From the acetone washings was obtained 0.60 g. more of this compound, total yield 37%, and a yellow solid, 0.70 g., 10% yield, *melting 102–104°, decomposing at 110°. Crystallization from* warm alcohol converted it to the white compound, m.p. *ca.* 150°.

Kinetic Studies.—The reaction vessel was a 100-ml. flask, essentially filled with toluene, immersed in an oil bath, the temperature of which was controlled to ± 0.03. The gas-outlet tube was in part insulated and the temperature in the remainder and in the gas burette was controlled to within $\pm 0.10°$ by circulating liquid from a second thermostat. The flask was evacuated several times and flushed with a stream of nitrogen, saturating the solvent. While nitrogen was flowing, the sample, about 1 mmole, in a Teflon holder, was placed in a neck of the flask, supported by an iron bar. The flask was sealed, lowered into the bath, and equilibrated, only the sample being above the bath level. An internal paddle stirrer coupled to an alnico magnet located in a submerged neck of the flask was rotated rapidly by a magnet placed above the bath. After equilibration, the support was withdrawn from under the sample by a magnet, the azo compound dissolved quickly, and nitrogen began to evolve. A sensitive manometer activated the gas volume measuring system. The manometer was a small Pyrex U-tube containing water, one arm connected to the gas-outlet tube, the other having one fixed and one movable contact in a Teflon bushing which was adjusted to a point slightly above the water level when the system was at atmospheric pressure. The effective diameters of the two arms of the manometer were different so that change in *liquid level in the contact arm was sensitive to change in pressure.* Contact activated a relay which started a motor and screw device which pulled on the plunger of a syringe which drew liquid from the gas buret maintaining the system at atmospheric pressure. The gas buret was read easily at frequent intervals.

Absorption spectra of the azo compounds were determined on a Perkin-Elmer Model 202 visible-ultraviolet spectrophotometer. For each of the compounds there is listed the solvent, absorption maxima, and log of the extenction coefficients. Phenylazotriphenylmethane: toluene, 284 mμ, 4.0; 424, 1.5; hexane, 267, 4.0; 272, 4.0; 421 2.3. *o*-Nitrophenylazotriphenylmethane: toluene, 283 mμ, 3.9; 424, 2.6; hexane, 267, 4.2; 273, 4.2; 425, 2.6. *p*-Nitrophenylazotriphenylmethane: toluene, 287 mμ, 4.0; 441, 2.5; hexane, 267, 4.2; 282, 4.2; 440, 2.7. 2,4-Dinitrophenylazotriphenylmethane: toluene, 284 mμ, 4.0; 439, 2.7; hexane, 267, 3.9; 273, 3.9; 442, 2.3. *o*-Nitrophenylazo-9-phenylfluorene: toluene, 285 mμ, 4.0; 406, shoulder, 2.5. *p*-Nitrophenylazo-9-phenylfluorene: toluene, 285 mμ, 4.5; 424, shoulder, 2.5; hexane, 267, 4.6; 273, 4.6; 429, 2.5; ethanol, 211, 5.0; 277, 4.8; 412, shoulder, 2.8. 2,4-Dinitrophenylazo-9-phenylfluorene: toluene 285 mμ, 4.3; 406, 2.8; hexane, 266, 4.3; 272, 4.3; ethanol, 209, 4.9; 275, 4.5; 412, shoulder, 2.8.

Some Reactions of Diazomethane with Carbon Dioxide and Ammonia in Aqueous Solution

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Carbon dioxide was allowed to react with diazomethane, prepared by alkaline hydrolysis of N-methyl-N-nitroso-N′-nitroguanidine. The different products were separated by gas chromatography, collected separately, and identified as dimethyl carbonate, methyl carbamate, N-methyl methyl carbamate, and N-dimethyl methyl carbamate. Methyl carbamate and its N-methyl derivatives were formed because of the presence of ammonia in the ethereal solution of diazomethane. Dimethyl carbonate, methyl carbamate, N-methyl methyl carbamate, and N-dimethyl methyl carbamate were found to be the products of the reaction between ammonia, carbon dioxide, and diazomethane, in aqueous solution. A mechanism for the formation of methyl carbamate and its N-methyl derivatives from ammonia, carbon dioxide, and diazomethane in the presence of water is proposed.

