SYNTHESIS AND HYDROLYSIS OF 5-HALO-2,3-DIHYDRO-1,3-6*H*-OXAZINE-2,6-DIONES

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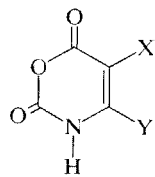
5-Chloro-, 5-bromo-, and 5-iodo-2,3-dihydro-1,3-6*H*-oxazine-2,6-diones *II*—*IV* were prepared by halogenation of 2,3-dihydro-1,3-6*H*-oxazine-2,6-dione (*I*). The rate of alkaline hydrolysis of the oxazine derivatives *I*—*V* is increased by substituents at position 5 in the order $CH_3 <$ $< H < I < Br < Cl$; the 3-carboxyaminoacrylic acids *VII*—*XI* are obtained as products. The acids *VII*—*IX* were characterised as methyl esters of the corresponding 3-methoxycarbonylaminoacrylic acids *XII*—*XIV*. The 3-carboxyaminoacrylic acids *VII*—*XI* are stable in media of pH 10—11; at pH < 10, the appropriate formylacetic acids are formed as decomposition products. The decarboxylation rate of acids *VII*—*XI* increases in the order $Cl < Br < I < H <$ $< CH_3$.

Remarkable antibacterial effects[1,2] of 2,3-dihydro-1,3-6*H*-oxazine-2,6-dione (*I*) (for nomenclature see ref.[3]) aroused our interest in preparation of the 5-halo derivatives of compound *I*. The isomeric 4-halo derivatives of compound *I* were prepared[4] by reaction of trimethylsilyl azide with substituted anhydrides of maleic acid. From the other derivatives of compound *I*, 4-methyl- and 5-methyl-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione[4,5] as well as 3-β-D-ribofuranosyl-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione[6] and its 2'-deoxy derivative[5] have been reported. In the present paper, we wish to describe the preparation of 5-chloro-, 5-bromo-, and 5-iodo-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione (*II*—*IV*) and their stability towards hydrolytical cleavage.

The chloro derivative *II* was obtained by chlorination of compound *I* with gaseous chlorine in ethyl acetate. The bromo derivative *III* was obtained by reaction of compound *I* with bromine in dioxane; in this solvent, the reaction is faster than in ethyl acetate. The iodo derivative *IV* resulted from the reaction of compound *I* with iodine monochloride in a mixture of acetic acid and anhydrous potassium acetate. Position of the halo atom in compounds *II*—*IV* was confirmed by ^{1}H-NMR spectra. The H-4 proton exhibits in compound *II*—*IV* an almost identical chemical shift as the corresponding proton in 5-methyl-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione (δ 7·35), the structure of which was unequivocally established by synthesis[5]. In 4-substituted derivatives[4] of compound *I*, the proton signal occurs in a higher field (δ 5·5). 5-Methyl-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione[5] (*V*), required for comparison of physical

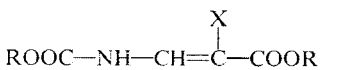
and chemical properties, was prepared by reaction of citraconic anhydride with trimethylsilyl azide in benzene. Under these conditions, compound *V* and the isomeric 4-methyl-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione (*VI*) are formed in the ratio of 1 : 1. Washburne and coworkers[4] obtained compound *VI* as the single product of the reaction of citraconic anhydride with trimethylsilyl azide in chloroform. The UV spectra of compounds *II*–*VI* are similar to those of the unsubstituted compound *I* (ref.[2]). Substituents at positions 4 and 5 of the oxazine ring cause a marked bathochromic shift in spectra taken both in acidic and alkaline media.



I, X = Y = H
II, X = Cl, Y = H
III, X = Br, Y = H
IV, X = I, Y = H
V, X = CH_3, Y = H
VI, X = H, Y = CH_3

In connection with the future biological tests, we have been also interested in the hydrolytical cleavage of compounds *II*–*V* under various reaction conditions with a special respect to the character of hydrolytical products. With the aim to trap the 3-carboxyaminoacrylic acids *VII*–*XI* (assumed as primary products of the cleavage of oxazine derivatives *I*–*V*), the hydrolysis was effected in a strongly alkaline medium. The concentration of the starting compounds and products was checked by spectrophotometry. The time dependence of UV spectra of compounds *II*–*V* in a strongly alkaline medium is similar to that reported[2] in the case of compound *I*. As it may be observed on Fig. 1, the absorbance decrease of the long wavelength maximum is accompanied by the formation of a new maximum at shorter wavelengths, belonging to 3-carboxyaminoacrylic acids *VII*–*XI*. (In the previous paper[2], the hydrolytical product of compound *I* was erroneously ascribed the structure of the enol form of formylacetic acid which exhibits in alkaline media an almost identical λ_{max} value as the acid *VII*). Since the attempted isolation (*cf.*[7–9]) of salts of the acids *VII*–*XI* failed, some of the acids (*VII*–*IX*) were characterised as methyl esters of 3-methoxycarbonylaminoacrylic acids *XII*–*XIV*. For this purpose, the cleavage of oxazine derivatives *I*–*III* was performed in 0·3M tetra-n-butylammonium hydroxide and the resulting salts of acids *VII*–*IX* were converted by reaction with methyl iodide in dimethylformamide into the esters *XII*–*XIV*. The reaction of sodium salts of acids *VII*–*IX* with methyl iodide in dimethylformamide afforded very low yields of the esters, probably because of the low solubility of sodium salts of carbamoic acids in dimethylformamide.

