m. p. 146°. The yields obtained by this method ranged from 50–70%; the reported yield[14] was 22%, m. p. 150–151°.

The residue from the ether extraction was apparently polymeric, failed to melt at 320°, was quite insoluble in water and organic solvents, but was soluble in base.

2-Hydroxy-4-chloromethylthiazole phenylurethan melted, after crystallization from pentane–ethyl ether, at 119.5–120.5°. *Anal.* Calcd. for $C_{11}H_9N_2O_2SCl$: C, 49.16; H, 3.38. Found: C, 49.35; H, 3.21.

Oxidation of 1-Methoxy-3-chloro-2-propanol (XV).—1-Methoxy-3-chloro-2-propanol[29] was oxidized by sodium dichromate and sulfuric acid.[30] The product was separated from the reaction mixture by ether extraction, and following conventional treatment of the extracts it was purified by fractional distillation. The main fraction boiled 72–73° (25 mm.), or 39–40° (6 mm.). Several refractionations gave a colorless lachrymatory liquid $n^{20}D$ 1.4470, the refractive index of which remained constant on further refractionation. On standing it darkened with evolution of hydrogen chloride; no satisfactory analysis was obtained, presumably because of this decomposition.

The oxidation product reacted readily to form typical ketone derivatives. On the basis of mixed m. p. observations these products were proved to be identical with the corresponding bis-derivatives of methyglyoxal. (The latter were prepared from chloroacetone by refluxing with an ethanol solution of the appropriate reagent.) The following types of derivatives were prepared from the oxidation product: *p*-nitrophenylhydrazone, m. p. 296–297° (dec.); 2,4-dinitrophenylhydrazone m. p. 292–293° (dec.); phenylhydrazone m. p. 145–146°; semicarbazone, m. p. 250–251.5° (dec.). The melting points of the corresponding bis-derivatives of methylglyoxal are reported in the literature as follows: *p*-nitrophenylhydrazone, m. p. (dec.) values given vary from 277 to 302°[31]; 2,4-dinitrophenylhydrazone, m. p. 299–300°[32] (dec.); phenylhydrazone, m. p. 148°[33]; semicarbazone, m. p. 254°.[34]

(29) Fourneau and Ribas, *Bull. soc. chim. France*, **39**, 1584 (1926); Koelsch, THIS JOURNAL, **65**, 2460 (1943); Flores-Gallardo and Pollard, *J. Org. Chem.*, **12**, 831 (1947).

(30) *Cf.* Conant and Quayle, "Organic Syntheses," Coll. Vol. I, pp. 211–213.

(31) See "Beilstein," Vol. XV, p. 472.

(32) Bülow and Seidel, *Ann.*, **439**, 48 (1924).

(33) Wohl and Lange, *Ber.*, **41**, 3612 (1908).

(34) Knöpfer, *Monatsh.*, **32**, 753 (1911).

ROCHESTER, NEW YORK RECEIVED MAY 6, 1950

Summary

1. 2-Methyl-4,5-dicarbethoxythiazole has been reduced to 2-methyl-4,5-bis-(hydroxymethyl)-thiozole, an analog of pyridoxine.

2. 2-Amino-4,5-dicarbethoxythiazole has been reduced with lithium aluminum hydride to 2-amino-4-hydroxymethyl-5-carbethoxythiazole. Both of these compounds have been reduced to 2-amino-4-hydroxymethyl-5-methylthiazole.

3. 2-Amino-4-methyl-5-carbethoxythiazole has been reduced with lithium aluminum hydride to 2-amino-4,5-dimethylthiazole, which has also been prepared by the reaction of sulfuryl chloride, methyl ethyl ketone and thiourea.

4. 2-Amino-4-carbethoxythiazole has been reduced to 2-amino-4-hydroxymethylthiazole by lithium aluminum hydride.

5. The ultraviolet absorption curves of a group of thiazoles have been observed and interpreted. These support the structures assigned to the reduction products of 2-amino-4,5-dicarbethoxythiazole. The effect of resonance interactions of substituents on the wave length of maximum absorption has been discussed. Modifications of this effect due to salt formation have also been considered.

