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.

The molar ratio of *p*-anisidine to aniline equalled 6.1. Since the fractionation was not a precise one, however, this figure is probably not as reliable as the others mentioned above, despite the substantial agreement. Thus the fact that the average neutralization equivalent for the mixed hydrochlorides turned out to be very nearly the theoretical value for pure *p*-anisidine hydrochloride was nothing more than a coincidence.

Since a *p*-ethoxyl group has nearly the same σ-value as a *p*-methoxyl group,[6] approximately the same molar ratio of acetophenone to *p*-ethoxyacetophenone should be obtained in the Schmidt reaction of 1-phenyl-1-*p*-ethoxyphenylethylene as the molar ratio of acetophenone to *p*-methoxyacetophenone obtained in the Schmidt reaction of 1-phenyl-1-*p*-anisylethylene, provided that the Hammett equation can indeed be applied to the results of these rearrangement reactions. After reaction of 1-phenyl-1-*p*-ethoxyphenylethylene with hydrazoic and sulfuric acids, the ketone fraction of the reaction mixture was separated and analyzed by an infrared method similar to that described above. The molar ratio of acetophenone to *p*-ethoxyacetophenone was found to be 6.2, in excellent agreement with the values found in the work with the *p*-methoxyl analog of the olefin.

On the basis of theoretical considerations elaborated by Winstein, *et al.*, for the Wagner–Meerwein rearrangement,[7] it would be anticipated that the relative rate of migration of the *p*-tolyl group with respect to the phenyl group in the Schmidt reaction of *p*-methylbenzhydrol would probably not be as great as the relative rate of migration of the *p*-tolyl group with respect to phenyl in the Schmidt reaction of 1-phenyl-1-*p*-tolylethylene. Yet, in a preliminary note, Arcus and Mesley[8] reported exclusive migration of the *p*-tolyl group in the Schmidt reaction of *p*-methylbenzhydrol, whereas the relative rate of migration of the *p*-tolyl group was found to be 5.0 in the Schmidt reaction of 1-phenyl-1-*p*-tolylethylene.[1,9] Since Arcus and Mesley did not follow up on their initial report of the *p*-methylbenzhydrol reaction in a later, more detailed article,[10] however, it may be assumed that the earlier note was in error. Studies on the Schmidt reactions of substituted benzhydrols have been in progress in this Laboratory for some time,[11] and we have found the relative rate of migration of the *p*-tolyl group to be 3.44 ± 0.09 in the Schmidt reaction of *p*-methylbenzhydrol.

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Experimental[12]

Schmidt Reaction of 1-Phenyl-1-*p*-anisylethylene.—To a suspension of 36.3 g. (0.56 mole) of sodium azide in 315 cc. of chloroform was added dropwise, with external ice cooling and mechanical stirring, 105 cc. of 90% sulfuric acid. The ice-bath was replaced by a water-bath maintained at about 25°, and 50.0 g. (0.238 mole) of 1-phenyl-1-*p*-anisylethylene[13] dissolved in 75 cc. of chloroform was added dropwise, with stirring, in the course of several hours. Stirring was continued for an additional hour after addition of the olefin had been completed, and then the reaction mixture was allowed to stand at room temperature for another 12 hours. The reaction mixture was poured into a slurry of ice water and an excess of sodium bicarbonate. The chloroform solution was separated from the aqueous solution, and the aqueous solution was extracted with several portions of ether. The combined ether–chloroform solution was extracted with several portions of dilute hydrochloric acid until no more amine was liberated on neutralization of a fresh acid extract. The ether–chloroform solution was dried over anhydrous sodium sulfate, filtered and the solvents distilled. The residual mixture of acetophenone, *p*-methoxyacetophenone and higher-boiling neutral products was distilled *in vacuo* in order to separate the two ketones from the higher boiling materials. There was obtained 17.2 g. of mixed ketones, b.p. 47–95° at 0.5 mm.

Anal. Found: CH_3O, 3.36, 3.32.

