PHYSICAL CONSTANTS AND ESTIMATION OF STEREOISOMERIC 2,3-BUTANEDIOLS¹

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Abstract

The refractive index - temperature relation for optically active and racemic diols is linear, with $n_{\rm D}^{t^*} = 1.4310 - 0.00036$ (t - 25) from 25° to 40°C.: for the meso-isomer $n_{\rm D}^t = 1.4372 - 0.00034(t - 25)$ from 25° to 50°C. The specific rotation $[a]_D^{25} \cong \pm 13.2^\circ$. Measurements of n_D^l and a_D^l in suitably purified mixtures of stereoisomers allow calculation of the percentage of each isomer present. Tests of the method on known mixtures of all three stereoisomers show an experimental error of $\pm 3\%$. Analyses of five diol samples from pilot plant fermentations of beet molasses by Aerobacter aerogenes reveal 65-87% meso-, from 2 to 16% dextro-, and from 0 to 33% racemic 2,3-butanediol.

Refractive indices from 1.4306 to 1.4322 at 25°C. have been reported for optically active and racemic 2,3-butanediols. Wide variations also occur in the recorded values of specific rotations and densities as shown in Table I. Specific rotations for the *levo*-isomer reportedly range from -12.85° to -13.34° . The present investigation aims to establish more accurate values for these constants and to develop a simple physical method, based on their use, for rapid estimation of isomeric ratios.

Two methods for the estimation of isomers in mixtures of isomeric 2,3butanediols have been described in the literature. Tipson (16) successfully used the procedure of conversion to isopropylidene derivatives followed by fractional distillation. The meso-derivative was readily separated in this way. Robertson and Neish (13) described a method in which the isomers in a 1 gm. sample were converted to their di-p-nitrobenzoates by a modified Schotten-Baumann reaction, dissolved in chloroform, measured polarimetrically to determine the concentration of optically active isomers, and fractionally crystallized for estimation of relative amounts of dl- and meso-esters. Four to six crops of crystals provided 90 to 95% recovery, from which the different components were separated by hand and weighed. This method has the unique advantage of simultaneously identifying the isomers, although several days are required for a determination. A simple procedure is described in the present communication which requires only 0.5 gm. of suitably purified diol, is accurate to $\pm 3\%$, and may be completed within half an hour.

The variations noted in the reported values of physical constants have probably arisen from measurements on impure or mixed isomers. Removal of acids, esters, ketones, and ketals as well as prevention of isomerization and absorption of water comprise the main difficulties in obtaining the pure com-

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TABLE I

PREVIOUSLY REPORTED PHYSICAL CONSTANTS OF STEREOISOMERIC 2,3-BUTANEDIOLS

Isomer	levo-	Ref.	dextro-	Ref.	racemic	Ref.	meso-	Ref.
	$n_{\rm D}^{25} = 1.4307$	19	$n_{\rm D}^{25} = 1.4306$	7	$n_{\rm D}^{25} = 1.4310$	11	$n_{\rm D}^{25} = 1.4364$	14
	$n_{\rm D}^{25} = 1.4308$	3					$n_{\rm D}^{18} = 1.4387^*$	8
Refractive	$n_{\rm D}^{25} = 1.4309$	7					$n_{\rm D}^{25} = 1.4384^*$	19
index	$n_{\rm D}^{25} = 1.4310$	22, 20					$n_{\rm D}^{35} = 1.4324$	7
	$n_{\rm D}^{26} = 1.4318$	9					$n_{\rm D}^{35} = 1.4325$	10
	$n_{\rm D}^{18} = 1.4340$	8		1)	1
	$[a]^{23} = -12.85$	8			$[a]^{26} = 0.000$	10	$[a]_{2}^{20} = +0.80^{*}$	3
Specific rotation	$[a]_{p}^{25} = -13.0$	19					$[a]_{p}^{25} = +1.0$ *	19
	$[a]_{\rm p}^{20} = -13.1$	3					$[\alpha]_{p}^{25} = +1.06^{*}$	8
	$[a]_{p}^{25} = -13.17$	5	,					
	$[a]_{\rm p} = -13.19$	7						
($[\alpha]_{D}^{26} = -13.34$	10						I
	-		WE 0.00 70	-	,		20	
	$d_{20}^{20} = 0.992(2)$	3	$d^{25} = 0.9872$	7			$d^{20} = 1.0033$]4
Density and specific gravity	$d_4^{25} = 0.9873$	3					$d^{20} = 1.004$	10
	$d^{25} = 0.9069$	7					$d^{25} = 0.990$	8
	$d_{15}^{26} = 0.9880$	9					$d^{25} = 0.9939$	7
	$d_{30}^{30} = 0.983(8)$	3						
• •	$d_{40}^{40} = 0.9750$	3						

