Antiviral Phenoxathiins and Their Analogs. Study of the Structure–Activity Relationships for Antiviral Activity and the Replacement Ability in Poliovirus Type III Dependent Variants

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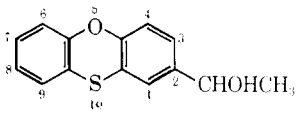
The antiviral activity of α-methyl-2-phenoxathiinmethanol (MPM) and its structural modifications has been investigated in tissue culture. During studies intended to measure antiviral activity against poliovirus type III, the appearance of drug-resistant variants, which were dependent on MPM, was noted. The ability of the structurally modified MPM derivatives to inhibit poliovirus type III and replace MPM in dependent mutants has been measured and related to the chemical structural changes performed.

In a search to find effective chemotherapeutic agents for viral diseases, α-methyl-2-phenoxathiinmethanol (MPM) (I) was found to be a potent inhibitor of poliovirus type III in tissue culture. Modifications of MPM were made and studied in order to define the



I

structure requirements for this activity.

In studies of the inhibition of poliovirus type III by MPM,[1] complete plaque reduction was obtained over a wide range of concentrations (0.7 to 12 μg of MPM/ml of agar overlay). Poliovirus type III yield was dependent upon drug concentration and virus input. For example, the 72-hr yield was reduced from $10^{7.5}$ plaque-forming units (PFU) per ml in untreated cultures to $10^{3.8}$ PFU/ml in cultures containing 6 μg/ml of MPM with an input of 1000 PFU/ml. MPM also gave good zones of protection, using a modification of the agar diffusion test.[2,3]

During these studies of the antiviral activity of MPM, we noted the development of stable variants of poliovirus type III that were dependent on MPM for maximum growth. Virus variants acting as resistant variants were also obtained, but we were unable to prove whether this state involved mixtures of sensitive and dependent variants. These observations allowed us to examine the structure–activity relationships (SAR) involved in inhibiting the sensitive virus and to compare this SAR with that involved in fulfilling dependency for the MPM-dependent variant virus.

Previous reports have described the isolation of variant strains of viruses which have become resistant to these virus inhibitors: guanidine,[4] α-hydroxybenzylbenzimidazole (HBB),[5] and 5-iodo-2-deoxyuridine (IDU).[6] In addition, virus variants showing drug dependency[4b,7] have been reported for guanidine[8] and HBB.[9] These reports include both DNA and RNA viruses, but they have usually involved single compounds or only a few related compounds. The potential importance of an understanding of the development of drug resistance and drug dependency encouraged us to examine the SAR of analogs of MPM. In addition, observation of the development of drug dependency assured us that these compounds were having a direct effect on virus multiplication.

Methods

Chemistry.—The compounds reported were prepared as shown in Scheme I. The desired Me and Et ketones were prepared in good yield by the Friedel–Crafts reaction. Inverse addition of the acid chloride–$AlCl_3$ complex, to the aromatic heterocycle at 5° in 1,2-dichloroethane avoided diacetylation side products. The structural assignments of the ketones were based on physical data reported in the literature. The ketones were reduced smoothly by $NaBH_4$ to the alcohols from which the remainder of the derivatives were obtained.

Assay for Virus Inhibition.—The ability of MPM-related compounds to inhibit virus multiplication was estimated by employing the plaque-reduction test. Three drug concentrations were selected, based on the known activity of MPM. These drug concentrations included one with excess drug and one known to be below the quantity needed for complete plaque reduction. All compounds were compared at equal concentrations on a weight basis. Three experiments were conducted on different days. The assay involved growing a confluent sheet of BSC-1 cells in tissue-culture flasks, infecting the monolayers with a countable number of PFU (40–200) of wild-type poliovirus type III, applying a nutrient agar overlay containing the drugs to the infected cells, incubating, inactivating the virus, staining, and counting the number of plaques per flask. Per cent inhibition was obtained by comparing counts

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(2) E. C. Herrmann, Jr., J. Gaöliks, C. Engle, and P. L. Perlman, *Proc. Soc. Exp. Biol. Med.*, **103**, 625 (1960).

