Throughout this paper the term denaturation has been used to denote in a general way the phenomenon of loss of various characteristics of the initial structure of a protein by more or less irreversible processes. It is probably not meaningful to attempt to attach a more precise definition since in specific cases the criteria by means of which it can be studied will vary widely and will not always indicate the same changes or extent of change. "Native" and "denatured" are biological terms to which we wish to give chemical meaning by investigation of the isomerizations, conformational changes and other rearrangements of the bonded structures which characterize these reactions of proteins.

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Fluorine-containing Pyrimidines and Purines: Synthesis and Properties of Trifluoromethyl Pyrimidines and Purines[1]

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RECEIVED DECEMBER 7, 1957

Syntheses of the following purines are described: 6- and 8-trifluoromethyl, 2-amino-6-trifluoromethyl, 2,6-diamino-8-trifluoromethyl and 2-amino-6,8-bis-trifluoromethyl. The syntheses of the 2- and 8-trifluoromethyl derivatives both of hypoxanthine and adenine also are described. A number of 6-trifluoromethylpyrimidines were prepared, some of which served as intermediates for the above purines. They include the uracil, 2-thiouracil, isocytosine and some 5-phenylazo derivatives. The 5-phenylazo compounds were converted to the amines by catalytic hydrogenolysis. The dissociation and ultraviolet absorption behavior of many of these compounds were studied and it was found that the trifluoromethyl group exerted a powerful inductive influence which was manifested by base-weakening and acid-strengthening effects on the parent pyrimidines and purines. The electron-withdrawing effect of the trifluoromethyl group was found to be quantitatively greater than the electron-donating effect of the amino group in certain of the purines. Evidence is presented that it probably is the pyrimidine moiety of the purine molecule which accepts the proton in acid solutions.

In 1939, May and Litzka[4] reported that 3-fluorotyrosine inhibited the growth of experimentally induced tumors in mice and rats. Since that time other compounds containing fluorine atoms in place of hydrogen have also shown striking biological effects. A few examples are the extremely toxic fluoroacetate,[5] the enhanced hormonal action of 9-α-fluorohydrocortisone[6] and the anti-microbial activity of 3-fluorophenylalanine.[7] Because of the special interest in the application of purines (such as 6-mercapto-[8] and 6-chloropurine[9]) to the control of neoplastic disease (see ref. 10), a program of synthesis of fluorine-containing purines (and pyrimidines) was begun in 1955 in the hope that other agents could be developed which might be useful in human cancer chemotherapy. A preliminary account of a portion of this work has appeared[11] and this paper presents the synthesis and properties of such compounds. Recently, several tumor-inhibiting fluorine-containing pyrimidines[12,13] and purines[14] have been prepared.

Synthetic Studies.—Because of the anti-tumor activity of purine and 6-chloropurine,[9] it was considered of importance to prepare 6-fluoropurine. Of especial interest was the failure[11] to synthesize this compound by application of the Schiemann reaction to adenine (6-aminopurine) despite the ready conversion of α- and β-aminopyridine (but not the γ-isomer) to the corresponding fluoropyridines by this method.[15]

The conversion of 6-hydrazinopurine[8] to 6-chloropurine upon reaction with ferric chloride in dilute hydrochloric acid[16] prompted the application of this diazotization-type reaction[17] to 6-hydrazinopurine in the presence of ferric fluoride and hydrofluoric or fluoroboric acid, but it was without success. Direct replacement of the halogen in 6-chloro- and 6-iodopurine[18] by means of a variety of fluorides was also unsuccessful. Accordingly, the synthesis of other analogs was undertaken.

6-Methylpurine[19] has been found to be highly toxic to mammals[20] and mouse and human tumor

(1) This investigation was supported by grants from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant CY-3190); the Atomic Energy Commission (Contract No. AT(30-1) 910); and the Ann Dickler League.

(2) Fellow of the Institute of International Education (1954–1956) and the Damon Runyon Memorial Fund (1956–1957).

(3) From the thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Sloan-Kettering Division of Cornell University Medical College.

(4) H. May and G. Litzka, *Z. Krebsforsch.*, **48**, 376 (1939).

(5) M. B. Chenoweth, *Pharmacol. Revs.*, **1**, 383 (1949).

(6) J. Fried and E. F. Sabo, THIS JOURNAL, **76**, 1455 (1954).

(7) H. K. Mitchell and C. Niemann, *ibid.*, **69**, 1232 (1947).

(8) G. B. Elion, E. Burgi and G. H. Hitchings, *ibid.*, **74**, 411 (1952).

(9) A. Bendich, P. J. Russell, Jr., and J. J. Fox, *ibid.*, **76**, 6073 (1954).

(10) G. H. Hitchings and C. P. Rhoads, *Ann. N. Y. Acad. Sci.*, **60**, art. 2, 185 (1954).

(11) A. Bendich, A. Giner-Sorolla and J. J. Fox in "The Chemistry and Biology of Purines," Wolstenholme and O'Connor editors, J. and A. Churchill, London, 1957, p. 3; a Ciba Foundation Symposium, held in London, May, 1956.

(12) C. Heidelberger, N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Pleven and J. Scheiner, *Nature*, **179**, 663 (1957).

(13) R. Duschinsky, E. Pleven and C. Heidelberger, THIS JOURNAL, **79**, 4559 (1957).

(14) J. A. Montgomery and K. Hewson, *ibid.*, **79**, 4559 (1957).

(15) A. Roe and G. F. Hawkins, *ibid.*, **69**, 2443 (1947).

(16) A. Giner-Sorolla and A. Bendich, *ibid.*, **80**, 3932 (1958).

(17) O. A. Seide, S. M. Scherlin and G. V. Bras, *J. prakt. Chem.*, **138**, 55 (1933).

(18) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **78**, 3510 (1956).

(19) S. Gabriel and J. Colman, *Ber.*, **34**, 1234 (1901).

(20) F. S. Philips, S. S. Sternberg, L. Hamilton and D. A. Clarke, *Ann. N. Y. Acad. Sci.*, **60**, art. 2 (1954).

TABLE I

PHYSICAL PROPERTIES

Compound	Melting point °C. (uncor.)	Solubility in water at 20° (±2°), 1 part in	pK_a (in water)[a]	Ultraviolet spectral data: pH	Species charge	λ_{max}, mμ	$\epsilon \times 10^{-3}$
6-Methylpurine[b,c]	235–236	5	9.02	0	+	265	7.73
			2.6	5.84	0	261	8.32
				11.53	−	271	8.52
6-Trifluoromethylpurine (I)	254–255		7.35	3 *N* HCl	Mainly +	267.5	8.40
			<0	3.23	0	270	8.11
				10.25	−	275	7.48
8-Methylpurine[b,c]	271–273	18	9.37	0	+	264	8.33
			2.85	5.85	0	266	10.2
				12.0	−	274	8.33
8-Trifluoromethylpurine (XXVII)	192	15	5.12	3 *N* HCl	Mainly +	267.5	7.04
			1.0	3.0	0	264	7.72
				7.98	−	270.5	8.80
2-Aminopurine[b,c]		120	9.93	−3.5	++	235, 325	6.46, 4.17
			3.80	1.84	+	237, 314	4.17, 3.98
			−0.28	7.0	0	236, 305	5.02, 6.03
				12.0	−	276, 303	4.08, 5.76
2-Amino-6-trifluoromethylpurine (XXIV)	360 dec.	6700	8.87	0.21	+	322.5	6.00
			1.85	5.14	0	323	5.94
				12.62	−	283, 323	4.78, 4.96
2-Amino-6,8-bis-trifluoromethylpurine (XXV)	230	705	5.02	3 *N* HCl	Mainly +	334	3.39
			ca. 0.3	2.14	0	327.5	7.32
				7.7 to 13	−	278, 327	4.75, 7.67
2,6-Diaminopurine[b,c]		420	10.77	−1.2	Partly ++	247, 296	10.2, 7.60
			5.09	3.0	+	241, 282	9.13, 10.5
			<1	7.38	0	246–247, 279–280	7.09, 8.92
				13.0	−	243, 284	4.68, 9.34
2,6-Diamino-8-trifluoromethylpurine (XXIX)	>350 dec.	2400	7.55	1.36	+	249, 287	8.37, 7.85
			3.68	5.87	0	284	10.8
				1 *N* NaOH	−	245, 288	2.61, 7.41
6-Hydroxypurine[b,c]		1400[d]	12.10	−0.75	+	248	10.5
			8.94	5.18	0	249	10.5
			1.98	10.35	−	258	11.2
				13.0	Mainly =	262	11.1
6-Hydroxy-2-trifluoromethylpurine (XXXV)	325 dec.	890	11.2	1 *N* HCl	Mainly +	247	9.05
			5.1	3.2	0	253	8.18
			ca. 1.1	7.03	−	258	9.10
				1 *N* NaOH	=	263	9.30
6-Hydroxy-8-trifluoromethylpurine (XXXI)	322–324	610	10.9	8.57	−	260.5	12.2
			ca. 5	1 *N* NaOH	=	268	12.1
Uracil	338	280[e]	>13[f]	7.2	0	259.5	8.20
			9.5[f]	12.0	−	284	6.15
				1 *N* NaOH	Partly =	276.5	6.38
6-Trifluoromethyluracil (III)	220–222	340	*ca.* 13	1 *N* HCl	Mainly 0	259	7.70
			5.7	8.28	−	291	10.20
				1 *N* NaOH	Mainly =	291	7.15

