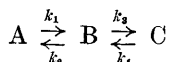


CCLXXIX.—*On the Modifications of Galactose.*

By CLAUS NISSEN RIIBER, JOSEF MINSAAS, and RALPH TAMBS  
LYCHE.

It has recently been shown by two of us (*Ber.*, 1926, **59**, 2266) that the volume of an aqueous solution of the ordinary form of galactose (known as  $\alpha$ -galactose) alters with time according to a law which requires for its expression an equation containing *two* exponential terms. Hence, we concluded that in such a solution more than two modifications of the sugar must be present. In order to simplify the hypothesis for the purpose of calculation, we suggested that an intermediate substance B appeared in the aqueous solution of galactose in addition to the two known modifications, designated respectively  $\alpha$ -galactose (A) and  $\beta$ -galactose (C) :



By means of our measurements of the alterations in volume, which take place respectively after  $\alpha$ -galactose and  $\beta$ -galactose have been dissolved in water, we calculated the values of the four velocity coefficients  $k_1$ ,  $k_2$ ,  $k_3$ , and  $k_4$ , and found

$$\begin{array}{ll} k_1 = 0.042777 \times \log_e 10 & k_3 = 0.006993 \times \log_e 10 \\ k_2 = 0.010333 \times \log_e 10 & k_4 = 0.002897 \times \log_e 10. \end{array}$$

From these figures we calculated the proportions of the three modifications in the equilibrium mixture to be A 6.61%, B 27.35%, and C 66.04%.

By means of the above values of the velocity coefficients, the volumes of the solutions of (a)  $\alpha$ -galactose and (b)  $\beta$ -galactose were calculated for definite times after dissolution of the sugars had taken place, and good agreement with the observed values was obtained, as shown by the curves marked I in Figs. 1 and 2.

Furthermore, we used the same values of the velocity coefficients in calculating the alteration of the specific rotations of these sugars, and in this case also we arrived at figures which, within the limits of experimental error, agreed with the observed figures. It ought, however, to be noticed that we intentionally took no observations of the polarimetric angle until 10 minutes after dissolution of the sugar, since experiments show that observations taken earlier are untrustworthy for several reasons (*e.g.*, imperfect heat transference, evolution of heat, rapid alteration of the polarimetric angle). Notwithstanding this, Smith and Lowry (*J.*, 1928, 666; see also Verschuur, *Rec. trav. chim.*, 1928, **47**, 445) have recorded measurements

of the change of the polarimetric angle taken in the interval between 2 and 10 minutes after solution had taken place, and accordingly their derived velocity coefficients suffer from a considerable amount of uncertainty. For this reason, their values for the velocity coeffi-

*Volumes of aqueous solutions of  $\alpha$ -galactose (Fig. 1) and  $\beta$ -galactose (Fig. 2).*

FIG. 1.

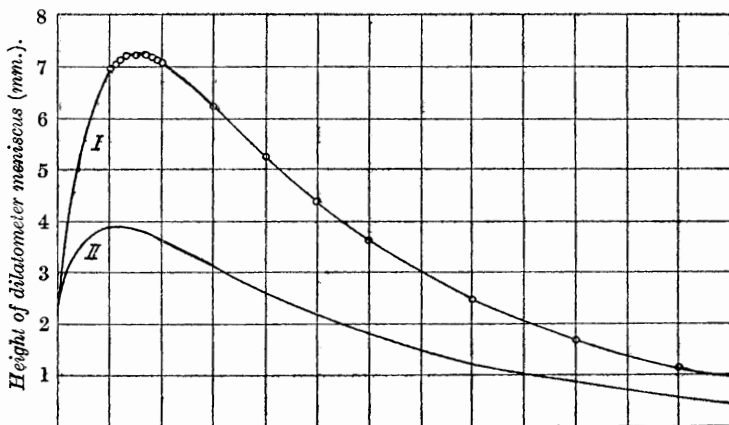
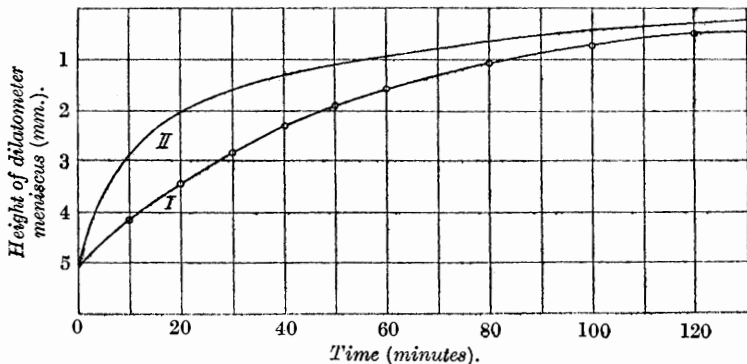


FIG. 2.



