*Z*)-5(*n*-propenyl)-2-isopropenyl-2,3-dihydrobenzofuran (0.39 g) (**7**) in pyridine (7.5 ml). The reaction mixture was stirred at room temp. for 15 hr. A solution of sodium metabisulphite (0.85 g) in a mixture of pyridine (10 ml) and water (15 ml) was added to the solution and stirred for a further 6 hr. The reaction mixture was extracted with warm EtOAc and the organic layer separated and washed with HCl (5%), $NaHCO_3$ (satd) and H_2O. Evapn afforded an oil (193 mg) which was subjected to CC over silica gel. Gradient elution with $CHCl_3$–MeOH (49:1 → 19:1) gave diastereomeric diols (**5**) as an oil. EIMS *m/z* 234 (M^+ 6%) 216 (3), 190 (16), 189 (100), 173 (10), 91 (12). UV λ_{max}^{MeOH} nm (log ε) 205 (4.3), 232 (3.8), 285 (3.4). ^1H NMR: δ *erythro* **5** 1.11 (3H, *d*, *J* = 6.6 Hz, Me-3″), 1.77 (3H, *s*, Me-1′), 3.03 (1H, *dd*, *J* = 15.8, 8.1 Hz, H_A-3), 3.32 (1H, *dd*, *J* = 15.7, 8.8 Hz, H_B-3), 3.97 (0.55H, *m*, H-2″), 4.54 (0.55H, *d*, *J* = 4.4 Hz, H-1″), 4.91, 5.08 (2H, 2 × *s*, $=CH_2$), 5.17 (1H, *dd*, *J* = 9.2, 8.8 Hz, H-2), 6.77 (1H, *d*, *J* = 8.0 Hz, H-7), 7.07–7.2 (2H, *m*, H-6, H-4). δ *threo* **5** 1.03 (3H, *d*, *J* = 6.6 Hz, Me-3″), 3.82 (0.45H, *m*, H-2″), 4.27 (0.45H, *d*, *J* = 7.7 Hz, H-1″), 6.75 (1H, *d*, *J* = 7.7 Hz, H-7) The signals for the isopropenyl moiety are identical with *erythro* **5**. ^{13}C NMR δ: Me-3″ 17.3 (*q*) *erythro*, CH_3-3″ 18.86 (*q*) *threo*, 17.73 (*q*), 34.65 (*t*), 71.36 (*d*), 79.5 (*d*), 86.04 (*d*), 108.86 (*d*), 112.19 (*t*), 123.19 (*t*), 127.0 (*d*), 132.52 (*s*), 133.23 (*s*), 143.89 (*s*), 159.63 (*s*).

Fraction VI (285 mg) was passed through a column of LH-20 with CH_2Cl_2 as eluent and the fraction R_f 0.34 afforded after CC on silica gel [eluent: $CHCl_3$–EtOAc (4:1)] a pale yellow oil (**4b**) (60 mg), $[\alpha]_D^{16}$ +35.14° ($CHCl_3$; *c* 1.0813) IR *v* 3380 (OH), 1678 (CO). EIMS *m/z* 222 (M^+ 18%) 173 (23), 148 (100), 135 (25), 119 (50). ^1H NMR: δ 1.18 (3H, *s*, 1′-Me), 3.32, 3.36 (2H, *dd* × 2, *J* = 9.0, 15.0 Hz, CH_2-3), 3.58, 3.72 (2H, *d* × 2, *J* = 11.0 Hz, CH_2OH), 9.88 (1H, *s*, CHO), 4.95 (1H, *dd*, *J* = 9.0, 9.0 Hz, H-2), 6.9 (1H, *dd*, *J*

= 8.0, 4.0 Hz, H-7), 7.7 (1H, *d*, *J* = 8.0 Hz, H-6), 7.76 (1H, *br s*, H-4), ^{13}C NMR: δ 18.99 (*q*), 29.60 (*t*), 66.98 (*t*), 73.53 (*s*), 86.32 (*d*), 109.57 (*d*), 126.07 (*d*), 128.67 (*s*), 130.62 (*s*), 133.02 (*d*), 164.96 (*s*), 190.8 (*d*).

