On cooling, the mother liquor from the diacetyl compound deposited 0.30 g. of colorless needles (yield 26%), melting at 189° after recrystallization from benzene, and showing no melting point depression when mixed with pure 2-acetylamino-3-fluoro-4,6-dibromophenol.

A repetition of this experiment in which the reaction mixture was set aside for twelve hours gave the triacetyl derivative (m. p. and mixed m. p. 125°), which on hydrolysis yielded the monoacetyl derivative. In this case also a second minor fraction consisting of the monoacetyl compound was obtained and identified.

Summary

When both acetyl and benzoyl groups were introduced into either 2-amino-3-fluoro-4,6-dibromophenol or 2,4-dibromo-3-fluoro-6-aminophenol, only one mixed acyl derivative was isolated, regardless of the sequence of introduction. On hydrolysis the benzoyl group was found on the nitrogen.

Acetylation of 2-benzoylamino-3-fluoro-4,6-dibromophenol with a large excess of acetic anhydride in pyridine caused the replacement of the benzoyl group by acetyl.

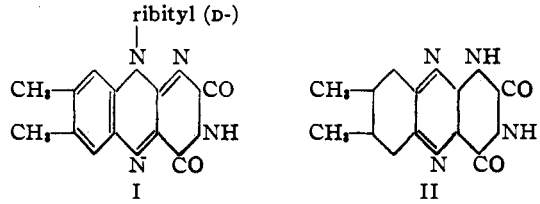
BATON ROUGE, LA. RECEIVED SEPTEMBER 11, 1945

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK AND CO., INC.]

The Preparation of Riboflavin.[1] III. The Synthesis of Alloxazines and Isoalloxazines

BY MAX TISHLER, J. W. WELLMAN[2] AND KURT LADENBURG[3]

During the past ten years, isoalloxazines have assumed importance because riboflavin, I, is a member of this class of compounds.



The only known, general synthesis of isoalloxazines involves the reaction of mono-N-substituted *o*-phenylenediamines with alloxan in acidic media.[4]

Kuhn and Cook,[5] in a search for other syntheses, found none of wide application and concluded that the reaction between ortho phenylenediamines and alloxan constitutes the only general method for making alloxazines (related to II) and isoalloxazines. Among the many routes unsuccessfully investigated by Kuhn and Cook was the reaction between ortho phenylenediamine and 5-bromobarbituric acid which according to them did not yield alloxazine. Contrary to this report we found that the reaction between halogenated barbituric acids and derivatives of *o*-phenylenediamine is a general method for preparing alloxazines and isoalloxazines. With the reagents employed by Kuhn and Cook, the yield of alloxazine is poor and the product difficult to isolate. This fact is not entirely unexpected as the halogen in 5-bromobarbituric acid is "positive"[6] and would, therefore, bring about oxidation and/or bromination of the diamine. The chlorobarbituric acids, 5-chloro- and 5,5-dichlorobarbituric acids, however, are better suited for reaction with ortho phenylenediamines as the chlorine atoms are "less positive".[7] It was also found that the nature of the diamine is important. Alkylated *o*-phenylenediamines behave better in the reaction with the halobarbituric acids than does ortho phenylenediamine itself. Thus, 4,5-dimethyl-*o*-phenylenediamine and 5,5′-dichlorobarbituric acid form 6,7-dimethylalloxazine, II, in almost quantitative yield whereas *o*-phenylenediamine under the same conditions is converted to alloxazine in 20% yield.

The condensation is best carried out in pyridine; the use of methanol and acetic acid as solvents gives unsatisfactory yields. With pyridine a striking color change occurs. A deep blue color is formed at the beginning and, as the reaction proceeds the mixture becomes orange-red. In most instances the product separates from the reaction mixture on cooling. With the appropriate diamines and the chlorobarbituric acids, we prepared alloxazine, 6,7-dimethylalloxazine, riboflavin, tetraacetylriboflavin, 1-araboflavin, 6,7-dimethyl-9-benzylisoalloxazine and 6,7-dimethyl-9-methylisoalloxazine.[8]

Although riboflavin is formed in excellent yields from the diamine III and 5,5′-dichlorobarbituric

(1) For other publications in this series, see Ladenburg, Tishler and Wellman, THIS JOURNAL, **66**, 1217 (1944); Tishler, Wendler, Ladenburg and Wellman, *ibid.*, **66**, 1328 (1944).

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(3) Present address: Wyeth, Inc., Philadelphia, Pennsylvania.

