cerning the significance of isosbestic points have appeared.[18,56]

Acknowledgments.—The authors wish to express their gratitude to Dr. George B. Brown for his advice and encouragement, and to Dr. Jack J. Fox for valuable discussions.

(56) J. J. Fox and D. Shugar, *ibid.*, **9**, 369 (1952).

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Synthesis of 5-Substituted Pyrimidines *via* Formaldehyde Addition[1]

BY RICHARD E. CLINE, ROBERT M. FINK AND KAY FINK

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A reinvestigation of the synthesis and properties of 5-hydroxymethyluracil has revealed that this compound is not unstable and can be prepared in 80% yield by the simple addition of formaldehyde to uracil. The 5-hydroxymethyl compounds, including those prepared from orotic acid, uridine and deoxyuridine, were oxidized to 5-formyl- and 5-carboxypyrimidines, hydrogenated to 5-methyl-, 5-hydroxymethyldihydro- and 5-methyldihydropyrimidines and condensed with alcohols and acids (including amino acids). Radioactive 5-hydroxymethyl derivatives of uracil and uridine were used in biological studies.

Formaldehyde and its addition products with pyrimidines are presently of considerable interest in connection with problems of nucleic acid metabolism. Wyatt and Cohen identified 5-hydroxymethylcytosine as a unique constituent of the DNA of T-even bacteriophage,[2] and Flaks and Cohen noted that phage-infected *E. coli* contain enzymes which can utilize formaldehyde to convert deoxycytidylic acid to the 5-hydroxymethyl derivative, or deoxyuridylic acid to thymidylic acid.[3] The latter transformation has also been studied by Friedkin using extracts of normal *E. coli*,[4] and a similar formation of thymidine from formaldehyde and deoxyuridine has been found to occur with extracts of thymus gland.[5,6] The addition product of formaldehyde with tetrahydrofolic acid appears to be an important cofactor in these biosyntheses. The conversion of 5-hydroxymethylcytosine to thymine has been observed in various bacteria.[7] Thymine biosynthesis has been reviewed recently.[8]

In a previous communication from this Laboratory[9] it was reported that incubation of C^{14}-labeled thymine with rat liver slices led to the formation of 5-hydroxymethyluracil (5-HMU) and 5-methyluridine as radioactive products. Because of puzzling literature reports concerning the preparation and properties of such compounds their proper identification required initiation of the studies reported in this paper. For example, it was surprising to find that 5-HMU prepared by deamination of 5-hydroxymethylcytosine was stable to prolonged boiling in aqueous solution, for the instability of 5-HMU and some other 5-hydroxymethylpyrimidines, in contrast to 6-hydroxymethylpyrimidines, appeared well documented.[10] Johnson and Litzinger, believing the lability of 5-HMU prevented its preparation from uracil and formaldehyde, devised a round-about synthesis involving deamination of thyminylamine,[11] although Kircher had been able to make 5-hydroxymethyl-6-methyluracil in good yield from formaldehyde and 6-methyluracil.[12]

The addition of formaldehyde to uracil (I) was reinvestigated with the aid of chromatographic and other techniques which were unavailable to the earlier workers and which greatly facilitate the detection and isolation of products present in complex reaction mixtures. After a mixture of paraformaldehyde and I were allowed to stand in warm 0.4 *N* KOH, there was obtained a 70–80% yield of product showing the elementary analysis expected for 5-HMU (II) and having R_f values which were identical with those of the 5-hydroxymethylcytosine deamination product in all chromatographic solvents tested. A lower yield and more by-products were obtained when the reaction was carried out in acidic media. Stability studies described in the Experimental section showed that although extreme conditions were generally required to reverse the simple addition of formaldehyde to pyrimidines, such as the conversion of II to I, much milder conditions sufficed to bring about self-condensation reactions of hydroxymethylpyrimidines with the evolution of a little formaldehyde.

The infrared absorption spectrum of II was measured by Ulbricht and interpreted as being consistent with the presence of the hydroxymethyl group.[13] Comparison of the ultraviolet absorption

(1) For a preliminary report on portions of this work see R. M. Fink, R. E. Cline and K. Fink, *Federation Proc.*, **15**, 251 (1956). A later report was presented before the Division of Organic Chemistry at the 133rd Meeting of the American Chemical Society, San Francisco, California, April, 1958. Financial support was provided in part by U. S. Public Health Service Grant C-1669 and by Cancer Research Funds of the University of California.

(2) G. R. Wyatt and S. S. Cohen, *Biochem. J.*, **55**, 774 (1953).

(3) J. G. Flaks and S. S. Cohen, *Biochim. et Biophys. Acta*, **25**, 667 (1957).

(4) M. Friedkin, *Federation Proc.*, **16**, 183 (1957).

(5) R. L. Blakley, *Biochim. et Biophys. Acta*, **24**, 224 (1957).

(6) E. A. Phear and D. M. Greenberg, THIS JOURNAL, **79**, 3737 (1957).

(7) F. Weygand, A. Wacker, A. Trebst and O. Swoboda, *Z. Naturforsch.*, **12b**, 184 (1957).

(8) M. Friedkin and A. Kornberg, in W. D. McElroy and B. Glass, "The Chemical Basis of Heredity," Baltimore, Md., 1957.