6. The oxidation of 1-methoxy-3-chloro-2-propanol gives a product which reacts to give derivatives of methylglyoxal.

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY AND VITAL ECONOMICS, UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE AND DENTISTRY]

The Synthesis of 6,7-Diethyl-9-(D-1¹-ribityl)-isoalloxazine[1]

BY JOHN P. LAMBOOY

Since the classical studies of flavin syntheses done by Kuhn and Karrer and their co-workers, which culminated in the synthesis of riboflavin[2] in 1935, no effort has been made to find additional analogs of riboflavin which might possess riboflavin-like activity. During this original period of interest a relatively large number of analogs were synthesized which differed from riboflavin in respect to the form, number or position of the substituents on the benzene ring portion or with respect to the form of the sugar portion of the molecule.

Of particular interest are 6-methyl-9-(D-1¹-ribityl)-isoalloxazine,[3] 7-methyl-9-(D-1¹-ribityl)-isoalloxazine,[4] 6-ethyl-7-methyl-9-(D-1¹-ribityl)-isoalloxazine,[5] 7-ethyl-9-(D-1¹-ribityl)-isoalloxazine,[5] 6,7-dimethyl-9-(D-1¹-arabityl)-isoalloxazine,[6] 6,7-dimethyl-9-(L-1¹-arabityl)-isoalloxazine[6,7,8] and 7-methyl-9-(D-1¹-sorbityl)-isoalloxazine[8] in that they have been found to have biological activity under certain conditions. In addition to riboflavin, 6-methyl-9-(D-1¹-ribityl)-isoalloxazine, 7-methyl-9-(D-1¹-ribityl)-isoalloxazine and 6-ethyl-7-methyl-9-(D-1¹-ribityl)-isoalloxazine have been found to support growth

(1) This study was supported in part by a grant-in-aid from the Fluid Research Fund Committee of the University of Rochester School of Medicine and Dentistry.

(2) Kuhn, Reinemund, Weygand and Ströbele, *Ber.*, **68**, 1765 (1935); Karrer, Becker, Benz, Frei, Salomon and Schöpp, *Helv. Chim. Acta*, **18**, 1435 (1935).

(3) Karrer and Strong, *ibid.*, **18**, 1343 (1935).

(4) Karrer, Salomon, Schöpp and Benz, *ibid.*, **18**, 1143 (1935).

(5) Karrer and Quibell, *ibid.*, **19**, 1034 (1936).

(6) Kuhn and Weygand, *Ber.*, **68**, 1282 (1935); Karrer and Meerwein, *Helv. Chim. Acta*, **19**, 264 (1936).

(7) Kuhn, Rudy and Weygand, *Ber.*, **68**, 166, 625 (1935).

(8) Karrer, Schöpp, Benz and Pfaehler, *Helv. Chim. Acta*, **18**, 69, 522 (1935).

as the sole source of flavin in rats[5,9,10] and in *L. casei* and *B. lactis acidi*.[11] 6,7-Dimethyl-9-(L-1¹-arabityl)-isoalloxazine may serve as the sole source of flavin for the rat,[12] but shows detectable activity only in the presence of suboptimal amounts of riboflavin when tested by growth methods on *L. casei* and *B. lactis acidi*.[11] Growth promoting activity has been found for 7-methyl-9-(D-1¹-sorbityl)-isoalloxazine and 7-ethyl-9-(D-1¹-ribityl)-isoalloxazine in the rat only when administered with suboptimal amounts of riboflavin.[13] 6,7-Dimethyl-9-(D-1¹-arabityl)-isoalloxazine possesses growth promoting activity in the presence of suboptimal amounts of riboflavin for *L. casei* and *B. lactis acidi*[11] while producing an inhibitor effect in rats.[14]

It appears that with the possible exception of 6,7-dimethyl-9-(L-1¹-arabityl)-isoalloxazine, only those flavins which serve as a sole source of flavin for *L. casei* and *B. lactis acidi* are capable of supporting growth in animals. To possess this activity the flavin must be a ribityl derivative, be substituted in either the 6 or 7 position or both by a methyl group or by an ethyl group in the 6 position and a methyl group in the 7 position, and cannot be substituted in any other position on the benzene ring. Isoriboflavin, 5,6-dimethyl-9-(D-1¹-ribityl)-isoalloxazine functions as an inhibitor in that it supresses growth in riboflavin deficient rats.[15]

The observations that 6-ethyl-7-methyl-9-(D-1¹-ribityl)-isoalloxazine can serve as the sole source of flavin while 7-ethyl-9-(D-1¹-ribityl)-isoalloxazine is effective only in the presence of suboptimal amounts of riboflavin, made it seem worth while to synthesize 6,7-diethyl-9-(D-1¹-ribityl)-isoalloxazine so that its activity could be investigated on rats and riboflavin requiring bacteria. This article is a report of the synthesis and preliminary biological study of this new flavin. The study was greatly facilitated by a generous gift of *o*-diethylbenzene from the National Advisory Committee for Aeronautics.

