[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT, UNIVERSITY OF OTAGO MEDICAL SCHOOL]

Chemistry of the Dihydropyrimidines. Ultraviolet Spectra and Alkaline Decomposition[1a]

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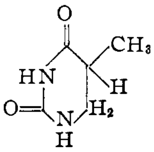
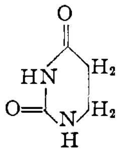
The substance previously regarded as hydroörotic acid is shown to be fumarylurea. Genuine 5,6-dihydroörotic acid has been synthesized and its decomposition in alkali, together with the breakdown of 5,6-dihydrouracil and 5,6-dihydrothymine under similar conditions, has been studied. All three compounds are hydrolyzed in 0.1 *N* alkali at the 3–4 linkage. The same lability has been observed with succinylurea, fumarylurea and maleylurea.

Recently the dihydropyrimidines have been implicated in the catabolism of the pyrimidine bases. Lieberman and Kornberg[2,3] reported the isolation of a cell-free enzyme system from an anaerobic bacterium which reduces orotic acid to dihydroörotic acid and converts this substance to ureidosuccinic acid. The breakdown of dihydrothymine to β-aminoisobutyric acid has been investigated in rats[4,5] while dihydrouracil has been isolated from beef spleen[6] and has been suggested as an intermediate in uracil catabolism in *Torula utilis*.[7,8]

During an investigation into the metabolism of this class of compounds, it was found that they were alkaline labile. This paper deals both with the nature of these decompositions and the ultraviolet absorption spectra of the dihydropyrimidines.

The compounds studied were 5,6-dihydrothymine (I), 5,6-dihydrouracil (II) and 5,6-dihydroorotic acid (III).


I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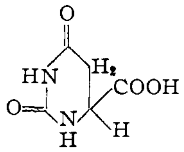
III

In our hands the synthesis of III according to the method reported by Bachstez and Cavallini[9] gave as the main product a substance which did not appear to contain a dihydropyrimidine ring system. It could be catalytically reduced to succinylurea and the infrared spectrum indicated a free $CO-NH_2$ grouping in the molecule. The product obtained by the catalytic reduction of orotic acid showed different chemical properties and the infrared spectrum did not reveal a free $CO-NH_2$ group.

The substances prepared by the above alternative methods were found to decompose rapidly in 0.1 *N* alkali. The hydrogenation product from orotic acid gave ureidosuccinic acid under these conditions while the compound prepared by the method of Bachstez and Cavallini gave urea and fumaric acid.

Maleylurea which was synthesized according to the method of Dunlap and Phelps[10] decomposed to urea and maleic acid in alkali and on reduction gave succinylurea. It is concluded, therefore, that the catalytic hydrogenation of orotic acid gives 5,6-dihydroörotic acid (III) and that the compound prepared by the method of Bachstez and Cavallini is, in fact, fumarylurea. These findings are significant in studies on dihydroörotic acid in metabolism.[3,7]

The three dihydropyrimidines rapidly decomposed in 0.1 *N* sodium hydroxide (Table I) and did not produce urea under these conditions. 5,6-Dihydrouracil gave β-ureidopropionic acid and, by analogy, 5,6-dihydrothymine would be expected to give β-ureidoisobutyric acid. The products formed from 5,6-dihydrouracil and 5,6-dihydrothymine by decomposition in 0.1 *N* alkali reverted to the original dihydropyrimidines on mild acid treatment, in agreement with earlier observations on β-ureidopropionic acid.[11] In the case of ureidosuccinic acid where ring closure could lead to the formation of the dihydropyrimidine or hydantoin ring, acid treatment causes preferential formation of the hydantoin ring.[12]

Ratner, *et al.*,[13] have prepared the anhydride of argininosuccinic acid, an intermediate in urea biosynthesis, and have reported that it is labile in alkali. They suggest either a dihydropyrimidine or a hydantoin structure for this substance. Investigation of its ultraviolet absorption spectrum should distinguish these two possibilities.

