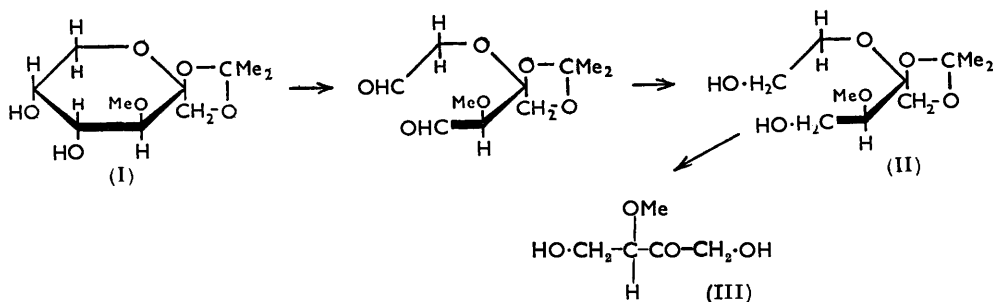


630. *Four-carbon Saccharinic Acids from the Alkaline Degradation of 3-O-Methyl-L-glycerotetralose and 4-O-Methyl-D-threose.*

By G. N. RICHARDS.

3-O-Methyl-L-glycerotetralose is degraded by lime-water mainly to $\alpha\gamma$ -dihydroxybutyric acid, but lactic and formic acid are also formed. Similar treatment of 4-O-methyl-D-threose yields $\alpha\beta$ -dihydroxy- α -methylpropionic acid.

PREVIOUS generalisations¹ indicate that alkaline degradation of the 3- and 4-O-methyltetroses should yield $\alpha\gamma$ -dihydroxybutyric and $\alpha\beta$ -dihydroxy- α -methylpropionic acid respectively. To verify this and to aid future work suitable mono-O-methyltetroses have been prepared.

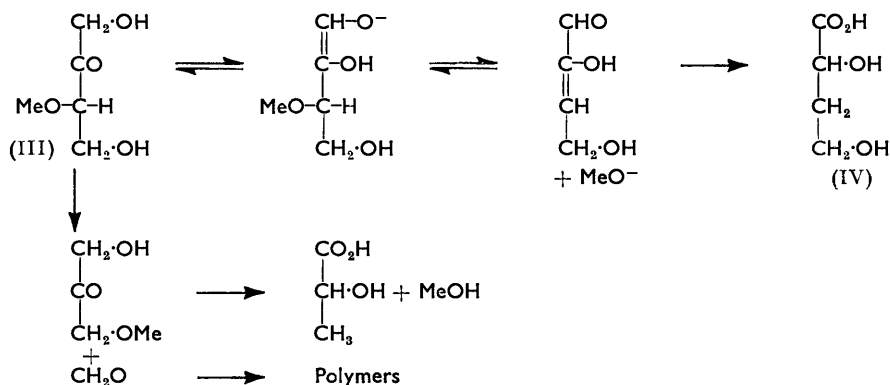


3-O-Methyl-L-glycerotetralose (III) was prepared from 3-O-methyl-1:2-O-isopropylidene-D-fructose (I) by a method similar to that reported² for L-glycerotetralose.