○ = Experimental observations.

Curves I are based on Riiber and Minsaas's velocity coefficients (Ber., 1926, 59, 2272, 2273).

Curves II are based on Smith and Lowry's velocity coefficients.

icients would be expected to differ to a certain extent from ours, but the actual differences are far too big to be explained in this way. The values of the velocity coefficients calculated from these measurements according to the same scheme of reaction are, as a matter of

fact, entirely different from those which we obtained from the change in volume, for Smith and Lowry found (their  $k_3$  being our  $k_4$  and *vice versa*)

$$\begin{aligned} k_1 &= 0.00899 \times \log_e 10 & k_3 &= 0.03422 \times \log_e 10 \\ k_2 &= 0.02145 \times \log_e 10 & k_4 &= 0.00691 \times \log_e 10. \end{aligned}$$

By means of these values they calculated also the quantities of the modifications A, B, and C which should be present in the equilibrium mixture, and found A 28.5%, B 12.0%, and C 59.5%.

We have repeated Smith and Lowry's polarimetric experiments with  $\alpha$ - and  $\beta$ -galactose and have obtained very similar results.

From their velocity coefficients, these authors calculate the alteration of the polarimetric angle, and, as may be expected, the calculated values agree with the observed values from which they are derived. When their velocity coefficients are used in the case of the alteration of the volume, curves are found which deviate considerably from the observed values, as will be apparent from the curves marked II in Figs. 1 and 2. On the other hand, the polarimetric angles calculated from our velocity coefficients, which are derived from the volumes, show much smaller deviations from the calculated values, and it is only within the interval 0—10 minutes that the deviations exceed the limits of experimental error (see the curves in Figs. 3 and 4).

The main result of Smith and Lowry's experiments as compared with our own is, therefore, that quite different values of the velocity coefficients are derivable from the mutarotation and from the alterations in volume. Both methods have the same justification, the supposition being that in the one case the optical rotation, and in the other the solution volume, is an additive property. An experimental investigation which we have carried out confirms this supposition, as it shows that the two physical properties mentioned are very nearly additive. In both methods the same scheme of reaction was used as a basis for calculation.

Since the application of these two methods has resulted in the ascription of quite different values to the velocity coefficients, the reason can only be *that the basis of calculation, viz., that the reaction between the modifications takes place according to the scheme*  $A \rightleftharpoons B \rightleftharpoons C$ , *is not in accordance with fact.*

Although the two methods do not agree as far as the individual velocity coefficients are concerned, yet there is in one respect a remarkable agreement between them. The alteration in polarimetric angle and in volume undergone by solutions of  $\alpha$ - and  $\beta$ -galactose in the course of  $t$  minutes after dissolution may be expressed, within the limits of experimental error, by the following

equation, where  $L$ ,  $M$ ,  $r_1$  and  $r_2$  are constants and  $r_1$  and  $r_2$  have in all cases practically the same values :

$$\Delta = Le^{-r_1 t} + Me^{-r_2 t}.$$

Thus, from the *optical rotation* for  $\alpha$ - and  $\beta$ -galactose, we found  $r_1 = 0.0083 \times \log_e 10$ ,  $r_2 = 0.060 \times \log_e 10$ , and Smith and Lowry found  $r_1 = 0.00817 \times \log_e 10$ ,  $r_2 = 0.0634 \times \log_e 10$ ; whereas from

*Mutarotation of  $\alpha$ -galactose (Fig. 3) and  $\beta$ -galactose (Fig. 4)—previous experiments (see Ber., 1926, 59, 2278, 2279) together with new values for the interval 2–10 mins.*

FIG. 3.

