using turbidity measurements and a 28–36 hr. growth period gave much less inhibition. That *L. casei* strains show markedly different sensitivities to potassium acetate inhibition has been discussed by Camien and Dunn.[7] These workers have indicated that strain differences may arise in this organism on normal subculture. The LLL tripeptide did not inhibit *L. casei* significantly.

TABLE I

CONCENTRATION FOR HALF-MAXIMUM INHIBITION OF GROWTH

| Compound | Mg. per ml. *S. plantarum* | Mg. per ml. *L. casei* | Mg. per ml. *L. citrovorum* mutant |
|---|---|---|---|
| L-Valyl-L-valyl-L-valine | N[a] | N | 0.6 |
| L-L-D[b] | N | See text | 0.05 |
| L-L-D (20 hr.) | 0.02–0.05 | See text | .. |
| L-D-D | N | 6 | 7 |
| D-D-D | N | 6 | N |
| D-L-D | N | N | N |
| L-L | N | S[c] | 2 |
| L-D | N | N | N |
| D-L | N | N | 3.5 |
| Phthalyl-L-L | 12 | 4 | 3 |
| Phthalyl-L-D | 8 | 4 | 8 |
| Phthalyl-D-L | 7 | 4 | 6 |
| Phthalyl-D-D | 8 | 2 | 6 |
| Phthalyl-L[d] | 6 ± 1 | 3 ± 1 | 3 ± 1 |
| Phthalyl-D | 4 | 2 | 3 |
| L-Methyl ester·HCl | N | S | 6 |
| D-Methyl ester·HCl | 9 | 7 | 8 |
| L-Valine | N | N | 2[d] |
| D-Valine | N | N | N |
| Glycine[e] | N | N | N |
| Amino acid mixture[f] | N | N | N |

[a] N = *no* inhibition at 8 mg./ml. [b] L-L-D represents L-valyl-L-valyl-D-valine, etc. [c] S = *slight* inhibition at 8 mg./ml. [d] No inhibition was obtained in some trials. [e] Control sample. [f] Mixture of amino acids used in medium.

In growth experiments of 20 hr. and with dilute inocula, it was possible to obtain 50% inhibition of growth of *S. plantarum* at 0.02 to 0.05 mg./ml. of the LLD tripeptide. For *L. citrovorum* parent, after reducing the total amino acid concentration to 1/3 that used previously,[5] in 20 hr. turbidity experiments the LLD tripeptide gave 50% inhibition of growth at 0.02 to 0.05 mg./ml.

The highly specific optical configuration LLD of the five tripeptides tested is noteworthy. Whether this structure is general for several amino acids or is specific for valine is now under investigation in our Laboratory. These compounds are also being presently screened for possible anti-tumor activity.

PASADENA, CALIFORNIA

(7) M. N. Camien and M. S. Dunn, *Proc. Soc. Exp. Biol. & Med.*, **95**, 697 (1957).

---

[CONTRIBUTION FROM THE LABORATORY OF PHARMACEUTICAL CHEMISTRY, THE UNIVERSITY OF KANSAS SCHOOL OF PHARMACY]

# The Amino- and Chloromethylation of Uracil[1]

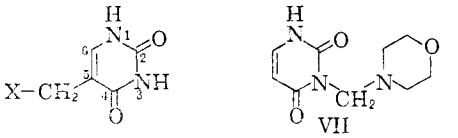
BY J. H. BURCKHALTER, ROBERT J. SEIWALD AND HOMER C. SCARBOROUGH

RECEIVED JULY 8, 1959

5-Morpholinomethyluracil (III) and 5-chloromethyluracil (V) have been synthesized, with results which are in disagreement with that of others. Both were converted through hydrogenolysis to thymine (I) as proof of structure. N-Chlorosuccinimide treatment of thymine (I), reported to give V, has been shown to yield 5-chloro-6-ethoxyhydrouracil (VIII).

