Intramolecular Migration of an Amino-group *via* a Transannular Process during the Reaction of *O*-Salicyloylglycolamides in Alkaline Solution: an Analogue of the Reverse of the Brenner Aminoacyl Insertion Reaction

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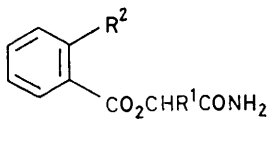
O-Salicyloylglycolamides undergo cleavage in the pH range 7—13 to yield salicylamide and salicylate anion in the high and low ranges respectively. Scanning the u.v. spectrum during reaction gives evidence for the accumulation and decay of intermediates at both ends of the pH range. Intermediates are not observed to accumulate during alkaline hydrolysis of *O*-benzoylglycolamides and benzamides are also not products. Amine is transferred intramolecularly in the salicyloyl reaction as judged from the absence of ammonia in the reactant solution. We postulate that the initial reaction involves the attack of the 2-oxyanion on the amido-group to yield a seven membered ring tetrahedral adduct: diacylamide and *O*-saliyloylisoamide intermediates are formed by transannular migration of oxygen or the amino-group from the tetrahedral centre to the ester carbonyl. The formation of the diacylamide is inhibited at low pH but when formed it cleaves to give salicylamide. The reaction of *O*-saliyloylglycolamides to produce salicylamide is essentially an analogue of the reverse of the Brenner aminoacyl insertion reaction. The driving force for the extrusion of the acid from the salicyloyl compound is probably the hydrolysis of the ester link. The effective molarity for the intramolecular attack of oxyanion on the amide has an estimated upper limit of *ca.* 10^8M.

As part of a project [1] concerned with models for component reactions of serine proteases in their catalytic action it was decided to investigate the effect of an *o*-hydroxy-group on the hydrolysis of *O*-benzoylhydroxymalonamides (A; $R^1 = CONH_2$, $R^2 = OH$). We were interested in the possibility of a concerted general base–electrophilic process (B) which is very close to a scheme postulated for the deacylation of acylserine proteases. However, by analogy with other systems of similar nature, it was anticipated that this simple scheme would not be preferred compared with nucleophilic attack of the *o*-oxyanion on the amido-function.[2] This turned out to be the case and the present paper reports a study

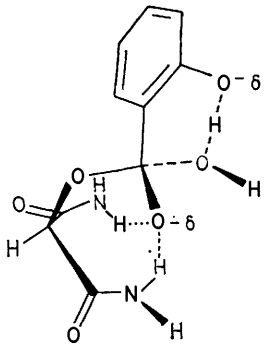
[1] See J. A. Boudreau and A. Williams, *J.C.S. Perkin II*, 1977, 1221 for a previous paper in this series.

[2] (*a*) T. C. Bruice and F. H. Marquardt, *J. Amer. Chem. Soc.*, 1962, **84**, 365; (*b*) S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Shalitin, *ibid.*, p. 2421; (*c*) Y. Shalitin and S. A. Bernhard, *ibid.*, 1966, **88**, 4711; (*d*) J. A. Davies, C. H. Hassell, and I. H. Rogers, *J. Chem. Soc.* (*C*), 1969, 1358; (*e*) T. C. Bruice and D. W. Tanner, *J. Org. Chem.*, 1965, **30**, 1668.

of the reactions of *O*-benzoylglycolamides (A) in alkaline solution to elucidate the hydrolytic pathway.



(A)



(B)

EXPERIMENTAL

Materials.—The *O*-acylglycolamides were prepared by the general methods previously outlined [1] from the α-halogenoamide and substituted benzoic acid in the presence of an equivalent of triethylamine. The α-halogenoamides are described elsewhere [1] and the esters were generally recrystallised from water–methanol or from acetone. The analytical and physical properties of these compounds are collected in Supplementary Publication No. SUP 22099 (2 pp.).* *N*-Salicyloylglycine methyl ester was prepared by coupling the ester of glycine with salicyloyl azide; the latter was synthesised from salicyloyl hydrazide and nitrous acid. The product amide was recrystallised from chloroform and then stirred with an excess of concentrated aqueous ammonia for 1 h at 0°. The ammonia was removed *in vacuo* and the residual solid washed with ether and then recrystallised from methanol. The structures of the materials synthesised for this report were confirmed by i.r. and n.m.r. ([$^2H_6$]DMSO or CCl_4) spectroscopy.

Materials other than the above were obtained from B.D.H. as analytical grades. Where only bench grade quality was available this was purified by distillation or recrystallisation. Acetonitrile was purified by the method of Lewis and Smyth [3] and stored over calcium hydride. Dioxan was purified by passage of the analytical grade material through an alumina column. Peroxides were shown to be absent by addition of aqueous potassium iodide to the effluent samples. The dioxan containing vessel was protected from light by aluminium foil. Water used throughout this study was doubly distilled from glass.

Methods.—N.m.r. spectra were recorded using a Perkin-Elmer R10 60MHz instrument or a JEOL 100 MHz machine operated by Dr. D. O. Smith. I.r. spectra were determined for Nujol mulls on a Perkin-Elmer 237 or 257 grating machine. M.p.s were determined using a Kofler hot stage Thermospan apparatus or in capillary tubes with a Gallenkamp MF-370 instrument. Microanalyses were carried out by Mr. G. M. Powell on a Hewlett–Packard model 185 CHN analyser which gave under ideal conditions a standard error of ±0.3% absolute. Mass spectra were determined by Mr. R. B. Turner using an A.E.I. MS 902 high resolution instrument.

Measurements of pH were performed with either a Radiometer pH meter 25b or a Pye-Dynacap machine. The meters were standardised before use with buffers made from E.I.L. buffer powders and were accurate to ±0.02 pH units.

