[CONTRIBUTION FROM THE SOUTHERN REGIONAL RESEARCH LABORATORY[1]]

Cuprammonium–Glycoside Complexes. VII. Glucopyranoside Ring Conformations in Amylose

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Amylose has been found to possess peculiar solubility properties in cuprammonium. Its direct solubility is limited, but increases in the presence of small amounts of added sodium hydroxide. Neutral amylose solutions are precipitated by the addition of cuprammonium, but alkaline solutions remain clear when diluted with this reagent. The gels which form when amylose is placed directly in cuprammonium, or when a neutral amylose solution is precipitated by cuprammonium, contain about 0.5 to 0.6 mole of copper per mole of glucose anhydride. Specific rotations were measured for amylose in aqueous sodium hydroxide solutions ranging from 0.008 to 4.6 normal, and for methyl β-maltoside, from 0.001 to 5 normal. Both of these substances show decreasing optical rotation with increasing alkali concentration. The rotations of methyl α-glucoside and methyl β-cellobioside were not significantly altered by alkali. The properties of amylose have been considered in the light of current knowledge regarding the stability of ring shapes and it has been concluded that amylose contains more than one ring conformation. Available evidence points toward two boat-form rings, one of which had already been proposed by Freudenberg and Cramer to represent the glucopyranoside ring shape in amylose. Senti and Witnauer's conclusion that alkali-amylose contains only one ring form is supported by the present experiments, but their suggestion that this is a chair-form ring is tentatively rejected in favor of a boat-form. It is suggested that the transformation from two ring forms to a single ring form under the influence of alkali is due to the tendency of a ring hydroxyl group oriented perpendicular to the plane of the ring to go to an equatorial position upon dissociation.

K. Meyers' speculation that cuprammonium reacts with the hydroxyl groups on positions 2 and 3 of the glucose units of cellulose has been confirmed in reports from this Laboratory.[2,3] The principal known structural difference between cellulose and amylose is that the glucopyranoside units are linked in α-glucosidic combination in amylose, in β-glucosidic combination in cellulose. Thus the two substances have unsubstituted hydroxyl groups at the same positions and might be expected to show similar behavior with respect to solubility in cuprammonium. However, it is reported in the literature that starch[4,5,6] and amylose[7] are insoluble in cuprammonium, and the fact that amylose has a very limited solubility in cuprammonium has been verified as will be shown below.

Amylose is decidedly more soluble than cellulose in water or dilute sodium hydroxide solutions, therefore, it can hardly be argued that amylose has the stronger intermolecular bonds. The available evidence points in the other direction, *i.e.*, that amylose has the weaker intermolecular bonds and should be the more readily soluble in cuprammonium unless some hitherto unrecognized factor makes its reaction different from the reaction of cellulose with cuprammonium. The present work was undertaken to explore this apparently anomalous behavior of amylose.

Experimental

The standard cuprammonium employed in this work contained 15.0 ± 0.1 g. of copper, 230 ± 15 g. of ammonia and 1 g. of glycerol per liter. This differs from the former standard, designated Cupra B,[8] only in the specifications for ammonia which were changed from 240 ± 5 g. per liter. Since no effects upon the reaction of cuprammonium with glucosides have been noted when ammonia concentration varied between 215 and 245 g. per liter, the designation Cupra B has been retained for the standard cuprammonium used in this work.

In addition to the standard Cupra B, a stock solution was prepared to the same specifications, but containing in addition 40 g. of sodium hydroxide per liter. By adding this stock solution to Cupra B, it was possible to prepare cuprammonium solutions having any desired sodium hydroxide concentration between zero and one normal.

Optical rotations were measured in a Gaertner polarimeter at 28 ± 2° with the sodium D line (589 mμ) or the mercury blue line (436 mμ), as noted in the text.

The recrystallized corn amylose used in this work had been prepared at the Northern Regional Research Laboratory by the butanol fractionation procedure.[9] It had an iodine binding capacity of approximately 200 mg. per g. The weights of amylose mentioned in this manuscript have been corrected to the anhydrous basis.

