Transmission of Substituent Effect.—We may write the structure of any set in the form XGY where X is the substituent, Y, the reaction site (if any), and G, the group to which X and Y are attached. The transmission of substituent effects may now be described by eq. 5[4,5] where ρ_G is the reaction constant for the group

$$\gamma = \frac{\rho_G}{\rho_G^\circ} \qquad (5)$$

G under consideration, undergoing some reaction under specified conditions, and ρ_G° is the reaction constant for the reference group G° undergoing the same reaction under the same conditions. Some values of γ for groups G requiring the use of σ_m in correlations are given in Table V.

TABLE V
VALUES OF γ

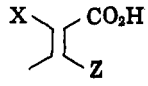
G	γ	G	γ
m-Phenylene	1.00[a]	Carbonylmethyl	3.34[c]
trans-Cyclopropylene	1.97[b]	*trans*-Vinylene	2.23[b]
Vinylidene	4.25[c]	Ethynylene	1.89[b]
Oximino	2.37[c]	Cyclopropylidene	5.36[b]

[a] ρ_G° is for the ionization of benzoic acids in water at 25°. [b] See ref. 5. [c] This work.

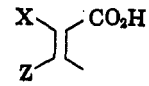
The value of γ for the oximino group is strikingly less than that for the vinylidene group. Unfortunately, no direct comparison can be made with the carbonyl group, as hydration of the carbonyl group in substituted carbonyl carboxylic acids apparently is quite extensive. Thus in these compounds the measured ionization constant is a composite. Presumably, however, γ of the carbonyl group will be greater than or at least the same magnitude as the value of γ for the carbonylmethyl group.

The vinylidene group is very much more sensitive to substituent effects than is the *trans*-vinylene group as is shown by their γ-values of 4.25 and 2.23, respectively. This is in accord with the molecular geometry of the two groups.

Effect of Constant Substituents.—A comparison of ρ-values for sets bearing the same constant substituent but differing in configuration (IVa,b) is of interest.



IVa



IVb

We may compare the sensitivity of the two configurations to substituent effects by means of the ratio below.

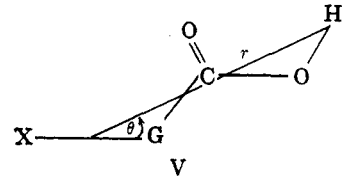
$$q = \frac{\rho_{cis}}{\rho_{trans}} \qquad (6)$$

Values of q are given in Table VI. To provide an additional value of q, ρ-values for the short sets *cis*- and *trans*-2-substituted crotonic acids (10, 11) were evaluated. The data used are given in Table I; the results are in Table III. The available values of q both show the *cis* configuration to be more sensitive to vinylidene

TABLE VI
VALUES OF q

Z	ρ_{cis}	ρ_{trans}	q
Ph	−4.42	−3.76	1.2
Me	−4.43	−3.95	1.1

substituent effects. This may be due to a decrease in the X–C-1–C-2 bond angle and/or a rotation of the CO_2H group out of the plane of the double bond. Either or both of these phenomena would affect the distance r and angle θ which determine the magnitude of ρ(V).[14]



V

(14) M. Charton, Abstracts, 137th National Meeting of the American Chemical Society, Cleveland, Ohio, April 1960, p. 92O; Abstracts, 140th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 961, p. 57T.

Studies on Methylglyoxal Bis(guanylhydrazone)[1] Analogs. IV. Acetylation Studies[2]

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Midwest Research Institute, Kansas City, Missouri

Received October 1, 1964

Acetylation of methylglyoxal bis(guanylhydrazone) (I) has been studied. The diacetylated derivative was prepared by the reaction of ketene with I. The structure of this product was confirmed by an alternate and unambiguous synthetic method using methylglyoxal dihydrazone and S-methylacetylthiourea hydroiodide. The tetraacetylated derivative of I was prepared by the reaction of I with either acetic anhydride or ketene, and its structure was substantiated with the aid of n.m.r. studies.

