REACTION OF 2,3-DIHYDRO-1,3-6*H*-OXAZINE-2,6-DIONE WITH ALIPHATIC AMINES AND AMINO ACIDS

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Received September 24th, 1981

Reaction of 2,3-dihydro-1,3-6*H*-oxazine-2,6-dione (*I*) with amines in aqueous medium leads predominantly to N-substituted amides of (*Z*)-3-carboxylamino-2-propenoic acid. Isomeric 3-(N′-alkylureido)-2-propenoic acids are formed as by-products in the reaction of compound *I* with ammonia or methylamine; tert-butylamine affords ureido derivative as the sole reaction product. Secondary amines react with compound *I* substantially more rapidly than primary amines. In both cases the branching on the α-carbon decreases the reactivity distinctly while the branching on the β-carbon does not have a significant effect on the rate of aminolysis. The results of kinetic measurements of the reaction of compound *I* with amino acids are discussed n connection with the possible interaction of compound *I* with enzymes.

2,3-Dihydro-1,3-6*H*-oxazine-2,6-dione (3-oxauracil, *I*) displays a clear bacteriostatic and cancerostatic activity[1]. In intact cells of *Escherichia coli* it elicits an unusually rapid onset of the inhibition of the biosynthesis of DNA and RNA. When using a ^{14}C-labelled compound *I* it was found that it is metabolised in the cells of *Escherichia coli* to the 5′-phosphate of 3-oxauridine and probably even to higher phosphates[2]. However, the molecular mechanism of the inhibitory effects of compound *I* has not yet been elucidated. Heidelberger[3] expressed the hypothesis that compound *I* acts as an irreversible inhibitor of the biosynthesis of pyrimidine nucleotides. A possible site of impact of this antimetabolite are free amino groups or the hydroxyl groups of the active site of the target enzyme. Both types of such acylation reactions are known in penicillins. Irreversible inhibition of bacterial D-alanine-carboxypeptidases is due to the formation of the ester-type binding of penicillins with the hydroxyl group of serine in active centre of the enzymes mentioned[4]. Benzylpenicillin is bound with an amide bond to the amino group of L-lysine, and the penicillin group thus bound is the main antigenic determinant of penicillin allergy (ref.[5] and the refs mentioned there).

In connection with the study of the mechanism of biological activity of compound *I* it seemed interesting to investigate its acylation ability with respect to nucleophilic

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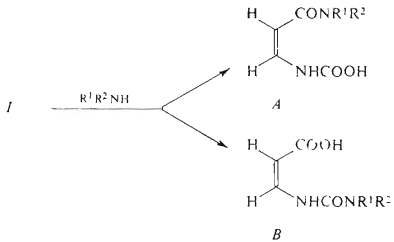
reagents. In this paper we shall discuss the mechanism of the reactions of compound *I* with aliphatic amines and amino acids.

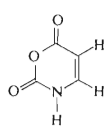
According to the analogy with the chemical behaviour of isatoic acid anhydride (2*H*-3,1-benzoxazine-2,4(1*H*)-dione)[6] and acylanthranils (2-alkyl-4*H*-3,1-benzoxazin--4-ones)[7] to amines and other nucleophilic reagents it may be considered that compound *I* will react with amines and amino acids under formation of amido derivatives *A* and ureido derivatives *B* (Scheme 1). We observed this reactional dichotomy in the reaction of compound *I* with ammonia and some amines.



SCHEME 1

On reaction of compound *I* with 5% aqueous ammonia we obtained (*Z*)-3-ureido--2-propenoic acid (*II*) in low yield. Since the amide of (*Z*)-3-carboxylamino-2-propenoic acid, which is formed simultaneously, is stable only in alkaline medium, we isolated it as its methyl ester. Therefore we converted the products of aminolysis of compound *I* to sodium salts which we submitted under anhydrous conditions to the reaction with iodomethane in dimethylformamide, obtaining thus the amide of (*Z*)-3-methoxycarbonylamino-2-propenoic acid (*III*) and the methyl ester of (*Z*)--3-ureido-2-propenoic acid (*IV*) in a 1 : 4 ratio and a total yield of 56%. In an analogous manner we also prepared earlier the methyl esters of 2-substituted (*Z*)-3-methoxycarbonylamino-2-propenoic acids[8]. The use of hexamethylphosphoric triamide[9] as a medium for the reaction of the sodium salts with iodomethane did not lead to an increase in the yield. On reaction of compound *I* with methylamine and subsequent reaction of the sodium salts with iodomethane we obtained N-methylamide of (*Z*)-3-methoxycarbonylamino-2-propenoic acid (*V*) and the methyl ester of (*Z*)-3--(N′-methylureido)-2-propenoic acid (*VI*) in a 2 : 1 ratio. While both types of products were formed, the reaction of compound *I* with dimethylamine led, after subsequent methylation, to N,N-dimethylamide of (*Z*)-3-methoxycarbonylamino-2-propenoic acid (*VII*) only. On reaction of tert-butylamine with compound *I* (applying the same working up procedure) a mixture of methyl (*Z*)- and (*E*)-3-(N′-tert-butylureido)-2-pro-

penoates (*XII*, *XIII*) was obtained. The *E*/*Z* isomerization evidently takes place during the reaction with iodomethane, because neutralization of the mixture after the reaction of compound *I* with tert-butylamine gave (*Z*)-3-(N′-tert-butylureido)--2-propenoic acid (*XI*) as the only product. The structure of these compounds *II* – *XIII*



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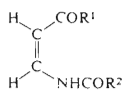
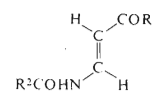