Ammonia was assayed using the Nessler method [4] and the reagent was prepared according to the following recipe: potassium iodide (10 g) was dissolved in ammonia-free water (10 ml) and a saturated solution of mercury(II) iodide (*ca.* 60 g l^{-1}) added in small quantities until a faint permanent precipitate formed. Potassium hydroxide (8 ml; 9M) was added and the mixture diluted to 200 ml with ammonia-free water. The clear supernatant liquid was decanted from the precipitate after allowing the mixture to stand overnight. The solution for the ammonia test was made up as follows: the buffer (2.5 ml) was mixed with sample solution (a stock in acetonitrile; 0.1 ml), kept for an appropriate time and then diluted with 1 ml freshly prepared Nessler's solution and ammonia-free water to 5 ml. Blanks were carried out and sensitivity tests on standard amounts of ammonia (prepared by Snell's method) [5] indicated the accuracy of the method. The solutions were compared visually and this approach was perfectly adequate for our purposes.

T.l.c. was carried out using either pre-made plates from Merck Ltd. or plates prepared by the technical services department of this laboratory. Only silica gel supports were used and compounds were ' spotted ' with 5 λ pipettes and separation achieved using the ascending solvent technique.[6] The eluant was ethyl acetate–methanol (4 : 1) and the resultant spots were developed by use of an iodine tank or by u.v. light. The procedure employed for product analysis was as follows for *O*-salicyloyl-2-hydroxymalonamide. Stock solutions of substrate in alcohol (10 ml; containing 6 mg) were mixed with 250 ml of each buffer and allowed to stand for 2 days to complete even the slowest reaction. Each solution was then acidified to pH 1 with dilute HCl and then extracted with chloroform (6 × 25 ml). The chloroform extracts were dried over $MgSO_4$ and evaporated to *ca.* 10 ml. Reference solutions of salicyclic acid and salicylamide were prepared at a concentration of 0.34 g l^{-1} in chloroform (the concentration equivalent to 100% conversion from *O*-salicyloyl-2-hydroxymalonamide).

Ionisation constants were measured by u.v. spectroscopy at different pH values by a method similar to that of Albert and Serjeant.[7a] The initial absorptions of solutions containing identical amounts of substrate were determined at different pH values and a plot of $A_{pH} - A_{low\ pH}$ *versus* pH made on two cycle semi-logarithmic graph paper. Comparison of the plot with a normalised standard curve enabled direct evaluation of the pK_a value.

Rates of hydrolysis were measured spectrophotometrically with a recording instrument (Unicam SP 800 with a repetitive scanning device SP 825 or Beckman DB-G spectrometer fitted with a linear logarithmic converter). Repeat

* For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin II*, 1976, Index issue. Items less than 10 pp. are supplied as full-size copies.

[3] G. L. Lewis and C. P. Smyth, *J. Chem. Phys.*, 1939, **7**, 1085.

[4] A. I. Vogel, ' Macro and Semi-micro Quantitative Inorganic Analysis,' Longman, London, 1959, 4th edn., p. 318.

[5] F. D. Snell and C. T. Snell, ' Colorimetric Methods of Analysis,' Van Nostrand, Princeton, New Jersey, 1948.

[6] I. Smith, ' Chromatographic and Electrophoretic Techniques,' Heinemann, London, 1969, 3rd edn.

[7] (*a*) A. Albert and E. P. Serjeant, ' Ionisation Constants of Acids and Bases,' Methuen, London, 1962; (*b*) S. Cerrini, W. Fedeli, and F. Mazza, *Chem. Comm.*, 1971, 1607; (*c*) G. Lucente and A. Romeo, *ibid.*, p. 1605; (*d*) M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, V. I. Shchelokov, and Z. E. Agadzhanyan, *Tetrahedron*, 1965, **21**, 3537; (*e*) A. Stoll, *Fortschr. Chem. Org. Naturstoffe*, 1952, **9**, 114; (*f*) A. Hofmann, A. J. Frey, and H. Ott, *Experientia*, 1961, **17**, 206; (*g*) M. Rothe and R. Steinberger, *Angew. Chemie Internat. Edn.*, 1968, **7**, 884; (*h*) M. Rothe, T. Toth, and D. Jacob, *ibid.*, 1971, **10**, 128.

scanning between pre-set wavelengths was used to determine the stoicheiometry of a reaction, the most suitable wavelength at which to determine kinetics and to provide evidence as to the nature of the reaction products. A typical

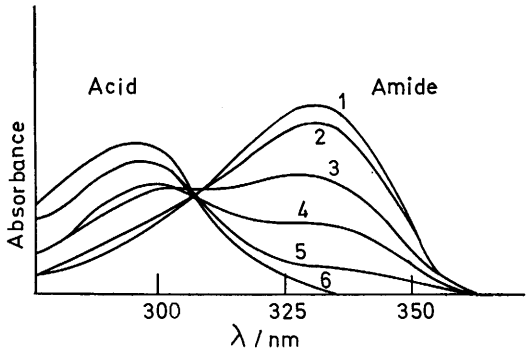


FIGURE 1 Change in u.v. spectrum of product with varying pH for the alkaline reaction of *O*-salicyloyl-2-hydroxymalonamide. Values of pH are: 1, 12.51; 2, 12.16; 3,10.95; 4, 10.06; 5, 9.13; 6, 7.84

kinetic run involved adding 20—200 λ of the stock solution of the substrate in acetonitrile, dioxan, or methanol on the flattened tip of a glass rod to buffer (2.5 ml) in a silica cell in the thermostatted cell compartment of the spectrophotometer. The solution was mixed by ' pumping ' the glass rod three times. The cell was then sealed with a Teflon stopper and the external potentiometric recorder (Servoscribe) was activated at the instant of mixing. First-order kinetics were determined from a plot of $A_\infty - A_t$ *versus* time on two-cycle semi-logarithmic graph paper.

