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Inhibition Studies with *Streptococcus faecalis.* Acridines and Acridones. (22238)

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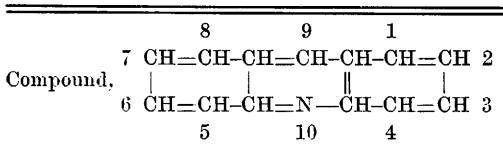
The investigations of Woods(1) led to the finding that the sulfonamides owed their chemotherapeutic activity to their property of interfering with the utilization of p-aminobenzoic acid by the microbial cell. This observation has stimulated the synthesis and testing of compounds related closely in structure to known metabolites for trial in the controlled interference with selected metabolic reactions. In the instance of folic acid, this has given rise to a series of folic acid blocking agents(2), some of which have proven usefulness(3), though limited, in a variety of ways. The interference with the utilization of folic acid was applied by Hitchings, among others, to develop compounds of biological interest. As a result Hitchings and his associates observed(4,5) that the rather active anti-folic, pyrimethamine, appeared also to possess some promise against the malarial parasite, though continued study may have modified this view somewhat(6,7).

In view of the findings that compounds which interfere with the utilization of folic acid may have application in other disorders for which agents are needed, a reinvestigation of 53 available acridines(8,9) and acridones (10), prepared initially for testing against the malarial parasite, was undertaken.

Methods. The folic acid assay medium utilized in this study was slightly modified from that of Teply and Elvehjem(11). The modifications consisted of omitting the xanthine and of utilizing "vitamin free" casein hydrolysate (Nutritional Biochemicals) in place of the charcoal treated hydrolysate originally suggested. When folic acid was added in amounts up to 1.0 mμg per ml of medium the acid production upon titration with N/10 alkali went up from blanks of about 0.5 ml of alkali per tube to 9.5 to 10 ml of alkali per tube at the higher levels of added folic acid. The test compounds were added to tubes containing 0.32 mμg of folic acid per ml of medium, a level designed to support half maximum growth as judged by acid production. This usually ranged about 4.5 to 5.0 ml of N/10 alkali per tube. For the assays, the test compounds were added to duplicate tubes usually at 4 levels of test: 1.6, 8,

TABLE I. Acridines Which Interfere with Utilization of Folic Acid by *S. faecalis*.

Compound,



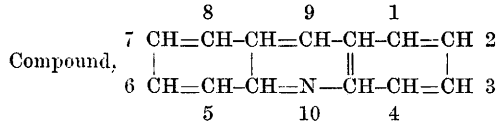
Substituent at 9	Derivatives	Activity vs. folic acid, μg/ml
9-NH_2 (aminacrine)	4-CH_3	8/ 40
9-$NH(CH_2)_3NHCH_2CH_2OH$	2-OCH_3	40/200
" $NHCH_2CHOHCH_3$	"	"
" $NH(CH_2)_2CHOHCH_3$	"	"
9-$NH(CH_2)_4N(CH_3)_2$	2-OCH_3 6-Cl	"
" $N(C_2H_5)_2$	"	"
9-$SCH(CH_3)(CH_2)_3N(C_2H_5)_2$	"	"
9-$NH(CH_2)_3S(CH_2)_2N(C_2H_5)_2$	"	"
9-NH $C_6H_4N(CH_3)_2$	"	"

40, and 200 μg per ml of final medium. The tubes were plugged, sterilized about 12 minutes at 120°C, cooled at room temperature, and inoculated(12), with a drop of a suspension of *Streptococcus faecalis* (A.T.C.C. No. 8043). The growth of the organism was estimated titrimetrically after 72 hours using brom thymol blue as the indicator.

Results. The acridines and acridones tested and their ability to interfere with the utilization of limiting levels of folic acid in the medium are summarized in Tables I-III. To characterize the inhibitory activity of the compounds, in instances where inhibitory activity was observed, an arbitrary ratio term has been used. The upper (and lesser) figure represents the amount of test compound which exercised little or no inhibition of acid

TABLE II. Acridines Which Do Not Interfere with Utilization of Folic Acid by *S. faecalis*.

