located in negative quadrants.[10] In this particular case, steric interference to drug-receptor association afforded by the axial allyl substituent overrides any facilitation of receptor binding due to the advantageous orientation of the phenyl group.[4,5]

Although the stereostructure-activity relationship of the highly potent (+)-1 is in conformity with the correlation found among the other more potent enantiomers (Table II), the magnitude of $\varphi_1$ and $\varphi_2$ are smaller. It seems unlikely that this difference alone can contribute to the greatly enhanced potency of (+)-1, particularly if the barrier for rotation of the phenyl group is not great. The possibility that the allylic double bond causes stabilization of the phenyl group through $\pi$-electron overlap is discounted because none of the distances between the allyl group and the aromatic system are shorter than normal van der Waal contacts.

The most plausible explanation for the high potency of (+)-1 is that the allylic double bond interacts with an accessory site adjacent to the receptor which confers enhanced affinity and stereoselectivity to the molecule. As the corresponding propyl analog[5] exhibits activity in the range of the other enantiomers, it appears that this is a highly specific interaction.

This study suggests the possibility of attaching the allyl group to a comparable position in other analgetic molecules as a probe to determine whether or not their mode of interaction[11,12] with receptors is similar. If, for example, a great enhancement of potency is observed this would suggest that the analgetics are complexing in a similar fashion.

Finally, it is important to point out that although all of the more potent antipodes possess similar conformations (Table II), this does not necessarily mean that drug-receptor association occurs in this conformational state. This study also underscores the fact that absolute configuration and conformation must be dealt with together in the analysis of stereostructure-activity relationships.

**Acknowledgment.** This investigation was supported by NIH Grants NS 05192 and CA 10104. The authors wish to thank Dr. Kevin H. Bell for preparing crystals of (±)-1 and (±)-2 and Ms. P. Sackman for her technical assistance.

**Supplementary Material Available.** A listing of the intramolecular bonding parameters (Table III) and of atomic coordinates (Table IV) (2 pp). Ordering information is given on any current masthead page.

## References and Notes

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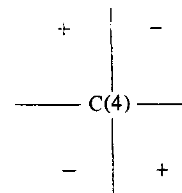


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# Chemistry and Antibacterial Activity of Nitrobenzofurans†

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Thirteen 2-methylbenzofurans were synthesized and their antibacterial activity was investigated. 2-Methyl-3-nitrobenzofuran and analogs containing 7-$NO_2$, 5-$NO_2$, 7-Br, 7-$CONH_2$, and 7-$CF_3$ substituents are bacteriostatic. The spectrum of activity of these compounds is similar to nitrofurazone; however, a strain of *E. coli* Br which has increased resistance to nitrofurazone did not show increased resistance to 3,7-dinitro-2-methylbenzofuran (1). The 3-nitro-2-methylbenzofurans are labile in solution ($T_{1/2}$ 0.8–3.5 hr at 37°, pH 7.0). The solvolysis product of 1 was identified as $\alpha$,6-dinitro-*o*-cresol (15). The 3-nitrobenzofurans are more potent in minimal media than in Penassay broth. This greater potency can be abolished by addition of casamino acids and tryptophan to the minimal media.

Many examples have appeared in the literature of antimicrobial activity of nitro heterocyclic compounds.[1] One of the initial reports of activity by this type of compound was Dodd and Stillman's report of the remarkable increase in antibacterial activity that results from a 5-nitro substituent in a series of 2-substituted furans.[2] Since this report several thousand 5-nitrofurans have been synthesized and evaluated as antibacterial and antiprotozoal agents. In the furan series it has been established that an $\alpha$-nitro substituent is necessary for antibacterial activity. $\beta$-Nitrofurans[3] as well as furans substituted with other electron-withdrawing substituents at the 5 position lack antibacterial activity.[3,4] In contrast to the thorough investigation of the antibacterial activity of substituted furans, the benzofuran ring system has received very little attention. We have reported the antibacterial activity of 3,7-dinitro-2-methylbenzofuran (**1**) and 3,5-dinitro-2-methylbenzofuran (**2**).[5] More recently, Royer and co-

† This manuscript is dedicated to Dr. Edward Smissman. While Ed is gone the mark which he leaves on Medicinal Chemistry is indelible.