All three dihydropyrimidines show similar spectra in acid and alkali (Fig. 1). Contrary to Austin,[14] the absorption spectrum of 5,6-dihydrouracil measured at *p*H 1 to 13 showed no peak over the wave length range 240 to 300 mμ. The spectra shown for the dihydropyrimidines are consistent with the view that these compounds exist in the enol form under alkaline conditions. The spectral characteristics of these compounds are being used as a method for their estimation.

Experimental

Preparations. 5,6-Dihydrothymine.—After recrystallization the material prepared by the method of Fischer and

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(1) A preliminary report of some of this work was presented in the *Proc. Univ. Otago Med. School*, **31**, 40 (1953). (b) Department of Chemistry, University of Otago, New Zealand.

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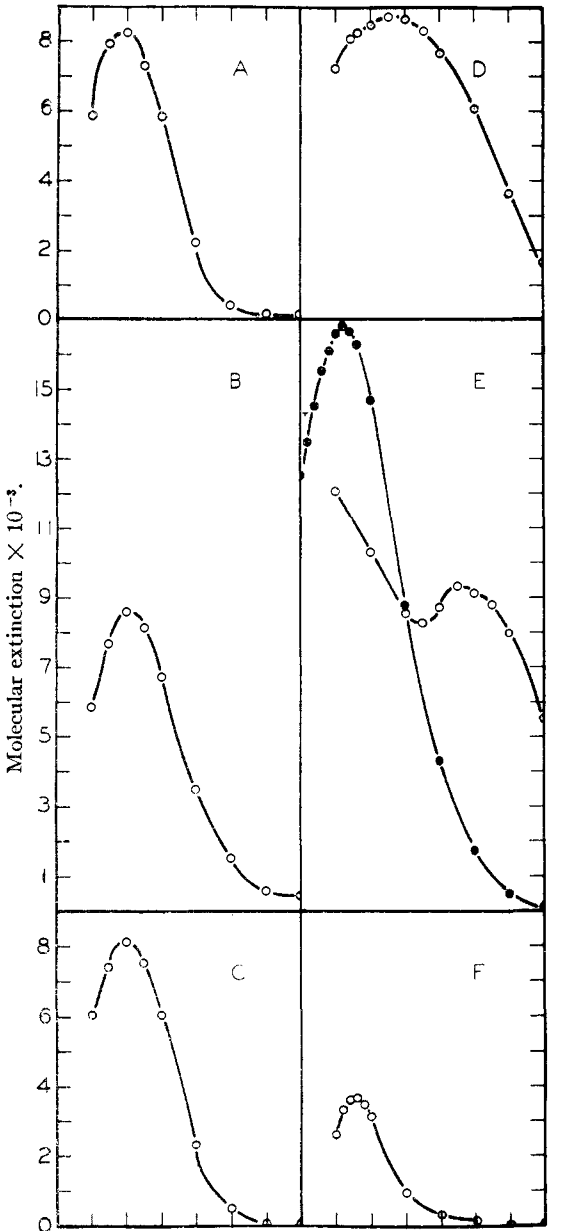

Fig. 1.—Absorption curves of: A, dihydrouracil; B, dihydroörotic acid; C, dihydrothymine; D, maleylurea; E, fumarylurea; F, succinylurea: O, in 0.10 *N* sodium hydroxide; and ●, in 0.10 *N* hydrochloric acid. The concentration of each solution was 10^{-4} *M*.

Roeder[15] had a m.p. of 261–263° (reported[15] m.p. 264–265°).[16]

Anal. Calcd. for $C_5H_8O_2N_2$: C, 46.82; H, 6.30; N, 22.23. Found: C, 47.14; H, 5.80; N, 22.04.

5,6-Dihydrouracil.—Uracil was hydrogenated with Adams catalyst in glacial acetic acid at atmospheric pressure and room temperature.[7] After recrystallization the product melted at 272–275° (reported[15] m.p. 275–276°).

