Antimalarial and Antischistosomal Effects of $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$-Hexachloroxylene Derivatives and Related Halogenated Hydrocarbons[1-3]

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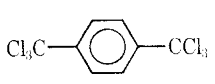
In 1960 Lämmler[4] reported that $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$-hexachloro-*p*-xylene (I)[5] was active against the liver fluke *Fasciola hepatica* in rats, rabbits, sheep, and cattle.



I

Subsequently, the drug was demonstrated to have exceptionally broad therapeutic effects against trematodes including *Dicrocoelium dendriticum* in golden hamsters,[6] *Schistosoma mansoni* in mice and golden hamsters,[6] *Schistosoma japonicum* in mice,[6] *Opisthorchis felineus* in cats,[6] *Clonorchis sinensis* in rats, rabbits, and dogs,[7,8] clonorchiasis in man,[8-10] and opisthorchiasis in children.[11] Thus I is strikingly different from other halogenated hydrocarbons in its spectrum of action.

In recent studies it was discovered that I (**18**, Table I) also possessed substantial suppressive antimalarial activity.[12] When administered subcutaneously in a single dose to mice infected with *Plasmodium berghei*,[13] the drug cured a majority of the animals at doses of 1280 or 640 mg/kg, and prolonged the survival time of treated mice from 8.3–9.7 days at 320 mg/kg to 1.7 to 6.4 days at 160 mg/kg.[12] Subsequent studies with a sample of fine particle size drug ($<25\ \mu$) prepared in these laboratories gave comparable overall results (**18**, Table I).[13,14] Additional studies carried out in these laboratories showed that **18** was also active against *P. berghei* when given continuously in the diet for 6 days.[15] The SD_{90} (daily dose required for 90% suppression of the parasitemia) for **18** and quinine was 36 and 74.5 mg/kg, respectively. Thus **18** was approximately twice as potent as quinine against *P. berghei* by drug diet.

Expanded antimalarial studies with **18** showed that the drug was effective orally against erythrocytic forms of *P. cynomologi* in monkeys at doses of 32–158 mg/kg,[12] but lacked appreciable effects against erythrocytic forms of *P. gallinaceum* in chicks at subcutaneous doses of 120 mg/kg.[16] Moreover, the drug did not suppress oocysts or sporozoites of *P. gallinaceum* when fed to infected mosquitoes (*Aedes aegypti*) at a concentration of 0.1% in sucrose.[17] It has been postulated that this substance may be metabolized to a terephthalic acid derivative *in vivo*.[12]

To enable a broader delineation of structure–antimalarial–antischistosomal relationships among $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$-hexachloroxylene derivatives and related halogenated hydrocarbons, a variety of known substances[18-35] (**1–14**, **16–24**, and **26–28**, Table I) were resynthesized or procured from commercial sources and purified for antiparasitic evaluation. α,α,α-Trichloro-*m*-tolunitrile (**15**), 3,5-bis(trichloromethyl)benzonitrile (**25**), and 4,4′-bis(trichloromethyl)benzophenone (**29**) were prepared in 43–79% yield by the chlorination of *m*-tolunitrile, 3,5-dimethylbenzonitrile,[36] and 4,4′-dimethylbenzophenone with Cl_2 in the presence of a floodlight. The progress of the chlorinations was monitored by nmr spectroscopy, and the reactions were

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(1) This is paper XXII of a series on antimalarial substances. For the previous paper, see M. P. Hutt, E. F. Elslager, and L. M. Werbel, *J. Heterocycl. Chem.*, in press.

(2) This is communication XV of a series relating to synthetic schistosomicides. For paper XIV, see E. F. Elslager, L. M. Werbel, and D. F. Worth, *J. Med. Chem.*, **13**, 104 (1970).

(3) This investigation was supported in part by U.S. Army Medical Research and Development Command Contract DA-49-193-MD-2754. This is Contribution No. 720 to the Army Research Program on Malaria.

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(15) For a description of the test methods, see P. E. Thompson, A. Bayles, and B. Olszewski, *Exp. Parasitol.*, **25**, 32 (1969).

(16) Antimalarial screening against *P. gallinaceum* in chicks was carried out by Dr. Leo Rane of the University of Miami, and test results were supplied through the courtesy of Dr. David P. Jacobus of the Walter Reed Army Institute of Research.

