Countercurrent Distribution of Mussel and Clam Poison.—A 6.5-mg. sample of purified mussel poison dihydrochloride (5500 MU per mg.) was dissolved in 8 ml. of the lower phase of the solvent system and placed in the first tube of the Craig apparatus with an equal volume of the upper phase. Twenty-four transfers were applied as described above. The distribution of poison was determined by bioassay[13] and the nitrogen distribution determined as described above. Results on the distribution are presented in Fig. 2. A portion of clam poison weighing 31 mg. also was distributed in the Craig apparatus and similar results were obtained.

The distribution coefficients of the two components of clam and mussel poisons sometimes varied as much as 25% in different experiments. This variability may be due in part to the difficulty in fitting the best theoretical curve to the observed data. An error of one tube in the placement of the maximum of curve A would produce a variation of about 25% in the calculated distribution coefficient. Some variation was also believed to be due to difficulties in controlling the temperature of the room in which the countercurrent distributions were performed.

Redistribution of Fractions A and B.—A sample of purified mussel poison dihydrochloride weighing 25 mg. was distributed through 24 transfers in the Craig apparatus at +6° with the solvent system described previously. At the conclusion of the experiment, the content of each tube was acidified prior to bioassay and analysis for nitrogen by the micro-Kjeldahl procedure. The poison appeared to be partially separated into a major component with a partition coefficient of 3.2 and a minor component with a partition coefficient of 1.2. The poison contained in tubes 18 through 24 had a toxicity of 23,200 MU per mg. of nitrogen or 5500 MU per mg. of poison. The bioassay on all tubes showed a quantitative recovery of the poison.

The contents of tubes 16 through 21 from the extraction described above, containing only the major component A, were concentrated to 5 ml. The *p*H of the poison solution was adjusted to 8 with concentrated potassium hydroxide, and the appropriate amounts of ethyl alcohol, *n*-butyl alcohol, α-ethylcaproic acid and aqueous potassium bicarbonate were added to give 32 ml. of the two-phase solvent system. This was divided equally between the first two tubes of the Craig apparatus and equal amounts of fresh solvent and aqueous layers added to the other tubes. Potassium chloride was added to each of the tubes which did not contain the poison in order to adjust the salt content to 1.4%, the amount present in the tubes containing the poison. Twenty-four transfers were applied.

The contents of tubes 9 through 15 from this extraction containing predominantly the minor component B were treated in a similar manner as described above for component A.

Paper Chromatography of Clam and Mussel Poison Dihydrochlorides and of Their Dihydro Analogs.—Four microliters of a solution of the test compound containing 50 mg./ml. was applied near the bottom of strips of Whatman No. 1 filter paper (12.5 × 38 cm.). After drying in air, the strips were dipped into the appropriate solvent contained in a cylindrical glass battery jar (13 × 45 cm.) to a depth of about 0.25 inch. The upper edge of the paper was attached by means of spring clips to a glass rod fastened to a ground glass plate which served as the cover. Yellow petrolatum was used to seal the cover. The solvent was allowed to ascend the paper for 16 hr. The strips were air-dried and sprayed with the Weber reagent[11] and Jaffé reagent[9] or cut into 2-cm. sections and eluted with water for bioassay (Table I).

Determination of Microgram Quantities of Nitrogen by a Kjeldahl Procedure.—The following digestion procedure was used for micro-Kjeldahl nitrogen determinations on samples containing up to 300 mg. of salt. Five-tenths ml. of 9 *N* sulfuric acid, containing 0.1% red mercuric oxide, was added to 2 ml. or less of a sample containing 0.5 to 10 μg. of nitrogen. The mixture was heated in an 18 × 150 mm. Pyrex test-tube at 150° on a sand-bath covered with an asbestos plate through which holes were bored to admit the tubes. Heating was continued until all of the water was driven off, then the tubes were covered with small beakers and digested at 270–300° for 18 hr. The entire operation was carried out in an ammonia-free atmosphere. After cooling to room temperature the colorimetric determination of the ammonia nitrogen produced was carried out directly on the digestive mixture according to the Borsook method,[14] or the solutions were made strongly alkaline (*ca.* 2 *M* sodium hydroxide), the ammonia diffused into one drop of 1 *N* sulfuric acid[15] and then determined either by the Borsook procedure or by Nesslerization.[16]

(13) Bioassay was carried out as described previously.[2] It was necessary to correct for the effect of the inorganic salts, alcohol and *p*H upon the bioassay in order to obtain reliable values. This was accomplished by the use of standard curves determined for the purified poison containing known amounts of these contaminants. After acidification of the contents of the tubes from the Craig extraction, it required several hours before the bioassay increased to its equilibrium value.