Reduction of **4b** *with* $NaBH_4$. Compound **4b** (28 mg), $NaBH_4$ (20 mg) and MeOH (5 ml) were stirred at room temp. for 30 min. The reaction mixture was subjected to CC on silica gel. The triol was an oil (25 mg) which on treatment with EtOAc afforded needles mp 124–129°. ^{1}H NMR: δ 1.13 (3H, *s*, 1′-Me), 3.21, 3.25 (2H, 2 × *dd*, *J* = 8.0, 16.0 Hz, CH_2–3), 4.48, 3.68 (2H, *d* × 2, *J* = 11.0, CH_2OH), 4.52 (2H, *s*, CH_2OH-5), 4.78 (1H, *ddd*, *J* = 8.0, 8.0, 2.0 Hz, H-2), 6.67 (1H, *d*, *J* = 8.0 Hz, H-7), 7.04 (1H, *d*, *J* = 8.0 Hz, H-6), 7.75 (1H, *s*, H-4). ^{13}C NMR: δ 20.1 (*q*), 30.7 (*t*), 64.6 (*t*), 68.3 (*t*), 73.9 (*s*), 86.3 (*d*), 108.9 (*d*), 124.6 (*d*), 127.1 (*s*), 128.4 (*s*), 135.4 (*d*), 159.9 (*s*).

Synthesis of racemic 5-formyl-2-(1′,2′-dihydroxyisopropyl)-2,3-dihydrobenzofuran **4b**. (±)-Fomannoxin (**2**) (657 mg) in pyridine (10 ml) and OsO_4 (1 g) were reacted together for 24 hr, a solution of $Na_2S_2O_5$ (3 g) in H_2O (50 ml) was added and stirring continued for a further 18 hr. The reaction mixture was extracted with EtOAc, and the organic layer washed successively with HCl (dil), $NaHCO_3$ (satd) and H_2O. Evapn afforded an oil (480 mg) which was purified on PLC [developer: MeOH (10%)–$CHCl_3$]. The solid was crystallized from $CHCl_3$ mp 80–86° EIMS *m/z* 222 (M^+ 13%), 148 (100), 131 (46), 119 (38). Identical UV, IR and ^{1}H NMR spectra with those of **4b**.

Fraction VII (151 mg) was examined but no metabolite was isolated.

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REFERENCES

1. Basset, C., Sherwood, R. T., Kepler, J. A. and Hamilton, P. B. (1967) *Phytopathology* **57**, 1046.
2. Kepler, J. A., Wall, M. E., Mason, J. E., Basset, C., McPhail, A. T. and Sim, G. A. (1967) *J. Am. Chem. Soc.* **89**, 1260.
3. Hirotani, M., O'Reilly, J., Donnelly, D. M. X. and Polonsky, J. (1977) *Tetrahedron Letters* 651.
4. Donnelly, D. M. X., O'Reilly, J., Polonsky, J. and van Eijk, G. W. (1982) *Tetrahedron Letters* 5451.
5. Donnelly, D. M. X., O'Reilly, J., Polonsky, J. and Sheridan, M. H. (1987) *J. Chem. Soc. Perkin Trans. I*, 1869.
6. Zalkow, L. H., Ekpo, B. A., Gelbaum, L. T., Harris III, R. N., Keinan, E., Novak, Jr. J. R., Ramming, C. T. and van Derveer, D. (1979) *J. Nat. Prod.* **42**, 203.
7. Heslin, M. C., Stuart, M. R., O'Murchú, P. and Donnelly, D. M. X. (1983) *Eur J. For. Path.* **13**, 11.
8. Bigi, F., Casiraghi, G., Casnati, G. and Sartori, G. (1983) *Tetrahedron* **39**, 169.
9. Martin, M. (1986) M.Sc. Thesis, The National University of Ireland.
10. Fukami, J., Shishido, T., Fukunaga, K. and Casida, J. E. (1969) *J. Agric. Food Chem.* **17**, 1217.
11. Fukami, J., Yamamoto, I. and Casida, J. E. (1967) *Science* **155**, 713.