Despite extensive investigations into the chemistry of the pyrimidines, particularly by Johnson and co-workers,[2] substitution reactions of uracil have not been thoroughly studied. In view of the occurrence of thymine (I), 5-methylcytosine and 5-hydroxymethylcytosine in nucleic acids,[3,4] it appears surprising that the 5-(substituted methyl)-uracils have received no more attention. A possible explanation is found in the instability imposed by the substitution of amino, chloro and hydroxyl groups at the 5-methyl of thymine (I).[5] Nevertheless, because of the importance of these substances as intermediates, or as such, in the search for antineoplastic and antiviral agents, we wished to investigate reactions which were expected to lead to desirable thymine derivatives (II–VI).[6]

| | X | | X |
|---|---|---|---|
| I | H | IV | $H_2N$– |
| II | piperidino- | V | $Cl^-$ |
| III | morpholino- | VI | HO |



VII

The reaction of morpholine, formalin and uracil has been reported by Bombardieri and Taurins to yield 3-morpholinomethyluracil (VII).[7] This report was in disagreement with our unpublished studies which had suggested structure III instead of VII. Also, synthesis of a compound designated

(1) This study was supported in part through the General Research Fund, University of Kansas.

(2) T. B. Johnson in Gilman's "Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1938, Vol. II, Chapter 11; and R. H. Wiley, *ibid.*, 1953, Vol. IV, p. 864.

(3) A. Bendich in "The Nucleic Acids," Academic Press, Inc., New York, N. Y., 1955, Vol. I, p. 86.

(4) Also, 5-hydroxymethyluracil (VI) is said to be a product of the bacterial oxidation of thymine; R. D. Batt and D. D. Woods, *Proc. Biochem. Soc.* in *Biochem. J.*, **49**, lxx (1951).

(5) Reference 3, p. 92.

(6) In previous studies in this Laboratory, a number of 5-alkyl- and 5-aryluracils were synthesized: J. H. Burckhalter and H. C. Scarborough, *J. Am. Pharm. Assoc.*, **44**, 545 (1955).

(7) C. C. Bombardieri and A. Taurins, *Can. J. Chem.*, **33**, 923 (1955).

by Barrett and West[8] as 5-chloromethyluracil (V) differed in properties from that prepared in our laboratory by a different method. The purpose of the present report is to outline the methods of synthesis of thymine derivatives II-VI and to resolve the differences between our results and those previously reported.[7,8]

5-Piperidinomethyluracil (II) was prepared in 73% yield from uracil, piperidine, paraformaldehyde and alcohol by the usual conditions of the Mannich reaction.[9] Similarly, 5-morpholinomethyluracil (III) was obtained in 63% yield. The product first obtained melted at over 300°, but after recrystallization from alcohol, melting occurred at 217°. It is presumed to be identical with Bombardieri and Taurins' product of m.p. 209° to which they assigned structure VII. All attempts to purify the high-melting substance converted it to the lower melting form. Both forms were converted by hydrochloric acid to the same hydrochloride, and both were transformed through hydrogenolysis to thymine. It is suggested that the products are different crystalline forms of the same compound.

Hydrogenolysis of 5-morpholinomethyluracil (III) to thymine (I) constitutes a proof of structure of III and indicates an incorrect structural assignment by Bombardieri and Taurins.[7]

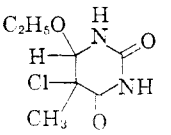
We have prepared 5-chloromethyluracil (V) by chloromethylation of uracil in yields ranging from 11 to 52%, depending upon the purity of the product. It was soluble in water and gave an immediate precipitate with cold aqueous silver nitrate solution.[10] The principal by-product is a high-melting, water-soluble, white solid presumed to be 5,5′-methylene-bis-uracil.