Where reactions gave clear evidence for an intermediate [equation (1)] first-order kinetics were observed for the

$$(A) \longrightarrow (B) \longrightarrow (C) \qquad (1)$$

(A) ⟶ (B) step by following the reaction at the isosbestic wavelength for the (B) ⟶ (C) step. A rough idea of this wavelength was obtained from repetitive scanning experiments but the final value was chosen by trial till the absorbance at the finish of the (A) ⟶ (B) step no longer drifted by virtue of interference from the (B) ⟶ (C) process. It was possible to obtain first-order kinetics for the (B) ⟶ (C) process only over the last part of the reaction because of the accumulation of the intermediate; the isosbestic wavelength for the (A) ⟶ (B) step was determined in a manner similar to the above except that the start of the reaction involved no absorbance drift.

RESULTS

Hydrolysis of the O-*Acyl-2-hydroxymalonamides.—Product studies.* Examination of the final spectrum obtained for hydrolysis of *O*-salicyloyl-2-hydroxymalonamide showed that the product varied with pH. As the pH decreased the absorbance maximum shifted from 330 to 295 nm (Figure 1). Check runs with salicylamide and salicyclic acid under identical conditions indicated that the major product at high pH is salicylamide and that at low pH is salicylate ion. Moreover, a solution of salicylamide at the strength calculated for 100% conversion of the ester for an experiment at pH 12.51 had an u.v. spectrum almost exactly superimposable on the final hydrolysis spectrum. The pH-dependence of the yield as measured by the ratio of amide to acid anion is illustrated in Figure 2 and documented in Table 1.

The product was also analysed by t.l.c.; hydrolysis of *O*-salicyloyl-2-hydroxymalonamide was carried out at various pH values (see Table 1). The results of the thin layer chromatographic analysis confirm the products as salicylate at low pH and salicylamide at high pH.

The presence of free ammonia in the hydrolysis reaction was assayed by the Nessler test at pH 11 and 7.8. Blanks were run to eliminate any sources of interference and each test solution was made in duplicate, one containing a standard ammonia solution at a concentration up to the amount present in solution given 100% cleavage of the substrate. The sample contained substrate at 3 g l^{-1}; no trace of ammonia was detected in the samples without the standard and we judge the accuracy of these determinations to be that we

TABLE 1

Results of product analysis by t.l.c. and u.v. spectrum analysis [a] for *O*-salicyloyl-2-hydroxymalonamide reaction

pH	Sample R_F	Control R_F Acid	Control R_F Amide	Acid (%) [b]	Amide (%) [b]
12.61	0.50	0.16	0.51	0	100
12.16	0.50 (0.13) [c]	0.15	0.51	18.6	81.4
11.62				45.8	54.2
10.95	0.51, 0.15	0.15	0.50	47.0	53.0
10.05	0.49, 0.15	0.15	0.48	64.6	35.4
9.13				88.2	11.8
7.8	0.17	0.17	0.53	100	0

[a] Conditions as in the Experimental section; product distributions similar to those for the malonamide were observed for the salicyloyl esters of lactic acid amide, glycolamide, and 2-phenylglycolamide and for the following aroyl-2-hydroxymalonamides: 2,5-dihydroxybenzoyl, 2,4-dihydroxybenzoyl, and 2-hydroxy-1-naphthoyl. [b] Determined from the product spectra. [c] Faint trace.

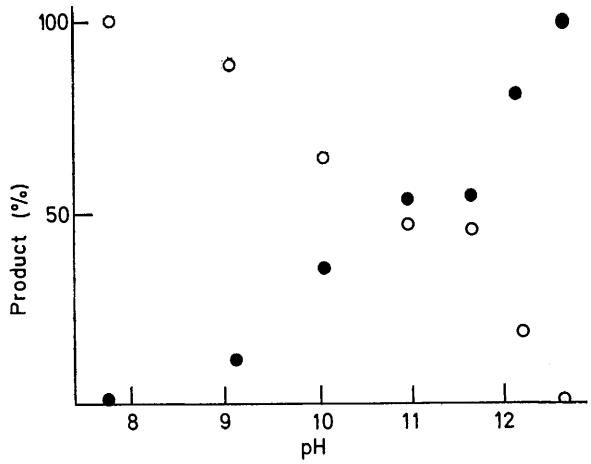


FIGURE 2 Dependence on pH of the product distribution expressed as the yield of acid (○) or amide (●) as a percentage of the total product (acid + amide) for the alkaline reaction of *O*-salicyloyl-2-hydroxymalonamide

would certainly have detected 1/20th of the total ammonia calculated on the substrate concentration even though the comparisons were made visually.

