

[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH, U. S. PUBLIC HEALTH SERVICE, DEPARTMENT OF HEALTH, EDUCATION AND WELFARE]

t-Amine Oxide Rearrangements. N,N-Dimethyltryptamine Oxide

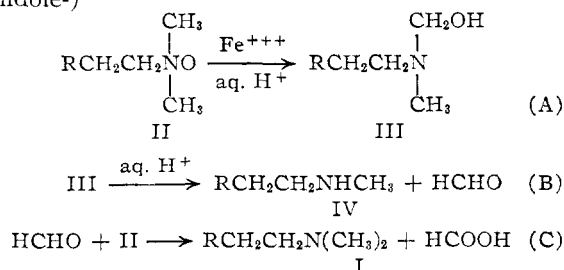
BY M. S. FISH, N. M. JOHNSON AND E. C. HORNING

RECEIVED JANUARY 30, 1956

It has been found that N,N-dimethyltryptamine oxide, a naturally occurring indole base from seeds of *Piptadenia macrocarpa* Benth., will undergo a ferric ion induced rearrangement in aqueous solution to give N-methyltryptamine and formaldehyde or formic acid. The reaction, which provides a model for biological N-dealkylation, was studied under a variety of conditions.

The function of *t*-amine oxides in biological systems is not known at the present time. The frequency with which they occur in living organisms suggests that they are something more than terminal oxidation products of amines, but in spite of this fact very little is known of their reactions. About 30 years ago the Polonovski's¹ found that oxides of the general structure (R₁)(R₂)(CH₃)NO were converted by boiling acetic anhydride into the corresponding secondary amines R₁R₂NH (as an acetyl derivative) and formaldehyde. It was recognized at the time that the over-all reaction was one of oxidative demethylation, and the suggestion was made that this reaction might be a model for naturally occurring demethylation processes. The conditions of the reaction, however, are not comparable to those of cellular systems. A few examples of acid-catalyzed or uncatalyzed N-oxide rearrangements are known; several such instances are summarized in Culvenor's review² and another has been reported recently.³ The final product or products of these reactions, after hydrolysis, are analogous to those expected from a Polonovski reaction.

We have found that it is possible to carry out a ferric ion induced rearrangement of *t*-amine oxides (R₁)(R₂)(CH₃)NO under relatively mild conditions in aqueous solution to yield as products a secondary amine, R₁R₂NH, and formaldehyde or formic acid. This reaction was studied under a variety of conditions with N,N-dimethyltryptamine oxide, a compound which was recently isolated from seeds of *Piptadenia macrocarpa* Benth.⁴ The reactions occurring in solution are presumed to be (R = 3-indole-)



The indole bases in the reaction mixture were separated by paper chromatography with a propanol-ammonia system. A typical reaction mixture analysis is shown in Fig. 1; this strip resulted from

heating a reaction mixture containing 10 μ moles of II, 30 μ moles of ferric nitrate nonahydrate and 37 μ moles of oxalic acid in 1 ml. of water for 15 minutes at 95–100°. The strip was sprayed with Ehrlich's reagent and examined with the aid of a densitometer. For purposes of identification the products indicated by the densitometer were cut and eluted separately in a run carried out for this purpose on heavier paper. The identification of the base in zone 1 as N,N-dimethyltryptamine followed the methods described in a recent paper.⁴ The N,N-dimethyltryptamine necessary for these experiments was prepared through the sequence methyl 3-indole acetate \rightarrow N,N-dimethyl-3-indoleacetamide \rightarrow N,N-dimethyltryptamine. Although the individual steps have no particular novelty, this route has not been employed previously, and it is a good method for obtaining the amine. The identification of material in zone 2 as N-methyltryptamine was made by comparison with an authentic specimen prepared by the reactions methyl 3-indoleacetate \rightarrow tryptophol \rightarrow 3-(2-bromoethyl)-indole \rightarrow N-methyltryptamine. The yield in the last step was poor, and this is not an attractive method for preparative work.