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(6) H. E. Renis, D. A. Bothala, *Ann. N. Y. Acad. Sci.*, **130**, 343 (1965).

(7) (a) H. J. Eggers and I. Tamm, *Int. Congr. Microbiol., 8th*, (Abstracts) 85 (1962); (b) H. J. Eggers, *Cold Spring Harbor Symp. Quant. Biol.*, **27**, 309 (1962); (c) N. Ledinko, *ibid.*, **27**, 309 (1962).

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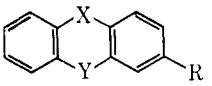
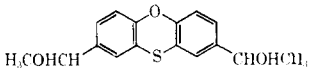
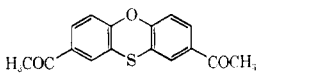
TABLE I

INHIBITION OF WILD-TYPE POLIO AND DEPENDENCY FULFILLMENT IN POLIO VARIANTS BY PHENOXATHIIN ANALOGS



No.	Formula	X	Y	R	Mp, °C	% inhibition of wild-type poliovirus type III (μg/ml)			% ability to fulfill dependency (μg/ml)			Class
						10[a]	2	0.4	10	2	0.4	
1	$C_{14}H_{12}O_2S$	O	S	$CHOHCH_3$	66–68[b]	100[c]	100	85	90	87	0	1
2	$C_{14}H_{12}OS_2$[d,e]	S	S	$CHOHCH_3$		100	24	12	89	0	0	2
3	$C_{14}H_{12}O_3S$	O	S→O	$CHOHCH_3$	105–107	15	15	12	0	0	0	3
4	$C_{14}H_{12}O_4S$	O	SO_2	$CHOHCH_3$	101–103	18	12	1	0	0	0	3
5	$C_{15}H_{14}O_2$	O	CH_2	$CHOHCH_3$	85–87	100	100	22	95	79	0	1
6	$C_{15}H_{14}O$		CH_2	$CHOHCH_3$	134–135	100	93	22	81	51	0	1
7	$C_{14}H_{10}O_2S$[f]	O	S	$COCH_3$	117–118	100	100	99	100	100	8	1
8	$C_{14}H_{10}OS_2$	S	S	$COCH_3$	70–71[g]	100	69	17	100	11	0	2
9	$C_{14}H_{10}O_2S$	O	S→O	$COCH_3$	128–130[e]	13	20	20	0	0	0	3
10	$C_{14}H_{10}O_4S$	O	SO_2	$COCH_3$	164–165	10	12	12	0	0	0	3
11	$C_{15}H_{12}O_2$	O	CH_2	$COCH_3$	103–104	T[h]	100	50	T	100	0	1
12	$C_{14}H_{10}O_2$	O		$COCH_3$	83–84[i]	100	100	33	81	85	0	1
13	$C_{14}H_{10}O_2$		O	$COCH_3$	144–145[j]	100	100	75	89	96	3	1
14	$C_{15}H_{12}O_2S$	O	S	$COCH_2CH_3$	77–79	100	62	18	34	0	0	2
15	$C_{15}H_{14}O_2S$[k]	O	S	$CHOHCH_2CH_3$		100	31	16	35	0	0	2
16	$C_{15}H_{14}O_2S$	O	S	$C(OH)(CH_3)_2$	95–96	100	22	14	3	0	0	3
17	$C_{14}H_{11}NO_2S$	O	S	$C(=NOH)CH_3$	152–154[l]	100	94	16	100	14	0	2
18	$C_{14}H_{11}NOS_2$	S	S	$CH(NH_2\cdot HCl)CH_3$	132–133[m]	61	22	18	55	0	0	3
19	$C_{14}H_{13}NOS\cdot HCl$	O	S	$CHCH_3$	245–246	50	23	13	0	0	0	3
20	$C_{15}H_{14}O_2S$	O	S	$CH(OCH_3)CH_3$	85	18	10	86	0	0	0	2
21	$C_{16}H_{15}NO_3S$	O	S	$CHCH_3$	124–126	T	22	24	T	0	0	3
22	$C_{25}H_{19}NO_3S$	O	S	$CHCH_3$	143–145	41	10	20	50	0	0	3
23	$C_{16}H_{14}O_3S$	O	S	$CHCH_3$		96	100	33	87	73	0	1
24	$C_{16}H_{16}O_3S$					47	20	21	3	0	0	3
25	$C_{16}H_{12}O_3S$				181–182	5	13	10	0	0	0	3

