and Pepper.[9] They assumed that the alkalinity of the permanganate solutions causes inversion, but in the osmium tetroxide reaction the medium is acidic throughout and yet the same products are obtained.

The author wishes to thank Professor and Mrs. L. F. Fieser for helpful advice during the course of this investigation.

Experimental

Oleic,[10] elaidic,[11] linoleic,[12] erucic[9] and brassidic[9] acids were prepared by the standard procedures. One gram lots of each were oxidized with osmium tetroxide by the method of Butenandt.[13] In each case there were obtained in about 60% yield the *hydroxyacids obtained also in the alkaline potassium permanganate oxidation* of the same acids. Their identity was established by melting point and mixed melting point. The mixture of stereoisomers melting at 156° obtained in the oxidation of linoleic acid was separated into the two isomers melting at 173° and 164°, respectively, by the method of Riemenschneider and co-workers.[14] The experimental data are summarized in Table I.

TABLE I

Compound oxidized	Crude product Wt. g.	Crude product M. p., °C.	Pure product Wt., g.	Pure product M. p., °C.	Pure product Mixed m. p., °C.
Oleic acid	0.86	126–129	0.67	132	132
Elaidic acid	.82	88–91	.62	94–95	94–95
Erucic acid	.90	124–127	.70	132	131–132
Brassidic acid	.79	94–96	.59	101	101
Lineoleic acid	.80	156–157	.28	172–173	172–173
			.17	164	164

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(9) Dorée and Pepper, *ibid.*, 477 (1942).

(10) Lapworth, Pearson and Mottram, *Biochem. J.*, **19**, 7 (1925).

(11) Bertram, *Chem. Weekblad*, **33**, 3 (1936).

(12) McCutcheon, "Organic Syntheses," **22**, 75 (1942).

(13) Butenandt, Schmidt-Thomé and Paul, *Ber.*, **72**, 1116 (1939).

(14) Riemenschneider, Wheeler and Sando, *J. Biol. Chem.*, **127**, 391 (1939).

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Chemical Degradation of Isotopic Succinic and Malic Acids[1a]

BY A. A. BENSON AND J. A. BASSHAM

The path of carbon in photosynthesis[1] may be traced by determination of the positions of labeled carbon atoms in the intermediates involved. Enzymatic degradation of succinic and malic acids have been reported[2] but require pure enzyme preparations. Unequivocal chemical degradations of these intermediates are reported in this note. Succinic and malic acids containing C^{14} were isolated from plant extracts using silica gel partition chromatography[3] and co-crystallized with convenient amounts of carrier acids.

The Curtius[4] degradation of succinic acid has been modified to give good yields on small amounts. Methyl succinate, prepared using diazomethane, is converted to the diazide through the dihydrazide.[4,5] Rearrangement of the diazide in ethanol gives ethylenediurethan which was hydrolyzed to give carbon dioxide from the carboxyl groups and ethylenediamine from the methylene groups of the original succinic acid.

$$HO_2C\cdot\overset{*}{C}H_2—\overset{*}{C}H_2—CO_2H \xrightarrow[\text{reaction}]{\text{Curtius}}$$

$$C_2H_5\text{-}O\text{-}CO\text{-}NH\text{-}\overset{*}{C}H_2\text{-}\overset{*}{C}H_2\text{-}NH\text{-}CO\text{-}OC_2H_5 \xrightarrow[\text{reflux}]{48\%\ \text{HBr}}$$

$$2CO_2 + H_3N^+—\overset{*}{C}H_2—\overset{*}{C}H_2—N^+H_3$$

With this method it is possible to determine the activity of the methylene carbon atoms[6] without interference from any amount of carboxyl carbon activity.

Malic acid has been oxidized with chromic acid to yield two molecules of carbon dioxide from the carboxyl groups and one molecule of acetic acid from the alpha and beta carbon atoms.

$$HOOC—\overset{*}{C}H_2—\overset{**}{C}HOH—COOH \xrightarrow{CrO_3}$$

$$2CO_2 + \overset{*}{C}H_3—\overset{**}{C}OOH$$

The same procedure has been used to determine radioactive carbon fixed in the alpha and beta carbon atoms of aspartic acid. Degradation of the acetic acid may then be performed by decarboxylation.[7]

Experimental

Ethylenediurethan.—A tracer amount of unequally C^{14}-labeled succinic acid synthesized in the dark by preilluminated *Chlorella* was diluted by crystallizing with 300 mg. of succinic acid. The 280 mg. yield of crystalline acid was converted to methyl succinate in the usual manner with diazomethane and distilled quantitatively into a conical reaction flask. The hydrazide was prepared from the ester in 90% yield and was converted to the diurethan using the methods of Shöfer and Schwan[5] and Curtius.[4] The product was recrystallized from water and sublimed *in vacuo* to give a yield of 117 mg. (36%). The specific activity of a thin (less than 0.2 mg./sq.cm.) sample was determined with a Geiger counter and found to be 21.7 ± 1 c./m./mg.

