tion was complete the ice–salt-bath was removed and the reaction mixture was stirred for 1–4 hr. at room temperature.

The thermometer was replaced by a condenser and a solution of 7.0 g. (0.3 g. atom) of sodium in 70 ml. of absolute methanol (dried over magnesium methoxide) was added dropwise at such a rate as to maintain gentle reflux. After the addition was complete the reflux was maintained by external heating to complete the reaction. The reaction was considered complete when a test portion of the reaction mixture gave a negative test with acidified starch–iodide paper (70 min.). The reaction mixture was cooled, and the precipitated sodium chloride was removed by filtration and washed three times with small portions of dry benzene. The filtrate was poured cautiously *into* a separatory funnel containing 150 ml. of 2 *N* hydrochloric acid solution.[34] The aqueous portion was separated and the benzene layer was extracted three times with 50-ml. portions of 2 *N* hydrochloric acid solution. The combined acid extracts were washed twice with 50-ml. portions of ether (which were discarded).

The aqueous solution was evaporated to dryness in a rotating evaporator at a temperature below 40°. The residue was extracted (under reflux) with 200-ml. and 150-ml. portions of isopropyl alcohol to which 1.0 ml. of concentrated hydrochloric acid per 100 ml. of alcohol had been added.[35] The precipitate which formed upon cooling the extracts separately was removed by filtration and washed with dry ether. The filtrates were either evaporated under reduced pressure to approximately half of their original volumes or were diluted with equal volumes of ether. Either treatment yielded a second crop of crystals (also washed with ether). The total yield of phenacylamine hydrochloride was 13.4 g. (78%), m.p. 186.6° dec.

The principal variations necessary in the preparations of the other amino ketone hydrochlorides were as follows. In the preparation of *p*-nitrophenacylamine hydrochloride the addition of the sodium methoxide solution was carried out in an ice–water-bath to maintain the temperature below 10°. Even so the color of the solution changed from yellow to green to brown as the addition progressed. After the addition was complete the mixture was stirred at room temperature until a negative test was obtained with starch–iodide paper (2 hours) or, less desirably, heated on the steam-bath (60 min.). The times required to obtain a negative test with starch–iodide paper for the other preparations are listed in Table I as reaction times. In the preparation of 2-amino cyclopentanone the aqueous acid extract was quite dark and was treated three times with charcoal before evaporation. Both this product and 2-aminocyclohexanone had limited shelf lives, but they could be kept up to several months.

Normally the benzene extracts were discarded after treatment with the aqueous acids. In the preparation of desylamine hydrochloride, however, the benzene was evaporated. The yellow-orange oil that remained deposited yellow crystals which were recrystallized from Skellysolve B yielding 200 mg. of yellow needles, m.p. 84–85°. This material was identified as benzil by determination of mixture m.p.'s and infrared spectrum.

cis-**2,3-Diphenylethyleneimine.**—Starting with 4.9 g. (0.025 mole) of 1,2-diphenylethylamine and proportionate quantities of other reagents, the procedure described above for the preparation of α-amino ketones was followed to the point at which a negative test for positive halogen was obtained with starch–iodide paper. The reaction mixture was cooled and the precipitated sodium chloride was removed by filtration and washed twice with small portions of dry benzene. The combined benzene solutions were poured with stirring into 150 ml. of ice-water. The benzene layer was separated and diluted with an equal volume of ether. The benzene–ether mixture was cooled in an acetone–Dry Ice-bath. After the benzene had crystallized, it was filtered off rapidly through a thoroughly chilled Buchner funnel. The essentially ethereal filtrate was dried over magnesium sulfate for 30 minutes.

To a slurry of 4.0 g. (0.1 mole) of lithium aluminum hydride in 1200 ml. of dry ether the above ethereal solution was added dropwise with stirring. After addition was complete, the reaction mixture was stirred at room temperature for 20 min. To the mixture the following were added dropwise: 4 ml. of water, 4 ml. of 15% sodium hydroxide and 12 ml. of water. The precipitate was removed by filtration and the ethereal solution was dried over magnesium sulfate. Evaporation of the ether gave 2.5 g. of yellow solid, which on recrystallization from 10 ml. of Skellysolve B yielded 2.0 g. (43%) of *cis*-2,3-diphenylethyleneimine, m.p. 82–84° (lit.[25] m.p. 81–82.5°). Repeated recrystallization from Skellysolve B raised the m.p. to 84–85°. The infrared spectrum of the product was nearly identical with that reported by Hatch and Cram.[25]

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(34) The reverse order of addition may lead to the formation of the self condensation products of the α-amino ketone.

(35) Inasmuch as sodium chloride is very nearly insoluble in hot isopropyl alcohol, the latter is the solvent of choice for such extractions. For less soluble amino ketone hydrochlorides, ethyl or methyl alcohols could be used, but the products may have been contaminated with traces of sodium chloride.

[CONTRIBUTION FROM THE KETTERING–MEYER LABORATORY,[1] SOUTHERN RESEARCH INSTITUTE]

Synthesis of Potential Anticancer Agents. XX. 2-Fluoropurines[2]

BY JOHN A. MONTGOMERY AND KATHLEEN HEWSON

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The 2-fluoro derivatives of a number of biologically active purines have been prepared from the corresponding 2-aminopurines by a modified Schiemann reaction. The properties of these compounds are discussed.