[a] Apparent pK_a determined spectroscopically [A. Bendich, P. J. Russell, Jr., and J. J. Fox, THIS JOURNAL, **76**, 6073 (1954)]. For those values given to two decimal places the experimental errors are within ±0.05; those given to one decimal place are within ±0.1. The values for 6-methyl-, 8-methyl-, 2,6-diamino- and 6-hydroxypurine, given for comparison, were determined by potentiometric titration; see ref. *b*. Determinations of dissociation constants of pyrimidines and purines by both methods are quite comparable: see Table II of A. Bendich, ref. 30. [b] The dissociation and solubility data were taken from A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954). [c] The spectral data are from S. F. Mason, *ibid.*, 2071 (1954). [d] At 23°; E. Fischer, *Ber.*, **30**, 2226 (1897). [e] At 25°; H. L. Wheeler and H. F. Merriam, *Am. Chem. J.*, **29**, 478 (1903). [f] D. Shugar and J. J. Fox, *Biochim. et Biophys. Acta*, **9**, 199 (1952). The first acidic pK_a of 6-methyluracil is 9.7, the second (probably above 13) was not reported: J. R. Marshall and J. Walker, *J. Chem. Soc.*, 1004 (1951).

cells in tissue culture.[21] The possibility that a relatively small alteration in the structure might result in a less toxic compound with retention of its antitumor activity made it of interest to prepare 6-trifluoromethylpurine (I).

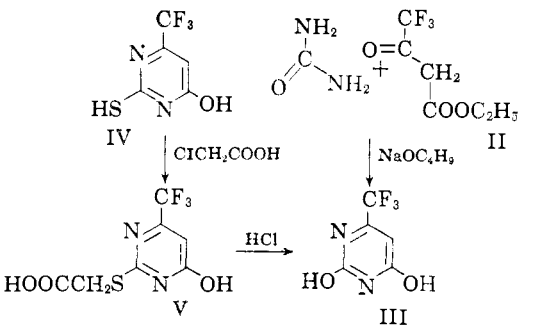
The method for the synthesis of 6-methylpurine from 6-methyluracil[19] proved not to be applicable to the synthesis of I since a suitable nitrogen function[22] could not be introduced into the 5-position of the analogous 6-trifluoromethyluracil (III) by nitration with fuming nitric acid or by nitrosation. An explanation of these failures probably resides in the strong inductive effect of the tri-

(21) J. J. Biesele, Proc. Third National Cancer Conf., 1957, p. 405.

(22) B. Lythgoe, A. R. Todd and A. Topham, *J. Chem. Soc.*, 315 (1944).

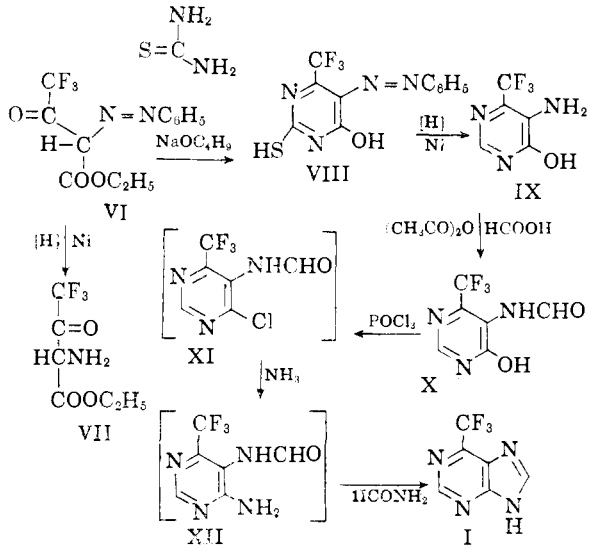
fluoromethyl group which results in electron depletion at C_5. Evidence for this effect on ring electron density is seen in the dissociation behavior of 6-trifluoromethyluracil (III) as compared with uracil: the first and second pK_a's of uracil[23] are 9.5 and >13, the first of 6-methyluracil is 9.7,[24] whereas those for III are (Table I) 5.7 and *ca.* 13.

SCHEME I



In any case, the synthesis of III by condensation of ethyl trifluoroacetoacetate (II) in the presence of sodium butoxide resulted in a very poor yield (*ca.* 2%); see Scheme 1. An alternative route to III from thiourea gave the 2-thio derivative IV in 55% yield.[25] Conversion of IV to III in the usual manner[26-28] *via* the hydrolysis of the intermediate carboxymethylthio derivative V afforded III in 57% yield. A method for the synthesis of I was investigated which was fashioned partly after a total synthesis of adenine[29] (see Scheme 2).

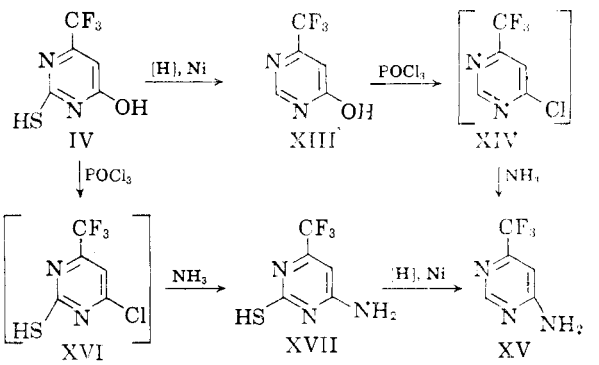
SCHEME 2



Ethyl trifluoroacetoacetate (II) was coupled with benzenediazonium chloride to give the phenylazo ester VI, which was reduced to a compound the analysis of which corresponded to the amino derivative VII. Condensation of thiourea and VI in the presence of sodium butoxide in boiling butanol gave 4-hydroxy-2-mercapto-5-phenylazo-6-trifluoromethylpyrimidine (VIII) in 91% yield. Concomitant desulfurization and hydrogenolysis with Raney nickel gave 5-amino-4-hydroxy-6-trifluoromethylpyrimidine (IX). It was not found possible to replace directly the 4-hydroxyl group of IX with chlorine. However, after formylation of IX to give 5-formylamino-4-hydroxy-6-trifluoromethylpyrimidine (X), reaction of X with $POCl_3$ gave the intermediate 4-chloro compound (XI) which was converted to 4-amino-5-formylamino-6-trifluoromethylpyrimidine (XII). Ring closure of XII gave the desired 6-trifluoromethylpurine (I) in 8% yield from IX. Several of the principles in this type of synthesis have been discussed.[30]

The Raney nickel desulfurization of 4-hydroxy-2-mercapto-6-trifluoromethylpyrimidine (IV) led to XIII which was chlorinated to the intermediate XIV and aminated, in turn, to give 4-amino-6-trifluoromethylpyrimidine (XV) in 58% yield from XIV (see Scheme 3). The same compound (XV) was obtained by prior chlorination of IV to give XVI which was aminated to XVII and then desulfurized, but the yields were lower by this route.

