was added dropwise for eight hours to a stirred solution of 29.0 g. (0.33 mole) of putrescine in 10 ml. of ether. After all acrylonitrile was added, the solution was stirred at room temperature for fifteen hours, on a steam-bath for one hour, and again at room temperature for one hour. During the time of heating, all the ether distilled out, and the weight of the final reaction product was 64 g. All attempts to distil the dinitrile, even at a pressure of 0.01 mm., resulted in decomposition. When the reaction mixture was allowed to stand for a longer period (forty to fifty hours), the yield of spermine tetrahydrochloride was decreased from 50 to 30%.

A small exploratory run, made as above, when treated with ethanolic hydrogen chloride, gave a 100% yield of N,N'-bis-(2-cyanoethyl)-putrescine dihydrochloride, m. p. 222–228° (dec.). This salt was recrystallized from ethanol–water (3:1) to give an 84% yield of N,N'-bis-(2-cyanoethyl)-putrescine dihydrochloride, m. p. 232–233° (dec.).

Anal. Calcd. for $C_{10}H_{20}N_4Cl_2$: Cl, 26.6. Found: Cl, 26.6.

Spermine Tetrahydrochloride.—A 270-ml. steel reaction vessel was charged with 18 g. (0.092 mole) of crude N,N'-bis-(2-cyanoethyl)-putrescine, 5 ml. of ether, 60 ml. of liquid ammonia, and 3 g. of W-2 Raney nickel catalyst. The contents of the bomb was reduced for thirty minutes at 140° under a hydrogen pressure of 4900 p. s. i. The catalyst-free reaction mixture was distilled to give 10 g. of viscous distillate that boiled at 100–170° (0.1–0.2 mm.), and 3 g. of a yellow residue.

The crude spermine was dissolved in 130 ml. of absolute ethanol, and to it was added 65 ml. of 20% ethanolic hydrogen chloride. The precipitated amine salt was filtered and air-dried to give 23.5 g. of white, impure spermine tetrahydrochloride that melted at 290–310° (dec.), with darkening at 270°. The crude spermine tetrahydrochloride was dissolved in 130 ml. of 12% hydrochloric acid and added to 1300 ml. of hot, absolute ethanol. After cooling the alcohol solution, the crystals were filtered, washed, and air-dried to give 17 g. of crystals, m. p. 300–310° (dec.). This material was placed in 119 ml. of 12% hydrochloric acid, and added to 357 ml. of hot, absolute ethanol. After six hours at room temperature, the spermine tetrahydrochloride was filtered off, rinsed, and dried to give 16.1 g. (51% yield) of light pink crystals, m. p. 310–311° (dec.), darkening at 300°. The value previously reported[9] was m. p. 310–311° (dec.).

Anal. Calcd. for $C_{10}H_{30}N_4Cl_4$: C, 34.5; H, 8.7; Cl, 40.8. Found: C, 34.2; H, 8.6; Cl, 40.8.

The picrate of spermine melted at 246–247° (dec.), darkened at 240°. The value previously reported[9] was m. p. 248–250° (dec.), with darkening at 242°.

Spermine chloroplatinate was also prepared, and found to melt at 242° (dec.), dark at 235°. The value previously reported[9] was m. p. 242–245° (dec.).

Acknowledgments.—I am grateful to Dr. Carnahan, formerly of this Laboratory, for suggesting this method as a possible synthesis of spermine; and to Drs. Adkins and Wilds, of this Laboratory, for advice which made this synthesis successful.

Summary

Spermine tetrahydrochloride has been prepared from succinonitrile through the formation of putrescine, N,N'-bis-(2-cyanoethyl)-putrescine, spermine, and spermine tetrahydrochloride. The yield of putrescine from succinonitrile was low (20%), but the over-all yield for the three subsequent reactions was 51% of the theoretical.

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(9) Dudley, Rosenheim and Rosenheim, *Biochem. J.*, **18**, 1263 (1924).

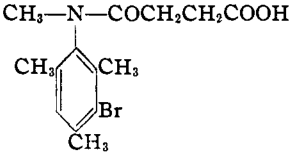
CORAL GABLES, FLORIDA RECEIVED APRIL 28, 1948

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

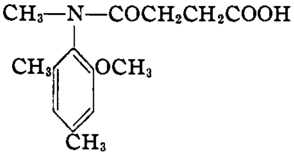
Restricted Rotation in Aryl Amines. IV. Preparation and Resolution of N-Succinyl-1-methylamino-2,4-dimethyl-6-substituted Benzenes

BY ROGER ADAMS AND N. K. SUNDHOLM[1]

A comparison of the relative interference effects of substituent groups, as deduced from the racemization rates of a series of analogous, optically active biphenyls,[2] showed these groups to fall in the following order: Br > CH_3 > Cl > NO_2 > COOH > OCH_3 > F. Two members of a series of aromatic amines in which restricted rotation exists have been previously described.[3] They are shown in I and II. Compound I, in optically active form, has a half-life of nine hours in boiling *n*-butanol (b.p. 117°) and compound II a half-life of two and seven-tenths hours in boiling methyl acetate (b.p. 57°). Although these values are not directly comparable due to the use of different solvents[4] and to the bromine atom in the former, qualitatively they can be accepted as indicative of the relative interference of the methyl and methoxyl groups in this type of molecule. These relative values coincide with those expected from the study of the biphenyls.



I



II

Several new analogs of I and II, as shown in III and IV, have now been synthesized, resolved and their optically active forms racemized. The half-life periods were as follows: IIIa, three and

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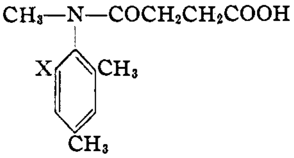
(1) An abstract of a thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Chemistry. Allied Chemical and Dye Corporation fellow second semester 1941–942, two semesters 1943–1944; New York Community Trust fellow 1942–1943.

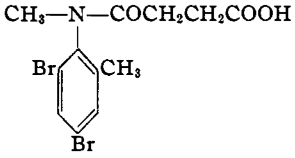
(2) Stoughton and Adams, THIS JOURNAL, **54**, 4426 (1932); Adams and Hale, *ibid.*, **61**, 2825 (1939).

(3) (a) Adams and Dankert, *ibid.*, **62**, 2191 (1940); (b) Adams and Stewart, *ibid.*, **63**, 2859 (1941); (c) Adams and Albert, *ibid.*, **64**, 1475 (1942).

(4) Li and Adams, *ibid.*, **57**, 1565 (1935).



