TABLE V

TRIARYLCYCLOPROPANE DERIVATIVES

$(C_6H_5)_2C$———CHC_6H_5
\ /
CHR

Compd.	R	B.p. (mm.) or m.p., °C.	Calcd., % C	Calcd., % H	Found. % C	Found. % H
XXV[a]	—COOEt	94–95				
XXVI[a]	—COOH	202–203				
XXVII	H	166–168 (0.2)	93.4	6.6	93.6	6.8

[a] H. Staudinger, E. Anthes, and F. Pfenninger, *Ber.*, **49**, 1928 (1916).

even its *p*-fluorophenyl isomer VIII was inactive. We have no explanation for this exceptional behavior.

Experimental[4]

General Method.—Many of the compounds reported in this study were prepared by means of a Grignard reaction according to the following procedure. To a Grignard reagent made by the interaction of slightly more than 1 g.-atom of Mg with slightly more than 1 equiv. of the appropriate benzyl halide in ether was added an ether solution of 1 equiv. of the appropriate ketone. The mixture was refluxed for 1–2 hr. and was finally cooled and treated with a slight excess of water or methanol. The reaction mixture was then extracted with ether, the ether solution was dried, and the ether was removed to give the desired alcohol. Some of the alcohols so prepared could be purified by distillation. Others lost a molecule of water when distilled either alone or with a few drops of sulfuric acid and gave the corresponding unsaturated derivatives. Some of the pertinent details are given in Table VI.

Triarylcyclopropane Derivatives (Table V). **1,1,2-Triphenylcyclopropane (XXVII).**—Six grams of 2,2,3-triphenylcyclopropanecarboxylic acid prepared according to the method of Staudinger[5] was heated above 290° until the evolution of CO_2 ceased. The reaction mixture was then distilled and the fraction boiling at 160–180° (0.2 mm.) was collected. Upon redistillation, the product was obtained in the form of a soft yellow glass boiling at 166–168° (0.2 mm.).

Miscellaneous. 4-Chloro-N-(4-chlorophenylphenylmethylene)aniline (XXIX).—A mixture of 22 g. of *p*-chlorobenzophenone, 25.6 g. of *p*-chloroaniline, and 5 drops of concentrated HCl was heated to 250° for about 20 min. The melt was cooled, extracted with hot benzene, and the benzene solution was filtered to remove an insoluble crystalline material varying in color from dark brown to dark blue. The benzene was removed and the residue was distilled twice to yield a viscous yellow oil, b.p. 190–192° (0.2 mm.).

Anal. Calcd. for $C_{19}H_{13}Cl_2N$: C, 70.0; H, 4.0; Cl, 21.8. Found: C, 70.2; H, 4.0; Cl, 21.5.

TABLE VI

Ketone	Reactants: Benzyl chloride	Yield of alcohol, %	$n^{25}D$	Yield of unsatd. compd., %	$n^{25}D$
Cyclopropylphenyl[a]	*p*-F	1			
Cyclobutylphenyl[b]	*o*-F	47 II	1.5618	60[c] V	
	p-F	47 III	1.5568	60[c] IV	
Cyclopentylphenyl[d]	*p*-F			48 VI	1.5709[e]
	o-F			50 VII	1.5766
Cyclohexylphenyl[d]	*p*-F			68 VIII	1.5760
	o-F			82 IX	1.5773
Fluorenone	*p*-F	31 XII		69[c] XVI	
10,11-Dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-one[f]	*p*-Cl		XIII		
	p-F	71 XIV		87[c] XX	
	o-F			73 XXI	
5*H*-Dibenzo[*a*,*d*]-hepten-5-one[g]	*p*-F	49 XV			
	p-F			93[c] XXIII	
	o-F			XXIV	

[a] M. Kishner, *Chem. Zentr.*, **83**, 1458 (1912). [b] D. E. Applequist and D. E. McGreer, *J. Am. Chem. Soc.*, **82**, 1965 (1960). [c] Yield obtained from the alcohol. [d] D. H. Hey and O. C. Musgrave, *J. Chem. Soc.*, 3156 (1949). [e] At 28°. [f] A. C. Cope and S. W. Fenton, *J. Am. Chem. Soc.*, **73**, 1676 (1951). [g] W. Treibs and H. J. Klinkhammer, *Ber.*, **84**, 671 (1951).

(4) All melting points are corrected; the boiling points are uncorrected.

(5) See Table V, footnote *a*.

Pyrimidine Derivatives. VI. 2,4,5-Triamino-6-chloro- and -6-mercaptopyrimidine and Related Compounds[1,2]

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The preparation of the title compounds has been accomplished from acyclic starting materials *via* a sequence of steps involving synthesis and reduction of the corresponding 5-phenylazo derivatives. As an alternate approach, nitrosation of two pyrimidine intermediates was attempted. Treatment of 2,4-diamino-6-chloropyrimidine with nitrous acid produced a multicomponent mixture which, by means of exhaustive paper chromatographic analysis, was found to consist of 4-amino-6-chloro-2-hydroxypyrimidine, 2-amino-6-chloro-4-hydroxypyrimidine, 4-amino-2,6-dihydroxypyrimidine, and a trace of unreacted starting material. Attempted nitrosation of 2,4-diamino-6-mercaptopyrimidine, however, resulted only in the oxidation of the sulfur with consequent formation of bis(2,4-diamino-6-pyrimidyl) disulfide. In the course of this work several previously unreported pyrimidines have been prepared. Quantitative ultraviolet absorption spectra are given for all compounds prepared, as well as a summary of growth-inhibitory properties in selected *in vitro* and *in vivo* bioassay systems.

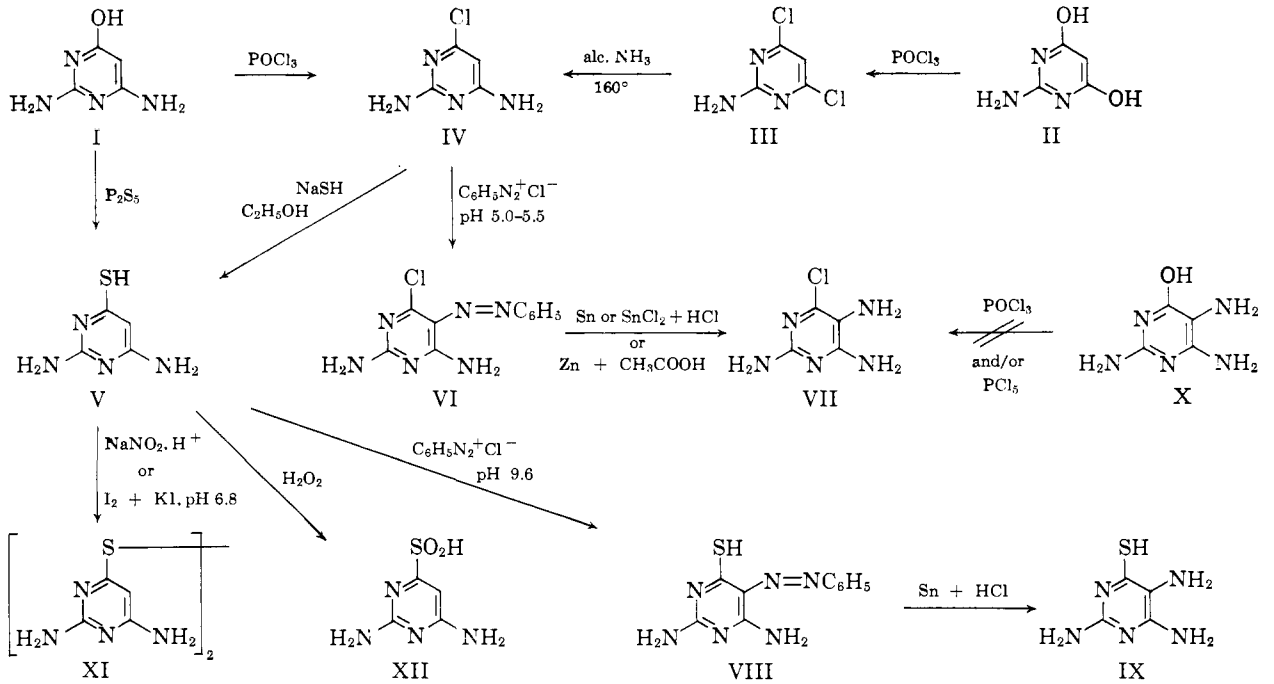
As part of a continuing program of cancer chemotherapy, a number of pyrimidine derivatives have been synthesized in these laboratories for biological evaluation and as precursors of condensed pyrimidine ring systems. We recently reported the synthesis of various substituted pyrimidines using 4-amino-6-chloro-2-methylthiopyrimidine as a versatile starting material.[2] We should like now to describe the preparation of the title compounds, as well as the synthesis and chemistry of some related substituted pyrimidines.

