mixed resin column. Unreacted hexonic acid was eluted from the column with 2 N NH_4OH and treated as before; this second treatment contributed about 20% of the final yield of sugar. The sugar mixture was dissolved in 0.2 molar Na phosphate buffer pH 7.5 (2.5% hexose w./v.) and epimerization effected by heating the solution on a steam-bath for 90 minutes. Glucose, mannose and fructose were partially separated by paper chromatography on four sheets of Whatman No. 1 filter paper (57 × 47 cm.) using phenol–water as solvent,[6] and the sugars located by exposing the dried sheets to X-ray film. After elution from the paper the sugars were further purified by paper chromatography in butanol–acetic acid–water.[6] The final products were shown by two-dimensional chromatography in phenol–water and butanol–ethanol–water[6] to contain no significant quantity of radioactive contaminants.

The yields of hexoses from 2.7 millimoles of starting $NaC^{14}N$ were: glucose 0.496, fructose 0.055 and mannose 0.077 millimole. The total hexose obtained (0.628 millimole) was 23% of the theoretical yield.

Degradation of glucose by heterolactic fermentation[7] after its isolation from the epimeric mixture showed that carbon atom 1 contained 100% of the total radioactivity in the molecule. Carbon atoms 2 to 6 contained no significant activity.

Acknowledgment.—We wish to thank Dr. W. Z. Hassid for his interest in this work.

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(7) I. C. Gunsalus and M. Gibbs, *J. Biol. Chem.*, **194**, 871 (1952).

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Reaction of Methylal with Some Acid Anhydrides

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RECEIVED MAY 26, 1954

Claisen[1] reported that the reaction of diethylacetal with acetic anhydride gave a good yield of α-ethoxyethyl acetate. He found it necessary to conduct the reaction in a sealed tube at 150°.

Recently we have studied this reaction with methylal and acetic, propionic and n-butyric anhydrides and find that it proceeds smoothly in the presence of an acid catalyst to give good yields of the corresponding methoxymethyl esters. Moreover, the use of a catalyst permits the reaction to take place merely by refluxing the mixture under atmospheric pressure. The products of the reaction are the methyl and methoxymethyl esters of the acid anhydride used and these can be separated readily by fractionation. The methoxymethyl esters have been prepared previously from monochloromethyl ether and the sodium salts of the corresponding acids.[2]

Attempts to extend the reaction to benzoic and phthalic anhydrides were not successful.

TABLE I

PROPERTIES OF METHOXYMETHYL ESTERS PREPARED

	This investigation	Literature[2]
Methoxymethyl acetate		
B.p., °C.	117–118	117–118
Mol. wt.	102.4	104 (calcd.)
$n^{25}D$	1.3917	1.3980
d^{20}_4	1.018	0.989
M_R	23.21	23.97 (calcd.)
Methoxymethyl propionate		
B.p., °C.	133	133
Mol. wt.	117	118 (calcd.)
n^{20}_4	0.9886	0.9872
Methoxymethyl n-butyrate		
B.p., °C.	152	151–152
Mol. wt.	131	132 (calcd.)
d^{20}_4	0.9740	0.9747

(1) L. Claisen, *Ber.*, **31**, 1018 (1898).

Experimental

Methoxymethyl Acetate.—The methylal used in this work was prepared from the methylal–methanol azeotrope by refluxing the latter over calcium chloride to remove most of the methanol and then distilling the product over sodium. The presence of methanol or moisture in the methylal results in decreased yield of the desired esters.

Acetic anhydride (102 g., 1 mole), methylal (76 g., 1 mole) and 1 g. of p-toluenesulfonic acid were refluxed gently for six hours. The mixture was then distilled through a 30-plate Oldershaw column and the cut boiling at 100–125° was collected. Redistillation gave 81 g. of a fraction boiling at 117–118° which was the desired product; $n^{25}D$ 1.3917, d^{20}_4 1.018. From the residue 14.5 g. of acetic anhydride was recovered. The yield of methoxymethyl acetate based on acetic anhydride consumed was 95%.

