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Structure-Activity Relationship of Bispyridyloxybenzene for Induction of Mouse Hepatic Aminopyrine *N*-Demethylase Activity

Chemical, Biological, and X-Ray Crystallographic Studies

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SUMMARY

1,4-bis-[2-(3,5-Dichloropyridyloxy)]-benzene (TCPOBOP) was previously shown to be an extremely potent phenobarbital-like inducer of hepatic microsomal monooxygenase activity in the mouse. To examine the structure-activity relationship, 31 congeners of TCPOBOP were synthesized and tested for their potency to induce hepatic aminopyrine *N*-demethylase activity in B6D2F$_1$/J mice. For biological activity, the minimum requirement is a) a central 1,4-dioxygenated benzene ring, b) lateral pyridine rings linked to the central ring by ether bonds, but with other lateral heteroaromatic rings, e.g., quinoline or pyrimidine, also active, c) 5,5′-substituents of Cl, Br, or NO_2 on the pyridine rings. For a series of 5,5′-substituted and 3,3′-dichloro,5,5′-substituted bispyridyloxybenzenes, no correlation was observed for Hansch π and σ_p values. To account for this lack of correlation and conformational variability produced by the two ether bonds, we performed x-ray structure determinations on three compounds: a) TCPOBOP, b) the 5,5′-dichloro analogue, and c) the biologically inactive, 3,3′-dichloro analogue. In the two biologically active congeners the positioning of the pyridine rings is *anti* to the plane of the central benzene ring, and the dihedral angle between the central ring and the pyridines is approximately 60°. In the inactive analogue the pyridine rings are *syn* and the dihedral angle is 84°. The x-ray crystallographic data are consistent with the ether oxygen having an sp^2-bonding conjugating with the heterodipolar bond of the pyridine C(2)—N(1), which strongly restricts rotation about the ether bonds. The potency of TCPOBOP and other bispyridyloxybenzene analogues to induce a phenobarbital-like pleiotropic response and the sharply defined structure-activity relationship among these congeners support the hypothesis that they act by binding to a specific recognition site.

INTRODUCTION

Induction of one or more species of hepatic cytochrome P-450 and the associated increase in microsomal monooxygenase activity, accompanied by an increase in other liver enzymes, are produced by the administration of a wide variety of foreign chemicals. Two distinct patterns of coordinate enzyme induction were recognized historically (1), one typified by phenobarbital, and the other by 3-methylcholanthrene; however, it is now recognized that many xenobiotics produce pleiotropic responses distinct from these two classical patterns. For 3-methylcholanthrene and like-acting compounds this coordinate induction has been shown to be mediated by their stereospecific reversible binding to a soluble protein receptor (2). The demonstration of this receptor was largely attributable to the availability of a potent agonist, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, with high affinity binding for the receptor. The mechanism by which phenobarbital and like-acting compounds produce their pleiotropic response is unknown. The wide variety of compounds reported to produce a phenobarbital-like response are all weak agonists (usually administered at doses of 10^{-3} to 10^{-4} mol/kg), and there is no discernible structural similarity among them, suggesting that they may not act on a specific saturable receptor.

We have previously described (4) a potent and long-

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acting phenobarbital-like agonist, TCPOBOP,[2] which is 650 times as potent as phenobarbital in inducing aminopyrine *N*-demethylase activity in mouse liver, with an ED_{50} of 4.9×10^{-7} mol/kg (3). Maximally effective doses of phenobarbital or TCPOBOP, or both compounds administered in combination induce murine hepatic NADPH-cytochrome *c* reductase and aminopyrine *N*-demethylase activities and the cytochrome P-450 concentration to the same extent, and both compounds induce epoxide hydrolase and glutathione *S*-transferase activities and the same major species of cytochrome P-450 (identified by sodium dodecyl sulfate-gel electrophoresis). A single maximally effective dose of TCPOBOP (7.5×10^{-6} mol/kg) induces hepatic aminopyrine *N*-demethylase activity for more than 20 weeks. Thus, in mice, TCPOBOP is a potent, long-acting inducer of the phenobarbital pleiotropic response. Dragani *et al.* (5) have recently shown that in $B6C3F_1$ female mice initiated with a single dose of *N*-nitroso-*N*-diethylamine at 7 days of age, TCPOBOP (3 mg/kg, once a week for 20 weeks) acts as a potent promoter of hepatocarcinogenesis.

The effect of TCPOBOP, in contrast to phenobarbital, is species specific. TCPOBOP is a very weak inducer of monooxygenase activity in the rat (4), despite a comparable long half-life for the compound in the rat and mouse.

In this report, we describe the synthesis of a series of TCPOBOP analogues and determine their potencies to induce murine hepatic aminopyrine-*N*-demethylase activity. The x-ray structure was determined for three of these compounds. This paper discusses the structure-activity relationships observed and examines the postulated topological requirements for biological activity.

MATERIALS AND METHODS

Animals and Treatments

$B6D2F_1/J$ female mice were purchased from the Jackson Laboratory, housed in plastic cages on softwood chip bedding with a 12-hr light/12-hr dark light cycle, and permitted unlimited access to laboratory chow and water. The mice were from 6 to 15 weeks of age when used, and all animals in a given experiment were the same age.

The test compounds were dissolved in corn oil and administered by intraperitoneal injection (8 ml of corn oil/kg), daily for 3 days. Biologically active compounds were administered at three or four dose levels with four animals per dose group. Inactive compounds were administered at the high concentration at which they were soluble in corn oil or, in some cases, as insoluble suspensions. All experiments contained a corn oil-treated group and TCPOBOP-treated group for maximal response.