The infrared spectrum of the mixture of ketones was measured in a 9.2 weight % chloroform solution in a 0.05-mm. cell. There was little absorbancy in the valley between the peaks at 836 cm.$^{-1}$ (due to *p*-methoxyacetophenone) and 689 cm.$^{-1}$ (due to acetophenone) other than a small peak at 809 cm.$^{-1}$ (due to *p*-methoxyacetophenone), and the minimum point of absorbancy in the valley was normalized to 100% transmittance. The ratio T 836 cm.$^{-1}$/T 689 cm.$^{-1}$, where T represents the percentage transmittance, for the ketone mixture was found to be 3.04. Reading from the plot of the ratio T 836 cm.$^{-1}$/T 689 cm.$^{-1}$ *vs.* weight % of acetophenone for known mixtures of acetophenone and *p*-methoxyacetophenone (data shown below), we could calculate that this value corresponds to a molar ratio of acetophenone to *p*-methoxyacetophenone of 6.6.

The hydrochloric acid extract of the ether–chloroform solution was made basic by addition of sodium bicarbonate and extracted with ether. The ether solution was dried over anhydrous sodium sulfate and then filtered. The mixed amine hydrochlorides were precipitated by passing anhydrous hydrogen chloride into the ether solution. After having been dried in a vacuum desiccator the mixed hydrochlorides weighed 25.7 g. The neutralization equivalent was found to be 159.8 by potentiometric titration with standard sodium hydroxide solution.

The mixture of hydrochlorides was made basic by addition of sodium carbonate solution, and the basic solution was extracted with ether. The ether solution was dried over anhydrous potassium carbonate, filtered and the ether distilled. The residue was partially fractionated by distillation through a Todd column. The first fraction, aniline, came over at 182–185° at 740 mm. and weighed 1.50 g. It gave a benzoyl derivative of m.p. 159–161°, undepressed by admixture with authentic benzanilide. The distilling pot was then removed from the Todd column, attached to a short Vigreux column, and the fractionation was continued *in vacuo*. The Todd column had also been washed with ether to recover any material held up by the packing, and the recovered material had been added to that in the still pot. The second fraction consisted of 12.01 g. of somewhat impure *p*-anisidine, b.p. 73–93° at 1–2 mm., m.p. 56–58°. The molar ratio of *p*-anisidine to aniline based on the above weights equals 6.1. The third fraction consisted of a dark oil, b.p. 104–190° at 1.5 mm., which was not further investigated.

Infrared Calibration Curve with Known Mixtures of Acetophenone and *p*-Methoxyacetophenone.—Chloroform solutions were prepared containing 9.2 ± 0.2 weight % of the mixed ketones, and the infrared spectra were measured in a 0.05-mm. cell. Following are the values of the ratio, T 836 cm.$^{-1}$/T 689 cm.$^{-1}$, followed in each case by the weight % of acetophenone in the corresponding binary mixture: 0.30, 17.2%; 0.55, 36.8%; 0.96, 48.0%; 1.34, 59.5%; 2.48, 78.5%; 2.54, 80.0%; 3.37, 86.7%.

1-Phenyl-1-*p*-ethoxyphenylethylene.—To the Grignard reagent prepared from 115 g. (0.81 mole) of methyl iodide and 19.5 g. of magnesium in 350 cc. of ether was slowly added an ether solution of 183 g. (0.81 mole) of *p*-ethoxy-

(6) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 188.

(7) S. Winstein, B. K. Morse, E. Grunwold, K. C. Schreiber and J. Corse, THIS JOURNAL, **74**, 1113 (1952).

(8) C. L. Arcus and R. J. Mesley, *Chem. and Ind.*, 701 (1951).

(9) Ege and Sherk[3] reported the relative rate of migration of the *p*-tolyl group to be 4.0 in the Schmidt reaction of 1-phenyl-1-*p*-tolylethylene.

(10) C. L. Arcus and R. J. Mesley, *J. Chem. Soc.*, 178 (1953).

(11) R. F. Tietz and W. E. McEwen, unpublished work.

(12) All m.p.'s are corrected. Analyses by Schwarzkopf Microanalytical Laboratory.