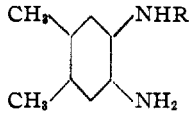
(4) Kuhn, Reinemund and Weygand, *Ber.*, **67**, 1460 (1934); Kuhn and Weygand, *ibid.*, **67**, 1939 (1934). Karrer, Salomon, Schopp and Schlitter, *Helv. Chim. Acta*, **17**, 1165 (1934). For a more complete bibliography, see H. R. Rosenberg, "Chemistry and Physiology of the Vitamins," Interscience Publishers, Inc., New York, N. Y., 1942, pp. 163-170.

(5) Kuhn and Cook, *Ber.*, **70**, 761 (1937).

(6) Hirst and Macbeth, *J. Chem. Soc.*, **121**, 904, 2169 (1921); Macbeth, *ibid.*, **121**, 116 (1921); Cox, Macbeth and Pennycuick, *ibid.*, 1870 (1931).

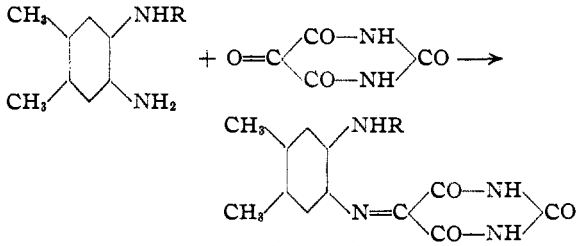
(7) The difference in nature of the halogen in 5-bromo- and 5-chlorobarbituric acids is manifested by the behavior of these compounds in acetone. Whereas the latter is resistant to change in warm acetone, the former rapidly brominates acetone and is converted to barbituric acid.

(8) Tishler and Wellman, U. S. Patent 2,261,608.



III, R is D-ribityl (from *d*-ribose)
IV, R is L-arabityl (from *l*-arabinose)

acid using pyridine as the solvent, no riboflavin is formed when alloxan replaces the halobarbituric acid. In the latter instance, a brilliant yellow compound is formed, the molecular formula of which differs from that of riboflavin by the presence of an additional molecule of water. This compound is isomeric and undoubtedly similar in structure to the so-called "anil," V, obtained by Kuhn and his collaborators[9] when the diamine, IV, and alloxan were allowed to react in a mixture of ethanol, water and acetic acid.



V, R is L-arabityl
VI, R is D-ribityl
VII, R is CH_3

Although some halogenated barbituric acid derivatives are known to be hydrolyzed to alloxans,[10] we do not believe that the formation of alloxazines and isoalloxazines in our synthesis is due to the reaction of diamines and alloxan formed by hydrolysis of the chlorobarbituric acids. We find that the reaction occurs readily when the solvent and reactants are scrupulously dried. In addition, alloxan and the diamine, III, in pyridine containing pyridine hydrochloride do not form significant amounts of riboflavin.[11]

It is interesting to note that the anil, V, could not be cyclized to the corresponding isoalloxazine.[4] Attempts by us to cyclize the anil, VI, to riboflavin were also unsuccessful. No significant amounts of riboflavin were formed when the anil was heated with tertiary amines or with organic and inorganic acids including hydrogen fluoride.[12] Treatment of the dihydroform of the anil[13] with acids and alkalies did not cause the desired cyclization.

(9) Kuhn, Rudy and Weygand, *Ber.*, **68**, 633 (1935).

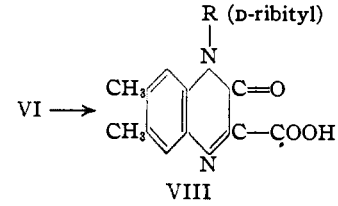
(10) Techow, *ibid.*, **27**, 3083 (1894).

(11) No riboflavin could be isolated when this mixture was treated in the manner in which riboflavin is obtained when dichlorobarbituric acid was used. Microbiological assays on the intractable residue obtained from the reaction mixture carried out by Dr. J. L. Stokes of the Research Laboratories of Merck and Co., Inc. indicated that some riboflavin was present (in the order of 10%). With dichlorobarbituric acid the yield of riboflavin is about 85%.

(12) In no instance was the yield of riboflavin greater than 2% as determined by microbiological assays. The anil itself showed about 0.2% the activity of riboflavin, possibly due to contamination with the latter.

