(and working up with barium hydroxide to remove ammonia in the usual manner), but the crude amino acid melted at 151–164°. When recrystallized from water to constant melting point, 166–168°, the yield was 10.4 g. of amino acid from one mole of acetamidocyanoacetic ester. This is essentially the same over-all yield as reported above. No attempt was made to isolate the second DL-stereoisomer which is presumably present.

5-Carboxy-3-methyl-2-pyrrolidone.—The pyrrolidone was obtained in an attempt to prepare the hydantoin. A solution of 10.4 g. of 4-methylglutamic acid in 100 ml. of water was brought to *p*H 7 with sodium hydroxide and heated with 7.1 g. of potassium cyanate on the steam-bath for one hour. It was then acidified with hydrochloric acid and heated for three hours more. The solvent was removed *in vacuo* and the residue extracted with hot alcohol. The product, recrystallized from water, melted at 173°.

Anal. Calcd. for $C_6H_9NO_3$: C, 50.43; H, 6.34; N, 9.79. Found: C, 49.92; H, 6.23; N, 9.53.

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RECEIVED APRIL 29, 1952

COMMUNICATIONS TO THE EDITOR

A NEW PROCEDURE FOR THE DETERMINATION OF THE FINE STRUCTURE OF POLYSACCHARIDES

Sir:

In a previous communication[1] it was reported that the dialdehydes obtained from simple glycosides by periodate oxidation could readily be reduced to the corresponding alcohols in almost quantitative yield.

We wish to report here that periodate oxidation followed by reduction with either hydrogen and a Raney nickel catalyst between 60 and 100° under pressure or with sodium borohydride in aqueous solution represents a general procedure which can also be applied to polysaccharides. Now, whereas the periodate-oxidized polysaccharides or "polyaldehydes" usually undergo profound decomposition when hydrolyzed even in the cold, the corresponding new "polyalcohols" can be subjected to hydrolysis with boiling dilute mineral acid with little or no decomposition to give cleavage products which can be separated by partition chromatography and determined quantitatively. Results obtained in this manner provide information concerning the nature and amount of glycosidic linkages in a polysaccharide.

Glucose residues linked so that free OH groups are present at C_2 and C_3 will give rise to erythritol and glycolic aldehyde when subjected to periodate oxidation followed by reduction and hydrolysis; this applies to residues linked through positions 1 and 4 or 1, 4, and 6. Glucose residues with free hydroxyl groups at C_3 and C_4 such as those in terminal positions and those joined through positions 1 and 6 or 1, 2 and 6 will provide glycerol instead of erythritol. However, any glucose residue linked so that no pair of adjacent hydroxyl groups is present will not be affected by periodate oxidation and will therefore appear as free glucose after the final hydrolysis step. Similar considerations, which are clearly not restricted to polyglucosans, will also apply to polysaccharides composed wholly or in part of furanose residues.

The deductions that can be made from the results of an examination of the polyalcohol produced from a given polysaccharide do not necessarily permit a clear cut solution to a structural problem but taken in conjunction with other experimental results such as, for example, those of methylation it is feasible to restrict greatly the number of structural possibilities.

The few typical examples given below will serve to illustrate the usefulness of the proposed new procedure. In the case of the branched chain polysaccharides amylopectin and glycogen which are composed of glucopyranose residues joined by 1,4 bonds and have branches at certain C_6 positions, the non-reducing terminal unit will give rise to glycerol while the glucose units of the main chain joined through positions 1 and 4 and those at which branching occurs with linkages at positions 1, 4 and 6 will all give erythritol. Hence the molecular ratio of glycerol to erythritol, as determined by the chromotropic acid procedure,[2] should equal the molecular ratio of terminal to non-terminal glucose residues. For glycogen a ratio of 1:10 has been found for the glycerol/erythritol ratio. This is in good agreement with the value of 1:11 for the molecular ratio of tetramethyl- to the sum of the trimethyl- and dimethyl-glucose components derived from methylation studies. The result is also in good agreement with the figure of 1:11 for the ratio of terminal to non-terminal residues as determined from the amount of formic acid liberated by periodate oxidation of glycogen itself.[3] Similar correlations have been obtained with amylopectin.

In a polysaccharide composed of hexopyranose residues joined by 1,6 and 1,4 linkages the ratio of the number of these two types of linkages should correspond to the mole ratio of the glycerol to the erythritol obtained from the corresponding polyalcohol by hydrolysis. By application of the new procedure reported herein to one type of dextran,[4] produced by *Leuconostoc mesenteroides* NRRL-B-512, followed by chromatographic separation and determination of glycerol and erythritol,[2] the ratio of 1,6 to 1,4 linkages has been found to be approximately 45:1.

Paper partition chromatographic investigation of

(1) Bertha Lewis, R. Montgomery, F. Smith and J. Van Cleve, 121st A.C.S. Meeting, Milwaukee, Wisconsin, April, 1952.

(2) Marguerite Lambert and A. C. Neish, *Can. J. Res.*, **B28**, 83 (1950).

(3) M. Abdel-Akher and F. Smith, THIS JOURNAL, **73**, 994 (1951).

(4) The authors thank Dr. Allene Jeanes of the Northern Regional Research Laboratory, Peoria, for the sample of dextran.

the hydrolysis fragments of the above dextran polyalcohol revealed also 2–2.5% glucose as determined by the phenol–sulfuric acid method.[5] This is believed to be derived from glucose residues which are immune to periodate oxidation as a consequence of being linked through positions 1 and 3, or 1, 2, and 4.

