benzene with 0.5 g. of boron trifluoride–ether catalyst for five and one-half hours at 88.5°). Products include 1 g. of monocyclic terpenes, $n^{20}\mathrm{D}$ 1.4798; 16 g. of b. p. 171–176° (10 mm.), $n^{20}\mathrm{D}$ 1.5217–1.5244 and $\alpha^{25}\mathrm{D}$ −10 to −16°, apparently monocyclic terpene ethers of 2,4-dimethylphenol; and 19 g. of higher boiling material and resin.

In another experiment boron trifluoride–ether catalyst was added to an ice–concd. hydrochloric acid-cooled mixture of 816 g. nopinene and 632 g. 2,4-dimethylphenol. On the addition of the seventh ml. of catalyst (four hours after beginning of catalyst addition) vigorous exothermic reaction suddenly took place; in three minutes the temperature rose from −1 to 140°. Ten minutes later the reaction was stopped by addition of 10% sodium hydroxide; the mixture had cooled to 57°. Petroleum ether was added to facilitate handling; unreacted 2,4-dimethylphenol (455 g.) was removed by extraction with aqueous alkali; the solvent and terpenes (39 g.) were removed by distillation with a short stripping column; and the reaction products were then distilled to give a 708-g. fraction, b. p. 185–220° (20 mm.), leaving 335 g. (41%) of nopinene polymer, a brittle brown resin. The distillate was fractionated through a 39 in. × 34 mm. spiral screen column[6, 7] having a 50 × 50 mesh stainless steel screen packing spiraled about a 17 mm. inner glass tube and having an efficiency of 29 theoretical plates. The physical properties of the products indicated them to include about 10% terpene dimer (b. p. 156–165° (10 mm.), $n^{20}\mathrm{D}$ 1.5121, $\alpha^{24}\mathrm{D}$ (10 cm.) +20.2°), 50% terpene ethers of 2,4-dimethylphenol (b. p. 172–174.5° (10 mm.), $n^{20}\mathrm{D}$ 1.5236–1.5248, $\alpha^{24}\mathrm{D}$ (10 cm.) −12.0 to −20.7°) and 30% 2,4-dimethyl-6-isobornylphenol (b. p. 186–198° (10 mm.), $n^{20}\mathrm{D}$ 1.5375–1.5412, $\alpha^{24}\mathrm{D}$ (10 cm.) +0.8 to +2.1°), a highly viscous liquid which crystallized on cooling. Recrystallized from petroleum ether, the last fraction had m. p. 79.6–80.6°; 2,4-dimethyl-6-isobornylphenol from camphene, m. p. 82.8–83.4°; m. p., mixed, 80.0–82.0°.

Alkylation of 2,4-Dimethylphenol with Dipentene.—A mixture of 408 g. of dipentene and 366 g. of 2,4-dimethylphenol (m. p. 18–20.5°) was stirred at 60–82° for six and one-half hours. Nine grams of boron trifluoride–ether catalyst was added slowly during the first four hours, after which heat was applied to keep the temperature up. The reaction mixture, worked up in the manner described for the previous experiments, yielded 191 g. (25%) of terpene ethers of 2,4-dimethylphenol having $n^{24}\mathrm{D}$ 1.5250–1.5261 and b. p. 169–176° (10 mm.), instead of the nuclear alkylation expected as the main product because of the tertiary olefin structure of dipentene.

The position of the isobornyl group in 2-isobornylphenol and the ether nature of several reaction products of nopinene and dipentene with 2,4-dimethylphenol were confirmed by infrared absorption spectra. The infrared data will be reported in a forthcoming paper.

Summary

Phenols condense with camphene at 0° with acid catalyst present to yield isobornyl ethers in good yield. The ethers rearrange at elevated temperatures, also in the presence of acid catalysts, to yield nuclearly substituted phenols. In the case of 2,4-dimethylphenol, where the number of rearrangement products is limited, the crystalline terpene phenol can be obtained in good yield. Nopinene reacts less smoothly with 2,4-dimethylphenol to give terpene ethers at 0° and ethers, along with some 2,4-dimethyl-6-isobornylphenol at elevated temperature. Dipentene yielded terpene ethers of 2,4-dimethylphenol instead of expected nuclear alkylation.

