cases the *ortho* is weaker than the *para* for the nitro group and stronger than the *para* for the methoxy group. This suggests that structure C would not be the predominant form in the ionization, since this form would be expected to be influenced by *o*-substituents.

TABLE II

pK_a'S OF *o*- AND *p*-SUBSTITUTED BENZOHYDROXAMIC, PHENYLPROPIOLIC AND BENZOIC ACIDS

Substituent	pK_a RCON-HOH	pK_a RC≡C—COOH[a]	pK_a RC≡C—COOH[b]	pK_a RCOOH[c]
C_6H_5–	8.8	3.58	3.24	4.20
o-$NO_2C_6H_4$–	8.2	3.39	2.83	2.17
p-$NO_2C_6H_4$–	8.0	3.26	2.57	3.42
o-$CH_3OC_6H_4$–	8.9	..	3.37	4.09
p-$CH_3OC_6H_4$–	9.0	..	3.44	4.47
o-ClC_6H_4–	..	3.51	3.08	2.92
p-ClC_6H_4–	8.6	3.47	3.07	3.98

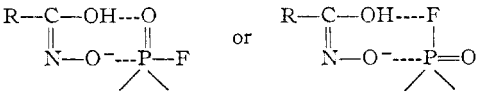
[a] Apparent pK_a's in 50% ethanol–50% water (by volume); Roberts and Carboni.[13] [b] Apparent pK_a's in 35% dioxane–65% water (by weight); Newman and Merrill.[14] [c] pK_a's in water; J. F. Dippy and J. E. Page, *J. Chem. Soc.*, 357, (1938). All pK_a's at 25°.

A linear relationship exists between the logarithms of the ionization constants and reaction rates of *o*-, *m*- and *p*-substituted phenylpropiolic acids with diphenyldiazomethane,[13] whereas ester saponification[13] and acid-catalyzed esterification[14] of *o*-substituted phenylpropiolic acids were faster than would be expected from the relationship between the rates and ionization of the *m*,*p*-substituted acids. This effect was ascribed to a decrease in the reaction site–ring distance, since both esterification and hydrolysis of esters involve attack of a nucleophilic agent at the carbonyl carbon.[13]

Since a linear relationship does exist between the logarithms of the ionization constants and the reaction rates of *o*-, *m*- and *p*-substituted benzohydroxamic acids with Sarin, it seems improbable that structure C is the reactive form because the *o*-substituents do not produce a deviation from linearity that would be expected if reaction occurred close to the ring.

Thus, tautomers A and/or B would appear to be the reactive forms of the anion in both ionization and reaction with electrophilic reagents, since both would take place at a site which is at a maximum distance from the *o*-substituent. However, if the inordinate reactivity of the hydroxamate ion (when compared to simple bases, *i.e.*, OH^-) is due to a concerted attack upon the Sarin molecule in a manner similar to catechol, the most probable reactive form is tautomer A.

Thus the interpretations presented above offer support to the mechanism originally postulated.[12c]



Experimental

Ionization Constants.—The pK_a's reported in Table I were obtained from conventional potentiometric titrations in the presence of 0.1 *M* potassium nitrate. All compounds reported except for *o*-hydroxybenzohydroxamic acid were either sufficiently soluble in water to titrate directly with standard base or stable in alkali so that excess standard base could be added and back titrated with standard acid. Due to the slight decomposition of *o*-hydroxybenzohydroxamic acid in alkali, the value reported is uncertain.

Kinetic Method.—The half-times of hydrolysis of Sarin in the presence of various hydroxamic acids are reported in Table I. The values were obtained by measuring the rate of addition of standard alkali taken up by the reaction mixture when maintained at a fixed *p*H by a Beckman autotitrator. The reaction was carried out as follows: a quantity of the hydroxamic acid was dissolved in 0.1 *M* potassium nitrate solution contained in a jacketed beaker through which water of 30.5 ± 0.2° was circulated from a thermostatically controlled bath. The final concentration of the hydroxamic acid in these experiments was 10^{-3} *M*. The solution was adjusted to *p*H 7.6 and the volume to 245 ml.

A stock solution of 0.65 ml. of Sarin (99% pure) in 100 ml. of water was prepared fresh daily. *Caution* should be exercised since Sarin is *extremely toxic* in both the liquid and vapor phase and must be handled in a hood of large capacity. In the *p*H range of 4 to 6, which the solution assumed, Sarin is resistant to hydrolysis. A 5-ml. aliquot was then added to the hydroxamic acid solution. The final concentration of the solution with respect to Sarin was 10^{-4} *M*. The quantity of standard 0.01 *N* sodium hydroxide delivered by the autotitrator *vs.* time was recorded.

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(13) J. D. Roberts and R. A. Carboni, THIS JOURNAL, **77**, 5554 (1955).

(14) M. S. Newman and S. H. Merrill, *ibid.*, **77**, 5552 (1955).

