TABLE II

VELOCITIES OF DEPOLYMERIZATION OF HEATED AND UNHEATED DNA BY RABBIT SERUM DNASE

| Date and rabbit, (no.) | Ml. rabbit serum per 19 ml. substrate–serum mixture | Slopes[a] Unheated (P) $v_1$ | Heated (H) $v_2$ | Mixture (P) + (H) $v_3$ | Probability (P) that $v_1 = v_3$ |
|---|---|---|---|---|---|
| 9/23 (1) | 1/8 | 0.25 ± 0.03 | 0.11 ± 0.02 | 0.21 ± 0.02 | >0.3 |
| 9/30 (1) | 1/6 | .35 ± .05 | .17 ± .03 | .33 ± .02 | > .7 |
| 11/17 (2) | 1/8 | .375 ± .025 | .11 | .371 ± .030 | > .9 |
| 11/12 (2) | 1/5 | .55 ± .07 | .19 ± .04 | .52 ± .05 | > .7 |
| 11/13 (2) | 1/6 | .78 ± .10 | 0.25 | .64 ± .03 | > .3 |
| 10/12 (2) | 1/4 | 1.06 ± .09 | .30 | .76 ± .09 | > .02 |
| 11/13 (2) | 1/2 | 1.62 ± .09 | .34 | 1.10 | < .001 |
| 8/25 (3) | 1/4 | 2.10 ± .19 | .70 ± .01 | 1.54 ± 0.06 | > .01 |
| 11/18 (4) | 1 | 2.65 ± .21 | .6 | 2.20 ± .04 | > .05 |
| 11/24 (5) | 3/4 | 4.42 ± .42 | 1.55 | 3.29 ± .27 | > .1 |
| 11/16 (6) | 1 | 5.06 ± .50 | 1.4 | 3.77 ± .21 | > .05 |
| 11/19 (7) | 3 | 5.70 ± .28 | 1.3 | 5.02 ± .41 | > .2 |

[a] See footnote to Table I.

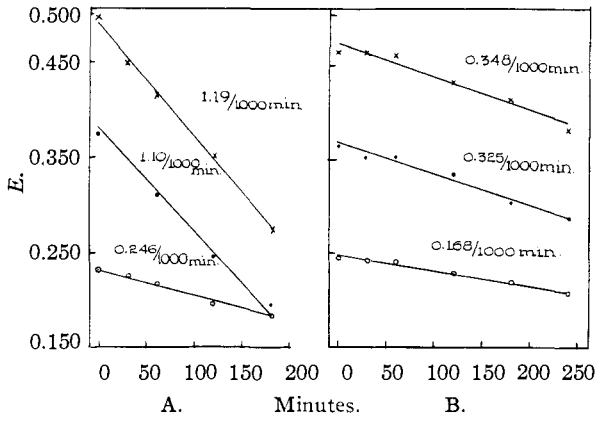


Fig. 1.—The slopes of the curves (given as a decimal along side each curve in the figure) indicate the rate of reduction in optical density at 640 mμ (= rate of depolymerization). In A, the enzyme is crystalline bovine pancreatic DNase. In B, the enzyme source is rabbit serum. In both A and B, the top curve (1) is for unheated substrate, the bottom curve (2) is for heated substrate, and the middle curve (3) is for the mixture of the heated and unheated substrates.

(Table I) and in only 3 of the 12 with rabbit serum (Table II) is the probability that the slopes of 1 and 3 are identical less than 0.05. It follows that in the mixture, the affinity of DNase for the unheated DNA is so much greater than for the heated that practically none of the DNase becomes bound up in the slower reaction with the heated substrate.

The ineffectiveness of heated DNA in competing for DNase with unheated DNA supports our previous suggestion[3] that heat causes intramolecular rearrangement (probably uncoiling) in the DNA molecule in addition to splitting it into smaller units, unlike the early effects of DNase depolymerization. Such steric alteration is more likely to account for the diminished affinity for DNase than is destruction of binding sites by heating.