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[6] H. S. Lecky and R. H. Ewell, *Ind. Eng. Chem., Anal. Ed.*, **12**, 544 (1940).

[7] W. D. Stallcup, R. E. Fuguitt and J. E. Hawkins, *Ind. Eng. Chem., Anal. Ed.*, **14**, 503 (1942).

[CONTRIBUTION FROM THE DEPARTMENT OF RESEARCH IN PURE CHEMISTRY, MELLON INSTITUTE]

Presence of D-(*levo*)-2,3-Butanediol in the Mixed 2,3-Butanediols Produced by Normal Fermentation of Glucose with *Aerobacter aerogenes*

BY R. STUART TIPSON

In the course of certain investigations in progress in this laboratory, it became desirable to obtain pure samples of the L-(*dextro*)[1]- and *meso*-isomers of 2,3-butanediol, respectively. The pure *dextro*-form is not commercially available but a mixture ($[\alpha]^{25}\mathrm{D}$ about +1.0°) supposedly consisting of the *meso*-form (about 90%) with the *dextro*-form (about 10%) is produced by the action of *Aerobacter aerogenes* on sucrose[2] or glucose.[3] It is said that the isomers may be separated from this mixture by fractional distillation[4] at atmospheric pressure, but, since the boiling points of the two diols differ by only 3 to 4°, this is presumably a rather tedious and unsatisfactory procedure, as evidenced by the specific rotations recorded by various authors, *viz.*, +5.0[5]; +2.41 (further purified,[6] +6.9); +10.15 (after fermentation of the *meso*-diol with *Acetobacter suboxydans*[7]); +13.0°.[8]

Having successfully used[9] the procedure of conversion to the isopropylidene derivatives, followed by fractional distillation, for the separation of mixtures of three closely related monosaccharides, it occurred to us that separation of the *dextro*- from the *meso*-diol might be achieved in the same manner. Consequently, the mixed diols were condensed with acetone in the presence of concentrated sulfuric acid; in some experiments, anhydrous copper sulfate was added as desiccant for the water liberated during the reaction.[9,10] On

[1] Morell and Auernheimer, THIS JOURNAL, **66**, 792 (1944).

[2] Fulmer, Christensen and Kendall, *Ind. Eng. Chem.*, **25**, 798 (1933).

[3] Lees, Fulmer and Underkofler, *Iowa State Coll. J. Sci.*, **18**, 359 (1944).

[4] Ward, Pettijohn, Lockwood and Coghill, THIS JOURNAL, **66**, 541 (1944).

[5] Böeseken and Cohen, *Rec. trav. chim.*, **47**, 839 (1928).

[6] Chappell, *Iowa State Coll. J. Sci.*, **11**, 45 (1936).

[7] Fulmer, Underkofler and Bantz, THIS JOURNAL, **65**, 1425 (1943).

[8] Knowlton, Schieltz and Macmillan, *ibid.*, **68**, 208 (1946).

[9] Levene and Tipson, *J. Biol. Chem.*, **115**, 731 (1936).

[10] Levene and Tipson, *ibid.*, **106**, 113 (1934); Tipson and Cretcher, *J. Org. Chem.*, **8**, 95 (1943).

careful fractional distillation, followed by refractionation, of the resulting mixture of isopropylidene 2,3-butanediols produced in either way, there was obtained a lower-boiling fraction (A) of constant boiling point, refractive index, and *dextro*-rotation which, from chemical analysis, had a composition corresponding to that of an isopropylidene butanediol. This was followed by a series of intermediate fractions having gradually increasing boiling point and refractive index but diminishing *dextro*-rotation, and finally by very pure isopropylidene *meso*-2,3-butanediol[11] (present in excess). The latter was readily obtained in exceedingly pure condition by a further fractional distillation. This method of purifying the *meso*-diol may be preferable to that involving recrystallization of the mixed diols from isopropyl ether,[3,8,12] a compound with explosive potentialities. Furthermore, it was found that the optically active diol reacts with acetone more rapidly than does the *meso*-diol, so that any unreacted *meso*-diol remains as still-residue on distilling off the isopropylidene derivatives and may be obtained pure by continuing the distillation. However, an attempt to obtain formation of the optically active isopropylidene-diol with concomitant minimal formation of the isopropylidene *meso*-diol (by reaction with only 10% of the theoretical amount of acetone during a shorter period of time, *viz.*, thirty minutes) still gave rise to the aforementioned low-boiling fraction (A), having the same properties as before. This material (A) presumably consisted either of an azeotrope of isopropylidene *meso*-2,3-butanediol with isopropylidene *dextro*-2,3-butanediol or of a mixture of isopropylidene *levo*-2,3-butanediol with excess isopropylidene *dextro*-2,3-butanediol.