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

Synthesis of Potential Anticancer Agents. IX. 9-Ethyl-6-substituted-purines[2]

BY JOHN A. MONTGOMERY AND CARROLL TEMPLE, JR.

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Several 9-ethyl-6-substituted-purines have been prepared from 6-chloro-9-ethylpurine. This compound was prepared by reaction of 5-amino-4,6-dichloropyrimidine with ethylamine and cyclization of the resulting 5-amino-6-chloro-4-ethylaminopyrimidine to the purine by the use of diethoxymethyl acetate.

Recently Robins and Lin reported[3] the synthesis of several 9-methyl-6-substituted purines. One of them, 6-chloro-9-methylpurine, has shown the same order of activity against Adenocarcinoma 755 in C57 black mice as 6-chloropurine,[4] and two other 9-methylpurines have shown lesser activity[4] against this tumor. These results make the investigation of other 6,9-disubstituted purines of great interest. This paper, the first of a series dealing with the synthesis of 6,9-disubstituted purines, is concerned with 9-ethyl-6-substituted purines.

Few 9-ethylpurines are reported in the literature,[5] and these are not prepared by methods which are generally applicable. For this series of 9-ethyl-6-substituted purines a procedure similar to that of

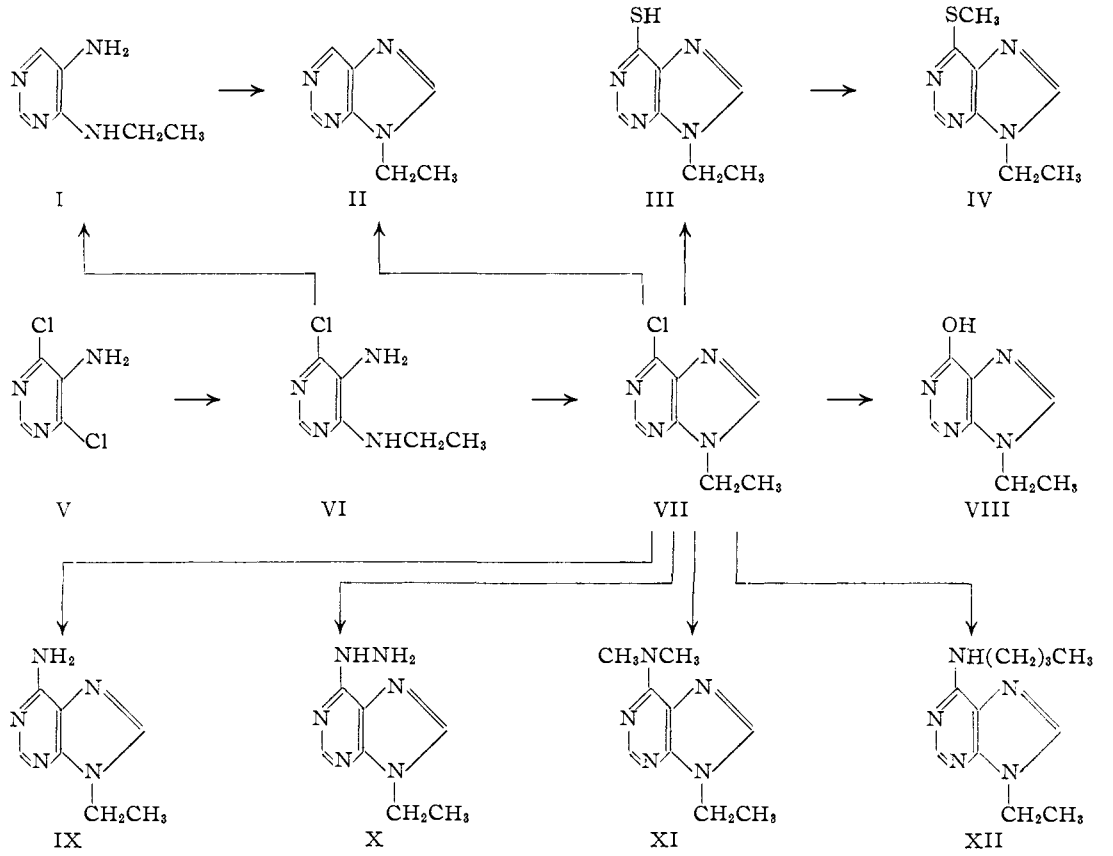
(1) Affiliated with the Sloan-Kettering Institute. This work was supported by funds from the C. F. Kettering Foundation.

(2) Part VIII, B. R. Baker and Kathleen Hewson, *J. Org. Chem.*, in press.

(3) R. K. Robins and H. H. Lin, THIS JOURNAL, **79**, 490 (1957).