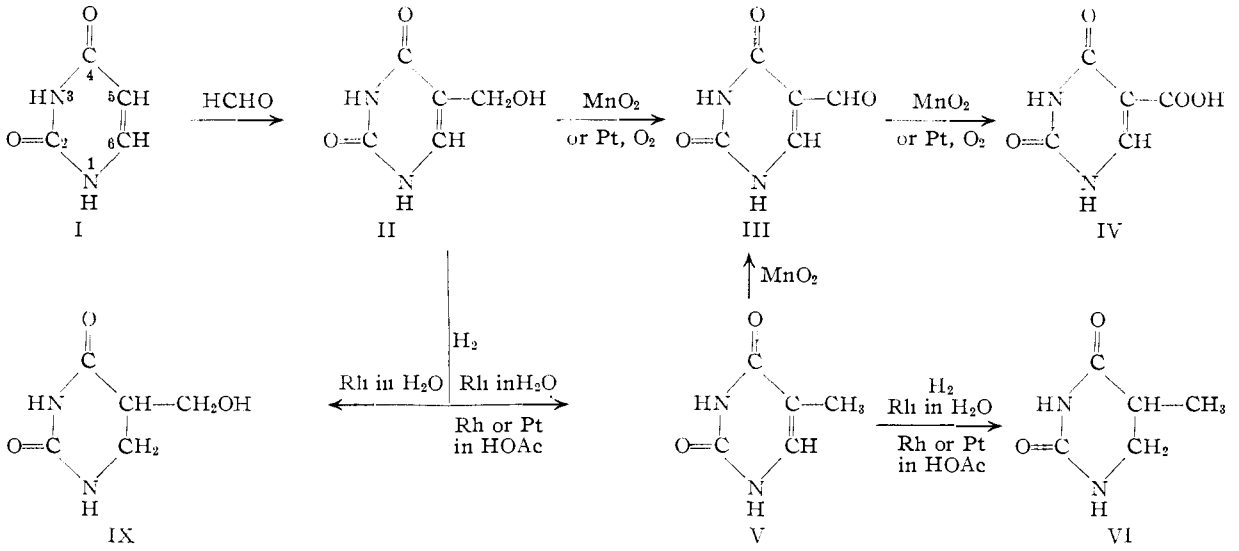
(9) R. M. Fink, R. E. Cline, R. B. Henderson and K. Fink, *J. Biol. Chem.*, **221**, 425 (1956).

(10) A. Bendich, "The Nucleic Acids," Vol. I, Academic Press, Inc., New York, N. Y., pp. 90–93.

(11) T. B. Johnson and A. Litzinger, THIS JOURNAL, **58**, 1940 (1936).

(12) W. Kircher, *Ann.*, **385**, 293 (1911).

(13) We are indebted to Dr. T. L. V. Ulbricht of Yale University for infrared data on 5-HMU (II). In common with two other 5-hydroxymethylpyrimidines, II shows a hydrogen-bond hydroxyl stretching vibration at 2.9 μ and a C–O vibration at 9.9 μ characteristic of pri-



data of the addition product II with similar data of Miller[14] on the deamination products of thyminylamine and 5-hydroxymethylcytosine (Table I) suggested that the three preparations were identical except for slight differences in purity. The addition of formaldehyde to I could conceivably produce four mono-hydroxymethyl derivatives (1-, 3-, 5-, or 6-HMU), of which only one, 5-HMU, was available for direct comparison. The ultraviolet data, however, could serve to distinguish between II and 1-HMU, for the large wave length shift in the absorption maximum of II (Table I) in changing from acidic to alkaline solutions is characteristic of pyrimidines unsubstituted in the 1-position.[15]

Replacement of the H at position 5 of pyrimidines with a hydroxymethyl group appears rather consistently to produce a bathochromic shift in the absorption peak that is about one-half as large as the shift produced by replacement with a methyl group. Thus in 0.01 *N* HCl the maxima of I, II and V are 258.5, 261 and 264 mμ, respectively. The data of Table II show opposite chromatographic effects produced by 5-substitution with hydroxymethyl and methyl groups, the former lowering R_f values and the latter raising them.

Two oxidation products were prepared from II, namely, 5-carboxyuracil (IV) identified chromatographically, and a new substance, 5-formyluracil or uracil-5-aldehyde (III) isolated in crystalline form. Although the acid IV was the major product produced when manganese dioxide was employed as oxidant, the oxidation appeared largely to stop at the aldehyde stage III when platinum and oxygen in 50% acetic acid were used as oxidant. The former oxidant converted thymine (V) to IV plus traces of III. If the addition of formaldehyde to I had provided 6-HMU, subsequent oxidation might have given orotic acid, a compound easily differentiated from IV by paper chromatography. The aldehyde III had an ultraviolet peak at 275 mμ in dilute HCl, gave a yellow color with the aldehyde reagent, dianisidine in acetic acid[16] and could be hydrogenated to thymine (V) in the presence of platinum oxide.

Hydrogenation of II with the use of platinum oxide in 50% acetic acid provided a 95% yield of thymine (V), which was differentiated by paper chromatography from the expected reduction products of other hydroxymethyluracils, namely, 1-methyl-, 3-methyl- and 6-methyluracil. Further reduction of V followed by hydrolyses led to dihydrothymine (VI), β-ureidoisobutyric acid (VII) and β-aminoisobutyric acid (VIII), which were identified chromatographically with the aid of previously described spray reagents.[17] The formation of VIII instead of β-alanine (two amino acids easily separated by paper chromatography) helped to differentiate II from 3-HMU, for in the sequence leading to VIII a 3-substituent would be lost.

While only V and VI were detected as products of the platinum catalyzed hydrogenation of II,[1] Green, Barner and Cohen[18] observed the formation of an additional product, IX, with the use of a rhodium–alumina catalyst in water for the hydrogenation of 5-HMU obtained from the deamination of 5-hydroxymethylcytosine. Our 5-HMU (II) also yielded IX in addition to V and VI when the re-

mary alcohols such as benzyl alcohol. In other regions II has spectral features in common with uracil and thymine as well. See T. L. V. Ulbricht, *Naturwissenschaften*, in press.

(14) The authors are grateful to Dr. Charles S. Miller of Sharp and Dohme for a sample of 5-hydroxymethylcytosine synthesized as described in THIS JOURNAL, **77**, 752 (1955), and for data on 5-HMU prepared by two methods.

(15) J. J. Fox and D. Shugar, *Biochim. et Biophys. Acta*, **9**, 369 (1952); D. Shugar and J. J. Fox, *ibid.*, **9**, 199 (1952).

(16) F. Feigl, "Spot Tests," Vol. II, Elsevier Publishing Co., New York, N. Y., 1954, pp. 148–151.

(17) R. M. Fink, R. E. Cline, C. McGaughey and K. Fink, *Anal. Chem.*, **28**, 4 (1956).

