PREPARATION AND DECOMPOSITION OF A C-14 LABELED MANNICH BASE

by

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PREPARATION AND DECOMPOSITION OF A C-14 LABELED MANNICH BASE

INTRODUCTION

As the opening part of a study of the mechanism of the Mannich Reaction, it was decided to incorporate radioactive paraformaldehyde in a standard Mannich Base. By pyrolysis and subsequent ozonolysis studies, it was hoped to prove whether or not there was any transposition of the carbon atoms in the reaction. Since the Mannich Reaction is used to prepare many compounds of biological and chemotherapeutical importance, it was hoped that this study would further serve to test the feasibility of incorporating carbon-14 in biologically important bases of this type. By this means, the mechanism of their physiological action could be ascertained. The historic Mannich Reaction, involving acetophenone and dimethylamine hydrochloride, was used in this work since it has been thoroughly studied and characterized. Additional experiments were made with negatively substituted acetophenones to ascertain the effect of these negative substituents.
HISTORICAL

The condensation of ammonia, or ammonia derivatives with formaldehyde and a compound containing a labile hydrogen was first reported by Tollens (2, pp. 304-5; 9, p. 1351) in 1903. He isolated primary, secondary, and tertiary amines corresponding to \( \text{C}_6\text{H}_5\text{COCH}_2\text{CH}_2\text{NH}_2\cdot\text{Cl}, \)
\( (\text{C}_6\text{H}_5\text{COCH}_2\text{CH}_2)\cdot2\text{NH}_2\cdot\text{Cl}, \)
and \( (\text{C}_6\text{H}_5\text{COCH}_2\text{CH}_2)_3\\text{NHCl}. \)

Between 1906 and 1917, a number of investigators studied the reaction, but failed to recognize it as a general reaction. In 1912, Mannich and Krosche (2, p. 306) reported that antipyrine, formaldehyde, and ammonium chloride condensed to form a tertiary amine analogous to the aminopropiophenones of Tollens. Mannich did little with the reaction during the next five years, but after 1917 he began a very detailed study of the condensation which now bears his name.

There has never been any completely acceptable mechanism proposed for the Mannich Reaction. There was a little done toward postulating a mechanism until 1933, when Bodendorf and Koralewski began a systematic investigation of the condensation mechanism. The first study involved the suggested addition product of formaldehyde and the amine, the methylolamine. Bodendorf and Koralewski (4, pp. 102-4) used the methylolamine in three syntheses that had been previously reported. In none of the cases was the yield as good as in the corresponding cases using the free amine and formaldehyde.
A second postulated intermediate was the methylol addition product of the formaldehyde with the active carbon position. Using the methylol compound as a starting material, some anomalous results were obtained (4, pp. 104–6) which suggested the possibility of the formation of an \( \alpha,\beta \)-unsaturated ketone. The amine would then add directly to the unsaturated position. However, since a number of compounds could not have the unsaturated intermediate, this was discounted.

Bodendorf and Koralewski then postulated another possible mechanism which seemed to answer the experimental needs (4, pp. 107–9). Since loose addition complexes had been recognized as forming between certain aldehydes and ketones (11, pp. 2057–8; 14, pp. 38–9), it was suggested that formaldehyde adds to active methyl, and methylene, compounds in an analogous manner.

\[
\begin{align*}
\text{R-C-H} & \rightarrow \text{R-C-H} + \text{OH} \\
\text{O-H} & \rightarrow \text{R-C-H} + \text{OH} \\
\text{O-H} & \rightarrow \text{R-C-H} + \text{OH} \\
\end{align*}
\]

These types of rings are known to be easily cleaved by conditions such as the aminolytic cleavage of ethylene oxide. This mechanism was supported by the known effect of substituents on the Mannich Reaction.

In the last few months, additional work has been reported by Lieberman and Wagner (6, pp. 1001–5) and by Alexander (1, pp. 4017). Although Bodendorf and Koralewski had indicated that the mechanism
could not proceed through an addition complex involving the formaldehyde and the amine. Lieberman and Wagner proposed the methylene-bis-amine as the intermediate. This methylene-bis-amine would protonate under the slightly acid conditions of the reaction, and the resulting cation would deaminate, giving an amino substituted carbonium ion. The active methylene compound would, in the near amphoteric conditions of the reaction, release a proton and form a carbonium. The carbanion and carbonium ions would then join in an essentially irreversible reaction to form the Mannich Base.