[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Rearrangement and Decarboxylation Reactions of N,N-Dimethylglycine Oxide

BY C. C. SWEELEY AND E. C. HORNING

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Ferric ion-catalyzed reactions of dimethylglycine oxide were studied in aqueous solution over the *p*H range 2–9. It was found that two modes of N-oxide rearrangement occurred; the products were formaldehyde and sarcosine, and dimethylamine and glyoxylic acid. In addition to these rearrangement reactions, a decarboxylation reaction also occurred, with a maximum rate near *p*H 8. The products of this reaction were carbon dioxide, formaldehyde and dimethylamine; these correspond to the products of oxidative decarboxylation of an α-amino acid.

A preliminary report from this Laboratory described a ferric ion-catalyzed rearrangement reaction of *t*-amine oxides.[1] This rearrangement (reaction A) was presumed to give a carbinolamine (II); the observed reaction products were a secondary amine (through reaction B) and formaldehyde or formic acid and the *t*-amine (reaction C). These products are equivalent to those ex-

(1) M. S. Fish, C. C. Sweeley and E. C. Horning, *Chemistry and Industry*, R. 24 (1956).

pected from a Polonovski reaction,[2] but the conditions are quite different. The Polonovski reaction is conducted in boiling acetic anhydride and is reported to occur by way of a free-radical mechanism[3]; the mechanism of the ferric ion induced reaction, which occurs in aqueous solution, is unknown.

$$\underset{\text{I}}{RCH_2N(CH_3)_2O} \xrightarrow[H_2O]{Fe^{+++}} \underset{\text{II}}{RCH_2N\begin{matrix}CH_3\\CH_2OH\end{matrix}} \quad (A)$$

$$\text{II} \longrightarrow \underset{\text{III}}{RCH_2NHCH_3} + HCHO \quad (B)$$

$$HCHO + \text{I} \longrightarrow RCH_2N(CH_3)_2 + HCOOH \quad (C)$$

The significance of reaction A lies in its relationship to what is known about biological methylation and demethylation reactions. It is known that the demethylation of drugs,[4] of N-methyltryptophan,[5] and of sarcosine and N,N-dimethylglycine[6] involves an oxidative sequence in which a methyl group is removed as formaldehyde or its cellular equivalent. It is also known that the *de novo* synthesis of the N-methyl groups of N,N-dimethylethanolamine is mediated by a folic acid derivative, in distinction from the methylation reaction which converts dimethylaminoethanol to choline and which requires methionine.[7] The methylation reactions leading to a secondary amine (N-methylethanolamine) and a tertiary amine (N,N-dimethylethanolamine) may require 5-hydroxymethyltetrahydrofolic acid.[8]

The successive steps of oxide formation, oxide rearrangement (reaction A) and hydrolytic cleavage of a methylol intermediate (reaction B) constitute a chemical model for demethylation, and methylation to a secondary or tertiary amine may be represented by the same sequence operating in a reverse direction. An enzyme-catalyzed amino acid oxide rearrangement corresponding to the transformation I → III has been observed[9] with a mouse liver homogenate for *l*-N,N-dimethyltyrosine oxide and *dl*-N,N-dimethyltryptophan oxide. These observations provide evidence that reaction A can occur as a cellular reaction in mammalian tissue, and it therefore seems likely that N-oxides are intermediates in biological methylation–demethylation reactions.

The enzymatic demethylation of N,N-dimethylglycine[6] to sarcosine and the cellular equivalent of formaldehyde is an oxidative sequence in which the amino acid is a normal component of mammalian metabolism.[10] It was therefore desirable to study the ferric ion induced reactions of N,N-dimethylglycine oxide with particular regard to the rearrangement leading to demethylation.

N,N-Dimethylglycine oxide was prepared from N,N-dimethylglycine by hydrogen peroxide oxidation. Its properties resembled those of most known *t*-amine oxides; it was a highly hygroscopic substance which tended to hold both water and hydrogen peroxide. No suitable derivatives were found. The expected relationship of the oxide to the parent amino acid was indicated by quantitative hydrogenation.

The ferric ion-catalyzed rearrangement reaction was studied in detail in two types of systems. Over the *p*H range 1–5 a ferric oxalate complex ion was employed; the results in this system have been summarized.[1] Over the *p*H range 2–9 a ferric tartrato complex ion was employed. Analyses of reaction mixtures were carried out by paper chromatography (system I). This work was aided by the development of an excellent spray procedure for detecting secondary amines in the presence of related tertiary (or primary) amines. The fact that the oxide gave a positive ninhydrin reaction[11] also allowed detection of the oxide in chromatographic analyses. Densitometer graph (B) in Fig. 1 shows a typical result obtained at *p*H 1.5, and this may be compared with (C) and (D) which represent reactions carried out at *p*H 6 and 4, respectively. The secondary amine in zone 1 was eluted from the appropriate zone in preparative runs and was identified as sarcosine by conventional biochemical procedures. Formaldehyde was detected in a usual way.[12] The secondary amine in zone 2 was found to be a volatile organic base whose concentration could be determined by a standard Kjeldahl distillation and titration procedure. The R_f value corresponded to that found for dimethylamine, but it was considered possible that this fraction might include some trimethylamine (arising by decarboxylation of the amino acid oxide to trimethylamine oxide); in separate experiments it was found that trimethylamine and/or trimethylamine oxide were not reaction products, and that therefore a direct decarboxylation to trimethylamine oxide was not occurring. A specific test procedure for glyoxylic acid[13] was applied to reaction mixtures containing the secondary amine of zone 2, and a spray procedure (adapted from the test) was used in chromatographic analyses to detect glyoxylic acid. Both sets of results indicated that glyoxylic acid and dimethylamine appeared simultaneously as reaction products when the rearrangement was carried out at *p*H 4–6; these data indicate that the ferric ion catalyzed reaction for N,N-dimethylglycine oxide occurred along both possible routes.