(4) H. E. Skipper, J. R. Thomson and R. K. Robins, unpublished data.

(5) (a) E. F. Armstrong, *Ber.*, **33**, 2314 (1900); (b) H. Biltz and K. Strufe, *Ann.*, **423**, 1215 (1921); (c) B. R. Baker, R. E. Schaub and J. P. Joseph, *J. Org. Chem.*, **19**, 639 (1954).



Robins and Lin[3] was independently devised. The parent compound, 6-chloro-9-ethylpurine (VII), was prepared by the action of diethoxymethyl acetate[6] on 5-amino-6-chloro-4-ethylaminopyrimidine (VI). Previously ethyl orthoformate and acetic anhydride had been used to cyclize chloro-4,5-diaminopyrimidines to purines in order to avoid hydrolysis of the chlorine atoms.[7] However, diethoxymethyl acetate was found to be superior to the ethyl orthoformate–acetic anhydride combination in the preparation of 2,6-dichloropurine,[8] so its application to the present cyclization seemed desirable. Comparative studies are now under way in this Laboratory to determine whether diethoxymethyl acetate is generally superior to the acetic anhydride–ethyl orthoformate combination as a cyclization reagent.

The 5-amino-6-chloro-4-ethylaminopyrimidine (VI) required for this synthesis was prepared by the reaction of 5-amino-4,6-dichloropyrimidine with aqueous ethylamine in a stainless steel bomb using the conditions Brown found successful for the preparation of 5-amino-6-chloro-4-methylaminopyrimidine.[9] The use of a bomb instead of a sealed tube[9] for this reaction makes the procedure more convenient and the yield obtained was actually higher.

Robins and Lin[3] prepared 5-amino-6-chloro-4-methylaminopyrimidine by treating 4,6-dichloro-5-nitropyrimidine with methylamine in aqueous acetic acid (to prevent di-replacement) followed by reduction of the intermediate nitro compound. This procedure avoids the use of a bomb, but the over-all yield is lower.

9-Ethylpurine (II) was prepared in two ways: by the cyclization of 5-amino-4-ethylaminopyrimidine (I) with diethoxymethyl acetate and by hydrogenolysis of 6-chloro-9-ethylpurine using 5% Pd-on-charcoal catalyst. This latter procedure, similar to that used by Bendich, Russell and Fox for the preparation of purine,[10] was found to be more satisfactory although hydrogenolysis of 5-amino-6-chloro-4-ethylaminopyrimidine (VI) to 5-amino-4-ethylaminopyrimidine went quite well. In both hydrogenolyses magnesium oxide was used as a proton acceptor.

Treatment of 6-chloro-9-ethylpurine with alcoholic ammonia in a bomb at 110° gave a good yield of 9-ethyladenine (IX), which shows that the replacement of the 6-chlorine atom with ammonia can be accomplished at a lower temperature than that used by Bendich, Russell and Fox,[10] or by Robins and Lin.[3] Reaction with anhydrous hydrazine took place readily at room temperature, whereas it was necessary to boil a solution of chloropurine in anhydrous *n*-butyamine to obtain 6-*n*-butylamino-9-ethylpurine (XII). 6-Dimethylamino-9-ethylpurine (XI) was prepared by boiling a solution of the chloropurine in aqueous dimethylamine.

Hydrolysis of 6-chloro-9-ethylpurine to 9-ethyl-6-hydroxypurine (VIII) was accomplished readily

(6) H. W. Post and E. R. Erickson, *J. Org. Chem.*, **2**, 260 (1937).
(7) J. A. Montgomery, THIS JOURNAL, **78**, 1928 (1956).
(8) J. A. Montgomery and L. B. Holum, to be published.
(9) D. J. Brown, *J. Appl. Chem.*, **4**, 72 (1954).
(10) A. Bendich, P. J. Russell and J. J. Fox, THIS JOURNAL, **76**, 6073 (1954).

in boiling 0.1 *N* hydrochloric acid. It was found that the hydrolysis could also be carried out in boiling 0.1 *N* sodium hydroxide.

This result was somewhat surprising since it is known that dilute aqueous base cleaves the imidazole ring of 6-chloro-9-β-D-ribofuranosylpurine and of 6-chloro-9-α-L-rhamnopyranosylpurine to give 6-chloro-5-formamido-4-ribosylaminopyrimidine and 6-chloro-5-formamido-4-rhamnosylaminopyrimidine, respectively.[2] It is also known that 9-β-D-ribofuranosylpurine,[11] 9-methylpurine[12] and 9-acetyl-2-chloropurine[7] are all cleaved in this manner. To investigate this matter further, the stability of 9-ethylpurine and 9-ethyl-6-hydroxypurine was determined. Although 9-ethyl-6-hydroxypurine was unaffected by boiling 0.1 *N* sodium hydroxide, 9-ethylpurine was cleaved to 5-amino-4-ethylaminopyrimidine in 16 hr. Since 9-β-D-ribofuranosylpurine is cleaved rapidly at room temperature,[11] these data show that although the alkyl group on the 9-nitrogen renders the imidazole ring base labile (purine itself is stable under these conditions),[12] sugars have a greater effect.