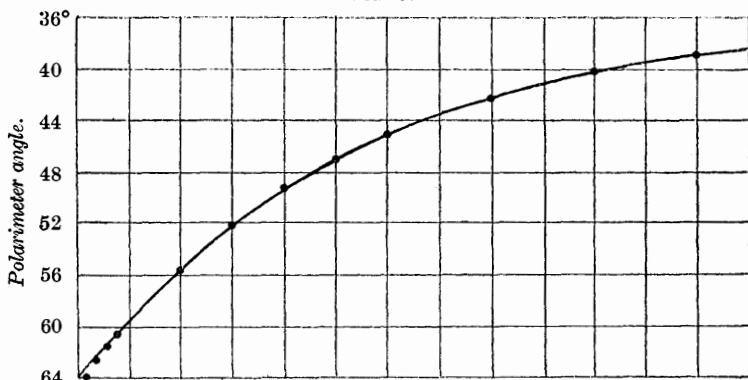
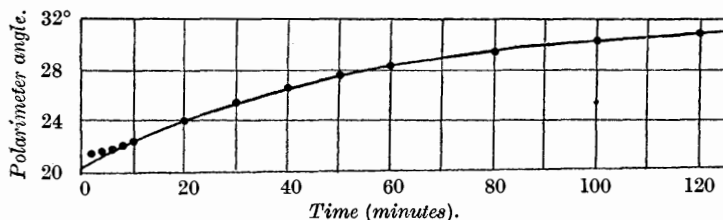


FIG. 4.



● = Experimental observations.

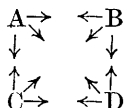
The curves are based on Riiber and Minsaas's velocity coefficients.

the alterations in volume we found  $r_1 = 0.00829 \times \log_e 10$ ,  $r_2 = 0.0547 \times \log_e 10$ .

Considering that even traces of impurities influence the values of these exponents, one is justified in presuming that in the case of pure substances dissolved in pure water the exponents  $r_1$  and  $r_2$  would be exactly the same both for mutarotation and for volume change.

When the question is treated mathematically (see Tambs Lyche, *Kgl. Norske Vid. Selskabs Skrifter*, 1928, No. 7), it appears that such a case might arise if we had three modifications reacting with one

another in accordance with the scheme  $A \rightleftharpoons B$   
 $\swarrow \searrow \nearrow \nwarrow$   
 $C$ , in which the possibility of direct reaction between A and C is postulated. On the other hand, the scheme of reaction



with four modifications will require changes of the properties in question which can be expressed by equations of either of the following forms :

$$\Delta = Le^{-r_1t} + Me^{-r_2t} + Ne^{-r_3t},$$

or

$$\Delta = Le^{-r_1t} + Me^{-r_2t} \cos(rt + s),$$

where  $L$ ,  $M$ ,  $N$ ,  $r_1$ ,  $r_2$ ,  $r_3$ ,  $r$ , and  $s$  are constants and  $r$ ,  $r_1$ ,  $r_2$ , and  $r_3$  are independent of the particular property in question.

It is then evident that if, for instance, the value of  $r_3$  lies close to  $r_2$ , it will, on account of the uncertainty associated with the determination of these figures, be impossible to decide whether two or three exponential terms should be used, *i.e.*, whether three or four modifications are present. This might also be the case when  $N$  is comparatively small or  $r_3$  very large. Also the last equation might be imagined to agree with the facts if, for instance,  $r$  was very small. Hence, the presence of a fourth modification will be concealed by the experimental errors if (1) its quantity is small, (2) its physical properties lie close to those of one of the other modifications, or (3) the velocity coefficients have such values as to cause any of the foregoing contingencies in the  $r$ -terms. Much more delicate experimental methods would be necessary to decide whether a fourth modification is actually present.

It is now of interest to compare the above-mentioned conclusions with those which can be drawn in another way. At present four penta-acetates of galactose are known as well-defined crystalline substances (*Ber.*, 1889, **22**, 2207; 1901, **34**, 976; *J. Amer. Chem. Soc.*, 1916, **38**, 1223) with the specific rotations (in chloroform)  $+107^\circ$ ,  $+23^\circ$ ,  $-42^\circ$ , and  $+61^\circ$ , and if the five acetyl groups were replaced by five hydrogen atoms, four different galactoses would be obtained. As the four penta-acetates do not give the aldehydic reactions, the four galactoses cannot be real aldehydes. If it is suggested that an aqueous solution of galactose contains four modifications, they must presumably correspond in structure to the four penta-acetates. Three methylgalactosides are known at present, two of which were prepared in a crystalline state several years ago, their rotations being respectively  $+197^\circ$  and  $0^\circ$ . Moreover, Haworth (*J.*, 1924, **125**, 2471)

has isolated a strongly lævorotatory form, which he supposes to be almost pure in spite of its being a liquid. Its specific rotation in methyl alcohol containing hydrogen chloride he found to be  $-58.1^\circ$ . To these three galactosides correspond three galactoses. By comparing the rotatory power of these compounds, reasoning by analogy, the following system is derived :

