4-Ethoxy-5-methoxyphthalic Anhydride from Oxidation of 7-Ethoxy-6-methoxy-3,4-dihydroisoquinoline.[12]—Seven hundred milligrams of 7-ethoxy-6-methoxy-3,4-dihydroisoquinoline, m.p. 83–84°, was dissolved in 50 ml. of water and, while this solution was stirred mechanically, a solution of 2.3 g. of potassium permanganate in 200 ml. of water was added gradually. The mixture was warmed on the steam-bath to finish the oxidation. The solution was acidified with hydrochloric acid and treated with sulfur dioxide. When the manganese dioxide was gone, the solution was extracted continuously with ethyl ether for 12 hours. The ether extract yielded 291 mg. of residue which was dissolved in 30 ml. of 5% ammonium hydroxide. Calcium chloride solution was added until precipitation was complete. The filtrate from the precipitate was acidified and extracted continuously with ether for 2 hours. The ether residue weighed 247 mg. It was sublimed at 7×10^{-4} mm. and 125–130° bath temperature. The yellow sublimate weighed 206 mg. and melted at 187–189°. After two recrystallizations from ether, the 4-ethoxy-5-methoxyphthalic anhydride melted constantly at 192–193°.

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.48; H, 4.53. Found: C, 59.55; H, 4.52.

(12) E. Späth and A. Dobrowsky, *Ber.*, **58**, 1274 (1925).

Summary

Erysopine and erysovine have been converted into apoerysopine by reaction with hydrobromic acid. Hofmann degradation of apoerysopine using dimethyl sulfate and alkali has yielded *des*-dimethylapoerysotrine after two stages. *des*-Dimethylapoerysotrine and tetrahydro-*des*-dimethylapoerysotrine were not degraded to nitrogen-free products. Alkali fusion of erysodine gave indole. Ethylation and oxidation of erysodine, erysovine and "erysocine" gave 4-ethoxy-5-methoxyphthalic anhydride. This phthalic derivative was also obtained from 7-ethoxy-6-methoxy-3,4-dihydroisoquinoline.

It was found that erythraline has the same ring system as erysopine and related erysoalkaloids by degradation of erythraline to apoerysopine.

Interpretation of these reactions and products permits structural formulations of erysopine, erysodine and erysovine, erythraline and other related alkaloids.

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[CONTRIBUTION FROM THE DIVISION OF PLANT BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF CALIFORNIA]

Starch. IV. The Molecular Constitution of Amylose Subfractions

BY A. L. POTTER AND W. Z. HASSID

If the amylose component of starch consists of unbranched chains, as commonly assumed, its molecular weight determined by the periodate oxidation end-group method should be equal to that estimated by osmotic pressure measurements. However, analytical results are not always in accord with this assumption. Examination of the data obtained for amyloses of seven different sources[1] showed that three of them, potato, Easter lily and apple, had essentially the same molecular weights by the two methods, indicating that these amyloses are apparently unbranched. The other four amyloses were found to possess lower degrees of polymerization when determined by the periodate oxidation method than by osmotic pressure measurements. These results imply the possible existence of branching in some amyloses.

In order to obtain further information regarding the question of branching in amylose, a number of subfractions from potato and corn amyloses were studied. The amylose subfractions were contributed by T. J. Schoch of the Corn Products Refining Company. Their method of preparation is given by Lansky, Kooi and Schoch.[2] The methods employed for the determination of end-groups, osmotic pressure and viscosity measurements are described in the previous papers.[1]

Comparison of the data obtained from the two methods showed that the molecular weights of the potato subfractions (Table I, Potato, P-7/9-A, 17a and 17f) and all of the corn amylose subfractions (C-148/150-A, 14a, 14b, 14c and 13c) were significantly higher when determined by osmotic pressure measurements than when estimated by the periodate oxidation method. Their average number of non-reducing terminal glucose units per molecule was considerably greater than 1.0.