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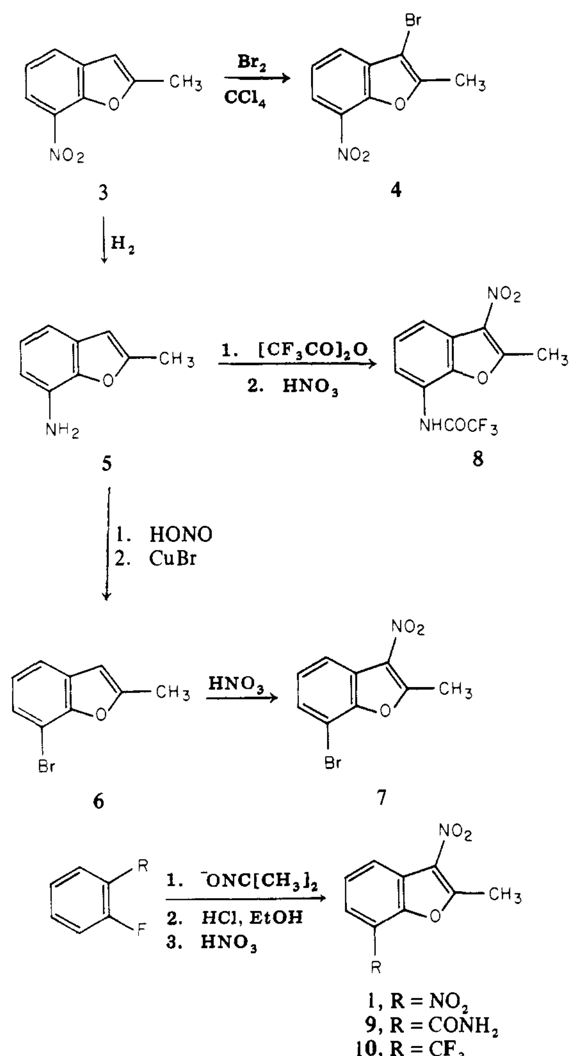
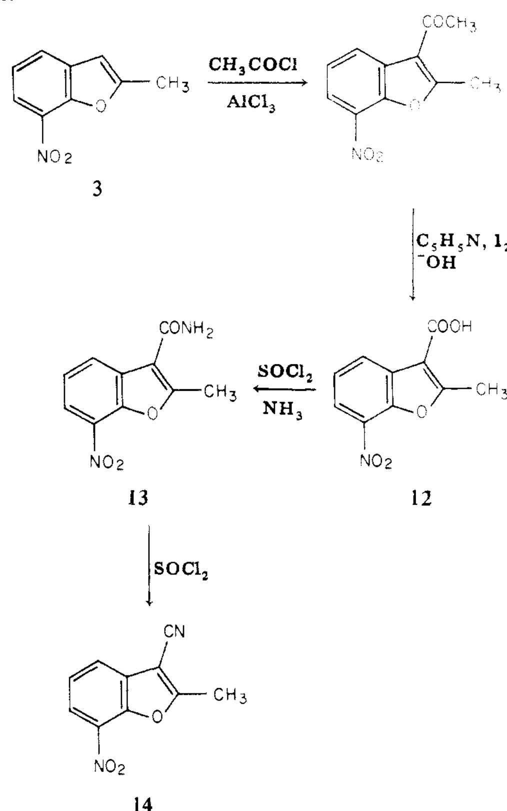
Scheme I




Scheme II




workers have described a series of 2-nitrobenzofurans with good antiparasitic activity.[6,7] The purpose of the following report is to consider the structure–activity relationships of this series, to compare the antibacterial activity of 1 to that of nitrofurazone, and to determine the solvolysis product of 1.

## Results and Discussion

**Synthesis.** The initial objective in the study of the structure–activity relationships of compounds related to 1 was to determine if either the nitro group on the homocycle or the nitro group on the heterocycle was essential for activity. Several 3-nitrobenzofurans were synthesized by nitration of 7-substituted 2-methylbenzofurans. The 7-substituted benzofurans were synthesized by the rearrangement of an ortho-substituted *O*-aryloxime[8–10] or by reduction of 3 followed by acylation to give 8 or a Sandmeyer reaction to give 6. The synthesis of the desired 3-nitro-7-substituted benzofurans (1 and 7–10) is summarized in Scheme I.

With the exception of the nitration of the 7-trifluoroacetamido-2-methylbenzofuran (7), the only nitration product isolated with the above series was the 3-nitro derivative. In the case of 7 three mononitro compounds were obtained and separated by chromatography. The first and the second isomers are products of nitration on the benzene ring. On the basis of the relative chemical shifts in the NMR spectrum of the proton at the 3 position, and the fact that the signals in the aromatic region appear as doublets with a coupling constant of 10 Hz, indicating that the two protons on the benzene ring are adjacent, the first component was assigned the structure of 2-methyl-6-nitro-7-trifluoroacetamidobenzofuran and the second component is assigned the structure 2-methyl-4-nitro-7-trifluoroacetamidobenzofuran. The NMR spectrum of the third component established that it was the desired 3-nitro isomer 8.

A corresponding series of 7-nitro-3-substituted benzofurans was also synthesized (Scheme II). Bromination of 3 gave a good yield of the 3-bromo derivative 4. Acylation of 3 gave the corresponding 3-acylbenzofuran 11. This was converted to the acid 12 by treatment with pyridine–iodine and alkali.[11] The acid was then converted to the amide 13 and nitrile 14 using standard methods.[12]

**Solvolysis of 3-Nitrobenzofurans.** During the course of this investigation it was observed that the 2-methyl-3-nitrobenzofurans are unstable in aqueous solution. The solvolysis in aqueous solution was accompanied by a change in the uv absorption spectrum and a loss in antibacterial activity. The solvolysis product was isolated by incubating 1 in 90% $Me_2SO$–$H_2O$ for 72 hr at 37°C. Dilution of the reaction mixture with water resulted in the precipitation of a product which was recrystallized from MeOH. The uv spectrum and TLC of this product are identical with that previously observed for the degradation of the stock solution. This degradation product has less than 2% of the antibacterial activity of 1, thus explaining the decrease of antibacterial activity of the stock solution upon standing.