III



IV

IIIa X = Br
IIIb X = I
IIIc X = NO$_2$

one-tenth hours; IIIb, twenty and one-half hours; IIIc, six-tenths hour; IV, one and one-tenth hours. All were taken in boiling *n*-butanol except IIIc which racemized so readily that boiling methyl acetate was employed. In these compounds, the bromine atom is only a third as effective as the methyl group (compare IIIa and I) in contributing to the restricted rotation of the molecule, and the nitro group is only one-fifth as effective as the small methoxyl (compare IIIc and II). It is thus obvious that factors other than the size of the groups are involved.

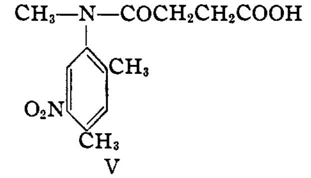
The basicity of aromatic amines is influenced profoundly by the presence of *o*-, *m*- or *p*-substituted electronegative groups as shown by the basic constants of *o*-toluidine, 2.9×10^{-10}; *o*-bromoaniline, 2.1×10^{-12}; *o*-iodoaniline, 0.36×10^{-12}; *o*-nitroaniline, 3.5×10^{-14}.[5a] The difference in basicity of these molecules is usually assigned to the effect of the bromine and iodine atoms or nitro group in inducing a positive nitrogen atom in the amino group. In the case of the nitro compound, a valence formula may be written which indicates the double-bond character of the carbon and amino-nitrogen linkage. For the halogens, an analogous structure cannot be written on a conventional basis but the mere fact that the basicity of the amines is reduced by the substitution of such atoms establishes the fact that the nitrogen is more positive, which condition is compatible with a linkage having double-bond character. For such a double bond to be produced the plane formed by the nitrogen and its two substituents must be coplanar with the ring. The racemization process, in which conversion of a *d*-form to an *l*-form and *vice versa* must take place through a coplanar configuration, should be facilitated by the presence in the optically active amines of groups favoring coplanarity. Thus the anomalous ease of racemization of hindered aromatic amines substituted with halogen or nitro groups in the *ortho* position is probably associated with their decreased basicity. The N-succinyl-1-methylamino-2-methyl-4,6-dibromobenzene (IV) was prepared in order to determine the effect of two electronegative bromine atoms in the *o*- and *p*-positions as compared with that of a single bromine in the *o*-position. The half-life of the former is only about a third of the latter, thus confirming the idea that the stability of these optically active aromatic amines is intimately connected with the base strength of the amino nitrogen atom and is greatly reduced by electronegative substituents. Apparently the larger size of the iodine atom overbalances the increase in ease of racemization induced by decrease in the basicity of the amine (compare I, IIIa, IIIb).

This relationship of basicity of the aromatic amines to the stability of their optically active hindered derivatives serves to explain the shorter half-life (four and one-tenth hours) of N-succinyl-1-methylamino-4-chloro-2-methylnaphthalene in comparison with that of N-succinyl-1-methylamino-2-methylnaphthalene (five and seven-tenths hours).[3c] It may also account for the non-resolvability of N-benzenesulfonyl-1-carboxymethylamino-2,4-dimethyl-6-nitrobenzene reported by Yuan.[5b]

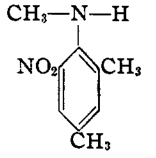
The increase in double-bond character of the carbon and amino-nitrogen bond would result in a decrease in the bond length. The groups on the nitrogen would then be closer to the ring and interfere more readily with the adjacent substituents on the ring. It is assumed that this effect is so small in comparison to that already discussed that it may be disregarded.

The best synthesis of the bromo and iodo derivatives (IIIa and IIIb) was effected by brominating or iodinating 1-amino-2,4-dimethylbenzene, then monomethylating the amino group with dimethyl sulfate. Iodination of 1-methylamino-2,4-dimethylbenzene failed, using conditions successful for the iodination of the primary amine. Succinic anhydride in dry benzene with a drop of 85% phosphoric acid as catalyst was a satisfactory procedure for succinylation of the 1-methylamino compounds although the reaction was slow in the case of the molecules substituted with electronegative groups. 1-Methylamino-2-methyl-4,6-dibromobenzene was prepared by brominating the 1-methylamino-2-methylbenzene.

The production of the nitro compound (IIIc) was attempted first by the action of cold yellow nitric acid upon N-succinyl-1-methylamino-2,4-dimethylbenzene. A mono nitro derivative was readily formed but further study indicated the nitro group to be probably in the 5- (V) rather than in the 6-position. This orientation was observed by Gnehm and Blumer,[6] who nitrated N-acetyl-1-methylamino-2-methylbenzene in sulfuric acid with a nitric acid–sulfuric acid mixture and obtained the 5-nitro compound. The same compound was produced upon using cold yellow



V



VI

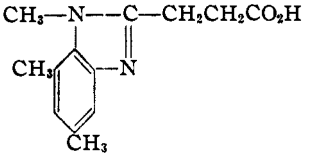
[5a] Williams and Soper, *J. Chem. Soc.*, 2469 (1930); Bennett, Brooks and Glasstone, *ibid.*, 1821 (1935); Myrbäck, *Z. physiol. Chem.*, **158**, 261 (1926).

[5b] Yuan, *J. Chinese Chem. Soc.*, **4**, 131 (1936).

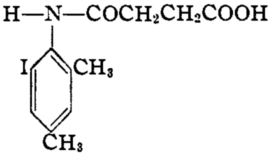
[6] Gnehm and Blumer, *Ann.*, **304**, 99 (1899).

nitric acid. Hydrolysis of the succinyl group of V resulted in the formation of a compound not identical with VI, which had been previously prepared by nitration of the N-nitroso-1-methylamino-2,4-dimethylbenzene followed by denitrosation. Compound IIIc was subsequently synthesized by succinylating VI.

The *d*-modification of the nitro compound (IIIc) was hydrogenated at room temperature in ether solution with platinum oxide as catalyst. The crude product was *dextro*-rotatory. After this product had been heated in boiling *n*-butanol for about an hour, a compound resulted which was optically inactive. This compound was not the amine (III, X = NH_2) since a molecule of water had been lost as shown by analysis. It must be the corresponding benzimidazole (VII). That this is so has been demonstrated by Betrabet and Chakravarti,[7] who obtained benzimidazole-2-β-propionic acid upon refluxing an absolute ethanol solution of N-succinyl-*o*-phenylenediamine. The product from the hydrogenation was undoubtedly the crude optically active amine which condensed to the benzimidazole during the subsequent treatment.



