The Paper Chromatography of Imidazoles

BY BRUCE N. AMES AND H. K. MITCHELL[1]

During the course of investigations on the accumulation of imidazole-like substances by histidineless mutants of *Neurospora*, methods have been developed for paper chromatography and detection on the paper of compounds of this type. Dent[2] reported the use of the Pauly reagent (diazosulfanilic acid) for this purpose. Using the modification of this reagent described below and the method of Jorpes[3] in which the acidic diazo reagent is mixed with the imidazole before developing the color with Na_2CO_3, excellent results have been obtained with a variety of imidazole derivatives.

Procedure and Results.—Air-dried chromatograms are sprayed so as just to wet the paper with the diazosulfanilic acid reagent, and then the color is developed by a light spray of 5% sodium carbonate solution. The diazo reagent is made by adding slowly and with stirring 25 ml. of a freshly made up 5% sodium nitrite solution to 5 ml. of a stock sulfanilic acid solution (0.9 g. sulfanilic acid and 9 ml. of concentrated HCl made up to 100 ml. with distilled water). Both the nitrite and sulfanilic acid solution must be brought to 0° before mixing.

The diazo reagent will keep for about 4–5 days at 0° but decomposes rapidly at higher temperatures. The stock sulfanilic acid solution keeps indefinitely at room temperature.

This method is considerably more sensitive than the ninhydrin method for histamine and histidine. The diazo reagent will indicate 0.3 of a microgram of histamine or histidine on a chromatogram. The sensitivity of the reagent is about the same for other imidazoles.

Using this procedure with known imidazoles several differently colored spots have been observed. In general imidazoles with a side chain at the 4-position, such as histidine, give a red color with the reagent. Substituents in addition to a side chain may alter the color considerably. Guanine gives a bright orange color and 4(5)-amino-5(4)-carboxamide imidazole gives first an orange color with the acid diazo solution and then a bright blue when the sodium carbonate is sprayed on.[4] This blue color is unique[5] and probably can be used for the quantitative estimation of this natural base.

The coupling reaction is not general for all imidazoles. Imidazoles with alkyl substituents on one of the ring nitrogens do not give the reaction[7]; *e.g.*, 1-methylhistidine and anserine. Some imidazoles with a carbonyl group next to the ring at the 4 position do not react; *e.g.*, [imidazolyl-4-(5)]-formaldehyde and [imidazolyl-4(5)]-glyoxylic acid. A weak yellow color is given by 4(5)-carboxyimidazole. Some condensed ring systems containing an imidazole nucleus also do not give the Pauly reaction; *e.g.*, most purines and benzimidazole.

In addition to imidazoles other compounds have been encountered in biological materials which react with diazosulfanilic acid giving colored dyes. Ammonium chloride as well as other ammonium salts gives yellow spots in fairly high dilution. Tyrosine has been found to give a red color under the conditions stated but only at much greater concentrations. Many phenols other than tyrosine are also known to couple with diazosulfanilic acid. This reaction has been used for their identification on paper chromatograms by Evans, *et al.*,[8] who reported various orange, brown and yellow colors from biochemically important phenols. A non-imidazole base that gives a red diazo reaction has been encountered by Hunter[9] in maize.

The R_f values and diazo colors of a number of imidazoles of biochemical and preparative importance are listed in Table I. Absorption spots under 2537 Å. ultraviolet and ninhydrin reaction color are also listed as they are useful in characterization.

TABLE I

R_f VALUES ON 3.5 HOURS, ASCENDING,[10] WHATMAN #1 CHROMATOGRAMS AT 25°

Compound	Diazo spot color	R_f with 3:1 propyl alcohol–0.2 *N* ammonia	R_f with 3:1 propyl alcohol–1 *N* acetic acid[11]	2537 Å. absorption	Ninhydrin color
Histidine	Red	0.22	0.12	–	Violet
4(5)-Carboxyimidazole[a]	Yellow	.23	.29	–	.....
Carnosine	Red	.24	.06	–	Violet
2-Thiol histidine[b]	Red	.25	.15	+	Violet
[Imidazolyl-4(5)]-lactic acid	Red	.26	.27	–	.....
Ergothionine	Red	.27	.26	+	.....
Guanine	Orange	.27	.35	+	.....
[Imidazolyl-4(5)]-pyruvic acid[c]	Red	.31	.31	–	.....
[Imidazolyl-4(5)]-acrylic acid	Red	.34	.69	+	.....
Ammonium chloride	Yellow	.39	.41	–	.....
4(5)-Amino-5(4)-carboxamide imidazole[d]	Blue	.52	.51	+	Yellow-Orange
Histamine	Red	.65	.10–0.28	–	Violet
Histidinol[e]	Red	.65	.10– .28	–	Violet
Histidine methyl ester[b]	Red	.75	.45	–	Violet
4(5)-Hydroxymethylimidazole[f]	Red	.75	.56	–	.....
Imidazole	Red-Orange	.88	.58	–	.....