Enzyme Assay

Aminopyrine *N*-demethylase activity was determined on the 10,000 × *g* supernatant fraction of mouse liver by the method of Cochin and Axelrod (6) with assay conditions previously described (3). In 12 experiments, the basal aminopyrine *N*-demethylase activity (in nanomoles of formaldehyde per minute per milligram wet weight of liver) averaged 0.72 ± 0.19 and the TCPOBOP maximally induced activity was 5.12 ± 0.61, with an average of 7.3-fold induction. The mean enzyme activity induced by each dose of a test compound was converted to a fractional response (control activity = 0, TCPOBOP-induced activity = 1.0), and from a plot of log dose versus fractional response, the potency (ED_{50}) of the compound was estimated. For inactive analogues the highest dose tested is reported.

Synthesis of Compounds

The compounds prepared in this study were typically synthesized by condensing the nucleophilic dihydroxybenzene with the reactive 2-halopyridine (or analogue). Three major variants of reaction conditions were used. These were: (A) aqueous sodium hydroxide in dimethylsulfoxide at 70–90°, (B) sodium methoxide in dimethylsulfoxide at 90–100°, or (C) potassium methoxide in dimethylsulfoxide at 110–120°. Details of these procedures are illustrated below. A few of the substances were prepared by reduction procedures (R), Sandmeyer reaction (S), or nucleophilic displacement reactions not involving a benzenediol (N). Table 1 shows the structures of these compounds along with their melting points and method of analytical characterization (combustion analyses or mass spectra). Analogues of TCPOBOP having an NO_2, I, or NH_2 substituent in the hydroquinone ring were synthesized as previously described (3). Partition coefficients of selected compounds in Table 1 were measured by distribution of the substance between hexane and ethylene glycol and measurement of concentrations by ultraviolet absorption spectroscopy in solvent.

Method A: 1,4-bis-[2-(5-chloropyridyloxy)]-benzene. To a magnetically stirred, 80° solution of 1.007 g (6.81 mmol) of 2,5-dichloropyridine and 375 mg (3.41 mmol) of hydroquinone in 11 ml of freshly distilled dimethylsulfoxide was added, dropwise, via syringe and under a nitrogen atmosphere, 0.90 ml of a nitrogen-purged sodium hydroxide solution (prepared from 903 mg of sodium hydroxide dissolved in water to a total volume of 2.8 ml). This addition required approximately 5 min, and the resulting yellow solution was then stirred at 93–95° for 16 hr, after which the mixture was allowed to cool and was then poured into a separatory funnel containing 100 ml of ethyl acetate and 100 ml of water. The organic phase was washed with four additional portions of water, dried, and evaporated, and the oily product was heated in a Kugelrohr apparatus (70°/0.2 mm Hg) to remove unreacted dichloropyridine. Thin layer analysis of the residue (silica gel, chloroform containing 2% methanol) showed it to consist of two components (R_f values of 0.7 and 0.3) which were separated by dissolving the residue in a small amount of chloroform and placing this solution at the top of a silica-gel column with 2% methanol in chloroform as eluent. The first component from the column was recrystallized from chloroform/hexanes to give 242 mg (21%) of 1,4-bis-(2-(5-chloropyridyloxy)]-benzene, m.p., 154–157°, mass spectrum *m/z* 334, 332, 306, 304, 278, 276, 271, 269, 243, and 241; exact mass, found *m/z* 332.0109.

Method B: 1,3-bis-(4-chloro-2-nitrophenoxy)benzene. To a 25-ml three-neck, round bottom flask equipped with an addition funnel, vacuum outlet with stopcock, septum cap, and stir bar magnet was placed 200 mg of resorcinol and 8 ml of MeOH. In the addition funnel was placed 1,4-dichloro-3-nitrobenzene (0.9 g, 2.3 eq) with 8 ml of freshly distilled dimethylsulfoxide. In a separate flask was placed 8 ml of freshly distilled dimethylsulfoxide. Nitrogen was bubbled through all three solutions for a minimum of 2 hr. Sodium methoxide (202 mg, 2.05 eq) was then added to the three-neck flask and stirred for 1–2 min. A vacuum, less than 0.1 mm Hg, was then applied with some heating to remove the methanol. When the disodium salt was dry, the dimethylsulfoxide in the separate flask was added with a transfer needle followed by the dropwise addition of the dichloronitrobenzene solution as the reaction mixture was brought to 95°. The reaction was left stirring at this temperature overnight. The solution was then taken up in ethyl acetate, washed three times in 30% NaOH and twice in H_2O, dried, and concentrated. The 1-g crude yield was streaked on 22-mm silica preparation plates and eluted twice with 50% chloroform/hexanes to afford 270 mg of the desired product (35% yield). The compound

[2] The abbreviation used is TCPOBOP, 1,4-bis-[2-(3,5-dichloropyridyloxy)]-benzene.