The key intermediate utilized in this investigation was 2,4-diamino-6-chloropyrimidine (IV). This com-

(1) This investigation was supported in part by research grants (CY3335 and C6516) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) For paper V in this series see M. Israel, H. K. Protopapa, H. N. Schlein, and E. J. Modest, *J. Med. Chem.*, **7**, 5 (1964).

SCHEME I



pound was prepared by two different approaches, one involving chlorination of a diaminohydroxypyrimidine, the other requiring amination of an aminodichloro compound. For the first route, 2,4-diamino-6-hydroxypyrimidine (I) was prepared by condensation of guanidine with ethyl cyanoacetate. When the usual Traube conditions[3,4] were followed, I was obtained in 45% yield along with an 8–12% yield of cyanoacetylguanidine. The latter compound, originally reported by Traube, is formed by reaction of the ester group of ethyl cyanoacetate as evidenced by nitrile absorption in the infrared. The total yield of I could be increased to 58–64% by conversion of the uncyclized by-product into I in refluxing 1 *N* sodium hydroxide. However, the preparation of I based upon the procedure of VanAllan,[5] further modified by precipitation of the product at pH 8,[6] was found to be superior to the Traube method and afforded I in 80–85% yield. Chlorination of I with phosphorus oxychloride (Scheme I) by modification of the procedure of Roth, *et al.*,[7] afforded IV in 62–71% yield.[6]

The alternate route to IV, *via* monoamination of 2-amino-4,6-dichloropyrimidine (III), involved first the preparation of 2-amino-4,6-dihydroxypyrimidine (II) from guanidine and diethyl malonate according to the method of Traube.[4] Refluxing II with phosphorus oxychloride in the absence of dimethylaniline[8] led to 2-amino-4,6-dichloropyrimidine (III) in 50% yield after purification by either recrystallization or sublimation. III was then monoaminated with ethanolic ammonia at 160° following the procedure of Büttner.[9] This route afforded IV in 20% over-all yield in three steps from acyclic materials, whereas the first described sequence led to a 57% over-all yield of IV in two steps and obviated the pressure reaction.

Refluxing IV with an excess of sodium hydrosulfide in 95% ethanol for 1 week afforded 2,4-diamino-6-mercaptopyrimidine (V), which was isolated as the sulfate salt in 74% yield. The product was purified by reprecipitation from an alkaline solution by the addition of sulfuric acid. Control of pH is important: at pH 5–6 the half-sulfate is obtained, below pH 4 the full sulfate is formed, and at pH 4–5 a mixture of sulfate and half-sulfate precipitates with the latter predominating. The free base of V was prepared by dissolving the half-sulfate in hot 1 *N* sodium hydroxide and adjusting the pH to 9.2 with 50% acetic acid. While this work was in progress, V free base was reported by Elion, *et al.*,[10] *via* a sealed-bomb reaction of IV with potassium hydrosulfide. More recently, V sulfate was obtained by reaction of IV with sodium hydrosulfide in ethylene glycol at 140–150°.[11] We have also prepared V in 20% yield by direct thiation[12] of I utilizing a highly reactive grade of phosphorus pentasulfide[13]; earlier attempts to thiate I to V by means of the usual commercial grade of phosphorus pentasulfide under various conditions proved unsuccessful.

The title compounds VII and IX were prepared by reductive cleavage of the corresponding 5-phenylazo

(3) D. J. Brown, "The Pyrimidines," Interscience Publishers, Inc., New York, N. Y., 1962, p. 71.

(4) W. Traube, *Ber.*, **33**, 1371 (1900).

(5) J. A. VanAllan, "Organic Syntheses," Coll. Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1963, p. 245.

(6) For successful and reproducible chlorination it is of critical importance to use I free base that is completely free of traces of acid salt. Material prepared according to the modified VanAllan procedure described herein, with precipitation at pH 8 and vacuum drying at 50° for 17 hr., is suitable for chlorination.

(7) B. Roth, J. M. Smith, Jr., and M. E. Hulquist, *J. Am. Chem. Soc.*, **72**, 1914 (1950).

(8) D. J. Brown, "The Pyrimidines," Interscience Publishers, Inc., New York, N. Y., 1962, p. 164.

(9) E. Büttner, *Ber.*, **36**, 2227 (1903).

(10) G. B. Elion, W. H. Lange, and G. H. Hitchings, *J. Am. Chem. Soc.*, **78**, 2858 (1956).

(11) G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel, and A. G. Beaman, *ibid.*, **82**, 2633 (1960).

(12) The direct thiation of I and various other 4-hydroxypyrimidines has been presented (E. J. Modest, S. Chatterjee, S. A. Lemlein, and D. M. Brun, Abstracts of Papers, 138th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1960, p. 4-O).

(13) The authors are indebted to Dr. Theodor Reetz and Dr. John W. Cross of the Monsanto Chemical Company, St. Louis, Mo., for generous samples of this reactive grade of phosphorus pentasulfide.

compounds VI and VIII, respectively. VI was obtained from the reaction of IV with benzenediazonium chloride in a sodium acetate–acetic acid buffer at pH 5–5.5.[14] However, the coupling of V with benzenediazonium chloride required specific alkaline conditions (pH 9.6 ammonium hydroxide–ammonium chloride buffer) for optimal quality of product.

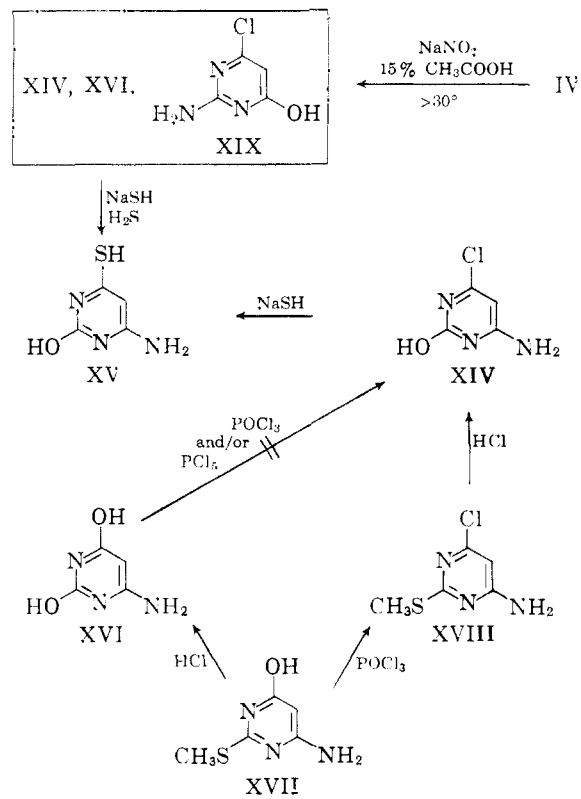
Analytical samples of VI and VIII were obtained with great difficulty; purification proved extremely troublesome and involved considerable loss in yield. Such losses had to be suffered, however, especially in the case of VI, for reduction of impure azo compound invariably led to black amorphous solids possessing no pyrimidine character in the ultraviolet. Reduction of highly purified VI by means of tin and hydrochloric acid, stannous chloride and hydrochloric acid, or zinc and acetic acid afforded 2,4,5-triamino-6-chloropyrimidine (VII)[15] in yields of 17–26%, the low yields probably resulting from the ease of hydrolysis of the 6-chloro substituent. VII could not be prepared directly from 2,4,5-triamino-6-hydroxypyrimidine (X) by chlorination with phosphorus oxychloride and/or phosphorus pentachloride.