The ester *XII* was ascribed the *Z*-configuration on the basis of a relatively low coupling constant[10] ($J_{2,3}$ = 6·2 Hz). In accordance with this conclusion, the IR spectrum of compound *XII* exhibits a band of the NH group connected by an intramolecular hydrogen bond with the more distant carbonyl group. On the basis of the same spectral evidence, the Z-configuration may also be attributed to compound *XIV*. On the other hand, the band of a free NH group in the IR spectrum of compound *XIII* suggests the *E*-configuration. The relatively low yields of compounds *XII*–*XIV* do not allow to to draw any conclusions on the isomerisation of (Z)-carboxyaminoacrylic acids that are primary products of the alkaline hydrolysis of oxazine derivatives.



VII, R = X = H
VIII, R = H, X = Cl
IX, R = H, X = Br
X, R = H, X = I
XI, R = H, X = CH_3
XII, R = CH_3, X = H
XIII, R = CH_3, X = Cl
XIV, R = CH_3, X = Br

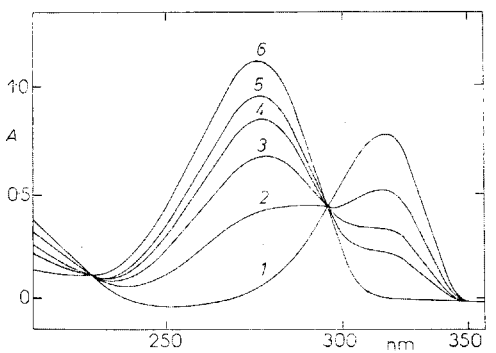


FIG. 1

Hydrolysis of the Chloro Derivative *II* in Alkaline Media as Checked by UV Spectra ($8·8 . 10^{-5}$M solution of compound *II* in 0·01M-NaOH at 25·00 ± 0·05°C)

1 Zero min, *2* to *5* measured in 6 min intervals, *6* 60 min.

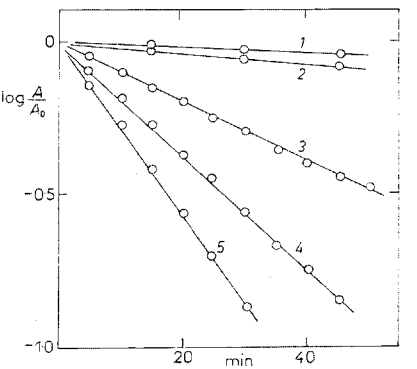


FIG. 2

Alkaline Hydrolysis of 5-Substituted Oxazine Derivatives *I*—*V*

Time dependence of the relative absorbance of $6·0 . 10^{-5}$M solutions of the oxazine derivatives in 0·01M-NaOH at 25·00 ± ± 0·05°C as measured at the following λ_{max} values: *1* 304 nm (compound *V*), *2* 296 nm (*I*), *3* 318·5 nm (*IV*), *4* 315·5 nm (*III*), *5* 315 nm (*II*)

Hydrolysis of oxazine derivatives *I* – *V* in 0·01M-NaOH at 25°C obeys the 1st order kinetics (Fig. 2, Table I). Substituents at position 5 of the oxazine ring enhance the rate of hydrolysis in the order $CH_3 < H < I < Br < Cl$. The electronegative substituents lower the electron density on carbon atoms of carbonyl groups and facilitate thus a nucleophilic attack by the hydroxylic ion; it is not possible to determine by a qualitative consideration which of the carbonyl groups reacts preferentially. The same order of substituents was determined in kinetic measurements of the hydrolysis of *para* substituted benzoic anhydrides[11]. An increase of the rate of hydrolysis due to introduction of an electronegative substituent is also visible from kinetic measurements of hydrolysis of mixed acetic and chloroacetic anhydrides and derivatives containing more chloro atoms[12].

TABLE I

Hydrolysis Half-Time (in min) of Oxazine Derivatives of the Initial Concentration $6·0 . 10^{-5}$M in 0·01M-NaOH at 25·00 ± 0·05°C and in Borate Buffer Solutions at 30·0 ± 0·1°C

Compound	X	0·01M-NaOH	Borate[a] pH	
			10·29	9·64
I	H	190·0	500	500
II	Cl	11·0	105	240
III	Br	16·5	160	480
IV	I	31·0	320	>500
V	CH_3	330	270	250[b]

[a] Spectrophotometrical measurements were performed on a Unicam 8000 apparatus; [b] the reaction was carried out at 29·85 ± 0·05°C.

TABLE II

Hydrolysis of Oxazine Derivatives *I* – *V* (Initial Concentration, $6·0 . 10^{-5}$M) in 0·1M-HCl at 29·85 ± 0·05°C; % of Absorbance at λ_{max} after 6·5 h of the Reaction

Compound	*I*[a]	*II*	*III*	*IV*	*V*
$100A/A_0$	100	74	78	84	98

[a] The 10^{-4}M solutions of compound *I* in 0·01M, 0·1M, and 1M hydrochloric acid heated at 98°C for 1 h afford the following $100A/A_0$ values: 79%, 61%, and 31%.