The structure of 5-chloromethyluracil (V) was confirmed by its hydrogenolysis in 90% yield to thymine, by its hydrolysis in 90% yield with silver carbonate to 5-hydroxymethyluracil (VI), which was oxidized by dichromate in 60% yield to the known uracil-5-carboxylic acid,[13] and by its conversion in 72% yield through its hexamethylenetetramine complex to the known 5-aminomethyluracil (IV) hydrochloride and sulfate, which had been made from uracil-5-acetic acid ester *via* the Curtius reaction.[14] 5-Chloromethyluracil (V) was treated with morpholine and piperidine to give, respectively, 5-morpholinomethyluracil (III) and 5-piperidinomethyluracil (II), and further confirmation of the structures II–VI was thereby afforded.

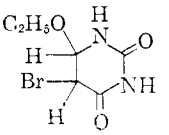
5-Hydroxymethyluracil (VI) was obtained as a hydrate by treatment of 5-chloromethyluracil (V) with an aqueous suspension of freshly prepared silver carbonate. Johnson and Litzinger[14] describe a preparation of the same alcohol by a different route, but because of its instability it was very impure and of doubtful value as a reference compound. Attempts by the other workers[4,14] and by us to prepare a satisfactory sample of the pure alcohol directly from uracil and formaldehyde have failed. Two recent reports mention the preparation of 5-hydroxymethyluracil but without giving properties or preparative details,[15–17] so that it is difficult to relate the various preparations.[18]

The lability of the 5-chloromethyluracil (V) which has just been described contrasts with the stability of a 5-chloromethyluracil of m.p. 222–224°, described by Barrett and West as having been prepared from the reaction of N-chlorosuccinimide and benzoyl peroxide with thymine.[8] Their product is characterized as being insoluble in cold water and stable to hot water and boiling alcoholic silver nitrate. As pointed out by these workers, such stability is not expected of 5-chloromethyluracil. Further doubt concerning the identity of their product arises from the fact that the calculated percentage for nitrogen is given as 19.38 when it should be 17.44, although the percentage found is given as 19.47.[8]

Attempts to repeat the preparation of Barrett and West gave a product possessing substantially the same properties described by them. However, we isolated two forms, one melting at 220–221° and the other at 227–228°. One form depressed the melting point of the other, and the higher-melting substance, upon standing or on repeated recrystallization from water, became identical with the lower-melting one. Elementary analysis of both forms disallows the structural assignment of 5-chloromethyluracil (V), and *cis-trans* isomers of 5-chloro-6-ethoxyhydrothymine (VIII) are suggested for the following reasons.




VIII




IX

The preparative reaction would apparently proceed only in certain grades of chloroform,[20] *e.g.*, chloroform U.S.P. which purposefully contains ethyl alcohol. Alcohol-free chloroform or carbon tetrachloride gave negative results.

The nuclear chlorine of VIII would be expected to be less active than that of 5-chloromethyluracil (V), in spite of the fact that it is attached to a

(8) H. Barrett and R. West, THIS JOURNAL, **76**, 3147 (1954).

(9) F. F. Blicke, "Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1942, Vol. I, Chapter 10.

(10) The related 5-chloromethyl-3,6-dimethyluracil[11] and 5-chloromethyl-6-methyluracil,[12] prepared by cyclizations, are described as unstable and readily hydrolyzable.

(11) K. Schmedes, *Ann.*, **441**, 192 (1925).

(12) M. M. Endicott and T. B. Johnson, THIS JOURNAL, **63**, 2063 (1941).

(13) H. L. Wheeler, T. B. Johnson and C. O. Johns, *Am. Chem. J.*, **37**, 392 (1907).

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

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

(16) R. M. Fink, R. E. Cline and K. Fink, *Federation Proc.*, **15**, 251 (1956).

(17) Also, a product so designated is marketed by California Corporation for Biochemical Research, Los Angeles, Calif. In a private communication, Dr. William Drell stated that his laboratory has prepared 5-hydroxymethyluracil from uracil and formaldehyde by the procedure of others.[16] After the writing of this manuscript, a report of the preparation of VI was noted. Base catalysis was used; R. E. Cline, R. M. Fink and K. Fink, THIS JOURNAL, **81**, 2521 (1959).

