Antibacterial Activity of Nitrobenzofurans[1]

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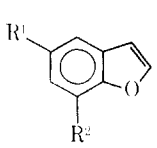
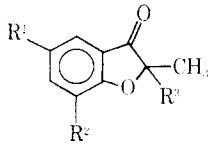
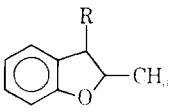
An investigation of the antibacterial activity of nitrobenzofurans was undertaken due to their close structural relationship to known antimicrobial agents. 3,7-Dinitro-2-methylbenzofuran was found to be as active against *Bacillus subtilis*, *Escherichia coli B*, *Bacillus cereus*, and *Staphylococcus aureus* as nitrofurazone. The isomeric 3,5-dinitro-2-methylbenzofuran was slightly active against *Bacillus subtilis*. The polarographic reduction of a series of nitrobenzofurans indicated that the 7-NO_2 group of 3,7-dinitro-2-methylbenzofuran is the most easily reduced.

Annaji and Subba[2] recently reported the fish toxicity, bacteriostatic activity, and fungicidal activity of several benzofuran derivatives including **1**–**4**. Of the compounds tested only the 5-halo-7-nitrobenzofurans **1** and **2** were "active in all cases." The nitrobenzofurans **3** and **4** were described as being partially active. Several nitrobenzofurans, which were closely related to these active antibacterials and were available in our laboratory, were screened for antibacterial activity in the hope of further defining the structure–activity relationships of this class of chemotherapeutic agents.





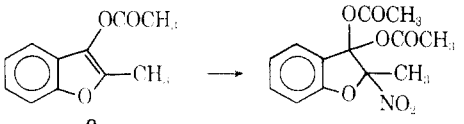


1. $R^1 = Br$; $R^2 = NO_2$
2. $R^1 = Cl$; $R^2 = NO_2$
3. $R^1 = H$; $R^2 = NO_2$
4. $R^1 = NO_2$; $R^2 = H$

5. $R^1 = H$; $R^2 = H$; $R^3 = H$
7. $R^1 = H$; $R^2 = H$; $R^3 = NO_2$
8a, $R^1 = NO_2$; $R^2 = H$; $R^3 = H$
8b, $R^1 = H$; $R^2 = NO_2$; $R^3 = H$

6a, R = OH
6b, $R = OCOCH_3$

Synthesis and Initial Screening.—In the course of another investigation the reaction of acetyl nitrate with 2-methylcoumaran-3-one (**5**) and with a mixture of the cis and trans stereoisomers of 3-acetoxy-2-methyl-2,3-dihydrobenzofuran (**6b**) was investigated. Treatment of **5** with a solution of HNO_3 in Ac_2O[3] gave 3 mononitrocoumaranones (**7**–**8**) which were separated by column chromatography. It is proposed that this nitration to give **7** results from the addition of acetyl nitrate to the enol acetate **9**, which would readily form in Ac_2O solution.[4] The acidic conditions of the isolation hydrolyze the resulting diacyl ketal (**10**) to 2-nitro-2-methylcoumaran-3-one (**7**).



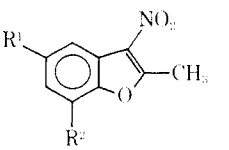
9 **10**

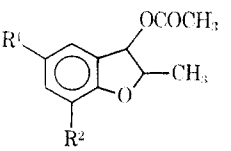
The structural assignments of **8a** and **8b** were made based on the aromatic region of the nmr spectra. The correlation of isomeric substitution to the nmr spectrum of nitrobenzofurans has been discussed in detail by Raulins.[5] The signals in the aromatic region of the nmr spectra of **7** and **8** are essentially identical with the pattern reported by Raulins.

5-Nitro-2-methylcoumaran-3-one (**8a**) was isolated in 20% yield and 7-nitro-2-methylcoumaran-3-one (**8b**) was isolated in 5% yield. It is believed that the low material balance in this reaction results from decomposition of these nitrocoumaranones during chromatography. While the nmr spectrum of the crude reaction mixture does not show any compounds other than the mononitrocoumaranones **7**, **8a**, and **8b**, only one-half of the material placed on the column could be eluted.