The rate of hydrolysis of the oxazine derivatives *I* – *V* decreases with the decreasins concentration of hydroxylic ions (Table I); the order of substituents is the same ga in the hydrolysis in a strongly alkaline medium except for the methyl derivative *V* since the rate of cleavage somewhat increases with a decreasing pH value. On the other hand in 0·1M-HCl, the methyl derivative *V* is almost as stable as compound *I*. When subjected to the above acidic hydrolysis, the halo derivatives *II*–*IV* are slowly decomposed; the rate order corresponds to the increasing electronegativity of substituents (Table II). The hydrolysis of compound *I* was also examined at 98°C and found to be markedly accelerated by an increasing concentration of hydrochloric acid (from 0·01M to 1·0M) (Table II).

In hydrolysis of the oxazine derivatives *I*–*V* in strongly alkaline media, the UV spectral curves exhibit an isosbestic point whereas this point is absent in hydrolyses at pH <10 as a consequence of the cleavage of 3-carboxyaminoacrylic acids *VII*–*XI*. This finding prompted us to a more detailed examination of the pH dependence with respect to the stability of acids *VII*–*XI*. Thus, solutions of 3-carboxyaminoacrylic acids *VII*–*XI* are stable in strongly alkaline media only; the absorbance of their solutions in 0·1M-NaOH at 25°C remains practically unchanged for 24 h while in 0·01M-NaOH a decrease by 5–11% may be observed within 17 h (Table III). The pH dependence of the decomposition rate was examined with the acid *VII*.

As shown on Fig. 3, the decarboxylation of acid *VII* obeys the 1st order kinetics and the rate constant strongly increases with the decreasing pH value. On the basis

TABLE III

UV Spectra of 2-Substituted 3-Carboxyaminoacrylic Acids in 0·1M-NaOH, Stability of the 5·0 . 10^{-3}M Solutions in 0·01M-NaOH at 25°C, and the Decarboxylation $t_{1/2}$ Value (in min) at 25·00 ± 0·05°C in a Phosphate Buffer Solution of pH 7·79

Compound	X	λ_{max}, nm	ε^a	$100A/A_0{}^b$	$t_{1/2}$, min
VII	H	259	18 760	91	7
VIII	Cl	273	12 960	95	36
IX	Br	276	13 540	91	33
X	I	275·5, sh 300	13 540, 5 800	89	28
XI	CH_3	266	13 900	89	<3

[a] The molar extinction coefficient ε was calculated on the basis of the molarity of the starting oxazine derivative; [b] A_0 designates the absorbance of a freshly prepared 5·0 . 10^{-3}M solution of the appropriate 3-carboxyaminoacrylic acid in 0·01M-NaOH at the λ_{max} value stated; A designates the absorbance of the same solutions after 17 h at 25°C.

of structural similarity with ethyl 3-aminocrotonate[13] and some keto enamines[14], the expected 3-aminoacrylic acid (as cleavage product of the acid *VII*) would exhibit a strong absorbance in the 260–270 nm region. No absorbance has been however observed in this region in the present case. It may be thus assumed that 3-aminoacrylic acid is unstable and undergoes hydrolysis with the formation of formylacetic acid. The presence of this acid in the reaction mixture after decarboxylation of the acid *VII* was established by the positive test with diazotized *p*-nitroaniline (the test is specific of β-ketocarboxylic acids[15,16]) as well as by conversion to 2,4-dinitrophenylhydrazone[17,18]. The formation of formylacetic acid was also demonstrated by ultraviolet spectrum. The nonabsorbing solutions resulting after the cleavage of acid *VII* in neutral media display, when adjusted to pH 10·5–11·0 a strong ultraviolet absorption at λ_{max} 258 nm, the pH dependence of which is identical with that of authentic formylacetic acid (Fig. 4). As suggested by spectrophotometrical measurements, the course of decarboxylation with acids *VIII–XI* and the acid *VII* is qualitatively the same. Marked differences may be observed in the stability of the resulting formylacetic acids. The attempted characterisation of formylchloroacetic acid in the form of the 2,4-dinitrophenylhydrazone resulted in isolation of chloroacetaldehyde 2,4-dinitrophenylhydrazone. Formylchloroacetic acid and its 2,4-dinitrophenylhydrazone thus undergo a rapid decarboxylation in acidic media. Decomposition of formylchloroacetic acid takes place even in neutral media as estab-

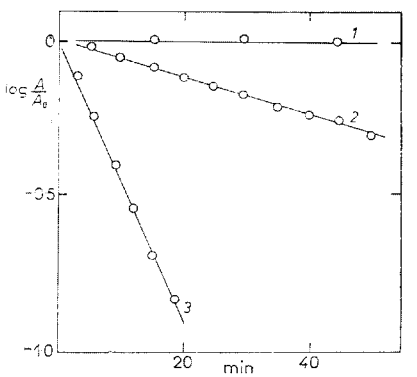


FIG. 3

pH-Dependence of the Decarboxylation of Acid *VII*

Relative absorbance (259 nm) of the starting $5{\cdot}0 \cdot 10^{-5}$M solution of the acid *VII* at 25·00 ± 0·05°C, time dependence: *1* borate buffer solution (pH 10·60), *2* borate buffer solution (pH 9·38), *3* phosphate buffer solution (pH 7·79).