(18) The instability of 5-(substituted-methyl)-uracils has been discussed in detail by Bendich.[5] As an illustration, 5-hydroxymethyluracil,[14] as well as 5-hydroxymethyl-6-methyluracil,[19] is readily decomposed by hot water, with formaldehyde and the methylene-bis-uracil apparently being the products.

(19) W. Kischner, *Ann.*, **385**, 293 (1911).

(20) Robert A. West, Ph.D. Thesis, The University of Kansas, 1954.

tertiary carbon. Treatment of VIII with silver carbonate resulted in the recovery of VIII, while authentic V was converted by silver carbonate to the corresponding alcohol.

The ultraviolet absorption spectrum of VIII in water shows a disappearance of the thymine peak (266 mμ),[21] with only end absorption remaining.[22]

Elementary analysis for carbon, hydrogen, chlorine and nitrogen agrees with the proposed structure VIII.

A compound, assigned structure VIII and prepared by treatment of an alcohol suspension of thymine with chlorine,[23] gave no depression in melting point with the lower melting isomer prepared by the procedure of Barrett and West.[8] Also, an analogous reaction involving uracil, bromine and alcohol yielded a compound which has been described as 5-bromo-6-ethoxy-hydrouracil (IX).[24]

## Experimental

**5-Piperidinomethyluracil** (II).—(a) A mixture of 11.2 g. (0.1 mole) of uracil, 3.3 g. (0.11 mole) of paraformaldehyde and 8.5 g. (0.1 mole) of piperidine in 500 ml. of alcohol was heated at reflux temperature for 6 hours. The clear solution, allowed to stand overnight, gave 14 g. of product, m.p. >320° dec. Concentration of the filtrate produced 5.5 g. more (total crude yield 93%). Two recrystallizations from alcohol gave 15 g. (73% yield) of white plates, m.p. >350° dec. (softens at 250°). The product was soluble in dilute hydrochloric acid.

*Anal.* Calcd. for $C_{10}H_{15}N_3O_2$: C, 57.40; H, 7.11. Found: C, 57.42; H, 7.21.

(b) A small quantity of 5-chloromethyluracil (V) was suspended in a little dry acetone and an excess of piperidine added. After it had been warmed for a few minutes, the reaction mixture was filtered to remove piperidine hydrochloride, the filtrate was concentrated to dryness and the residue triturated with cold alcohol. The white insoluble solid was collected and recrystallized from alcohol, m.p. >330° (sinters at 228°), not depressed by II (a). Also, infrared spectra of the two preparations were identical.

**5-Morpholinomethyluracil** (III).—(a) A mixture of 11.2 g. (0.1 mole) of uracil. 3.3 g. (0.11 mole) of paraformaldehyde and 8.7 g. (0.1 mole) of morpholine in 600 ml. of 95% alcohol was heated at reflux temperature for 15 hours. Filtration of the warm mixture removed 1.6 g. of undissolved uracil. Concentration of the filtrate gave a white solid, m.p. >300° (sinters at 217°). Concentration of the second filtrate to a thick sirup and dilution with alcohol gave a white solid of m.p. 217° dec. After treatment of the high-melting solid with boiling alcohol, a total of 13.3 g. (63% yield) of 5-morpholinomethyluracil was obtained, m.p. 217° dec. Further recrystallizations from alcohol brought no change in melting point; $\lambda_{max}^{H_2O}$ 264 mμ, ε 7.2 × 10³.[25]

*Anal.* Calcd. for $C_9H_{13}N_3O_3$: C, 51.18; H, 6.20. Found: C, 51.10; H, 6.39.

(b) **Room Temperature Reaction.**—A solution of 3.36 g. (0.03 mole) of uracil in 400 ml.[26] of hot water was cooled to 30°, and to it a mixture of 2.7 ml. (0.033 mole) of 38% formalin and 2.8 ml. (0.03 mole) of morpholine was added. After the solution had stood for 10 days at room temperature, the water was removed at 30–40° under reduced pressure, and the white residue dissolved in 150 ml. of boiling alcohol. A yield of 4 g. (63%) of white solid was obtained, m.p. >300°. The product was not analyzed because recrystallization from alcohol converted it to the material of m.p. 217° described in (a).