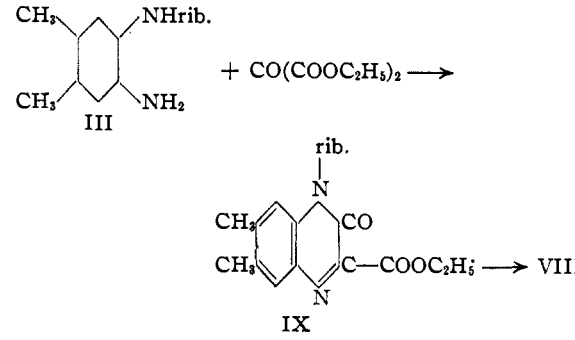
(13) On catalytic hydrogenation the anil VI absorbs one mole of hydrogen yielding a white compound melting at 185°. Exposure of a solution of the latter to air regenerates the anil.

The inability of the anils to cyclize to isoalloxazines is difficult to understand and implies that they are not intermediates in the synthesis of isoalloxazines from *o*-phenylenediamines and alloxan. The anil related to riboflavin, VI, behaves like the N-methyl anil, VII,[14] in that on prolonged alkaline hydrolysis the elements of urea are lost and cyclization to a dihydroquinoxaline derivative occurs.



VIII

The structure of the keto acid, VIII, was established by synthesis from 1-N-D-ribitylamino-2-amino-4,5-dimethylbenzene, III, and ethyl oxomalonate followed by hydrolysis of the resulting keto ester.



Experimental

Alloxazine.—A mixture of 1 g. of *o*-phenylenediamine and 1.2 g. of monobromobarbituric acid in 25 cc. of pyridine was heated at 100° for one hour. A transient purple color develops which changes to brown in a few minutes. The reaction mixture was concentrated to dryness and the residue was washed well with water, and then with *N* hydrochloric acid. The washed residue was purified by three recrystallizations from hot 50% acetic acid. The weight was 0.31 g. (20.7% yield).

Anal. Calcd. for $C_{10}H_8O_2N_4$: C, 55.55; H, 3.75. Found: C, 55.66; H, 3.98.

6,7-Dimethylalloxazine.—A suspension of 3.5 g. of 5,5-dichlorobarbituric acid, 2.0 g. of 4,5-dimethyl-*o*-phenylenediamine[15] in 100 cc. of glacial acetic acid was boiled gently under reflux for forty-five minutes. A considerable amount of product separated during the heating period. The mixture was stored at room temperature for six hours and then filtered. Recrystallization of the crude product from hot acetic acid gave 2.1 g. of pure lumichrome (6,7-dimethylalloxazine); 59% yield.

Anal. Calcd. for $C_{12}H_{10}O_2N_4$: C, 59.50; H, 4.13; N, 23.18. Found: C, 59.42; H, 4.21; N, 24.31.

When the reaction was carried out in warm pyridine the yield of pure lumichrome was 90%. Using 5-bromobarbituric acid in place of the dichlorobarbituric acid and

(14) Kuhn and Reinemund, *Ber.*, **67**, 1934 (1934).

(15) Noelting, Braun and Thesman, *ibid.*, **34**, 2252 (1901). Instead of reducing the requisite nitroamine by tin and hydrochloric acid as indicated by these authors, catalytic hydrogenation with Raney nickel catalyst was employed.

pyridine as the solvent a 30% yield of lumichrome was obtained.

6,7,9-Trimethylisoalloxazine (Lumiflavin).—The reaction between N-methyl-4,5-dimethyl-*o*-phenylenediamine[14] (2.0 g.) and 4 g. of 5,5-dichlorobarbituric acid was carried out by heating at 100° in 45 cc. of pyridine for thirty minutes. The reaction mixture was concentrated to dryness and the residue was washed with 0.5 *N* hydrochloric acid. The product was recrystallized from hot 50% acetic acid; weight 3 g.

Anal. Calcd. for $C_{13}H_{12}O_2N_4$: C, 60.93; H, 4.69; N, 21.87. Found: C, 61.21; H, 4.81; N, 21.65.

6,7-Dimethyl-9-benzylisoalloxazine.—The required diamine, N-benzyl-5,4-dimethyl-*o*-phenylenediamine, was synthesized by the reaction of 1,2-dinitro-4,5-dimethylbenzene with benzylamine and reducing the resulting compound.

(a) **1-Benzylamino-2-amino-4,5-dimethylbenzene.**—A mixture of 25 g. of 1,2-dinitro-4,5-dimethylbenzene,[16] 35 g. of benzylamine and 40 cc. of isoamyl alcohol was refluxed for fifteen hours during which time the product separated. After cooling the resulting mixture for three hours the solid was collected and recrystallized from methanol. The product, 1-benzylamino-2-nitro-4,5-dimethylbenzene, was obtained as bright red rods melting at 102–103°; weight 28.5 g.

