For normal butane: $\log P = -\frac{1224.5}{T} + 7.3948$.

Heats of Evaporation of Propane, Propylene and Normal Butane.

The heats of evaporation over the temperature range studied (calories per gram molecule) were calculated from the Clausius-Clapeyron equation:

$$Q = \frac{(d \ln p)\, RT^2}{dT}$$

By integrating this equation and assuming that Q is a constant, one obtains:

$$\ln p = -\frac{Q}{RT} + \text{const.}$$

Using the values 1006.1, 983.7 and 1224.5 in the above equation and from common to natural logarithms, one finds:

For propylene: $Q = (1006.1 \times 4.571) = 4599$ calories.

For propane: $Q = (983.7 \times 4.571) = 4496$ calories.

For normal butane: $Q = (1224.5 \times 4.571) = 5597$ calories.

Summary.

The vapor pressures of propane, propylene and *N*-butane at low temperatures are shown. For propane the vapor pressure ranges from 760 mm. at —44.1° to 3 mm. at —124.2°. For propylene the vapor pressure ranges from 760 mm. at —47.8° to 3 mm. at —127.4°. For *N*-butane the vapor pressure ranges from 760 mm. at —0.3° to 1 mm. at —99.9°

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THE ESTIMATION OF RAFFINOSE BY ENZYMOTIC HYDROLYSIS.

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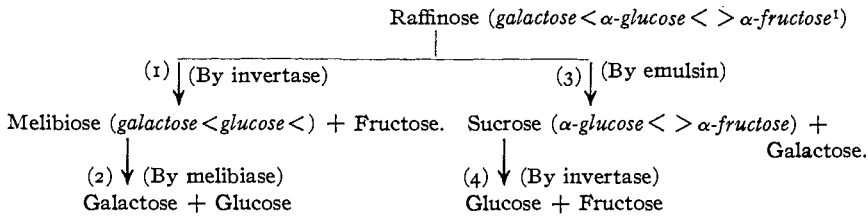
In a recent article[2] we have called attention to the fact that it is possible to prepare from top-fermentation yeast by a specified procedure an extract which is rich in the enzyme invertase but contains no melibiase, while bottom-fermentation yeast yields an extract which contains both enzymes. Each extract hydrolyzes sucrose to invert sugar, and each also has a hydrolyzing action upon raffinose, but the top yeast extract produces melibiose and fructose from it, while the bottom yeast extract converts it to galactose, glucose and fructose. These relations are better understood from the accompanying diagram which indicates the various steps in the hydrolysis of raffinose by enzymes.

[1] Contribution from the Carbohydrate Laboratory, Bureau of Chemistry, United States Department of Agriculture.

[2] THIS JOURNAL, **36**, 1570 (1914).



The action upon raffinose of that mixture of enzymes from almonds which is called emulsin is a peculiar one. Neuberg[2] has shown that the products of hydrolysis are sucrose and galactose, which he isolated in crystalline condition. He considers that the splitting takes place in this manner because of the presence of a melibiase in emulsin and the absence of an invertase. It is to be emphasized that the partial hydrolyses represented as (1) and (3), which lead to the disaccharides melibiose and sucrose, can be carried to completion by use of the appropriate enzymes without any occurrence of the splittings represented by (2) and (4), respectively. It is indeed remarkable that either of the glucosidic unions in raffinose may be completely and accurately hydrolyzed by its specific enzyme without any detectable action upon the other union. Such very specific action is not observed if the catalyst of the hydrolysis is an acid but rather it is found that both unions are attacked, though the glucose-fructose linkage is split far more rapidly than the other.