The structure of the solvolysis product was established as $\alpha$,6-dinitro-*o*-cresol (15) by ir, NMR, and mass spectra as well as elemental analysis. This product probably results from the initial attack of hydroxide ion at the 2 position of the benzofuran ring followed by opening of the furan ring by either the breaking of the $-OC_2$ bond or the $C_2C_3$ bond (Scheme III).

This type of solvolysis has previously been described by Perold[13] for 3-nitrobenzofuran. Also, Pene et al.[14] have

Scheme III

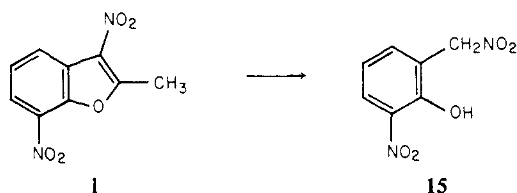



Table I. Rates of Solvolysis of 3-Nitro-2-methylbenzofurans in 5% $Me_2SO$–Jensen's Minimal Media at pH 7.0

| Compd no. | Substituent[a] | λ[b] | $k$, $hr^{-1}$ [c] | $T_{1/2}$, hr | Correlation coeff[d] |
|---|---|---|---|---|---|
| **1** | $7\text{-}NO_2$ | 310 | 0.504 ± 0.067 | 1.37 | 0.9972 |
| **2** | $5\text{-}NO_2$ | 310 | 0.772 ± 0.045 | 0.89 | 0.9970 |
| 7 | 7-Br | | *e* | *e* | |
| 8 | $7\text{-}CF_3\text{-}$CONH- | 335 | 0.240 ± 0.015 | 2.88 | 0.9996 |
| 9 | $7\text{-}CONH_2$ | 280 | 0.361 ± 0.031 | 1.91 | 0.9984 |
| 10 | $7\text{-}CF_3$ | 280 | 0.467 ± 0.062 | 1.48 | 0.9966 |
| **16** | None | 335 | 0.207 ± 0.032 | 3.34 | 0.9981 |

[a] Substituents on the 3-nitro-2-methylbenzofuran ring system. [b] Wavelength at which rate of solvolysis was determined. [c] Confidence limits are based on 0.05 percentile of Student's t test. [d] Correlation coefficient for linearity of log $C$ vs. time plot. [e] Because of the low solubility and low extinction coefficient of 7 as well as the small difference between the spectra of 7 and its solvolysis product, it was not possible to obtain an accurate measure of the solvolysis rate.

Table II. Effect of $Me_2SO$ on Solvolysis of 3,7-Dinitro-2-methylbenzofuran at pH 6[a,b]

| % $Me_2SO$ | $k$, $hr^{-1}$ | $T_{1/2}$, hr | Correlation coeff |
|---|---|---|---|
| 5 | 0.330 ± 0.008 | 2.10 | 0.9999 |
| 10 | 0.354 ± 0.012 | 1.95 | 0.9997 |
| 20 | 0.389 ± 0.018 | 1.78 | 0.9993 |
| 40 | 0.460 ± 0.034 | 1.51 | 0.9982 |

[a] McIlvaine's buffer was used.[22] [b] For explanation of column headings see Table I.

recently described the reaction of 3-acyl- and cyanobenzofurans with guanine to form 5-arylpyrimidines via a similar reaction.

In order to determine if this reactivity was directly related to antibacterial activity, the rates of solvolysis of a series of 3-nitrobenzofurans were determined at 37°C in 5% $Me_2SO$–Jensen's minimal media[15] at pH 7.0 (Table I). Under these reaction conditions, the benzofurans (**4** and **11–14**) which contained a substituent other than a nitro group in the 3 position did not undergo solvolysis. The $Me_2SO$ was necessary in order to obtain solutions concentrated enough to analyze spectroscopically. As illustrated in Table II, an increase in the percentage of $Me_2SO$ in the solution increases the rate of reaction.

**Antibacterial Activity.** The action of **1** and nitrofurazone on a relatively small inoculum of bacteria (i.e., under the conditions used to generate the data in Table III, see Experimental Section) increases the lag period. There is no apparent decrease in the rate of growth of bacteria in the presence of increasing concentrations of either agent. This has been noted previously for nitrofurazone.[16] If the antibacterial effect is to prolong the lag phase of growth rather than suppress the rate of growth, a linear relationship between the concentration of the agent and the lag time should exist.[17] This is illustrated in Figure 1 for **1** against three species of bacteria and for nitrofurazone against *Escherichia coli* Br. The variation in slope in comparing the activity of **1** against the three species of bacteria reflects the relative efficiency which the particular species can overcome the inhibition by **1** under the test conditions. The lag times for the various cultures studied in the absence of drug were in the range of 1.7–1.8 hr. The lag time vs. concentration plots (Figure 1) for **1** all extrapolate through this point at zero concentration. The graft for nitrofurazone, although linear, does not extrapolate through the control culture (zero concentration) lag time. Comparison of the plots of **1** and nitrofurazone suggests that for nitrofurazone there exists a threshold concentration below which there is no effect or lag time. In contrast it appears that **1** has no threshold concentration. A determination of whether this is a valid interpretation or if the apparent differences are simply an artifact of the assay system will require further investigation of the effects of low levels of **1** on bacteria using more sensitive assay methods (such as $O_2$ uptake). Similar activity exists against all species of bacteria which are sensitive to **1** or its analogs.