The direct solubility of amylose in cuprammonium, with and without added alkali, was investigated. Samples of amylose of known weight (12 to 15 mg.) were placed on a rotating wheel with 5 ml. of Cupra B or cuprammonium containing sodium hydroxide. The amounts dissolved were estimated by means of optical rotation measurements on the clear solutions after centrifugation, taking the specific rotation of amylose in cuprammonium to be −750°. The results of these experiments were as follows

Concn. NaOH in cuprammonium, *N*	Amylose dissolved, % After 5 hr.	After 16 hr.
None	22	37
0.1	37	45
0.2	53	65
0.5	91	Pptd. CuO
1.0	6	Pptd. CuO

After 16 hours the solutions having 0.5 and 1 *N* sodium hydroxide had decomposed to give black precipitates of cupric oxide.

Dilution of aqueous amylose solutions with Cupra B yielded clear solutions or precipitates, depending upon the sodium hydroxide concentration. In one set of experiments amylose was dissolved in *N* sodium hydroxide and portions of this solution were neutralized or partially neutralized with hydrochloric acid. After adjusting the alkalinity the solutions containing known weights of amylose (14 to 18

Final NaOH concn. in diluted cupra, *N*	Per cent. amylose dissolved
0.0058	17
.016	31
.033	93
.052	100
.20	100

(1) One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) R. E. Reeves, *Science*, **99**, 148 (1944).

(3) R. E. Reeves, THIS JOURNAL, **71**, 215 (1949).

(4) E. Schweizer, *J. prakt. Chem.*, **72**, 109 (1857).

(5) T. F. Hanausek and A. L. Winton, "The Microscopy of Technical Products," John Wiley & Sons, Inc., New York, N.Y., 1907, p. 33.

(6) H. Staudinger and H. Eilers, *Ber.*, **69**, 819 (1936).

(7) K. H. Meyer, P. Bernfeld and E. Wolff, *Helv. Chim. Acta*, **23**, 854 (1940).

(8) R. E. Reeves, *Advances in Carbohydrate Chem.*, **6**, 107 (1951).

(9) T. J. Schoch, THIS JOURNAL, **64**, 2957 (1942).

mg. per ml.) were diluted with 4 volumes of Cupra B. The specific rotation of the amylose in the clear solutions was $-750 \pm 10°$. The solubilities of the amylose in the solutions where precipitation occurred were calculated from the observed optical rotations after centrifugation.

Copper bound by the insoluble amylose was estimated in two different ways. In one experiment 0.126 g. of amylose was wetted with 1 ml. of water, 4 ml. of Cupra B was added and the mixture was placed on a rotating wheel for 16 hours. The clear solution obtained after centrifuging was analyzed for copper, and soluble amylose was estimated by its optical rotation. Only 8.7 mg. of amylose was found to have dissolved in this slightly diluted cuprammonium, and the total copper content of the solution had decreased from 60.4 to 31.8 mg. This indicated that the insoluble amylose gel representing 117.3 mg. (0.725 mmole of glucose anhydride) of amylose had bound 28.6 mg. (0.448 mmole) of copper. The copper to glucose combining ratio was thus found to be 0.62 for the undissolved amylose.

In another experiment 0.1137 g. of amylose was dissolved in 2 ml. of *N* sodium hydroxide by shaking for several hours. This solution was exactly neutralized with 2.1 ml. of hydrochloric acid and 4 ml. of Cupra B was added. A blue gel precipitated immediately and after centrifugation the solution contained 3 mg. of amylose and 38 mg. of copper. In this case 110.7 mg. of amylose (0.682 mmole of glucose anhydride) in the gel had bound 22.4 mg. (0.352 mmole) of copper and the copper to glucose combining ratio was found to be 0.52.

In both of the above experiments the presumption was made that liquid adhering to or imbibed by the amylose gel had essentially the same composition as the clear liquid obtained by centrifugation.

The Effect of Sodium Hydroxide upon the Optical Rotation of Amylose in Aqueous Solutions.—Sufficient amylose to make a 2.9% solution was dissolved in 10 ml. of normal sodium hydroxide and diluted with water to exactly 20 ml. total volume. From this stock solution other solutions were prepared, either by partially neutralizing the sodium hydroxide with standard hydrochloric acid, or by diluting the solution with strong caustic. The optical rotations were measured on solutions containing between 1 and 2.9% amylose. It was not possible to make observations when the sodium hydroxide was in excess of 5 normal because the solutions gelled before mixing could be completed. The highest concentration of sodium hydroxide allowing an observation was 4.6 *N*. This amylose solution was fluid when it was first observed in the polarimeter, but it set to a stiff, clear gel within an hour. The optical rotation of the gel was the same as that observed for the fluid solution. The results of these observations are shown in Fig. 1 (curve A).