TABLE 1

Bispyridyloxybenzenes: Structure, method of synthesis, chemical analysis, and biological potency

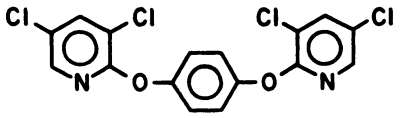
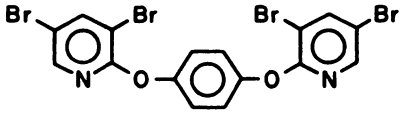
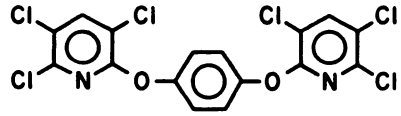
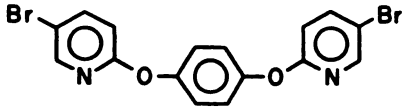
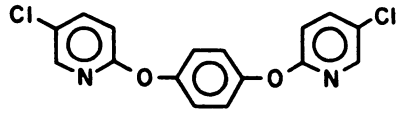
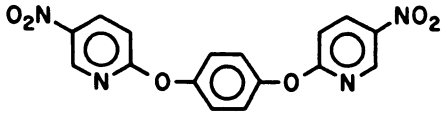
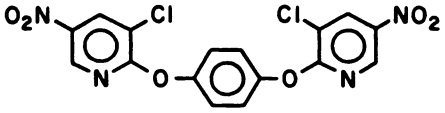
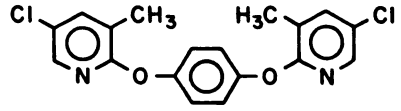
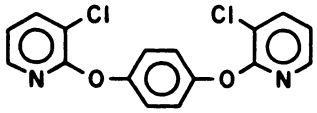
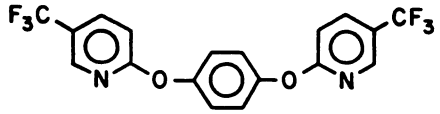
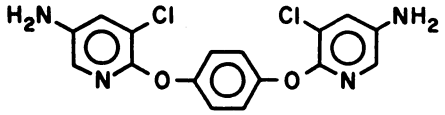
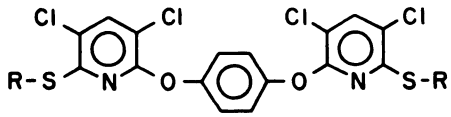
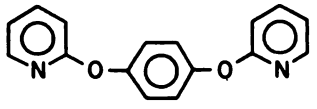
			log P[a]	ED_{50}[b]
				μmol/kg/day
1		m.p. 155–156.5° C,H 47.83%, 2.01% Method A yd 68%	1.68	0.16
2		m.p. 187.5–188° C,H 32.71%, 1.38% Method B yd 30%	1.79	0.54
3		m.p. 244–246°S C,H 40.65%, 1.32% Method A yd 47%	1.96	0.43
4		m.p. 157–158° *m/z* = 419.9081 Method C yd 33%	0.41	0.72
5		m.p. 155–156° *m/z* = 332.0109 Method A yd 21% Method C yd 34%	0.37	1.4
6		m.p. 176–177° *m/z* = 354.0565 Method C yd 19%		1.5
7		m.p. 225–226° *m/z* = 421.9802 Method C yd 58%		1.0
8		m.p. 144–145° *m/z* = 360.0420 Method C yd 15%		3.4
9		m.p. 166–167° *m/z* = 332.0119 Method A	0.24	>40
10		m.p. 80–83° *m/z* = 400.0591 Method A	−1.70	>40
11		m.p. 176–177° *m/z* = 362.0299 Method R yd 68%		>60
12		m.p. 187–188.5° C,H 44.76%, 3.17% Method N yd 25% R = $CH_2COOC_2H_5$	1.21	>20
13		m.p. 123–124° *m/z* = 264.0874 Method C yd 47%		>20

