[CONTRIBUTION FROM THE CARBOHYDRATE LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

CLERGET-INVERTASE HYDROLYSIS CONSTANTS OF SUCROSE AND RAFFINOSE[1]

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Recent advances in methods of sugar analysis depending upon enzymic hydrolysis emphasize the importance of the relation between the polarizations before and after enzymic action as a useful physical constant, and render apparent the need for its precise evaluation. In addition to a procedure[2] for the determination of sucrose by invertase inversion which can be operated in the same amount of time as the rapid form of the acid-inversion Clerget method, the writers have recently described a procedure[3] for simultaneous determination of sucrose and raffinose in such products as beet sugar-house liquors. The latter method depends upon the action of the enzymes invertase and melibiase.

As invertase hydrolyzes sucrose with production of invert sugar and causes hydrolytic cleavage of raffinose with production of fructose and melibiose, accurate data on the Clerget hydrolysis constants of invertase, with respect to both sucrose and raffinose, are highly desirable. Clerget invertase-inversion constants for a few sucrose concentrations have been reported by a number of investigators, but no systematic measurements covering a wide range of sucrose concentration have been made.

Previous Data on Clerget Constants

The belief that the presence of hydrochloric acid, used as a hydrolytic agent for sucrose, accounted for the variability of the Clerget constant, which would entirely disappear if invertase were employed in its place, was erroneously held until 1912, even though it was known long before that the specific rotation of fructose in pure water varies with its concentration. Tolman[4] strongly expressed this view and Hudson, in 1910,[5] apparently took it for granted and assumed that the value which he determined, namely, 131.7 for a 7% solution at 20°, held for all concentrations below a 0.5 *N* weight solution. Ogilvie,[6] in 1911, applying the yeast method of O'Sullivan and Tompson[7] to the analysis of beet molasses polarizing approximately 50° Ventzke, determined the Clerget constant

[1] Presented before the Sugar Division, at the 69th Meeting of the American Chemical Society, Baltimore, Maryland, April 6–10, 1925.

[2] Paine, *J. Assoc. Official Agr. Chem.*, **8**, 362 (1925).

[3] Paine and Balch, *Ind. Eng. Chem.*, **17**, 240 (1925).

[4] Tolman, THIS JOURNAL, **24**, 523 (1902).

[5] Hudson, *J. Ind. Eng. Chem.*, **2**, 143 (1910).

[6] Ogilvie, *J. Soc. Chem. Ind.*, **30**, 62 (1911).

[7] O'Sullivan and Tompson, *J. Chem. Soc.*, **59**, 46 (1891).

to be 131.6. These two values agree closely with those of the authors, namely, for 6.5 g. in 100 cc., our inversion constant is 131.64, and for 7 g. in 100 cc., 131.68 instead of 131.6 and 131.7, respectively, as found by Ogilvie and by Hudson.

Saillard[8] was apparently the first to disclose the fact that even with invertase hydrolysis of sucrose, the Clerget constant varies with the concentration. Saillard's[9] results are summarized in the relation, constant = 130.74 + 0.0664 c where c = sucrose concentration.

Browne,[10] in 1916, likewise pointed out the necessity of taking into consideration the concentration of sucrose present in determining the constant used in calculating the percentage of sucrose from the polariscopic readings before and after hydrolysis with invertase. No data were presented but he established the formula $S = [100\ (P - I)]/\{132 - 0.0065\ [132 - (P - I)]\}$ for 20°. His constant may also be expressed as, constant = 131.14 + 0.0660 m, where m = g. of solids in 100 cc. Jackson and Gillis,[11] in 1920, reported the value —32.00° for the negative constituent of the Clerget constant based upon the Bates and Jackson conversion factor, which is equivalent to —31.97° Ventzke, on the Herzfeld-Schönrock standardization of the sugar scale. This value was determined from results obtained with mixtures of invert sugar and acid by extrapolation to zero acid.