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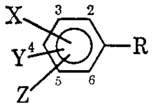
TABLE I

EFFECTS OF $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$-HEXACHLOROXYLENE DERIVATIVES AND RELATED HALOGENATED HYDROCARBONS AGAINST *Plasmodium berghei* IN MICE



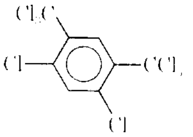
No.	R	X, Y, Z	Formula	Ref	Drug diet, 6 days			Single s.c. dose ΔMST; *T* or C^c after dose				
					No. of mice	SD_{90},[a] mg/kg per day	Q^b	640	320	160	80	40
1	CCl_3	2,4,5-Cl_3	$C_7H_2Cl_6$	18,19	7	>71	<1.1					
2	CCl_3	3,4-Cl_2	$C_7H_3Cl_5$	18,19	7	>79	<1.0					
3	CCl_3	2,4-Cl_2	$C_7H_3Cl_5$	20,21				5.4		1.2		0.8
4	CCl_3	2-Cl	$C_7H_4Cl_4$	21,22				3.6	1.0	0.8	0.6	0.0
								1.4		0.2		0.4
5	CCl_3	4-Cl	$C_7H_4Cl_4$	20,21				0.8; *T* 2		0.6		0.2
6	$CHCl_2$	2,6-Cl_2	$C_7H_4Cl_4$	21,23				2.2		1.4		1.0
7	CCl_3	H	$C_7H_5Cl_3$	21,24				1.0		0.6		0.6
8	CCl_3	2,4-Cl_2,5-CCl_3	$C_8H_2Cl_8$	19	14	59	1.3					
9	CCl_3	2,3-Cl_2,4-CCl_3	$C_8H_2Cl_8$	19	28	41	1.8					
10	CCl_3	2,5-Cl_2,4-CCl_3	$C_8H_2Cl_8$	19,25	7	70	1.1					
11	CF_3	3-Br,5-CF_3	$C_8H_3BrF_6$	26				*T* 5		*T* 5		*T* 5
12	CCl_3	2-Cl,5-CCl_3	$C_8H_3Cl_7$	19,27	14	85	0.9					
13	CCl_3	2-Cl,4-CCl_3	$C_8H_3Cl_7$	19,27	14	62	1.2					
14	CF_3	3-NO_2,5-CF_3	$C_8H_3F_6NO_2$	26,28				1.5		1.3		1.3
15	CCl_3	3-CN	$C_8H_4Cl_3N$		14	263	0.3	2.9			2.1	
								2.4			1.8	
16	CCl_3	4-CN	$C_8H_4Cl_3N$	29	14	180	0.4	4.8	3.6	1.2	0.6	0.2
								3.8	3.0	1.6		
17	CCl_3	3-CCl_3	$C_8H_4Cl_6$	30				C 3, *T* 1		2.7		1.7
18	CCl_3	4-CCl_3	$C_8H_4Cl_6$	24	28	36	2.1	C 5	8.9	6.7	0.3	0.3
								C 5		6.9		0.3
19	CF_3	3-CF_3	$C_8H_4F_6$	26,31				0.4		0.4		0.2
20	CF_3	4-CF_3	$C_8H_4F_6$	26,32				0.4		0.2		0.2
21	CCl_3	4-COOH	$C_8H_5Cl_3O_2$	33	7	>329	<0.2					
22	CCl_3	2-$CHCl_2$	$C_8H_5Cl_5$	24				9.8; *T* 1	4.3; *T* 1	3.2	2.8	2.7
								5.8; *T* 1	2.0	1.8		2.0
								4.5		3.1		
23	CCl_3	4-$CHCl_2$	$C_8H_5Cl_5$	34	14	340	0.2					
24	CCl_3	4-$CONH_2$	$C_8H_6Cl_3NO$	33	7	>354	<0.2					
25	CCl_3	3-CN,5-CCl_3	$C_9H_3Cl_6N$		14	120	0.6	11.9; *C* 2	7.9	2.1	0.5	0.3
								5.9; *C* 2		1.7		0.3
26	CF_3	3-CN,5-CF_3	$C_9H_3F_6N$	26				0.8; *T* 3		0.2		0.2
27	CCl_3	4-$CO_2C_2H_5$	$C_{10}H_9Cl_3O_2$	33	7	>347	<0.2					
28	CCl_3	3-$CONHC_6H_5$	$C_{14}H_{10}Cl_3NO$	35	7	>421	<0.2					
29	CCl_3	4-COC_6H_4-*p*-CCl_3	$C_{15}H_8Cl_6O$		7	>41	<1.8	5.7	5.5	4.7	1.9	0.7
								5.5		4.5		0.5