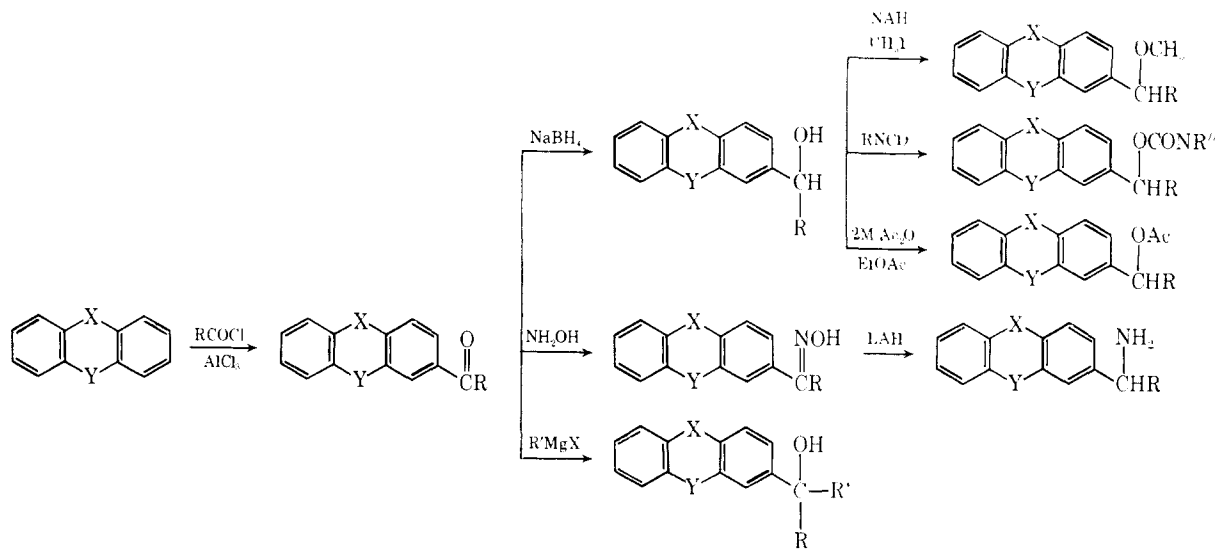
[a] Concentration of drug in agar overlay. [b] R. G. Flowers and L. W. Flowers, *J. Amer. Chem. Soc.*, **71**, 3102 (1949) report mp 65–67°. [c] Values represent the average of three separate tests, each test value being the average of three counts from different cultures. [d] Yielded an oil having a good combustion analysis. [e] Purified by chromatography. [f] J. F. Nobis, A. J. Blardinelli, and D. J. Blaney [*J. Amer. Chem. Soc.*, **75**, 3384 (1953)] report a 25.8% yield. [g] M. Janczewski and M. Dec, *Rocz. Chem.*, **37**, 745 (1961); *Chem. Abstr.*, **55**, 23541*i* (1961) report mp 73–74°. [h] Toxic level. [i] I. V. Andreeva and M. M. Koton [*Zh. Obshch. Khim.*, **27**, 671 (1957); *Chem. Abstr.*, **51**, 16409*e* (1957)] report mp 81°. [j] J. N. Chatterjea [*J. Indian Chem. Soc.*, **33**, 447 (1956); *Chem. Abstr.*, (1957)] **51**, 6594*f* reports mp 144°. [k] Gave a mass spectrum consistent with the structure. [l] E. Lescot, Ng. Ph. Buu-Hoi, N. D. Xuong [*J. Chem. Soc.*, 2408 (1956)] report mp 153°. J. F. Nobis, A. J. Blardinelli, D. J. Blaney [*J. Amer. Chem. Soc.*, **75**, 3384 (1953)] report a crude mp 154–157°, recrystd 158–159.5°. [m] M. Janczewski and M. Dec [*Rocz. Chem.*, **35**, 745 (1961); *Chem. Abstr.*, **55**, 23541*i* (1961)] report mp 135–136°.

from drug-treated flasks with counts from untreated flasks.