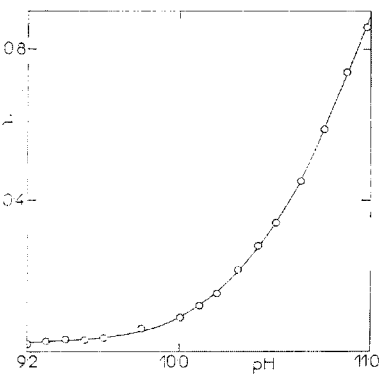


FIG. 4

pH-Dependence of the UV-Spectrum of Formylacetic Acid

Absorbance (at 259 nm) of an about 10^{-4}M solution of formylacetic acid as measured in borate buffer solutions at 25°C.

lished by removal of chloroacetaldehyde from the neutral reaction mixture by distillation under diminished pressure at 10°C. The stability of formylacetic acids was measured spectrophotometrically in 0·1M-NaOH at 25°C (Table IV). Whereas formylacetic acid and formylpropionic acid are stable in alkaline media, the formylhaloacetic acids are decomposed, presumably with the formation of haloacetaldehydes that do not absorb in the 240–300 nm region. In the course of the decomposition of formyliodoacetic acid in a borate buffer solution of pH 9·94, a growing ultraviolet maximum at 227 nm may be observed; the position corresponds to that of an iodide anion (Fig. 5). Formyliodoacetic acid or iodoacetaldehyde formed from this acid react with hydroxylic ions under substitution of the halo atom.

TABLE IV

UV Spectra of 2-X-Substituted Formylacetic Acids in 0·1M-NaOH and $t_{1,2}$ Value (in min) of Their Decomposition at 25·00 ± 0·05°C in 0·1M-NaOH

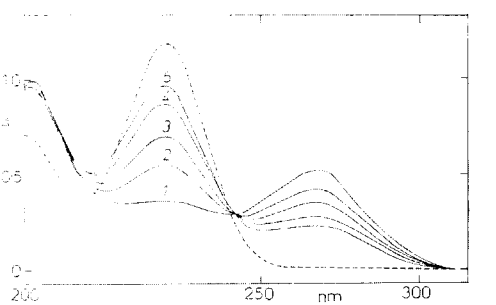
X	λ_{max}	ε^a	$t_{1/2}$
H	258	13 200	stable
Cl	267	7 200	21·0
Br	267	5 300	3·4
I	263	6 400	7·4
	227[b]	7 900	
CH_3	266	7 800	stable

[a] For conditions of the UV spectral measurements see the Experimental. The molar extinction coefficient was calculated on the basis of the molarity of the starting oxazine derivative. [b] The absorption maximum belongs to iodide ions produced by decomposition of formyliodoacetic acid.

FIG. 5

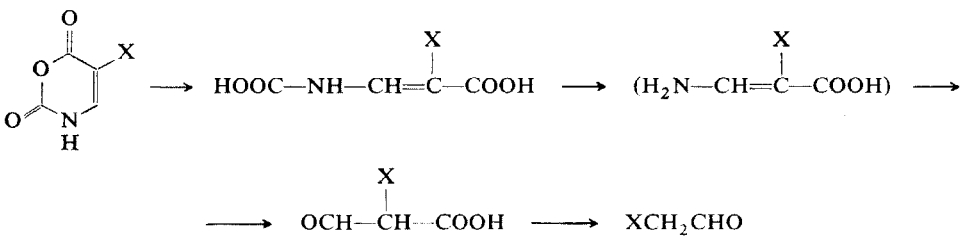
Decomposition of Formyliodoacetic Acid

Time dependence of the UV spectrum of a $5 \cdot 10^{-5}$M solution of formyliodoacetic acid at 25·00 ± 0·05°C due to decomposition of the acid *X* in a borate buffer solution of pH 9·94: ——— *1* zero min, *2* 3 min, *3* 6 min, *4* 12 min, *5* 21 min (------ UV spectrum of a $9{\cdot}6 \cdot 10^{-5}$M solution of potassium iodide).



It may be inferred from kinetic measurements on decarboxylation of acids *VII*–*XI* (Table III) in a phosphate buffer solution of pH 7·8 at 25°C that substituents at position 2 enhance the reaction rate in the order Cl < Br < I < H < CH_3. This order is consistent with results from decarboxylation of N-substituted carbamoic acids[19,20]. According to the latter investigations, the rate constant of hydroxonium-ion-catalysed decarboxylation of N-substituted carbamoic acids is capable of a linear correlation with pK_a values of the corresponding amines provided that the pK_a value is lower than 5. The pK_a values of the basic component of 3-aminoacrylic acid and its 2-substituted derivatives are not accessible to a direct measurement. It may be however assumed that weak bases are involved and that substituents at position 2 affect the pK_a values in a similar manner as in the series of substituted anilines[21]. According to this assumption, the pK_a value of the acid *XI* should be higher than of the remaining acids *VII*–*X*; the acid *XI* was virtually found to exhibit the fastest decarboxylation rate of the whole series.

