Isolation and Structure Determination of Cotonefuran, an Induced Antifungal Dibenzofuran from *Cotoneaster lactea* W.W.Sm.

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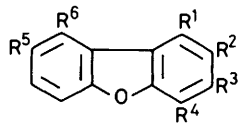
An antifungal compound, cotonefuran, has been isolated from necrotic wood of a fungal-infected specimen of *Cotoneaster lactea* W.W.Sm. (Rosaceae). Spectroscopic evidence demonstrates that cotonefuran is a trimethoxydibenzofurandiol which is trisubstituted in one aromatic ring and disubstituted in the other. Comparison of the ^{1}H n.m.r. spectrum with the ^{1}H n.m.r. spectra of suitable model dibenzofurans provided some information on the positioning of the substituents, but an *X*-ray analysis was necessary for a full structural elucidation. Cotonefuran was thus shown to be 3,4,6-trimethoxydibenzofuran-2,7-diol (**9**). The compound was not found in healthy sapwood and is probably a phytoalexin.

We have recently reported[1] the isolation and characterisation by total synthesis of α- (**1**) and β-pyrufuran (**2**), two new antifungal dibenzofurans from fungus-infected perry pear wood. We now report the isolation and characterisation by *X*-ray crystallography of another dibenzofuran, cotonefuran, from *Cotoneaster lactea* W.W.Sm. (Rosaceae).

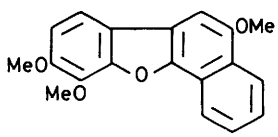
Cotoneaster lactea grows as a small tree. The specimen examined exhibited symptoms of a wilt disease and after the tree was felled and the trunk sectioned, a narrow (*ca.* 1 mm) pigmented zone was observed in the sapwood beneath the bark. A similar zone is one of the most characteristic symptoms of Dutch Elm Disease, caused by the fungal pathogen *Ceratocystis ulmi*.[2] Ethanol extraction of the sapwood from this zone followed by column and thin layer chromatography led to the isolation of cotonefuran. This was shown to be the principal antifungal component of the extract by a t.l.c. plate *Cladosporium cucumerinum* bioassay.[3] The compound was absent from extracts of healthy sapwood.

Cotonefuran was shown to have a molecular formula of $C_{15}H_{14}O_6$ (mass spectrum). The ^{1}H n.m.r. spectrum revealed the presence of three aromatic protons which produced a singlet at δ 7.02 and a pair of *ortho* doublets at δ 6.88 and 7.30 (*J* 8.5 Hz). Three methoxy groups were responsible for singlets at 3.97 (3 H) and 4.24 (two degenerate, each 3 H) while acetylation and methylation revealed the presence of two phenolic hydroxy groups. No signal in the i.r. spectrum was assignable to carbonyl, and the sixth oxygen was therefore considered to be present as an ether linkage within a $C_{12}O$ moiety containing nine double bond equivalents. The u.v. spectrum showed absorption maxima at 226, 266, and 306 nm in a pattern very similar to that observed for α- and β-pyrufuran.[1] Hence it is probable that cotonefuran is a dibenzofuran which is trisubstituted in one aromatic ring and 6,7-, 6,9-, or 8,9-disubstituted in the other. This, however, leads to 12 possible individual structures for the fully methylated derivative (*i.e.* all five substituents methoxy) and to no less than 120 for the parent trimethoxydibenzofurandiol.

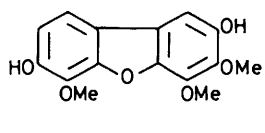
Many of the possible structures may be eliminated from a consideration of the ^{1}H n.m.r. data. In our studies with α- and β-pyrufuran we found that multiple substitution of one aromatic ring had little effect on the chemical shifts of the protons of the other ring. We also synthesised[1] the 1,2,3-, 1,2,4-, 1,3,4-, and 2,3,4-trimethoxydibenzofurans [(**3**)—(**6**) respectively] and these provide suitable model compounds for comparative ^{1}H n.m.r. spectroscopy. The aromatic singlets in these model trimethoxydibenzofurans appear at δ 6.82, 6.65, 6.42, and 7.08, respectively,



	R^1	R^2	R^3	R^4	R^5	R^6
(**1**)	OMe	OH	OMe	OMe	H	H
(**2**)	OMe	OMe	OH	OMe	H	H
(**3**)	OMe	OMe	OMe	H	H	H
(**4**)	OMe	OMe	H	OMe	H	H
(**5**)	OMe	H	OMe	OMe	H	H
(**6**)	H	OMe	OMe	OMe	H	H
(**7**)	OMe	H	H	OMe	OMe	OMe



(**8**)



(**9**)

as compared to the singlet at δ 6.98 in the fully methylated derivative of cotonefuran. From this it appears that methylated cotonefuran possesses either the 1,2,3- or the 2,3,4-substitution pattern in one aromatic ring.

