[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION, CORNELL UNIVERSITY MEDICAL COLLEGE]

Synthesis and Properties of Some 6-Substituted Purines[1]

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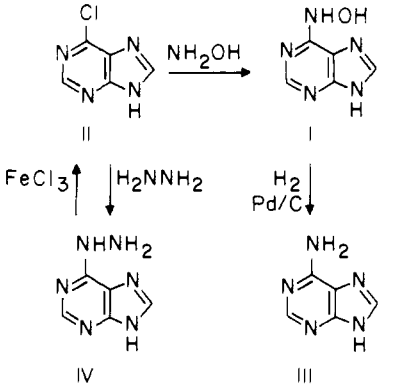
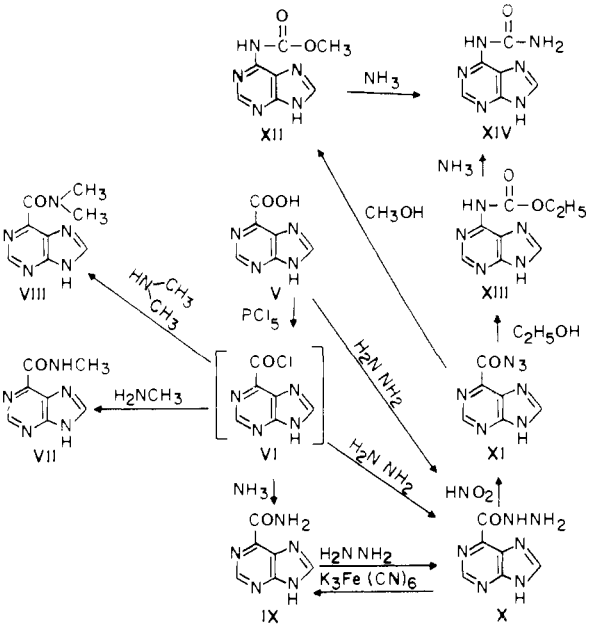
6-N-Hydroxylaminopurine, synthesized from 6-chloropurine and hydroxylamine, was reduced to adenine. The oxidation of 6-hydrazinopurine with ferric chloride led to a new synthesis of 6-chloropurine, in low yield. Purine-6-carboxylic acid was transformed to the acid chloride from which the mono- and dimethyl amides and acid hydrazide were obtained. The latter, purine 6-carbohydrazide, which was also synthesized from the amide or directly from the carboxylic acid and hydrazine, was transformed into the acid azide upon reaction with nitrous acid. The acid azide was converted to the methyl- and ethylurethans which, in turn, were aminated to 6-ureidopurine. 6-Cyanopurine was converted into N-hydroxy- and N-amino-6-purinylamidine. The latter also could be obtained from purine-6-thiocarboxamide. The catalytic hydrogenation of 6-cyanopurine led to 6-aminomethylpurine. Some of the physicochemical properties of these compounds are recorded. A discussion of the dissociation behavior of these purines is given.

It can be said that the discovery of the anti-tumor and antimetabolite activities of such adenine analogs as 6-mercaptopurine[4,5] and 6-chloropurine[6] stemmed from the central role of adenine in the nucleic acids and in numerous biologically important co-enzymes, as well as the demonstration[7,8] that exogenous adenine serves as an efficient precursor of the purines of nucleic acids (compare Rhoads[9]). Although the mode of action of these inhibitors (see ref. 10) has not yet been elucidated, the usefulness of such agents is attested to by their widespread clinical use in human neoplastic disease[11,12] and by the fact that a large number of explorations dealing with the synthesis of other purine (or pyrimidine) analogs have been made and are under way. The present paper deals with the synthesis of such 6-substituted purine derivatives. Certain of their properties were studied in attempts to determine whether a relationship between biological activity and structural or other features might be discerned (see ref. 13 and 14). A preliminary account of some of these studies has appeared.[15]



A study of the structural features of a large number of purines led to a suggestion[6] that certain changes in the structure of adenine (*i. e.*, a single change at the 6-NH_2, C_2 or C_8) might lead to an anti-tumor agent.[16] Accordingly, 6-N-hydroxylaminopurine (I) was prepared by reaction of 6-chloropurine (II)[6] with hydroxylamine in refluxing



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

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

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

(7) G. B. Brown, P. M. Roll, A. A. Plentl and L. F. Cavaleri, *J. Biol. Chem.*, **172**, 467 (1948).

(8) G. B. Brown and P. M. Roll, in "The Nucleic Acids," ed. Chargaff and Davidson, Vol. 2, Academic Press, Inc., New York, N. Y., 1955, p. 341.

(9) C. P. Rhoads, *Science*, **119**, 77 (1954).

(10) G. B. Brown, in "Antimetabolites and Cancer," C. P. Rhoads, editor, Amer. Assoc. Adv. Science, Washington, D. C., 1955, p. 283.

(11) J. H. Burchenal, in Current Research in Cancer Chemotherapy, Comm. on Cancer Chemotherapy, Report No. 4, p. 3, (Feb. 1956) in "Medical Clinics of North America," Vol. 40, No. 3, W. B. Saunders, Philadelphia, Pa., p. 935 (May, 1956).