Both 5-nitro-2-methylcoumaran-3-one (**8a**) and 7-nitro-2-methylcoumaran-3-one (**8b**) are white crystalline solids when freshly recrystallized; the dry solids are stable if stored in a dark, cold place. However, on standing for several days at room temperature the compounds turn to yellow solids and eventually decompose to yellow gums. The nature of this instability is not known; however, photosensitivity seems to be a contributing factor. No attempts were made to identify the decomposition products.

Reduction of the coumaran-3-one (**5**) with $NaBH_4$ and acetylation of the resulting mixture of alcohols (**6a**) with Ac_2O gave **6b**. The crude mixture of *cis*- and *trans*-**6b** was nitrated without further purification. The yellow oil which resulted was chromatographed over silica gel (5% $CHCl_3$–C_6H_6). The elemental analyses of the first compound eluted from the column corresponded to the empirical formula $C_9H_6N_2O_4$. The ir spectrum contained no CO absorption, indicating that the 3-AcO substituent had been cleaved. The nmr spectrum is characteristic of 7-nitrobenzofurans[5] in the aromatic region, and contains a sharp singlet at δ 3.05. The compound was assigned the structure 3,7-dinitro-2-methylbenzofuran (**11**) on the basis of these data. The isomeric 3,5-dinitro-2-methylbenzofuran (**12**) was the second compound eluted from the column.





11, $R^1 = H$; $R^2 = NO_2$
12, $R^1 = NO_2$; $R^2 = H$

13, $R^1 = NO_2$; $R^2 = H$
14, $R^1 = H$; $R^2 = NO_2$

The nmr resonance for the C-4 proton is further downfield (9.04) than in most 5-nitrobenzofurans. This

(1) Supported by an NDEA Title IV Predoctoral Fellowship, 1966–1969, to L. J. P. and by Grant IK3-CA-10739 from the National Cancer Institutes, National Institutes of Health. Inquiries should be directed to L. J. P. at the Department of Molecular and Quantum Biology, The University of Tennessee Medical Units, Memphis, Tenn. 38103.

(2) R. A. Annaji and R. N. V. Subba, *Symp. Syn. Heterocycl. Compounds Physiol. Interest, Proc.*, **1964**, 26–30 (1966); *Chem. Abstr.*, **69**, 18955 (1968).

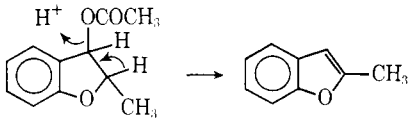
(3) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Wiley New York, N. Y., 1968, p 13.

(4) L. F. Fieser and M. Fieser, ref 3, p 530.

(5) N. R. Raulins, W. G. Kruggel, D. D. Titus, and D. C. Van Landuyt, *J. Heterocycl. Chem.*, **5**, 1 (1968).

deshielding effect is probably due to the proximity of the 3-NO_2 substituent.

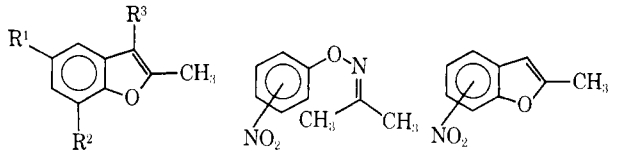
As 3-acetoxy-2,3-dihydrobenzofurans (**15**) readily eliminate AcOH under acidic conditions to form benzofurans (**16**)[6,7] it is likely that the 3-NO_2 substituent results from nitration of this elimination product. Whether elimination of AcOH occurs before or after nitration of the benzene ring is not known. It is possible that both routes are important.



15 **16**

Continued elution of the silica column gave a mixture of *cis*- and *trans*-5-nitro-3-acetoxy-2-methyl-2,3-dihydrobenzofurans (**13**) followed by fractions containing a mixture of the *cis*- and *trans*-7-nitro-3-acetoxy-2-methyl-2,3-dihydrobenzofurans (**14**). The separation and purification of these stereoisomers and the assignment of cis–trans stereochemistry to the pure diastereomers will be discussed in a subsequent paper.