Anal. Calcd. for $C_4H_6O_2N_2$: C, 42.10; H, 5.42; N, 24.56. Found: C, 42.25; H, 4.98; N, 24.86.

5,6-Dihydroörotic Acid.—Anhydrous orotic acid (300 mg.) was dissolved in 97% ethanol (500 ml.) and Adams catalyst (200 mg.) added. The mixture was submitted to a pressure of 24 atmospheres of hydrogen for 36 hours at 80° by which time all the orotic acid had disappeared. The reaction mixture was cooled, filtered and concentrated under high vacuum. The crystalline material, recrystallized from water and dried *in vacuo* over phosphorus pentoxide, yielded the hemihydrate, 75 mg., m.p. 250–253°.

Anal. Calcd. for $C_5H_6O_4N_2 \cdot {}^1/_2H_2O$: C, 35.94; H, 4.22; N, 16.76; neut. equiv., 167. Found: C, 35.86; H, 4.53; N, 16.60; neut. equiv., 169.

Fumarylurea.—When the method of Bachstez and Cavallini[9] was used for the synthesis of hydroörotic acid from urea and maleic acid, the product described by these authors was obtained in good yield; m.p. 250–251° (reported[9] m.p. 247–249°).

Anal. Calcd. for $C_5H_6O_4N_2$: C, 37.98; H, 3.83; N, 17.62; neut. equiv., 158. Found: C, 38.25; H, 3.67; N, 17.60; neut. equiv., 160.

Reduction of Fumarylurea.—The material (158 mg.) prepared as above was dissolved in glacial acetic acid (100 ml.) containing Adams catalyst (133 mg.). The mixture was hydrogenated at 40° and atmospheric pressure. After 12 hours the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized from water; yield, 72 mg. A further 35 mg. was obtained from the mother liquors. The substance melted at 206°; mixed m.p. with succinylurea (m.p. 204°) prepared by the method of Pike[17] was 203°. It was also identified by its absorption spectrum in alkali.

Maleylurea.—This compound was prepared from urea and maleic anhydride by the method of Dunlap and Phelps[10]; one recrystallization from water resulted in a product melting at 156–160° dec. (reported[10] m.p. 167.5–168°). The maleylurea was catalytically reduced with Adams catalyst giving succinylurea in quantitative yield (m.p. 204°).

Ureidosuccinic Acid.—The mixture obtained by the reaction of potassium cyanate with aspartic acid according to the conditions described by Nye and Mitchell,[12] on careful acidification to *p*H 2.0, gave a substance melting at 171–172°. The reported[12] m.p. for ureidosuccinic acid is 178–180°.

β-Ureidopropionic Acid.—Following the method of Lengfeld and Stieglitz,[11] β-ureidopropionic acid was synthesized from β-alanine and potassium cyanate; m.p. 169–170° (reported[11] m.p. 170–171°). On evaporation of the compound in 0.1 *N* HCl, 5,6-dihydrouracil was obtained in quantitative yield.

Alkaline Decompositions. (a) **5,6-Dihydrothymine.**—To 40 ml. 0.2 *N* $Ba(OH)_2$ was added 256 mg. of 5,6-dihydrothymine and the solution left to stand at room temperature until all the compound had disappeared as shown spectroscopically (*ca.* 1 hour). The solution was titrated to *p*H 2.0 with 2 *N* H_2SO_4 and the barium sulfate removed by centrifuging. The supernatant was concentrated under high vacuum and the residue recrystallized from ethanol. The product (258 mg.) which melted at 98–100° did not give a positive ninhydrin reaction. Heating the substance in an acid solution gave 5,6-dihydrothymine.