When 6-chloropurine is substituted on the 9-nitrogen, the nature of the substituent actually determines the course of the reaction of the compound with dilute base, so that when the 9-substituent is a sugar ring cleavage predominates; when the 9-substituent is an alkyl group hydrolysis of the 6-chlorine atom results. It is also interesting that the hydroxy group in the 6-position counteracts the alkyl group in the 9-position so that 9-ethyl-6-hydroxypurine is stable to the base treatment.

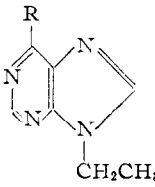
9-Ethyl-6-mercaptopurine (III) was obtained by reaction of 6-chloro-9-ethylpurine with thiourea in boiling ethanol.[3,10] Methylation of this compound (III) with dimethyl sulfate produced 9-ethyl-6-methylmercaptopurine (IV) in good yield.

In general, 6-chloro-9-ethylpurine undergoes the reactions typical of 6-chloropurine[10] with equal facility.

The maxima of the ultraviolet spectra of the 9-ethylpurines are listed in Table I. As was expected, the ultraviolet spectra of the 9-ethylpurines agree well with those of the 9-methylpurines.[3] They are also very similar to the spectra of the corresponding purines unsubstituted in the 9-position.[13] However, a difference between the spectra of the 9-ethylpurines and the parent purines is evident in 0.1 *N* sodium hydroxide solution. In this basic solution the maxima of the spectra of the 9-ethylpurines, with the exception of 9-ethyl-6-mercaptopurine, occur 4–8 mμ lower than those of the corresponding purines. This difference is undoubtedly due to the fact that the presence of the ethyl group in the 9-position prevents the acidic ionization which normally occurs in unsubstituted purines. In the case of 9-ethyl-6-mercaptopurine and 6-mercaptopurine the acidic ionization of the mercapto group masks this effect.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA



R	0.1 *N* HCl λ_{max}, mμ	0.1 *N* HCl $\varepsilon M \times 10^{-3}$	pH 7 λ_{max}, mμ	pH 7 $\varepsilon M \times 10^{-3}$	0.1 *N* NaOH λ_{max}, mμ	0.1 *N* NaOH $\varepsilon M \times 10^{-3}$
H	261.5	5.85	263	7.82	263	7.82
Cl	265	9.46	266	9.40	266	9.40
OH	250	10.8	250	11.65	250	12.4
SH	225	9.20	227[a]	9.20	232	14.4
	325.5	19.6	320	20.0	310	21.8
SCH_3	221.5	10.6	221[a]	11.8	222	11.6
	296	16.5	286	17.6	286	17.7
			290[a]	17.1	290[a]	17.4
NH_2	259	13.8	262	14.1	262	14.1
	264[a]	13.1				
$NHNH_2$	263	15.8	265	14.3	...	...[b]
$(CH_3)_2N$[c]	270	17.4	275	20.6	277	18.2
$CH_3(CH_2)_3NH$	266	18.2	269	17.5	269	17.6

[a] Shoulder. [b] Unstable in 0.1 *N* NaOH. [c] These values are in agreement with those reported by Baker, Schaub and Joseph, see ref. 5c.

The important maxima of the infrared spectrum of each compound is reported in the Experimental section under the preparation of that compound. As in the case of the ultraviolet spectra, the infrared spectra of the 9-ethylpurines are very similar to those of the corresponding purines with some notable exceptions. These exceptions are, of course, due to the presence of the 9-ethyl group. Absorption from aliphatic CH bonds occurs between 3000–2800 kaysers, around 1450 kaysers, and around 1375 kaysers in the spectra of the 9-ethylpurines. These maxima are absent from the spectra of the parent purines, except in those cases in which the group in the 6-position contains aliphatic CH bonds, *i.e.*, 6-*n*-butylaminopurine. The absorption between 2800–2500 kaysers, which is due to acidic H bonds and which is found in the spectra of all purines unsubstituted in the 9-position, is missing from spectra of the 9-ethylpurines, except in the case of 9-ethyl-6-mercaptopurine and 9-ethyl-6-hydroxypurine. The spectra of these two compounds do exhibit absorption in the 2800–2500 region due to acidic hydrogen bonds. The acid hydrogen bonds, however, arise from the group in the 6-position. In the case of 9-ethyl-6-hydroxypurine the acidic hydrogen resides on the 1-nitrogen of the purine ring, whereas the position of the acidic hydrogen of 9-ethyl-6-mercaptopurine is open to question.[14]