The compounds indicated were kindly supplied by [a] Dr. R. G. Jones, [b] Dr. M. Fling, [c] Mr. P. Thayer, [d] Dr. E. E. Snell, [e] Dr. B. Davis,[12] [f] Dr. P. M. Ruoff.

Chromatograms have been made from the reaction mixture of the Parrod[13] synthesis of imidazoles from glucose.[14]

(1) This work was supported in part by funds from the Rockefeller Foundation and by funds from the Atomic Energy Commission administered through contract with the Office of Naval Research, U. S. Navy, Contract N6 onr-244, Task Order V.

(2) C. E. Dent, *Biochem. J.*, **43**, 169 (1948).

(3) E. Jorpes, *ibid.*, **26** 1507 (1932).

(4) A darker color is obtained if the sodium carbonate is sprayed on before the diazo reagent.

(5) A blue diazotization product has also been reported by Hunter[6] with 4(5)-guanidinoimidazole.

(6) G. Hunter, *Biochem. J.*, **30**, 1183 (1936).

(7) M. Guggenheim, "Die Biogenen Amine," S. Karger, Basel, 1940, p. 407.

(8) R. A. Evans, W. Parr and W. Evans, *Nature*, **164**, 674 (1949).

(9) G. Hunter, *Biochem. J.*, **48**, 265 (1951).

(10) R. J. Williams and H. Kirby, *Science*, **107**, 481 (1948).

(11) Histamine and histidinol give streaks with this solvent.

(12) H. J. Vogel, B. D. Davis and E. S. Mingioli, THIS JOURNAL, **73**, 1897 (1951).

(13) J. Parrod, *Ann. Chim.*, **19**, 233 (1933).

(14) A modification of the Parrod procedure is listed in ref. 15 for the synthesis of 4(5)-hydroxymethylimidazole, an intermediate in histidine synthesis.

(15) *Org. Syntheses*, **24**, 64 (1944).

Parrod reported imidazole and 4(5)-(*D-arabino*)-tetrahydroxybutaneimidazole as the products. The chromatograms indicate that fair quantities of 4(5)-hydroxymethylimidazole and two other diazo reacting substances were obtained as well.

Chromatograms have been made of many other complex materials, such as deproteinized liver fractions, the products of the action of L-aminoacid oxidase on DL-histidine, and culture filtrates from *Neurospora* and *Penicillium* histidineless mutants, with good separation of imidazoles and reproducible colors and R_f values.

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The Preparation of Tropic Acid

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It has been found that tropic acid, which is required for the synthesis of atropine and certain synthetic medicaments, can be obtained very conveniently, and in good yield, by the addition of formaldehyde to the Ivanov reagent[1] prepared from phenylacetic acid and isopropylmagnesium chloride

$$C_6H_5CH_2COOH + 2(CH_3)_2CHMgCl \longrightarrow C_6H_5CH(MgCl)COOMgCl + 2C_3H_8$$

$$C_6H_5CH(MgCl)COOMgCl + CH_2O \longrightarrow$$

(Ivanov reagent)

$$C_6H_5CH(CH_2OMgCl)COOMgCl \xrightarrow[H_2SO_4]{} C_6H_5CHCOOH \quad (CH_2OH)$$

Magnesium (97.3 g.) and 2 liters of anhydrous ether were placed in a 5-liter, 3-necked flask fitted with a stirrer, dropping funnel and a 2-ft. reflux condenser. About 2 cc. of ethyl bromide and 10 cc. of isopropyl chloride were added, and the mixture was warmed to initiate the reaction. Isopropyl chloride (330 g.) was then added, dropwise, at such a rate that the mixture refluxed. After all of the chloride had been added, the mixture was refluxed for one-half hour.

Phenylacetic acid (240 g.), dissolved in 2 liters of dry benzene, was added to the stirred solution at such a rate that the mixture refluxed. After the addition was completed, the material was refluxed (about 12 hours) until no more gas was evolved. Since a large volume of propane was evolved during and after the addition of the phenylacetic acid, this operation should be carried out in a hood or the gas should be passed through a tube which carries it out of the laboratory.

