In another experiment, carbon dioxide from the bromination mixture was collected in sodium hydroxide solution and precipitated as barium carbonate. This barium carbonate showed an activity of 118 counts per minute; the background count was 20.3 counts per minute.

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Mechanisms of Elimination Reactions. XIV. Solvent and Salt Effects in the Alkaline Dehydrohalogenation of Chloro- and Bromomaleate and -Fumarate[1a]

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In a recent kinetic study[2] of the elimination reactions it was noted that, at 0.1 *M* ionic strength, the

$$OH^- + \ominus OCOCH{=}CXCOO\ominus \longrightarrow \ominus OCOC{\equiv}CCOO\ominus + H_2O + X\ominus \quad (1)$$

(*cis* or *trans*; X = Cl, Br)

second-order rate constants were greater in 54.2% aqueous ethanol than in water. As the transition state in these reactions has a greater concentration of charge than the reactants, the results appeared to contradict the electrostatic theory of solvent effects.[3,4]

We now wish to report an extrapolation of the available ionic strength-kinetic data[2,5] for the reactions indicated in equation 1 to zero ionic strength. The resulting second-order rate constants at 70° are summarized in Table I, together with the respective quantities of activation. It may be seen that, at zero ionic strength, the specific rates in water are about three times those in 54.2% ethanol, in qualitative agreement with electrostatic theory.[6] However, at ionic strengths even as low as 0.1 *M*, the solvent effect is masked by the large interionic effects, which are more important in the solvent of lower dielectric constant. The results show that estimates of solvent effects for ionic reactions may be in error, not only in magnitude, but even in direction, when working at moderate ionic strengths.

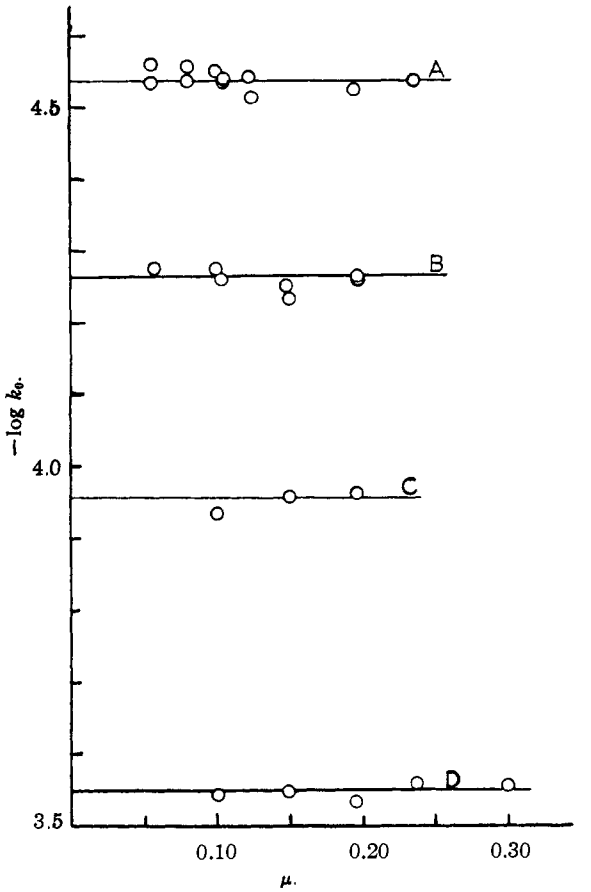

Fig. 1.—Plots of extrapolated rate constants, log k_0 *vs.* ionic strength, μ, for the alkaline dehydrohalogenation of: bromomaleate in water, 45.12° (A); in 54.2% ethanol, 61.40° (B); chlorofumarate in 54.2% ethanol, 72.95° (C); in water, 71.02° (D).

