creased. The small amount of LAD available for this study made it impossible to test the activity at higher concentrations.[20]

The results with both enzymatic assay systems give further convincing evidence of the remarkable ability of enzyme systems to distinguish between closely related substances. In this respect, it would be of interest to isolate typical flavoprotein enzymes from *L. lactis* cells, and to determine the relative efficiency of Rb and Lx nucleotides in these systems. Since Lx nucleotides satisfy the requirements of all of the flavoprotein enzymes of this organism that are essential for its growth, they may approach their Rb counterparts more closely in activity. Even in this organism Rb promotes growth at substantially lower concentrations than Lx,[6] and it is unlikely that the Lx coenzymes function as efficiently as the Rb coenzymes.

(20) The present evidence does not constitute unequivocal proof that the material in question is actually lyxoflavin-*adenine* dinucleotide. By all criteria of paper and column chromatography, the material is clearly a lyxoflavin *dinucleotide*. After acid degradation, LMN could be identified by paper chromatography, but the equally minute amount of purine mononucleotide could not be located on the paper chromatograms.

SEATTLE 5, WASHINGTON
AUSTIN, TEXAS

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, LOUISIANA STATE UNIVERSITY SCHOOL OF MEDICINE, AND THE DEPARTMENT OF CHEMISTRY, TULANE UNIVERSITY]

The Shape of Pyranoside Rings. II. The Effect of Sodium Hydroxide upon the Optical Rotation of Glycosides[1]

BY RICHARD E. REEVES AND FLORINE A. BLOUIN

RECEIVED OCTOBER 25, 1956

The optical rotation of many glycosides is essentially the same in neutral aqueous solution or in *N* sodium hydroxide solution. However, there are some glycosides which show relatively large, reversible changes in optical rotation at sodium hydroxide concentrations greater than approximately 0.01 *N*. The observation that these changes are reversible argues against their being due to hydrolysis or to degradation reactions. The possibility is considered that these alkali-sensitive substances might undergo an alteration in the fine structure (conformation) of the molecules arising as a result of a tendency of axially oriented hydroxyl groups to shift toward an equatorial orientation under strongly alkaline conditions.

In a continuing search for an understanding of the effect of alkali upon the optical rotation of amylose,[2] we have been led to a general investigation of the effect of alkali upon the rotation of simple glycosides. The literature contains few observations of an effect of alkali upon the rotation of "alkali-stable" sugar derivatives, and no survey of this effect seems to have been reported. An unusual temperature dependence of optical rotation has been noted for a class of glycosides, the acylated *o*-nitrophenyl glycosides, and this had led to the development of ideas regarding the fine structure of these substances.[3] However, no significance has hitherto been attached to the fact that some glycosides are quite alkali-sensitive while others are not.

Experimental

The glycosides employed in this work possessed melting points and specific rotations in close agreement with the best values reported in the literature.

To avoid the possibility that the different optical rotations observed for neutral and alkaline aqueous solutions might be due to ionic strength differences, we have observed optical rotations in molar sodium chloride and in normal sodium hydroxide. When a substance with different rotations in water and in alkali was encountered, solutions of equal concentration were prepared in molar saline and in normal sodium hydroxide, and the solutions were mixed in various proportions in a sealed-end polarimeter tube made with a side-arm bulb to allow for mixing. Additive volumes were assumed for these solutions. In some instances observations were made at alkali concentrations greater than normal and these solutions were separately prepared in volumetric flasks. The reversibility of the alkali-induced shifts in rotation was checked by neutralizing normal or half-normal sodium hydroxide solutions with hydrochloric acid and observing the rotations of the neutralized solutions.

The observations were made with the sodium D-line at 27.5 ± 2.5° using either a 1- or a 2-dm. polarimeter cell. Because of the fixed volume requirements of the cells and the widely different availability of pure samples of the substances employed, the measurements are not uniformly precise. However, it seems to us that the greatest uncertainty due to experimental error in the difference between specific rotation in saline and in normal sodium hydroxide should not exceed 1°.

In the preparation of a solution of methyl β-D-altropyranoside the crystalline tetraacetate was dissolved in an excess of aqueous sodium hydroxide at room temperature. Back titration with standard hydrochloric acid provided assurance that all four acetate groups had been saponified. The saline and alkaline solutions were prepared from this neutralized stock solution and contained 0.53 *M* sodium acetate in addition to the sodium chloride and sodium hydroxide.

