Table II
Comparison of ΔF^* for C–N Rotation in $CD_3C(X)N(CH_3)_2$ with ^{13}CH Coupling Constants for the NCH_3 Protons[a]

Registry no.	X	ΔF^* (25°), kcal/mol	$J(^{13}CH)$, Hz[b]
44364-33-2	NH	(<17)	135
20255-66-7	O	17.3	138
34302-08-4	S	20.3	140
50600-26-5	NH_2^+	22	141

[a] Values of ΔF^* from Table I except for X = NH, which comes from ref 3. [b] The ^{13}CH coupling constants for NCH_3 protons; see ref 3 and 13.

should depend on the extent of C–N double-bond character and since in turn the rotational barrier should also depend on this, we were disappointed that an inconsistency appeared based on our kinetic data using DMSO-d_6.[3] We are now gratified to be able to report that the rotational barriers determined in the nonpolar solvents at low concentration do correlate with the ^{13}CH coupling constants for the NCH_3 protons (Table II).

Experimental Section

Compounds. Syntheses and properties of all of the compounds have been previously described.[3]

Solvents. Isooctane (spectroquality, Matheson Coleman and Bell), carbon tetrachloride (spectrophotometric grade, Mallinckrodt), decalin (spectrophotometric grade, Aldrich), *n*-decane (99%, gold label grade, Aldrich), and 1,1,2,2-tetrachloroethane (Matheson Coleman and Bell) were used as received in sealed bottles.

Variable-Temperature Spectra, Temperatures, and Line Shape Analyses. The procedures followed were essentially those which we have previously used and described.[1,3] Kinetic data were obtained in all cases by total line shape analysis.[1,3]

References and Notes

(1) Part IX: R. C. Neuman, Jr., and V. Jonas, *J. Org. Chem.*, **39**, 925 (1974).
(2) Support by the U. S. Public Health Service (National Institute of General Medical Sciences) through Grant GM-13342 is gratefully acknowledged.
(3) R. C. Neuman, Jr., and V. Jonas, *J. Phys. Chem.*, **75**, 3532 (1971).
(4) R. C. Neuman, Jr., W. Woolfenden, and V. Jonas, *J. Phys. Chem.*, **73**, 3177 (1969).
(5) M. Rabinovitz and A. Pines, *J. Amer. Chem. Soc.*, **91**, 1585 (1969).
(6) A. Calzolari, F. Conti, and C. Franconi, *J. Chem. Soc. B*, 555 (1970).
(7) R. C. Neuman, Jr., W. Snider, and V. Jonas, *J. Phys. Chem.*, **72**, 2469 (1968).
(8) Calculations were performed in a manner similar to that described in ref 7.
(9) These results for dimethylacetamide are qualitatively similar to those reported by Calzolari, *et al.*[6]
(10) J. Sandström, *J. Phys. Chem.*, **71**, 2318 (1967).
(11) See ref 1 for a discussion of analysis methods and leading references.
(12) See R. C. Neuman, Jr., and V. Jonas, *J. Phys. Chem.*, **75**, 3550 (1971).
(13) R. C. Neuman, Jr., and L. B. Young, *J. Phys. Chem.*, **69**, 2570 (1965).
(14) P. Haake, W. B. Miller, and D. A. Tysee, *J. Amer. Chem. Soc.*, **86**, 3577 (1964).

Synthesis and Fourier Transform Carbon-13 Nuclear Magnetic Resonance Spectroscopy of New Toxic Polyhalodibenzo-*p*-dioxins

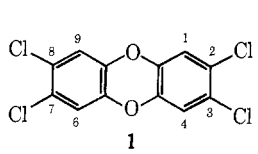
Andrew S. Kende,* James J. Wade, David Ridge, and Alan Poland

Department of Chemistry, The University of Rochester, Rochester, New York 14627

Received October 24, 1073

The extraordinary toxicity and potential environmental significance of certain polyhalodibenzo-*p*-dioxins has led us to carry out regiospecific syntheses of these compounds by condensation of catechol derivatives with various polyhalobenzenes. Electrophilic halogenation of 2,3-dihalodibenzo-*p*-dioxins, available by the above route, leads mainly to 2,3,7,8-tetrahalo derivatives, but these are more cleanly obtained by direct condensation of 4,5-dichlorocatechol with 1,2,4,5-tetrahalobenzenes. Fourier transform ^{13}C spectroscopy is shown to be a useful structural probe in this series. Some structure–activity relations for enzyme induction by polyhalodibenzo-*p*-dioxins are outlined.

The surprisingly high toxicity of certain halogenated dibenzo-*p*-dioxins has been demonstrated in a number of recent investigations.[1] The most thoroughly studied member of this group is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (**1**, TCDD), which has been shown to be the cause of several outbreaks of chloracne among workers in factories which manufacture the herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid).[2] It is now recognized that structure **1** represents perhaps the most lethal small molecule known.[1d] Although present in only trace amounts during the manufacture of 2,4,5-T, the toxicity of this xenobiotic is so extraordinarily high that even these minute quantities constitute a potentially serious health hazard.



1 **2**

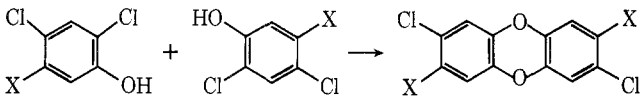
The identification of a toxic contaminant in poultry feed, 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (**2**), as a probable cause of the "chick edema" which has caused widespread loss of chickens in the United States since 1957[3] also demonstrates the environmental significance of certain polyhalogenated dibenzo-*p*-dioxins, apparently formed as by-products in the commercial synthesis of a number of chlorinated phenols. Thus the tetrachloro derivative **1** formed during the manufacture of 2,4,5-trichlorophenol is responsible for the contamination of 2,4,5-T, since the phenol is an intermediate in the manufacture of the herbicide. Because of the widespread use of chlorinated phenols, the extreme potency and environmental persistence of the chlorinated dibenzo-*p*-dioxins which may be present as impurities, and the teratogenic[4] and possible mutagenic effects of these contaminants at sublethal concentrations, further chemical and toxicological characterization of these compounds is urgently needed.

Until recently little was known about the biochemical mechanisms of toxicity for these compounds, and no systematic studies had attempted to relate molecular structure to toxicity or other biological properties in the dibenzo-*p*-dioxin series. In 1973, however, Poland and Glov-

er established that 1 (and certain congeners) are powerful inducers of the enzymes δ-aminolevulinic acid synthetase and aryl hydrocarbon hydroxylase in the chick embryo.[5] Enzymatic assays based on these properties now provide a means of detecting highly toxic dioxins at the nanogram level and, moreover, appear to demonstrate a parallel between enzyme-inducing activities and toxicities of various dioxins toward test animals. With the availability of such assays it became both feasible and imperative to try to define the structural parameters responsible for the extraordinary biological activities shown by some members of this series.

The present investigation has four main objectives: (1) to develop practical and regiospecific syntheses of certain polyhalodibenzo-*p*-dioxins, (2) to discover spectroscopic correlations among such compounds which may aid in identifying new members of the series, (3) to prepare radioactively labeled compounds of high purity for localization and metabolic studies and (4) to elucidate structural requirements for toxicity within this series.