(12) S. Farber, R. Toch, E. M. Sears and D. Pinkel, *Advances in Cancer Res.*, **4**, 1 (1956).

(13) A. Albert, *Pharmacol. Revs.*, **4**, 136 (1952).

(14) A. Bendich, *Proc. Royal Soc. Med.*, **50**, 6 (1957).

(15) A. Bendich, A. Giner-Sorolla and J. J. Fox, in "The Chemistry and Biology of Purines," A Ciba Foundation Symposium (Wolstenholme and O'Connor, editors), J. and A. Churchill, London, p. 3 (1957). See also: A. Giner-Sorolla and A. Bendich, THIS JOURNAL, in press.

(16) It may be argued that anti-tumor agent 4-aminopyrazolo[3,4-d]pyrimidine is an exception to this guiding principle since it can be visualized as a compound which had arisen from adenine as a result of two structural changes (*cf.* H. E. Skipper, R. K. Robins and J. R. Thompson, *Proc. Soc. Exptl. Biol. and Med.*, **89**, 594 (1955)).

alcoholic solution, in quantitative yield. As expected, compound I was hydrogenated to adenine (III) in presence of a palladium–charcoal catalyst.

When 6-hydrazinopurine (IV) was treated with ferric chloride in dilute acid solution, a 6% yield of 6-chloropurine (II) was obtained. This reaction, fashioned after the analogous transformation of phenylhydrazine to chlorobenzene[17] can be visualized as proceeding *via* a diazonium chloride intermediate. 8-Hydrazinocaffeine recently has been converted[18] to 8-chlorocaffeine in this manner.

A number of other 6-substituted purines were prepared from 6-carboxypurine (V).[19,20] The acid chloride VI prepared from V and PCl_5 proved to be so reactive that its isolation in pure form was not feasible. However, crude VI was transformed to the monomethyl- (VII) and dimethylamide (VIII) as well as to the known purine-6-carboxamide (IX) which had previously been obtained[19] from 6-cyanopurine by partial alkaline hydrolysis. Interest in the purine-6-carbohydrazide (X) was stimulated by its structural analogy to the tuberculostatic isonicotinic acid hydrazide.[21] The hydrazide X was prepared in nearly quantitative yield by refluxing the amide IX with aqueous hydrazine[22] and in less satisfactory yields either from the acid chloride VI in ethanolic hydrazine solution or directly from the acid V in boiling anhydrous hydrazine. Direct formation of acid hydrazides from hydrazine and aromatic or pyridine carboxylic acids has been observed previously. The treatment of purine-6-carbohydrazide (X) with an ammoniacal solution of potassium ferrocyanide led to purine-6-carboxamide (IX) with liberation of nitrogen. The hydrazide X served as an excellent precursor of the acid azide XI which was obtained in nearly quantitative yields upon reaction with nitrous acid (*cf.* ref.[23]). Attempts to prepare XI from purine-6-carboxylic acid chloride and sodium azide were not successful. In contrast, 6-azidopurine (or its tetrazolo isomer) could be obtained from 6-chloropurine and sodium azide, but the yields were lower than those resulting from the action of nitrous acid on 6-hydrazinopurine.[15]

Because of the leukotoxic activity of urethan[24] and the anti-tumor and anti-leukemic action of other carbamic esters,[25] it was of interest to prepare urethans derived from adenine. Accordingly, purine-6-carboazide (XI) was refluxed in methanol and ethanol and the methyl- (XII) and ethyl- (XIII) urethans were obtained. The formation of 6-N-(ethoxycarbamyl)-purine (XIII) from XI was attended by the expected liberation of nitrogen. The identity of these urethans was established by their transformation into 6-ureidopurine (XIV) by treatment with aqueous ammonia as in the preparation of aromatic urea derivatives.

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

(18) H. Priewe and A. Poljack, *Chem. Ber.*, **88**, 1932 (1955).

(19) L. B. Mackay and G. H. Hitchings, THIS JOURNAL, **78**, 3511 (1956).

(20) A convenient synthesis of 6-carboxypurine from 6-methylpurine has been developed by A. Hampton (*ibid.*, in preparation).

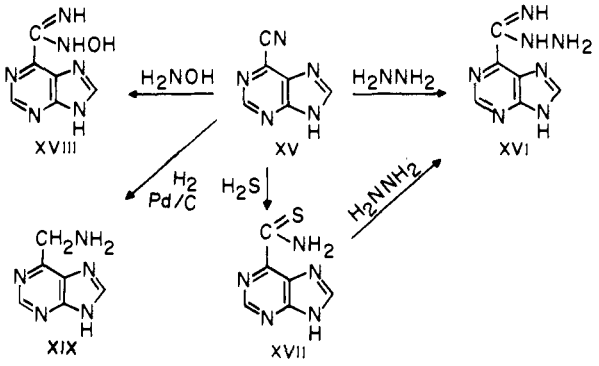
(21) H. H. Fox, *Trans. N. Y. Acad. Sci., II*, **15**, 234 (1953).