SCHEME 3



In another series leading to 2-aminopurine derivatives, three reactions were employed in attempts to introduce a nitrogen function at C_5 of the requisite pyrimidine intermediates. Ethyl trifluoromethylacetoacetate was condensed with guanidine to give 2-amino-4-hydroxy-6-trifluoromethylpyrimidine (XVIII) in 72% yield (see Scheme 4). By bromination of XVIII in either carbon tetrachloride[31] (77% yield) or in glacial acetic acid (62% yield), the 5-bromo derivative XIX was obtained, but this could not be aminated. Direct nitration of XVIII with fuming nitric acid could not be effected. The synthesis of XX was carried out by direct condensation of ethyl-(α-phenylazo)-trifluoroacetoacetate (VI) and guanidine by refluxing in 1-butanol in the presence of sodium butoxide.

Chlorination of XX with phosphorus oxychloride gave the intermediate 4-chloro compound XXI

(23) D. Shugar and J. J. Fox, *Biochim. et Biophys. Acta*, **9**, 199 (1952).

(24) J. R. Marshall and J. Walker, *J. Chem. Soc.*, 1004 (1951).

(25) This compound (4-hydroxy-2-mercapto-6-trifluoromethylpyrimidine) was synthesized earlier by W. H. Miller, A. M. Dessert and G. W. Anderson, THIS JOURNAL, **70**, 500 (1948).

(26) H. L. Wheeler and L. M. Liddle, *Am. Chem. J.*, **40**, 547 (1908).

(27) A. Bendich, J. F. Tinker and G. B. Brown, THIS JOURNAL, **70**, 3109 (1948).

(28) G. H. Hitchings and P. B. Russell, *J. Chem. Soc.*, 2454 (1949).

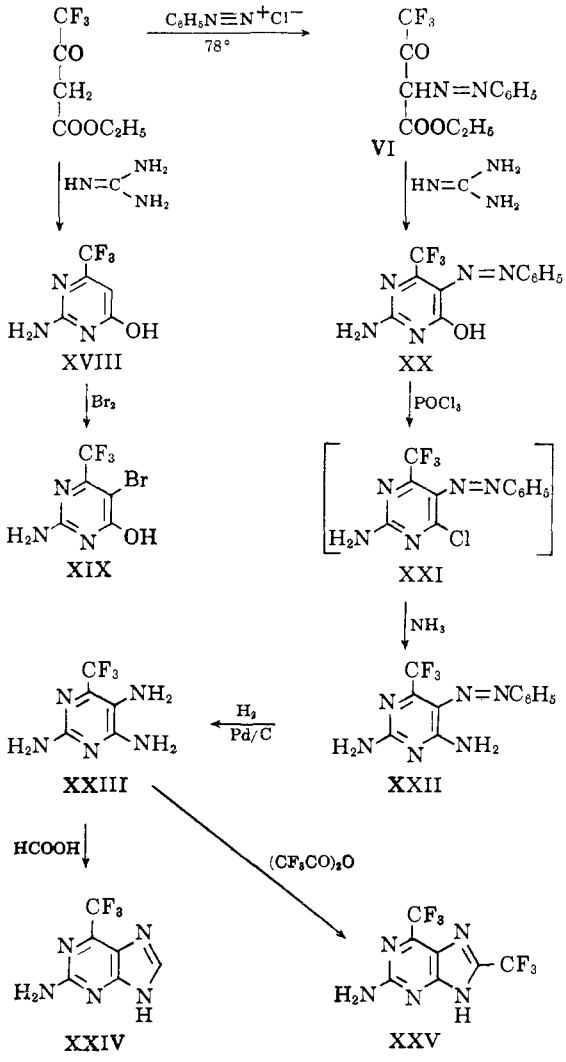
(29) J. Baddiley, B. Lythgoe and A. R. Todd, *ibid.*, 386 (1943).

(30) A. Bendich, in "The Nucleic Acids," Vol. 1, Chargaff and Davidson, eds., Academic Press, Inc., New York, N. Y., 1955, p. 81.

(31) A. Bendich and G. C. Clements, *Biochim. et Biophys. Acta*, **12**, 462 (1953).

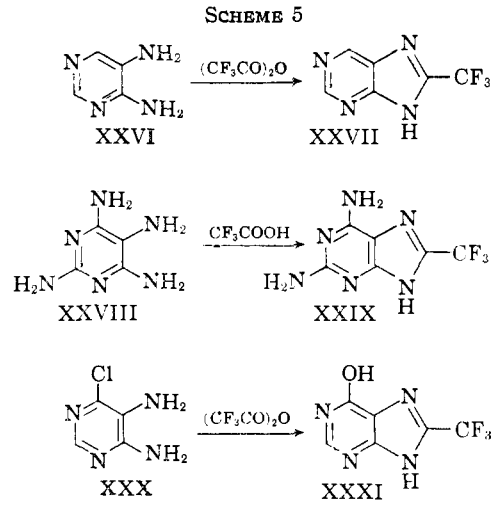
which was converted to the 4-amino derivative XXII in alcoholic ammonia. Catalytic hydrogenolysis afforded 2,4,5-triamino-6-trifluoromethylpyrimidine (XXIII). 2-Amino-6-trifluoromethylpurine (XXIV) and 2-amino-6,8-bis-trifluoromethylpurine (XXV) were obtained therefrom by refluxing with trifluoroacetic acid and trifluoroacetic anhydride (22 and 28%, respectively).

SCHEME 4



It was of interest to determine whether the antitumor activity of certain purines might be affected by the presence of the trifluoromethyl group in the 8-position. The following syntheses were therefore carried out (see Scheme 5). The reaction of 4,5-diaminopyrimidine (XXVI) with refluxing trifluoroacetic anhydride gave 8-trifluoromethylpurine (XXVII). Analogously, 2,4,5,6-tetraaminopyrimidine (XXVIII) afforded 2,6-diamino-8-trifluoromethylpurine (XXIX) in boiling trifluoroacetic acid. When, however, 6-chloro-4,5-diaminopyrimidine (XXX) was refluxed with trifluoroacetic anhydride, the hypoxanthine (XXXI) was obtained instead of the desired 6-chloropurine derivative. The conversion of XXX to hypoxanthine rather than 6-chloropurine was observed

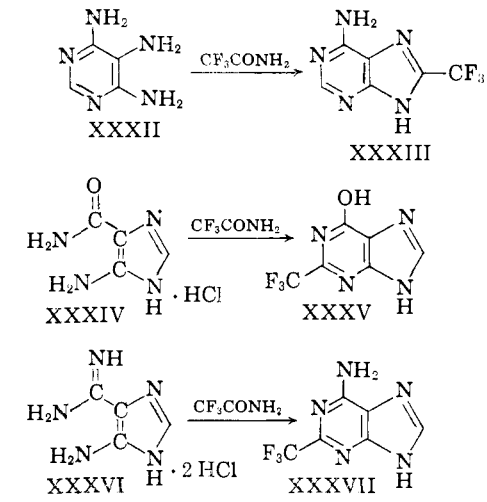
SCHEME 5



previously in boiling formic acid[9] and formamide.[32]

The reaction of 4,5,6-triaminopyrimidine (XXXII) (see Scheme 6) with boiling trifluoroacet-

SCHEME 6



amide gave 8-trifluoromethyladenine (XXXIII) in 80% yield. To prepare the 2-trifluoromethyl derivatives of hypoxanthine and adenine, 4-amino-5-imidazole carboxamide hydrochloride (XXXIV) was refluxed with trifluoroacetamide to give 6-hydroxy-2-trifluoromethylpurine (XXXV) and, in similar fashion, 4-amino-5-imidazole carboxamidine dihydrochloride (XXXVI) gave 6-amino-2-trifluoromethylpurine (XXXVII). The compounds XXXV and XXXVII are of interest because they are analogs of 2-methylhypoxanthine[32] and 2-methyladenine[33] which occur in pseudovitamin B_{12}.[34,35]

Physico-chemical Considerations.—A measure of the strong inductive effect of the trifluoromethyl group on the pyrimidine nucleus is seen in the dissociation behavior of 6-trifluoromethyl-

(32) R. K. Robins, R. J. Dille, C. H. Willits and B. E. Christensen, THIS JOURNAL, **75**, 263, 6359 (1953).

(33) For an ingenious synthesis of 2-methyladenine and related purines, see O. Vogl and E. C. Taylor, *ibid.*, **79**, 1518 (1957).