Sazavsky,[12] in 1924, determined the negative constituent to be —31.97° Ventzke apparently on the Herzfeld-Schönrock scale, which likewise does not agree with the authors' results nor with the results of Zerban[13] calculated from the most recent data on the specific rotation of fructose and dextrose, namely, —32.09. Zerban[14] also obtained experimentally the value 132.10 ± 0.025 at a sucrose concentration of 13.0 g. in 100 cc. Sufficient evidence was obtained to warrant the adoption of the constant 132.1 for this concentration by the Association of Official Agricultural Chemists in 1925.

Preparation and Testing of Purified Sucrose

An approximately 50% solution of the highest grade commercial granulated sugar was clarified with salt-free alumina cream and decolorizing carbon. After the addition of diatomaceous earth, it was filtered through hardened filter paper and concentrated in a vacuum to 78–80% sucrose at a temperature not exceeding 35° (usually under 32°). This sirup was slowly stirred during crystallization and after several hours the mother

[8] Saillard, Eighth International Congress of Applied Chemistry, **25**, 541 (1912); *Cir. Hebd. Synd. Fabr. Sucr. France*, **1915**.

[9] Saillard, *J. Fabr. Sucre*, **57**, No. 2 (1916).

[10] Browne, *J. Assoc. Official Agr. Chem.*, **2**, 140 (1916).

[11] Jackson and Gillis, *U. S. Bur. Standards Sci. Paper*, No. **375**, **1920**.

[12] Sazavsky, *Z. Zuckerind. Cechoslovak. Rep.*, **48**, 259 (1924).

[13] Zerban, THIS JOURNAL, **47**, 1104 (1925).

[14] Zerban, *J. Assoc. Official Agr. Chem.*, **8**, 384 (1926).

liquor was separated from the crystals by centrifuging. The sugar was washed in the centrifuge with neutral dilute alcohol and finally with neutral 95% alcohol. It was dried by exposure to the air for a day and was then placed in a low-temperature vacuum oven through which a slow current of dry air was passed.

Five lots of sucrose were prepared. Their composition is shown in Table I. Three of these lots were of nearly the same degree of purity. The percentage of ash was determined by incinerating 5 g. of sugar in a platinum dish. Copper-reducing substances were determined by a modification of the Soldaini method.

TABLE I

ANALYSIS OF SUCROSE USED FOR DETERMINING CLERGET INVERSION CONSTANTS

Preparation no.	Source and treatment	Ash, %	Invert sugar, %	Water, %	Sucrose[a] wt. in air, brass weights (corr.) g./100 cc.	Polarization[a] at 20°, °V.	Polarization[a] of 26 g. in 100 cc. at 20°, °V.
1	Highest grade commercial sucrose recrystallized once from water after treatment with carbon	0.007	<0.01	0.003	26.0068	99.980	99.954
2	Same as 1, but recrystallized twice from water	.001	< .005	.005	25.9958	99.836	99.852
3	Same as 2	.002	< .001	Not weighable	26.0410	100.042	99.884
4	Same as 2	.002	< .001	Not weighable	25.9870	99.851	99.901
5	Same as 2	.002	< .001	.018	25.9617	99.729	99.877
4	Same as No. 4, but using a different saccharimeter				26.0064	99.943	99.918
							Av. 99.898

[a] Each of these values is the average of a number of determinations.

TABLE II

PROPERTIES OF INVERTASE PREPARATIONS

Source	Solids, g./100 cc.	Ash, g./100 cc.	Polarization, °V.	Activity[a] constant, K_s
Bottom yeast[b]	6.43	Not weighable	26.65	0.40
Top yeast No. 1[b]	1.60	0.20	7.00	.09
No. 2[b]	1.58	.01	6.80	.17
No. 3[c]	0.49	.09	1.95	.014
No. 4[b]	..	..	..	.10

[a] $K_s = (1/t) \log_{10} (P - 1)/(I_t - I)$ using 100 cc. of sugar solution (containing 10 g. of sucrose) plus 10 cc. of invertase solution and inverting at 20°, where P = direct polarization = 34.96°; I = theoretical invert polarization = —11.08°; I_t = polarization of solution after time t; or $K_s = (1/t) \log_{10} 46.04/(I_t + 11.08)$.

[b] Concentrated and purified by Reynolds' method.

[c] Extracted and purified by Hudson's method.