*Aerobacter diol.

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pounds. The preparation of stereoisomeric diols of high purity is described in the experimental section. All purified samples gave negative tests for acetyl groups (2), esters (4), ketones, and ketals (6). Water contents, estimated by the Karl Fischer method (15) did not exceed 0.046%. Accurately determined physical constants of the isomers are reported in Table II.

Close agreement between the refractive indices of optically active and *racemic* diols prepared by different methods (Table II) is evidence for their accuracy to the fourth place. The value of n_D^{25} for the *dextro-*, *levo-*, and *racemic* forms is therefore 1.4310. The refractive index of *meso*-diol at 25°C. was obtained by direct reading on the supercooled liquid. The same value (± 0.0001) is obtained (a) by extrapolation of readings on a series of *meso-dl*-

TABLE II

Physical constants of	STEREOISOMERIC 2,3-BUTANEDIOLS
FOUND IN THE	PRESENT INVESTIGATION

Loomor		levo-		race			
isomer	Sample A	Sample B	Sample C Sample A		Sample B	111050-	
Water, %	0.046	0.028		0.040		0.042	
Refractive index $n_{\rm D}^{25}$	1.43095	1.43100	1.4310	1.43109	1.43095	1.43719	
Optical rotation a_D^{25} (1 dm.)	-12.75.	-12.73	-12.99	-0.005	0.00	0.00	
Specific rotation $[\alpha]_{D}^{25}$	-12.92	-12.90	-13.16	-0.005	0.00	0.00	
Density d_4^{25}	0.9873						

and *meso-levo*- mixtures (Fig. 1) as well as (b) by extrapolation of a series of readings on the pure diastereoisomer at temperatures above the melting point (Fig. 2). The value of $n_{\rm D}^{25}$ for *meso*-diol is therefore 1.4372 ± 0.0001 .

Changes in refractive index with temperature have not previously been determined. Values of n_D^t for the *racemic*, *levo*-, and *meso*-forms over the range from 25° to 50°C. are given in Table III. These readings are plotted versus



FIG. 1. Refractive index vs. per cent meso-2,3-butanediol in meso-levo-mixtures (open circles) and meso-dl-mixtures (solid circles).



FIG. 2. The temperature - refractive index relation for racemic, levo-, and meso-2,3-butanediols.

temperature in Fig. 2 and show strictly linear relations. Conversion coefficients for the refractive indices of (a) *levo-* and *racemic* forms from 25° to 40°C. and (b) the *meso-*isomer from 25° to 50°C. are therefore -0.00036 and -0.00034 respectively per degree Centigrade rise in temperature.