An initial screen for antibacterial activity in these compounds using inpregnated filter disks on agar media which had been inoculated with *Staphylococcus aureus* showed that **11** was the only active antibacterial agent (Table I). Based on this information an attempt was made to define further the structure–activity relationships of the nitrobenzofurans by synthesizing the 3 mononitrobenzofurans **17**, **18**, and **19**.



17, $R^1 = NO_2$; $R^2 = H$; $R^3 = H$ **21** **22**
18, $R^1 = H$; $R^2 = NO_2$; $R^3 = H$
19, $R^1 = H$; $R^2 = H$; $R^3 = NO_2$
20, $R^1 = H$; $R^2 = H$; $R^3 = H$

TABLE I
ANTIBACTERIAL ACTIVITY AGAINST *Staphylococcus aureus*

No.	Compd	Wt/10 ml of EtOH	Zone, mm
	Control		0
11	3,7-Dinitro-2-methylbenzofuran	11.2	4.5
12	3,5-Dinitro-2-methylbenzofuran	10.2	0
8b	7-Nitro-2-methylcoumaran-3-one	10.5	0
8a	5-Nitro-2-methylcoumaran-3-one	11.7	0
7	2-Nitro-2-methylcoumaran-3-one	10.5	0
	Penicillin G	1 unit	6.0

The syntheses of **17** and **18** have been reported recently by Kaminsky, *et al.*,[8] as well as by Mooradian.[9,10] They involve the displacement of an activated halide from a ring by acetone oxime anion in DMF. The *O*-aryl oxime **21** is rearranged in EtOH–HCl to give the substituted 2-methylbenzofuran **22**. This synthesis gives good yields, and the products are easy to isolate and purify. However, an alternate route was used due to the availability of 5-nitro and 7-nitro-3-acetoxy-2-methyl-2,3-dihydrobenzofurans **13** and **14**, respectively. Dissolving the mixture of cis and trans isomers of **13** or **14** in CF_3COOH at room temperature gives the corresponding nitro-2-methylbenzofurans (**17** or **18**). The melting points of the compounds prepared in this manner correspond with the values reported in the literature.[8]

While 2-methylbenzofuran (**20**) has been prepared by several methods,[6] the synthesis which was used differs from the previously reported syntheses. Again, the synthetic route was chosen because of the availability of the required intermediate, 3-acetoxy-2-methyl-2,3-dihydrobenzofuran (**6b**). Refluxing this dihydrobenzofuran with CF_3COOH gives 2-methylbenzofuran (**20**) in good yield. Nitration of **20** in Ac_2O gives a complex mixture of products which was chromatographed on silica gel. The nmr spectrum of the first nitrobenzofuran eluted from the column contained a sharp resonance at δ 2.92, which integrated for 3 protons. Of the possible mononitro-2-methylbenzofurans only in the case of the 3-nitro isomer (**19**) is the Me group a singlet. If NO_2 is substituted on the benzene ring, the proton at C-3 couples with the Me at δ 2.61. This coupling (1.4 Hz) splits the Me group into a doublet in the case of the 7-NO_2 isomer (**18**) and the 5-NO_2 isomer (**17**).

The reaction of 7-nitro-2-methylbenzofuran, obtained from either the above elimination reaction or in larger scale preparation by the *O*-aryl oxime method,[8-10] can be nitrated to give a 24% yield of 3,7-dinitro-2-methylbenzofuran (**11**). As no other products were observed, it is likely that a more careful work-up of the nitration reaction would increase the yield.

Antimicrobial Activity.—Using the previously described agar plate–filter disk technique, 6 compounds were screened for antibacterial activity against *B. subtilis*, *E. coli B*, *B. cereus*, and *S. aureus*. Nitrofurazone (5-nitro-2-furaldehyde semicarbazone) was used as a standard. The results of this screen are shown in Table II. The 3,7-dinitro isomer **11** was active against all 4 organisms to about the same degree as nitrofurazone.