TABLE 1—*Continued*

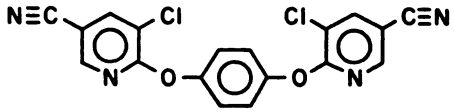
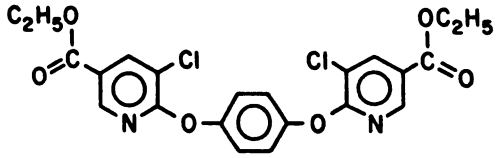
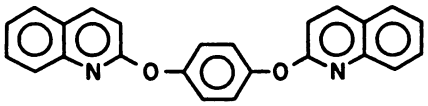
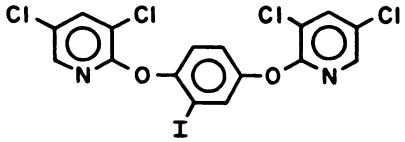
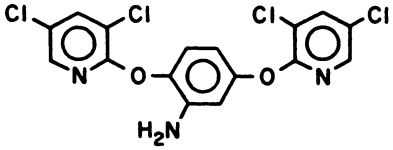
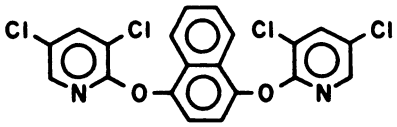
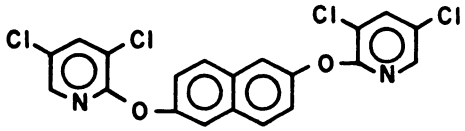
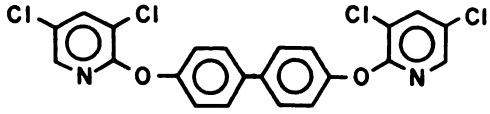
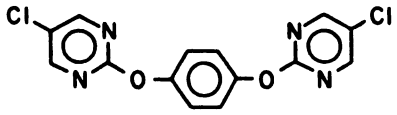
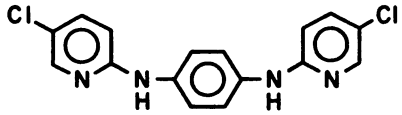
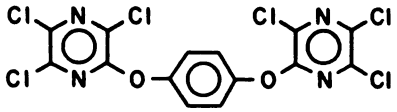
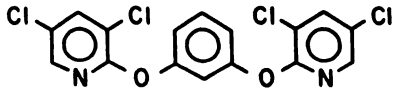
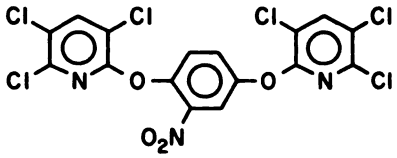
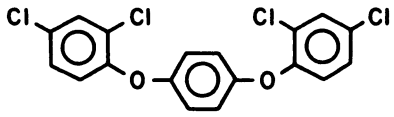
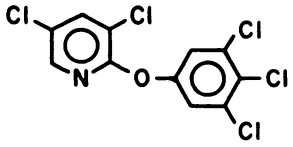
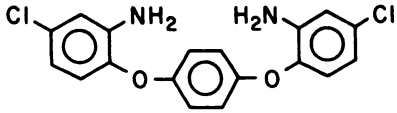
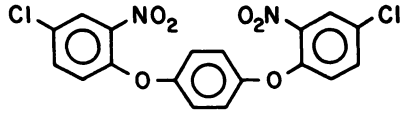
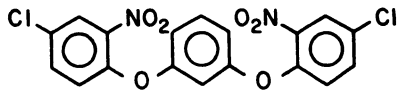
			log P[a]	ED_{50}[b]
				μmol/kg/day
14		m.p. 321–323° C,H 55.23%, 2.24% Method C yd 52%		>60
15		m.p. 197–198° *m/z* = 476.0512 Method NaH, DMSO yd 57%		>60
16		m.p. 197–198° *m/z* = 364.1193 Method C yd 35%		2.5
17		m.p. 172.5–174° C,H 36.37%, 3.23% Method S yd 11%	1.62	1.2
18		m.p. 169–170° C,H 46.38%, 2.66% Method R yd 96%	0.20	10
19		m.p. 169–170° *m/z* = 449.9465 Method C yd 13%		8.8
20		132–134° *m/z* = 449.9497 Method B yd 73%		56
21		187–188° Method C yd 13%		>60
22		m.p. 259–260.5° *m/z* = 333.9980 Method B		5.4
23		m.p. 238–241° *m/z* = 330.0425 Method N yd 19%		>60
24		m.p. 259–260° *m/z* = 469.8502 Method C yd 10%		>60
25		m.p. 101–102° C,H 47.60%, 2.17% Method B yd 25%		>60

TABLE 1—*Continued*

			log P[a]	ED_{50}[b]
				μmol/kg/day
26		m.p. 221–222° *m/z* = 512.8403 Method HNO_3	1.07	0.5
27		m.p. 111–113° C,H 54.50%, 2.78% Method S yd 70%	1.51	>66.7
28		m.p. 109–111° C,H 38.52%, 1.27% Method B yd 49%		>60
29		m.p. 146–148.5° C,H 59.74%, 4.60% Method R yd 64%		>66.7
30		m.p. 200–202° C,H 51.09%, 2.33% Method B yd 36%	0.03	>60
31		m.p. 129.5–131° C,H 51.32%, 2.42% Method B yd 35%		>40

[a] Log partition coefficient, hexane/ethylene glycol.

[b] Daily dose for 3 days which produces one-half the maximal induction of hepatic aminopyrine-*N*-demethylase activity.

was recrystallized in ethanol to an m.p. of 129.5–131°. Confirming analysis, NMR, and mass spectra were obtained:

Theoretical: C 51.32% H 2.38%

Found: C 51.32% H 2.42%

Method C: 1,4-bis-[2-(3-chloro-5-nitropyridyloxy)]-benzene. To a flame-dried three-neck flask purged with nitrogen and equipped with stir bar was added 0.572 g of hydroquinone (5.2 mmol) in 26 ml of dry, freshly distilled dimethylsulfoxide. The solution was degassed (through nitrogen bubbling) for 1 hr. Potassium methoxide, 0.801 g (11.4 mmol), was added, causing the solution to turn yellow. After ½ hr, 2.5 g of 2,3-dichloro-5-nitropyridine were added and the resulting solution was heated to 80° for 24 hr. The solution was cooled to room temperature, and 250 ml of water were added and a precipitate was formed. This was filtered to give 1.62 g of a tan solid. Recrystallization from ethyl acetate gave a white crystalline solid m.p. of 225–226°, 1.28 g (58% yield). NMR and mass spectral data confirmed that 1,4-bis-[2-(3-chloro-5-nitropyridyloxy)]-benzene had been produced.

Exact Mass Theor: 421.9820

Found: 421.9802

Method N: 1,4-bis-[2-(3,5-dichloro-6-carbethoxymethylthiopyridyloxy)]-benzene. In a 100-ml, two-neck round bottom flask equipped with septum, condenser, and magnetic stir bar was placed the solid disodium salt of mercaptoacetic acid (prepared from 2.2 eq of sodium in methanol) (63.5 mg, 2.2 eq) and 16 ml of dry, freshly distilled dimethylsulfoxide (previously purged with nitrogen). 1,4-bis-[2-(3,5,6-trichloropyridyloxy)]-benzene (100 mg) was dissolved in a separate flask in 50 ml of freshly distilled dimethylsulfoxide (also previously purged with nitrogen). This solution was then added slowly to the disodium salt solution and the temperature was brought to 90°. The reaction was left stirring at this temperature overnight. With the aid of a vacuum pump, most of the dimethylsulfoxide and excess mercaptoacetic acid was removed on the rotary evaporator after the reaction was neutralized with H_2SO_4. In a 100-ml round bottom flask this crude product was combined with 20 ml of benzene, 15 ml of 99% ethanol, and 5 ml of H_2SO_4. This mixture was refluxed under N_2 in a Dean Stark apparatus overnight. Ethanol and benzene were then removed on the rotary evaporator and the residue was taken up in ethyl acetate. This solution was then washed with saturated sodium bicarbonate and dried. The 107-mg crude yield was then recrystallized once with a mixture of $CHCl_3$/95% ethanol to yield 35 mg of the desired product. Further recrystallizations gave a white fluffy solid that melted at 187–188.5°. Confirming analyses, NMR, and mass spectra were obtained.