\[
\begin{align*}
\text{HNR}_2 + \text{HCHO} & \rightarrow \text{R}_2\text{NCH}_2\text{NR}_2 + \text{H}^+ \rightarrow \text{R}_2\text{NCH}_2^+ + \text{HNR}_2 \\
\text{RCOCH}_3 & \rightarrow \text{RCOCH}_2 + \text{R}_2\text{NCH}_2 \rightarrow \text{RCOCH}_2\text{CH}_2\text{NR}_2
\end{align*}
\]

Alexander's proposed mechanism was similar to that of Lieberman and Wagner's, with the exception that he returned to the proposed methylolamine as an intermediate. Using ethylmalonic acid as the active methylene compound, he proposed a complex formation between the catalyst acid and the methylol amine. The addition complex would polarize to place a residual positive charge on the formaldehyde carbon. Tautomerism of the ethylmalonic acid and resultant polarization of the olefin would lead to a residual negative charge on the methylene carbon. The junction would then take place between these polar groups and the resultant complex would eliminate water and the catalyst acid to form the Mannich Base.

\[
\text{HCHO} + \text{M}_2\text{NH} \rightarrow \text{R}_2\text{NHCHOH}
\]
\[ R_2NCH_2OH + HA \rightarrow R_2NCH_2OH-\text{HA} \quad \text{EtCH(COOH)}_2 \rightarrow \]
\[ \text{Et} = C(OH)_2 + R_2NCH_2OH-\text{HA} \rightarrow R_2NCH_2CH(Et)COOH \quad \text{EtCOOH} + H_2O + HA \]

Both of these proposals were supported by considerable experimental evidence. Lieberman and Wagner gave tables of compounds showing the similarity of yields of reactions using the methylene-bis-amine as a starting material as compared with the addition of formaldehyde and amine separately. Alexander supported his suggested course by reference to kinetic studies. He made no attempt to carry the study to a general conclusion, but limited his study to the one compound.
GENERAL DISCUSSION

As a part of a study to determine the mechanism, and extend the usefulness of the Mannich Reaction, this program of incorporating radioparaformaldehyde in the reaction medium was undertaken. The problem involved, first of all, the determination of a means, whereby, if possible, the yield of the classical Mannich Reaction might be improved (unless otherwise stated, the reaction concerned will be the condensation of acetophenone, dimethyamine hydrochloride, and paraformaldehyde).

It was first noted by Mannich and Heilner (6, p. 359) and has since been noted by Blicke (2, p. 326) that variation of the amounts of the constituents apparently has little effect on the yield of the final product. In fact, as Blicke noted, too great a variation in the amounts frequently has an inhibiting effect on the yield. However, since it was noted that the optimum conditions usually required a slight excess of the amine and the active methylene compound, it was decided to run the condensation in a considerable excess of these two, to try and increase the yield with respect to formaldehyde.

A number of experiments in varying the concentrations of the reactants failed to significantly vary the yield of the final product. The average reported yield in the literature, was 60%, and this investigator exceeded that figure once in obtaining a 70% yield. The remainder of the time, the yields ranged from 50-60%. It appears
that there must be some type of side reaction involving the for-
mation of a complex with the formaldehyde. This complex has to
be stable for the conditions of the reaction, but it is still suf-
ficiently unstable that it is possible to obtain positive tests for
formaldehyde with the resorcinol, Schiff, and dimedon* tests.

The next group of experiments were suggested by the study made
by Plati and Wenner (10, pp. 546-7). They found that the results of
the condensation involving methylamine were greatly affected by the
solvent conditions. The most satisfactory yields were obtained by
the use of excess acetophenone as a solvent, and by asolvent pro-
cedures. The asolvent conditions were shown by Plati and Wenner
to be impractical, in spite of the increased yields; and, as a
result, this experiment was not tried.

Two experiments were attempted to try and increase the yield by
varying the solvent conditions. The first, using excess acetophenone,
resulted in an almost quantitative recovery of dimethylamine hydro-
chloride. The Mannich Base, if any, was undetectable in the excess
of amine. The second experiment involved the use of a very small
amount of ethanol plus excess acetophenone as the solvent. Yields
in this experiment were comparable to those originally reported, and
the smaller volume of material led to the adoption of these conditions.