Assay for Fulfilling Dependency.—The assay conditions for the ability of MPM-related compounds to fulfill dependency were similar to those used for inhibition; however, a variant of poliovirus type III[1] was used that grew at a normal rate in the presence of MPM, but not in the absence of MPM or MPM-related compounds. Formation of virus plaques indicated ability to fulfill dependency for MPM. This ability was measured by comparing the plaque counts of drug-treated flasks with average counts from flasks treated with those five compounds which gave the highest counts.

Statistical Analysis.—A statistical analysis was used in an attempt to quantify and reduce the data into ratings which hopefully would aid the interpretation of the relationship of structure to biological activity.

Scheme I



Compounds were compared at an equal concentration and were ranked according to the degree of inhibition and the degree of fulfilling the dependency requirement. A combination of the number of runs plus the sum of rankings allowed assignment of a probability. The probabilities were then combined for all concentrations for each compound. These statistics were reparameterized into ratings in which 1.0 reflects the highest obtainable activity over these levels of drugs and 0 the lowest. The compounds in this study fell into three clusters: 0.6 (class 1), 0.3 (class 2), and 0 (class 3), reflecting the relative activity at these doses.

SAR Relationships.—The SAR studies indicate that ability to inhibit wild-type poliovirus type III multiplication and the ability to fulfill the dependency requirement of an MPM-dependent variant of poliovirus type III are directly related. Any compound in this series that inhibits virus multiplication also fulfills the dependency requirement. This is in contrast to other nonrelated poliovirus inhibitors such as aranotin, guanidine, HBB, fluorophenylalanine, and antiserum, all of which inhibit wild-type virus and the dependent variant in the presence of MPM but will not replace MPM.[1]

One objective of the SAR study was to find compounds which would carry out one function and not the other, but this was not accomplished on a qualitative basis. At some drug concentrations that caused inhibition, dependency is not fulfilled, and *vice versa;* this appears to be due to the shape of the dose–response curve. Comparison of drug potency for the SAR includes both ability to inhibit virus multiplication and ability to fulfill dependency.

The most active compounds (class 1) had structures in which R was $CHOHCH_3$, $COCH_3$, or $CH(OCOCH_3)CH_3$ (**1**, **5**, **6**, **7**, **11**, **12**, **13**, **23**). All other R modifications led to less active compounds. Class 1 compounds include only the above R groups when the X and Y bridging heteroatoms are different. When X = Y = S, as in **2** and **8**, the activity decreased to a class 2 type activity. Oxidation of bridging S atoms at Y to sulfones or sulfoxides led in all cases to less active class 3 derivatives (**3**, **4**, **9**, **10**).

Increasing the aliphatic chain length of class 1 compounds like **1** and **7** by one CH_2 (**14**, **15**) reduced their activity to that of class 2.

Changing **1** (class 1) from a secondary to a tertiary alcohol (**16**) caused a decrease in activity to that of a class 3 compound. Conversion of alcohol **1** into a methyl ether (**20**) led to decreased activity.

The inactivity of **24** was disappointing since this compound was designed to act as a bidentate analog of MPM. If the inhibiting action of MPM analogs is in any way due to interaction of the molecules with macromolecules, an additional functional group at a distal position could be considered a logical extension to increase activity. Further studies are in progress to elaborate the mode of action of this series.

Anderson, *et al.*,[10,11] have reported that mono- and disubstituted phenoxathiin glyoxals substituted in the 2 and 8 positions like **24** and **26** are active against Newcastle Disease Virus and PR8 influenza virus in eggs. This series of glyoxals has a virus spectrum different from the compounds we have examined and, presumably, involves a different mechanism.[12] In addition, the bisglyoxal was more active than the monoglyoxal,[10] which is in contrast to the results reported here for the phenoxathiin ketones (**7**, **25**) and alcohols (**1**, **24**). We found the 2-phenoxathiin glyoxal to be inactive in wild-type poliovirus type III.