When it is sought to base upon these properties of raffinose a method for its quantitative estimation in the presence of other sugars, preference must be given to the hydrolysis by enzymes rather than by acids. On first consideration it might appear possible to estimate raffinose in a complex mixture of sugars, such as are obtained in the extraction of most plants, by measuring the hydrolytic change which emulsin produces, but it is to be remembered that emulsin contains, in addition to melibiase, other enzymes which hydrolyze many of the naturally occurring glucosides. This source of uncertainty makes the method of little value unless it is possible to know that no glucosides are present in the material under examination. Neuberg has proposed this method and has found it useful in estimating the percentage of raffinose in mixtures of sucrose and raffinose such as low-grade beet sugars. Bourquelot and Bridel[3] have proposed the use of invertase, followed by that of emulsin, as a useful indication of the presence of raffinose, but here again the action of emulsin upon glucosides introduces an uncertainty. The method which we have developed for the determination of raffinose depends, briefly

[1] The symbol < indicates the carbonyl group of the glucosidic linkage. See THIS JOURNAL, 31, 661 (1909).

[2] *Biochem. Z.*, 3, 526 (1907).

[3] *Compt. rend.*, 147, 361 (1909).

stated, upon the measurement of step (2) in the enzymotic hydrolysis (see diagram). This is indeed a measurement of the cleavage of the same union which Neuberg, and also Bourquelot and Bridel, have selected, but there is an important difference between the methods because the melibiase which we employ, prepared from bottom yeast, is free from enzymes that hydrolyze glucosides. Tests have also shown that the melibiase solution does not contain maltase, cellase, lactase, trehalase, inulase or diastase. It does contain a very powerful invertase and so far it has not been possible to remove this enzyme. But removal is not necessary since top yeast yields an extract which is rich in invertase and quite free from melibiase, and this top yeast extract can be used to complete step (1), whereupon an accurate measurement of step (2), and the consequent estimation of raffinose from it, may be carried out. The only interfering substance of which we are aware is melibiose itself,[1] which would be estimated as raffinose by the method. Since melibiose reduces Fehling's solution and raffinose does not, it is possible to exclude it from consideration in some cases. Thus an aqueous extract from cottonseed, or its meal, contains almost no reducing sugar but much raffinose; and low-grade beet sugars are nearly free of reducing substances but often contain raffinose. The method appears sufficiently conclusive, therefore, for the estimation of raffinose in these products which are the best-known sources of the sugar. In the analysis of plant material and sugar mixtures in general, the method serves for the determination of raffinose and melibiose but does not distinguish them.

Directions for Performing the Analysis.—The solution in which raffinose is to be estimated is first clarified, if necessary, with neutral lead acetate and the excess of lead removed as oxalate or sulfide. It is advisable to dilute the solution, if necessary, so that the total sugars are not over 13%, because the enzymotic hydrolysis becomes very slow at higher sugar concentrations. The solution must be slightly acid to permit the action of the enzymes, but any free mineral acidity is to be avoided. It is recommended, in general, that solutions be accurately neutralized and subsequently acidified with one or two drops of glacial acetic acid per 100 cc. To 95 cc. of the sugar solution which has been prepared in the manner specified, 5 cc. of top yeast invertase solution, prepared as directed (see first reference), are added, a few cc. of toluene are shaken with the mixture to prevent the growth of microörganisms and it is kept at room temperature until its rotation becomes constant. The time which is required will depend primarily upon the activity of the enzyme solution, but as a rule constant rotation is reached over night or in twenty-four hours. In

[1] It may be found that stachyose contains a union which is hydrolyzed by melibiase, since Bourquelot and Bridel (*loc. cit.*) have observed its hydrolysis by emulsin. Up to the present we have not been able to secure any stachyose for the necessary experiments.