The specific rotation $[a]_{D}^{25} = -12.92^{\circ}$, obtained for the first supposedly pure sample (A, Table II) of *levo*-diol was viewed with suspicion. The experience of other investigators indicated that this value was probably too low (see Table I). Accordingly the entire procedure of preparation and purification was repeated, with care being taken to prevent metal contamination and isomerization (21). Polarimetric measurement of the second sample (B, Table II) merely served to confirm the value obtained for the specific rotation of Sample A. A third sample, prepared with special care from an-

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REFRACTIVE INDICES OF ISOMERS AT VARIOUS TEMPERATURES

Temp., °C.	levo-	racemic	meso-
25 - 30	$1.4310 \\ 1.4293$	$\begin{array}{c}1.4310\\1.4293\end{array}$	1.4372
35 40 45	1.4256	1.4256	1.4339 1.4323 1.4202
45 50	1.4220	1.4220	1.4303 1.4287

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other fermentation of wheat by *B. polymyxa* (23) had $n_D^{25} = 1.4310$, $[a]_D^{25} = -13.16^\circ$. In view of the fact that the refractive indices of these three samples are almost identical it seems probable that *B. polymyxa* produces a small variable amount of the *dextro*-isomer and that the true specific rotation is nearer -13.16° (9). The recent work of Garner and Lucas (5) gives $[a]_D^{25} = -13.17^\circ$ for the *levo*-isomer. Although this constant cannot yet be accepted as established, any error which may remain in its measurement is too small to effect a variation of more than $\pm 2\%$ in the results of a physical method for the estimation of isomeric ratios.



FIG. 3. Relation between optical rotation and per cent levo-2,3-butanediol in meso-levo-mixtures.

The density of the pure *levo*-isomer was stated by Clendenning (3) to be $d_4^{25} = 0.9873$. Repetition of this measurement using Sample A of *levo*-diol exactly confirmed the previous determination as shown in Table II. Moreover, the value reported for the density of *dextro*-diol is almost identical (see Table I), so that $d_4^{25} = 0.9873$ was used in calculations of the specific rotation.

The refractive indices of a series of *meso-levo-* and *meso-dl-* mixtures are given in Tables IV and V respectively. These values are plotted in Fig. 1 and show a linear relation between concentration and refractive index at 25°C. The weight per cent of *meso-2,3-*butanediol in a mixture of stereoisomers whose D-line refractive index at 25.0°C. = x may therefore be calculated from the expression,

$$\% \text{ meso-} = \frac{x - 1.4310 \times 100}{0.0062}$$
.

The comparatively narrow gap of 0.0062 divisions between the refractive indices at 25°C. of the internally compensated and other isomers makes

TABLE IV

REFRACTIVE INDICES OF meso-levo-MIXTURES

No.	levo-, gm.	meso-, gm.	levo-, %	meso-, %	$n_{\rm D}^{25}$
1 2 3 4 5 6 7 8 9	$\begin{array}{c} 100.\ 0\\ 3.\ 1350\\ 0.\ 8799\\ 2.\ 3342\\ 0.\ 8919\\ 0.\ 6443\\ 2.\ 0422\\ 1.\ 6279\\ 1.\ 1632\\ 0.\ 1062\end{array}$	$\begin{array}{c} 0.\ 6471\\ 0.\ 2088\\ 1.\ 4113\\ 0.\ 6263\\ 0.\ 4552\\ 1.\ 8016\\ 2.\ 1102\\ 2.\ 8177\\ 0.\ 7807\end{array}$	$100. 0 \\ 82. 9 \\ 80. 8 \\ 62. 3 \\ 58. 7 \\ 58. 6 \\ 53. 1 \\ 43. 5 \\ 29. 2 \\ 10. 0 \\ 10. 0 \\ 100 \\$	$\begin{array}{c} 0.0\\ 17.1\\ 19.2\\ 37.7\\ 41.3\\ 41.4\\ 46.9\\ 56.5\\ 70.8\\ 80.1 \end{array}$	$\begin{array}{c} 1.4310\\ 1.4320\\ 1.4322\\ 1.4334\\ 1.4336\\ 1.4336\\ 1.4336\\ 1.4339\\ 1.4345\\ 1.4355\\ 1.455\\ 1.455\\ 1.455\\ 1.455\\ 1.455\\ 1.455\\ 1.455\\ 1.$
10 11 12	0. 1903 0. 0518 0. 0	0.8393 100.0	5.8 0.0	94.2 100.0	$ \begin{array}{r} 1.4301 \\ 1.4368 \\ 1.4372 \end{array} $

