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5-Trifluoromethyl-6-azauracil (1,2)

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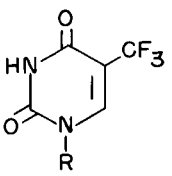
Sir:

The synthesis and biological activity of 5-trifluoromethyluracil (Ia), the corresponding deoxyriboside (Ib), and 5-difluoromethyluracil (II) have been reported recently (3,4). In an effort to study further changes in the structure and the effects on biological activity the analogous aza compound, 5-trifluoromethyl-6-azauracil (III, 6-trifluoromethyl-*as*-triazine-3,5-(2*H*,4*H*)-dione), has been synthesized.

5-Carboxy-6-azauracil (IV) was prepared by a modification of the procedure of Barlow and Welch (5). Treatment of the acid IV with a 4 molar excess of water and a 40 molar excess of sulfur tetrafluoride at 50° for 24 hours followed by recrystallization from chloroform gave a 53% yield of 5-trifluoromethyl-6-azauracil (III) m.p. 153°. (Calcd. for $C_4H_2F_3N_3O_2$: C, 26.53; H, 1.11; N, 23.21; F, 31.48. Found: C, 26.62; H, 1.15; N, 23.04; F, 31.23) U. V. λ max, *p*H 1.0, 262 mμ (ε = 6,500); *p*H 12.4, 292 mμ (ε = 7,500). R_f values in butanol: acetic acid: water (5:2:3) on Whatman No. 1 were 0.85 (ascending) and 0.86 (descending). The pK_{a_1} of III by titration was found to be 5.9. The apparent pK_{a_1} obtained by plotting *p*H *vs.* absorbance at 282 mμ was approximately 10.9.

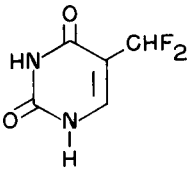
The biological activity of 5-trifluoromethyl-6-azauracil (III) was examined. Thymidylate synthetase, purified from *E. coli* and assayed according to the procedure of Wahba and Friedkin (6), was not inhibited by III at a concentration of $6.7 \times 10^{-4}M$ (deoxyuridine-5'-monophosphate = $4.2 \times 10^{-5}M$). Horse serum nucleoside phosphorylase was assayed according to the procedure of Friedkin and Roberts (7) and was not inhibited by a $2 \times 10^{-4}M$ concentration (thymidine = $1.6 \times 10^{-4}M$). Dihydrofolate reductase was purified from chicken livers and assayed by the method of Matthews and Huennekens (8). At a substrate (dihydrofolic acid) concentration of $5.5 \times 10^{-4}M$ the enzyme was not inhibited by III at a concentration of $1 \times 10^{-4}M$.

The results of *in vivo* studies directed by the Cancer Chemotherapy National Service Center are listed in Table I. Studies are continuing on the synthesis of the deoxyriboside of III.

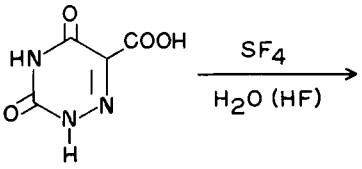


Ia. R = H

b. R = deoxyribosyl



II



IV III

Acknowledgments.

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REFERENCES

(1) This work was generously supported by grants Nos. CA-5639 and CA-6536 from the National Cancer Institute, U. S. Public Health Service.

(2a) The synthesis of III was reported at the 112th meeting of the American Pharmaceutical Association in March, 1965. M. P. Mertes, Abstract A-III, Symposium on Newer Concepts of Structure Activity Relationships, Scientific Section of the American Pharmaceutical Association, Detroit, 1965. (b) C. Heidelberger and A. Dipple also announced the synthesis of III. Abstracts of the 150th meeting of the American Chemical Society, September, 1965, p. 13D.

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(7) M. Friedkin and D. Roberts, *ibid.*, 207, 245; 257 (1954).

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TABLE I

5-Trifluoromethyl-6-azauracil (NSC 91365)

Test System	Dose	Results	
KB Cells	1 μg/ml	ED_{50} > 100 μg/ml	
Leukemia L-1210	250 mg/Kg	6/6 Survivors	Tumor Weight: Test 8.5 / Control 8.7