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# Streptomyces Antibiotics. XXII. Configuration of Streptose and Streptobiosamine

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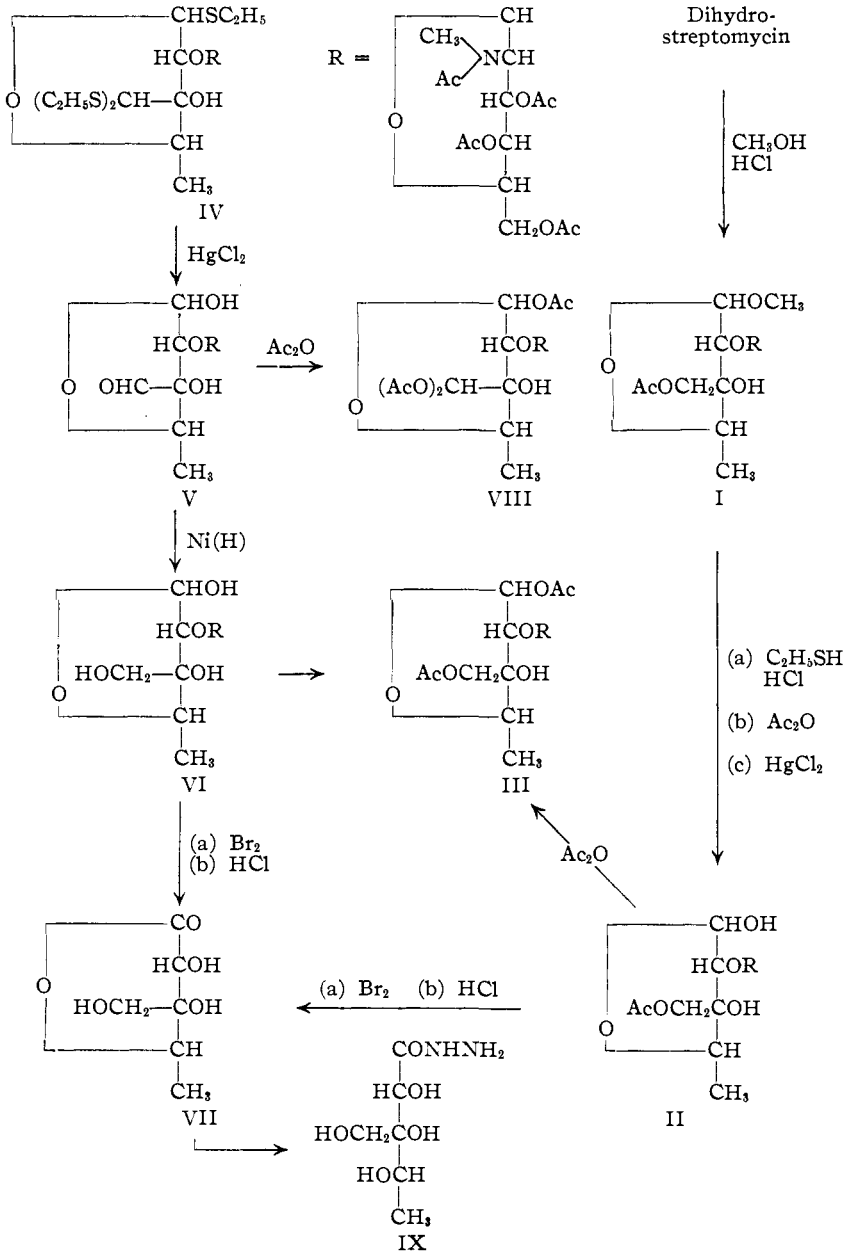
The degradative preparation of dihydrostreptosonic acid lactone and the configuration of streptose and streptobiosamine have been communicated[1]; the details of these studies and a new improved preparation of the lactone are described herein. The improvement also made available crystalline tetraacetyldihydrostreptobiosamine.

Although the structural formula[2] of streptobiosamine was determined, certain stereochemical aspects of the structure remained to be elucidated. The configuration of the glycosidic linkage between streptose and N-methyl-L-glucosamine has been calculated[3] to be $\alpha$-L on the basis of the rotations of tetraacetylbisdesoxystreptobiosamine[4] and its components.[5,6,7] The stereochemical features of streptobiosamine remaining unknown concerned the streptose moiety, and for the elucidation of this aspect of the molecule, the lactone and the hydrazide of dihydrostreptosonic acid have been prepared to determine the sign of their optical rotations.