TABLE I

COMPARISON OF DEGREE OF POLYMERIZATION OF AMYLOSE SUBFRACTIONS OBTAINED BY OSMOTIC PRESSURE MEASUREMENTS AND PERIODATE OXIDATION END-GROUP METHOD

Source	Subfraction	No additional *n*-butanol repptns.	DP, osmotic pressure	DP, periodate oxidation	Average no. of non-reducing terminal glucose units per molecule
Potato	P-7/9-A, 17a		1600	1320	1.6
Potato	P-7/9-A, 17a	2	1600	1540	1.1
Potato	P-7/9-A, 17b		1230	1140	1.2
Potato	P-7/9-A, 17c		970	1000	0.9
Potato	P-7/9-A, 17d		900	940	0.9
Potato	P-7/9-A, 17e		890	900	1.0
Potato	P-7/9-A, 17f		930	630	2.4
Potato	P-7/9-A, 17f	2	880	880	1.0
Corn	C-148/150-A, 14a		1150	380	7.2
Corn	C-148/150-A, 14a	2	1150	450	5.6
Corn	C-148/150-A, 14b		890	360	5.4
Corn	C-148/150-A, 14b	2	890	510	3.2
Corn	C-148/150-A, 14b	3	890	510	3.2
Corn	C-148/150-A, 14c		670	470	2.3
Corn	C-148/150-A, 14c	2	670	540	1.7
Corn	C-148/150-A, 13c		560	340	2.9

If these discrepancies were due to the presence of amylopectin impurities, it should be possible to eliminate the latter by repeated recrystallization of the amyloses with *n*-butanol. In order to check

(1) (a) A. L. Potter and W. Z. Hassid, THIS JOURNAL, **70**, 3488 (1948); (b) **70**, 3774 (1948); (c) A. L. Potter, W. Z. Hassid and M. A. Joslyn, *ibid.*, **71**, 4075 (1949).

(2) S. Lansky, M. Kooi and T. J. Schoch, *ibid.*, **71**, 4066 (1949).

this hypothesis, the amylose subfractions, 17a, 17f, 14a, 14b and 14c, whose molecular weights did not agree by the two methods, were selectively precipitated twice with *n*-butanol according to Schoch's method.[3] About 5% of subfraction 17f and only very small amounts of subfractions 17a, 14a, 14b and 14c were removed when these samples were twice recrystallized by this method. Recrystallization of subfraction 14b a third time with this reagent did not produce any noticeable precipitate when an excess of methyl alcohol was added to the supernatant.

Inspection of the data in Table I reveals that the degree of polymerization obtained from the periodate oxidation end-group method of the subfractions that were twice reprecipitated with *n*-butanol is greater than that of the original subfractions. This indicates that some branched material was removed from these subfractions. However, only the molecular weights of the two subfractions, 17a and 17f, approached the same value when determined by the end-group method and osmotic pressure measurements, after being twice reprecipitated with *n*-butanol. A third recrystallization of subfraction 14b showed no increase in the degree of polymerization over that obtained after the second recrystallization.

It is to be noted that the osmotic pressure molecular weight of subfraction 17f decreased after the two recrystallizations with *n*-butanol, showing that the molecular weight of the material removed must have been greater than that of the major portion of the amylose subfraction. Since the branching in subfraction 17f was eliminated by recrystallization with *n*-butanol, it is reasonable to assume that the material which had been removed is amylopectin. This assumption was experimentally confirmed by subjecting the material to periodate oxidation. An end-group value of 27 glucose residues per terminal glucose unit was obtained, corresponding to that of potato amylopectin.[1a]

The results from the investigation of whole amyloses[1] and the amylose subfractions indicate that amylose prepared from some starches such as potato, Easter lily and apple, by Schoch's method using pentasol and *n*-butanol, are linear. However, the amyloses separated from starches of other plant sources may contain branched material. It appears that there is no uniformity of branching in the amyloses; some of the subfractions are branched to a greater extent than others.

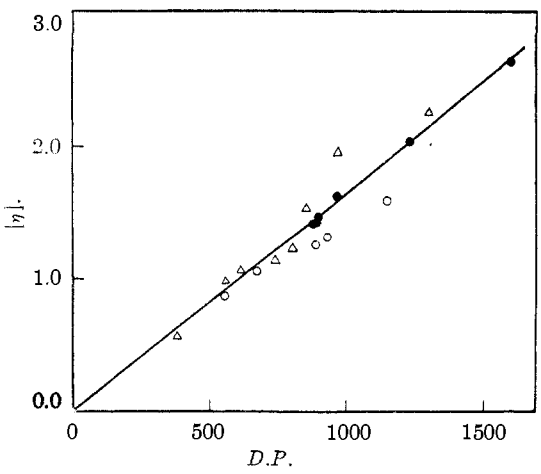

Fig. 1.—Intrinsic viscosity–molecular weight relationship of amyloses: ●, unbranched amylose subfractions; ○, branched amylose subfractions; △, unfractionated amyloses.