Spectral differences are not the only differences noted between purines and 9-ethylpurines. Physical properties such as solubilities and melting points are also different. The 9-ethylpurines are generally more soluble in water and in organic solvents and melt considerably lower than the corresponding purines. Indeed one of the 9-ethylpurines, 6-*n*-butylamino-9-ethylpurine, was purified by distillation *in vacuo*.

(11) M. P. Gordon and G. B. Brown, *J. Biol. Chem.*, **220**, 927 (1956). NOTE ADDED IN PROOF.—Recently a more detailed account of the instability of certain purine ribosides to dilute base has been published; see M. P. Gordon, V. S. Weliky and G. B. Brown, THIS JOURNAL, **79**, 3245 (1957).

(12) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

(13) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

(14) D. J. Brown and S. F. Mason, *ibid.*, 682 (1957).

Acknowledgment.—The authors are indebted to Mr. V. Minic for technical assistance, to Mr. J. P. Holmquist and Mr. J. W. Murphy for the microanalytical results reported, to Mr. W. A. Rose and Mr. L. D. Norton for the spectral determinations and to Dr. W. C. Coburn, Jr., for his aid in the interpretation of the spectral data.

Experimental[15]

5-Amino-4-ethylaminopyrimidine (I).—A solution of 5-amino-6-chloro-4-ethylaminopyrimidine (1.5 g., 8.69 mmoles) in water (50 ml.) was hydrogenated over a palladium catalyst (0.5 g., 5% Pd) in the presence of magnesium oxide (1 g.). The required hydrogen absorption occurred in 3.5 hr. The catalyst was removed by filtration, the solution evaporated to dryness *in vacuo* and the residue extracted in a Soxhlet extractor with chloroform. Evaporation of the chloroform gave 590 mg. of a light yellow solid. The residual material in the thimble of the Soxhlet was dissolved in water (40 ml.), a 10% solution of sodium carbonate added (10 ml.) and the whole evaporated to dryness *in vacuo*. The residue was boiled in methyl isobutyl ketone (50 ml.), the insoluble residue removed by filtration and the filtrate evaporated to dryness under diminished pressure to give 195 mg. of a light brown solid: total yield 785 mg. (65.5%), m.p. 196–198°. Recrystallization of a small sample from benzene–ethanol with Norit treatment gave a light yellow solid, m.p. 195–198°; λ_{max} in mμ ($a_M \times 10^{-3}$): 0.1 *N* HCl, 288 (11.65); *p*H 7, 258.5 (7.35), 289 (8.28); 0.1 *N* NaOH, 255 (8.2), 285 (7.1); $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3345 and 3200 (NH); 2950 and 2850 (CH); 1660 (NH); 1585, 1555 and 1508 (C=C, C=N); 1475 (CH); 1466 and 1372 (C-CH_3).

Anal. Calcd. for $C_6H_{10}N_4$: C, 52.23; H, 7.25; N, 40.52. Found: C, 52.07; H, 6.90; N, 40.35.

9-Ethylpurine (II). Method A.—A solution of 5-amino-4-ethylaminopyrimidine (135 mg., 0.98 mmole) in diethoxymethyl acetate (2 ml.) was heated at 120° for 1 hr. and the volatiles removed under reduced pressure. The residue was sublimed (60° (0.3 mm.)) to give 70 mg. (48.5%) of a white solid, m.p. 52–53°; $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3040 and 2950 (CH); 1590, 1575 and 1500 (C=C, C=N); 1455 (shoulder) (CH); 1445 and 1375 (shoulder) (C-CH_3).

Anal. Calcd. for $C_7H_8N_4$: C, 56.76; H, 5.40; N, 37.85. Found: C, 56.90; H, 5.52; N, 37.75.

Method B.—A solution of 6-chloro-9-ethylpurine (80 mg., 0.44 mmole) in a 1:1 mixture of ethanol–water (10 ml.) containing 5% palladium-on-charcoal catalyst (40 mg.) and magnesium oxide (50 mg.) was hydrogenated at atmospheric pressure. The required hydrogen absorption occurred in 17 minutes. The catalyst was removed by filtration, sodium carbonate solution (10 ml., 2.5%) added and the mixture evaporated to dryness *in vacuo*. The residue was extracted with cold methyl isobutyl ketone (5 ml.).

