3-Chloro-6,6a-dihydro-2,6-dimethyl-11H-isoindolo[1,2-*c*]-[1,2,4]benzothiadiazin-11-one 5,5-dioxide (XIV). A.—A mixture of 3.5 g. of 2-amino-5-chloro-N-methyl-*p*-toluenesulfonamide, 2.0 g. of phthalaldehydic acid, 3 drops of concentrated sulfuric acid, and 40 ml. of dimethoxyethane was heated under reflux for 3 hr. Upon cooling, a crystalline solid, 2.0 g., m.p. 206–208°, precipitated.

B.—To a cold solution of XIII (5.0 g.) in 150 ml. of acetone and an aqueous sodium hydroxide solution (made from 5 g. of sodium hydroxide and 70 ml. water), methyl sulfate (5 ml.) was gradually added. The mixture was stirred in an ice bath for 1 hr. The solid was collected and recrystallized from acetone to afford 2.6 g. of product identical with that made by method A.

Anal. Calcd. for $C_{16}H_{13}ClN_2O_3S$: C, 55.10; H, 3.75; Cl, 10.11; N, 8.02; S, 9.20. Found: C, 54.85; H, 3.50; Cl, 10.10; N, 7.75; S, 9.20.

Acknowledgment.—We are grateful to Dr. Gordon Ellis and his associates for the microanalyses and to Mr. Bruce Hofmann for helpful comments on the spectra. Mr. Carl Gochman gave valuable technical assistance.

Studies on the Thiation of Purines[1]

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Direct thiation of purine with elementary sulfur afforded 8-mercaptopurine in good yield; lower yields of the 8-mercapto derivatives were obtained from 2-amino- and 6-methylpurine. Similarly, benzimidazole was smoothly converted to 2-mercaptobenzimidazole upon reaction with elementary sulfur. 6-Methylpurine 1-N-oxide was transformed by reaction with either thiolacetic acid or diacetyl sulfide into 2- and 8-mercapto-6-methylpurine. N-Acetylpurine-6-thiocarboxaldehyde was prepared from purine-6-carboxaldehyde hydrazone or the oxime by reaction with thiolacetic acid or diacetyl sulfide. The thioaldehyde, from which purine-6-carboxylic acid was obtained upon oxidation with potassium permanganate, gave the corresponding hydrazone and thiosemicarbazone with the appropriate carbonyl reagent. Reduction of the thioaldehyde to 6-methylpurine was effected by prolonged reaction with hydrazine *via* the intermediate hydrazone, and by treatment with Raney nickel. Conversion of 6-hydrazino-, 6-N-hydroxylamino-, and 6-halogenopurine to 6-mercaptopurine was achieved by refluxing with thiolacetic acid.

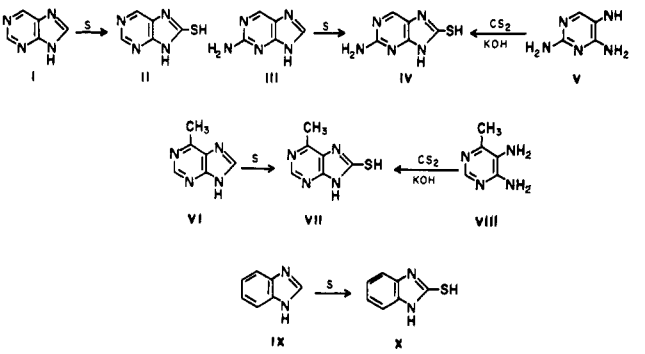
The direct thiation of heterocyclic compounds with sulfur was first described by Edinger and Arnold,[2] who synthesized 5-thioacridine by heating acridine with sulfur. This thiation reaction failed with pyridine, although with α-picoline a polycyclic thiated base formed.[3] The treatment of γ-picoline with sulfur[4] led to thiation as well as the coupling of the heterocyclic nuclei. The thiation of indole to yield diindole-polysulfides has been studied.[5] Stilbene and tetraphenylthiophene were obtained in the reaction between sulfur and toluene.[6] Glass and Reid[7] succeeded in introducing the thiol function into aromatic compounds with elemental sulfur without catalysts; thiophenol and several phenyl sulfides were obtained.

