[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Studies on Condensed Pyrimidine Systems. XVIII. Substances Related to 6-Purinecarboxylic Acid

BY LOTTIE BOHM MACKAY AND GEORGE H. HITCHINGS

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6-Cyanopurine was synthesized from 6-iodopurine by reaction with cuprous cyanide. Alkaline hydrolysis of 6-cyanopurine gave the 6-carboxamide and the 6-carboxylate; acid hydrolysis resulted in the formation of hypoxanthine. The 6-thiocarboxamide was formed from the cyanopurine by treatment with ammonium hydrosulfide. The iminoester, 6-purinecarboxamidine and 2-(6′-purinyl)-imidazoline also were prepared.

In view of the effectiveness of a number of 6-substituted purines as antimetabolites in nucleic acid metabolism[1-3] it was of interest to prepare 6-purinecarboxylic acid and related compounds for testing as antimetabolites. Several of these compounds were prepared and are being tested for biological activity.

A large number of purine derivatives are known, with a wide variety of substituents on either or both of the condensed rings. There are, however, only a few reports in the literature of purinecarboxylic acids[4] or their derivatives. In no case is the carboxyl (or potential carboxyl) group on the 6-position.

Pyrimidinecarboxylic acids are somewhat more common, orotic acid (4-uracilcarboxylic acid) being the best known instance. 4-Pyrimidinecarboxylic acid, the closest pyrimidine analog to 6-purinecarboxylic acid, was prepared by Gabriel and Coleman.[5]

The method employed for the preparation of 6-cyanopurine is an adaptation of the standard preparation of aromatic nitriles from halides with cuprous cyanide. 6-Chloropurine[6] failed to react under a variety of conditions. 6-Iodopurine[7] (I) did not react in the desired manner at high temperatures, but on refluxing in a large volume of pyridine with cuprous cyanide, 6-cyanopurine (II) was formed in 50% yield.

Alkaline hydrolysis of this nitrile proceeded in the normal manner. Refluxing with an equivalent amount of alkali yielded 6-purinecarboxamide (III), while with an excess of alkali 6-purinecarboxylic acid (IV) was formed. The latter decarboxylates at 198° to form purine which was identified by mixed melting point with an authentic sample, and by its ultraviolet spectrum. When 6-cyanopurine was hydrolyzed in hot 2 *N* sulfuric acid, two products were formed. These were separated by paper chromatography and one of them was identified as hypoxanthine (V) by its R_f value and ultraviolet absorption spectrum (Table I); the other hydrolysis product was not identified.

REACTION SCHEME

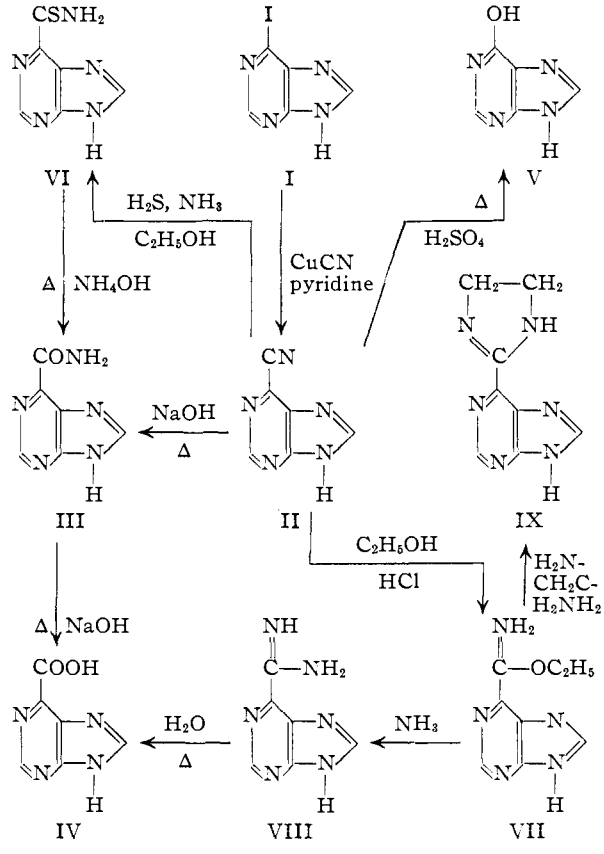

TABLE I

HYDROLYSIS OF 6-CYANOPURINE IN 2 *N* SULFURIC ACID

		Hydrolysate	
Response to w.v. light	Hypoxanthine	Spot 1	Spot 2
R_f	0.64	0.64	0.48
λ_{max} at *p*H 1, mμ	248	248	332 (broad)
λ_{max} at *p*H 11, mμ	258	258	325 (broad)