The mercaptotriamine IX, isolated as the dihydrochloride salt, was obtained by tin and hydrochloric acid reduction of purified VIII. This compound has been reported previously by Elion, *et al.*,[10] *via* an analogous sequence of reactions involving the formation and reduction with zinc and hydrochloric acid of the corresponding 5-*p*-chlorophenylazo compound.

In connection with the synthesis of these triamines, the possibility of preparing the 5-nitroso derivatives of IV and V was first investigated. It was hoped that these compounds might then be reduced easily to VII and IX, respectively. The factors governing the ease of nitrosation of pyrimidines have been generalized.[16] With sodium nitrite and acetic acid, the introduction of a nitroso group requires that electron-releasing groups (such as NH_2, OH, SH, SCH_3) be present at the 2-, 4-, and 6-positions; without activation at position 2, mineral acid must be used in place of acetic acid to promote the nitrosation. On the basis of this empirical rule, it was expected that V would undergo facile nitrosation but that IV would be nitrosated with difficulty, if at all.

Below 20° sodium nitrite failed to produce any significant reaction with IV in either mineral acid or 15% aqueous acetic acid. However, above 30° in aqueous acetic acid, a reaction was achieved; a sandy brown solid, possessing none of the physical or chemical characteristics expected for a nitroso compound, began to precipitate midway through the reaction. This product contained a considerable quantity of 4-amino-6-chloro-2-hydroxypyrimidine (XIV), as indicated by the isolation in 56% yield of 4-amino-2-hydroxy-6-mercaptopyrimidine (XV) when the crude material was treated with sodium hydrosulfide in water in the presence of hydrogen sulfide gas (Scheme II). Identification of XV was made by microchemical analysis and by comparison of melting and mixture melting points, ultraviolet absorption spectra, and paper chromatographic behavior with an authentic sample which had been prepared by direct thiation of 4-amino-6-chloro-2-hydroxypyrimidine (XIV).[2] An alternate synthesis of XV *via* chlorination of 4-amino-2,6-dihydroxypyrimidine (XVI) was attempted. However, neither mono- nor dichlorination was observed with phosphorus oxychloride and/or phosphorus pentachloride under a variety of conditions.

SCHEME II



The presence of XIV in the reaction mixture of IV and nitrous acid was also demonstrated by paper chromatographic analysis. Descending paper chromatography of samples of the reaction product, run in a 4:1:1 1-butanol–acetic acid–water solvent system with adenine used as an internal standard, afforded three ultraviolet absorbing spots, one of which showed the same R_{Ad} value (1.15) as authentic XIV. Streaked chromatograms were then run and the strip corresponding to R_{Ad} 1.15 was cut from the paper and eluted with methanol. The ultraviolet absorption spectra of the material obtained on evaporation of the eluate matched those of authentic XIV in methanol, pH 1, and pH 10 buffer solutions. The 56% yield of XV on thiation of the crude product from the reaction of nitrous acid with IV indicates that XIV is the major component of the reaction mixture and, therefore, that the preferred site of attack of nitrous acid on IV is at the 2-amino group. However, since we have previously shown that nitrous acid converts 4-amino-6-chloropyrimidine into 6-chloro-4-hydroxypyrimidine,[2] it was

(14) A detailed study has been made in these laboratories of the pH conditions for optimal diazo coupling with substituted pyrimidines (H. N. Schlein, G. E. Foley, and E. J. Modest, Abstracts of Papers, 131st National Meeting of the American Chemical Society, Miami, Fla., April, 1957, p. 3-N).

(15) VII has recently been prepared by reduction of the corresponding 5-*p*-chlorophenylazo derivative [Y. F. Shealy, J. D. Clayton, C. A. O'Dell, and J. A. Montgomery, *J. Org. Chem.*, **27**, 4518 (1962)]. Prior to his publication and when our work was nearly complete, Dr. Montgomery kindly made available to us a sample of his material (m.p. 227° dec.), which proved to be identical with our own.

(16) B. Lythgoe, A. R. Todd, and A. Topham, *J. Chem. Soc.*, 315 (1944); B. Lythgoe, *Quart. Rev.* (London), **3**, 205 (1949).

considered that attack at the 4-amino group of IV would also be likely to occur. The identification of the other components of the reaction mixture was attempted by comparison of R_{Ad} values and ultraviolet spectra of eluted material with authentic samples of the various replacement and hydrolysis products of IV (Table I). In this way, XVI was shown also to be

TABLE I

R_{Ad} VALUES AND ULTRAVIOLET ABSORPTION MAXIMA IN METHANOL FOR 2,4-DIAMINO-6-CHLOROPYRIMIDINE AND ITS HYDROLYSIS AND/OR REPLACEMENT PRODUCTS

	Pyrimidine	R_{Ad}[a]	$\lambda_{max}^{CH_3OH}$, mμ
I	2,4-Diamino-6-hydroxy	0.57	268
II	2-Amino-4,6-dihydroxy	0.41	257
IV	2,4-Diamino-6-chloro	1.54	283
XIV	4-Amino-6-chloro-2-hydroxy	1.15	283
XVI	4-Amino-2,6-dihydroxy	0.59	263
XIX	2-Amino-6-chloro-4-hydroxy	1.50	226, 288
	Barbituric acid	0.56	256
	6-Chlorouracil	1.36	261

[a] Descending paper chromatography at 24° with a 4:1:1 1-butanol–acetic acid–water solvent system for 17 hr. with adenine used as an internal standard. Spot locations are expressed as R_{Ad} units with adenine at 1.00.

present (R_{Ad} 0.59). Elution of the uppermost absorbing streak (R_{Ad} 1.51) afforded material, the ultraviolet spectrum of which did not permit differentiation between IV and 2-amino-6-chloro-4-hydroxypyrimidine (XIX),[17] both of which exhibit R_{Ad} values of approximately 1.51 in 4:1:1 1-butanol–acetic acid–water. A solvent system (1-butanol–ammonia–water, 100:2:16) was found that afforded a sharp separation of IV and XIX (R_{Ad} 1.99 and 0.90, respectively). Samples of the IV–nitrous acid reaction mixture run in this weakly alkaline solvent system clearly showed the presence of both XIX and a small amount of unreacted IV. These results indicate that nitrous acid attacks IV at both the 2- and 4-amino groups and presumably that one of the products, XIV, is susceptible to hydrolytic replacement of the chlorine. No trace of any of several other possible hydrolysis and/or replacement products, *e.g.*, 6-chlorouracil,[18] 2-amino-4,6-dihydroxypyrimidine (II), or barbituric acid, could be detected.