The sucrose was transferred to weighed volumetric flasks of high-precision type and was weighed in air with brass weights which had been recently checked against standards. It was dissolved in recently-boiled distilled water, the solution being then diluted to 100 cc. at 20° according to the directions of the International Commission for Unifying Methods of Sugar Analysis.[15]

[15] Proceedings of Paris Meeting, July 24, 1900; *Z. Ver. deut. Zucker-Ind.*, 50 (N. F. 37), 357 (1900).

Polarizations were made at 20° in a 2dcm. tube, correction for tube length being made when required. In some cases polarizations were made in two saccharimeters. The saccharimeters were checked with standard quartz control plates at frequent intervals over the range of sugar scale used and found to be in excellent agreement therewith. The instruments were placed in a dark room maintained at 20–21°. Precise control of temperature was effected by rapidly pumping water from a large constant-temperature bath through the jacket of the observation tube. The source of illumination was a Mazda lamp, the light from which passed through 3 cm. of a 3% solution of potassium dichromate. The polarization of the 1 N weight solution of sucrose was determined by calculation by direct proportion from the polarization of sucrose solutions containing approximately 26 g. per 100 cc.

Determination of the Clerget Constants

The final drying and weighing of the sucrose for the determination of the inversion constants was accomplished in the manner described above. Direct polarizations were calculated from the polarization of a 1 N weight solution. The weighed sugar was dissolved in approximately 50 cc. of recently-boiled distilled water, a definite quantity of enzyme solution added, and water was added nearly to the mark on the volumetric flask. The solutions were allowed to stand overnight at about 20° before diluting to 100 cc. at 20°. After thorough mixing, the polarizations were made in a 4dcm. tube at 20°, the average of 10 to 20 settings of the saccharimeter being recorded. In many cases readings, the average of which is reported, were made by two observers. The zero point of the instrument was determined frequently by observation through the same tube filled with water.

Invert polarizations were determined at frequent intervals of concentration corresponding to sucrose concentrations ranging from 2.5 to 26.0 g. in 100 cc. The measurements made represent 44 sucrose concentrations within this range. The invert polarizations were corrected for variation in the zero point of the instrument, for deviation in tube length and for the optical activity of the enzyme solution before dividing by 2 to convert to 2dcm. tube length polarization. The Clerget inversion constant for each sucrose concentration was calculated from the relation, constant = $100\ (P - I)/S$, where P is the direct polarization, I is the corrected invert polarization and S = g. of sucrose in 100 cc./0.26. The relation of the Clerget constants to sucrose concentration in the data obtained is expressed by the linear equation constant = $131.17 + 0.073c$, where c is the number of grams of sucrose in 100 cc. The experimental data with corresponding invert polarizations and Clerget constants calculated from this equation are shown in Table III. The linear character of the variation of the Clerget constant with change in sucrose concentration is shown in Fig. 1. With a few exceptions, the calculated values agree with the observed values within the limit of error of polariscopic reading.