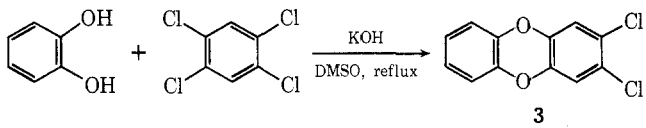
Chemical Background. The classical preparations of polyhalodibenzo-*p*-dioxins are limited to two types of processes: (1) self-condensation of a polyhalophenol and (2) direct halogenation of the parent dibenzo-*p*-dioxin or a monohalo derivative. The former method, exemplified by the reaction through which 1 arises as a commercial impurity, normally proceeds in moderate yield and is practical only when a single condensation product can be formed. Yields of 10–20% have been reported for the Ullman-type self-condensation of 2,4-dichlorophenol to 2,7-dichlorodibenzo-*p*-dioxin,[6] while Aniline[7] has obtained 1 in over 30% yield by a modified Ullman reaction from 2,4,5-trichlorophenol.



X = H or Cl

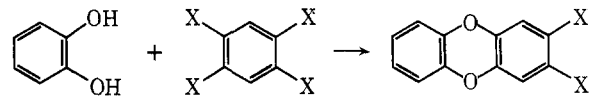
The second method, involving direct halogenation, is of limited scope and has been shown to give mixtures of products. Direct bromination of dibenzo-*p*-dioxin gives both 2,8- and 2,7-dibromodibenzo-*p*-dioxin, while under forcing conditions the 2,3,7,8-tetrabromo derivative is formed.[8] Direct chlorination of dibenzo-*p*-dioxin proceeds in poor yield to give first the 2-chloro and then the 2,7-dichloro derivative.[8] Further chlorination using catalysis by iodine and ferric chloride does produce 1 in moderate yield, but the product is mixed with tri- and pentachlorodibenzo-*p*-dioxins and isolation of pure 1 is extraordinarily difficult.[9]

Catechols in Dibenzo-*p*-dioxin Syntheses. Most of the drawbacks of the conventional syntheses outlined above can be avoided by carrying out the condensation between a catechol dianion and a polyhalobenzene in boiling dimethyl sulfoxide. Using this method, Pohland and Yang[6a] found that the dipotassium salt of catechol in refluxing DMSO reacts smoothly with certain tri- and tetrachlorobenzenes to give good yields of the corresponding dibenzo-*p*-dioxin, *e.g.*



3

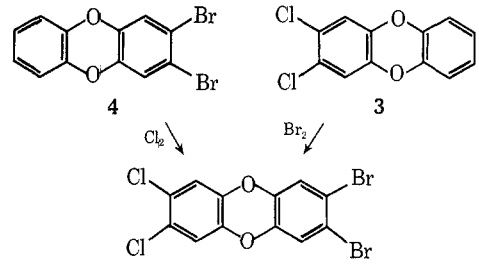
Our own work has systematically extended the scope of this condensation to more highly substituted catechols and to a broad range of polyhalobenzene acceptors. For example, the use of 1,2,4,5-tetrabromobenzene as acceptor gave 2,3-dibromodibenzo-*p*-dioxin (4), and 1,2,4,5-tetrafluorobenzene yielded the 2,3-difluoro derivative (5).



3, X = Cl
4, X = Br
5, X = F

The catechol dianion also reacts with other chlorinated benzenes under the above conditions. Reaction with 1,2,3,5-tetrachlorobenzene gives 1,3-dichlorodibenzo-*p*-dioxin (6), while hexachlorobenzene as acceptor produced 1,2,3,4-tetrachlorodibenzo-*p*-dioxin in good yield. The condensations of catechol with 1,2,3,4-tetrachlorobenzene or with pentachlorobenzene likewise occur readily, but each yields as expected two isomeric products which have not been individually characterized to date.

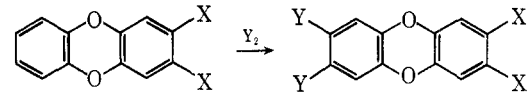
Electrophilic Substitution of 2,3-Dihalodibenzo-*p*-dioxins. The apparent generality of the reaction of catechol dianions with various polyhalobenzenes led us to examine the further halogenation of the primary condensation products. Chlorination of 2,3-dichlorodibenzo-*p*-dioxin (3) in the presence of iodine and ferric chloride gave a crystalline material which was predominantly 1, but contained varying amounts of tri- and pentachlorodibenzo-*p*-dioxins. Although extensive purification produced 1 of above 98% purity, the overall yield of pure 1 by this route was unsatisfactory, owing in part to variable yields in the initial condensation with catechol and also to the extensive purification necessary to obtain 1 pure enough for biological study. Chlorination of the dibromo compound 4 gave 2,3-dibromo-7,8-dichlorodibenzo-*p*-dioxin (7), identical with the product prepared by bromination of the dichloro compound 3. The dibromo compound 4 was also brominated to give 2,3,7,8-tetrabromodibenzo-*p*-dioxin (8). Finally, the difluoro derivative 5 was converted in a



4 3

7

similar manner to the 2,3-dichloro-7,8-difluoro- and 2,3-dibromo-7,8-difluorodibenzo-*p*-dioxins (9 and 10, respectively).



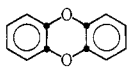
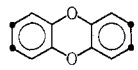
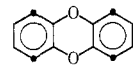
3, X = Cl
4, X = Br
5, X = F

1, X = Y = Cl
7, X = Br; Y = Cl
8, X = Y = Br
9, X = F; Y = Cl
10, X = F; Y = Br

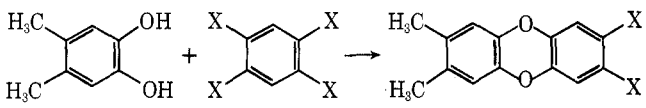
The dichlorodibenzo-*p*-dioxin 3 could also be nitrated using nitronium tetrafluoroborate. The product appeared to consist of two isomeric dichlorodinitrodibenzo-*p*-dioxins, however, and was not characterized further.

Substituted Catechols as Nucleophilic Components. The catechol condensation reaction was slightly modified in order to prepare an isostere of 1. This was conveniently achieved by reaction of 4,5-dimethylcatechol[10] dianion with 1,2,4,5-tetrachlorobenzene to yield 2,3-dichloro-7,8-dimethyldibenzo-*p*-dioxin (11). The 2,3-dibromo counterpart (12) is similarly available from 1,2,4,5-tetrabromobenzene.