(2) M. Polonovski and M. Polonovski, *Bull. soc. chim. France*, 1190 (1927), and earlier papers.

(3) V. Boekelheide and D. L. Harrington, *Chemistry and Industry*, 1423 (1955).

(4) B. B. Brodie, J. Axelrod, J. R. Cooper, L. Gaudette, B. N. LaDu, C. Mitoma and S. Udenfriend, *Science*, **121**, 603 (1955).

(5) T. Yoshida and S. Fukuyama, *J. Biochem. (Japan)*, **34**, 429 (1941).

(6) C. Mackenzie, in "Amino Acid Metabolism," Johns Hopkins University Press, Baltimore, Md., 1955, p. 684.

(7) J. A. Stekol, S. Weiss and E. I. Anderson, THIS JOURNAL, **77**, 5192 (1955).

(8) This tetrahydrofolic acid derivative was proposed as a cellular equivalent for formaldehyde by A. D. Welch and C. A. Nichol (*Ann. Rev. Biochem.*, **21**, 633 (1952)).

(9) M. S. Fish, C. C. Sweeley, N. M. Johnson, E. P. Lawrence and E. C. Horning, *Biochim. Biophys. Acta*, **21**, 196 (1956).

(10) The amino acids of ref. 9 are not known to be cellular components.

(11) *t*-Amine oxides give positive ninhydrin reactions. The nature of the reaction is unknown.

(12) M. S. Fish, N. M. Johnson and E. C. Horning, THIS JOURNAL, **78**, 3668 (1956).

(13) F. Feigl, "Spot Tests," Vol. II, Elsevier Publishing Co., New York, N. Y., 1954, p. 255.

$$ON(CH_3)_2CH_2COOH \xrightarrow[pH\ 4\text{–}6]{Fe^{+++}} \begin{cases} \longrightarrow HOCH_2N(CH_3)CH_2COOH \longrightarrow CH_3NHCHCOOH + HCHO \\ \longrightarrow (CH_3)_2NCHOHCOOH \longrightarrow (CH_3)_2NH + \begin{matrix} CHO \\ | \\ COOH \end{matrix} \end{cases}$$

Separate experiments have provided evidence for the enzymatic formation of *t*-amine oxides[14] and for the rearrangement of N,N-dimethyltyrosine oxide and N,N-dimethyltryptophan oxide by an amino acid oxide hydroxytransferase in mouse liver.[10] From these observations, we suggest that the enzymatic demethylation of dimethylglycine described by Mackenzie[6] involves the intermediate steps of N-oxide formation and N-oxide rearrangement. This view is supported by the results presented in this paper for the non-enzymatic demethylation of dimethylglycine oxide, under conditions which approximate those of the cell.

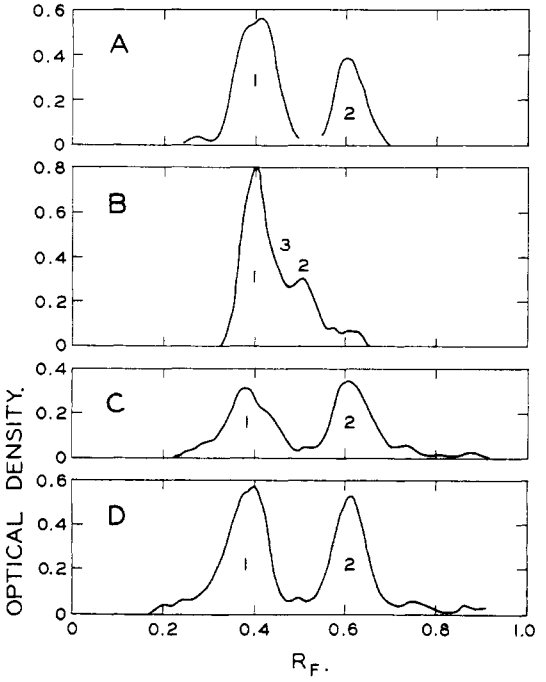
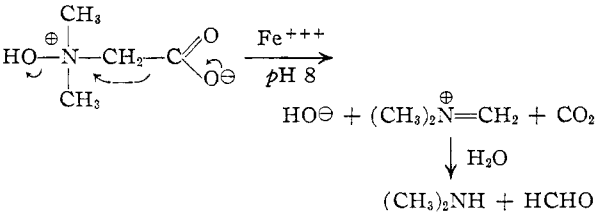


Fig. 1.—Densitometer data: A, reference compounds sarcosine (1), dimethylamine (2); B, sarcosine (1) and N,N-dimethylglycine oxide (2) in rearrangement with oxalic acid (*p*H 1.5), N,N-dimethylglycine-1-C^{14} (3) detected on radioautographs; C, sarcosine (1) and dimethylamine (2) in rearrangement reaction with tartaric acid (*p*H 6); D, sarcosine (1) and dimethylamine (2) in rearrangement reaction with tartaric acid (*p*H 4). The paper chromatograms were developed in *t*-butyl alcohol–acetic acid–water (3:1:1) and sprayed with ninhydrin reagent.