(22) T. Curtius and H. Struve, *J. prakt. Chem.*, [2] **50**, 205 (1895).

(23) T. Curtius, *Ber.*, **23**, 3029 (1890).

(24) J. A. Hawkins and J. B. Murphy, *J. Exptl. Med.*, **42**, 609 (1925).

(25) A. Haddow and W. A. Sexton, *Nature*, **157**, 500 (1946).



The recently published synthesis of 6-cyanopurine (XV) by Mackay and Hitchings[19] has made available a useful intermediate for the preparation of a number of otherwise difficultly accessible purines. Upon treatment with alcoholic hydrazine, XV was transformed smoothly into N-amino-6-purinylamidine (XVI) which also was obtained in this manner from the known[19] thiocarboxamide (XVII). The related 6-hydroxyamidinopurine-(XVIII) was prepared in excellent yield from the nitrile XV upon refluxing with alcoholic hydroxylamine. Analogous reactions in the aromatic series were described earlier.[26]

The catalytic reduction of cyanopyrimidines by Heyl, *et al.*,[27] has led to the corresponding aminomethyl derivatives. By similar treatment of an alcoholic solution of 6-cyanopurine (XV) with hydrogen (1 atmosphere) in the presence of a palladium–charcoal catalyst, the relatively unstable 6-aminomethylpurine (XIX) was obtained in 75% yield, and characterized as the crystalline picrate. This compound (XIX) is of interest because it is related to the extremely toxic 6-methylpurine.[28]

Physicochemical Studies

The four purines listed after adenine in Tables I and II can be considered to be derivatives of adenine in which a proton of the 6-amino group is replaced by the hydroxyl, carboalkoxy and carboxamino moieties. These substitutions have resulted in a lowering of the melting point (dec.) of about 30 to 135°, and a decrease in solubility in water at 20 ± 2°. Except for 6-ureidopurine (XIV), the basic dissociation of which is about 1.8 units of pK lower than that of adenine, the other three compounds (I, XII and XIII) exhibited an additional acidic dissociation with pK_{a_3} equal to or greater than 12. At the dilute concentrations employed (*ca.* 10^{-4} M) for determination of the ultraviolet absorption spectra, the urethans XII and XIII were sufficiently stable to permit accurate measurement of the spectra and the determination of apparent dissociation constants therefrom. However, under these conditions of concentration and above pH 9.5, the strongly reducing 6-N-hydroxylaminopurine (I) was found to be quite unstable (undoubtedly due to oxidation by the oxygen dissolved in the aqueous media) and this prevented an

(26) F. Tiemann and P. Krueger, *Ber.*, **17**, 1685 (1884).

(27) D. Heyl, E. Luz and S. A. Harris, THIS JOURNAL, **78**, 4474 (1956).

(28) F. S. Philips, S. S. Sternberg, L. D. Hamilton and D. A. Clarke, *Ann. N. Y. Acad. Sci.*, **60**, 283 (1954).

accurate recording of its spectral properties. Hence the spectral data for the anionic species for I are not listed in Table II. However, the in-

TABLE I

PHYSICAL PROPERTIES OF SOME 6-SUBSTITUTED PURINES

	Melting point, °C. (uncor.)	Solubility in water at 20° (±2°) 1 part in	pK_a (in water)[a]
Adenine	360 d.	1,100[b]	9.80 4.15[c]
6-N-Hydroxylaminopurine (I)	260 d.	1,660	>12[d] 9.83 3.80
6-N-(Methoxycarbamyl)-purine (XII)	282–284 d.	1,600	12.1 9.68 2.27
6-N-(Ethoxycarbamyl)-purine (XIII)	225–230 d.	4,200	12.2 9.63 2.4
6-Ureidopurine (XIV)	330–335 d.	3,300	9.95 2.35
6-Cyanopurine[e] (XV)	177–178[e]	Very sol.	6.88[f] *ca.* 0.3
N-Amino-6-purinylamidine (XVI)	325–330 d.	3,100	*ca.* 10[g] 5.12
6-Hydroxyamidinopurine (XVIII)	270–272 d.	13,100	9.4 *ca.* 2
Purine-6-N-methylcarboxamide (VII)	308–310 d.	1,360	8.9 *ca.* 1.0
Purine-6-N,N-dimethylcarboxamide (VIII)	210–211 d.	Very sol.	7.9 *ca.* 0

[a] Apparent pK_a, determined spectroscopically. For those values given to two decimal places, the experimental errors in the determinations are within ±0.05. Those values given to one decimal place are within ±0.1. [b] At 40°; A. Kossel, *Z. physiol. Chem.*, **10**, 254 (1886). [c] Determined by titration; H. F. W. Taylor, *J. Chem. Soc.*, 765 (1948). [d] Determined both by potentiometric titration and spectroscopically. The instability at high values of *p*H rendered a more accurate determination of $pK_a > 12$ difficult. [e] L. B. Mackay and G. H. Hitchings, THIS JOURNAL, **78**, 3511 (1956). [f] Determined also by potentiometric titration. No groups titrating from *p*H *ca.* 9 to 12.5 could be found. [g] Instability at high values of *p*H prevented a more accurate determination of pK_a *ca.* 10.

stability was not readily apparent in the more concentrated solution (c, 7×10^{-2} M) during the short time required for the potentiometric determination of dissociation constants,[29] and it was possible to demonstrate the apparent pK_a's of 9.83 and > 12 (in addition to 3.80). The potentiometric titration made it possible to decide that 6-N-hydroxylaminopurine can exist, although momentarily, in the monoanionic form at *p*H 8.8 to 10.8 and as a species carrying two negative charges above *p*H 11.