Many organic fluorine compounds have shown interesting biological activity. An excellent review of fluorine-containing drugs was published in 1954.[3] Since that time, a large number of such compounds have appeared in the literature; for example, fluorobarbiturates,[4] fluoroacetylcholine,[5] fluoro-anesthetics,[6] 9α-fluorohydrocortisone,[7] Vesprin,[8] and some potential anticancer agents (fluorourethans[9] and fluoroaromatics[10]). More recently, several 5-fluoropyrimidines[11] showing marked tumor-inhibiting properties[12] and some trifluoromethylpurines[13] have been prepared.

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(2) For paper XIX of this series, see J. A. Montgomery, L. B. Holum and T. P. Johnston, THIS JOURNAL, **81**, 3963 (1959).

(3) P. Tarrant in J. H. Simon, ed., "Fluorine Chemistry," Academic Press, Inc., New York, N. Y., 1954, p. 213.

(4) W. F. Bruce and R. de V. Huber, THIS JOURNAL, **75**, 4668 (1953).

(5) T. R. Blohm, *ibid.*, **73**, 5445 (1951).

(6) G. A. Olah, A. E. Pavlath, J. A. Olah and F. Herr, *J. Org. Chem.*, **22**, 879 (1957).

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

(8) H. L. Yale, F. Sowinski and J. Bernstein, *ibid.*, **79**, 4375 (1957).

(9) G. A. Olah, S. J. Kuhn and G. Kovacs-Bruckner, *J. Org. Chem.*, **22**, 979 (1957).

(10) Ng. Ph. Buu-Hoi, N. D. Xuong and R. Rips, *ibid.*, **22**, 193 (1957).

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

(12) C. Heidelberger, L. Griesbach, B. J. Montag, D. Mooren and O. Cruz, *Cancer Research*, **18**, 305 (1958).

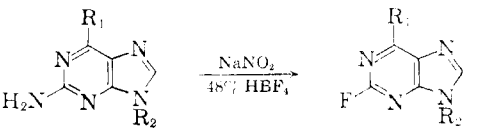
(13) A. Giner-Sorolla and A. Bendich, THIS JOURNAL, **80**, 5744 (1958).

Adenine is important in metabolism because it occurs in many co-enzymes as well as in nucleic acids. The anticancer activity of 6-purinethiol and certain other purines may be due to this fact. One of the smallest steric changes that can be made in the structure of adenine is the replacement of a hydrogen by a fluorine atom,[14] although this change may have a pronounced effect on the electron configuration of the molecule.[14] This type of change might be ideal for the production of an antimetabolite since the resulting compound should be able to fit an active enzyme site and yet it might possess biochemical properties quite different from the normal substrate. It has been suggested, on the basis of screening data on a large number of purines, that a slight change at a single site in the structure of adenine might be expected to produce a biologically active compound.[16] Indeed, the introduction of an amino group or a chlorine atom at C_2 of adenine resulted in compounds with some anticancer activity.[16] The present paper deals with the preparation of 2-fluoroadenosine and other 2-fluoropurines. A preliminary report on some of this work has appeared.[17]

Although a number of fluoroheterocycles have been prepared by the Schiemann reaction,[3,18] almost all of these contain only one heteroatom, usually nitrogen. Indeed, the failure of such compounds as adenine to undergo the Schiemann reaction[19] or other normal diazotization reactions has been reported,[16,20] even though adenine can be "deaminated" successfully.[21] However, 2-aminopyrimidine can be diazotized and, in fact, 2-chloropyrimidine[22] and 2-fluoropyrimidine[23] have both been prepared *via* diazotization of 2-aminopyrimidine. Because of these observations, we first studied the diazotization of 2-aminopurine in concentrated hydrochloric acid, obtaining a small yield of 2-chloropurine and a lesser amount of 2-purinol from the reaction. Diazotization of 2-aminopurine in 48% hydrobromic acid yielded only 2-purinol, but a relatively good yield (40%) of 2-fluoropurine (I) was obtained when the reaction was carried out in 48% fluoboric acid,[17] and no 2-purinol was found in the reaction mixture.

In an effort to extend our observations to 8-aminopurine,[24] we studied the behavior of this compound in both concentrated hydrochloric and fluoboric acids. In neither case could we find any evidence of the formation of the 8-halopurine or 8-purinol, although we did obtain a Bratton–Marshall test with 8-aminopurine, indicating successful diazotization of this compound as reported.[25]

Next, the behavior of a number of 2-amino-6-substituted purines was investigated. Because of the successful preparation of 2-chloro- and 2-fluoropurine from 2-aminopurine and because it is known that treatment of 2,6-diaminopurine with sodium nitrite in acetic acid solution gives isoguanine and no guanine,[26] we assumed that other diazotization–replacement reactions of 2,6-diaminopurine would give 2-substituted adenines. However, the insolubility of 2,6-diaminopurine in concentrated hydrochloric acid caused us to attempt instead the diazotization of its ribonucleoside; a reaction mixture composed principally of unchanged 2,6-diaminopurine ribonucleoside and 2-chloroadenosine, but also containing small amounts of 2-chloradenine, isoguanine and crotonoside, was obtained. From this mixture, 2-chloroadenosine was isolated in about 5% yield. 2-Fluoroadenosine (II) was then prepared in 8% yield using 48% fluoboric acid instead of concentrated hydrochloric acid. After the procedure for the preparation of 2-fluoroadenosine was developed, it was applied, with modifications, to the preparation of 2-fluoroadenine (III), 2-fluorohypoxanthine (IV), 2-fluoro-6-purinethiol (V) and its ribonucleoside VI, 2-fluoro-6-(methylthio)-purine (VII), 6-dimethylamino-2-fluoropurine (VIII), 2-fluoro-6-methylpurine (IX) and 6-chloro-2-fluoropurine (X).