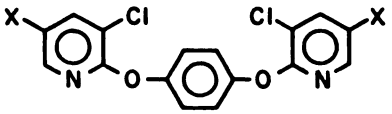
Theoretical: C 45.16% H, 3.16% N 4.39%

Found: C 44.76% H, 3.17% N 4.31%

Method S: 1,4-bis-(2,4-dichlorophenoxy)benzene. 1,4-bis-(2-Amino-4-chlorophenoxy)benzene (200 mg) was dissolved in 1 ml of HCl and 1 ml of H_2O in a 25-ml round bottom flask. The mixture ws cooled at 0°. $NaNO_2$ (267 mg) dissolved in 0.5 ml of water was cooled to 0° and then was added slowly to reaction mixture. After 10 min of stirring, this diazonium salt solution was added to an ice bath-chilled solution of CuCl (prepared from 0.3 g of NaCl and 1.167 g of hydrated copper sulfate) and 2 ml of HCl. The reaction was then allowed to warm to

TABLE 2

3,3′-Dichloro,5,5′-diX-bispyridyloxybenzenes: Lack of correlation of the Hansch π and σp values for 5,5′-substitutes with biological potency[a]



	π (for X)	σ_p (for X)	ED_{50}
			μmol/kg/day
X = —Cl	0.71	0.23	0.16
—NO_2	0.28	0.78	1.0
—H	0.00	0.00	>40
—NH_2	−1.23	−0.66	>60
—C≡N	0.57	0.66	>60
—$COOC_2H_5$	0.51	0.45	>60

[a] π and σp substituent values were taken from Ref. 7.

room temperature, followed by gentle reflux for 5–10 min. This solution was then taken up in ether, washed with 30% NaOH, H_2O, and dried to afford 184 mg of crude product. This was then purified on a silica preparation plate (35% $CHCl_3$/hexane, two elutions) m.p. 111–113°. The desired compound was subsequently recrystallized using 10% $CHCl_3$/ethanol. Confirming analysis, NMR, and mass spectra were obtained.

Theoretical: C 54.02 H 2.50

Found: C 54.50 H 2.78

Method R: 1,4-bis-(2-amino-4-chlorophenoxy)benzene. To a 50-ml round bottom flask equipped with nitrogen line and magnetic stir bar was placed 400 mg of the corresponding nitro precursor, 600 mg of Fe powder, and 20 ml of acetic acid. The mixture was then stirred for 1½ days under N_2 at room temperature. The reaction vessel was then put on a rotary evaporator to remove the acetic acid. The residue was taken up in ethyl acetate, neutralized with sodium bicarbonate, washed with H_2O, and dried. The crude product was spread on a 2-mm preparation plate (silica) and eluted with 2% acetone/chloroform to yield 200 mg of the desired product (64% yield). This compound was recrystallized from ethanol to give an m.p. of 146–148.5°. Confirming analyses, NMR, and mass spectra were obtained.

Theoretical: C 59.85% H 3.88%

Found: C 59.39% H 4.10%

RESULTS

Table 1 lists the structural formulae of the compounds synthesized, their melting points, method of synthesis and yield, mass spectral data or C,H analysis, and log partition coefficients, potency to induce hepatic aminopyrine *N*-demethylase activity. For biological activity the 1,4-dioxygenated benzenoid ring as the center unit appears mandatory; replacement of this unit by a 1,4-diaminobenzene ring (compound **23**), a 1,3-dioxygenated benzene ring (compounds **25** and **31**) or a 4,4′-dioxygenated diphenyl ether (compound **21**) deletes activity. In the lateral rings, a pyridine unit is essential, although substantial activities of the bisquinoline analogue (compound **16**) and of the bis(chloropyrimidine) analogue (compound **22**) are noteworthy. A number of analogues with lateral benzene rings (compounds **29–31**) were found to be inactive.

Monosubstitutions on the central benzene ring (compounds **17–19**, and **26**) are well tolerated. Substituents on the lateral pyridine rings, especially at the 5,5′-positions, are important and are not readily rationalized by simple Hansch-type substituent effects (7, 8). The 3,3′-dichloro analogue (compound **9**) is inactive. For the limited number of compounds with varying 3,3′-substituents (all with 5,5′-chloro- or bromo-substitutions), the structure-activity response is rather flat, with the potencies in the order Cl > Br > H > CH_3 (compounds **1**, **2**, **5**, and **8**). All 1,4-bispyridyloxybenzenes with biological activity had 5,5′-substituents of bromine, chlorine, or nitro groups. Analogues with other 5,5′-substituents such as H, CH_3, CF_3, NH_2, CN, or $COOC_2H_5$ were inactive.