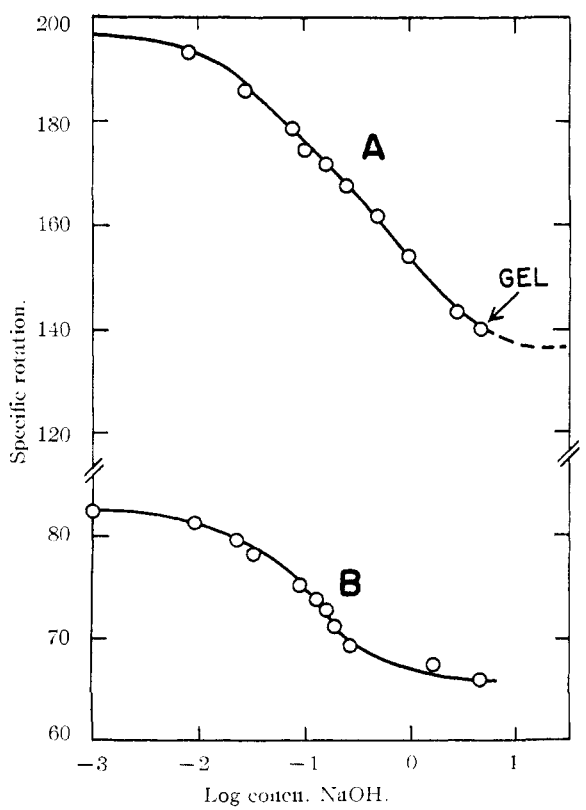


Fig. 1.—The specific rotation (D line) of amylose (curve A) and methyl β-maltoside (curve B) in aqueous sodium hydroxide solutions.

The Effect of Sodium Hydroxide upon the Optical Rotation of Some Glucopyranosides.—The specific rotations were determined in water and 5 *N* sodium hydroxide solution for the following glucopyranosides

	Specific rotation (D line), deg. In water	In 5 *N* NaOH	Difference, deg.
Methyl α-D-glucopyranoside	+158.6	+155.5	3
Methyl β-maltoside	+ 82.2	+ 66.0	16.2
Methyl β-cellobioside	− 18.2	− 19.5	1

Other observations were made on methyl β-maltoside at intermediate alkali concentrations and these are shown graphically in Fig. 1 (curve B).

Discussion

Whistler, Deane and Hilbert[10] had already observed the limited solubility of amylose in cuprammonium, and their observations have been confirmed in the present work. Recrystallized corn amylose swells, but only partially dissolves, in the standard cuprammonium. If amylose is dissolved first in a small amount of aqueous sodium hydroxide the resulting solution remains clear when diluted with cuprammonium. But if the alkaline amylose solution is neutralized and then diluted with cuprammonium almost all of the amylose precipitates in the form of a dark blue gel. The amount of copper bound in the insoluble cuprammonium–amylose gels was estimated to be about 0.5 to 0.6 mole per mole of glucose anhydride.

The swelling and limited solubility of recrystallized amylose in cuprammonium, and its low copper to glucose combining ratio suggest that only part of the glucose units of amylose react with cuprammonium. Existing information regarding amylose structure provides no reason for the observed behavior, but it is possible that a satisfactory explanation may be found by consideration of the glucopyranoside ring conformations in amylose.

There is a large body of evidence to indicate that the chair conformation, Cl, is the usual ring form for glucopyranoside units in cellulose,[11] sucrose[12] and many simple glucosides.[3] However serious objections to the Cl conformation become apparent on joining scale models of α-glucopyranoside through the 1,4′-type of glucosidic linkage. Space conflicts occur at almost every position of rotation about the glucose–glucose bond. These difficulties were recognized by Freudenberg and Cramer[13] who suggested the boat form ring 3B to represent the glucose units in amylose. But glucose units oriented in either the Cl or 3B conformation should react with one mole of cuprammonium.