1,4,8,9-Tetramethoxydibenzofuran[4] (**7**) is a suitable model for the disubstituted ring in cotonefuran as it contains two of the three possible disubstitution patterns. From a comparison of the ^{1}H n.m.r. spectra, the substituents in methylated cotonefuran clearly do not occupy the 6- and 9-positions as the observed resonances of the *ortho*-aromatic protons (δ 6.88 and 7.30) are at much lower field than the equivalent protons in compound (**7**) [2-H and 3-H appear at δ 6.53 and 6.76, respectively[4]]; the alternative 8,9-substitution is more likely [6-H and 7-H appear at δ 7.14 and 6.96 respectively in (**7**)[4]], as is 6,7-substitution. A model for the latter is provided by 5,9,10-trimethoxybenzonaphthofuran (**8**). The relevant pair of *ortho* doublets in this compound occur at δ 6.85 and 7.34,[5] and compare particularly well with the signals observed for cotonefuran.

However, there are limitations in the use of such comparative ^{1}H n.m.r. methods, and no single structure for methylated cotonefuran could be arrived at with any confidence. Cotonefuran itself is even more difficult to elucidate, although a negative test with Gibbs reagent (2,6,*N*-trichlorobenzoquinone

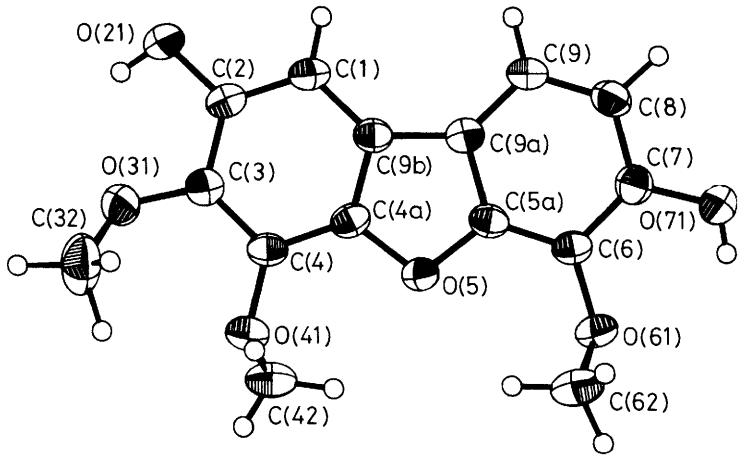


Figure. ORTEP drawing of the molecule of cotonefuran showing the non-hydrogen atoms as their 50% thermal ellipsoids and displaying the crystallographic numbering scheme

monoimine) suggested that the molecule does not have an unsubstituted position *para* to either of the free hydroxy groups.

Because of these difficulties, a single crystal *X*-ray diffraction study of cotonefuran was undertaken. Cotonefuran formed colourless prisms, m.p. 150—151 °C from ethyl acetate–n-hexane, and the molecular structure was found to be 3,4,6-trimethoxydibenzofuran-2,7-diol as shown in the Figure [structure (**9**) with conventional dibenzofuran numbering]. The atomic co-ordinates are as shown in Table 1 and the bond lengths and angles (Table 2) are similar to those found in other dibenzofuran derivatives.[6] The molecule forms an approximate plane, excluding the methyl groups which are rotated about 60° from the coplanar position on the same side of the molecule. The benzene rings are tilted by about 1° on opposite sides of the plane of the central furan ring, while the hydroxy H atoms are also close to the molecular plane and both take part in the hydrogen bonding scheme. Each molecule is hydrogen-bonded to four others, forming a three-dimensional network. The O • • • O distances (Table 2) indicate that the two independent H bonds are not of equal strength.