At this stage of the investigation, a publication by Neish[13] appeared, reporting the *successful* separation of the two diols by an acetonation procedure somewhat similar to the above, although the optical rotation (+15°) found for the supposed isopropylidene *dextro*-diol isolated was too low. However, since his results were invalidated through contamination of his starting material (supposedly *dextro*- plus *meso*-2,3-butanediol) with *levo*-isomer "picked up in the pilot plant when it was distilled through columns previously used for *levo*-2,3-butanediol,"[13] it was still not feasible to decide which of the two above possibilities represented the composition of the low-boiling fraction (A). Accordingly, the reaction was repeated under his experimental conditions, but using as starting material a 2,3-butanediol mixture, produced by normal fermentation of glucose with *Aerobacter aerogenes*, which had *not* become contaminated by *levo*-diol during isolation procedures.[14] On careful fractional distillation of the product, followed by refractionation, there was isolated an isopropylidene diol fraction having the same properties as fraction (A) obtained under our conditions. This material had the same boiling point and refractive index as pure isopropylidene *levo*-2,3-butanediol,[15] suggesting that it contained no isopropylidene *meso*-derivative (of higher boiling point and refractive index). Hence, if the low optical rotation was occasioned by the presence of *levo*-diol, the latter must be an integral part of the original diol mixture and not a contaminant accidentally introduced.

In order to check this conclusion, a sample of pure isopropylidene *levo*-2,3-butanediol was mixed with an equal volume of pure isopropylidene *meso*-2,3-butanediol and the mixture fractionally distilled. The first fractions consisted of practically pure *levo*-derivative, the last fractions of practically pure *meso*-derivative, and the intermediate fractions of mixtures of the two (successively richer in the *meso*-derivative). Hence the behavior observed is not the result of formation of an azeotrope of the *meso*- with the optically active derivative, but is attributable to the presence of D-(*levo*)-2,3-butanediol in the mixed diols produced by normal fermentation of glucose with *Aerobacter aerogenes*.[16]

Experimental

The "*meso*"-2,3-butanediol was technical grade 2,3-butanediol[17] originally described as consisting of approximately 90% of the *meso*-isomer and 10% of the *dextro*-isomer. It contained 0.22 g. of water per 100 cc. and had $n^{25}\mathrm{D}$ 1.4333, $\alpha^{22}\mathrm{D}$ +0.47(5)°.[18]

Condensation of "*meso*"-2,3-Butanediol with Acetone.—(a) Sulfuric Acid Catalyst.—"*meso*"-2,3-Butanediol (200 g.) was placed in a bottle, 200 cc. of acetone was added, and the mixture shaken; this solution had $\alpha^{22}\mathrm{D}$ +0.24(5)°. Concentrated sulfuric acid (0.8 cc.) was now added, the bottle stoppered, and the mixture shaken until the acid had dissolved. After one hour, the solution had $\alpha^{22}\mathrm{D}$ +0.70°; constant thereafter. After a total of ninety minutes at room temperature, 5 g. of finely powdered anhydrous calcium hydroxide was added and the suspension shaken mechanically for one hour; it was then filtered with suction (rubber dam, Dry Ice–chloroform trap) through a thin layer of anhydrous calcium hydroxide and the insoluble material was washed with a little acetone.