Results

The simple glycopyranosides so far examined in saline and in normal sodium hydroxide solution have fallen into two classes: those which show differences in specific rotations amounting to less than 2.5° and those which show differences greater than 7.0°. These substances are listed in Table I together with the approximate concentrations employed, their observed rotations in molar sodium chloride solution, in normal sodium hydroxide solution and the difference, sp. rot. (NaCl) minus sp. rot. (NaOH). For the first class of substances some of the differences are within the experimental error of the observations, but others, for example that of methyl β-D-glucoside, appear to lie slightly outside the experimental error. Almost an order of magnitude greater are the differences attributed to the second class of substances, described as being alkali-sensitive. These five substances have spe-

(1) Some of the data reported herein were presented before the Division of Carbohydrate Chemistry at the 127th Meeting of the American Chemical Society, Cincinnati, Ohio, March–April, 1955.

(2) R. E. Reeves, THIS JOURNAL, **76**, 4595 (1954).

(3) W. W. Pigman, *J. Research Natl. Bur. Standards*, **33**, 129 (1944).

cific rotations which are appreciably altered by the presence of alkali. Upon neutralization each of these specific rotations in alkali returned to the value characteristic of a directly prepared neutral solution. Observations on the disaccharides sucrose[4] and trehalose are included in the table for comparison with the simple glycosides. An interesting aspect of the rotation of sucrose in alkaline solution will be considered below.

In Fig. 1 is plotted the change in specific rotation *versus* the log sodium hydroxide concentration for

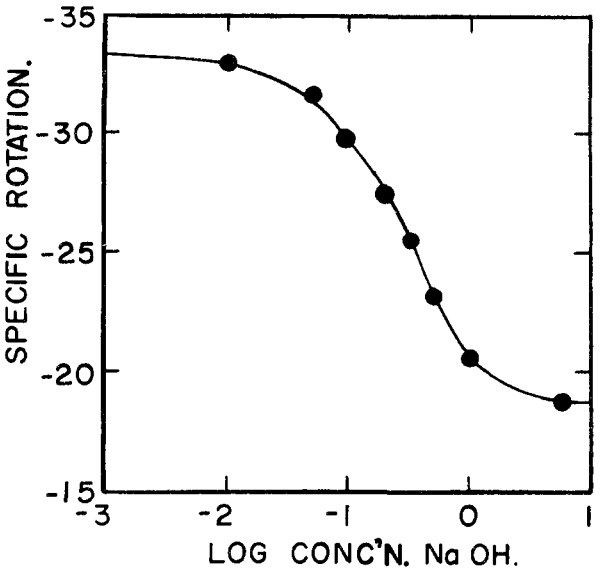


Fig. 1.—The effect of sodium hydroxide upon the optical rotation of methyl β-D-altropyranoside.

methyl β-D-altropyranoside, a member of the group possessing alkali-sensitive rotations. All observations save the one at the highest alkali concentration were made at constant ionic strength. The point of inflection of this curve lies in the vicinity of log *N* NaOH, −0.4. All other members of this group gave curves of similar shape and location on the *x*-axis.

In Fig. 2 is plotted the change in specific rotation *versus* the log sodium hydroxide concentration for sucrose. The solid circles represent observations made in the present work, at unit ionic strength up to the normal sodium hydroxide concentration. The triangles represent data reported by Thomsen in 1881.[5] Although Thomsen did not comment upon the fact, his data clearly showed the minimum occurring at 1.57 *N* alkali concentration. The highest alkali concentration employed by this worker was 2.14 *N*, and at this point the specific rotation had risen only 0.1° above the minimum; the pronounced rise at still higher alkali concentrations apparently was not observed. Thomsen did note an evolution of heat when sucrose was added to sodium hydroxide solution.

(4) It is noted that the specific rotation of 10% sucrose in molar sodium chloride is one degree lower than its rotation in water as required by the formula proposed by Brown, *Ind. Eng. Chem.*, **17**, 39 (1925).

(5) Th. Thomsen, *Ber.*, **14**, 1647 (1881).