TABLE III

OBSERVED AND CALCULATED CLERGET INVERSION CONSTANTS

Preparation no.	Sucrose: Wt., g. per 100 cc.	Sucrose: Normal wt., %	Direct polarization[a] (calcd.), °V.	Invert polarization: Obs., °V.	Invert polarization: Calcd.,[b] °V.	Inversion constant: Obs.	Inversion constant: Calcd.[c]	Invertase preparation used
			Polarizations made with a Fric saccharimeter					
1	2.4906	9.579	9.575	− 3.025	− 3.007	131.54	131.35	Bottom yeast
	5.0070	19.258	19.249	− 6.085	− 6.083	131.55	131.54	
	9.9643	38.324	38.307	−12.263	−12.242	131.95	131.90	
	13.5176	51.991	51.967	−16.726	−16.744	132.12	132.16	
	14.9730	57.588	57.562	−18.618	−18.610	132.28	132.27	
	19.4798	74.922	74.888	−24.397	−24.459	132.52	132.60	
	19.9627	76.780	76.745	−25.134	−25.088	132.69	132.63	
	25.9584	99.840	99.794	−33.073	−33.063	133.08	133.07	
	3.9913	15.351	15.344	− 4.854	− 4.836	131.58	131.46	Top yeast No. 1
	6.9870	26.873	26.861	− 8.525	− 8.525	131.68	131.68	
	12.0049	46.173	46.152	−14.836	−14.819	132.09	132.05	
	17.0375	65.529	65.499	−21.319	−21.275	132.49	132.42	
	23.0008	88.465	88.424	−29.200	−29.102	132.96	132.85	
	6.3828	24.549	24.538	− 7.796	− 7.778	131.71	131.64	Top yeast[d] No. 3
	12.4147	47.749	47.727	−15.355	−15.340	132.11	132.08	
	12.5945	48.440	48.418	−15.596	−15.566	132.15	132.09	
2	8.4751	32.597	32.549	−10.428	−10.411	131.84	131.79	Bottom yeast
	10.9881	42.262	42.200	−13.547	−13.577	131.91	131.98	
	15.9377	61.299	61.209	−19.937	−19.914	132.38	132.34	
3	13.1248	50.480	50.422	−16.266	−16.277	132.11	132.13	Top yeast No. 2
4	5.0000	19.231	19.212	− 6.062	− 6.084	131.42	131.54	Top yeast No. 2
	6.4651	24.866	24.841	− 7.889	− 7.893	131.63	131.64	
	9.5908	36.885	36.848	−11.790	−11.792	131.86	131.87	
	10.0000	38.462	38.424	−12.295	−12.307	131.87	131.90	
	12.0000	46.153	46.107	−14.818	−14.838	132.01	132.05	
	14.0673	54.104	54.050	−17.456	−17.475	132.17	132.20	
5	11.5889	44.573	44.518	−14.318	−14.327	132.00	132.02	Top yeast No. 4
	16.5979	63.838	63.759	−20.754	−20.750	132.39	132.38	
	17.4414	67.082	66.999	−21.838	−21.851	132.43	132.45	
	21.0305	80.887	80.788	−26.550	−26.557	132.70	132.71	
	21.9729	84.511	84.407	−27.801	−27.807	132.77	132.78	
	24.9394	95.921	95.803	−31.766	−31.766	132.99	132.99	
			Polarizations made with a Peters saccharimeter					
4	6.0037	23.091	23.071	− 7.298	− 7.319	131.52	131.61	Top yeast No. 2
	7.9892	30.728	30.701	− 9.754	− 9.786	131.66	131.76	
	8.0088	30.803	30.776	− 9.808	− 9.810	131.76	131.76	
	8.9893	34.574	34.544	−11.047	−11.035	131.87	131.83	
	9.0078	34.645	34.615	−11.023	−11.058	131.73	131.83	
	17.9852	69.174	69.114	−22.543	−22.535	132.50	132.49	
	18.9920	73.046	72.983	−23.862	−23.847	132.58	132.56	
	19.0120	73.123	73.059	−23.898	−23.873	132.59	132.56	
	19.0454	73.252	73.188	−23.820	−23.915	132.43	132.56	
	21.4978	82.684	82.612	−27.121	−27.143	132.72	132.74	
	23.8988	91.918	91.839	−30.410	−30.338	133.00	132.92	
	25.9715	99.890	99.804	−33.108	−33.121	133.06	133.07	

(The average polarization error = ±0.023 °V.; if only 4 of the determinations in greatest error are discarded, the average polarization error becomes ±0.016 °V.)

[a] P = polarization of 26 g. of sucrose in 100 cc. × $S/100$.

[b] I (calcd.) = (S × const.) − P.

[c] Const. (calcd.) = 131.17 + 0.073 m.

[d] Inversion occurred at 46°.

The work of several investigators indicates that the 100° point of the saccharimeter, based on the Herzfeld-Schönrock standardization, is in error to a certain extent. This would cause an error in the evaluation of the inversion constant for 13 g. of sucrose in 100 cc. if calculated from the negative constituent plus 100, for the exact polarization of 26 g. of sucrose in 100 cc. is yet in doubt. The negative constituent, or twice the polarization of 13.6842 g. of invert sugar in 100 cc., calculated from the authors' data, is —32.22, which, when combined with the positive constituent or the average polarization, 99.90°, of 26 g. of sucrose in 100 cc. as determined by the authors, yields the value 132.12 for the complete constant at this concentration.