Relationship between Intrinsic Viscosity and Degree of Polymerization of Amyloses.—It has been shown by a number of investigators that the relation between intrinsic viscosity $[\eta]$ and the molecular weight M for linear molecules can be expressed by the formula[4]: $[\eta] = KM^a$, where K and a are constants. The value for a is usually from 0.5 to 1.0.

If the molecules are not linear, but cross-linked, a different dependence on the molecular weight will prevail. Under these circumstances polymers of high molecular weights may possess relatively low intrinsic viscosities. Since viscosity and osmotic pressure measurements give "viscosity average" and "number average" molecular weights, respectively, in order to obtain a good correlation between the intrinsic viscosity and the molecular weight determined by osmotic pressure measurements, it is necessary for the material to be fairly homogeneous with respect to its molecular size. Wagner[5] demonstrated this experimentally with polyvinyl acetate polymers. He showed that repeated fractionations of the polymers resulted in fractions in which the intrinsic viscosities agreed well with their molecular weights.

For this reason, only amylose subfractions that were shown to be linear were used in the determination of the relationship between the intrinsic viscosity and osmotic pressure molecular weights. (See Table I, subfractions 17b, 17c, 17d, 17e and the twice reprecipitated subfractions, 17a and 17f.) Plotting the intrinsic viscosities of six such potato amylose subfractions against their degrees of polymerization, a straight line passing through the origin was obtained (Fig. 1). From the slope of this line, the relationship of the viscosity to the molecular weight was found to be $[\eta] = 0.00166DP$, where $[\eta]$ is the intrinsic viscosity and DP is the degree of polymerization in glucose units.

In Fig. 1 are also plotted corn amylose subfractions which are either slightly branched themselves, or contain a small proportion of branched material. The intrinsic viscosity of such molecules would be expected to be lower than that of linear molecules of similar size. This is confirmed by the fact that all the points from these subfractions lie below the line.

From the intrinsic viscosities of unfractionated amyloses and from the intrinsic viscosity–molecular weight relationship obtained here the degrees of polymerization of the amyloses were determined. By comparing these values with those obtained by osmotic pressure measurements, a variation in differences is observed by the two methods ranging from

(3) T. J. Schoch, "Advances in Carbohydrate Chemistry," edited by Pigman and Wolfrom, Academic Press, Inc., New York, N. Y., Vol. I, 1945, pp. 258–261.

(4) H. Staudinger and K. Fischer, *J. prakt. Chem.*, **157**, 19 (1941); M. L. Huggins, THIS JOURNAL, **64**, 2716 (1942); P. J. Flory, *ibid.*, **65**, 372 (1943).

(5) R. H. Wagner, *J. Polymer Sci.*, **2**, 21 (1947).

3.2% for Easter lily amylose to 20.6% for potato amylose (Table II). Although the unfractionated amyloses are not homogeneous with respect to their molecular size, approximate molecular weights can be obtained from their intrinsic viscosity measurements.

TABLE II

COMPARISON OF THE DEGREE OF POLYMERIZATION OF AMYLOSES FROM VARIOUS PLANT SOURCES OBTAINED FROM OSMOTIC PRESSURE AND INTRINSIC VISCOSITY MEASUREMENTS

Plant source	*DP* from osmotic pressure	*DP* from intrinsic viscosity	Difference, %
Tapioca	1300	1360	4.6
Potato	970	1170	20.6
Wheat	860	930	8.1
Corn	800	740	7.5
Sago	740	680	8.1
Easter lily	620	640	3.2
Apple	560	600	7.1
Acid modified corn	390	340	12.8

Acknowledgment.—The authors are grateful to the Corn Industries Research Foundation for their support of this work and to Dr. T. J. Schoch for his generous contribution of the starch samples.

Summary

The molecular weights of potato and corn amylose subfractions were determined by osmotic pressure measurements and by estimation of their chain-lengths with the periodate oxidation end-group method. Comparison of the molecular weights by the two methods indicates that, like in the parent amylose, the molecules of the potato subfractions constitute single chains. However, the corn subfractions, like the unfractionated material, appear to be slightly branched, or may contain branched components, which cannot be removed by the usual procedures of separation.