(34) E. L. Smith, ref. 11, p. 160.

(35) H. W. Dion, D. G. Calkins and J. J. Pfiffner, THIS JOURNAL, **76**, 948 (1954).

uracil (III) (Table I) as determined spectrophotometrically (Fig. 1). Reference to this was made above in an explanation of the failure to nitrate III with fuming nitric acid despite the readiness with which uracil or 6-methyluracil can be nitrated.[19,36] It thus can be visualized that the electron depletion of the ring, brought about by the trifluoromethyl group, was sufficient to prevent reaction with the electrophilic nitronium ion.[37]

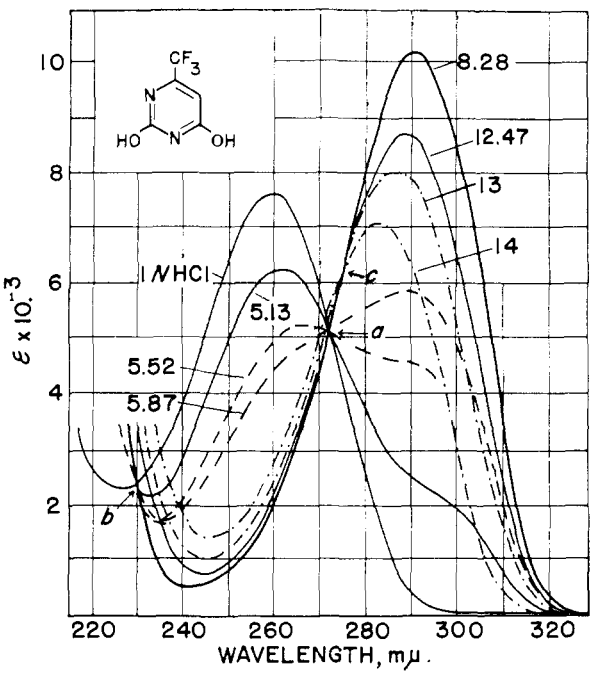


Fig. 1.—Ultraviolet absorption spectra of 6-trifluoromethyluracil; for explanation see text.

The ultraviolet absorption spectrum of III as a function of *p*H is given in Fig. 1. Since its first pK_a is 5.7 and the second *ca.* 13, the curve for 1 *N* HCl (and those, not shown, up to *p*H *ca.* 3.7) represents the neutral species, and shows a single peak at 259 mμ, ε 7,700. As the *p*H is increased above 3.7, this maximum decreases and its position shifts to longer wave lengths, and at *p*H 8.28 (the curve of which is due to the monoanionic species) there is a new single peak at 291 mμ, ε 10,200. It is clear that any curve at an intermediate *p*H will be a result of contributions from both absorbing species and that all curves in this region will cross at isosbestic points *a* and *b*,[38] because at these points both species have the same extinction coefficients. There are no changes in the spectrum as the *p*H is increased from 8.28 to about 11. With still further increases in *p*H, a second acidic association (pK_a about 13) is manifest and finally at *p*H 14 (1 *N* NaOH) the curve mainly for the dianionic species is seen, the curves in this region of *p*H crossing at isosbestic point *c*. As the monoanionic form is in equilibrium with both the neutral and doubly charged species, its curve (*p*H 8.28) passes through the three isosbestic points.

It was previously concluded[23] from ultraviolet spectral studies that the first dissociation of uracil (pK_a 9.5) was due to the 2-hydroxyl (more specifically the lactim–lactam system at N_1–C_2) and the second due to the 4-hydroxyl. The convincing conclusion stemmed from a comparison of the spectral changes of uracil and suitably selected derivatives (*i.e.*, 3- and 1-methyluracil) in which the dissociation of the 2- or 4-hydroxyl was blocked. The analogous derivatives of 6-trifluoromethyluracil were not available for the present study; hence a direct comparison of spectral changes (due to *p*H) of uracil and III was made. The striking similarity in spectral behaviors of uracil[23] (*p*H 4 to 12), due to the first dissociation, 3-methyluracil[23] and III (*p*H 0 to 8) suggests the same order of dissociation in III, *i.e.*, first the 2-hydroxyl, then the 4-. The introduction of the 6-trifluoromethyl group, however, caused a 6,000-fold increase in the acid strength of uracil. It can be anticipated that the placement of the trifluoromethyl group at C_5 would not show so large an effect as it would be removed from the N_1–C_2 system by an additional carbon atom. Note the effect of the position of the fluorine atom on the dissociation behavior of pyridine: the values of pK_a for pyridine and its 2- and 3-fluoro derivatives are, respectively, 5.17, −0.44 and 2.97.[39]

Another example of the effect of the position of an electron-attracting group on acid and base strength is seen in the differential influence of the trifluoromethyl group in position 6 as compared to position 8 of purine (Table I). 8-Trifluoromethylpurine (XXVII) is both a stronger acid and a stronger base than its 6-isomer I. The placement of the trifluoromethyl group in the 6-position of purine has a greater base-weakening effect than when substituted at C_8 as might be expected if the pyrimidine moiety (rather than the imidazole) of these purines were the site of proton capture leading to the formation of the cationic species. See ref. 11 for a more extensive treatment and discussion of this interpretation.

The introduction of the electron-donating amino group into position 2 of purine (pK_a 2.52 and 8.92[9]) causes an expected increase in base strength and a decrease in acid strength as seen in an elevation of the pK_a's to 3.80 and 9.93.[40] The increase in electron density of the ring due to the amino group is such as to allow measurement[40] of an additional basic dissociation, pK_a −0.28 at *p*H's below zero. Introduction of the 6-trifluoromethyl group (compound XXIV) into the 2-aminopurine nucleus was more than sufficient to counteract the increased electron density due to the 2-amino group, and as a consequence there is a decrease in pK_a's to values lower than those of purine. A second basic dissociation was apparent when the ultraviolet absorption spectrum was examined in 3 *N* HCl, but it was too feeble to permit accurate measurement of the dissociation constant. It can there-

(36) D. J. Brown, *J. Appl. Chem.*, **2**, 239 (1952).

(37) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell Univ. Press, Ithaca, N. Y., 1953, p. 269.

(38) For discussions of the significance of isosbestic points in the spectra of pyrimidines, and purines, see J. J. Fox and D. O. Shugar, *Biochim. et Biophys. Acta*, **9**, 369 (1952), and Ref. 30.

(39) H. C. Brown and D. H. McDaniel, THIS JOURNAL, **77**, 3752 (1955).

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

fore be inferred from the data on these compounds that the electron-withdrawing effect of the trifluoromethyl group is quantitatively greater than is the electron-donating effect of the amino group.

The introduction of a second trifluoromethyl group gives 2-amino-6,8-bis-trifluoromethylpurine (XXV) the dissociation behavior of which (pK_a 0.3, 5.02) is very similar to that of 8-trifluoromethylpurine (XXVII) (pK_a 1.0, 5.12). The ultraviolet absorption spectra of XXIV and XXV (Table I), still show marked similarities in shape to that of 2-aminopurine.[41]

When a second amino group is placed in position 6 of 2-aminopurine, the resulting compound, 2,6-diaminopurine, as can be anticipated, is a stronger base and a weaker acid ($pK_a > 1$, 5.09 and 10.77)[40] than 2-aminopurine. Again, the introduction of the trifluoromethyl group to give 2,6-diamino-8-trifluoromethylpurine (XXIX) has more than offset the increased ring electron density due to the second amino group: the compound XXIX is a stronger acid (pK_a 7.55) than 2-aminopurine and a slightly weaker base (pK_a 3.68). The base-weakening activity of the trifluoromethyl group in XXIX was sufficient to lower the first basic pK_a of 2,6-diaminopurine so that the very feeble first dissociation of XXIX could not be demonstrated below *p*H 1.36. The ultraviolet absorption spectra of XXIX at *p*H 1.36 and in 3 *N* HCl were indistinguishable.