For biological activity, the bispyridyloxybenzenes require: 1) a 1,4-dioxybenzene central unit, 2) lateral pyridine rings, and 3) 5,5′-substituents of Br, Cl, or NO_2 on the pyridine rings. We have previously defined the structural requirements for halogenated dibenzo-*p*-dioxin binding to the Ah receptor and induction of monooxygenase activity (2). The dibenzo-*p*-dioxin ring is fairly rigid and planar, permitting one to estimate the absolute molecular conformation. In contrast, the bispyridyloxybenzenes congeners have the potential for rotation about the bonds to the ether oxygens, giving rise to many possible conformations. Changes in substituents may be accompanied by subtle conformational preferences in these molecules that could substantially affect their binding affinity to a receptor.

For six 3,3′,5,5′-tetrasubstituted dipyridyloxybenzenes shown in Table 2, we failed to observe any clear correlation between Hansch π values, σ_p values, or any simple combination of these values and biological potency (7). A similar lack of correlation was seen for the 5,5′-disubstituted series (data not shown). This absence of a Hansch-type continuum of substituent effects at 5,5′-positions in the lateral pyridine ring, and the above-mentioned potential for conformation variability, led us to perform x-ray structure determinations of three similar compounds listed in Table 1. The test compounds were: compound **1**, TCPOBOP (3,3′,5,5′-tetrachloro-analogue), ED_{50} = 0.16 μmol/kg/day; compound **5**, 5,5′-dichloro-analogue, ED_{50} = 0.37 μmol/kg/day; and compound **9**, 3,3-dichloro-compound, ED_{50} > 40 μmol/kg/day. Our goal was to find any conformational differences that might exist between active and inactive analogues.

The x-ray structures (Fig. 1, Table 3) of the two active compounds, **1** and **5**, show almost identical conformations but differ considerably from the inactive compound, **9**, in two significant aspects. The more striking of these is the positioning of the pyridine rings; in compounds **1** and **5** the pyridine rings are *anti* with respect to the plane of the central ring, whereas in compound **9** they are *syn*. The other major difference occurs in the dihedral angle between the central ring and the pyridine rings. In compounds **1** and **5** this angle is approximately 60°. In compound **9** this angle is about 84°. These conformational differences are both defined by rotation about the two C—O bonds at the *para*-positions of the benzene ring, 'O'(1)—C(7) and 'O'(1′)—C(2′) in compound **9**, and 'O'(1)—C(7) and 'O'(1′)—C(7′) in compounds **1** and **5**.

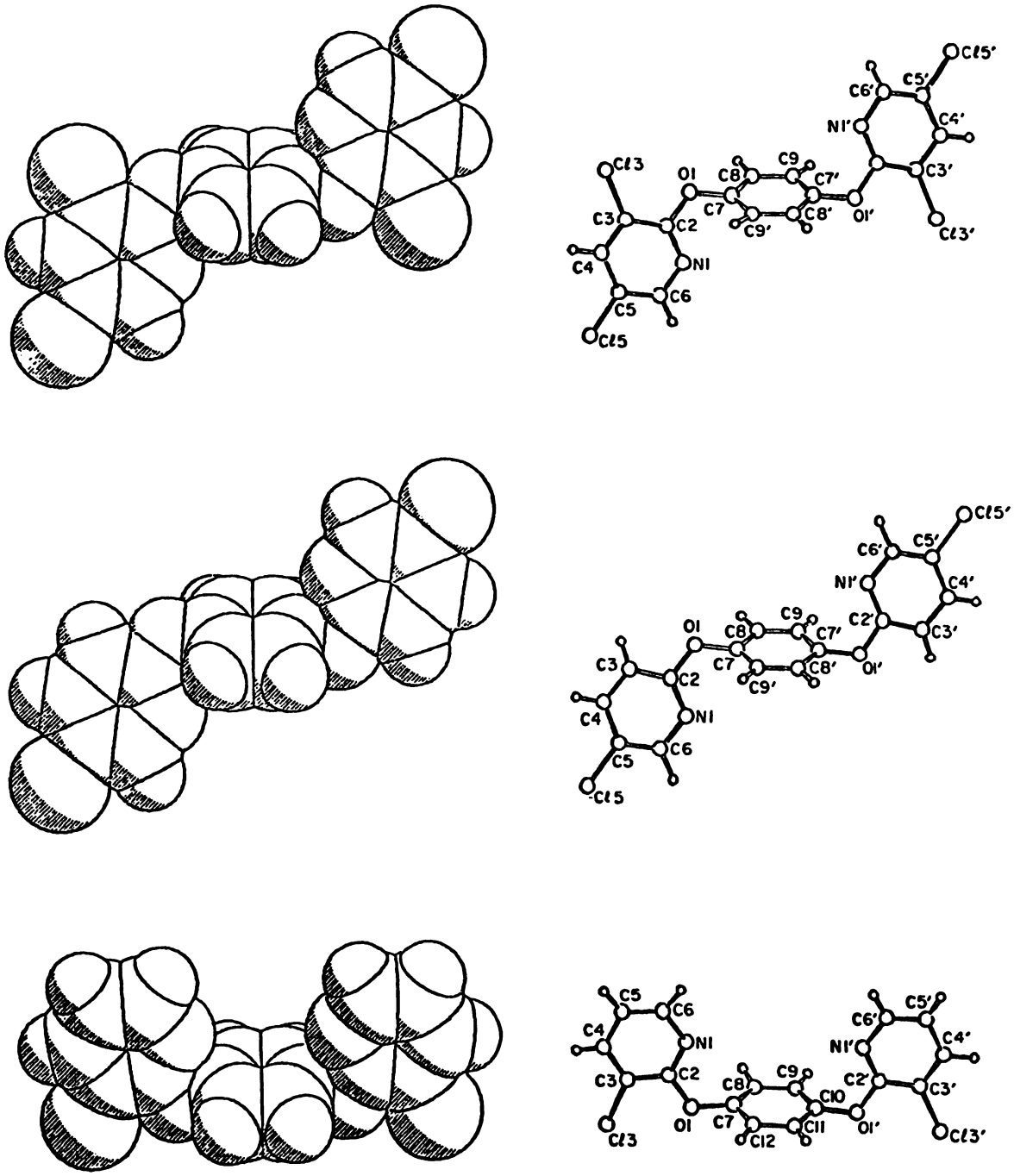


FIG. 1. *Space filling and ball and stick diagrams of compounds 1, 5, and 9 shown with the central ring in a fixed orientation.*