I, $R_1 = R_2 = H$
II, $R_1 = NH_2$, R_2 = 9-β-D-ribofuranosyl
III, $R_1 = NH_2$, $R_2 = H$
IV, $R_1 = OH$, $R_2 = H$
V, $R_1 = SH$, $R_2 = H$
VI, $R_1 = SH$, R_2 = 9-β-D-ribofuranosyl
VII, $R_1 = SCH_3$, $R_2 = H$
VIII, $R_1 = N(CH_3)_2$, $R_2 = H$
IX, $R_1 = CH_3$, $R_2 = H$
X, $R_1 = Cl$, $R_2 = H$
XI, $R_1 = NH_2$, $R_2 = CH_2C_6H_5$

Because of the very low yields and difficulties encountered in the preparation of 2-fluoroadenine and 2-fluorohypoxanthine, alternative routes to these compounds were investigated. Since the major problem seemed to lie in the isolation of the fluoropurine, and since the less polar purines such as 2-fluoropurine could be isolated efficiently by ether extraction, one promising alternative route would involve the following steps: benzylation of the 2-aminopurine (*i.e.*, 2,6-diaminopurine or guanine) to be converted to the 2-fluoropurine, conversion to the N-benzyl-2-fluoropurine, isolation of this material by ether extraction, and, finally, debenzylation to the desired 2-fluoropurine.

(14) The unusually small van der Waals radius of the fluorine atom (1.35 Å.) is very close to that of hydrogen (1.2 Å.), while at the same time, fluorine is the most electronegative atom ordinarily found in organic compounds.[15]

(15) L. Pauling, "Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1944.

(16) A. Bendich, P. J. Russell, Jr., and J. J. Fox, THIS JOURNAL, **76**, 6073 (1954).

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

(18) A. Roe in R. Adams, ed., "Organic Reactions," Vol. 5, John Wiley and Sons, Inc., New York, N. Y., 1949, p. 193.

(19) A. Bendich, A. Giner-Sorolla and J. J. Fox, in G. E. W. Wolstenholme and C. M. O'Connor, eds., "The Chemistry and Biology of Purines" (A Ciba Foundation Symposium), J. and A. Churchill, Ltd., London, 1957, p. 3.

(20) A. G. Beaman, THIS JOURNAL, **76**, 5633 (1954).

(21) A. Kossel, *Ber.*, **18**, 1928 (1885).

(22) I. C. Kogon, R. Minin and C. G. Overberger, *Org. Syntheses*, **35**, 35 (1955).

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

(24) A. Albert, reporting the work of D. J. Brown, has stated that 8-aminopurine could be diazotized and coupled like an aromatic amine, whereas 2-aminopurine and adenine could not.[25]

(25) A. Albert, ref. 19, p. 97.

(26) J. Davoll, THIS JOURNAL, **73**, 3174 (1951).

2,6-Diaminopurine was smoothly benzylated in dimethylformamide to 2,6-diamino-N-benzylpurine. That this material is actually 2,6-diamino-9-benzylpurine is inferred from the similarity of its ultraviolet absorption spectrum to that of 2,6-diaminopurine ribonucleoside[27] (see Table I); no rigorous proof of this was undertaken. The reaction of 2,6-diamino-9-benzylpurine by the modified Schiemann procedure and isolation of the product by ether extraction both proceeded well, as did the debenzylation with sodium in liquid ammonia. The over-all yield of *pure* 2-fluoroadenine by this route is much better than that obtained by direct reaction of 2,6-diaminopurine, mainly because of the efficacy of the purification procedure employed. Further proof of the position of benzylation of 2,6-diaminopurine can be derived from a comparison of the ultraviolet spectrum of N-benzyl-2-fluoroadenine with the spectra of 7-[28] and 9-methyladenine[29] (see Table I). The comparison shows quite definitely that this compound is 9-benzyl-2-fluoroadenine (XI), which therefore must have been derived from 2,6-diamino-9-benzylpurine.

TABLE I

Compound	*p*H 1 λ_{max}, mμ	*p*H 1 $\epsilon \times 10^{-3}$	*p*H 7 λ_{max}, mμ	*p*H 7 $\epsilon \times 10^{-3}$	*p*H 13 λ_{max}, mμ	*p*H 13 $\epsilon \times 10^{-3}$
2,6-Diamino-N-benzylpurine	254	10.8	256	8.95	256	8.97
	292	10.6	281	10.9	281	11.0
2,6-Diaminopurine ribonucleoside	252	10.9	255	9.64	255	9.54
	290	10.3	278	10.2	278	10.2
9-Methyladenine	261[a]	14.6[a]	..	..	262[a,b]	11.9[a]
9-Benzyl-2-fluoroadenine	264	13.9	263	15.7	262	15.7
7-Methyladenine	272[c]	15.05[c]	..	..	270[b,c]	10.55[c]

[a] Ref. 29. [b] *p*H 11. [c] Ref. 28.