The isomeric 3,5-dinitro compound **12** is active against only *B. subtilis*. As this is the organism which is most susceptible to **11**, it is probable that **12** would be active against the other organisms at a higher concentration. The mononitrobenzofurans (**17–19**) did not inhibit bacterial growth. As Annaji and Subba[2] reported that 5- and 7-nitrobenzofuran (**4** and **3**) have partial activity against *E. coli* and *S. aureus*, it is possible that the mononitrobenzofurans (**17–18**) would show some activity, however, they were not tested at higher concentrations.

Polarographic Reduction of Nitrobenzofurans.—Hirano[11] proposed that the NO_2 substituent of nitrofuran antibacterials acts as an electron acceptor to interfere with bacterial metabolism. If this proposal is correct, it is likely that only one of the two NO_2 substituents in **11** is required as an electron acceptor. The function of the other NO_2, which the data in Table II

(6) R. C. Elderfield and V. B. Meyer, *Heterocycl. Compounds*, **2**, Chapter 1 (1951).
(7) L. H. Zalkow and M. Gosal, *J. Org. Chem.*, **34**, 1646 (1969).
(8) D. Kaminsky, F. Shavel, and R. I. Meitzer, *Tetrahedron Lett.*, 859 (1967).
(9) A. Mooradian, *ibid.*, 407 (1967).
(10) A. Mooradian and P. E. Dupont, *J. Heterocycl. Chem.*, **4**, 441 (1967).
(11) K. Hirano, S. Yoshina, K. Okamura, and I. Suzuka, *Bull. Chem. Soc. Jap.*, **40**, 2229 (1967).

Table II

Antibacterial Activity of Nitrobenzofurans against Four Organisms[a]

No.	Compd	Concn, mg/10 ml of acetone	Zone of inhibition			
			B.s.	*E.c.*	*B.c.*	*S.a.*
	Nitrofurazone	9.9	4.0	3.0	4.0	4.0
17	5-Nitro-2-methylbenzofuran	11.0	0	0	0	0
18	7-Nitro-2-methylbenzofuran	9.5	0	0	0	0
12	3,5-Dinitro-2-methylbenzofuran	10.8	1.0	0	0	0
19	3-Nitro-2-methylbenzofuran	9.0	0	0	0	0
11	3,7-Dinitro-2-methylbenzofuran	10.4	6.0	3.0	4.0	3.0
7	2-Nitro-2-methylcoumaran-3-one	9.8	0	0	0	0
	Control		0	0	0	0

[a] *B. subtilis* (*B. s.*), *E. coli B.* (*E. c.*), *B. cereus* (*B. c.*), *S. aureus* (*S. a.*).

Table III

Polarography of Nitrobenzofurans

No.	Compd[a]	pH	$E_{1/2}$ relative to Ag|AgCl		Wave height (mm)[b] (sensitivity 5×10^{-8} A/mm)	
			1	2	1	2
23	NF	4.9	−0.31		105	
23	NF	6.1	−0.38		105	
23	NF	7.4	−0.48		105	
11	3,7-DNB	4.9	−0.38	−0.65	100	100
11	3,7-DNB	6.1	−0.46	−0.74	100	100
11	3,7-DNB	7.4	−0.52	−0.79	100	100
12	3,5-DNB	4.9	−0.49	−0.67	100	100
12	3,5-DNB	6.1	−0.55	−0.75	100	100
12	3,5-DNB	7.4	−0.60	−0.84	100	100
18	7-NB	4.9	−0.52		105	
18	7-NB	6.1	−0.61		105	
18	7-NB	7.4	−0.68		105	
19	3-NB	4.9	−0.62		100	
19	3-NB	6.1	−0.69		100	
19	3-NB	7.4	−0.77		100	

[a] Abbreviations: NF, nitrofurazone; DNB, dinitro-2-methylbenzofuran; NB, nitro-2-methylbenzofuran. [b] Concentration of all solutions 6.77×10^{-4} *M*.

indicate is required, might be either to facilitate complex formation with the biological reducing agent or to make the other NO_2 more electrophilic. As the reduction potential of NO_2 is apparently important in the antimicrobial activity of nitrofurans,[11,12] the NO_2 of **11** with the highest (most positive) reduction potential is probably the site of reaction with a biological reducing agent. In order to determine if the reduction potentials of the two NO_2 substituents in **11** are different and if so which is the most easily reduced, the polarograms of 4 nitrobenzofurans were compared (Table III).