Data relating to a novel decarboxylation reaction of the oxide are in Fig. 2. This reaction was discovered when the possibility of a direct decarboxylation of the oxide to trimethylamine oxide and carbon dioxide was considered. Carbon dioxide was found as a reaction product when the oxide was subjected to ferric ion catalysis above about *p*H 5, but trimethylamine and trimethylamine oxide were found to be absent. A quantitative study of the yields of key reaction products (formaldehyde, dimethylamine and radioactive carbon dioxide) as a function of *p*H was carried out with N,N-dimethylglycine-1-C^{14} oxide. The observation that the increase in carbon dioxide yield, which rose to a maximum near *p*H 8, was paralleled by increasing yields of formaldehyde and dimethylamine, suggests strongly that the decarboxylation may be represented by



The reaction may be represented as a β-elimination in which the concerted separation of carbon dioxide and hydroxide ion is facilitated by formation of a ferric ion–amino acid oxide complex.

In this connection, recent work on the oxidative decarboxylation of amino acids should be mentioned. Spenser, Crawhall and Smyth[15] recently proposed a new reaction pathway to explain the known effects of oxidizing agents on α-amino acids.[16]

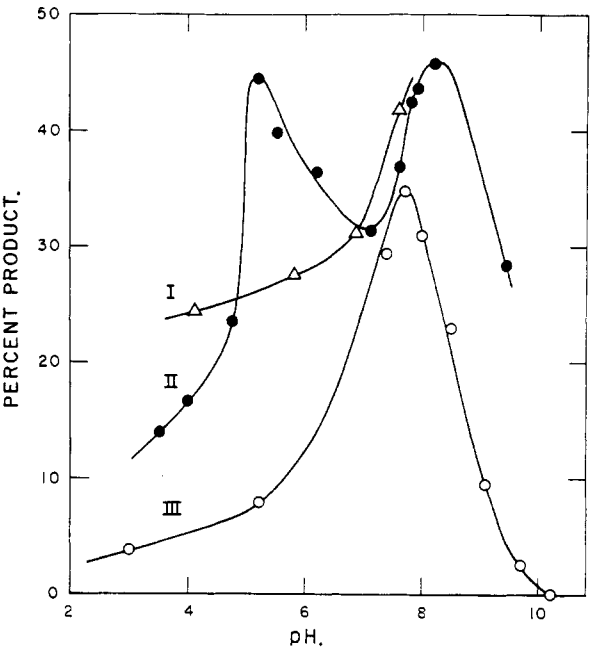


Fig. 2—Variations in yield of dimethylamine (I), formaldehyde (II) and carbon dioxide (III) with *p*H for reactions with ferric-tartrato complex ion at 22–23° for 90 minutes.

(14) M. S. Fish, N. M. Johnson, E. P. Lawrence and E. C. Horning, *Biochim. et Biophys. Acta*, **18**, 564 (1955).

(15) I. D. Spenser, J. C. Crawhall and D. G. Smyth, *Chemistry and Industry*, 796 (1956).

(16) Hypotheses under consideration up to 1934 (including the Wieland–Bergel, the Bergel–Bolz and the Haber–Willstätter proposals) were reviewed by Bergel (*Hoppe-Seyler's Z. physiol. Chem.*, **223**, 66 (1934)). The Herbst–Clarke mechanism is described in *J. Biol. Chem.*, **104**, 769 (1934).

The new experimental data leading to this proposal included isotope tracer studies which showed that the hydrogen on the α-carbon atom was not involved in the reaction, and oxidation experiments with α-N-diphenylglycine in which a Schiff base was found as the reaction product. The results were interpreted in terms of an oxidative ($OH^{\oplus}$) attack on the α-carbon atom, with concurrent loss of carbon dioxide. The immediate product, a carbinolamine, would then be expected to hydrolyze to an amine and an aldehyde or ketone (the normal products of oxidative decarboxylation of an α-amino acid) or to yield a Schiff base (as found for α,N-diphenylglycine). Data cited by Spenser, Crawhall and Smyth included the fact that the reaction fails for trialkyl (betaine) structures, and that it is successively more effective with substitution on the amino group (through mono and dimethyl stages). The critical point of this hypothesis lies in the assumption that a carbinolamine must be postulated as an intermediate product. The isolation of a Schiff base supports this view only indirectly. Our experimental observations with N,N-dimethylglycine oxide suggest that an alternate explanation should be considered. The reaction may involve oxidative ($OH^{\oplus}$) attack on the amino group, followed by concurrent β-elimination and loss of carbon dioxide, and leading to hydroxide ion, carbon dioxide and a Schiff base as the products. It is not necessary to assume that carbinolamine formation is a prerequisite for Schiff base formation. This explanation is supported by the data of Spenser, Crawhall and Smyth and by the observations in this paper.

The place of oxidative decarboxylation in plant biosynthesis has been reserved by Spenser, Crawhall and Smyth for future discussion. If both oxidative decarboxylation and oxidative demethylation occur as cellular reactions *via* amino acid oxides, it may be possible to demonstrate an enzyme-catalyzed rearrangement reaction for N-oxides. This possibility is under study.