It is clear[30] (see also ref.[6]) that acidic dissociation of unsubstituted purine and of adenine involves removal of a proton from the imidazole nucleus. Hence, alterations in the acidic dissociation of amino-substituted adenines may be ascribed to an influence of the substituent on the electron configuration of the imidazole part (assuming the first acidic dissociation to be concerned with the imidazole). The first acidic dissociations of I, XII, XIII and XIV (pK_{a_2} of 9.63 to 9.95, Table I) are very nearly equal to that of adenine (9.80), and it may thus be argued that the substituents on the 6-amino group probably do not exert a significant effect on the electron density of the imidazole moieties. However, the basic pK_a's of XII, XIII and XIV are about 1.8 units lower than that of adenine. The substituents of these three purines would therefore appear to exert an electron-withdrawing effect on the pyrimidine nucleus consistent with the conclusion[15] that it is the pyrimidine moiety (rather than the imidazole) of purine which accepts the proton in sufficiently acidic solution.[31]

When the dissociation behavior of unsubstituted purine (pK_a 8.92, 2.52[6]; see also ref[30]) is compared to that of 6-cyanopurine[19] (Table I), it is seen that the cyano group exerts an acid-strengthening and base-weakening effect (of about two pK units). The inductive effect of the cyano group is thus about as strong as that of the trifluoromethyl group in 6-trifluoromethylpurine (pK_a 7.39 and < 0[15,31]). The hydroxy- and aminoamidino groups in XVI and XVIII did not show an analogous acid-strengthening effect, although the base strengths were not particularly different from that of 6-cyanopurine.

Ultraviolet absorption properties of several of these purines are given in Table II.

Biological Activity.—These purines have been tested in the Division of Experimental Chemotherapy, against Sarcoma 180 and Adenocarcinoma 755 in mice. No significant inhibition of growth was observed. In tissue culture, 6-N-hydroxylaminopurine has been found to be toxic to cells of mouse Sarcoma 180 as seen in mitotic inhibition and induction of nuclear degeneration when compared to normal embryo skin fibroblasts over a concentration range of 0.001 to 0.1 mM.[32]

Experimental[33]

6-N-Hydroxylaminopurine (I).—Hydroxylamine hydrochloride (95% purity) (12 g., 0.16 mole) was dissolved in 200 ml. of boiling absolute ethanol and a solution of potassium hydroxide (11.2 g., 0.20 mole) in 40 ml. of hot absolute ethanol was added. The precipitated KCl was filtered and washed three times with 20 ml. of hot ethanol. 6-Chloropurine[6] (II) (3.0 g., 0.019 mole), dissolved in 70 ml. of absolute ethanol, was added to the solution (total volume, 300 ml.) of hydroxylamine. The mixture was refluxed for six hr.; a white precipitate appeared after 0.5 hr. The mixture was allowed to stand overnight at room temperature and filtered. The precipitate was washed thoroughly with water and dried *in vacuo* over pellets of NaOH; yield 2.9 g. (quantitative), m.p. 252° dec. An analytically pure sample of the product was prepared in the same manner except that the volume of the solvent was increased fourfold and the precipitate washed repeatedly with water and ethanol. The sample melted at 260° dec.

Anal. Calcd. for $C_5H_5N_5O$: C, 39.73; H, 3.33; N, 46.34. Found: C, 40.00; H, 3.23; N, 46.30.

The compound was soluble in acetic acid and insoluble in the usual organic solvents. It was not recrystallized easily from water since it decomposed on boiling to give a deeply

(29) It is of interest that the three pK_a's (Table I) of 6-N-hydroxylaminopurine (I) are numerically almost the same as those of guanine, [*i.e.*, 12.3, 9.2 and 3.3 (H. F. W. Taylor, *J. Chem. Soc.*, 765 (1948)] Guanine, however, is about 1/120th as soluble in water at 20 ± 2° than is 6-N-hydroxylaminopurine [see A. Albert and D. J. Brown, *ibid.*, 2060 (1954)], it is infusible and there are large differences in the ultraviolet absorption spectra [compare the data in Table I and those in S. F. Mason, *ibid.*, 2071 (1954)].

(30) A. Albert and D. J. Brown, *ibid.*, 2060 (1954).

(31) *Cf.* discussion of S. F. Mason in "The Chemistry and Biology of Purines," A Ciba Foundation Symposium (Wolstenholme and O'Connor, editors), J. and A. Churchill, London, 1957, p. 72.