Attempts to prepare 2,4-diamino-6-mercapto-5-nitrosopyrimidine by direct nitrosation of V with nitrous acid or isoamyl nitrite were also unrewarding, although the corresponding methylthiopyrimidine is nitrosated smoothly in 88% yield.[19] Despite the fact that the substitution pattern in V allows for facile nitrosation,[16] the product was always a yellow-tan solid which showed a hypsochromic shift of 21.5 mμ (from λ_{max} 322.5 to 301 mμ) in the ultraviolet at pH 1. The product caused a gradual, rather than instantaneous, decolorization of Feigl's iodine–azide reagent[2,20] and, on reduction, returned starting material V in high yield; both properties are characteristic of a disulfide linkage. The structure was conclusively established as bis-(2,4-diamino-6-pyrimidyl) disulfide (XI) by comparison with an authentic sample prepared by oxidation of V by iodine in potassium iodide solution at pH 6.8.[21] In contrast to the reaction of IV, paper chromatographic analysis of the reaction mixture of V and nitrous acid failed to show any indication of possible replacement and/or hydrolysis products of V.

The ease of oxidation of the sulfur in V was further demonstrated by the formation of 2,4-diaminopyrimidine-6-sulfinic acid (XII) on treatment of the mercapto compound with hydrogen peroxide (30%) at room temperature, this being completely analogous to the case of the 2-unsubstituted compound.[2] This product showed the properties of an internal salt, being soluble at pH 1 and pH 10 but relatively insoluble in distilled water.

Quantitative ultraviolet absorption spectra of compounds prepared during the course of this investigation have been determined and are given in Table II.

TABLE II

ULTRAVIOLET ABSORPTION SPECTRA

	pH 1		pH 10	
Compd.	λ_{max}, mμ	$\epsilon \times 10^{-3}$	λ_{max}, mμ	$\epsilon \times 10^{-3}$
I	265	20.07	267	13.91
II	257	11.18	261	13.22
III	233	15.25	233	14.83
	298	5.75	299	5.59
IV	*a*		229[b]	9.33
			282	7.76
V[c]	244	9.83	226	17.40
	322.5	31.02	304	19.68
VI	358	29.85	233	13.52
			290[b]	7.54
			364	21.11
VII[d]	228	12.68	236[e]	
	305.5	5.15	302	5.67
VIII[f]	*g*		*g*	
IX[h]	225[b]	19.36	224	15.17
	310	27.37	325	13.72
XI[i]	214	32.55		
	230	33.15	*g*	
	301	18.08		
XII	272.5	6.68	229	11.42
			292	7.11
XVI	264	17.21	263	16.85

[a] pH 2: 227 (9.55), 299 (7.43). [b] Inflection. [c] Lit.[10] pH 1: 242 (8.80), 322 (23.80); pH 11: 235 (15.30, infl.), 297 (16.60). Lit.[11] pH 1: 244 (8.10), 320 (28.10); pH 11: 237 (16.50), 298 (17.50). [d] Lit.[15] pH 1: 229 (13.3), 306 (5.6); pH 13: 236 sh, 304 (6.2). [e] Shoulder. [f] Dimethylacetamide: 286 (13.54), 393 (26.20). [g] Insoluble. [h] Lit.[10] pH 1: 310 (21.80); pH 11: 240 (13.60). 320 (12.10). [i] Methanol: 287 (13.87).

The spectrum of the disulfide XI displays a similar behavior to that of its 2-unsubstituted analog; both are insoluble at pH 10 and show hypsochromic shifts in pH 1 solution for long and short wave peaks as compared to the thiol compounds from which they are derived. For the long wave peak, this shift amounts to 23 mμ for both compounds. Koppel, *et al.*,[22] have

(17) We are grateful to Dr. C. C. Cheng and Mr. Fred Baiocchi of the Midwest Research Institute, Kansas City, Mo., for the purified sample of XIX used for comparison in this work.

(18) The comparison sample of 6-chlorouracil was supplied through the courtesy of Dr. R. B. Ross of the Cancer Chemotherapy National Service Center.

(19) E. J. Modest, H. Kangur, H. N. Schlein, and S. P. Bhattacharya, Abstracts of Papers, 131st National Meeting of the American Chemical Society, Miami, Fla., April, 1957, p. 4-N.

(20) F. Feigl, "Spot Tests in Organic Analysis," 5th English Ed., Elsevier Publishing Co., Amsterdam, 1956, p. 228.

(21) These reaction conditions are essentially those described by W. H. Miller, R. O. Roblin, Jr., and E. B. Astwood [*J. Am. Chem. Soc.*, **67**, 2201 (1945)] and were used more recently to prepare thymidine disulfide and 4-thiouridine disulfide [J. J. Fox, *et al.*, *ibid.*, **81**, 178 (1959)].

(22) H. C. Koppel, R. H. Springer, R. K. Robins, and C. C. Cheng, *J. Org. Chem.*, **26**, 792 (1961).

generalized that the major absorption maximum of a 4-mercaptopyrimidine, appearing in the range 300–320 mμ at pH 1, undergoes a hypsochromic shift from pH 1 to pH 10. The behavior of IX disagrees with this generalization in that the absorption maximum at 312 mμ at pH 1 undergoes a bathochromic shift to 325 mμ when the spectrum of a freshly prepared solution in pH 10 buffer is immediately determined. This finding indicates that Koppel's generalization does not extend to 5-substituted mercaptopyrimidines.

Biological Activity.—The twelve pyrimidine derivatives listed in Table II have been examined in various *in vitro* and *in vivo* bioassay systems at the Children's Cancer Research Foundation.[23] All except VI and VII have been evaluated in the *Streptococcus faecalis* No. 8043–folic acid bioassay system in the presence of 0.001 γ/ml. of folic acid.[24] Six compounds (II, III, V, VIII, IX, and XII) were found to be active at a 50% inhibiting dose (ID_{50}) of less than 100 γ/ml. (ID_{50} = 60, 30, 27, 3, 50, and 80 γ/ml., respectively). Further details of the antimetabolite activity of 2,4-diamino-6-mercapto-5-phenylazopyrimidine (VIII) and reversal of inhibition in microbiological systems have been reported.[25] Of four compounds (VII, VIII, IX, and XII) investigated for activity against KB cells (human epidermoid carcinoma) in culture,[26] the sulfinic acid XII was active at an ID_{50} of 66 γ/ml. *In vivo* activity has been evaluated in one or more transplantable mouse tumor systems according to the standard procedures employed in this foundation.[27] Compounds II, V, VI, and XVI exhibited slight to moderate tumor inhibition in the C1498 tumor system when administered intraperitoneally at nontoxic dosages. I, II, and XVI also demonstrated slight tumor inhibition *vs.* P1534 lymphatic leukemia.

We are indebted to Dr. George E. Foley for the determination of the biological activity of these compounds in selected bacterial and mammalian cell culture systems and to Dr. Charlotte L. Maddock and Dr. Sidney Farber for the biological data against transplantable mouse tumors.

Experimental[28]

Ultraviolet absorption spectra were measured at pH 1 (0.1 *N* HCl) and at pH 10 (0.05 *M* sodium carbonate–sodium borate buffer) with a Cary Model 11 spectrophotometer. Infrared spectra were determined with a Perkin-Elmer Model 137B spectrophotometer by the KBr disk technique. Paper chromatograms were run on Whatman No. 1 paper for 17 hr. at room temperature and examined on a viewing box equipped with a 15-w. General Electric germicidal lamp and a Corning filter, No. 9863. Descending chromatography was carried out in a Research Specialties Co. Chromatocab; ascending chromatography was performed in a specially constructed constant-temperature cabinet. Melting points were taken by the capillary method at a rate of heating of 2°/min. in a modified Wagner-Meyer melting point apparatus.[29] Decomposition points are not reproducible unless conditions are rigidly controlled. If not otherwise specified, drying of analytical samples was carried out at 70–100° for 17 hr. *in vacuo* over phosphorus pentoxide.