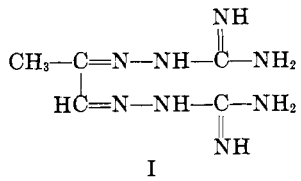
Since the discovery of the antileukemia activity of methylglyoxal bis(guanylhydrazone)[3] (I), a systematic investigation of compounds related to I has been undertaken in our laboratories.[4] One phase of the study centered about the preparation of acylated derivatives of I, which by alteration of the polarity of the molecule

(1) The *Chemical Abstracts* name for this compound is 1,1′-[(methyl)-ethanediylidenedinitrilo]diguanidine.

(2) This investigation is supported by the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Public Health Service, Contract SA-43-ph-3025.

(3) (a) B. L. Freedlander and F. A. French, *Cancer Res.*, **18**, 360 (1958); (b) F. Freireich and E. Frei, III, *Proc. Am. Assoc. Cancer Res.*, **3**, 319 (1962).

(4) (a) E. G. Podrebarac, W. H. Nyberg, F. A. French, and C. C. Cheng, *J. Med. Chem.*, **6**, 283 (1963); (b) F. Baiocchi, C. C. Cheng, W. J. Haggerty, Jr., L. R. Lewis, T. K. Liao, W. H. Nyberg, D. E. O'Brien, and E. G, Podrebarac, *ibid.*, **6**, 431 (1963); (c) E. G. Podrebarac and C. C. Cheng, *ibid.*, **7**, 806 (1964).



I

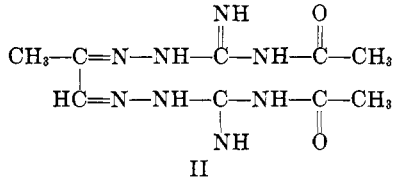
would change its lipid solubility and absorption.[5] These derivatives would undoubtedly modify the intercellular transport characteristics of I, and thus perhaps yield compounds with better therapeutic indices.

Although direct acylation of amidines with acyl halides has been reported,[6] a search of the literature reveals that the reaction has been successfully employed only with the N-substituted amidines. Arylsulfonyl chloride, on the other hand, reacts with unsubstituted amidines to form both mono- and disulfonamidines, the proportion varying with the amount of the sulfonyl chloride used and with the temperature.[7]

The preparation of monoacetylguanidine hydrochloride, according to Korndörfer,[8] can be achieved by the reaction of acetyl chloride with guanidine hydrochloride. In our laboratories no acetylated product was formed when acetyl chloride was used as the acetylating agent with either the hydrochloride salt or the free base[4b] of methylglyoxal bis(guanylhydrazone) under various conditions.

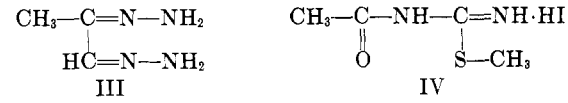
Wolff[9] treated the aminoguanidine derivative of dextrose with a mixture of acetic anhydride and sodium acetate and reportedly obtained acetylation of the amino group of the aminoguanidine moiety in addition to the hydroxyl groups of the sugar.[10] However, when these reaction conditions were employed by us for the preparation of acetylated derivatives of I, the reaction failed and unchanged starting material was recovered.

Under strictly anhydrous conditions, free base of I could be acetylated by means of acetic anhydride in pyridine to form a diacetylated derivative II, which melted around 210°. Since the yield was extremely low and the experimental work could not be duplicated each time, the method is quite impractical. Consequently, other approaches have also been explored.