[a] SD_{90} represents the daily dose (mg/kg) required for 90% suppression of the parasitemia in treated mice relative to control mice. The SD_{90} was estimated graphically using semi-log paper. [b] The quinine equiv *Q* is the ratio of the SD_{90} of quinine·HCl (74.5 mg base/kg per day) to the SD_{90} of the test substance under comparable experimental conditions. [c] ΔMST is the mean survival time (days) of treated mice (MSTT) minus the mean survival time (days) of control mice (MSTC). In the present study the MSTC ranged from 6.1 to 6.5 days. *T* signifies the number of toxic deaths occurring on days 2–5 after infection which are attributed to drug action. *C* indicates the number of mice surviving at 60 days post infection and termed "cured"; data to establish parasitological cure based on subinoculation is unavailable. Each entry at each dose level represents results with a 5-animal group.

terminated upon the disappearance of all aliphatic proton absorption.

Antimalarial Studies.—The $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$-hexachloroxylene derivatives and related halogenated hydrocarbons (**1–29**, Table I) were subjected to broad antimalarial evaluation. In studies utilizing normal drug-sensitive strains of *P. berghei* in mice, the compounds were administered in single subcutaneous doses of 40–640 mg/kg[13,14] or given continuously in the diet for 6 days at daily doses ranging from 36 to 421 mg/kg.[15] $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$-Hexachloro-*m*-xylene (**17**) and 3,5-bis(trichloromethyl)benzonitrile (**25**) exhibited curative activity subcutaneously at 640 mg/kg, but neither was more active than **18**. By drug diet, $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$-4,6-octachloro-*m*-xylene (**8**), $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$,2,3-octachloro-*p*-xylene (**9**), $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$,2,5-octachloro-*p*-xylene (**10**), and $\alpha,\alpha,\alpha,\alpha',\alpha',\alpha'$,2-heptachloro-*p*-xylene (**13**) were 1.1–1.8 times as potent as quinine against *P. berghei*, but none was more active than **18**. It is noteworthy that α,α,α-trichloro-*p*-toluic acid (**21**), a likely metabolite of **18**, was inactive against *P. berghei* when administered to mice in the diet at 329 mg/kg, a dose nearly 10 times the SD_{90} of the parent drug.

Compounds **3**, **11**, **22**, and **25** were also tested against a normal drug-sensitive strain of *P. gallinaceum* in chicks, but each lacked appreciable antimalarial effects when administered in single subcutaneous doses ranging from 120 to 480 mg/kg.[16] None of the compounds tested (**4**, **5**, **7**, **11**, **14**–**16**, **22**, **25**, **26**, **29**) suppressed oocysts or sporozoites of *P. gallinaceum* when fed to infected mosquitoes at a concentration of 0.1% in sucrose.[17] α,α,α,α′,α′,α′,4,6-Octachloro-*m*-xylene (II) (**8**) was examined for therapeutic action against patent *P. cynomolgi* infections in two Rhesus monkeys.[15] The drug was given orally twice daily for 10 days. A dose of 200 mg/kg per day suppressed the parasitemia rapidly and cured the infection, while a dose of 50 mg/kg per day partially suppressed the infection but failed to cure.

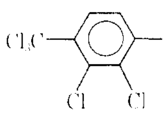


II

α,α,α,α′,α′,α′,4,6-Octachloro-*m*-xylene (II) (**8**) was subsequently tested against representative drug-resistant lines of *P. berghei* in the mouse to determine whether or not the hexachloroxylene derivatives might represent a unique chemical type with respect to apparent mode of action. In two parallel studies **8** was administered by gavage at doses of 12, 50, and 200 mg/kg daily to mice infected with the sensitive parent line P and the following drug-resistant lines: line T, completely (>300-fold) resistant to cycloguanil; line S, completely (>600-fold) resistant to 4,4′-sulfonyldianiline (DDS); and line C, 77-fold resistant to chloroquine.[15] Groups of 10 mice were employed throughout, and treatment extended over a 4-day period starting the day before infection. The SD_{85} (daily dose required for 85% suppression of the parasitemia) for each line was as follows: line P, 114 mg/kg; line T, 57 mg/kg; line S, 132 mg/kg; line C, 150 mg/kg. Thus the chloroquine- and DDS-resistant lines were nearly as sensitive to **8** as the parent line, and there was an indication that the cycloguanil-resistant line was hypersensitive to the drug. These results suggest that the principal mode of action of **8** and the other hexachloroxylenes may be different from that of chloroquine, cycloguanil, and DDS, and encourage further chemical work aimed toward the development of more potent analogs.