VII



VIII

Compound VIII, which differs from compound IIIb by having the N-methyl group missing, was not resolvable through its crystalline brucine salt. If the compound truly proves to be unresolvable, this would show the important contribution the N-methyl group makes to the restricted rotation in these compounds.

The optically active forms of the nitro compound (IIIc) and the dibromo compound (IV) were partially racemized during the process of crystallizing the crude acids obtained from the decomposition of their cinchonidine salts. For this reason the specific rotations of the crude optically active acids are reported since they should approximate the maximum specific rotations.

No particular complications were encountered in the resolution of the various compounds except in the case of the iodo derivative (IIIb). The salt tended to dissociate so that it was impossible to obtain a product of maximum rotation. The technique employed in the racemization determinations was that described in a previous communication.[3c]

Experimental

1-Methylamino-2,4-dimethyl-6-bromobenzene.—A mixture of 40 g. of 1-amino-2,4-dimethyl-6-bromobenzene,[8] 25 g. of dimethyl sulfate and 100 ml. of water was stirred with gentle warming on a steam-bath until homogeneous. The temperature was not permitted to go over 70°. The cooled solution was acidified with 30 ml. of concentrated hydrochloric acid, cooled to below 10°, and a solution of 14 g. of sodium nitrite in 50 ml. of water added dropwise with stirring, keeping the temperature between 5–10°. Stirring was continued for fifteen minutes after the addition of the sodium nitrite. The cold solution was extracted with ether, the ether layer washed with 5% aqueous sodium hydroxide, and then with water. The ether was evaporated by directing a stream of air over the surface of the solution.

The nitrosamine was reduced by careful addition in portions to a solution of 136 g. of stannous chloride dihydrate in 132 ml. of concentrated hydrochloric acid. The stannous chloride solution was heated to 40° before the first addition of the nitrosamine. In order to keep the temperature under 60° after several portions of the nitrosamine were added, cooling was necessary. After each addition the flask was shaken by hand. A precipitate of the tin complex slowly settled out. After standing overnight, the mixture was cooled in ice, and made strongly alkaline by addition of a solution of 176 g. of sodium hydroxide in 250 ml. of water. The mixture was steam-distilled until the distillate came over clear. The distillate was saturated with sodium chloride, extracted with ether, the extract dried over solid potassium hydroxide and the ether distilled. The secondary amine was distilled *in vacuo*, b. p. 92–94° (4.5 mm.); yield, 21 g. (49%); n^{20}_D 1.5682; d^{20}_4 1.3379.

Anal. Calcd. for $C_9H_{12}BrN$: C, 50.50; H, 5.60; *M*D, 52.6. Found: C, 50.54; H, 5.78; *M*D, 52.4.

N-Succinyl-1-methylamino-2,4-dimethyl-6-bromobenzene.—To a solution of 13 g. of succinic anhydride in 100 ml. of dry benzene was added 15 g. of 1-methylamino-2,4-dimethyl-6-bromobenzene and a drop of 85% phosphoric acid. The solution was refluxed for four hours, cooled, diluted with 100 ml. of ether, and extracted with 5% aqueous potassium hydroxide. The alkaline extract was washed with ether and acidified with 1:2 hydrochloric acid. The product separated as an oil which solidified on stirring in ice. It was recrystallized from a mixture of three volumes of carbon tetrachloride and one volume of petroleum ether (b. p. 60–110°); white crystals, m. p. 115.5–116.5° (cor.); yield, 9 g. (41%).

Anal. Calcd. for $C_{13}H_{16}BrNO_3$: C, 49.68; H, 5.09. Found: C, 49.79; H, 5.16.

Resolution of N-Succinyl-1-methylamino-2,4-dimethyl-6-bromobenzene.—A solution of 5 g. of N-succinyl-1-methylamino-2,4-dimethyl-6-bromobenzene and 4.68 g. of cinchonidine in 180 ml. of 9:1 ethyl acetate–methanol by volume was filtered and evaporated to 145 ml. by directing a stream of air over its surface. After four days in the refrigerator, 3.1 g. of salt had crystallized. These crystals were collected and the filtrate evaporated to 137 ml. Refrigerated, it yielded a second crop of 1.4 g. At 125 ml., 1.3 g. of salt crystallized; at 110 ml., 0.1 g.; at 90 ml., 1.3 g. The first three fractions were combined and recrystallized in the same manner to constant rotation; this produced white feathery crystals m. p. 164–165° (cor.); yield, 1.8 g.

Anal. (*l*B*d*A) Calcd. for $C_{13}H_{16}BrNO_3 \cdot C_{19}H_{22}N_2O$: C, 63.36; H, 6.27. Found: C, 63.44; H, 6.60. *Rotation.* (*l*B*d*A) 0.03 g. made up to 10 ml. with absolute ethanol at 28° gave α_D −0.13°; *l*, 1; $[\alpha]^{28}_D$ −43°.

***d*-N-Succinyl-1-methylamino-2,4-dimethyl-6-bromobenzene.**—To 75 ml. of 1:1 hydrochloric acid at 0° was added 1.65 g. of the less-soluble salt. The mixture was stirred for fifteen minutes and then put in a refrigerator overnight. The next day the gummy material had solidified and it was broken up. The mixture was filtered and the solid material again stirred with cold 1:1 hydrochloric acid and put in a refrigerator overnight. This treatment was repeated until the filtrate gave a negative test with Folin's reagent.[9] The residue was dried in a vacuum desiccator; yield, 0.49 g. The acid was crystallized

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[7] Betrabet and Chakravarti, *J. Indian Chem. Soc.*, **7**, 191 (1930).

[8] Noelting, Braun and Thesmar, *Ber.*, **34**, 2242 (1901).

[9] Folin and Denis, *J. Biol. Chem.*, **12**, 239 (1912).

from a mixture of benzene and petroleum ether (b. p. 60–110°). The rotation was unchanged by the crystallization.

The *d*-acid was obtained from the less-soluble salt; white crystals, m. p. 118.5–120.5° (cor.).

Anal. (*d*-acid) Calcd. for $C_{13}H_{16}BrNO_3$: C, 49.68; H, 5.09. Found: C, 49.95; H, 5.18. *Rotation.* (*d*-acid) 0.05 g. made up to 10 ml. with absolute ethanol at 28° gave α_D +0.125°; *l*, 1; $[\alpha]^{28}_D$ +25°.