Diazomethane has been used in our laboratory in the search for unstable intermediates in the carboxylation reaction of photosynthesis—*i.e.*, the carboxylation of ribulose-1,5-diphosphate to give phosphoglyceric acid, the first stable product in the carbon reduction cycle of photosynthesis. It usually was prepared by alkaline hydrolysis of N-methyl-N-nitroso-N′-nitroguanidine.[2] In all the experiments with diazomethane ^{14}C-bicarbonate was present in the reaction mixture. It is known that the product of the reaction of bicarbonate with diazomethane is dimethyl carbonate. However, by the use of paper and vapor phase chromatography for the separation of the products of the reaction of ^{14}C-bicarbonate and diazomethane, three other ^{14}C-labeled compounds were found in addition to dimethyl carbonate. These were identified as methyl carbamate, N-methyl methyl carbamate, and N-dimethyl methyl carbamate. The carbamate nitrogen atom was found to have its origin in ammonia present in the solution inadvertently at first (as a by-product of the hydrolysis of the nitroguanidine) and later deliberately added. The mechanism of the formation of the methyl carbamates has been investigated.

Experimental

Preparation of Diazomethane.—An ethereal solution of diazomethane was prepared either by alkaline hydrolysis of N-methyl-N-nitroso-N′-nitroguanidine as described by McKay,[2] or from N-methyl-N-nitroso-*p*-toluenesulfonamide (Diazald) as described by Backer and de Boer.[3] Both compounds were obtained from Aldrich Chemical Co.

Reaction of $H^{14}CO_3^-$ with Diazomethane.—A 0.50-ml. solution of $H^{14}CO_3^-$ (1.98 mc./ml.; 0.06 *M*) was mixed with an ethereal solution of diazomethane until the yellow color persisted. The reaction mixture was left at room temperature for a few hours, and overnight in the deep freeze (−16°). The solution was concentrated under a current of air at 5° and the different radioactive components of the mixture were separated by gas chromatography in conjunction with a proportional counter.[4–7]

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[1] (a) Abstracted from a thesis submitted by George A. Akoyunoglou, August, 1962, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California, Berkeley; (b) partially supported by the Greek State Scholarship Foundation; (c) Ames Research Center, National Aeronautics and Space Administration, Moffett Field, Calif.; (d) The preparation of this paper was sponsored by the U. S. Atomic Energy Commission.

[2] A. F. McKay, *J. Am. Chem. Soc.*, **70**, 1974 (1948); A. F. McKay, W. L. Taylor, M. N. Buchanan, and J. F. Crooker, *Can. J. Res.*, **28B**, 683 (1950).

[3] H. J. Backer and Th. J. de Boer, *Proc. Koninkl. Ned. Akad. Wetenschap.*, **54B**, 191 (1951); *Chem. Abstr.*, **46**, 1961 (1952).

[4] I. M. Whittemore, University of California Radiation Laboratory Quarterly Report, UCRL-9408, p. 49, September, 1960.

[5] G. Akoyunoglou, thesis, University of California, Berkeley, UCRL-10352, August, 1962.

[6] R. Wolfgang and F. S. Rowland, *Anal. Chem.*, **30**, 903 (1958).

[7] R. Wolfgang and C. F. McKay, *Nucleonics*, **16**, No. 10, 69 (1958).