(32) J. J. Biesele, unpublished results.

(33) All melting points are uncorrected. Microanalyses were carried out by Dr. J. Alicino, Metuchen, N. J.

TABLE II

ULTRAVIOLET SPECTRAL PROPERTIES

	pH	Species charge	$\lambda_{max.}$, mμ	$\epsilon \times 10^{-3}$
Adenine[a]	2.10	+	262	13.2
	7.03	0	260	13.5
	12.01	−	267	12.0
6-N-Hydroxylaminopurine[b] (I)	1.23	+	271	13.3
	6.73	0	268	11.8
6-(Methoxycarbamyl)-purine (XII)	0.13	+	275	15.5
	7.07	0	274	13.4
	11.3	Mainly −	278	12.8
	1 *N* NaOH	Mainly =	289	14.1
6-(Ethoxycarbamyl)-purine (XIII)	0.13	+	274	15.6
	7.07	0	273	13.3
	11.3	Mainly −	277	12.9
	1 *N* NaOH	Mainly =	289	14.3
6-Ureidopurine (XIV)	2.9	+	266	16.6
	8.18	0	267	14.8
	10.25	−	269	15.8
6-Cyanopurine[c] (XV)	6 *N* HCl	Mainly +	247, 284	3.80, 9.10
	4.91	0	288	9.10
	9.11	−	292	7.63
N-Amino-6-purinylamidine (XVI)	3.2	+	291	8.05
	6.18	0	275, 320	7.22, 6.34
	11.20[d]	−	295	8.15
6-Hydroxyamidinopurine (XVIII)	0.13	+	274, 285[e]	7.15, 6.79
	7.34	0	272, 305	7.91, 6.70
	11.72	−	326	8.44
Purine-6-N-methylcarboxamide (VII)	0.13	+	276	10.7
	7.72	0	287	10.0
	12	−	292	7.26
Purine-6-N,N-dimethylcarboxamide (VIII)	3 *N* HCl	Mainly +	268	8.41
	6.60	0	268	8.71
	14	−	277	7.67

[a] The data for adenine were taken from S. F. Mason, *J. Chem. Soc.*, 2071 (1954). [b] Instability at values of *p*H above 9 prevented accurate determination of spectra. [c] L. B. Mackay and G. H. Hitchings, THIS JOURNAL, **78**, 3511 (1956), report molar extinction values (ε) of 7510 at 289 mμ and *p*H 1, and 6530 at 292 mμ and *p*H 11. [d] Broad maximum. [e] Shoulder.

colored solution. This decomposition, probably due to oxidation, was more rapid in the presence of charcoal.

6-N-Hydroxylaminopurine (I) reduced alkaline phosphomolybdate reagent as well as ammoniacal silver nitrate, and produced a deep blue color when mixed with a dilute solution of ferric chloride.[34] The compound (I) was not altered by the action of concentrated HCl or HF even after prolonged contact at 10°. It effervesced strongly on treatment with 6 *M* HNO_3 and, on cooling, crystals of hypoxanthine nitrate appeared. Adenine (III) was obtained from I upon heating with an alkaline solution of sodium dithionite or by hydrogenation in aqueous solution at atmospheric pressure using 5% palladium–charcoal as catalyst.

6-Chloropurine from 6-Hydrazinopurine.—To a solution of 0.50 g. (0.003 mole) of 6-hydrazinopurine[35] in 25 ml. of 2 *N* hydrochloric acid, 20 ml. of 10% ferric chloride was added. The initial blue color turned to brown after a few minutes at room temperature, and the solution effervesced strongly. The aqueous reaction mixture was allowed to stand for 0.5 hr. and then extracted 6 times with 10-ml. portions of ether. The combined extracts were dried with anhydrous sodium sulfate and evaporated to yield 30 mg. (6%) of a product identical with 6-chloropurine as determined by paper chromatography and ultraviolet spectroscopy.[6] When the remainder of the aqueous reaction mixture was heated for 0.5 hr. on a steam-bath, more gas was evolved and the color darkened, but re-extraction with ether yielded no more 6-chloropurine. Lower yields of 6-chloropurine were obtained in the reaction between 6-hydrazinopurine and a mixture of potassium chlorate and hydrochloric acid at room temperature.