Table I
Fourier Transform ^{13}C Nmr Data for Some Chlorinated Dibenzo-*p*-dioxins[a]

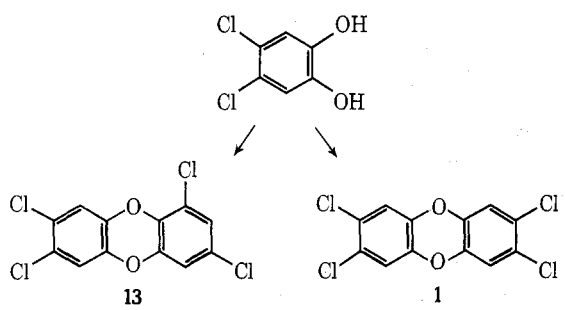
Cl substitution pattern			
None	142.2 (33%)	123.6 (100%)	116.2 (90%)
2,3	141.1 (46%)	126.3 (28%) 124.2 (100%)	117.5 (85%) 116.3 (84%)
1,2,3,4	140.4 (39%) 138.9 (12%)	126.9 (11%) 125.0 (100%)	119.9 (14%) 116.6 (91%)
1,3[b]	143.3 (8%) 141.2 (8%) 141.1 (11%)	124.5 (100%) 124.3 (70%)	116.7 (51%) 116.3 (52%) 115.2 (48%)
1[b]	143.1 (16%) 141.6 (8%)	124.6 (87%) 124.3 (100%) 124.0 (89%) 123.2 (81%)	116.6 (50%) 116.2 (62%) 114.7 (62%)
1,2,4[b]	140.7 (24%)	127.2 (10%) 124.9 (100%) 124.8 (100%) 124.1 (57%)	119.6 (8%) 116.9 (97%)
2,7[b]		123.9 (100%)	116.6 (81%) 117.0 (79%)
Average	141.4 ± 2.5 ppm	124.8 ± 2.5 ppm	116.8 ± 3.0 ppm

[a] Chemical shifts are given in parts per million downfield from TMS. The relative peak heights are given in parentheses. [b] Weak peaks missing or unresolved.



11, X = Cl
12, X = Br

Although Pohland and Yang[6a] report that tetrachlorocatechol does not condense with 1,2,4,5-tetrachlorobenzene, we have found that both 4-chlorocatechol[11] and 4,5-dichlorocatechol[11] dianions are useful nucleophilic partners toward tetrachlorobenzenes. The former reacts smoothly with 1,2,4,5-tetrachlorobenzene to give 2,3,7-trichlorodibenzo-*p*-dioxin. Reaction of 4,5-dichlorocatechol dianion with 1,2,3,5- and 1,2,4,5-tetrachlorobenzene yields the new 1,3,7,8-tetrachlorodibenzo-*p*-dioxin (**13**) and **1**, respectively.



13 **1**

The synthesis of **1** from 4,5-dichlorocatechol provides a one-step route, giving material of very high purity. Despite the low yield, this is the preferred synthesis of pure, uniformly labeled [^{14}C]-TCDD, and we have prepared the latter by this route to give material having a specific activity of 147.5 mCi/mmol.

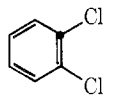
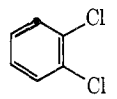
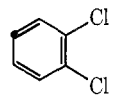
Cmr Spectroscopy of Halogenated Dibenzo-*p*-dioxins. A major problem for any complete and systematic study of the halogenated dibenzo-*p*-dioxins is the reliable identification of the structures of compounds in the series. The evidence for the structures of compounds we have synthesized is based primarily on the method of synthesis and, where possible, upon comparison with a sample of known structure (*e.g.*, by X-ray crystallography for **1** itself).[9] A combination of gas chromatography and mass spectroscopy is of utmost importance in assessing the homogeneity and degree of halogenation of the compounds, and both techniques were used extensively in the present study. These techniques, however, do not reliably define the positions of halogenation in this series. Pohland and Yang have described the possible use of infrared and phosphorescence spectra in regard to this problem and have tabulated spectroscopic data using these methods.[6a] These investigators have also examined the esr spectra of radical cations derived from chlorinated dibenzo-*p*-dioxins.[12]

It is clear from the spectroscopic studies cited that a need remains for rapid identification of the substitution pattern of an unknown polyhalodibenzo-*p*-dioxin. Proton magnetic resonance spectroscopy appears to be remarkably useless in this context because the aromatic resonances for these compounds occur at virtually identical chemical shifts, making assignment of peaks difficult and integration unsatisfactory. For example, the 100-MHz pmr spectrum of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin in tetrachloroethylene solvent appears as a sharp singlet at δ 7.10, that of the 2,3-dichloro compound as singlets at δ 6.97 and 7.07, and the spectrum of **1** as a singlet at δ 7.00.

It seemed possible that natural abundance ^{13}C Fourier transform magnetic resonance spectroscopy (cmr) might be useful for structural assignments in this series. A brief study of several dibenzo-*p*-dioxins suggests that this technique may be of special value for such structural studies. As summarized in Table I, our data reveal chemical shift parameters and relative peak heights which carry substantial information bearing on the substitution pattern of the dibenzo-*p*-dioxin system.

Two main features of the cmr data should be noted. First of all, the resonances due to the lateral carbons (2, 3, 7, and 8), the "peri" carbons (1, 4, 6, and 9), and the junction carbons (11, 12, 13, and 14) each appear in one of three separate and characteristic regions of the spectrum. Although chlorine substitution anywhere may effect slight changes in the chemical shifts of all the carbons, any given shift is relatively small, and each carbon resonance remains in its characteristic region. This observation regarding the minute effect of chlorine on ^{13}C chemical shifts is in accord with published data (Table II).[13]

Table II
^{13}C Chemical Shifts for Symmetrical *o*-Dichlorobenzenes[a,b]

		
3.7	2.0	−0.5

[a] Reference 13. [b] Shifts are given in parts per million relative to benzene.

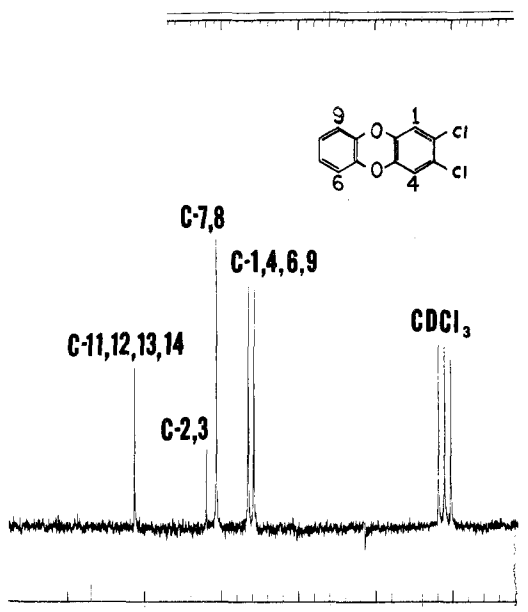


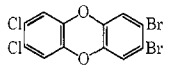
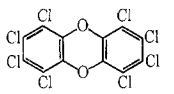
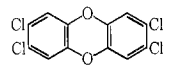
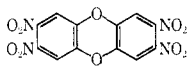
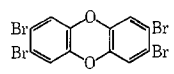
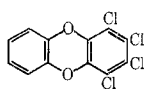
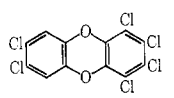
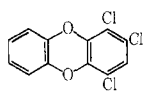
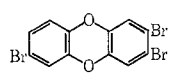
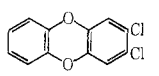
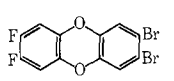
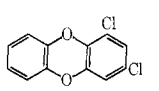
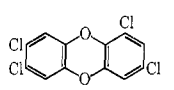
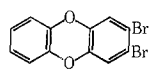
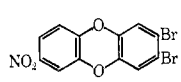
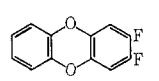
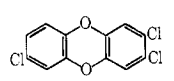
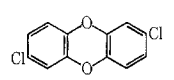
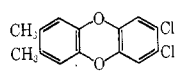
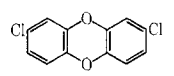
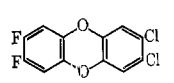
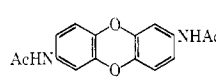
Figure 1. Fourier transform ^{13}C nmr spectrum of 2,3-dichlorodibenzo-*p*-dioxin, 3.6% in $CHCl_3$, with $CDCl_3$ internal reference. Chemical shifts are in Table I.