Reaction of Gaseous Carbon Dioxide with Diazomethane.—Gaseous carbon dioxide (100%) was bubbled through an inlet tube into the ethereal solution of diazomethane at 2° until the solution became colorless. The flask containing the ethereal solution of diazomethane had also a small amount of water (2.5 ml.). The inlet tube was dipped below the surface of the water layer. The solution was concentrated at 5° under a current of air, and the different components of the reaction mixture were separated by vapor phase chromatography and collected separately.

Vapor Phase Chromatography.—A Wilkens standard gas chromatograph, Model A90, connected with a Brown recorder and a 10-ft. EGS (ethyleneglycol polyester of succinic acid) column, were used for the separation and identification of the different compounds. A collector, in conjunction with the chromatography unit, was used to collect the different compounds separately. The collector was cooled by a Dry Ice–acetone bath or by liquid nitrogen.

Preparation of Methyl Carbamate (Methylurethan).—Methyl carbamate was prepared from methyl chlorocarbonate and ammonia as described by Hartman and Brethen.[8] The methyl chlorocarbonate (carbomethoxychloride) was prepared by the procedure described by Bergmann and Zervas for the preparation of carbobenzoxychloride.[9] The phosgene, necessary for the preparation of methyl chlorocarbonate, was prepared by the procedure described by Vogel.[10]

Anal. Calcd. for $C_2H_5O_2N$: C, 32.00; H, 6.66; N, 18.66; O, 42.66. Found: C, 31.79; H, 6.58; N, 17.95.

Preparation of N-Methyl Methyl Carbamate.—N-Methyl methyl carbamate was prepared from dimethyl carbonate and methylamine as described by Delepine and Schving.[11]

Anal. Calcd. for $C_3H_7O_2N$: C, 40.45; H, 7.86; N, 15.73; O, 35.95. Found: C, 40.57; H, 7.94; N, 15.60.

Preparation of N-Dimethyl Methyl Carbamate.—N-Dimethyl methyl carbamate was prepared from dimethyl carbonate and dimethylamine as described by Delepine and Schving.[11]

Anal. Calcd for $C_4H_9O_2N$: C, 46.60; H, 8.74; N, 13.59, O, 31.07. Found: C, 46.85; H, 8.52; N, 13.17.

Results

Vapor phase chromatography in conjunction with a proportional counter was used for the separation of the different radioactive products of the ^{14}C-bicarbonate and diazomethane[12] reaction. It was found that the mixture consisted of five radioactive compounds as shown in Fig. 1. These were identified as carbon dioxide, dimethyl carbonate (DMC), N-dimethyl methyl carbamate (III), N-methyl methyl carbamate (NMMCR), and methyl carbamate (MCR).

Gas and paper chromatography of the untreated aqueous solution of $NaH^{14}CO_3$ showed that there were no radioactive impurities present, and that the bicarbonate was the precursor of all (four) compounds which were formed by action of diazomethane on $NaH^{14}CO_3$.

The same compounds were formed when gaseous carbon dioxide ($C^{12}O_2$) reacted with diazomethane in solution, as is shown in Fig. 2 and 3.

Identification of the Products.—For the identification of the products, larger amounts (nonradioactive) were prepared from gaseous carbon dioxide and diazomethane. The components of the reaction mixture were separated and purified by gas chromatography and collected separately.

The identification of them was based on:

(1) The analysis for C, H, N, O, and determination of molecular weight; (2) the study of the mass and infra-