the solution at this stage all sucrose has been inverted, and all raffinose has been hydrolyzed to melibiose and fructose by the invertase. The next step consists in hydrolyzing the melibiose with melibiase and measuring accurately the accompanying change in rotation. The rotation of the solution should be accurately determined and since it may now contain considerable fructose its temperature must be carefully controlled for the polariscopic observation. It is recommended that all readings be made at 20°, and the data of this article were obtained at this temperature. There is now added to 95 cc. of the solution which has been hydrolyzed by invertase, 5 cc. of bottom yeast extract, prepared as directed, and the rotation is read immediately after mixing. It should correspond to the rotation that may be calculated from those of the bottom yeast extract and the solution to which it was added, since the reading is made before there has been sufficient time for the hydrolysis of melibiose to proceed to a measurable extent. The solution should be preserved with toluene, kept at room temperature and its reading measured from day to day. A change of rotation in the levo direction indicates the hydrolysis of melibiose. The specific rotation of this sugar is $+143°$[1] and since one gram of it yields on hydrolysis 0.527 g. glucose and the same quantity of galactose, which have the specific rotations $+52.5°$ and $+81°$, respectively, the specific rotation of melibiose changes from $+143°$ to $+70.4°$ on hydrolysis. If the solutions are read in a 2 dcm. tube in a saccharimeter, a solution containing 1 g. of melibiose per 100 cc. will change in rotation during hydrolysis 4.18 degrees Ventzke, as may be calculated from the above data, using the relation that one degree Ventzke equals 0.347[2] angular degrees. Each degree Ventzke change of rotation indicates, therefore, 0.239 g. melibiose per 100 cc. in the solution as finally constituted, a value which corresponds to 0.352 g. anhydrous raffinose. The percentage of raffinose may be thus determined.

The method requires careful manipulation and several days' waiting, and it is advisable to determine occasionally the activity of the enzyme solutions, but the care which must be exercised in carrying out all details is fully repaid by the accuracy and certainty of the final results.

Analyses of Mixtures of Sucrose and Raffinose.—Thirteen solutions were prepared containing known amounts of sucrose and raffinose, the total sugar strength being in each case near 13 g. per 100 cc. solution. In Table I are recorded the rotations of the original solutions (Column 6), the rotations after inversion by invertase from top yeast (Column 7), correction having been made for the rotation of the enzyme solution and for dilution, and the rotations after the subsequent hydrolysis with bottom yeast extract containing melibiase (Column 8), with similar corrections.

[1] Bau, *Chem. Z.*, **26**, 69 (1902).

[2] Browne, "Handbook of Sugar Analysis," p. 145 (1912).

TABLE I.—ESTIMATION OF RAFFINOSE IN THE PRESENCE OF SUCROSE.

	Sugars in 100 cc. solution (grams).				Polarizations (corrected).		
		Raffinose.					
Number.	Sucrose added.	Added.	Found (H. & H.).	Found (Creydt).	Original.	After first hydrolysis.	After second hydrolysis.
1	12.98	0.032	0.042	0.047	+50.12	—16.09	—16.21
Duplicate	12.98	0.032	0.039	0.047	+50.12	—16.09	—16.20
2	12.94	0.065	0.049	0.070	+50.19	—15.97	—16.11
Duplicate	12.94	0.065	0.060	0.072	+50.16	—15.95	—16.12
3	12.88	0.130	0.127	0.148	+50.39	—15.57	—15.93
Duplicate	12.88	0.130	0.144	0.148	+50.40	—15.57	—15.98
4	12.75	0.260	0.239	0.268	+50.87	—15.01	—15.69
5	12.62	0.390	0.363	0.403	+51.25	—14.33	—15.36
6	12.35	0.649	0.648	0.675	+52.16	—13.00	—14.84
7	11.71	1.30	1.27	1.26	+53.25	— 9.85	—13.46
8	11.05	1.95	1.89	1.97	+56.31	— 6.64	—12.02
9	10.40	2.59	2.48	2.60	+58.45	— 3.56	—10.61
10	9.10	3.89	3.72	3.89	+62.65	+ 2.81	— 7.75

The top yeast extract read +4.97° in a 2 dcm. length, the bottom yeast extract +2.56°. The tube length was 2 dcm. for all the readings, the temperature was accurately 20°, and the rotations refer to degrees Ventzke. Column 4 is calculated from the polarization differences between the last two columns, as has been described. By comparison of Columns 3 and 4, it will be seen that the estimation of raffinose is quite satisfactory, and that it appears possible to determine raffinose with fair accuracy even in case 100 parts of sucrose are mixed with only 0.5 part of raffinose.