II; $R^1 = OH$, $R^2 = NH_2$
III; $R^1 = NH_2$, $R^2 = OCH_3$
IV; $R^1 = OCH_3$, $R^2 = NH_2$
V; $R^1 = NHCH_3$, $R^2 = OCH_3$
VI; $R^1 = OCH_3$, $R^2 = NHCH_3$
VII; $R^1 = N(CH_3)_2$; $R^2 = OCH_3$
XI; $R^1 = OH$, $R^2 = NHC(CH_3)_3$
XII; $R^1 = OCH_3$, $R^2 = NHC(CH_3)_3$
XIV; $R^1 = OH$, $R^2 = OCH_3$
XV; $R^1 = R^2 = OCH_3$

VIII; $R^1 = OH$, $R^2 = OCH_3$
X; $R^1 = R^2 = OCH_3$
XIII; $R^1 = OCH_3$, $R^2 = NHC(CH_3)_3$

was proved by means of IR and ^1H NMR spectra. In addition to this amides *III*, *V* and *VII* were prepared for the sake of comparison from (*Z*)-3-methoxycarbonylamino-2-propenoic acid (*XIV*) and the corresponding amine in the presence of N,N′--dicyclohexylcarbodiimide. Compound *XIV* was prepared on reaction of compound *I* with sodium methoxide in methanol. The preparation of acid *XIV*, described earlier, using Hoffmann's degradation of maleic acid monoamide, did not lead to reproducible results[10]. After having assigned *E*/*Z* configuration to derivatives of N-substituted 3-amino-2-propenoic acids on the basis of the coupling constants of the protons $H_{(2)}$ and $H_{(3)}$ we also prepared as a model substance (*E*)-3-methoxycarbonylamino-2-propenoic acid (*VIII*) by photoisomerization of the (*Z*)-isomer *XIV*. Reaction of the (*Z*)-isomer *XIV* with thionyl chloride and subsequent hydrolysis gave the (*E*)-isomer *VIII* in low yield. The (*E*)-isomer of the methyl ester *X* was obtained on action of sodium methoxide in methanol on the (*Z*)-isomer *XV*.

Aminolysis of compound *I* was investigated kinetically in aqueous medium (pH 9·2) at 25°C, using a considerable excess of the nucleophilic reagent. Under the conditions

mentioned the rate of hydrolytic cleavage of the oxazine ring of compound *I* was relatively low. The reaction of compound *I* with amines is a second order reaction and the corresponding rate constant k_2 was calculated according to relation (*1*), the inference of which starts from the assumption that the reactive species is a non-protonated form of amine[11]:

$$k_2 = \frac{k_1}{c}\left(1 + \frac{[H_3O^+]}{K_a}\right), \qquad (1)$$

where k_1 means the apparent first order rate constant, comprizing a correction for parallel solvolysis (k_s), c is the analytical concentration of the amine, K_a is the dissociation constant of the conjugated acid and $[H_3O^+]$ is the activity of hydroxonium ions. The k_2 values should be invariant with respect to the analytical concentration of the base, but in the case of some primary amines the observed k_2 values increase significantly with increasing concentration of the amine. For example an approximately ten-fold increase in methylamine concentration causes a 15% increase in the rate constant k_2 value. For the determination of the amide : ureide ratio in kinetic experiments the different stability of the products in acetate buffer has been made use of. In consequence of easy decarboxylation, products *A* decompose almost immediately while ureido derivatives *B* are stable in weakly acid medium. In the investigated series of amines and amino acids aminolysis of compound *I* takes place regioselectively under formation of products *A*. Only ammonia, tert-butylamine and bis(2-cyanoethyl)amine afford mixtures of products *A* and *B* (Table I).

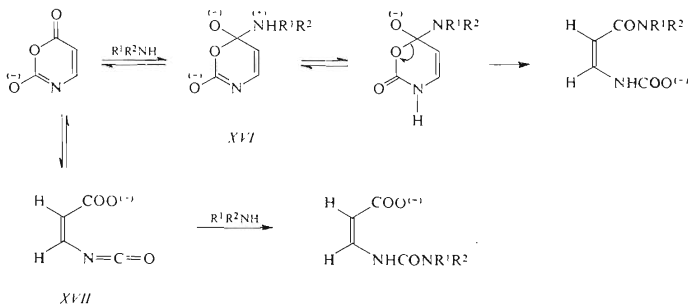
The opening of the oxazine ring of compound *I* probably takes place by a mechanism which was proposed some time ago for the reaction of 2*H*-3,1-benzoxazine-2,4(1*H*)-dione (isatoic anhydride) with amines[12], and later on confirmed by kinetic studies[13]. The first step in the formation of amides *A* consists in an attack of the amino group on the electrophilic centre of the anion of compound *I*, in the position 6

TABLE I

Ratio amide/ureide and the total yield in the reaction of compound *I* (0·1 mmol) with amines (10 mmol) in 100 ml of 0·1M sodium tetraborate at 25°C, after 24 h

Base	Amide/Ureide	Yield, %
Ammonia	48/52	42·5
Tert-butylamine	49/51	39·2
Bis(2-cyanoethyl)amine	60/40	54·8

(Scheme 2). The intermediate *XVI* formed splits off a proton and opens the cycle under formation of amide. The splitting off of the proton from intermediate *XVI* takes place under participation of the amine or hydroxide anion present. The participation



SCHEME 2

of amine in proton transfer is evidently the reason for the catalytic effect which is evident especially in the case of methylamine and ethylamine. A catalytic effect of this type was also observed in the aminolysis of benzylpenicillin[5]. In the case of the reaction of compound *I* with diethylamine a reproducible drop in the value k_2 with increasing amine concentration was observed. However, we have no acceptable explanation of this fact.