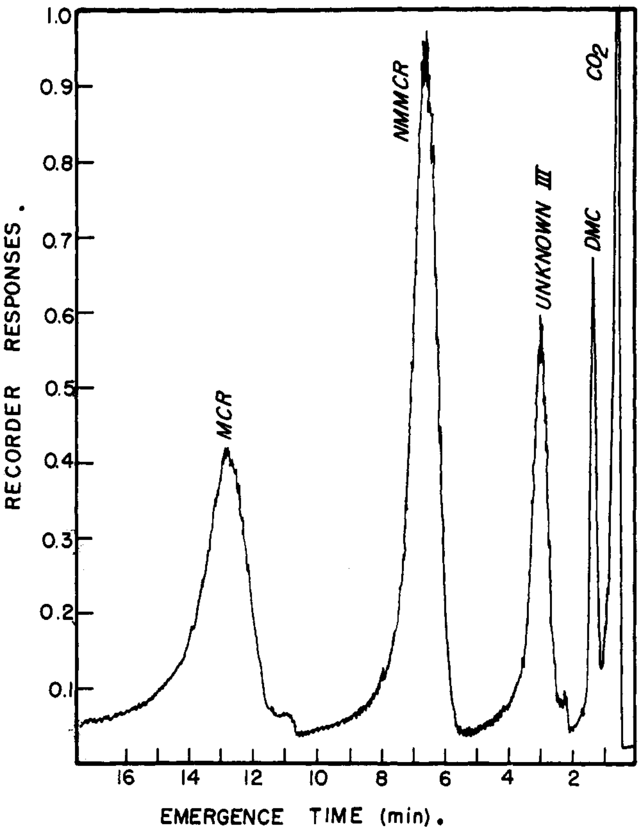

Fig. 1.—Gas chromatogram of the radioactive mixture of the products of the reaction $NaH^{14}CO_3$ with diazomethane prepared from N-methyl-N-nitroso-N′-nitroguanidine. The recorder response is proportioned to radioactivity. DMC is dimethyl carbonate; unknown III is N-dimethyl methyl carbamate; NMMCR is N-methyl methyl carbamate; and MCR is methyl carbamate.

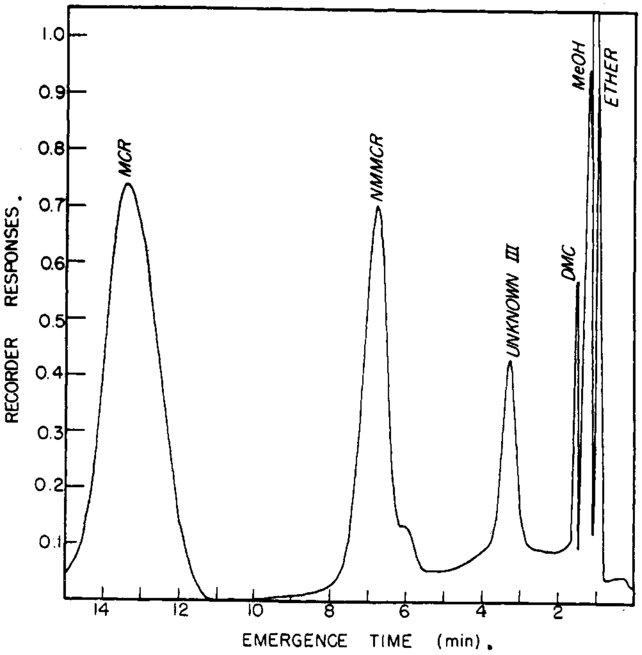

Fig. 2.—Gas chromatogram of the nonradioactive mixture of the products of the reaction of carbon dioxide with diazomethane prepared from N-methyl-N-nitroso-N′-nitroguanidine. The recorder response is proportional to the mass of material. DMC is dimethyl carbonate; unknown III is N-dimethyl methyl carbamate; NMMCR is N-methyl methyl carbamate; and MCR is methyl carbamate.

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[8] W. W. Hartman and M. R. Brethen, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 278.

[9] M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

[10] A. I. Vogel, "Textbook of Practical Organic Chemistry," Longmans Green, New York, N. Y., 1956, p. 185.

[11] M. Delepine and F. Schving, *Soc. chim. France*, **7**, 894 (1910).

[12] If it is not otherwise stated, the ethereal solution of diazomethane was prepared by alkaline hydrolysis of N-methyl-N-nitroso-N′-nitroguanidine.