Cotonefuran is an addition to the small number of dibenzofurans isolated from higher plants.[1] Its antifungal activity and presence in necrotic wood resulting from fungal infection suggests that it may function as a tree phytoalexin.

Experimental

All isolated and synthesised compounds were shown to be pure by both t.l.c. and g.l.c. Electron impact (e.i.) mass spectra were measured from direct insertion probe samples on a Kratos MS 80 or 950 and a Finnigan 4021 MS-DS instrument. 90 MHz ^{1}H N.m.r. spectra were recorded on a Perkin-Elmer R32 spectrometer. M.p.s were measured on a Köfler block and are uncorrected. Kieselgel 40 (Merck: 70-230 mesh) was used for column chromatography and Kieselgel 60 F254 plates (Merck: 0.25 mm) were employed for t.l.c. G.l.c. was carried out on a 1 m × 2 mm i.d. stainless steel column packed with 1% Dexsil 300 on 100—120 mesh Supelcoport, temperature programmed 130—350 °C at 6° min^{-1}, injector 250 °C, flame ionisation detector 300 °C, and carrier gas N_2 at 40 ml min^{-1}. Antifungal activity was detected by a t.l.c. *Cladosporium cucumerinum* bioassay.[3]

Isolation of Cotonefuran.—A tree of *Cotoneaster lactea*, infected with an unidentified fungal disease, was felled and the trunk sectioned. Immediately beneath the bark there was a dark brown pigmented necrotic band which was removed with a plane. The shavings (250 g) were soaked in ethanol at 20 °C for 10 days, after which the solvent was removed under reduced pressure. The residue was dissolved in chloroform (5 ml) and applied to a column (60 cm × 3 cm i.d.) of Kieselgel 40 and eluted with 50% chloroform–n-hexane followed by chloroform alone. Biologically active fractions gave an antifungal zone at R_F 0.37 (2% ethanol–chloroform). These were combined and the solvent evaporated under reduced pressure. The residue was chromatographed on t.l.c. (2% ethanol–chloroform) and the biologically active zone removed. Crystallisation from ethyl acetate–n-hexane afforded colourless prisms (85 mg) of *cotonefuran* (**9**), m.p. 150—151 °C (Found: M^+, 290.0779 $C_{15}H_{14}O_6$ requires M, 290.0790); $\lambda_{max.}$(EtOH; 1 cm) 226 [$\log_{10}$ (ε dm^3 mol^{-1}, cm^{-1}) 4.52], 246sh (4.38), 266 (4.23), 306 (4.18), and 318sh nm (4.10); $\nu_{max.}$($CHCl_3$; 0.5 mm) 3 530m (OH), 3 020w (Ar), 2 940w, 2 840w (CH_3), 1 605m, 1 508m, 1 495m (Ar), 1 460s, 1 432m, 1 422m, 1 390w, 1 355m, 1 298w, 1 160s, 1 102m, 1 063m, and 1 035m cm^{-1}; δ_H (90 MHz; $CDCl_3$; Me_4Si) 3.97, 4.24, 4.24 [each 3 H, s (2 degenerate), together 3-, 4-, and 5-OMe], 6.88 (1 H, d, *J* 8.5 Hz, 8-H), 7.02 (1 H, s, 1-H), and 7.30 (1 H, d, *J* 8.5 Hz, 9-H); *m/z* (e.i. 40 eV) 291 (12.1%), 290 (M^+, 69.5), 276 (12.4), 275 (100), 232 (23.8), 229 (17.7), 214 (10.3), 145 (10.8), and 133 (10.3); R_F (2% EtOH–$CHCl_3$) 0.37. Spots of cotonefuran on t.l.c. when sprayed with Gibbs reagent (2,6,*N*-trichlorobenzoquinone monoimine) and exposed to ammonia vapour gave a grey and not a bright blue colouration. R_t 13.3 min.