(13) R. Stoermer and M. Simon, *Ber.*, **37**, 4163 (1904).

benzophenone.[14] The mixture was heated on the steam-bath for an additional 30 minutes, then it was cooled and hydrolyzed with an aqueous solution of ammonium chloride. The ether solution was separated from the aqueous solution, and the aqueous solution was extracted with fresh ether. Evaporation of the ether from the combined organic material afforded an oil. This was mixed with 200 cc. of water plus 50 cc. of concentrated sulfuric acid and then heated under reflux for one hour. Solid 1-phenyl-1-*p*-ethoxyphenylethylene was filtered from the cooled solution and crystallized from ethanol. There was obtained 168 g. (0.75 mole, 93%) of the olefin, m.p. 78.8–79.4°. Since the reported[15] m.p. is 71°, the material was analyzed.

Anal. Calcd. for $C_{16}H_{16}O$: C, 85.68; H, 7.19. Found: C, 85.83; H, 7.41.

Schmidt Reaction of 1-Phenyl-1-*p*-ethoxyphenylethylene.—The reaction was carried out as described above for 1-phenyl-1-*p*-anisylethylene, 50.0 g. (0.223 mole) of 1-phenyl-1-*p*-ethoxyphenylethylene being employed. A total of 20.98 g. of mixed ketones was obtained by distillation, a major portion distilling at 83–107° (13 mm.) and the remainder at 111–170° (16 mm.). The infrared method of analysis was identical with that described above. The ratio T 840 cm.$^{-1}$/T 689 cm.$^{-1}$ equalled 3.48 for the ketone fraction, and this corresponds to a molar ratio of acetophenone to *p*-ethoxyacetophenone of 6.2.

Infrared Calibration Curve with Known Mixtures of Acetophenone and *p*-Ethoxyacetophenone.—*p*-Ethoxyacetophenone was prepared by the procedure of Hartough and Kosak.[16] Chloroform solutions were prepared containing about 9 weight % of the mixed ketones, and the infrared spectra were measured in a 0.05-mm. cell. Following are the values of the ratio T 840 cm.$^{-1}$/T 689 cm.$^{-1}$, followed in each case by the weight % of acetophenone in the corresponding binary mixture: 1.33, 52.3%; 2.38, 68.8%; 3.35, 80.9%; 4.93, 89.8%.

(14) W. E. Bachmann and J. W. Ferguson, THIS JOURNAL, **56**, 2081 (1934).

(15) G. Busignies, *Compt. rend.*, **151**, 515 (1910).

(16) H. D. Hartough and A. I. Kosak, U. S. Patent 2,475,564, July 5, 1949.

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COMMUNICATIONS TO THE EDITOR

TOTAL SYNTHESIS OF THE ANTIBIOTIC PUROMYCIN[1]

Sir:

The structure of puromycin has been elucidated as 6-dimethylamino-9-(3′-*p*-methoxy-L-phenylalanylamino-3′-deoxy-β-D-ribofuranosyl)-purine.[2] Recently the conversion of the antibiotic to the highly biologically active 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (I) and the resynthesis of the antibiotic from I were described.[1] This communication outlines the synthesis of I from D-xylose, thus completing a total synthesis of the antibiotic.