(c) A small quantity of 5-chloromethyluracil (V) was stirred into an excess of morpholine. After the vigorous reaction had subsided, morpholine was removed under reduced pressure and the white residue recrystallized from alcohol, m.p. 217° dec. and not depressed by III (a).

**5-Morpholinomethyluracil hydrochloride** was prepared by treating 1 g. of the high- or low-melting form of III with 10 ml. of boiling concentrated hydrochloric acid. In both cases, cooling gave a yield of 90% of white solid hydrochloride, m.p. 265–267°. Recrystallization from dilute alcohol did not change the melting point, and the samples did not depress each other.

*Anal.* Calcd. for $C_9H_{13}N_3O_3 \cdot HCl \cdot H_2O$: C, 40.68; H, 6.07. Found: C, 40.75; H, 5.77.

**5-Chloromethyluracil** (V).—Hydrogen chloride gas was passed for two hours into a solution containing 5.6 g. (0.05 mole) of uracil, 4.9 ml. of 38% formalin and 20 ml. of concentrated hydrochloric acid. After the solution was well cooled, 3.6 g. (45% yield) of white crystalline V was obtained, m.p. 355° dec.; $\lambda_{max}^{dioxane}$ 265 mμ, ε 6.35 × 10³. No further purification was carried out since attempts led to decomposition.

*Anal.* Calcd. for $C_5H_5ClN_2O_2$: C, 37.40; H, 3.14; Cl, 22.08. Found: C, 37.36; H, 3.17; Cl, 21.42.

Retaining the same volume of concentrated acid but doubling the quantities of the other reactants gave yields varying from 24 to 52%. However, samples from the larger yields analyzed 97% pure based upon chloride content and appeared to contain a small amount of water-insoluble material, assumed to be 5,5′-methylene-bis-uracil.

**Hydrogenolysis of 5-Chloromethyluracil (V) to Thymine.**—A suspension of 1.6 g. (0.01 mole) of 5-chloromethyluracil (97% pure based upon chlorine analysis) and 0.1 g. of 10% palladium-on-charcoal in 180 ml. of 85% alcohol was subjected to hydrogenation at three atmospheres pressure. Within a few minutes time the theoretical amount of hydrogen was absorbed. The solution heated to boiling was filtered and the filtrate reduced on the steam-bath to a volume of about 50 ml. Upon cooling, 1.05 g. of thymine was obtained, m.p. 318–320°. Upon concentration and charcoaling of the filtrate, a total yield of 1.13 g. (90%) of thymine, based upon 100% pure chloromethyluracil, was realized, m.p. 318–320°; $\lambda_{max}^{H_2O}$ 266 mμ, ε 6.8 × 10³.

A reference sample of thymine[27] did not depress the melting point of the thymine derived from chloromethyluracil. Ultraviolet and infrared spectra of the two are identical. The infrared spectra are also identical with that of Blout and Fields,[28] except that use of potassium bromide pellets gave us greater resolution in the 3 μ region, with bands at 3.12 (NH), 3.28 (=C—H), 3.4 ($CH_3$—C) and 3.52 ($CH_2$). The band at 7.26 is almost certainly associated with $CH_3$–C group vibrations.[28] Other distinctive bands are at 5.76 (CO) and 5.97 (CO–NH).

**Hydrogenolysis of 5-(4-Morpholinylmethyl)-uracil(III) to Thymine.**—The directions of the experiment just described were applied to 2.11 g. (0.01 mole) of low-melting base (III). After several recrystallizations from hot water followed by sublimation, 0.41 g. (32% yield) of thymine was obtained, m.p. 320–321°. Mixed with authentic thymine,[27] there was no depression in melting point. Also, infrared spectra of the two samples of thymine are identical.

Another experiment employing the high-melting form of base III gave a product shown by melting-point and infrared spectra to be identical with authentic thymine.