Methyl pentaacetyldihydrostreptobiosaminide[8] (I), which is prepared from dihydrostreptomycin, was converted into ethyl pentaacetylthiodihydrostreptobiosaminide by reaction with ethyl mercaptan and hydrogen chloride. The crude thioglycoside was reacetylated, and the product was treated with mercuric chloride and strontium carbonate for the preparation of pentaacetyldihydrostreptobiosamine[9] (II). The hy-




(1) Kuehl, Bishop, Flynn and Folkers, THIS JOURNAL, **70**, 2613 (1948).

(2) Kuehl, Flynn, Brink and Folkers, *ibid.*, **68**, 2679 (1946).

(3) Lemieux, De Walt and Wolfrom, *ibid.*, **69**, 1838 (1947).

(4) Kuehl, Flynn, Brink and Folkers, *ibid.*, **68**, 2096 (1946); Hooper, Klemm, Polglase and Wolfrom, *ibid.*, **68**, 2120 (1946). In the latter reference, this compound is called tetraacetyldidesoxydihydro-L-streptobiosamine.

(5) Brink, Kuehl, Flynn and Folkers, *ibid.*, **68**, 2405 (1946).

(6) Kuehl, Flynn, Holly, Mozingo and Folkers, *ibid.*, **68**, 536 (1946).

(7) Wolfrom and Thompson, *ibid.*, **69**, 1847 (1947).

(8) Brink, Kuehl, Flynn and Folkers, *ibid.*, **68**, 2557 (1946).

(9) Stavely, Wintersteiner, Fried, White and Moore, *ibid.*, **69**, 2742 (1947).

drolysis of this thioglycoside by mercuric chloride is similar to that used in the preparation of tetraacetylstreptobiosamine.[2] The amorphous pentaacetyldihydrostreptobiosamine (II) was acetylated to give the crystalline hexaacetyldihydrostreptobiosamine (III). This derivative is identical with hexaacetyl-$\alpha$-dihydrostreptobiosamine which has been described by others.[9]

Oxidation of pentaacetyldihydrostreptobiosamine (II) with bromine in the presence of strontium carbonate followed by reacetylation gave amorphous hexaacetyldihydrostreptobiosamic acid lactone, which was not further characterized. Hydrolysis of this oxidation product with 5% hydrochloric acid gave N-methyl-L-glucosamine and a new crystalline compound, $C_6H_{10}O_5$, which was designated dihydrostreptosonic acid lactone. Although dihydrostreptosonic acid lactone may be obtained by this series of reactions, the process is laborious and another degradative series was sought.

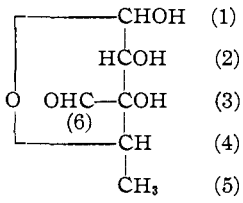
Streptomycin has been converted stepwise in good yields to ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal (IV) and to tetraacetylstreptobiosamine (V).[2] It has now been found that crystalline tetraacetylstreptobiosamine (V) may be hydrogenated with Raney nickel catalyst to tetraacetyldihydrostreptobiosamine (VI). In this hydrogenation, the preferential reduction of the formyl group takes place to an extent of at least 65%. Proof of this preferential reaction was secured by acetylation of the tetraacetyldihydrostreptobiosamine (VI) to give the known hexaacetyldihydrostreptobiosamine (III).

Tetraacetylstreptobiosamine (V) reacted with acetic anhydride in pyridine solution to give a crystalline product of the composition $C_{27}H_{39}NO_{17}$, which has been formulated as heptaacetylstreptobiosamine (VIII).

Tetraacetyldihydrostreptobiosamine (VI) was oxidized also with bromine, and then after hydrolysis the dihydrostreptosonic acid lactone (VII) was obtained. The preparation of the lactone by the latter series of reactions was more satisfactory.

Dihydrostreptosonic acid lactone was easily separated from N-methyl-L-glucosamine, because of its solubility in acetone. The compound behaved as a lactone upon potentiometric titration, and showed an absorption band at 5.65$\mu$ in the infrared corresponding to the lactone carbonyl group. Titration of dihydrostreptosonic acid lactone with sodium periodate showed that oxidation ceased in fifteen minutes after the consumption of two moles of oxidant. The oxidation liberated formaldehyde which was isolated as the dimedone derivative. These data, in conjunction with the known structure of streptobiosamine,[2] permit structure VII to be written for dihydrostreptosonic acid lactone.