The formation of ureido derivatives *B* may also be explained by the equilibrium between the cyclic and the open form of deprotonated compound *I*. The existence of the open form of the anion of isatoic anhydride is also supported by Staiger and Miller[12] on the basis of the analogy with the behaviour of anions of N-carboxyanhydrides of amino acids in which the existence of the open form of the anion was proved by IR data[14]. The intermediate anion *XVII* is capable of reacting with poorly reactive amines, since its transition state has a trigonal character and the steric effects of the alkyl group of the amine participate to a lesser extent.

From kinetic data in the series of the investigated amines and amino acids (Table II and III) some qualitative relationships follow, concerning the reactivity of the bases during the aminolysis of compound *I*: *1*) Secondary amines react much more rapidly than primary amines unless their reactivity is affected by polar effects, as for example in the case of bis(2-cyanoethyl)amine. *2*) The reactivity of primary and secondary amines is strongly decreased by the branching on the α-carbon, while the branching on the β-carbon is practically without effect. *3*) Ammonia has a special

position in the investigated series, since its reactivity with compound *I* is substantially lower than would correspond to its basicity. *4*) No linear correlation exists between the values of the logarithms of the rate constants k_2 and the pK_a values of bases due probably to the considerable sensitivity of the reaction to steric effects. This result contrasts with the results of Friedman and coworkers[15] who found a satisfactory linear correlation between the mentioned constants for the reaction of acrylonitrile with amino acids.

Among amino acids the greatest reactivity with compound *I* was observed in L-proline and then L-lysine, the reactivity of which is probably due to the terminal amino group since the rate constants k_2 in α-amino acids are substantially lower. The mentioned amino acids are the most probable sites of the attack of the target enzyme by compound *I*. A direct proof of the covalent bond of compound *I* with enzymes is difficult, owing to the low stability of the bonded residue of 3-carboxylamino--2-propenoic acid.

TABLE II

Rate constants k_2, defined by the equation (*1*) for the reactions of compound *I* (at a $3{\cdot}22 \cdot 10^{-5}$ mol l^{-1} concentration) with amines of concentration *c* in 0·1M-sodium tetraborate, at 25·00 ± ± 0·01°C. The pH values were measured at the beginning of the reaction. The pK_a values are from ref.[22].

Compound	pK_a	$10^2 \cdot k_2$, mol^{-1} l s^{-1} [a]		
		(1)	(2)	(3)
Methylamine	10·62	50·04	46·72	43·59[b]
Ethylamine	10·63	18·07	17·02	16·79
n-Propylamine	10·53	17·94	16·96	16·55
n-Butylamine	10·59	19·56	18·82	18·78
Isobutylamine	10·43	18·66	18·40	17·76
Neopentylamine	10·21	19·23	18·44	18·54
Isopropylamine	10·63	3·58	3·58	3·53
Tert-butylamine	10·45	0·067	—	—
Dimethylamine	10·64	512·0[c]	509·0[d]	513·0[e]
Diethylamine	10·98	81·17	87·96[c]	—
2-Cyanoethylamine	7·80[f]	1·92	—	—
Bis(2-cyanoethyl)amine	5·26[g]	0·003	—	—
Ammonia	9·21	0·238	—	—

[a] Rate constants k_2, given in columns (1) to (3) were obtained at the following concentrations of amines (unless stated otherwise): *c* (mol l^{-1}) 0·06 (9·25); 0·03 (9·22); 0·015 (9·19). The numbers in brackets are the pH values of the corresponding reaction mixtures; [b–e] measured at the following concentrations of amines: *c* (mol l^{-1}) 0·006; 0·02; 0·01; 0·005; [f] ref.[23]; [g] ref.[24].

EXPERIMENTAL

The melting points were determined on a Kofler block. Analytical samples were dried at 25°/6·5 Pa for 8 h. The UV spectra were recorded on a Specord UV VIS and the IR spectra on a UR-20 spectrophotometer (both from Zeiss, Jena). The ^{1}H NMR spectra were measured on a Tesla 467 (60 MHz) instrument with tetramethylsilane (in deuteriochloroform) and hexamethyldisiloxane (in hexadeuteriodimethyl sulfoxide) as internal standards; chemical shifts, δ-scale, are in ppm. The measurements of pH were carried out with a Beckman Research pH-Meter, using standard buffers from Beckman.

Starting Compounds

Compound *I* (ref.[16]), neopentylamine[17], 2-cyanoethylamine[18] and bis(2-cyanoethyl)amine[18] were prepared by known procedures. Hydrochlorides of amines were prepared by neutralization of aqueous solutions of amines with 10% hydrochloric acid. The evaporated solution of the hydrochloride was codistilled with ethanol and crystallized from ethanol–ethyl acetate to constant melting point. Before use the hydrochlorides were dried at 100°C/13 Pa for 5 h. The hydro-

TABLE III

Rate constants k_2 defined by the equation (*I*) for the reactions of compound *I* ($3{\cdot}22 \cdot 10^{-5}$ mol . l^{-1}) with amino acids ($2{\cdot}0 \cdot 10^{-2}$ mol l^{-1}) in 0·1M-sodium tetraborate at 25·00 ± 0·01°C; the pH values of the reaction mixture was 9·20; λ_{max} are absorption maxima of the products. The pK_a values are from the literature cited