The estimation of raffinose in the presence of sucrose may also be made according to the method of Creydt[1] from a knowledge of the polarizations before and after the first hydrolysis. The fifth column of the table records the amounts of raffinose which may be calculated from the Herzfeld formula of the Creydt method, $R = \frac{0.3266\ P + P'}{1.554}$, in which R is the percentage of raffinose, P the direct polarization of the normal weight (26 g. per 100 cc. solution), and P′ the polarization after the first hydrolysis. The agreement with the known values may be somewhat better by the Creydt method than by the procedure which we propose, but it is to be remembered that the Creydt method is not applicable in case any optically active substances other than sucrose and raffinose are present. On the other hand, there is apparently no reason for supposing that the estimation of raffinose in the presence of any other sugars

[1] *Z. Ver. d. Zuckerind.*, **37**, 153 (1887). For a full discussion of this method, see C. A. Browne's "Handbook of Sugar Analysis," 1912 edition, p. 282. The authors express their thanks to Dr. Browne for calling their attention to the fact that Creydt's method, with Herzfeld's modification, is quite applicable to the data recorded in the table, although that method presupposes an inversion of the sucrose and raffinose by acid hydrolysis, and the data result from enzymotic hydrolysis.

now known, excepting melibiose and possibly stachyose, cannot be carried out by the method of a double enzymotic hydrolysis with as much accuracy as has been obtained for the sucrose and raffinose mixtures. In the experiments given in Table II this conclusion is tested.

TABLE II.—THE ESTIMATION OF RAFFINOSE IN THE PRESENCE OF SUCROSE AND OTHER SUGARS.

	Sugars in 100 cc. solution (grams).				Polarizations (corr.).		
		Raffinose.					
No. Expt.	Sucrose added.	Added.	Found.	Other sugars.	Original.	After first hydrolysis.	After second hydrolysis.
1	3.00	None	None	None	+11.53	— 3.69	— 3.69
2	None	3.00	2.95	None	+21.42	+11.04	+ 2.65
3	1.50	1.50	1.49	None	+16.47	+ 3.68	— 0.56
4	1.50	1.50	1.53	1.50 glucose	+21.21	+ 8.39	+ 4.05
5	1.50	1.50	1.50	1.50 fructose	+ 8.25	— 4.64	— 8.91
6	1.50	1.50	1.46	1.50 invert	+14.05	+ 1.96	— 2.18
7	1.50	1.50	1.45	1.50 lactose	+21.07	+ 8.28	+ 4.16
8	1.50	1.50	1.53	1.50 maltose	+27.59	+14.77	+10.42
9	1.50	1.50	1.46	0.75 invert 0.75 maltose	+21.30	+ 8.46	+ 4.32
10	1.50	1.50	1.46	0.75 invert 0.75 lactose	+18.00	+ 5.16	+ 1.00
11	1.50	1.50	1.50	1.50 trehalose	+31.43	+18.60	+14.35
12	1.50	1.50	1.52	1.50 cellose	+19.46	+ 6.61	+ 2.27

Table II records the data on the estimation of raffinose in admixture with sucrose and glucose, fructose, invert sugar, lactose, maltose, cellose or trehalose. A comparison of the third and fourth columns indicates that the accuracy of the method is quite sufficient.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY.]

STUDIES ON THE CULTURE MEDIA EMPLOYED FOR THE BACTERIOLOGICAL EXAMINATION OF WATER.

III. THE COMPOSITION OF THE GASES FORMED IN LACTOSE-PEPTONE FERMENTATION TUBES.

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In our second paper we have shown[1] that when lactose-peptone media are inoculated with the mixed flora of sewage or with bacteria from the feces of man or animals, the volumes of the gases formed by the fermentative action of these microörganisms are proportional to the amount of peptone, meat or liver present in the media inoculated.

Since in routine water examinations the "gas ratios" are generally ascertained for the purpose of record and diagnosis, it became essential to learn whether the composition of the gases was affected by concentration changes in the media, as well as the total gas volumes.

[1] THIS JOURNAL, 37, 1949 (1915).