Racemization of *d*-N-Succinyl-1-methylamino-2,4-dimethyl-6-bromobenzene.—The racemization was carried out in a polarimeter tube constructed from Pyrex tubing (25 mm. outside diameter, 100 mm. internal length) and fitted at the center to a reflux condenser by means of a ground-glass joint.[3c] A solution of 0.15 g. of the *d*-acid made up to 25 ml. with *n*-butanol was added to the polarimeter tube. The rotation was observed, several small carborundum boiling chips added, and the polarimeter tube with its contents weighed. The polarimeter tube was then placed on a hot-plate and the solution concentrated to 15 ml. by boiling without reflux. The reflux condenser was attached and the solution refluxed until the total boiling period was one-half hour. The solution was cooled rapidly by plunging the tube into cold water, made up to the previously determined weight with *n*-butanol, and the rotation observed. By repetition of this process, the following α_D values were obtained: at the start, +0.148°; after one-half hour, +0.133°; after one and one-half hours, +0.105°; after two and one-quarter hours, +0.091°; after three and one-half hours, +0.068°; after five hours, +0.050°; after seven hours, +0.030°. Calculated for a reversible unimolecular reaction, the half-life was three and one-tenth hours. A check racemization gave a half-life of three and two-tenths hours.

1-Amino-2,4-dimethyl-6-iodobenzene.—This procedure was modeled after that for the iodination of *p*-toluidine used by Wheeler and Liddle.[10] To 28.2 g. of 1-amino-2,4-dimethylbenzene 59.5 g. of ground iodine was added with stirring. The addition of the iodine took about forty-five minutes. The reaction mixture was kept below 45° by cooling with cold water. After the completion of the addition of the iodine, the stirring was continued until the heat of the reaction had subsided. After the addition of 60 ml. of ether, 60 ml. of water and 28 g. of calcium carbonate, the mixture was refluxed on a steam-bath until the evolution of carbon dioxide ceased. This took several hours. The ether was distilled on the steam-bath and the residue steam-distilled. The first portion of the distillate was a red oil but after a short time material began to solidify in the lower part of the condenser. The water in the condenser had to be allowed to run out occasionally to permit the solid material to melt and run into the receiver. The solid product was collected on a filter, dried and crystallized twice from ethanol; white crystals that darkened on contact with the air, m. p. 66–67° (cor.); yield, 24 g. (42%). Kerschbaum[11] reported a m. p. of 65°.

1-Methylamino-2,4-dimethyl-6-iodobenzene.—The procedure described for the methylation of 1-amino-2,4-dimethyl-6-bromobenzene was used with several changes. The dimethyl sulfate was added dropwise to a stirred mixture of the 1-amino-2,4-dimethyl-6-iodobenzene and water at 70°. About a 20% excess of dimethyl sulfate was used. It was found that the nitrosamine was not reduced appreciably at 50° in the stannous chloride-hydrochloric acid mixture. The optimum temperature for the reduction was 70°. Above 80° an appreciable amount of the iodine was reduced off. From 83 g. of the primary amine, 24 g. (27.4%) of the secondary amine was obtained; b. p. 108–110° (4 mm.); d^{20}_4 1.5588; n^{20}_D 1.6050.

Anal. Calcd. for $C_9H_{12}IN$: C, 41.38; H, 4.60; *M*D, 57.7. Found: C, 41.43; H, 4.82; *M*D, 57.5.

N-Succinyl-1-methylamino-2,4-dimethyl-6-iodobenzene.—To a solution of 6.6 g. of succinic anhydride in 40 ml. of dry benzene was added 16 g. of 1-methylamino-2,4-dimethyl-6-iodobenzene and a drop of 85% phosphoric acid. The solution was refluxed for four hours, cooled, ether added, and the mixture extracted with 5% aqueous potassium hydroxide. The alkaline extract was acidified with 1:2 hydrochloric acid. After cooling and stirring for an hour, the product remained oily, so it was dissolved in ether and the ether solution dried with anhydrous magnesium sulfate. After removal of the magnesium sulfate by filtration and evaporation of the ether, first on a steam-bath, then *in vacuo*, the product slowly crystallized. The product was recrystallized from a mixture of three volumes of carbon tetrachloride and one volume of petroleum ether (b. p. 60–110°); white crystals, m. p. 103–104° (cor.); yield, 6.6 g. (30%).

Anal. Calcd. for $C_{13}H_{16}INO_3$: C, 43.21; H, 4.43. Found: C, 43.35; H, 4.57.

Resolution of N-Succinyl-1-methylamino-2,4-dimethyl-6-iodobenzene.—A solution of 5.6 g. of N-succinyl-1-methylamino-2,4-dimethyl-6-iodobenzene and 4.56 g. of cinchonidine in 55 ml. of 9:1 ethyl acetate-methanol by volume was filtered and evaporated to 45 ml. by directing a stream of air to the top of the flask. After four days in the refrigerator, 4 g. of salt had crystallized. Upon concentrating the mother liquor to 40 ml. and refrigerating, 1.5 g. of salt crystallized; at 30 ml., 1.6 g.; at 19 ml., 1.0 g.; at 4 ml., 0.5 g. It had been found while working with this salt previously that during recrystallization of the salt it dissociated, so the first crop was not recrystallized further; white crystals, m. p. 140–145° (cor.).

Anal. (*l*B*d*A) Calcd. for $C_{13}H_{16}INO_3 \cdot C_{19}H_{22}N_2O$: C, 58.62; H, 5.85. Found: C, 58.53; H, 5.97. *Rotation.* (*l*B*d*A) 0.05 g. made up to 10 ml. with ethanol at 26° gave α_D −0.23°; *l*, 1; $[\alpha]^{26}_D$ −46°.

The fourth and fifth fractions were combined and crystallized once; white crystals; yield, 1.1 g.

Rotation. (*l*B*l*A) 0.05 g. made up to 10 ml. with ethanol at 31° gave α_D −0.315°; *l*, 1; $[\alpha]^{31}_D$ −63°.

***d*- and *l*-N-Succinyl-1-methylamino-2,4-dimethyl-6-iodobenzene.**—The salts were decomposed in the same manner as the salt of the 6-bromo compound. From 4 g. of the less-soluble salt, 1.2 g. of the *d*-acid was obtained after one crystallization from a mixture of three volumes of carbon tetrachloride and one volume of petroleum ether (b. p. 60–110°); white crystals, m. p. 105–106° (cor.).