Carbon atom 4 of streptose (VIII) was shown to be L, since streptobiosamine was degraded to

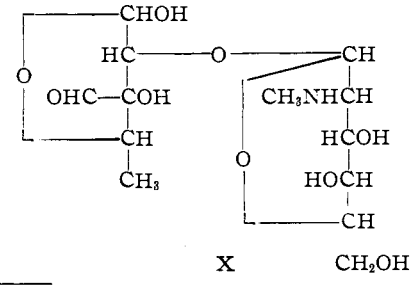



VIII

4-desoxy-L-erythrose[10]; by convention then, streptose is an L-sugar. The hydroxyl groups at carbon atoms 2 and 3 of streptose are known to have *cis* configuration, because bisdesoxystreptose formed an acidic complex with boric acid.[11] The knowledge of the configuration about either $C_2$ or $C_3$ would complete the elucidation of the total configuration of streptose.

The application of Hudson's rule[12] of optical rotation to streptosonic acid diamide,[2] which is dextrorotatory, indicates that the hydroxyl group at $C_2$ lies on the right. The application of the rule to this compound may be questioned, since the substance is a diamide. However, the application of the rule to dihydrostreptosonic acid amide would be more satisfactory, but the lactone was recovered after attempts to prepare the amide. The application of the hydrazide rule of Levene and Hudson to the hydrazide of dihydrostreptosonic acid (IX) was possible, since the lactone reacted readily with hydrazine in alcoholic solution to give the hydrazide (IX) in almost quantitative yield. Since the hydrazide was dextrorotatory, the hydroxyl group at $C_2$ may be assumed to be on the right; this confirms the configuration deduced from the rotation of the diamide of streptosonic acid. Credence in the applicability of these rules to those branched chain compounds is strengthened by the observation that both streptosonic acid lactone[2] and dihydrostreptosonic acid lactone are levorotatory. These lactones must be levorotatory if Hudson's lactone rule is applicable, since it is established conclusively that the configuration about $C_4$ of this lactone is L. The nature of the $\gamma$-lactone group of dihydrostreptosonic acid lactone is established by the above-mentioned periodate oxidation results.

The configuration of streptobiosamine may now be represented by structure X.




(10) Fried, Walz and Wintersteiner, *ibid.*, **68**, 2746 (1946).
(11) Brink, Kuehl, Flynn and Folkers, *ibid.*, **68**, 2405 (1945).
(12) Hudson, *ibid.*, **40**, 813 (1918).

## Experimental

**Pentaacetyldihydrostreptobiosamine (II) from Dihydrostreptomycin.**—A 5.43-g. sample of methyl pentaacetyldihydrostreptobiosaminide[2] (prepared from dihydrostreptomycin) was suspended in 50 ml. of ethyl mercaptan and the solution was saturated with dry hydrogen chloride. The crystals dissolved after several hours and the product deposited from the solution as an oil after standing overnight at room temperature. The ethyl mercaptan was removed *in vacuo* and the product was reacetylated by reaction with acetic anhydride in the presence of sodium acetate for one-half hour on the steam-bath. The acetylated product, after removal of excess acetic anhydride *in vacuo*, was separated from inorganic matter by extraction with chloroform. The chloroform extractives were dissolved in 200 ml. of 50% acetone and the solution was refluxed with 20 g. of strontium carbonate and 8 g. of mercuric chloride for one-half hour. After cooling, the reaction mixture was filtered and concentrated *in vacuo* to remove the acetone. The resulting aqueous solution was extracted continuously with chloroform to give 5.641 g. of amorphous pentaacetyldihydrostreptobiosamine.

**Hexaacetyldihydrostreptobiosamine (III) from Dihydrostreptomycin.**—A 621-mg. portion of pentaacetyldihydrostreptobiosamine was reacetylated in 8 ml. of acetic anhydride and 15 ml. of pyridine by allowing the mixture to stand for two days at 5°. The acetylation product was obtained as an amorphous powder, 532 mg. A solution of this material in 10 ml. of 3:7 chloroform–ether was passed over 25 g. of acid-washed alumina. The column was developed with 25 ml. of 3:7 chloroform–ether, 25 ml. of 1:1 chloroform–ether, 25 ml. of chloroform, and finally with 25 ml. of 1:9 methanol–chloroform. The product was removed from the column by elution with 25 ml. of 1:5 methanol–chloroform. It weighed 323 mg. and when it was dissolved in chloroform–ether, the solution deposited 102 mg. of crystalline hexaacetyldihydrostreptobiosamine, m. p. 141–143°. After a second recrystallization, the product melted at 146–147°.