	I $\alpha$ .	Diff.	I $\beta$ .	Diff.	II $\alpha$ .	Diff.	II $\beta$ .	Diff.
Penta- acetates	$+107^\circ$	$\left. \begin{array}{l} \} \\ \} \end{array} \right\} -38^\circ$	$+23^\circ$	$\left. \begin{array}{l} \} \\ \} \end{array} \right\} -31^\circ$	$+61^\circ$	$\left. \begin{array}{l} \} \\ \} \end{array} \right\} -37^\circ$	$-42^\circ$	$\left. \begin{array}{l} \} \\ \} \end{array} \right\} -33^\circ$
Modific- ations	$+145^\circ$		$+54^\circ$		$(ca. +98^\circ)$		$(ca. -9^\circ)$	
Methyl- galactosides	$+197^\circ$	$\left. \begin{array}{l} \} \\ \} \end{array} \right\} +52^\circ$	$0^\circ$	$\left. \begin{array}{l} \} \\ \} \end{array} \right\} -54^\circ$	$(ca. +150^\circ)$	$\left. \begin{array}{l} \} \\ \} \end{array} \right\} +52^\circ$	$(ca. -61^\circ)$	$\left. \begin{array}{l} \} \\ \} \end{array} \right\} -52^\circ$

As will be seen, the comparison of the rotatory powers leads to the conclusion that, besides the two modifications of galactose already known, two more should be found with rotations of about  $+98^\circ$  and  $-9^\circ$ . Of the methylgalactosides, besides the two hitherto-known crystalline forms, two more should be found, with rotations of about  $+150^\circ$  and  $-61^\circ$ . The last is probably present in Haworth's strongly lævorotatory syrup.

In the scheme given above it is presumed that the substances in the columns I $\alpha$  and I $\beta$  are related to each other in the same manner as  $\alpha$ - to  $\beta$ -glucose. They will differ only in the way in which hydroxyl groups and hydrogen atoms are bound to carbon atom 1. According to Hudson's rule of optical superposition, the mean value of the rotation should in that case be the same whether the free sugars or the methylgalactosides are considered. The mean rotation of the modifications I $\alpha$  and I $\beta$  is  $99^\circ$  both for the free sugars and for the methylgalactosides; and similarly, the corresponding means for the substances of type II $\alpha$  and II $\beta$  are  $45^\circ$  in each case.

The difference between the substances in columns I and II is considered (in agreement with Haworth's experiments on the oxidation of the methylated galactoses) to be due to the difference of the oxygen rings, and hence must be the same whether the free sugars or the methylgalactosides are considered. This is evidently the case, for the difference is  $99^\circ - 45^\circ = 54^\circ$  for each pair. It is therefore apparent that in the above system the agreement between the various angles of rotation is very good. It should be noted, however, that the values of the two unknown modifications, *viz.*,  $+98^\circ$  and  $-9^\circ$  (average  $+45^\circ$ ), do not agree with Haworth's rule for the direction of rotation of the aldoses (J., 1926, 2303). The angle of rotation of  $\gamma$ -galactonolactone is *negative* ( $-78^\circ$ ) and this is in agreement with Hudson's rule for the lactones. Now Haworth's rule would lead one to expect that the average value of the angles of the two modifications also should be *negative*, but according to our

scheme they are *positive*. The rotatory powers for the two new modifications (and for the two corresponding methylgalactosides\*) in the above table are therefore given with all reserve.

Smith and Lowry suppose that in an aqueous solution of galactose, in addition to the ring-containing modifications, a real aldehydic form is present in a not inappreciable quantity (12%). It may be objected, however, that an aqueous solution of galactose does not give the characteristic reactions of real aldehydes; thus Angeli's reagent (Angeli and Marchetti, *Atti R. Accad. Lincei*, 1908, **17**, ii, 362), which is a sensitive and characteristic test for aldehydes and oxy-aldehydes, gives a negative result, and Schiff's reagent gives a red colour only after many hours, and even then the colour is much lighter than that given by the equivalent quantity of glyceraldehyde. The real aldehydic form must therefore be present only in a very small quantity.