Anal. (*d*-acid) Calcd. for $C_{13}H_{16}INO_3$: C, 43.21; H, 4.43. Found: C, 43.22; H, 4.41. *Rotation.* (*d*-acid) 0.435 g. made up to 25 ml. with *n*-butanol at 33° gave α_D +0.21°; *l*, 1; $[\alpha]^{33}_D$ +12°.

This *d*-acid did not have the maximum specific rotation since it was found that recrystallizing the less-soluble salt of another resolution several times, in spite of the dissociation of the salt, and then decomposing gave an acid with a specific rotation of +43° and a m. p. of 108° (cor.).

The decomposition of the more-soluble fraction did not give the pure *l*-acid; white crystals, m. p. 105–106° (cor.).

Rotation. (*l*-acid) 0.05 g. made up to 10 ml. with ethanol at 31° gave α_D −0.09°; *l*, 1; $[\alpha]^{31}_D$ −18°.

Racemization of *d*-N-Succinyl-1-methylamino-2,4-dimethyl-6-iodobenzene.—The procedure described for the racemization of *d*-N-succinyl-1-methylamino-2,4-dimethyl-6-bromobenzene was used. A solution of 0.435 g. of the *d*-acid made up to 25 ml. with *n*-butanol was racemized. The following α_D values were obtained: at the start, +0.210°; after four hours, +0.182°; after nine hours, +0.154°; after fifteen hours, +0.127°; after twenty-one and one-half hours, +0.100°; after thirty and one-half hours, +0.075°; after forty-one and one-half hours, +0.052°. Calculated for a reversible unimolecular reaction, the half-life was twenty and one-half hours. A check racemization gave a half-life of nineteen and four-tenths hours.

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[10] Wheeler and Liddle, *Am. Chem. J.*, **42**, 441 (1909).

[11] Kerschbaum, *Ber.*, **28**, 2798 (1895).

1-Methylamino-2-methylbenzene.—By the same general procedure described below for the preparation of 1-methylamino-2,4-dimethylbenzene, 214 g. of *o*-toluidine was methylated to give 89 g. (37%) of product having a b. p. of 95° (15 mm.). Monnet, Reverdin and Nölting[12] report a b. p. of 207–208°.

1-Methylamino-2-methyl-4,6-dibromobenzene.—This compound was prepared by the method of Fries.[13] One minor change was made in his procedure in that anhydrous sodium acetate was added to bring the reaction to completion as well as heating. From 89 g. of 1-methylamino-2-methylbenzene was prepared 170 g. (82.5%) of product having a b. p. of 122–123° (1 mm.). Fries reports a b. p. of 187° (50 mm.).

N-Succinyl-1-methylamino-2-methyl-4,6-dibromobenzene.—To a solution of 27 g. of succinic anhydride in 210 ml. of dry benzene was added 69 g. of 1-methylamino-2-methyl-4,6-dibromobenzene and a drop of 85% phosphoric acid. This mixture was refluxed for twenty-two hours. The reaction mixture was worked up in the manner described in the preparation of N-succinyl-1-methylamino-2,4-dimethyl-6-iodobenzene. The product was recrystallized from a carbon tetrachloride–petroleum ether (b. p. 60–110°) mixture; white crystals, m. p. 116–117° (cor.); yield, 19 g. (20.2%).

Anal. Calcd. for $C_{12}H_{13}Br_2NO_3$: C, 38.00; H, 3.43. Found: C, 38.11; H, 3.66.

Resolution of N-Succinyl-1-methylamino-2-methyl-4,6-dibromobenzene.—A solution of 18 g. of N-succinyl-1-methylamino-2-methyl-4,6-dibromobenzene and 13.97 g. of cinchonidine in 200 ml. of 9:1 ethyl acetate–methanol by volume was filtered. This solution was concentrated by means of a gentle air stream to 170 ml. The crop of salt which had crystallized was collected. The dried product weighed 13.3 g. Concentration of the mother liquor to 130 ml. produced a second crop of 3.9 g. At 115 ml., 2.0 g. of salt crystallized; at 80 ml., 3.8 g.; and at 40 ml., 3.7 g. The first crop was recrystallized in the same manner to constant rotation. White feathery crystals were obtained; m. p. 161–163° (cor.); yield, 2.9 g.

Anal. (*l*B*d*A) Calcd. for $C_{12}H_{13}Br_2NO_3 \cdot C_{19}H_{22}N_2O$: C, 55.27; H, 5.20. Found: C, 55.63; H, 5.32. *Rotation.* (*l*B*d*A) 0.05 g. made up to 10 ml. with ethanol at 32° gave α_D −0.245°; *l*, 1; $[\alpha]^{32}_D$ −49°.

Recrystallization of the fifth crop failed to change its rotation.

Rotation. (*l*B*l*A) 0.05 g. made up to 10 ml. with ethanol at 30° gave α_D −0.285°; *l*, 1; $[\alpha]^{30}_D$ −57°.

***d*- and *l*-N-Succinyl-1-methylamino-2-methyl-4,6-dibromobenzene.**—The salts were decomposed in the same manner as the salt of the 6-bromo compound. From the less-soluble salt the *d*-acid was obtained. The rotation was observed before the acid was crystallized from a carbon tetrachloride–petroleum ether (b. p. 60–110°) mixture.

Rotation. (*d*-acid) 0.10 g. made up to 10 ml. with *n*-butanol at 32° gave α_D +0.07°; *l*, 1; $[\alpha]^{32}_D$ +7°.

Crystallization of this acid produced white crystals; m. p. 118° (cor.).

Anal. (*d*-acid) Calcd. for $C_{12}H_{13}Br_2NO_3$: C, 38.00; H, 3.43. Found: C, 38.14; H, 3.52. *Rotation.* (*d*-acid) 0.51 g. made up to 15 ml. with *n*-butanol at 32° gave α_D +0.13°; *l*, 1; $[\alpha]^{32}_D$ +3.8°.

Decomposition of the more-soluble salt gave the *l*-acid, which was not entirely pure.

Rotation. (*l*-acid) 0.45 g. made up to 10 ml. with ethanol at 30° gave α_D −0.245°; *l*, 1; $[\alpha]^{30}_D$ −5.4°.

