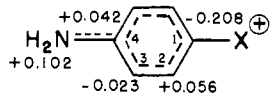
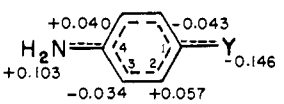
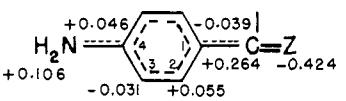
hetero-atoms is small and can be shown[4,5] to exert only a second-order effect on charge distribution. Since the orbitals of the central atom of the $-X^{\oplus}$ group in IV (like N in $-N(CH_3)_3^{\oplus}$) are used in four sp^3-bonds, the π-electron resonance integral for the C_1–$X^{\oplus}$ bond was assumed to be zero. Thus, the only electrical effect considered for $-X^{\oplus}$ was inductive generation of a positive charge on C_1, increasing the electron affinity of that atom and to a lesser extent the electron affinities of C_2 and C_6. The values of the parameters used are summarized in Table I. The resulting π-electron charge distributions in the *para*-amino derivatives are shown in VII, VIII and IX.



VII



VIII



IX

TABLE I

COULOMB INTEGRALS USED IN CALCULATIONS

System	Coulomb integral
IV and VII	$C_1 = \alpha + 0.50\beta$
	$C_2 = C_6 = \alpha + 0.05\beta$
V and VIII	$Y = \alpha - 1.40\beta$
	$C_1 = \alpha - 0.14\beta$
VI and IX	$Z = \alpha + 0.80\beta$
	$C_7 = \alpha + 0.08\beta$
VII, VIII and IX	$N = \alpha + 2.00\beta$[a]
	$C_4 = \alpha + 0.20\beta$
IV–IX	Carbon atoms not otherwise designated $= \alpha$

[a] The value chosen for the coulomb integral of nitrogen is not critical; the value of $\alpha + 2\beta$ commonly is used, although it is probably not the best value for nitrogen.[4]

The calculated charges on the amino nitrogens for VII–IX are essentially the same.[6] Furthermore, the magnitudes of the calculated resonance energies for VII–IX and the corresponding anilinium ions indicate that compounds with charge distributions like VII–IX should have practically equal base strengths. Thus, $-X^{\oplus}$, $-Y$ or $-\overset{|}{C}=Z$ are calculated to be equally effective in causing delocalization of *para*-amino electrons provided that C_4 initially has the same charge in each case. It is concluded, therefore, that so far as the LCAO method is concerned, the relative basicities of amino groups *para* located with respect to electron-attracting substituents provide a reasonable measure of the charge on C_4 as postulated previously.[7] The failure of *p*-amino- and *p*-dimethylaminophenyltrimethylammonium salts to be weak bases relative to the corresponding *m*-isomers is thus regarded as strong experimental evidence against important preferential induction by the trimethylammonium group of charged centers in the 2- and 4-positions.[7b,8]

(5) G. W. Wheland, THIS JOURNAL, **64**, 900 (1942).

(6) If the charges on C_2, C_4 and C_6 in the $-X^{\oplus}$ and $-\overset{|}{C}=Z$ systems are initially +0.05 instead of +0.05 of an electron charge, the calculated charges on a *para*-amino group are again practically equal (approximately +0.14 of an electron charge). A comparable calculation has not been made for the —Y system, but there is no reason to expect that the result would be different.

(7) (a) J. D. Roberts, E. A. McElhill, R. A. Armstrong, THIS JOURNAL, **72**, 408 (1950); (b) J. D. Roberts, R. A. Clement and J. J. Drysdale, *ibid.*, **73**, 2181 (1951).

(8) A somewhat different interpretation recently has been expressed by C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., pp. 254, 732.

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Reaction of Fumaric Acid with Cysteine

BY LEO PINE AND CARL L. PEACOCK

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In recent studies of growth requirements of the yeast phase of *Histoplasma capsulatum* in liquid media,[1] it was observed that the presence of fumarate in a cysteine-containing medium did not affect growth of the fungus when the medium was sterilized by filtration. When the medium was autoclaved, however, growth was completely inhibited. Substitution of pyruvic acid for fumarate completely inhibited growth whether the medium was sterilized by heat or by filtration. These results appeared to demonstrate a requirement of the fungus for —SH groups in the medium, since the reaction of pyruvate with cysteine in the cold[2] and the reaction of mercaptans with unsaturated compounds[3] are known. Although Morgan and Friedmann[4] have reported the reaction of maleic acid with cysteine at low temperatures, fumaric acid did not react with cysteine under their conditions. The amorphous addition product of maleic acid and cysteine was isolated by these workers and was identified as S-cysteinosuccinic acid.