It has been pointed out[40] previously that the cationic dissociation constant of hypoxanthine (6-hydroxypurine), *i.e.*, pK_a 1.98, had escaped attention prior to 1954 because the ultraviolet absorption spectra of the cationic and neutral species are very nearly the same.[41] The spectra of the corresponding species of 6-hydroxy-2-trifluoromethylpurine (XXXV) were sufficiently distinct to make possible spectroscopic determination of both the basic and the first acidic dissociations (Table I). This was not so for its 8-trifluoromethyl isomer XXXI and it was difficult to obtain convincing spectral data below *p*H *ca.* 4. It is clear, however, that in both the 2- and 8-position the trifluoromethyl group exerts a powerful acid-strengthening effect as evidenced by a decrease in the first anionic dissociation constants (decrease of about 3.8 pK_a units from that of hypoxanthine). If the electron-withdrawing effect of the 2-trifluoromethyl group were not about equal to that of the 8-trifluoromethyl in these hypoxanthine derivatives, it might have been possible to decide whether the first acidic dissociation had resulted in proton removal from the 6-hydroxyl or from the imidazole. Evidence from the potentiometric titration of hypoxanthine in the presence of heavy metal ions has led to the conclusion[42] that the first acidic dissociation of hypoxanthine concerns the 6-hydroxyl rather than the imidazole nucleus.

Experimental[43]

Ethyl (α-Phenylazo)-trifluoroacetoacetate (VI).—The procedure of Bulow and Neber[44] for the preparation of ethyl (α-phenylazo)-acetoacetate was employed with some modifications. Aniline (52 g., 0.56 mole) was dissolved in 160 ml. of concentrated HCl and diazotized at 0° with a solution of sodium nitrite (43.5 g., 0.63 mole) in 90 ml. of water. A solution of 450 g. of sodium acetate in one liter of water was added slowly with continuous stirring, and the temperature was kept at 0°. Ethyl trifluoroacetoacetate (II)[45] (112 g., 0.60 mole) in 90 ml. of ethanol was added dropwise with stirring at 0°. After a few minutes, a yellow crystalline precipitate appeared which turned red and increased in amount. The mixture was stirred at 0° for 4 hours and was allowed to stand at room temperature overnight. The precipitate was collected, washed with cold water and dried *in vacuo* over pellets of sodium hydroxide; yield 136 g. (78%), m.p. 77–78°. The product was sparingly soluble in water and easily soluble in common organic solvents. Its identity was established by hydrogenation (in ethanolic solution at room temperature, atmospheric pressure, over Raney nickel) to afford, presumably, ethyl α-aminotrifluoroacetoacetate (VII), colorless prisms from ethanol, m.p. 200–202°.

Anal. Calcd. for $C_6H_8NO_3F_3$ (VII): C, 36.19; H, 4.03; N, 7.03. Found: C, 36.33; H, 3.99; N, 6.75.

4-Hydroxy-2-mercapto-5-phenylazo-6-trifluoromethylpyrimidine (VIII).—To a solution of sodium (0.46 g., 0.02 g. atom) in 30 ml. of 1-butanol, 1.52 g. (0.02 mole) of thiourea was added. The mixture was stirred and refluxed until complete solution was effected and then ethyl (α-phenylazo)-trifluoroacetoacetate (5.76 g., 0.02 mole) was added. The stirred reaction mixture was refluxed for 3 hr. and allowed to stand at room temperature overnight. The mixture was concentrated *in vacuo* and 200 ml. of water was added. The solution was filtered to eliminate some resinous material and the filtrate was acidified to *p*H 5 with 20% acetic acid. The red precipitate which had formed was filtered, washed with cold water and dried *in vacuo* over solid sodium hydroxide to yield 5.5 g. (91%) (orange needles from ethanol), m.p. 168–172° dec.

Anal. Calcd. for $C_{11}H_7N_4F_3OS$: C, 44.01; H, 2.35; F, 18.99. Found: C, 44.39; H, 3.80; F, 18.53.

5-Amino-4-hydroxy-6-trifluoromethylpyrimidine (IX).—To a suspension of 4-hydroxy-2-mercapto-5-phenylazo-6-trifluoromethylpyrimidine (3.5 g., 0.012 mole) in 100 ml. of water containing 3.5 ml. of concentrated ammonia was added 18 g. of freshly prepared Raney nickel.[46,47] The mixture was stirred and refluxed for one hour. The suspension was filtered when hot and the residue was boiled with 30 ml. of water and filtered. The filtrate was yellow and gave a positive color test for a 5-amino-4-hydroxypyrimidine with alkaline phosphomolybdate reagent.[31] The combined filtrates were further refluxed with 10 g. of fresh Raney nickel and 1 ml. of concentrated ammonia for 45 minutes and filtered. The residue was boiled with 80 ml. of water and filtered. The combined filtrates were concentrated *in vacuo* on a steam-bath to dryness to give 1.1 g. (50%) of yellow crystals, m.p. 190°. On recrystallization from water, yellow needles were obtained, m.p. 222°. The m.p. remained unchanged after three recrystallizations from water.

Anal. Calcd. for $C_5H_4N_3OF_3$: C, 33.52; H, 2.25; N, 23.46. Found: C, 33.61; H, 2.24; N, 23.88.

6-Trifluoromethylpurine (I).[27,32]—A solution of 5-amino-4-hydroxy-6-trifluoromethylpyrimidine (IX) (7.0 g., 0.039 mole) in a mixture of 16 ml. of 98% formic acid and 32 ml. of acetic anhydride was heated at 40° for five minutes and then at 70° for 30 minutes. The solution was concentrated *in vacuo*. The crude residue (8.6 g.), of 5-formylamino-4-hydroxy-6-trifluoromethylpyrimidine (X) melted at 190–195°. A sample of crude 5-formylamino-4-hydroxy-6-trifluoromethylpyrimidine was recrystallized three times from ethanol to give needles with m.p. 195–196°. The product gave a negative phosphomolybdic test.[31]

(41) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

(42) A. Albert, *Biochem. J.*, **54**, 646 (1953).

(43) All melting points are uncorrected. Elementary microanalyses were performed by Dr. J. F. Alicino, Metuchen, N. J.; Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and Spang Microanalytical Laboratory, Ann Arbor, Mich. In all cases, paper chromatographic homogeneity was demonstrated prior to elemental analysis. In certain instances further reliance on homogeneity was placed on the sharpness of isosbestic points in the ultraviolet absorption spectra.[30] In all instances fluorine was present. When percentage of fluorine is omitted, the figures were a few units off.

(44) C. Bulow and P. Neber, *Ber.*, **45**, 3736 (1912).

(45) Supplied by Peninsular ChemResearch Inc., Gainesville, Fla.

(46) R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole and J. R. Vaughan, Jr., THIS JOURNAL, **67**, 292 (1945).

(47) D. J. Brown, *J. Soc. Chem. Ind.*, **69**, 353 (1950).

Anal. Calcd. for $C_6H_4N_3O_2F_3$: C, 34.79; H, 1.95; N, 20.29. Found: C, 34.91; H, 2.35; N, 20.77.

The dried material was dissolved in 30 ml. of phosphorus oxychloride and refluxed for one hour with stirring. The reaction mixture was concentrated *in vacuo* to about two-thirds of the initial volume and cooled. The resulting dark sirup containing 4-chloro-5-formylamino-6-trifluoromethylpyrimidine (XI) was poured onto 150 g. of crushed ice and stirred for 15 minutes. The resulting suspension was filtered and the solid poured into 200 ml. of a cold saturated solution of ammonia in ethanol. The mixture was kept in a refrigerator overnight, concentrated *in vacuo* and the residue (3.7 g. of a black, tarry product) of crude 4-amino-5-formylamino-6-trifluoromethylpyrimidine (XII) was boiled with 15 ml. of formamide for 15 minutes using an air condenser provided with a calcium chloride tube. After cooling, 5 ml. of water was added and the mixture chilled overnight. The solution was filtered to eliminate some tarry material and the filtrate was evaporated *in vacuo*. The brown residue was washed with a little cold water and dried *in vacuo* over P_2O_5 to yield 0.6 g. (8%) of a yellow product, m.p. 225–230° dec. A sample was sublimed at 150–160° (1 mm.) yielding white needles which melted at 254–255° dec.

Anal. Calcd. for $C_6H_3N_4F_3$: C, 38.30; H, 1.61; N, 29.78; F, 30.30. Found: C, 38.59; H, 2.01; N, 29.41; F, 29.93.

