PREPARATION OF 3,5-DIOXOHEXAHYDRO-1,2,4-TRIAZINE-1-CARBOXYLIC ACID (DIHYDRO-6-AZAOROTIC ACID) DERIVATIVES*

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Reaction of hexahydro-1,2,4-triazine-3,5-dione (*I*) with ethyl chloroformate in pyridine or benzyl chloroformate in aqueous sodium hydroxide affords a mixture of the corresponding ethyl or benzyl 3,5-dioxohexahydro-1,2,4-triazine-1-carboxylates (*IIa*, *IIb*) with ethyl or benzyl 3,5-dioxohexahydro-1,2,4-triazine-1,2-dicarboxylates (*IIIa*, *IIIb*). In the presence of sodium hydrogen carbonate or sodium carbonate, only the monocarboxylates *IIa* or *IIb* are obtained. From the benzyl ester *IIb*, the sodium salt *IId* or the lithium salt *IIe* resulted on alkaline hydrolysis in anhydrous media while the reduction with sodium in liquid ammonia yielded the sodium salt *IId*.

From dihydroorotic acid, an intermediate in the biosynthesis of pyrimidine components of nucleic acids, two aza analogues may be theoretically inferred. One of them, the previously known 2,4-dioxohexahydro-1,3,5-triazine-6-carboxylic acid alias dihydro-5-azaorotic acid, proved some time ago[1] as the first specific inhibitor of dihydroorotate-dehydrogenase, an enzyme by which the dehydrogenation of dihydroorotic acid to orotic acid is catalysed. It was therefore of interest to examine the biological activity of the other aza analogue, namely, dihydro-6-azaorotic acid (3,5-dioxohexahydro-1,2,4-triazine-1-carboxylic acid). As a carbamoic acid, the 6-aza acid may exist in the form of esters or salts only, some of which are reported in the present paper (*IIa*–*IIe*).

The esters were prepared by carboxylation of hexahydro-1,2,4-triazine-3,5-dione (*I*) with esters of chloroformic acid in the presence of alkali. The reaction could theoretically take place on all the three NH groups; on the basis of the assumed basicity and analogy to acetylation[2] of compound *I*, a preferential substitution at positions N^1 and N^2 may be expected. Virtually, a mixture of the corresponding monocarboxylates (*IIa*, *IIb*) with dicarboxylates (*IIIa*, *IIIb*) was obtained by reaction of the dione *I* with ethyl chloroformate in pyridine or benzyl chloroformate in aque-

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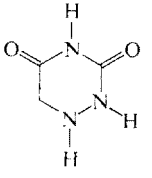
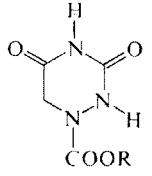
ous NaOH. Alcoholysis of the ethyl ester *IIIa* in the presence of sodium ethoxide results in removal of one from the two ethoxycarbonyl groups with the formation of the ethyl ester *IIa*. Position of the ethoxycarbonyl group in compound *IIa* was determined as follows: reaction of the ester *IIa* with diazomethane and reaction of 2,4-dimethylhexahydro-1,2,4-triazine-3,5-dione (*IVa*) with ethyl chloroformate afforded an identical ethyl 2,4-dimethyl-3,5-dioxohexahydro-1,2,4-triazine-1-carboxylate (*IVb*).

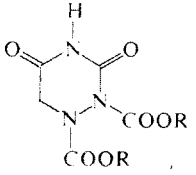
The esters *IIa* and *IIb* are thus derivatives of the required dihydro-6-azaorotic acid. As shown by the above experiments, a preferential substitution takes place on the most nucleophilic NH groups at positions 1 and 2. Consequently, a lowered basicity of the reaction medium should result in suppression of a further substitution and in a selective formation of monosubstituted derivatives. Accordingly, the reaction of compound *I* with benzyl chloroformate afforded almost exclusively the 1-substituted product *IIb* when performed in the presence of a theoretical amount of sodium hydrogen carbonate or sodium carbonate. On the other hand, the 2-substituted derivative *V* was obtained as the principal product when N-ethylpiperidine was present in the reaction mixture. Position of the alkoxycarbonyl may also be inferred from IR spectra shown in Table I. Compounds *IIa*–*IIc* exhibit characteristic bands of valence vibrations due to NH groups at positions 2 and 4 similarly to 3,5-dioxohexahydro-1,2,4-triazine[3] (*I*); however, the $\nu(N^2—H)$ values are somewhat



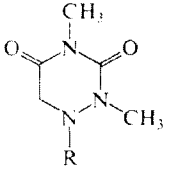
I



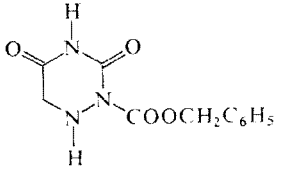
IIa, R = C_2H_5
IIb, R = $CH_2C_6H_5$
IIc, R = CH_3
IId, R = Na
IIe, R = Li