Antischistosomal Studies.—Eighteen compounds (**3**, **5**, **7**–**11**, **13**, **15**, **17**–**20**, **22**–**24**, **26**, **27**, Table I) were tested in mice against a Puerto Rican strain of *Schistosoma mansoni*[37] by Dr. Paul E. Thompson and coworkers of these laboratories. Drugs were administered in a powdered diet for 14 days or by gavage in 10 ml/kg of aqueous 1% hydroxyethyl- or CM-cellose for 10 days. α,α,α,α′,α′,α′,4,6-Octachloro-*m*-xylene (**8**), α,α,α,α′,α′,α′,2,3-octachloro-*p*-xylene (**9**), and α,α,α,α′,α′,α′,2-heptachloro-*p*-xylene (**13**) possessed significant schistosomicidal activity and effected a 65–74% reduction of live schistosomes in infected mice at daily doses ranging from 200 to 327 mg/kg. However, none was appreciably more active than **18** which produced a 64% reduction of live schistosomes when given in the diet at 223 mg/kg per day for 7 days. All other compounds lacked significant antischistosome effects at drug–diet dose levels ranging from 105 to 346 mg/kg per day for periods of 7 or 14 days.

α,α,α,α′,α′,α′,2,3-Octachloro-*p*-xylene (III) (**9**) was also tested against the Puerto Rican strain of *S. mansoni* in Rhesus monkeys.[37] The drug was given orally by gavage twice daily 5 days a week for 1 or 2 weeks to three monkeys. A regimen of 400 mg/kg per day for 5 days produced only a slight, temporary suppression of egg production. Regimens comprising 200 mg/kg per day for 5 days followed by 400 mg/kg per day for 5 days or 400 mg/kg per day for 10 days effected a partial permanent suppression of egg production but were not curative.



III

Experimental Section[38,39]

α,α,α-**Trichloro-*m*-tolunitrile** (**15**).—Cl_2 was bubbled into 98.6 g (0.84 mol) of *m*-tolunitrile at bath temp of 180° for 15.5 hr. The apparatus was irradiated with a 75-W floodlight. Distillation of the resulting product yielded 145.4 g (79%) of **15**, bp 74–76° (0.10 mm), mp 37–39°. *Anal.* ($C_8H_4Cl_3N$) C, H, N.

3,5-Bis(trichloromethyl)benzonitrile (**25**).—Cl_2 was bubbled into 41.0 g (0.313 mol) of 3,5-dimethylbenzonitrile[36] at 180° (oil bath temperature) for 21.5 hr. The reaction flask was irradiated with a 75-W floodlight during the chlorination. Four recrystallizations from aqueous EtOH gave 43.1 g (43%) of the desired product, mp 90–92°. *Anal.* ($C_9H_3Cl_6N$) C, H, N.

4,4′-Bis(trichloromethyl)benzophenone (**29**).—Cl_2 was bubbled into a melt of 20.0 g (0.095 mol) of 4,4′-dimethylbenzophenone for 15.25 hr as above. The bath temperature was steadily increased from 160 to 220° during the course of the reaction to maintain the reaction mixture as a melt. Recrystallization from cyclohexane yielded 20.4 g of product mp 202–204° and a second crop of 7.8 g, mp 197–202°, which combined represent a 71% yield. A 4.5-g sample recrystallized for analysis from 500 ml of EtOH yielded 3.4 g of material, mp 203–204°. *Anal.* ($C_{15}H_8Cl_6O$) C, H.

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(37) For a description of test methods, see P. E. Thompson, J. E. Meisenhelder, and H. Najarian, *Amer. J. Trop. Med. Hyg.*, **11**, 31 (1962).

(38) Melting points (corrected) were taken on a Thomas–Hoover capillary melting point apparatus.

(39) Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

Coumarin Derivatives as Coronary Vasodilators

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Many naturally occurring coumarin compounds have been shown to possess strong coronary vasodilating activity (visnadin, samidin, and dihydrosamidin).[1,2]

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