Comparisons of the products with reference samples were made in four chromatographic systems and by paper ionophoresis in a 0.01 *M* borate buffer solution. The latter procedure proved to be particularly helpful in identifying indole bases, and Table I contains migration ratios for several related indoles. N-Methyltryptamine was found to be a stronger base than N,N-dimethyltryptamine or tryptamine, and the oxide, as would be expected, was found to be a weak base.

TABLE I
IONOPHORETIC MOBILITY RATIOS FOR INDOLE BASES

Compound	R _m	Cm. from origin ^a
Urea	0.03	3.5 \pm 0.3
N,N-Dimethyltryptamine oxide	.00	3.2 \pm .1
N,N-Dimethyltryptamine	.93	14.3 \pm .1
Tryptamine	.87	13.7 \pm .3
N-Methyltryptamine	1.00	15.2 \pm .1

^a Conditions: 300 v., 3 ma., for 5 hours with 0.01 *M* pH 9.3 sodium borate buffer solution and washed Whatman 1 paper, at room temperature (22–23°).

The detection of formaldehyde or formic acid was carried out in separate experiments by a chromotropic acid test (method of Eegriev⁵).

The products of these reactions are equivalent to those obtained from a Polonovski rearrangement. We believe that the first reaction product is the car-

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

(2) C. C. J. Culvenor, *Rev. Pure Appl. Sci.*, **3**, 84 (1953).

(3) H. Tsuyuki, M. A. Stahmann and J. E. Casida, *Biochem. J.*, **59**, iv (1955).

(4) M. S. Fish, N. M. Johnson and E. C. Horning, *THIS JOURNAL*, **77**, 5892 (1955).

(5) F. Feigl, "Spot Tests," Vol. II, Elsevier Publishing Co., New York, N. Y., 1954, p. 240.

binolamine III (reaction A) and that hydrolysis of this intermediate leads to the secondary amine IV and formaldehyde (reaction B). Since formic acid and the parent amine (I) are also products, reaction (C) must be included in the sequence. A recent study by Boekelheide⁶ on the rearrangement of 2-methylpyridine oxide in boiling acetic anhydride (Polonovski conditions) indicated that this reaction followed a free-radical mechanism, and it was suggested that the same mechanism held for *t*-amine oxide rearrangements in acetic anhydride. The considerable difference in conditions makes it difficult to compare the Polonovski reaction and that described here. Until conclusive data on the mechanism of the ferric ion induced reaction are at hand, it is probably best to regard the two rearrangements as being related but not necessarily identical in mechanism. A more extensive discussion of the Polonovski reaction (with projected mechanisms) has been published.⁷

Numerous experiments were carried out to determine the effect of variations in the reaction conditions. These are summarized in the Experimental section. It was found that other metal ions could not replace Fe^{+++} ; those tried were Co^{++} , Ni^{++} , Cu^{++} , Mg^{++} , Mn^{++} and Zn^{++} . The effect of adding Fe^{+++} in different chelated forms will be described in a following paper. By using a ferric-tartrato complex ion in solution, it is possible to work at a higher pH (4–8) with less ferric ion than is required at pH 1–2. These circumstances do not alter the yield of N-methyltryptamine, but the possibility of isolating an intermediate (as III) is increased. Other amine oxides are being studied to obtain information about the mechanism and degree of generality of this reaction.⁸