The specimen of  $\beta$ -galactose used by us in our previous experiments and in the above-mentioned control measurements was prepared in essentially the same way as that used by Smith and Lowry, *i.e.*, by pouring a concentrated aqueous solution of galactose into well-cooled alcohol (Hudson and Janowsky, *J. Amer. Chem. Soc.*, 1917, **39**, 1020), filtering off the sugar, redissolving and reprecipitating it, and then extracting the precipitate with 80% alcohol (yield, 2—3 g. from 20 g. of galactose). We have, however, endeavoured to find a better method, since the precipitation method would not be likely to yield a product free from other modifications; in fact, the floury preparation thus obtained is seen under the microscope to have an indistinct crystalline appearance, and it rapidly changes into the stable modification, which fact would appear to indicate the presence of crystal nuclei of other modifications.

We have succeeded in finding a very simple method [Riiber, *Kongl. Norske Videnskabers Selskab* (forhandl.), 1926—28, I, No. 4] by means of which ordinary galactose can be quantitatively transformed into  $\beta$ -galactose, namely, by evaporating at room temperature (in a vacuum over calcium chloride) an aqueous solution of galactose, which has been previously heated in order to destroy all the crystal nuclei present, and taking precautions to prevent extraneous nuclei from reaching the solution. In this way  $\beta$ -galactose was obtained in well-developed, water-clear crystals, which Dr. E. Berner had the kindness to measure. The results are as follows: Class of symmetry: monoclinic prismatic. Axial angle:  $\beta = 106^\circ 25'$ . Ratio of axes:  $a : b : c = 0.827 : 1 : 0.775$ . Crystallises in prisms of the following forms: frequently,  $m\{110\}$ ,  $p\{120\}$ ,

\* Haworth's rule for the free sugars should consequently be extended to the glucosides.

and  $s\{101\}$ ; less frequently,  $c\{001\}$ . Etched figures, by means of alcohol, on  $m\{110\}$  show the monoclinic prismatic symmetry. The crystals do not show any well-defined cleavages.

The  $\beta$ -galactose prepared in this way remains unaltered for an unlimited time even if it is not dry, provided that precautions be taken to prevent the entrance of extraneous nuclei. If, however, a paste of powdered  $\beta$ -galactose and a little water is seeded with the much less soluble  $\alpha$ -galactose, the latter form is rapidly produced with evolution of heat. The pure  $\beta$ -galactose has the following physical properties, calculated for an infinitely dilute solution at  $20^\circ$ :  $[\alpha]_D^{20} = +54.20^\circ$ ;  $[R_G]_D = 62.62^\circ$ ; molecular solution volume (see Traube, "Ueber den Raum der Atome," Stuttgart, 1899)  $V_m = 108.94$  ml.

As these values deviate not inconsiderably from those which we previously found with samples prepared according to Hudson and Janovsky, we at first thought that we were dealing with a fourth modification [*Kongl. Norske Videnskabers Selskab* (forhandl.), 1926—8, Vol. I, No. 21], but more detailed examination has convinced us that the differences in the physical properties are due to the fact that Hudson and Janovsky's preparation is not pure  $\beta$ -galactose.

*Dilatometric Measurements.*—The apparatus and procedure have already been described (*Ber.*, 1922, **55**, 3138). From our experiments (see Table I), we find that at  $t = \infty$ ,  $d_4^{20}$  is 1.038582, corresponding to  $c = 10.30032$  g./dl. The calculated positions are

TABLE I.

*Dilatometer measurements with  $\beta$ -galactose, crystallised from water.*

		Temp. of bath $20.000^\circ$ .		Expansion.	
Time (mins.).	Position of meniscus (in mm.),		Time (mins.).	Position of meniscus (in mm.),	
	obs.	calc.		obs.	calc.
0	—	—13.514	35	—2.68	—2.68
10	—6.39	—6.34	40	—2.41	—2.41
15	—4.85	—4.90	45	—2.18	—2.18
20	—4.01	—4.02	50	—1.98	—1.97
25	—3.43	—3.43	60	—1.58	—1.62
30	—3.02	—3.02	1440	0.00	0.00

derived from the equation  $\Delta = 5.100 \times 10^{-0.0083t} + 8.414 \times 10^{-0.0597t}$ . The content of the dilatometer is 75.434 ml. and the diameter of the capillary tube 1.134 mm. The pycnometer used has already been described (Riiber, *Z. Elektrochem.*, 1923, **29**, 334).

*Calculation of the solution volume of  $\beta$ -galactose (i.e., when  $t = 0$ ).* For  $c = 10.0000$  and  $t = \infty$ ,  $d_4^{20}$  is 1.037413. The displacement of the meniscus is —13.210 mm., corresponding to 13.251 ml. The



volume of the solution at  $t = \infty$  is 75.434000 ml., and at  $t = 0$ , 75.420749 ml.; hence, at  $t = 0$ ,  $d_4^{20^\circ}$  is 1.0375953 and  $c = 10.0017569$ . From this is calculated  $V_m = 109.4105$  ml. or, for infinite dilution,  $V_m = 108.9370$  ml.