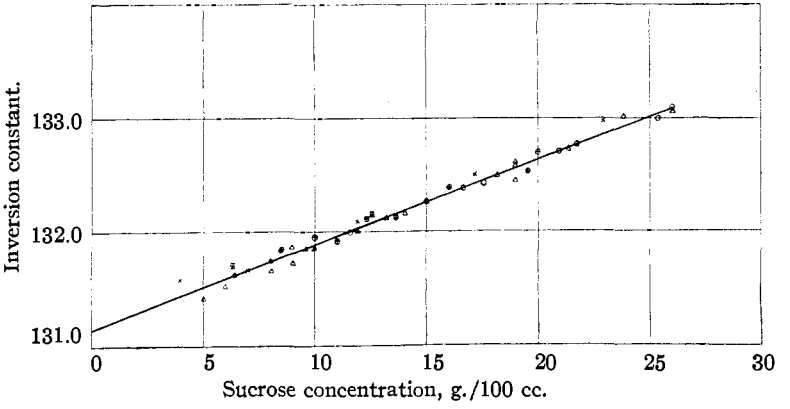

Fig. 1.—Relation between invertase-inversion constants and sucrose concentrations. ⊕ = bottom yeast invertase; × = top yeast invertase 1; △ = top yeast invertase 2; ⊞ = top yeast invertase 3; ○ = top yeast invertase 4.

It is to be noted that our concentration factor 0.0730, the amount the Clerget constant varies per unit of concentration of sugar, is somewhat higher than the value 0.0676 determined by Herzfeld[16] in 1890. Jackson and Gillis,[11] in 1920, stated that they were in agreement with Herzfeld's value of 0.0676, and likewise did Browne (1916),[10] but a recalculation of this factor for the latter's formula yields a value of only 0.0660, expressed in terms of grams of sucrose in 100 cc. From Saillard's (1916) results[9] the concentration factor is found to be 0.0664. In the cases where the data obtained by the investigators mentioned are reported in full, it is seen that the number of observations at the various concentrations is very limited, especially at concentrations above 13 g. of sucrose in 100 cc., in which range the most accurate polariscopic observations may be made. It is possible that this factor has not been evaluated as accurately as may be done from the authors' more extensive data.

The question has been raised as to whether the same Clerget constant

[16] Herzfeld, *Z. Rübenzuckerind.*, **27** (N. F.), 167 (1890).

is obtained with invertase solutions of different activity.[13] A variation might result from the fact that toward the conclusion of inversion the percentage of sucrose present decreases asymptotically to the time, thereby causing some uncertainty as to completion of inversion when invertase solutions of low activity are used. Most of our data have been obtained with invertase solutions of high activity prepared by Reynolds' ultrafiltration method.[17] However, in some cases an invertase solution of lower activity prepared according to Hudson's directions was used.

Five different invertase preparations were employed, four being obtained from top yeast and one from bottom yeast. These invertase preparations are described in Table II. Their activity values, measured by the unimolecular reaction-velocity constant k, according to our standard testing method,[18] were 0.014, 0.09, 0.10, 0.17 and 0.40. The activities of these invertase preparations under identical conditions may be regarded as proportional to the k values. Regarding the activity of the weakest invertase preparation as unity, the activities of the other preparations were approximately 6.5, 7, 12 and 29. The invertase preparation of lowest activity was prepared by Hudson's method[5] (using chloroform) and that of the highest activity by Reynolds' ultrafiltration method.[17]

These invertase preparations were used with different lots of sucrose, and the conditions also were varied by changing the temperature at which hydrolysis occurred from a minimum of about 20° to a maximum of 47°. Fig. 1 shows that the constants obtained with different invertase preparations do not in general deviate from the linear relation to any greater extent than do values obtained with the same invertase preparation.