From these results, the reaction of substituted purines with elementary sulfur would be expected to result in coupling of purine nuclei or in the formation of sulfur-containing purines.

8-Mercaptopurine (II) was obtained in 75% yield when an equimolar mixture of purine (I) and sulfur was heated at 245° for 45 min.; the yields were lower when the hydrochloride and the sodium salt of purine were used (Scheme I). Lower yields of 8-mercaptopurine resulted when the reaction was carried out at 220° or in hexachlorobenzene at 180°.[8]

2-Amino-8-mercaptopurine (IV) was prepared in 15% yield by heating 2-aminopurine (III) with 2 equiv. of sulfur at 250° for 1 hr.; IV was also prepared from 2,4,5-triaminopyrimidine (V) by condensation with carbon disulfide.[9]

SCHEME I



The reaction of sulfur with 6-methylpurine (VI) at 230 to 240° proved difficult to control, but 8-mercapto-6-methylpurine (VII) could be isolated in very small yield.[10] Direct thiation of hypoxanthine, guanine, 6-methylpurine 1-N-oxide, 6-chloropurine, uracil, 6-methyluracil, thymine, pyrimidine, quinoline, and isoquinoline was not achieved by interaction with elementary sulfur.

(1) This investigation was supported by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. CY-3190), the Atomic Energy Commission (Contract No. AT[30-1], 910), and the American Cancer Society (Grant No. T-128B). This paper was presented in part at the 137th National Meeting of the American Chemical Society, Cleveland, Ohio, April, 1960, Abstracts, p. 11N.

(2) A. Edinger and H. Arnold, *J. prakt. Chem.*, **64**, 182 (1901).

(3) B. Emmert and M. Groll, *Chem. Ber.*, **86**, 205 (1953).

(4) H. I. Thayer and B. B. Corson, *J. Am. Chem. Soc.*, **70**, 2331 (1939).

(5) B. Oddo and L. Raffa, *Gazz. chim. ital.*, **69**, 362 (1939); W. Carpenter, M. S. Grant, and H. R. Snyder, *J. Am. Chem. Soc.*, **82**, 2739 (1960).

(6) L. Aronstein and A. S. von Nierop, *Rec. trav. chim.*, **21**, 448 (1902).

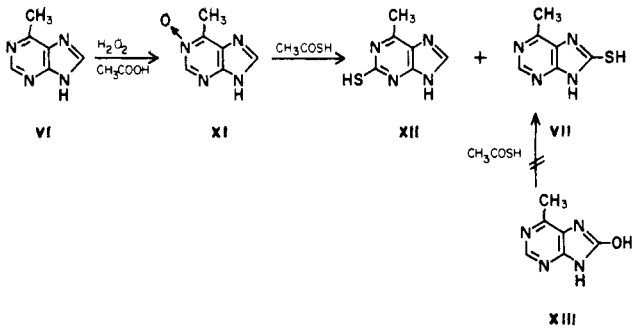
(7) H. B. Glass and E. E. Reid, *J. Am. Chem. Soc.*, **51**, 3428 (1929).

(8) The synthesis of 8-mercaptopurine from 4,5-diaminopyrimidine and thiourea was described by O. Isay, [*Ber.*, **39**, 257 (1906)].

(9) An independent synthesis of 2-amino-8-mercaptopurine (IV) from thiourea and 2,4,5-triaminopyrimidine (V) was reported by A. F. Lewis, A. G. Beaman, and R. K. Robins [*Can. J. Chem.*, **41**, 1807 (1963)].