The extract was evaporated to dryness to give 33 mg. (51%) of a white solid, m.p. 53–56°. The ultraviolet and infrared spectra were practically identical with those described in method A.

9-Ethyl-6-mercaptopurine (III).—A solution of 6-chloro-9-ethylpurine (500 mg., 2.74 mmoles) and thiourea (208 mg., 2.74 mmoles) in absolute ethanol (7 ml.) was refluxed for 1 hr. The white solid which deposited was collected by filtration, dissolved in sodium hydroxide (1 *N*, 7 ml.) and the solution acidified with acetic acid. The precipitate was collected by filtration and dried *in vacuo* over P_2O_5 at 100° for 4 hr.; yield 415 mg. (84%), m.p. 333–337° dec. (taken from 300°); $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3050 and 2975 (CH); 2900–2500 (SH); 1590 and 1550 (C=C, C=N); 1485 (CH); 1440 and 1375 (C-CH_3).

Anal. Calcd. for $C_7H_8N_4S$: C, 46.67; H, 4.44; N, 31.10. Found: C, 46.84; H, 4.37; N, 30.76.

9-Ethyl-6-methylmercaptopurine (IV).—A mixture of 9-ethyl-6-mercaptopurine (300 mg., 1.7 mmoles) and dimethyl sulfate (210 mg., 1.7 mmoles) in water (10 ml.) containing sodium hydroxide (66.5 mg., 1.7 mmoles) was stirred for 1 hr. Unreacted 9-ethyl-6-mercaptopurine (25 mg.) which had deposited was removed by filtration.

The filtrate was neutralized with dilute sodium hydroxide, evaporated to dryness *in vacuo* and the residue extracted with ether. Evaporation of the ether gave a white residue which was sublimed at 85° (0.2 mm.); yield 150 mg. (51% based on mercaptan reacted), m.p. 116–118°; $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3020, 2960 and 2900 (CH); 1565 and 1545 (C=C, C=N); 1460 (CH); 1435 and 1380 (C-CH_3).

Anal. Calcd. for $C_8H_{10}N_4S$: C, 49.48; H, 5.15; N, 28.85. Found: C, 49.32; H, 5.04; N, 28.80.

5-Amino-6-chloro-4-ethylaminopyrimidine (VI).—A solution of 5-amino-4,6-dichloropyrimidine (4 g., 24.2 mmoles) in alcoholic ethylamine (50 ml., 11%) was heated in a stainless steel bomb for 6 hr. at 125–130°, evaporated to dryness *in vacuo* and the residue dissolved in hot benzene (560 ml.). The solid which deposited was collected by filtration and dried *in vacuo* over P_2O_5; yield 1.0 g., m.p. 140–145°. Concentration of the filtrate under reduced pressure gave an additional 2.47 g., m.p. 145–147°; total yield 3.47 g. (83%).

Two recrystallizations of the crude material (500 mg.) from benzene–Skellysolve C gave 210 mg., m.p. 148–149°; λ_{max} in mμ ($a_M \times 10^{-3}$): 0.1 *N* HCl, 272 (8.1), 301 (11.5); *p*H 7, 262 (8.8), 288 (9.0); 0.1 *N* NaOH, 261.5 (8.62), 287 (8.86); $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3310 and 3200 (NH), 2935 and 2880 (shoulder) (CH), 1640 (NH), 1576, 1559 (shoulder), 1545 (shoulder) (C=C, C=N), 1450 and 1382 (CH).

Anal. Calcd. for $C_6H_9ClN_4$: C, 41.74; H, 5.21; N, 32.45. Found: C, 42.03; H, 5.30; N, 31.96.

6-Chloro-9-ethylpurine (VII).—A solution of 5-amino-6-chloro-4-ethylaminopyrimidine (16 g., 92.7 mmoles) in diethoxymethyl acetate (130 ml.) was heated at 120° for 1.5 hr. and the volatiles removed under diminished pressure. Recrystallization of the residue from Skellysolve C gave 9 g. (53%) of a white solid, m.p. 81–84°; $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3060 and 2960 (CH); 1591, 1555 and 1545 (C=C, C=N); 1465 (CH); 1441 and 1390 (C-CH_3).

Anal. Calcd. for $C_7H_7ClN_4$: C, 46.03; H, 3.84; N, 30.69. Found: C, 46.16; H, 3.66; N, 30.37.

9-Ethyl-6-hydroxypurine (VIII).—A solution of 6-chloro-9-ethylpurine (1 g., 5.48 mmoles) in hydrochloric acid (10 ml., 0.1 *N*) was refluxed for 2 hr., evaporated to dryness *in vacuo* and the residue triturated with ethanol. The ethanol was removed under diminished pressure leaving 1 g. (91%) of 9-ethyl-6-hydroxypurine hydrochloride (83% pure based on ultraviolet spectrum), m.p. 224–227° dec. (taken from 200°).

