2,4-DIHYDROXY-5,6-DIHYDROPYRIMIDINE DERIVATIVES

IV.* EFFECT OF THE STRUCTURE OF AMINES ON THE FORMATION OF AMIDES OF DIHYDROOROTIC OR UREIDOSUCCINIC ACIDS

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The reaction of dihydroorotic acid and its butyl ester with amines was studied. Depending on the basicity and three-dimensional structure of the amines, the amidation of the ester group may be accompanied by cleavage of the pyrimidine ring at the 3,4-bond to form ureidosuccinic acid or its monoamide.

In previous communications [1,2], we described the synthesis of esters of DL-dihydroorotic acid and their reaction with amines. In the present research, this reaction was studied in greater detail and used for the preparation of optically active dihydroorotic acid derivatives.

The more reactive ester group and the less active ring $C_4=O$ group undergo nucleophilic attack by amines. For a qualitative evaluation of the different reactivities, we investigated the reaction of butyl dihydroorotate (Ia) with amines of different basicity and structure under identical conditions. Methanol was used as the solvent. The pK_b values are presented in Table 1 in order of decreasing basicity of the amines used [3,4], and the final product obtained is indicated.

*See [1] for communication III.

TABLE 1. Results of the Reaction of the Butyl Ester of DL-Dihydroorotic Acid with Amines

Amines	pK_b [3, 4]	Reaction products	
		dihydroorotic acid amide	ureidosuccinic acid diamide
Dibutylamine	2,75	–	–
Piperidine	2,8	+	–
Ethylamine*	3,3	–	+
Methylamine*	3,4	–	+
Butylamine*	3,4	–	+
Allylamine	4,5	–	+
Ethanolamine	4,6	+‡	+
Benzylamine	4,7	+	–
Ammonia	4,8	+	–
Hydrazine*	5,5	+	–
Morpholine†	5,6	+	–
Phenylhydrazine†	8,8	+	–

*The reaction products have been previously described [1].
† The amination was carried out for 5-7 days (the reaction time with the other amines was 24 h).
‡ The reaction time was 6 h.

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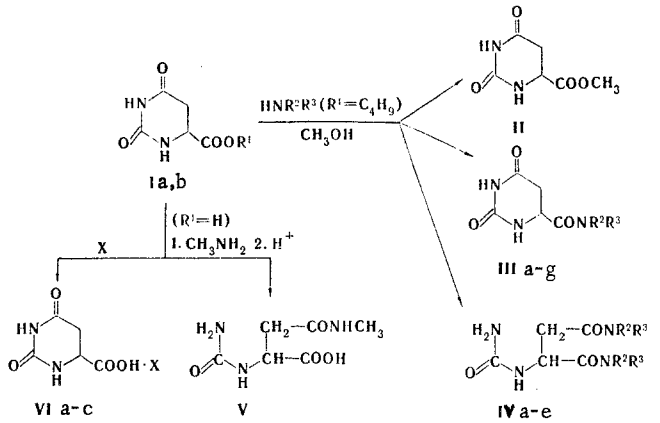
As expected, the less basic amines, commencing with benzylamine, form only amides of dihydroorotic acid (IIIb,d-g). The more basic amines ($pK_b \leq 4.6$) attack the $C_4=O$ group, thereby cleaving the ring. The sequence of the reaction can be followed graphically in the reaction of Ia with ethanolamine (pK_b 4.6). After 6 h, dihydroorotic acid amide (IIIc) precipitates and then reacts with a second molecule of ethanolamine, cleaving the ring to form a new precipitate of ureidosuccinic acid diamide (IVb). Secondary amines, the reactivities of which depend to a greater extent on the steric effect than on the basicity of the amine, constitute an exception to this. Thus piperidine attacks the ester group with difficulty, and cleavage of the 3,4-bond is not observed. The steric effect for dibutylamine is manifested so strongly that nucleophilic attack does not occur at either the ester group or at the $C_4=O$ group of the amide groupings. Only the previously observed [2] transesterification of Ia to methyl dihydroorotate (II) is observed in absolute methanol, even in the presence of a 40-fold excess of dibutylamine. Transesterification occurs with piperidine, morpholine, and diphenylhydrazine in the presence of a 10-fold excess of the imine, and amidation occurs slowly only in the presence of a 40-fold excess, despite the steric effect exerted by piperidine and morpholine and the reduced basicity of the phenylhydrazine.