*Note: The term dimedon refers to 5,5-dimethylhydrosorcinol
(5,5-dimethylcyclohexandione), a quantitative reagent for Formalde-
hyde. (15, pp. 263-6; 16, p. 365)
The mechanism proposed by Bodendorf and Koralewski (1, pp. 107-109) which appeared to them to best satisfy the experimental results involved the formation of an enol type structure as the first step. A parallel investigation was started to study the effect of enol promoting substituent groups. The effect of ortho or para-nitro substituents on acetophenone should be to greatly increase the amount of enolization. By this effect, it should also increase the amount of base obtained as a product, and this effect was noted. The use of ortho-nitroacetophenone increased the yield to 87% and the para had an even greater effect. It was then proposed to use the 2,4-dinitroacetophenone in an attempt to obtain nearly quantitative results. However, the instability of the compound caused an explosion during the preparation, and that study was abandoned. Although this study of the effect of negative substituents gave promise of fruitful results, the problems inherent in the preparation of the starting materials caused us to abandon this approach.

The next problem involved finding a method for accurately locating the radioformaldehyde in the product molecule. Mannich and Heilner (8, pp. 361-2) reported the pyrolysis of $\beta$-dimethylaminopropiophenone hydrochloride by steam distillation to give vinyl phenyl ketone. Ozonolysis of this product would give phenyl glyoxal and formaldehyde which could greatly aid in locating the final position of the radioactive carbon.

\[
\begin{align*}
\text{CH}_2\text{CH}_2\text{N(Me)}_2\text{HCl} & \rightarrow \text{OCH} = \text{CH}_2 & \text{O}_3 & \rightarrow \text{H}_2\text{O} & \text{Zn} & \rightarrow \text mass + \text{O}_2
\end{align*}
\]
The pyrolysis was performed by dissolving the base in a great excess of water and distilling the mixture to near dryness. This procedure was repeated until the distillate was clear, and the vinyl phenyl ketone was extracted with chloroform.

The ozonolysis was performed by a modification of the procedure of Weinberger (15, pp. 365-9) and Smyth and Pease (12, pp. 11-3). Only one ozonizer tube was used; and instead of using the water bath and jacket, the tube was wrapped in heavy lead foil to which the electrical connection was made. This foil was, in turn, wrapped with heavy kraft paper, and that covered with strips of electrician's rubber tape. A Cenco-Thordarson transformer was used which produced 15,000 volts across the secondary. The delivery tube from the ozonizer led directly into the reaction vessel.

Ozone was passed through the solution of the vinyl phenyl ketone for a period of approximately three to four hours; following which, the mixture was cooled and poured into an evaporating dish full of water. Since hydrogen peroxide is one of the products of the hydrolysis of any ozonide, granulated zinc was added to the solution to prevent oxidation of the phenyl glyoxal and formaldehyde.

Smyth's procedure called for evaporation of the hydrolysate to remove the acid. However, since the formaldehyde would be lost, the hydrolysate was filtered to remove the phenyl glyoxal. The filtrate was then treated with ammonium carbonate to raise the pH and increase the sensitivity of the dimedon precipitation.
saturated solution of dimedon in 95% ethanol was added to the solution and the mixture allowed to remain at room temperature for one day. After chilling, the methylene-bis-dimedon was recovered as a fine precipitate.

\[
\begin{align*}
\text{HO} & \quad + \quad \text{HCHO} \\
\text{Me}_2 & \quad \rightarrow \quad \text{Me}_2 \\
\text{OH} & \quad \text{OH} & \quad \text{CH}_2 & \quad \text{HO} & \quad \text{Me}_2
\end{align*}
\]

After the development of this technique, the reaction was run incorporating the radioparaformaldehyde. The paraformaldehyde was that prepared by Spencer (12, pp. 20-6). A number of combustion and counting analyses were performed, and the data are summarized on page thirteen. During the course of the study, one limiting factor became apparent. Due to the dilution of the activity of the radioactive carbon in the preparation of the paraformaldehyde, little quantitative data would be available on the activity of the final products. The data would necessarily be qualitative in the detection of final location of the activity.

Following the initial reaction involving the radioparaformaldehyde, it was found necessary to chill the mixture to below zero temperatures before the product could be isolated. Although the yield was comparatively low, being 51%, addition of dimedon solution to the mother liquor failed to indicate the presence of unreacted formaldehyde. The Mannich Base was dissolved in water, subjected to steam distillation, and the vinyl phenyl ketone was isolated by chloroform extraction. Use of a monitor indicated considerable
activity in the extract. The isolated unsaturated ketone was subjected to the ozonolysis procedure and upon hydrolysis the crystalline phenyl glyoxal monohydrate precipitated.