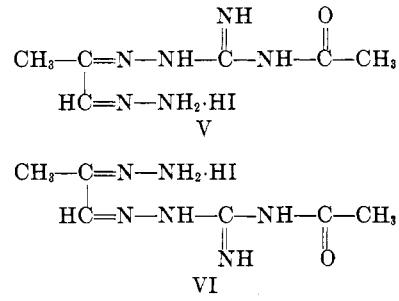


II

Methylglyoxal dihydrazone (III), readily obtained by the reaction of pyruvic aldehyde with an excess of hydrazine, was treated with 2 equiv. of N-acetyl-S-methylthiourea hydriodide (IV). The product isolated melted at 165.5–167° with decomposition. The ultraviolet absorption maximum of the product was 289 mμ at pH 1 and 301 mμ at pH 11. The product gave a positive ionizable halide test. Elemental analysis indicated that the product was not the expected II but rather one of the following.



III IV



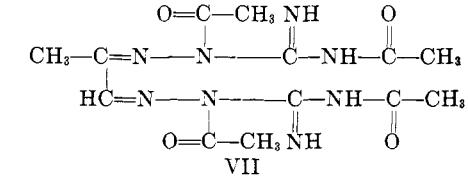
V

VI

The possibility of the product being a mixture of V and VI was ruled out by n.m.r. studies. Only two sharp and distinct CH_3 signals were observed at 1.97 and 2.13 p.p.m. On the basis of inductive effect of the glyoxal methyl moiety, the structure of the product is assigned as V.

The desired diacetylated derivative II was finally obtained when compound III was treated, at low temperature, with 2 equiv. of IV in the presence of sodium bicarbonate. The product, which melted at 215° with decomposition, had ultraviolet absorption maxima of 296 mμ at pH 1 and 335 mμ at pH 11. The n.m.r. spectra clearly indicated a glyoxal methyl absorption at 2.15 and two terminal acetyl methyls at 2.20 and 2.22 p.p.m.

An alternate synthesis of II has also been achieved by the reaction of the methylglyoxal bis(guanylhydrazone) free base (I) with ketene. The highly pure diacetylated derivative obtained by this process was found to be identical in every respect with that obtained from the previously described condensation method. When the ketene reaction was carried out for a longer period, a different solid product was obtained. This product, which melted at 201–203°, was also prepared in better yield by reaction of acetic acid-free acetic anhydride with the free base of I at 70–90°. On the basis of elemental analysis, ultraviolet absorption (312 mμ at pH 1 and 238 and 340 mμ at pH 11),[11] and n.m.r. studies[12] the product was assigned the following tetraacetyl structure, VII.



VII

(5) *Cf.* L. R. Lewis, R. K. Robins, and C. C. Cheng, *J. Med. Chem.*, **7**, 200 (1964), and references cited therein.

(6) R. L. Shriner and F. W. Neumann, *Chem. Rev.*, **35**, 351 (1944), and references cited therein.

(7) E. H. Northey, A. E. Pierce, and D. J. Kertesz, *J. Am. Chem. Soc.*, **64**, 2763 (1942).

(8) G. Korndörfer, *Arch. Pharm.*, **241**, 449 (1903).

(9) H. Wolff, *Ber.*, **27**, 971 (1894).

(10) The structure of Wolff's product was not proven. The analytical data—the product was reported to contain 1 equiv. of water—suggested that the product could conceivably be a monoacetate salt of (pentaacetyl-*d*-glucosyl)guanylhydrazone.

(11) A study of the ultraviolet absorption behavior of methylglyoxal bis(guanylhydrazone) derivatives will be published shortly by Dr. T. S. Hermann and co-workers from our institute.

(12) The n.m.r. spectrum of this compound was taken at 25° in $CDCl_3$ (internal standard, tetramethylsilane). Sharp singlets were observed at 8.13 (aldimine CH) and 2.28 p.p.m. (glyoxal methyl). The four acetyl CH_3 absorptions were noted at 2.20, 2.24, 2.37, and 2.46 p.p.m. (integral of protons, in each case, ~3). This rules out the possibility that all four acetyl groups are present at the terminal amidine groups.