6-Purinethiocarboxamide (VI) was prepared by passing hydrogen sulfide through a cold suspension of the nitrile in alcoholic ammonia. The thioamide was stable to boiling water; boiling with ammonium hydroxide hydrolyzed it to the amide III, and boiling with dilute alkali to the carboxylic acid IV.

The ethyliminoester of 6-purinecarboxylic acid (VII) was prepared in the usual way. Conversion to the amidine VIII required elevated temperatures, as did conversion to a substituted cyclic amidine IX. Boiling water or boiling dilute

(1) G. B. Elion, G. H. Hitchings and H. VanderWerff, *J. Biol. Chem.*, **192**, 505 (1951).

(2) G. H. Hitchings and G. B. Elion, *Ann. N. Y. Acad. Sci.*, **60**, 195 (1954).

(3) G. H. Hitchings and G. B. Elion, 3*eme* Congrès International de Biochemie Rapports, page 185, August 1–6, 1955.

(4) (a) German Patent 115,121; 153,121, *Chem. Centralbl.*, **75**, II, 625 (1904); (b) W. Traube, *Ann.*, **432**, 266 (1923).

(5) S. Gabriel and J. Coleman, *Ber.*, **32**, 1525 (1899).

(6) A. Bendich, P. J. Russell, Jr., and J. J. Fox, THIS JOURNAL, **76**, 6073 (1934).

(7) G. B. Elion and G. H. Hitchings, *ibid.*, **76**, 3508 (1956).

alkali hydrolyzed the free amidine to 6-purinecarboxylic acid.

The ultraviolet absorption spectra of the compounds described are listed in Table II.

TABLE II

ULTRAVIOLET ABSORPTION SPECTRA

Compound	pH 1 λmax, mμ	pH 1 Em	pH 11 λmax, mμ	pH 11 Em
6-Cyanopurine	289	7,510	292	6,530
6-Purinecarboxylic acid	280	7,720	279	7,720
6-Purinecarboxamide	240	4,520	292	6,680
	279	7,910		
6-Purineethiocarboxamide	285	8,800	294	8,700
	(335)[a]	5,990		
6-Purinecarboxamidine	294	8,100	300	7,170
6-(2′-Imidazolinyl)-purine	286	12,650	(245)	(2,540)
	295.5	11,850	299	14,690
	338	6,440	311	13,750
	(353)	(5,260)	(342)	(4,420)

[a] Figures in parentheses indicate a shoulder in the absorption curve.

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Experimental

6-Cyanopurine (II).—A mixture of 29.5 g. (0.12 mole) of 6-iodopurine and 16 g. (0.18 mole) of cuprous cyanide in 300 ml. of pyridine (dried over potassium hydroxide and barium oxide) was heated under reflux conditions, with a drying tube on the condenser, in a bath kept at 125–135° for two hours. The black reaction mixture was chilled and filtered with suction; the residue was thoroughly washed with ether and then discarded. To the combined pyridine filtrate and ether washings an additional 1 l. of ether was added and this suspension was briefly stirred. After being chilled for several hours, the cold suspension was filtered and the residue thoroughly washed with ether before being discarded. The combined filtrate and washings were evaporated to dryness on the steam-bath *in vacuo* to yield a brown partly crystalline residue weighing 21 g. This was finely ground, and 100 ml. of water added. The resulting brown suspension was filtered and the filtrate extracted five times with ether. The ether extracts were dried over calcium sulfate and evaporated to dryness to yield 7.1 g. (41%) of 6-cyanopurine, m.p. 177–178°. By saturating the aqueous layer with salt and re-extracting with ether an additional 1.7 g. (10%) of 6-cyanopurine was obtained. A small amount of the product was twice recrystallized from benzene, the crystals washed with petroleum ether and dried *in vacuo* at 75° over phosphorus pentoxide. The pure crystals melted at 177–178°.