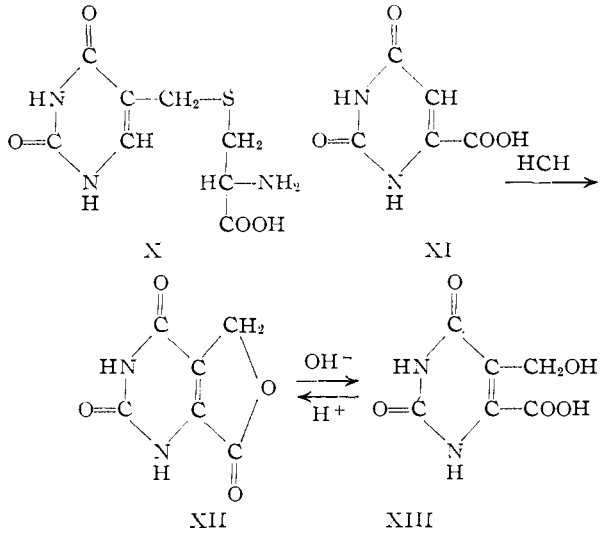
(18) M. Green, H. D. Barner and S. S. Cohen, *J. Biol. Chem.*, **228**, 621 (1957).

duction was carried out under the latter conditions, and it was found that the *p*H of the solvent appears to be important in determining whether the rhodium–alumina hydrogenation of II occurs first at the ring double bond (to give the hydrogenation-resistant IX) or first at the hydroxymethyl group (to give V, which is easily reduced further to VI). For example, from chromatographic estimation, it appeared that IX was formed in approx. 50% yield when the rhodium–alumina catalyst was used in water, in much lower yield in 5% acetic acid, and hardly at all in 50% acetic acid. The 5-hydroxymethylpyrimidine nucleosides showed similar *p*H effects on hydrogenation.

Ethers prepared from 5-hydroxymethylpyrimidines are reminiscent of glucosides with regard to ease of formation (by refluxing in alcohol containing a little concd. HCl) and hydrolysis; and they are similar to benzyl ethers in being readily cleaved by hydrogenation. It is noteworthy that the members of a homologous series of alkoxy derivatives of 5-HMU are well separated by paper chromatography although melting points are close together (Tables I and II); therefore 5-HMU may be of value in the identification of alcohols. Esters of 5-HMU were also easily prepared and hydrolyzed. Treatment of 5-HMU with cysteine and homocysteine in 2 *N* HCl produced compounds having properties expected for thioethers, the cysteine derivative giving the correct elementary analysis for X. These derivatives gave a purple color with ninhydrin and displayed absorption spectra (Table I) with longer wave length maxima than shown by 5-HMU. By contrast, conversion of 5-HMU to oxygen ether derivatives did not change the wave length maxima (Table I). A thyminylamine derivative may have been obtained on heating 5-HMU with glycine in concd. aqueous solution, for this product yielded a yellow color with ninhydrin, had essentially the same ultraviolet absorption spectra (acid and alkali) as 5-HMU and could be converted by hydrogenation to glycine, thymine and dihydrothymine.

When a mixture of orotic acid (XI) and paraformaldehyde in water was refluxed for many hours the solids gradually dissolved. A chromatogram prepared from the resulting solution and viewed under an ultraviolet lamp then showed a product with a white fluorescence, and the fluorescence could be intensified by exposure of the chromatogram to ammonia fumes. This product was isolated in crystalline form and appeared to be the lactone of 5-hydroxymethylorotic acid (XII) on the basis of elementary analysis, saponification equivalent, ultraviolet spectra, chromatographic properties and its conversion to 5-methylorotic acid by platinum oxide hydrogenation. After mild alkaline hydrolysis of XII, a new spot appeared on chromatograms which is probably the uncyclized 5-hydroxymethylorotic acid (XIII), for this spot had a lower R_f value than XI in weakly acidic solvents and could be made fluorescent (presumably because of recyclization to XII) by spraying with dilute HCl followed by drying and exposing to ammonia fumes.

Addition of formaldehyde to pyrimidine nucleosides required somewhat more vigorous conditions



X XI XII XIII

than addition to 1-unsubstituted pyrimidines. After refluxing a mixture of uridine and formaldehyde in 0.5 *N* HCl for 24 hours, 5-hydroxymethyluridine was isolated in 20% yield with the aid of ion-exchange resin. Hydrogenation of this product yielded 5-methyluridine which appears to be identical, on the basis of a mixed melting point and ultraviolet absorption spectra (Table I), with the 5-methyluridine synthesized by Fox[19] from thymine and ribose. Hydrolysis of the hydrogenation product gave thymine on chromatograms. Other derivatives of 5-hydroxymethyluridine, prepared essentially as described for 5-HMU and detected by chromatography, were two ethers, two dihydropyrimidines and an oxidation product.

Treatment of uridine with formaldehyde in 6 *N* HCl at room temperature for several days led to the formation of 5-hydroxymethyluridine and an interesting by-product, with yields estimated chromatographically as approx. 50% for each compound. Indications that the by-product was a derivative of 5-hydroxymethyluridine were provided by the following studies conducted on a microgram scale: (a) the by-product had absorption spectra (Table I) closely resembling those of 5-hydroxymethyluridine; (b) the by-product could be hydrolyzed to 5-hydroxymethyluridine and 5-HMU; (c) it could be oxidized in the presence of platinum (as described for 5-HMU) to an unknown ultraviolet absorbing compound giving a characteristic aldehyde reaction with dianisidine; and (d), the by-product could be hydrogenated in the presence of platinum oxide. The hydrogenated by-product, which could be hydrolyzed to thymine, was also obtained by treatment of 5-methyluridine with formaldehyde in 6 *N* HCl at room temperature. The high R_f values of the by-product in all solvents including one containing boric acid (see Experimental and Table II) suggest that it has a ribose moiety combined with or altered by formaldehyde. The ribosides of pyrimidines (uracil, thymine, 5-HMU, cytosine) were found to differ from most available pyrimidine

(19) Data and a sample of 5-methyluridine were kindly contributed by Dr. J. J. Fox of Sloan-Kettering. For the synthesis of this compound and a comparison of its properties with the properties of preparations by others see: J. J. Fox, N. Yung, J. Davoll and G. Brown, THIS JOURNAL, **78**, 2117 (1956).