The filtrate and washings were combined, and freed from acetone by distillation at atmospheric pressure through an electrically heated, air-jacketed column (110 cm. high), packed with 3-mm., single-turn glass helices and provided with a total-condensation, variable take-off, still-head. The still-residue was then fractionally distilled at atmospheric pressure. The first fractions consisted of an azeotrope (b. p., 84–85° at 743 mm.) of isopropylidene compounds with water, which separated into two layers on cooling (vol. of isopropylidene compounds, 40 cc.; water, 21.5 cc.). A further 123 cc. of distillate (a colorless, mobile liquid with a menthol-like odor and b. p., 105–117° at 743 mm.) and a still residue of 85 g. of unreacted diol were

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[11] Backer, *Rec. trav. chim.*, **55**, 1036 (1936).

[12] Wilson and Lucas, THIS JOURNAL, **58**, 2396 (1936).

[13] Neish, *Can. J. Research*, **25B**, 423 (1947).

[14] V. E. Sohns, private communication.

[15] Neish and Macdonald, *Can. J. Research*, **25B**, 70 (1947).

[16] We were then unaware that investigators at the Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria 5, Illinois, had previously found that the mixed diols "may contain as much as 3 to 4% of the *levo*-diol"; unpublished result (V. E. Sohns, private communication).

[17] Kindly presented by Mr. V. E. Sohns, Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria 5, Illinois.

[18] In the present paper, $\alpha^{t}\mathrm{D}$ means the *observed* optical rotation for 1 dm., although low rotations were actually measured in a 2-dm. tube.

obtained. The highest optical rotation observed for a fraction was α^{22}D +4.32° (n^{25}D 1.3915); the lowest was zero. In an experiment in which the reaction-time was five days, the yield of isopropylidene derivatives was not much greater (200 g. from 200 g. of diol), because in the absence of enough desiccant the reaction reaches an equilibrium.

The fractions having zero optical rotation and n^{25}D 1.4005 were set aside and a total of 207 cc. of the fractions having optical activity from +0.075 to +4.32° (from this and another experiment) was now dissolved in pentane, dried with anhydrous sodium sulfate, filtered, freed from pentane and refractionated. A series of seventeen 6-cc. fractions was collected having α^{22}D +3.98, 4.49, 4.58, 4.61, 4.58, 4.50, 4.48, 4.49, 4.41, 4.45, 4.00 (b. p., 107° at 743 mm.), 3.22, 1.68, 1.02, 0.75, 0.37, 0.09(5) (b. p., 109–117° at 744 mm.). In all redistillations, a small first fraction, consisting of a yellow, mobile liquid with no menthol-like odor, was obtained.

A total of 304 cc. of fractions having *low* optical activity was refractionated. After removal of 6-cc. fractions having α^{22}D +3.50 (very pale yellow), 3.46 (colorless), 3.06, 2.54, 1.93, 1.49, 1.13, 0.82, 0.61, 0.39, 0.29, 0.14, 0.09, 0.07 and 0.04°, a series of fractions having zero optical rotation was obtained. This isopropylidene *meso*-2,3-butanediol boiled at 117° at 744 mm. and had n^{25}D 1.4010. Backer[11] gave b. p., 117.5–118.5°; n^{18}D 1.4033.

In a modification of the above procedure, intermediate isolation of the aqueous azeotrope could be eliminated by neutralization of the sulfuric acid by shaking with excess saturated barium hydroxide solution, followed by extraction of the isopropylidene derivatives with pentane, drying the pentane extract, and evaporating off the pentane–acetone azeotrope and pentane. Unchanged diol was recovered from the aqueous layer by saturating with carbon dioxide, heating to boiling under reflux, cooling, filtering, evaporating the filtrate to dryness, extracting with ether, drying the ether extract and evaporating it to dryness. After distillation, it had zero optical rotation and the correct analysis and refractive index for the *meso*-diol.