The mixture was cooled in an ice-bath, and the dropping funnel was replaced by the side-arm of a distillation flask. The side-arm was made of wide glass tubing and was bent at a right angle. The horizontal section of the side-arm should be about 6 inches long and the vertical section should be long enough so that after it has been inserted into the reaction flask the end of the side-arm is about 1 inch above the surface of the stirred liquid. Paraformaldehyde (140 g.) (Mallinckrodt), which had been dried for two days in a desiccator over phosphorus pentoxide, was placed in the distillation flask which was heated in an oil-bath at 180–200°. The formaldehyde was carried into the vigorously-stirred mixture by a slow stream of dry nitrogen. The latter operation required from three to three and one-half hours. The reaction mixture was then poured into a mixture of 300 cc. of concd. sulfuric acid and 3 liters of finely crushed ice which had been placed in a 2-gal. crock, and the mixture was stirred for one-half hour. The solid material was removed by filtration, the organic layer separated, and the aqueous layer, and also the filtered solid, were placed in the original reaction flask. The mixture was warmed on a steam-bath for one-half hour, or until most of the solid had disappeared, then thoroughly cooled, filtered, and extracted with three 300-cc. portions of ether. The ether extracts and the organic layer were combined, and the solvents were removed under reduced pressure until the volume in the distillation flask had been reduced to about 500 cc. The mixture was then cooled for 12 hours, the precipitated tropic acid filtered, the filtrate concentrated to one-half of its original volume, cooled and more tropic acid removed by filtration. The crude tropic acid was heated with 500 cc. of benzene to dissolve unchanged phenylacetic acid, cooled, filtered, and washed with a small amount of cold benzene. The air-dried acid melted at 116–117°; yield 207–245 g. (71–83%).

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(1) D. Ivanov and S. Spassov, *Bull. soc. chim.*, [4] **49**, 19 (1931).

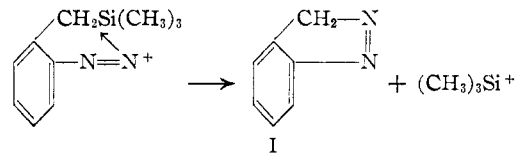
Aminoaryltrialkylsilanes

BY ROBERT A. BENKESER AND PHILIP E. BRUMFIELD

The successful isolation and identification of the isomeric nitro compounds from phenyltrimethylsilane and benzyltrimethylsilane[1] has provided a convenient approach to the preparation and study of the corresponding amines. Very few reports[2] of such amines are found in the literature, probably because compounds of this type suffer easy fission of their aromatic carbon–silicon bond which renders their preparation rather difficult. This tendency for cleavage of the silicon group becomes especially pronounced in acidic media, when the amine group is situated on the aromatic nucleus either *ortho* or *para* to the silicon. The instability of these aromatic silanes to strong acid renders the so-called "wet" reductions of the corresponding nitro compounds rather difficult since these procedures normally employ an acidic medium. In the work herein reported low pressure hydrogenation over a Raney nickel catalyst was found quite satisfactory for obtaining the aminosilanes. These compounds can be acetylated in the usual manner with little or no cleavage of the silicon group.

Five of these amines (*o*-aminophenyltrimethylsilane excepted) were diazotized and then coupled with β-naphthol to yield the corresponding azo compound. The formation of considerable amounts of tar *during* the diazotization indicated that the diazonium salts were unstable.

During the diazotization and subsequent reaction of *o*-aminobenzyltrimethylsilane there was isolated a 16% yield of indazole (I). This product can be explained if one assumes an intramolecular displacement of the trimethylsilyl group.



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Experimental

Aminosilanes.—Weighed portions of up to 50 g. of the nitrosilanes[1] obtained from phenyltrimethylsilane and benzyltrimethylsilane were dissolved in 95% or absolute ethanol and reduced with hydrogen over Raney nickel. A Parr hydrogenator was used, with maximum pressures of 60

(1) R. A. Benkeser and P. E. Brumfield, THIS JOURNAL, **73**, 4770 (1951).

(2) See F. S. Kipping and N. W. Cusa, *J. Chem. Soc.*, **79**, 1088 (1935); also B. N. Dolgov and O. K. Panina, *J. Gen. Chem.*, **18**, 1129 (1948), *C. A.*, **43**, 1737 (1949); and R. F. Fleming, U. S. Patent 2,386,452 (1945).