IIIa, R = C_2H_5
IIIb, R = $CH_2C_6H_5$



IVa, R = H
IVb, R = $COOC_2H_5$



V

shifted to lower frequencies. Compounds *IIIa*, *IIIb*, and *V* exhibit only a band of the NH group at position 4. The 2-benzyloxycarbonyl derivative *V* displays an additional weak band at 3313 cm^{-1} which might be ascribable to the N^1—H group. The 2- and 4-methyl derivatives of 3,5-dioxohexahydro-1,2,4-triazine exhibit similar very weak bands at about 3270 cm^{-1} with the half-width comparable to that of the free N^2—H and N^4—H group bands. On the other hand, neither the parent compound *I* nor hexahydro-3-pyridazinone exhibit similar bands. In the carbonyl region, the ν(C=O) bands of the particular carbonyl groups of compounds *IIa*–*IIc* (in chloroform solution) are not resolved. The alkoxycarbonyl groups at position 2 of compounds *IIIa*, *IIIb*, and *V* manifest themselves by the presence of an additional ν(C=O) band at about 1790 cm^{-1}.

The salts of dihydro-6-azaorotic acid (required for biological activity assays) were prepared with the use of the benzyl ester *IIb* as the starting compound. The sodium salt *IId* was obtained by hydrolysis with ethanolic sodium hydroxide or by reduction with sodium in liquid ammonia and the lithium salt *IIe* resulted from hydrolysis with methanolic lithium hydroxide. The latter reaction was accompanied by the formation of a little methyl 3,5-dioxohexahydro-1,2,4-triazine-1-carboxylate (*IIc*) on transesterification. The attempted purification of salts *IId* and *IIe* by crystallisation or precipitation from non-aqueous solvents failed. In an aqueous solution (as shown by time dependence of ultraviolet spectra), a relatively rapid decomposition of the two salts occurs; consequently, the chromatographic purification cannot be taken into consideration. The structural proof of the two salts was therefore limited to measurement of infrared spectra in solid state and in dimethyl sulfoxide as solvent (Table II). Owing to the lower purity of the particular samples of the two salts, there were some differences in position of the characteristic infrared spectral bands. The biological assays were performed with crude samples. Compound *IId* did not inhibit the growth of *Escherichia coli* B in glucose-containing mineral medium in concentrations up to 1000 μg/ml while with 5-azadihydroorotate a complete growth inhibition has been encountered in the concentration of 200 μg/ml under otherwise analogous conditions (ref.[1]). In view of this negative finding and instability of the test substance *IId*, it did not appear desirable to perform assays in other biological systems.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Analytical samples were dried at room temperature and 0·1 Torr for 8 h. Column chromatography was performed on the Pitra macroporous silica gel (particle size, 60—120 μ), produced by Service Laboratories of this Institute. Thin-layer chromatography was performed on ready-for-use fluorescent indicator containing Silufol UV_{254} silica gel sheets (Kavalier Glassworks, Votice, Czechoslovakia); spots were detected by viewing under ultraviolet light (Chromatolite). The IR spectra were taken on Zeiss UR-20 and Perkin-Elmer 621 spectrophotometers.

TABLE I

IR Spectra of Alkyl 3,5-Dioxohexahydro-1,2,4-triazine-1-carboxylates (cm^{-1})

Compound	Solvent	$\nu(N^2—H)$[a]	$\nu(N^4—H)$[a]	$\nu(N^1—H)$[a]	$\nu(C{=}O)$		$\nu(C—O—C)$ region	
I[b]	chloroform	3 439 m	3 394 m	—	—	1 740 w	1 722 m	—
IIa	chloroform[c]	3 418 m, sh[d]	3 390 s	—	—	1 736 m, br		1 267 w [e]
IIb	chloroform[c]	3 416 m, sh	3 391 s	—	—	1 739 m		1 274 w [e]
	dimethyl sulfoxide[f]		[g]			1 723 s, br	1 713 s, sh	1 275 m, br 1 216 m
IIc	chloroform[c]	3 419 m sh	3 393 s	—	—	1 738 m, br		1 277 w [e]
	dimethyl sulfoxide[f]		[g]		—	1 723 vs, br	1 714 s, sh	1 276 m 1 223 m
IIIa	chloroform[h]	—	3 379 s	—	1 803 s	1 751 vs, br		1 250 s [g]
	dimethyl sulfoxide[f]		[g]		1 798 s	1 756 s	1 736 s, br	1 250 s 1 213 s
IIIb	chloroform[h]	—	3 381 s	—	1 803 s	1 748 s, br		1 249, s br [e]
	dimethyl sulfoxide[f]		[g]		1 798 m	1 756 m, sh	1 739 s, br	1 249 m 1 211 m
IVb	chloroform[h]	—	—	—	—	1 732 vs	1 688 vs	[e]
	dimethyl sulfoxide[f]		[g]		—	1 730 s	1 689 s	1 228 m
V	chloroform[c]	—	3 386 m	3 313 w	1 793 w	1 745 w, br		1 269 w, br [e]
	dimethyl sulfoxide[f]	—	[g]		1 781 s	1 740 s	1 722 s	1 267 s 1 254 s, sh