The second important feature is that the peaks due to carbon atoms bearing a hydrogen are significantly enhanced in intensity relative to those lacking a hydrogen substituent. Such enhancement (heteronuclear Overhauser effect) is anticipated and derives from the relatively short spin lattice relaxation times of carbons comprising a CH unit. Other things being equal, then, one can assume that an intense singlet probably denotes CH, and a weak singlet defines either CO or CCl, assuming no degeneracy for the spectrum. Figure 1 illustrates these intensity differences for the cmr spectrum of 2,3-dichlorodibenzo-*p*-dioxin. In some samples the weak signals could not always be distinguished from the noise level.

Considering these characteristics of the cmr spectrum it should often be possible, in conjunction with mass spectral data, to employ cmr in defining the substitution pattern of an unknown dibenzo-*p*-dioxin. Although the assignment of each individual peak to a specific position is not always unambiguous, especially for an unsymmetrical system such as the 1,3-dichloro compound **6**, the overall pattern of the spectrum will generally be indicative of a particular structure in view of the substantial chemical shift differences distinguishing the three sets of carbon atoms already defined.

Certain structures, however, are still ambiguous using the cmr technique. Thus we probably could not distinguish *a priori* between the 1,3-dichloro compound and the 1,7- or 1,8-dichloro isomer, nor between the 1,2,3,7,8,9-hexachloro compound and its 1,2,3,6,7,8-hexachloro isomer. A further drawback is the very low solubility of some of the highly chlorinated dioxins, necessitating prolonged scanning of the spectrum. Nevertheless, Fourier transform cmr spectroscopy appears to be a useful adjunct to other techniques for structure elucidation in this series.

Table III
Structure–Activity Relations for AHH Induction in Chick Embryo Liver, Expressed Relative to TCDD Activity = 1.0

	1.1		0
	1.0		0
	1.0		0
	0.8		0
	0.6		0
	0.2		0
	0.2		0
	0.01		0
	0.02		0
	0.01		0
	0.01		0

The data lead to the following rules, based on the AHH-inducing activities of some two dozen compounds: (1) positions 2, 3, 7, and 8 must contain at least three halogen substituents; (2) substituents at these lateral positions have the order of activity Br > Cl > F > NO_2; (3) at least

Enzyme Induction by Halogenated Dibenzo-*p*-dioxins. The dibenzo-*p*-dioxins prepared in this study were assayed for their ability to induce aryl hydrocarbon hydroxylase (AHH) in the chick embryo according to the published procedure.[5] A summary of relative activities (1 = 1.00) for all compounds available to us in this series is shown in Table III.

Several trends can be noted from the collected data. The halogen-free compounds are inert, as is the 1,2,3,4-tetrachloro derivative. When three of the four lateral positions of the dibenzo-*p*-dioxin system are halogenated, the compound becomes active, but is not as active as when all four lateral positions are halogenated. Replacement of halogen by nitro at all four lateral positions destroys activity. The 2,3-dichloro-7,8-dimethyl compound, roughly isosteric with TCDD, is inactive. The fluorinated compounds are less active than their tetrachloro or tetrabromo counterparts. Furthermore, the 2,3,7-tribromo compound is significantly more active than the corresponding trichloro compound.

Our data lead to the following rules, based on the AHH-inducing activities of some two dozen compounds: (1) positions 2, 3, 7, and 8 must contain at least three halogen substituents; (2) substituents at these lateral positions have the order of activity Br > Cl > F > NO_2; (3) at least

one hydrogen atom must remain on the dibenzo-p-dioxin nucleus.

Further Investigations. Further chemical and biological studies on halogenated tricyclic aromatic systems are clearly warranted because of the potential health hazard which they represent and because of the extraordinary potency of certain members of this class as inducers of selected enzyme systems. Investigations continue in these laboratories using ^{14}C-labeled materials to study metabolism, distribution, and storage of such xenobiotics in animals. Chemical studies to extend our synthetic methodology to related aromatic systems are also in progress.

Experimental Section

General Comments. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Ultraviolet (uv) spectra were obtained on a Cary 118 spectrophotometer. Proton magnetic resonance (pmr) spectra were determined with a Jeolco C60HL or JNM HL100 spectrometer and are given in parts per million (δ) downfield from tetramethylsilane as an internal standard. Mass spectra were obtained using a direct (solid) inlet at 70 eV on a Hitachi Perkin-Elmer RMU-6E instrument. The glpc data were obtained on a Perkin-Elmer 900 or a Hewlett-Packard 700 instrument with a hydrogen flame ionization detector, using a 6 ft × 0.125 in. 10% SE-30 cloumn at a nitrogen flow rate of 30 ml/min. Glc–mass spectra were obtained by Varian Associates on a MAT-111 instrument and by the Du Pont Co. on a 490-B mass spectrometer. The Fourier-transform ^{13}C nmr spectra were determined with a Jeolco JNM-PS-100 spectrometer.

Preparative thin layer chromatography (tlc) was carried out on commercially prepared 20 × 20 cm silica gel plates (E. Merck) having the thickness indicated. Dimethyl sulfoxide solvent was dried by distillation at reduced pressure from calcium hydride. Microanalyses were performed by Chemalytics, Inc., Tempe, Ariz. The purity of all halogenated dibenzo-p-dioxins was shown by glc as at least 98% unless otherwise indicated in the text.

Caution: Many of the compounds described here are highly toxic. All of the halogenated dibenzo-p-dioxins should be handled with extreme care, using precautions which parallel work with radioactive compounds. Arrangements should be made for prompt and safe disposal of wastes, all glassware should be rigorously cleaned with chromic–sulfuric acid mixture after use, and disposable gloves, aprons, and absorbent bench-top and hood lining should be used. Contact or absorption of toxic dioxins may lead to acne, porphyria, and irreversible liver damage.

2,3-Dichlorodibenzo-p-dioxin (3). This compound was prepared according to the procedure of Pohland and Yang[6a] in 81% yield after recrystallization from methanol. Three successive recrystallizations from isooctane yielded a colorless solid, mp 159–160° (lit. mp 163–164°), glc retention time (220°) 5.2 min.

Anal. Calcd for $C_{12}H_6O_2Cl_2$: C, 56.95; H, 2.39; Cl, 28.02. Found: C, 57.02; H, 2.41; Cl, 28.25.