The intrinsic viscosities and the molecular weights of the linear amylose subfractions show a relationship which can be expressed by the following formula: $[\eta] = 0.00166\ M$. Using this expression, the approximate molecular weight of an amylose from various plant sources can be determined from its intrinsic viscosity.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Degradation of Glycogen to Isomaltose[1]

BY M. L. WOLFROM, E. N. LASSETTRE AND A. N. O'NEILL[2]

Considerable evidence exists that glycogen is a two-dimensional polymer composed of α-D-glucopyranose units joined through the 1,4- and 1,6-positions in the frequency ratio of 12:1, respectively. This is based upon the establishment of D-glucose[3] and maltose[4] as acid hydrolytic products of glycogen; upon the isolation from the hydrolyzate of trimethylglycogen of the 2,3-, 2,3,6- and 2,3,4,6-methyl ethers of D-glucose[5]; and upon periodate assay.[6] It was desirable to place this structure on a more definitive basis through the isolation of the disaccharide containing the 1,6-α-D-glucopyranosyl linkage from an acid hydrolyzate of glycogen. This disaccharide, 6-α-D-glucopyranosyl-D-glucose or isomaltose, has been characterized as its crystalline β-D-octaacetate obtained from an acid-hydrolyzed dextran (from *Leuconostoc dextranicum*)[7] and from the hydrolysis of amylopectin with "Takadiastase"[8] (but not with malt diastase[7,9]) and with acid.[10]

Since preliminary experiments failed to yield isomaltose (as its β-octaacetate) from glycogen acetolyzates, it was deemed advisable to calculate the degree of hydrolysis required to give the maximum yield of isomaltose from glycogen. Some evidence existed that the maltose glycosidic linkage was more readily acid-hydrolyzable than that of isomaltose.[11] This was directly determined in our Laboratory and the data of Table I demonstrate that maltose hydrolyzes four times as fast as isomaltose in 2% concentration in 0.050 *N* sulfuric acid at 99.5°.

Information on the dependence of the nature of the depolymerization product upon the degree of hydrolysis of a polymer is obtainable from a statistical treatment of the depolymerization reaction and leads to equations representing the distribution of all possible chain lengths at different degrees of hydrolysis. From the results of a kinetic investigation of the hydrolytic degradation of starch, Meyer, Hopff and Mark[12] reported that such a degradation could be followed with a fair degree of accuracy by assuming that all hydrolyzable bonds in the large molecules were broken at approximately the same rate. Hence the total process could be calculated by a comparatively simple equation.

(1) A preliminary report of the experimental portion of this work appeared in THIS JOURNAL, **71**, 3857 (1949).

(2) Supported in part by a fellowship grant from the Corn Industries Research Foundation, New York, N. Y.

(3) W. N. Haworth, E. L. Hirst and J. I. Webb, *J. Chem. Soc.*, 2479 (1929).

(4) P. Karrer, C. Nägeli (and H. Hoffmann), *Helv. Chim. Acta*, **4**, 267 (1921); W. N. Haworth and E. G. V. Percival, *J. Chem. Soc.*, 1342 (1931).

(5) W. N. Haworth and E. G. V. Percival, *ibid.*, 2277 (1932); D. J. Bell, *Biochem. J.*, **29**, 2031 (1935); W. N. Haworth, E. L. Hirst and F. A. Isherwood, *J. Chem. Soc.*, 577 (1937); W. N. Haworth, E. L. Hirst and F. Smith, *ibid.*, 1914 (1939).

(6) T. G. Halsall, E. L. Hirst and J. K. N. Jones, *ibid.*, 1399 (1947).

(7) M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, **69**, 473 (1947); **71**, 125 (1949).

(8) Edna M. Montgomery, F. B. Weakley and G. E. Hilbert, *ibid.*, **69**, 2249 (1947); **71**, 1682 (1949).

(9) M. L. Wolfrom, L. W. Georges, A. Thompson and I. L. Miller, *ibid.*, **71**, 2873 (1949).

(10) M. L. Wolfrom, J. T. Tyree, T. T. Galkowski and A. N. O'Neill, *ibid.*, **72**, 1427 (1950).

(11) Marjorie A. Swanson and C. F. Cori, *J. Biol. Chem.*, **172**, 797 (1948); K. Myrbäck, B. Örtenblad and K. Ahlborg, *Biochem. Z.*, **307**, 53 (1940); K. Ahlborg and K. Myrbäck, *ibid.*, **308**, 187 (1941).

(12) K. H. Meyer, H. Hopff and H. Mark, *Ber.*, **62**, 1103 (1929).