Hydrolysis of Ethylenediurethan.—In a 30-ml. two-neck flask equipped with a reflux condenser and nitrogen inlet tube was refluxed a solution of 104 mg. of ethylenediurethan in 5 ml. of 48% hydrobromic acid for two hours. A slow stream of nitrogen gas during this period carried the evolved carbon dioxide through the condenser into a sodium hydroxide bubbler from which it was recovered as barium carbonate, 210 mg., specific activity 10.0 c./m./mg. The ethylenediamine was obtained upon evaporating excess acid *in vacuo* and adding excess methanolic potassium hydroxide. After evaporating the methanol, ethylenediamine was distilled *in vacuo* and converted to the dihydrochloride, 52 mg. (theoretical yield 68 mg.) by addition of methanolic hydrogen chloride. After recrystallization from methanol–water, the specific activity of the pure ethyl-

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(1) Calvin and Benson, *Science*, **105**, 648 (1947); **107**, 476 (1948).

(1a) This paper is based on work performed under contract No. W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, California.

(2) Wood, Werkman, Hemingway and Nier, *J. Biol. Chem.*, **135**, 7890 (1940).

(3) Isherwood, *Biochem. J.*, **40**, 688 (1946).

(4) Curtius, *J. prakt. Chem.*, [2] **52**, 222 (1895).

(5) Shöfer and Schwan, *J. prakt. Chem.*, [2] **51**, 190 (1895).

(6) An activity of one count per minute determined on a 1 mg. sample of ethylenediamine dihydrochloride by the Nucleometer corresponds to 0.89 c./m./mg. or $8\cdot10^{-10}$ millicurie/mg. for the succinic acid.

(7) Aronoff, Haas and Fries, private communication.

enediamine dihydrochloride was found to be 0.86 c./m./mg. This indicates that 2.6% of the succinic acid radioactivity was in the methylene carbon atoms.

Oxidation of Malic Acid.—To a solution of 109.7 mg. of C^{14}-labeled malic acid (275 c./m./mg. or 30,100 c./m. total.) in 10 ml. of 1.0 *N* sulfuric acid in a 100-ml. flask equipped with nitrogen inlet bubbler, reflux condenser and dropping funnel was added 20 ml. of 0.15 *M* chromic acid solution during two hours on the steam-bath.

The carbon dioxide evolved was collected in carbonate-free sodium hydroxide solution and precipitated with barium chloride to give 326 mg. (101%) of barium carbonate. The specific activity was found to be 81.5 c./m./mg. or 26,600 c./m. total. Acetic acid was obtained from the residual solution upon steam distillation. It was converted to barium acetate (80% yield based on titer of steam distillate) which was recrystallized from water. From the specific activity (2.0 c./m./mg.) and the theoretical yield (112 mg.), the activity in the α and β carbon atoms of the malic acid, 224 c./m., can be determined accurately without interference from carboxyl activity. Incomplete recovery of the carbon dioxide and overoxidation of the malic acid is assumed to have caused the loss of carboxyl activity.

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Acetylation of D-Psicose

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In connection with our studies[2] on the chromatography of acetylated molasses fractions, it became of interest to investigate the behavior of the rare ketose D-psicose (synonyms D-allulose and D-erythrohexulose) under mild acetylating conditions since Zerban and Sattler[3] have shown that this ketose may be a constituent of some molasses fractions. Under conditions of mild acetylation the ketoses may form acetates of the cyclic or acyclic types.[4] In the work herein described D-psicose was acetylated at low temperature with zinc chloride and acetic anhydride and the resultant product was subjected to chromatographic analysis. Five fractions (Table I) were obtained and from the major fraction there was isolated the previously described[5] crystalline *keto*-D-psicose pentaacetate. The main product then of the direct acetylation of D-psicose under mild conditions is the acyclic acetate.