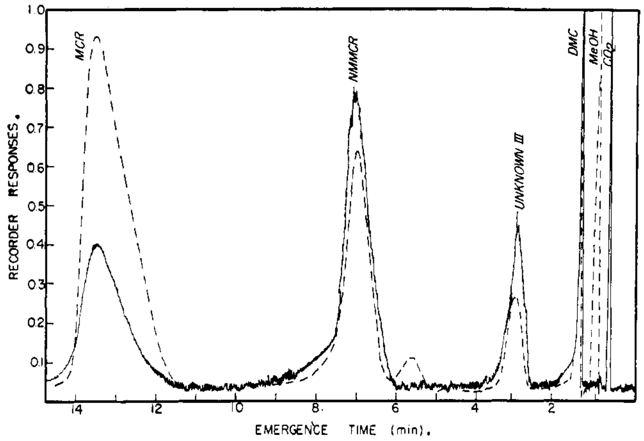

Fig. 3.—Gas cochromatogram of the radioactive mixture of the products of the reaction $NaH^{14}CO_3 + CH_2N_2$, and the nonradioactive products of the reaction $CO_2 + CH_2N_2$. Diazomethane was prepared from N-methyl-N-nitroso-N′-nitroguanidine. Solid line is the radioactivity recording; dotted line is the mass recording. DMC is dimethyl carbonate; unknown III is N-dimethyl methyl carbamate; NMMCR is N-methyl methyl carbamate; and MCR is methyl carbamate.

red spectra, and their comparison with the mass and infrared spectra of authentic marker compounds; and (3) the cochromatography of the unknown compounds or their hydroxamate derivatives with authentic marker compounds (*e.g.*, Fig. 3).

Gas cochromatography of the radioactive products, which were collected separately, with authentic dimethyl carbonate, N-dimethyl methyl carbamate, N-methyl methyl carbamate, and methyl carbamate, respectively, showed in each case an exact coincidence of the radioactivity with the mass peaks.

Analysis of the products for C, H, N, and O content and determination of molecular weight are shown in Table I.

TABLE I

Analysis for	% C	% H	% N	Mol. wt.
Found for DMC	39.81	6.72	...	88.0
Calcd. for $C_3H_6O_3$	40.00	6.66	...	90.0
Found for NDMMCR	46.48	8.86	13.25	109.0
Calcd. for $C_4H_9NO_2$	46.60	8.74	13.59	103.0
Found for NMMCR	40.63	7.82	15.49	84.0
Calcd. for $C_3H_7NO_2$	40.45	7.86	15.73	89.0
Found for MCR	31.61	6.71	17.75	74.0
Calcd. for $C_2H_5NO_2$	32.00	6.66	18.66	75.0

The comparison of the mass spectra of the products with the mass spectra of dimethyl carbonate, N-dimethyl methyl carbamate, N-methyl methyl carbamate, and methyl carbamate, respectively, showed an exact coincidence in the place and number of peaks and in the relative abundance of each peak. Identification of the products was finally confirmed by the identity of their infrared spectra with that of authentic dimethyl carbonate, N-dimethyl methyl carbamate, N-methyl methyl carbamate, and methyl carbamate, respectively. The relative yield of each product is approximately 40% dimethyl carbonate, 35% methyl carbamate, 20% N-methyl methyl carbamate, and 5% N-dimethyl methyl carbamate.

Discussion

The appearance of compounds containing only one nitrogen atom as a result of a reaction of diazomethane was so unusual as to prompt us to seek some other source for that nitrogen atom than diazomethane itself. This was established by demonstrating the absence of carbamates in reactions of diazomethane prepared from N-methyl-N-nitroso-*p*-toluenesulfonamide, and suggested as the source of the single atom of nitrogen a by-product of the N-nitroso-N-methyl-N′-nitroguanidine hydrolysis. This was easily demonstrated to be free ammonia. The presence of ammonia can be accounted for by the alkaline hydrolysis of the imide group of N-methyl-N-nitroso-N′-nitroguanidine. The hydrolysis can take place before or after the formation of diazomethane. In the first case N-methyl-N-nitroso-N′-nitrourea is formed according to the reaction

$$CH_3N(NO)C(NH)NHNO_2 + H_2O \xrightarrow{HO^-} CH_3N(NO)CONHNO_2 + NH_3$$

which is further hydrolyzed to yield diazomethane. In the second case, diazomethane is formed first, and the residue is further hydrolyzed to give ammonia.