These polarograms are similar in many respects to the reported polarographic characteristics of the nitrofurans. The reduction potentials of all the compounds become more negative as the pH of the media increases. This is the same trend as seen in the nitrofuran series.[12] The values of the diffusion current, i_d, indicate that the same number of electrons are transferred per nitro group in each compound. This value has been determined to be 4 in the case of nitrofurazone.[12] The reduction potentials of the two antibacterial dinitrobenzofurans **11** and **12** support correlations made for the nitrofurans. The reduction potential of the more active compound, **11**, is more positive than that of **12**. The reduction potential of **12** is more positive than the reduction potential of the inactive mononitrobenzofurans.

The polarograms of the dinitrobenzofurans are bipartite waves. As the reduction potential of 7-nitro-2-methylbenzofuran **18** is more positive than the reduction potential of 3-nitro-2-methylbenzofuran (**19**), the most positive reduction wave of **11** is assigned to the reduction of the 7-NO_2 substituent.

Since nitrofurazone and **11** are similar in both antibacterial activity and polarographic reduction, it is proposed that they have a similar mode of antibacterial action. The 7-NO_2 substituent of **11** appears to be the more easily reduced NO_2 and is probably the more important for antibacterial activity. The synthesis of nitrobenzofurans having different substituents at the 3 or 7 positions is currently underway to test this hypothesis.

Experimental Section[13]

2-Methylcoumaran-3-one (5).—2-Bromo-2′-hydroxypropiophenone[14] (111 g, 0.48 mole), $NaHCO_3$ (50 g, Fischer certified), and DMF (500 ml) were heated at 55° with stirring for 6 hr. The reaction mixture was poured into H_2O (1.5 l.) and extracted with Skelly B (bp 60–70°, 6 × 500 ml). The residue (80 g) after evapn was distd in an apparatus protected from light to give 46.5 g of 2-methylcoumaran-3-one (**5**) (65%) as a colorless oil: bp 128–130° (30 mm) [lit.[15] bp 119° (19 mm)].

(12) T. Sasaki, *Chem. Pharm. Bull.*, **2**, 104 (1954).

(13) All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Elemental analyses were carried out either by Midwest Microlab, Inc., Indianapolis, Ind., or by Mrs. H. Kristiansen at the University of Kansas. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Spectra were recorded on a Beckman IR-10 or a Varian A-60A.

(14) A. V. Dombrovskii, M. I. Shebchuk, and V. P. Kravets, *Zh. Obshch. Khim.*, **32**, 2278 (1962); *J. Gen. Chem. USSR*, **32**, 2246 (1962).

(15) "Dictionary of Organic Compounds," Vol. 3, Oxford University Press, New York, N. Y., 1965, p 1720.

Nitration of 2-Methylcoumaran-3-one.—2-Methylcoumaran-3-one (**5**, 25 g, 0.17 mole) was added slowly to a cold (0°) soln of concd HNO_3 (17.5 ml, 0.28 mole) in Ac_2O (100 ml). The reaction mixture was stirred under N_2 in a low actinic flask at 25°. After 48 hr the reaction mixture was cooled (0–5°) and 5% HCl was added. When the hydrolysis of the Ac_2O was complete (1 hr), the mixture was poured into H_2O (200 ml) and extracted with Et_2O (3 × 250 ml). The combined Et_2O extracts were washed with H_2O (6 × 500 ml), 5% $NaHCO_3$ (5 × 500 ml), and a satd soln of NaCl (500 ml). The organic layer was dried ($MgSO_4$) and the solvent evapd to give a yellow oil (21.5 g). This oil was chromatographed on 1 kg of silica (5% $CHCl_3$–C_6H_6). After a forerun (2 l.), 200-ml fractions were collected. Evaporation of fractions 3–7 gave a yellow solid (3.5 g) which was recrystd from CCl_4 to give 3.2 g (10%) of the 2-NO_2 isomer **7**: mp 125–126°; ir (KBr) 1740 (C=O), 1560 and 1340 cm^{-1} (NO_2); nmr ($CDCl_3$) δ 2.07 (s, 3, CH_3), 7.1–7.9 (m, 4, aromatic protons). *Anal.* ($C_9H_7NO_4$) C, H, N.