Methoxymethyl Propionate and n-Butyrate.—Methoxymethyl propionate was prepared as described above, using an equivalent amount of propionic anhydride. The cut boiling at 130–135° was washed with 10% sodium bicarbonate followed by water, dried over magnesium sulfate and refractionated. The product boiled at 133°, d^{20}_4 0.9886. Thirty-one per cent. of the propionic anhydride was recovered and the yield of ester based on anhydride consumed was 94%.

Methoxymethyl n-butyrate was similarly prepared. The ester boiled at 155°, d^{20}_4 0.9740. Of the n-butyric anhydride taken, 27.6% was recovered and the yield of ester on this basis was 86%.

(2) F. E. Clark, S. F. Cox and E. Mack, THIS JOURNAL, **39**, 712 (1917).

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The Reduction of Streptomycin with Sodium Borohydride

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RECEIVED JUNE 7, 1954

The generally accepted procedure for the reduction of the aldehyde group of streptomycin involves a catalytic hydrogenation with platinum or other metals as catalyst.[1–3] An electrochemical procedure for the reduction of streptomycin has also been reported.[4] Chaikin and Brown[5] demonstrated the reduction of organic aldehydes in aqueous solution with sodium borohydride. We have found sodium borohydride to be a very effective reagent with which to convert the aldehyde group of streptomycin and hydroxystreptomycin to their respective derivatives.

The completeness of the reduction of streptomycin by sodium borohydride was investigated with variations in time of reaction, temperature, pH of the solution, quantity of sodium borohydride, and purity of the streptomycin.

(1) R. L. Peck, C. E. Hoffhine and K. Folkers, THIS JOURNAL, **68**, 1390 (1946).

(2) Q. R. Bartz, J. Controulis, H. M. Crooks, Jr., and M. C. Rebstock, *ibid.*, **68**, 2163 (1946).

(3) R. A. Carboni and P. P. Regna, U. S. Patent 2,522,858 (1950).

(4) R. Ohdake, Y. Kojima and H. Kusakabe, *Repts. Sci. Research Inst. (Japan)*, **28**, 103 (1952).

(5) S. W. Chaikin and W. G. Brown, THIS JOURNAL, **71**, 122 (1949).

Time, temperature and *p*H when greater than 7.5, had little or no effect on the course of the reaction.

The concentration of sodium borohydride greatly influenced the completeness of reaction up to a point of maximum reduction. One equivalent of sodium borohydride only partially reduced an equivalent of streptomycin. With increasing quantities of sodium borohydride, the reduction of streptomycin rapidly approached completion until a maximum value (as determined by assay for residual streptomycin by the maltol method[6]) was reached. Beyond this point a very great excess of borohydride reagent only slightly lowered the residual streptomycin value.

The amount of borohydride needed for maximum reduction varied with different samples of streptomycin and appeared to be associated to some degree with the purity or previous processing of the material. Samples of pure commercial grade streptomycin sulfate were readily reduced to a satisfactory level (less than 1% residual streptomycin by maltol test) with only a small excess of the sodium borohydride reagent. Partially purified materials and ion-exchange resin eluates, however, often gave unsatisfactory reduction with even very large excesses of sodium borohydride. This apparent resistance to complete reduction of some streptomycin samples has also been observed in the catalytic hydrogenation procedure.

One sample of dihydrostreptomycin sulfate which was treated with a large excess of sodium borohydride still contained 20% of residual streptomycin on the basis of the maltol test. When this material was submitted to paper strip chromatographic analysis according to the procedure of Winsten,[7] only a single dihydrostreptomycin zone was observed; no streptomycin zone was present. Thus, it would appear that sodium borohydride completely reduced the streptomycin component of the sample.

The presence of substances in streptomycin broth which are not active against microörganisms but which hydrolyze to give an absorption at 322 mμ like that of maltol has been postulated.[8] The data obtained in our experiments indicate that there may be associated with crude streptomycin some biologically inactive substance which gives a positive maltol test similar to that of streptomycin. This entity may be carried along in streptomycin processing and continue to give a positive color test after treatment with reducing agents, thus causing an apparent incomplete reduction of streptomycin by sodium borohydride or by catalytic hydrogenation.