Anal. Calcd. for $C_6H_3N_5$: C, 49.66; H, 2.06; N, 48.28. Found: C, 49.70; H, 2.12; N, 48.45.

6-Purinecarboxylic Acid (IV).—To 3 g. (0.02 mole) of 6-cyanopurine was added 20 ml. of 2 *N* sodium hydroxide and the mixture refluxed for one hour. The resulting clear solution was cooled and acidified to *p*H 2 with concentrated hydrochloric acid. The precipitate was filtered off and washed with water to yield 3.3 g. (90%) of 6-purinecarboxylic acid. A small sample of the carboxypurine was recrystallized from water and dried *in vacuo* at 75° over phosphorus pentoxide. The pure acid melted at 198° dec. and was obtained as the monohydrate. The anhydrous compound was obtained after two hours at 110°.

Anal. Calcd. for $C_6H_4N_4O_2 \cdot H_2O$: C, 39.82; H, 3.53; N, 30.64; H_2O, 9.9. Found: C, 39.56; H, 3.32; N, 30.76; H_2O, 9.9.

Decarboxylation of 6-Purinecarboxylic Acid.—A sample of 6-carboxypurine was heated in a 300° bath and the sublimate collected on a water-cooled finger. The colorless crystalline sublimate melted at 212–213° and showed no melting point depression when mixed with an authentic sample of purine,[8] m.p. 214–215°.

6-Purinecarboxamide (III).—To 3 g. (0.02 mole) of 6-cyanopurine was added 9 ml. of 2.2 *N* sodium hydroxide (0.02 mole) and the mixture refluxed for one hour. The cooled suspension was neutralized to *p*H 7 with dilute hydrochloric acid, filtered, and the crystals freed of chloride ion by repeated washing with water; yield 3.2 g. (95%). A sample of the product was recrystallized twice from water and dried at 110°. The pure crystals melted at 315–320° dec.

Anal. Calcd. for $C_6H_5N_5O$: C, 44.17; H, 3.07; N, 42.94. Found: C, 44.33; H, 2.95; N, 43.43.

6-Purinethiocarboxamide (VI).—Hydrogen sulfide was passed through a solution of 1.45 g. (0.01 mole) of 6-cyanopurine in 20 ml. of absolute ethanolic ammonia for four hours. The resulting yellow suspension was evaporated to dryness on the steam-bath to yield 1.75 g. (98%) of yellow crystals. These were recrystallized once from methanol and dried at 100°; m.p. 240–242° dec.

Anal. Calcd. for $C_6H_5N_5S$: C, 40.22; H, 2.79; N, 39.11; S, 17.88. Found: C, 40.10; H, 2.74; N, 39.38; S, 17.69.

Ethyl 6-Purineiminocarboxylate Hydrochloride (VII).—Dry hydrogen chloride was passed through a suspension of 4.5 g. (0.03 mole) of 6-cyanopurine in 25 ml. of absolute ethanol for 5 hours. The suspension was stoppered and kept in the refrigerator overnight. It was then filtered, washed once with absolute ethanol, and dried to constant weight in a vacuum desiccator. This material appeared to be the monoethanolate of ethyl 6-purineiminocarboxylate hydrochloride. Its instability prevented further purification. The yield was 6.5 g. (80%).

Anal. Calcd. for $C_8H_9N_5O \cdot HCl \cdot C_2H_5OH$: N, 25.55. Found: N, 25.35.

6-Purinecarboxamidine (VIII).—A mixture of 5.5 g. (0.02 mole) of the above imino ester and 100 ml. of absolute ethanolic ammonia was placed in a bomb and left at 100° for 16 hours. The contents of the bomb were chilled and filtered. The crude amidine hydrochloride (4.3 g.) was dissolved in dilute hydrochloric acid and the free amidine precipitated by the addition of 5% sodium bicarbonate solution. After being washed with water and dried at 110°, the amidine weighed 2.4 g. (75%). A sample of the amidine was again dissolved in dilute hydrochloric acid, precipitated with sodium bicarbonate, thoroughly washed with water and dried at 110°; m.p. 316–320° dec.

Anal. Calcd. for $C_6H_6N_6$: C, 44.42; H, 3.73; N, 51.85. Found: C, 44.83; H, 3.75; N, 51.59.