[a] c 0·003M, 1 cm; [b] ref.[3]; [c] saturated solution, 0·5 mm; [d] after separation of bands on a computer, 3424 cm^{-1}; [e] solvent absorption: [f] c 4%, 0·04 mm; [g] the ν(NH) region not measured; [h] c 2%, 0·1 mm.

TABLE II

IR Spectra of Sodium and Lithium 3,5-Dioxohexahydro-1,2,4-triazine-1-carboxylates (cm^{-1})

Compound	Medium	ν(C=O)	$\nu_a(COO^-)$
IId	dimethyl sulfoxide	1 695 ± 1	1 626 ± 1
IId	KBr	1 701	1 626 ± 4
IIe	dimethyl sulfoxide	1 698 ± 3	1 626 ± 3
IIe	KBr	1 701 ± 1	1 621 ± 5
I	dimethyl sulfoxide	1 709, 1 695 sh	—

Diethyl 3,5-Dioxohexahydro-1,2,4-triazine-1,2-dicarboxylate (*IIIa*)

To a solution of hexahydro-1,2,4-triazine-3,5-dione (*I*; 11·5 g; 0·1 mol) in pyridine (100 ml) there was added dropwise over 30 min ethyl chloroformate (21·7 g; 19·1 ml; 0·2 mol). The mixture was stirred at 60°C for 2 h, evaporated, and the residue taken up into chloroform. The chloroform solution was washed with water, dilute hydrochloric acid, and aqueous sodium hydrogen carbonate, dried over anhydrous sodium sulfate, and evaporated. The residue was crystallised from ethanol to afford 11·6 g (47%) of the ester *IIIa*, m.p. 108–110°C. For $C_9H_{13}N_3O_6$ (259·2) calculated: 41·70% C, 5·06% H, 16·2% N; found: 41·79% C, 4·76% H, 16·40% N.

Ethyl 3,5-Dioxohexahydro-1,2,4-triazine-1-carboxylate (*IIa*)

A solution of the ester *IIIa* (5·18 g; 20 mmol) in ethanol (100 ml) was added to ethanolic sodium ethoxide (from 0·92 g *i.e.* 40 milligramatom of sodium and 600 ml of ethanol), the whole mixture refluxed for 1 h, cooled down, and diluted with water to dissolve the gel. Sodium ions were removed with Amberlite IRC 50 (H^+) ion exchange resin (50 g). Crystallisation from water yielded, 1·5 g (41·5%) of the ester *IIa*, m.p. 151–152°C. For $C_6H_9N_3O_4$ (187·2) calculated: 38·51% C, 4·85% H, 22·45% N; found: 38·31% C, 4·97% H, 22·55% N.

Ethyl 2,4-Dimethyl-3,5-dioxohexahydro-1,2,4-triazine-1-carboxylate (*IVb*)

A. A mixture of the ester *IIa* (187 mg; 1 mmol), dimethylformamide (5 ml), and ethereal diazomethane (0·3M; 25 ml) was kept at room temperature for 24 h, evaporated, and the residue purified by column chromatography on alumina in benzene–ethyl acetate (95 : 5) to afford the ester *IVb*, m.p. 71–72°C (ethanol–ether). For $C_8H_{13}N_3O_4$ (215·2) calculated: 44·65% C, 6·09% H, 19·53% N; found: 44·34% C, 6·21% H, 19·28% N.

B. To a stirred solution of 2,4-dimethylhexahydro-1,2,4-triazine-3,5-dione[4] (*IVa*; 715 mg; 5 mmol) in pyridine (20 ml) there was added dropwise ethyl chloroformate (0·651 g; 0·57 ml; 6 mmol), the whole mixture stirred at 50°C for 2 h, and evaporated. The residue was taken up into chloroform, the solution washed with water, dilute hydrochloric acid, and aqueous sodium hydrogen carbonate, dried over anhydrous sodium sulfate, evaporated, and the residue crystal-

lised from ethanol–ether to afford the ester *IVb*, m.p. 70—71°C, undepressed on admixture with a specimen obtained by procedure *A*.