2,4-Diamino-6-hydroxypyrimidine (I).—According to the Traube procedure,[3] an alcoholic solution of guanidine (free base, prepared in absolute ethanol from guanidine hydrochloride and an equimolar amount of sodium) was allowed to stand with ethyl cyanoacetate overnight. Insoluble by-product, cyanoacetylguanidine (8–12% yield, nitrile absorption at λ_{max}^{KBr} 4.43 μ), was separated by suction filtration. The alcoholic filtrate was evaporated and the residue was taken up in dilute alkali. The product was precipitated by partial neutralization of the alkaline solution to pH 8. The yield was 45%, m.p. 280–285° dec. Recovered cyanoacetylguanidine was converted into I in 60% yield on refluxing with 1 *N* NaOH for 30 min., the product being isolated by partial neutralization of the solution to pH 8, m.p. 280–287° dec. More product could be obtained from the pH 8 mother liquors by reducing the pH to 2 with sulfuric acid. The half-sulfate salt of I precipitated in 14–20% yield and was converted into the free base by dissolution at pH 12, followed by partial neutralization to pH 8 (60% recovery, m.p. 278–283° dec.).

In the VanAllan modification,[3] a 1 *M* run was refluxed for 2 hr. and left at room temperature overnight. The reaction mixture was evaporated to dryness and the residue was dissolved in hot water. The aqueous solution was treated with 5 g. of Darco[30] and filtered and the product was precipitated at pH 8.0 (pH meter) at *ca.* 40° (required about 50 ml. of glacial acetic acid). After overnight refrigeration, the solid was collected and dried *in vacuo* at 50° for 17 hr.; off-white prismatic rods, 80–85% yield, m.p. 285–290° dec. (heating rate 5°/min.) (lit.[5] m.p. 280–283°). This material can be used directly for chlorination.

2-Amino-4,6-dichloropyrimidine (III).—2-Amino-4,6-dihydroxypyrimidine (II), prepared in 62% yield by the method of Traube[3] from guanidine and diethyl malonate in refluxing ethanol, was chlorinated in 50% yield in refluxing phosphorus oxychloride.[8] The product was purified by either crystallization from benzene or high vacuum sublimation at 200°, m.p. 220–221° (lit.[9] m.p. 221°).

2,4-Diamino-6-chloropyrimidine (IV). A. By Chlorination of I.—This procedure is a modification of that described by Roth, *et al.*[3] To 50.5 g. (0.4 mole) of 2,4-diamino-6-hydroxypyrimidine (free base)[5] was added with stirring 240 ml. of phosphorus oxychloride. After 2–3 hr. at reflux, *ca.* 25% of the phosphorus oxychloride was removed by vacuum distillation from the deep red-brown solution. The residual brown syrup was poured while still warm over 400 g. of crushed ice in a 2-l. beaker and the resulting strongly acidic solution (*ca.* 600 ml. including water rinses) was neutralized to pH 8.5–9.0 (pH meter) by the addition of concentrated ammonium hydroxide (required *ca.* 600 ml.). After overnight refrigeration the solid was separated by suction filtration, sucked dry on the funnel without washing, and finally dried in an oven at 60°. The dry powder containing product and inorganic salts was extracted five times with boiling acetone (1–5 ml./g. of solids); evaporation of the acetone left 36–41 g. (62–71%) of off-white product melting at 197–201°. For purification, this material was ground in a mortar under concentrated ammonium hydroxide (1.5 ml./g.), transferred to a suction funnel with additional ammonia, washed once with ammonia and once with cold water, and dried. The recovery was 88–90% of white product, m.p. 199–201°. This product is suitable for most purposes. Analytically pure colorless prisms, m.p. 200–201° (lit. m.p. 198°,[3] 197–200°[3]) were obtained by recrystallization from acetone or 10% ethanol, or by high vacuum sublimation at 120–140°.

B. By Monoamination of III.—A stainless steel bomb, charged with 5 g. (0.031 mole) of 2-amino-4,6-dichloropyrimidine and 50 ml. of absolute ethanol saturated with ammonia at 0°, was heated

(23) Of the other pyrimidine compounds discussed in this report, bioassay data pertaining to compounds XIV, XV, XVII, and XVIII have been summarized in an earlier communication.[2]

(24) G. E. Foley, R. E. McCarthy, V. M. Binns, E. E. Snell, M. Guirard, G. W. Kidder, V. C. Dewey, and P. S. Thayer, *Ann. N. Y. Acad. Sci.*, **76**, 413 (1958).

(25) E. J. Modest, H. N. Schlein, and G. E. Foley, *J. Pharm. Pharmacol.*, **9**, 68 (1957).

(26) G. E. Foley and H. Eagle, *Cancer Res.*, **18**, 1012 (1958); H. Eagle and G. E. Foley, *ibid.*, **18**, 1017 (1958).

(27) The four-tumor screen includes the following transplantable mouse tumors: L1210 ascitic lymphatic leukemia in the DBF/1 hybrid, P1534 lymphatic leukemia in the DBA/2 inbred, C1498 myelogenous leukemia in the C57BL/6 inbred, and DBRB mammary carcinoma in the DBA/1 inbred strain. The standard assay procedures have been described [C. L. Maddock, G. J. D'Angio, S. Farber, and A. H. Handler, *Ann. N. Y. Acad. Sci.*, **89**, 386 (1960)].

(28) Analyses were performed by the Scandinavian Microanalytical Laboratory, Herlev, Denmark, and Dr. Carol K. Fitz, Needham Heights, Mass.

(29) E. C. Wagner and J. F. Meyer, *Ind. Eng. Chem., Anal. Ed.*, **10**, 584 (1938).

(30) Darco G-60 activated carbon, Atlas Chemical Industries, Inc., Wilmington, Del.

at 160° for 3 hr., following the procedure of Büttner.[9] The ethanol solution was evaporated to dryness and the residue was crystallized from water, yielding 2.73 g. (62%) of IV as prisms, m.p. 200°. A mixture melting point determination of this product with material prepared according to procedure A showed no depression.

2,4-Diamino-6-mercaptopyrimidine (V). A. From IV.—A suspension of 33.2 g. (0.23 mole) of 2,4-diamino-6-chloropyrimidine and 127 g. (1.15 moles, 5 *M* excess) of sodium hydrosulfide trihydrate in 500 ml. of 95% ethanol was refluxed and stirred for 7 days. The hot suspension was filtered from residual sodium chloride and evaporated to dryness *in vacuo*. The brown sticky residue was dissolved in 250 ml. of water and filtered free of unreacted IV (3.0 g.). The resulting dark filtrate was clarified with Darco and the pH of the clear amber filtrate was adjusted to 3 with concentrated sulfuric acid. After overnight refrigeration, the yellow solid that had precipitated was separated and washed with water. The product was redissolved in 200 ml. of 1 *N* NaOH at steam bath temperature and again treated with Darco. The pH of the clear filtrate was adjusted to 4 with concentrated sulfuric acid, and the solid which formed upon overnight refrigeration was collected, washed well with water, and dried at 50° *in vacuo* overnight. The yield of yellow solid ($V \cdot H_2SO_4$) was 40.7 g. (74%).