Purine-6-carboxamide (IX).[19] Method A.—A mixture of purine-6-carboxylic acid (0.66 g., 0.004 mole) prepared according to Hampton[20] and of phosphorus pentachloride (0.83 g., 0.004 mole) was heated under anhydrous conditions at 115–120° for 15 minutes. The phosphorus chlorides present at the end of the reaction were removed by distillation *in vacuo*. The residual product consisted of 0.7 g. of an amorphous brown material which charred between 250–270°. This crude material (undoubtedly the purine-6-carboxylic acid chloride) could not be purified because of its instability. It was allowed to stand at room temperature for one hour in 50 ml. of saturated ethanolic ammonia and the resulting mixture filtered from an insoluble residue. The residue was extracted three times in the cold with 20 ml. of ethanolic ammonia and then washed with cold water; yield 0.39 g. (60%), m.p. 305–310° dec. On recrystallization from water, colorless needles, m.p. 315–320° dec., were obtained which were identical with an authentic specimen of purine-6-carboxamide.[19]

Method B.—To a suspension of purine-6-carbohydrazide (X) (see below) (0.20 g., 0.0011 mole) in 1.5 ml. of water at room temperature was added a solution of potassium ferricyanide (0.70 g., 0.002 mole) in 3.5 ml. of concentrated ammonium hydroxide. There was a strong evolution of gas and when this ceased an additional 0.35 g. (0.001 mole) of potassium ferricyanide in 1.5 ml. of concd. ammonia was added. The reaction mixture was allowed to stand for 10

(34) A. Hantzsch and C. H. Desch, *Ann.*, **323**, 23 (1902).

(35) J. A. Montgomery and L. B. Holum, THIS JOURNAL, **79**, 2185 (1957).

minutes at room temperature, evaporated *in vacuo* to dryness and the residue extracted with 90% ethanol; 0.12 g. (65%) of prisms was obtained, m.p. 310–312° dec. Thin needles, m.p. 315° dec., were obtained upon recrystallization from water. The product was chromatographically identical with an authentic sample of purine-6-carboxamide (IX).

Purine-6-carbohydrazide (X). Method A.—A mixture of 2.40 g. (0.15 mole) of purine-6-carboxamide (IX) and a solution of 3.2 ml. (0.1 mole) of anhydrous hydrazine in 15 ml. of water was refluxed for 30 minutes. A copious white precipitate appeared within 5 to 10 minutes. The reaction mixture was cooled, filtered and the precipitate washed with cold ethanol to yield 1.85 g. of colorless needles, m.p. 290–292° dec. An additional amount of 0.65 g., m.p. 290–292° dec., was obtained from the filtrate; total yield 96%. Needles were obtained after three recrystallizations from water; m.p. 292–294° dec.

Anal. Calcd. for $C_6H_6N_6O$: C, 40.45; H, 3.39; N, 47.18. Found: C, 41.01; H, 3.00; N, 47.93.

Compound X was sparingly soluble in cold water and ethanol. It reduced alkaline phosphomolybdate solution in the cold and ammoniacal silver nitrate on warming, but had little or no effect on Fehling reagent.

Method B.—This method gave lower yields (about 30%) than method A. To a specimen of purine-6-carboxylic acid chloride (VI), suspended in ethanol, was added an excess of a 25% solution of hydrazine in ethanol. After filtration and evaporation of the filtrate *in vacuo*, colorless needles were obtained, m.p. 282–285° dec.

Method C.—This method also gave a lower yield (10 to 20%) than that of method A. Purine-6-carboxylic acid (V) was refluxed for 10 hours with an excess of anhydrous hydrazine. The reaction mixture was evaporated *in vacuo* to give a crystalline product, m.p. 283–285° dec. All three products were indistinguishable by paper chromatography and by mixed melting points.

Purine-6-carboazide (XI).—To a suspension of purine-6-carbohydrazide (X) (0.71 g., 0.004 mole) in 40 ml. water at 25°, 0.55 g. (0.008 mole) of sodium nitrite was added at room temperature. The suspension thickened almost immediately following the dropwise addition of 3 ml. of 20% acetic acid and the appearance of the crystals changed from thin, long needles to rosettes of short needles. The reaction mixture was stirred for 30 minutes. The cream-colored product was washed thoroughly with cold water and dried *in vacuo*.

The yield was 0.75 g. (quantitative); m.p. 156° (dec., with explosion; the white dust remaining on the walls of the melting point tube decomposed by further heating at 305–310°). This material decomposed in boiling water or ethanol; it was difficult to recrystallize from ethanol due to the loss of nitrogen and the resulting transformation into 6-N-(ethoxycarbamyl)-purine (XIII).

Anal. Calcd. for $C_6H_3ON_7$: C, 38.10; H, 1.60; N, 51.84. Found: C, 38.48; H, 1.76; N, 51.92.

6-N-(Methoxycarbamyl)-purine (XII).—A suspension of 1.5 g. (0.008 mole) of purine-6-carboazide (XI) was refluxed with 150 ml. of absolute methanol for 5 hours. Evolution of nitrogen was observed during the first three hours by which time complete solution had occurred. The solution was treated with charcoal, filtered, cooled and the crystalline product collected and washed with cold methanol. White needles, 0.75 g. (49%), were obtained, m.p. 282–284°. (The filtrate on concentration yielded 0.6 g. of a unidentified product with m.p. 207–210°). The melting point (282–284°) was unchanged after recrystallization from water.

Anal. Calcd. for $C_7H_7N_5O_2$: C, 43.52; H, 3.65; N, 36.25. Found: C, 43.59; H, 3.64; N, 36.23.