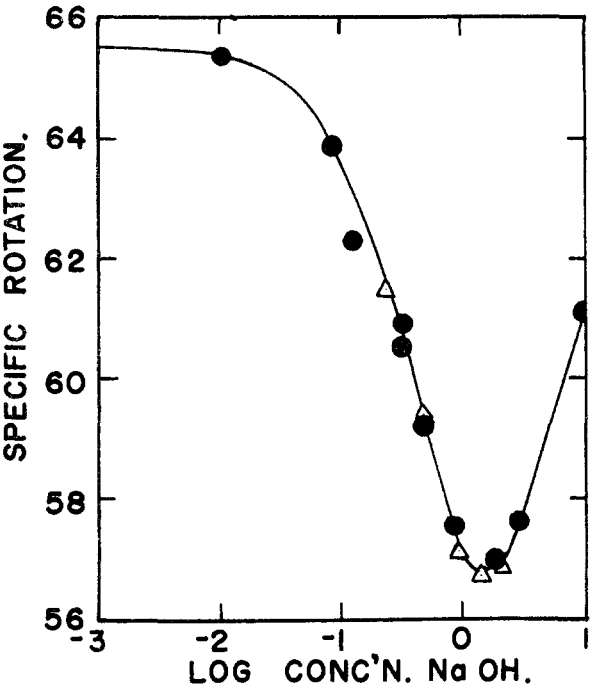


Fig. 2.—The effect of sodium hydroxide upon the optical rotation of sucrose; triangles represent observations made by Thomsen.[5]

Discussion

The position on the alkalinity scale and the general shape of the curves of alkali-sensitive substances suggest the possibility that the ionization of hydroxyl groups may be involved in these rotational shifts. Yet there are closely related glyco-

TABLE I

OPTICAL ROTATIONS OF GLYCOSIDES IN NEUTRAL AND ALKALINE SOLUTIONS

Substance	Approx. concn., %	Specific rotation, degrees *M* NaCl	*N* NaOH	Diff.
Alkali-stable pyranosides				
Methyl α-D-glucoside	2	157.8	157.8	0.0
Methyl β-D-glucoside	2	−32.8	−31.1	−1.7
Cyclohexyl α-D-glucoside	1.2	140.1	139.2	0.9
Phenyl α-D-glucoside	2.3	178.7	179.7	−1.0
Phenyl β-D-glucoside	3	−71.5	−71.0	−0.5
Methyl α-D-xyloside	11	155.9	154.3	1.6
Methyl β-D-xyloside	13	−63.7	−64.9	1.2
Methyl α-D-mannoside	2	76.1	76.6	−0.5
Methyl α-L-rhamnoside	2	−61.0	−60.5	−0.5
Methyl α-D-altroside	1	123	121	2
Methyl α-D-lyxoside	5	58.0	60.4	−2.4
Methyl α-D-guloside·H_2O	2	104.4	103.0	1.4
Alkali-sensitive pyranosides				
Methyl α-D-galactoside·H_2O	4.7	177.3	170.0	7.3
Methyl β-D-galactoside	2	3.0	− 6.6	9.6
Methyl β-D-mannoside	1.2	−69.4	−60.1	− 9.3
Methyl β-D-altroside	2.6	−33.2	−20.5	−12.7
Methyl β-L-arabinoside	2	243	233	10
Disaccharides				
Sucrose	10	65.4	57.6	7.8
Trehalose·$2H_2O$	2	179.4	180.8	−1.4

sides which are not alkali-sensitive. In searching for an explanation for the difference between the two classes of glycosides, it was considered possible that axially oriented hydroxyl groups might tend to produce a ring strain or a conformation shift at high alkali concentrations. Since charged RO^- groups would be more strongly solvated and have a greater effective volume than neutral ROH groups, it might be supposed that under ionizing conditions there would be a tendency for the ring to adopt a shape placing as many as possible of the ring hydroxyls in the equatorial positions where there is less steric hindrance than is afforded by the axial positions. We have chosen to accept the hypothesis that hydroxyl groups which are axially oriented in neutral solution may produce reversible rotational changes due to their tendency to move to equatorial positions at high alkalinity and to examine the implications of this hypothesis in the light of the present observations.

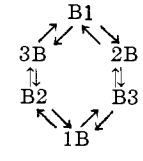
Except for the α-1,4-linked glucose polysaccharides, there is no reason to doubt that the C1 conformation is the favored shape of the D-glucopyranoside ring. In this conformation there are no axially oriented hydroxyl groups and, in agreement with the hypothesis, all the simple glucosides (and xylosides) yet examined have exhibited optical rotations which were similar in neutral and alkaline solution.

In the D-galactose (L-arabinose) series the favored conformation again appears to be C1,[6] but in this series there is an axial hydroxyl group at carbon atom 4. Both α- and β-galactosides and the arabinoside were alkali-sensitive. In agreement with the hypothesis this might be due to a strain imposed upon the C1 ring, or to displacement of an equilibrium in the direction of a form possessing fewer axial hydroxyl groups. In the latter event the effect produced by alkali might be to shift a C1 $\rightleftarrows$ B2 equilibrium to the right. In the B2 conformation the hydroxyl groups at carbon atoms 2, 3 and 4 are equatorial in the D-galactose series.