Fractions 14–27 contained 6.6 g (20%) of the 5-NO_2 isomer **8a**: mp 57–58° (CCl_4); ir (KBr) 1730 (C=O), 1535 and 1345 cm^{-1} (NO_2); nmr ($CDCl_3$) δ 1.58 (d, 3, J = 7, $CHCH_3$), 4.85 (q, 1, J = 7, $CHCH_3$), 7.3 (m, 1, H-7), 8.5 (m, 2, H-4 and 6). *Anal.* ($C_9H_7NO_4$) C, H, N.

Fractions 30–50 contained 1.7 g (5%) of the 7-NO_2 isomer **8b**: mp 117.5–119° (CCl_4); ir (KBr) 1725 (C=O), 1520 and 1325 cm^{-1} (NO_2); nmr ($CDCl_3$) δ 1.66 (d, 3, J = 7 Hz, $CHCH_3$), 4.92 (q, 1, J = 7 Hz, $CHCH_3$), 7.28 (t, 1, J = 7 Hz, aromatic H-5), 8.08 (q, 1, J = 2 and 7 Hz, aromatic H-4), 8.50 (q, J = 2 and 7 Hz, aromatic H-6). *Anal.* ($C_9H_7NO_4$) C, H, N.

3-Hydroxy-2-methyl-2,3-dihydrobenzofuran (6a).—2-Methylcoumaran-3-one (**5**, 7.2 g, 0.05 mole) was dissolved in 95% EtOH (75 ml) and $NaBH_4$ (1.0 g, 0.026 mole) was added. After the addition was complete (5 min), the reaction mixture was allowed to stir for 2 hr. Glacial AcOH was added dropwise to destroy the excess $NaBH_4$, the EtOH removed, and the residue slurried in Et_2O. The resulting suspension was washed with 5% $NaHCO_3$ (100 ml) and a satd soln of NaCl (50 ml). Evaporation of the solvent gave 7.5 g of a colorless oil (98%) which nmr and ir indicated was a mixture of *cis*- and *trans*-**6a**.

3-Acetoxy-2-methyl-2,3-dihydrobenzofuran (6b).—3-Hydroxy-2-methyl-2,3-dihydrobenzofuran (**6a**, 20 g, 0.15 mole), dry C_6H_6 (50 ml), fused NaOAc (5.5 g), and Ac_2O (21.6 g, 0.21 mole) were refluxed with stirring for 4 hr. After cooling, the reaction mixture was poured into H_2O (200 ml) and the C_6H_6 layer was sepd, stirred with 10% Na_2CO_3 (50 ml) for 1 hr, and washed with H_2O (50 ml), a satd soln of NaCl (50 ml), and dried ($MgSO_4$). Evapn of the solvent gave 24 g of a residual oil (88%) which nmr and ir indicated was a mixture of *cis*- and *trans*-**6b**.