6-(2′-Imidazolinyl)-purine (IX).—A mixture of 2.74 g. (0.01 mole) of 6-carboxypurine ethyliminoester hydrochloride monoethanolate, 1.8 g. (0.03 mole) of ethylenediamine, and 25 ml. of absolute ethanol was heated in a bomb tube at 110–120° for 1 to 3 days. The contents of the bomb were chilled, filtered, and washed with ether followed by petroleum ether; the resulting crude hydrochloride weighed 1.95 g. (85%). A portion (1.5 g.) of the hydrochloride was dissolved in water and adjusted to about *p*H 8 with 5% sodium bicarbonate. The small amount of precipitate was filtered and washed twice with small amounts of cold water, followed by ethanol and ether. The washed material was recrystallized from absolute ethanol and dried at 110° to yield 0.5 g. of free imidazoline (40% recovery from the crude hydrochloride), m.p. 287–288° dec.

Anal. Calcd. for $C_8H_8N_6$: C, 51.06; H, 4.26; N, 44.68. Found: C, 51.21; H, 4.44; N, 45.13.

Acid Hydrolysis of 6-Cyanopurine.—6-Cyanopurine (20 mg.) was dissolved in 2 *N* sulfuric acid (3 ml.) and warmed in a boiling water-bath for 1 hour. Additional heating produced no further change in the ultraviolet absorption spectrum which showed maxima at 248 mμ (*p*H 1) and 258 mμ (*p*H 11) with weaker bands at 328 mμ (*p*H 1) and 325 mμ (*p*H 11). An amount of aqueous solution equivalent to 40 γ of starting material was spotted on paper (S and S #597)

(8) O. Isay, *Ber.*, **39**, 250 (1906).

and developed in ascending fashion with isopropyl alcohol–ammonium sulfate (5% of each in water). Hypoxanthine (20 γ) was spotted as a control. The materials were located by visual inspection under ultraviolet light and the spots were eluted with water. The pertinent data are recorded in Table I.

Ultraviolet Absorption Spectra.—The ultraviolet absorption spectra were measured on a Beckman model DU spectrophotometer at a concentration of 10 mg. per l. For *p*H 1, 0.1 *N* hydrochloric acid was used, for *p*H 11, a Sørensen glycine–sodium hydroxide buffer.

TUCKAHOE, NEW YORK

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Alkaloids from *Senecio tomentosus*. Observations on the Alkaloid Jacobine and on the Structure of Jaconecic Acid

BY ROGER ADAMS, MAURIZIO GIANTURCO AND BENJAMIN L. VAN DUUREN

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Extraction of *Senecio tomentosus* affords a new alkaloid, $C_{19}H_{27}O_7N$, isomeric with the previously described otosenine. Hydrogenation with platinum or palladium as catalyst gives a tetrahydro derivative. Hydrolysis of the alkaloid yields an acid, $C_{10}H_{16}O_6$, identical with jaconecic acid first obtained by the hydrolysis of jacobine. Tomentosine with hydrochloric acid gives a chlorinated dehydrated jaconecic acid, presumably the same compound as that derived from otosenine by similar treatment. The structure of jaconecic acid is discussed in light of its physical and chemical properties. A synthetic approach to jaconecic acid involved the oxidation of isoseneciphyllic acid by perbenzoic acid. From the product, a pure dilactone and a mixture of isomeric dilactones were isolated. The first was reduced to two dihydro derivatives which were isolated in pure form. The second was reduced to a mixture of dihydro compounds. The dihydro compounds from the first were stable to thionyl chloride, the dihydro product from the second reacted with replacement of an hydroxyl with chlorine. The infrared spectrum of the resulting material is very similar to that of the product obtained by treatment of tomentosine with hydrochloric acid. The structures of the synthetic lactones are discussed.