Comparison of the various acetylation methods used in this study indicates that the ketene method is to be recommended for the preparation of the diacetylated derivative of I. The methylglyoxal dihydrazone condensation method is not only unequivocal but may be readily extended to the synthesis of other acylated compounds. For the preparation of tetraacetylated derivatives, the direct acetylation using acetic anhydride is the method of choice. Preparation of other acylated compounds by this method, obviously, depends upon the availability of the corresponding anhydrides.

Experimental[13]

A Convenient Preparation of Methylglyoxal Bis(guanylhydrazone) Free Base.—Preparation of the free base has been reported in one of our previous publications.[4b] We have since then developed a simpler and less time-consuming method, which is described as follows.

To 26.6 g. (0.1 mole) of finely powdered methylglyoxal bis(guanylhydrazone) dihydrochloride was added 100 ml. of 8% (0.2 mole) warm, freshly prepared sodium hydroxide solution. The resulting slightly yellowish, clear solution was rapidly flash evaporated to dryness, and the residue was triturated with 150–200 ml. of boiling anhydrous methanol. The insoluble inorganic salt was filtered and the filtrate was again evaporated to dryness. The residue was stirred with a small amount of 95% ethanol and filtered to give 14.5–17.5 g. (75–91% yield) of the free base, which melted at 230–232° with decomposition.

Methylglyoxal Dihydrazone (III).—To a solution of 156 g. (4.7 moles) of 95% hydrazine in 300 ml. of 95% ethanol cooled in an ice–water bath was added dropwise, with vigorous stirring, 336 g. (2 moles) of 43% pyruvic aldehyde. The resulting solution was flash evaporated at 50–60° to a sirupy liquid. On standing, a solid product formed. The crude compound was filtered and recrystallized from absolute ethanol to give 75 g. of pure III, m.p. 96–97°. An additional 20–25 g. of product was obtained from the filtrate to give a total yield of 48–50%: $\lambda_{max}^{pH\ 1}$ 292 mμ (ε 10,100), $\lambda_{max}^{pH\ 11}$ 266 mμ (ε 14,400).

Anal. Calcd. for $C_3H_8N_4$: C, 36.0; H, 8.00; N, 56.0. Found: C, 36.3; H, 8.14; N, 56.3.

N-Acetyl-S-methylthiourea Hydroiodide (IV).—To a partial solution of 118 g. (1 mole) of N-acetylthiourea in 300 ml. of anhydrous methanol was added 142 g. (1 mole) of methyl iodide in one portion. The resulting solution was stirred under anhydrous conditions for 3–4 hr. and then flash evaporated at room temperature to dryness. The white residue was washed with absolute ethanol to give 200–210 g. (78–80% yield) of pure IV, m.p. 148–149°.

Anal. Calcd. for $C_4H_8N_2OS \cdot HI$: C, 18.4; H, 3.46; N, 10.8. Found: C, 18.2; H, 3.04; N, 10.6.

Methylglyoxal 1-Hydrazone-2-(4-acetylguanylhydrazone)[14] (V).—To a suspension of 5 g. (0.05 mole) of III in 80 ml. of absolute ethanol was added 26.4 g. (0.1 mole) of IV. The resulting mixture was stirred vigorously at room temperature for 2 hr. Evolution of methylmercaptan began immediately. The resulting precipitate was filtered and washed repeatedly with cold ethanol and finally with ether to give 12 g. of light yellow powder. The crude product melted at 151° dec. Recrystallization from a mixture of ethanol and water raised the melting point to 165.5–167° dec.

Anal. Calcd. for $C_6H_{12}N_6O \cdot HI$: C, 23.1; H, 4.17; N, 26.9. Found: C, 23.6; H, 4.46; N, 26.9.

The free base was obtained as yellow prisms by triturating the hydriodide salt with concentrated aqueous ammonia and recrystallizing from 95% ethanol: m.p. 181.5–183°; $\lambda_{max}^{pH\ 1}$ 290 mμ (ε 14,850), $\lambda_{max}^{pH\ 11}$ 298 mμ (ε 10,500); R_f (25°, descending) 0.54 (5% NH_4HCO_3), 0.52 (80% C_2H_5OH–10% concentrated HCl–10% H_2O), and 0.45 (BuOH saturated with acetic acid).