Experimental

N,N-Dimethylglycine hydrochloride was prepared in 84% yield by reductive methylation of glycine with formaldehyde,[17] using Adams platinum catalyst in a low pressure apparatus.

N,N-Dimethylglycine Oxide.—A solution of 0.80 g. of N,N-dimethylglycine hydrochloride in 15 ml. of water was adjusted to *p*H 6 with Amberlite resin (IR-45, hydroxyl phase). The resin was removed by filtration, and 14 ml. of 0.2 *M* barium hydroxide solution and 10 ml. of 15% hydrogen peroxide were added. The solution (*p*H 12) was allowed to stand at room temperature for 1.5 hours (the *p*H dropped to 8). Barium ion was removed as barium sulfate and the filtrate was concentrated under reduced pressure to 5 ml. Ethanol (20 ml.) was added and the solution was again reduced in volume to 2 ml. After the addition of 5 ml. of ethanol, 100 ml. of acetone and 100 ml. of ether, the product was allowed to crystallize. The yield (after washing with ether and drying) was 0.35 g. (40%) of the amino acid oxide.

Anal. Calcd. for $C_4H_9NO_3.0.5H_2O.0.5H_2O_2$: C, 33.10; H, 7.63; N, 9.65. Found: C, 32.95; H, 7.36; N, 9.43.

The oxide was a colorless, highly hygroscopic substance. Repeated preparative runs gave material whose melting points were identical but not sharp (68–73° dec., Kofler). The infrared spectra of these samples were identical and different from that of N,N-dimethylglycine (specific bands due to the N-oxide functional group have not been located in N-oxides under study in this Laboratory.[1,9,12] The oxide gave a positive ninhydrin reaction[11] and it was detected in chromatographic analyses in this way. All systems (Table I) gave single, well-defined areas for the oxide. The ionophoresis data (Table II) indicated that the amino acid oxide was a stronger acid than glycine or sarcosine.

TABLE I

PAPER CHROMATOGRAPHIC DATA (R_f VALUES)

Compound	Solvent systems I	II	III	IV
Glycine	0.29	0.49	0.30	..
Sarcosine	.40	.58	.74	0.13
N,N-Dimethylglycine oxide	.52	.70	.95	..
Glyoxylic acid	.25	..	..	..

TABLE II

PAPER IONOPHORETIC DATA[a]

Compound	R_g[b]	Distance (cm.)
Urea	0.10	0.8
N,N-Dimethylglycine oxide	.13	1.0
Sarcosine	.76	6.2
Glycine	1.00	7.9

[a] Conditions: 300 v., 3.5 ma., 4 hours at 22–23°, with 25% aqueous acetic acid as electrolyte. [b] Ratio calculated for distance from origin.

No satisfactory derivatives were found. The chloroaurate, chloroplatinate, picrate and flavianate were not suitable derivatives. The thiouronium salt from *p*-bromobenzylthiouronium bromide retained the generally unsatisfactory physical properties of the oxide.

The formulation of the oxide as a solvate with both water and hydrogen peroxide is in accord with current knowledge of *t*-amine oxide properties. Aliphatic *t*-amine oxides often crystallize with water and/or hydrogen peroxide,[18] and one-half mole equivalent of hydrogen peroxide is not uncommon.[19] In accordance with Bodendorf and Binder,[19] the material was found to liberate iodine from potassium iodide solution. The structure of the oxide was verified by quantitative reduction to N,N-dimethylglycine. A 10-mg. quantity of Pd-C (10%) catalyst in 5 ml. of ethanol was saturated with hydrogen in a micro apparatus for quantitative hydrogenations. A solution of 70 mg. of the oxide in 30 ml. of ethanol was added, and the subsequent hydrogenation required 15.7 ml. (97% of the requirement for reduction of the oxide and peroxide of solvation) of hydrogen. This method is preferred over that of Bodendorf and Binder.[19] The product was isolated as the hydrochloride and it was found to be authentic N,N-dimethylglycine hydrochloride (infrared and m.p. data).

N,N-Dimethylglycine-1-C^{14} oxide was required for decarboxylation studies. It was prepared from N,N-dimethylglycine-1-C^{14} by the usual procedure. The infrared spectrum (Nujol) showed no changes from non-radioactive preparations, and the paper chromatographic and paper ionophoretic behavior showed no changes. The specific activity of the preparation was compared with that of the parent compound, and this was used as an experimental determination of the molecular weight of the solvated oxide: calcd. for formula above, mol. wt., 146; found 146.

Mild heating (to obtain a non-solvated product) resulted in decomposition of the oxide.

Paper Chromatographic Systems and Sprays.—Systems used for the examination of reaction mixtures and for identification of products were: (I) *t*-butyl alcohol–acetic acid–water (3:1:1); (II) propanol–acetic acid–water (10:1:9); (III) phenol–water (4:1);[20] (IV) propanol–1 *N* ammonium hydroxide (5:1). System I gave excellent results in qualitative analyses of reaction mixtures. The time required for separations was usually 16 hours at room temperature (22–23°). The papers were washed Whatman 1 and washed Whatman 3 MM.