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are uncorrected. Ir bands, nmr, and mass spectra were consistent for the proposed structures. All compounds were analyzed for C and H and were within ±0.4% of the theoretical value.

Methyl 2-Phenoxathiinyl Ketone (7).—A soln of AcCl (78.5 g, 1.0 mol) in 100 ml of 1,2-dichloroethane (DCE) was added to 350 ml of DCE containing $AlCl_3$ (133.0 g, 1.0 mol). This mixture was stirred at room temp for 30 min and then was added dropwise with stirring to a chilled (5°) soln containing phenoxathiin (200 g, 1.0 mol) in 750 ml of DCE. The reaction mixture was allowed to return slowly to room temp and was then poured into a mixture of 600 ml of 12 *N* HCl and 500 ml of ice. The organic layer was sepd, and the aq layer was extracted twice with

(10) E. L. Anderson, J. E. Casey, Jr., M. Enas, E. E. Force, E. M. Jensen, R. S. MacG. and D. E. Rivard, *J. Med. Chem.*, **6**, 787 (1963).
(11) E. L. Anderson, U.S. Patent 3,117,121, Jan 30, 1961.
(12) G. E. Underwood and S. D. Weed, *Virology*, **13**, 138 (1961).

$CHCl_3$. The organic layers were combined, dried, and evapd *in vacuo*. The product was recrystd from a C_6H_6–hexane solvent mixture to give 174.9 g (72%), mp 117–118°.

Methyl 2-Phenoxathiinyl Ketone 10-Oxide (9).—A solution of *m*-chloroperbenzoic acid (5.3 g, 0.026 mol) in 25 ml of CH_2Cl_2 was added to 5.0 g (0.026 mol) of **7** dissolved in 100 ml of CH_2Cl_2. This was maintained at −70° for 30 min. The reaction mixture was washed with aq $NaHCO_3$, and the solvent was evapd *in vacuo*. Tlc of the residue indicated a mixture of sulfoxide and sulfone. The components were separated by chromatography over silica gel. The sulfone was eluted with 4:1 C_6H_6–EtOAc, and the desired sulfoxide with EtOAc alone. The column afforded 3.3 g (49.3%) of the sulfoxide, mp 128–130°.

Methyl 2-Phenoxathiinyl Ketone 10,10-Dioxide (10).—To compd **7** (24.2 g, 0.1 mol) in 250 ml of glacial AcOH at 60° was added, rapidly, with stirring, 30% H_2O_2 (24 ml, 0.24 mol). This was heated at 80° for 4 hr. Upon cooling, the product crystallized from AcOH. After filtration and drying, 24.5 g (89.5%) of the product was obtained, mp 168–170°.

2-(1-Methoxyethyl)phenoxathiin (20).—In a flame-dried apparatus under N_2, 50% NaH in oil suspension (1.0 g, 0.0204 mol) was added to 24 ml of PhMe. A soln of **1** (5.0 g, 0.0204 mol) in 100 ml of PhMe was added. The reaction mixture was refluxed for 2 hr and then cooled to room temp. MeI (3.0 g, 0.0204 mol) was added dropwise with stirring. The reaction mixture was washed with H_2O to remove the NaI formed, dried, filtered, and evapd *in vacuo*. The residue proved to be two-spot material by tlc. The residue was dissolved in C_6H_6–EtOAc and passed over 300 g of silica gel. Short-path distillation of the eluate from the column yielded 2.9 g (54.6%) of 2-(1-methoxyethyl)phenoxathiin.

2-(Methylcarbamate-1-ethyl)phenoxathiin (21).—A mixture of **1** (6.8 g, 0.028 mol), K_2CO_3 (0.83 g, 0.006 mol), CH_2Cl_2 (20 ml), 1 drop of H_2O, 1 drop of MeOH, and MeNCO (1.87 g, 0.033 mol) was refluxed overnight. The solvent was removed *in vacuo*, and the crude product was recrystd from Me_2CO–hexane to give 5.1 g (60.8%), mp 124–126°.