Anal. Calcd. for $C_6H_{12}N_6O$: C, 39.2; H, 6.52; N, 45.7. Found: C, 39.5; H, 6.84; N, 46.0.

Methylglyoxal Bis(ω-acetylguanylhydrazone)[15] (II). Method A.—To a mixture of 5.2 g. (0.05 mole) of III and 10 g. of sodium bicarbonate suspended in 120 ml. of anhydrous methanol was added, with stirring and cooling, 26.4 g. (0.1 mole) of IV. The slurry was stirred for 40 min. and filtered; the inorganic solid was washed well with 50 ml. of methanol. The combined filtrate was flash-evaporated at room temperature to a viscous residue, which was taken up with 60 ml. of ethyl acetate. The ethyl acetate solution was allowed to stand for 3 hr. at room temperature during which time a solid separated. The solid was filtered and then washed well with concentrated aqueous ammonia to give 3.8 g. of II, which decomposed at 188°. The product, after recrystallization from a mixture of methanol and water, melted at 215° with decomposition: $\lambda_{max}^{pH\ 1}$ 296 mμ (ε 43,700), $\lambda_{max}^{pH\ 11}$ 334 mμ (ε 36,000); R_f (25°, descending) 0.14 (5% NH_4HCO_3), 0.11 (80% C_2H_5OH–10% concentrated HCl–10% H_2O), and 0.30 (BuOH saturated with acetic acid).

Anal. Calcd. for $C_9H_{16}N_8O_2$: C, 40.3; H, 6.00; N, 41.7. Found: C, 40.2; H, 6.34; N, 41.5.

Method B.—Ketene, prepared in quantities of 0.64 mole/hr.,[16] was passed into a stirred suspension of 5.0 g. (0.027 mole) of finely powdered methylglyoxal bis(guanylhydrazone) free base in 100 ml. of anhydrous methanol.[17] As ketene was passed through the solution, all the solid rapidly dissolved followed by gradual formation of a yellow solid. After 1 hr. the generation of ketene was discontinued and 2.2 g. of pale yellow crystals melting at 213–214° with decomposition were collected by filtration. An additional 1.0 g. of the product was isolated from the refrigerated filtrate. Total yield was 44%. Recrystallization from methanol raised the melting point to 214–215° dec. The product was found to be identical in every respect with that obtained by the previous method.

Method C.—To a slurry of 9.2 g. (0.05 mole) of methylglyoxal bis(guanylhydrazone) free base in 10.2 g. (0.1 mole) of acetic anhydride was added dropwise, with stirring, 7.9 g. (0.1 mole) of freshly distilled pyridine. A moderate exothermic reaction soon started and the reaction temperature was kept <45°. The reaction mixture gradually changed from a yellow slurry to a brown, viscous solution. It was heated on a steam bath for 2 hr. and then cooled. The solid product was filtered and recrystallized from a mixture of ethanol and water to yield 0.5 g. of product. The product was identical in every respect with that prepared by methods A and B.

Methylglyoxal Bis(1,3-diacetylguanylhydrazone)[18] (VII). Method A.—When ketene was introduced to a stirred suspension of 5.0 g. (0.027 mole) of I (free base) in 100 ml. of anhydrous methanol, the solution became clear in about 10 min. As more ketene was passed through the solution, a yellow solid gradually formed and after about 1 hr. the solid slowly redissolved. The stream of ketene was continued for an additional 3 hr. (a longer period of time does not increase the yield). The resulting yellow solution was evaporated to half of its original volume under reduced pressure. After refrigerating overnight, 1.7 g. (18% yield) of pale yellow crystals were isolated: m.p. 201–203° dec.; $\lambda_{max}^{pH\ 1}$ 312 mμ (ε 21,120), $\lambda_{max}^{pH\ 11}$ 238 mμ (ε 13,730) and 340 mμ (ε 25,340); R_f (25°, descending) 0.74 (5% NH_4HCO_3), 0.05 (80% C_2H_5OH–10% concentrated HCl–10% H_2O), and 0.52 (BuOH saturated with acetic acid).