Ia $R^1=C_4H_9$, b $R^1=H$; IIIa $R^2,R^3=-(CH_2)_5-$, b $R^2,R^3=-(CH_2)_2-O-(CH_2)_2-$, c $R^2=H$, $R^3=CH_2CH_2OH$, d $R^2=H$, $R^3=CH_2C_6H_5$, e $R^2=H$, $R^3=NHC_6H_5$; IV a $R^2=H$, $R^3=CH_2-CH=CH_2$, b $R^2=H$, $R^3=CH_2CH_2OH$, c $R^2=H$, $R^3=CH_3$, d $R^2=H$, $R^3=C_2H_5$, e $R^2=H$, $R^3=n\text{-}C_4H_9$; VIa X = morpholine, b X = L-arginine, c X = adenine

The structures of the ureidosuccinic and dihydroorotic acid derivatives were confirmed by paper chromatography with development by Erlich's reagent (the dihydro derivatives give yellow coloration only after treatment with 1 N NaOH) and by the IR spectra. The IR spectra of the amides of ureidosuccinic acid contain ν_{CO} bands below 1660 cm^{-1} and a clearly expressed additional maximum above 3430 cm^{-1}; the vibrations of the ring C=O group of the amides of dihydroorotic acid are found at 1715-1720 cm^{-1}.

It was assumed that the reaction of dihydroorotic acid with amines that cleave the ring would be a convenient method for the preparation of ureidosuccinic acid amides. However, the COOH group considerably inhibits the ring-opening reaction in absolute methanol. Ureidosuccinic acid N-methylamide (V) can be obtained only on prolonged reaction (5 days). It was assumed that the formation of slightly soluble (in methanol) salt Ib · CH_3NH_2 and of the unprecipitated final product V · CH_3NH_2 affects the course of the reaction. The reaction with butylamine (Ib · $C_4H_9NH_2$ and ureidosuccinic acid dibutylamide are soluble in methanol) was used to compare the reactivities of ester Ia and acid Ib under conditions that were identical with respect to the solubilities of the starting and final products. However, the opening of the ring of acid Ib proceeds so slowly that ureidosuccinic acid monobutylamide could not be isolated. Its formation is assumed only from the results of electrophoresis. In aqueous media, ring cleavage under the influence of amines is accelerated considerably, but the reaction is complicated by the formation of ureidosuccinic acid (VII) during the action of OH^- ions on Ib. Depending on the structure of the amine, the latter reaction may be the major one. By qualitatively evaluating the electrophoresis data, one may conclude that ring cleavage by amine predominates in the reaction with methylamine, and the rates of both reactions with butylamine are approximately identical, while only VII is formed with piperidine.

In the cyclic system under discussion, δ^+ on C_4 is extremely insignificant, and, moreover, the substituent in the 6 position sterically hinders the approach of the nucleophilic agent and the formation of an intermediate complex. Ring opening occurs in the presence of a considerable excess and for a favorable three-dimensional structure of the nucleophilic component in proton-containing solvents. In our opinion, the chief reason that hinders opening of the ring of dihydroorotic acid is hindrance to the approach of the amine to the $C_4=O$ group because of the negatively charged carboxyl ion.

TABLE 2. Derivatives of DL-Dihydroorotic and DL-Ureidosuccinic Acids

Comp.	mp, °C*	R_f †	Empirical formula	Found, %			Calc., %			Yield, %
				C	H	N	C	H	N	
IIIa	272—273	0,68—0,70	$C_{10}H_{15}N_3O_3$	53,0	6,7	18,5	53,3	6,7	18,7	46
IIIb	298—299	0,48—0,54	$C_9H_{13}N_3O_4$	47,3	5,7	18,4	47,6	5,8	18,5	38
IIIc	191—192	0,43—0,45	$C_7H_{11}N_3O_4$	41,4	5,5	21,3	41,8	5,5	20,9	42
IIId	226—228	0,75	$C_{12}H_{13}N_3O_3$	58,7	5,2	16,9	58,3	5,3	17,0	92
IIIe	245—247 (dec.)	0,25—0,30	$C_5H_7N_3O_3$	38,2	4,4	26,5	38,2	4,5	26,7	86
IIIg	255—257	—	$C_{11}H_{12}N_4O_3$	53,5	4,8	22,9	53,2	4,9	22,6	60
IVa	209—211	0,85	$C_{11}H_{18}N_4O_3$	52,1	7,2	22,2	52,0	7,1	22,0	84
IVb	153—154	0,40—0,45	$C_9H_{18}N_4O_5$	41,4	6,9	21,3	41,2	6,9	21,4	73
V	176—177	0,60	$C_6H_{11}N_3O_4$	37,9	5,9	22,1	38,1	5,9	22,2	71

*Compounds IIIa,b,d-g, and IVa were recrystallized from water, while IIIc, IVb, and V were recrystallized from ethanol.
† With butanol—water—acetic acid (2 : 1 : 1) and ascending chromatography on LS paper.