The glyoxal was filtered, washed with water, and ground to a fine powder with a spatula. It was then deposited in a planchet for counting analysis. The 110 milligrams of phenyl glyoxal indicated an activity of 150 counts per minute above background on the initial two minute count. This was approximately one-third the activity, per equivalent, of the initial formaldehyde. Since none of the proposed mechanisms could explain the presence of activity in that portion of the molecule, it was believed due to occlusion of formaldehyde from the initial precipitation. Successive grindings and washings produced the following counting data:

<table>
<thead>
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<th></th>
<th>initial count</th>
<th>activity</th>
<th>mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>150 counts/ minute</td>
<td>110 mg.</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>115 counts/ minute</td>
<td>104 mg.</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>92 counts/ minute</td>
<td>98 mg.</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>65 counts/ minute</td>
<td>87 mg.</td>
<td></td>
</tr>
</tbody>
</table>

Since the successive washings reduced the activity more than could be accounted for by the loss of weight, this was felt to conclusively indicate that the activity was due to occlusion rather than a molecular transposition. A further indication of this was shown by the formation of a precipitate on the addition of dimedon solution to the wash water. Since heating would destroy the crystalline monohydrate and leave only an unworkable oil, it was believed
to be impractical to attempt to purify the sample to complete inactivity.

The molecular weight of the methylene-bis-dimedon is approximately three-halves that of barium carbonate. As a result, the specific activity of a given sample should be approximately two-thirds that of a corresponding weight of barium carbonate. The specific activity of a 50 milligram sample of barium carbonate was approximately five counts per minute per milligram. A 50 milligram sample of the methylene-bis-dimedon from the ozonolysis showed a specific activity of three counts per minute per milligram, the required activity to be well within the experimental error of this work. The yield of the methylene-bis-dimedon based on the Mannich Base was 37%.

This work indicates that there is no transposition of the carbons in the Mannich Reaction. Since the greater part of the dimethylanine hydrochloride can be recovered, either as the product, or as unchanged excess amine, it would also indicate that any complex formed would involve the formaldehyde and the acetophenone. This latter possibility is further supported by the failure to recover any of the unreacted formaldehyde from the mother liquor.
### Consolidated Counting Data

<table>
<thead>
<tr>
<th>Sample</th>
<th>mg.</th>
<th>mg/cm²</th>
<th>c/min*</th>
<th>c/min/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia**</td>
<td>56.8</td>
<td>11.2</td>
<td>310</td>
<td>5.46</td>
</tr>
<tr>
<td>IIa</td>
<td>70.5</td>
<td>13.9</td>
<td>312</td>
<td>4.42</td>
</tr>
<tr>
<td>IIIa</td>
<td>15.9</td>
<td>3.4</td>
<td>189</td>
<td>11.9</td>
</tr>
<tr>
<td>IV</td>
<td>14.2</td>
<td>2.8</td>
<td>212</td>
<td>14.9</td>
</tr>
<tr>
<td>V</td>
<td>36.5</td>
<td>7.2</td>
<td>288</td>
<td>7.9</td>
</tr>
<tr>
<td>VI</td>
<td>35.9</td>
<td>7.1</td>
<td>284</td>
<td>7.9</td>
</tr>
<tr>
<td>Ib</td>
<td>53.2</td>
<td>10.5</td>
<td>276</td>
<td>5.2</td>
</tr>
<tr>
<td>IIb</td>
<td>22.5</td>
<td>4.4</td>
<td>234</td>
<td>10.4</td>
</tr>
<tr>
<td>IIIb</td>
<td>38.9</td>
<td>7.7</td>
<td>282</td>
<td>7.25</td>
</tr>
</tbody>
</table>

* Samples were counted for two minutes and corrected for two minute background before these data were prepared.

** Samples Ia through VI were determined from one combustion. A second combustion was run to prepare the "b" samples which were to prove the values to be reproducible.

These self-absorption and counting data are summarized in the following graphs. In the first, the self-absorption of the barium carbonate is indicated by plotting counts per minute per milligram as a function of milligrams per centimeter squared. The second shows the maximum activity for the geometry of the system. Counts per minute are plotted versus milligrams per centimeter squared.
SELF-ABSORPTION OF BARIUM CARBONATE

C/MIN/MG vs MG/CM²

Counts per Minute

Per 9

Per 8

Per 7

Per 6

Per 5

Per 4

Per 3

Per 2

Per 1

Milligram/Centimeter²
EXPERIMENTAL

The Preparation of β-dimethylaminopropiophenone Hydrochloride

The macro procedure for the preparation of this base has been well described in the literature (8, p. 360). In an attempt to adapt the synthesis for the semi-micro scale required for the use of radioactive material, a number of changes were tried.