6-N-(Ethoxycarbamyl)-purine (XIII).—A suspension of purine-6-carboazide (0.75 g., 0.004 mole) (X) in 60 ml. of absolute ethanol was refluxed under anhydrous conditions. The rate of evolution of nitrogen gas was rapid at the start and diminished with the disappearance of the azide. After 2 hr. of refluxing, thin needles appeared. At this point the evolution of nitrogen had ceased and the mixture was refluxed for an additional half-hour. The volume of the nitrogen evolved in the reaction was 90% of theory. The reaction mixture was allowed to stand overnight at room temperature and the crystalline urethan was collected, yield 0.57 g. (70%); the compound melted at 225–230° to a thick white sirup which charred and decomposed at 315–320°. Concentration of the mother liquor *in vacuo* yielded a second crop, 0.065 g. (8%), m.p. 225–230°. Recrystallization from ethanol did not affect the melting point.

Anal. Calcd. for $C_8H_9N_5O_2 \cdot {}^1/_2H_2O$: C, 44.44; H, 4.66; N, 32.39. Found: C, 44.74; H, 4.36; N, 32.04.

6-Ureidopurine (XIV).—A solution of 6-N-(ethoxycarbamyl)-purine (XIII) (0.10 g., 0.00046 mole) in 10 ml. of concentrated aqueous ammonia was heated at 100° in a sealed tube for 3 hr. The tube was cooled, its contents filtered and the filtrate evaporated *in vacuo* to yield 0.070 g. (85%) of white needles, m.p. 330–335° dec.

Anal. Calcd. for $C_6H_6N_6O$: C, 40.45; H, 3.39; N, 47.18. Found: C, 40.40; H, 3.47; N, 47.22.

The same yield also was obtained by permitting the 6-N-(ethoxycarbamyl)-purine to stand in concentrated aqueous ammonia at room temperature for one week. A complete recovery of the starting material was obtained when ethanolic ammonia was used instead of aqueous. 6-Ureidopurine also was obtained from the methylurethan XII by treatment with aqueous ammonia at room temperature.

Purine-6-N-methylcarboxamide (VII).—A mixture of purine-6-carboxylic acid (V) (0.8 g., 0.005 mole) and of phosphorus pentachloride (1.2 g., 0.006 mole) was heated under anhydrous conditions at 120–130° for 10 minutes. The crude purine-6-carboxylic acid chloride (VI) which formed was dried *in vacuo* at 110° and poured into 20 ml. of a cold saturated solution of methylamine in ethanol, and the mixture allowed to stand for 1 hour at room temperature. The reaction mixture was filtered and the filtrate evaporated to dryness *in vacuo*. The residue was washed with 5 ml. of cold water and dried to give 0.28 g. (33%) of crude material, m.p. 298–300° dec. After three recrystallizations from water short colorless needles, m.p. 308–310° dec., were obtained.

Anal. Calcd. for $C_7H_7N_5O$: C, 47.45; H, 3.98; N, 39.53. Found: C, 47.59; H, 3.84; N, 39.56.

Purine-6-N,N-dimethylcarboxamide (VIII).—A mixture of purine-6-carboxylic acid (V) (0.65 g., 0.004 mole), phosphorus pentachloride (0.84 g., 0.004 mole) and 0.5 ml. of phosphorus oxychloride was heated under anhydrous conditions at 130–140° for 15 minutes. The crude purine-6-carboxylic acid chloride (VI) was dried *in vacuo* at 110° and poured into 30 ml. of a cold saturated solution of dimethylamine in absolute ethanol and the mixture was processed in the same way as with its analog; yield 0.15 g. (20%), m.p. 208–210° dec. Upon two recrystallizations from water colorless needles, m.p. 210–211° dec., were obtained.

Anal. Calcd. for $C_8H_9N_5O$: C, 50.25; H, 4.74; N, 36.63. Found: C, 50.29; H, 4.53; N, 36.84.

N-Amino-6-purinylamidine (XVI).[36] **Method A.**—A solution of 6-cyanopurine (0.20 g., 0.0014 mole) (XV)[19] in 20 ml. of absolute ethanol and anhydrous hydrazine (0.1 g., 0.003 mole) was refluxed for one hour. After 10–15 minutes, yellow crystals appeared. The reaction mixture was allowed to stand overnight at room temperature, the precipitate was collected, washed thoroughly with cold ethanol and dried to yield 0.19 g. (78%) of yellow prisms, m.p. 325–330° dec.

Anal. Calcd. for $C_6H_7N_7$: C, 40.67; H, 3.98; N, 55.34. Found: C, 40.87; H, 3.97; N, 55.41.

Method B.—A solution of purine-6-thiocarboxamide (XVII)[19] (0.50 g., 0.0028 mole) in 30 ml. of methanol and anhydrous hydrazine (0.5 g., 0.015 mole) was refluxed for 3 hours during which time there was a strong evolution of H_2S and ammonia. The solution was cooled, and the filtrate evaporated to dryness *in vacuo*. A yield of 0.41 g. (81%) was obtained. The product was recrystallized from water to give colorless needles, m.p. 325–330° dec. The mixed m.p. with the compound obtained above showed no depression. The compounds made by both methods were indistinguishable by paper chromatography and ultraviolet spectra.

