estadiene, $\Delta^{3,5}$-cholestadiene, β-7-hydroxycholesterol, and trans-cholestane-3,5,6-triol.

Formation of Polymeric Hydrocarbons

Cholesterol.—A solution of 10 g. of cholesterol in 100 cc. of chloroform was shaken with 10 g. of a 70% perchloric acid. After the initially formed crystals had been digested and the clear liquid was dark reddish-brown, the solution was washed neutral with water, dried and the solvent evaporated. The tough, light brown residue was free of cholesterol and was dissolved in 175 cc. of hot ethyl acetate. Upon cooling, 4.4 g. of a resinous, light orange precipitate was obtained, obviously not homogeneous and also containing ash, m. p. 144–150°.

Anal. Calcd. for $(C_{27}H_{44})_2$: C, 87.96; H, 12.04; mol. wt., 737. Found: C, 87.34; H, 11.75; mol. wt., 718; ash.

The hydrocarbon was brominated in ether solution.

Anal. Calcd. for $(C_{27}H_{44}Br_2)_2$: Br, 30.26; mol. wt., 1056. Found: Br, 29.2; mol. wt. (Barger-Rast), 1074.

In another preparation, the reaction mixture was kept for one hour after the liquid had become clear and was then washed neutral with water. The precipitate obtained from the ethyl acetate solution was recrystallized twice from a mixture of toluene and ethyl acetate. The slightly yellow material seemed to darken at 128° and melted completely at about 200–205°.[19]

Anal. Calcd. for $(C_{27}H_{44})_3$: C, 87.96; H, 12.04; mol. wt., 1105. Found: C, 87.77; H, 11.89; mol. wt. (Rast-Tiedcke), 1032.

$\Delta^{3,5}$-Cholestadiene.—Five grams of the hydrocarbon in 250 cc. of chloroform was shaken with 5 g. of a 70% perchloric acid for one minute. The solution was washed neutral with water and processed further as has just been described. The slightly yellow recrystallized material softened at 152° and was completely melted at 203°.[19]

Anal. Calcd. for $(C_{27}H_{44})_x$: C, 87.96; H, 12.04. Found: C, 87.57; H, 12.00; mol. wt. (Barger-Rast), 1140.

$\Delta^{2,4}$-Cholestadiene.—The reaction product, prepared similar to that of the 3,5-diene, was slightly yellow, softened at 132° and was completely melted at about 200°.[19]

Anal. Calcd. for $(C_{27}H_{44})_x$: C, 87.96; H, 12.04. Found: C, 87.72; H, 11.95; mol. wt. (Barger-Rast), 926.

Fractional crystallizations of the various preparations of the polymeric hydrocarbon gave products with very good analytical data for C and H. While molecular weights differed between values for a dimeric and trimeric compound, no fractions higher than a trimer have been observed. The specific rotation of the fractions in chloroform was positive.

Acknowledgment.—We wish to express our thanks to Miss Ruth Daniels for help with the spectrophotometric measurements.

Summary

1. Common sterols containing the 3(β)-hydroxy-Δ^5-ene grouping are precipitated instantaneously from sufficiently concentrated chloroform solutions in the form of well-crystallized, colorless salts, with the composition Sterol·$HClO_4$ or Sterol·HPF_6, by the addition of sufficiently concentrated perchloric or hexafluorophosphoric acids.

2. The sterolium salts hydrolyze instantaneously upon contact with an excess of water, with quantitative recovery of the unchanged sterols.

3. An excess of acid over the stoichiometric quantity results in slow digestion of the crystals and formation of strongly purple colored halochromic compounds, with final degradation of the color, probably in the following steps: (a) colorless sterolium salt, dissolved in dilute solution, (b) colorless cholestadiene, (c) unstable halochromic cholestadiene salt, (d) stable, discolored halochromic salt of a polymeric hydrocarbon $(C_{27}H_{44})_x$, with the trimer being the highest polymer observed.

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[19] See Fig. 1 for absorption spectrum.

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

Cuprammonium-Glycoside Complexes. IV. The Conformation of the Galactopyranoside Ring in Solution

BY RICHARD E. REEVES

In a preceding communication of this series[2] it was shown that the behavior in cuprammonium solution of twelve complex-forming D-glucopyranoside derivatives can be interpreted in terms of a single ring conformation, namely, the Sachse strainless ring chair form in which the sixth carbon atom and the ring oxygen atom project on the same side of the plane formed by carbon atoms 1, 2, 4 and 5. This ring conformation has been designated C1, and its mirror image, 1C. Examination of nineteen substances belonging or related to the galactose series now reveals that the D-galactopyranoside ring can also be regarded as ordinarily possessing the C1 conformation.