TABLE V

REFRACTIVE INDICES OF meso-dl-mixtures

No.	dl-, gm.	meso-, gm.	dl-, %	meso-, %	n _D ²⁵
$ \begin{array}{c} 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7 \end{array} $	$\begin{array}{c} 100.\ 0\\ 1.\ 0625\\ 0.\ 5471\\ 0.\ 4811\\ 0.\ 3326\\ 0.\ 3114\\ 0.\ 0\end{array}$	$\begin{array}{c} 0.0\\ 0.2934\\ 0.3432\\ 0.4353\\ 0.5200\\ 0.9676\\ 100.0 \end{array}$	$100. 0 \\78.4 \\61.5 \\52.5 \\39.0 \\24.4 \\0.0$	$\begin{array}{c} 0.0\\ 21.6\\ 38.5\\ 47.5\\ 61.0\\ 75.6\\ 100.0 \end{array}$	$\begin{array}{c} 1.\ 43102\\ 1.\ 43219\\ 1.\ 43334\\ 1.\ 43396\\ 1.\ 43396\\ 1.\ 43486\\ 1.\ 43592\\ 1.\ 43719 \end{array}$

accurate determination of the fourth place necessary. Without special precautions this may be obtained under ordinary conditions from a series of readings with an Abbé refractometer.

Specific rotation is independent of dilution (Fig. 3). Since errors in polarimetry need not exceed $\pm 0.02^{\circ}$, and a range of approximately 13.0° occurs between the rotations (1 dm.) of optically active and inactive diols, direct estimates of the percentage of an optically active isomer present in a mixture in excess of its enantiomer may be made with an error $< \pm 2\%$. The optical rotations (1 dm.) of a series of *levo-dl*-mixtures at 25°C. are shown in Table VI.

TABLE VI Optical rotations (1 dm.) of *levo-dl-mixtures*

No.	<i>levo-</i> , gm.	<i>dl-</i> , gm.	levo-, %	dl-, %	a ²⁵
$ \begin{array}{c} 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7 \end{array} $	$\begin{array}{c} 0.0\\ 0.8628\\ 1.6648\\ 2.1663\\ 2.5917\\ 3.3890\\ 100.0 \end{array}$	$\begin{array}{c} 100.\ 0\\ 2.\ 9149\\ 2.\ 3446\\ 1.\ 7983\\ 1.\ 3668\\ 0.\ 6834\\ 0.\ 0\end{array}$	$\begin{array}{c} 0.0\\ 22.8\\ 41.5\\ 54.7\\ 65.5\\ 83.2\\ 100.0 \end{array}$	$\begin{array}{c} 100.\ 0\\ 77.\ 2\\ 58.\ 5\\ 45.\ 3\\ 34.\ 5\\ 16.\ 8\\ 0.\ 0\end{array}$	$\begin{array}{r} 0.00 \\ - 3.04 \\ - 5.32 \\ - 6.98 \\ - 8.30 \\ - 10.58 \\ - 12.74 \end{array}$

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These values are plotted in Fig. 3, and show a linear relation between concentration and optical activity. The weight per cent of either optically active isomer in a mixture whose optical rotation (1 dm.) at 25° C. = $\pm y$ may therefore be obtained from the equation:

$$\%$$
 optically active isomer $= \frac{\pm y \times 100}{\pm 13.0}$.

The percentage of the *racemic* mixture is obtainable by difference.

Tests of the method on mixtures containing all three stereoisomers, with refractive indices measured in room air, are reported in Table VII. The isomeric composition, calculated from measurements of the refractive index and optical rotation, are within $\pm 3\%$ of the known values.