Mechanism of the Reaction.—Presumably dimethyl carbonate was formed by the action of diazomethane on carbonic acid (reaction of diazomethane with acidic hydrogen) according to Arndt,[13] Eistert,[14] or Roberts[15] mechanisms. Methyl carbamate was formed by the action of diazomethane on ammonium carbamate, which is present in a mixture of carbon dioxide and ammonia. Three hypotheses were considered to account for the formation of N-methyl methyl carbamate and N-dimethyl methyl carbamate from ammonia, carbon dioxide, and diazomethane.

(a) Methyl carbamate may be formed first, which then reacts with diazomethane to give its N-methyl derivatives.

(b) Diazomethane may react with ammonia to give methylamine and dimethylamine, which form the salts of N-methyl carbamic acid and N-dimethyl carbamic acid in the presence of carbon dioxide. The acids may be further methylated by the action of diazomethane to yield the corresponding methyl esters.

(c) The carbamic acid, present in a mixture of ammonia and carbon dioxide, may react with diazomethane to form its N-methyl derivatives (N-methyl carbamic acid and N-dimethyl carbamic acid), and the products are further methylated to yield the corresponding methyl esters.

The first (a) hypothesis is not correct, because: (1) continued action of diazomethane on the reaction mixture did not show an increase in the amount of NMMCR and NDMMCR, as would be the case if they were formed by methylation of methyl carbamate; (2) action of diazomethane on ^{14}C-MCR, or unlabeled MCR, showed no formation of any N-methyl derivative.

The second (b) hypothesis also is not correct because: (1) the same relative amount of each product was

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[13] F. G. Arndt, "Organic Analysis," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1953, p. 221.

[14] B. Eistert, *Angew. Chem.*, **54**, 99 (1941); translated and revised by F. W. Spangler in "Newer Methods of Preparative Organic Chemistry," 1st Ed., Interscience Publishers, Inc., New York, N. Y., 1948, pp. 513-570.

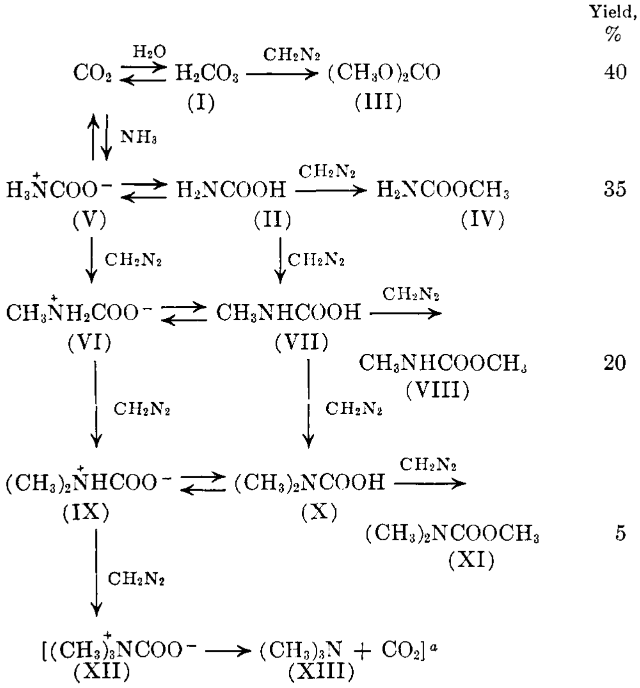
[15] G. D. Roberts, W. Watamore, and R. E. McMahon, *J. Am. Chem. Soc.*, **73**, 760 (1951).

formed when either a new or an old preparation of diazomethane was used; (2) all attempts to prepare methyl derivatives of ammonia by the action of diazomethane on gaseous ammonia, or aqueous ammoniacal solution or solutions of ammonium salts were unsuccessful.