Compound	pK_a	Ref.	$10^2 \cdot k_2$ (mol^{-1} l s^{-1})	λ_{max} (in nm)
L-Proline	10·64	25	330·0	278
L-Lysine	10·81	26	64·7	270
Sarcosine	10·11	27	31·8	276
6-Aminohexanoic acid	10·75	28	24·8	—
3-Aminopropanoic acid	10·24	29	12·2	269
Glycine	9·78	30	9·68	—
L-Glutamic acid	9·95	31	4·06[a]	—
L-Isoleucine	9·76	32	3·67	270
L-Valine	9·72	32	3·31	270
L-Alanine	9·87	32	2·81	270
L-Leucine	9·74	32	2·66	270
Glycylglycine	8·27	33	1·34	—
L-Histidine	9·16	34	0·89	272
(D,L)-3,3-Dimethyl-2-aminopropanoic acid	—	—	3·50[b]	—

[a] Concentration of amino acid $c = 0{\cdot}01$ mol l^{-1}; [b] the value of k_2 was calculated for the tentative value of p$K_a = 9{\cdot}70$.

chlorides of the amines used were already described, with the exception of 2-cyanoethylamine hydrochloride, m.p. 167–168°C. $C_3H_7ClN_2$ (106·6) calculated: 33·81% C, 6·62% H, 33·27% Cl, 26·29% N; found: 34·05% C, 6·62% H, 33·02% Cl, 26·91% N.

Kinetic Measurements

The investigated substance or its solution (5 ml) was added to a solution of compound *I* in 0·1M sodium tetraborate in a calibrated flask (25, 50, 100 ml). After filling up to the mark with a solution of 0·1M sodium tetraborate and agitation, part of the measured solution was transferred into a measuring cell and the remaining solution was employed for the determination of pH. The resulting concentration of compound *I* was equal in all experiments ($3{\cdot}22 \cdot 10^{-5}$ mol l^{-1}), while the base was used in a 200 to 2 000 fold molar excess. The decrease in absorbance of compound *I* was measured at 305 nm in a cell 2 cm long, thermostated at 25·00 ± 0·01°C. In several cases the reaction course was also followed on the basis of absorbance increase of products (in the case of diethylamine at 272 nm, in the case of sarcosine, proline, glycyl-glycine and alanine at 272 nm). Both methods afforded the same values for the rate constants k_1 (within the limits of experimental errors). The values of absorbance with the corresponding time *t* were recorded at equidistant time intervals (20 to 3 600 s) on a perforated band using a digital device connected to the emitting potentiometer of the Specord UV VIS spectrophotometer. The reaction was observed for 4 half-times and 30 to 60 points were read. Three to 6 measurements were carried out for each substance at various concentrations of bases. The reproducibility of the rate constants k_1 was about 1%. The k_1 values were found as one of the parameters of the exponential $A_t = A_0 \exp(-k_1 t)$, which was constructed through the experimental field of the points (A_t, *t*) using the method of least squares[19]. For the calculation Späth's algorithm[20] was employed and the transcendental relationship in this alogorithm was solved using the regula falsi method[21]. In the same manner the value of k_s for the solvolysis of compound *I* in 0·1M sodium tetraborate at 25·0°C was determined, when the base was not added. In all the computations the value $k_s = 1{\cdot}55 \cdot 10^{-5}\ s^{-1}$ was used. The constants k_2, given in Tables I and II, were calculated using the relation (*1*).

Spectrophotometric Determination of the Ratio Amide/Ureide in Mixtures after Aminolysis of Compound *I*

Compound *I* (11·3 mg; 0·1 mmol) was added under stirring to a solution of dimethylamine hydrochloride (810 mg; 10 mmol) in 0·1M sodium tetraborate (100 ml) and the mixture was allowed to stand at 25°C for 24 h. 2 ml of the mixture were pipetted into 0·1M acetate buffer (pH 4·2) and 0·1M sodium tetraborate buffer, and the solutions were made up to 50 ml. After 2 min the absorbance at 270 nm in the acetate buffer was zero, while in the borate buffer the mixture had an absorbance of 1·48 at 275 nm. In the same manner the reactions with ammonia, tert-butylamine and bis(2-cyanoethyl)amine were also carried out. For the calculation of the concentration of amides the molar exctinction coefficient ($\varepsilon_{270} = 3{\cdot}61 \cdot 10^4$) was used, which was determined for the product of the reaction of *I* with dimethylamine, for which a quantitative course was assumed. For the calculation of the concentration of ureides the ε_{270} values determined for compounds *II* and *XI* ($1{\cdot}56 \cdot 10^4$ and $2{\cdot}02 \cdot 10^4$) were used. For the ureide formed on reaction with bis(2-cyanoethyl)amine the value ε_{270} for compound *II* was used. The percentual compositions of mixtures, determined spectrophotometrically, are given in Table I.

Isolation of 3-Ureido-2-propenoic Acids *II* and *XI*

Compound *I* (1·13 g; 0·01 mol; well ground) was added to a solution of ammonia or amine (0·1 mol) in water (30 ml) at 5°C and under stirring over 10 min. When compound *I* was dissolved the mixture was allowed to stand at room temperature for 12 h (in the case of tert-butylamine for 48 h). After addition of 1M-NaOH (10 ml) the mixture was evaporated at 30°C (bath temperature) and *in vacuo* to dryness. The residue was dissolved in 50 ml of water and then Dowex 50 (H^+) ion exchanger was added to it, under cooling with ice, until the reaction was weakly acid. In the case of tert-butylamine the residue was dissolved in 100 ml of 50% aqueous methanol. The ion exchanger was filtered off, washed with 50 ml of water and evaporated under reduced pressure from a bath 30°C warm. The residue was dissolved in a small volume of methanol at room temperature. After addition of an equal volume of water the product crystallized out. Analytical samples were obtained by crystallization from methanol. Crystallization from hot water is connected with considerable losses in consequence of the easy hydrolysis of 3-ureido-2-propenoic acids.