Because fumaric acid and cysteine are used in the preparation of certain culture media and in many biological systems their reaction at elevated temperatures is reported here. A simplified procedure for the isolation of S-cysteinosuccinic acid as a crystalline salt of acetic acid and some of the properties of this compound are also given.

Experimental

Ten grams of L-cysteine (free base) and 11.5 g. of fumaric acid were added to 200 ml. of 95% ethanol. The mixture was heated to near boiling until the reactants dissolved and the presence of —SH groups could no longer be detected with nitroprusside and strong alkali. The time required for the reaction to go to completion was approximately two hours. The ethanol lost by evaporation during this time was replaced with water. The solution was placed in a refrigerator at 5° overnight. The following morning, any cystine or fumaric acid which crystallized from solution was filtered off and the volume of the filtrate was reduced to approximately 25 ml. by vacuum distillation. One hundred ml. of distilled water was added, the solution was cooled to 5°, and the excess fumaric acid which crystallized from solu-

(1) L. Pine, *J. Bacteriol.*, **68**, 671 (1954).

(2) M. P. Schubert, *J. Biol. Chem.*, **114**, 341 (1936).

(3) T. Posner, *Ber.*, **40**, 4788 (1907).

(4) E. J. Morgan and E. Friedmann, *Biochem. J.*, **32**, 733 (1938).

tion was filtered off, and the filtrate lyophilized. Approximately 20 g. of an amorphous hygroscopic product was obtained. The product was added to 100 ml. of glacial acetic acid, heated to 95° and kept warm until crystalline. After cooling to room temperature, the product (18 g.) was filtered off and was washed several times with ether. The compound was recrystallized as bundles of microscopic needles from approximately 50 times its weight of hot glacial acetic acid. The compound is assumed to be the acetic acid salt of S-cysteinosuccinic acid.

Anal. Calcd. for $C_9H_{15}NO_8S$: C, 36.47; H, 5.05; N, 4.88; S, 10.78. Found: C, 35.90; H, 5.10; N, 4.85; S, 10.77.

After drying over $CaCl_2$ and KOH for two weeks, the total acid equivalency of a sample based on a mol. wt. of 297 was 2.88 (theory 3.0), equivalents volatile acid 0.85 (theory 1.0), and equivalents non-volatile acid (calculated) was 2.03 (theory, 2.00). Upon heating the crystals became moist at 118 to 120°; they contract from 120 to 124°, and then swell until they decompose with bubbling at 132 to 134°. The compound is very soluble in ethanol and water but only slightly soluble in ether. It gives a negative —SH or —S—S— spot test but a positive test for thioether[5] and a positive ninhydrin test for amino acids.

Although the addition of sterile fumaric acid to agar media containing cysteine stimulates growth of small inocula of the yeast phase of *Histoplasma capsulatum* on agar media,[6] the addition of the S-cysteinosuccinic acid salt instead of fumaric acid has no effect. In liquid media the compound does not substitute for the cysteine requirement.

Acknowledgment.—Appreciation is expressed for the helpful suggestions given by Dr. Evan C. Horning for the isolation and identification of the fumarate–cysteine addition compound.

(5) G. Toennies and J. J. Kolb, *Anal. Chem.*, **23**, 823 (1951).
(6) L. Pine, unpublished results.

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3,4,5-Triiodobenzoyl Chloride as a Reagent for Identifying Mercaptans

BY DAVID C. O'DONNELL, HENRY A. MARIANI AND DENIS J. DOWNING[1]

RECEIVED FEBRUARY 2, 1955

We have reported previously[2,3] the use of 3,4,5-triiodobenzoyl chloride for the identification of cellosolves, carbitols and alcohols. The increasing use of mercaptans has prompted us to extend the use of this acid chloride to the identification of these compounds. The acid chloride was prepared by the method previously described.[4] All of the mercaptans were obtained commercially.

Experimental

With all but two of the mercaptans, 1 ml. of the mercaptan was added to 1 g. of the acid chloride in a 15-cm. test-tube and gently heated with a micro-burner for 10 minutes. Methyl and isopropyl mercaptans, because of their low boiling points, were treated differently. In these two cases 1 g. of the acid chloride was dissolved in 50 ml. of ether, 1 g. of the mercaptan added and the solution was allowed to stand for 10 minutes. It was then heated for 5 minutes, the ether evaporated and the residue crystallized from a solvent. The lower molecular weight thioesters were crystallized from 40 ml. of either methyl or ethyl alcohol. The higher molecular weight aliphatic and the aromatic thioesters were crystallized from 20 ml. of *n*-butyl alcohol and after filtration the crystals were washed with a small amount of ether. All of the derivatives crystallized in the form of fine white needles with the exception of the two noted in Table I. All melting points are corrected.

