trated female rats. The melting points of the di-ethers show regular alternation; those of the mono- do so from the decyl up.

Experimental

These ethers were prepared in the conventional manner by heating stilboestrol in alcoholic solution with alkali and the required alkyl bromide (or iodide for the methyl). The preparation of the di-ethers presented no difficulty. These (particularly the high alkyl di-ethers) are much less soluble than the corresponding mono-ethers and can be purified by two or three recrystallizations from alcohol. To get rid of traces of mono-ether, alkali was usually added in the first recrystallization. The yields were high, usually above 90%. The mono-ethers proved to be difficult to prepare. The exclusive formation of a mono-ether could not be obtained by any attempted modification of the alkylation procedure. With less than one equivalent of alkyl halide and alkali the product always contained di-ether along with unreacted stilboestrol.

Although stilboestrol is soluble in 0.1 N aqueous alkali and the mono-ethers only in alcoholic alkali, the separation, theoretically simple, is tedious in practice. Since the solubilities change greatly with the size of the alkyl, each mono-ether required special study. In some cases the separation was repeated as many as 10 times before a satisfactory product was obtained. For the mono-methyl ether 0.4 N potassium hydroxide in 50% alcohol was used; the higher mono-ethers required stronger alcohol.

The monomethyl ether was recrystallized from 70% ethanol and then distilled in vacuo, b. p. 185–195° (0.3 mm.).

Summary

1. The normal mono- and di-alkyl ethers of stilboestrol from methyl to octadecyl have been prepared.

2. Their estrogenic activities have been determined.

The Effect of Temperature on the Validity of Hudson's Rules of Isorotation

BY WALTER KAUZMANN*

Over thirty years ago, Hudson[1] proposed his "rules of isorotation" for use in calculating the optical rotatory powers of carbohydrate derivatives. Although these have since been of great use to the carbohydrate chemist in determining the structure of new derivatives, their reliability has been seriously reduced by the existence of a number of definite instances, notably but not solely in the mannose series, in which calculated rotations fail to agree at all satisfactorily with those observed. It has been felt that if the source of these discrepancies could be determined, the value of the rules as a tool in carbohydrate structure determination might be considerably enhanced. So far, the effects of different solvents and concentrations[2] and of wave length[3] on carbohydrate rotations have been studied, but the behavior of the anomalous cases with respect to these variables has not led to any clue as to the true nature of the difficulty. Previous theoretical considerations,[4] based upon the physical origin of optical rotation have led us to suspect that the real clue to the problem is to be found in temperature dependence of the rotation. In this paper we shall give the reasoning which leads to this conclusion and show by means of experimental data that there is indeed good reason to believe that this is actually the case.

In a previous paper[4] it was shown by relatively simple, essentially geometrical arguments that Hudson's rules may be expected to be valid if two conditions are fulfilled. (1) The vicinal actions between any two groups whose locations, conformations and relative orientations in a molecule are fixed must be unaffected by change in the spacial configurations of other groups in the molecule.[5] In order to state this more explicitly,
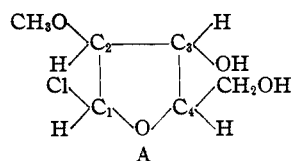
* Westinghouse Research Fellow. The experimental work in this paper was carried out at Frick Chemical Laboratory, Princeton University, Princeton, New Jersey.

(1) C. S. Hudson, THIS JOURNAL, 31, 66 (1909).

(2) P. Levene and I. Bencowitz, J. Biol. Chem., 73, 685 (1927).

(3) T. L. Harris, E. L. Hirst and C. E. Wood, J. Chem. Soc., 2108 (1932).

(4) E. Gorin, W. Kauzmann and J. Walter, J. Chem. Phys., 7, 327 (1939).