(*Z*)-3-Ureido-2-propenoic Acid (*II*)

Yield, 12·3%, m.p. 145—150°C (water–methanol), under decomp. UV spectrum (0·025-HCl): λ_{max} 266 nm (log ε = 4·25); (acetate buffer pH 4·2): λ_{max} 261 nm (log ε = 4·26); (0·05M-NaOH); λ_{max} 256·5 nm (log ε = 4·29). IR spectrum (KBr), cm^{-1}: 3 505, 3 478, 3 330 sh, 3 512 (NH); 2 765, 2 590 (OH dimer); 1 739, 1 694, 1 681 sh (C=O, amide I); 1 633 (NH_2); 1 599 (C=C); 1 443, 693 (CH).

^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): δ 4·73 (d, 1 H, $H_{(2)}$, $J_{2,3}$ = 9·0 Hz); 6·80 (s, 2 H, NH_2); 7·32 (dd, 1 H, $H_{(3)}$, $J_{3,2}$ = 9·0 Hz, $J_{3,NH}$ = 12·0 Hz); 9·75 (d, 1 H, NH, $J_{NH,3}$ = 12·0 Hz). For $C_4H_6N_2O_3$ (130·1) calculated: 36·83% C, 4·65% H, 21·53% N; found: 36·95% C, 4·76% H, 21·62% N. Half-time of hydrolysis of compound *II* at 45°C in 0·1M-acetate buffer (pH 5·2): 450 min (measured by absorbance decrease at 270 nm).

(*Z*)-3-(N'-Tert-Butylureido)-2-propenoic Acid (*XI*)

Yield, 34·4%, m.p. 168°C (methanol–water). UV spectrum (0·025M-HCl): λ_{max} 273 nm (log ε = 4·25); (in 0·1M-acetate buffer of pH 4·2): λ_{max} 269·5 nm (log ε = 4·31); in 0·05M NaOH): λ_{max} 262 nm (log ε = 4·35). IR spectrum (KBr), cm^{-1}: 3 305, 3 280 (NH); 1 689, 1 718 (C=O dimer COOH, and amide I); 1 610 (C=C); 1 566, 1 556 (amide II). ^{1}H NMR spectrum (hexadeuteriodimethyl sulfoxide): δ 1·22 (s, 9 H, 3 CH_3); 3·42 (d,1 H, $H_{(2)}$, $J_{2,3}$ = 9·0 Hz); 6·03 (dd, 1 H, $H_{(3)}$, $J_{3,2}$ = 9·0 Hz, $J_{3,NH}$ = 9·0 Hz); 6·27 (s, 1 H, NH—$C(CH_3)_3$); 8·43 (d, 1 H, NH, $J_{NH,3}$ = 12·0 Hz). For $C_8H_{14}N_2O_3$ (186·2) calculated: 51·60% C, 7·58% H, 15·04% N; found: 51·71% C, 7·57% H, 15·35% N. Half-time of hydrolysis of compound *XI* at 25°C in 0·1M acetate buffer (pH 4·2): 51 min (measured spectrophotometrically as absorbance decrease at 273 nm).

Isolation of 3-Ureido-2-propenoic Acids and Amides of 3-Carboxylamino-2-propenoic Acids in the Form of their Methyl Esters (Compounds *III*—*VII*, *XII* and *XIII*)

1M-NaOH (20 ml) was added to a mixture after aminolysis of compound *I* under the above-mentioned conditions and the mixture was evaporated in a vacuum at 30°C. The residue was codistilled under reduced pressure with a mixture of ethanol (25 ml) and toluene (25 ml) (three times). The residue was ground and suspended in dimethylformamide (50 ml). Iodomethane (7·14 g; 0·05 mol) was added dropwise at room temperature to the stirred suspension and the

mixture was stirred at this temperature for 6 h. After dilution with water (50 ml) the mixture was saturated with solid ammonium sulfate and the solution was extracted with ethyl acetate (4 × 50 ml). The extract was dried over sodium sulfate, evaporated and codistilled in a vacuum with toluene (3 × 50 ml). The residue was chromatographed on a silica gel column (30–60 μm) in benzene–ethyl acetate (2 : 1).

Methyl (*Z*)-3-Ureido-2-propenoate (*IV*)

Yield, 45·5%, m.p. 163°C (methanol). UV spectrum (0·1M-HCl): λ_{max} 266 nm ($\log \varepsilon = 4{\cdot}40$); (0·1M-NaOH): λ_{max} 266 nm ($\log \varepsilon = 4{\cdot}33$). IR spectrum (in KBr), cm^{-1}: 3 392, 3 356 sh, 3 311, 3 212 (NH); 1 751 (C=O), urea); 1 683 (C=O), ester); 1 643 (NH_2); 1 610 (C=C); 1 500 (NH). ^1H NMR spectrum (hexadeuteriodimethyl sulfoxide): δ 3·59 (s, 3 H, CH_3); 4·85 (d, 1 H, $H_{(2)}$, $J_{2,3} = 9{\cdot}0$ Hz); 6·82 (br s, NH_2); 7·28 (dd, 1 H, $H_{(3)}$, $J_{3,2} = 9{\cdot}0$ Hz, $J_{3,NH} = 12{\cdot}0$ Hz); 9·64 (br d, 1 H, NH). For $C_5H_8N_2O_3$ (144·1) calculated: 41·66% C, 5·59% H, 19·44% N; found: 41·78% C, 4·71% H, 19·33% N.