(10) The synthesis of 8-mercapto-6-methylpurine (VII), from 4,5-diamino-6-methylpyrimidine (VIII) and thiourea, was first reported by S. Gabriel and J. Colman [*Ber.*, **34**, 1234 (1901)]; VII was also prepared by reaction of VIII and carbon disulfide in the presence of potassium hydroxide and pyridine [*Cf.* C. W. Noell and R. K. Robins, *J. Am. Chem. Soc.*, **81**, 5997 (1959)].

SCHEME II



As a model experiment, treatment of benzimidazole (IX) with 1 equiv. of sulfur gave an 82% yield of 2-mercaptobenzimidazole (X).[11]

The selective attack of sulfur on the 8-position of purine is in accord with the theoretical considerations of Pullman,[12] which indicate that the activation energy for any type of attack on the unsubstituted purine molecule should be lowest for the C-8 position.

Treatment of 6-methylpurine 1-N-oxide (XI) with diacetyl sulfide invariably afforded a mixture of 2- (XII) and 8-mercapto-6-methylpurine (VII) (Scheme II). Similar reaction of XI with thiolacetic acid resulted in the formation of both VII and XII; on prolonged treatment only VII could be recovered. This reaction was suggested by the acetic anhydride induced rearrangement of 2-methylpyridine 1-N-oxide to 2-acetoxymethylpyridine[13] recently used in the rearrangement of 6-methylpurine 1-N-oxide with acetic anhydride to 6-acetoxymethylpurine.[14]

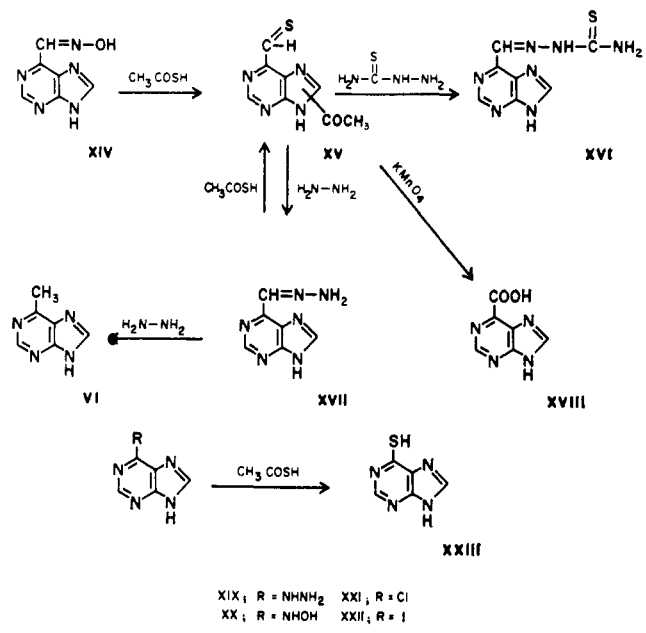
A possible mechanism for the reaction between 6-methylpurine 1-N-oxide (XI) and thiolacetic acid or diacetyl sulfide to give VII and XII could involve the initial rearrangement of XI to 2- or 8-hydroxy-6-methylpurine which is then thiated. To check this possibility, 8-hydroxy-6-methylpurine (XIII) was boiled for several hours either with thiolacetic acid or diacetyl sulfide. The starting material was recovered with no trace of thiated purines. It was observed by Ulrich[15] that thiolacetic acid decomposes at the boiling point (93°) liberating sulfur and hydrogen sulfide. Moreover, direct treatment of XI with elementary sulfur led to an explosive reaction.

In the present work, no evidence of formation of the acetyl derivative of 6-mercaptomethylpurine (thio analog of 6-acetoxymethylpurine[14]) was obtained. This thio analog has recently been prepared by a different route.[16] It is unlikely that the hydrogen sulfide that is produced reduces the 1-N-oxide (XI) to 6-methylpurine (VI) which subsequently undergoes the thiation discussed above, since VI does not react with thiolacetic or diacetyl sulfide. It is more plausible that the 1-N-oxide function activates the purine nucleus, thus making it susceptible to attack by the thiolacetate ion.