Chemical Properties.—Our observation that pure 2-fluoroadenine hydrochloride, after standing in a stoppered vial for five months, contained about 10% isoguanine prompted us to prepare this compound as the free base and also to investigate the stability of the 2-fluoropurines. 2-Fluorohypoxanthine was found to contain a small amount of xanthine after standing for fourteen months, but the storage life of the other 2-fluoropurines, including 2-fluoroadenine as the free base, in the dry state was found to be satisfactory.

Hydrolysis studies also showed that the fluorine atom of these compounds is very reactive. For example, 2-fluorohypoxanthine on standing for 23 hours in 0.1 *N* hydrochloric acid at 32° was hydrolyzed to xanthine to the extent of 84%. At 70° hydrolysis was complete in 1.25 hours. By comparison, 2-chlorohypoxanthine was essentially unchanged after standing for 23 hours at 32° in 0.1 *N* hydrochloric acid and only about 20% hydrolyzed after 23 hours at 70°. It was also found that 2-fluorohypoxanthine was hydrolyzed in boiling distilled water (64% at the end of 4 hours), but remained essentially unchanged in boiling *p*H 7 buffer solution and in 0.1 *N* sodium hydroxide solution after 4 hours, indicating that the hydrolysis is autocatalytic.[30] The fluorine atom of 2-fluoroadenine is somewhat less reactive, being completely hydrolyzed in 4 hours in 0.1 *N* hydrochloric acid at 70°.

2-Fluoroadenosine was converted to 2-butylaminoadenosine by refluxing it for three hours in ethanol containing one equivalent of butylamine, whereas it was necessary to heat 2-chloroadenosine with excess dimethylamine[32] in aqueous methanol for 16 hours at 100° (in a bomb) to convert it to 2-dimethylaminoadenosine,[34] showing again the greater reactivity of the 2-fluoropurines compared to the 2-chloropurines.

In connection with some other work, it was of interest to determine the position of alkylation of 6-dimethylamino-2-fluoropurine. Ultraviolet data established that benzylation took place in the 9-position instead of the 7-position, as was found to be the case with 6-dimethylaminopurine.[35] This same effect upon the position of glycosidation by the 2-methylthio group has been observed by Baker, Joseph and Williams.[36]

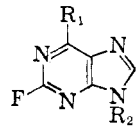
Chromatographic Behavior and Spectral Data.—Table II lists the R_{ad} values and ultraviolet spectral data for the 2-fluoropurines. With a few exceptions the 2-fluoropurines and the corresponding 2-chloropurines travel about the same distance in four solvent systems and both series travel further than the corresponding unsubstituted purines, which in turn travel much further than the 2-aminopurines from which the 2-fluoropurines were prepared. This chromatographic behavior made it possible to follow readily the conversion of the 2-aminopurine to the corresponding 2-fluoropurine.

The introduction of a fluorine atom into the 2-position of a purine results in a small bathochromic shift in the position of the maxima of the ultraviolet spectra of that purine and, except in the case of 6-purinethiol and its ribonucleoside, a smaller, usually hyperchromic, shift in the intensity of the maxima. In general, the bathochromic shift is smaller and the hyperchromic shift larger than those caused by a chlorine atom at C_2.[37]

An interesting observation resulted from the determination of the ultraviolet spectra of 2-fluoro-6-purinethiol and its ribonucleoside. The data in Table III show that the position of the maxima of these two compounds in methanol or ethanol occurs at the same wave length as that of 6-(methylthio)-purine, which is quite different from that of 1-methyl-6-(1H)purinethione, indicating that in alcohol they exist in the thiol XXIV rather than the thione form. It has already been established from this type of comparison that in aqueous solution at various *p*H values 6-purinethiol exists in the thione form XXII[38] (the same thing is true for 6-purinethiol ribonucleoside and for

(27) J. Davoll and B. A. Lowy, THIS JOURNAL, **73**, 1650 (1951).
(28) R. N. Prasad and R. K. Robins, *ibid.*, **79**, 6401 (1957).
(29) R. K. Robins and H. H. Lin, *ibid.*, **79**, 490 (1957).
(30) A similar observation has been made with 4-chloroquinazoline.[31]
(31) A. J. Tomisek and B. E. Christensen, THIS JOURNAL, **67**, 2112 (1945).
(32) A stronger nucleophilic reagent than butylamine.[33]
(33) J. F. Bunnett and R. E. Zahler, *Chem. Revs.*, **49**, 273 (1951).
(34) H. J. Schaeffer and H. J. Thomas, THIS JOURNAL, **80**, 3738 (1958).
(35) B. R. Baker, R. E. Schaub and J. P. Joseph, *J. Org. Chem.*, **19**, 638 (1954).
(36) B. R. Baker, J. P. Joseph and J. H. Williams, *ibid.*, **19**, 1780 (1954).
(37) *Cf.* Table II; S. F. Mason, *J. Chem. Soc.*, 2071 (1954); and ref. 48 and 49.
(38) G. B. Elion, ref. 19, p. 46.