Amide of (*Z*)-3-Methoxycarbonylamino-2-propenoic Acid (*III*)

Yield, 11·7%, m.p. 97–99°C (benzene). UV. spectrum (0·1M-HCl): λ_{max} 255 nm ($\log \varepsilon = 4{\cdot}34$); (0·1M-NaOH): λ_{max} 256·5 nm and 303 nm sh ($\log \varepsilon = 4{\cdot}26$ and 3·67, resp.). IR spectrum (10^{-1} mol l^{-1}, in chloroform, 0·1 cm cell), cm^{-1}: 3 530, 3 415 (free NH_2 group); 3·305 (NH, intramolecular hydrogen bond); 1 738 (C=C, carbamate); 1 671–1 673 (C=O, amide I); 1 626 (C=C); 1 582 (C=O), amide II); 1 505 (NH); ($3 \cdot 10^{-3}$ mol l^{-1} in chloroform, 1 cm cell), cm^{-1}: 3 531, 3 415·5 (free NH_2 group); 3 305 (NH, intramolecular hydrogen bond); 1 740 (C=O, carbamate); 1 673 (C=O, amide I); 1 626 (C=C); 1 586 (C=O, amide II). ^1H NMR spectrum (hexadeuteriodimethyl sulfoxide): δ 3·78 (s, CH_3); 4·95 (d, 1 H, $H_{(2)}$, $J_{2,3} = 9{\cdot}5$ Hz); 7·14 (dd, 1 H, $H_{(3)}$, $J_{3,2} = 9{\cdot}5$ Hz, $J_{3,NH} = 11{\cdot}5$ Hz); 5·58 (br s, 2 H, NH_2); 10·4 (br d, 1 H, NH). For $C_5H_8N_2O_3$ (144·1) calculated: 41·66% C, 5·59% H, 19·44% N; found: 41·70% C, 5·60% H, 18·89% N. The analytical sample was sublimated at 100°C/2 kPa.

N-Methylamide of (*Z*)-3-methoxycarbonylamino-2-propenoic Acid (*V*)

Yield, 21·3%, m.p. 61–62°C (sublimated at 100°C/2 kPa). UV spectrum (0·1M-HCl): λ_{max} 258 nm ($\log \varepsilon = 4{\cdot}40$); (0·1M-NaOH): λ_{max} 260 nm ($\log \varepsilon = 4{\cdot}35$). IR spectrum ($3 \cdot 10^{-1}$ mol l^{-1} in chloroform, 1 cm cell), cm^{-1}: 3 463 (free NH group); 3 300 (NH, intramolecular hydrogen bond); 1 736 (C=O, amide I, carbamate); 1 665 (C=O, amide I, CH_3NHCO); 1 622 (C=C); 1 541 (C=O, amide II, CH_3NHCO); 1 481 and 1 468 sh (C=O, amide II, carbamate); 1 454 sh and 1 379 (CH_3); 1 054, 962. ^1H NMR spectrum (deuteriochloroform); δ 2·83 (d, 3 H, CH_3—NH, $J_{CH_3,NH} = 5{\cdot}0$ Hz); 3·75 (s, CH_3O); 4·87 (d, 1 H, $H_{(2)}$, $J_{2,3} = 9{\cdot}0$ Hz); 5·5 (m, CH_3NH); 7·08 (dd, 1 H, $H_{(3)}$, $J_{3,2} = 9{\cdot}0$ Hz, $J_{3\cdot NH} = 10{\cdot}5$ Hz); 10·5 (br d, 1 H, NH—CO_2.CH_3). For $C_6H_{10}N_2O_3$ (158·2) calculated: 45·56% C, 6·37% H, 17·71% N; found: 45·83% C, 6·46% H, 17·43% N.

Methyl (*Z*)-3-(N′-methylureido)-2-propenoate (*VI*)

Yield, 12·6%, m.p. 105–106°C (benzene–diethyl ether). UV spectrum (0·1M-HCl): λ_{max} 271 nm ($\log \varepsilon = 4{\cdot}35$); (0·1M-NaOH): λ_{max} 271 nm ($\log \varepsilon = 4{\cdot}28$). IR spectrum ($3 \cdot 10^{-3}$ mol l^{-1} in chloroform, 1 cm cell), cm^{-1}: 3 457 (NH, free group); 3 320 (NH, intramolecular hydrogen bond); 1 710 (C=O, ester); 1 680 (C=O, amide I, urea); 1 629 (C=C); 1 565, 1 550, 1 535 (C=O,

amide II); 1 436, 1 384 (CH_3); 1 012, 981, 912. ^{1}H NMR spectrum (deuteriochloroform): δ 2·86 (d, 3 H, CH_3—NH, $J_{CH_3,NH}$ = 4·0 Hz); 3·67 (s, CH_3O); 4·96 (d, 1 H, $H_{(2)}$, $J_{2,3}$ = 9·0 Hz); 6·08 (br m, CH_3NH; 7·51 (dd, 1 H, $H_{(3)}$, $J_{3,2}$ = 9·0 Hz, $J_{3,NH}$ = 11·5 Hz); 9·95 (br d, 1 H, $C_{(3)}$—NH, $J_{NH,3}$ = 11·0 Hz). For $C_6H_{10}N_2O_3$ (158·2) calculated: 45·56% C, 6·32% H, 17·71% N; found: 45·66% C, 6·42% H, 17·84% N.