The rate constants were extrapolated to zero ionic strength by means of the equation

$$\log k = \log k_0 + 4S\sqrt{\mu}/(1 + Aa\sqrt{\mu}) \quad (2)$$

where μ is the ionic strength; S is the Debye–Hückel limiting slope and is equal to $1.825 \times 10^6/(DT)^{3/2}$; $A = 50.30 \times 10^8/(DT)^{1/2}$ cm.$^{-1}$; and a is an average of the ion-size parameters for reactants and transition state.[8] The values chosen for a were 5.0 Å. for the halomaleates in both solvents, and 4.0 Å. for the halofumarates in both solvents. Values of log k_0 computed in this way were satisfac-

TABLE I

SPECIFIC REACTION RATE CONSTANTS AND QUANTITIES OF ACTIVATION FOR REACTIONS 1 AT 70.0°, EXTRAPOLATED TO ZERO IONIC STRENGTH

Compound	$10^4 k_0$, l./mole/sec.		$10^4 k$ at μ = 0.1 *M*, l./mole/sec.		E_{act}, kcal./mole.		$\Delta S^{\ddagger}$, cal./deg.	
	H_2O	54.2% EtOH	H_2O	54.2% EtOH	H_2O	54.2% EtOH	H_2O	54.2% EtOH
Chlorofumarate	2.54	0.845	8.15	9.96	21.0	23.1	−16	−12
Chloromaleate	0.279	0.088	0.809	0.832	24.9	24.0	− 9	−14
Bromofumarate	43.3	19.2	139[a]	204	17.9	19.7	−19	−16
Bromomaleate	3.84	1.30	11.3	12.3	22.6	22.5	−11	−13

[a] The activation energy, extrapolated rate constant at 70°, and entropy of activation reported previously[2] should be corrected to 18.5 kcal./mole, 139 × 10^{-4} l./mole/sec., and −15 e.u., respectively, for bromofumarate in water.

(1) (a) Previous paper in series: S. J. Cristol, W. Barasch and C. H. Tieman, THIS JOURNAL, **77**, 583 (1955). (b) Florida State University.

(2) S. J. Cristol and A. Begoon, THIS JOURNAL, **74**, 5025 (1952).

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(5) A. Begoon, Ph.D. thesis, University of Colorado, 1950.

(6) This correction now removes the requirement for any unusual solvation effects with these compounds.[7]

(7) E. D. Hughes, C. K. Ingold and R. Pasternak, *J. Chem. Soc.*, 3832 (1953).

(8) See, for example, G. Scatchard, THIS JOURNAL, **52**, 52 (1930); V. K. La Mer, *J. Franklin Inst.*, **225**, 709 (1938).

torily constant and showed no systematic trends with ionic strength, as is illustrated by the data in Fig. 1. Dielectric constants for 54.2% ethanol were computed from the equation, $\log D = 1.6806 - 0.00244\ [t(°C.) - 20]$, which is based on the data of Akerlöf.[9] Dielectric constants for water were taken from the work of Wyman.[10]

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Hydroxylysine in Proteins

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Hydroxylysine was first recognized among the acid hydrolytic products of gelatin by Van Slyke and Hiller[1] and later isolated and identified by Van Slyke, Hiller, Dillon and MacFadyen.[2] Van Slyke, Hiller and MacFadyen[3] determined the hydroxylysine content of various proteins and found that collagen and gelatin were characterized by approximately 1% of their total nitrogen as hydroxylysine. Cotton seed globulin, casein, zein and aleuronate appeared to have approximately 0.2 to 0.5% of total nitrogen as hydroxylysine nitrogen. Other proteins appeared to have negligible amounts of hydroxylysine. MacPherson[4] concluded that hydroxylysine was present only in collagen and gelatin. Desnuelle and Antonin[5] were in essential agreement with Van Slyke, Hiller and MacFadyen.[3] They failed to find evidence for hydroxylysine in casein but claimed that small amounts (0.1%) were present in beef albumin, edestin, ovalbumin, rat muscle and Bence-Jones protein. Middlebrook[6a] reported 0.18% of total nitrogen in sheep's wool as hydroxylysine; Simmonds[6b] found 0.7% in sheep's wool. Inskip[7] could find no evidence for hydroxylysine in casein, lactalbumin, glycinin or zein. It was of doubtful occurrence in keratin (human hair) and the evidence for its presence in wool (sheep) was inconclusive.