A number of biological and chemical questions are raised by these results. If reaction A is reversible, it may provide a key to biological N-alkylation and N-dealkylation reactions in which a one-carbon fragment at the oxidation level of formaldehyde is involved.⁹ The demonstration of a specific catalytic effect, the occurrence of the reaction in aqueous solution and the analogy between the products and what is known of cellular dealkylations suggest that an enzyme-induced rearrangement may be possible. Studies on the enzymatic formation and reactions of N-oxides are in progress to test these possibilities.¹⁰ Chemical questions relating to other products are also important. Reaction (A) might be accompanied by a reaction leading to $\text{RCH}_2\text{-CHOHNMe}_2$ as a product; this would give $\text{RCH}_2\text{-CHO}$ and dimethylamine on hydrolysis. Work by Lecoq,¹¹ in extension of the Polonovski reaction, has shown that acetaldehyde and acetic acid result from the rearrangement of suitable N-ethylamine

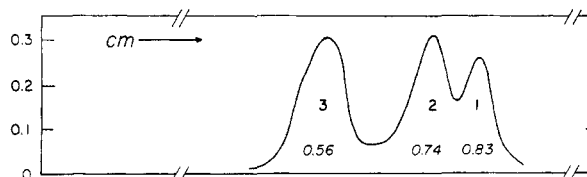


Fig. 1.—Densitometer data for rearrangement of N,N-dimethyltryptamine oxide. Compounds indicated are: (1) N,N-dimethyltryptamine; (2) N-methyltryptamine; (3) N,N-dimethyltryptamine oxide. R_f values are for maximum absorption.

oxides, and it seems unlikely that the ferric ion induced rearrangement will be limited to N-oxide \rightarrow methylolamine reactions. Another problem involves the products to be expected from III. In the case described here, the reaction conditions are such as to lead to hydrolysis to IV. Under other conditions the carbinolamine III might cyclize to N-methyltetrahydronorharman; this compound is not detectable with Ehrlich's reagent and consequently would not appear in the analyses employed here. To explore this problem, work is proceeding on the rearrangement of bufotenine oxide⁴ under circumstances where 6-hydroxy-N-methyltetrahydronorharman can be detected.

Experimental¹²

Ferric Ion Induced Rearrangement.—A typical reaction mixture, brought to a total of 1 ml., contained 10 μ moles of the N-oxide, 30 μ moles of ferric nitrate nonahydrate and 37 μ moles of oxalic acid and had a pH of 1.3. After heating at 95–100° for 15 minutes, the mixture was cooled and aliquot portions (0.05 ml. was usually found to be most suitable) were placed on paper. The developing system was propanol–ammonia (5:1, propanol:1 N ammonium hydroxide) with a developing time of 16 hr. at room temperature (22°). The spray was 0.5% *p*-dimethylaminobenzaldehyde in 1 N hydrochloric acid. The indole areas were brought out at room temperature; usually 15–20 minutes were required for the development of the sprayed areas. Rates of development and shades of color were compared routinely by visual observation. Under these circumstances, the ferric ion present in the original solution was deposited at the origin, and the separation of the indole bases normally gave R_f values nearly the same as those observed for pure compounds. Figure 1 shows a densitometer (Photovolt Model 510, 545 $m\mu$ filter) record obtained by examination of a typical paper strip. The reaction was conducted at 95–100° for 15 minutes; the paper was washed S&S 589R, and the strip was examined 1.5 hr. after spraying. Visual examination was usually satisfactory for detecting the presence of N-methyltryptamine in zone 2.

Identification of Products.—Several sheets of washed Whatman 3 MM paper were loaded with a reaction mixture and were developed with propanol–ammonia. The product zones were cut and eluted with ethanol. The material from zone 1 was compared with an authentic sample of N,N-dimethyltryptamine, using the paper chromatographic solvent systems I, II, III and IV specified in a previous paper.⁴ An authentic sample of N-methyltryptamine was compared with the compound from zone 2. We find that washed S&S 589R paper gives excellent results in the identification of indole bases in these systems, and it may therefore be helpful to record R_f values for this combination of paper and systems.

	R_f values for 589R paper—			
	I	II	III	IV
N,N-Dimethyltryptamine	0.83	0.95	0.72	0.66
N-Methyltryptamine	.75	.94	.74	.70
N,N-Dimethyltryptamine oxide	.59	.68	.80	.74

(12) All melting points were taken with a Kofler hot stage. Infra-red spectra were obtained with a Perkin-Elmer Model 21 instrument.