TABLE II



R_1	R_2	0.1 N HCl λ_{max}, mμ	0.1 N HCl $\epsilon \times 10^{-3}$	pH 7 λ_{max}, mμ	pH 7 $\epsilon \times 10^{-3}$	0.1 N NaOH λ_{max}, mμ	0.1 N NaOH $\epsilon \times 10^{-3}$	R_{ad}[a] A	B	C	D
H	H	264	8.3	268	8.4	273	8.8	1.66	1.26	1.48	1.85
CH_3	H	262	9.9	266	9.8	272	10.4	1.52	1.25	1.55	1.81
Cl	H	270	9.7	272	9.5	275	9.0	1.69	1.31	1.66	1.68
SCH_3	H	296	18.6	295	18.8	296	16.3	2.02	1.36	1.68	1.13
$N(CH_3)_2$	H	280	15.3	273	18.5	280	18.1	1.85	1.32	1.53	1.14
$N(CH_3)_2$	$CH_2C_6H_5$	276	17.7	274	21.8	274	21.8	2.01	..	1.75	0
		283	17.1								
OH	H	..[f]	..	257	11.0	262	11.0	0.24	1.00	1.07	1.35
SH	H	328[g]	14.2	316	14.6	314	16.2	1.81	1.36	1.12	0.92
SH	9-β-D-Ribofuranosyl	326[h]	13.7	315	14.7	315	16.0	1.16	1.17	1.02	1.86
NH_2	H	266	11.8	262	12.6	269	12.7	1.42	1.20	1.16	1.07
NH_2	$CH_2C_6H_5$	264	13.9	263	15.7	262	15.7	1.85	1.37	1.58	0
NH_2	9-β-D-Ribofuranosyl	260.5	13.7	260.5	14.3	260.5	14.8	0.90	1.01	1.30	1.39

[a] The paper chromatograms were run by the descending technique on Whatman No. 1 paper in the following solvent systems: A, water-saturated butyl alcohol[b]; B, butyl alcohol–acetic acid–water (5/2/3)[c]; C, isopropyl alcohol–ammonium hydroxide–water (70:5:25)[d]; D, 5% disodium hydrogen phosphate.[e] Adenine was used as a standard on all chromatograms and arbitrarily assigned a value of 1.00. Other spots were assigned R_{ad} values with reference to adenine. [b] J. G. Buchanan, C. A. Dekker and A. G. Lowy, *J. Chem. Soc.*, 3162 (1950). [c] D. M. Brown, A. Todd and S. Varadarajan, *ibid.*, 2388 (1956). [d] R. Markham and J. B. Smith, *Nature*, **168**, 406 (1951). [e] C. E. Carter, THIS JOURNAL, **72**, 1466 (1950). [f] Hydrolyzes in 0.1 *N* HCl to xanthine. [g] Converted slowly to the thiol form in 0.1 *N* HCl; see Table III. [h] Converted rapidly to thiol form in 0.1 *N* HCl; see Table III.

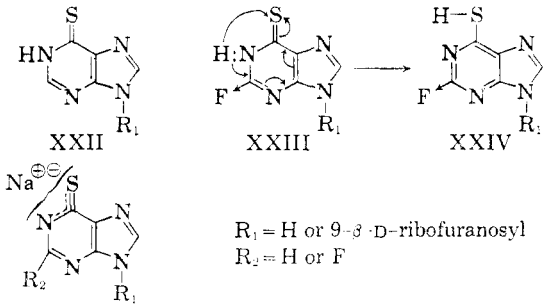
both these compounds in ethanol solution; see Table III). If 2-fluoro-6-purinethiol ribonucleoside is dissolved in an equivalent of 0.1 *N* sodium hydroxide and this solution diluted at once with enough hydrochloric acid to give a 0.1 *N* hydrochloric acid solution, the position of the ultraviolet maximum of this solution shifts from an initial wave length of 326 mμ to a final wave length of 296 mμ, indicating a tautomeric shift from thione to thiol form which takes place at a measurable rate in acid solution.[39]

TABLE III

Compound		λ_{max}, mμ pH 1	pH 7	pH 13	EtOH
1-Methyl-6(1H)-purinethione		321[a]	320[a]	321[a,b]	..
6-Purinethiol		325[a]	323[a]	312	329
6-(Methylthio)-purine		294[a]	290[a]	290	295
2-Fluoro-6-purinethiol		328[c]	316	314	296
2-Fluoro-6-purinethiol ribonucleoside	1[d]	326	315	315	294[e]
	2[f]	296	316	315	294
6-Purinethiol ribonucleoside		322[g]	319	312[g]	323

[a] Data taken from G. B. Elion; see ref. 38. [b] pH 11. [c] Peak shifts slowly to 294 mμ. [d] Reading taken immediately after acidifying solution. [e] CH_3OH reading the same. [f] Reading taken after five minutes. [g] See ref. 47.