An analytical sample was prepared by dissolving the hydrochloride in alcohol, treating the solution with Norit and precipitating the hydrochloride by the addition of ether: m.p. 233–236° dec. (taken from 200°).

Anal. Calcd. for $C_7H_8N_4O$·HCl: C, 41.90; H, 4.48; N, 27.90. Found: C, 42.18; H, 4.97; N, 28.04.

The free base was prepared by dissolving a small portion of the hydrochloride in water and adding an equivalent amount of 10 *N* sodium hydroxide. The solution was evaporated to dryness *in vacuo*, the residue triturated with water and the solid collected by filtration.

Recrystallization from methyl isobutyl ketone gave pure 9-ethyl-6-hydroxypurine, m.p. 263–265° dec. (taken from 200°); $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3040 and 2958 (CH); 2850–2500 (acidic H); 1679 (C=O); 1590, 1545 and 1520 (C=C, C=N); 1475 (CH); 1455 and 1356 (C-CH_3).

Anal. Calcd. for $C_7H_8N_4O$: C, 51.22; H, 4.88; N, 34.10. Found: C, 51.24; H, 4.77; N, 34.02.

6-Amino-9-ethylpurine (IX).—A solution of 6-chloro-9-ethylpurine (500 mg., 2.74 mmoles) in ethanolic ammonia (50 ml., saturated at 0°) was heated in a stainless steel bomb at 110° for 16 hr. The solution was evaporated to dryness under diminished pressure and the residue extracted with hot benzene (3 × 20 ml.). The combined extracts were chilled and the solid which deposited was collected by filtration; yield 190 mg., m.p. 194–195°; $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3250 (NH); 3085, 2970 and 2850 (CH); 1662 (NH); 1591 and 1569 (C=C, C=N); 1479 (CH); 1451 and 1383 (C-CH_3).

Anal. Calcd. for $C_7H_9N_5$: C, 51.53; H, 5.52; N, 42.95. Found: C, 51.17; H, 5.21; N, 42.57.

Concentration of the filtrate gave an additional 120 mg.; m.p. 190–194°. The total yield was 310 mg. (70%).

9-Ethyl-6-hydrazinopurine (X).—6-Chloro-9-ethylpurine (500 mg., 2.74 mmoles) was added in small portions with

(15) All melting points are uncorrected. All infrared spectra were determined in pressed KBr disks.

stirring to anhydrous hydrazine (2.5 ml.). A large quantity of white solid deposited after stirring for 30 minutes. The mixture was evaporated to dryness *in vacuo* and the residue extracted with boiling benzene (100 ml.). The white solid which deposited from the extract was collected by filtration; yield 340 mg. (70%), m.p. 160–162°; $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3125 (NH); 2960 and 2860 (CH); 1612 (NH); 1570, 1562 and 1528 (C=C, C=N); 1479 (CH); 1460 and 1374 (C-CH_3).

Anal. Calcd. for $C_7H_{10}N_6$: C, 47.19; H, 5.62; N, 47.19. Found: C, 47.16; H, 5.68; N, 48.35.

6-Dimethylamino-9-ethylpurine[5c] **(XI).**—A solution of 6-chloro-9-ethylpurine (500 mg., 2.74 mmoles) in aqueous dimethylamine (10 ml., 25%) was refluxed for 1 hr., evaporated to dryness *in vacuo* and the residue extracted with ether (3 × 25 ml.). The combined extracts were dried over magnesium sulfate, the drying agent removed by filtration and the filtrate evaporated to dryness; yield 390 mg.

Recrystallization of this material from Skellysolve C gave 380 mg. (73%) of a white solid, m.p. 82–84° (lit.[5c] 79–80°); $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3065 and 2950 (CH); 1590, 1559 and 1547 (C=C, C=N); 1485 (CH); 1430 and 1370 (C-CH_3).

Anal. Calcd. for $C_9H_{13}N_5$: C, 56.54; H, 6.81; N, 36.65. Found: C, 56.87; H, 7.02; N, 36.39.

6-*n*-Butylamino-9-ethylpurine (XII).—After a solution of 6-chloro-9-ethylpurine (500 mg., 2.74 mmoles) in *n*-butylamine (10 ml.) was refluxed for 2 hr., the excess amine was removed *in vacuo* and the residue triturated with ether. The solid (*n*-butylamine hydrochloride) which deposited was removed by filtration. The residual oil obtained from the evaporation of the ether filtrate was dissolved in hydrochloric acid (8 ml., 1 *N*) by heating on a hot-plate for several minutes and the solution evaporated to dryness *in vacuo;* yield 400 mg.