(5) Vicinal actions are the optical interactions between groups in a molecule, the sum total of which results in the molecule's being able to rotate the plane of polarized light. For further details, see W. Kauzmann, J. Walter and H. Eyring, Chem. Rev., 26, 339 (1940).

consider the molecule shown as A. This condition then requires that the contribution to the optical rotation due to the interactions of, say, the methoxyl on C_2 and the chlorine on C_1 shall not be influenced by the configurations of the groups about C_3 and C_4, either directly, through the "higher order perturbations" considered in reference 5, or indirectly through, for instance, solvent and steric effects[6] on the methoxyl which might depend on the orientation of the groups about C_3. (2) The second condition which must be fulfilled in order that Hudson's rules may be valid is that the framework of the molecule have a plane of symmetry, such that when the configurations about any two asymmetric centers in the compound are simultaneously reversed, all of the contributions to the optical activity resulting from the interactions of the groups about the two centers with one another change their signs but not their absolute values. Referring again to compound A, this condition means that if we change the configurations about C_1 and C_2, the new *average* conformation which the molecule assumes must be merely the mirror image of the old one as far as C_1 and C_2 by themselves are concerned. In a plane, five-membered ring with tetrahedral carbon atoms this will be automatically true; in a puckered, six-membered ring this will only be true if the various possible "chair conformations" occur in equal amounts, and similarly if "bed conformations" are also present, so that the *average* conformation of the ring will be symmetrical.

As far as the vicinal actions themselves are concerned, condition (1) will very probably be well satisfied in the sugars. Evidence for this has been discussed elsewhere.[7] On the other hand, the effect of steric repulsions, solvent effects and other structural factors may not be such as to allow the molecule to conform with these requirements, and it is probably here that we should seek the reason for the observed discrepancies to the rules, as well as the failure of optical superposition rules more generally.

Now let us inquire what will be the effect of an increase in the temperature on these structural

factors which tend to invalidate Hudson's rules. First of all, solvent effects are known to tend to decrease with increasing temperature, so that any trouble from this source must also decrease with increasing temperature. Secondly, increasing temperatures tend more or less to equalize the frequency of occurrence of all possible conformations of a molecule. This will tend to reduce the effects of "higher order steric interactions" which tend to invalidate the rules. It will also probably tend to equalize the amounts of the two possible chair forms of a six-membered ring, and it will tend to do the same for the bed forms. In short, except for the purely optical interactions (which would probably not lead to serious discrepancies anyway), an increase in the temperature should tend to bring about those conditions which result in Hudson's rules being valid.

Experimental.—The rotations of a number of sugar derivatives were measured at different temperatures and wave lengths. Since there was nothing particularly striking about the rotatory dispersion, however, these data will not be given here. The results at three temperatures for Na D light are given in Table I for various sugar derivatives. These derivatives were supplied by Dr. E. Pacsu, of Princeton University, or were prepared by standard procedures starting with materials furnished by him. The α-methyl mannofuranoside was supplied by Dr. A. Scattergood. The author wishes to express his gratitude for these materials.

The rotations recorded in Table I have been corrected for changes in the densities of the solutions on which the measurements were made. In all cases, rotations at room temperature were taken both before and after the readings at high temperatures in order to make sure that the observed changes with temperature were reversible. Practically all of the rotations changed very nearly linearly with the temperature in the range studied here.

Discussion.—It is obvious from the data of Table I that perhaps the conformations of, and certainly the solvent effects on, many of the sugars change with temperature sufficiently to cause, in some instances, very considerable changes in the optical rotation. Thus we see that there is no real justification for applying Hudson's rules indiscriminately to data obtained at room temperature, and serious errors might be expected from such a course.

(6) By the solvent effect here is meant the difference between the rotation in a given solvent and the rotation at the same temperature in the dilute vapor or (neglecting the internal field effect given by the factor $(n^2 + 2)/3$—see ref. 5) in an inert solvent.

(7) W. Kauzmann and H. Eyring. *J. Chem. Phys.*, **9**, 41 (1941).