If the solution is made basic and reacidified, the same shift from 326 to 296 mμ is observed again indicating that the change is easily reversible and reproducible. The same changes, but at a slower rate, were observed in the ultraviolet spectrum of 2-fluoro-6-purinethiol. Apparently the fluorine at C_2 withdraws electrons from N_1 to such an extent that in alcohol or acid solution the electrons of the sulfur at C_6 are more available to protons than the electrons at N_1 (XXIII), whereas the reverse is true in the case of 6-purinethiol or its ribonucleoside. In base the spectra are those of the anionic species XXV in both cases and therefore are the same.



XXII XXIII XXIV

R_1 = H or 9-β-D-ribofuranosyl
R_2 = H or F

The infrared spectra of the 2-fluoropurines were very similar to those of the parent purines with small shifts toward higher frequencies in the 1800–1500 cm.$^{-1}$ range. No bands due to C–F vibrations could be positively identified.

Potentiometric Titrations.—Table IV gives the ionization constants of three of the 2-fluoropurines and the corresponding unsubstituted purines. The introduction of a fluorine atom into the purine nucleus has a much smaller effect on the ionization constants than the introduction of a trifluoromethyl group.[13] This follows since the trifluoromethyl group is known to be a stronger electron-withdrawing group than the fluorine atom. The effect is greater on the basic strength of adenine than on its acid strength since the former is reduced to such an extent that the protonation of 2-fluoroadenine cannot be detected in 50% aqueous ethyl alcohol,[41]

(39) This type of phenomena is well known in the enol–keto system of β-dicarbonyl compounds.[40]

(40) B. Eistert and W. Reiss, *Ber.*, **87**, 92 (1954); A. M. Stock, W. E. Donahue and E. D. Amstütz, *J. Org. Chem.*, **23**, 1840 (1958).

(41) The fluoropurines are not soluble enough to permit the potentiometric determination of their ionization constants in water.

whereas the acidic ionization constant is essentially unchanged. The acidic ionization constants of 2-fluoropurine and 6-dimethylamino-2-fluoropurine are both measurably greater than those of the corresponding unsubstituted purines (the basic ionization constants were not determinable).

TABLE IV

Compound	—0.15 *M* NaCl— pK_{a1}	pK_{a2}	50 vol. % EtOH pK_{a1}	pK_{a2}
Purine	2.39[a]	8.93[a]	..	8.82
2-Fluoropurine	..	...	..	8.17
Adenine	4.22[a]	9.80[a]	3.59	9.63
	3.93	9.36		
2-Fluoroadenine	..	...	..	9.58
6-Dimethylaminopurine	3.87[a]	10.50[a]	..	10.54
	2.33	9.86		
6-Dimethylamino-2-fluoropurine	..	....	..	9.97

[a] See ref. 44.

Biological Activity.—The high degree of cytotoxicity of 2-fluoroadenosine has been reported.[17] This toxicity carries over to the whole animal since the maximum tolerated dose in C57 black mice implanted with Adenocarcinoma 755 is only 1 mg./kg.[42] Unfortunately, the compound is inactive at this level against Ad755 and also against leukemia L1210 and the Ehrlich ascites tumor. The introduction of a fluorine atom into the 2-position of 6-purinethiol ribonucleoside destroyed completely the activity of this compound against Ad755 but did not raise the toxicity.[42] 9-(5′-Fluoro-β-D-ribofuranosyl)-6-purinethiol was also found to be devoid of anticancer activity.[43] It is noteworthy that the same small change in two widely different positions of this highly active nucleoside results in complete loss of anticancer activity. None of the other 2-fluoropurines tested thus far have shown any anticancer activity.[42]

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Experimental

Melting points below 260° were determined on a Kofler Heizbank and are corrected; those above 260° were determined in a capillary tube in an aluminum block and are uncorrected. The ultraviolet spectra were determined with a Beckman model DK-2 spectrophotometer, but the optical densities at the maxima were measured with a Beckman DU. The infrared spectra were determined in pressed potassium bromide disks with a Perkin–Elmer model 21 spectrophotometer. Potentiometric titrations were carried out with Beckman model H2.

2-Aminopurines.—2-Aminopurine,[44] 2-amino-6-methylpurine,[45] 2-amino-6-chloropurine,[46] thioguanosine,[47] 2,6-diaminopurine ribonucleoside,[27] 2-amino-6-(methylthio)-purine[48] and 2-amino-6-dimethylaminopurine[49] were all prepared by known methods. Guanine,[50] thioguanine[51] and 2,6-diaminopurine[52] were purchased.

2,6-Diamino-9-benzylpurine.—2,6-Diaminopurine (150 mg.) was dissolved in dimethylformamide (5 ml.) containing potassium carbonate (138 mg.), and benzyl chloride (0.23 ml.) was added to the mixture, which was stirred for 18 hours at room temperature. It was necessary to heat the reaction mixture at 85° for 40 minutes to complete the reaction, after which it was filtered to remove the insoluble sodium chloride. The filtrate was then evaporated to dryness *in vacuo* and the residue triturated with absolute ethyl alcohol. Evaporation of the ethyl alcohol solution gave a white crystalline solid; yield 130 mg. This material was recrystallized from water; yield 53 mg., m.p. 180–181°; $\lambda_{max}^{pH\ 7,13}$ 256, 281 mμ (ε 8,950, 10,900).

Anal. Calcd. for $C_{12}H_{12}N_6$: N, 35.00. Found: N, 35.13.