*Anal.* Calcd. for $C_{25}H_{35}O_{15}N$: O-acetyl, 36.5. Found: O-acetyl, 36.6.

**Dihydrostreptosonic Acid Lactone (VII) from Pentaacetyldihydrostreptobiosamine.**—A solution of 2.5 g. of pentaacetyldihydrostreptobiosamine in 100 ml. of water containing 3 g. of bromine and 12 g. of strontium carbonate was maintained at 25° overnight in the dark. The reaction mixture was then filtered and concentrated *in vacuo* until all the bromine was removed. The resulting colorless solution was shaken with excess silver carbonate, filtered, then treated with hydrogen sulfide and filtered once more. The aqueous solution was concentrated *in vacuo* to a residue which was acetylated in pyridine–acetic anhydride. The crude acetylation product, 1.984 g., failed to crystallize. A 1.6-g. portion of this product was hydrolyzed by dissolving in 30 ml. of 5% hydrochloric acid and refluxing the solution for two hours. The hydrolysate was clarified by treatment with Darco and concentrated *in vacuo* to a residue. This dried residue was dissolved in 4 ml. of methanol and 75 ml. of acetone was gradually added. The precipitated N-methyl-L-glucosamine hydrochloride was removed by filtration and the filtrate was concentrated *in vacuo* to a residue. This product was further purified by dissolving it in 1 ml. of methanol and again adding 60 ml. of acetone. A final purification was accomplished by dissolving the acetone soluble material in dioxane and filtering the traces of the methylamino sugar. The product, 402 mg., was dissolved in 2 ml. of dioxane and treated with 20 ml. of chloroform. The dihydrostreptosonic acid lactone crystallized; yield, 190 mg., m. p. 135–138°. After recrystallization from chloroform-methyl ethyl ketone, the lactone melted at 143–144°, $[\alpha]_D$ −32° (*c*, 0.40 in water).

*Anal.* Calcd. for $C_6H_{10}O_5$: C, 44.45; H, 6.22; mol. wt., 162. Found: C, 44.02; H, 5.87; eq. wt., 167 (potentiometric titration).

**Tetraacetylstreptobiosamine[2] (V).**—A solution of 45.9 g. of crude ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal which was obtained by the acetylation of 35.9 g. of crystalline ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride, 75.4 g. of mercuric chloride, and 92 g. of cadmium carbonate in 920 ml. of 50% acetone was heated under reflux for forty minutes. Vigorous mechanical stirring was required to prevent bumping. The resulting slurry was cooled, filtered and concentrated *in vacuo* to *ca.* 250 cc. The product was removed from the aqueous concentrate by continuous chloroform extraction. The chloroform extract, dried and adjusted to a volume of 100 ml., was poured onto a column of 100 g. of acid washed alumina. Elution of the column with 350 ml. of chloroform gave, after evaporation of the solvent, 27.7 g. of amorphous material. Crystallization of this material from acetone–absolute ether yielded 11.765 g. of product, m. p. 184–187°. After several recrystallizations from the same solvent, the tetraacetylstreptobiosamine melted at 188–189°, $[\alpha]_D$ −78.4° (*c*, 1.00 in chloroform).

*Anal.* Calcd. for $C_{21}H_{31}O_{13}N$: C, 49.89; H, 6.19; N, 2.77; acetyl, 34.0; O-acetyl, 25.5. Found: C, 49.90; H, 6.48; N, 2.73; acetyl, 34.3; O-acetyl, 25.3.

**Tetraacetyldihydrostreptobiosamine (VI).**—Twenty-four grams of tetraacetylstreptobiosamine was dissolved in 150 ml. of methanol and 400 ml. of water and shaken with 18 g. of Raney nickel catalyst. The mixture was then centrifuged and the filtrate was hydrogenated in the presence of another 18-g. portion of Raney nickel catalyst at 40 lb. pressure of hydrogen. Hydrogen ceased to be absorbed after three and one-half hours. The nickel was removed by filtration, and the solvent was removed *in vacuo*. A solution of the residue in 200 ml. of chloroform was dried, filtered, and concentrated *in vacuo* leaving 24 g. of a gum. To a solution of the gum in 100 ml. of methanol, was added 200 ml. of ether and 150 ml. of petroleum ether. After standing overnight, 10.4 g. of product was removed by filtration, m. p. 188–190° (cor.). The mother liquor was concentrated to 25 ml., and 25 ml. of ether and 25 ml. of petroleum ether were added. After cooling the solution overnight, 5.0 g. of additional product was obtained, m. p. 188–189°. After recrystallization from methanol–ether, the tetraacetyldihydrostreptobiosamine melted at 190–191°, $[\alpha]^{25}_D$ −86.5° (*c*, 1.0 in chloroform).