In all three compounds the pyridine-oxygen bond, ranging from 1.35 to 1.37 Å, is shorter than the benzene-oxygen bond, which ranges from 1.39 to 1.40 Å. The bond angle at the ether oxygen atom ranges from 119.1° to 121.0°. This is consistent with an sp^2 hybridized oxygen atom, conjugating with the pyridine ring rather than the benzene ring. Preference for conjugation with pyridine is probably due to the distinct electronic and steric properties that nitrogen confers upon the pyridine ring. The conjugation strongly restricts rotation about the C(2)—'O'(1) and C(2′)—'O'(1′) bonds. This could be an important constraint since for both the *syn* and the *anti* conformations it has the effect of placing the two pyridine rings in a common plane with one another and with the C—O bonds of the ether linkages. This is strictly observed for compounds **5** and **9**, but in the case of compound **1** there is a slight rotation of about 9° around the C(2)—'O'(1) and C(2′)—'O'(1′) bonds, so that the pyridine rings are no longer exactly coplanar, but do lie in parallel planes. The importance of conjugation between the ether oxygens and the lateral rings is illustrated in Fig. 2. It is evident that conjugation between the ether oxygens and the central ring would lead to a completely different placement and orientation of the rings and their substituents.

The requirement for a pyridine unit in the lateral rings

TABLE 3

Molecular parameters for the compounds

Parameter	3,3′,5,5′-Tetrachloro-BPB[a], **1**	5,5′-Dichloro-BPB, **5**	3,3′-Dichloro-BPB, **9**
Dihedral angles between central and lateral rings	58.7(2)	60.1(1)	83.7(3) 83.9(3)
Molecular dimensions (Å)	15.3 × 5.4 × 3.5	15.4 × 4.7 × 3.5	13.4 × 4.7 × 4.1
Cl(3)—Cl(3′) (Å)	11.2		11.2
Cl(5)—Cl(5′) (Å)	15.3	15.4	
CL(3)—Cl(5′) (Å)	12.3		
N(1)—N(1′) (Å)	7.4	7.5	5.9
Pyridine-pyridine (center to center) (Å)	9.6	9.7	8.7

[a] BPB, bispyridyloxybenzene.

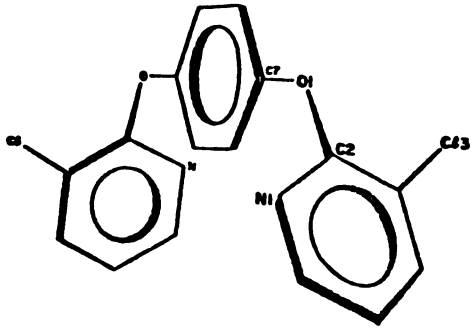


FIG. 2. *Hypothetical conformation (shown for compound 9) that would result from benzene-oxygen conjugation, as opposed to pyridine-oxygen conjugations.*

may be explained by the above observation and emphasizes the need for conjugation between the ether oxygens and the lateral rings. The pyridine ring, however, may also play a more direct role, in which the pyridine nitrogen is specifically involved in recognition by a putative receptor site. This would necessitate some degree of exposure and accessibility of the nitrogen atoms. In all three molecules the nitrogens are proximal to the face of the central ring, i.e., the torsion angle, C(7)—'O'(1)—C(2)—N(1), is approximately 0°. For the nitrogen to assume the more exposed position occupied by C(3), a rotation of 180° about the C(2)—'O'(1) bond would be required (Fig. 3). This would bring the chlorine atom on C(3) into steric conflict with the benzene ring; thus, such a conformation is not favored. A hydrogen atom on C(3), however, might be accommodated proximal to the benzene ring, via suitable rotation about the 'O'(1)—C(7) bond, but even then the permissible rotation about C(2)—'O'(1) bond would be considerably less than 180°; thus, the conjugation between 'O'(1) and the pyridine ring would be disrupted.

In compound **9**, exposure of the nitrogens is further limited by the *syn* orientation of the pyridine rings. In this case, access to the nitrogen edge of each pyridine ring is restricted not only by the central ring, but also by the other pyridine ring. This is evident in Table 3, which compares various molecular parameters of the three compounds. Note that the distances between the two pyridine nitrogens and between the pyridine rings themselves are significantly shorter in compound **9**.