Anal. Calcd. for $C_{13}H_{20}N_8O_4$: C, 44.3; H, 5.70; N, 31.8. Found: C, 44.5; H, 6.00; N, 32.0.

Method B.—A mixture of 5 g. of I (free base) and 30 g. of acetic anhydride (freshly redistilled over calcium carbonate) was heated in an oil bath at 70–90° for 40 min. A clear solution was obtained within 15 min. after which a precipitate gradually formed. The reaction mixture was refrigerated overnight and the pale yellow crystalline product was filtered, washed well with ethanol, and dried at 80° to give 3.5–4 g. (37–43% yield), m.p. 201–203°. The product was found to be identical in every respect with that prepared by the preceding procedure.

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(13) All melting points (corrected) were taken on a Thomas–Hoover melting point apparatus. The ultraviolet absorption spectra were determined with a Beckman DK-2. The infrared spectra were taken with a Perkin-Elmer Infracord No. 137, and the n.m.r. was measured with the Varian A-60 high resolution n.m.r. spectrometer.

(14) 3-Acetyl-1-(1-methyl-2-hydrazonoethylidenenitrilo)guanidine.

(15) 3,3′-Diacetyl-1,1′-[(methyl)ethanediylidenedinitrilo]diguanidine.

(16) G. Quadbeck, in "Newer Methods of Preparative Organic Chemistry," Vol. II, W. Foerst, Ed., F. K. Kirchner, Trans., Academic Press Inc., New York, N. Y., 1963, p. 133.

(17) During the reaction ketene slowly converted methanol into methyl acetate. When methyl acetate was used as the initial reaction solvent, however, the yield of acetyl derivative was drastically reduced.

(18) 1,1′,3,3′-Tetraacetyl-1,1′-[(methyl)ethanediylidenedinitrilo]diguanidine.

A much lower yield (1.7 g.) was obtained when the diacetate of I (7.0 g.) was used in place of the free base.

Acknowledgment.—The authors wish to express their appreciation to Dr. Ralph L. Shriner of Southern Methodist University, Drs. Verne C. Bowen, Jack D. Davidson, and Harry B. Wood, Jr., of the National Cancer Institute, Mr. Frederic A. French of Mt. Zion Hospital and Medical Center, Dr. William E. McEwen of the University of Massachusetts, and Dr. Eugene G. Podrebarac of our institute for their interest and suggestions. They are also indebted to Dr. James J. Downs for the n.m.r. interpretations, and to Mr. Hal P. Van Fossen, Mrs. Margaret L. Rounds, and Mr. John R. Gravatt for their analytical and instrumental measurements.

Acetophenone Mesitylhydrazone in the Fischer Indole Synthesis. Migration of a Phenacyl Group[1]

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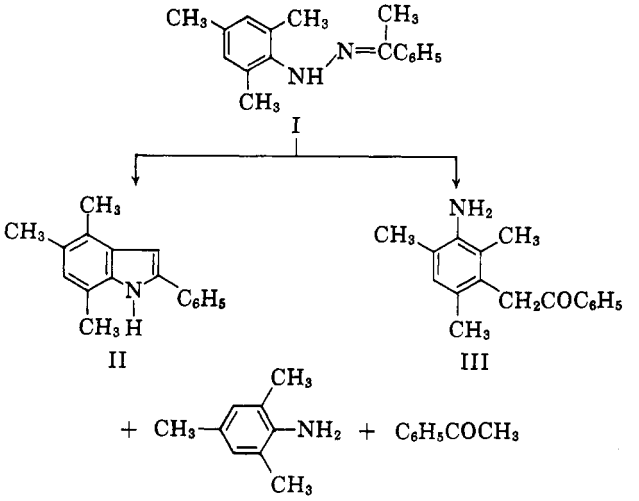
Received October 28, 1964