Repetitive scanning of the u.v. spectrum during hydrolysis of the salicyloyl derivative revealed two reactions, a fast one followed by a slow step. At a wavelength not coincident with an isosbestic value the formation and decay of the intermediate is observed and this is illustrated in Figure 3 which

also shows the detection of the fast initial reaction and the slower subsequent one. The spectra of the intermediates were readily obtained from the repetitive scans and they proved to vary as the pH increased and two spectral types

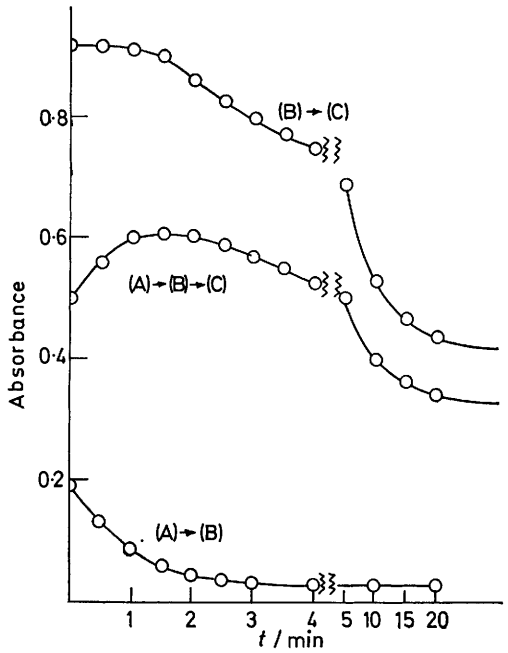


FIGURE 3 Formation and decay of an intermediate in the alkaline reaction of *O*-salicyloyl-2-hydroxymalonamide. The reaction (B) ⟶ (C) is followed at the isosbestic wavelength between (A) and (B) (356.5 nm); (A) ⟶ (B) at the isosbestic wavelength for (B) and (C) (338.5 nm); (A) ⟶ (B) ⟶ (C) is followed at 380 nm. The conditions are: pH 12.5, 0.1M ionic strength, 25°

were observed: one for low and one for high pH. The former had maxima at 240 and 310 nm (pH 7.84) while the latter (pH 12.51) had a shoulder at 250 nm and a maximum at 340 nm. Attempts to isolate the intermediates at low and high pH were not successful. The difficulty appears to be that extraction into organic solvents of the intermediates (present in low concentrations of *ca.* 300 mg l^{-1}) after acidification of the reaction mixture is not feasible owing to their electrophilic nature. The kinetic data indicate that a substantial fraction of the material is present as intermediate after a given time (see Figure 3 for example) but solubility problems in water preclude increased concentrations for analysis by n.m.r. techniques. The u.v. spectra of the intermediates, however, are consistent with their identity as salicyloyl species rather than some more complicated function.

The first-order rate constants for formation of intermediate are given in Table 2 and illustrated in Figure 4 as a function of pH. The pH-dependence exhibits a sigmoidal portion corresponding to the ionisation of the phenolic moiety (Table 3) and a portion linearly dependent on hydroxide ion concentration. The pH dependence of the decomposition of the intermediate is also given in Table 2 and illustrated in Figure 4.

Hydrolysis of the other *O*-benzoyl-2-hydroxymalonamides gave only the salicylate ion at high pH as judged from the n.v. spectrum and t.l.c. There was no evidence for the accumulation of intermediates as judged from the repetitive u.v. spectral scans. The second-order rate constants for hydroxide ion attack are collected in Table 4.

TABLE 2

pH Dependence for the rate constant for formation and decay of the intermediate in the alkaline reaction of *O*-salicyloyl-2-hydroxymalonamide [a]

pH	λ_i/nm	10^4k/s^{-1}	λ_i/nm	10^4k/s^{-1}
12.51	338.5	173	356.5	36.7
11.66	279.5	35.0	358.0	16.4
10.95	276.5	23.0	359.0	9.67
10.06	319.5	10.4	359.0	3.67
9.13	312.5	7.2	354.0	1.80
7.84	271.0	3.20		
7.20	267.0	1.80		
	(A) ⟶ (B)		(B) ⟶ (C)	

[a] Conditions: 25°, 0.1M ionic strength made up with sodium chloride. Substrates mentioned in footnote *a* of Table 1 also gave evidence for intermediates.

TABLE 3

Ionisation constants for some phenolic substrates [a]

Ester	pK_a	λ/nm [b]
O-(3-Hydroxybenzoyl)-2-hydroxymalonamide	8.7	380
O-(4-Hydroxybenzoyl)-2-hydroxymalonamide	7.8	310
O-Salicyloyl-2-hydroxymalonamide	8.65	360
O-Salicyloyl-lactic acid amide	8.9	338
N_α-Salicyloylglycinamide	7.2	330
O-Salicyloyl-*N*-ethylglycolamide	9.25 [c,d]	
O-Salicyloyl-*NN*-diethylglycolamide	9.80 [c,d]	
O-Salicyloyl-*N*-phenylglycolamide	9.20	335

[a] Conditions: 25°, 0.1M ionic strength made up with NaCl. [b] Wavelength for determination of pK_a. [c] Measured with a Radiometer pH-titration assembly (REC 61 Servograph, REA160 Titratigraph, PHM 62 standard pH meter, TTT 60 titrator, and ABU 11 autoburette). [d] 23.5°.

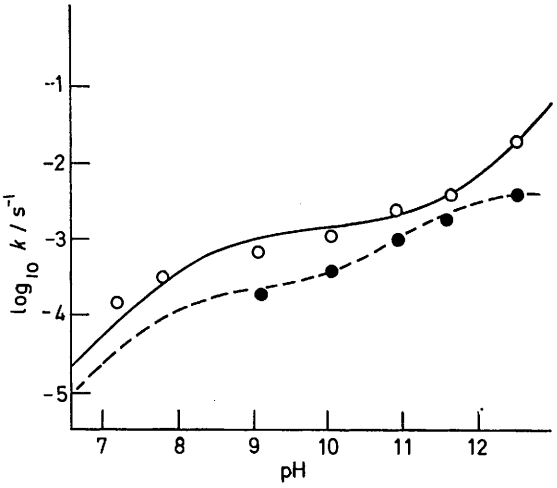
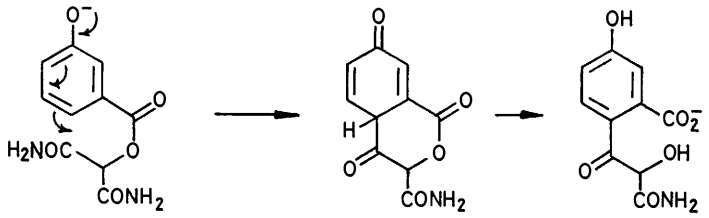