N,N-Dimethylamide of (*Z*)-3-Methoxycarbonylamino-3-propenoic Acid (*VII*)

A) Yield, 24·5%, m.p. 92—93°C (sublimated at 100°C/2 kPa). UV spectrum (0·1M-HCl): λ_{max} 266 nm (log ε = 4·38); (0·1M-NaOH): λ_{max} 269 nm (log ε = 4·37). IR spectrum (2% solution in chloroform), cm^{-1}: 3 291 (NH, intramolecular hydrogen bond); 1 737 (C=O, carbamate); 1 646, 1 595 (C=O, amide I and C=C); 1 503, 1 472 (C=O, amide II, carbamate); 1 453, 1 385 (CH_3). ^{1}H NMR spectrum (deuteriochloroform): δ 2·98 (s, 6 H, $(CH_3)_2$); 3·75 (s, CH_3O); 5·31 (d, 1 H, $H_{(2)}$, $J_{2,3}$ = 9·0 Hz); 7·16 (dd, 1 H, $H_{(3)}$, $J_{3,2}$ = 9·0 Hz, $J_{3,NH}$ = 12·0 Hz); 10·8 (d, 1 H, $C_{(3)}$—NH, $J_{NH,3}$ = 12·0 Hz). For $C_7H_{12}N_2O_3$ (172·2) calculated: 48·83% C, 7·03% H, 16·27% N; found: 49·03% C, 7·30% H, 15·98% N.

B) N,N'-dicyclohexylcarbodiimide (0·186 g; 3 mol) was added at room temperature to a solution of acid *XIV* (0·290 g; 2 mmol) in acetonitrile (10 ml) and the mixture allowed to stand for 20 min. It was then cooled in an ice bath and dimethylamine (1·0 g; 22 mmol) in acetonitrile (5 ml) was added. After 12 h standing at room temperature the mixture was evaporated in a vacuum, the residue was mixed with ethyl acetate (2 ml) and N,N'-dicyclohexylurea was filtered off and the filtrate evaporated. Crystallization of the residue from diisopropyl ether gave 0·19 g (37%) of compound *VII*, m.p. 93—94°C, undepressed on admixture of the sample from experiment *A*).

Using the same procedure amides *III* and *V* were also prepared. The crude products were chromatographed on silica gel (30—60 μm) in benzene–ethyl acetate (1 : 1).

Methyl (*Z*)-3-(N'-Tert-butylureido)-2-propenoate (*XII*)

Yield, 18·7%, m.p. 118°C (ethyl acetate–light petroleum). UV spectrum (0·1M-acetate buffer, pH 4·2): λ_{max} 272 nm (log ε = 4·38); (0·05M-NaOH): λ_{max} 272 nm (log ε = 4·35). IR spectrum (2% solution in chloroform), cm^{-1}: 3 438 (free NH group); 3 348 (NH, intramolecular hydrogen bond); 1 713, 1 681 (C=O), amide I and ester); 1 630 (C=C); 1 525, 1 452 sh, 1 557 sh (C=O, amide II); 1 395, 1 367 ($(CH_3)_3C$); 1 395 (CH vinyl groups); 1 380 (characteristic band of the (*Z*)-isomer); (10^{-3} mol l^{-1} in chloroform, 1 cm cell), cm^{-1}: 3 434 (free NH group); 3 331 (NH, intramolecular hydrogen bond). ^{1}H NMR spectrum (deuteriochloroform); δ 1·38 (s, 9 H, $(CH_3)_3$.C); 3·68 (s, CH_3O); 4·95 (d, 1 H, $H_{(2)}$, $J_{2,3}$ = 9·0 Hz); 5·21 (s, 1 H, NH—$C(CH_3)_3$); 7·45 (dd, 1 H, $H_{(3)}$, $J_{3,2}$ = 9·0 Hz, $J_{3,NH}$ = 12·0 Hz); 9·75 (d, 1 H, NH—$C_{(3)}$, $J_{NH,3}$ = 12·0 Hz). For $C_9H_{16}N_2O_3$ (200·2) calculated: 63·98% C, 8·06% H, 13·99% N; found: 54·07% C, 5·03% H, 13·94% N.

Methyl (*E*)-3-(N'-Tert-butylureido)-2-propenoate (*XIII*)

Yield, 5·8%, m.p. 138—139°C (ethyl acetate–light petroleum). UV spectrum 0·1M-acetate buffer, pH 4·2): λ_{max} 267 nm (log ε = 4·50); (0·05M-NaOH): λ_{max} 267 nm (log ε = 4·40) and 315 nm (log ε = 3·62). IR spectrum (2% solution in chloroform), cm^{-1}: 3 428 (free NH group); 3 390, 3 319, 3 227 (associated NH group); 1 690 (C=O, amide I); 1 635 (C=C); 1 555, 1 540, 1 519 (C=O, amide II); 1 395, 1 367 ($(CH_3)_3C$); 1 310, 994, (CH of the vinyl group); 1 270, 1 152

(characteristic bands of the (*E*)-isomer); (10^{-3} mol l^{-1}) solution in chloroform, 1 cm cell): 3 437 (free NH group). ^{1}H NMR spectrum (deuteriochloroform): δ 1·37 (s, 9 H, $(CH_3)_3C$); 3·68 (s, 3 H, CH_3O); 5·03 (d, 1 H, $H_{(2)}$, $J_{2,3}$ = 12·0 Hz); 5·77 (s, 1 H, NH—$C(CH_3)_3$); 7·93 (dd, 1 H, $H_{(3)}$, $J_{3,2}$ = 13·0 Hz, $J_{3,NH}$ = 12·0 Hz); 8·40 (d, 1 H, NH—CH=). For $C_9H_{16}N_2O_3$ (200·2) calculated: 53·98% C, 8·06% H, 13·99% N; found: 53·78% C, 8·14% H, 14·01% N.