Table III. MIC of Nitrofurazone and **1** after 6 Hr of Incubation at 37°C

| Organism | MIC, μg/ml: 1 | MIC, μg/ml: Nitrofurazone |
|---|---|---|
| *E. coli* B | 1.87 | 5.0 |
| *E. coli* Br | 1.87 | 5.0 |
| *E. coli* Br 207 | 1.87 | 18.7 |
| *Staph. aureus* | 2.5 | 5.0 |
| *Strept. faecalis* | 3.75 | 50 |
| *Proteus mirabilis* | 6.25 | 50 |
| *Proteus rettgeri* | 7.5 | >100 |
| *Pseudomonas aeruginosa* | >10 | >100 |
| *Klebsiella pneumonia* | 5.0 | 10.0 |

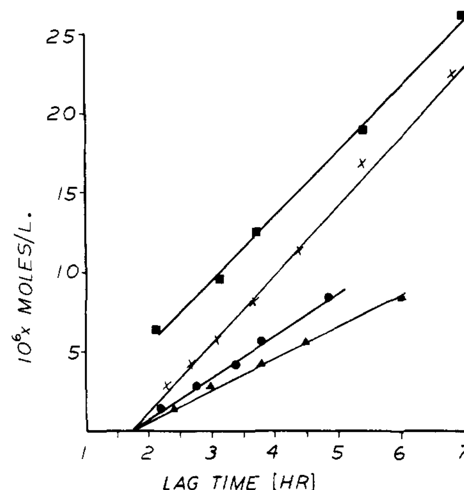



Figure 1. Effect of varying concentrations of **1** and nitrofurazone on the lag time of various bacterial cultures in Penassay broth (■), nitrofurazone on *E. coli* Br; (×), **1** on *K. pneumonia;* (●), **1** on *Staph. aureus;* (▲), **1** on *E. coli* Br.

If **1** is added to a culture of bacteria in the early or late lag phase of growth, growth is arrested. As illustrated in Figure 2, an increasing bacterial density decreases the effectiveness of **1**. That is, while a $4.4 \times 10^{-5}$ $M$ solution of **1** will suppress growth of *E. coli* Br for more than 6 hr if added in the early log phase, it has only a transient (ca. 3 hr) effect if added to the late log phase of growth. Determination of viable counts, using standard plating methods[18] after exposure of *E. coli* Br to $7.7 \times 10^{-5}$ $M$ **1** for 2 hr, shows no decrease in viability, indicating that **1** is a bacteriostatic agent at this concentration.

Comparison of the activity of **1** and nitrofurazone against several gram-positive and gram-negative bacteria in Penassay broth (Table III) indicated that the spectrum of activity is similar for the two agents. Neither compound is effective against *Pseudomonas*. Against other bacteria,

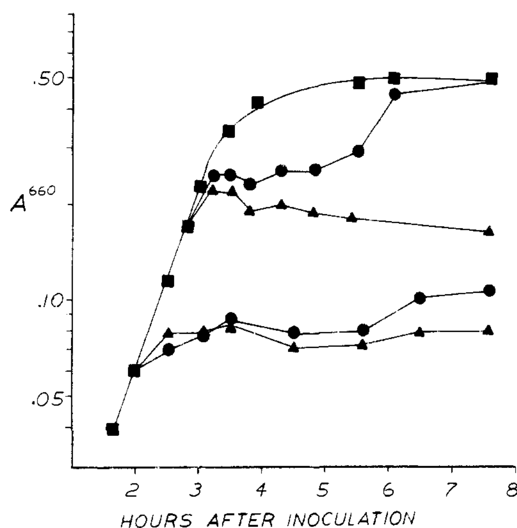



Figure 2. Effect of adding 1 to a culture of *E. coli* Br in Penassay broth in the log phase of growth: (▲) final concentration $7.7 \times 10^{-5}$ *M*; (●) final concentration $4.5 \times 10^{-5}$ *M*; (■) control, no 1 added.

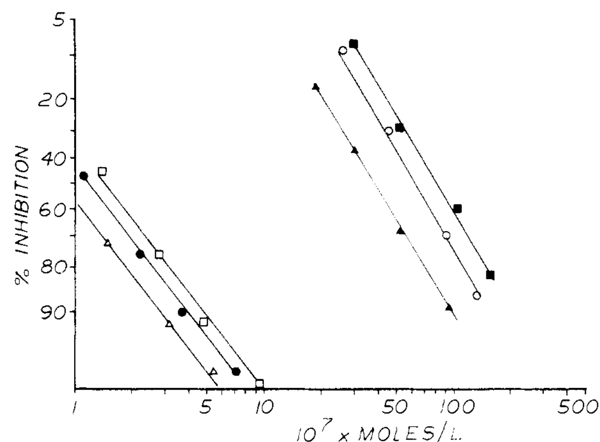



Figure 3. Dose–response lines for 3-nitrobenzofurans against *E. coli* B in minimal media: 7 (△); 1 (●); 10 (□); 16 (▲); 2 (○); and 9 (■).

the benzofuran 1 is somewhat more active. The short incubation time of 6 hr was used as solubility limited the concentration of 1 to no greater than 10 μg/ml.