2-(1-Acetoxyethyl)phenoxathiin (23).—By the method of Fritz and Schenk[13] a soln of 2 *M* Ac_2O in EtOAc was made. To 5.0 g (0.0204 mol) of **1** was added 33 ml of 2 *M* Ac_2O at 5°. This was stirred for 10 min. The soln was washed with satd $NaHCO_3$, dried, filtered, and evapd *in vacuo*. The crude product was short-path distd to give 5.8 g of product (97.6%).

Acknowledgment.—We wish to thank Dr. C. E. Redman for statistical analysis of biological data.

(13) J. S. Fritz and G. H. Schenk, *Anal. Chem.*, **31**, 1808 (1959).

Structure–Activity Correlations for the Central Nervous System Depressant 2-Imidazolidinones

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The CNS depressant activities of a series of substituted 2-imidazolidinones are found to be highly dependent on the log of the octanol–water partition coefficient. Chlorpromazine fits the same regression lines. Exceptions to this dependence on the lipohydrophilic character are discussed in terms of steric factors.

The dependence of biological activity in a set of congeneric drugs on lipohydrophilic character has been shown in many types of drug action.[2] In particular, a study by Hansch and his coworkers[2a] pointed out that variation in the CNS depression activity of several types of hypnotics depends parabolically upon the relative lipohydrophilic character as represented by the 1-octanol–water partition coefficient (log *P*). The hypnotics reported included barbiturates, thiobarbiturates, tertiary alcohols, carbamates, substituted amides, and *N,N'*-diacylureas. In all of these compounds a value of 2 was found for log *P* for maximum activity (log P_0). Similar log P_0 values for this wide range of structurally dissimilar compounds are taken as a strong indication that these compounds have the same rate-determining step in producing CNS depression. This step may lie in the penetration of the inter- and/or intracellular membranes or one of the prior barriers such as the blood–brain barrier.

As an extension of this type of study, interesting and ample biological data published by Wright, *et al.*,[3] were analyzed by regression analysis.

Methods

The set of congeners analyzed was a series of substituted 2-imidazolidinones (ethyleneurea derivatives). Chlorpromazine was included for comparison (see Table I and Figure 1). To place the drugs on a common basis for quantitation the biological activity, given originally in milligrams per kilogram, was converted into log $1/c$ where c is the effective concentration in moles per kilogram.

Three different measurements of CNS depression were used by Wright, *et al.*,[3] (1) reduction of motor activity of mice by 50% as measured by an actophotometer (MDD_{50}), (2) inhibition of 50% of the test group of mice to walk from the midpoint of a horizontal steel rod to a platform at the ends (RWD_{50}), and (3) inability of 50% of the test mice to remain on a 60° inclined screen (ISD_{50}). All injections were intraperitoneal.

The log *P* values were calculated from the experimental value of 1-(2-dimethylaminoethyl)-3-(*m*-methoxyphenyl)-2-imidazolidinone and the π constants reported by Hansch and his coworkers[2,4] (see Table II).

The Hammett's σ constant, a measure of the electronic effect of a substituent, was given adequate analysis, and the steric effect was not considered quantitatively. σ values were included only for the substituents on the phenyl ring. Since the alkyl sub-

(1) (a) To whom requests for reprints should be addressed; (b) R. M. and J. L. Converse Fund Fellow; (c) NSF–URP participant, summer, 1969, GY-5829.

(2) (a) C. Hansch, A. R. Steward, S. M. Anderson, and D. Bentley, *J. Med. Chem.*, **11**, 1 (1968); (b) E. J. Lien, C. Hansch, and S. M. Anderson, *ibid.*, **11**, 430 (1968); (c) E. J. Lien, *J. Agr. Food Chem.*, **17**, 1265 (1969); (d) E. J. Lien, Symposium on Fungitoxic Action, 61st Annual Meeting of American Phytopathological Society, August 19, 1969, Spokane, Washington.

(3) W. B. Wright, Jr., H. J. Brabander, R. Hardy, and A. C. Osterberg, *J. Med. Chem.*, **9**, 852 (1966).

(4) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).