It is observed that the methyl α-pyranosides of D-mannose, D-altrose, D-lyxose and L-rhamnose fall into the alkali-stable class. Each of these configurations would possess one or more axial hydroxyl groups if it existed in a chair form. But for each of these α-anomers there is a boat-form conformation with *no* axial substituents: D-mannose and D-lyxose, B1; D-altrose, 2B; and L-rhamnose, 1B. These forms may be reconciled with the cuprammonium-complexing behavior of these glycosides as well as the previously suggested chair forms.[7]

To argue, as the present hypothesis appears to require, that boat-form rings represent the lowest energy state for some pyranosides brings into question a previous hypothesis that "Pyranose rings assume a chair form in preference to any boat form whenever both are structurally possible."[7] It may be the case that although chair forms are generally of lower energy than boat forms, the difference is more than offset when a boat form allows *all* ring substituents (other than hydrogen) to be equatorial. It should be remarked that in simple pyranosides geometrically regular boat forms are unlikely because of the high energy resulting from the eclipsed valence angles on some of the adjacent atoms. Therefore, the suggestion that methyl α-D-mannoside may prefer the B1 conformation should be construed to mean that it may take a shape approximating that of the pure B1 form but distorted into the region where the tendency of the large groups to move into the equatorial plane is balanced by the repulsion brought about by approaching the eclipsed valence position. There is already experimental evidence that some of these glucosides do not take a pure boat form, for to do so would place two adjacent hydroxyl groups in the true *cis* position where they should be almost instantly cleaved (titratable) with lead tetraacetate. It has been shown that this does not occur in the case of methyl α-D-mannoside, α-D-lyxoside or α-D-altroside.[7]

Boat-form rings are unlike chair forms in possessing a flexibility so that positions intermediate between Reeves' designated forms can occur without greatly increased ring strain. There is a sequence in which one boat form may be changed into another, as is shown in the following diagram. Unless certain of the forms are prohibited by steric interference of the substituents, boat forms may shift in either direction around the ring shown in the diagram.



Faced with increasing evidence that boat-form rings may have an important role in carbohydrate chemistry, it would be desirable to give further consideration to the problem of designating the various ring shapes. It now seems clear that Reeves' designations B1, 1B, etc., will not be sufficiently precise. However, until a better system is devised it is proposed to describe the boat forms as *e.g.* "approximating" a particular conformation; or as intermediate between B1 and 3B, or between such other forms as are adjacent to each other in the above diagram.

The β-mannose and β-altrose configurations differ from their anomers in having no conformation which places all ring substituents in the equatorial position. Without attempting, for the present, to assign most stable ring conformations to these substances, it would appear that neutral aqueous solutions might contain some finite proportion of axial hydroxyl groups and that this proportion might be reduced by ring shifts at high alkali concentrations.

The alkali-stability of the optical rotation of methyl α-D-guloside presents some unusual aspects. Any of the conformations compatible with the cuprammonium complexing behavior of this substance[7] require at least one axial hydroxyl group. As far as the hitherto recognized factors governing ring stability are concerned, the guloside could as well shift from C1 toward 1B under the influence of alkali as the galactosides might shift from C1 toward B2. However, inspection of scale

(6) R. E. Reeves, THIS JOURNAL, **71**, 1737 (1949).
(7) R. E. Reeves, *ibid.*, **72**, 1499 (1950).

models of α-D-hexosides reveals extensive interference between the hydrogen atom of carbon 4 and the oxygen atom at carbon 1 in the 1B conformation, an interference not present in the proposed galactoside shift C1 ⇄ B2. It may be that the 1B conformation is prohibited for steric reasons in the α-D-hexose (and pentose) series, or its energy level is so much greater than that of the other forms that it cannot be overcome by the tendency of a charged axial hydroxyl group to move to the equatorial position. It is noted that methyl β-D-guloside might be expected to show an alkali-sensitive rotation due to the shift of a C1 ⇄ 1B equilibrium.

The hypothesis that hydroxyl groups which are axially oriented in neutral solution move to equatorial positions under conditions of high alkalinity requires further confirmation. Work to prove or disprove the concept is in progress. No attempt will be made at this time to interpret the alkali-sensitive behavior of the disaccharide sucrose, beyond observing that since the simple α-glucopyranosides (and trehalose) fail to show the same type of behavior, it would be logical to attribute the observed effect to changes in the fructofuranoside portion of the sucrose molecule.