FIGURE 4 The pH dependence of the rate constant for formation (○) and decay (●) of intermediate in the alkaline decomposition of *O*-salicyloyl-2-hydroxymalonamide. Data is from Table 2 and the lines are theoretical. Intermediate formation: $k = 0.54\ [OH^-] + 1.3 \times 10^{-3}/(1 + a_H/10^{-8.65})$. Intermediate decay: $k = 2.5 \times 10^{-4}/(1 + a_H/10^{-8}) + 4 \times 10^{-3}/(1 + a_H/10^{-11.5})$

O-(3-Hydroxybenzoyl)-2-hydroxymalonamide unexpectedly gave very poor isosbestic wavelengths when its hydrolysis was studied at alkaline pH by repetitive u.v. spectral scanning. The alkaline solution was also observed to develop a yellow colour during the course of the reaction which returned to colourless on completion. This was

confirmed by the appearance and disappearance of a visible absorption maximum at 450 nm. The final spectrum indicated that 3-hydroxybenzoic acid was present in the product but in conjunction with other materials; there was no evidence for amide production and the acid product was confirmed by t.l.c. An unsatisfactory attempt was made to isolate the yellow intermediate and the product from a preparative scale experiment at 300 mg l^{-1} had m.p. 130—167° and t.l.c. showed a major and a minor spot at R_F 0.53 and 0.33 respectively. Mass spectral analysis gave peaks continuously over *m/e* 150—400. We tentatively propose Scheme 1 which would not be possible with the other 2-hydroxymalonamides. No similar intermediate is observed with the *O*-(3-hydroxybenzoyl)lactic acid amide where, presumably, the amide is not sufficiently activated for reaction with the carbanion.



SCHEME 1

TABLE 4

Reaction of hydroxide ion with some *O*-benzoylglycolamides [a]

Ester	M.p. (°C)	λ/nm [b]	k_{OH}/l mol^{-1} s^{-1}
O-Benzoyl-2-hydroxymalonamides			
Parent			2.63[1]
2-Methyl	210—213	240	0.60 (8)
2-Methoxy	198—201	305	6.7 (6)
4-Hydroxy	228—230	310	0.4 (3)
O-Benzoyl-lactic acid amides			
Parent			0.13[1]
3-Hydroxy	171—172.5	345	2.1×10^{-2} (3)
2-Methoxy	125—128	300	1.3×10^{-1} (3)
4-Hydroxy	218—222	313	1.7×10^{-3} (3)
O-Benzoylglycolamides			
Parent			9.12[1]
2-Methoxy	104.5—106.5	305	0.4 (3)
O-Benzoyl-*N*-ethylglycolamides			
Parent			1.4[1]
2-Methoxy	58—60	300	0.31 (3)
O-Benzoyl-*NN*-diethylglycolamides			
Parent			0.12[1]
2-Methoxy	41—42	245	0.1 (3)
O-Benzoyl-*N*-phenylglycolamides			
Parent			0.59[1]
2-Methoxy	112—113	300	0.5 (3)

[a] Conditions: 25°, ionic strength maintained at 0.1M with NaCl; figures in parentheses equal the number of kinetic runs; ' parent ' is the unsubstituted benzoate. [b] Wavelength for the kinetic study.

Hydrolysis of the Lactic Acid Amide Series.—The hydrolysis of *O*-salicyloyl-lactic acid amide exhibited an intermediate as judged from the u.v. scanning spectrum; the product spectra indicated salicylate at low and salicylamide at high pH and this was confirmed by t.l.c. on the products. Repetitive scanning for the other lactic acid amides indicated clean 1 : 1 stoicheiometry with acid as the product at all pH values. The rate constants for the reactions are given in Table 4.

Hydrolysis of the Glycolamide Series.—Repetitive scanning of the u.v. spectrum during alkaline hydrolysis of the salicyloyl esters indicated the accumulation of an intermediate; the final spectrum showed the formation of salicylate ion and salicylamide as major components at low and high pH respectively. The other benzoate esters gave normal 1 : 1 stoicheiometry for their hydrolyses and the rate constants are collected in Table 4.

Alkaline Hydrolysis of Other Esters.—The alkaline hydrolysis of *O*-salicyloyl-2-phenylglycolamide was found to involve an intermediate leading to the acid and amide at low and high pH respectively but the salicyloyl esters of *N*-ethyl-, *NN*-diethyl-, and *N*-phenyl-glycolamide had clean 1 : 1 stoicheiometry. The rate constants for these esters are given in Table 5 and illustrated in Figure 5. The hydrolysis of N_α-salicyloylglycinamide failed to exhibit any evidence of salicylamide in the product even at 2-M-NaOH at 60°. Only faint amounts of salicylate were observed by t.l.c.