B. From I.—A suspension of 2.0 g. (0.014 mole) of 2,4-diamino-6-hydroxypyrimidine monohydrate and 6.2 g. (0.028 mole) of phosphorus pentasulfide[13] in 60 ml. of reagent grade pyridine was refluxed, with vigorous stirring, for 50 min. The reaction mixture was evaporated to dryness and the gummy solid was hydrolyzed in 12 ml. of water on the steam bath. The light yellow solid that precipitated on cooling was separated by suction filtration and dried; yield, 1.21 g. This solid was dissolved in 1 *N* NaOH and filtered to remove insoluble impurity. Adjustment of the pH of the filtrate to 3 with 9 *N* sulfuric acid returned 0.66 g., equivalent to 20% yield, of V as the sulfate salt. The ultraviolet spectrum of this material was identical with that of the material obtained by method A.

The free base of V was prepared from the half-sulfate as follows. 2,4-Diamino-6-mercaptopyrimidine half-sulfate (6 g.) was dissolved in 60 ml. of 1 *N* NaOH (steam bath) and filtered free of insoluble impurity. The filtrate was cooled to approximately 5° and the pH was adjusted to 9.2 by the addition of 50% acetic acid. After refrigeration, the pale yellow solid that had formed was collected, washed with cold water, and dried for 4 hr. at room temperature *in vacuo*. This solid (3.87 g., 87%) was crystallized three times from water (once with Darco). The colorless prismatic needles melted at 298° dec. with previous darkening above 220° (lit.[10] 309–310°).

Anal. Calcd. for $C_4H_6N_4S$: C, 33.79; H, 4.25; N, 39.41; S, 22.55. Found: C, 33.79; H, 4.41; N, 39.17; S, 22.62.

2,4-Diamino-6-chloro-5-phenylazopyrimidine (VI).—A solution containing 0.1 mole of benzenediazonium chloride was prepared by treating a cold (0°) solution of 13.0 g. (0.1 mole) of aniline hydrochloride in 80 ml. of 3 *N* HCl dropwise with a solution of 7.6 g. (0.11 mole) of sodium nitrite in 20 ml. of water at such a rate that the reaction temperature did not rise above 3°. During the preparation of the benzenediazonium chloride solution, a cold solution of 14.5 g. (0.1 mole) of 2,4-diamino-6-chloropyrimidine in 200 ml. of 20% acetic acid was prepared by warming on the steam bath, clarifying with Darco, and cooling to 0°.

The benzenediazonium chloride solution, after decomposition of excess nitrous acid with urea, was filtered into the flask containing the cold pyrimidine solution. With continued cooling and vigorous mechanical stirring, solid NaOH was added to bring the pH to 4, followed by a large quantity of sodium acetate to buffer the solution at pH 5.5 (short-range indicator paper); the resulting brown suspension was left stirring at room temperature overnight. Filtration, followed by thorough washing with cold water, afforded 17.8 g. of mustard brown solid (72% yield), m.p. 145–165° dec. Purification was achieved by repeated recrystallization of the product from 50% aqueous ethylene glycol, with the aid of Darco, until the product was bright yellow-orange in color and had a decomposition point of over 230°. For analysis, material thus purified and melting at 241–243° dec. was recrystallized twice more from 95% ethanol (crude product will not crystallize from alcohol) affording yellow-orange solid, m.p. 242–243° dec.

Anal. Calcd. for $C_{10}H_9ClN_6$: C, 48.29; H, 3.65; Cl, 14.26; N, 33.80. Found: C, 48.41; H, 3.90; Cl, 14.04; N, 33.76.

2,4,5-Triamino-6-chloropyrimidine (VII).—Purified 2,4-diamino-6-chloro-5-phenylazopyrimidine (m.p. 240–243° dec., 1.0 g., 4 mmoles) was suspended in a mixture of 20 ml. of ethanol, 15 ml. of water, and 2.5 ml. of glacial acetic acid. A gentle stream of nitrogen was introduced and the mixture was warmed to 70° with stirring. Granulated zinc (30 mesh, 2.62 g., 40 mmoles) was added in small portions over a period of 20 min. The resulting yellow solution was stirred at 70° for 1 hr. longer and then cooled and filtered to remove excess zinc. The filtrate was concentrated to 10 ml. in a nitrogen atmosphere, neutralized to pH 7 with anhydrous sodium carbonate, and lyophilized. The resulting solid was extracted with 30 ml. of warm (60°) ethanol, and the ethanol solution was clarified with Darco and evaporated to dryness *in vacuo*. The pale orange residue was sublimed under high vacuum at 125–130° for 48 hr. yielding 168 mg. (26%) of white solid. Resublimation returned 110 mg. of white powder, m.p. 226–227° dec. (lit.[15] 227° dec.).

Anal. Calcd. for $C_4H_6ClN_5$: C, 30.10; H, 3.79; Cl, 22.22; N, 43.89. Found: C, 30.33; H, 3.84; Cl, 21.85; N, 44.00.

Impure samples of VII showed a tendency to turn pink within a few days in air; high-purity resublimed samples, however, remained colorless for long periods of time.

VII was also prepared by reduction of VI by means of "Stannochlor" (Metal and Thermit Co. grade of anhydrous stannous chloride) in 1.2 *N* HCl at 50°. The yield after purification by high vacuum sublimation was 13%.

2,4-Diamino-6-mercapto-5-phenylazopyrimidine (VIII).—A cold (0–5°) solution of benzenediazonium chloride was prepared by dropwise addition of a cold (0–5°) solution of sodium nitrite (4.56 g., 0.066 mole, in 12 ml. of water) to 5.58 g. (0.06 mole) of freshly distilled aniline in 52 ml. of 3.6 *N* HCl, the temperature not being permitted to rise above 3°. Excess nitrous acid was decomposed with urea until the starch–iodide test became negative. At the same time, a buffered solution of 2,4-diamino-6-mercaptopyrimidine was prepared from 12.6 g. (0.066 mole) of the half-sulfate in 1.2 l. of 2 *N* NH_3 to which 88 ml. of concentrated HCl had been added to bring the pH from 11.0 to 9.6. The pyrimidine solution was cooled to 12° and, with stirring, the solution of benzenediazonium chloride was added to it below the surface *via* a long-stem separatory funnel. A bright yellow precipitate began to form immediately. The addition required 10 min. and, at the end of the reaction, the pH of the mixture was 9.5. After standing at room temperature for 24 hr., the solid was collected, washed well with water, and sucked dry with the aid of a rubber dam. It was further dried under vacuum for 17 hr. at room temperature and for 5 hr. at 50°. The yield of orange-yellow solid was 9.94 g. (67%), m.p. 210° dec. with progressive shrinking and preliminary melting from 115°.

The crude solid was purified from acetone–water (2:1) by dissolving 2.55 g. in 678 ml. of acetone at room temperature and filtering free of a small insoluble residue. Water (339 ml.) was added to the clear orange filtrate to precipitate the product. The suspension was refrigerated overnight at −30° and the solid was collected, washed with cold acetone–water (2:1) and dried at 45° *in vacuo* for 4 hr. The pale yellow solid (1.11 g., 44% recovery) decomposed at 248–250° with progressive shrinking and darkening above 178°.

The acetone–water precipitation afforded VIII of sufficient purity to be used for the subsequent reduction step. For analysis, a sample of material thus purified was precipitated a second time from acetone with water (recovery this time was of the order of 86%). This twice purified product (200 mg.) was dissolved in 300 ml. of ether at room temperature and the solution was filtered free of insoluble residue. The volume was reduced very slowly on a steam bath to a few milliliters; crystals appeared after the volume had been reduced to approximately one-half. The crystals were collected, washed with ether, and air-dried. The pale orange prismatic plates (recovery 31%) decomposed at 257–259° with previous shrinking at 253–256°. The analytical sample was dried at 100° *in vacuo* for 48 hr.

