

When deacetylation was allowed to occur at the same pH but at $5.0 \pm 0.2^\circ$ and the spectrum was scanned from 270–230 $m\mu$ both before and, at a series of times, after the addition of base, the resulting difference spectra (inset to Fig. 2) show the rapid appearance of a peak with its maximum at 245 $m\mu$ which slowly declines with time, corresponding closely to that described for acetyl-imidazole.⁴ According to the published extinction coefficient of this compound⁴ ($\epsilon = 3 \times 10^3$), the observed maximum increase and subsequent decrease at 245 $m\mu$ is equivalent to 0.41–0.42 mole acetyl-imidazole per mole of reactive acetyl in the enzyme. Similar results were obtained in glycine buffer, but in phosphate the magnitude of the change at 245 $m\mu$ was reduced.

It is postulated, therefore, that as indicated in Fig. 1, the deacetylation of mono-acetyl- δ -chymotrypsin occurs by a rapid intramolecular transfer of acetyl- from serine hydroxyl to imidazolyl- followed by a slower hydrolysis of acetyl-imidazolyl-. The first order rate constant for the disappearance of the E_{245} compound corresponds closely with that observed for the rate of deacetylation of δ -chymotrypsin as measured by the reappearance of enzyme activity,⁵ which in turn corresponds to the rate of base catalyzed hydrolysis of acetyl-imidazole in a model system.⁸

(8) M. L. Bender and B. W. Turnquest, *THIS JOURNAL*, **79**, 1656 (1957).

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SYNTHESIS OF POTENTIAL ANTICANCER AGENTS. X. 2-FLUOROADENOSINE¹

Sir:

Recently the biological activity of three fluoro derivatives of naturally occurring pyrimidines has been reported.²

Of these three fluoropyrimidines, 5-fluorouracil and 5-fluoro \ddot{o} rotic acid have shown appreciable tumor-inhibitory activity against a variety of rat and mouse tumors^{2a} and 5-fluorouracil was selected for clinical trials.^{2b} The biological activity of the fluoropyrimidines increased our interest in the preparation of fluoropurines and their ribosides, especially fluoro derivatives of naturally occurring purines. Although Bendich, Giner-Sorolla and Fox were unable to prepare 6-fluoropurine from adenine by the Schiemann reaction,³ Weisbach successfully prepared 2-fluoropyrimidine from 2-aminopyrimidine by this method.⁴ The success of the

(1) This work was supported by funds from the C. F. Kettering Foundation. Part IX, John A. Montgomery and Carroll Temple, Jr., *THIS JOURNAL*, in press.

(2) (a) C. Heidelberger, D. Morren, L. Griesbach, B. J. Montag, R. Duschinsky, E. Plevin and R. Schnitzer, *Proc. Am. Ass. Cancer Research*, **2**, 212 (1957); (b) F. A. McIver, A. R. Curreri, O. O. Meyer, R. F. Schilling and H. Waisman, *ibid.*, **2**, 230 (1957); (c) C. Heidelberger, L. Bosch, N. K. Chaudhuri and P. B. Danneberg, *Federation Proc.*, **16**, 194 (1957); (d) J. M. Scheiner, E. Kostelak and R. Duschinsky, *ibid.*, **16**, 242 (1957); (e) T. Wong and W. M. Benson, *ibid.*, **16**, 348 (1957).

(3) A. Bendich, A. Giner-Sorolla and J. J. Fox, "The Chemistry and Biology of Purines" (A Ciba Foundation Symposium), J. and A. Churchill Ltd., London, England, 1957, p. 7.

(4) D. E. Weisbach, M. S. Thesis, University of North Carolina, 1954.

latter reaction led us to attempt the preparation of 2-fluoroadenosine from 2,6-diaminopurine riboside by diazotization in fluoboric acid.

An aqueous solution of sodium nitrite (360 mg. in 2.4 ml.) was added with stirring to a solution of 2,6-diaminopurine riboside⁵ (846 mg.) in 48% fluoboric acid (9.6 ml.) at -10° . The solution was stirred at -10° to 0° for 15 minutes, cooled to -20° and neutralized with 50% sodium hydroxide solution. The water was removed *in vacuo* and the residue chromatographed on a Celite column using water-saturated butanol. The crude 2-fluoroadenosine obtained (149 mg.) was recrystallized from absolute ethanol and dried *in vacuo* over P_2O_5 at 70° for several hours: yield, 75 mg. (8.7%), dec. at 200° ; (α)^{26D} -60.3 ± 11.1 (0.127% in ethanol); $\lambda_{\max}^{pH 1}$ 260.5 $m\mu$ (a_M 13,700); $\lambda_{\max}^{pH 13}$ 260.5 $m\mu$ (a_M 14,300); $\lambda_{\max}^{pH 13}$ 260.5 (a_M 14,800). *Anal.* Calcd. for $C_{10}H_{12}FN_5O_4 \cdot \frac{1}{4}C_2H_5OH$: C, 42.45; H, 4.60; N, 23.60. Found: C, 42.34; H, 4.93; N, 23.40. A qualitative test for fluorine was positive. The ratio of the R_f values of 2-fluoroadenosine and adenine in butanol-water on a descending paper chromatogram (Watman No. 1) was 0.9.

2-Fluoropurine was prepared in the same manner from 2-aminopurine⁶ (850 mg.): yield, 254 mg. (41%) dec. at 216° ; $\lambda_{\max}^{pH 1}$ 264 $m\mu$ (a_M 8,300) $\lambda_{\max}^{pH 7}$ 266.5 $m\mu$ (a_M 8,400), $\lambda_{\max}^{pH 13}$ 272 $m\mu$ (a_M 8,800). *Anal.* Calcd. for $C_5H_3FN_4$: C, 43.48; H, 2.20; N, 40.60. Found: C, 43.52; H, 2.01; N, 40.37. A qualitative test for fluorine was positive.

In preliminary tests 2-fluoroadenosine inhibits the growth of Human Epidermoid Carcinoma (HE 2) at 10^{-8} g./ml. Five times this concentration is required to inhibit monkey kidney cells. Azaserine and 6-diazo-5-oxo-L-norleucine inhibit the growth of these tissues at 10^{-7} g./ml.

The preparation of other 2-fluoropurines is now under way in this laboratory.

(5) J. Davoll and B. A. Lowy, *THIS JOURNAL*, **73**, 1650 (1951).

(6) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

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THE SYNTHESIS OF 5-FLUOROPYRIMIDINES

Sir:

We wish to report the synthesis of a new class of compounds, some of which were designed to function as nucleic acid antagonists, by substituting fluorine for hydrogen in naturally occurring pyrimidines.

The 5-fluoropyrimidines (III) were obtained from pseudourea and pseudothiourea salts (I) and α -fluoro- β -keto ester enolates (II) by adaptation of the Wheeler synthesis.¹

Crystalline IIa was prepared by the addition at 0° of 2.4 moles of methyl formate and 1.2 moles of ethyl fluoroacetate (IV) to 1.2 moles of potassium ethoxide in 800 ml. of toluene and letting the mix-

(1) H. L. Wheeler and H. F. Merriam, *Am. Chem. J.*, **29**, 478 (1903); A. Dornow, F. Boberg and L. Schürer, *Arch. Pharm.*, **286**, 494 (1953).