In considering what ring conformations might oc-

(10) R. L. Whistler, R. A. Deane and G. E. Hilbert, unpublished results.

(11) W. T. Astbury and M. M. Davies, *Nature*, **154**, 84 (1944).

(12) C. A. Beevers and W. Cochran, *ibid.*, **157**, 872 (1946).

(13) K. Freudenberg and F. Cramer, *Ber.*, **83**, 296 (1950).

cur in amylose it should be borne in mind that the large substituents on carbon atoms 1 and 4 will tend to occupy equatorial positions.[14] Examination of models reveals that in only two conformations, those previously designated B1 and 3B, do the carbon–oxygen valences of both carbon atoms 1 and 4 project in the equatorial position. In an earlier publication[3] it has been shown that conformation 3B would be expected to yield a strongly levorotatory complex with cuprammonium, while B1 would not react with cuprammonium because of the excessive distance between the hydroxyl groups on positions 2 and 3. The assumption that amylose contains glucopyranoside units oriented in these two different ring conformations provides an explanation for the swelling, the limited solubility, and the low copper to glucose combining ratio of amylose in cuprammonium.

Unlike recrystallized amylose, alkali-amylose is readily and completely soluble in a large volume of cuprammonium.[15] The work of Senti and Witnauer[16] has indicated that all the glucopyranoside units in alkali-amylose are oriented in a single conformation. The form proposed by these workers was the "symmetrical chair configuration" (C1 conformation), but for reasons mentioned above it will be assumed that the ring form in alkali-amylose is 3B. Either 3B or C1 would fulfill the requirement of reacting with cuprammonium to form a levorotatory complex.

If recrystallized amylose contains two ring conformations, while alkali-amylose contains but one, a possible explanation is provided for the changes in the optical rotation of amylose with changing alkali concentration. In Fig. 1 (curve A) the specific rotation (D line) of amylose is plotted along the ordinate; the logarithm of the sodium hydroxide concentration, along the abscissa. The specific rotation of amylose in neutral aqueous solution is generally accepted to be about $+200°$,[17] and approximately this value was observed for amylose in the most dilute alkali solution. With increasing sodium hydroxide concentration the rotation decreases sharply, and the curve passes very close to the value of $+155°$ which has been reported for the rotation of amylose in N sodium hydroxide solution.[18] The curve continues downward until the alkali concentrations become so great that amylose is no longer soluble. This effect of alkali upon the optical rotation of amylose is reversed by neutralization of the alkali.

(14) A substituent is said to be in the equatorial position when it projects, approximately, into the plane which most closely approaches the position of all of the ring atoms. A substituent projecting perpendicular to this plane is said to be in the axial position. Since axial valences are parallel while equatorial valences are diverging the larger substituents will tend to prefer equatorial positions. The sterically hindered nature of axial (polar) positions has been discussed by D. H. R. Barton, *Experientia*, **6**, 316 (1950).

(15) In order to dissolve alkali-amylose in cuprammonium it is necessary to employ sufficient cuprammonium to reduce the final alkali concentration to about 0.5 normal, or less. At higher alkali concentrations amylose, like cellulose, forms an insoluble blue gel which is probably cross-linked through copper.

(16) F. R. Senti and L. P. Witnauer, THIS JOURNAL, **70**, 1438 (1948).

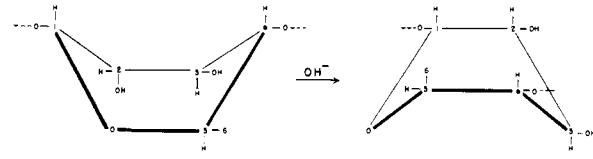
(17) R. W. Kerr, Ed., "Chemistry and Industry of Starch," Academic Press, Inc., New York, N. Y., 1944.

(18) W. Z. Hassid and R. M. McCready, THIS JOURNAL, **65**, 1157 (1943).

Simple glucosides do not, in general, resemble amylose in this behavior. The specific rotation of methyl α-glucopyranoside is changed only 3°, that of methyl β-cellobioside only 1° from neutral solution to 5 N sodium hydroxide. Methyl β-maltoside, however, resembles amylose to the extent that its rotation is decreased from 82° in water to 66° in 5 N sodium hydroxide, the major portion of the change occurring between alkali concentrations of 0.01 and 0.3 normal. The results obtained with the maltoside are also shown in Fig. 1 (curve B).