Anal. Calcd. for $C_{10}H_{10}N_6S$: C, 48.76; H, 4.09; N, 34.13; S, 13.02. Found: C, 49.39; H, 3.94; N, 33.62; S, 12.70.

2,4,5-Triamino-6-mercaptopyrimidine (IX) Dihydrochloride.—To a suspension of 0.7 g. (2.84 mmoles) of 2,4-diamino-6-mercapto-5-phenylazopyrimidine in 800 ml. of 70% ethanol was added, in aliquots, 5.6 ml. (0.067 mole) of concentrated HCl. Most of the solid dissolved immediately. Granulated tin (0.52 g.) was added and the internal temperature was maintained at approximately 80° for 14 hr. with stirring. During this time the

reaction mixture changed from an orange solution to red, and then to yellow. After 14 hr., the ultraviolet absorption spectrum of the solution indicated essentially complete conversion to IX; the mixture was filtered free of tin and saturated with H_2S until no more precipitate formed. The tin sulfide was separated by filtration and the clear yellow filtrate was evaporated to dryness under vacuum (45–53° bath temperature). The pale orange residue was dissolved in 80 ml. of anhydrous methanol. Addition of 400 ml. of anhydrous ether precipitated a nearly colorless solid. After refrigeration, the solid was separated, washed with ether, and air-dried. The yield was 245 mg. (37%); no m.p. below 360° but darkening above 230°. Two crystallizations from 2 *N* HCl, the first with Darco, afforded analytically pure, pale yellow prismatic rods which on heating darkened progressively above 200° but failed to melt below 360°.

Anal. Calcd. for $C_4H_7N_5S \cdot 2HCl$: C, 20.88; H, 3.94; Cl, 30.81; N, 30.44; S, 13.93. Found: C, 20.88; H, 4.05; Cl, 31.06; N, 30.23; S, 13.92.

Bis(2,4-diamino-6-pyrimidyl) Disulfide (XI).—A suspension of 2.0 g. (0.0105 mole) of 2,4-diamino-6-mercaptopyrimidine half-sulfate in 110 ml. of water containing 30 ml. of pH 6.8 NaOH-KH_2PO_4 buffer (0.1 *M*) was warmed on a steam bath and filtered by gravity to remove a small insoluble residue. At this point the pH of the filtrate had to be readjusted to 6.8 by the addition of a few drops of 10% NaOH. The solution was cooled to 5° and to it was added dropwise, with stirring, a 1 *N* iodine–potassium iodide solution. During the addition, the temperature was maintained at 5–7°, and the pH kept constant at 6.8 by the addition of 1 *N* potassium carbonate solution. The iodine–potassium iodide solution was added until the iodine color was no longer discharged; the amount of iodine added (8.0 ml.) was less than the theoretical amount (10.5 ml. required). The white precipitate was separated by suction filtration and air-dried; yield, 1.18 g. (80%). The crude disulfide was recrystallized from anhydrous methanol to yield white prismatic rods which began to decompose at 160° and which melted into an orange liquid at 179°. The product analyzed for XI with one molecule of methyl alcohol.

Anal. Calcd. for $C_8H_{10}N_8S_2 \cdot CH_3OH$: C, 34.38; H, 4.50; N, 35.65; S, 20.39. Found: C, 34.08; H, 4.79; N, 35.51; S, 20.57.

This product was also obtained when a solution of V in 20% acetic acid was treated with an equivalent amount of sodium nitrite at 15° or when a cold solution of V in 1 *N* NaOH containing an equivalent amount of sodium nitrite was acidified. This identity was established by melting and mixture melting point determination, by comparison of infrared and ultraviolet spectra, and by behavior on ascending paper chromatography (R_{Ad} 0.45 in 4:1:1 1-butanol–acetic acid–water and R_{Ad} 0.70 in 14:1:5 2-propanol–ammonia–water).

2,4-Diaminopyrimidine-6-sulfinic Acid (XII).—2,4-Diamino-6-mercaptopyrimidine half-sulfate (3.0 g., 0.016 mole) was dissolved in 60 ml. of 1 *N* NaOH and the resulting solution cooled to 5°. With stirring and continued cooling, 6 ml. of "Superoxol" (30% hydrogen peroxide) was added dropwise. The reaction was maintained at 5° for 10 min., and the solution was acidified with 6 *N* HCl to pH 4. The ice bath was removed and the resulting suspension was stirred for 45 min. The white product was collected, washed with cold water, pressed as dry as possible and dried at 70°; 1.23 g. (45%). Recrystallization from a large volume of hot water returned 0.5 g. of pale yellow microcrystalline solid. The product failed to melt below 360° but, on heating, gradually darkened, turning red at 210° and black at 285°.

Anal. Calcd. for $C_4H_6N_4O_2S$: C, 27.58; H, 3.47; N, 32.17; S, 18.41. Found: C, 27.82; H, 3.82; N, 32.15; S, 18.64.

4-Amino-2,6-dihydroxypyrimidine (XVI). A.—As described by Conrad,[31] ethyl cyanoacetate (33.93 g., 0.3 mole) was added to a solution of 19.47 g. (0.324 mole) of urea in 300 ml. of absolute ethanol which had been previously treated with 10.33 g. (0.45 g.-atom) of sodium. The mixture was refluxed with stirring for 1.5 hr., during which time a colorless precipitate began to form. After cooling for a few min., the solid was collected, washed with absolute ethanol, and dried in a steam cabinet. The crude product was dissolved in 240 ml. of water with gentle warming and to this solution concentrated HCl (37.5 ml., 0.45 mole) was added. The colorless solid that precipitated immediately was collected and washed with water. The product was crystallized, while still damp, from 3.5 l. of boiling water with approximately 10 g. of Darco. After overnight refrigeration, the crystals were collected, washed with water, and dried at 93° for 17 hr. The yield of long, colorless shiny needles was 16.3 g. (43%); no m.p. below 360° (lit.[32,33] 325°). Two crystallizations from water followed by drying at 120° under vacuum afforded colorless shiny needles of analytical purity.

Precipitation of the product at pH 7.6 by the addition of glacial acetic acid to a dilute alkaline solution of XVI provided an alternate method of purification with approximately the same per cent recovery.

B. From XVII.—A mixture of 0.5 g. (3.2 mmoles) of 4-amino-6-hydroxy-2-methylthiopyrimidine[2] and 10 ml. of concentrated HCl was warmed on a steam bath and the resulting solution was allowed to stand at room temperature, in a stoppered flask, for 5 days. The mixture was evaporated to dryness on a steam bath and the residue was dissolved in 20 ml. of 0.5 *N* NaOH. The solution was clarified with Darco and the pH was adjusted to 7.0–7.2 by means of dilute acetic acid. After overnight refrigeration, the crystals were collected and dried; yield, 0.165 g. (41%). This product, after one more purification by the same method but without the aid of Darco, was shown to be identical with the previous sample of XVI by comparison of their quantitative ultraviolet absorption spectra.

Reaction of IV with Nitrous Acid.—A solution of 10 g. (0.069 mole) of 2,4-diamino-6-chloropyrimidine in 150 ml. of 15% aqueous acetic acid was treated at 35° with a solution of 7.15 g. (0.104 mole, 50% excess) of sodium nitrite in 40 ml. of water added dropwise over a period of 0.5 hr. A tan solid began to precipitate midway through the addition and the resultant suspension was stirred for an additional 1.5 hr., the temperature being maintained at 35°. Stirring was continued overnight at room temperature. The tan solid was collected, washed well with cold water, and dried in a vacuum desiccator.