The structure of 2-mercapto-6-methylpurine (XII) was established by X-ray crystallography by Cochran and Srinivasan.[17]

(11) *Cf.* E. Lellmann, *Ann.*, **221**, 9 (1883).
(12) B. Pullman, *J. Chem. Soc.*, 1621 (1959).
(13) V. Boekelheide and W. J. Lynn, *J. Am. Chem. Soc.*, **76**, 1286 (1954).
(14) M. S. Stevens, A. Giner-Sorolla, H. W. Smith, and G. B. Brown, *J. Org. Chem.*, **27**, 567 (1962).
(15) E. Ulrich, *Ann.*, **109**, 274 (1859).
(16) A. Giner-Sorolla, unpublished results.

SCHEME III



XIX, R = $NHNH_2$ XXI, R = Cl
XX, R = NHOH XXII, R = I

Direct introduction of sulfur into the methyl group of 6-methylpurine was not achieved by any of the reactions described. However, this was accomplished indirectly by treatment of purine-6-carboxaldehyde hydrazone (XVII),[18] and the corresponding oxime (XIV)[18] (Scheme III), with thiolacetic acid to give the thioaldehyde (XV) as an acetyl derivative. This reaction is analogous to the synthesis of N-acetylpurine-6-carboxaldehyde from its hydrazone with acetic anhydride.[16] Acetylpurine-6-thiocarboxaldehyde (XV) afforded the thiosemicarbazone (XVI) with thiosemicarbazide[18] and was oxidized with $KMnO_4$ to the known purine-6-carboxylic acid (XVIII).[18,19]

The acetylthioaldehyde (XV) was smoothly transformed to 6-methylpurine (VI) by treatment with Raney nickel; however, similar treatment of purine-6-carboxaldehyde does not lead to reduction.[16] Thioaldehyde (XV) was transformed into the hydrazone (XVII) by refluxing with aqueous hydrazine for 15 min., but further refluxing (3 hr.) effected reduction to 6-methylpurine (VI). This type of reduction by prolonged boiling with aqueous hydrazine solution has also been observed with 2-aminopurine-6-carboxaldehyde hydrazone.[16] The reaction is analogous to a Wolff–Kishner reduction[20] in which aqueous hydrazine is the alkaline catalyst.

Reaction of 6-hydrazinopurine (XIX)[21] with thiolacetic acid afforded 6-mercaptopurine[22] (XXIII) in 60% yield. Similar treatment of N-hydroxylaminopurine (XX)[23] gave lower yields of 6-mercaptopurine. 6-Chloropurine (XXI)[24] and iodopurine[25] (XXII) also

(17) W. Cochran, R. Srinivasan, and P. Tollin in "Crystallography and Crystal Perfection," G. N. Ramachandran, Ed., Academic Press, London, 1963, p. 67.
(18) A. Giner-Sorolla, I. Zimmerman, and A. Bendich, *J. Am. Chem. Soc.*, **81**, 2515 (1959).
(19) L. B. Mackay and G. H. Hitchings, *ibid.*, **78**, 3511 (1956).
(20) N. Kishner, *J. Russ. Phys. Chem. Soc.*, **43**, 582 (1911); L. Wolff, *Ann.*, **394**, 86 (1912).
(21) J. A. Montgomery and L. B. Holum, *J. Am. Chem. Soc.*, **79**, 2185 (1957).
(22) G. B. Elion and G. H. Hitchings, *ibid.*, **69**, 2138 (1947).
(23) A. Giner-Sorolla and A. Bendich, *ibid.*, **80**, 3932 (1958).
(24) A. Bendich, P. J. Russell, Jr., and J. J. Fox, *ibid.*, **76**, 6073 (1954).
(25) G. B. Elion and G. H. Hitchings, *ibid.*, **78**, 3508 (1956).

reacted with thiolacetic acid with formation of 6-mercaptopurine.