TABLE II.

*Polarimetric measurements with  $\beta$ -galactose, crystallised from water.*

Values of  $\alpha_D^{20^\circ}$ ;  $l = 4$ .

Time (mins.).	Obs.	Calc.	Time (mins.).	Obs.	Calc.
0	—	22.26°	25	25.04°	25.03°
1	—	22.21	30	25.73	25.74
2	22.21°	22.20	40	26.95	26.99
3	22.22	22.22	50	27.99	28.04
4	22.28	22.28	60	28.88	28.91
5	22.30	22.32	80	30.26	30.23
10	22.84	22.84	100	31.15	31.13
15	23.51	23.53	1440	33.06	33.06
20	24.32	24.29			

*Polarimetric Measurements.*—The calculated values in Table II are obtained from the equation  $x = 33.06 - \Delta$ , where  $\Delta = 13.058 \times 10^{-0.0083t} - 2.255 \times 10^{-0.060t}$ . At  $t = \infty$ ,  $d_4^{20^\circ}$  is 1.038435, corresponding to  $c = 10.2625$ , and  $\alpha = 33.06^\circ$ . Calculated for  $c = 10.0000$ ,  $d_4^{20^\circ}$  is 1.037413,  $\alpha = 32.21^\circ$ ; and accordingly  $[\alpha]_D^{20^\circ}$  (at  $t = \infty$ ) = + 80.54°. At  $t = 0$ ,  $d_4^{20^\circ}$  is 1.037595,  $c = 10.00176$ , and  $\alpha = 21.69^\circ$ ; and therefore  $[\alpha]_D^{20^\circ}$  (at  $t = 0$ ) = + 54.21°.

TABLE III.

*Interferometer measurements with  $\beta$ -galactose, crystallised from water.*

Temp. 20°. Length of chamber = 40 mm. Old solution in the right, fresh solution in the left chamber. Decreasing  $n$ .

Time (mins.).	Number of drum divisions.			Time (mins.).	Number of drum divisions.		
	Obs.	Corr.	Calc.		Obs.	Corr.	Calc.
0	—	—	1331.07	30	1171.0	1127.98	1128.76
6	1294.2	1240.47	1240.25	40	1147.2	1106.12	1106.37
10	1257.5	1207.09	1206.57	50	1128.0	1088.43	1088.15
15	1227.0	1179.27	1178.39	60	1112.0	1073.69	1073.08
20	1203.9	1158.14	1158.21	70	1098.0	1060.76	1060.56
25	1186.7	1142.38	1142.25	1440	1031.0	998.67	998.67

*Interferometer Measurements.*—The apparatus has already been described by Löwe (*Physikal. Z.*, 1910, **9**, 1047; *Chem.-Ztg.*, 1921, 405) and by Riiber (*Ber.*, 1923, **56**, 2190). The readings on the drum of the interferometer used were not accurately proportional to the changes in the refractive index, and were therefore corrected according to an empirical curve and are shown in col. 3. The values in col. 4 are calculated from the equation  $x = 998.67 + \Delta$ , where  $\Delta = 224.68 \times 10^{-0.0080t} + 107.72 \times 10^{-0.071t}$ . From  $t = 0$  to  $t = \infty$  the displacement is  $1331.07 - 998.67 = 332.4$  drum divisions. At

$t = \infty$ ,  $d_{43}^{20}$  is 1.039493, corresponding to  $c = 10.5345$ . Accordingly the 332.4 drum divisions correspond to 315.54 drum divisions for a solution with  $c = 10.0000$ , and  $\Delta n = 315.54 \times 3.8535 \times 10^{-7} = 1215.93 \times 10^{-7}$ . For  $c = 10.0000$  and  $t = \infty$ ,  $d_{43}^{20}$  is 1.037413 and  $n = 1.3474582$ . At  $t = 0$ ,  $c = 10.0017569$ ,  $d_{43}^{20} = 1.0375953$  and  $n = 1.3475798$ . From these figures the molecular refractivity of  $\beta$ -galactose in aqueous solution of  $c = 10.000$  is  $[R_G]_D = 62.687$ , or in infinitely dilute solution,  $[R_G]_D = 62.618$ .

