Inhibition Studies with *Streptococcus faecalis*. Xanthones and Thiaxanthones. (22108)

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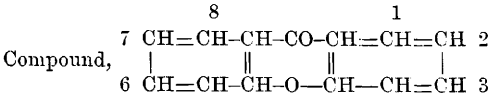
Woods' finding(1), that the chemotherapeutic properties of sulfonamides resided in their ability to interfere with the utilization of p-aminobenzoic acid by the organism, has given tremendous impetus to the study of antimetabolites. Such studies, of course, may be approached from more than one direction. For example, anti-folics were prepared on the basis of their studied dissimilarity to the structure of folic acid. This gave rise to the useful agents aminopterin(2) and amethopterin(3). From another approach we have examined compounds prepared(4-6) for use against schistosomiasis(7) for their ability to interfere with utilization of folic acid by *Streptococcus faecalis*. The results of such tests on 16 xanthones and 63 thiaxanthones are discussed below.

Methods. The medium used for these studies with *S. faecalis* (ATCC No. 8043) was slightly modified from that of Teply and Elvehjem(8). The modifications consisted of omitting xanthine and of using "vitamin-free" casein hydrolysate (Nutritional Biochemicals) in place of charcoal-treated material suggested by the authors. Under these conditions the blanks required about 0.5 ml of N/10 alkali per tube for neutralization. Upon addition of 1 mμg of folic acid per ml of medium, growth of the organism, as judged by acid production after 72 hours, was represented by 9.5 to 10 ml of N/10 alkali per tube. Half maximum growth of the organism, the level against which the test compounds were run, resulted from addition of 0.32 mμg of folic acid per ml of medium. For the tests the compounds were added to duplicate tubes at levels of 1.6, 8.0, 40 or 200 μg per ml of medium. Tubes were plugged, sterilized at 120°C for about 12 minutes, cooled, inoculated with suspension of the organism(9), and incubated at 37°C for 3 days. Growth of the organism was estimated titrimetrically.

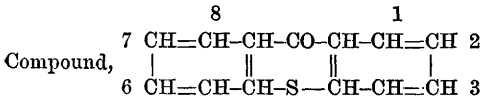
Results. The results summarized in Tables I-III are expressed as an arbitrary ratio term. The upper (and smaller) value represents μg of compound per ml of medium which had little or no inhibitory effect on growth of the organism. The lower (and larger) figure represents amount of test compound which completely or essentially completely inhibited growth of the organism. By way of reference, the ratio terms for some known anti-folics under these conditions of test were: (μg/ml) aminopterin 0.008/0.04, amethopterin 0.00032/0.0016, and pyrimeth-

TABLE I. Results of Tests with 16 Xanthones against *S. faecalis* in the Presence of Limiting Amounts of Folic Acid.



Compound,	Derivatives	Activity vs. folic acid, μg/ml
1-$NH(CH_2)_2N(C_2H_5)_2$	4-CH_3 6-OCH_3, 4,6-$(CH_3)_2$	8/ 40
$N(C_2H_5)$ CH_2CHOH	4-Cl 6-CH_3, 4,6-$(CH_3)_2$, 4-CH_3 6-Cl, 4-CH_3 6-OCH_3(4)	"
	4-CH_3(4)	40/200
$NHCH_2CHOHCH_3$	"	"
	4-CH_3 6-Cl(4)	8/ 40
$N(CH_3)$ CH_2CHOH CH_3	"	0
NH CH_2CHOH C_2H_5	4-CH_3(4)	40/200
	6-Cl(4)	8/ 40
$NHCH_2C(OH)$ $(CH_3)_2$	(4)	"
$N(C_2H_5)$ $CH_2CHOHCH_3$	6-Cl(4)	0
$N(C_4H_9)$ $CH_2CHOHCH_3$	"	20/100
$N(C_2H_5)CH_2C(OH)$ $(CH_3)_2$	"	0

TABLE II. Thiaxanthones Which Inhibit *S. faecalis* in the Presence of Limiting Amounts of Folic Acid.