TABLE I

PROPERTIES OF 5-SUBSTITUTED PYRIMIDINES[a]

Pyrimidine	M.p., °C.	Spectra in HCl: pH	λ_{max}	λ_{min}	ϵ_{max}	ϵ_{min}	250/260	280/260	Spectra in NaOH: pH	λ_{max}	λ_{min}	ϵ_{max}	ϵ_{min}	250/260	280/260
5-HMU (II)	300d	2	261	231	8000	1940	0.77	0.32	12	286	245	7375	1900	0.62	1.94
5-Methoxymethyluracil	203	2	261	230.5	7950	1870	.81	.31	12	285.5	244.5	8275	1680	.59	2.22
5-Ethoxymethyluracil	212	2	261	230.5	8080	2010	.78	.30	12	286	244	8380	1500	.57	2.35
5-*n*-Butoxymethyluracil	215	2	261	231	8080	1925	.79	.31	12	286	244.5	8450	1640	.58	2.28
5-Formyluracil (III)	305d	2	275.5	248	11850	3500	.51	1.57	12	297.5	267	15700	3850	1.51	1.30
Lactone (XII)[b]	300d	2	282.5	242.5	7060	1610	.57	2.05	12	288	248	9100	1575	0.59	3.00
5-Hydroxymethyluridine	167	2	264	233	9450	2325	.70	0.52	12	263.5	243.5	6950	4950	.79	0.45
Uridine–formaldehyde by-product		2	263	233			.74	.44	12	262	246			.87	.41
5-Hydroxymethyldeoxyuridine	176	2	264	233	9600	2200	.68	.52	12	264	243	7030	4800	.78	.44
5-Hydroxymethyldeoxyuridine[c]		1.7	264	234			.69	.53	12.3	264.5	244			.78	.53
5-Methyluridine	183	2	266.5	234.5	9300	2200	.65	.71	12	266.5	245.5	7090	4600	.74	.67
5-Methyluridine[d]	183	3	267.5	235	9880	2470	.65	.72	12	267.5	245	7370	4720	.75	.69
5-HMU (II)		1	260.5	230	7900	1750	.77	.28	13	285.5	245.5	7250	2100	.64	1.90
5-HMU prepd. from HMC[e]		1	261	231	9520	2420			13	285.5	245.5	8570	2440		
5-HMU prepd. from thyminylamine[f]		1	261	231	8510	2100			13	285.5	245.5	7680	2190		
5-Hydroxymethyl-6-methyluracil[g]	Over 300	1	263.5	233	8950	2050	.69	.43	13	284.5	246	7260	2450	.62	1.66
S-*Thyminylcysteine (X)*	*241*	2	*265.5*	*237*	*7610*	*2950*	.71	.64	*12*	*291.5*	*252*	*6850*	*2425*	*.83*	*1.93*
S-Thyminylhomocysteine	261	2	266	237	6375	2575	.71	.67	12	291.5	252	6140	2240	.87	1.89

[a] Spectra were determined with a model DU, Beckman spectrophotometer. The ratios 250/260 and 280/260 are ratios of absorbances at those wave lengths. [b] Lactone ring probably opened at *p*H 12. [c] Sample furnished by M. Friedkin.[23] [d] Data of J. J. Fox.[19] [e,f] Data of C. S. Miller[14] on products prepared from 5-hydroxymethylcytosine by deamination and from thyminylamine using Johnson's procedure[11]; both products had the same infrared spectrum and the thyminylamine product contained 0.19% ash. [g] Prepared by Kircher's alkaline method.[12]

bases and deoxyuridine in giving by-products, having higher R_f values than the starting compounds in the *sec*-butyl alcohol solvent, on treatment with formaldehyde in 6 *N* HCl at room temperature.

The evidence now indicates that 5-methyluridine synthesized in two steps starting with uridine and formaldehyde is identical with two or more biological preparations. Previous work in this Laboratory[9] demonstrated its identity with a radioactive metabolite obtained by incubation of radiothymine in the presence of rat liver slices. Its identity with a compound prepared by Lampen by the action of a nucleosidase of *E. coli* on a mixture of thymine and D-ribosyl phosphate[20] may be inferred from the data of Fox[19] relating his 1-β-D-ribofuranosylthymine to Lampen's preparation, together with above evidence relating the formaldehyde derived product to Fox's compound. Different properties were noted[19] for the thymine–ribose product of Roberts and Visser.[21] Littlefield and Dunn have recently reported the presence of 5-methyluridine in hydrolysates of RNA from yeast and bacteria.[22]

After treatment of deoxyuridine with formaldehyde in strong HCl at near room temperature 5-hydroxymethyldeoxyuridine was isolated in 19% yield with the aid of ion-exchange resin. This product was found to have chromatographic properties and ultraviolet absorption spectra (Table I) essentially the same as a product prepared enzymically by Friedkin[23] from deoxyribose and 5-HMU. Thymidine was detected in high yield after hydrogenation of the deoxyuridine–formaldehyde product using the rhodium–alumina catalyst. Two other reduction products and an oxidation product of the hydroxymethyl compound have been prepared in microgram quantities.

Two radioactive hydroxymethylpyrimidines have been incubated with rat liver slices and the products detected on radioautographs of chromatograms. Labeled 5-HMU, prepared from formaldehyde and 2-C^{14}-uracil, was found to be metabolized to 5-carboxyuracil, 5-hydroxymethyluridine and an unknown compound with chromatographic properties which suggest that it may be a condensation product of 5-HMU with an amino acid. The radioautographs did not reveal the presence of thymine or any of the available thymine derivatives. After incubation of 5-hydroxymethyluridine, prepared from uridine and C^{14}-formaldehyde, only radioactive 5-carboxyuracil and 5-HMU were identified as metabolites.

Experimental[24]

5-Hydroxymethyluracil (II). (a) **Base Catalysis.**—To 500 ml. of 0.42 *N* KOH was added 36 g. (0.32 mole) of uracil and 12 g. of paraformaldehyde (0.4 mole of formaldehyde) and the mixture allowed to stand 73 hr. at 50°. After dilution with 1400 ml. of water the solution was stirred with 120 g. of freshly washed Dowex-50 (H form, 100–200 mesh) and filtered to remove resin and a little insoluble product. The slightly acidic filtrate was concentrated by vacuum distillation to a volume of 80 ml., refrigerated and filtered to remove product. After recrystallization from 200 ml. of water, 36.6 g. (0.26 mole, 81%) of fine powder was obtained, showing a very minor amount of impurity by chromatography. Final recrystallization from acetone and water (2:1) afforded microcrystals decomposing at 260–300°.

Anal. Calcd. for $C_5H_6N_2O_3$: C, 42.25; H, 4.26; N, 19.72. Found: C, 42.24; H, 4.29; N, 19.89.