Methylation of Cotonefuran.—Cotonefuran (15 mg) was quantitatively methylated with an excess of diazomethane in 10% methanol–diethyl ether (10 ml). The product 2,3,4,6,7-*pentamethoxydibenzofuran* was isolated as an oil by t.l.c. (2% EtOH–$CHCl_3$) (Found: M^+, 318.1079. $C_{17}H_{18}O_6$ requires M, 318.1103); $\lambda_{max.}$(EtOH; 1 cm) 226 (log ε 4.57), 229 (4.48), 264 (4.26), 302 (4.23), and 314sh nm (4.13); δ_H (90 MHz; $CDCl_3$; Me_4Si) 3.92, 3.92, 3.92, 4.17, 4.22 [each 3 H, s, (3 degenerate), together 2-, 3-, 4-, 6-, and 7-OMe], 6.89 (1 H, d, *J* 8.2 Hz, 8-H), 6.98 (1 H, s, 1-H), 7.36 (1 H, d, *J* 8.2 Hz, 9-H); R_F (2% EtOH–$CHCl_3$) 0.68; R_t 14.3 min.

Acetylation of Cotonefuran.—Cotonefuran (20 mg) was acetylated at 20 °C with acetic anhydride (0.2 ml) and pyridine (0.2 ml). 2,7-*Diacetoxy*-3,4,6-*trimethoxydibenzofuran* was obtained as an oil by t.l.c. (2% EtOH–$CHCl_3$) (Found: M^+, 374.0993. $C_{19}H_{18}O_8$ requires M, 374.1001); $\lambda_{max.}$(EtOH; 1 cm)

Table 1. Fractional co-ordinates, $\times 10^4$ for the non-hydrogen atoms, $\times 10^3$ for the H atoms, and equivalent isotropic temperature factor U_{eq} ($\times 10^4$ Å^2) defined as $\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* (a_i \cdot a_j)$

Atom	x	y	z	U_{eq}
C(1)	906(2)	3 067(3)	−745(2)	364(6)
C(2)	−174(2)	3 627(2)	−1 198(2)	359(6)
C(3)	−807(2)	5 134(3)	−1 047(2)	355(6)
C(4)	−367(2)	6 128(2)	−424(2)	337(6)
C(4a)	696(2)	5 526(2)	27(2)	338(6)
O(5)	1 215(1)	6 330(2)	688	352(4)
C(5a)	2 231(2)	5 325(2)	960(2)	338(6)
C(6)	3 053(2)	5 680(2)	1 602(2)	340(6)
C(7)	4 069(2)	4 557(3)	1 774(2)	383(7)
C(8)	4 203(2)	3 106(3)	1 339(2)	401(7)
C(9)	3 341(2)	2 757(2)	712(2)	372(6)
C(9a)	2 353(2)	3 901(2)	507(2)	330(6)
C(9b)	1 335(2)	4 038(2)	−113(2)	333(6)
O(21)	−626(2)	2 670(2)	−1 812(1)	479(6)
O(31)	−1 893(2)	5 507(2)	−1 524(1)	451(5)
C(32)	−1 929(5)	7 097(4)	−1 868(3)	760(12)
O(41)	−973(2)	7 596(2)	−270(1)	402(5)
C(42)	−75(3)	8 982(3)	−357(2)	502(9)
O(61)	2 881(2)	7 017(2)	2 093(1)	390(5)
C(62)	3 097(3)	8 587(3)	1 742(2)	520(9)
O(71)	4 942(2)	4 808(2)	2 389(1)	489(5)
H(1)	129(3)	204(3)	−86(2)	
H(8)	494(3)	232(3)	149(2)	
H(9)	343(3)	179(3)	43(2)	
H(21)	−135(4)	316(5)	−195(3)	
H(32a)	−272(4)	713(5)	−225(2)	
H(32b)	−102(4)	744(5)	−193(3)	
H(32c)	−208(4)	812(5)	−147(3)	
H(42a)	−57(4)	982(5)	−39(3)	
H(42b)	46(5)	894(5)	−84(3)	
H(42c)	64(4)	910(5)	4(3)	
H(62a)	305(3)	943(5)	215(2)	
H(62b)	240(5)	866(4)	130(3)	
H(62c)	401(4)	878(5)	154(3)	
H(71)	488(4)	568(4)	257(3)	

234 (log ε 4.63), 266 (4.28), 290 (4.26), and 308sh nm (3.61); δ_H (90 MHz; $CDCl_3$; Me_4Si) 2.33, 2.33 [each 3 H, s (degenerate), 2- and 7-COMe], 3.91, 4.19, 4.21 (each 3 H, s, together 3-, 4-, and 6-OMe), 6.95 (1 H, d, *J* 8.2 Hz, 8-H), 7.20 (1 H, s, 1-H), and 7.37 (1 H, d, *J* 8.2 Hz, 9-H); R_F (2% EtOH–$CHCl_3$) 0.64; R_t 17.2 min.