(b) **5,6-Dihydrouracil.**—Dihydrouracil (228 mg.) was dissolved in 0.2 *N* $Ba(OH)_2$ (40 ml.) and the solution treated as in (a). The decomposition product was recrystallized from water; m.p. 169–170°, yield 230 mg. The mixed m.p. with synthetic β-ureidopropionic acid (m.p. 169–170°) was 168–169°.

(c) **5,6-Dihydroörotic Acid.**—The product (50 mg.) obtained from the high pressure, high temperature hydrogenation of orotic acid was dissolved in 2 *N* NaOH (1 ml.). After standing for 24 hours the reaction mixture was acidified with 2 *N* HCl to *p*H 2.0. The crystalline product was filtered and recrystallized from water; yield 30 mg., m.p. 173°. Mixed m.p. with synthetic ureidosuccinic acid (m.p. 171–172°) was 171–173°.

(d) **Fumarylurea.**—Fumarylurea (316 mg.) was dissolved in 0.5 *N* NaOH (10 ml.) and the solution left for 24 hours. It was then acidified with 2 *N* HCl and concentrated under reduced pressure. A crystalline product (210 mg.) appeared which, after recrystallization from water, melted at 293–296°. The melting point was not depressed on admixture with authentic fumaric acid.

Anal. Calcd. for $C_4H_4O_4$: C, 41.38; H, 3.45; neut.

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(15) E. Fischer and G. Roeder, *Ber.*, **34**, 3751 (1901).

(16) All melting points were taken on a copper block with the temperature increasing at a rate of two degrees per minute.

(17) W. H. Pike, *Ber.*, **6**, 1104 (1873).

equiv., 116. Found: C, 41.53; H, 3.68; neut. equiv., 116.

(e) **Maleylurea.**—Maleylurea (100 mg.) was dissolved in 0.1 *N* NaOH (5 ml.) and left to stand for 24 hours. On acidification with sulfuric acid and repeated extraction with ether, an acid was obtained which melted at 135°. Mixed m.p. with maleic acid (m.p. 137°) was 136°.

Rate of Alkaline Decomposition of the Dihydropyrimidines and Related Compounds.—The rate at which the dihydropyrimidines and some related compounds disappeared in 0.1 *N* NaOH was followed spectroscopically at their respective ϵ_{max} values. Table I summarizes the times taken to reach 50% of the initial concentrations.

TABLE I

DECOMPOSITION AND ABSORPTION CHARACTERISTICS OF THE DIHYDROPYRIMIDINES AND RELATED SUBSTANCES

Substance	Time of $^1/_2$ decomp.,[a] minutes	λ_{max}, mμ	ϵ_{max}, $\times 10^{-3}$	Solvent
5,6-Dihydrouracil	5.0	230	8.22	0.1 *N* NaOH
5,6-Dihydroörotic acid	11.1	230	8.60	.1 *N* NaOH
5,6-Dihydrothymine	10.3	230	8.13	.1 *N* NaOH
Maleylurea	34.0	235	8.72	.1 *N* NaOH
Fumarylurea	9.4	255	9.36	.1 *N* NaOH
		222	16.82	.1 *N* HCl
Succinylurea	5.4	226	3.67	.1 *N* NaOH

[a] 10^{-4} *M* solution in 0.1 *N* NaOH at 18°.

Urea Production during Alkaline Decomposition of Ureido Compounds.—Solutions of the substances to be tested were prepared containing 0.7 mg. N/ml. To 1 ml. of such solutions was added 1 ml. of 0.2 *N* NaOH and after standing at room temperature for 24 hours the alkali was neutralized with 0.2 *N* HCl. To the system was added 5 ml. of 0.1 *M* phosphate buffer, *p*H 8.0, together with 1 ml. of urease solution (10 BDH tablets/10 ml. of phosphate buffer *p*H 8.0). After 2 hours incubation at 37° the volume was made up to 23 ml. with distilled water and 2 ml. of Nessler reagent added The solutions were centrifuged and the optical densities of the supernatants were read at 490 mμ in a photoelectric colorimeter.