(b) **Acid Catalysis.**—A mixture consisting of 2.24 g. (20 mmoles) of uracil, 1.20 g. of paraformaldehyde (40 mmoles of formaldehyde) and 15 ml. of 0.5 *N* HCl was refluxed for 25 hr. After dilution with 200 ml. of water the

(20) J. O. Lampen, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," Vol. II, The Johns Hopkins Press, Baltimore, Md., 1952, p. 368.

(21) M. Roberts and D. W. Visser, THIS JOURNAL, **74**, 668 (1952).

(22) J. W. Littlefield and D. B. Dunn, *Nature*, **181**, 254 (1958). Dr. Dunn, in a personal communication, stated that they had obtained a sample of Fox's synthetic compound and found that it corresponded with their 5-methyluridine isolated from RNA.

(23) The authors wish to thank Dr. Morris Friedkin of Washington University, St. Louis, for data and a sample of 5-hydroxymethyldeoxyuridine prepared enzymically.

(24) Analyses were performed by Dr. Adalbert Elek, Los Angeles, Calif. Melting points are corrected and were determined on a Fischer–Johns melting point block.

mixture was heated to boiling and filtered to remove 1.2 g. of highly insoluble material which was discarded. The filtrate was added to a Dowex-1 column (80 g., 100–200 mesh, OH form, 1.8 cm. diameter), the column was washed thoroughly with water to remove formaldehyde, and the product was then eluted slowly with 0.012 *N* HCl (product emerged from column after 10–15 liters of effluent had been collected). On concentration by vacuum distillation of the appropriate eluate fraction, there was isolated 769 mg. (5.4 mmoles, 27%) of 5-HMU shown to be about 95% pure by chromatographic and spectrophotometric techniques.

5-Hydroxymethyluridine.—A mixture consisting of 1.22 g. (5 mmoles) of uridine, 0.3 g. of paraformaldehyde (10 mmoles of formaldehyde) and 4 ml. of 0.5 *N* HCl was refluxed 24 hr. and then fractionated on a Dowex-1 column as described for the isolation of 5-HMU. That portion of the eluate shown chromatographically to contain largely 5-hydroxymethyluridine was concentrated by vacuum distillation, alternately diluted with ethanol and concentrated several times, and finally diluted with ethyl acetate and ether to bring about crystallization of the product. The yield was 282 mg. (1 mmole, 20%) of 5-hydroxymethyluridine contaminated with a very small amount of uridine, as shown by chromatography. Recrystallization from ethanol and ether provided 146 mg. of needles melting at 167–168°.

Anal. Calcd. for $C_{10}H_{14}N_2O_7$: C, 43.79; H, 5.14; N, 10.21. Found: C, 44.10; H, 5.14; N, 9.52.

5-Hydroxymethyldeoxyuridine.—To 1.2 ml. of 3 *N* HCl was added 0.4 g. (1.75 mmoles) of deoxyuridine and 0.4 g. of paraformaldehyde (13.3 mmoles of formaldehyde), and the mixture incubated at 50° for four days. Deoxyuridine, 5-hydroxymethyldeoxyuridine and an unknown compound in the ratios of 1:4:2 were then detected on a chromatogram as ultraviolet spots giving color with the Dische reagent.[25] The mixture was combined with two similar reaction mixtures, absorbed on a Dowex-1 column (OH form, 100–200 mesh, 130 g., 2.5 × 51 cm.), washed well with water and eluted with 0.012 *N* HCl. The appropriate portion of the elutate was concentrated by vacuum distillation and alternately diluted with ethanol and taken to near dryness until addition of ethyl acetate to the alcohol solution precipitated the product as a fine powder. The yield was 250 mg. (0.97 mmole, 19%) based on 1.2 g. of deoxyuridine, the total starting material involved in the combined reaction mixtures. The product melted at 170–174° and showed the presence of very minor amounts of impurities by chromatographic analysis. Recrystallization from ethanol, ethyl acetate and a few drops of water furnished 110 mg. of microcrystals melting at 176–179°.

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.64; H, 5.55; N, 11.26.

Ester and Ether Derivatives of 5-HMU (II).—To 50 ml. of each alcohol or acid was added 0.1 ml. of concd. HCl and 400 mg. of 5-HMU. The mixture was refluxed, diluted with ether and pet. ether, refrigerated and filtered to remove the microcrystalline product. The alcohols or acids used, hours of reflux, yields before recrystallization and melting points of recrystallized products were, respectively: methanol, 2, 36%, 203–204°; ethanol, 24, 68%, 212–213°; *n*-butanol, 0.5, 56%, 215–217°; acetic acid, 1, 71%, over 300°; propionic acid, 1, 47%, over 300°. These derivatives were all easily degraded to 5-HMU by refluxing with water, the time required for complete degradation of 5-methoxymethyluracil in 0.01 *M* solution being 6–8 hours. The 5-ethoxymethyluracil gave thymine on reduction with platinum oxide and hydrogen.

Anal. of 5-ethoxymethyluracil. Calcd. for $C_7H_{10}N_2O_3$: C, 49.43; H, 5.92; N, 16.46. Found: C, 48.98; H, 5.95; N, 16.09.

Amino Acid Derivatives of 5-HMU (II).—A solution of 142 mg. (1 mmole) of 5-HMU and 157 mg. (1 mmole) of cysteine hydrochloride in 5 ml. of 2 *N* HCl was incubated at 50° for 44 hr. After cooling and filtering, S-thyminylcysteine (202 mg., 0.82 mmole, 82%) was collected as a fine powder. The product, recrystallized from ethanol and water, melted at 241–242° and appeared on paper chromatograms as an ultraviolet spot giving a purple color with ninhydrin.

Anal. Calcd. for $C_8H_{11}N_3O_4S$: C, 39.17; H, 4.52; N, 17.13; S, 13.07. Found: C, 39.59; H, 4.38; N, 16.58; S, 12.49.

Substitution of homocysteine in the above procedure led to the isolation of a powder melting at 261–262° having chromatographic and spectrophotometric properties indicative of S-thyminylhomocysteine.