(6) V. Boekelheide and D. L. Harrington, *Chem. and Ind.*, 1423 (1955).

(7) E. Wenkert, *Experientia*, **10**, 346 (1954). Expected products from amine oxide rearrangements and their possible part in the biogenesis of alkaloids are discussed.

(8) M. S. Fish, C. C. Sweeley and E. C. Horning, *Chem. and Ind.*, R 24 (1956).

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

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

(11) H. Lecoq, *Bull. soc. roy. sci. Liege*, **12**, 484 (1943).

The time required for development, at room temperature, varied from 16 hr. for I to 64 hr. for IV. Comparisons were made in the usual way, with concurrent development of an eluted sample, a reference compound and a mixture.

The effect of hydrogen peroxide on the amines was compared. N,N-Dimethyltryptamine may be oxidized on paper with a drop of 3% hydrogen peroxide immediately before developing. The resultant chromatogram always shows an N-oxide area, and this is one of the best means of confirming the identity of the tertiary amine. The secondary amine is not affected by peroxide under these conditions. Samples suspected to be mixtures, if treated in this way, will show the oxide area if the tertiary amine is present. When the components of zones 1 and 2 were examined in this way, the material eluted from zone 2 gave no oxide spot, while the component of zone 1 gave the expected reaction.

Paper ionophoretic experiments confirmed the identity of the component of zone 2. The apparatus was a variation of a hanging-paper design; a 0.01 M sodium borate buffer solution was employed as the electrolyte. The solvent was permitted to rise by capillary action to wet the entire paper, and the separation was carried out at 300 v. and 3 milliamperes for 5 hr. at 22–23°. Urea was used as a neutral reference compound. The values in Table I are distance ratios for each compound, based on N-methyltryptamine = 1.00. For convenience, typical cm. measurements are also indicated; these data were obtained with a pH 9.3 borate solution and washed Whatman 1 paper. The point of maximum optical density, after spraying with Ehrlich's reagent, was taken for distance measurements. Comparisons for establishing identity were made in the usual way, with concurrent running of the eluted sample, the reference compound and a mixture, together with the ionophoretic references.

Effects of Temperature, Time and Acid Ions.—A number of experiments were carried out under varying conditions of temperature and time, with a ferric ion to oxalate ratio of 3:1 in an oxalate mixture. At 90–95°, runs were made for 3, 5, 15, 30 and 60 minutes. No effect on the yield of secondary amine was noted. Runs at room temperature (22–23°) for 1–5 hr. were compared with those at 55–60°. The yield of secondary amine was materially lowered in the room temperature runs. No attempt was made to carry out rate studies in greater detail. The effect of anions was studied by employing comparable reaction conditions (55–60°, 15 minutes, 3:1 ratio) for mixtures containing formic acid, oxalic acid or hydrochloric acid. In the case of both formic and hydrochloric acids, zone 1 at times contained a barely detectable amount of unidentified by-products. Two such by-products, both of which were neutral substances, were observed. These were not found when oxalic acid was present, and consequently most runs were made in the presence of oxalate ion.

A series of runs was also made to determine the effect of variations in the Fe^{+++} /oxalate ratio. With a constant amount of oxide (10 μ moles), varying quantities of Fe^{+++} and oxalic acid were employed. The total volume was 1.0 ml.; the mixture was heated at 60° for 15 minutes; the pH was measured after the reaction was completed. The first two runs showed a slight reduction in yield of N-methyltryptamine, but no appreciable difference was found in the other cases.