It is noted that the curve showing change in rotation of methyl β-maltoside *versus* the log of the concentration of sodium hydroxide is definitely S shaped. If it be presumed that at some high alkali concentration the rotation of amylose levels off then its curve would also become S shaped. An S-shaped curve in these coördinates is in agreement with the idea that the dissociation of a weakly acidic hydroxyl group is involved in the rotational changes. But dissociation alone is not sufficient to explain the behavior of amylose or methyl β-maltoside, for no significant change was observed in the rotation of methyl α-glucoside or methyl β-cellobioside. Some structural changes must accompany the dissociation of methyl β-maltoside, and to a much greater extent, amylose, in order to account for their behavior in alkali.

That the above mentioned structural changes might involve the transformation of B1 conformations to 3B (or C1) is suggested by the following considerations. In the B1 conformation the hydroxyl group on position 2 is located in an axial position. So long as this hydroxyl is not dissociated, the B1 form may be stable. But when this hydroxyl becomes dissociated, at high alkalinity, it assumes a greater effective volume and requires the greater space which is available in an equatorial position. Conformation 3B (or C1) would place both of the ring hydroxyls in an equatorial position. The shift from B1 to 3B is illustrated below. In the diagrams equatorial ring substituents are attached by horizontal lines; axial substituents, by vertical lines.



Since the solubility of recrystallized amylose in water is slight, it must be supposed that it has a regular structure which permits extensive cross-linkage by hydrogen bonds. This regularity might result, for example, from alternating ring conformations. When amylose is exposed to low concentrations of alkali some of the B1 rings shift to another form (as evidenced by changed optical rotation), the regularity is disrupted, and the amylose becomes soluble. But when the alkali concentrations become great enough to cause all the B1 rings to shift to the other form the amylose again has a regular structure and becomes insoluble.

Conclusion

The behavior of amylose supports the conclusion

that its glucopyranoside rings exist in more than a single ring conformation. There are structural reasons for believing that two boat forms, B1 and 3B, may be the principal conformations involved. The conclusion is not entirely dependent upon the argument favoring these particular ring forms. It is recognized that other pairs of ring conformations might account for the behavior of amylose, provided one of the forms were reactive, the other unreactive with cuprammonium.

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NEW ORLEANS, LOUISIANA

[CONTRIBUTION FROM THE MCPHERSON CHEMICAL LABORATORY, OHIO STATE UNIVERSITY]

Attempted Syntheses of Nitrogen Analogs of Adamantane[1]

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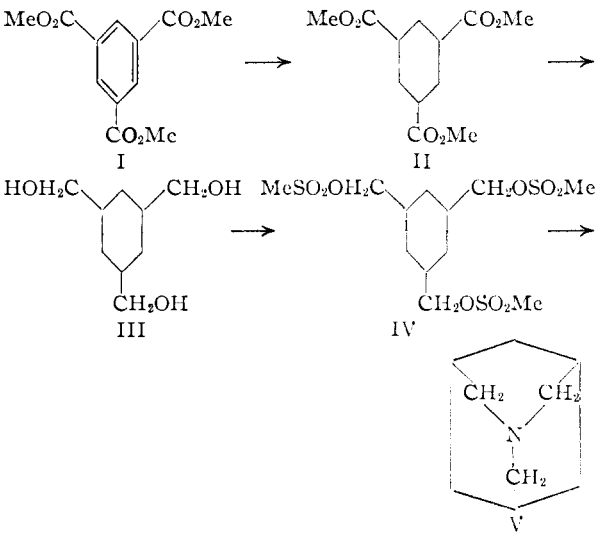
Attempts to prepare nitrogen analogs of adamantane from 1,3,5-trisubstituted cyclohexanes failed. A number of these cyclohexanes were related in configuration, postulated to be *cis*.