Hydrogenation of Hydroxymethylpyrimidines. (a) **5-HMU (II).**—To 10 ml. of 50% acetic acid was added 56.8 mg. (0.4 mmole) of 5-HMU and an equal weight of platinum oxide. After hydrogenation at 1.5 atm. for 1 hr. in a Parr apparatus, a paper chromatogram showed much unreduced 5-HMU, so an additional 57 mg. of fresh catalyst was added and hydrogenation resumed for 9 hr. at 3 atm. A 95% yield of thymine was then determined by paper chromatographic and spectrophotometric techniques. Further reduction of 5-HMU to dihydrothymine was brought about by using about 10 times as much platinum catalyst and double the reaction time used for thymine preparation. Dihydrothymine was formed in near quantitative yield as shown by chromatography when 80 mg. of 5-HMU in 20 ml. of 50% acetic acid was added to 100 mg. of 5% rhodium on alumina catalyst (Baker and Co. Inc., Newark) and hydrogenated at 3.3 atm. for 5 hr. When water was used in place of the acetic acid in this reduction about equal amounts of two dihydropyrimidines were detected on chromatograms, dihydrothymine and 5-hydroxymethyldihydrouracil, the latter having properties in agreement with those reported by Green, Barner and Cohen.[18] Dihydropyrimidines were detected on chromatograms by spraying with alkali followed by Ehrlich's *p*-dimethylaminobenzaldehyde reagent.[17]

(b) **5-Hydroxymethyluridine.**—To 137 mg. (0.5 mmole) of 5-hydroxymethyluridine in 50 ml. of 50% acetic acid was added 100 mg. of platinum oxide, and the mixture was hydrogenated in a Parr apparatus for 15 minutes at 1.5 atm. pressure. The catalyst was removed by filtration and the filtrate, shown by chromatography to contain 5-methyluridine and a very minor amount of dihydropyrimidine, was taken to dryness by vacuum distillation. The residue was crystallized from ethanol, ether and petr. ether, providing 63 mg. (0.24 mmole, 48%) of small needles melting at 182–183°. A mixed melting point with a sample of 5-methyluridine furnished by Fox[19] showed no depression.

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46. Found: C, 47.09; H, 5.56.

Following hydrolysis of the product in 4 *N* HCl at 100° for 22 hr. thymine was identified on chromatograms. After hydrogenation of 1 mg. of hydroxymethyluridine and an equal weight of the rhodium–alumina catalyst in 0.8 ml. of water at 3 atm. for 3 hr., two products, presumably 5-hydroxymethyldihydrouridine and 5-methyldihydrouridine (about 50% yields), were detected on chromatograms by spraying with the dihydropyrimidine reagent combination.[17]

(c) **5-Hydroxymethyldeoxyuridine.**—Hydrogenation of 1 mg. of 5-hydroxymethyldeoxyuridine and an equal weight of the rhodium–alumina catalyst in 0.8 ml. of 50% acetic acid at 0.7 atm. for 30 min. produced thymidine (gave thymine on hydrolysis) as the major product and only traces of by-products detectable by paper chromatography. By substituting water for acetic acid in the hydrogenation conducted at 3 atm. for 3.3 hr., about equal amounts of two compounds giving typical color reactions for dihydropyrimidines[17] and deoxyribosides[25] were detected on chromatograms, one product being dihydrothymidine and the other being most likely 5-hydroxymethyldihydrodeoxyuridine.

Oxidation of Hydroxymethylpyrimidines. (a) **5-HMU (II).**—Following a procedure similar to that used in the oxidation of a pyridine alcohol,[26] manganese dioxide (80 g., 93.1%, powder, J. T. Baker Chemical Co. lot No. 8246) was kept suspended for 51 hr. by mechanical stirring in 800 ml. of 0.2 *N* HCl containing 8 g. (56 mmoles) of 5-HMU. The oxide was removed by filtration, the filtrate concentrated to 180 ml. by vacuum distillation, cooled and filtered to remove 4.3 g. of crude product demonstrated by paper chromatography to consist of 5-formyluracil, 5-carboxyuracil and unreacted 5-HMU in approx. ratios of 3:4:1, resp. Two recrystallizations from acetone and water provided material with a ratio of formyluracil to carboxyuracil of about 7:1. The mixture was added to a column of Dowex-1 (acetate form, 50 g., 100–200 mesh), washed with

(25) J. G. Buchanan, *Nature*, **168**, 1091 (1951).

(26) D. Metzler, M. Ikawa and E. E. Snell, THIS JOURNAL, **76**, 648 (1954).

water and eluted with 10% acetic acid. The crystals obtained on concentration of the appropriate eluate fractions weighed 594 mg. (4.2 mmoles, 7.5%) but still contained a little carboxyuracil. After recrystallization from water and then from 5% acetic acid, 345 mg. (2.5 mmoles, 4.5%) of tiny prisms of 5-formyluracil was obtained which decomposed above 300° (darkened below 300°) and showed no impurities on chromatograms examined under the ultraviolet lamp and sprayed with the aldehyde reagent, dianisidine.[16]

Anal. Calcd. for $C_5H_4N_2O_3$: C, 42.86; H, 2.87; N, 19.99. Found: C, 42.37; H, 3.30; N, 19.89.

A higher yield of 5-formyluracil was indicated by chromatographic analysis after oxidation of 5-HMU following the method described by Sneeden and Turner.[27] Platinum oxide (25 mg.) in 2 ml. of 50% acetic acid was hydrogenated in a test-tube held in the bottle of a Parr apparatus at 3.3 atm. for 1 hr. The tube was removed and oxygen was bubbled slowly into the mixture for 30 min., after which 4 mg. of 5-HMU was added, and slow oxygenation continued for 18 hr. A chromatogram then showed the presence of 5-formyluracil with only traces of 5-HMU and 5-carboxyuracil. When water was substituted for 50% acetic acid in this oxidation the major product (about 80%) was 5-carboxyuracil.

(b) Other 5-Hydroxymethylpyrimidines.—Using the above platinum–acetic acid procedure all available 5-hydroxymethylpyrimidines have been converted to dianisidine-reacting products; the R_f values of those assumed to be 5-formylpyrimidines are found in Table II. The production of 5-carboxypyrimidines by this procedure appeared to be inappreciable. The 5-hydroxymethyl derivatives of orotic acid, cytosine and deoxyuridine each furnished only one chromogenic product; 5-hydroxymethyluridine provided two such products, of which the one obtained in larger yield had chromatographic properties which would be expected for 5-formyluridine; and 5-hydroxymethyl-6-methyluracil formed two products neither of which is likely to be a 5-formyl derivative.