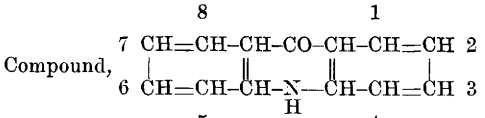
Compound,



Substituent at 9	Derivatives
9-NH_2	2-OCH_3 6-Cl, 3-NO_2 6,7-$(OCH_2)_2$
9-$NHCH_2CH_2OH$	3-NO_2 6,7-$(OCH_2)_2$
9-$NHCH_2CH_2NH_2$	2-OCH_3 6-Cl
9-(3′-C_5H_4N)	"
9-(6′-OCH_3-8′-quinolyl-NH_2)	"
9-$NH(CH_2)_2NH(CH_2)_2CH_2OH$	" (unsub.), 6-NO_2 2,3-$(OCH_3)_2$
" $NHCH_2CHOHCH_3$	2-OCH_3, 2-OCH_3 6-Cl, 2,3-$(OCH_3)_2$ 6-Cl
" $NHCH_2CHOHCH_2OH$	2-OCH_3, 6-Cl, 2-OCH_3 6-Cl
" $NHCH_2C(OH)(CH_3)_2$	2-OCH_3, 2-OCH_3 6-Cl
9-$S(CH_2)_2N(C_2H_5)_2$	2-OCH_3 6-Cl
9-$O(CH_2)_2N(C_2H_5)_2$	"
9-$NH(CH_2)_3NHCH_2CH_2OH$	"
" $NHCH_2CHOH\ CH_3$	"
" $NH(CH_2)_2CHOH\ CH_3$	"
" $NHCH_2C(OH)(CH_3)_2$	2,3-$(OCH_3)_2$ 6-NO_2
" $N(C_2H_5)CH_2CHOH$	2-OCH_3 6-Cl
9-$NHCH_2CHOHCH_2N(C_2H_5)_2$	2,3-$(OCH_3)_2$6-NO_2 (entozon)
9-$NHCH(CH_3)(CH_2)_3N(C_2H_5)_2$	2-OH 6-Cl, 2-OCH_3 6-Cl (quinacrine), 2,3-$(OCH_3)_2$ 6-Cl, 2-OCH_3 6-CH_3, 2-F 6-Cl
9-$NHC_6H_4N(CH_3)_2$	
9-$NHCH_2CH(C_6H_5)(CH_2)_2N(CH_3)_2$	2-OCH_3 6-Cl
" $(CH_2)_2N(C_2H_5)_2$	"
9-$NHCH_2(C_6H_5)C_5H_8NCH_3$	"
" $C_5H_8N\ CH_2C_6H_5$	"

TABLE III. Effect of Acridones on Utilization of Folic Acid by *S. faecalis*.

Compound,



Substituent at 1	Derivatives	Activity vs. folic acid, μg/ml
1-NH$(CH_2)_2N(C_4H_9)_2$	4-CH_3, 4-$CH_3$6-Cl	None
1-NH$(CH_2)_3N(C_2H_5)_2$	3-Cl, 4,6-Cl_2	8/ 40
"	4-Cl	40/200
1-$NHCH_2CHOHCH_2N(C_2H_5)_2$	4,6-Cl_2	"
1-$NHCH(CH_3)$ $(CH_2)_3N(C_2H_5)_2$	4-Cl	"

production (growth) of the organism. The lower (and larger) value represents the amount of test substance which appreciably or completely inhibited acid production by the organism. This method of evaluating the test compounds has proven more reproducible than the calculation of the 50% molar inhibition index. The latter has been observed to vary to a disturbing extent following small changes in the test conditions, whereas the ratio term has remained constant in the face of minor modifications in the testing of the compounds.

The results summarized in Tables I-III probably may be rendered more meaningful if compared with those obtained under these conditions of test on some known effective anti-folics. The inhibition ratios against folic acid and citrovorum factor, respectively, were: aminopterin 0.008/0.04 and 0.2/1.0, amethopterin 0.00032/0.0016 and 0.04/0.2, and pyrimethamine 0.0016/0.008 and 25/100.