TABLE I

CHROMATOGRAM OF ACETYLATED D-PSICOSE

Zone	Approx. time, sec., for distinct zone formation	Zone position in mm. from top of column	Zone yield, g.
Top	60	0–3	0.017
1	180	21–27	.064
2	180	63–73	.095
3	30	115–122	.183
4	10	126–129	.037
		Total	.396 (84.6%)

Experimental

The D-psicose sirup obtained from the deacetylation of 500 mg. of *keto*-D-psicose pentaacetate[5] was treated with 10 ml. of acetic anhydride and 0.3 g. of freshly fused zinc chloride for twenty hours at 0–3°. The reaction mixture was then poured onto 40 g. of ice and water under good agitation and after thirty minutes of stirring the *p*H of the mixture was adjusted to 6 with sodium bicarbonate. The mixture was then extracted with three 25-ml. portions of chloroform. A sirup was obtained on solvent removal; yield 470 mg.

The above sirup (468 mg.), dissolved in 10 ml. of benzene, was added at the top of a 170 × 44 mm. (diam.) column of 100 g. of 5 parts Magnesol[6]/1 part Celite (by wt.) pre-wet with benzene. The chromatogram was developed with 600 ml. of 100 parts benzene/1 part ethanol (by vol.). Five zones were detected on the extruded column with the permanganate streak indicator (1% potassium permanganate in 2.5 *N* sodium hydroxide) and were eluted with acetone (Table I). Well-formed elongated prisms were obtained from a 95% ethanolic solution of zone 3 and were identified as *keto*-D-psicose pentaacetate; yield 90 mg., m. p. 64–65° (mixed melting point unchanged), $[\alpha]^{23}\text{D}$ $-21.0°$ (*c* 4, chloroform). Accepted values[5] for *keto*-D-psicose pentaacetate are: m. p. 63–65°, $[\alpha]^{29}\text{D}$ $-21.5°$ (*c* 3, chloroform). No crystalline products were obtained from the other zones.

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[1] Sugar Research Foundation Fellow of The Ohio State University Research Foundation (Project 190).

[2] W. W. Binkley, M. Grace Blair and M. L. Wolfrom, THIS JOURNAL, **67**, 1789 (1945); W. W. Binkley and M. L. Wolfrom, *ibid.*, **69**, 664 (1947); **70**, 290 (1948).

[3] F. W. Zerban and L. Sattler, *ibid.*, **64**, 1740 (1942); *Ind. Eng. Chem.*, **34**, 1180 (1942).

[4] See, for example, M. L. Wolfrom and A. Thompson, THIS JOURNAL, **56**, 880 (1934); Yvonne Khouvine and G. Aaragon, *Bull. soc. chim.*, [5] **5**, 1404 (1938); Yvonne Khouvine and Y. Tomoda, *Compt. rend.*, **205**, 1414 (1937).

[5] M. L. Wolfrom, A. Thompson and E. F. Evans, THIS JOURNAL, **67**, 1793 (1945).

[6] W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *ibid.*, **67**, 527 (1945).

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Rearrangement of Alkyl Sulfites to Alkanesulfonate Esters

BY WILLIAM E. BISSINGER, FREDERICK E. KUNG AND CHARLES W. HAMILTON

Although a number of references on the formation of *salts* of alkanesulfonic acids from the reaction of alkyl sulfites with sodium or potassium hydroxides have appeared,[1,2,3,4] only one direct conversion of an alkyl sulfite to an *ester* of an alkanesulfonic acid has been described.[3] In that case, only a very small amount of methyl methanesulfonate resulted when dimethyl sulfite and excess methyl iodide were heated in a sealed tube for twelve hours. We have now found that *catalytic* amounts of tertiary amines, such as tri-*n*-butylamine, are excellent agents for promoting the rearrangement of dimethyl sulfite to methyl methanesulfonate, in yields as high as 56%. Dimethyl ether and sulfur dioxide were the other products. Diethyl sulfite, with the same amine, gave lower yields of either ethyl ethanesulfonate or

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[1] Warlitz, *Ann.*, **143**, 72 (1867).

[2] Rosenheim and Liebnicht, *Ber.*, **31**, 405 (1898).

[3] Arbuzov, *J. Russ. Phys.-Chem. Soc.*, **41**, 429 (1909).

[4] Baggesgaard and Rasmussen, *Ber.*, **52B**, 1069 (1919).