Fe^{+++} , μ moles	Oxalic acid, μ moles	pH
9	5	2.5
	9	2.3
	18	2.1
	27	1.5
	54	1.5
27	13	1.4
	27	1.3
	54	1.0
	81	1.0
	162	1.0

It is likely that Fe^{+++} in these experiments was present as an oxalato complex ion,¹³ but the independence of yield and Fe^{+++} /oxalate ratio, together with the fact that other an-

ions may be used, does not indicate a dependency on a specific complex ion.

Effect of Metal Ions.—Using a formate or chloride system (oxalate was generally not suitable) experiments were carried out with a 3:1 mole ratio of metal ion to oxide at 55–60° and at 90–100° for 15 minutes. The reaction mixtures were analyzed on washed S&S 589R paper with a propanol-ammonia system. No rearrangement resulted with Co^{++} , Cu^{++} , Ni^{++} , Mg^{++} , Mn^{++} or Zn^{++} .

Under similar conditions, with an oxalate system of constant composition (37 μ moles/ml.), the Fe^{+++} /oxide ratio was varied through 0.3:1, 1:1, 3:1 and 9:1. The best yield of secondary amine was obtained with a 3:1 ratio, but the yield with a 1:1 ratio was only very slightly lower. The 0.3:1 ratio gave only a small quantity of products, while at 9:1 the oxide was destroyed.

Product Measurements.—Attempts were made to determine the amount of each base in the reaction mixture. For this purpose, paper chromatographic reference areas were developed and sprayed in a standardized way, and densitometer measurements were made after a fixed time interval. It was found that rough estimates could be made in this way for total concentrations up to about 40 μ g. for each case. With this information, a typical run was analyzed at the appropriate dilution. It was found that the ratio of tertiary amine to secondary amine was approximately 1:1 and that the total amount of indole bases accounted for less than half of the starting oxide. Since the oxide was partially destroyed during the reaction, the present conditions are not well adapted for rate or yield studies, and no further work of this kind was done.

Identification of Formaldehyde or Formic Acid.—A few ml. was distilled from a typical reaction mixture. The distillate was treated with dilute hydrochloric acid and magnesium shavings to reduce formic acid to formaldehyde, and the latter compound was detected by a chromotropic acid color test. The procedure, essentially due to Eegriwe, was that described by Feigl.⁶ With runs of 50 μ moles of oxide, the first few drops of distillate usually gave the color test directly, indicating that traces of formaldehyde were present. In separate runs it was determined that oxalate ion in the original solution caused no interference. No attempt was made to show, in separate experiments, that the oxide and formaldehyde would react; such studies were carried out by Lecoq¹¹ for other examples.

3-(2-Methylaminoethyl)-indole (N-Methyltryptamine).

A.—Methyl 3-indoleacetate was obtained from 3-indoleacetic acid following the esterification procedure of Jackson.¹⁴ The yield from 30.0 g. of acid was 28.6 g. (89%) of colorless oil, b.p. 160–163° (0.9 mm.), reported¹⁴ in the neighborhood of 180° (2 mm.).

B. Tryptophol.—Reduction of the methyl ester with lithium aluminum hydride was carried out in a usual way to yield 96% of colorless solid melting at 58.5–59.5°. Recrystallization from benzene raised the m.p. to 59–60°. This material has been prepared by reduction of the ester with sodium in alcohol¹⁵ and by reduction of the acid with lithium aluminum hydride in 65% yield¹⁶ (reported m.p. 58–59°, 57–58°).

C.—3-(2-Bromoethyl)-indole was prepared according to Hoshino.¹⁵ From 3.0 g. of tryptophol there was obtained 3.5 g. (81%) of colorless material melting at 100–102°, reported¹⁵ m.p. 98–99°, yield 60%.

D.—N-Methyltryptamine was prepared by the method of Hoshino¹⁵ by heating the halide with methylamine at 100° in a sealed apparatus. The yield of pure amine was very poor (ca. 5–8%), m.p. 89–90°. The reported m.p. was 89–90°, with no yield given.¹⁵

The orange *picrate* melted at 193–195° after recrystallization from methanol; reported¹⁵ m.p. 190–191°.