**5-Hydroxymethyluracil** (VI).—To a solution of 1.6 g. (0.01 mole) of 5-chloromethyluracil (V) of 97% purity in 100 ml. of cold water, there was added with stirring a slight excess of freshly prepared silver carbonate. After most of the yellow silver salt had disappeared, the mixture was filtered through a charcoal mat and the filtrate allowed to evaporate at room temperature. The residue of white felted needles weighed 1.33 g. (94% yield). The product had no definite melting point but sintered below 200° and decomposed over 300°. An analytical sample was prepared by adding cold alcohol to a concentrated aqueous solution

(21) *Cf.* D. Shugar and J. J. Fox, *Biochim. et Biophys. Acta*, **9**, 199 (1952).

(22) *Cf.* S. Y. Wang, THIS JOURNAL, **80**, 6198 (1958).

(23) T. B. Johnson and J. M. Sprague, *ibid.*, **59**, 2436 (1937).

(24) B. Kurtev and M. Kirilov, *C. A.*, **47**, 1607 (1953). The fact that IX does not depress the melting point of 5-bromouracil suggests that the latter compound is formed from IX by *trans* elimination and that IX can be assigned a DL-*cis* configuration.

(25) Ultraviolet spectra in this paper were determined by Mr. J. L. Brannon.

(26) Uracil could not be dissolved in the volume of water suggested by Bombardieri and Taurins and kept at 0° without precipitation of the uracil.

(27) Identified lot 4827, Nutritional Biochemicals, Cleveland 28, O.

(28) E. K. Blout and M. Fields, THIS JOURNAL, **72**, 480 (1950).

of the compound. It sintered at about 250° and foamed at about 300° (lit.[17] gives 260–300°); $\lambda_{max}^{H_2O}$ 262 mμ, ε 8.44 × 10³.

*Anal.* Calcd. for $C_5H_6N_2O_3$: C, 42.25; H, 4.26. Found[29]: C, 40.58; H, 4.65.

**Uracil-5-carboxylic Acid.**—A suspension of 0.71 g. (0.005 mole) of 5-hydroxymethyluracil in 12 ml. of water was cooled and 1.9 ml. of concentrated sulfuric acid added slowly with stirring. A slurry of 1.47 g. (0.005 mole) of potassium dichromate in 3 ml. of water was added portionwise, care being taken that the temperature did not rise above 20°. At the conclusion of the addition, the solution was heated on the steam-bath for 5 minutes and filtered hot. The filtrate on standing in the cold overnight deposited 0.23 g. of greenish-white crystals. A second crop of 0.26 g. was obtained by concentrating the filtrate under vacuum. The total yield was 0.49 g. (63%) of off-white solid which melted at 278–279° (lit.[13] gives 278°) and did not depress a reference sample (identified lot 9501, Nutritional Biochemicals Corp.). The infrared spectra are also identical, with significant bands at 2.83 (OH), 6.2 and 8.43 μ which did not appear in the spectrum of uracil.[28]

**5-Aminomethyluracil (IV) Hydrochloride.**—A solution of 1.65 g. (0.01 mole) of 5-chloromethyluracil (V) and 1.55 g. (0.011 mole) of hexamethylenetetramine in 10 ml. of water was allowed to stand for 3 days. The solution was filtered to remove a small amount of solid and the filtrate diluted with alcohol and ether to precipitate 2.75 g. of the intermediate complex. The solid was suspended in 20 ml. of concentrated hydrochloric acid and 140 ml. of ethanol. After about 10 minutes of heating at reflux on the steam-bath, the solution cleared and after about one hour a white crystalline precipitate started to form. Refluxing was continued for a total of 6 hours and the reaction mixture cooled overnight in the ice-box. The 2.4 g. of solid was fractionally recrystallized from alcohol and water to remove the ammonium chloride. A total of 1.27 g. of product (72% yield) was obtained, m.p. 250–254°. The best sample obtained melted at 252–253° (lit.[14] gives 242–243°); $\lambda_{max}^{H_2O}$ 261.5 mμ, ε 6.3 × 10³.