In order to determine if there is any difference in the antibacterial activity of 1 and nitrofurazone, the activity of these compounds against *E. coli* Br and *E. coli* Br 207 was compared. *E. coli* Br 207 is a mutant strain originally isolated by McCalla[19] and coworkers. It has been established that this strain has increased resistance to the 5-nitrofuran class of antibacterials due to a lack of a nitrofuran reductase which is essential for metabolic activation of nitrofurans. As shown in Table III we also found *E. coli* Br 207 to be more resistant to nitrofurazone than *E. coli* Br. The activity of the benzofuran 1, however, is the same against both strains suggesting some difference in mode of action.

The antibacterial activities of the various 3-nitrobenzofurans (1, 2, 7–10, and 16) were determined against *E. coli* B in Jensen's[15] minimal broth. The activities of the 3-nitrobenzofurans were compared using the log–probability plot introduced by Treffers.[20] Percent inhibition was calculated by subtracting percent growth (for calculation see Kavanagh[21]) from 100. A comparison of the activities (Figure 3) shows that the substances fall into two groups which differ by about an order of magnitude in potency. Compound 8 did not suppress growth at concentrations below $2 \times 10^{-5}$ *M*. It was also observed that the compounds are more potent in minimal media than in Penassay broth by a factor of more than tenfold. The addition of vitamin-free casamino acids (1.2 g/l.) and tryptophan (200 mg/l.) to minimal broth overcame this greater potency. That is, the activity in minimal media plus amino acid supplement is the same as the activity in Penassay broth. The benzofurans which do not have a nitro group in the 3 position do not have any antibacterial activity at the limit of their solubility.

## Conclusions

The structural requirements for potent antibacterial activity of 2-methylbenzofurans require a nitro substituent in the 3 position. The substituent in the 7 position can be varied; however, lipophilic, electron-withdrawing substituents are apparently favored. All of the compounds that have measurable antibacterial activity are labile in solution. The relative rates of solvolysis do not correlate directly with antibacterial activity. Indeed 8, which is solvolyzed in solution at a faster rate than 16, has no measurable activity, and 2 which is the most labile in solution has low antibacterial activity. This might reflect the balance of the need for a minimal reactivity in this series of compounds which is required to suppress bacterial growth in competition with the inactivation of the antibacterial substance by solvolysis. However, the lack of antibacterial activity of benzofurans which are stable in solution (4 and 11–14) does suggest that lability of the furan ring plays some role in the expression of antibacterial activity.

Data presented here do not allow determination of whether (a) the intact benzofuran is responsible for the antibacterial activity, (b) an intermediate in the solvolysis of the benzofuran is responsible for activity, or (c) the antibacterial activity is due to the ability of the 3-nitro-2-methylbenzofurans to acetylate biologically significant nucleophiles. The loss of antibacterial activity of the stock solutions on standing, as well as the low antibacterial activity found for 15, suggests that the final solvolysis product is not the active antibacterial agent.

## Experimental Section

The synthesis of 3,7-dinitro-2-methylbenzofuran, 3,5-dinitro-2-methylbenzofuran, and 3-nitro-2-methylbenzofuran has been previously described.[5] Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Nuclear magnetic resonance spectra were determined in $CDCl_3$ ($Me_4Si$) solution on a Varian A-60A analytical spectrometer. Infrared spectra were determined on a Perkin-Elmer Model 257 spectrophotometer. Mass spectra were obtained on a Finnigan 1015 gas chromatograph–mass spectrometer with dual chemical-ionization and electron-impact ionization sources and vacuum manifolds equipped with a Finnigan 6000 interactive data system and zeta plotter. Samples were admitted via the direct introduction solid probe. The electron-impact spectrum was recorded at 70 eV. The chemical ionization spectrum was obtained with methane (1 Torr) as the reagent gas, with a source temperature of 110°C. TLC was determined on EM Reagents precoated silica gel F-254 plates (5 × 10 cm).

**3-Bromo-2-methyl-7-nitrobenzofuran (4).** 3 (5.0 g, 0.028 mol), bromine (5.0 g, 0.061 mol), and iron powder (0.1 g) were refluxed in $CCl_4$ for 3 hr. The $CCl_4$ solution was cooled, filtered, and washed with 5% $NaHCO_3$ and a saturated solution of NaCl. Evaporation of the solvent gave a residue which was recrystallized from MeOH to give yellow needles (mp 123–124°, 5.4 g, 75%). Anal. ($C_9H_6BrNO_3$) C, H, N, Br.

**7-Amino-2-methylbenzofuran (5).** 3 (7.38 g, 0.041 mol) was dissolved in THF and reduced in a Brown hydrogenator using 5% Pd/C as a catalyst. After 31 ml of 1 *M* $NaBH_4$ had been consumed the reaction mixture was filtered and the solvent

evaporated. The residue, 5.3 g of a light brown oil, was used in subsequent reactions without further purification.