Reaction of Hexahydro-1,2,4-triazine-3,5-dione (*I*) with Benzyl Chloroformate

A. *In aqueous sodium hydroxide.* To a precooled (0—5°C) solution of compound *I* (2·3 g; 20 mmol) in 0·5M sodium hydroxide (75 ml) there was added dropwise over 20 min a solution of benzyl chloroformate (3·4 g; 2·8 ml; 20 mmol) in tetrahydrofuran (35 ml), the whole mixture stirred at room temperature for 1 h, and neutralised with hydrochloric acid to afford a mixture (3·66 g) of compounds *IIIb* and *IIb*. The mixture was separated by column chromatography on silica gel (180 g). Elution with benzene–ethyl acetate (4 : 1) yielded 1·3 g of compound *IIIb*, m.p. 141—142°C (ethanol). For $C_{19}H_{17}N_3O_6$ (383·4) calculated: 59·53% C, 4·47% H, 10·96% N; found: 59·61% C, 4·44% H, 11·08% N. The subsequent elution with methanol yielded 1·93 g of compound *IIb*, m.p. 190—192°C. For $C_{11}H_{11}N_3O_4$ (249·2) calculated: 53·01% C, 4·45% H, 16·86% N; found: 52·83% C, 4·43% H, 16·91% N.

B. *In aqueous sodium carbonate.* A mixture of compound *I* (0·23 g; 2 mmol), sodium carbonate (0·105 g; 1 mmol), water (8 ml), and benzyl chloroformate (0·341 g; 0·28 ml; 2 mmol) was stirred at room temperature for 90 min to deposit a solid which was crystallised from ethanol. Yield, 0·45 g (92·4%) of compound *IIb*, m.p. 190—192°C, undepressed on admixture with a specimen obtained by procedure *A*.

C. *In aqueous sodium hydrogen carbonate.* A mixture of compound *I* (1·15 g; 10 mmol), sodium hydrogen carbonate (0·84 g; 10 mmol), water (70 ml), and benzyl chloroformate (1·7 g; 1·4 ml; 10 mmol) was stirred at room temperature for 2 h to deposit a solid which was crystallised from ethanol. Yield, 1·4 g (56·2%) of compound *IIb*, m.p. 190—192°C, undepressed on admixture with a specimen obtained by procedure *A*.

Benzyl 3,5-Dioxohexahydro-1,2,4-triazine-2-carboxylate (*V*)

A mixture of compound *I* (0·46 g; 4 mmol), water (12 ml), N-ethylpiperidine (0·452 g; 0·55 ml; 4 mmol), and benzyl chloroformate (0·68 g; 0·56 ml; 4 mmol) was stirred at room temperature for 1 h to deposit a solid which was crystallised from ethyl acetate–light petroleum. Yield, 0·55 g (55·2%) of the ester *V*, m.p. 177—179°C. For $C_{11}H_{11}N_3O_4$ (249·4) calculated: 53·01% C, 4·45% H, 16·86% N; found: 53·28% C, 4·50% H, 17·21% N.

Sodium 3,5-Dioxohexahydro-1,2,4-triazine-1-carboxylate (*IId*)

A. A mixture of the ester *IIb* (0·498 g; 2 mmol), methanol (22 ml), 1·15M methanolic sodium methoxide (1·74 ml; 2 mmol), and water (0·036 g; 2 mmol) was kept at room temperature for 24 h, concentrated, and the concentrate precipitated with ether to afford 0·35 g (96·6%) of the crude salt *IId* which was purified by reprecipitation from methanol with ether.

B. A mixture of the ester *IIb* (0·498 g; 2 mmol), liquid ammonia (100 ml), and sodium metal (0·046 g; 2 milligramatom) was kept at room temperature until the ammonia evaporated. The residue was washed with dioxane to afford 0·33 g (91%) of the salt *IId*.

Lithium 3,5-Dioxohexahydro-1,2,4-triazine-1-carboxylate (*IIe*)

A mixture of the ester *IIb* (0·498 g; 2 mmol), methanol (22 ml), and lithium hydroxide (0·048 g; 2 mmol) was kept at room temperature for 4 days, concentrated, and the concentrate precipitated with ether to afford 0·3 g (91%) of the crude salt *IIe*.

Methyl 3,5-Dioxohexahydro-1,2,4-triazine-1-carboxylate (*IIc*)

Column chromatography of the crude lithium salt *IIe* on silica gel (100 parts by weight) in ethyl acetate yielded about 10% of the methyl ester *IIc*, m.p. 145–146°C. For $C_5H_7N_3O_4$ (173·1) calculated: 34·69% C, 4·08% H, 24·27% N; found: 34·68% C, 4·13% H, 24·08% N.

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