The kinetic measurements allow to express the course of the hydrolysis of oxazine derivatives *I*–*V* by Scheme 1 proposed by Rinkes[22] for the hydrolysis of compound *I*.



X = H, Cl, Br, I, CH_3

SCHEME 1

On the basis of the present investigation on the stability of intermediates in decomposition of the oxazine derivatives *I*–*V*, only formylacetic acid or substituted formylacetic acids and the corresponding aldehydes may occur in significant concentrations under conditions of biological assays. The biological results should not be affected by the presence of readily metabolisable substances such as formylacetic acid, formylpropionic acid and the corresponding aldehydes; on the other hand, the presence of formylhaloacetic acids and haloacetaldehydes as potent alkylation agents must undoubtedly be taken into consideration.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block). Analytical samples were dried at 25°C/0·05 Torr for 8 h. The UV spectra were measured on a Specord UV VIS (Carl Zeiss, Jena) apparatus. The IR spectra were recorded on a UR-10 (Carl Zeiss, Jena) spectrophotometer. The ^{1}H-NMR spectra were determined on a Varian HA 100 apparatus (100 MHz;

hexamethyldisiloxane as internal standard; chemical shifts δ in p.p.m.). The mass spectra were measured on a double focussing MS-902 spectrometer. The pH values were determined on a MV 85 pH-meter (Präcitronic, Dresden, German Democratic Republic); for the preparation of buffer solutions see ref.[23]. Thin-layer chromatography was performed on ready-for-use Silufol UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in 1 : 1 benzene–ethyl acetate. Spots were detected by viewing under UV light (Chromatolite) and by spraying with 0·5% aqueous potassium permanganate. Electrophoresis was performed on paper Whatman No 1 (30 V/cm, 1 h).

5-Chloro-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione (*II*)

A mild stream of chlorine gas was introduced at 15°C into a stirred suspension of compound *I* (2·26 g; 20 mmol) in ethyl acetate (50 ml) until the solid dissolved (10 min) and the faintly yellow persisted for about 1 min (the course of chlorination was checked by thin-layer chromatography). The mixture was evaporated under diminished pressure, the residue dissolved in water (10 ml), and the aqueous solution extracted with three 20 ml portions of ethyl acetate. The extract was dried over anhydrous magnesium sulfate and evaporated under diminished pressure. The residue was triturated with ether and collected with suction. Crystallisation from ethyl acetate yielded 0·75 g (25%) of compound *II*, m.p. 163—164°C (ethyl acetate). UV spectrum in 0·01M-HCl: λ_{max} 206 and 281 nm (ε 5930 and 7080); in 0·05M sodium tetraborate: λ_{max} 214 and 314·6 nm (ε 5480 and 9170). IR spectrum (in KBr): 1799, sh 1779, 1730, 1711 (C=O); 1634 (C=C); 3091 (C—H); 3163 cm^{-1} (N—H). ^{1}H-NMR spectrum (in hexadeuteriodimethyl sulfoxide): δ 7·82 (s, 1 H, H-4). For $C_4H_2ClNO_3$ (147·5) calculated: 32·57% C, 1·37% H, 24·03% Cl, 9·49% N; found: 32·86% C, 1·59% H, 24·08% Cl, 9·59% N.

5-Bromo-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione (*III*)

Bromine (1·6 g; 10 mmol) in dioxane (5 ml) was added dropwise at 20°C to a stirred solution of compound *I* (1·13 g; 10 mmol) over 5 min. The mixture was kept at room temperature for 3 h and evaporated under diminished pressure (bath temperature, 35°C). The residue was dissolved in ethyl acetate (100 ml), the solution washed with two 25 ml portions of 1% aqueous sodium thiosulfate, dried over anhydrous sodium sulfate, and evaporated under diminished pressure. Crystallisation from ethyl acetate yielded 0·62 g of compound *III* (32%). Work-up of mother liquors afforded additional 0·36 g (19%) of compound *III* of the same purity; m.p. 191—192°C (ethyl acetate). UV spectrum in 0·01M-HCl: λ_{max} 206 and 283 nm (ε 7225 and 7920); in 0·05M sodium tetraborate: λ_{max} 217 and 315·5 nm (ε 6830 and 10590). IR spectrum (in KBr): 1788, 1743, 1727 (C=O); 1644, 1617 (C=C); 1501 (N—H); 1373, 1328, 1004 (oxazine ring); 3080, 1191 (C—H); 3235, 3150, 2965 cm^{-1} (N—H). ^{1}H-NMR spectrum (in hexadeuteriodimethyl sulfoxide): δ 7·97 (s, 1 H, H-4). The pK value, 6·23 $\pm$ 0·02 (determined spectrophotometrically at 25°C). For $C_4H_2BrNO_3$ (192·0) calculated: 25·02% C, 1·05% H, 41·63% Br, 7·29% N; found: 25·18% C, 1·25% H, 41·92% Br, 7·65% N.