Extraction of *Senecio tomentosus* with ethanol[1] yielded 0.006% of a mixture of two or possibly three alkaloids. By chromatography of the crude bases on Florisil and elution with carbon tetrachloride, a crystalline product was obtained. This was identified as senecionine by melting point, optical rotation, infrared spectrum and R_f value.[2,3] A chloroform fraction yielded a new alkaloid, m.p. 232°, $[\alpha]_D$ +14° ($CHCl_3$), picrate m.p. 251° dec. From analyses of the pure alkaloid and of its picrate, the empirical formula $C_{19}H_{27}NO_7$ for the alkaloid was deduced. An alkaloid with the same empirical formula, m.p. 218–219°, $[\alpha]_D$ +20.8° ($CHCl_3$), picrate m.p. 233–235° dec., has been isolated from *Senecio othonnae*[4] and *Senecio renardi*[5] and was named otosenine. Since the physical properties of otosenine and its picrate are so different from those of the alkaloid obtained from *Senecio tomentosus* and its picrate, these two alkaloids are assumed not to be identical. The name tomentosine is consequently suggested for the alkaloid isolated in the present investigation.

Tomentosine, on hydrogenation with a palladium–strontium carbonate catalyst, absorbed two moles of hydrogen to give tetrahydrotomentosine, $C_{19}H_{31}NO_7$, m.p. 157–158°, $[\alpha]_D$ −9.2° ($CHCl_3$). With platinum as catalyst, hydrogenation proceeded to an uptake of about 2.5 moles of hydrogen indicating perhaps the partial reduction of a ketone group. From the oily reaction product a small amount of tetrahydrotomentosine was all that could be isolated.

The infrared spectrum of tomentosine shows a band at 1745 cm.$^{-1}$ which may indicate a small ring ketone structure.[6] Zhdanovich and Menshikov[4] identified the presence of a ketone in the basic moiety from otosenine by the formation of an oxime of reduced otonecine. The spectrum of tomentosine shows other bands, an hydroxyl at 3540, an ester carbonyl at 1725 and a carboxyl (zwitterion) at 1612 cm.$^{-1}$. In addition, bands in the vicinity of 880, 1160, 1210 and 1260 cm.$^{-1}$ in the spectrum of the alkaloid are consistent with the assumption of the presence of an epoxide function in the molecule.[7] A band at 830 cm.$^{-1}$ corresponds to a CHR=CR′R″ type of carbon–carbon double bond. This band is present in the spectra of all Senecio alkaloids whose necine contains the RCH=C(R′)CH_2OH grouping.

Significant features of the absorption spectrum of the tetrahydrotomentosine (Table I) are: complete absence of an ester C=O and C=C bands and the presence of hydroxyl, keto C=O and carboxyl (zwitterion) bands as in tomentosine. The four bands from 880 to 1260 cm.$^{-1}$ previously discussed for tomentosine and assigned to an ethylene oxide function are also present in the spectrum of tetrahydrotomentosine. When tetrahydrotomentosine in aqueous sodium bicarbonate was continuously extracted with ether and the ether evaporated, an oil, which could not be crystallized, re-

(1) The method employed was that described in a previous paper: R. Adams, K. E. Hamlin, C. F. Jelinek and R. F. Phillips, THIS JOURNAL, **64**, 2760 (1942). An earlier plant sample yielded 0.07% tomentosine unaccompanied by any other alkaloids.

(2) The determination of melting points alone is not a reliable guide for purity or identity of a Senecio alkaloid, since mixtures frequently melt at the same temperature as a single component.

(3) The carbon tetrachloride–chloroform fraction yielded more crystalline material, also identified as senecionine, together with a dark resinous material, from which, after chromatography, the picrate of a base isomeric with tomentosine was isolated (see Experimental).

(4) E. S. Zhdanovich and G. P. Menshikov, *J. Gen. Chem. U.S.S.R.*, **11**, 835 (1941).

(5) A. V. Danilova and R. A. Konovalova, *ibid.*, **20**, 1921 (1950).

(6) The frequencies of absorption of the carbonyl of cyclobutanone, cyclopentanone and cyclohexanone are 1775, 1740 and 1715 cm.$^{-1}$, respectively (R. S. Rasmussen "Progress in Chemistry of Natural Products," Vol. 5, Springer Verlag, Vienna, 1948, p. 331). 17-Ketosteroids show characteristic absorption around 1743 cm.$^{-1}$ (L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd edition, Reinhold Publishing Corp., New York, N. Y., 1949).

(7) J. E. Field, J. O. Cole and D. E. Woodford, *J. Chem. Phys.*, **18**, 1298 (1950).