Lactone (XII) of 5-Hydroxymethylorotic Acid.—In 400 ml. of water was dissolved 15.6 g. (0.1 mole) of orotic acid and 20 g. of paraformaldehyde (0.66 mole of formaldehyde) by refluxing for 40 hr. The solution was concentrated to a volume of 50 ml. by vacuum distillation and 4.0 g. of crude product was removed by filtration. Product and filtrate were fractionated separately on a Dowex-2 column (acetate form, 200 g., 100–200 mesh), the procedure involving thorough washing of the column with water to remove formaldehyde, followed by elution with 5% acetic acid. The lactone was isolated from the appropriate eluate fractions by vacuum distillation followed by cooling and filtration. It was recrystallized from 2% acetic acid and acetone (1:2), providing 1.35 g. (0.008 mole, 8%) of chromatographically pure needles melting over 300°.

Anal. Calcd. for $C_6H_4N_2O_4$: C, 42.87; H, 2.39; N, 16.66. Found: C, 43.20; H, 2.63; N, 16.66; mol. wt., 168; sapon. equiv., 169.

TABLE II

Pyrimidines	R_f values in various solvents: *sec*-Bu[a]	Formic[b]	Ammonia[c]	HCl[d]	Phenol[e]
Uracil (I)	0.54	0.50	0.36	0.70	0.81
5-Hydroxymethyluracil (II)	.45	.37	.34	.60	.72
Thymine (V)	.72	.59	.54	.88	.92
5-Formyluracil (III)	.47	.43	.54	.71	.77
5-Carboxyuracil (IV)	.08	.43	.08	.70	.34
5-Methoxymethyluracil	.66	.59	.49	.85	.90
5-Ethoxymethyluracil	.79	.72	.57	.96	.91
5-*n*-Butoxymethyluracil	.92	.88	.86	.92	.93
N-Thyminylglycine[f]	.10	.12	.11	.14	.53
S-Thyminylcysteine (X)	.06	.15	.14	.21	.54
S-Thyminylhomocysteine	.09	.22	.15	.34	.58
1-Methyluracil	.62	.65	.47	.82	.95
3-Methyluracil	.57	.49	.35	.72	.81
6-Methyluracil	.65	.59	.46	.79	.90
5-Hydroxymethyl-6-methyluracil	.43	.42	.38		.83
5,6-Dimethyluracil	.74	.67	.69	.91	.93
5,6-Dihydrouracil	.47	.50	.64	.73	.90
5-Hydroxymethyl-5,6-dihydrouracil (IX)[f]	.42	.41	.62	.71	.79
5,6-Dihydrothymine (VI)	.67	.65	.77	.89	.94
Orotic acid (XI)	.12	.34	.18	.78	.31
5-Hydroxymethylorotic acid (XIII)[f]	.13	.25	.20		.34
Lactone (XII)	.38	.45		.59	.83
5-Methylorotic acid	.18	.50	.26	.94	.45
5-Formylorotic acid[f]	.00	.15	.19		.20
Cytosine	.35	.44	.45	.35	.88
5-Hydroxymethylcytosine	.28	.38	.36	.35	.82
5-Methylcytosine	.43	.50	.50	.46	.93
5-Formylcytosine[f]	.40	.39	.49	.32	.89
Uridine	.40	.34	.33	.67	.73
Uridine–formaldehyde byproduct	.50	.50	.43	.78	.90
5-Hydroxymethyluridine	.31	.21	.28	.55	.64
5-Methyluridine	.54	.42	.40	.77	.82
5-Formyluridine[f]	.34	.30	.43	.62	.74
5-Methoxymethyluridine[f]	.47	.44	.37	.76	.86
5-Ethoxymethyluridine[f]	.64	.55	.51	.92	.89
5,6-Dihydrouridine	.31	.27	.52	.61	.76
5-Hydroxymethyl-5,6-dihydrouridine[f]	.27	.22	.49	.57	.67
5-Methyl-5,6-dihydrouridine[f]	.42	.37	.63	.73	.83
Deoxyuridine	.58	.49	.40	.80	.85
5-Hydroxymethyldeoxyuridine	.48	.35	.37	.70	.79
Thymidine	.71	.59	.55	.90	.90
5-Formyldeoxyuridine[f]	.53	.44	.62	.78	.85
5,6-Dihydrodeoxyuridine	.46	.44	.64	.68	.89
5-Hydroxymethyl-5,6-dihydrodeoxyuridine[f]	.42	.38	.62	.68	.82
5,6-Dihydrothymidine	.58	.54	.75	.83	.92

[a] Upper phase from a mixture of water and *sec*-butyl alcohol. [b] *t*-Butyl alcohol, methyl ethyl ketone, formic acid, water (40:30:15:15). [c] *t*-Butyl alcohol, methyl ethyl ketone, water, ammonium hydroxide (40:30:20:10). [d] *t*-Butyl alcohol, methyl ethyl ketone, water, hydrochloric acid (40:30:20:10). [e] Lower phase from phenol and water. [f] Tentative identification based largely on chromatographic behavior and color reactions.

The fluorescent lactone was hydrolyzed by allowing it to stand overnight in concd. ammonia at room temperature, yielding a product having properties consistent with structure XIII. In weakly acidic chromatographic solvents the hydrolytic product traveled as an ultraviolet spot with lower R_f value than orotic acid, and this spot could be made fluorescent by spraying with 0.5 *N* HCl, drying and exposing to ammonia fumes.

Stability of 5-Hydroxymethylpyrimidines.—Only a trace of uracil was found on a chromatogram run in the *sec*-butyl alcohol solvent after refluxing a 0.004 *M* aqueous solution of 5-HMU for 67 hr.; two ultraviolet absorbing spots at R_f's of 0.32 and 0.25 represented unknown products formed in estimated yields of 20 and 10%, resp. Only about a 10% yield of uracil was detected by chromatography after heating a sealed tube containing a 0.002 *M* solution of 5-HMU in 6 *N* HCl at 100° for 47 hr. The unknown compounds appear to be self-condensation products since they are produced in better yields, along with some highly insoluble materials, on heating more concentrated solutions of 5-HMU. After refluxing 8 ml. of aqueous 0.25 *M* 5-HMU for 16 hr. and then distilling to reduce the volume to 1.2 ml., chromatograms showed the presence of unknown

(27) R. P. A. Sneeden and R. B. Turner, THIS JOURNAL, **77**, 130, 190 (1955).

products (estimated 50% yield) but no uracil. However, a 0.6% yield of formaldehyde was obtained from this reaction by bubbling nitrogen into the solution throughout the heating period, passing the gases through a water trap (initial volume of 2 ml.) and assaying the trap solution with chromotropic acid.