Because of the ease of resolving mixtures of basic amino acids on short columns of ion exchange resins as described by Moore and Stein,[8] it seemed worthwhile to examine a number of protein hydrolysates. The analytical procedure employed is capable of detecting 0.05 micromole of amino acid (0.001 mg. approximately of hydroxylysine nitrogen) with certainty. Where zero values are reported (Table I), not even a trace of hydroxylysine was indicated in the position on the chromatogram normally occupied by hydroxylysine. The traces of hydroxylysine encountered by Van Slyke, Hiller and MacFadyen[3] in proteins other than the collagen group would seem likely attributable to the difficulties inherent in the quantitative specific precipitation of hydroxylysine at very low concentrations. From the evidence presented it appears that collagen is the only protein discovered so far that contains hydroxylysine.

TABLE I

	Lit. reference number (3)	(4)	(5)	(7)	Present paper
	Hydroxylysine N as % of toal N, %				
Collagen (ox cortical bone)	0.9				1.0–1.3
Collagen (human cortical bone)					0.8
Cartilage (beef)					1.5
Skin—Beef (raw)					1.0
Calf (raw)					1.3
Pork (raw)					0.7
Gelatin (from skin or bone)	0.9	1.1–1.2	1.0–1.1		1.0–1.2
Icthyocol (Sturgeon)					0.8
Elastin			0		0
Albumin (bovine plasma)			0.1		0
Fibrin (beef blood)					0
Plasma (human)					0
Hemoglobin (horse)	0	0			0
Serum (horse)					0
Casein (Hammarsten)	0.33	0	0	0	0
Lactalbumin	0.03	0		0	0
β-Lactoglobulin	0.02	0			...
Myosin		0			...
Muscle (rat)			0.1		...
Insulin		0			...
Bence-Jones protein			0.2		...
Keratin (hair, human)				?	0
Keratin (horn, cow)[a]					0
Keratin (hoof, cow)					0
Keratin (feathers, duck)					0
Keratin (wool, sheep)	0.11	0		+?	0[b]
Ovalbumin	0.09	0	0.1		0
Salmine		0			...
Protamine					0
Fibroin (silk)		0			0
Tobacco mosaic virus		0			...
Edestin		0			0
Gliadin	0.12	0	0.1		...
Zien (corn)	0.33	0		0	0
Gluten (wheat)					0
Globulin (pumpkin seed)	0.10				0
Aleuronate					0
Glycinine (soy bean)			0	0	
Peptone (Wittes)					0
Muscle (cod)					0[c]

[a] Some commercial preparations of keratin contain ornithine, but fresh untreated tissue, *i.e.*, hair, horn, hoof, feathers or wool, do not; its presence in the processed material is presumably an artifact.[9] [b] Middlebrook[6a] reported 0.18% of total nitrogen. [c] G. Agren (*Acta Physiol. Scand.*, 7, 134 (1944)) reported 1.1% of total nitrogen in cod muscle (Swedish).

Experimental

Hydrolysates were prepared by refluxing approximately 1 g. of protein with 100 ml. of 6 *N* hydrochloric acid for 20 hours. The nitrogen content of each hydrolysate was determined by macro Kjeldahl. From a portion of hydrolysate, excess acid was evaporated and the residue dissolved in water so that approximately 1.5 mg. of nitrogen was contained in each ml. of solution. One ml. of solution was placed on an 0.9 × 15 cm. column of Dowex 50, 8% cross-linked, 200 to 400 mesh, operated in the sodium form. The columns were jacketed at 25°. The method was that of Moore and Stein,[8] but the buffer sequence employed to develop the column was that described by Hamilton and

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