(17) D. E. Pearson and J. D. Bruton, THIS JOURNAL, **73**, 864 (1951).

(18) C. C. J. Culvenor, *Rev. Pure appl. Chem.*, **3**, 83 (1953).

(19) K. Bodendorf and B. Binder, *Arch. pharm.*, **287**, 326 (1954).

(20) H. K. Berry and L. Cain, *Arch. Biochem.*, **24**, 179 (1949).

The ninhydrin spray was a 0.2% solution in 50% aqueous pyridine. The strips were heated to develop the colored areas, and the chromatograms were preserved by spraying with a dilute cupric nitrate solution,[21] followed by exposure to ammonia to remove the excess nitric acid.

For secondary amines, the chromatogram was sprayed with a freshly prepared 1% sodium nitroprusside solution containing 10% (v./v.) of acetaldehyde. This was followed immediately by a spray with 2% sodium carbonate solution. A slowly developing deep blue spot on a light pink or colorless background constituted a positive test for secondary amines. This method was a modification of a spot test procedure for detecting secondary amines.[22] It gave no reaction with components of the reaction mixtures other than sarcosine and dimethylamine.

A spray to detect glyoxylic acid was developed from a qualitative test procedure.[13] The paper chromatogram was sprayed with a freshly prepared solution of 1% phenylhydrazine hydrochloride in 0.5 *N* hydrochloric acid. After drying (heat), the paper was sprayed with 3% hydrogen peroxide in 0.5 *N* hydrochloric acid. A red color was formed with glyoxylic acid.

Rearrangement of N,N-Dimethylglycine Oxide. A. Oxalate System.—In a typical experiment, a solution containing 3.0 mg. (20 μmoles) of N,N-dimethylglycine oxide, 200 μmoles of oxalic acid and 60 μmoles of ferric nitrate nonahydrate, in a total volume of 1.3 ml. (*p*H 1.5–2.0), was kept at 60° for 30 minutes. Paper chromatography of 50–100 μg. of organic constituents with system I, followed by a ninhydrin spray, gave a pattern as shown in the densitometer (Photovolt Model 520, 545 mμ filter) graph of Fig. 1B. Similar experiments were run at five intervals in the *p*H range 1–5; above *p*H 2 the yield of sarcosine decreased slowly.

In these experiments, the ferric ion was presumably present as an oxalato complex ion.[23] Above *p*H 5 a slow precipitation of ferric hydroxide interfered with the experiments.

B. Tartrate System.—A solution containing 1.0 mg. (6.9 μmoles) of N,N-dimethylglycine oxide, 40 μmoles of tartaric acid and 2.0 μmoles of ferric nitrate nonahydrate was adjusted to the appropriate *p*H with 10% sodium carbonate solution (final volume of 1.0 ml.). It was heated at 60° for 15 minutes. Paper chromatography, using system I with a ninhydrin spray or a secondary amine spray, was used for qualitative analysis of the reaction mixture. The densitometer records in Fig. 1C-1D were obtained with a ferric ion:oxide ratio of 0.3:1 at *p*H 6.0 and 3.0:1 at *p*H 4.0, respectively. A ninhydrin spray was used for both records.

A series of experiments were run at *p*H 2–11, with heating for 15 minutes at 60°. Visual examination of the chromatograms indicated that for runs in the *p*H range 4–8, all of the oxide was consumed, and approximately the same yield of the two secondary amines was obtained. Below *p*H 4 the yield fell, and unreacted oxide was present. At *p*H 8–9 the yield also fell, and at *p*H 10–11 there was no rearrangement.

The ferric ion was present in these solutions as a tartrato complex ion. When iron tartrate solutions of the concentrations used here were irradiated in the absence of air, an oxidation–reduction reaction occurred, and all of the iron was reduced to the ferrous state (as judged by the complete disappearance of the characteristic yellow color of ferric ion). This reaction may occur during the rearrangement, but its effect was kept at a minimum by using freshly prepared solutions for each experiment. The ferrous–tartrato complex seems to be of no great significance in these reactions. Some oxidation of the tartrate ion (by air or the amine oxide) may also occur.

Identification of Sarcosine and Dimethylamine.—A preparative run was carried through chromatography on Whatman 3MM paper. Zone 1 was eluted (water) and the amine was compared with an authentic sample of sarcosine in four solvent systems. The eluted material, sarcosine, and a mixture were run concurrently; the R_f values are in Table I, together with data for glycine and N,N-dimethylglycine oxide. Chromatographic identity was observed.

A comparison of the reaction product with sarcosine was also carried out by paper ionophoresis on washed Whatman 1 paper with 25% acetic acid as the electrolyte. The data are in Table II, with comparative values for glycine. Ionophoretic identity was observed for the authentic and eluted samples.

The reaction product gave a positive test (blue color) with a secondary amine spray; the test result was identical with that produced by authentic sarcosine.

Dimethylamine was recognized by chromatographic separation (zone 2) in system I, followed by spraying with ninhydrin or the secondary amine reagent (in alkaline systems, the amine is lost by evaporation). With system I, the R_f of dimethylamine was 0.61; chromatograms were run with the reaction mixture, an authentic sample at approximately the same concentration, and a mixture. In all cases the R_f values and the colors with both sprays were identical for these comparative runs. Quantitative determinations were also made as described.