Acknowledgment.—Some of the glycosides employed in this work were supplied by Drs. N. K. Richtmyer and H. S. Isbell.

NEW ORLEANS, LA.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF UTAH]

4-Allylveratrole from *Anemopsis californica*

BY W. J. HORTON AND E. G. PAUL

RECEIVED NOVEMBER 5, 1956

The steam distillable oil from rhizomes of *Anemopsis californica* contains 4-allylveratrole which contributes the pungency to the odor of the oil. A second compound of unknown nature greatly masks the spice-like odor. A number of 4-alkylveratroles and their 5-sulfonamides were prepared in an effort to identify the sulfonamide (2-propyl-4,5-dimethoxybenzenesulfonamide) derived from the natural oil.

Anemopsis californica has a distinctive odor with a faint undertone of a spicy material. Both leaves and rootstock contain the odorant which resides in the steam distillable oil. Since the woody rhizome was the richer source, oil was obtained from this portion of the plant and submitted to fractional distillation using a small Vigreux column. A redistilled sample gave analytical data in excellent agreement with $C_{12}H_{16}O_2$. Catalytic hydrogenation indicated a single olefinic bond and oxidation produced veratric acid which was confirmed by the preparation of veratramide. The ultraviolet absorption also suggested the veratryl ring[1] with the olefinic bond in a non-conjugated position. Synthetic work was therefore designed so as to determine the nature of the butyl group in hydrogenated *Anemopsis* oil. A comparison of synthetic 4-butylveratroles (Table II) with the alkylveratrole derived from the oil was facilitated by the preparation of a crystalline sulfonamide from the hydrogenated oil. However, re-examination of the original analysis became necessary when none of the synthetic 4-butylveratroles nor isoamylveratrole gave a sulfonamide identical to the unknown sulfonamide (Table III). Furthermore the analytical values obtained from the unknown sulfonamide indicated a propyl group rather than a butyl group. Subsequent samples of the oil submitted to analysis pointed to contamination in the original oil by compounds of higher carbon content so as to make the excellent agreement with the formula $C_{12}H_{16}O_2$ fortuitous. The preparation of 4-propylveratrole (Table II) and its sulfonamide together with the ultraviolet spectra identify this part of the original oil as 4-allylveratrole.

The acyl-3,4-dimethoxyphenones were prepared by the excellent procedure of Gardner[2] and are collected in Table I. Clemmensen reduction gave the corresponding 4-alkylveratroles (Table II) whose sulfonamides are presented in Table III.

We are greatly indebted to Prof. Walter P. Cottom of our Department of Botany for discussions and to Mr. L. Woodbury at Dixie College, St. George, Utah, who so kindly provided the plant material.

Experimental[3]

The plant material collected in the vicinity of St. George, Utah, was divided into rhizomes and stems, the rhizomes were washed in water to remove adhering soil and thoroughly air-dried. They were then passed through a food chopper to produce material of the appearance of a brown sawdust. A 200-g. portion was steam distilled until 6 l. of distillate was obtained. The distillate was saturated with sodium chloride and extracted with ether. The residual oil on distillation of the dried ether extract weighed 17.4 g. (8.7%). This oil was divided into three fractions by distillation: (a) 6.22 g. of pale yellow, b.p. 137–141° (23 mm.); (b) 5.38 g. of yellow green, b.p. 141–146° (23 mm.); and (c) 2.44 g. of green, b.p. 147–155° (23 mm.). All fractions gave a brown color with tetranitromethane and a negative test with ferric chloride except (c) which was dubious. Fraction (a) gave a red complex with picric acid but a solid picrate could not be obtained.

In a similar manner from 500 g. of rhizomes the following fractions were collected by distillation at 23 mm. through a 25-cm. Vigreux column: 0.40 g., 90–106°; 16.16 g., 135–141.5°; "Fraction C" 8.09 g., 141–143°; 11.67 g., 144–151° (total yield 7.3%).

By distillation of fraction b above, a sample was obtained for analysis.

(1) *Cf.* R. Adams, C. K. Cain and H. Wolff, THIS JOURNAL, **62**, 732 (1940), who give the absorption curves for 4-methyl- and 4-*n*-amylveratrole.

(2) P. D. Gardner, *ibid.*, **76**, 4550 (1954).

(3) Melting points on analytical samples are corrected. Microanalyses are by Dr. K. W. Zimmerman, University of Melbourne.