Attempts were made to extend the direct thiation reaction of purines as described here to the synthesis of selenopurines by using elementary selenium. However, selenium proved to be more reactive than sulfur, and extensive decomposition of the purine nucleus was noted.[26]

Experimental[27]

8-Mercaptopurine (II).—Finely powdered purine (I, 3.60 g., 0.03 mole) and sulfur (0.96 g., 0.03 mole) were thoroughly mixed and heated at 245° for 45 min. The initially pink melt turned brown and there was an evolution of hydrogen sulfide. The reaction mixture was cooled, pulverized, thoroughly washed with carbon disulfide, and dried. The resulting solid was thoroughly extracted with boiling water and filtered. A dark residue (0.35 g.) remained. From the chilled filtrate, 2.15 g. of yellow needles, m.p. 284–285° dec., were obtained; concentration of the mother liquors afforded a further 0.85 g. (over-all yield 75%). Further evaporation of the mother liquors yielded 0.35 g. of unreacted purine. After repeated recrystallization from 50% aqueous ethanol, pale yellow needles, m.p. 295–297° dec., were obtained which gave no depression of decomposition point when admixed with an authentic sample of 8-mercaptopurine.[8] The product was indistinguishable from 8-mercaptopurine as judged by ultraviolet and infrared spectroscopy and paper chromatography.

Anal. Calcd. for $C_5H_4N_4S$: C, 39.46; H, 2.65; N, 36.82; S, 21.06. Found: C, 39.51; H, 2.77; N, 36.94; S, 20.92.

2-Amino-8-mercaptopurine (IV).—Finely powdered 2-aminopurine (III, 0.35 g., 0.25 mmole) and sulfur (0.16 g., 0.5 mmole) were mixed and heated for 1 hr. at 250°. The reaction mixture was cooled, ground, washed with carbon disulfide, and dried. The resulting solid was thoroughly extracted with hot water, and 60 mg. (15%) of a colorless, crystalline product was obtained by sublimation at 10 mm. and 280°, m.p. 350°.

Anal. Calcd. for $C_5H_5N_5S$: C, 35.92; H, 3.01; N, 41.89; S, 19.18. Found: C, 36.20; H, 3.38; N, 42.27; S, 19.46.

This product was identical, in terms of ultraviolet spectra, mixture melting point, and chromatographic properties, with a specimen prepared from 2,4,5-triaminopyrimidine (V), carbon disulfide, and potassium hydroxide in pyridine according to the method of Robins, *et al.*[10]

8-Mercapto-6-methylpurine (VII). Method A.—A mixture of 6-methylpurine (VI, 3.0 g., 0.022 mole) and sulfur (1.2 g., 0.037 mole) was heated at 230–240° for 50 min. The reaction mixture was worked up as above and yielded 0.15 g. of a yellow product. After repeated recrystallization from water, microneedles were obtained, m.p. >350°.

Anal. Calcd. for $C_6H_6N_4S$: C, 43.37; H, 3.64; N, 33.73; S, 19.30. Found: C, 42.98; H, 3.29; N, 33.40; S, 19.06.

Method B.—A mixture of 4,5-diamino-6-methylpyrimidine (VIII, 0.50 g., 4 mmole) in pyridine (15 ml.) and carbon disulfide (0.92 ml.) containing NaOH (0.2 g., 5 mmole) was refluxed for 5 hr. with stirring. The reaction mixture was cooled, filtered, and washed with cold ether and afforded 0.62 g. (92%) of a white crystalline product, m.p. >350°.

Method C.—A suspension of 6-methylpurine 1-N-oxide (XI,[14] 0.50 g., 3.3 mmole) in thiolacetic acid (5 ml.) was refluxed for 12 hr., cooled, and poured into 25 ml. of ethanol. The resulting precipitate was collected, washed thoroughly with cold ethanol, and dried. A yield of 0.35 g. of light cream-colored needles was obtained (69%), m.p. >350°.