TABLE VII Test of method on known mixtures

Weight % of isomers calcu-Known weight % of lated from $n_{\rm D}^{25}$ and $a_{\rm D}^{25}$ No. $n_{\rm D}^{25}$ a_D^{25} isomers in mixture dllevolevodlmesomeso-1.4312 56.855.23.2-5.2941.41.841.5 $\frac{2}{3}$ 1.4324-3.6849.021.228.922.629.848.540.9 1.4328 -3.8230.6 28.531.4 39.6 29.0 $-4.86 \\ -2.42$ 40.0 20.7 4 1.4331 31.1 38.2 27.933.9 28.91.4338 537.142.219.0 45.26 1.4340 -5.9950.92.4 46.847.048.3

The method was applied to the mixed 2,3-butanediols produced by Aerobacter aerogenes. Walpole (18) originally reported that this organism formed a meso-racemic mixture with a small positive rotation. Böeseken and Cohen (1) also showed that certain samples may contain an unstated percentage of the racemic mixture; and Tipson (16) recently reported the presence of D-(levo)-2,3-butanediol in the mixed 2,3-butanediols from the normal fermentation of glucose by A. aerogenes. Pilot plant samples of 2,3-butanediol, all obtained from the fermentation of beet molasses by A. aerogenes Strain M148, were purified (see experimental section). Karl Fischer determinations on two of the purified samples showed 0.02% and 0.065% of water respectively. Isomeric ratios obtained by the physical method were checked for one sample by fractional crystallization of the di-*p*-nitrobenzovl esters. Results are shown in Table VIII. The percentage of *racemic* diol reported by the ester method for Sample 2 is known to be too high because of the difficulty of removing fine meso-crystals from dl-buttons. The proportion of meso-diol reported by the physical method varies from 65-87%, of the dextro- isomer from 2 to 16%, and of the *racemic* mixture from 0 to 33%. Whether this variation exists in the diol produced by the organism, or whether it arises during the recovery process in the pilot plant has not been determined.

TABLE VIII

ISOMERIC COMPOSITION OF Aerobacter DIOLS

No.	$n_{\rm D}^{25}$	$\begin{array}{c c} - & - & - & - & - & - & - & - & - & - $	Weight $\%$ of isomers calculated from $n_{\rm D}^{25}$ and $a_{\rm D}^{25}$			Weight % of isomers from di-p-nitrobenzoyl esters (13)		
			meso-	dextro-*	racemic	meso-	dextro-*	racemic
$ \begin{array}{c} 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{array} $	$\begin{array}{c} 1.4350\\ 1.4359\\ 1.4362\\ 1.4364\\ 1.4367\end{array}$	+0.31 +0.4 +2.09 +1.66 +0.8		$ \begin{array}{c} 2 \\ 3 \\ 16 \\ 13 \\ 6 \end{array} $	$ \begin{array}{r} 33 \\ 18 \\ 0 \\ 0 \\ 2 \end{array} $	73	2	25

* In excess of the enantiomer.

Experimental

Preparation of Isomers

1. D-(levo-)-2,3-Butanediol

Levo-diol, produced in the pilot plant from the fermentation of wheat by Bacillus polymyxa (23) was refluxed overnight at pH 8 with an equal volume of reagent benzene, the water removed in a Barrett distilling receiver, and the benzene distilled off through a Vigreux column at atmospheric pressure. The residual diol was distilled *in vacuo* (0.2 to 0.5 mm. Hg) at pH 8-9, treated with activated charcoal (Anachæmia H-73), and redistilled without fractionation under a pressure of 0.2 to 0.5 mm. Hg into a dry receiver that was sealed off under vacuum. The receiver was so designed that the distillate could be evaporated without ebullition into a dry test tube, which was sealed off in turn. The supposedly pure isomer had $n_{\rm D}^{25} = 1.43095$; $[a]_{\rm D}^{25} = -12.92^{\circ}$; and contained 0.046% water. Other samples of *levo*-diol prepared in the same way had the physical constants and water contents shown in Table II.