*Anal.* Calcd. for $C_6H_7N_3O_2 \cdot HCl$: Cl, 19.94. Found: Cl, 19.88.

**5-Aminomethyluracil (IV) Sulfate.**—A small quantity (0.2 g.) of the hydrochloride salt was dissolved in a minimum amount of warm water and a few drops of hot 12 *N* $H_2SO_4$ added. On cooling, white plates precipitated which, after three recrystallizations from alcohol and water, melted at 254–255° (lit.[14] gives 245–246° for the monohydrate).

*Anal.* Calcd. for $C_6H_7O_2N_3 \cdot {}^1/_2H_2SO_4 \cdot H_2O$: C, 28.86; H, 4.81. Found: C, 28.89; H, 4.71.

**5-Chloro-6-ethoxyhydrothymine (VIII) from Thymine and N-Chlorosuccinimide.**—The experiment of Barrett and West, who assigned a different structure (V) to the product,[8,20] was repeated. To a solution of 1.47 g. (0.011 mole) of N-chlorosuccinimide and 0.4 g. of benzoyl peroxide in 50 ml. of chloroform (Merck U.S.P.), 1.26 g. (0.01 mole) of thymine was added. The suspension was heated at reflux temperature for 10 hr. during which time the appearance of the suspended solid changed. The mixture was filtered to give 1.2 g. of white solid, m.p. 215–219°. Concentration of the filtrate and cooling yielded a further 1.2 g. of solid which, after treatment with cold water to remove succinimide, yielded an additional 0.25 g. of product; total crude yield 1.45 g. (72%). Recrystallization from hot water gave a first crop of crystalline VIII, m.p. 220–221° (lit.[8,20] gives 222–224° and erroneously assigns structure V). A mixed melting point with the product as prepared by Johnson and Sprague[23] showed no depression. The ultraviolet absorption spectrum of VIII in water showed a disappearance of the thymine peak at 266 mμ,[21] with only end absorption remaining[22]; $\lambda_{max}^{H_2O}$ 204 mμ, ε 8.19 × 10³.

*Anal.* Calcd. for $C_7H_{11}ClN_2O_3$: C, 40.70; H, 5.36; Cl, 17.15; N, 13.56. Found: C, 40.73; H, 5.26; Cl, 17.04; N, 13.58.

A second crop of crystals was obtained, m.p. 227.5°. Admixture with first crop product depressed the melting point. Recrystallization from hot water gave needles, m.p. 227.5°.

*Anal.* Found: C, 41.05; H, 5.08.

Upon further recrystallization or standing for several weeks, the product was converted to the lower melting form, m.p. 223–224°.

An attempt to effect a reaction between the lower melting form of VIII and silver carbonate resulted in a recovery of unchanged material, m.p. 221–222°. It did not depress the melting point of starting material but did depress that of the 227.5° compound.

*Anal.* Found: C, 41.16; H, 5.32.

LAWRENCE, KANS.

(29) Agrees with the presence of about one-third mole of water. Because of the ready decomposition of the substance to give water, a water determination would probably be valueless.

# COMMUNICATIONS TO THE EDITOR

## THE CONFIGURATION OF DEUTERIO-L-MALIC ACID PRODUCED ENZYMATICALLY. SYNTHESIS OF *THREO*-3-DEUTERIO-DL-MALIC ACID

*Sir:*

The recent extensive work of Alberty and his co-workers[1–3] has established that the hydration of fumaric acid in deuterium oxide, catalyzed by fumarase, proceeds stereospecifically to give a single deuterio-L-malic acid (I). From a broad-line n.m.r. study[2] of the crystalline acid the *threo*

(1) R. A. Alberty and P. Bender, THIS JOURNAL, **81**, 542 (1959).

(2) T. C. Farrar, H. S. Gutowsky, R. A. Alberty and W. G. Miller, *ibid.*, **79**, 3978 (1957).

(3) H. F. Fisher, C. Frieden, J. S. McKinley McKee and R. A. Alberty, *ibid.*, **77**, 4436 (1955).