Nitration of 3-Acetoxy-2-methyl-2,3-dihydrobenzofuran.—3-Acetoxy-2-methyl-2,3-dihydrobenzofuran (**6b**, 40 g, 0.21 mole) was added slowly to a cold (0°) soln of HNO_3 (26 ml, 0.42 mole) in Ac_2O (100 ml). The reaction mixture was stirred under N_2 in a low actinic flask at 25°. After 36 hr the reaction mixture was cooled (0–5°) and 5% HCl (30 ml) was added. The reaction mixture was stirred 2 hr at 5–20°, poured into H_2O (300 ml), and extd with Et_2O (3 × 300 ml). The combined Et_2O exts were washed with H_2O (6 × 500 ml), 5% $NaHCO_3$ (4 × 500 ml), H_2O (6 × 500 ml), and a satd soln of NaCl (200 ml). The organic layer was dried ($MgSO_4$) and the solvent removed to give a yellow oil (50.4 g) which was chromatographed over 2 kg of silica (5% $CHCl_3$–C_6H_6). Evaporation of the solvent from fractions 9–10 (500 ml each) gave a yellow solid (2.3 g, 4.9%) which was recrystd from MeOH to give 3,7-dinitro-2-methylbenzofuran (**11**): mp 150.0–151.5°; ir (KBr) 1519 and 1334 cm^{-1} (NO_2); nmr ($CDCl_3$) δ 3.05 (s, 3, CH_3), 7.60 (t, 1, J = 8 Hz, aromatic H-5), 8.27 (q, 1, J = 8 and 1.5 Hz, aromatic H-4), 8.54 (q, 1, J = 8 and 1.5 Hz, aromatic H-6). *Anal.* ($C_9H_6N_2O_5$) C, H, N.

Fractions 12–15 contained 2.4 g (5.1%) of 3,5-dinitro-2-methylbenzofuran (**12**) mp 164–166° (MeOH); ir (KBr) 1523 and 1340 cm^{-1} (NO_2); nmr ($CDCl_3$) δ 3.00 (s, 3, CH_3), 7.67 (d, 1, J = 9 Hz, aromatic H-7), 8.37 (q, 1, J = 9 and 2 Hz, aromatic H-6), 9.04 (d, 1, J = 2 Hz, aromatic H-4). *Anal.* ($C_9H_6N_2O_5$) C, H, N.

Evaporation of the solvent from fractions 21–42 and distillation of the residue gave 22.5 g (45%) of a mixture of *cis*- and *trans*-5-nitro acetates (**13**) as a yellow oil. Fractions 44–86 contained 17.2 g (35%) of the *cis*- and *trans*-7-nitro acetates (**14**).

2-Methyl-5-nitrobenzofuran (17).—5-Nitro-3-acetoxy-2-methyl-2,3-dihydrobenzofuran (**13**, crude mixture of isomers, 2.5 g, 9.5 mmoles) was dissolved in CF_3COOH (10 ml). After 5 min the soln was poured into H_2O (50 ml) and the suspension was extracted with Et_2O (3 × 50 ml). The Et_2O extract was washed with 5% NaOH (2 × 50 ml), H_2O (50 ml), and a satd soln of NaCl (50 ml). The Et_2O was removed to give a white solid which was recrystd from 95% EtOH to give an impure solid (1.5 g); mp 89–93°. This was chromatographed over 200 g of silica (20% C_6H_{12}–C_6H_6). Evaporation of fractions 3–8 (150 ml each) and recrystallization of the residue from MeOH gave 1.1 g (61%) of **17** as white needles: mp 94–95° [lit.[8] 97–98°]. *Anal.* ($C_9H_7NO_3$) C, H, N.

2-Methyl-7-nitrobenzofuran (18).—7-Nitro-3-acetoxy-2-methyl-2,3-dihydrobenzofuran (**14**, crude mixture of isomers, 1.7 g, 7.0 mmoles) was dissolved in CF_3COOH (10 ml). After 4 min the soln was poured into H_2O (75 ml) and the H_2O suspension was extracted with $CHCl_3$ (3 × 50 ml). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and the $CHCl_3$ removed to give a green-brown residue which was recrystd from MeOH to give 0.6 g (50%) of **18** as light yellow needles: mp 100–101° (lit.[8] 101–102°). *Anal.* ($C_9H_7NO_3$) C, H, N.