**7-Bromo-2-methylbenzofuran (6).** 5 (7.0 g, 0.047 mol) was added to a cold (0–5°) solution of 45% HBr (30 ml) to give a suspension of the HBr salt. The cold (0–5°) suspension was added to a cold (0–5°) solution of $NaNO_2$ (3.4 g, 0.049 mol) in 6.5 ml of water. The dark suspension was allowed to stir for 5 min and then added to a hot (80°) mixture of CuBr (6.8 g, 0.047 mol) in 48% HBr (18 ml). The reaction mixture was allowed to stir at 80° for 30 min and then steam distilled. The distillate (1 l.) was extracted with $Et_2O$. Drying and evaporation of the solvent gave a light yellow oil which was distilled [bp 138–140° (15 mm)] to give 3 g (30%) of product. Anal. ($C_9H_7BrO$) C, H, Br.

**7-Bromo-2-methyl-3-nitrobenzofuran (7).** Concentrated $HNO_3$ (0.9 ml, 0.02 mol) was added to cold (0–5°) $Ac_2O$ (20 ml). 6 (2.5 g, 0.118 mol) in $Ac_2O$ (20 ml) was then added. The reaction mixture was cooled again and 5% HCl (20 ml) was added slowly maintaining the temperature below 10°. The resulting suspension was extracted with $Et_2O$ and the solvent removed to give a solid. Recrystallization of this compound from MeOH gave 2.0 g of white needles (mp 152–153°, 66%). Anal. ($C_9H_6BrNO_3$) C, H, N, Br.

**7-Trifluoroacetamido-2-methylbenzofuran.** 5 (4.0 g, 0.027 mol) was dissolved in a mixture of pyridine (3 ml) and dry $Et_2O$. The reaction mixture was cooled (0–5°) and stirred while trifluoroacetic anhydride (10% excess) was added. The reaction mixture was allowed to stir 4 hr at 0–5°. The reaction mixture was poured into dilute HCl (0°) and the resulting suspension extracted with $Et_2O$. The $Et_2O$ layer was washed with dilute HCl and a saturated solution of NaCl and dried ($MgSO_4$), and the solvent was evaporated. The residue was recrystallized from $C_6H_{12}$: yield 3.29 g; mp 91–92° (48%). Anal. ($C_{11}H_8F_3NO_2$) C, H, N.

**Nitration of 7-Trifluoroacetamido-2-methylbenzofuran.** The nitration procedure described for 1 was used. Hydrolysis of the $Ac_2O$ and the usual work-up gave 5.2 g of a dark oil. This oil was chromatographed on silica using hexane and benzene (0–60%) as the eluent. Fractions were combined on the basis of TLC (benzene) and the solvent was removed to yield three compounds. The first compound to elute was 2-methyl-6-nitro-7-trifluoroacetamidobenzofuran which was recrystallized from MeOH–$H_2O$ (250 mg, 6%): mp 120–122°; NMR $\delta$ 2.55 (m, $CH_3$), 6.65 (m, H-3), 7.55 (d, $J$ = 10 Hz, H-4), 8.0 (d, $J$ = 10 Hz, H-5); ir ($CHCl_3$) 1745 $cm^{-1}$ (C=O). Anal. ($C_{11}H_7F_3N_2O_4$) C, H, N. The second combined fraction was recrystallized from $C_6H_6$–$C_6H_{12}$ to give 2-methyl-4-nitro-7-trifluoroacetamidobenzofuran (300 mg, 8%): mp 175–176°; NMR $\delta$ 2.65 (m, $CH_3$), 7.20 (m, H-3), 7.70 (d, $J$ = 10 Hz, H-6), 8.20 (d, $J$ = 10 Hz, H-5); ir ($CHCl_3$) 1735 $cm^{-1}$ (C=O). Anal. ($C_{11}H_7F_3N_2O_4$) C, H, N. The third fraction yielded 2-methyl-3-nitro-7-trifluoroacetamidobenzofuran (200 mg, 5%) from $C_6H_6$–$C_6H_{12}$: mp 178-179°; NMR 2.91 (s, $CH_3$), 7.45 (m, H-5 and H-6), 8.04 (q, $J$ = 2 Hz and $J$ = 8 Hz, H-4); ir ($CHCl_3$) 1730 $cm^{-1}$ (C=O). Anal. ($C_{11}H_7F_3N_2O_4$) C, H, N.

**2-Methyl-3-nitrobenzofuran-7-carboxamide (9).** To a stirred solution of acetoneoxime (7.88 g, 0.108 mol) in $Me_2SO$ (150 ml) was added 12.1 g of potassium butoxide. A solution of *o*-fluorobenzamide (15 g, 0.108 mol) in $Me_2SO$ (150 ml) was added in a steady stream. The reaction mixture was stirred for 24 hr at 25° and then poured into a saturated solution of NaCl. The resulting precipitate was washed with water and dissolved in $Et_2O$. The $Et_2O$ solution was dried and evaporated to give a white solid, 10.25 g. This product was dissolved in 20% HCl–EtOH and the solution refluxed for 3 hr. The reaction mixture was poured onto ice and extracted with $Et_2O$ (300 ml). The $Et_2O$ was washed with $H_2O$ (1 × 200 ml) and a saturated solution of NaCl. The $Et_2O$ was evaporated and the residue recrystallized from $C_6H_6$–$C_6H_{12}$: yield 5.0 g. This product (5.0 g, 0.028 mol) was dissolved in freshly distilled $Ac_2O$ and $HNO_3$ (0.2 ml) was added. After stirring for 12 hr at 25°, an additional 0.2 ml of $HNO_3$ was added. After 18 hr the reaction mixture was poured onto ice and the resulting slurry extracted with $Et_2O$. The $Et_2O$ was evaporated to give an oil which was chromatographed on silica gel ($CHCl_3$). The resulting product was recrystallized from EtOAc–hexane to give white crystals (500 mg, 8%), mp 200–202°. Anal. ($C_{10}H_8N_2O_4$) C, H, N.