Reaction of crude methyl D-xylofuranoside[3] with acetone in the presence of copper sulfate and 0.002 *N* sulfuric acid afforded a readily separable mixture of 41% (based on D-xylose) of methyl 3,5-O-isopropylidene-α-D-xylofuranoside, b.p. 85° (0.1 mm.), $[\alpha]^{24}D$ +17.6° ($CHCl_3$) and the β-anomer, b.p. 108° (0.1 mm.), $[\alpha]^{24}D$ −64° ($CHCl_3$) in 31% yield.[4] Found: (α), C, 53.1; H, 8.06; (β), C, 52.3; H, 7.93. Each anomer was then used separately in the synthesis until the O-methyl was removed. 2-O-Mesylation, deacetonation in 70% acetic acid, and oxide formation with sodium methoxide gave methyl 2,3-anhydro-D-lyxofuranoside: α-anomer (74% yield), m.p. 80–82°, $[\alpha]^{26}D$ + 67° (H_2O); β-anomer (71%), m.p. 74–75°, $[\alpha]^{25}D$ −102° (H_2O).[5] Found: (α), C, 48.9; H, 6.90; (β), C, 49.5; H, 7.07. Ring-opening of the anhydro sugar with ammonia at 100° occurred with Walden inversion to give methyl 3-amino-3-deoxy-D-arabinofuranoside, isolated as the crystalline N-isopropylidene derivative[6]: α-anomer (54%), m.p. 157–159°, $[\alpha]^{26}D$ +98° (H_2O); β-anomer (47%), m.p. 155–157°, $[\alpha]^{25}D$ −96° (H_2O). Found: (α), C, 53.1; H, 7.96; N, 7.12; (β), C, 52.9; H, 8.69; N, 7.05. Acetylation in water with acetic anhydride formed methyl 3-acetamino-3-deoxy-D-arabinofuranoside: α-anomer (90%), m.p. 115–116°, $[\alpha]^{24}D$ +102° (H_2O); β-anomer (98%), m.p. 155°, $[\alpha]^{24}D$ −119° (H_2O). Found: (α), C, 47.2; H, 7.30; N, 6.88; (β), C, 47.2; H, 7.56; N, 6.41. Mesyl chloride in pyridine gave methyl 2,5-di-O-mesyl-3-acetamino-3-deoxy-D-arabinofuranoside: α-anomer (84%), m.p. 125–126°, $[\alpha]^{28}D$ +104° (Pyr.); β-anomer (84%), m.p. 169–170°, $[\alpha]^{25}D$ −88° (Pyr.). Found: (β), C, 33.6; H, 5.36; N, 3.93; (β), C, 33.5; H, 5.48; N, 3.89. Treatment with sodium acetate in boiling 95% Methyl Cellosolve caused displacement of the 2-mesylate by the neighboring 3-acetamino group with Walden inversion via an oxazoline[7] and the 5-mesylate by acetate. Isolation of the product by acetylation afforded methyl 2,5-di-O-acetyl-3-acetamino-3-deoxy-D-ribofurano-

(1) This communication is derived from papers VIII and IX of the series Puromycin, Synthetic Studies; for paper VII, *cf.* B. R. Baker, J. P. Joseph and J. H. Williams, THIS JOURNAL, **76**, 2838 (1954).

(2) (a) C. W. Waller, P. W. Fryth, B. L. Hutchings and J. H. Williams, THIS JOURNAL, **75**, 2025 (1953); (b) N. Y. Meeting-in-miniature, Feb., 1954.

(3) P. A. Levene, A. L. Raymond and R. T. Dillon, *J. Biol. Chem.*, **95**, 699 (1932).

(4) E. E. Percival and R. Zobrist, *J. Chem. Soc.*, 4306 (1952), have reported for this preparation, b.p. 110° (0.1 mm.) and $[\alpha]D$ −26°, indicating a mixture of 54% α-anomer and 46% β-anomer.

(5) E. E. Percival and R. Zobrist, *J. Chem. Soc.*, 564 (1953), deacetonated crystalline methyl 2-O-tosyl-3,5-O-isopropylidene-β-D-xylofuranoside with 1% methanolic hydrogen chloride which caused extensive anomerization. Oxide formation afforded them 23% of methyl 2,3-anhydro-α-D-lyxofuranoside, m.p. 81°, $[\alpha]D$ +57° (H_2O).

(6) Hydrolysis of either anomer gave 3-amino-3-deoxy-D-arabinose hydrochloride, m.p. 159° dec., $[\alpha]^{24}D$ −112°. This gave a negative ninhydrin test in 3% alkali and is isomeric to the other possible oxide ring-opening product, 2-amino-2-deoxy-D-xylose hydrochloride, described by M. L. Wolfrom and K. Anno, THIS JOURNAL, **75**, 1038 (1953).

(7) B. R. Baker and R. E. Schaub, *J. Org. Chem.*, **19**, 646 (1954).