TABLE 5

Dependence on pH of the rate constant for the hydrolysis of some salicyloyl esters [a] of glycolamides

NN-Diethyl [b]		*N*-Ethyl [c]		*N*-Phenyl [b]	
pH	10^5k/s^{-1}	pH	10^5k/s^{-1}	pH	10^4k/s^{-1}
12.40	11.2	12.80	38.9	12.41	5.6
12.05	7.6	12.58	27.8	12.05	2.7
11.15	7.9	11.81	14.5	11.68	1.7
10.43	7.4	11.0	12.6	10.97	1.3
9.87	4.9	10.2	12.0	10.43	1.3
9.29	2.1	9.6	9.55	9.87	1.1
9.15	1.5	9.0	7.76		

[a] Conditions: 25°, ionic strength made up to 0.1M with NaCl. [b] Wavelength for kinetic study = 340 nm. [c] Wavelength for kinetic study = 330 nm.

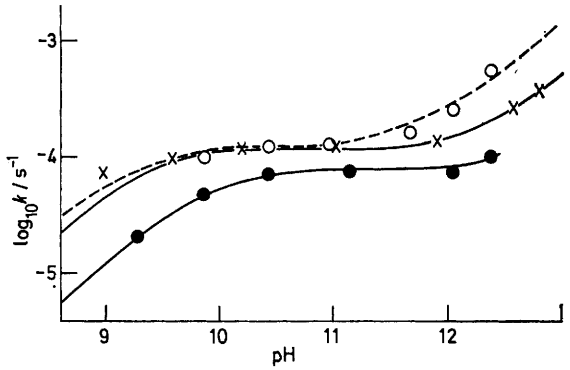


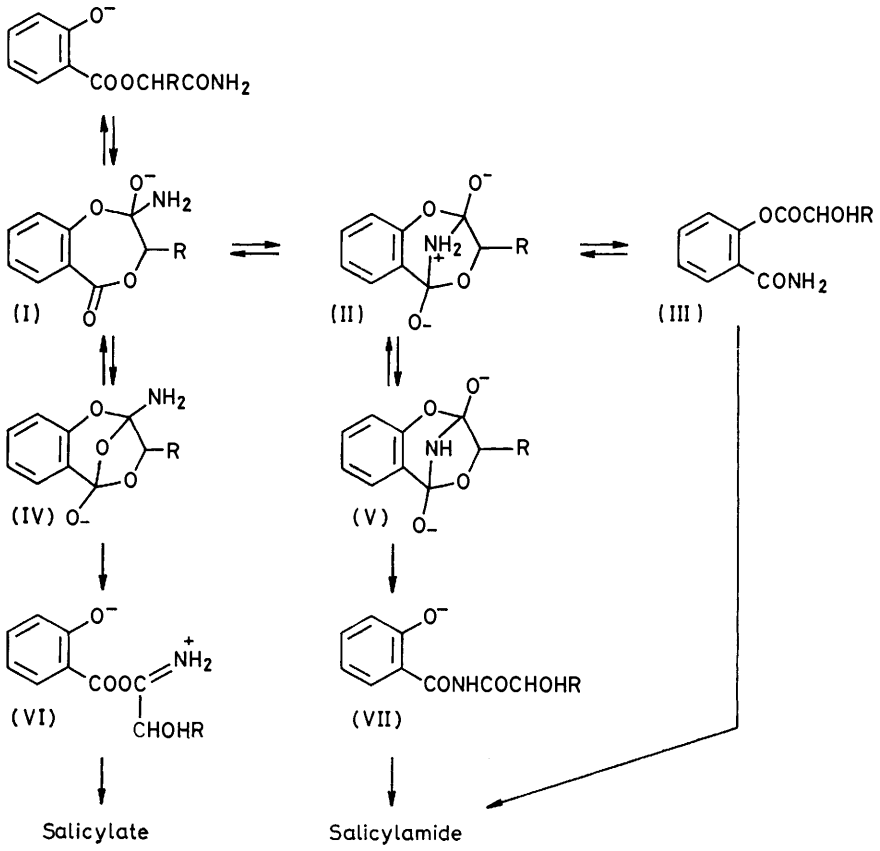
FIGURE 5 pH Dependence of the hydrolysis of the *O*-salicyloyl esters of *N*-ethyl- (×), *NN* diethyl- (○), and *N*-phenyl-glycolamide (●). Data is from Table 5 and the lines are theoretical: *N*-ethyl, $k = 6.3 \times 10^{-3}$ [OH$^-$] + 1.1×10^{-4}/(1 + a_H/K_a); *NN*-diethyl, $k = 2.5 \times 10^{-2}$ [OH$^-$] + 1.1×10^{-4}/(1 + a_H/K_a); *N*-phenyl, $k = 3.5 \times 10^{-3}$ [OH$^-$] + 6.9×10^{-5}/(1 + a_H/K_a). The respective K_a values are given in Table 3

No buffer effects on the hydrolysis rate constants were observed throughout this study.

The pK_a values of the substrates containing free phenolic groupings come close to those expected for other similar phenols and are recorded in Table 3.

DISCUSSION

We propose that *O*-salicyloylglycolamides are hydrolysed in alkali *via* the pathway in Scheme 2. This mechanism is consistent with the variation in product distribution with pH. Figures 1 and 2 and Table 2 illustrate that as the pH is raised the major product changes from salicylic acid to salicylamide and this is confirmed by t.l.c.