The required starting material, 4,5-diethyl-2-nitroaniline was prepared by a previously described procedure[16] and by a better procedure involving a new series of intermediates. In the second method, *o*-diethylaniline was brominated and the 4-bromo-*o*-diethylbenzene was aminated. Nitration of the acetylated 3,4-diethylaniline followed by alkaline hydrolysis gave the desired compound in higher yields. Starting with *o*-diethylbenzene, the yield of 4,5-diethyl-2-nitroaniline by the second method is 36% as compared to 19% for the older procedure.

The 4,5-diethyl-2-nitroaniline was condensed with *d*-ribose to produce 4,5-diethyl-2-nitroaniline-N-D-ribopyranoside which in turn was reduced catalytically to 2-(D-ribitylamino)-4,5-diethylaniline. This *o*-phenylenediamine was not isolated but used directly in the condensation with alloxan to produce 6,7-diethyl-9-(D-1¹-ribityl)-isoalloxazine.

The rotation of 6,7-diethyl-9-(D-1¹-ribityl)-isoalloxazine was found to be $[\alpha]^{24}D$ −26.3° which is considerably less than that found for riboflavin,[17] $[\alpha]^{20}D$ −114°, with the same solvent.

A comparison of the ultraviolet absorption spectra of riboflavin and the diethyl analog is given (Fig. 1).

The fluoresence of the diethyl analog was found to be 84% of that of riboflavin at 4360 Å., which is in agreement with expectations in consideration of the extinction coefficients for these two compounds at this wave length.

6,7-Diethyl-9-(D-1¹-ribityl)-isoalloxazine has been tested biologically for its ability to support growth in *L. casei*. Not only has it been found to be capable of supporting growth as the sole source of flavin in this organism but preliminary studies indicate that it may be equal to riboflavin in this respect at limiting levels. A detailed study of its biological properties will appear at a later date.

Experimental

4-Bromo-*o*-diethylbenzene.—*o*-Diethylbenzene,[18] 50 g. (0.373 mole) was brominated with 50 g. (0.312 mole) of bromine by the procedure outlined by Wisansky and Ansbacher.[19] Following the isolation of the product from the steam distillate it was fractionated by the use of the column described[16] and only that portion boiling from 120–122° at 20 mm. was used. This fraction weighed 61.4 g. which is 86% of the theoretical amount, and was identified as 4-bromo-*o*-diethylbenzene; colorless oil, b. p. 120–122° at 20 mm. pressure, $d^{27.5}$ 1.2625, $n^{22}D$ 1.5440.

Anal. Calcd. for $C_{10}H_{13}Br$: C, 56.35; H, 6.15; Br, 37.5. Found: C, 56.09; H, 6.34; Br, 37.3.

The 4-bromo-*o*-diethylbenzene was characterized by nitric acid oxidation to 4-bromophthalic acid and by its following conversion to 3,4-diethylaniline.

3,4-Diethylaniline.—Some of this material was prepared by a method already described,[16] and the rest was made by the method outlined by Wisansky and Ansbacher.[20] 4-Bromo-*o*-diethylbenzene, 31.8 g. (0.15 mole), 2.8 g. of flattened copper wire and 118 ml. of 28% ammonia water containing 2.4 g. of cuprous chloride contained in a brass liner were heated for 14 hours at 188–200° in the bomb. At the conclusion of the reaction the oil phase was separated and the water phase was extracted with ether, the ether being combined with the oil. Following evaporation of the ether the product was treated with 400 ml. of 5% hydrochloric acid and the acid solution extracted with ether to remove unreacted 4-bromo-*o*-diethylbenzene. This recovered 10.3 g. of the starting material. The acid solution was made alkaline, extracted with ether and the aniline recovered in the usual manner to yield

(9) Kuhn, Vetter and Rzeppa, *Ber.*, **70**, 1302 (1937).

(10) Karrer, Salomon, Schöpp, Benz and Becker, *Helv. Chim. Acta*, **18**, 908 (1935).

(11) Snell and Strong, *Enzymologia*, **6**, 186 (1939).

(12) Kuhn and Rudy, *Ber.*, **69**, 2557 (1936); Kuhn, *Angew. Chem.*, **49**, 6 (1936).

(13) Karrer, *Helv. Chim. Acta*, **19**, E33 (1936).