After heating a sealed tube containing 0.004 *M* aqueous 5-hydroxymethyluridine at 100° for 65 hr., chromatograms revealed the formation of only traces of uridine and an unknown product. Using 0.002 *M* hydroxymethyluridine in 3 *N* HCl and a heating period of 24 hr., there was extensive breakdown to 5-HMU and three unknown compounds but no uridine or uracil. After heating sealed tubes containing 0.004 *N* 5-hydroxymethylcytosine in water solution or 0.002 *N* hydroxymethylcytosine in 0.1 *N* HCl at 100° for 24 hr., the breakdown products consisted of 5-HMU and unknown products but neither uracil nor cytosine. Sealed tubes containing 0.004 *M* aqueous solutions of 5-hydroxymethyl-6-methyluracil and the lactone of 5-hydroxymethylorotic acid (XII) were heated at 100° for 23 hr. By chromatography it was then estimated that about 40% of the first compound was degraded to 6-methyluracil, but none of the lactone was converted to orotic acid (XI) although much of it was hydrolyzed to XIII.

Paper Chromatography.—A filter paper sheet (Whatman No. 1, 20 × 22 cm.) was spotted 2.5 cm. from the edge and formed into a cylinder with the vertical edges of the sheet held nearly in contact by glass ringlets. The cylinder was then placed in a wide-mouth, half gallon fruit jar containing 50 ml. of solvent for ascending chromatography at a constant temperature of 28°. For two-phase solvent systems, the aqueous phase was placed in a 20-ml. beaker within the jar. Most of the pyrimidines were detected as dark spots on chromatograms held in front of an ultraviolet lamp (2537 Å.). Ribosides could generally be distinguished from other pyrimidine derivatives by their markedly lowered R_f values in the presence of borate,[28] employing *sec*-butyl alcohol saturated with 5% boric acid, and an inner beaker containing equal amounts of *sec*-butyl alcohol and 1.5 *N* ammonia.

Acknowledgment.—Valuable technical assistance has been provided by Philip Anthony, now at the University of Hawaii.

(28) I. A. Rose and B. S. Schweigert, THIS JOURNAL, **73**, 5903 (1951).

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[CONTRIBUTION FROM THE CHEMISTRY DIVISION, LAKESIDE LABORATORIES, INC.]

Aminolysis and Hydrazinolysis Products of N-Methyl-3-chloropiperidine. Non-mercurial Diuretic Agents

BY JOHN H. BIEL, WALLACE K. HOYA AND HELEN A. LEISER

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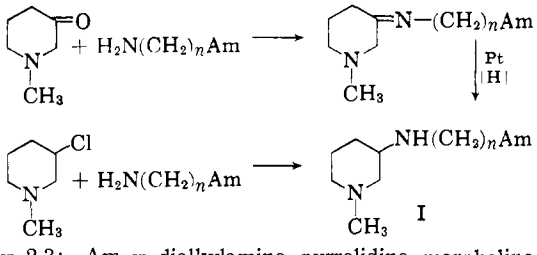
The initial finding that maleic acid had diuretic properties in dogs prompted the synthesis of a number of maleic acid salts of alkylenediamine derivatives. This paper deals with the nature of the reaction products obtained from the aminolysis, hydrazinolysis of N-methyl-3-chloropiperidine, the unequivocal synthesis of N-(1-methyl-3-piperidyl)-N,N′-disubstituted alkylenediamines and N-(1-methyl-3-piperidyl)-N′-(w-aminoalkyl)-hydrazines, and the physiologic properties of the maleate salts. Optimum diuretic properties were obtained with the 2-pyrrolidylmethylethylenediamines. The isomeric 3-piperidyl derivatives and the isosteric hydrazines were considerably less potent. The more active diuretic agents also displayed pronounced blood pressure lowering effects in the dog.

The finding in our laboratories that the diuretic effect of maleic acid could be markedly enhanced when the acid was administered in the form of certain diamine salts[1] prompted the synthetic investigation of a variety of alkylene diamines.

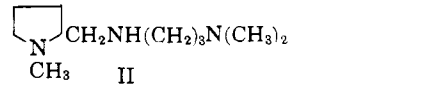
The present paper deals with the reaction of N-methyl-3-chloropiperidine with certain alkylene diamines as well as hydrazine, the nature of the reaction products and the pharmacologic properties of these new derivatives.

Two modes of preparation were employed in an effort to synthesize compounds of structure I

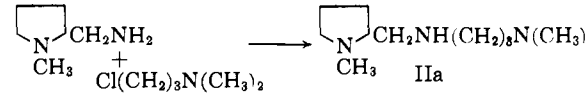


n = 2,3; Am = dialkylamino, pyrrolidino, morpholino, 4-methylpiperazino and *m*-chlorobenzylamino

Since the reaction products obtained from the condensation of 1-methyl-3-piperidone and 1-methyl-3-chloropiperidine with N,N-dimethylpropylenediamine were not identical as shown by their chemical and physiologic properties, we suspected that a ring contraction had occurred (in the case of the N-methyl-3-chloropiperidine reaction) to a pyrrolidylmethyl derivative (II). Such a phenomenon had been previously described by Reitsema[2] for N-ethyl-3-chloropiperidine, when the latter was allowed to react with such strongly basic nucleophilic agents as benzylamine and ammonia.



The structure of II was proved by treating 1-methyl-2-pyrrolidylmethylamine[2] with 3-dimethylaminopropyl chloride



The identity of product II and IIa was established *via* the mixed melting points of the picrate and maleate salts and the similarity of their physiologic properties: While product I produced a 53% increase in the diuretic response with a 50 mg./kg. dose (oral, dog), products II and IIa produced a 300-330% diuresis increase at the same dose.

A similar ring contraction was observed when 1-methyl-3-chloropiperidine was treated with hy-

(1) R. R. Rowland and P. A. Nuhfer, unpublished report.

(2) R. H. Reitsema, THIS JOURNAL, **71**, 2041 (1949).