The ultraviolet spectra and chromatographic properties of these products were indistinguishable from those of an authentic sample of 8-mercapto-6-methylpurine (VII).[10]

2-Mercapto-6-methylpurine (XII). Method A.—A suspension of 6-methylpurine 1-N-oxide (XI, 1.0 g., 6.6 mmole) in thiolacetic acid (10 ml.) was refluxed for 4 hr., then kept at room temperature overnight. The solids were collected and washed thoroughly with ether to give 0.65 g. of a mixture of two products, m.p. >325°. The crude material was recrystallized from ethanol–water (1:1, v./v.) to yield 0.30 g. of a mixture of long slender needles and short prisms. A sample of this recrystallized material (100 mg.) was subjected to chromatography on a column of Whatman standard grade cellulose powder, using butanol saturated with water as eluent. Two components were obtained, one of which (38 mg.) was identified as 8-mercapto-6-methylpurine (VII) by its ultraviolet spectrum and chromatographic properties. The second component, colorless slender needles (20 mg.), was an isomer of VII. It was identified as 2-mercapto-6-methylpurine (XII) by X-ray crystallography.[17]

Anal. Calcd. for $C_6H_6N_4S$: C, 43.37; H, 3.64; N, 33.73; S, 19.30; Found: C, 43.44; H, 3.58; N, 33.62; S, 19.19.

2-Mercapto-6-methylpurine (XII) showed maxima (pH 14) at 246 and 316 mμ (A_M 13,700 and 3420) and (pH 9.27) at 259 mμ (A_M 11,050).

Method B.—A suspension of 6-methylpurine 1-N-oxide (XI, 3.0 g., 0.02 mole) in a solution of diacetyl sulfide[28] (b.p. 153–155°, 4.5 ml.) in dry dioxane (10 ml.) was refluxed with stirring. After 1 hr. the solid dissolved, and after 2 hr. a yellow precipitate appeared. The refluxing was continued for a total of 4 hr. The suspension was kept at room temperature overnight and then filtered, and the precipitate was washed thoroughly with ether. An additional 0.1 g. was obtained when the mother liquors were poured into ether; total yield of pale yellow needles was 0.45 g. (16%). Upon recrystallization from aqueous ethanol, needles were obtained, m.p. >320°. This product was identified as 2-mercapto-6-methylpurine (XII) by comparison of ultraviolet spectra and chromatographic properties. 8-Mercapto-6-methylpurine could be isolated from the mother liquors.

6-Methylpurine from Either 8-Mercapto-6-methylpurine (VII) or 2-Mercapto-6-methylpurine (XII).—Samples of each purine (VII and XII, 100 mg. each) were separately suspended in water (2 ml.) and Raney nickel (100 mg.) was added. After refluxing for 1 hr., the filtrates were evaporated *in vacuo* to yield, in each case, a product which was identified as 6-methylpurine (VI) by comparison of ultraviolet spectra and chromatographic properties.

Reaction of Benzimidazole with Sulfur.—A mixture of benzimidazole (1.18 g., 0.01 mole) and sulfur (0.32 g., 0.01 mole) was heated at 230–240°. The mixture melted and turned brown; after 10 min. a solid appeared. The mixture was heated an additional 35 min., then cooled and washed with carbon disulfide (3 times, 5 ml. each) affording 1.25 g. (83%) of a product, m.p. 288–290°. The mixture melting point with an authentic sample of 8-mercaptobenzimidazole (X) showed no depression.[11]

N-Acetylpurine-6-thiocarboxaldehyde (XV).[29] **Method A.**—Purine-6-carboxaldehyde hydrazone (XVII,[18] 0.48 g., 3 mmole) was suspended in thiolacetic acid (5 ml.). After refluxing for 1 hr., the solution was evaporated to dryness *in vacuo*, and the residue was thoroughly washed with ethanol. A yellow crystalline precipitate (0.19 g., 34%) was obtained which yielded orange needles upon repeated recrystallization from ethanol, m.p. 182–184°.