Acetophenone mesitylhydrazone (I) was transformed by zinc chloride in nitrobenzene at 120–135° into 2-phenyl-4,5,7-trimethylindole (II, 10%), product of a 1,2 methyl migration; 3-phenacylmesidine (III, 2%), product of a 1,2 phenacylimino group migration; mesidine (16%); and acetophenone (8%). The indole II and 3-phenacylmesidine (III) were identified with products of independent synthesis. Their formation is rationalized by postulating an intermediate analogous to those proposed to account for similar reactions encountered previously; in this case the intermediate leads in part to II and in part to III. Mesidine and acetophenone are postulated to be derived from the tautomer of I by disproportionation of hydrogen.

The Fischer reaction of acetophenone 2,6-xylylhydrazone, conducted in nitrobenzene with zinc chloride as the promoter, led to 2-phenyl-3a,5-dimethyl-3a,4,7,-7a-tetrahydro[3H]pseudoindolone-4 and 2-phenyl-4,7-dimethylindole.[3] No methyl group migration was involved in the formation of the ketonic product; a 1,2-methyl migration must have accompanied that of the indole. Cyclohexanone mesitylhydrazone, on the other hand, was converted in acetic acid into 6,7,8-trimethyl-1,2,3,4-tetrahydrocarbazole, the result of an apparent 1,4-methyl migration.[4]

The differences in behavior of these two *ortho* dimethylated arylhydrazones under the conditions of the Fischer reaction might be ascribed to the structural differences in the ketone and/or the aryl groups, or differences in the medium might be chiefly responsible. For the purpose of deriving evidence bearing on the structural factors, acetophenone mesitylhydrazone (I) was next selected for investigation in the Fischer reaction; this substance, of course, comprises the ketone fragment of one of the arylhydrazones previously studied and the arylhydrazine moiety of the other. The observations derived from this investigation form the subject of this paper.

Acetophenone mesitylhydrazone (I), prepared in 92% yield from acetophenone and mesitylhydrazine,[4] was not stable in air and accordingly had to be used promptly after its isolation. Like acetophenone 2,6-xylylhydrazone, I underwent reaction in nitrobenzene in the presence of zinc chloride at 120–135°. The reaction mixture afforded four products: 2-phenyl-4,5,7-trimethylindole (II, 10%), a product of 1,2-methyl migration; 3-phenacylmesidine (III, 2%); mesidine (16%); and acetophenone (8%).



Mesidine was characterized through its known N-acetyl derivative, acetophenone by its refractive index and infrared spectrum. The indole II was synthesized independently by the Fischer reaction of acetophenone 2,4,5-trimethylphenylhydrazone.

In addition to II, the Fischer reaction of acetophenone 2,4,5-trimethylphenylhydrazone yielded a compound whose composition and spectroscopic properties were consistent with those to be expected of a higher homolog of the ketonic substance which was the principal product derived from acetophenone 2,6-xylylhydrazone.[3] The ultraviolet, infrared, and n.m.r. spectra left little doubt that the compound was one of the isomers IV or V. The single, broad, vinyl proton signal at τ 4.16 in the n.m.r. spectrum might be consistent with V as well as with IV, since models indicate that the vinyl and the adjoining tertiary hydrogens in V are inclined at a dihedral angle of nearly 90°, so that splitting of the vinyl proton signal might not occur.[5] More

(1) Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Carnegie Institute of Technology.

(2) Esso Foundation Fellow, 1961–1962.

(3) R. B. Carlin and D. P. Carlson, *J. Am. Chem. Soc.*, **79**, 3605 (1957); **81**, 4673 (1959).

(4) R. B. Carlin and M. S. Moores, *ibid.*, **81**, 1259 (1959); **84**, 4107 (1962).

(5) *Cf.* M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); *J. Am. Chem. Soc.*, **85**, 2870 (1963).