2,3-Dibromodibenzo-p-dioxin (4). The dipotassium salt of catechol was prepared by dissolving 110 mg (1.00 mmol) of catechol in 2.0 ml (2.00 mmol) of 1.00 *N* KOH solution. The solution was evaporated to dryness *in vacuo*, 400 mg (1.01 mmol) of tetrabromobenzene was added, and the mixture was refluxed in 2 ml of dry dimethyl sulfoxide under nitrogen for 4 hr. The cooled reaction mixture was poured into 40 ml of cold water and extracted with three 30-ml portions of chloroform. The combined extracts were washed successively with two 30-ml portions of 2% NaOH solution, 30 ml of water, and 30 ml of brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified by tlc (2 mm, elution with hexane, R_f 0.5) to yield 87 mg (25%) of the dibromo compound. Three recrystallizations from isooctane gave a colorless solid: mp 157.5–158°; nmr ($CDCl_3$) δ 6.88 (s, 4), 7.03 (s, 2); uv max ($CHCl_3$) 308 nm (ϵ 3890); mass spectrum (70 eV) *m/e* (rel intensity) 344 (48), 342 (100), 340 (53), 263 (4), 261 (4), 182 (36); glc retention time (200°) 18.3 min.

Anal. Calcd for $C_{12}H_6O_2Br_2$: C, 42.14; H, 1.77; Br, 46.73. Found: C, 42.44; H, 1.72; Br, 46.53.

2,3-Difluorodibenzo-p-dioxin (5). This dioxin, prepared from 1,2,4,5-tetrafluorobenzene by the above procedure, was obtained in 41% yield as a colorless solid: mp 174–176°; nmr ($CDCl_3$) δ 6.88 (s), 6.60 (d, J = 8 Hz); uv max ($CHCl_3$) 296 nm (ϵ 4140); mass spectrum (70 eV) *m/e* (rel intensity) 220 (100), 191 (6), 173 (16), 164 (16); glc retention time (200°) 5.1 min.

Anal. Calcd for $C_{12}H_6O_2F_2$: C, 65.46; H, 2.74. Found: C, 65.50; H, 2.54.

1,3-Dichlorodibenzo-p-dioxin (6). The dipotassium salt of catechol was prepared by dissolving 156 mg (1.42 mmol) of catechol in 2.38 ml (2.88 mmol) of 1.21 *N* KOH solution. This solution was evaporated to dryness at 50° *in vacuo*, 281 mg (1.30 mmol) of 1,2,3,5-tetrachlorobenzene was added, and the mixture was refluxed under nitrogen in 3 ml of dry dimethyl sulfoxide for 19 hr. The cooled reaction mixture was then poured into 15 ml of cold water and extracted with four 10-ml portions of chloroform. The combined extracts were washed successively with three 10-ml portions of 2% NaOH solution, two 10-ml portions of water, and 10 ml of brine, dried ($MgSO_4$), and concentrated *in vacuo*. The concentrate was purified by tlc (0.5 mm, elution with hexane, R_f 0.3) to yield 100 mg (31%) of colorless solid: mp 113.5–114.5°; uv max ($CHCl_3$) 296 nm (ϵ 3100); mass spectrum (70 eV) *m/e* (rel intensity) 254 (68), 252 (100), 217 (5), 189 (24), 161 (6), 126 (20); glc retention time (220°) 4.1 min.

Anal. Calcd for $C_{12}H_6O_2Cl_2$: C, 56.95; H, 2.39; Cl, 28.02. Found: C, 56.95; H, 2.36; Cl, 28.07.

1,2,3,4-Tetrachlorodibenzo-p-dioxin. This substance was formed in 41% yield from hexachlorobenzene, employing the above procedure, as a colorless, crystalline solid: mp 188–190° (lit.[5a] mp 189°); uv max ($CHCl_3$) 315 nm (ϵ 2720); mass spectrum (70 eV) *m/e* (rel intensity) 324 (52), 322 (100), 320 (81), 259 (13), 257 (13, 194 (10); glc retention time (220°) 8.8 min.

Anal. Calcd for $C_{12}H_4Cl_4O_2$: C, 44.76; H, 1.25. Found: C, 45.02; H, 1.17.

Condensation of Catechol with 1,2,3,4-Tetrachlorobenzene. The dipotassium salt of catechol was prepared by dissolving 115 mg (1.05 mmol) of catechol in 1.73 ml (2.09 mmol) of 1.2 *N* KOH solution. This solution was evaporated to dryness *in vacuo*, 217 mg (1.01 mmol) of 1,2,3,4-tetrachlorobenzene was added, and the mixture was refluxed in 0.75 ml of dimethyl sulfoxide under nitrogen for 18 hr. The cooled reaction mixture was poured into 10 ml of cold water and extracted with four 10-ml portions of chloroform. The combined extracts were washed successively with two 10-ml portions of 1% NaOH solution, 10 ml of water, and 10 ml of brine, dried ($MgSO_4$), and concentrated *in vacuo*. The concentrate was purified by tlc (0.5 mm, elution with CCl_4, R_f 0.4) to yield 102 mg (40%) of colorless solid, a mixture of two compounds by glc analysis: mp 80–115°, uv max ($CHCl_3$) 293 nm (ϵ 2440); glc–mass spectral data—retention time (200°) 6.9 min [64%, mass spectrum (70 eV) *m/e* (rel intensity) 256 (10), 254 (64), 252 (100), 217 (8), 191 (9), 189 (27), 161 (10), 127 (12), 126 (28)], 7.7 min [36%, mass spectrum (70 eV) *m/e* (rel intensity) 256 (10), 254 (62), 252 (100), 217 (10), 191 (13), 189 (33), 161 (12), 127 (15), 126 (30)].

Condensation of Catechol with Pentachlorobenzene. This reaction was carried out as described above for the 1,2,3,4-tetrachlorobenzene case, using 117 mg (1.06 mmol) of catechol, 1.76 ml (2.12 mmol) of 1.21 *N* KOH solution, and 256 mg (1.02 mmol) of pentachlorobenzene. Purification of the product by tlc (0.5 mm, elution in CCl_4, R_f 0.4) yielded 103 mg (35%) of a colorless solid, a mixture of two compounds by glc analysis: mp 93–104°; uv max ($CHCl_3$) 296 nm (ϵ 2200); glc–mass spectral data—retention time (200°) 12.2 min [66%, mass spectral data (70 eV) *m/e* (rel intensity) 290 (30), 288 (100), 286 (100), 253 (8), 251 (11), 225 (20), 223 (29), 197 (7), 195 (11), 162 (7), 160 (171)], 13.6 min [34%, mass spectrum (70 eV) *m/e* (rel intensity) 290 (35), 288 (99), 286 (100), 253 (8), 255 (12), 225 (27), 223 (38), 197 (8), 195 (9), 162 (9), 160 (23)].