Anal. Calcd. for $C_8H_7N_4OS$: C, 46.36; H, 3.40; N, 27.03; S, 15.47. Found: C, 46.34; H, 3.42; N, 27.02; S, 15.27.

Ultraviolet Spectral Properties.—N-Acetylpurine-6-thiocarboxaldehyde (XV) showed (pH 7.65) λ_{max} 269 mμ (A_M 7300), λ_{min} 248 mμ (A_M 4530), λ_{sh} 233 mμ (A_M 5450); in 1 *N* HCl, λ_{max} 263 mμ (A_M 9250), λ_{min} 241 mμ (A_M 6200); in 1 *N* NaOH, λ_{max} 275 mμ (A_M 6750), λ_{min} 241 mμ (A_M 2500).

Method B.—Purine-6-carboxaldehyde hydrazone (XVII, 75 mg.) was suspended in diacetyl sulfide (0.40 ml.) and refluxed for 2 hr. and cooled. A tarry product was obtained which, by washing with ether, afforded 26 mg. of crude product with ultraviolet spectra and chromatographic properties identical with those of the material obtained by method A.

Method C.—A suspension of purine-6-carboxaldehyde oxime[18] (XIV, 96 mg.) in thiolacetic acid (2 ml.) was refluxed for 1 hr., cooled, and evaporated *in vacuo*; the crude residue was washed with ethanol to give 34 mg. of a material identical (in ultraviolet spectral properties and R_f values in several solvent systems) with the material prepared by methods A and B.

N-Acetylpurine-6-thiocarboxaldehyde (XV) was reduced to 6-methylpurine (VI) when refluxed with an aqueous suspension of

(26) *Cf.* H. G. Mautner, *J. Am. Chem. Soc.*, **78**, 5292 (1956).

(27) All melting points uncorrected. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich., and by the Microanalytical Laboratory of the University of Cambridge.

(28) Prepared according to S. H. Davies, *Ber.*, **24**, 3519 (1891).

(29) Although many aromatic thioaldehydes exist in the trimeric form; molecular weight determinations reveal that compound XV is monomeric [*cf.* E. Campaigne, *Chem. Rev.*, **39**, 1 (1946); E. E. Reid, "Organic Chemistry of Bivalent Sulfur," Vol. III, Chemical Publishing Co., New York, N. Y., 1960, p. 148].

Raney nickel. Compound XV gave the known[18] thiosemicarbazone (XVI) and hydrazone (XVII) upon refluxing with solutions of thiosemicarbazide and hydrazine, respectively. Oxidation of XV with $KMnO_4$ afforded purine-6-carboxylic acid (XVIII).[18,19]

6-Mercaptopurine from 6-Hydrazinopurine.—6-Hydrazinopurine (XIX)[21] (0.20 g., 1.3 mmole) was dissolved in thiolacetic acid (2 ml.) and refluxed for 12 hr. The reaction product was washed with ether, dried, dissolved in dilute ammonia, treated with charcoal, and filtered. Upon neutralization with dilute acetic acid, 0.12 g. (60%), light cream-colored crystals were obtained, m.p. 298–301°, dec. The resulting product was identical with an authentic sample of 6-mercaptopurine (XXIII)[22] in ultraviolet spectral and chromatographic properties.

6-Mercaptopurine was also obtained by similar treatment from 6-N-hydroxylaminopurine (XX)[23] in 30% yield, from 6-chloropurine (XXI)[24] and 6-iodopurine (XXII)[25] in almost quantitative yield.

Acknowledgment.—The authors wish to express their gratitude to Drs. R. Srinivasan and W. Cochran of the Cavendish Laboratory, University of Cambridge, for X-ray crystallographic studies of 2-mercapto-6-methylpurine. They also wish to thank Drs. George B. Brown, Peter S. Fitt, and D. M. Brown for valuable discussions and advice, and Mrs. S. Sirlin for assistance. One of us (A. G-S.) would like to express his gratitude to Professor A. R. Todd and Dr. D. M. Brown for the use of their laboratory.