Recrystallization (380 mg.) from methyl isobutyl ketone (75 ml.) gave 270 mg. (39%) of 6-*n*-butylamino-9-ethylpurine hydrochloride, m.p. 176–178°.

Anal. Calcd. for $C_{11}H_{17}N_5$·HCl: C, 51.66; H, 7.05; N, 27.45. Found: C, 51.67; H, 7.12; N, 27.45.

In a second run of 500 mg. of 6-chloro-9-ethylpurine, the oil obtained from the evaporation of the ether was distilled at approximately 164° (0.1 mm.). Crystallization of the distillate took place on standing overnight; yield 340 mg. (57%) of 6-*n*-butylamino-9-ethylpurine, m.p. 60–61.5°; $\tilde{\nu}_{max}$ in cm.$^{-1}$: 3265 (NH); 3030, 2917 and 2845 (CH); 1615 (NH); 1580, 1560, 1538 (C=C, C=N); 1487 (CH); 1469 and 1375 (C-CH_3).

Anal. Calcd. for $C_{11}H_{17}N_5$: C, 60.03; H, 7.76; N, 31.97. Found: C, 59.73; H, 7.70; N, 32.02.

BIRMINGHAM, ALABAMA

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Indazole Analog of Tryptamine: A New Synthesis of Indazoles

BY C. AINSWORTH

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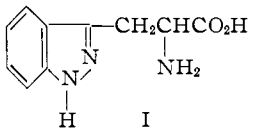
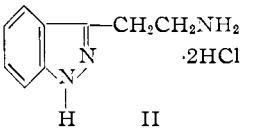
A new synthesis of indazoles involving the dehydrogenation of readily available tetrahydroindazoles, and the application of this method to the preparation of 3β-aminoethylindazole dihydrochloride (II) are described.

Indazole compounds have been known for a long time and have been synthesized in several different ways. In recent years there has been a certain interest in indazole analogs of some biologically important indole derivatives. For example, compound I, isosteric with tryptophane, has been reported.[1]

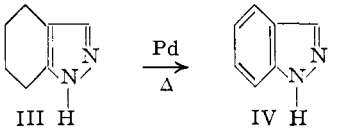


I

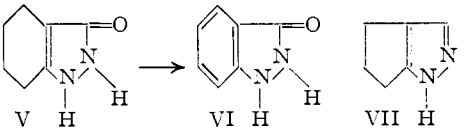


II

This paper describes a new and fairly general route to the synthesis of indazoles. In particular it describes the preparation of compound II, which is the indazole analog of tryptamine, by this new method. It has been found that indazole (IV) can be prepared easily and in good yield by the catalytic dehydrogenation of the readily available pyrazole compound III.

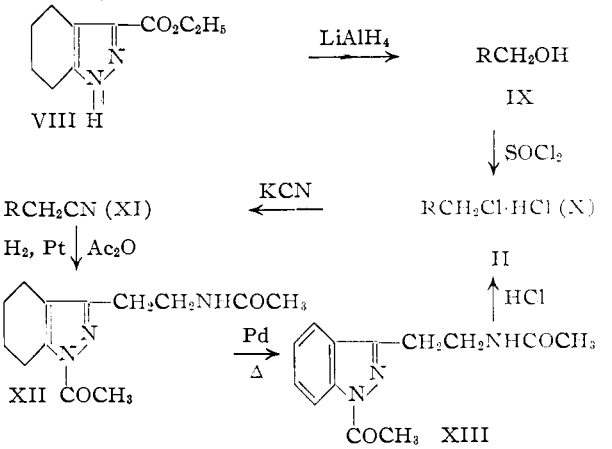


The reaction is carried out in boiling decalin using 5% palladium-on-carbon. Some related dehydrogenations were studied, and it was shown that 4,5-dihydronaphtho[1,2]pyrazole gave naphtho[1,2]-pyrazole, and compound V formed VI. However, the pyrazole derivative VII was recovered un-



changed when subjected to the dehydrogenation conditions.[2]

The method of synthesizing II is outlined by the following series of reactions.[3] Compound VIII



where the radical R is 4,5,6,7-tetrahydroindazole-3

(1) H. R. Snyder, C. B. Thompson and R. L. Hinman, THIS JOURNAL, **74**, 2009 (1952).

(2) This observation is consistent with the finding of J. D. Roberts and W. F. Gorham, *ibid.*, **74**, 2278 (1952), that tetrahydropentalene failed to dehydrogenate to pentalene.

(3) This synthetic approach was proposed by Dr. Reuben G. Jones.