**2-Methyl-3-nitro-7-trifluoromethylbenzofuran (10).** To a stirred solution of acetoneoxime (11.9 g, 0.164 mol) and potassium *tert*-butoxide (18.4 g, 0.164 mol) in $Me_2SO$ (100 ml) was added *o*-fluorobenzotrifluoride (27 g, 0.164 mol). The reaction was stirred for 24 hr at 25° and then poured into a saturated solution of NaCl (100 ml). The resulting slurry was extracted with $CHCl_3$ (3 × 100 ml), the combined $CHCl_3$ layers were dried ($Na_2SO_4$), and the $CHCl_3$ was removed. The yellow residue was dissolved in 20% HCl–EtOH and the mixture refluxed for 3 hr. The reaction mixture was poured onto ice and the resulting slurry extracted with $Et_2O$. The $Et_2O$ layer was dried ($Na_2SO_4$) and the $Et_2O$ removed to yield 15.55 g of a dark oil. This was distilled [bp 100–103° (30 mm)] to give 10.0 g of a colorless oil. To 1 g of this oil in freshly distilled $Ac_2O$ (5 ml) was added 1 equiv of $HNO_3$ in $Ac_2O$ (40 ml). The reaction mixture was worked up as described for 7 and the crude product recrystallized from MeOH: yield 300 mg (25%); mp 97.5–98.5°. Anal. ($C_{10}H_6F_3NO_3$) C, H, N.

**3-Acetyl-2-methyl-7-nitrobenzofuran (11).** $AlCl_3$ (30 g) was placed in dry $Cl_2CHCHCl_2$ (100 ml) under $N_2$ and at 0° and AcCl (10 ml) was added dropwise. After this addition was complete, 3 (20 g, 0.115 mol) was added in $Cl_2CHCHCl_2$ to give a red slurry. The reaction mixture was allowed to warm to 25° and stir for 12 hr. 10% HCl (200 ml) was added to the cooled (0°) reaction mixture. The resulting slurry was transferred to a separatory funnel and extracted with $CHCl_3$ (3 × 250 ml). The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was recrystallized from $C_6H_6$ to give white needles: 22 g (89%); mp 145–146°. Anal. ($C_{11}H_9NO_4$) C, H, N.

**2-Methyl-7-nitrobenzofuran-3-oic Acid (12).** 11 (2.5 g, 0.0114 mol), $I_2$ (2.89 g), and pyridine (10 ml) were allowed to stand for 2 days at 25°. The reaction mixture was diluted with $H_2O$ (10 ml) and NaOH (1.5 g) was added. This reaction mixture was heated at 100° for 30 min. The acidic fraction was isolated using standard methods and the crude product (1.5 g) was recrystallized from EtOAc–hexane to give white needles: 1.0 g (40%); mp >300°. Anal. ($C_{10}H_9NO_5$) C, H, N.

**2-Methyl-7-nitrobenzofuran-3-carboxamide (13).** 12 (0.4 g, 0.002 mol) was refluxed with $SOCl_2$ (7 ml) and the acid chloride converted to the amide by adding the crude acid chloride to 1 *N* $NH_4OH$. The precipitate was isolated, dried, and recrystallized from hexane–EtOAc: yield 350 mg (88%); mp 240–241°. Anal. ($C_{10}H_8N_2O_4$) C, H, N.

**3-Cyano-3-methyl-7-nitrobenzofuran (14).** 13 (200 mg) was refluxed for 72 hr in $SOCl_2$ (5 ml) and $C_6H_6$. The excess $SOCl_2$ was hydrolyzed and the $C_6H_6$ was evaporated. The residue was extracted with acetone (2 × 5 ml) and the acetone evaporated to give a residue which was recrystallized from MeOH to give 14: mp 140–141°; 80 mg (44%). Anal. ($C_{10}H_6N_2O_4$) C, H, N.

**Solvolysis of 3,7-Dinitro-2-methylbenzofuran.** 1 (200 mg, 0.9 mmol) was dissolved in 90% $Me_2SO$–$H_2O$ (10 ml) and the solution placed in an incubator (37°). After standing for 72 hr, TLC ($C_6H_5$) indicated that 1 ($R_f$ 0.63) had been completely solvolyzed (one product, $R_f$ 0.41). The solution was poured into water (100 ml) to give a cloudy solution which yielded crystals on scratching. The solid was separated by filtration and dried: yield 125 mg (70%); mp 114–116°. This was recrystallized from MeOH to give yellow platelets: mp 115–116°; NMR (acetone-$d_6$) 5.80 (s, 2), 7.19 (t, $J$ = 8 Hz, 1), 7.91 (q, $J$ = 1 Hz, $J$ = 8 Hz, 1), 8.26 (q, $J$ = 1 Hz, $J$ = 8 Hz, 1) 10.7 (s, 1); ir ($CHCl_3$) 3220 (broad), 1617, 1561, 1545, 1458, 1370, 1331 $cm^{-1}$; EI mass spectrum (rel intensity) 198 (1.1, $M^+$), 153 (11.6), 152 (100), 135 (34.1); CI mass spectrum (rel intensity) 227 (3.2, M + 29), 199 (0.5, M + 1), 181 (12.4), 153 (24.2), 152 (100). Anal. ($C_7H_6N_2O_5$) C, H, N.