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## A Resin for the Selective Retention of Sulfhydryl Compounds

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A resin has been prepared by mercuration of a phenol–formaldehyde polymer that will selectively remove mercaptans from aqueous solutions. The mercaptans can be recovered by elution with dilute 2-mercaptoethanol or hydrogen sulfide solutions. Experiments 1 and 3 in Table I show that 13 and 20 mg., respectively, of glutathione (GSH) and cysteine are quantitatively retained by the resin and are recovered by elution with 2-mercaptoethanol. Oxidized glutathione (GSSG) is not retained by the resin at *p*H 7.0 (experiment 5) though it is partially retained at *p*H 3.0–3.5 (experiment 4). The retention of GSSG under the latter conditions is probably made possible by a reducing action of the resin which causes some reduction of GSSG to GSH. This view is supported by the observation that water extracts of the resin cause a reduction of equivalent amounts of iodine and GSSG. Experiment 6 shows that coenzyme A (CoA) is also retained by the resin and may be recovered by elution with solutions of potassium sulfide (0.1 *M*, *p*H 7.7). CoA is not eluted from the resin with 2-mercaptoethanol or quantitatively by acid solutions

TABLE I

| Expt. | Compound | Milligrams put on resin | Milligrams recovered in water effluent | Milligrams recovered in 2-mercaptoethanol eluate |
|---|---|---|---|---|
| 1 | GSH | 13.5 | 0[a] | 12.0[a] |
| | | | | 12.8[b] |
| 2 | GSH (*p*H 7) | 11.0 | 0.2[a] | ...[c] |
| 3 | Cysteine·HCl | 22.8 | 0 | 21.7 |
| 4 | GSSG | 31.1 | 21.7[a] | 7.6[a] |
| | | | 0.0[b] | 7.4[b] |
| 5 | GSSG (*p*H 7) | 11.7 | 11.7[a] | ...[c] |
| 6 | CoA | 21.5 | 0.76 | 21.2[d] |
| 7 | DL-Lysine | 6.7 | 5.8 | ...[e] |
| 8 | DL-Serine | 8.2 | 8.0 | ...[e] |
| 9 | L-Alanine | 9.8 | 8.1 | ...[e] |

[a] Determined by ninhydrin method. [b] Determined by alloxan method. [c] No elution with 2-mercaptoethanol was performed [d] Eluted with 0.1 *M* potassium sulfide (*p*H 7.7).

of hydrogen sulfide. The selectivity of the resin in removing mercaptans is indicated by experiments 7, 8 and 9 showing that amino acids are not retained.

In view of the extreme importance of various naturally occurring mercaptans in biological chemistry, it seems probable that a resin of the type described will find wide application in the separation and isolation of these substances from biological mixtures. The marked influences of *p*H on the retention of different compounds by the resin, as well as the variations in effectiveness of different eluting solutions, suggest that the resin may be used for the chromatographic separation of one mercaptan from another.

The resin was prepared by adding 200 g. of mercuric acetate in 500 ml. of hot ethanol to a solution of 300 g. of phenol–formaldehyde polymer[1] in 950 ml. of absolute ethanol at 30–35°. The yellow precipitate, which formed in five minutes, was filtered after two days and washed with hot ethanol and then with water until free of soluble mercury salts. The mercury content[2] of the air dried (50°) resin was 35.5%.

In the experiments described above, 3 g. of 2:1 resin–Celite mixture were packed into a glass tube (column size about 12 × 80 mm.) and after washing with *ca.* 100 ml. of water, 20 ml. of solution containing 10–30 mg. of the compound to be adsorbed was placed on the column and washed through with 100 to 300 ml. of distilled water. The water effluents were analyzed for the solute. The mercaptans retained by the resin were finally eluted with dilute solutions of 2-mercaptoethanol (0.05–1%) or, in the case of CoA, with a potassium sulfide solution (0.1 *M*, *p*H 7.7). The 2-mercaptoethanol eluate was analyzed for the mercaptans after extraction with ethyl acetate to remove the 2-mercaptoethanol, which interfered with the analytical methods. The eluting solutions were unbuffered except in experiments 2 and 5 when 0.33 *M* phosphate buffer was used. The *p*H of the unbuffered eluates was about 3–4. GSH, the amino acids, and GSSG were determined by the ninhydrin method.[3] Cysteine also was determined by the ninhydrin method after oxidation to cystine with hydrogen peroxide (0.02 ml. of 3% solution per ml. of cysteine solution). The excess hydrogen peroxide was destroyed with sulfur dioxide. The excess of the latter was removed by degassing with helium. GSH was determined also by the specific alloxan method.[4] CoA was determined by the phosphotransacetylase assay method.[5]

(1) The material used here was an ethanol solution of polymer (BV-1600) made by the Bakelite Co. Effective resins were also made from our own polymer, prepared from phenol and formaldehyde.