Paper chromatographic analysis of the reaction product showed a mixture, the components of which were identified by comparison of R_{Ad} values with authentic samples. Samples of the reaction product, starting material, the seven possible hydrolysis and replacement products of IV, and adenine (used as an internal standard) were each dissolved in glacial acetic acid and each was applied as a discrete spot with capillary pipets to Whatman No. 1 chromatography grade filter paper (46 × 57 cm.). Descending chromatograms were run in a 4:1:1 1-butanol–acetic acid–water solvent system. The nitrous acid reaction product showed three distinct spots at R_{Ad} values of 1.50, 1.15, and 0.59; R_{Ad} values of the comparison samples are given in Table I.

Streaked chromatograms were then run with the aid of a Research Specialties Co. streaking pipet for the application of a thin line of the reaction product in acetic acid solution across the entire base line. The resulting three bands were cut from the paper and each eluted with anhydrous methanol in a micro-Soxhlet extractor. The methanol extracts were concentrated to 4 ml. and the ultraviolet absorption spectra of the solutions were determined. The methanol was then evaporated to dryness and the ultraviolet spectra of the residues were obtained at pH 1 and pH 10. The presence of 4-amino-6-chloro-2-hydroxypyrimidine (XIV) and 6-amino-2,4-dihydroxypyrimidine (XVI) was established by comparison of R_{Ad} values and ultraviolet absorption spectra in methanol (Table I) and in pH 1 and pH 10 buffer solutions (Table II). Elution of the band equivalent to R_{Ad} 1.50 afforded an extract, the ultraviolet spectrum of which did not compare definitively for either 2-amino-6-chloro-4-hydroxypyrimidine (XIX) or starting material (IV), both of which possess R_{Ad} values of 1.51. Descending chromatography in 100:2:16 1-butanol–ammonia–water showed that both IV (R_{Ad} 1.90) and XIX (R_{Ad} 0.90) were present.

4-Amino-2-hydroxy-6-mercaptopyrimidine (XV).—2,4-Diamino-6-chloropyrimidine was treated with nitrous acid as in the above procedure and the product was suspended, while still moist, in 345 ml. of water containing 38 g. of sodium hydrosulfide trihydrate (0.345 mole, 5 *M* excess). The mixture was saturated with H_2S for 15 min., and, with a continuing slow stream of H_2S, was warmed to 75–80° (internal temperature) for 3 hr. with constant stirring. The reaction solution was cooled to below room temperature and the pH was adjusted to 3 with 20 ml. of 9 *N* sulfuric acid. The mixture was evaporated to dryness *in vacuo* and the residue was dissolved directly in 170 ml. of 3 *N*

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ammonia by warming on a steam bath. The dark amber solution was clarified with Darco and, after cooling to room temperature, the pH was adjusted to 7–8 by the addition of 5 ml. of 9 *N* sulfuric acid. After overnight refrigeration the solid was collected, washed with cold water, and dried. The yellow prismatic rods weighed 5.5 g. (56% yield). Three more precipitations from ammonia, the last without Darco, afforded the analytical sample; no m.p. below 360° (lit. 355° dec.,[22] >360°[2]).

A sample of XV prepared by this route was shown to be identical with material prepared by thiation of authentic 4-amino-6-chloro-2-hydroxypyrimidine (XIV) obtained by acid hydrolysis of 4-amino-6-chloro-2 methylthiopyrimidine (XVIII).[2] This identity is based upon melting points, comparison of ultraviolet and infrared spectra, and behavior on paper chromatography.

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Notes

Synthesis of Indomethacin Metabolites

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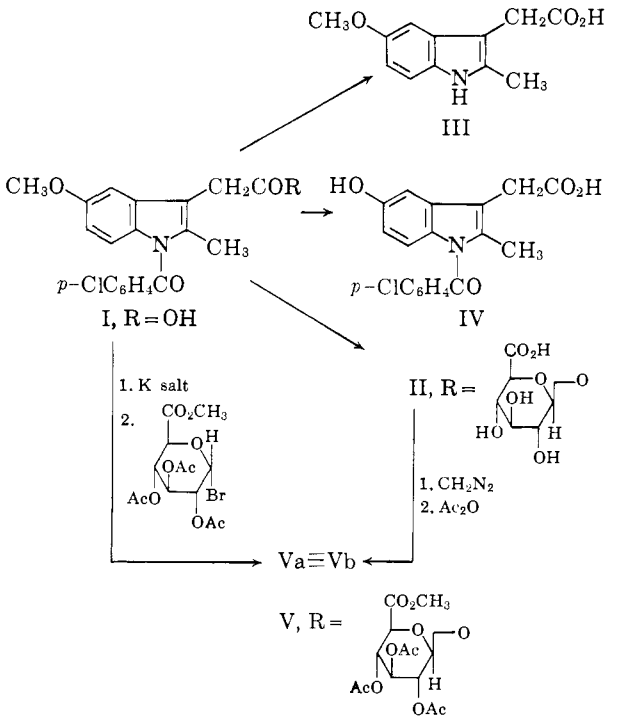
The isolation and characterization of the metabolites of indomethacin (I), a new nonsteroidal antiinflammatory agent,[1] have been reported recently.[2] In man, the acyl glucuronide of indomethacin (II) is rapidly excreted in the urine, whereas in several animal species the N_1-deacylated derivative III, the *O*-desmethyl analog IV, and the corresponding acyl glucuronides are major urinary metabolites.

During the study of indomethacin analogs the ease of hydrolytic removal of the N_1-aroyl group under mildly acidic or alkaline conditions was noted,[3] thus the *in vivo* N_1-deacylation of iodomethacin to the known acid III, previously described by Shaw,[4] was not totally unexpected.

For the preparation of the *O*-desmethyl analog IV, a preferential demethylation was effected in about 50% yield with pyridine hydrochloride at 180° without extensive concomitant N_1-deacylation.

The chemical lability of acyl glucuronides is well known.[5] Facile hydrolytic cleavage of the glycosidic linkage of II was also observed during its isolation.[2] To effect the synthesis of II, the potassium salt of indomethacin was converted to the ester glucuronide derivative Va by condensation with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate[6] in acetone. Several exploratory attempts to convert Va into II were abortive. Nevertheless, the crystalline derivative Va proved to be identical with a sample (Vb) prepared from the *in vivo* metabolite II by esterification and acetylation,[7] thus establishing the structure of II.



Experimental

1-*p*-Chlorobenzoyl-5-hydroxy-2-methyl-3-indolylacetic Acid (IV).—Indomethacin (I, 10 g., 0.028 mole) was added to 50 g. of molten pyridine hydrochloride (Eastman, practical grade) under nitrogen at 180°. The mixture was stirred at 180° for 0.25 hr., cooled slightly, and poured into 200 g. of ice. The solid product was filtered, dried, and digested successively with 50 ml. of methylene chloride and 150 ml. of ether. Recrystallization from acetone (75 ml.)–water (125 ml.) yielded 4.8 g. (50%) of IV, m.p. 208–210° dec.

Anal. Calcd. for $C_{18}H_{14}ClNO_4$: C, 62.89; H, 4.10; Cl, 10.31. Found: C, 63.02; H, 4.40; Cl, 10.32.

Compound IV was compared with a sample of the metabolite[2] as shown in Table I.

(Methyl 2′,3′,4′-Tri-*O*-acetyl-β-D-glucuronosyl)-1-*p*-chlorobenzoyl-5-methoxy-2-methyl-3-indolylacetate (V). A. From Indomethacin (Va).—1-*p*-Chlorobenzoyl-2-methyl-5-methoxy-2-indolylacetic acid (5 g., 0.014 mole) was suspended in 15 ml. of anhydrous methanol, and 32.8 ml. of a solution of 0.427 *N* (0.014

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