(a) Excess Acetophenone as a Solvent:

Following the work of Plati and Wenner (10, pp. 546-9), the reaction was run in an excess of acetophenone. To ten milliliters of acetophenone was added 0.6 grams of paraformaldehyde (0.02 moles calculated as pure formaldehyde) and 2.43 grams (0.3 moles) of dimethyleamine hydrochloride. All materials dissolved on initial heating, and within ten minutes a new crystalline form precipitated. These crystals dissolved, and heating at 100°C was continued for one and one-half hours. The solution became very dark and viscous, and crystallization took place rapidly upon cooling. The product, re-crystallized from acetone solution, was identified as dimethylamine hydrochloride. The Mannich Base prepared, if any, was undetectable.

(b) Acetophenone-Ethanol Solvent:

Twice the quantities of reactants used in the above experiment were added to a mixture of 10 milliliters of acetophenone and 10 milliliters of 95% ethanol. The reaction was heated under vigorous reflux conditions for two hours, and the mixture cooled in a refrigerator overnight. The yields varied from 50-70% with the average
(based on formaldehyde) being 60%. The base was recrystallized from acetone to remove the unreacted dimethylamine hydrochloride. The filtrate from the reaction mixture was treated with dimedon to precipitate the unreacted formaldehyde.

The Preparation of o- and p-nitroacetophenone:

The ortho and para-nitroacetophenones were prepared by the procedure of Ford-Moore and Rydon (5, pp. 680-1). A three-neck, two-liter flask was equipped with a high speed stirrer and placed in a running water bath which maintained the temperature at 19°. In this flask was placed 867 grams of ethylbenzene. A mixture of 436 milliliters of concentrated HNO$_3$ and 436 milliliters of concentrated H$_2$SO$_4$ was placed in a separatory funnel and allowed to drop slowly into the ethylbenzene. The rate of addition was controlled so that the temperature of the reaction mixture was maintained at, or below, 30° throughout the addition period. This temperature was found to be fairly critical, since too low a temperature slowed the reaction to such an extent that a large concentration of acid accumulated in the flask and an explosive reaction ensued. Following the addition of the acid, the bath was heated by a steam line for two hours, with stirring. The nitrated material was separated from the acids and dried over magnesium sulfate. The products were separated by fractional distillation through a Vireaux Column under two millimeters pressure. The yields were: o-nitroethylbenzene, 383 grams (35%), b.p. 85-90°/2 mm., Index of refraction 1.5390 (reported 1.5388);
p-nitroethylbenzene, 296 grams (27%), b.p. 93-100°/2 mm., Index of refraction 1.5453 (reported 1.5159); and dinitroethylbenzene, 93.5 grams (7%), b.p. 120-5°/2 mm.

These compounds were converted to the oxime by treatment with tertiary butyl nitrite and sodium t-butoxide in t-butyl alcohol. The excess alcohol was removed by steam distillation, and the oxime was extracted from the residue with chloroform. Shaking the chloroform solution with 10% sodium hydroxide removed the oxime as the salt. The oxime was precipitated from the basic solution by bubbling CO₂ through the liquid. The melting point of the ortho oxime was 113-114° (reported 117°), and the melting point of the para oxime was 172-3° (reported 174°). No results were obtained with the dinitroethylbenzene, although Ford-Moore and Rydon reported a small yield of the oxime.

The oximes were hydrolyzed by heating under reflux with dilute sulfuric acid. The ketones were extracted from the acid solution with chloroform, dried over magnesium sulfate, and distilled under reduced pressure. The yields of the acetophenones were: o-nitroacetophenone 105.8 grams (25%) b.p. 126° at three millimeters, and p-nitroacetophenone, 65.6 grams (20%), m.p. 50°. An attempt to prepare additional dinitroethylbenzene by nitration of the mononitro compounds failed. The material exploded during the distillation.