Racemization of *d*-N-Succinyl-1-methylamino-2-methyl-4,6-dibromobenzene.—A solution of 0.7 g. of the *d*-acid made up to 15 ml. with *n*-butanol was racemized. The solution was kept at this concentration throughout the racemization. This was done by weighing the polarimeter tube before the first period of refluxing and then making the tube up to this weight after each period of refluxing by adding *n*-butanol. Otherwise the procedure was the same as described for the racemization of *d*-N-succinyl-1-methylamino-2,4-dimethyl-6-bromobenzene. The following α_D values were obtained: at the start, +0.313°; after one-quarter hour, +0.268°; after one-half hour, +0.231°; after one hour, +0.166°; after one and one-half hours, +0.124°; after two and one-half hours, +0.066°. Calculated for a reversible unimolecular reaction, the half-life was one and one-tenth hours. This value was checked by repeating the racemization.

N-Succinyl-1-amino-2,4-dimethyl-6-iodobenzene.—To a solution of 9 g. of succinic anhydride and 21 g. of 1-amino-2,4-dimethyl-6-iodobenzene in 150 ml. of dry benzene was added one drop of 85% phosphoric acid. This mixture was refluxed for one hour, a white solid separating after fifteen minutes of refluxing. The mixture was cooled and allowed to stand at room temperature for several hours to complete the crystallization of the product. The solid was separated from the mother liquor by filtration and dried. It was recrystallized from a mixture of five volumes of ethanol and three volumes of water; white powder, m. p. 207–208° (cor.); yield, 8 g. (27%).

Anal. Calcd. for $C_{12}H_{14}INO_3$: C, 41.52; H, 4.04. Found: C, 41.87; H, 4.19.

Attempted Resolution of N-Succinyl-1-amino-2,4-dimethyl-6-iodobenzene.—A solution of 5 g. of N-succinyl-1-amino-2,4-dimethyl-6-iodobenzene and 5.68 g. of brucine in 230 ml. of ethyl acetate was filtered and concentrated to 210 ml. by directing a stream of air to the top of the flask. At this volume, 3.0 g. of salt had crystallized. At 190 ml., 2.8 g. crystallized and at 185 ml., 0.8 g. All of these crops showed the same rotation. The first two crops were combined and recrystallized in the same manner with no change of rotation; white crystals, m. p. 103–110° (cor.).

Anal. Calcd. for $C_{12}H_{14}INO_3 \cdot C_{23}H_{26}N_2O_4$: C, 56.67; H, 5.39. Found: C, 56.18; H, 5.62. *Rotation.* 0.05 g. made up to 10 ml. with ethanol at 25° gave α_D −0.140°; *l*, 1; $[\alpha]^{25}_D$ −28°.

Decomposition of this salt in the previously described manner produced an optically inactive acid.

1-Methylamino-2,4-dimethylbenzene.—The procedure of Fichter and Müller[14] was improved.

A well-stirred mixture of 108.6 g. of 1-amino-2,4-dimethylbenzene and 450 ml. of water was heated to 50°. To this was added dropwise 100 g. of dimethyl sulfate over a period of about forty-five minutes. Cooling was applied to keep the temperature from rising above 50°. After the solution had become homogeneous, it was stirred for a half hour. When cool, it was acidified with 135 ml. of concentrated hydrochloric acid. It was cooled to below 10° and a solution of 62.3 g. of sodium nitrite in 175 ml. of water added dropwise. The cold solution was extracted with ether and the ether evaporated with a stream of air. The nitrosamine was reduced by adding it in portions to a solution of 610 g. of stannous chloride dihydrate in 630 ml. of concentrated hydrochloric acid. The temperature was kept below 60° during the reduction. A heavy precipitate of the tin complex settled to the bottom. After standing overnight, the mixture was made strongly alkaline with 40% aqueous sodium hydroxide and steam-distilled. The distillate was saturated with sodium chloride and extracted with ether. The ether extract was dried with solid potassium hydroxide, the ether removed by distillation and the amine distilled *in vacuo*, b. p. 89–91° (7 mm.); yield, 43 g. (35.5%). Pinnow and Oesterreich[15] report a b. p. of 220.5–221.5° (760 mm.).

N-Succinyl-1-methylamino-2,4-dimethylbenzene.—To a solution of 14.9 g. of succinic anhydride in 200 ml. of dry benzene was added 20 g. of 1-methylamino-2,4-dimethylbenzene and a drop of 85% phosphoric acid. The solution was refluxed for six hours, cooled, diluted

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(12) Monnet, Reverdin and Nölting, *Ber.*, **11**, 2278 (1878).

(13) Fries, *Ann.*, **346**, 180 (1906).

(14) Fichter and Müller, *Helv. Chim. Acta*, **8**, 290 (1925).

(15) Pinnow and Oesterreich, *Ber.*, **31**, 2926 (1898).

with ether and extracted with 5% aqueous sodium hydroxide. The alkaline extract was acidified with 1:2 hydrochloric acid. The product separated as an oil which solidified on stirring and cooling. It was recrystallized from benzene; white crystals, m. p. 133–134° (cor.); yield, 22 g. (63%).

Anal. Calcd. for $C_{13}H_{17}NO_3$: C, 66.38; H, 7.23. Found: C, 66.79; H, 7.42.

Nitration of N-Succinyl-1-methylamino-2,4-dimethylbenzene; N-Succinyl-1-methylamino-2,4-dimethyl-5-nitrobenzene.—To 25 ml. of well-stirred fuming nitric acid (sp. gr. 1.49) in a small flask cooled in an ice-bath was added in small portions 6 g. of N-succinyl-1-methylamino-2,4-dimethylbenzene. The mixture was allowed to stand for fifteen minutes and then poured onto ice. The mixture was made alkaline with concentrated aqueous ammonia, cooled with ice, and made acid to litmus with hydrochloric acid. The product settled out as an oil which solidified on standing a day in the refrigerator. The product was treated with Norit in methanol and recrystallized from benzene; light yellow crystals, m. p. 144.5–145.5° (cor.) with sintering at 139°; yield, 2.9 g. (40%).

Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.71. Found: C, 55.98; H, 5.48.

Hydrolysis of the Product of the Nitration of N-Succinyl-1-methylamino-2,4-dimethylbenzene; 1-Methylamino-2,4-dimethyl-5-nitrobenzene.—To 20 ml. of 1:1 hydrochloric acid was added 2 g. of the nitro compound. This mixture was refluxed for two hours, cooled, and made alkaline with 10% aqueous sodium hydroxide. The precipitate was removed by filtration, washed with water, and recrystallized from ethanol; orange-red needles, m. p. 140–141° (cor.).

Anal. Calcd. for $C_9H_{12}N_2O_2$: C, 60.00; H, 6.66. Found: C, 60.18; H, 6.55.