2,3,7,8-Tetrachlorodibenzo-p-dioxin (1). To a solution of 16 mg (0.063 mmol) of 2,3-dichlorodibenzo-p-dioxin in 2 ml of chloroform in a 12-ml conical test tube were added a small crystal of ferric chloride and a small crystal of iodine. Chlorine gas was slowly bubbled through the solution for 21 hr at room temperature. The mixture was then concentrated to 0.8 ml by evaporation under a stream of nitrogen. The chloroform-soluble material was separated by centrifugation; the precipitate was shaken with another 0.75 ml of chloroform and separated by centrifugation. The precipitate was then shaken with 1.5 ml of chloroform and 0.5 ml of water, the mixture was centrifuged, the aqueous phase was drawn off by pipet, and the chloroform was evaporated to dryness under a stream of nitrogen. The residue was recrystallized from anisole to yield 8.4 mg (41%) of colorless needles. Glc analysis (230°) indicates that the material consists of 85% 2,3,7,8-tetrachlorodibenzo-p-dioxin [retention time 8.3 min; mass spectrum (70 eV) *m/e* 322] and that the remainder is 2,3,7-trichlorodibenzo-p-dioxin [retention time 5.1 min; mass spectrum (70 eV) *m/e* 286] and, presumably, 1,2,3,7,8-pentachlorodibenzo-p-dioxin (retention time 13.2 min). Three successive recrystallizations gave material of >98% purity by glc analysis: mp 305–307° (lit.[6a] mp 305°); uv

max ($CHCl_3$) 306 nm (ϵ 6030); mass spectrum (70 eV) *m/e* (rel intensity) 324 (48), 322 (100), 320 (74), 259 (23), 257 (50), 194 (23).

2,3-Dibromo-7,8-dichlorodibenzo-*p*-dioxin (7). To a solution of 25 mg (0.099 mmol) of 2,3-dichlorodibenzo-*p*-dioxin in 1 ml of chloroform in a 12-ml conical test tube were added a small crystal of iodine, a small crystal of ferric chloride, and 10 drops of bromine. The mixture was allowed to stand for 3 days, during which time several drops of bromine were occasionally added. The chloroform-soluble material was then separated by centrifugation; the precipitate was shaken with another 0.75 ml of chloroform and separated by centrifugation. The precipitate was then shaken with 1.5 ml of chloroform and 0.5 ml of 5% sodium thiosulfate solution, the mixture was centrifuged, the aqueous phase was drawn off by pipet, and the chloroform was evaporated to dryness under a stream of nitrogen. The residue was recrystallized from anisole to yield 31.2 mg (76%) of colorless needles. Glc analysis (230°) indicates that the material is 92% dibromodichloro compound [retention time 17.8 min; mass spectrum (70 eV) *m/e* 408] and 8% bromodichloro compound [retention time 7.7 min; mass spectrum (70 eV) *m/e* 330]. Three successive recrystallizations from anisole gave material of >98% purity by glc analysis. This material had the same retention time as a sample of material prepared by chlorination of 2,3-dibromodibenzo-*p*-dioxin: mp 316–317.5°; uv max ($CHCl_3$) 307 nm (ϵ 4600); mass spectrum (70 eV) *m/e* (rel intensity) 414 (32), 412 (85), 410 (100), 408 (38), 349 (4), 347 (5), 345 (4), 305 (3), 303 (6), 301 (3).

2,3,7,8-Tetrabromodibenzo-*p*-dioxin (8). This compound was prepared as described above for 2,3-dibromo-7,8-dichlorodibenzo-*p*-dioxin (7), using 25.5 mg (0.0745 mmol) of 2,3-dibromodibenzo-*p*-dioxin to yield 24.9 mg (67%) of white crystals. Glc analysis indicates that the material consists of 95% tetrabromo compound [retention time 31.8 min; mass spectrum (70 eV) *m/e* 496] and presumably 5% tribromo compound (retention time 13.5 min). Two successive recrystallizations from anisole gave material of >98% purity by glc analysis: mp 334–336° (lit.[8] mp 334°); uv max ($CHCl_3$) 308 nm; mass spectrum (70 eV) *m/e* (rel intensity) 504 (19), 502 (69), 500 (100), 498 (69), 496 (19), 423 (9), 421 (23), 417 (9), 342 (9), 340 (18), 338 (9).

2,3-Dichloro-7,8-difluorodibenzo-*p*-dioxin (9). To a solution of 26 mg (0.118 mmol) of 2,3-difluorodibenzo-*p*-dioxin (5) in 1 ml of chloroform in a 12-ml conical test tube were added a small crystal of iodine and a small crystal of ferric chloride. Chlorine gas was bubbled slowly through the solution for 22 hr. The solution was shaken with 0.5 ml of water and centrifuged, the aqueous layer was drawn off using a pipet, and the chloroform was evaporated to dryness under a stream of nitrogen. The residue was recrystallized from a small amount of anisole to yield 8.2 mg (24%) of fluffy white crystals: mp 223–225°; uv max ($CHCl_3$) 300 nm (ϵ 3900); mass spectrum (70 eV) *m/e* (rel intensity) 290 (66), 288 (100), 227 (6), 225 (18), 162 (100); glc retention time (200°) 6.5 min.

2,3-Dibromo-7,8-difluorodibenzo-*p*-dioxin (10). Bromination of 2,3-difluorodibenzo-*p*-dioxin (5) by the procedure described above for compound 7 gave the dibromo derivative **10:** mp 210–212°; uv max ($CHCl_3$) 301 nm (ϵ 4600); mass spectrum (70 eV) *m/e* (rel intensity) 380 (50), 378 (100), 376 (52), 299 (3), 297 (3), 271 (7), 269 (7), 218 (32), 188 (5); glc retention time (200°) 11.4 min (97%).

4,5-Dimethylcatechol. This compound was prepared in 33% yield by the method of Teuber and Staiger,[10] mp 83–85° (lit. mp 87–88°).

2,3-Dichloro-7,8-dimethyldibenzo-*p*-dioxin (11). The dipotassium salt of 4,5-dimethylcatechol was prepared by dissolving 24.6 mg (0.178 mmol) of the catechol in 0.30 ml (0.363 mmol) of 1.21 *N* KOH solution. The solution was evaporated to dryness *in vacuo*, 36 mg (0.167 mmol) of 1,2,4,5-tetrachlorobenzene was added, and the mixture was refluxed in 2 ml of dry dimethyl sulfoxide under nitrogen for 18 hr. The cooled reaction mixture was poured into 6 ml of cold water and extracted with four 10-ml portions of chloroform. The combined extracts were washed successively with 10 ml of 1% NaOH solution, 10 ml of water, and 10 ml of brine, dried ($MgSO_4$), and concentrated *in vacuo* to 47 mg of solid. Recrystallization from anisole yielded 22.9 mg (49%) of colorless solid: mp 231–231.5°; nmr ($CDCl_3$) δ 2.15 (s, 6), 6.60 (s, 2), 6.95 (s, 2); uv max ($CHCl_3$) 301 nm (ϵ 4200); mass spectrum (70 eV) *m/e* (rel intensity) 282 (65), 280 (100), 267 (13), 265 (22); glc retention time (220°) 11.5 min.

Anal. Calcd for $C_{14}H_{10}O_2Cl_2$: C, 59.81; H, 3.59; Cl, 25.22. Found: C, 60.05; H, 3.50; Cl, 24.94.