**Hexaacetyldihydrostreptobiosamine (III).**—Ten grams of tetraacetyldihydrostreptobiosamine was dissolved in 60 ml. of pyridine, and 60 ml. of acetic anhydride was added. After standing overnight, the solvents were removed *in vacuo*. The residue was dissolved in 50 ml. of methanol and the solution was filtered. To the filtrate was added 100 ml. of ether and then petroleum ether to the point of incipient cloudiness. After crystallization began, 300 ml. of petroleum ether was added, and the mixture was cooled overnight. The hexaacetyldihydrostreptobiosamine was removed by filtration; 7.7 g., m. p. 141–143°. After several recrystallizations from methanol–ether, it melted at 146–147°, $[\alpha]^{25}_D$ −113° (*c*, 0.5 in chloroform).

*Anal.* Calcd. for $C_{25}H_{37}O_{15}N$: C, 50.76; H, 6.30; N, 2.37. Found: C, 50.68; H, 6.23; N, 2.54.

**Heptaacetylstreptobiosamine (VIII).**—A solution of 413 mg. of tetraacetylstreptobiosamine in 4 ml. of pyridine and 2 ml. of acetic anhydride was allowed to stand for eighteen hours at 0°. The solvent was removed *in vacuo* and the residue was dissolved in ice water. Extraction with chloroform removed 460 mg. of an oily product. This material was dissolved in 4 ml. of 1:3 acetone–absolute ether and poured onto a column of 10 g. of acid-washed alumina. The column was then developed with 15 ml. of the same solvent mixture and then eluted with 20 ml. of 1:1 acetone–ether to give 236 mg. of an oil. A solution of this oil in a mixture of acetone, ether, and petroleum ether deposited 28 mg. of heptaacetylstreptobiosamine; m. p. 151–152°, $[\alpha]_D$ −106° (*c*, 0.70 in chloroform). Further recrystallizations did not alter the melting point.

*Anal.* Calcd. for $C_{27}H_{39}O_{17}N$: C, 49.94; H, 6.06; O-acetyl, 39.7. Found: C, 49.98; H, 6.40; O-acetyl, 39.5.

**Dihydrostreptosonic Acid Lactone (VII) from Tetraacetyldihydrostreptobiosamine.**[1]—Oxidation of 5.7 g. of crystalline tetraacetyldihydrostreptobiosamine yielded

after hydrolysis 433 mg. of dihydrostreptosonic acid lactone.

**Determination of Periodate Consumption by Dihydrostreptosonic Acid Lactone.**—A 5-ml. aqueous solution of 12.0 mg. of the lactone and 78.0 mg. of sodium metaperiodate was allowed to stand at room temperature. The periodate consumption was determined by the standard arsenite method.[13] A 1-ml. aliquot required 7.975 ml. of sodium arsenite solution (1 ml. ≅ 1.09 mg. of sodium meta periodate) corresponding to 2.1 moles after fifteen minutes. No further consumption was observed after one hour.

**Oxidation of Dihydrostreptosonic Acid Lactone (VII).**—A 36.6-mg. quantity of dihydrostreptosonic acid lactone was dissolved in 11.7 ml. of water containing 98 mg. of paraperiodic acid (determined by titration with standard sodium arsenite solution). After two hours, a 1-ml. aliquot was removed and treated with strontium carbonate. The excess strontium carbonate and strontium iodide was removed by filtration. The aqueous filtrate was adjusted to a volume of 1.5 ml. and then treated with 1.5 ml. of an alcoholic solution containing 50 mg. of dimedone. After two hours at 5°, the crystalline product was removed by filtration; yield, 5.5 mg. (97% theory), m. p. 193–194°. After recrystallization of the product from alcohol–water, the melting point did not change. When this derivative was mixed with the dimedone derivative of formaldehyde, no depression of melting point was observed; m. p. 193–194°.