Previously (*Ber.*, 1926, 59, 2271) we measured the refractive index of an aqueous solution of galactose, which was in the equilibrium state, by means of a Pulfrich refractometer ("Neukonstruktion") to an accuracy of  $\pm 0.00001$ . As greater accuracy was required, Dr. E. Berner kindly determined the refractive index by means of a Hallwachs prism [compare *Kongl. Norske Videnskabers Selskab* (forhandl.), 1926—28, Vol. I, No. 22; *Z. physikal. Chem.*, 1929, 141, 91], and found the following relation between refraction and concentration :

$$n_D^{20} - v_D^{20} = 0.00145081c - 0.000000499c^2,$$

where  $v_D^{20}$  is the refractive index of water and  $c$  is the concentration of the solution (g./dl.). The sodium light was filtered so as to give a very narrow interval of high intensity; a sharp limit of total reflexion was thereby obtained, and the accuracy in the refractive index was of the order  $2 \times 10^{-7}$  (see *Ber.*, 1927, 60, 2407; *Z. physikal. Chem.*, 1929, A, 141, 100).

As will be seen, a binomial exponential equation has to be used in order to express, within the limits of experimental error, the variation of the three foregoing physical properties with time. In the case of the mutarotation experiments, it is especially to be remarked that, according to the empirical equation found for  $\Delta$ , a minimum should be found at 1.9 minutes after solution has taken place. A number of special experiments showed that there actually was a minimum of rotation at about 2 minutes. The fact that  $d\Delta/dt$  became negative at  $t = 0$  shows that the solution contains a modification which has either a lower positive rotation than the  $\beta$ -galactose ( $+ 54^\circ$ ) or a negative rotation.\*

In the case also of  $\alpha$ -galactose we have searched for a better method of preparation. The specimen used in our previous investigation was prepared in a way essentially the same as that described by Hudson and Janovsky (*J. Amer. Chem. Soc.*, 1917, 39, 1020; *Ber.*, 1926, 59, 2271), *i.e.*, by pouring a concentrated aqueous solution of galactose into boiling alcohol and letting the solution cool in the

\* The equation deduced empirically for the mutarotation of  $\beta$ -galactose by Smith and Lowry (*loc. cit.*, p. 682) has also a minimum (at  $t = 0.7$  min.) and gives at  $t = 0$  a negative differential coefficient.

presence of crystals of  $\alpha$ -galactose. In order to remove the other modifications present the crystal powder was extracted with 60% alcohol for a week. Dr. E. Berner has kindly examined under the polarisation microscope the crystals obtained by crystallisation from alcohol. They consisted mainly of badly developed tables ( $\alpha$ -galactose), which under crossed Nicols gave vivid interference colours, mixed with small quantities of well-developed prisms; the latter were recognised by microscopic examination as  $\beta$ -galactose, and their identity was confirmed by measurements of the angles.

By using the method of evaporating the solution of galactose at room temperature, crystals of  $\alpha$ -galactose only were formed if  $\alpha$ -crystals had been previously introduced. In this way a crystal paste is obtained, which on filtration gives a crystal powder consisting solely of microscopically small, well-developed tables. The physical constants found for this preparation do not deviate noticeably from those we had found for the specimen prepared by our first method.

It is of special interest to compare the constants found for  $\alpha$ - and  $\beta$ -galactose with those of the methylgalactosides, which are known in the crystalline state. The oxidation of the methylated hexoses, carried out during recent years, can of course only establish the ring system of the methylated sugars themselves. Another question then arises: which of the free modifications correspond to the methyl compounds? Only when this question has been answered can the ring system of the free sugar modifications be cleared up. We have therefore determined as accurately as possible the constants of the so-called  $\alpha$ - and  $\beta$ -methylgalactosides. A sample of the latter, prepared according to E. Fischer (*Ber.*, 1895, **28**, 1154) and recrystallised many times from alcohol, gave the following figures for an aqueous solution ( $c = 10 \cdot 10006$ ):  $d_{45}^{20} = 1 \cdot 031257$ ,  $n_D^{20} = 1 \cdot 3468516$ ,  $\alpha_D^{20} = 0^\circ$ .