Anal. Calcd. for $C_{15}H_{16}O_2N_2$: C, 70.31; H, 6.25. Found: C, 70.14; H, 6.20.

The reduction of the above nitro compound to the diamine was carried out by shaking a solution of 10 g. in 100 cc. of methanol containing 2 g. of Raney nickel catalyst with hydrogen at room temperature. The reduction was complete in one hour. The product was isolated by concentrating the filtered solution to dryness and dissolving the residue in petroleum ether. The product separated as fluffy needles and melted at 59°.

Anal. Calcd. for $C_{15}H_{16}N_2$: C, 79.64; H, 7.96. Found: C, 79.81; H, 7.91.

When platinum oxide is used as a catalyst for the hydrogenation, an additional mole of hydrogen is absorbed and 4,5-dimethyl-*o*-phenylenediamine is formed.

(b) **Condensation with 5,5-Dichlorobarbituric Acid.**—A mixture of 5 g. of the diamine, 4 g. of 5,5-dichlorobarbituric acid and 50 cc. of pyridine was heated at 100° for one hour, chilled and filtered. The crude product was recrystallized from hot 5% acetic acid solution from which it separated as microscopic orange-yellow needles melting at 280° with dec. (yield 78%).

Anal. Calcd. for $C_{19}H_{16}O_2N_4$: C, 68.77; H, 4.82; N, 16.86. Found: C, 68.50; H, 4.99; N, 16.52.

Using 5-chlorobarbituric acid in place of the dichlorobarbituric acid the yield of the N-benzylisoalloxazine was 70%.

Riboflavin (I).—The required diamine, III, was prepared by the method of Karrer and Meerwein.[17] A mixture of 3 g. of 4,5-dimethyl-N-*d*-ribityl-*o*-phenylenediamine, 3 g. of 5,5-dichlorobarbituric acid and 50 cc. of pyridine was maintained at 100° for thirty minutes. A deep blue color formed when the reactants were mixed which soon changed to orange-yellow. At the end of the heating period some product had separated. The mixture was concentrated to dryness and the residue was washed with dil. hydrochloric acid. The residue was recrystallized from boiling water; weight 3.5 g.; m. p. 280° dec. (85% yield).

Anal. Calcd. for $C_{17}H_{20}O_6N_4$: C, 54.25; H, 5.32. Found: C, 54.02; H, 5.50. $[\alpha]^{25}D$ +115° (0.5% in 0.1 *N* NaOH).

On acetylation with acetic anhydride in the presence of pyridine, tetraacetylriboflavin (m. p. 236–238°) was obtained.

6,7-Dimethyl-9-[1,L′-arabityl]-isoalloxazine (1-Araboflavin).—A mixture of 0.5 g. of 2-L-arabinitylamino-4,5-dimethylaniline prepared by the method of Karrer and Meerwein[17] and 0.5 g. of 5,5-dichlorobarbituric acid in 20 cc. of pyridine was heated at 100° for one hour. The product was isolated in the manner described for riboflavin. The product (0.51 g.) melted at 295–297° (dec.).

Anal. Calcd. for $C_{17}H_{20}O_6N_4$: C, 54.25; H, 5.32. Found: C, 54.06; H, 5.42.

Tetraacetylriboflavin.—The preparation of the required diamine was accomplished by the catalytic hydrogenation of the desired ortho amino azo compound.

(a) **1-(Tetraacetyl-D-ribitylamino)-2-*p*-nitrophenylazo-4,5-dimethylbenzene.**—A filtered solution of diazotized *p*-nitroaniline, prepared from 27 g. of the nitro amine was added to a solution of 50 g. of 1-N-(tetraacetyl-D-ribitylamino)-3,4-dimethylbenzene[1] in 1250 cc. of acetic acid at 10°. The mixture was stirred for three hours and filtered. The crude azo compound was recrystallized from hot ethyl alcohol from which it separates as brick red needles melting at 168–169°. The yield of pure product was 50 g.

Anal. Calcd. for $C_{27}H_{32}N_4O_{10}$: C, 56.63; H, 5.61; N, 9.76. Found: C, 56.82; H, 5.68; N, 9.70.