By a conductometric method it was established that the D-glucopyranosides form cuprammonium complexes only with the hydroxyl pairs on the second and third or third and fourth carbon atoms. This same situation is believed to apply to the galactose series. Examination of suitably substituted methyl and phenyl D-galactopyranosides has revealed the formation of levorotatory complexes at both of these sites, but no indication of reaction involving any other pair of hydroxyl groups.

Since reaction is limited to adjacent hydroxyl groups, the projected angle between carbon-oxygen valences for each complex-forming pair of hy-

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[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

[2] Reeves, THIS JOURNAL, 71, 215 (1949).

droxyl groups was calculated for all D-galactopyranoside ring conformations in the same manner as was done for the D-glucopyranosides.[2] The calculated angles are given in Table I together with the anticipated behavior for a simple D-galactopyranoside in cuprammonium if it were oriented in each of the conformations. The observed behavior for galactopyranosides and related glycosides is given in Table II. The grouping of D-galactosides and L-arabinosides in the D-galactose series, and D-arabinosides and L-fucosides in the L-galactose series is adequately justified by prior work,[3,4] and confirmed by the present results.

TABLE I

CALCULATED ANGLE BETWEEN ADJACENT HYDROXYL GROUPS FOR D-GALACTOPYRANOSIDES IN EACH RING CONFORMATION

Conformation	Glycol 2–3	Glycol 3–4	Anticipated behavior in cuprammonium solution
C1	− 60°	−60°	Levo complex
1C	180°	+60°	Dextro complex
B1	−120°	−60°	Levo complex
1B	−120°	+60°	Dextro complex
B2	− 60°	0°	Levo complex[a]
2B	180°	0°	[a]
B3	180°	+60°	Dextro complex
3B	− 60°	−60°	Levo complex

[a] The only optically active pyranoside known to possess the 0° angle is methyl 2,6-anhydro-α-D-altropyranoside. This substance exhibited a very high conductance effect and a low rotational shift, see footnote 5.

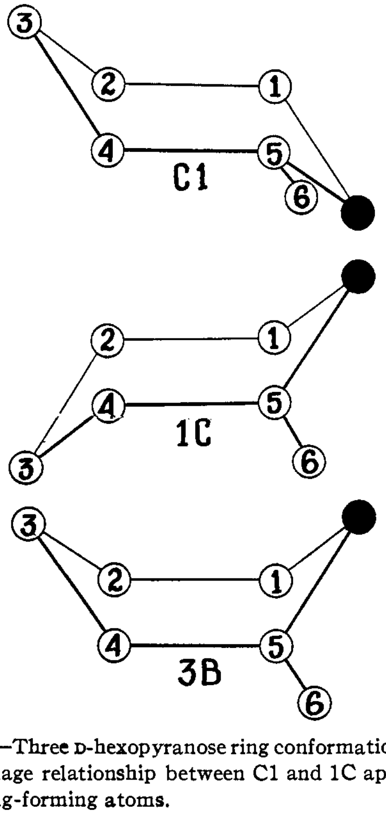

Fig. 1.—Three D-hexopyranose ring conformations. The mirror image relationship between C1 and 1C applies only to the ring-forming atoms.

The data of Table II allow direct conclusions regarding ring conformation only with the D-galactopyranosides. The case for the ring conformation of the arabinosides and fucosides rests entirely on analogy with the galactosides. Proceeding on the hypothesis[5] that strongly levorotatory complexes form at the −60° angle, strongly dextrorotatory at the +60° angle, and no complexes at the 120° and 180° angles it appears that D-galactopyranoside ring conformations C1, B1, B2 and 3B could give strongly levorotatory complexes. But only C1 and 3B would give the levorotatory complexes observed for both 2-3 and 3-4 hydroxyl pairs. Thus, if a single ring conformation is to be selected to represent the D-galactopyranosides it must be either Cl or 3B.