TABLE I

VARIATION IN THE OPTICAL ROTATIONS OF CARBOHYDRATE DERIVATIVES WITH TEMPERATURE AND SOLVENT

Substance	Solvent	Concn., g./100 ml.	$[M]^{20}_D$	$[M]^{70}_D$	$[M]^{80}_D$
α-Glucose pentaacetate	$C_2H_2Cl_4$	5.07	394.5	394.5	394.5
β-Glucose pentaacetate	$C_2H_2Cl_4$	4.98	16	25	26.5
α-Mannose pentaacetate	$C_2H_2Cl_4$	3.12	190.5	223	230.5
β-Mannose pentaacetate	$C_2H_2Cl_4$	5.04	−97.5	−90	−88.5
α-Methyl glucoside tetraacetate	$C_2H_2Cl_4$	4.80	439	461	465.5
α-Methyl glucoside tetraacetate	C_6H_6	5.30	623	583	(578)
β-Methyl glucoside tetraacetate	$C_2H_2Cl_4$	5.17	−80	−58.5	(−55)
β-Methyl glucoside tetraacetate	C_6H_6	4.85	−83.5	−73	(−70.5)
α-Methyl mannoside tetraacetate	C_6H_6	4.94	160.5	180	182.5
α-Methyl mannoside tetraacetate	$C_2H_2Cl_4$	5.06	221	218.5	(219)
α-Methyl glucopyranoside	H_2O	5.12	302	301.5	301
β-Methyl glucopyranoside	H_2O	5.00	−64.5	−61	−60.5
α-Methyl mannopyranoside	H_2O	4.93	150.5	152.5	153
α-Methyl mannofuranoside	H_2O	5.26	212	199	196.5

In order to see if our previous theoretical considerations are valid, values of $2A$ for the penta-acetates of glucose and mannose in acetylene tetrachloride were calculated at 20, 70 and 80°. The results are given in Table II. It is apparent that by increasing the temperature from 20 to 80° the agreement with Hudson's rules is improved by nearly 50%, and since at even the highest temperature the rotations were still rapidly changing with the temperature, it is easy to believe that at sufficiently high temperatures the agreement would be very nearly complete.

In Table III the values of $2A_{OMe}$ are given as determined from the methyl glucoside tetraacetates in benzene and acetylene tetrachloride as solvents. It is apparent that as the temperature is increased, the discrepancy due to the very large solvent effects here is considerably reduced. It is also apparent from Table I that when there are large differences in the rotation of one substance in different solvents, these differences tend to be reduced at higher temperatures, in accordance with a rule previously given.[7]

In the previous paper[4] it was recalled that there is a corollary to Hudson's rules[8] which predicts that, for instance, the difference in the rotations of α-methyl glucoside and α-glucose should be the same as that between α-methyl mannoside and α-mannose. It was shown that this corollary should and does tend to be very accurate when the partial rotations being compared (here A_{OH} and A_{OMe}) are due to optically similar groups, but that when optically dissimilar groups are compared (such as CH_3—O and CH_3—CO—O)[9] no

good agreement should be expected, as is indeed found to be the case (Table VI, ref. 4). In Table IV this conclusion is tested further, and it is again seen that when dissimilar groups are involved, no general improvement in the discrepancy is found at higher temperatures. Even the apparent convergence for the glucose derivatives here is only temporary, since at the higher temperatures the rotations appeared to be still changing rapidly enough so that at about 100° their differences will probably become equal and then start to diverge.

The considerable temperature effects on the rotation of α-methyl mannofuranoside in water is of significance in connection with the poor agreement found for the mannofuranosides with respect to Hudson's rules.[10]

The temperature variation of the optical rotation offers a method for evaluating the F factors introduced by Pacsu[10,11] in order to improve the agreement with observed rotations of the rotations calculated using Hudson's rules: these factors at any given temperature should be given by the difference between the limiting rotation at high temperatures (insofar as the frequency of occurrence of all conformations is actually equalized at high temperatures) and the rotations at the given temperature. Pacsu[11] originally suggested that the factors arise from new types of isomerism, perhaps involving chair and bed forms of the sugar rings. More recently Scattergood and Pacsu[11] have suggested that they arise through "different

(see ref. 5, p. 351) are similar. Thus the first excited states in hydroxyl and methoxyl groups involve predominantly the same orbitals on the oxygen atom, so the groups are similar to one another in this sense, while the first excited state in the acetyl group involves the double bond of C=O, so this group is dissimilar to hydroxyl and methoxyl.