N^1-Succinyl-1-methylamino-2,4-dimethyl-5-aminobenzene.—To a solution of 13 g. of N-succinyl-1-methylamino-2,4-dimethyl-5-nitrobenzene in 150 ml. of absolute ethanol was added 0.2 g. of platinum oxide. the catalyst. At room temperature and an initial pressure of 45 lb. the calculated amount of hydrogen was absorbed. The catalyst was removed by filtration and the ethanol evaporated. The product was recrystallized from absolute ethanol; almost transparent crystals, m. p. 169–172° (cor.); yield, 9.3 g. (80%).

Anal. Calcd. for $C_{13}H_{18}N_2O_3$: C, 62.40; H, 7.20. Found: C, 62.61; H, 7.35.

Methyl Ester of N^1-Succinyl-1-methylamino-2,4-dimethyl-5-aminobenzene.—A solution of 2 g. of N^1-succinyl-1-methylamino-2,4-dimethyl-5-aminobenzene in 40 ml. of methanol containing 5% of dry hydrogen chloride by weight was allowed to stand for two days at room temperature. The methanol was evaporated at room temperature *in vacuo*. The resulting oil was poured onto a watch glass and allowed to stand one week. This did not cause complete crystallization, so it was shaken with dilute aqueous ammonia, the material solidifying nicely. The solid was removed by filtration, washed with dilute aqueous ammonia and with water, and recrystallized from a mixture of one volume of ethanol and two volumes of water; white crystals, m. p. 109–110° (cor.); yield, 1.5 g. (72%).

Anal. Calcd. for $C_{14}H_{20}N_2O_3$: C, 63.63; H, 7.60. Found: C, 64.37; H, 8.07.

N-Acetyl-1-methylamino-2,4-dimethylbenzene.—This was prepared by the acetylation of 1-methylamino-2,4-dimethylbenzene with acetic anhydride in acetic acid. The product had a m. p. of 63–63.5° (cor.). Pinnow and Oesterreich[15] report a m. p. of 65°.

N-Acetyl-1-methylamino-2,4-dimethyl-5-nitrobenzene.—Nitration of 25 g. of N-acetyl-1-methylamino-2,4-dimethylbenzene by the previously described procedure (acidification was unnecessary after treatment of the reaction mixture with concentrated aqueous ammonia) gave 6.3 g. (20%) of product; light yellow crystals from a mixture of three volumes of petroleum ether (b. p. 60–110°) and one volume of benzene, m. p. 109–110° (cor.).

Anal. Calcd. for $C_{11}H_{14}N_2O_3$: C, 59.46; H, 6.30. Found: C, 59.40; H, 6.31.

N^1-Acetyl-1-methylamino-2,4-dimethyl-5-aminobenzene.—To a solution of 16 g. of N-acetyl-1-methylamino-2,4-dimethyl-5-nitrobenzene in 150 ml. of absolute ethanol 0.1 g. of platinum oxide was added. At room temperature and an initial pressure of 47 lb. the calculated amount of hydrogen was absorbed. The catalyst was removed by filtration and the ethanol evaporated. The residue was recrystallized from benzene; white crystals, m. p. 134–134.5° (cor.); yield,.11 g. (80%).

Anal. Calcd. for $C_{11}H_{16}N_2O$: C, 68.75; H, 8.33. Found: C, 68.81; H, 8.50.

N-Acetyl-1-methylamino-2-methyl-5-nitrobenzene.—N-Acetyl-1-methylamino-2-methylbenzene[12] was nitrated in the previously described manner (acidification was unnecessary after treatment of the reaction mixture with ammonia). The product was recrystallized from dilute ethanol; light yellow crystals, m. p. 119° (cor.). Gnehm and Blumer[6] report a m. p. of 119° for the product they received on nitrating the same compound in sulfuric acid with a nitric acid–sulfuric acid mixture.

N-Nitroso-1-methylamino-2,4-dimethyl-6-nitrobenzene.—The method of Pinnow and Oesterreich[15] for the preparation of the same compound was used.

Methylation and nitrosation of 217 g. of 1-amino-2,4-dimethylbenzene following the procedure given for the preparation of 1-methylamino-2,4-dimethylbenzene gave 106 g. of wet, crude N-nitroso-1-methylamino-2,4-dimethylbenzene. Nitration in acetic acid gave 65 g. of N-nitroso-1-methylamino-2,4-dimethyl-6-nitrobenzene (17.4% based on the 1-amino-2,4-dimethylbenzene used); light yellow crystals (not pure), m. p. 58–61° (cor.). Pinnow and Oesterreich report a m. p. of 63°.

1-Methylamino-2,4-dimethyl-6-nitrobenzene.—The same procedure that Pinnow and Oesterreich used for the removal of the nitroso group was followed. From 60 g. of the nitrosamine, 25.4 g. (49%) of the secondary amine was obtained. The product was purified by steam distillation and recrystallization from methanol; dark red plates, m. p. 56–57° (cor.). Pinnow and Oesterreich report a m. p. of 58°.

N-Succinyl-1-methylamino-2,4-dimethyl-6-nitrobenzene.—To a solution of 28 g. of succinic anhydride and 24 g. of 1-methylamino-2,4-dimethyl-6-nitrobenzene in 100 ml. of dry benzene was added one drop of 85% phosphoric acid. This mixture was refluxed for seventy-two hours, cooled, diluted with ether and extracted with 5% aqueous sodium hydroxide. The alkaline extracts were acidified with dilute hydrochloric acid. The oil which was formed slowly solidified on standing. It was recrystallized from benzene. One treatment with Norit in benzene removed a tan color from the crystals; light yellow crystals, m. p. 129–131° (cor.); yield, 9 g. (24%).

Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.71. Found: C, 55.89; H, 5.92.

Resolution of N-Succinyl-1-methylamino-2,4-dimethyl-6-nitrobenzene.—A solution of 5.5 g. of N-succinyl-1-methylamino-2,4-dimethyl-6-nitrobenzene and 5.775 g. of cinchonidine in 100 ml. of 9:1 ethyl acetate–methanol by volume was filtered. The filtrate was evaporated to 65 ml. by directing a stream of air to the top of the flask. It was seeded with crystals obtained by allowing 1 ml. of the solution to evaporate slowly in a small test-tube. After five days in the refrigerator, 1.9 g. of salt had crystallized. Upon concentrating the mother liquor to 35 ml. and refrigerating, 4.7 g. of salt crystallized; at 20 ml., 1.8 g.; and at 5 ml., 0.25 g. The first two fractions were combined and recrystallized in the same manner to constant rotation. White feathery crystals were obtained; m. p. 141–143° (cor.); yield, 1.5 g.