Identification of Glyoxylic Acid.—Reaction mixtures were tested for the presence of glyoxylic acid by a spot test procedure.[13] One drop each of the mixture, 12 *N* hydrochloric acid and a freshly prepared 1% solution of phenylhydrazine hydrochloride were mixed and heated at 80° for 5 minutes. The mixture was chilled and treated with one drop each of 12 *N* hydrochloric acid and 3% hydrogen peroxide. An intense red color constituted a positive test. Blank runs, omitting the oxide, gave faint pink colors.

This procedure was modified for use as a spray, and in system I it was found that authentic glyoxylic acid (prepared from dichloroacetic acid)[24] and the reaction mixture gave corresponding colored areas (R_f 0.25).

Formaldehyde and Formic Acid Tests.—These were carried out as described in a previous study of the rearrangement of N,N-dimethyltryptamine oxide.[12] With a Fe^{+++} to oxide ratio of 0.03:1, it was found possible to detect formaldehyde directly in the reaction mixture by a chromotropic acid color test.

Metal Ions.—With two sets of standard conditions (a) an oxalate system at *p*H 2 with a metal ion to oxide ratio of 3:1, and (b) a tartrate system at *p*H 5–6 with a metal ion to oxide ratio of 0.3:1, it was found that Co^{++}, Ni^{++}, Cu^{++}, Mg^{++} and Cr^{+++} were without catalytic effect. No reaction was observed in a tartrate system at *p*H 4–6 with $[Fe(CN)_6]^{---}$ or an 8-hydroxyquinoline-5-sulfonic acid complex with ferric ion. With Fe^{++} in a 3:1 ratio, and with a tartrate system at *p*H 5, the oxide reacted as usual and the two amine products were observed in normal yield.

Quantitative Determination of Products.—In a series of experiments, conducted at 23° for 90 minutes with a 0.6:1 mole ratio of ferric ion to oxide, the *p*H of the reaction was varied. Formaldehyde, dimethylamine and carbon dioxide were determined quantitatively. The results are shown in Fig. 2.

A. Determination of Formaldehyde.—In a typical experiment, 1.0 mg. (6.9 μmoles) of N,N-dimethylglycine oxide in 1.0 ml. of water was adjusted to *p*H 5.0 with 0.1 *N* sodium hydroxide solution. A solution (1.0 ml.) of tartaric acid (50 μmoles) and ferric nitrate nonahydrate (4 μmoles) was adjusted to *p*H 5.0 with 0.1 *N* sodium hydroxide and was added to the solution of the oxide. After 90 minutes at 23° the reaction mixture was pipetted into a 50-ml. flask fitted with a distilling head and condenser. The solution was acidified with 2.0 ml. of 8.5% phosphoric acid and the mixture was distilled at atmospheric pressure into 1.0 ml. of ice-water. The tip of the condenser was immersed in the ice-water. An additional 3.0 ml. of water was added to the residue and distillation was repeated, collecting 7.0 ml. of distillate in all. Formaldehyde was determined on an aliquot of the distillate by a modified Hantzsch reaction with acetylacetone and ammonium acetate.[25] Optical density measurements were made with a Beckman DU spectrophotometer.

B. Determination of Dimethylamine.—A solution of oxide (13.7 μmoles), tartaric acid (100 μmoles) and ferric nitrate nonahydrate (8.0 μmoles), adjusted to *p*H 4.5 as described in the previous section for formaldehyde determination, was allowed to stand at 23° for 90 minutes. The solution was pipetted into a Kjeldahl still with 8.0 ml. of 20% sodium hydroxide solution. The volatile base was steam distilled for 4 minutes into 5.0 ml. of 5% boric acid

(21) R. J. Block, E. L. Durrum and G. Zweig, "A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press, New York, N. Y., 1955, p. 90.

(22) Ref. 13, p. 191.

(23) N. V. Sidgwick, "Chemical Elements and their Compounds," Oxford University Press, 1951, p. 1365.

(24) O. Doebner, *Ann.*, **311**, 129 (1900).

(25) T. Nash, *Biochem. J.*, **55**, 416 (1953).

containing 3 drops of methyl red (0.1%)-brom cresol green (0.1%) indicator. The dimethylamine was determined by titration of the boric acid solution with 0.01 *N* hydrochloric acid.

In separate experiments, the volatile organic base fraction was examined for the presence of trimethylamine by the sensitive quantitative procedure of Cromwell.[26] Trimethylamine was not present. The oxide of trimethylamine was prepared; it gave a positive ninhydrin reaction[11] and it was found to be absent in chromatographic analyses of reaction mixtures at *p*H 4–8.

C. Determination of Carbon Dioxide.—A reaction mixture (*p*H 7.0), containing N,N-dimethylglycine oxide-1-C^{14} (6.9 μmoles), tartaric acid (50 μmoles) and ferric nitrate nonahydrate (4 μmoles), was prepared in the usual way and allowed to react for 90 minutes at 23° in a flask connected to a sodium hydroxide trap. The solution was acidified with 1.0 ml. of 8.5% phosphoric acid and the system was flushed with nitrogen for 15 minutes. The carbon dioxide collected in the sodium hydroxide trap was counted[27] as barium carbonate. Runs at other *p*H values were made in the same way.