A second run in which 1.5 g. of 2,6-diaminopurine was used gave 1.34 g. (56%) of recrystallized 2,6-diamino-9-benzylpurine, m.p. 180–181°.

Preparation of the 2-Fluoropurines. A. The Reaction.—To a suspension of the 2-aminopurine in 48% fluoboric acid (2 – 3.5 ml./mmole purine) maintained at −10° was added a saturated aqueous solution of sodium nitrite (1.7 mmoles/mmole of purine) at a rate of 0.1 ml. per minute. After the addition was complete, the mixture was stirred for 15 minutes at −10 to 0° and then it was neutralized (<0°) to *p*H 5–7 with 50% sodium hydroxide solution.

B. Isolation. 1. 2-Fluoropurine (I), 2-Fluoro-6-(methylthio)-purine (VII), 6-Dimethylamino-2-fluoropurine (VIII), 2-Fluoro-6-methylpurine (IX) and 9-Benzyl-2-fluoroadenine (XI).—The neutral slurry from A was evaporated to dryness *in vacuo* and the crude purines isolated by extraction of the dry residues with ether in a Soxhlet extractor. The analytical samples were then obtained by recrystallization of the crude purines from ethyl alcohol. Details are given in Table V.

2. 2-Fluoroadenosine (II).—The neutral slurry obtained from the treatment of 846 mg. of 2,6-diaminopurine ribonucleoside as described in A was evaporated to dryness *in vacuo*. The dry residue was then partitioned on a Celite column (8.5 × 35 cm.) using water-saturated butyl alcohol as the eluant. The eluant was collected in 15-ml. fractions and the 2-fluoroadenosine was found in fractions 107–162. These fractions were combined and evaporated to dryness *in vacuo* giving 173 mg. of crude product (*ca.* 90% pure). It was necessary to rechromatograph this material on a second Celite column to obtain the analytical sample. This material was dried *in vacuo* for 3 hr. at 78° and overnight at room temperature; yield 137 mg. (16%), m.p. 200° dec., $[\alpha]^{26}D$ −60.3 ± 11.1° (0.127% in ethanol).

Anal. Calcd. for $C_{10}H_{12}FN_5O_4$: C, 42.11; H, 4.24; F, 6.67; N, 24.56. Found: C, 42.28; H, 4.93; F, 7.33; N, 24.13.

3. 2-Fluoroadenine (III).—The neutral slurry obtained from the treatment of 16.8 g. of 2,6-diaminopurine hydrate as described in A was filtered. The solid that was collected was resuspended in water (200 ml.), the *p*H of the slurry was adjusted to 7 with 1 *N* sodium hydroxide, 30 g. of Celite was mixed in thoroughly, and this mixture was collected by filtration and dried.

The dried solids were then partitioned on a Celite column (8.5 × 35 cm.) using water-saturated butyl alcohol as the eluant. Fractions 107–171 of the eluant (975 ml). were combined and evaporated to dryness *in vacuo* giving 935 mg. (6%) of 2-fluoroadenine approximately 90% pure. Four recrystallizations from water gave 112 mg. of pure material, which on heating decomposed but did not melt below 350°. A qualitative test for fluorine was positive.

Anal. Calcd. for $C_5H_4FN_5$: C, 39.22; H, 2.64; N, 45.75. Found: C, 38.93; H, 2.98; N, 45.81.

In one run the material obtained from the Celite column (349 mg., 34%) was dissolved in 1 *N* hydrochloric acid and

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(51) Francis Earle Laboratories, Inc.

(52) Dougherty Chemical Co.

TABLE V

Cpd.	2-Aminopurine, mg.	Ether extraction, hr.	Yield of 2-fluoropurine—Crude, mg.	Yield of 2-fluoropurine—Pure, mg.	Yield of 2-fluoropurine—Pure, %	Recrystd. volume EtOH, ml.	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
I	850	4	422	353	40	6	216[a]	43.48	43.52	2.20	2.01	40.60	40.37
VII	2000	16	960	470	23	50	260	39.13	39.23	2.74	3.03	30.45	30.01
VIII	2250	72	664	532	23	110	>280[a]	46.41	46.26	4.46	4.40	38.65	38.22
IX	923	8	405	223	26	5	194	47.37	47.48	3.32	3.90	36.01	35.75
XI	500	48	147	83	16	20	272[a]	59.27	59.23	4.16	4.26	28.80	28.80

[a] Decomposition point.

adsorbed on a column of Dowex 50 (5 g. Dowex 50, 200–400 mesh). The column was washed with water (700 ml.) and then 0.1 *N* hydrochloric acid (400 ml.) which eluted a small amount of xanthine. The eluant was changed to 0.5 *N* hydrochloric acid and the 2-fluoroadenine appeared in the eluant between 200–430 ml. Evaporation of this solution gave 2-fluoroadenine hydrochloride, which after trituration with ethyl alcohol and drying, weighed 147 mg. (12%), m.p. >350°.

Anal. Calcd. for $C_5H_4FN_5 \cdot HCl$: C, 31.74; H, 2.67; N, 37.04. Found: C, 31.64; H, 3.15; N, 37.22.