4-Amino-2-mercapto-6-trifluoromethylpyrimidine (XVII).—4-Hydroxy-2-mercapto-6-trifluoromethylpyrimidine[25] (IV) (10 g., 0.051 mole) was slowly added to 40 ml. (0.43 mole) of phosphorus oxychloride which was maintained at 20 to 25°. Diethylaniline (13 ml., 0.087 mole) was added dropwise and the temperature kept at 20 to 25°. An additional amount of phosphorus oxychloride (20 ml., 0.22 mole) was poured into the mixture which was then stirred and refluxed for 2 hr. About half of the phosphorus oxychloride was removed by distillation *in vacuo*. The crude reaction product, 4-chloro-2-mercapto-6-trifluoromethylpyrimidine (XVI), was cooled, poured onto 500 g. of cracked ice and stirred for 15 minutes. The white precipitate was collected, washed with cold water and poured into 150 ml. of a saturated solution of ammonia in ethanol and chilled overnight. A precipitate of ammonium chloride was filtered off and the filtrate evaporated to dryness *in vacuo*. The dry residue was extracted with ether and the extracts were poured into petroleum ether. The white precipitate was collected and dried to yield 3.0 g. (33%) of product, m.p. 198–203°. By recrystallization from water, colorless needles were obtained, m.p. 203–205°.

Anal. Calcd. for $C_5H_4N_3F_3S$: C, 30.77; H, 2.06; N, 21.53; F, 29.20; S, 16.40. Found: C, 30.44; H, 2.06; N, 21.45; F, 29.49; S, 16.04.

4-Hydroxy-6-trifluoromethylpyrimidine (XIII).—4-Hydroxy-2-mercapto-6-trifluoromethylpyrimidine (IV) (3.4 g., 0.017 mole) was suspended in a solution of 2 ml. of concentrated ammonia in 50 ml. of water. Raney nickel (7 g.) was added and the mixture refluxed with continuous stirring for 1.5 hr. The suspension was filtered when hot and the precipitate extracted with 15 ml. of boiling water. The combined filtrates were evaporated to dryness *in vacuo* to give 2.5 g. (88%) of white needles, m.p. 160–162°. A sample, sublimed twice at 120° and 0.1 mm., melted at 162–163°.

Anal. Calcd. for $C_5H_3N_2OF_3$: C, 36.59; H, 1.84; N, 17.07. Found: C, 36.33; H, 1.87; N, 17.20.

4-Amino-6-trifluoromethylpyrimidine (XV). Method A.—To a solution of 4 g. (0.025 mole) of 4-hydroxy-6-trifluoromethylpyrimidine (XIII) in 30 ml. (0.32 mole) of phosphorus oxychloride, 8 ml. of diethylaniline (0.05 mole) was added dropwise at 20–25°. An additional amount of phosphorus oxychloride (15 ml., 0.16 mole) was poured into the mixture, which was stirred and refluxed for 1.5 hours. About half of the phosphorus oxychloride was removed by distillation *in vacuo*. The crude 4-chloro-6-trifluoromethylpyrimidine (XIV) was poured, after cooling, onto 250 g. of cracked ice. The greenish oil which formed was separated from the aqueous layer, and after washing with cold water was poured into 30 ml. of a saturated solution of ammonia in ethanol. The combined aqueous layers and washings were extracted with ether and the dried ether extracts also treated with ethanolic ammonia. The ammoniacal solutions were combined and evaporated to dryness *in vacuo*. The residue was washed with water and dried to yield 2.3 g. (58%) of yellow prisms, m.p. 165–170°. The mixed m.p. with XIII was 120°.

Anal. Calcd. for $C_5H_4N_3F_3$: C, 36.82; H, 2.47; N, 25.76. Found: C, 36.98; H, 2.17; N, 26.13.

Method B.—A suspension of 1 g. (0.005 mole) of 4-amino-2-mercapto-6-trifluoromethylpyrimidine (XVII) and 10 g. of Raney nickel in 50 ml. of 1 *M* ammonia was stirred and refluxed for four hours. The suspension was chilled and filtered and the insoluble was washed twice with 20 ml. of boiling water and the combined filtrates were evaporated *in vacuo* to dryness to give 0.3 g. (36%) of colorless prisms, m.p. 168–170°. A mixed m.p. with the product obtained by method A (above) showed no depression.

2,4-Dihydroxy-6-trifluoromethylpyrimidine (III) (6-Trifluoromethyluracil). Method A.—4-Hydroxy-2-mercapto-6-trifluoromethylpyrimidine (IV) (1.96 g., 0.01 mole) was suspended in a solution of monochloroacetic acid (0.95 g., 0.01 mole) in 20 ml. of water. The mixture was refluxed for 4 hr. Upon chilling, there was obtained a 60% yield (1.55 g.) of colorless needles of 2-carboxymethylthio-4-hydroxy-6-trifluoromethylpyrimidine (V), m.p. 175–178° (*Anal.* Calcd. for $C_7H_5N_2SF_3O_3$: N, 11.02. Found: N, 10.92). The melting point rose to 205–207° after 3 recrystallizations from water. A suspension of 1.0 g. (0.004 mole) of this product in 15 ml. of 6 *N* HCl was refluxed for 5 hours and then chilled. The crystalline 2,4-dihydroxy-6-trifluoromethylpyrimidine (III) was collected, washed with water and dried; yield 0.68 g. (95%), m.p. 210–215°. After three recrystallizations from water colorless plates or prisms were obtained, m.p. 218–220°.

Anal. Calcd. for $C_5H_3N_2O_2F_3$: C, 33.34; H, 1.68; N, 15.56; F, 31.65. Found: C, 33.13; H, 2.03; N, 15.95; F, 31.91.

Method B.—Urea (3.0 g., 0.05 mole) was dissolved in a solution of 1.15 g. (0.05 mole) of sodium in 20 ml. of 1-butanol. The mixture was refluxed and stirred until solution was complete. Ethyl trifluoroacetoacetate (II) (9.6 g., 0.05 mole) was added and the mixture refluxed with continuous stirring for three hours. The solution was cooled, filtered and the *p*H of the filtrate was adjusted to 5 by the dropwise addition of concentrated HCl.

The precipitate was collected by filtration, washed with cold water and dried *in vacuo* over sodium hydroxide pellets to give 0.3 g. (3%) of colorless prisms, m.p. 220–222°. The mixed m.p. with crystals prepared by method A was 220–222°. The product was not obtained when ethanol was used instead of butanol.

2-Amino-4-hydroxy-6-trifluoromethylpyrimidine (XVIII).—To a solution of 4.60 g. (0.20 g. atom) of sodium in 80 ml. of 1-butanol, 9.55 g. (0.10 mole) of dry guanidine hydrochloride was added. The mixture was stirred and refluxed for 15 minutes. Ethyl trifluoroacetoacetate (18.4 g., 0.10 mole) was added and the mixture was refluxed for four hours. After chilling, the solution was decolorized with charcoal, and the filtrate adjusted to *p*H 5 by the addition of glacial acetic acid. The precipitate which formed was collected, washed with cold water and dried; 13.0 g. (72%) of colorless needles, m.p. 282°, were obtained.

Anal. Calcd. for $C_5H_4N_3OF_3$: C, 33.53; H, 2.25; N, 23.46. Found: C, 33.27; H, 2.22; N, 23.96.

Nitration of XVIII was unsuccessful, although it could be brominated.

2-Amino-5-bromo-4-hydroxy-6-trifluoromethylpyrimidine (XIX). Method A.[31]—2-Amino-4-hydroxy-6-trifluoromethylpyrimidine (XVIII) (2.7 g., 0.015 mole) was suspended in 25 ml. of carbon tetrachloride, 1.6 g. (0.02 mole) of bromine was added dropwise and the suspension was refluxed for 26 hr. The crude yellow material which remained after the evaporation of the CCl_4 and bromine weighed 6.2 g. The product was dissolved in 50 ml. of 2 *N* sodium hydroxide and the solution decolorized with charcoal. The product was precipitated by the addition of glacial acetic acid to *p*H 5. This procedure was repeated twice and the resulting compound melted at 298° dec. The yield was 3.0 g. (77%). After recrystallization from water, colorless needles were obtained, m.p. 303° dec.