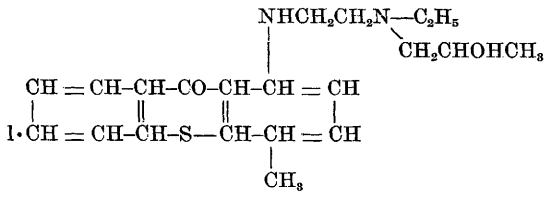


Compound,	Derivatives	Activity vs. folic acid, μg/ml
1-NH(CH$_2$)$_2$NHCH$_2$CH$_2$OH	4-CH$_3$	8/ 40
NHCH$_2$CHOH CH$_3$	(5), 4-CH$_3$ 6-Cl	"
NHCH$_2$CHOH CH$_2$OH	4-CH$_3$(6)	"
NH CH$_2$CHOH C$_2$H$_5$	4-CH$_3$	"
"	6-Cl(5)	1.6/ 8
NH CH$_2$C(OH) (CH$_3$)$_2$	(5)	8/ 40
"	6-Cl	1.6/ 8
N(CH$_3$) CH$_2$CHOHCH$_3$	(5)	8/ 40
NC$_5$H$_{10}$	6-Cl	1.6/ 8
N(CH$_3$)$_2$	4-CH$_3$	8/ 40
N(C$_2$H$_5$)$_2$	(6), 4,6-(CH$_3$)$_2$	"
N(C$_2$H$_5$)CH$_2$CH$_2$OH	(5), 6-Cl(6), 4,6-(CH$_3$)$_2$, 4-CH$_3$ 6-OCH$_3$, 4-CH$_3$6-Br	"
"	6-Cl(5)	1.6/ 40
N(C$_2$H$_5$) CH$_2$CHOH CH$_3$	"	1.6/ 8
N(C$_4$H$_9$) CH$_2$CH$_2$OH	(5)	8/ 40
N(C$_4$H$_9$)$_2$	4,7-(CH$_3$)$_2$(6)	40/200
N C$_5$H$_{10}$	4-CH$_3$	8/ 40
1-NH(CH$_2$)$_3$N(C$_6$H$_{13}$)$_2$	(6)	"
1-NHCH(CH$_3$) CH$_2$N(C$_2$H$_5$)$_2$	"	"
1-NH(CH$_2$)$_3$N(C$_2$H$_5$)$_2$	"	"
"	4-CH$_3$ 7-Cl (6)	1.6/ 8
1-NH(CH$_2$)$_3$N(C$_2$H$_5$) CH$_2$CH$_2$OH	(6)	8/ 40
1-NHCH$_2$CHOH CH$_2$N(C$_2$H$_5$)$_2$	4-CH$_3$ 7-Cl (6)	"
"	2,4-(CH$_3$)$_2$(6)	40/200
1-NH(CH$_2$)$_3$ NH C$_3$H$_7$	4-CH$_3$(6)	8/ 40
N(C$_3$H$_7$)$_2$	"	"
N(C$_5$H$_{11}$)$_2$	"	"
1-NH(CH$_2$)$_4$N(C$_2$H$_5$)$_2$	"	"

amine 0.0016/0.008.

From the data summarized in the Tables it may be seen that 13 of the 16 xanthones and 35 of the 63 thiaxanthones manifested some degree of toxicity to the organism. Since thymidine has been shown to abolish toxicity of some compounds which block utilization of folic acid(10), thymidine at 2 μg per ml of medium, was added in place of folic acid. The nucleoside level was otherwise adequate for optimum growth with the above medium.

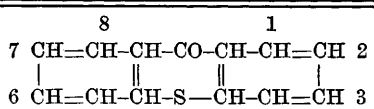
In the thymidine-containing medium all xanthones and thiaxanthones inhibitory in the presence of folic acid were still inhibitory, with one exception. The exception was



Further tests showed that thymine(11,12) was unable to replace thymidine in abolishing the toxicity of this compound. This thiaxanthone thus differed from aminopterin, amethopterin, and pyrimethamine, which in the medium used, exhibited no adverse effects on growth of *S. faecalis* in the presence of thymine. The latter fact has been noted previously by others(13). On the other hand, the behavior of this thiaxanthone may be considered similar to that noted by Shive *et al.* (10) with another organism, who observed that thymidine, but not thymine, was able to abolish toxicity of either the sulfonamides or 2,4-diamino-6,7-diphenylpteridine for *L. arabinosus* 17-5. Whether or not the unusual metabolic blocking property of 6-chloro-1-{2-[ethyl - (2 - hydroxypropyl)amino]ethylamino}-4-methylthiaxanthone will prove useful alone or in combination with other blocking agents must await further studies under way.

In contradistinction to the unusual proper-

TABLE III. Thiaxanthones Which Do Not Interfere with the Utilization of Folic Acid by *S. faecalis*.

Compound, 	Derivatives
1-$NH(CH_2)_2N(CH_3)CH_2CHOHCH_3$	4-CH_3 6-Cl (5)
NC_4H_8O	4-CH_3 6-Cl (6)
$N(C_2H_5)_2$	4-NO_2 6-Cl (6)
$N(C_2H_5)CH_2CH_2OH$	4-CH_3 5-Cl (6)
$N(C_2H_5)CH_2C(OH)(CH_3)_2$	6-Cl (5)
$N(C_3H_7)_2$	(6)
$N(C_4H_9)CH_2CHOHCH_3$	4-CH_3 6-Cl (5)
$N(C_4H_9)_2$	4-NO_2, 4,6-Cl_2, 4,6-$(CH_3)_2$, 5-Cl(6), 4-CH_3 6-Cl, 4-CH_3 7-Cl(6), 7-NO_2(6), 4,5,7-$(CH_3)_3$, 4-Cl 5-CH_3(6), 4-Cl 5,7-$(CH_3)_2$, 4-Cl 7-NO_2, 4-$NHCOCH_3$(6), 4-$NHCOC_6H_5$(6), 4-$NHSO_2C_6H_5$(6)
$N(C_4H_9)_2$	4-CH_3 (6)
$N(C_5H_{11})_2$	"
$N(C_6H_{13})_2$	"
1-$NH(CH_2)_3N(C_4H_9)_2$	"

ties of thiaxanthone above, its xanthone analog given in Table I exhibited no adverse effects on the organism in the presence of folic acid.