5-Iodo-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione (*IV*)

Anhydrous potassium acetate (1·19 g; 15 mmol) was added to the solution of compound *I* (1·13 g; 10 mmol) in acetic acid (30 ml). When the solid dissolved, the solution of monochloro iodide (2·43 g; 15 mmol) in acetic acid (5 ml) was introduced, the whole mixture kept at room temperature for 2 h, poured into ice-cold water (100 ml), and the product extracted with three 20 ml portions of ethyl acetate. The extracts were combined, washed with three 20 ml portions of 10% aqueous

sodium thiosulfate, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was chromatographed on a column of silica gel in the solvent system 1 : 1 benzene–ethyl acetate to afford 0·74 g (31%) of compound *IV*, m.p. 149—150°C (ethanol) (decomp.). UV spectrum in 0·01M-HCl: λ_{max} 210 and 292 nm (ε 9890 and 6900); in 0·05M sodium tetraborate: λ_{max} 217 and 318·5 nm (ε 9110 and 11770). IR spectrum (in KBr): 1790, sh 1780, 1724, 1711 (C=O); 1621 (C=C); 3235, 3148 (N—H); 3080 cm^{-1} (C—H). ^{1}H-NMR spectrum (in hexadeuteriodimethyl sulfoxide): δ 7·82 (s, 1 H, H-4). Mass spectrum (high resolution): M^+ 238·9082; for $C_4H_2INO_3$ calculated: 238·9079. For $C_4H_2INO_3$ (239·0) calculated: 20·10% C, 0·84% H, 53·10% I, 5·86% N; found: 20·97% C, 1·05% H, 54·49% I, 6·80% N. Attempts (repeated crystallisation from ethanol) to obtain a specimen giving more accurate analytical data failed, probably because of an easy decomposition (strong darkening on day-light).

5-Methyl- and 4-Methyl-2,3-dihydro-1,3-6*H*-oxazine-2,6-dione (*V* and *VI*)

A mixture of citraconic anhydride (8·1 g; 0·072 mol), trimethylsilyl azide[24] (9·0 g; 0·078 mol), and benzene (20 ml) was kept at room temperature for 14 days to deposit a solid which was collected with suction and crystallised from ethanol. Yield, 0·476 g of compound *VI*, m.p. 173 to 174°C (reported[4], 176·5°C). UV spectrum in 0·01M-HCl: λ_{max} 206 and 266 (ε 5700 and 9250); in 0·05M-NaOH: λ_{max} 216 and 295 (ε 5800 and 11330). ^{1}H-NMR spectrum (in hexadeuteriodimethyl sulfoxide): δ 2·05 (s, 3 H, CH_3), 5·38 (s, 1 H, H-5).

The above benzene filtrate (after removal of compound *VI*) was diluted with light petroleum (200 ml) and water (2 ml), and the mixture shaken for 1 min to deposit a solid which was collected on a porous plate and then crystallised from ethyl acetate. Yield, 0·599 g of compound *V*, m.p. 136°C (reported[5], m.p. 134·0—134·5°C). UV spectrum in 0·1M-HCl: λ_{max} 206 and 271 nm (ε 5460 and 7290); in 0·01M-NaOH: λ_{max} 212 and 304 nm (ε 5950 and 9420). IR spectrum (in KBr): 1784, 1739 (C=O); 3285, 3238, 1657 (N—H); 3075 cm^{-1} (C—H). ^{1}H-NMR spectrum (in hexadeuteriodimethyl sulfoxide): δ 1·77 (d, 3 H, CH_3, J = 1·5 Hz); 7·35 (m, 1 H, H-4).

Alkaline Solutions of 3-Carboxyaminoacrylic Acids *VII*—*XI*

Compound *I* (1 mmol) was added at room temperature with stirring to 0·1M-NaOH (20 ml). The solid dissolved within 15 min. The mixture was allowed to stand at room temperature for additional 15 min and made up with water to the volume of 200 ml. A solution of the acid *XI* was prepared similarly. The acids *VIII*—*X* (1 mmol) were prepared in 200 ml of 0·01M-NaOH. For the purpose of UV spectral measurement, 0·5 ml of the solution was transferred into a 50 ml volumetric flask and made up to the volume of 50 ml by the addition of 0·1M-NaOH. The absorbance of solutions in 0·1M-NaOH ($5·0 . 10^{-5}$M) did not change within 24 h at 25°C. The stability of stock solutions (concentration $5·0 . 10^{-3}$M) was determined spectrophotometrically after 17 h at 25°C (Table III). For the electrophoretic mobility of acids *VII*—*XI* see Table V.

Proof of Formylacetic Acid

A solution (200 ml; $5·0 . 10^{-3}$M) of the acid *VII* in 0·01M-NaOH was adjusted to pH 6·5 a portionswise addition of 0·067M-KH_2PO_4. After 10 min at room temperature (when the absorbance of the mixture at 259 nm was zero), the mixture was treated with a 0·1% solution (5 ml) of 2,4-dinitrophenylhydrazine in 0·1M-HCl. The precipitate (containing on thin-layer chromatography on silica gel in 9 : 1 benzene–ethyl acetate 2,4-dinitrophenylhydrazones of acetaldehyde and formylacetic acid) was extracted with ethyl acetate. The ethyl acetate solution was then ex-

tracted with three 10 ml portions of 5% aqueous sodium carbonate. The alkaline extracts were combined and acidified with 2M-HCl to deposit a solid which was collected with suction and crystallised from ethyl acetate–ether. Yield, 85 mg of formylacetic acid 2,4-dinitrophenylhydrazone. m.p. 135°C (reported[17], 136°C).