(14) von Euler and Karrer, *ibid.*, **29**, 353 (1946).

(15) Emerson and Tishler, *Proc. Soc. Exptl. Biol. Med.*, **55**, 184 (1944).

(16) Lambooy, THIS JOURNAL, **71**, 3756 (1949).

(17) Kuhn and Rudy, *Ber.*, **68**, 169 (1935).

(18) The *o*-diethylbenzene used in this study was generously furnished by the National Advisory Committee for Aeronautics.

(19) Wisansky and Ansbacher, "Org. Syn.," **28**, 22 (1948).

(20) Wisansky and Ansbacher, *ibid.*, **28**, 46 (1948).

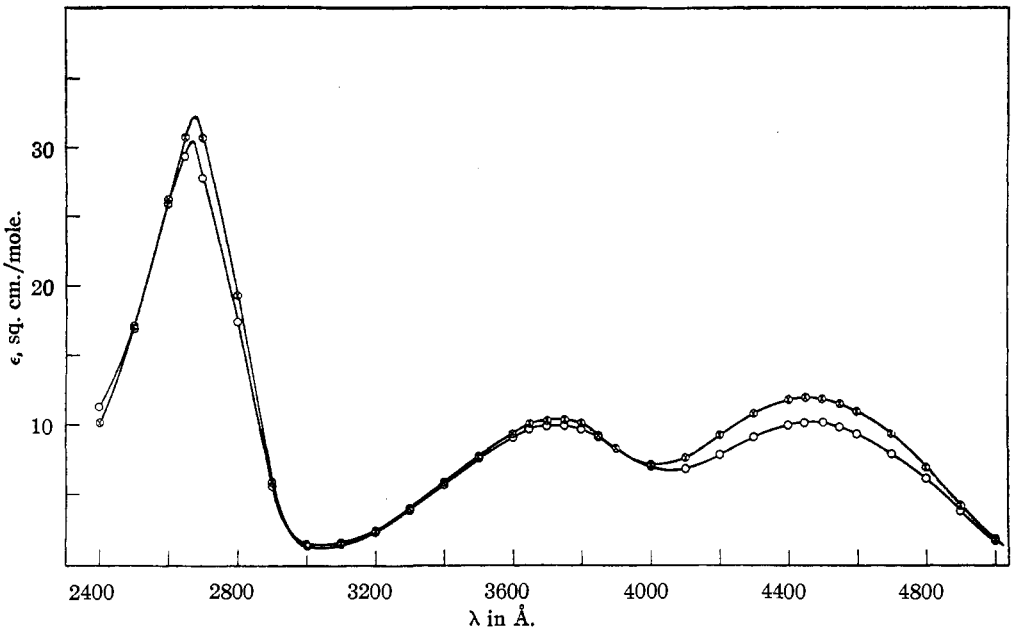

Fig. 1.—Ultraviolet absorption spectra of the diethyl analog –O– and riboflavin –⊗–; samples dissolved in distilled water.

11.7 g. of 3,4-diethylaniline, b. p. 129–130° at 20 mm. This represents a yield of 78% of the theoretical amount based on the 21.5 g. of 4-bromo-*o*-diethylbenzene actually used by the reaction.

3,4-Diethylacetanilide.—The 3,4-diethylaniline was refluxed for one minute with an equal weight of 99–100% acetic anhydride to produce the acetanilide in yields which varied from 95–98% of theoretical, m. p. 118°.[21] When mixed with the same compound prepared previously, the melting point showed no depression.

4,5-Diethyl-2-nitroacetanilide.—Since the extremely small size of the crystals of 4,5-diethyl-2-nitrocarbethoxyanilide made filtration difficult it was not easily purified. In an effort to avoid this difficulty the nitration was performed on the acetanilide instead of the carbethoxyanilide. The resulting 4,5-diethyl-2-nitroacetanilide was easily purified. A mixture of 100 ml. of concentrated nitric acid and 38 ml. of concentrated sulfuric acid was cooled to −10°. 3,4-Diethylacetanilide, 21.7 g. (0.112 mole), was ground to a fine state and divided roughly into ten portions. Following the addition of each portion of the acetanilide the reaction mixture became orange-brown. A subsequent addition was delayed until the color had returned to a bright yellow-orange. The addition required approximately one and one-half hours and at no time did the temperature exceed −5°. The mixture was finally stirred an additional thirty minutes in the cooling bath. The product was poured on ice and it solidified. The material was dissolved in ether, washed three times with water, once with 10% sodium bicarbonate and again three times with water. After drying and removal of the solvent the product was recrystallized from ethyl alcohol to yield 15.6 g. of yellow needles, m. p. 76–77°, which is 58% of the theoretical amount.