From the x-ray crystallographic structure of these compounds one may draw inferences about their binding to the putative receptor, but it must be noted that crystal structure does not necessarily guarantee the same conformation in solution. Because of their lipophilic nature, these compounds might be expected to bind to a strongly hydrophobic pocket in a putative receptor. In considering such potential interactions, it is useful to note that the major packing forces found in the crystals involve π-π interactions between the aromatic rings of neighboring molecules. Similar π-π interactions might occur with the side chains of aromatic amino acids (e.g., phenylalanine, tyrosine, or tryptophan) of a hypothesized receptor. In the crystals these interactions are restricted to benzene-benzene and pyridine-pyridine stacking. Again there are notable contrasts between the biologically active and inactive compounds. In compounds **1** and **5** there is partial overlap of the benzene rings, with an interplanar distance of about 3.4 Å. In compound **9** there is less overlap of neighboring benzenes and the interplanar distance is approximately 3.3 Å. In the case of pyridine-pyridine stacking, compounds **1** and **5** exhibit only partial overlap of the rings with interplanar separations of about 3.5 and 3.4 Å, respectively. In compound **9**, however, there is more extensive overlap of the pyridine rings, but with a somewhat greater interplanar distance of about 3.65 Å. The variations observed in crystal packing may reflect, in addition to conformational differences, contrasts in the type and extent of π-π interactions

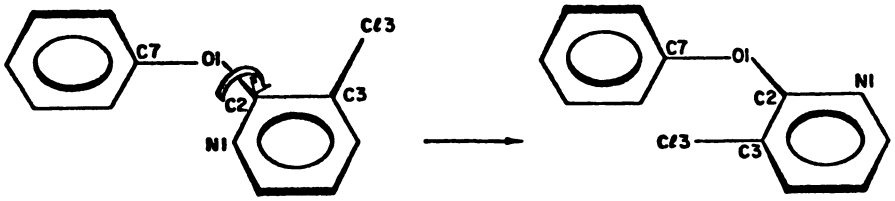


FIG. 3. *Relationship of the pyridine and benzene rings in the crystal structure*

The three crystal structures (*left*) all show N1(N1′) to be proximal to the benzene ring, i.e., the torsion angle N(1)—C(2)—'O'(1)-C(7) is approximately 0°. A rotation of 180° about the C(2)—'O'(1) bond places N1 in a more exposed position, but Cl(3) is then in steric conflict with the benzene ring (*right*) (see the text).

preferred by the molecules. If such differences extend to the interactions of these molecules with a receptor, this would help to account for their relative activities.

DISCUSSION

1,4-bis-[2-(3,5-Dichloropyridyloxy)]-benzene has been previously shown to be a potent, long-acting phenobarbital-like inducer of hepatic monooxygenase activity. In this report we have examined the structure-activity relationship among 31 congeners to induce murine hepatic aminopyrine *N*-demethylase activity. Comparison of the biological potency of these congeners *in vivo* (prompted by the observation that many defined hepatic cell culture systems fail to respond to phenobarbital-like compounds) is complicated by their relative rates of metabolism and elimination. However, the long biological half-life of TCPOBOP and the similar structure of many of the congeners (e.g., 5,5′- and 3,3′,5,5′-halosubstituted bispyridyloxybenzenes) suggest that this may not be an important consideration.

We have noted that the minimum requirements for biological activity are: a) a central 1,4-dioxygenated benzene ring, b) lateral pyridine rings (or other heteroaromatic rings, e.g., pyrimidine or quinoline) having a heteropolar double bond at the site of the ether bond attachment, namely C(2)═N(1), and c) for pyridine rings, 5,5′-chlorine, bromine, or nitro substituents. The importance of the ether-heteroaromatic double bond attachment is emphasized by the x-ray crystal structures showing for all three examples a short pyridine oxygen bond and geometries consistent with an sp^2 oxygen atom conjugating with lateral rather than central rings.

The observed structure-activity data are consistent with a putative receptor containing one or more binding sites that coordinate to 5,5′-Cl or NO_2 but not to C═N, NH_2, $COOC_2H_5$. The location of these binding sites in the receptor may also be fixed and therefore exquisitely sensitive to the equilibrium conformation of the ligand. The current x-ray crystal studies demonstrate a wholly unexpected conformational dichotomy between the active compounds **1** and **5** and the closely related isomer **9.** It is only the active compounds that exhibit an *anti* relationship between the two pyridine nitrogens relative to the central benzene ring. Moreover, it is only in these equilibrium conformations that the two C-5 chlorine substituents are above and below the plane of that benzene ring at an interatomic separation of approximately 15 Å; the corresponding separation in compound **9** would be approximately 12 Å.

On the basis of the structure-activity and x-ray crystallographic data, we propose that the putative receptor for bispyridyloxybenzene congeners is relatively specific, having two similar and complementary binding sites. These sites have a high affinity for Cl, Br, and NO_2 substituents and are located some 17–18 Å apart so as to accommodate a centrosymmetric ligand molecule **1** or **5,** but not the less symmetric molecule **9.** Perhaps the receptor itself has some degree of centrosymmetry. One test of this hypothesis would be the synthesis of asymmetric bisdipyridyloxybenzenes related to the above series, but with different substituents in their 5- and 5′-position. Phenobarbital and other phenobarbital-like inducers which are presumed to compete for this same receptor are far less potent and bear no obvious structural similarity to the bisdipyridyloxybenzenes. These weak compounds may bind two ligands to the receptor site occupied by one bisdipyridyloxybenzene molecule.

TCPOBOP is the most potent agonist for the phenobarbital-like pleiotropic response yet identified. At the ED_{50} dose of TCPOBOP (4.9×10^{-7} mol/kg), the hepatic concentration of the compound in the mouse was estimated to be 1×10^{-7} M (4). This value serves as a rough estimate of the affinity of TCPOBOP for its putative receptor, and it probably is too large to observe specific binding by nonequilibrium methods. The potency of TCPOBOP to evoke this response and the well defined structure-activity relationships among bisdipyridyloxybenzene congeners support the hypothesis that these compounds act by virtue of binding to a specific recognition site. However, identification of this hypothesized receptor must await more potent agonists or an equilibrium method of identifying the putative ligand-receptor complex (e.g., photoaffinity labeling).

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