SCHEME 2

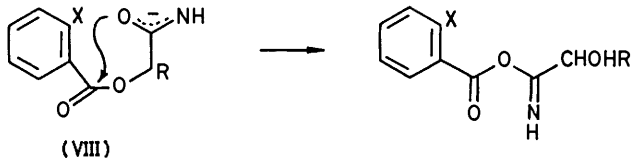
We propose that the initial tetrahedral adduct and subsequent ones (I)—(V) are present in small concentrations compared with reactant and do not accumulate. This is consistent with the known instability of such species although recently fused ring adducts have been isolated as stable entities from solution. The species (II), (IV), and (V) are sterically feasible as judged from the construction of Corey, Pauling, and Koltun models; moreover they are closely similar in structure to the naturally occurring tropane system and to the stable cyclol type species whose structures and existence are now confirmed.[7b-h] The intermediates (VI) and (VII) probably represent a much stabler situation than the fused species (IV) and (V) thus preventing the latter from accumulating. We propose that the intermediates (I)—(III) are in equilibrium with the starting ester throughout the pH range studied but that further reaction of (II) to yield (V) and eventually (VII) requires base catalysis to remove the proton from the bridging nitrogen. We also suggest that the formation of (III) is much less efficient than the pathway through (IV) to (VI) otherwise amide would be seen as product at low pH. Thus at low pH the major pathway involves the *O*-salicyloylisoamide (VI) but as (II) is deprotonated (in increasing pH) the diacylamide (VII) takes the majority of the reaction flux. The sigmoidal nature of the pH-dependence for intermediate formation is due to ionisation of the *o*-hydroxy-group of the substrate and the rate-limiting steps are after the formation of (I)—(III) otherwise no change in mechanism could occur. When the decomposition of (II) is no longer blocked the rate of intermediate formation will increase proportionally to hydroxide ion concentration and the pathway *via* (I), (II), (V), and (VII) will carry the major part of the reaction flux.

The nature of the accumulating intermediates (VI) and (VII) is clearly similar as judged from their u.v. spectra (see Results section) but there are sufficient differences to assign the spectra to *two* different intermediates. These species would be expected to have spectra closely similar

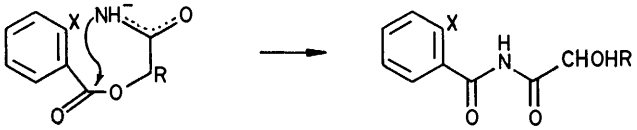
to salicylic acid derivatives; the spectra are characterised by a low and a high wavelength peak and the former does not appear to be diagnostic. The high wavelength peaks [310 nm for (VI) and 340 nm for (VII)] seem to be close to the peaks observed in the starting ester (308 nm) and salicylamide (330 nm) at their respective pH values. The latter species would seem to be reasonable structural models for the intermediates which therefore appear to have consistent u.v. spectra.

The decomposition of the intermediates as a function of pH varies in a complicated manner with an apparent slope of *ca.* 0.4 (Figure 4, filled circles). This behaviour is quite consistent with the proposed mechanism: as the pH increases the decomposition of the isoamide should follow a sigmoid pH-dependence with cleavage of the CO–O bond depending on the ionisation of the *o*-hydroxy-group. The mechanism changes on further increase in pH and the decomposition observed will be that of the diacylamide which should also exhibit a sigmoidal pH-dependence (due to the ionisation of the NH group). The inflection points corresponding to the pK_a values of the diacylamide (11.5) and the phenolate oxyanion (VI) (8.0) are sufficiently close that if the rate constants were similar for the ' plateau ' hydrolysis of these species then the pH profile could have an apparently linear form of low slope (allowing for error in rate constant determination). Also consistent with the mechanistic scheme are rate constants expected for the hydrolysis of the intermediates and the observed reactivity. The parameters used to fit the observed rate data [(VI), pK_a 8.0, $k_{plateau}$ $2.5 \times 10^{-4} s^{-1}$ and (VII), pK_a 11.5, $k_{plateau}$ $4.0 \times 10^{-3} s^{-1}$] are similar to those to be expected from the hydrolysis of model compounds. Cleavage of the diacylamide will be at the more reactive aliphatic moiety [8,9] to yield salicylamide and the rate constant for hydroxide attack at neutral diacetylamide [9] is 0.89 l mol^{-1} s^{-1} which compares quite well with the value 1.3 l mol^{-1} s^{-1} calculated from the above parameters for (VII). The intermediate (VII) would be expected to hydrolyse faster than the diacetyl species owing to the presence of electrophilic substituents. The pK_a which fits the data (11.5) is lower than that for diacetylamide (12.9) [9] presumably due to the influence of the benzoyl group. The parameters for the hydrolysis of (VI) fitting the data yield a hydroxide term for attack on the species with un-ionised phenoxide group of 250 l mol^{-1} s^{-1}. This value agrees with the rate constant for the alkaline cleavage of *N*-phenylphthalisoimide studied by Ernst and Schmir; [10a] the hydrolytic pathway for acylisoimides involves CO–O cleavage in alkaline solution [10b,c] but there appears to be no closer example in the literature for the rate comparison.

The mechanism is also consistent with the absence of detectable ammonia at both low and high pH values. Transfer of the amine *via* its release into solution by cleavage of the amide followed by reattack on the ester carbonyl would be very inefficient indeed at the low concentrations employed in the kinetics (10^{-3}—10^{-4}M). Migration of the amine intermolecularly may also be discounted because the rate constant for amide cleavage at pH 10 is 8.5×10^{-8} s^{-1} for malonamide at 25° [11a] and is not able to support a reaction flux of the magnitude observed for the salicyloyl substrate (see Figure 3).



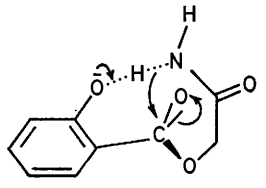
(VIII)



(IX)

The mechanism involving direct attack of the amido-function on the ester carbonyl [by O or N attack, (VIII) and (IX) respectively] has been excluded by the observation that benzoylglycolamides not possessing an *o*-hydroxy-function do not undergo the amine migration or involve observable intermediates in their alkaline cleavage reactions.