TABLE 3. Derivatives of L- and D-Dihydroorotic and L- and D-Ureidosuccinic Acids

Comp.	mp, °C*	$[\alpha]_D^{20-23}$, deg*	Empirical formula	Found, %			Calc., %			Yield, %
				C	H	N	C	H	N	
L-Ia	153—156	+59,3 (0,51 C_2H_5OH)	$C_9H_{14}N_2O_4$	50,6	6,7	13,0	50,5	6,6	13,1	81
D-Ia	154—156	−60,1 (0,76 C_2H_5OH)		50,5	6,6	13,0				80
L-IIIe	223—225	+41,2 (0,89 H_2O)	$C_5H_7N_3O_3$	38,3	4,6	26,6	38,2	4,5	26,7	70
D-IIIe	224—227	−42 (0,84 H_2O)		38,1	4,5	26,4				88
L-IVc	223—224	+23,4 (1,21 H_2O)	$C_7H_{14}N_4O_3$	41,3	6,9	27,7	41,6	7,0	27,7	85
D-IVc	223—224	−20,0 (1,07 H_2O)		41,8	7,1	27,9				92
L-VIa	200—203	+17,34 (1,36 H_2O)	$C_5H_6N_2O_4 \cdot C_4N_9NO$	44,1	6,2	16,9	44,1	6,2	17,1	96
L-VIb	244—246	+20,38 (1,10 H_2O)	$C_5H_6N_2O_4 \cdot C_6H_{14}N_4O_2$	39,4	6,2	25,4	39,8	6,1	25,3	99
L-VIc	>275 (dec.)	+19,15 (0,23 H_2O)	$C_5H_6N_2O_4 \cdot C_5H_5N_5$	41,2	3,8	33,5	41,0	3,8	33,4	85

*The melting point and [α] values were determined for uncrystallized products, except for L-Ia and D-Ia, which were recrystallized from ethanol.

All of the reactions mentioned above were carried out with racemic derivatives. Since L-dihydroorotic acid participates in the biosynthesis of pyrimidines, while the D-form is its antimetabolite [5], we were interested in the possibility of using the developed methods for the synthesis of optically active derivatives. L- and D-Dihydroorotic acids were obtained by a known method [5], while the L-form was also obtained in good yields (up to 76%) by the cyclization of L-N^{α}-carbobenzoxyasparagine under similar conditions. The reactions of the optically active forms at the COOR groups and at $C_4{=}O$ were carried out in the same way as the reactions of DL-dihydroorotic acid derivatives. The characteristics of the synthesized L- and D-dihydroorotic acid derivatives and D- and L-ureidosuccinic acid derivatives are presented in Table 3.

The salts of DL-dihydroorotic acid have been previously synthesized [6] for physiological tests for regeneration of liver function, and the salts of morpholine and L-arginine [7] had the greatest activity. The salts of the metabolite of L-dihydroorotic acid with morpholine, L-arginine, and adenine, the physicochemical constants of which are presented in Table 3, were synthesized for further physiological tests.

EXPERIMENTAL

The melting points were determined with a Boetius apparatus. The specific rotation was measured with a Perkin-Elmer-141 polarimeter. The IR spectra of mineral oil suspensions were obtained with a UR-20 spectrophotometer. The R_f values of the L- and D-derivatives are not presented in Table 3, since they coincide with the values of the previously synthesized DL-forms [1,2]. The electrophoresis was carried out on FILTRAK-FN-16 paper in a pyridine acetate buffer (pH 5.0) at 400/30 V/cm for 1.5 h. The electrophoretic mobilities of the compounds were characterized as the ratio of the distance traveled by the substance relative to the distance traveled by ureidosuccinic acid, synthesized via the method in [8].

DL-Dihydroorotic Acid Piperidide (IIIa). A 0.5-g (2.3 mmole) sample of Ia was dissolved in 15 ml of absolute methanol, 10 ml (101 mmole) of piperidine was added, and the mixture was stirred at room temperature for 6 h and allowed to stand overnight (the overall reaction time was 24 h). The precipitate was removed by filtration and washed with ether to give 0.24 g of IIIa.

Compounds IIId, IIIe (by reaction with 19 ml of 16.5% absolute methanol saturated with ammonia), and IVa,b were similarly obtained.

Compound IVe did not precipitate, but was isolated after evaporation of the methanol. In the preparation of IIIb and IIIg, the reaction time at room temperature was 5-7 days.

To obtain IIIc, the reaction time should be shortened to 6 h, and the precipitate should be removed by filtration. When the reaction is carried out for a longer period, the precipitated IIIc dissolves, and a new precipitate of IVb forms (reaction time 24 h).