(b) **Sulfuric Acid–Copper Sulfate Catalyst–Desiccant.**—The yield of isopropylidene *meso*-diol is increased by lengthening the time of reaction and by removal of water formed during the reaction (and formation of the water azeotrope is avoided). In a typical experiment, 400 g. of mixed diols was placed in an Erlenmeyer flask, and 400 cc. of acetone was added. To the solution, which became much colder, was added 160 g. of anhydrous copper sulfate and 1.6 cc. of concentrated sulfuric acid. Owing to evolution of heat, the flask was placed under a reflux condenser (Drierite tube) and cooled in cold water during thirty minutes. It was then shaken mechanically during eighteen hours at room temperature. The copper sulfate was now filtered off (rubber dam, Dry Ice–chloroform trap) and washed with acetone, and the filtrate plus washings treated with anhydrous calcium hydroxide as previously described. After distilling off the acetone, the product was fractionated, giving 256 cc. of isopropylidene derivatives having α^{22}D +4.10 to 0.02°, 99 cc. having zero rotation, and a still-residue of 96.5 g. of unreacted diols. (In experiments in which the reaction time was greater, the total yield of isopropylidene derivatives increased, *e. g.*, 440 cc. from 400 g. of diol after forty-four hours, owing to the formation of a larger amount of the isopropylidene *meso*-diol.) The fractions displaying optical rotation (256 cc.) were combined and refractionated. Seventeen successive 6-cc. fractions had α^{22}D +3.40 (yellow), 4.30 (colorless), 4.56, 4.65 (b. p. 107–108° at 747 mm.), 4.64, 4.64, 4.70, 4.86, 4.90, 4.91, 4.90, 4.84, 4.72 (b. p., 108° at 744 mm.), 4.43, 3.64, 2.50, 1.40° (b. p., 108–115° at 744 mm.).

A total of 144 cc. of fractions having α^{22}D above +4.0°, from several such experiments, was now refractionated. Two 6-cc. fractions, having αD +4.08° (very pale yellow) and 4.65° (colorless), followed by *sixteen* successive 6-cc. fractions boiling at 107.5° at 745 mm. and having α^{22}D +4.8 to 4.9° (n^{25}D, 1.3915) were obtained. This material largely crystallized in colorless needles on cooling in Dry Ice–chloroform.

Anal. Calcd. for $C_7H_{14}O_2$: C, 64.56; H, 10.8. Found: C, 64.71; H, 10.3.

Since it appeared obvious that the optically active diol reacts more rapidly with acetone than does the *meso*-diol, an attempt was made to isolate the pure isopropylidene *dextro*-diol by using only sufficient acetone to react with the *dextro*-diol said to be present in the mixed diols and shortening the reaction-time to thirty minutes. Thus, 300 g. of mixed diols was treated with 24.5 cc. of acetone plus 1 cc. of concentrated sulfuric acid during thirty minutes and the product was isolated as usual. However, a total of 41 cc. of isopropylidene derivatives was obtained, the first six 6-cc. fractions of which had successively α^{22}D +4.64, 4.66, 4.47, 1.94, 0.15, 0.01(5)°. On refractionation of the first three fractions by addition to the still-residue of unreacted diols, the fraction of highest rotation had α^{22}D +4.69° (n^{25}D 1.3915).

Repetition of Neish's Experiment.[13]—The conditions differ from those described above in that an exactly equimolar amount of acetone is employed, a much greater proportion of concentrated sulfuric acid is added, and unreacted glycol is removed by extraction with water before distillation of the product. Because of the paucity of details in Neish's description, the exact conditions of our experiment are given.

Mixed diols (200 g., 205 cc.) were weighed into a 500-cc. graduated, cylindrical separating funnel, 163 cc. of acetone was added, and the mixture shaken until dissolved. The temperature fell from 26 to 20°. Concentrated sulfuric acid (15 cc.) was now added and the funnel was immediately placed under a reflux condenser (Drierite tube). The mixture was gently swirled until the lower layer had practically all disappeared, and then left undisturbed. A lower layer rapidly reappeared (85 cc., seven minutes) and increased in volume to 200 cc. (9 minutes), decreasing to 155 cc. after two hours. The upper layer decreased from 300 cc. (seven minutes) to 182.5 cc. (nine minutes), increasing to 217.5 cc. after two hours (constant thereafter). After sixty-eight hours, the lower layer was withdrawn and the upper layer was shaken with saturated, aqueous sodium bicarbonate solution until alkaline to red litmus and then with 25 cc. of water. The upper layer (vol., 182.5 cc.) was dried with anhydrous sodium sulfate and then had α^{22}D +1.32°. In another experiment it had α^{22}D +1.39°.