(*Z*)-3-Methoxycarbonylamino-2-propenoic Acid (*XIV*)

Compound *I* (22·6 g; 0·2 mol) was added in portions over 20 min, under stirring at 0°C, to a solution of 0·5M-sodium methoxide in methanol (800 ml). After 6 h standing at 0°C methanol was evaporated *in vacuo* at 40°C bath temperature. The residue was dissolved in 200 ml of water, cooled to 5°C and acidified with 30% sulfuric acid to pH 1, under cooling the mixture with ice. The solution was extracted with ethyl acetate (4 × 100 ml), dried over sodium sulfate and evaporated. The residue was crystallized from water. Yield, 16·5 g (57%), m.p. 126°C (water), undepressed in admixture with a sample prepared from maleic acid monoamide according to ref.[10].

Methyl (*Z*)-3-Methoxycarbonylamino-2-propenoate (*XV*)

An ethereal 0·5M-diazomethane solution was added in several portions to a solution of the (*Z*)-isomer *XIV* (1·50 g) in methanol (10 ml), cooled with ice, until the solution remained yellow. After 30 min standing at room temperature the mixture was evaporated and the residue distilled, to yield 1·22 g (74·2%) of product *XV*, b.p. 110—120°C/2 kPa, which solidified at 20°C. UV spectrum (5% ethanol): λ_{max} 261 nm (log ε = 4·24); (0·01M-HCl): λ_{max} 261·5 nm (log. ε = 4·25); (0·01M-NaOH): λ_{max} 251 nm (log ε = 4·25). IR spectrum (2% solution in chloroform), cm^{-1}: 3 344 (NH, intramolecular hydrogen bond); 1 744 (C=O, carbamate); 1 691 (C=O, ester); 1 640, 1 635, 1 652 (C=C); 1 502 (NH); (3 . 10^{-3} mol l^{-1} in chloroform): 3 344 (NH, intramolecular hydrogen bond); 1 745 (C=O, carbamate); 1 692 (C=O, ester); 1 641, 1 634, 1 652 (C=C). ^{1}H NMR spectrum (deuteriochloroform): δ 3·62 (s, 3 H, CH_3-ester); 3·69 (s, 3 H, CH_3-carbamate); 4·96 (d, 1 H, $H_{(2)}$, $J_{2,3}$ = 9·0 Hz); 7·16 (dd, 1 H, $H_{(3)}$, $J_{3,2}$ = 9·0 Hz, $J_{3,NH}$ = 12·0 Hz); 9·64 (br d, 1 H, NH). For $C_6H_9NO_4$ (159·1) calculated: 45·28% C, 5·70% H, 8·80% N; found: 45·23% C, 5·69% H, 8·88% N.

(*E*)-3-Methoxycarbonylamino-2-propenoic Acid (*VIII*)

A) Photochemical isomerization of compound *XIV*: (*Z*)-Isomer *XIV* (200 mg) in 65 ml of water was irradiated at room temperature with a 150 W high-pressure mercury lamp, using a liquid filter (20% dimethylformamide solution), for 20 min. After concentration to about 10 ml volume practically pure (*E*)-isomer (*VIII* crystallized out, m.p. 194°C (water). Yield, 0·071 g (36%). The mother liquors contain a mixture of both stereoisomers (according to TLC in ethyl acetate). UV spectrum (0·01M-HCl): λ_{max} 258 nm (log ε = 4·24); (0·01M-NaOH): λ_{max} 250 nm (log ε = 4·20). For $C_5H_7NO_4$ (145·1) calculated: 41·38% C, 4·86% H, 9·67% N; found: 41·15% C, 4·89% H, 9·51% N.

B) Isomerization of compound *XIV* with thionyl chloride: (*Z*)-isomer *XIV* (1·45 g) was refluxed in a mixture of diethyl ether (5 ml), thionyl chloride (5 ml) and dimethyl formamide (0·1 ml) for 1 h. The mixture was evaporated in a vacuum and the residue decomposed with 3 ml of water under cooling with ice. The solid fraction was crystallized from water. Yield, 0·12 g (8·3%) of compound *VIII*, m.p. 194—196°C, undepressed on admixture with a sample obtained as under *A*).

Methyl (*E*)-3-Methoxycarbonylamino-2-propenoate (*X*)

(*Z*)-Isomer *XV* (0·90 g) was refluxed with 0·1M sodium methoxide in methanol (10 ml) for 3 h. After cooling a product crystallized out which was crystallized from methanol, to give 0·18 g (20%) of compound *X*, m.p. 173—174°C (methanol). UV spectrum (50% ethanol): λ_{max} 259 ($\log \varepsilon = 4{\cdot}32$). IR spectrum (saturated solution in chloroform), cm^{-1}: 3 432 (free NH group); 1 754 (C=O carbamate); 1 708, 1 695 sh (C=O, ester); 1 652 (C=C); 1 511 (NH); ($3 \cdot 10^{-3}$ mol l^{-1} in chloroform): 3 430 (NH); 1 755 (C=O, carbamate); 1 709, 1 695 (C=O, ester); 1 653 (C=C). ^{1}H NMR spectrum (deuteriochloroform): δ 3·59 (s, 3 H, CH_3-ester); 3·69 (s, 3 H, CH_3-carbamate); 5·39 (d, 1 H, $H_{(2)}$, $J_{2,3} = 14{\cdot}5$ Hz); 7·69 (dd, 1 H, $H_{(3)}$, $J_{3,2} = 14{\cdot}5$ Hz, $J_{3,NH} = 11{\cdot}0$ Hz); 9·72 (d, 1 H, NH, $J_{NH,3} = 11{\cdot}0$ Hz). For $C_6H_9NO_4$ (159·1) calculated: 45·28% C, 5·70% H, 8·80% N; found: 45·49% C, 6·09% H, 8·60% N.

The authors thank Dr J. Pospíšek for the kind donation of a sample of rac. 2,2-*dimethyl-2-aminopropanoic acid and Dr A. Vítek for writing the programme for the computation of the rate constants.*

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Translated by Ž. Procházka.