Anal. Calcd. for $C_{12}H_{16}N_2O_3$: N, 11.86. Found: N, 11.9, 12.1.

4,5-Diethyl-2-nitroaniline.—4,5-Diethyl-2-nitroacetanilide, 10 g., in 200 ml. of 50% alcohol was heated to 80° on the steam-bath and 20 g. of sodium hydroxide in 40 ml. of water was added. The mixture was kept at 80° for thirty minutes and then placed in the refrigerator. The product is filtered to yield 7.7–8.1 g., which is 94–98% of the theoretical amount of the desired material. The product thus prepared melts at 65–66° and the melting point can not be raised by recrystallization from dilute alcohol. It seemed desirable to have especially pure material for the next reaction so it was recrystallized.

4,5-Diethyl-2-nitroaniline-N-D-ribopyranoside.—4,5-Diethyl-2-nitroaniline, 2.000 g., and 1.500 g. of crystalline *d*-ribose were treated by the general procedure for the preparation of pyranosides as described by Berger and Lee.[22] When 95% alcohol was used the yield was approximately 1.1 g. or 35% of the theoretical amount. The use of absolute alcohol increased the yield to 2.2–2.3 g. or 67–70% of the theoretical amount based on ribose. The product is fine yellow needles melting with decomposition between 171 and 177°; $[\alpha]^{28}D$ −85° (0.5% pyridine). The material was analyzed without further treatment and was found to be stable for at least eight months if kept cold.

Anal. Calcd. for $C_{15}H_{22}O_6N_2$: C, 55.20; H, 6.80; N, 8.59. Found: C, 55.24; H, 7.14; N, 8.6.

The accumulated filtrates and washings were evaporated and the residue subjected to the hydrolysis procedure described by Berger and Lee[23] for the recovery of unused 4,5-diethyl-2-nitroaniline.

6,7-Diethyl-9-(D-1¹-ribityl)-isoalloxazine.—The procedure for the preparation of the flavin was based on that described by Kuhn and Ströbele.[24] Two grams of 4,5-diethyl-2-nitroaniline-N-D-ribopyranoside was dissolved in approximately 300 ml. of 72% alcohol and 57 ml. of a solution of primary sodium borate was added to furnish 0.83 g. of NaH_2BO_3. After the addition of 5 g. of the catalyst[24] in the form of palladium on calcium carbonate containing trace amounts of zinc and cupric hydroxides, the mixture was reduced at 30 atmospheres of hydrogen for six hours at 70–80°. To the mixture was added 200 mg. of ascorbic acid and the material evaporated to dryness under reduced pressure at a temperature not exceeding 35°. The product was treated repeatedly with absolute alcohol followed by evaporation to ensure complete dryness. The dry material was dissolved in 20 ml. of glacial acetic acid and to it was added a suspension of 1.4 g. of

(21) All melting points given were observed on thermometers calibrated against U. S. P. Melting Point Reference Standards.

(22) Berger and Lee, *J. Org. Chem.*, **11**, 84 (1946).

(23) Berger and Lee, *ibid.*, **11**, 91 (1946).

(24) Kuhn and Ströbele, *Ber.*, **70**, 773 (1937).

boric acid and 1.4 g. of alloxan in 20 ml. of glacial acetic acid. The flavin synthesis began immediately and the mixture was shaken at 40–50° for thirty minutes. The product was allowed to stand at room temperature for forty-eight hours and then evaporated to dryness as above. The crude product was suspended in 250 ml. of 5% acetic acid, heated to boiling and filtered. On cooling, 0.82 g. of fine orange needles are deposited which is 33% of the theoretical amount. The flavin melts with decomposition at 255–256°, darkening at 245°. The product was recrystallized from water for analytical purposes. The purified material resembles riboflavin in crystalline form but the color is yellow-orange.

Anal. Calcd. for $C_{19}H_{24}N_4O_6$: C, 56.34; H, 5.98; N, 13.85. Found: C, 55.9; H, 5.9; N, 14.0.

Physical Data.—The ultraviolet absorption spectra were measured on solutions containing 10.3 micrograms of riboflavin[25] and 11.1 micrograms of the diethyl analog per milliliter, or 2.74×10^{-5} mole per liter made up in ordinary distilled water. The optical density was read directly from the spectrophotometer and plotted as extinction coefficients.