A total of 347.5 cc. of such material was now fractionally distilled. Acetone (41 cc.) was obtained as the first fraction. This was followed by two 6-cc. fractions having α^{22}D +4.08 and 4.45°, ten successive 6-cc. fractions having α^{22}D above +4.5°, and five 6-cc. fractions having α^{22}D +4.03, 3.69, 2.55, 1.61 and 0.79°, respectively. The fractions having α^{22}D above 4° were combined and refractionated, giving two 6-cc. fractions having α^{22}D +2.81° (pale yellow) and +4.64° (colorless), followed by *six* 6-cc. fractions boiling at 107.5° at 744 mm. and having α^{22}D +4.8 to +4.9° (n^{25}D, 1.3915), and a 6-cc. fraction having α^{22}D +4.40°. The non-*meso* part of the diol mixture examined behaved as a mixture of about 62.5% of *dextro*-diol with about 37.5% of *levo*-diol.

Anal. (fraction of α^{22}D +4.9°). Calcd. for $C_7H_{14}O_2$: C, 64.56; H, 10.85. Found: C, 64.62; H, 10.58.

Neish[13] obtained material boiling at 109.5 to 110.5° and having an observed optical rotation of +15°. Its refractive index and chemical analysis were not recorded.

Isopropylidene D-(*levo*)-2,3-Butanediol.—The diol used was technical grade, *levo*-2,3-butanediol.[17] It had n^{25}D 1.4300, α^{23}D −12.0° (no solvent) and $[\alpha]^{23}$D −10.74° ($c = 5.5848$, in acetone). On adding one drop of concentrated sulfuric acid to this acetone solution, the specific rotation changed to −26.41° (calculated as diol) or −18.29° (calculated as isopropylidene derivative) after twenty-six minutes; constant thereafter. Readings at intermediate time-intervals could not be taken because of eddy-currents.

In order to obtain the maximum yield, and because of the considerable heat evolution, the reaction was performed as follows. *levo*-2,3-Butanediol (100 g.) was weighed into a 500-cc. Erlenmeyer flask, 200 cc. of acetone was added, and the mixture swirled, causing a pronounced fall in temperature. Concentrated sulfuric acid (0.8 cc.) was added and the solution immediately placed under a reflux condenser (Drierite tube). On swirling, much heat was evolved; after ninety minutes, the solution had cooled to room temperature and a small layer of colorless liquid had separated out below the main solution. Anhydrous copper sulfate (20 g.) was now added, the flask stoppered and vigorously shaken, and then replaced under the reflux condenser because of further heat evolution. At intervals, 20 g. and then 40 g. of anhydrous copper sulfate were similarly added and, after the mixture had finally cooled to room temperature, the flask was stoppered, vigorously shaken, and kept overnight at room temperature.

The copper sulfate was now filtered off and the filtrate plus washings treated with anhydrous calcium hydroxide (5 g.) as previously described. The filtrate and washings were combined and freed from acetone by distillation at atmospheric pressure through the column. The acetone-free still-residue was acid to moist blue litmus paper; it was cooled to room temperature (vol., 152 cc.) and shaken with 100 cc. of dilute aqueous sodium bicarbonate solution. The aqueous layer (108.5 cc.) was withdrawn and the upper layer dried with anhydrous sodium sulfate, filtered, and fractionally distilled. After removal of a 4-cc. fraction (containing acetone and moisture) having $\alpha^{22}\mathrm{D}$ −1.45° and 6 cc. having $\alpha^{22}\mathrm{D}$ −17.15°, *ten* 6-cc. fractions having $\alpha^{22}\mathrm{D}$ −18.4 to −18.7° ($n^{25}\mathrm{D}$ 1.3910, b. p., 107° at 737 mm.) were obtained, followed by a fraction of $\alpha^{22}\mathrm{D}$ −18.28° ($n^{25}\mathrm{D}$ 1.3908). For isopropylidene *levo*-2,3-butanediol, Neish and Macdonald[15] found $\alpha^{25}\mathrm{D}$ −19.1° or $[\alpha]^{25}\mathrm{D}$ −22.1°; $n^{25}\mathrm{D}$ 1.3914; b. p., 110° at 760 mm. It gave[15] a "diphasic azeotrope with water, b. p., 86°." They obtained a yield of only 52% of the theoretical.