**Hydrazide of Dihydrostreptosonic Acid (IX).**—A solution of 183 mg. of dihydrostreptosonic acid lactone in 10 ml. of ethanol and 0.5 ml. of hydrazine hydrate was allowed to stand for two hours at room temperature. Fifteen milliliters of chloroform was then added and the solution was allowed to stand for one and one-half hours at 5°. The crystalline hydrazide was removed by filtration; yield, 204 mg., m. p. 135–136°. After several recrystallizations from methanol–water, it melted at 137–139°, $[\alpha]^{25}D + 23°$ ($c$, 0.9 in water).

(13) Jackson, "Organic Reactions," Vol. II, p. 361.

*Anal.* Calcd. for $C_6H_{14}N_2O_5$: C, 37.09; H, 7.26; N, 14.4. Found: C, 36.91; H, 6.96; N, 14.8.

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## Summary

Methyl pentaacetyldihydrostreptobiosaminide was converted into amorphous pentaacetyldihydrostreptobiosamine, through the intermediate ethyl thioglycoside, which gave a crystalline hexaacetyldihydrostreptobiosamine. Oxidation of pentaacetyldihydrostreptobiosamine with bromine gave amorphous hexaacetyldihydrostreptobiosamic acid lactone which was hydrolyzed to N-methyl-L-glucosamine and the new dihydrosteptosonic acid lactone.

Tetraacetylstreptobiosamine was hydrogenated selectively with Raney nickel catalyst to the new crystalline tetraacetyldihydrostreptobiosamine. Oxidation and hydrolysis of the tetraacetyldihydro derivative gave also dihydrostreptosonic acid lactone. The lactone gave a hydrazide.

Application of rules of rotation to streptosonic acid diamide and dihydrostreptosonic acid hydrazide indicates that the configuration at $C_2$ is D. The configuration of streptose and streptobiosamine are now known.

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

# Reactions of Free Hydroxyl Radicals with Organic Compounds

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In previous publications on hydroxylation of organic compounds in solution with hydrogen peroxide in the presence of catalysts[3] or ultraviolet light[4] we postulated the formation and subsequent reaction of free hydroxyl radicals with organic compounds.[5,6] Hydroxylations of this type have not been attempted previously in the gaseous phase in spite of the fact that numerous reactions have been studied with free hydroxyl radicals usually obtained by the dissociation of water or mixtures of water and oxygen in glow discharges.[7–10]

The present investigation describes some experiments in which free hydroxyl radicals were allowed to react in the gaseous phase with saturated and unsaturated hydrocarbons. It has been found that free hydroxyl radicals are much more reactive in the gaseous phase than in solution. For example, in solution hydroxyl radicals are not known to react with saturated aliphatic hydrocarbons, while in the gaseous phase they react readily with methane to form chiefly carbon dioxide and formic acid, small amounts of methanol, and traces of ethanol. With ethylene and a large excess of hydroxyl radicals, formaldehyde, formic acid and carbon dioxide were the chief products with possible traces of ethylene glycol. When ethylene was used in large excess so that it entered the discharge tube, besides formaldehyde, acetylene and ethane were found among the condensa-

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(3) Milas and Sussman, THIS JOURNAL, **58**, 1302 (1936); **59**, 2345 (1937); Milas, *ibid.*, **59**, 2352 (1937); Milas, Sussman and Mason, *ibid.*, **61**, 1844 (1939); Milas and Maloney, *ibid.*, **62**, 1841 (1940).

(4) Milas, Kurz and Anslow, Jr., *ibid.*, **59**, 543 (1937).

(5) Weiss, *Trans. Faraday Soc.*, **36**, 856 (1940).

(6) Waters, *Ann. Reports*, **42**, 131 (1946).

(7) Rodebush and Campbell, *J. Chem. Phys.*, **4**, 293 (1936); Rodebush, Keizer, McKee and Quagliano, THIS JOURNAL, **69**, 538 (1947).

(8) Frost and Oldenberg, *J. Chem. Phys.*, **4**, 642 (1936); Oldenberg and Rieke, *ibid.*, **6**, 169, 439 (1938); **7**, 485 (1939).

(9) Kondrat'ev and Ziskin, *Acta Physicochim. U. S. S. R.*, **5**, 301 (1936); Kondrat'ev, *ibid.*, **8**, 315 (1938); **10**, 791 (1939).

(10) Smith, *J. Chem. Phys.*, **11**, 110 (1943).