From the data of Tables I and III it may be seen that 40 to 200 μg of 10 acridines and of 5 acridones per ml of medium proved to be inhibitory. These amounts were 1000-fold (or more) those needed by the anti-folics given above. The acridine and acridone inhibitors thus appear to be primarily of theoretical interest at this time. The activities of the inhibitory acridines and the acridones were further differentiated. With the same organism and with 2.0 mμg of citrovorum factor(13) per ml of medium as the limiting nutrient 6-chloro-9(4-diethylamino-1 methylbutylthio)-2-methoxy acridine and 1-(3-diethylamino-2-hydroxypropylamino)-4, 6-dichloroacridone were no longer inhibitory. In the presence of 2.5 μg of thymine(14,15) per ml of medium and with *S. faecalis* as the test organism, 9-[3-(3-hydroxybutylamino)propylamino]-2-methoxyacridine no longer inhibited. In the presence of 2.5 μg of thymidine(16) and with *S. faecalis* as above, 2-methoxy-6-chloro-9 - (4'-dimethylaminophenylamino)-acridine, 6-chloro-2-methoxy-9-(4-dimethylaminobutylamino)acridine, and 9-[3-(2-hydroxyethylamino) propylamino] - 2-methoxyacridine no longer inhibited the organism.

The remaining 5 acridines of Table I (9-aminoacridine, 9-amino-4-methylacridine, 2-methoxy-6-chloro - 9(4 - diethylaminobutylamino)propylamino)-acridine, 9-[3-(2-hydroxypropylamino]-2-methoxy acridine, 6-chloro-2-methoxy-9(γ-(β'-diethylaminoethylthio)-propylamino) acridine, and 4 of the acridones of Table III (3-chloro-1-(3-diethylaminopropylamino)-acridone, 4-chloro-1-(3-diethylaminopropylamino)-acridone, 4, 6-dichloro-1 - (3-diethylaminopropylamino)-acridone, 4-chloro-1-(4-diethylamino-1-methylbutylamino)-acridone) which inhibited *S. faecalis* in the presence of limiting amounts of folic acid were inhibitory whichever of the above nutritiles were limiting in the medium. Whether the inhibitory activity of these compounds was due to interference in the metabolic sequence of nucleic acid metabolism or whether it was due to blocking some other metabolic sequence is not known at this time.

Another point of interest arises from these studies. It may be noted that the effective antimalarial, quinacrine (mepacrine), does not

inhibit the organism in the presence of limiting amounts of folic acid. Parenthetically, a number of salts of quinacrine were tested so that the results are not limited to one salt. Thus, the acridine anti-malarials appear to owe their anti-malarial activity to some property other than interference in nucleic acid metabolism. This must be qualified, however, for the reason that it is not proven that results with *S. faecalis* may be carried over *in toto* to the malarial parasite.

Like quinacrine, the anti-bacterial Entozon (nitroacridine 3582, Hoechst) did not inhibit the test organism under the conditions of the test with folic acid as the limiting nutrilite. Contrariwise, the anti-bacterial 5-aminoacridine (aminacrine) inhibited the organism whichever nutrilite was present in limiting concentrations.

Thus, the studies with *S. faecalis* demonstrate interesting selectivity with various acridines and acridones. Whether they will remain of theoretical interest only remains to be determined.

Summary. 1. 46 Acridines and 7 acridones were tested against *Streptococcus faecalis* in the presence of limiting amounts of folic acid. It was observed that 10 of the acridines and 5 of the acridones inhibited the organism. However, the amounts of test compounds needed were 1000 times (or more) those needed by aminopterin, amethopterin, and pyrimethamine. 2. Further study demonstrated that inhibition exhibited by acridines and acridones could be overcome by citrovorum factor, thymine, or thymidine in some instances. The toxicity of 5 of the acridines and 4 of the acridones was not overcome by any of the nutrilites tested which suggests that their inhibition was not due to interference with the nucleic acid metabolism of the organism. 3. An additional observation was that the anti-malarial quinacrine and the anti-bacterial Entozon did not inhibit *S. faecalis* in the presence of limiting amounts of folic acid. The anti-bacterial 5-aminoacridine was inhibitory whether the limiting nutrilite was folic acid, citrovorum factor, thymine, or thymidine.

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