2,3-Dibromo-7,8-dimethyldibenzo-*p*-dioxin (12). This dioxin was formed by condensation of 4,5-dimethylcatechol and 1,2,4,5-tetrabromobenzene according to the procedure reported for the dichloro analog **11:** mp 229–230°; uv max ($CHCl_3$) 302 nm (ϵ 4800); mass spectrum (70 eV) *m/e* (rel intensity) 372 (48), 370 (100), 368 (52), 357 (7), 355 (14), 353 (7), 210 (16); glc retention time (230°) 11.6 min.

4-Chlorocatechol. This intermediate was prepared in 44% yield by the method of Willstätter and Müller,[11] mp 86–87.5° (lit. mp 90–91°).

4,5-Dichlorocatechol. This substance was prepared in 24% yield by the method of Willstätter and Müller,[11] mp 115.5–117.5° (lit. mp 116–117°).

2,3,7-Trichlorodibenzo-*p*-dioxin. The dipotassium salt of 4-chlorocatechol was prepared by dissolving 20.0 mg (0.138 mmol) of the catechol in 0.24 ml (0.29 mmol) of 1.21 *N* KOH solution. The solution was evaporated to dryness *in vacuo*, 26.5 mg (0.123 mmol) of 1,2,4,5-tetrachlorobenzene was added, and the mixture was refluxed under nitrogen in 0.5 ml of dry dimethyl sulfoxide for 22 hr. The cooled reaction mixture was poured into 7 ml of cold water and extracted with three 10-ml portions of chloroform. The combined extracts were washed successively with two 10-ml portions of 2% NaOH solution, 10 ml of water, and 10 ml of brine, dried ($MgSO_4$), and concentrated by evaporation of the chloroform under a stream of nitrogen to leave 26.8 mg of brown solid. This material was sublimed (120°, 1 mm) to yield 16.0 mg (50%) of a colorless solid. A second sublimation yielded 14.8 mg of solid. Glc analysis (230°) indicated that the material consists of 86% of the desired trichloro compound (retention time 3.9 min) and 14% 2,3-dichlorodibenzo-*p*-dioxin (retention time 2.3 min): mp 153–158°; uv max ($CHCl_3$) 304 nm (ϵ 3460); mass spectrum (70 eV) *m/e* (rel intensity) 290 (34), 288 (100), 286 (100), 253 (10), 251 (10), 225 (28), 223 (46). Further crystallizations or sublimations gave no improvement in homogeneity of this sample.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (1). The dipotassium salt of 4,5-dichlorocatechol was prepared by dissolving 29.0 mg (0.162) mmol) of the catechol in 0.29 ml (0.29 mmol) of 1.00 *N* KOH solution. The solution was evaporated to dryness *in vacuo*, the last traces of water were removed by azeotroping with mixtures of benzene and ethanol, 27.1 mg (0.126 mmol) of 1,2,4,5-tetrachlorobenzene was added, and the mixture was refluxed under nitrogen in 1.5 ml of dry dimethyl sulfoxide for 17 hr. The cooled reaction mixture was poured into 10 ml of cold water and extracted with ten 10-ml portions of chloroform. The combined extracts were washed successively with two 20-ml portions of 2% NaOH solution, 20 ml of water, and 20 ml of brine, dried (Na_2SO_4), filtered, and evaporated to dryness under a stream of nitrogen. The residue was washed with two 0.5-ml portions of chloroform, using a centrifuge to concentrate the solid and pipeting off the solvent. The solid was sublimed (240–250°) to give 7.6 mg (19%) of colorless needles. Glc analysis (230°) indicated that this material is 99% pure: mp 306–307° (lit.[6a] mp 305°); uv max ($CHCl_3$) 306 nm (ϵ 6000); mass spectrum (70 eV) *m/e* (rel intensity) 324 (48), 322 (100), 320 (74), 259 (23), 257 (50), 194 (23); glc retention time (230°) 8.3 min.

1,3,7,8-Tetrachlorodibenzo-*p*-dioxin (13). Condensation of 4,5-dichlorocatechol with 1,2,3,5-tetrachlorobenzene by the above procedure gave 40% of colorless dioxin **13:** mp 193.5–195°; uv max ($CHCl_3$) 304 nm (ϵ 4160); mass spectrum (70 eV) *m/e* (rel intensity) 324 (54), 322 (100), 320 (84), 287 (5), 285 (5), 259 (19), 257 (19), 194 (11); glc retention time (220°) 10.8 min.

Anal. Calcd for $C_{12}H_4O_2Cl_4$: C, 44.76; H, 1.25. Found: C, 45.13; H, 1.51.

Acknowledgment. We are grateful to Dr. Albert Pohland (Food and Drug Administration) and Dr. Wesley A. Muelder (Dow Chemical Co.) for providing samples and technical information prior to publication, to Professor Henry Gilman for furnishing samples of dibenzo-*p*-dioxins from his laboratory, to Professor T. Krugh for advice on cmr spectroscopy, and to Mr. Edward Glover for technical assistance. This work was supported in part by NIH Postdoctoral Grants 1-FO2-CA 55053-01 (to J. J. W.) and 5-FO3-ES 46196 (to A. P.), an NIH Center Grant for Toxicology Research and Training (2P-11-GM 15190-06A1), and research grants CA 11326 from the National Cancer Institute and ES-00965 from the National Institute of Environmental Health Sciences, U. S. Public Health Service.

Registry No.—**1**, 1746-01-6; **3**, 29446-15-9; **4**, 50585-37-0; **5**, 50585-38-1; **6**, 50585-39-2; **7**, 50585-40-5; **8**, 50585-41-6; **9**, 50585-

42-7; **10**, 50585-43-8; **11**, 50585-44-9; **12**, 50585-45-0; **13**, 50585-46-1; catechol dipotassium salt, 50585-47-2; 1,2,4,5-tetrafluorobenzene, 327-54-8; 1,2,3,4-tetrachlorodibenzo-*p*-dioxin, 30746-58-8; hexachlorobenzene, 118-74-1; 1,2,3,4-tetrachlorobenzene, 634-66-2; pentachlorobenzene, 608-93-5; 4,5-dimethylcatechol dipotassium salt, 50585-48-3; 2,3,7-trichlorodibenzo-*p*-dioxin, 33857-28-2; 4-chlorocatechol dipotassium salt, 50585-49-4; 4,5-dichlorocatechol dipotassium salt, 50585-50-7.

References and Notes

(1) (a) NIEHS Conference on the Toxicity of the Chlorinated Dibenzo-*p*-dioxins and Dibenzofurans, April 2–3, 1973, Research Triangle Park, N. C.; *Environ. Health Perspec.*, (5) (1973). (b) Report on 2,4,5-T, A Report of the Panel on Herbicides of the President's Science Advisory Committee, U. S. Government Printing Office, Washington, D. C., March 1971. (c) Report of the Advisory Committee on 2,4,5-T to the Administrator of the Environmental Protection Agency, U. S. Government Printing Office, Washington, D. C., May 1971. (d) B. A. Schwetz, J. M. Norris, G. L. Sparschu, V. K. Rowe, P. J. Gehring, J. L. Emerson, and C. G. Gerbig, *Advan. Chem. Ser.*, **120**, 55 (1973).