(2) We are indebted to Dr. W. C. Alford of the National Institute of Arthritis and Metabolic Diseases for the mercury analysis.

(3) S. Moore and W. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

(4) A. Lazarow, J. W. Patterson and S. J. Copperstein, personal communication.

(5) E. R. Stadtman, G. D. Novelli and F. Lipmann, *J. Biol. Chem.*, **191**, 365 (1951).

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## Relative Rates of Migration of Aryl Groups in the Schmidt Reaction of Unsymmetrical Diarylethylenes

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The Schmidt reaction of 1-phenyl-1-*p*-anisylethylene has been carried out in the past by three different groups of chemists. McEwen, Gilliland and Sparr,[1] using a distillation analysis of the ketone fraction of the reaction mixture, found a molar ratio of acetophenone to *p*-methoxyacetophenone of 6.1. Kuhn and Di Domenico[2] analyzed the mixture of ketones by an unspecified infrared method and reported a value of about 3.5 for the molar ratio of acetophenone to *p*-methoxyacetophenone. Ege and Sherk[3] analyzed the amine fraction of the reaction mixture by titration of the crude, mixed hydrochlorides. They reported a molar ratio of *p*-anisidine to aniline of 99 or greater. Thus, in the Schmidt reaction of 1-phenyl-1-*p*-anisylethylene, there are three widely different values for the relative rate of migration of the *p*-anisyl group as compared to the phenyl group.

Since one of the main aspects of our previous work on the Schmidt reaction consisted of an attempt to correlate relative rates of migration of aryl groups by a suitable adaptation of the Hammett equation,[4] and since our previously reported success along this line would have little meaning unless the relative rate of migration of the *p*-anisyl group were about 6 or 7, we decided to repeat this work and use more than one method of analysis to determine the product ratios. The reaction of 1-phenyl-1-*p*-anisylethylene with hydrazoic and sulfuric acids was carried out essentially as previously described.[1] The ketone fraction of the reaction mixture was separated and analyzed by the following infrared method: known mixtures of acetophenone and *p*-methoxyacetophenone were used to establish a calibration curve. A plot was made of the per cent. composition *vs.* the ratio $T$ 836 cm.$^{-1}$/$T$ 689 cm.$^{-1}$, where $T$ represents percentage transmittance. Pure acetophenone shows negligible absorbancy at 836 cm.$^{-1}$ and *p*-methoxyacetophenone shows negligible absorbancy at 689 cm.$^{-1}$. From this plot and the infrared spectrum of the mixed ketones obtained from the Schmidt reaction it was possible to determine that the molar ratio of acetophenone to *p*-methoxyacetophenone was 6.6. As a check on this value, a duplicate methoxyl determination was carried out on the mixed ketones by the standard Zeisel method. The percentage methoxyl was found to be 3.36 and 3.32; by calculation, the molar ratio of acetophenone to *p*-methoxyacetophenone was determined to be 6.5. Thus, including the value of 6.1 obtained by distillation analysis,[1] we have found the relative rate of migration of the *p*-anisyl group to be 6.4 ± 0.2 as a result of three fundamentally different methods of analysis.

In order to further clarify the matter, the method of analysis of the amine fraction employed by Ege and Sherk[3] was repeated. In complete agreement with them we found a neutralization equivalent of 159.8 for the mixed hydrochlorides.[5] Yet, when the crude, mixed hydrochlorides were neutralized and the amines subjected to distillation, an appreciable amount of aniline was obtained, as well as *p*-anisidine and an unidentified higher boiling amine.

(1) W. E. McEwen, M. Gilliland and B. I. Sparr, THIS JOURNAL, **72**, 3212 (1950).

(2) L. P. Kuhn and J. Di Domenico, *ibid.*, **72**, 5777 (1950).

(3) S. H. Ege and K. W. Sherk, *ibid.*, **75**, 354 (1953).

(4) W. E. McEwen and N. B. Mehta, *ibid.*, **74**, 526 (1952).

(5) *Anal.* Calcd. for *p*-anisidine hydrochloride: neut. equiv., 159.6; calcd. for aniline hydrochloride: neut. equiv., 129.6.