In an analogous manner it has been shown that glycogen and amylopectin contain about 1.0 and 0.5% glucose, respectively, which is immune to prolonged treatment with sodium periodate. This glucose, which still remains intact even when the derived polyalcohols themselves are treated with sodium periodate, could arise because of incomplete oxidation or because of fixed *trans* OH groups arising from stereochemical strain[6] but the present evidence suggests that it arises from glucose residues in the polysaccharides linked by 1, 3 bonds. While it is probable that these same glucose residues correspond to those which give rise to the 2,6-dimethyl-D-glucose fragment produced from the methylated polysaccharides by hydrolysis, the possibility exists that they correspond to glucose residues joined through positions 2 and 4.

In similar experiments on amylose and cellulose the indications are that these two polysaccharides contain approximately 0.2 to 0.5 and 0.1 to 0.2% glucose, respectively, which is immune to periodate oxidation. It is believed, therefore, that the possibility of a hitherto unrecognized linkage in these polysaccharides is worthy of some consideration. The details and constitutional significance of these and similar experiments on other polysaccharides such as fructosans, hemicelluloses, fungus glucosans, plant gums and degraded plant gums will be published later.

(5) M. Dubois, K. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Nature*, **168** 167 (1951).

(6) B. H. Alexander, R. J. Dimler and C. L. Mehltretter, THIS JOURNAL, **73**, 4658 (1951).

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RECEIVED AUGUST 4, 1952

X-RAY EXAMINATION OF IRON BISCYCLOPENTADIENYL

Sirs:

Crystals of iron biscyclopentadienyl, prepared by Dr. E. O. Brimm of Linde Air Products Co., and forwarded to us by Professor W. C. Fernelius, were examined by X-ray methods at the suggestion of the latter. Rotation and Weissenberg photographs, using MoKα radiation, revealed a monoclinic cell, space group $P2_1/n$, with $a = 9.00$ Å; $b = 7.52$ Å., $c = 5.94$ Å., $\beta = 92.5°$. The measured density of 1.516 g./cc. showed 2 molecules per cell, and thus required the two iron atoms to be at the cell corner and body center. The cell symmetry requires that the molecule is centrosymmetric, with the iron atom at its center.

The molecular structure was determined automatically on X-RAC, the electronic computer for X-ray analysis,[1] by use of the non-negativity criterion, as previously utilized by us on the structure of fructose[2] and in several other analyses.[3] The iron atoms contribute positive phases to $(h,k,0)$ terms with $h + k = 2n$. All such structure factors were inserted into X-RAC with positive phases, and the strongest term with $h + k$ odd was also entered as positive. The effects of phase permutation of the remaining odd terms were examined consecutively and in order of decreasing amplitude, and phases were assigned so as to minimize negative excursions of the density function. A projection on the (x,y) plane concomitant with the "sandwich" structures proposed by Wilkinson, Rosenblum, Whiting and Woodward[4] and Woodward, Rosenblum and Whiting[5] immediately appeared. The density projection did not indicate that the cyclopentadiene groups were rotating, and the center of symmetry then demanded the anti-prismatic structure of Wilkinson, *et al.*[4]

A correct form factor for iron as it occurs here is not known, and thus a refinement of carbon positions has not yet been possible. Using an empirical Fe^{++} curve with an approximate temperature factor, an R-factor of 0.17 was found for a planar carbon ring with C–C distances of 1.41 Å. and Fe–C distance of 2.0 Å. A three-dimensional analysis is in progress, to establish the nature of the bonding and the electronic configuration of the iron atom.

We are grateful to Dr. Brimm and Prof. Fernelius for suggesting the problem and supplying the crystalline material.

(1) R. Pepinsky, *J. Appl. Phys.*, **18**, 601 (1947).

(2) P. F. Eiland and R. Pepinsky, *Acta Cryst.*, **3**, 160 (1950).

(3) X-RAC Computations supported by Office of Naval Research.

(4) G. Wilkinson, M. Rosenblum, M. C. Whiting and R. B. Woodward, THIS JOURNAL, **74**, 2126 (1952).

(5) R. B. Woodward, M. Rosenblum and M. C. Whiting, *ibid.*, **74**, 3458 (1952).

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RECEIVED AUGUST 12, 1952

CHROMATOGRAPHIC SEPARATION OF THE ADRENOCORTICOTROPIC HORMONE ON PARTITION COLUMNS[1]

Sir:

Fractionation of pig and sheep pituitary extracts on oxycellulose columns[2] has yielded materials with ascorbic acid depleting activities up to 100 u./mg.[3] After *peptic* digestion of such fractions of porcine origin, Brink, *et al.*,[4] applied the countercurrent distribution technique in the isolation of an apparently homogeneous material with an activity of 300 u./mg.

(1) This work was aided in part by grants to Professor C. H. Li from the National Institutes of Health, the United States Public Health Service, the Armour Laboratories, Merck and Company, Inc., and the Eli Lilly Laboratories.

(2) (a) E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951); (b) C. H. Li, *ibid.*, **74**, 2124 (1952).

(3) Assays reported here were performed by the adrenal ascorbic acid depletion method of M. A. Sayers, G. Sayers and L. A. Woodbury, *Endocrinology*, **42**, 379 (1948). Results are expressed in U. S. P. units per milligram.

(4) N. G. Brink, F. A. Kuehl, J. W. Richter, A. W. Bazemore, M. A. P. Meisinger, D. E. Ayer and K. Folkers, THIS JOURNAL, **74**, 2120 (1952).