N,N-Dimethyl-3-indoleacetamide.—A mixture of 16.0 g. of methyl 3-indoleacetate, 100 ml. of ethylene glycol and 19.4 g. of anhydrous dimethylamine was stirred at room temperature for 40 hr. The mixture was poured into 100 ml. of water and extracted with five 100-ml. portions of ether-ethyl acetate (1:1). This extract was washed with a little water, dried and evaporated to give a red oil. This was taken up in warm ethyl acetate and, on chilling, three

(14) R. W. Jackson, *J. Biol. Chem.*, **88**, 659 (1930).

(15) T. Hoshino and K. Shimodaira, *Ann.*, **520**, 19 (1935).

(13) N. V. Sidgwick, "Chemical Elements and their Compounds," Oxford University Press, Vol. II, 1951, p. 1364.

(16) H. R. Snyder and F. J. Pilgrim, *This Journal*, **70**, 3770 (1948).

crops of colorless crystals weighing a total of 12.5 g. (70%) were obtained; m.p. 126–128°. Samples melting at 116–117° were also obtained by recrystallization from ethyl acetate. Late samples gave only the higher m.p.

Anal. Calcd. for $C_{12}H_{14}ON_2$: C, 71.26; H, 6.98; N, 13.85. Found: C, 71.07; H, 6.82; N, 13.37.

The infrared spectra in chloroform of the two samples with different m.p. were identical, with a strong amide CO band at 6.10μ .

3-(2-Dimethylaminoethyl)-indole (N,N-Dimethyltryptamine).—A suspension of finely-divided amide (2.1 g.) in 100 ml. of ether was added to a slurry of 0.8 g. of lithium alumi-

num hydride in 50 ml. of ether, and the mixture was heated under reflux for 4 hr. The mixture was treated in the usual way, and the final organic extract of the amine gave, after recrystallization from hexane, 1.6 g. (85%) of material melting at 47–49°. This material was converted to a higher melting form (71–73°) by crystallization from hexane after seeding with an authentic specimen of m.p. 73–74°. The infrared spectra in chloroform of the two samples were identical.

(17) We are indebted to Dr. M. E. Speeter of the Upjohn Co. for this sample.

BETHESDA 14, MARYLAND

[CONTRIBUTION FROM THE MCARDLE MEMORIAL LABORATORY, THE MEDICAL SCHOOL, UNIVERSITY OF WISCONSIN]

Studies on the Structure of the Skin Protein-bound Compounds Following Topical Application of 1,2,5,6-Dibenzanthracene-9,10- C^{14} . II. Nature of the 2-Phenylphenanthrene-3,2'-dicarboxylic Acid-Protein Bond^{1,2}

BY P. M. BHARGAVA AND CHARLES HEIDELBERGER

RECEIVED NOVEMBER 21, 1955

We have previously shown that 2-phenylphenanthrene-3,2'-dicarboxylic acid (PDA) is obtained on the alkaline hydrolysis of the protein-bound compounds following the application of 1,2,5,6-dibenzanthracene-9,10- C^{14} to the skin of mice. By hydrazine treatment of the proteins and by carrier experiments, it has now been demonstrated that 25% of the protein-bound radioactivity involves the binding of PDA to the protein, partly through the diamide and partly through a monoamide of the acid.