The above azo compound was deacetylated by heating a mixture in methanol containing 0.05 equivalent of sodium methoxide. The product, 1-[D-ribitylamino]-2-nitrophenylazo-3,4-dimethylbenzene, melted at 180–182°. Karrer and Meerwein[17] prepared this azo compound by coupling 1-(N-ribitylamino)-3,4-dimethylbenzene with diazotized *p*-nitroaniline but did not record its melting point.

(b) **Coupling Reaction.**—The azo compound in methanol was readily reduced catalytically using platinum oxide as the catalyst. As we were not able to obtain the diamine crystalline the reduction mixture was filtered, concentrated to dryness and the residue was used as such for condensation with dichlorobarbituric acid. Thus the residue obtained by reducing 5 g. of the amino azo compound was heated at 100° with 4 g. of 5,5-dichlorobarbituric acid in 60 cc. of pyridine for half an hour. The mixture was poured into an excess of water and the product was extracted with chloroform. The chloroform extracts were extracted several times with 5% hydrochloric acid to remove a red colored by-product. The chloroform solution was concentrated to dryness, and the residue was dissolved in a minimum amount of boiling methanol. The hot solution was treated with charcoal, filtered and cooled. About 2.4 g. of pure tetraacetylriboflavin (m. p. 236–238°) was obtained.

Anal. Calcd. for $C_{25}H_{28}O_{10}N_4$: C, 55.14; H, 5.14; N, 1029. Found: C, 55.02; H, 5.38; N, 10.50.

A sample of tetraacetylriboflavin was deacetylated to riboflavin by heating a suspension in methanol containing a small amount of calcium methylate.

5-(6-*d*-Ribitylamino-3,4-dimethylphenylimino)-barbituric Acid Anil, VI.—A suspension of 4 g. of 1-N-ribitylamino-2-amino-4,5-dimethylbenzene,[17] 3 g. of alloxan in 50 cc. of pyridine was heated at 100° for half an hour. As in the case with dichlorobarbituric acid a blue color was first formed which gradually changed to yellow. The product which separated during the heating period was filtered and recrystallized from 50% acetic acid. The product weighing 4.2 g. melted at 237–240° and was identical with the product obtained when the same reactants are heated in ethanol, water and acetic acid in the same manner that Kuhn, *et al.*,[6] carried out the preparation of the arabityl anil, V. The anil, unlike riboflavin, forms a deep red color when added to concentrated hydrochloric acid.

Anal. Calcd. for $C_{17}H_{22}O_7N_4$: C, 51.77; H, 5.58; N, 14.21. Found: C, 51.85; H, 5.72; N, 14.05.

Hydrolysis of Anil, VI.—The hydrolysis of the anil takes place in several steps as is evidenced by the isolation of several partially hydrolyzed products depending on the conditions of the hydrolysis. When the anil dissolved in 2.5 *N* sodium hydroxide is held a few minutes and acidified, a product melting at 148° was isolated (not analyzed). On boiling a mixture of this substance with water a compound melting at 218° was isolated (*Anal.* Found: C,

(16) Noelting and Thesman, *Ber.*, **35**, 631 (1902).

(17) Karrer and Meerwein, *Helv. Chim. Acta*, **18**, 1133 (1935).

50.56; H, 5.71; N, 13.80). When the anil in a 27% solution of sodium carbonate was heated under reflux until ammonia was no longer evolved, the keto acid VIII was isolated on neutralizing the reaction mixture with acetic acid. The keto acid melted with decomposition at 175°.

Anal. Calcd. for $C_{16}H_{20}N_2O_7$: C, 54.54; H, 5.68. Found: C, 54.30; H, 5.91.

Ethyl 1-D-Ribityl-2-oxo-6,7-dimethyl-1,2-dihydro-3-quinoxalinecarboxylate, IX.—A mixture of 5 g. of the diamine, III, 3 g. of ethyl oxomalonate in 35 cc. of ethanol was refluxed for thirty minutes. The product crystallized after storage in the refrigerator for twelve hours. The product was recrystallized from ethanol from which it separated as pale yellow, microscopic needles melting at 167–168°. The yield was 5 g.

Anal. Calcd. for $C_{18}H_{24}O_7N_2$: C, 56.85; H, 6.32; N, 7.38. Found: C, 56.60; H, 6.26; N, 7.27.

The above keto ester was hydrolyzed by dissolving in absolute methanol containing 1 equivalent of sodium methylate and adding a few drops of water. The sodium salt of the free acid precipitated shortly. The salt was dissolved in a minimum amount of water and was treated with a small excess of acetic acid. The keto acid, which separated, melted at 174–176° (dec.) and when mixed with a sample of the keto acid obtained by the exhaustive hydrolysis of the anil, VI, the melting point was not depressed.