pH-Dependence of the UV Spectrum of Formylacetic Acid

A solution (2 ml; $5{\cdot}0 \cdot 10^{-3}$M) of the acid *VII* in 0·01M-NaOH was treated with 0·067M-KH_2PO_4 (5 ml), the mixture kept at room temperature for 20 min, and made up to the volume of 100 ml with 0·05M sodium tetraborate. This solution was gradually treated (stirring) with 1M-NaOH and samples were withdrawn for measurement of pH values and absorbances at 258 nm. The pH-dependence of the UV spectrum of an authentic sample of formylacetic acid[25] was examined similarly (Fig. 4).

Proof of Chloroacetaldehyde

A solution (20 ml; $5 \cdot 10^{-3}$M) of the acid *VIII* in 0·01M-NaOH was adjusted to pH 7 by additions of 5% aqueous KH_2PO_4 and kept at room temperature for 20 min. A portion (50% by volume) of the mixture was evaporated at 1 Torr and bath temperature of 10°C. The distillate was cooled by a mixture of Dry Ice and methanol. 2,4-Dinitrophenylhydrazine (5 ml of a 0·1% solution in 1M-HCl) was added to the distillate. After 1 h, the precipitate (15 mg) was collected with suction and crystallised from ethanol to chloroacetaldehyde 2,4-dinitrophenylhydrazone, m.p. 155°C (reported[26], m.p. 156°C). In another experiment, the stock solution (20 ml) of the acid *VIII* was treated with 5 ml of the above 2,4-dinitrophenylhydrazine solution and the precipitate processed analogously to isolation of formylacetic acid 2,4-dinitrophenylhydrazone. The alkaline extract was free of any formylchloroacetic acid 2,4-dinitrophenylhydrazone. The neutral portion yielded 3 mg of chloroacetaldehyde 2,4-dinitrophenylhydrazone.

Methyl (*Z*)-3-Methoxycarbonylaminoacrylate (*XII*)

Compound *I* (1·13 g; 10 mmol) was dissolved with stirring at room temperature in 0·3M tetra-n-butylammonium hydroxide (132 ml). The course of the reaction was checked by thin-layer chromatography in ethyl acetate; the R_F value of compound *I* is 0·6 whereas the salt of acid *VII* remains on the start line. The mixture was kept at room temperature for 1 h and evaporated under diminished pressure at the bath temperature of 30°C. The residue was coevaporated with

TABLE V

Electrophoretical Mobility in 0·033M-Na_2HPO_4 (in cm)

I	8·1	*V*	3·0	*X*	19·1
II	13·0	*VII*	21·7	*XI*	20·1
III	11·1	*VIII*	20·7	*XV*[a]	20·4
IV	11·3	*IX*	20·3	*XVI*[b]	9·3

[a] Formylacetic acid. [b] Picric acid as standard.

three 50 ml portions of ethanol and finally dissolved in dimethylformamide (30 ml). Methyl-iodide (3 ml) was added at 0°C, the mixture kept in an ice bath for 1 h and then at room temperature overnight, and evaporated at 1 Torr and bath temperature of 45°C to remove the unreacted methyl iodide and the solvent. The residue was dissolved in water (50 ml) and the aqueous solution extracted with three 50 ml portions of ethyl acetate. The extracts were combined, dried over anhydrous magnesium sulfate, evaporated, and the residue chromatographed on a column of silica gel (100 g) in 9 : 1 benzene–ethyl acetate. The main chromatographic fraction yielded by distillation 556 mg (35%) of compound *XII*, b.p. 110–115°C/15 Torr, solidifying into crystals at 0°C. IR spectrum (2% solution in chloroform, 0·1 cm cell): 1744 (C=O carbamate); 1691 (C=O ester); 1640, sh 1535, 1652 (C=C); 1502, 3344 cm^{-1} (N—H); $3 . 10^{-3}$M solution in chloroform, 1 cm cell (3200–3600 cm^{-1}), 0·1 cm (1600–1800 cm^{-1}): 1745 (C=O carbamate) 1692 (C=O ester); 1641, sh 1634, 1652 (C=C); 3340 cm^{-1} (intramolecular NH bond). UV spectrum (in ethanol): λ_{max} 264 nm (log ε 4·12). ^{1}H-NMR spectrum (in deuteriochloroform): δ 3·62 (s, 3 H, CH_3 ester); 3·71 (s, 3 H, CH_3 carbamate); 4·94 (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 (broad s, 1 H, NH). For $C_6H_9NO_4$ (159·1) calculated: 45·28% C, 5·70% H, 8·80% N; found: 45·25% C, 5·88% H, 8·67% N. An authetic sample of compound *XII* was obtained by reaction of 3-methoxycarbonylaminoacrylic acid[27] with ethereal diazomethane; its identity with the present specimen was established by IR spectrum.