In addition to theoretical arguments favoring the chair form (C1) over the boat form (3B)[6,7,8] there is the following argument based on the behavior of the substance D-galactosan$<1,5>\beta<1,6>$: D-galactosan is limited, by reason of the spatial requirements of the 1,6-anhydro ring, to two possible pyranose ring conformations, the 1C or the 3B form. The behavior of D-galactosan in cuprammonium solution excludes the 3B form and agrees with the 1C conformation. If the boat form 3B were the stable conformation for the simple D-galactopyranosides then it might have been expected to persist without change in the galactosan. The three D-hexopyranose conformations C1, 1C and 3B are shown diagrammatically in Fig. 1. Note that the mirror image relationship between forms 1C and C1 applies only to the six ring-forming atoms. The carbon atoms outside the pyranose rings are not in mirror image relationship in these two forms.

These results indicate that in the galactopyranoside series both members of an anomeric pair possess the same ring shape or conformation. Furthermore, the conformations involved are C1 in the D-galactopyranoside series, and, of course, the mirror image form 1C would apply to the L-galactopyranoside series.

The behavior of the glycosides of arabinose and fucose indicates that the L-arabinosides resemble in ring conformation the D-galactosides, while the D-arabinosides and L-fucosides resemble the L-galactosides.

Experimental

Optical rotations were measured at 25° with the sodium D line or the mercury blue line (436 mμ) as indicated. Rotations in cupra B—a standard cuprammonium hydroxide solution containing 15 g. copper, 240 g. ammonia, and 1 g. glycerol per liter[9]—were measured in 0.5 d. tubes.

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(3) Isbell and Frush, *J. Research Natl. Bur. of Standards*, **24**, 125 (1940).

(4) Hockett, Phelps and Hudson, THIS JOURNAL, **61**, 1658 (1939).

(5) Reeves, Part II, *ibid.*, **71**, 212 (1949).

(6) Gorin, Kauzmann and Walter, *J. Chem. Phys.*, **7**, 327 (1939).

(7) Scattergood and Pacsu, THIS JOURNAL, **62**, 903 (1940).

(8) Hassel and Ottar, *Acta Chemica Scandinavica*, **1**, 929 (1947).

(9) Reeves, *J. Biol. Chem.*, **154**, 49 (1944).

TABLE II

BEHAVIOR OF GALACTOPYRANOSIDES AND RELATED GLYCOSIDES IN CUPRAMMONIUM SOLUTION

Substance	Specific rotation, degrees (g./100 ml.)			Rotational shift,[a] degrees	Δ sp. res.[b]	Remarks	Ref.
	D line, in water	436 mμ, in water	436 mμ, in cupra B				
(D-Galactopyranoside series)							
Methyl α-D-galactoside[c]	+198 (0.64)	+385 (0.64)	− 660 (0.70)	−2027	75	....	*d*
Methyl β-D-galactoside	− 1 (0.60)	− 5 (0.60)	−1030 (0.64)	−1989	90	....	*d*
Phenyl α-D-galactoside	+207 (0.97)	+416 (0.97)	− 362 (0.66)	−1992	...	....	*e*
Phenyl β-galactoside	− 41 (2.34)	− 91 (2.34)	− 890 (1.16)	−2045	90	....	*f*
Methyl 4,6-benzylidene-α-D-galactoside	+163 (1.22)	+316 (1.22)	− 354 (1.09)	−1889	...	Complex at 2–3	*g*
Methyl 4,6-benzylidene-β-D-galactoside	− 7 (1.23)	− 11 (1.23)	− 658 (1.13)	−1825	61	Complex at 2–3	*g*
Phenyl 4,6-benzylidene-β-D-galactoside	Insol.	Insol.	− 708 (0.20)	....	...	Complex at 2–3	*f*
Methyl 2,6-dimethyl-β-D-galactoside	0 (1.06)	− 2 (1.06)	− 682 (1.15)	−1510	...	Complex at 3–4	*h*
Methyl 3,4-isopropylidene-β-D-galactoside	− 21 (0.90)	− 38 (0.90)	+ 14 (1.33)	+ 121	11	No complex at 2–6	*i*
Phenyl 2,3-dimethyl-β-D-galactoside	− 31 (1.03)	− 60 (1.03)	− 3 (0.62)	+ 162	4	No complex at 4–6	*f*
Methyl-β-L-arabinoside	+243 (0.56)	+470 (0.56)	− 397 (0.56)	−1422	75	....	*d*
Methyl-α-L-arabinoside	+ 17 (0.59)	+ 32 (0.59)	−1000 (0.54)	−1692	78	....	*d*
Methyl D-manno-α-D-galaheptoside	+171 (0.67)	+332 (0.68)	−1025 (0.56)	−3040	...	(A complex at 6–7	*j*
Methyl D-manno-β-D-galaheptoside	− 5 (0.90)	− 14 (0.90)	−1160 (0.67)	−2567	...	may contribute to rotation	*j*
D-Galactosan <1,5>β<1,6>	− 22 (0.83)	− 47 (1.37)	+ 620 (0.79)	+1081	193	Definite 1C conformation	*k*
(L-Galactopyranoside series)							
Methyl-β-D-arabinoside	−243 (0.53)	−465 (0.53)	+ 396 (0.58)	+1412	78	....	*l*
Methyl-α-L-fucoside	−194 (0.64)	−377 (0.64)	+ 648 (1.01)	+1825	63	....	*m*
Methyl-β-L-fucoside	+ 13 (0.72)	+ 24 (0.72)	+1100 (0.60)	+1915	...	....	*m*
Phenyl-β-D-arabinoside	−242 (0.59)	−475 (0.59)	+ 212 (0.87)	+1533	...	....	*n*