A standard curve was prepared using ammonium sulfate and standard urea solutions were run simultaneously as a check on the activity of the urease preparation. The results are summarized in Table II.

TABLE II

UREA PRODUCTION FROM THE ALKALINE DECOMPOSITION OF THE DIHYDROPYRIMIDINES AND RELATED COMPOUNDS

Substance	Urea produced mol./mol. initial substrate
5,6-Dihydrouracil	0
5,6-Dihydrothymine	0
5,6-Dihydroörotic acid	0
Succinylurea	1.0
Fumarylurea	1.1
Maleylurea	0.9

Ultraviolet Spectra (Fig. 1 and Table I).—All measurements were made on a Beckman model DU spectrophotometer. The instrument was calibrated with potassium dichromate according to Gridgeman.[18] For determining spectra in alkaline solutions, readings for a particular wave length were taken at various time intervals after adding alkali and a zero time value obtained by extrapolation. No values are reported below 220 mμ because of the high absorption of sodium hydroxide below this wave length. In acid solution only the spectrum of fumarylurea is reported. Peaks between 200 and 210 mμ were observed for all the dihydropyrimidines, but these were probably due to stray light effects.[19]

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Addendum on the Infrared Spectra of the Dihydropyrimidines

BY JAMES MURRAY

Fumarylurea shows strong absorption bands at 3400 and 3250 cm.$^{-1}$, originating in a primary amide group, as in maleylurea and succinylurea. This contrasts with the single –CONHR absorption band at about 3240 cm.$^{-1}$, in the cyclic compounds. There appear to be four strong bands in the 1800–1600 cm.$^{-1}$ region, but although this corresponds with the number to be expected (2 for $-CONH_2$, 1 for CO–NH and 1 for –COOH) it was not possible to assign them with certainty. A strong band at 985 cm.$^{-1}$ is evidently due to the *trans*-RCH:CHR′ group (found at 980 cm.$^{-1}$ in diethyl fumarate, but not evident in fumaric acid itself or the other compounds mentioned). A prominent feature of the spectrum is a strong band at about 660 cm.$^{-1}$. Among the above compounds, a similar band was found only in fumaric acid (650 cm.$^{-1}$) and diethyl fumarate (666 cm.$^{-1}$).

The spectrum of dihydroörotic acid clearly shows the absence of a primary amide group, and fairly closely resembles that of dihydrothymine. Dihydrouracil appears anomalous in showing carbonyl absorption at 1760 cm.$^{-1}$. Since a five-membered ring structure appears to be eliminated by other evidence, this suggests the presence of a β,γ-unsaturated six-membered ring amide. Such a structure would be obtained by enolization of one carbonyl and some support for this is provided by bands at 3090 (enolic OH) and 758 cm.$^{-1}$ (O—CR═CHR′). These bands are weak or absent in the spectra of dihydrothymine and dihydroörotic acid. They may, however, be due to a crystal structure effect (spectra were run as Nujol mulls), but the insolubility of the compounds in suitable solvents precluded measurements of solution spectra.

INFRARED ABSORPTION BANDS OF UREIDO COMPOUNDS (IN NUJOL MULLS)

Compound	OH and NH stretching frequencies, cm.$^{-1}$	CO and C═C stretching frequencies, cm.$^{-1}$
Maleic acid	3100	1710, 1600
Fumaric acid	3100	1680, 1640–1620
Succinylurea	3400 3230	1720, 1605
Maleylurea	3410 3250	1720, 1680–1670
Fumarylurea	3400 3250 3210	1724–1700 1660 1595
5,6-Dihydrothymine	3240	1740–1720
5,6-Dihydrouracil	3220 3090	1760, 1680 1620, 1600
5,6-Dihydroörotic acid	3260	1722, 1678, 1610

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[19] E. R. Holiday and G. H. Beaven, *ibid.*, No. 3, October (1950).