Anal. (*l*B*d*A) Calcd. for $C_{13}H_{16}N_2O_5 \cdot C_{19}H_{22}N_2O$: C, 66.90; H, 6.67. Found: C, 66.32; H, 6.60. *Rotation.* (*l*B*d*A) 0.05 g. made up to 10 ml. with ethanol and 26° gave α_D −0.20°; *l*, 1; $[\alpha]^{26}_D$ −40°.

_d_-N-Succinyl-1-methylamino-2,4-dimethyl-6-nitrobenzene.—The less-soluble salt was decomposed with 0.5% hydrochloric acid in the same manner as the salt of the 6-bromo compound. Before crystallization from benzene the specific rotation was determined.

Rotation. (*d*-acid) 0.05 g. made up to 10 ml. with ethanol at 25° gave α_D +0.125°; *l*, 1; $[\alpha]^{25}_D$ +25°.

Crystallization from benzene produced light yellow crystals; m. p. 130–131° (cor.).

Anal. (*d*-acid) Calcd. for $C_{13}H_{15}N_2O_5$: C, 55.71; H, 5.71; N, 10.00. Found: C, 56.22; H, 5.87; N, 10.07. *Rotation.* (*d*-acid) 0.05 g. made up to 10 ml. with ethanol at 25° gave α_D +0.05°; *l*, 1; $[\alpha]^{25}_D$ +10°.

Racemization of _d_-N-Succinyl-1-methylamino-2,4-dimethyl-6-nitrobenzene.—The *d*-acid was completely racemized in a half hour in boiling *n*-butanol.

A solution of 0.25 g. of the *d*-acid made up to 15 ml. with methyl acetate was racemized using the technique described for the racemization of *d*-N-succinyl-1-methylamino-2-methyl-4,6-dibromobenzene. The following α_D values were obtained: at the start, +0.215°; after fifteen minutes, +0.164°; after thirty minutes, +0.121°; after forty-five minutes, +0.091°; after sixty minutes, +0.067°; after ninety minutes, +0.037°. Calculated for a reversible unimolecular reaction, the half-life was thirty-six minutes. A check racemization gave a half-life of thirty-eight minutes.

Catalytic Hydrogenation of _d_-N-Succinyl-1-methylamino-2,4-dimethyl-6-nitrobenzene; 1,5,7-Trimethylbenzimidazole-2-β-propionic Acid.—A solution of 0.25 g. of *d*-N-succinyl-1-methylamino-2,4-dimethyl-6-nitrobenzene in 125 ml. of ether was hydrogenated at room temperature and 40 lb. pressure with 0.1 g. of platinum oxide as catalyst. At the end of the reduction, the catalyst was removed by filtration and the ether evaporated by directing a stream of air onto the solution. The residue was dried in a vacuum desiccator.

No quantitative specific rotation was determined for this crude product. However, a qualitative reading showed the material to be *dextro*-rotatory.

When a solution of the crude product in *n*-butanol was refluxed for about an hour, and cooled, white crystals separated. Recrystallized from ethanol, the material melted at 265–267° (cor.). It was soluble in 5% aqueous sodium bicarbonate and was optically inactive.

Anal. Calcd. for $C_{13}H_{16}N_2O_2$: C, 67.24; H, 6.89; N, 12.06. Found: C, 67.22; H, 6.82; N, 12.06.

Summary

1. Several new N-succinyl-1-methylamino-2,4-dimethyl-6-substituted benzenes and N-succinyl-1-methylamino-2-methyl-4,6-dibromobenzene have been prepared, resolved and the half-lives of the optically active forms determined.

2. The half-lives of the series are as follows: bromine, three and one-tenth hours; iodine, twenty and one-half hours; nitro, six-tenths hour; and the dibromo compound, one and one-tenth hours. Boiling *n*-butanol was used as solvent except in the case of the nitro compound where boiling methyl acetate was employed. The corresponding methyl and methoxyl analogs have been prepared previously and their half-lives are nine hours in boiling *n*-butanol and two and seven-tenths hours in boiling methyl acetate, respectively.

3. From these values, after comparison with the interference effects produced by the same groups in the biphenyl series, it was concluded that factors other than size of the groups were influencing the rates of racemization.

4. The basicity of aryl amines is reduced by the substitution of electronegative groups in the *o*-, *m*- or *p*-positions. A decrease in the expected stability of the optically active amines is observed with similar substituents. From these facts it appears that the increased double-bond character of the carbon–nitrogen bond in these amines of decreased basicity facilitates racemization. The tendency to form a double bond will aid in forcing the substituents on the amino nitrogen into a coplanar configuration with the ring.

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[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

The Reaction of Formaldehyde with Proteins. V. Cross-linking between Amino and Primary Amide or Guanidyl Groups

BY HEINZ FRAENKEL-CONRAT AND HAROLD S. OLCOTT

According to the opinion of most experts in the field, the tanning or hardening action of formaldehyde is probably not due to its primary addition to the amino or any other type of protein group, but rather to a secondary condensation reaction which transforms the methylol (—CH_2OH) groups into cross-linking methylene (—CH_2—) bridges. The first experimental evidence for the occurrence of condensation was supplied by Nitschmann and his co-workers, who showed that there was a loss of water during the reaction of casein with gaseous formaldehyde.[2,3] Proof for cross-linking was obtained in studies from this Laboratory, in which it was demonstrated that the average molecular weights of salmine[4] and other proteins[5] could be increased by formaldehyde treatment.

The question arose: Which protein group or groups are available for such condensation reactions with formaldehyde? Nitschmann, *et al.*,[2] showed that the amino groups were directly concerned.[6] Thus casein, in which the amino groups were largely protected with acetyl groups, bound formaldehyde only by addition, while condensation occurred when unmodified casein was used. However, experiments with salmine,[4] which con-

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[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

[2] Nitschmann and Hadorn, *Helv. Chim. Acta*, **27**, 299 (1944).

[3] Nitschmann and Lauener, *ibid.*, **29**, 174 (1946).

[4] Fraenkel-Conrat and Olcott, THIS JOURNAL, **68**, 34 (1946).

[5] Mecham and Fraenkel-Conrat, in preparation.

[6] See also Gustavson, *J. Int. Soc. Leather Trades Chem.*, **24**, 377 (1940); *Kolloid Z.*, **103**, 43 (1943); *J. Biol. Chem.*, **169**, 531 (1947).