Synthesis of a Renin Substrate

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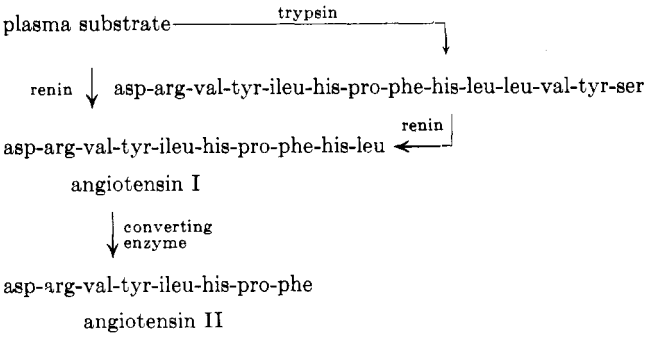
The synthesis of L-prolyl-L-phenylalanyl-L-histidyl-L-leucyl-L-leucyl-L-valyl-L-tyrosyl-L-serine, a substrate for the enzyme renin, is described. A significant improvement in the azide coupling procedure has been found.

A subject of intense study in recent years has been the renin–angiotensin system as it may be involved in normal homeostasis of blood pressure and in certain forms of hypertension.[1] One function of renin is to cleave a plasma substrate found in the α-globulin fraction to angiotensin I, a decapeptide.[2] The latter is then changed by a converting enzyme to the active hormone, angiotensin II, an octapeptide. Preliminary treatment of plasma substrate with trypsin gives a tetradecapeptide, asp-arg-val-tyr-ileu-his-pro-phe-his-leu-leu-val-tyr-ser,[3,4] which reacts with renin to liberate angiotensin I. The structure of the tetradecapeptide was confirmed by synthesis.[5] The anomalous reaction of trypsin to produce a peptide with C-terminal serine has never been explained. Chart I shows the

CHART I



sequence of reactions. Recent evidence indicates that the renin–angiotensin system may also control aldosterone synthesis.[6] If this is true, renin becomes an even more important enzyme than previously thought.

The present work stems from a program to find inhibitors of renin. The search for a renin inhibitor represents one rational approach to the discovery of antihypertensive drugs and possibly also to new types of antialdosterone compounds.[7,8] It is easily seen that an effective and selective renin inhibitor could have profound blood pressure effects. For purposes of reproducibility and control of variables, it was decided that an *in vitro* assay would be desirable and that a synthetic substrate would be preferable to one isolated from blood.[9] For a successful screening test, the substrate must be available in reasonable quantity and equally important its synthesis must permit repetition as new supplies are needed without each occasion requiring a major research effort.

Reports have appeared concerning a renin substrate, pro-phe-his-leu-leu-val-tyr-ser (**11**), which represents the C-terminal octapeptide from the tryptic tetradecapeptide.[10] Both substrates are cleaved by renin at the same position, at the leu–leu bond, although in the case of the octapeptide, the product, pro-phe-his-leu, has no pressor activity. Because of the same position of enzymic hydrolysis in the two peptides, it is reasonable to hope that renin inhibitors found by utilizing the octapeptide substrate might be active in intact animals. The octapeptide was first synthesized by coupling Z-pro-phe and his-leu-leu-val-tyr-ser-OMe with subsequent re-

(1) I. H. Page and F. M. Bumpus, *Physiol. Rev.*, **41**, 331 (1961).

(2) A. S. Plentl, I. H. Page, and W. W. Davis, *J. Biol. Chem.*, **147**, 143 (1943); A. S. Green and F. M. Bumpus, *ibid.*, **210**, 281 (1954).

(3) Abbreviations are based on the proposals of E. Brand and J. T. Edsall, *Ann. Rev. Biochem.*, **16**, 223 (1947). In addition, Z = carbobenzoxy, OMe = methyl ester, ONB = *p*-nitrobenzyl ester, and ONP = *p*-nitrophenyl ester.

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