It may be noted that the 4 thiaxanthones indicated by Berberian, Dennis, and Freele (7) to be schistosomacidal behaved quite differently from each other when examined for toxicity with *S. faecalis*. Thymidine was unable to reverse the toxicity of one (6-chloro-1-{2-[ethyl-(2-hydroxyethyl)amino]ethylamino}-4-methylthiaxanthone). Thymidine abolished the toxicity of one (6-chloro-1-{2-[ethyl(2-hydroxypropyl)amino]ethylamino}-4-methylthiaxanthone), as indicated above. The remaining two (1-(2-dibutylaminoethylamino)-4-methylthiaxanthone) and (6-chloro-1-[2-ethyl-2-(2-hydroxy-2-methylpropylamino)ethyl-amino]-4-methylthiaxanthone) exhibited no adverse effects on growth of the organism. Presumably, therefore, the schistosomacidal properties of the foregoing 4 thiaxanthones do not reside in their ability to interfere in the nucleic acid metabolism of *S. mansoni*.

We may conclude, accordingly, that one compound of interest, 6-chloro-1-{2-[ethyl(2-hydroxypropyl)amino]ethylamino}-4-methylthiaxanthone, has been brought to the fore by our study. While it does not compare favorably as an anti-folic with known anti-folics, being only 1/200, or less, as active as some of the better known ones, its differing pattern of inhibition points it up as a compound of possible interest and application, alone or in combination with other blocking agents.

Summary. 1. A series of xanthones and thiaxanthones initially prepared for tests against schistosomiasis was tested for toxicity with *S. faecalis* in the presence of limiting amounts of folic acid. Under these conditions 13 of the 16 xanthones and 35 of the 63 thiaxanthones tested were toxic. The toxicity of only one thiaxanthone could be abolished. 2. 6-Chloro-1-{2-[ethyl(2-hydroxyproyl)amino]ethylamino}-4-methylthiaxanthone was inert with thymidine in the medium. Thymine was not able to replace thymidine in this respect. 3. Separately, it may be indicated that no correlation was discerned between activity against *S. faecalis* and *S. mansoni* infection in mice.

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Neutralizing Antibody to Viruses of Poliomyelitis in Sera of Domestic Animals. (22109)

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The explanation for the origin of seasonal outbreaks of poliomyelitis in the temperate zone is unknown, although it is now evident that man to man transmission is responsible for maintenance of an epidemic. Viral isolation studies have, as yet, failed to implicate animal-reservoir-insect-vector pattern which is so characteristic of many other neurotropic viruses. However, most isolation studies were done prior to the development of tissue culture technics. and intensive surveys were limited. Immunological studies have been carried out by Hammon(1) and others(2,3) in which "neutralizing factors" or "protective substances" have been found in sera of various animals. The status of these "protective substances" is uncertain for they were demonstrable only in low dilution and against one strain (Lansing, type 2), the only strain readily available in most laboratories. Whether one is here dealing with a non-specific neutralizing substance or specific antibody is a critical point, for identification of these substances as specific antibodies to viruses of poliomyelitis would point strongly to the existence of either (1) an extra-human reservoir of viruses of poliomyelitis or (2) a serologically related group of viruses. In the present study animal sera were tested for presence of neutralizing antibody to all 3 types of poliomyelitis virus using the HeLa cell. The protective substance existing in certain cases was identified as a true antibody. To determine the origin of this antibody, extensive though unsuccessful attempts were made to isolate the agents responsible for production of this antibody.

Methods. The HeLa Cell. Techniques employed in the management of the HeLa cell have been adequately described(4), and were followed in the present study. The HeLa cells were routinely grown in rubber stoppered test tubes in a growth medium consisting of 40% human adult serum, 2.5% chick embryo extract and 57.5% Hanks' balanced salt solution. Before use in neutralization test, the cells were washed 3 times with balanced salt solution and 0.9 ml maintenance solution was added to each test tube. The maintenance medium was composed of 90% maintenance solution as described by Syverton, Scherer and Ellwood(4) and 10% chicken serum. *Strains of Poliovirus.* The Brunhilde (type 1), YSK (type 2) and Leon (type 3) strains were employed as representatives of the 3 known antigenic types of poliovirus. These viruses were used in a 100 tissue culture dose concentration against each animal sera tested. *Source and Preparation of Serum Samples.* Table I illustrates the source of sera and geographical origin of animals from which sera were obtained. Sam-

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