2-Methylbenzofuran (20).—3-Acetoxy-2-methyl-2,3-dihydrobenzofuran (**6b**) (crude mixture of isomers, 20 g, 0.11 mole) was dissolved in CF_3COOH (40 ml) and the reaction mixture refluxed for 30 min. The cooled reaction mixture was poured into Et_2O (250 ml), washed with H_2O (4 × 200 ml), 5% NaOH (2 × 100 ml), and H_2O (200 ml). The Et_2O solution was dried ($MgSO_4$) and the solvent evaporated. The residue (17 g) was distd to give 13.4 g 2-methylbenzofuran (**20**, 80%); bp 80° (15 mm) [lit.[16] 78° (12 mm)].

3-Nitro-2-methylbenzofuran (19).—2-Methylbenzofuran (**20**, 3.0 g, 20 mmoles) was added slowly to a cold (0°) soln of HNO_3 (1.2 ml) in Ac_2O (10 ml). When the addition was complete, the ice bath was removed and the reaction mixture was allowed to stir at 25° under N_2 in a low actinic flask. After 24 hr the reaction mixture was cooled (0–10°) and 5% HCl (30 ml) was added. The reaction mixture was stirred for an additional 30 min and then poured into H_2O (50 ml). The aq suspension was extd with Et_2O (100 ml), 5% $NaHCO_3$ (2 × 100 ml), H_2O (2 × 100 ml), and a satd soln of NaCl (50 ml). The Et_2O layer was dried and the solvent evapd to give a yellow residual oil (3.0 g). This oil was chromatographed over 150 g of silica gel (Skelly B to 20% C_6H_6–Skelly B). Fractions (20 ml) were combined on the basis of tlc (50% Skelly B–C_6H_6, silica gel). The residue (0.5 g, 14%) from fractions 90 to 108 was recrystd from C_6H_{12} to give **19**: mp 125–126°; ir (KBr) 1500 and 1380 cm^{-1} (NO_2); nmr ($CDCl_3$) δ 2.92 (s, 3, CH_3) 7.2–8.2 (m, 4, aromatic H). *Anal.* ($C_9H_7NO_3$) C, H, N.

3,7-Dinitro-2-methylbenzofuran (11).—7-Nitro-2-methylbenzofuran (**18**, 1.0 g, 5.0 mmoles) in Ac_2O (10 ml) was added to a cold (0°) soln of HNO_3 (0.5 ml, 0.49 g, 7.8 mmoles) in Ac_2O (5 ml), and the reaction was allowed to stir at 25° for 48 hr in a low actinic flask under N_2. The reaction mixture was poured into ice to give a yellow gum which was crystallized from MeOH to give 0.3 g (24%) of the dinitro compound **11**, mp 149–151°. A mixture melting point with the material obtained from the nitration of 3-acetoxy-2-methyl-2,3-dihydrobenzofuran showed no depression.

Antibacterial Testing.—Sterile nutrient agar tubes were melted in a steam bath and then transferred to a water bath (58°). The agar tubes (after equilibrating to 58°) were inoculated with enriched media cultures (1 ml) and plated. Solutions of the compds to be tested were made by dissolving *ca.* 10 mg of the compd in 10 ml of Me_2CO or 95% EtOH. Filter disks (15 mm) were dipped into these solns and allowed to dry. As each disk is assumed to absorb about 0.03 ml of solution,[17] each filter disk contains approximately 0.03 mg of nitrobenzofuran. The impregnated disk is placed on an inoculated agar plate, and the plate is incubated for 24 hr at 37°. The side of the zone of inhibition, if any, is then measured from the edge of the filter disk to the edge of the bacterial growth.

Polarography.—The compds were dissolved in 95% EtOH (50 ml). Citric acid (0.1 *M*) and 0.2 *M* Na_2HPO_4[18] were added and the pH of the soln determined on a Leeds and Northrup pH meter, which had been standardized at pH 4 and pH 10. The polarograms were determined on Metrohm Polarecord E261-R using a rapid dropping Hg electrode with an impulse rate of 4/sec. The voltage range scanned was 0 to −2 V relative to the Ag/AgCl electrode.

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