Crystal Data.—$C_{15}H_{14}O_6$, *M* 290.3. Orthorhombic, a = 9.870(5), b = 8.163(3), c = 16.836(5) Å, U = 1 356.5(9) Å^3, Z = 4, D_c = 1.42 g cm^{-3}, $F(000)$ = 608, space group $Pna2_1$ (C_{2v}^9 No. 33). No molecular symmetry is required, Mo-K_α radiation (λ 0.7107 Å), μ 0.104 mm^{-1}, no absorption correction, crystal size 0.50 × 0.38 × 0.38 mm. 3 261 Planes were measured on a CAD4 diffractometer using the ω–2θ scan method, with $1.5 < \theta < 28°$, and with $h \geqslant 0$ and $k \geqslant 0$. Three control reflections measured every 2 h of exposure time were unchanged over the data collection period. Equivalent reflections were averaged (R = 0.063 for 1 576 pairs) to give 1 685 planes. The weak and negative intensities were treated [7] to give better estimates of their intensities and e.s.d.s. The structure was solved by direct methods [8] and full matrix refinement carried out using all 1 685 planes giving R = 0.040 and R_w = 0.035. Weights $\propto \sigma^{-2}(F_o)$ gave a satisfactory weighting analysis. Phenyl H atoms were refined with a common U_{iso} which refined to 0.040(4) Å^2, and the rest treated similarly, giving U_{iso} = 0.094(4) Å^2. The C and O atoms were given anisotropic temperature factors. The maximum shift/e.s.d. on the final cycle was 0.9, and the largest peaks (<0.2 e Å^{-3}) on the final ΔF map were close to the methyl C atoms, indicating an alternative position for the methyl H atoms of low occupation. Scattering factors were calculated using the analytical approximation in Table 2.2B of ref. 9. ORTEP [10] was used for the diagrams and local programs for the data reduction and geometry calculations on a PRIME 550 computer. Anisotropic temperature factors and full geometry tables have been deposited as a Supplementary Publication * (SUP No. 23910, 16 pp.). Fractional co-ordinates are given in Table 1, and selected bond lengths and angles in Table 2.

Table 2. Selected interatomic distances and angles

(*a*) Bond lengths (Å)

C(2)–O(21)	1.370(3)	C(7)–O(71)	1.363(3)
C(3)–O(31)	1.374(3)	C(9a)–C(9b)	1.454(3)
C(4)–O(41)	1.363(3)	O(31)–C(32)	1.421(3)
C(4a)–O(5)	1.390(3)	O(41)–C(42)	1.445(3)
O(5)–C(5a)	1.375(3)	O(61)–C(62)	1.427(3)
C(6)–O(61)	1.380(3)		

(*b*) Bond angles (°) (mean e.s.d. 0.2°)

O(5)–C(4a)–C(9b)	112.5	C(4a)–C(9b)–C(9a)	105.0
C(4a)–O(5)–C(5a)	104.7	C(3)–O(31)–C(32)	117.4
O(5)–C(5a)–C(9a)	112.2	C(4)–O(41)–C(42)	113.5
C(5a)–C(9a)–C(9b)	105.6	C(6)–O(61)–C(62)	116.4

(*c*) Torsion angles (°) (mean e.s.d. 0.3°)

C(4)–C(3)–O(31)–C(32)	50	C(5a)–C(6)–O(61)–C(62)	−66
C(4a)–C(4)–O(41)–C(42)	63		

(*d*) Hydrogen-bonding geometry

A–B···C	A–B	A···C	B···C	A–B–C
O(21) H(21)O(61)^{I}	0.85(4) Å	2.901(3) Å	2.21(4) Å	138(4)°
O(71) H(71) O(21)^{II}	0.78(4)	2.779(3)	2.07(4)	153(4)

Symmetry operations: I, $-x$, $1-y$, $z-\frac{1}{2}$; II, $\frac{1}{2}-x$, $\frac{1}{2}+y$, $\frac{1}{2}+z$

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* For details of the Supplementary Publications scheme, see Instructions for Authors (1984), *J. Chem. Soc., Perkin Trans. 1*, 1984, Issue 1.

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