The rotation was determined on a solution containing the equivalent of 0.826 g. per 100 ml. in tenth normal sodium hydroxide and found to be $[\alpha]^{24}D$ −26.3°.

Fluorescence measurements were made at 4360 Å.[26] on solutions containing 0.0773 microgram of riboflavin and 0.0833 microgram of the diethyl analog per milliliter, or 2.055×10^{-7} mole per liter.

Biological Data.—The biological activity of the diethyl analog was determined by the use of *Lactobacillus casei* 7469. Growth curves were compared to those obtained by the use of riboflavin at equimolecular levels. The curves for the diethyl analog and riboflavin were superimposable within the limits of error of the method, throughout the range from zero to 8.22×10^{-10} mole per milliliter.

(25) U. S. P. Riboflavin which had been recrystallized from water was used.

(26) This filter is provided with the Model 12-A Coleman Electronic Photofluorometer for riboflavin determinations.

Acknowledgment.—I am happy to acknowledge the help given me by Drs. V. Boekelheide and D. S. Tarbell of the Department of Chemistry, by making available the high pressure hydrogenator and for their interest and discussion. I also want to thank Dr. K. Altman of the Atomic Energy Commission for permitting me to use the ultraviolet spectrophotometer and advising me in its use.

Summary

1. The complete synthesis of 6,7-diethyl-9-(D-1′-ribityl)-isoalloxazine has been described.

2. The steps involved begin with *o*-diethylbenzene and proceed through 4-bromo-*o*-diethylbenzene, 3,4-diethylaniline, 3,4-diethylacetanilide, 4,5-diethyl-2-nitroacetanilide, 4,5-diethyl-2-nitroaniline, 4,5-diethyl-2-nitroaniline-N-D-ribopyranoside to 6,7-diethyl-9-(D-1′-ribityl)-isoalloxazine.

3. Comparisons are made to riboflavin with regard to rotation, fluorescence and ultraviolet spectra.

4. The new riboflavin analog, 6,7-diethyl-9-(D-1′-ribityl)-isoalloxazine has been found capable of serving as the sole source of flavin in the growth of *Lactobacillus casei*.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Cyclic Polyolefins. XI. Carbonyl-bridged Compounds Derived from the Adduct of α-Carbethoxycyclohexanone and Acrolein

BY ARTHUR C. COPE AND MARTIN E. SYNERHOLM

The success of syntheses of phenyl-substituted cyclooctadienes from carbonyl-bridged intermediates[1,2] has led us to investigate similar methods for the preparation of eight-membered ring compounds substituted by carboxyl groups.

The first route which was found to yield an intermediate useful in the projected synthesis was alkylation of the potassium enolate of α-carbethoxycyclohexanone with β-chloropropionaldehyde diethyl acetal in hydrocarbon solvents. This reaction produced β-(1-carbethoxy-2-ketocyclohexyl)-propionaldehyde diethyl acetal (I) in 31% yield. Mild acid hydrolysis of I yielded a viscous liquid which proved to be isomeric with the expected aldehyde, but boiled higher than the acetal from which it was prepared. This product is believed to be the cyclic aldol, II, formed by intramolecular condensation of the aldehyde III. Evidence in favor of this interpretation was obtained by development of a direct synthesis of the aldehyde III by the Michael addition of α-carbethoxycyclohexanone to acrolein. This reaction yielded only polymeric products under usual conditions for the Michael reaction, but it was possible to isolate the aldehyde III in 65–70% yield when the addition was conducted at −70° in the presence of a small amount of sodium ethoxide in ethanol. The product obtained in this manner was fluid rather than viscous, and boiled lower than the diethyl acetal I. The structure of this product (the aldehyde III) was established by oxidation with silver oxide, which yielded β-(1-carbethoxy-2-ketocyclohexyl)-propionic acid, identified by comparison with an authentic sample. When the aldehyde III was treated with acids under conditions similar to those used for hydrolysis of the acetal I, a viscous liquid was formed which appeared to be identical with the cyclic aldol II prepared from the acetal. Both II and III yielded 1-carbethoxybicyclo[3.3.1]non-3-en-9-one (IV) on treatment with concentrated sulfuric acid. Less drastic

(1) Cope, Fawcett and Munn, THIS JOURNAL, **72**, 3399 (1950).

(2) Cope and Hermann, *ibid.*, **72**, 3405 (1950).