[a] ($[\alpha]_{436}$ cupra B − $[\alpha]_{436}$ water) × mol. wt./100. [b] The increase in specific resistance due to an 0.01 molar glycoside concentration in cupra A. [c] The optical rotations were determined on methyl α-D-galactoside·H_2O and recalculated to the anhydrous basis. [d] "Polarimetry, Saccharimetry and the Sugars," F. J. Bates and Associates, U. S. Govt. Printing Office, Washington, 1942, p. 718. [e] Helferich and Appel, *Z. physiol. Chem.*, **205**, 231 (1932). [f] Reeves, THIS JOURNAL, **70**, 3963 (1948). [g] See footnote 11. [h] See footnote 12. [i] Micheel, *Ber.*, **62**, 687 (1929). [j] Montgomery and Hudson, THIS JOURNAL, **64**, 253 (1942). [k] Hann and Hudson, *ibid.*, **64**, 2435 (1942). [l] Neuberg and Wohlgemuth, *Z. physiol. Chem.*, **35**, 31 (1902). [m] See footnote 8. [n] Helferich, Appel and Gootz, *Z. physiol. Chem.*, **215**, 277 (1933).

Resistance measurements were observed in cupra A—a standard dilute cuprammonium solution containing 0.01 mole copper, 3.0 moles ammonia, and 10 ml. of ethanol per liter.[10] Measurements at a series of glycoside dilutions were plotted on coördinate paper, and the value for 0.01 molar glycoside concentration was read from the smooth curve through the experimental points. The cell employed had a constant of 0.116 as determined against 0.1 *M* potassium chloride solution.

All of the substances included in Table II are well-characterized crystalline compounds. The D line specific rotations indicate the purity of the samples employed. A reference to a previous characterization is included for each substance. Satisfactory agreement was noted between the properties of the preparations here employed and the properties recorded in the literature with the following two exceptions:

Methyl 4,6-benzylidene-α-D-galactoside gave m. p. 168–169°, $[\alpha]^{25}\mathrm{D}$ +142° (*c* 2.54, $CHCl_3$) although Sorkin and Reichstein[11] report m. p. 177–178°, $[\alpha]\mathrm{D}$ +162° ($CHCl_3$) for this substance.

Methyl 2,6-dimethyl-β-D-galactoside melted at 67–69°, $[\alpha]^{25}\mathrm{D}$ −27° (*c* 1.15, $CHCl_3$); Oldham and Bell[12] report m. p. 45–47°, $[\alpha]\mathrm{D}$ −23° ($CHCl_3$) for this substance.

Summary

Nineteen substances belonging or related to the galactopyranosides have been investigated in cuprammonium solution. It has been observed that the behavior of D-galactopyranosides and L-arabopyranosides is compatible with ring conformation C1, L-fucosides and D-arabinopyranosides with the mirror image conformation, 1C.

The pyranose ring of D-galactosan<1,5>β<1,6> exists in the 1C conformation; thus the ring shape of the anhydride is different from that of the methyl and phenyl D-galactopyranosides.

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(10) Reeves and Jung, THIS JOURNAL, **71**, 209 (1949).

(11) Sorkin and Reichstein, *Helv. Chim. Acta*, **28**, 1 (1945).

(12) Oldham and Bell, THIS JOURNAL, **60**, 323 (1938).