Table II shows the proposed mechanism for the formation of the products of the reaction of ammonia, carbon dioxide, and diazomethane in the presence of water, and the different reactions that may take place, according to the third hypothesis.

Table II

Mechanism of the Reaction of Diazomethane with an Aqueous Solution of a Mixture of Carbon Dioxide and Ammonia



[a] It is not known if trimethylamine is really formed because no attempts were made to detect it.

Carbon dioxide reacts either with water to form carbonic acid (I) or with ammonia to give carbamic acid (II). Compound I with diazomethane gives dimethyl carbonate (III). Compound II is in equilibrium with compound V, its zwitterion. Diazomethane reacts with compounds II and V to give methyl carbamate (IV) and N-methyl carbamic acid (VII) or the N-methyl derivative of the zwitterion (VI), respectively. Compound VI is in equilibrium with compound VII, and diazomethane reacts with both of them to give N-methyl methyl carbamate (VIII) and N-dimethyl carbamic acid (X) or compound IX, respectively, and so on. It is not known whether diazomethane reacts with compound IX to give the betaine (XII), which, being unstable, would give trimethylamine (XIII) and carbon dioxide, because no attempts were made to detect trimethylamine. If trimethylamine is formed, the yield would be very low, as can be seen from the low yield of its precursors.

The mechanism of the methylation of the various intermediates (Table II) with diazomethane is probably similar to the mechanism proposed by Arndt[13] or Roberts[15] for the methylation of compounds containing acidic hydrogen.

The proposed mechanism finds support in the observation by Kuhn and Ruelius[16] that extended action of diazomethane on the aqueous solution of an amino acid produced, as final product, the corresponding betaine. Some intermediates (*e.g.*, the methyl ester of the amino acid) were present in the case when the reaction was allowed to proceed for a short time only. Because of the basicity of the amino group, and the presence of water, a self-hydrolysis of the methyl esters occurred, reforming the free acids, thus permitting the quantitative transformation of the amino acid to betaine. In the case of the carbamates the methyl ester of carbamic acid, and methyl esters of the N-methyl derivatives of carbamic acid (compounds IV, VIII, and XI) once formed, cannot be self-hydrolyzed, even in the presence of water, because the amide group is not so strong a basic group as the amino group of amino acids. Therefore, a mixture of all these compounds is the final product of the above reaction.

Summary

Carbon dioxide was allowed to react with diazomethane, prepared by alkaline hydrolysis of N-methyl-N-nitroso-N′-nitroguanidine. The different products were separated by gas chromatography, collected separately, and identified as dimethyl carbonate, methyl carbamate, N-methyl methyl carbamate, and N-dimethyl methyl carbamate. Methyl carbamate and its N-methyl derivatives were formed because of the presence of ammonia in the ethereal solution of diazomethane.

Dimethyl carbonate, methyl carbamate, N-methyl methyl carbamate, and N-dimethyl methyl carbamate were found to be the products of the reaction between ammonia, carbon dioxide, and diazomethane, in aqueous solution. A mechanism for the formation of methyl carbamate and its N-methyl derivatives from ammonia, carbon dioxide, and diazomethane in the presence of water is proposed.

It is thus to be expected that any experiments designed to trap unstable products formed from radioactive carbon dioxide in the presence of any enzyme protein will lead to the appearance of large amounts of carbon dioxide fixed as methyl carbamate on the free amino groups of the protein as well as any amino acid which may be present. In fact, such observations have been made with carboxydismutase.[5]

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(16) R. Kuhn and H. W. Ruelius, *Ber.*, **83**, 420 (1950).