Methyl (*E*)-2-Chloro-3-methoxycarbonylaminoacrylate (*XIII*)

The chloro derivative *II* (0·70 g; 4·8 mmol) was dissolved with stirring at room temperature in 0·3M tetra-n-butylammonium hydroxide (62 ml) and the solution processed analogously to preparation of compound *XII*. The crude *XIII* was chromatographed on a column of silica gel in 9 : 1 benzene–ethyl acetate and the residue obtained from the main fraction was sublimed at 80°C/1 Torr. Yield, 181 mg (19%) of compound *XIII*, m.p. 83–84°C. IR spectrum (in chloroform): 1757 (C=O amide I, carbamate); 1725 (C=O ester); 1651 (C=C); 1481 (C=O amide II); 3420 cm^{-1} (N—H). ^{1}H-NMR spectrum (in deuteriochloroform): δ 3·72 (s, 3 H, CH_3 ester); 3·75 (s, 3 H, CH_3 carbamate); 8·03 (d, 1 H, H-3, $J_{3,NH}$ = 12·0 Hz). For $C_6H_8ClNO_4$ (193·6) calculated: 37·23% C, 4·16% H, 18·32% Cl, 7·24% N; found: 37·34% C, 4·19% H, 18·35% Cl, 7·33% N.

Methyl (*Z*)-2-Bromo-3-methoxycarbonylaminoacrylate (*XIV*)

The reaction of the bromo derivative *III* (1·90 g; 10 mmol) with tetra-n-butylammonium hydroxide (132 ml) and the subsequent esterification was performed analogously to the preparation of compound *XII*. Yield (after chromatography), 0·283 g (11·8%) and 0·249 g (after sublimation at 80°C/1 Torr) of compound *XIV*, m.p. 102–103°C. IR spectrum (1·5% solution in chloroform, 0·1 cm cell): 1749 (C=O amide I, carbamate); 1683 (C=O ester); 1627 (C—H); 1479 (C=O amide II); 3346 (NH intramolecular hydrogen bond); 3422 cm^{-1} (N—H free); $3 . 10^{-3}$M solution in chloroform, 1 cm cell: 3344 (NH intramolecular hydrogen bond); 3419 cm^{-1} (NH free). The weak band at 3419 cm^{-1} might be attributable to the NH group of the (*E*)-isomer of compound *XIV*, the content of which is lower than 10% as estimated from the band intensity. ^{1}H-NMR spectrum (in deuteriochloroform): δ 3·76 (s, 6 H, 2 CH_3); 7·96 (d, 1 H, H-3, $J_{3,NH}$ = = 12·0 Hz). For $C_6H_8BrNO_4$ (238·05) calculated: 30·27% C, 3·38% H, 33·57% Br, 5·88% N; found: 31·94% C, 3·67% H, 32·64% Br, 6·10% N.

Kinetic Measurements

Spectrophotometric measurements of concentrations were performed on a Specord UV VIS recording spectrophotometer (Carl Zeiss, Jena) equipped with a device for an automatic record of spectra in required time intervals. Quartz cells were placed into blocks maintained at a constant temperature (accuracy, ±0·05°C) by means of the Wobser U 2 thermostat. The water bath for reaction mixtures was thermostatted by the Wobser U 8 apparatus (accuracy, ±0·05°C).

Preparation of 10^{-3}M *stock solutions of compounds* I–V. The appropriate oxazine derivative (0·05 mmol) was dissolved in 1 ml of methanol and the solution made up to the volume of 50 ml by the addition of water. Fresh stock solutions were prepared every day.

Hydrolysis of compounds I–V. The stock 10^{-3}M solution of the appropriate oxazine derivative *I–V* (3 ml) was transferred into a volumetric flask and made up to 50 ml by the addition of 0·01M-NaOH or a buffer solution. The UV spectra were taken in thermostatted cells. Concentration of the starting compound was determined on the basis of the long-wavelength absorption maximum height. The acidic hydrolysis was examined in volumetric flasks immersed into thermostatted baths; samples for spectrophotometrical measurements were withdrawn in time intervals stated. Concentration of the starting compound was determined on the basis of the absorption maximum height in the 260–290 nm region (Fig. 1, Table I).

Decarboxylation. The $5 . 10^{-4}$M solution (5 ml) of the acid *VII–XI* in 0·01M-NaOH was made up to the volume of 50 ml by the addition of the appropriate buffer solution and the mixture immediately transferred into a thermostatted cell. The reaction course was checked spectrophotometrically (Fig. 3, Table III).

Decomposition of formylacetic acids. Two volumetric flasks were charged with a $5 . 10^{-4}$M solution (5 ml each) of the appropriate 3-carboxyaminoacrylic acid in 0·05M-NaOH and glacial acetic acid (0·02 ml each) and kept at room temperature for 10 min. The contents were made up to the volume of 50 ml by the addition of (1) water and (2) 0·1M-NaOH. The neutral solution did not exhibit any absorption in the 230–300 nm region. The alkaline solution from the other flask was transferred into a thermostatted (25°C) cell and the time dependence of the absorbance was measured. The λ_{max} values of alkaline solutions and the decomposition half-time are shown in Table IV. The solution of formyliodoacetic acid (prepared by the above method) was made up to the volume of 50 ml with a buffer solution of pH 9·94. The time dependence of the UV spectrum is shown on Fig. 5.

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