Testing the Clerget Invertase Constants with Known Quantities of Purified Sucrose

Two procedures were employed for checking the Clerget invertase constants against known quantities of specially purified sucrose in the presence of invert sugar. These are designated as the 50–55 cc. and the 50–100 cc. procedures. Only the 50–100 cc. method will be described here. In this procedure the direct polarization of the original solution was determined after diluting 50 cc. to 100 cc. in order that the concentration of the original invert sugar may be the same during both polarizations. For inversion, 50 cc. of the original solution was transferred to a 100cc. flask to which was added 5 cc. of an invertase preparation. For maximum enzyme activity, the P_H value of the solution must be 4.3 to 4.6, that is, just distinctly acid to methyl red indicator. This was accomplished by adding a drop or two of glacial acetic acid. Inversion was allowed to take place at approximately 20° overnight. The volume

[17] Reynolds, *Ind. Eng. Chem.*, **16**, 169 (1924).

[18] "Methods of Analysis," Assoc. Official Agr. Chem., **1925**, p. 184.

was then adjusted to 100 cc. and after thorough mixing, the polarization was determined in a 4dcm. tube. The polarization was corrected for the optical activity of the enzyme solution, and the percentage of sucrose was calculated by the formula $S = [(P - I)\ 100]/(131.17 + 0.073\ m) = [(P - I)\ 100]/[132.12 + 0.073\ (m - 13)]$, where P = direct polarization, I = invert polarization and m = g. of solids in 100 cc. of solution polarized after inversion.[18] The data obtained are given in Table IV.

TABLE IV

DETERMINATION OF SUCROSE IN INVERT SUGAR-SUCROSE MIXTURES BY THE 50–100 CC. PROCEDURE

Sucrose, g. per 100 cc.	Invert sugar, g. per 100 cc.	Direct polarization, 50 cc. dil. to 100 cc., 4dcm. tube 20° (*P*) °V.	Invert polarization (*I*), °V.	Sucrose Found, %[b]	Sucrose Taken, %	Deviation, %
24.700	0.13	94.70	−30.70	94.96	95.00	−0.04
			−30.75			
22.100	0.26	84.64	−27.55	85.00	85.00	± .00
			−27.54			
19.500	1.30	73.40	−25.48	74.95	75.00	− .05
			−25.49			
19.500	1.30	73.58	−25.30	74.95	75.00	− .05
			−25.30			
15.639	..	60.12[a]	−19.16	60.18	60.15	+ .03
15.600	2.60	57.00	−22.09	59.99	60.00	− .01
			−22.09			
13.000	5.20	44.00	−21.96	50.03	50.00	+ .03
			−21.95			
13.000	5.20	44.14	−21.72	49.95	50.00	− .05
			−21.71			
7.800	7.80	21.01	−18.45	29.95	30.00	− .05
			−18.45			
6.757	..	25.96[a]	− 8.19	25.99	25.99	± .00
						Av. − .019

[a] Measured in a 2dcm. tube without diluting.

[b] $S = [(P - I)100]/(131.17 + 0.073\ m)$ or $[(P - I)100]/[132.12 + 0.073\ (m - 13)]$.

Ratio of Polarization of Raffinose after Invertase Hydrolysis to Original Polarization

Two preparations of raffinose which had been carefully purified and contained close to 15.15% of water as required for raffinose pentahydrate were used for determining this value. In determining direct polarizations, quantities of raffinose were transferred to 100cc. volumetric flasks and weighed in air with brass weights. Solution was effected with recently-boiled distilled water and the volume was adjusted to 100 cc. at 20°. The polarization was determined directly in 2dcm. and 4dcm. tubes after diluting 25 cc. to 50 cc. The average of the two polarizations was taken

as the true value. Very close agreement indicated that the specific rotation of raffinose does not materially change with concentration, which substantiates the observations of other investigators.

The procedure for the determination of the polarization ratio was similar to the foregoing. Hydrolysis was accomplished by an invertase solution prepared from top yeast, which was tested in order to make certain that it contained no melibiase. The data on these measurements are shown in Table V. The values obtained, which included several determinations for each lot of raffinose, showed acceptable agreement, the average for this ratio being 0.521. Data obtained for concentrations of raffinose corresponding to direct polarizations varying from a minimum of 17.43° to a maximum of 63.20° Ventzke in a 2dcm. tube at 20° under otherwise identical conditions showed only an extremely small variation in the value of the ratio with variation in initial concentration of raffinose, and the value 0.521 may be accepted as applying to the entire range mentioned. This value is a little higher than the value 0.514 found by Browne and Gamble[19] for acid hydrolysis of raffinose with formation of levulose and melibiose.