**Determination of Rates of Solvolysis.** The benzofurans were dissolved in $Me_2SO$ and the solutions diluted with Jensen's minimal media[15] (pH 7). The final $Me_2SO$ concentration was 5% and the initial concentration of the compounds was in the range of 0.7 to 2.0 × $10^{-4}$ *M*. Preliminary solvolysis experiments were conducted to determine the optimal wavelength for monitoring the reaction. The change in absorption as a function of time was monitored using a Gilford Model 2400-S spectrophotometer. The reaction temperature was maintained at 37 ± 0.5° using a Forma Scientific Model 2095 water bath. The rate constants were determined from the change in absorption values using a Hewlett Packard Model 9800 calculator. The concentration of the benzofuran was calculated at half-hour intervals from the equation

$$C^s = (A - C_0^s\epsilon^p)/(\epsilon^s - \epsilon^p)$$

where $C^s$ is the concentration of the benzofuran at time $T$, $A$ is the measured absorption at time $T$, $C_0^s$ is the initial concentration of the benzofuran, $\epsilon^p$ is the extinction coefficient of the product (determined from $A$ measured after >25 half-lives of reaction), and $\epsilon^s$ is the extinction coefficient of the benzofuran. The calculator program also fit the data points to a first-order rate law equation, determined the correlation coefficient of this fit, and calculated the 95% confidence range (t test) of the rate constant (Table I).

**Determination of Antibacterial Activity of 1 and Nitrofurazone.** Cultures of *Staphylococcus aureus*, *Klebsiella pneumonia*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Proteus rettgeri* were obtained from the culture file of the Department of Microbiology, UTCHS. Cultures of *E. coli* Br and *E. coli* Br 207 were kindly provided by Dr. D. R. McCalla, McMaster University. *E. coli* B was obtained from ATCC. These cultures were maintained on Penassay agar (Difco) at 25°. An overnight culture of the organism in Penassay broth was adjusted to 60% transmittance and 10 ml of this culture used to inoculate 900 ml of Penassay broth in an Oxford pipettor. The inoculated broth (4.5 ml) was added to a solution of the benzofuran in 10% $Me_2SO$ (0.5 ml) and the tubes were incubated at 37°. All determinations were in stationary culture except *Pseudomonas aeruginosa*. For each assay the turbidity of tubes containing concentrations of benzofuran (dilution series 1, 0.75, 0.5, 0.375, 0.25, 0.187, 0.125, 0.062, and 0.031, four replicates each) and appropriate positive and negative controls was determined after 6 hr (660 nm, Spectronic 20). The minimal inhibitory concentration at 6 hr was defined as the lowest concentration which had a % $T$ of greater than 90%. In order to obtain the data plotted in Figure 1, the cultures were monitored at 20-min intervals for 9 hr after inoculation. The log $A^{660}$ vs. time plots for each concentration were extrapolated to $A = 0.05$. The elapsed time between inoculation and the time of initiation of growth (i.e., when $A = 0.05$) was defined as the lag time for the concentration. The lag times so obtained were then plotted against concentration.

**Comparison of Antibacterial Activity of Various Benzofurans in Minimal Media.** An overnight culture of *E. coli* B in Jensen's minimal media was used to inoculate (10 ml) 90 ml of Jensen media in a Nephelo culture flask. This culture was incubated in a shaker–water bath incubator at 37° until the growth had reached 70% $T$. This culture (1 ml) was added to 8 ml of Jensen's broth (pH 7) and 1 ml of benzofuran in 10% $Me_2SO$. The cuvettes were placed in a J-Y Biophotometer and turbidity changes monitored (with agitation). Five concentrations of benzofuran (dilution series 1, 0.67, 0.33, 0.2, and 0.1) and a control culture containing 1% $Me_2SO$ without benzofuran were monitored in each assay. Plots of log $A$ vs. time were used to calculate percent inhibition as a function of concentration. Log concentration vs. probability plots of these values were linear (Figure 1). Slopes of these lines as well as $IC_{50}$ values obtained from the plots were reproducible to within ±5%.

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# Correlation Analysis of Baker's Studies on Enzyme Inhibition. 1. Guanine Deaminase, Xanthine Oxidase, Dihydrofolate Reductase, and Complement[†,1a]

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Five correlation equations are presented which relate inhibitory activity of 578 inhibitors of guanine deaminase, xanthine oxidase, dihydrofolate reductase, and complement to their chemical structures. The use of correlation analysis in enzyme studies for drug development is discussed. The importance of indicator variables in such studies is emphasized.

Starting about 1964, the late B. R. Baker and his students published over 100 papers studying the effect of over 1500 inhibitors on various enzymes. It was Baker's view that one should be able to develop more selective and more effective drugs through the selective control of enzymes. Baker demonstrated that one can rather quickly develop extremely potent enzyme inhibitors by more or less sys-

† This paper is dedicated to the memory of Edward Smissman and Bernard R. Baker.