The Preparation of o and p-nitro(β-dimethylamino)propiophenone:

The 2- and 4-nitro substituted bases were prepared by the method
of Mannich and Danneh (7, pp. 210-2). Twenty-three grams (.14 moles) of the nitroacetophenone, 13 grams (.15 moles) of dimethylamine hydrochloride, and 9 grams of paraformaldehyde (.10 moles) were dissolved in 150 milliliters of glacial acetic acid. The solution was heated under reflux for three hours after which the excess acetic acid was removed by vacuum distillation. Water was added to the residue, and the solution was extracted with ether. Evaporation of the ether left a residue of fine yellow crystals which were then recrystallized from acetone. The yield of the ortho nitro compound was 22.2 grams (87%) m.p. 178° (reported 180°). The yield of the para nitro base was 23.3 grams (90%) m.p. 199-200° (reported 204°).

Pyrolysis and Ozonolysis of \( \beta \)-dimethylaminopropiophenone:

The Mannich Base was pyrolyzed by steam distillation. Since the base was water soluble, it was dissolved in a large excess of water and distilled to near dryness. This procedure was repeated until the distillate was clear. The pyrolysate, vinyl phenyl ketone, separated as a clear, pale yellow liquid with a very sharp odor and lacrymator properties. Since the separation was not complete, a number of solvents were used to extract the material. The most satisfactory extraction solvent was chloroform. The chloroform solution was dried over calcium chloride, and the chloroform removed by distillation. The vinyl phenyl ketone was then dissolved in glacial acetic acid and subjected to ozonolysis by the procedure of Smyth and Pease (12, pp. 11-3). The ozone was passed through the solution,
which was maintained at approximately 80°, for a period of three hours. After cooling, the solution was poured into an evaporating dish with water and a small amount of granulated zinc. The phenyl glyoxal separated either as the viscous oil, or as the crystalline monohydrate. The formaldehyde was then isolated as the dimeredon derivative.

Preparation of the C-14 Labeled Base

The radioparaformaldehyde prepared by Spencer (13, pp. 20-6) weighed 1.84 grams, after the combustion samples were removed. This was 0.06 moles on the basis of pure formaldehyde. To this was added 9.70 grams (0.12 moles) of dimethylamine hydrochloride, 10 milliliters of acetophenone, and 10 milliliters of 95% ethanol, in a 250 milliliter flask. The material was heated under vigorous reflux for two hours and ten minutes. The flask was removed from the hotplate, stoppered, and allowed to remain overnight to crystallize.

The first precipitate was filtered on a sintered glass funnel, washed with alcohol and dried. The recovery was 2.155 grams (0.01 moles) or only 17% based on the formaldehyde. The mother liquor was chilled to below 0°, seeded with a crystal of the base, and a second crop of crystals was recovered from the cold solution. This raised the total recovery to 6.62 grams, (0.031 moles) or a 51% yield. The melting point of the product was 155-7° (reported 157-8°).

Decomposition of the C-14 labeled Base:

The base was dissolved in 150 milliliters of water and distilled
to near dryness. The process was repeated, and the distillate was clear after the second distillation. The vinyl phenyl ketone was extracted with five extractions of 25 milliliters each of chloroform. The chloroform solution was dried over calcium chloride and the solvent removed by distillation. The unsaturated ketone was left in the distillation flask and 100 milliliters of glacial acetic acid was added. The flask was then connected to the ozone delivery tube, and ozone was passed through the solution for a period of four hours. The reaction material was cooled and poured into a beaker containing 100 milliliters of water and two grams of granulated zinc. The mixture was then allowed to sit overnight to enable the glyoxal precipitate to flocculate.

The phenyl glyoxal was filtered, washed and counted. The filtrate was treated with 10 grams of ammonium carbonate; and, after the ebullition had ceased, 5 grams of dimedon in a saturated ethanol solution were added. The solution was chilled after remaining at room temperature for 48 hours and being seeded. The cold solution was filtered and the methylene-bis-dimedon was recovered. The final recoveries were: phenyl glyoxal monohydrate, 110 milligrams, m.p. 90° d.; methylene-bis-dimedon, 320 milligrams, m.p. 182-5° (reported 189°) or 37% based on the Mannich Base.

Note: All melting points reported were uncorrected. All organic chemicals were Eastman White Label.
SUMMARY

1. The mechanism of the Mannich Reaction is not such that it involves any transposition of the carbon atoms.

2. The mechanism may involve enolization of the carbonyl as an intermediate, since enol type structures react readily, and since substituents that would increase enolization also increase the yield of the reaction.

3. The incorporation of radioparaformaldehyde in the Mannich Reaction may lead to the synthesis of compounds having chemotherapeutic importance.
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