(8) C. S. Hudson, THIS JOURNAL, 47, 271 (1925).

(9) By optically similar groups it is meant that the electronic transitions which play a predominant role in determining the contribution to the rotatory power by these groups acting as chromophores

(10) A. Scattergood and E. Pacsu, THIS JOURNAL, 62, 903 (1940).

(11) E. Pacsu, ibid., 61, 2669 (1939).

orientation [about the C—O bond] of the hydroxyl groups in the α- and β-isomers of certain sugars." In the light of the observed large solvent effects for some substances, it does not, however, seem profitable to ascribe all of these variations to any one such physical cause.

When the temperature dependence of the rotations of β-methyl mannoside tetraacetate in benzene and in acetylene tetrachloride and of β-methyl manno-pyranoside and -furanoside in water are known, it will be possible, with the data presented here, to find out if the values of $2A$ for these compounds, too, tend to agree any better at higher temperatures with the corresponding values of the glucose derivatives, just as we have shown is the case for the pentaacetates of the two sugars.

TABLE II
(FROM DATA ON THE PENTAACETATES)

t, °C.	$2A_{OAc}$(glucose)	$2A_{OAc}$(mannose)	Discrepancy
20	378.5	288	90.5
70	369.5	313	56.5
80	368	319	49

TABLE III
(FROM DATA ON THE METHYL GLUCOSIDE TETRAACETATES)

t, °C.	$2A_{OMe}$ (in acetylene tetrachloride)	$2A_{OMe}$ (in benzene)	Discrepancy
20	519	706.5	187.5
70	519.5	656	136.5
80	(520.5)	(648.5)	(128)

TABLE IV
(FROM DATA ON SOLUTIONS IN ACETYLENE TETRACHLORIDE)

t, °C.	α-Me gluc. tetraac.-α gluc. pentaac.	−(β-Me gluc. tetraac.-β gluc. pentaac.)	α-Me mann. tetraac.-α mann. pentaac.
20	44.5	96	−30
70	66.5	83.5	−43
80	71	82	(−48)

Conclusion.—From the evidence presented here it should be clear that for many of the derivatives of the carbohydrates there is a considerable dependence of the rotation on the temperature, so that structural influences on Hudson's

rules must be important in affecting their validity, and it is not at all unlikely that these structural influences lie at the root of the major part of the observed discrepancies in those rules. It would be very desirable if, when a test is being made concerning the validity of Hudson's rules in a series of compounds, measurements of the effect of the temperature on the rotations as well as the rotations themselves were made. Then, if the rotations were found to be in disagreement with the rules, the influence of the temperature in making the rules better or worse could be ascertained, and we would gain considerably more insight into the origin of the discrepancy.

It would also be very interesting to have such temperature data for the menthyl amines investigated by Read,[12] since these should show superposition in the ordinary van't Hoff sense at higher temperatures. Similarly, the recent application of superposition rules to the γ-lactones by Hudson[13] should become more exact at higher temperatures, and no division of the γ-lactones into two classes should be necessary. By working at higher temperatures, superposition rules would undoubtedly be found to apply to many new types of compounds, possibly even to some open chain compounds.

Summary

It is shown from theoretical considerations that the clue to the discrepancies observed on the application of Hudson's rules of isorotation to the calculation of the optical rotations of carbohydrates is probably to be found in the temperature variation of the optical rotation for these compounds. Experimental measurements are made which support the prediction that as the temperature is increased, the rules should with certain restrictions tend to become more and more accurate.

(12) J. Read, Trans. Faraday Soc., 26, 441 (1930).
(13) C. S. Hudson, THIS JOURNAL, 61, 1525 (1939).