Distillation of a 50:50 Mixture of Isopropylidene *levo*-2,3-Butanediol plus Isopropylidene *meso*-2,3-Butanediol.—Isopropylidene *levo*-2,3-butanediol (63 cc.) was mixed with 63 cc. of the *meso*-derivative, giving a solution having $\alpha^{24}\mathrm{D}$ −9.45°. This was fractionally distilled through the column previously described, yielding two 3-cc. fractions of $\alpha^{23}\mathrm{D}$ −9.88° (yellow) and −17.81° (pale yellow; $n^{25}\mathrm{D}$ 1.3909), followed by five 3-cc. fractions of $\alpha^{23}\mathrm{D}$ −18.16° to −18.11° ($n^{25}\mathrm{D}$ 1.3914, b. p., 107° at 735 mm.), and a series of fractions of gradually diminishing optical rotation and increasing refractive index and boiling point. The last fraction collected boiled at 117° at 735 mm. and had $\alpha^{23}\mathrm{D}$ −0.12°, $n^{25}\mathrm{D}$ 1.4005.

Summary

Separation of *meso*-2,3-butanediol from its optical isomers *via* the isopropylidene derivatives is readily achieved. Owing to the presence of some *levo*-2,3-butanediol together with the *dextro*-isomer in technical grade "*meso*"-2,3-butanediol, only the *meso*-diol derivative can be isolated in pure condition by *distillation* of the mixed isopropylidene derivatives obtained by reaction of the mixed diols with acetone. Some properties of pure isopropylidene *meso*-2,3-butanediol are given.

The preparation of isopropylidene *levo*-2,3-butanediol in practically quantitative yield is described.

The presence of *levo*- or *dextro*- or of D,L-2,3-butanediol in admixture with the *meso*-isomer may be detected and the proportions ascertained.

levo-2,3-Butanediol is a natural concomitant in the bacterial formation of the mixed 2,3-butanediols produced by a normal fermentation of glucose by *Aerobacter aerogenes*.

PITTSBURGH 13, PA. RECEIVED JUNE 18, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY]

Studies on the Structure of ζ-Carotene[1]

BY H. A. NASH,[2] F. W. QUACKENBUSH AND J. W. PORTER

The occurrence, isolation, absorption spectrum and biological inactivity of ζ-carotene have been reported in previous papers.[3,4,5] In the present study information is presented on the structure of this carotene.

Chemical studies on ζ-carotene were preceded by purification as previously described.[3] Removal of a wax-like impurity, which persisted after chromatographic purification, by sodium ethylate saponification did not promote crystallization nor did subsequent molecular distillation. The molecular distillation was carried out in a small still of the pot type at 110° and was designed to remove more-volatile impurities which might be present. After chromatographic removal of the large amount of steroisomers which were formed at this temperature, a solution of ζ-carotene was obtained which showed a specific absorption coefficient at 3560 Å., 2% higher than that previous to molecular distillation. Specific absorption coefficients at 3560 Å. before and after molecular distillation were 54.3 and 55.4, respectively. The failure of these attempts to effect crystallization necessitated further work with non-crystalline material.

Because of the extreme ease of oxidation on exposure to air, ζ-carotene was kept under carbon dioxide or under high vacuum when not dissolved in hexane. Estimates of sample weight were obtained from light absorption values of the ζ-carotene in hexane solution. This method of obtaining sample weights introduced errors of ±2%.

The studies reported in this paper indicate that the most probable formula of ζ-carotene is $C_{40}H_{64}$ and that it possesses an open chain structure similar to lycopene. The typical 40-carbon atom structure of the carotenoids is indicated by the molecular weights, although the experimental values obtained were slightly high, 582, 578 and 543. The polyisoprenic structure is corroborated by the C-methyl determinations. An open chain structure is indicated by the isopropylidene determina-

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[1] Journal Paper No. 340 of the Purdue University Agricultural Experiment Station.

[2] Present address: Pitman-Moore Co., Indianapolis, Indiana.

[3] Nash and Zscheile, *Arch. Biochem.*, **7**, 305 (1945).

[4] Porter, Nash, Zscheile and Quackenbush, *ibid.*, **10**, 261 (1946).

[5] Porter and Zscheile, *ibid.*, **10**, 537 (1946).