Method B.[48]—The same material was prepared by gentle warming (steam-bath) of 2-amino-4-hydroxy-6-trifluoromethylpyrimidine (XVIII) (3.98 g., 0.022 mole) with bro-

(48) *Cf.* H. L. Wheeler and T. B. Johnson, *Am. Chem. J.*, **29**, 501 (1903).

mine (3.3 g., 0.04 mole) in 20 ml. of glacial acetic acid. The product was isolated by evaporation *in vacuo* and upon recrystallization from water, 3.6 g. (62%) of white needles was obtained, m.p. 298° dec.

Anal. Calcd. for $C_5H_3N_5OBrF_3$: C, 23.28; H, 1.17; Br, 30.97; F, 22.09. Found: C, 23.61; H, 1.45; Br, 30.74; F, 21.83.

The compound was stable in strongly alkaline solutions. No alteration was observed when the compound was refluxed with 10 *N* sodium hydroxide for 0.5 hr. Replacement of the 4-hydroxyl group by chlorine was not successful when attempted with $POCl_3$ in the usual manner. By treatment of the compound with concentrated aqueous ammonia at 160° in a sealed tube for 2 hr. needles were obtained which gave a negative test for fluorine, but the product was not further identified.

2-Amino-4-hydroxy-5-phenylazo-6-trifluoromethylpyrimidine (XX).—Guanidine hydrochloride (6.8 g., 0.071 mole) was added to a solution of sodium (3.6 g., 0.15 g. atom) in 80 ml. of 1-butanol and the mixture stirred and refluxed for 15 minutes. Ethyl (α-phenylazo)-trifluoroacetoacetate (VI) (20.6 g., 0.071 mole) was added and the mixture was refluxed with stirring for 3 hr. The crystalline precipitate was collected and dissolved in 300 ml. of boiling water, treated with charcoal, and the filtrate adjusted to *p*H 5 with glacial acetic acid. A yellow-brown precipitate was obtained. An additional crop of yellow material was obtained from the filtrate following the removal of the butanol by distillation *in vacuo*. The total yield was 16.4 g. (80%), m.p. 280–282°.

Anal. Calcd. for $C_{11}H_8N_6OF_3$: C, 46.64; H, 2.85; N, 24.86. Found: C, 46.93; H, 2.85; N, 25.08.

2,4-Diamino-5-phenylazo-6-trifluoromethylpyrimidine (XXII).—To a mixture of 2-amino-4-hydroxy-5-phenylazo-6-trifluoromethylpyrimidine (XX) (6.7 g., 0.024 mole) in phosphorus oxychloride (32 ml., 0.35 mole), diethylaniline (8.3 ml., 0.05 mole) was added dropwise while the temperature was maintained between 20–25°. An additional amount of phosphorus oxychloride (16 ml., 0.175 mole) was poured into the mixture which was then stirred and refluxed for 1.5 hr. About two-thirds of the phosphorus oxychloride was removed by distillation *in vacuo*. After cooling, the residue, presumably 2-amino-4-chloro-5-phenylazo-6-trifluoromethylpyrimidine (XXI), was poured onto cracked ice (300 g.). The dark precipitate was collected and washed three times with ice-cold water and dissolved in 150 ml. of a cold solution of ethanol saturated with ammonia. The mixture was allowed to stand overnight at room temperature, filtered and the brown precipitate (presumably starting material) was washed with cold water and discarded. The filtrate gave a tarry residue together with ammonium chloride upon concentration *in vacuo*. After removal of the ammonium chloride and water-soluble impurities, 3.0 g. (44%) was obtained, m.p. 215–220°. Upon sublimation at 160° and 0.1 mm., yellow prisms, m.p. 235–236°, were obtained.

Anal. Calcd. for $C_{11}H_9N_6F_3$: C, 46.80; H, 3.24; N, 29.78. Found: C, 46.89; H, 2.94; N, 30.06.

2-Amino-6-trifluoromethylpurine (XXIV).—A solution of 5.0 g. (0.018 mole) of 2,4-diamino-5-phenylazo-6-trifluoromethylpyrimidine (XXII) in 200 ml. of absolute ethanol was hydrogenated at atmospheric pressure and room temperature in the presence of 5.0 g. of 5% palladium–charcoal. The filtered reaction mixture was concentrated to dryness *in vacuo* and 10 ml. of 98% formic acid was added. After evaporation *in vacuo*, 3.5 g. of crude material was obtained. (A sample of crude 2,4,5-triamino-6-trifluoromethylpyrimidine (XXIII) was recrystallized three times from ethanol to give prisms, with m.p. 196–198°. The product gave a positive phosphomolybdic test.[31] *Anal.* Calcd. for $C_5H_6N_5F_3$: C, 31.09; H, 3.13. Found: C, 31.54; H, 3.66.) The crude, unstable 2,4,5-triamino-6-trifluoromethylpyrimidine (XXIII) (1.5 g., 0.008 mole) was dissolved in 10 ml. of 98% formic acid and refluxed at 110° for 0.5 hour under a stream of carbon dioxide. The mixture was concentrated to dryness and kept at 210–215° for 45 minutes. The resulting brown residue was refluxed for 3 hr. with 50 ml. of ethanol and 0.3 g. of calcium carbonate,[40] and the suspension was filtered after the addition of charcoal. The undissolved residue was re-extracted for 0.5 hr. with 30 ml. of boiling ethanol. The combined filtrates were concentrated *in vacuo* to a volume of about 15 ml. On cooling, 0.35 g. (22%) of colorless needles formed which melted at 360° dec., after recrystallization from ethanol. A sample, sublimed *in vacuo*, gave the same m.p.

Anal. Calcd. for $C_6H_4N_5F_3$: C, 35.47; H, 1.98; N, 34.48. Found: C, 35.54; H, 1.81; N, 34.78.

2-Amino-6,8-bis-trifluoromethylpurine (XXV).—A solution of 2 g. (0.01 mole) of crude 2,4,5-triamino-6-trifluoromethylpyrimidine (XXIII) in 10 ml. of trifluoroacetic acid and 2 ml. of trifluoroacetic anhydride was refluxed at 110° for 1 hr. under a stream of carbon dioxide. The solution was then evaporated to dryness and kept at 210–215° for 1 hr. The dark residue was refluxed for 3 hr. with 30 ml. of water and 0.5 g. of calcium carbonate; some charcoal was added and the mixture filtered. On cooling, 0.8 g. (28%) of colorless needles appeared which melted at 230°. After four recrystallizations from water, the melting point remained unchanged. By sublimation *in vacuo*, colorless needles were obtained with m.p. 230°.

The same compound was obtained in lower yield by reaction of XXIII with trifluoroacetamide at 180° for 1 hr. and then removal of the excess of the trifluoroacetamide by distillation *in vacuo* or by thorough washing with ether.

Anal. Calcd. for $C_7H_4N_5F_6$: C, 31.00; H, 1.15; N, 25.83; F, 42.04. Found: C, 30.59; H, 1.49; N, 26.01; F, 41.86.

8-Trifluoromethylpurine (XXVII).—A solution of 2 g. (0.018 mole) of 4,5-diaminopyrimidine (XXVI)[49,50] in 10 ml. of trifluoroacetic anhydride was refluxed for two hours under a stream of carbon dioxide. The temperature then was raised and the anhydride removed by distillation. The residue was kept at 210° for 45 minutes in a carbon dioxide atmosphere. The last traces of anhydride were eliminated by distillation *in vacuo* at 210°. The crystalline residue weighed 2.8 g. (82%), m.p. 192°. For purification, the compound was extracted with boiling ethanol (40 ml.) to which calcium carbonate (0.5 g.) and charcoal were added and the mixture filtered through diatomaceous earth. The extraction with ethanol was repeated. The combined filtrates, concentrated to 15 ml., were kept in the refrigerator for 5 hr. and 1.2 g. of cream-colored needles, m.p. 192°, was obtained. The product was sublimed at 90° and 0.1 mm. yielding crystals, m.p. 192°.

Anal. Calcd. for $C_6H_3N_4F_3$: C, 38.30; H, 1.61; N, 29.78. Found: C, 38.51; H, 1.85; N, 29.49.