6-Hydroxyamidinopurine (XVIII).[36]—To a solution of 6-cyanopurine (XV)[19] (6 g., 0.04 mole) in 300 ml. of absolute ethanol was added 300 ml. of ethanol containing 0.17 mole of hydroxylamine. The mixture was refluxed on a steam-bath for 45 minutes. After a few minutes, a copious crys-

(36) These names conform with the nomenclature given in the introduction of the Subject Index, *C. A.*, **39**, 5913 (1945), and **46**, 12412 (1952).

talline precipitate appeared. The reaction mixture was cooled and filtered to give 7.3 g. (96%) of colorless needles, m.p. 270–272° dec. A sample of the product was recrystallized from water and dried *in vacuo* over P_2O_5 at 100° for 3 hours; m.p. 270–272° dec.

Anal. Calcd. for $C_6H_6N_6O \cdot {}^1/_3H_2O$: C, 39.12; H, 3.65; N, 45.62. Found: C, 39.19; H, 3.61; N, 45.59.

6-Aminomethylpurine (XIX).—A solution of 6-cyanopurine (XV) (0.29 g., 0.002 mole) in 15 ml. of absolute methanol was hydrogenated at room temperature and atmospheric pressure in the presence of 0.2 g. of 5% palladium–charcoal. The suspension was filtered, the precipitate washed with methanol and the combined filtrates evaporated *in vacuo* to yield 0.23 g. (75%) of needles, m.p. 181–183° dec. The reaction product, which turned pink on exposure to air, was very soluble in water, ethanol, methanol and acetic acid and sparingly soluble in ether and benzene. A sample was purified by pouring a concentrated ethanol solution into ether. This treatment was repeated twice to yield a pale pink product, m.p. 183–185° dec. An ethanolic solution of this compound produced an intense crimson coloration on acidification. The picrate of XIX was prepared from aqueous solution in cold water. After four crystallizations from water, a light yellow product, m.p. 190° dec. was obtained.

Anal. Calcd. for $C_6H_7N_5 \cdot C_6H_3N_3O_7$: C, 38.10; H, 2.66; N, 29.62. Found: C, 37.94; H, 2.86; N, 29.47.

Spectrophotometric and Dissociation Studies.—Spectrophotometric measurements were made with a Cary model 11 ultraviolet recording spectrophotometer (Applied Physics Corporation, Pasadena, Calif.) using matched 1-cm. silica cells and techniques and buffers previously described.[37] The apparent pK_a values were determined using the methods described by Fox and Shugar,[38,39] and Parke and Davis.[40]

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 and Mr. Herbert S. Rosenkranz for assistance.

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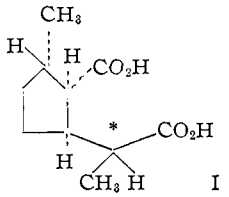
Studies in the Iridomyrmecin Series. Abnormal Ring Closure of a 1,6-Keto Aldehyde

BY N. L. WENDLER AND H. L. SLATES

RECEIVED MARCH 6, 1958

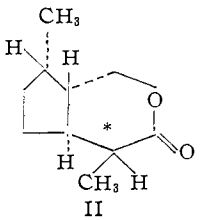
An unusual ring closure of a 1,6-keto aldehyde derivative of a tetrahydrofuran is described.

The original work by Fusco, Trave and Vercellone[1] on the structure of iridomyrmecin and related terpenoid compounds isolated from various *Iridomyrmex* species of ants led these authors to the proposal of a part structure for this substance. This part structure was based on the oxidation of iridomyrmecin to a nepetalinic acid whose structure had presumably been established by McElvain and Eisenbraun[2] to be I (unknown configuration at carbon designated by asterisk). Some time there-

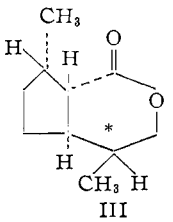


I

after Cavill, Ford and Locksley[3] reported summary findings permitting the assignment of either structure II or III to iridomyrmecin and its epimer (epimeric at the carbon designated by an asterisk).



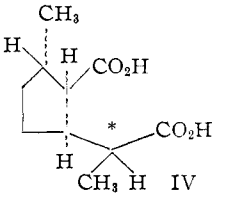
II



III

Recently McElvain[4] succeeded in converting nepetalic acid to isoiridomyrmecin in such a way as presumably to have established structure II for these substances.

On the basis of structure II we undertook to synthesize iridomyrmecin as well as its epimer, and the present account reports our experience in this direction. Since the inception of our work, however, a further article by Cavill[5] has appeared in which it is now disclosed that through private communication with McElvain a change of group configurations in nepetalinic acid is necessary whereby formula IV now represents the structure of this key compound. Details concerning the chemistry necessitating revision of the previously



IV

accepted structure I for nepetalinic acid which would have greatly enlightened this rather confused field were not revealed.

We prepared the Diels–Alder adduct V[6] from isoprene and maleic anhydride and reduced it with lithium aluminum hydride to the diol VI.

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