The interest shown in adamantane[3] and in quinuclidine[4] led us to seek a synthesis of the adamantane skeleton which could be modified to introduce a hetero atom at a bridgehead. The success of triple condensations for preparing bicyclic compounds[5] suggested the use of the *cis* isomers of 1,3,5-trisubstituted cyclohexanes for this synthesis. We therefore attempted the following sequence[6]



Trimethyl 1,3,5-cyclohexanetricarboxylate was prepared by reduction of trimethyl trimesate, and a predominant isomer, II, m.p. 48.0–49.0°, was separated.[7] Reduction by lithium aluminum hydride afforded an alcohol, III, which was converted to the trismethane sulfonate, IV, and treated with ammonia.

An amine hydrochloride XIII·HCl was obtained in very low yield (*ca.* 2%) from each of three runs along with large amounts of polymeric material. The analysis agreed with that calculated for V·HCl, but the compound was destroyed by alkaline permanganate,[8] and reacted with bromine to yield a monobromo derivative. Insufficient material forced us to abandon further work on the structure.

On hydrolysis, either the solid isomer, II, or the isomeric mixture of trimethyl 1,3,5-cyclohexanetricarboxylates gave only a single isomer, VI, of the parent acid, as found by earlier workers.[7b] Reaction of VI with diazomethane afforded II, which proves identical configurations. Since the lithium aluminum hydride reduction should not cause isomerization of II,[9] the alcohol, III, and its methanesulfonate, IV, should have the same configuration as II. VI, as the most stable isomer, should be of *cis* configuration[6c]; therefore, II, III and IV should also be *cis*. It thus seems likely that failure to obtain the tricyclic amine, V, was due to side reactions[6d] and an unfavorable conformation[6c] of the substituents of IV rather than to a *trans* configuration in IV.

The ready availability of the triacid, VI, prompted us to attempt the preparation of the tricyclic triamide, IX, since this compound would constitute a most unusual case of an amide involving a "bridgehead" nitrogen.[10]

(1) Taken in part from the Ph.D. thesis of H. S. Lowrie, Ohio State University, 1952.

(2) Cincinnati Chemical Works Fellow, 1951–1952.

(3) C. Prelog and R. Sciwerth, *Ber.*, **74**, 1769 (1941), and references mentioned therein.

(4) H. C. Brown and N. R. Eldred, THIS JOURNAL, **71**, 445 (1949).

(5) V. Prelog, D. Kohlbach, E. Carkovnikov, A. Rezik and M. Piantanida, *Ann.*, **532**, 69 (1937).

(6) The preparation of predominantly the *cis* isomer by the reduction of mesitylene (a) and evidence that this was the more stable of the pair (b) made us expect, when this work was begun in 1949, that the isomeric mixture obtained by the reduction of trimethyl trimesate (I) would consist mainly of the *cis* form. Later work (c) has substantiated these beliefs, but has indicated that even though the substituents are in the necessary *cis* configuration, they are bound by equatorial bonds and are thus widely separated, favoring side-reactions (e) from the trismethanesulfonate, IV, rather than the intramolecular condensation desired. (a) C. E. Bowers, M. S. Thesis, Ohio State University, 1949; (b) C. W. Beckett, K. S. Pitzer and R. Spitzer, THIS JOURNAL, **69**, 2488 (1947); (c) E. M. Fry, *ibid.*, **76**, 284 (1954), and references mentioned therein; (d) D. D. Reynolds and W. O. Kenyon, *ibid.*, **72**, 1597 (1950), and references therein.

(7) (a) R. C. Fuson and C. H. McKeever, *ibid.*, **62**, 2088 (1940), give m.p. 42–44° for II; (b) J. M. Van der Zanden and G. DeVries, *Rec. trav. Chim.*, **67**, 998 (1948), found m.p. ranges between 43–47° for II.

(8) Both adamantane[1] and quinuclidine (a) are stable to these conditions; by analogy, V should be. (a) K. Löffler and F. Stiezel, *Ber.*, **42**, 124 (1909).

(9) W. G. Brown in R. Adams, "Organic Reactions," Vol. 6, J. Wiley and Sons, Inc., New York, N. Y., 1951, p. 469.

(10) The attempts to prepare this type of compound, and the properties expected of it have been reviewed recently. (a) F. S. Fawcett, *Chem. Revs.*, **47**, 219 (1950); see also Y. J. Topper, Ph.D. Dissertation, Harvard, 1946; S. M. McElvain and L. W. Bannister, THIS JOURNAL, **76**, 1126 (1954).