Experimental

Preparation of Dihydrostreptomycin Sulfate with Sodium Borohydride.—Five grams of streptomycin sulfate assaying 719 μg. base/mg. was dissolved in 35 ml. of water at room temperature to give a solution containing approximately 100,000 μg/ml. The *p*H was adjusted to 8.0 with triethylamine and 0.15 g. of sodium borohydride[9] in 5 ml. of water was added with stirring. A slight elevation in temperature and mild gas evolution occurred. After thirty minutes, 6 *N* H_2SO_4 was added slowly to *p*H 1.5. Some gas was evolved. The acidified solution was kept at room temperature for 10 minutes and then added to 175 ml. (5 volumes) of methanol with vigorous stirring. The resulting precipitate was collected, washed with methanol, and dried *in vacuo* over P_2O_5 to give 5.1 g. of boron-free dihydrostreptomycin sulfate with a biological potency of 700 μg./mg. and a residual maltol assay of 4.8 μg. streptomycin/mg. The amorphous dihydrostreptomycin sulfate obtained from the above process was readily crystallized from water–methanol solution.

Effect of Time on Completeness of Reduction.—Thirty milliliters of a partially purified streptomycin sulfate solution at a concentration of 100,000 μg./ml. and at *p*H 7.0 was mixed with 1.2 ml. of a solution containing 192 mg. of sodium borohydride. At these concentrations, three equivalents of sodium borohydride were present for each equivalent of streptomycin aldehyde. The determination of residual streptomycin by maltol determination was made after 7 minutes, 30 minutes, and 18 hours. Values obtained for unreduced streptomycin were 3.1, 2.9 and 3.2%, respectively. Similarly, no significant difference in degree of reduction was observed when the streptomycin solution was allowed to stand in the presence of 8 equivalents of sodium borohydride for 7 minutes or for 18 hours.

Effect of Temperature on Reduction with Sodium Borohydride.—Two 20-ml. portions of a partially purified streptomycin sulfate solution of 100,000 μg./ml. concentration were treated with 4 equivalents of sodium borohydride. One sample was held at 25° overnight, the other at 45°. After acidification with sulfuric acid, maltol assays indicated 3.5 and 3.3% residual streptomycin, respectively.

Influence of Nature of the Sample upon Completeness of Reduction with Sodium Borohydride.—Aliquots of a solution of crude streptomycin sulfate containing 50,000 μg./ml. were treated at *p*H 8.5 with 2, 4 and 8 equivalents of sodium borohydride solution. After 30 minutes the solutions were acidified to destroy excess borohydride and assayed for residual streptomycin, giving values of 4.2, 5.0 and 4.2%, respectively.

A partially purified streptomycin was treated similarly. After reduction with 2, 4, 8 and 16 equivalents of sodium borohydride, residual streptomycin values of 2.0, 3.6, 2.7 and 2.2% were obtained.

Solutions of pure commercial streptomycin sulfate were treated in the same manner with 1 and 2 equivalents of sodium borohydride. Unreduced streptomycin values following such treatment were 2.5 and 0.9%.

Catalytic Reduction *vs.* Borohydride Reduction.—A sample of partially purified streptomycin sulfate at a concentration of 200,000 μg./ml. was placed in a Parr hydrogenator. Activated platinum catalyst (2.5 g./100 g. streptomycin sulfate) was added and the mixture shaken continuously for 15 hours at 25° under 50 lb. pressure of hydrogen. The solution was added to 5 volumes of methanol to give solids assaying 5.7 μg. streptomycin/mg. To an aliquot of the above starting solution before reduction was added 6 equivalents of sodium borohydride. Fifteen minutes after mixing, the solution was acidified to *p*H 1.5 with 6 *N* H_2SO_4 and added to 5 volumes of methanol to give solids with an apparent streptomycin content of 9.7 μg./mg.

In a repeat run comparing catalytic *vs.* sodium borohydride reduction in which pure streptomycin sulfate was used as starting material, it was found that by both procedures the unreduced streptomycin was less than one-half per cent.

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(6) *Federal Register*, **15**, 9460 (1950).

(7) W. A. Winsten and E. Eigen, THIS JOURNAL, **70**, 3333 (1948).

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(9) Metal Hydrides, Inc., Beverly, Mass.

The Deuteration of Aniline in the Presence of Raney Alloy

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RECEIVED APRIL 19, 1954

Methods for the introduction of deuterium into aniline have been investigated since deuterated anilines in which deuterium was in a specific position were required for a separate study. Accordingly,