2,6-Diamino-8-trifluoromethylpurine (XXIX).[40,51]—A solution of 2,4,5,6-tetraaminopyrimidine (XXVIII)[52] (2.5 g., 0.018 mole) in trifluoroacetic acid (20.5 g., 0.18 mole) was refluxed for 1 hr. under a stream of carbon dioxide. The trifluoroacetic acid then was distilled off *in vacuo* and the residue was heated under CO_2 on a metal-bath at 210° for 1 hr. to give a yellow crystalline product. Recrystallization from a mixture of acetic acid and ethanol (3:2, v./v.) gave 1.85 g. (47%) of colorless crystals, m.p. 230–235°. The compound was sublimed at 250° and 0.1 mm. Thin needles (which showed a blue iridescence under the polarizing microscope) and bright rosettes were obtained by recrystallization of the sublimed material from water. Both crystal forms had the same m.p. >350°. They were identical when examined by paper chromatography in the systems water saturated with 1-butanol and 1-butanol saturated with water. Both showed a blue fluorescence on paper chromatograms under ultraviolet light. This was probably an example of dimorphism.

Anal. Calcd. for $C_6H_5N_5F_3 \cdot 2H_2O$: N, 33.07. Found: N, 32.93.

6-Hydroxy-8-trifluoromethylpurine (XXXI).—A solution of 2 g. (0.014 mole) of 6-chloro-4,5-diaminopyrimidine[53,54] in 20 ml. of trifluoroacetic acid and 10 ml. of trifluoroacetic anhydride was refluxed for 3 hours. The reaction mixture was concentrated to dryness *in vacuo* and the crystalline residue then heated to 260° in a stream of carbon dioxide. The residue was heated *in vacuo* for 1 hr., and the brown

(49) Supplied by the Francis Earle Laboratories, Inc., Peekskill, New York.

(50) *Cf.* O. Isay, *Ber.*, **39**, 250 (1906).

(51) *Cf.* C. K. Cain, M. F. Mallette and E. C. Taylor, THIS JOURNAL, **68**, 1996 (1946); **69**, 1814 (1947).

(52) Supplied by H. M. Chemical Co., Ltd., Santa Monica, Calif.

(53) Kindly supplied by I. Wempen, Sloan-Kettering Institute, New York.

(54) A. Albert, D. J. Brown and G. Cheeseman, *J. Chem. Soc.*, 4219 (1952).

solid (2.8 g.) was extracted with 100 ml. of ethanol to which 0.4 g. of calcium carbonate was added. The filtrate was concentrated *in vacuo* to about 20 ml. On cooling, 1.2 g. of colorless prisms, m.p. 305–310° dec., was obtained. From the filtrate, an additional 1.0 g. of colorless crystals, m.p. 306–308°, was recovered. The m.p. rose to 322–324° dec. after three recrystallizations from water; total yield 2.2 g. (78%).

Anal. Calcd. for $C_5H_3N_4OF_3$: C, 35.30; H, 1.48; N, 27.45; F, 27.93. Found: C, 35.11; H, 1.47; N, 28.20; F, 27.57.

6-Amino-8-trifluoromethylpurine (XXXIII).—4,5,6-Triaminopyrimidine (XXXII)[29,52] (1.5 g., 0.012 mole) was dissolved in melted trifluoroacetamide (8.1 g., 0.072 mole) and refluxed. After a few minutes, a copious white precipitate formed and ammonia was evolved. The refluxing (oil-bath temp. 175–180°) was continued for 2 hr. and the white product washed thoroughly with ether and water to yield 1.95 g. (80%) of colorless crystals which charred at 330–335°. Recrystallization from 50% aqueous ethanol gave prisms with the same m.p.

Anal. Calcd. for $C_6H_4N_5F_3$: C, 35.47; H, 1.98; F, 28.06. Found: C, 35.57; H, 1.98; F, 27.94.

2-Trifluoromethyl-6-hydroxypurine (XXXV).—4-Amino-5-imidazolecarboxamide hydrochloride (XXXIV) (1 g., 0.006 mole) prepared by the method of Shaw,[55] was refluxed with trifluoroacetamide (6.8 g., 0.06 mole) for 4 hr. The solid product was washed thoroughly with ether and water. The yield of crude material was 1 g., m.p. 313–315°. Recrystallization from methanol gave 0.8 g. (78%) of colorless needles, m.p. 324–326° dec.

(55) E. Shaw, *J. Biol. Chem.*, **185**, 444 (1950).

Anal. Calcd. for $C_6H_3N_4OF_3$: C, 35.30; H, 1.48; N, 27.45. Found: C, 35.73; H, 1.69; N, 27.44.

2-Trifluoromethyl-6-aminopurine (XXXVII).—A mixture of 4-amino - 5 - imidazolecarboxamidine dihydrochloride (XXXVI) (2.4 g., 0.012 mole), prepared by the method of Shaw,[55] and trifluoroacetamide (13.5 g., 0.12 mole), was refluxed (oil-bath temp. 175–180°) for 2 hr. The reaction mixture was cooled, washed thoroughly with ether and recrystallized from 50% aqueous ethanol; yield 1.4 g. (56%), m.p. 360–362° dec. After three recrystallizations from 50% aqueous ethanol, colorless needles were obtained with the same m.p.

Anal. Calcd. for $C_6H_4N_5F_3$: C, 35.47; H, 1.98; N, 34.48. Found: C, 35.28; H, 1.84; N, 34.59.

Spectrophotometric and Dissociation Studies.—Spectrophotometric measurements were made with a Cary model 11 ultraviolet recording spectrophotometer (Applied Physics Corp., Pasadena, Calif.) using matched 1-cm. silica cells and techniques and buffers previously described.[56] The apparent pK_a values were determined using the methods described by Fox and Shugar[57,23] and Parke and Davis.[58]

Acknowledgments.—The authors wish to thank Dr. George B. Brown, Dr. J. J. Fox, Dr. C. Chester Stock and Dr. D. A. Clarke for valuable advice and discussion. They wish to express their gratitude to Mr. Janis Vitols for assistance.

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NEW YORK 21, N. Y.

[CONTRIBUTION FROM THE INSTITUT FÜR KRISTALLOGRAPHIE UND PETROGRAPHIE OF THE EIDG. TECHN. HOCHSCHULE]

The Determination of *cis–trans* Isomerism by Length Measurements of Molecules in Urea or Thiourea Adducts

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RECEIVED MARCH 22, 1958

In X-ray photographs of single crystals of urea or thiourea adducts taken by the Laue technique continuous layer lines appear. The distance between these lines enables one to compute the length of the adducted molecule. This length measurement has been used to differentiate between *cis* and *trans* isomers. A *cis* double bond shortens a molecule 0.9 ± 0.1 Å. while a *trans* double bond shortens it 0.15 ± 0.1 Å. compared to the length of the saturated molecule. The method can be applied to mono- and to some polyunsaturated substances, to some trisubstituted double-bonded systems, and, to some extent, to mixtures.

When a *cis* double bond is present in a hydrocarbon chain, the length of the most extended structural conformation is somewhat less than is that of the corresponding *trans* isomer. This fact has been used to elucidate the structures of rubber and guttapercha, two isomeric, acyclic, polyisoprenoid hydrocarbons. Thus, on the basis of the X-ray work of Bunn[2] and earlier investigators,[3,4] the double bonds in rubber were assigned the *cis* configuration while those of guttapercha were given the *trans* configuration, since the periodicity in stretched rubber was found to be shorter than it was in guttapercha.

Recently it was shown[5–8] that the over-all molecular length of certain compounds can be measured if they can be made to form an inclusion type of complex with urea or thiourea known as urea or thiourea adducts. These adducts yield continuous layer lines when Laue photographs of single crystals are taken, and the distance between these lines can be used to compute the molecular length of the included compound. In the present work this length measurement has served as a basis for differentiating between *cis* and *trans* isomers.[9]

Materials.—To ensure length measurements as exact as the method permits, every attempt was made to employ only substances of the highest purity. Samples of stearic, oleic and elaidic acids of very high purity were kindly provided by R. R. Allen[10] of the Research Division of Armour and Co., Chicago, Ill.

(1) Western Fish Nutrition Laboratory, Fish and Wildlife Service, Cook, Wash. Guggenheim Fellow for 1955–1956 during which time most of this work was performed.

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