Acknowledgment.—We are indebted to Drs. R. T. Major and J. R. Stevens[18] for their interest and suggestions. We are also indebted to Dr. J. L. Stokes for the microbiological assays and to Mr. R. N. Boos for the microanalyses.

Summary

A new synthesis of alloxazines and isoalloxazines from orthophenylenediamines and halobarbituric acids has been described. Pyridine is an outstanding solvent for the reaction. Evidence has been presented to indicate that the reaction does not depend on the hydrolysis of the halobarbituric acids to alloxan.

(18) At present Director of Research of J. T. Baker Co., Phillipsburg, N. J.

RAHWAY, N. J. RECEIVED SEPTEMBER 27, 1945

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT LABORATORIES OF UNIVERSAL OIL PRODUCTS COMPANY]

Synthesis of Methanol from Carbon Dioxide and Hydrogen over Copper–Alumina Catalysts. Mechanism of Reaction

BY V. N. IPATIEFF AND G. S. MONROE

The reactions of hydrogen and carbon monoxide over a great range of experimental conditions have been studied and reported by various investigators. However, the literature on the reactions of hydrogen and carbon dioxide, especially under high pressure, is relatively scant. The subject is usually dismissed by the statement that the carbon dioxide reduces to the monoxide and from this point follows the usual behavior of hydrogen and carbon monoxide.

This paper deals, for the most part, with the reactions of carbon dioxide and hydrogen over copper–alumina catalysts at pressures of about 410 atm. at which pressure the effects of change in catalyst composition, hydrogen:carbon dioxide ratio, and temperature were investigated. A few experiments, however, were made at 117–410 atm. to study the influence of pressure on the reaction. Some experiments were also made with carbon monoxide and hydrogen and with carbon monoxide–carbon dioxide mixtures and hydrogen to obtain parallel data for comparison.

Experimental

Materials.—The carbon dioxide and hydrogen for these experiments were of commercial grade. The carbon monoxide was prepared by the dehydration of 85% commercial formic acid with concentrated phosphoric acid (85%). The purity of the carbon monoxide was 98+%. Formalin was used for the experiments with formaldehyde and water.

Catalysts.—The copper–alumina catalysts were prepared by the precipitation at 60° of basic copper carbonate on alumina (hydroxide calcined at 350°) by the addition of ammonium carbonate solution to cupric nitrate solution containing the alumina held in suspension by vigorous agitation. After thorough washing, the precipitate was dried (final temperature 240°), ground to 40-mesh, mixed with 4% rosin, and pilled to $1/8'' \times 1/8''$ size (cylindrical). After burning out the rosin with air at 220°, the pills were reduced in hydrogen at 250°. The catalyst containing copper alone was prepared by the same method employed for the copper–alumina catalysts, precipitating the copper in the absence of alumina. The catalyst consisting only of alumina was made by pilling 40-mesh alumina (calcined at 350°) according to the procedure previously described.

Apparatus and Procedure.—All experiments were made in a flow-type apparatus. This equipment consisted essentially of four parts: a high-pressure gas charger for hydrogen and hydrogen–carbon monoxide mixture; a high-pressure liquid charger with feed pump for charging liquid carbon dioxide; a high-pressure vertical reaction tube with a copper liner heated by an electric furnace; and a pressure regulator attached to the exit end of the reaction tube.

The hydrogen or hydrogen–carbon monoxide mixture from the high-pressure gas charger was discharged into the preheater section of the reaction tube with water displacement by means of a hydraulic pump. From the amount of water pumped from graduated cylinders into the chargers, the amount of gas discharged into the reaction tube could be determined.

The carbon dioxide was pumped as liquid from a charger with a calibrated gage glass into the top preheater section of the reaction tube, where it contacted and mixed with the hydrogen from the gas charger before reaching the catalyst zone.

The reaction tube, made from stainless steel, was 86 cm. long and had an inside diameter of 2.38 cm. with the liner inserted. The top section (first 38 cm. filled with copper punchings) served as a preheater. The middle section (25.4 cm.), with a capacity of 100 cc., served as the catalyst zone. The lower section (22.6 cm.) contained a loose-fitting copper bar or filler to serve as a support for the catalyst and at the same time to hasten the exit of the products from the reaction tube. A thermocouple pocket