4. 2-Fluoro-9-β-D-ribofuranosyl-6-purinethiol (VI).—The neutral slurry resulting from the treatment of thioguanosine (6.7 g.) as described in A above was allowed to stand in an ice-bath for several hours before the yellow solid was collected by filtration, washed thoroughly with two portions of ice-water followed by ethanol and then ether giving 2.6 g. of crude product. Recrystallization of this material from water (330 ml.) with charcoal treatment gave the pure product which was collected by filtration, washed with water, and dried *in vacuo* at 78°; yield 505 mg. (7%), m.p. 187–188°, $[\alpha]^{22}D$ 70.4 ± 2.5° (0.1 *N* NaOH).

Anal. Calcd. for $C_{10}H_{11}FN_4O_4S \cdot {}^1/_2\ H_2O$: C, 38.58; H, 3.90; N, 18.01; S, 10.29. Found: C, 38.52; H, 4.00; N, 18.11; S, 10.47.

5. 2-Fluoro-6-purinethiol (V).—The neutral slurry obtained from the treatment of thioguanine (400 mg.) as described in A was filtered and the solid was washed and dried giving a crude yield of 665 mg. This material was partitioned on a Celite column (8.5 × 35 cm.) using water-saturated butanol as the eluant. Fractions 107–153 (690 ml.) containing the 2-fluoro-6-purinethiol were combined and evaporated to dryness *in vacuo*. The residue (217 mg., 54%) was recrystallized from ethyl alcohol (30 ml.); yield 49 mg. (12%), m.p. >360°. Qualitative tests for fluorine and sulfur were positive.

Anal. Calcd. for $C_5H_3FN_4S$: C, 35.30; H, 1.78; N, 32.96. Found: C, 35.48; H, 2.23; N, 33.46.

6. 2-Fluorohypoxanthine (IV).—The thick slurry resulting from the treatment of guanine (5 g.) as described in A was concentrated to one-third volume *in vacuo*, and the residue was triturated with absolute alcohol (100 ml.). The insoluble material was removed by filtration and washed with 75% ethyl alcohol and then with absolute alcohol. The filtrate and washes were combined and evaporated to dryness *in vacuo*. The residue was triturated with absolute ethyl alcohol (75 ml.) and the insoluble inorganic salts removed by filtration. Evaporation of the filtrate to dryness gave 945 mg. of crude 2-fluorohypoxanthine, which was partitioned on a Celite column (8.5 × 35 cm.) using butyl alcohol–water as the eluent.

Fractions 107–150 (645 ml.) were combined and evaporated to dryness, giving 2-fluorohypoxanthine (114 mg., 2.2%).

Purified material (244 mg.) from two runs was dissolved in warm absolute ethyl alcohol (20 ml.) and an insoluble residue removed by filtration. The filtrate was evaporated to dryness *in vacuo*, and the residue was triturated with ether, collected by filtration, and dried; yield 204 mg. This material decomposed without melting above 260°.

Anal. Calcd. for $C_5H_3FN_4O \cdot {}^1/_8C_2H_5OH$: C, 39.38; H, 2.37; F, 11.87; N, 35.00. Found: C, 39.53; H, 2.68; F, 11.88; N, 34.91.

7. 6-Chloro-2-fluoropurine (X).—The neutral solution from the treatment of 2-amino-6-chloropurine (385 mg.) as described in A was extracted with ether in a continuous liquid–liquid extractor for 10 hours. Evaporation of the ether extract gave 277 mg. of crude 6-chloro-2-fluoropurine which was recrystallized from ethyl alcohol (1.5 ml.); yield 41 mg. (13%), m.p. 174°.

Anal. Calcd. for $C_5H_2ClFN_4$: C, 34.80; H, 1.17; N, 32.47. Found: C, 34.84; H, 1.11; N, 32.88.

Preparation of 2-Fluoroadenine by the Debenzylation of 9-Benzyl-2-fluoroadenine.—To a suspension of 9-benzyl-2-fluoroadenine (100 mg.) in 10 ml. of liquid ammonia was added metallic sodium (52 mg.) in several pieces. After the solution was stirred for 10 minutes, it was neutralized with ammonium chloride and then allowed to evaporate at room temperature. The residue was slurried with ether, and the ether distilled off to remove the last traces of ammonia. The residue was dissolved in water and extracted with ether. The extraction of ether-soluble materials caused precipitations of a solid which was removed by filtration, washed, and dried *in vacuo* over phosphorus pentoxide; yield 41 mg. (66%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 266 (11.5); *p*H 7, 262 (12.2); *p*H 13, 269 (12.3).

9-Benzyl-6-dimethylamino-2-fluoropurine.—Benzyl chloride (64 mg., 0.5 mmole) was added to a suspension of potassium carbonate (34.5 mg., 0.25 mmole) and 6-dimethylamino-2-fluoropurine (45 mg., 0.25 mmole) in dimethyl sulfoxide (2.5 ml.), and the reaction mixture was stirred at 50° for 4 hours. After standing overnight at room temperature, the reaction mixture was diluted with water (7.5 ml.), and the solid that separated from solution was collected by filtration, washed with water, and dried *in vacuo* at 78°; yield 38 mg. (57%), m.p. 137°.

Anal. Calcd. for $C_{14}H_{14}FN_5$: C, 61.99; H, 5.22; N, 25.83. Found: C, 62.06; H, 5.52; N, 25.34.

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