The constants calculated from these figures are shown together with the corresponding values for  $\beta$ -galactose:

	$[\alpha]_D^{20}$ .	$V_m$ .	$[R_\alpha]_D$ .
$\beta$ -Methylgalactoside .....	$0^\circ$	130.88	70.20
$\beta$ -Galactose .....	+54.2	109.41	62.69
Diff. ....	-54.2	+21.47	+7.51

For comparison the corresponding values for  $\beta$ -methylglucoside and  $\beta$ -glucose are given:

$\beta$ -Methylglucoside .....	-34.2°	133.26	70.55
$\beta$ -Glucose .....	+19.3	111.65	63.07
Diff. ....	-53.5	+21.61	+7.48

The  $\beta$ -methylgalactoside is evidently related to  $\beta$ -galactose as  $\beta$ -methylglucoside is to  $\beta$ -glucose.

The sample of  $\alpha$ -methylgalactoside, purified by many recrystallisations from alcohol, gave in 10% solution the following values :

	$[\alpha]_D^{20}$ .	$V_m$ .	$[R_a]_D$ .
$\alpha$ -Methylgalactoside .....	+196.6°	130.66	69.83
$\alpha$ -Galactose .....	+145.0	109.79	62.27
Diff. ....	+ 51.6	+20.87	+7.56

The corresponding values for  $\alpha$ -methylglucoside and  $\alpha$ -glucose are

$\alpha$ -Methylglucoside .....	+158.9°	132.61	70.17
$\alpha$ -Glucose .....	+110.1	111.23	62.68
Diff. ....	+ 48.8	+21.38	+7.49

As will be seen, the difference between the solution volumes of  $\alpha$ -methylgalactoside and  $\alpha$ -galactose is unexpectedly small, *viz.*, 20.87, since by analogy it should be 21.4.

From what has been recorded above, the difference between  $\alpha$ -methylgalactoside and  $\alpha$ -galactose is normal, as far as the rotation and molecular refractivity are concerned. The unexpectedly small difference between the solution volumes would not of itself under these conditions be sufficient evidence against the conclusion that the so-called  $\alpha$ -methylgalactoside corresponds to  $\alpha$ -galactose in regard to both ring structure and configuration. One has not at present, as a matter of fact, sufficient knowledge of all the factors which affect the solution volume of a substance.

#### Summary.

(1) The curves for the alterations with time of optical rotation and of volume of aqueous solutions of galactose prove that at least three modifications are present in such solutions.

(2) By comparison of the two classes of curve, the conclusion is reached that, in the case of three modifications, A, B, and C, these curves do not correspond to the simple scheme of reaction  $A \rightleftharpoons B \rightleftharpoons C$ .

(3) Moreover, a mathematical consideration shows that the curves might be explained by means of the more complicated

scheme 
$$\begin{array}{ccc} A & \rightleftharpoons & B \\ & \swarrow \quad \searrow & \\ & C & \end{array}$$

(4) This result does not preclude the possibility that under certain conditions the presence of a fourth modification may be concealed by the experimental errors. More delicate experimental methods would be required to detect this.

(5) Whilst the physical measurements now known do not enable us to decide whether three or four modifications are present in aqueous solutions of galactose, the existence of four crystalline well-defined penta-acetates makes it probable that there exist four modi-

fications of galactose, of which only the  $\alpha$ - and  $\beta$ -forms have been isolated.

(6) Reasoning by analogy, the rotations of the two new modifications and of the two corresponding methylgalactosides are calculated.

(7) The behaviour of aqueous solutions of galactose towards Angeli's and Schiff's reagents do not support the view that such solutions contain a noticeable amount of a real oxy-aldehyde.

(8)  $\alpha$ -Galactose can be obtained in quantitative yield by evaporating an aqueous solution of galactose which is in contact with crystalline nuclei of  $\alpha$ -galactose.

(9)  $\beta$ -Galactose may be obtained in quantitative yield by evaporation of an aqueous solution of galactose if the presence of extraneous crystal nuclei is prevented. Its crystallographic data are given.

(10) The optical rotation, solution volume and refractivity of  $\beta$ -galactose thus obtained and of  $\alpha$ - and  $\beta$ -methylgalactoside have been determined.

(11) By means of the physical constants mentioned under (10) it has been shown that  $\beta$ -methylgalactoside is related to  $\beta$ -galactose in the same way as  $\beta$ -methylglucoside to  $\beta$ -glucose. On the other hand, since the solution volume of  $\alpha$ -galactose is unexpectedly large, it cannot be definitely asserted that  $\alpha$ -methylgalactoside is related to  $\alpha$ -galactose in the same way as  $\alpha$ -methylglucoside is to  $\alpha$ -glucose.

We tender our sincere thanks to Dr. E. Berner for the crystallographic examination of  $\alpha$ - and  $\beta$ -galactose and for his measurements of the refractive index of solutions of galactose.

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