TABLE I

THIOESTERS OF 3,4,5-TRIIODOBENZOIC ACID

Mercaptan used	M.p., °C.	Yield, %	Formula	Iodine, % Calcd.	Found
Methyl	153.6–154.6[a]	43	$C_8H_5OSI_3$	71.85	71.56
n-Propyl	97.8–98.8[a]	37	$C_{10}H_9OSI_3$	68.24	68.38
Isopropyl	153.4–155[a]	28	$C_{10}H_9OSI_3$	68.24	68.40
n-Butyl	90–91.4[a]	48	$C_{10}H_{11}OSI_3$	66.57	66.96
Isobutyl	89.8–90.8[a]	37	$C_{11}H_{11}OSI_3$	66.57	66.39
n-Amyl	83.2–84.4[b,d]	34	$C_{12}H_{13}OSI_3$	64.97	64.63
n-Hexyl	64.3–65.2[b]	49	$C_{13}H_{15}OSI_3$	63.63	63.63
n-Heptyl	70–70.8[b]	41	$C_{14}H_{17}OSI_3$	62.00	62.13
n-Octyl	67–68.2[b]	46	$C_{15}H_{19}OSI_3$	60.61	60.50
n-Nonyl	70–70.8[c]	64	$C_{16}H_{21}OSI_3$	59.29	59.03
n-Decyl	76–77.2[b]	55	$C_{17}H_{23}OSI_3$	58.04	58.09
n-Undecyl	78.6–79.8[c]	63	$C_{18}H_{25}OSI_3$	56.81	57.08
n-Dodecyl	78.4–78.8[c]	53	$C_{19}H_{27}OSI_3$	55.65	55.59
n-Tetradecyl	86.4–87.4[c,e]	39	$C_{21}H_{31}OSI_3$	53.46	53.76
Hexadecyl	91.0–91.8[c]	62	$C_{23}H_{35}OSI_3$	51.48	51.65
o-Thiocresol	98.4–99.0[c]	30	$C_{14}H_9OSI_3$	62.83	62.44
Benzyl	116.2–117.0[c]	60	$C_{14}H_9OSI_3$	62.83	62.88
β-Phenylethyl	99.8–100.6[c]	63	$C_{15}H_{11}OSI_3$	61.41	61.62
α-Phenylpropyl	133.4–134.2[c]	59	$C_{16}H_{13}OSI_3$	60.05	59.83

[a] Methyl alcohol as solvent. [b] Ethyl alcohol as solvent. [c] *n*-Butyl alcohol as solvent. [d] White plates. [e] White granules.

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Preparation of Pure 2-Aminonitropyridines and 2-Aminonitropicolines. Rapid Separations by Sublimation

BY LEWIS N. PINO AND WINFIELD S. ZEHRUNG III[1]

RECEIVED DECEMBER 16, 1954

Considerable information exists on the nitration of 2-aminopyridine and the 2-aminopicolines. However, there is reported no simple, rapid method of separating the isomeric nitroamines of the pyridine series. This paper gives the results of a study of sublimation as a means of rapid, clean separation.

Separation of aminonitropyridine has been accomplished by steam distillation and fractional crystallization.[2a,b,3] In some instances, no attempt at separation was made until the mixture of isomers had completed a series of additional reactions after which one modified isomer was recovered and the other lost.[2,3]

Separation by sublimation seemed feasible since a vicinal nitroamine is capable of chelation with a resulting increase in molecular symmetry and vapor

(1) Taken from theses submitted in partial fulfillment for the M.S. degree.
(2) D. O'Donnell and R. Carey, THIS JOURNAL, **68**, 1865 (1946).
(3) D. O'Donnell, J. Kelley, R. O'Malley and R. Upham, *ibid.*, **70**, 1657 (1948).
(4) C. Klemme and J. Hunter, *J. Org. Chem.*, **5**, 508 (1940).

(1) From a thesis submitted to the faculty of Allegheny College in partial fulfillment of the requirements for the M.S. degree, June, 1954.
(2) (a) A. E. Chichibabin and B. A. Razorenov, *J. Russ. Phys. Chem. Soc.*, **47**, 1286 (1915); *J. Chem. Soc.*, **108**, I, 992 (1915); (b) W. T. Caldwell and E. C. Kornfeld, THIS JOURNAL, **64**, 1695 (1942).
(3) M. A. Phillips, *J. Chem. Soc.*, 13 (1944).