(2) (a) J. Bleiberg, M. Wallen, R. Brodkin, and I. Appelbaum, *Arch. Dermatol.*, **89**, 793 (1964); (b) J. von Kinnig and K. Schultz, *Dermatologica*, **115**, 540 (1957); (c) K. Schultz, *Arch. Klin. Exp. Dermatol.*, **206**, 589 (1957); (d) J. von Kinnig and K. Schultz, *Naturwissenschaften*, **44**, 337 (1957).

(3) (a) A. Yartzoff, D. Firestone, D. Barnes, W. Horwitz, L. Friedman, and S. Neshwin, *J. Amer. Oil Chem. Soc.*, **38**, 60 (1961); (b) J. S. Cantrell, N. C. Webb, and A. J. Mabis, *Acta Crystallogr., Sect. B*, **25**, 150 (1969).

(4) (a) G. L. Sparschu, F. L. Dunn, and V. K. Rowe, *Food Cosmet. Toxicol.*, **9**, 40S (1971); (b) K. D. Courtney and J. A. Moore, *Toxicol. Appl. Pharmacol.*, **20**, 396 (1971); (c) K. D. Courtney, D. W. Gaylor, M. D. Hogan, H. L. Falk, R. R. Bates, and I. Mitchell, *Science*, **168**, 864 (1970); (d) S. Hussain, L. Ehrenberg, G. Loforth, and T. Gejvall, *Ambio*, **1**, 32 (1972).

(5) A. Poland and E. Glover, *Science*, **179**, 476 (1973).

(6) (a) A. E. Pohland and G. C. Yang, *J. Agr. Food Chem.*, **20**, 1093 (1972); (b) W. W. Muelder and L. A. Shadoff, *Advan. Chem. Ser.*, **120**, 1 (1973).

(7) O. Aniline, *Advan. Chem. Ser.*, **120**, 126 (1973).

(8) H. Gilman and J. J. Dietrich, *J. Amer. Chem. Soc.*, **79**, 1439 (1957).

(9) F. P. Boer, F. P. van Remoortere, P. P. North, and M. A. Newman, *Acta Crystallogr., Sect. B*, **28**, 1023 (1972); F. P. Boer, F. P. van Remoortere, and W. A. Muelder, *J. Amer. Chem. Soc.*, **94**, 1006 (1972).

(10) H. J. Teuber and G. Staiger, *Chem. Ber.*, **88**, 802 (1955).

(11) R. Willstätter and H. E. Müller, *Chem. Ber.*, **44**, 2182 (1911).

(12) A. E. Pohland, G. C. Yang, and N. Brown, Paper No. 2 presented at the NIEHS Conference on the Toxicity of the Chlorinated Dibenzo-*p*-dioxins and Dibenzofurans, April 1973, Research Triangle Park, N. C.; *Environ. Health Perspec.*, (5), 9 (1973).

(13) A. R. Tarphy, Jr., and J. H. Goldstein, *J. Mol Spectrosc.*, **37**, 432 (1971).

Synthesis of Some Tricyclic Nucleosides Related to the "Y" Base of tRNA[1a,b]

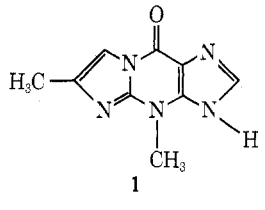
Gary L. Anderson,[1c] Boshra H. Rizkalla, and Arthur D. Broom*

Department of Biopharmaceutical Sciences, College of Pharmacy, University of Utah, Salt Lake City, Utah 84112

Received September 27, 1973

The synthesis of three tricyclic nucleosides, 5*H*(7*H*)-9-oxo-3-β-D-ribofuranosyl-1,2,4-triazolo[2,3-*a*]purine (**3**), 6,7-dimethyl-10-oxo-3-β-D-ribofuranosyl-1,2,4-triazino[2,3-*a*]purine (**4**), and 10-oxo-3-β-D-ribofuranosyl-1,2,4-triazino[2,3-*a*]purine (**5**), is reported. These are structural analogs of the "Y" base of tRNA. The use of the nuclear Overhauser effect in proton assignment of **3** is described, as well as the fluorescence of **3** and **4**. Covalent hydration of **5** is discussed.

Transfer ribonucleic acids specific for phenylalanine ($tRNA^{Phe}$) from a variety of sources have recently been shown to contain certain highly fluorescent heterocyclic bases, the simplest of which is the tricyclic derivative **1**.[2] Other tricyclic fluorescent derivatives of naturally occurring purines, exemplified by 1,N^6-ethenoadenosine (ε-adenosine), have recently been synthesized[3] and shown to enter into a number of biochemical pathways.[4] The availability of 1-aminoguanosine (**2**) in our laboratories[5] led us to undertake the synthesis of certain tricyclic nucleosides derived from guanosine and structurally related to the "Y" base (**1**).



1

Results and Discussion

Synthetic Aspects. The cyclization procedures used to obtain the tricyclic nucleosides are shown in Scheme I. Attempts to prepare the triazolopurine ribonucleoside **3** using diethoxymethyl acetate[6] gave complex mixtures from which **3** could be isolated only with great difficulty. The procedure of Clark and Lister[7] using DMF-$POCl_3$ has been widely used for cyclization of weakly basic 1,2-diamino compounds. Application of this procedure to 1-aminoguanosine (**2**) gave the desired **3** in good yield. The structure of **3** was confirmed by elemental analysis and uv and pmr spectra. The uv spectra (Table I) reveal substantial bathochromic shifts relative to starting material **2** and are very similar to those reported for the imidazo[1,2-*a*]purine ribonucleoside obtained by the reaction of guanosine with glycidaldehyde.[8]

The condensation of 1,2-diaminopyrimidines with 1,2-dicarbonyl compounds (the Isay synthesis) has found extensive use in the synthesis of pteridines.[9] Application of this reaction with **2** using biacetyl and glyoxal gave 6,7-dimethyl-10-oxo-3-β-D-ribofuranosyl-1,2,4-triazino[2,3-*a*]purine (**4**) and its unmethylated derivative **5**, respectively. Nucleoside **4** was found to have elemental analysis and uv and pmr spectra consistent with the assigned structure (Scheme I). The uv spectra of **5**, however, were grossly different from those of its dimethyl counterpart **4** (Table I), the pmr spectra were incompatible with structure **5** (Table II), and elemental analysis revealed the presence of 2.5 equiv of water/mol of **5**. The data were consistent with the existence in solution of **5** as a covalent hydrate and **4** as the anhydrous molecule. The interpretation receives support from the observation by Clark[10] that ethyl pteridine-4-carboxylate readily forms a covalent hydrate in solution whereas its 6,7-dimethyl derivative is only slightly hydrated at equilibrium, and is confirmed by the pmr data discussed below.

Pmr Considerations. Examination of the aromatic region of the pmr spectrum of the triazolo[2,3-*a*]purine nucleoside **3** revealed two one-proton singlets downfield 0.30 and 0.80 ppm from the H-8 signal of 1-aminoguanosine (**2**)