N,N-Dimethylglycine hydrochloride-1-C^{14} (7.1 μmoles) and glyoxylic acid (400 μmoles) gave no carbon dioxide under similar conditions (catalyst, tartrate, 23°, 90 minutes).

D. *p*H Effects.—Over the *p*H region where decarboxylation was the primary reaction it was observed that the *p*H rose as the reaction proceeded. Initial and final *p*H values were averaged to locate the points near *p*H 8 in Fig. 2.

(26) B. T. Cromwell, *Biochem. J.*, **46**, 578 (1950).

(27) C. V. Robinson, *Science*, **112**, 198 (1950).

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[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF IOWA STATE COLLEGE]

Some Reactions of *o*-Halophenyllithium Compounds

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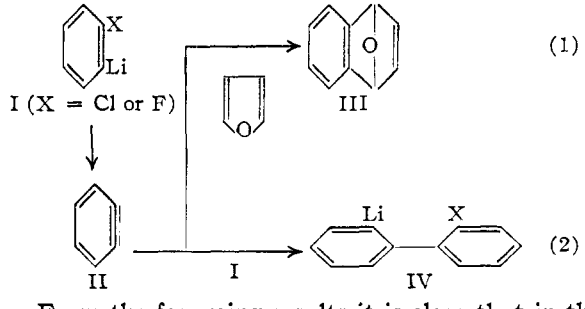
When *o*-chloro- and *o*-fluorophenyllithium are prepared at −90 and −60°, respectively, in the presence of furan and then carbonated, the main product is 1,4-dihydronaphthalene-1,4-endoxide (III) while the corresponding 2-carboxy-2′-halobiphenyl (IV) is obtained in small yields. *o*-Dibromobenzene and *n*-butyllithium in the presence of furan also give III under modified conditions. Only III is obtained when an RLi is added to a mixture of the *o*-bromohalobenzene and furan at a temperature above that at which the *o*-halophenyllithium compound is stable. Methyllithium and phenyllithium are equally effective in reacting with an *o*-halobromobenzene in the presence of furan to give excellent yields of III. 2-Chloro-2′-(triphenylsilyl)-biphenyl has been synthesized and compared with the bromo-isomer prepared by a less ambiguous method. The results are discussed and correlated.

As a continuation of some of our earlier studies concerned with *o*-halophenyllithium compounds,[1] we have investigated further some of their reactions and possible routes by which these organometallic compounds couple to form new organolithium compounds. In order to obtain a better understanding of the various transformations, the elegant procedure of Wittig and Pohmer[2] was employed in this present work. These workers successfully interacted *o*-bromofluorobenzene with lithium amalgam in furan to obtain 1,4-dihydronaphthalene-1,4-endoxide (III), a strained molecule which has been formulated as arising *via* a Diels–Alder reaction between furan and the benzyne intermediate (II). Furthermore, the reactive species II has been postulated as being formed from *o*-fluorophenyllithium after the latter has been generated by the interaction of *o*-bromofluorobenzene and lithium amalgam.[3] In view of these results it was hoped that furan could be used in a similar fashion to trap any intermediate which might arise during the break-down of *o*-halophenyllithium compounds (I). *o*-Fluoro- and *o*-chlorophenyllithium were prepared at −60 and −90°, respectively. A relatively large excess of furan was added in each case and then the mixtures were allowed to warm to −50° for the chloro-isomer and −10° for the fluoro-isomer. Since Color Test I[4] was positive in both cases at these temperatures, the mixtures were carbonated. The run involving *o*-bromochlorobenzene afforded 41% of III and 11% of 2-carboxy-2′-chlorobiphenyl; with *o*-bromofluorobenzene the yield of III was 67% while 4% of 2-carboxy-2′-fluorobiphenyl was isolated.

A modified procedure was used with *o*-dibromobenzene since it was found in previous studies that *o*-bromophenyllithium is a short-lived intermediate.[1] Consequently, *o*-dibromobenzene was added to a mixture of furan and butyllithium. The yield of III was 68%; however, as might be expected and will be explained later, no acid was isolated.



From the foregoing results it is clear that in the case of *o*-chloro- and *o*-fluorophenyllithium some reactive intermediate is competitively reacting with furan and with I. Furthermore, evidence substantiates that such a competitive reaction occurs only when the organolithium compound is formed first independently of the other reactants. In order to ascertain that no metalation reaction

(1) H. Gilman and R. D. Gorsich, THIS JOURNAL, **78**, 2217 (1956); this reference contains a general survey of relevant literature citations.

(2) G. Wittig and L. Pohmer, *Angew. Chem.*, **67**, 348 (1955). See G. Wittig and L. Pohmer, *Chem. Ber.*, **89**, 1334 (1950), for details about which we have just learned, after returning proof, of study mentioned in their prior communication.

(3) It is interesting to note that the same reaction in the absence of furan gives diphenylene and triphenylene; G. Wittig and W. Herwig, *Ber.*, **87**, 1511 (1954).

(4) H. Gilman and F. Schulze, THIS JOURNAL, **47**, 2002 (1925).