2. Meso-2,3-Butanediol

Diol obtained from a pilot plant fermentation of beet molasses by Aerobacter aerogenes N.R.C. strain M148, was adjusted to the phenolphthalein end point with sodium hydroxide, and distilled through a Vigreux column at about 1 mm. pressure. Mixed propionals, prepared from this diol by Neish and MacDonald's procedure (12), were fractionated through a 100-plate Podbelniak Hyper-Cal column. From 1230 ml. of mixed propionals, 230 nl. of pure meso-propional (b.p. 134.5°C.; $a_D < + 0.005^\circ$) was obtained. Mesopropional (2-ethyl, 4,5-dimethyl, 1,3-dioxacyclopentane) refluxed with 530 ml. of 1.65% hydrochloric acid for about two hours, was distilled to remove propionaldehyde, and the mixture neutralized with sodium hydroxide. Glycerol was added as a still-pot base, propionaldehyde and water were removed by distillation, and the diol finally distilled through a semimicro-Todd (17) column into a dry receiver. The product had $a_{26}^{26} = -0.005: n_{25}^{25} = 1.4371$. A second sample of pure *meso*-diol, prepared from dimethyl ketals (12) by a similar procedure, had $n_{\rm D}^{25} = 1.4373$.

The two samples of crystalline *meso*-diol were combined and recrystallized at 10°F. from three and one-half volumes of isopropyl ether. The crystals were finally distilled *in vacuo* through a semimicro-Todd column (17) into a dry receiver. The final product had $a_D^{25} = 0.00 \pm 0.003$; $n_D^{25} = 1.43719$; water content = 0.042% (Karl Fischer); m.p. = 34.5° C. (24).

3. Racemic 2,3-Butanediol

Pure racemic diol was prepared by hydrolysis of the *dl*-propional. Samples of the propional, obtained during fractionation of the mixed propionals from *Aerobacter* diol, were mixed and refractionated through the Podbelniak column to $a_D^{26.5} = \pm 4.05$; $n_D^{25} = 1.4010$. *Levo*-propional ($a_D = -14.0$) was added until the optical rotation of the mixture was 0.00 in a 2 dm. tube. Propionaldehyde was removed continually by fractional distillation during the hydrolysis. The final distillate had $a_D = \pm 0.23$ (2 dm.). Pure *levo*-diol was added until the mixture became optically inactive ($a_D^{25} = 0.00$). The diol was finally redistilled and stored as previously described ($a_D = -0.005^{\circ}$ $\pm 0.003^{\circ}$ (1 dm.); $n_{D}^{25} = 1.43109$; water content $= 0.040 \pm 0.006\%$.

Measurement of Physical Constants

The pure isomers were stored in glass tubes sealed from distillation receivers *in vacuo*. The tubes were opened, and transfers to polarimeter tubes and precision pycnometers (25) were made in a specially constructed air-tight box-desiccator containing Anhydrone and fitted with a Plexiglass window and neoprene gloves. A current of dry air was passed over the prisms of the Phoenix precision refractometer while refractive indices were being measured.

Purification of Aerobacter Diols and Estimation of Isomers

Five samples of diol from the pilot plant fermentation of beet molasses by A. aerogenes were prepared as follows: 2,3-butanediol (50 ml.), reagent benzene (50 ml.), and several glass beads were placed in a 250 ml. round-bottom flask fitted with a Barrett distilling receiver and reflux condenser. The mixtures were refluxed overnight to remove water. Benzene was largely removed by distillation at atmospheric pressure through an 18 in. Vigreux column. The pH of the diol was maintained at 8.5–9.0 during subsequent distillation (without fractionation) at 1–5 mm. pressure, and the distillate collected in a dry receiver. Karl Fischer determinations on two samples prepared in this way showed 0.065% and 0.020% of water respectively. Refractive indices at 25°C. were measured in room air with an Abbé refractometer, and optical rotations were determined with a Hilger polarimeter.

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