TABLE V

RATIO OF POLARIZATION OF RAFFINOSE AFTER INVERTASE HYDROLYSIS TO THE ORIGINAL POLARIZATION

Raffinose preparation no.	Water, %	Ash, %	Specific rotation, $[\alpha]_{20}$ (uncorr.)	Direct polarn., P, 2dcm. tube °V.	Invert polarn., 2dcm. tube (corr.) °V.	Polarization ratio, A/P
1	15.15	0.03	104.72	30.147	15.749	0.5224
				24.257	12.652	.5216
				17.425	9.109	.5227
					Av.	.5222
2	15.10	.02	104.70	27.272	14.187	.5194
				42.583	22.119	.5202
				63.200	32.874	.5202
					Av.	.5199
					Grand av.	.521

In the procedure which we have developed for the simultaneous determination of sucrose and raffinose the use of the value 0.521 instead of 0.514 for the polarization ratio for raffinose will result in the formula $S = (P - 2.202A + 1.202B)/1.3212$ instead of $S = (P - 2.219A + 1.219B)/1.3213$ (as orginally given), where S = per cent. of sucrose and P = direct polarization, and A and B denote the polarizations after hydrolysis by invertase and invertase plus melibiase, respectively. The general formula thus becomes $S = [(P - 2.202A + 1.202B)\ 100]/-\{132.12 - 0.00718\ [132.12 - (P - 2.202A + 1.202B)]\}$.

[19] Browne and Gamble, *J. Ind. Eng. Chem.*, **13**, 793 (1921).

Summary

The Clerget-invertase hydrolysis constant of sucrose was determined. The relation between sucrose concentration and this constant is expressed by the equation, constant = 131.17 + 0.073 c, where c is the number of g. of sucrose in 100 cc.

The value 0.521, which shows negligible variation over a considerable range of concentration, was found for the ratio of polarization of raffinose after invertase hydrolysis to the original polarization. The above values are applicable in enzymic analytical methods for the determination of sucrose and of sucrose and raffinose in mixture.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF SWARTHMORE COLLEGE]

ACTION OF ANHYDROUS ALUMINUM CHLORIDE ON CRESYL BENZOATES

BY EDWARD H. COX

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The action of anhydrous aluminum chloride on monohydric phenolic esters (the Fries reaction) as a method for preparing aromatic hydroxy ketones has been successfully applied.[1] Thus far, the method has been almost entirely limited to the action of aluminum chloride on the fatty acid esters of phenols. The yields of the resulting products, acyl phenols, have been variously reported as from poor to excellent. The present work deals with the action of aluminum chloride on the benzoic acid esters of the cresols as a method for preparing benzoyl cresols.

That this method possesses advantages over that of Friedel and Crafts for the preparation of benzoyl cresols there seems little doubt, from a review of the literature. The reaction of benzoyl chloride with phenols in the presence of anhydrous aluminum chloride often fails altogether or results in low yields of the benzoyl phenols,[1a,2] unless the hydroxyl group has been previously protected or the reaction carried out in nitrobenzene. On the other hand, benzoyl cresols are produced in excellent yields by the action of aluminum chloride directly on the cresyl benzoates.

The procedure for the conversion of each cresyl benzoate into its respective benzoyl cresol is described, but since they are all known and found in Beilstein,[3] only a tabular review of them is given. Although the structure of one of the benzoyl cresols has been assumed, their constitutional

[1] (a) Fries and Finck, *Ber.*, **41**, 4276 (1908). (b) Fries and Pfaffendorf, *Ber.*, **43**, 214 (1910). (c) Auwers, *Ber.*, **47**, 3319 (1914); **49**, 813 (1916); (d) *Ann.*, **421**, 36 (1920); **447**, 162 (1926). (e) Fries and co-workers, *Ber.*, **54**, 717 (1921); **56**, 1304 (1923).

[2] Heller, *Ber.*, **46**, 1498 (1913).

[3] Beilstein, "Handbuch der organischen Chemie," Julius Springer, Berlin, **1925**, 4th ed., vol. 8.