DL-α-Ureidosuccinic Acid β-N-Methylamide (V). A mixture of 0.65 g (4.1 mmole) of Ib and 50 ml of 20% absolute methanol saturated with CH_3NH_2 (260 mmole) was stored in a sealed ampule for 5 days until the precipitate (Ib · CH_3NH_2) had dissolved completely. The solution was evaporated to dryness, and the residue was dissolved in the minimum amount of water. The aqueous solution was acidified to pH 1-2 with concentrated HCl to give 0.55 g of V with E_{VII} 0.65 (pH 5.0).

Reaction of Ib with Methylamine in Aqueous Media. A 0.32-g (2 mmole) sample of Ib was dissolved in 20 ml of 20% aqueous methylamine (pH 10.45), and the mixture was allowed to stand for 24 h. The solution was evaporated to dryness, and the residue was dissolved in the minimum amount of water. The aqueous solution was acidified to pH 1-2 with concentrated HCl, but no precipitate was obtained. Electrophoresis of the reaction mixture immediately after the standing period revealed two spots (treatment with Erlich's reagent): 1) a substance corresponding to VII, according to the electrophoretic mobility; 2) a substance corresponding to V, according to the electrophoretic mobility, i.e., E_{VII} 0.65 (pH 5.0). From the volume of the spots, the ratio of VII to V was 1:2.

Reaction of Ib with n-Butylamine in Absolute Methanol. A 9-ml (92 mmole) sample of butylamine was added to 0.32 g (2 mmole) of Ib in 20 ml of water. The solution was allowed to stand for 24 h and was then analyzed by electrophoresis. Treatment with Erlich's reagent revealed two spots of equal intensity and dimensions, which, according to the electrophoretic mobilities, correspond to E_{VII} 1 and E_{VII} 0.65 (pH 5.0). In analogy with the reaction with methylamine, we propose that the substances are VII and the butylamide of VII.

Reaction of Ib with Piperidine in Aqueous Solution. A 4-ml (40 mmole) sample of piperidine was added to 0.32 g (2 mmole) of Ib in 10 ml of water, and the mixture was allowed to stand for 24 h and analyzed by electrophoresis [E_{VII} 1 (pH 5.0)]. The solution was evaporated to dryness, and the residue was dissolved in the minimum amount of water. The aqueous solution was acidified to pH 1-2 with concentrated HCl to give 0.1 g of VII with mp 181°, which did not depress the melting point of an authentic sample.

Butyl Ester of L-Dihydroorotic Acid (L-Ia). A 0.1-ml sample of concentrated H_2SO_4 was added to 4 g (25.3 mmole) of L-Ib in 140 ml of butanol, and the mixture was refluxed for 1 h and cooled. The precipitate was removed by filtration to give 4.4 g of L-Ia, which was dried over P_2O_5 in vacuo and recrystallized from ethanol. Compound D-Ia was similarly obtained, but the reflux time was 2.5 h.

L-Dihydroorotic Acid Amide (L-IIIe) and D-Dihydroorotic Acid Amide (D-IIIe). These compounds were obtained by the method used to prepare IIIe (the reaction time was ~18 h). When the reaction time was increased (for example, to 2 days), a certain amount of racemization was observed (~5-6%). The melting points of L-IIIe and D-IIIe were lower than the melting point of DL-IIIe. However, the melting point of a mixed sample (containing equal amounts of the L- and D-forms) corresponded to the melting point of the racemate.

L-Ureidosuccinic Acid Di(N-methylamide) (L-IVc). A total of 20 ml of 26% absolute methanol saturated with methylamine was added to 0.5 g (2.3 mmole) of L-Ia, and the mixture was allowed to stand at room temperature for 18 h. The precipitate was removed by filtration and washed with water to give 0.4 g of L-IVc.

D-Ureidosuccinic Acid Di(N-methylamide) (D-IVc). This compound was similarly obtained (the reaction time was about 40 h). This explains the lack of agreement between the angles of specific rotation, since a basic medium promotes racemization.

L-Dihydroorotic Acid Morpholine Salt (L-VIa). A 0.79-g (5 mmole) sample of L-Ib was suspended in 10 ml of water, 0.43 ml (5 mmole) of morpholine was added, and the mixture was stirred until L-Ib dissolved. The water was evaporated at room temperature to give an oily substance, which was treated with ethanol. The resulting precipitate was removed by filtration to give 1.15 g of L-VIa. Compound L-VIb was similarly obtained.

L-Dihydroorotic Acid Adenine Salt (L-VIc). A 0.79-g (5 mmole) sample of L-Ib and 0.67 g (5 mmole) of adenine were suspended in 40 ml of water, and the suspension was heated to the boiling point. The solution was then cooled, and the resulting precipitate was removed by filtration to give 1.25 g of L-VIc.

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