In 1947, the Millers³ demonstrated that following the feeding of the potent hepatic carcinogen, *p*-dimethylaminoazobenzene, to rats, some dye was bound to liver proteins. Subsequently, it has been found that several hydrocarbons and 2-acetylaminofluorene are also bound to the proteins of susceptible tissues.^{4–9} These findings have led to the formulation of the "protein deletion" hypothesis of carcinogenesis, which states that as a result of the chemical interaction of the carcinogen with the proteins, an enzyme system important to the control of growth is deleted, thus initiating the production of a cancer cell.⁵ In view of the probable importance of this chemical binding in the carcinogenic process, it is essential to determine the structure of the carcinogen-protein complex. Work along these lines, using 1,2,5,6-dibenzanthracene-9,10- C^{14} (DBA) has been in progress in this Laboratory for some time, and it has been reported¹⁰ that 2-phenylphenanthrene-3,2'-dicarboxylic acid (PDA) is obtained following rather drastic hydrolysis of several fractions of skin proteins. It then became important to determine whether this acid was

produced as an artifact during the isolation procedure or is bound directly to the proteins. The work reported here demonstrates that PDA is bound to the proteins through its carboxyl groups in amide linkage.

Results and Discussion

It is known that imides of certain dicarboxylic acids, such as phthalic acid,¹¹ give cyclic hydrazides on treatment with hydrazine. Sheehan and Frank¹² have further shown that treatment with hydrazine hydrate of phthalimides substituted on the nitrogen with peptide groups, gives the cyclic hydrazide of phthalic acid and the free peptide. Similarly, it is also known that amides (*e.g.*, benzamide^{13,14}) and esters (*e.g.*, the dimethyl and diethyl esters of diphenic acid^{15,16}) of aromatic acids give their hydrazides on treatment with hydrazine. If, therefore, PDA were bound to the protein(s) through its carboxyl groups as an ester, amide or imide, hydrazine treatment of the protein would result in cleavage of the peptide-metabolite bond and thus yield radioactivity extractable into organic solvents. According to Akabori,¹⁷ hydrazine treatment of proteins splits the amino acid chain, giving rise to the hydrazides of free amino acids, although his conditions were somewhat different from those of Sheehan and Frank. The extent of degradation of the protein was, however, immaterial to us, as we are concerned only with the structure of the DBA metabolite

(1) This work was supported in part by a research grant, C-1132, from the National Cancer Institute, National Institutes of Health, Public Health Service, and in part by a grant from the Wisconsin Section of the American Cancer Society.

(2) An abstract of part of this work appears in *Proc. Am. Assoc. Cancer Research*, **2**, 5 (1955).

(3) E. C. Miller and J. A. Miller, *Cancer Research*, **7**, 468 (1947).

(4) E. C. Miller, *ibid.*, **11**, 100 (1951).

(5) E. C. Miller and J. A. Miller, *ibid.*, **12**, 547 (1952).

(6) W. G. Wiest and C. Heidelberg, *ibid.*, **13**, 246, 250, 255 (1953).

(7) M. M. Moodie, C. Reid and C. A. Wallick, *ibid.*, **14**, 367 (1954).

(8) D. S. Tarbell, E. G. Brooker, P. Seifert, A. G. Fluka and T. J. Hall, Abstracts Am. Chem. Soc., 126th Meeting, Sept. 12–17, 1954, p. 5N.

(9) C. Heidelberg and M. G. Moldenhauer, *Proc. Am. Assoc. Cancer Research*, **2**, 24 (1955).

(10) P. M. Bhargava, H. I. Hadler and C. Heidelberg, *THIS JOURNAL*, **77**, 2877 (1955).

(11) H. D. K. Drew and H. H. Hatt, *J. Chem. Soc.*, 16 (1937).

(12) J. C. Sheehan and V. S. Frank, *THIS JOURNAL*, **71**, 1855 (1949).

(13) G. Struve, *J. prakt. Chem.*, **50**, 295 (1894).

(14) W. J. Hickinbottom, "Reactions of Organic Compounds," Longmans, Green and Co., Ltd., London, 1948, pp. 230–231.

(15) L. Kalb and O. Gross, *Ber.*, **59**, 736 (1926).

(16) W. Borsche, W. Müller and C. A. Bodenstein, *Ann.*, **475**, 120 (1929).

(17) S. Akabori, K. Ohno and K. Narita, *Bull. Chem. Soc. Japan*, **25**, 214 (1952).