The diacylamine intermediate may be attained by either a stepwise path *via* removal of the amide proton by the phenoxide group followed by attack of the amido-anion on the ester [analogous to (IX)] or a concerted process. The stepwise mechanism may be excluded because the concentration of the amide anion will be no different at a given pH from that of *O*-benzoylglycolamides with no *o*-hydroxy; these esters do not hydrolyse in alkali *via* the diacylamine. A concerted process is quite feasible as far as stereoelectronic requirements are concerned where the HOMO (a π orbital on the amide) overlaps with the LUMO (a vacant π orbital on the ester) as indicated in structure (X). The scheme, however, does



(X)

not provide for the existence of *two* accumulating intermediates (for high and low pH) because the corresponding

[8] A. H. Lamberton and A. E. Standage, *J. Chem. Soc.*, 1960, 2957.

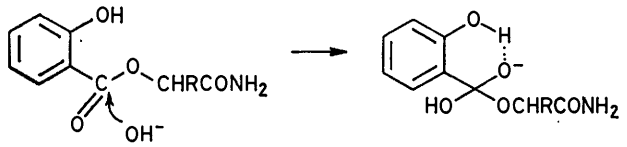
[9] J. T. Edward and K. A. Terry, *J. Chem. Soc.*, 1957, 3527.

[10] (*a*) M. L. Ernst and G. L. Schmir, *J. Amer. Chem. Soc.*, 1966, **88**, 5001; (*b*) D. D. Davidson and H. Skovronek, *ibid.*, 1958, **80**, 376; (*c*) R. M. Topping and D. E. Tutt, *J. Chem. Soc.* (*B*), 1967, 1346.

[11] (*a*) This value is calculated from data reported by A. Bruylants and F. J. Kezdy, *Records Chem. Progr.*, 1960, **21**, 213; (*b*) L. Pekkarinnen and E. Tommila, *Acta Chem. Scand.*, 1959, **13**, 1019.

concerted process for attack of the amido-oxygen on the ester [analogous to the stepwise mechanism (VIII)] is ruled out on steric grounds. Neither the stepwise processes (VIII) and (IX) nor the concerted ones explain product variation with pH.

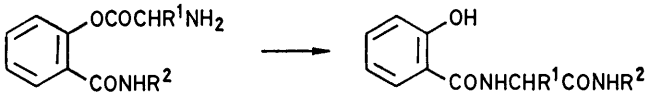
At pH values below significant ionisation of the phenol group in *O*-salicyloylglycolamides one could postulate a direct attack of hydroxide on the ester carbonyl group. However, it is not likely that the *o*-hydroxy-group could stabilise the tetrahedral adduct sufficiently for it to accumulate. We may also exclude this mechanism by a kinetic argument: ethyl salicylate [11b] reacts with hydroxide ion an order of magnitude faster than does ethyl benzoate but the enhancement in the case of the glycolamides is *ca.* 1 000-fold leaving a factor of *ca.* 100-fold to



explain. Some of this may be taken up in the relative leaving abilities of glycolamide anion and ethoxide ion but this will not amount to a factor of 100.

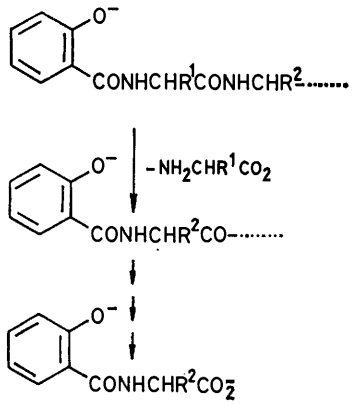
Effective Molarity for Intramolecular Phenoxide Attack.—It is difficult to obtain a precise estimate of the effective molarity of the intramolecular nucleophilic attack of phenoxide ion on the amido-group but this situation is common to other similar ones.[12] The rate constant for attack of hydroxide ion on malonamide is 8.5×10^{-4} l mol^{-1} s^{-1} at 25°.[11a] Allowing a β_{Nuc} of unity for the attack of oxyanions on amides leads to a value 1.2×10^{-11} l mol^{-1} s^{-1} for a phenolate ion of pK_a 8.65 (Table 3) and a value for water of 16.5.[13] The plateau rate constant (corresponding to intramolecular attack of phenoxide ion) for the formation of the intermediate for the *O*-salicyloyl-2-hydroxymalonamide is 1.3×10^{-3}s^{-1}, thus the effective molarity has an upper limit of *ca.* 10^8M. The lower limit for β_{Nuc} is probably not less than *ca.* 0.5 [14] and this leads to the lower limit for the effective molarity of *ca.* 10^4M. This range of values fits the expected effective molarity for an intramolecular nucleophilic substitution with a tight transition-state as obtained with acyl group transfer.[12]

Relationship with the 'Brenner' Aminoacyl Insertion Reaction.—The reaction of *O*-salicyloylglycolamides at high pH involves the *extrusion* of an acyl function in contrast to the *insertion* of the aminoacyl function in the type of rearrangement studied extensively by Brenner,[15] Topping,[16] and Shemyakin.[7d,17] The direction taken by the two processes depends on the driving force available



and this, in the 'Brenner' reaction, is the cleavage of the phenyl ester to yield the stabler amide link. In our extrusion reaction the hydrolysis of the ester is the driving force as there is no net gain of amide bond; there is also a favourable entropy term for the extrusion process.

Possibility for Sequential Peptide Cleavage.—The extrusion of the acyl function in the present alkaline reaction makes it a candidate for the sequential extrusion of aminoacid residues from a peptide. This was tested on N_α-salicyloylglycinamide under forcing conditions but no salicylamide was produced. This result is not surprising since the replacement of the oxygen by an NH group to yield the glycinamide destroys the potential



driving force caused by converting ester to amide in the original reaction.

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