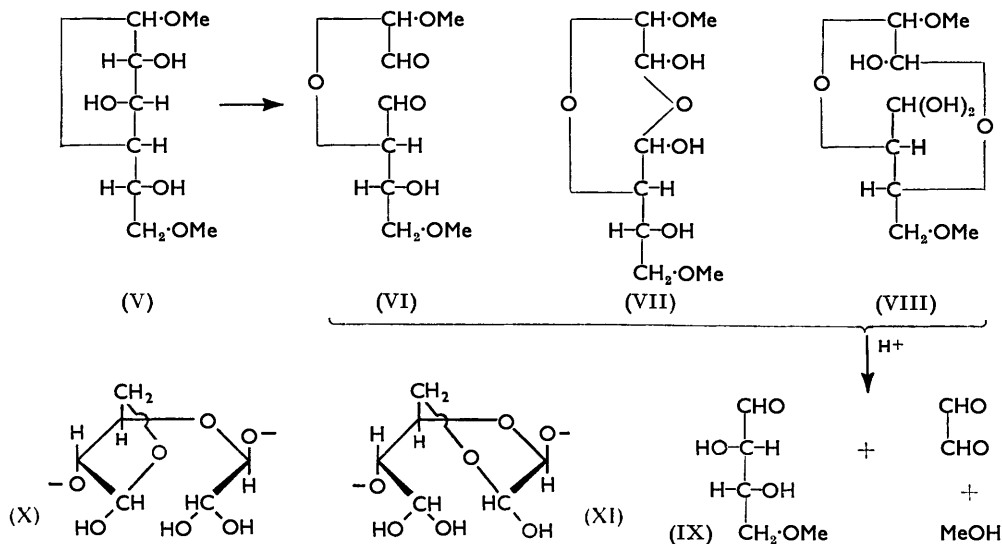
¹ Kenner and Richards, *J.*, 1955, 1810.

² Gorin, Hough, and Jones, *J.*, 1955, 2699.

Degradation of 3-O-methyl-L-glycerotetrolucose (III) by lime-water yielded mainly $\alpha\gamma$ -dihydroxybutyric acid (IV) with smaller amounts of lactic and formic acid. In analogy with the alkaline degradation of 3-O-methyl-D-fructose,³ the annexed reaction scheme is postulated. $\alpha\gamma$ -Dihydroxybutyric acid (IV) was separated from the other acidic products and characterised as its brucine salt and anilide, but the present procedure appears less suitable than earlier methods⁴ for its preparation.



The preparation of 4-O-methyl-D-threose (IX) by periodate oxidation of methyl 6-O-methyl-D-galactofuranoside (V) was difficult because of resistance of the product (VI) to acid hydrolysis and because of decomposition of the ether (IX) produced. In the light of



recent work two stable compounds (VII) and (VIII) may be derived from the oxidation product (VI). For example, Mester⁵ has proposed the structure (X) for periodate oxycellulose. Comparison with the results of Jayme and Maris⁶ indicates that structure (XI) proposed by Head⁷ may be more likely, but both possibilities provide an analogy for

³ Kenner and Richards, *J.*, 1954, 278.

⁴ *E.g.*, Glattfeld and Sander, *J. Amer. Chem. Soc.*, 1921, **43**, 2675.

⁵ Mester, *ibid.*, 1955, **77**, 5452.

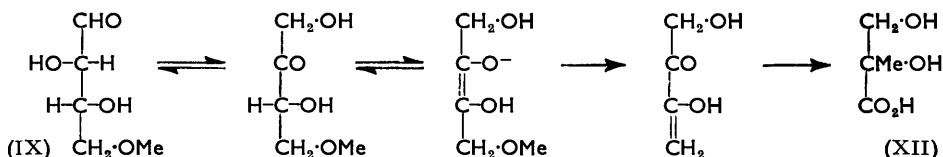
⁶ Jayme and Maris, *Ber.*, 1944, **77**, 483.

⁷ Head, *Shirley Inst. Mem.*, 1951, **25**, 220.

structure (VIII). However, earlier work^{8,9} on oxidised monosaccharides indicates that structures of type (VII) may also be formed. In the present case there is insufficient evidence to decide between the two possibilities but both formulæ (VII) and (VIII) contain a 6-membered hemiacetal ring which may be connected with a resistance to acid hydrolysis more reminiscent¹⁰ of a pyranoside than of the original furanoside (V). The resistance to hydrolysis may also depend on the inductive effect of the aldehyde group or its hydrate.

The difficulties inherent in hydrolysing this type of periodate oxidation product when the hydrolysis products are subject to further degradation by acid, have been emphasised by Smith and his co-workers,¹¹ and it appears to be a general rule that the resistance to acid hydrolysis, which must be connected either with the presence of the aldehyde groups or with some stable ring-form derived from them, is much diminished by reduction of the aldehyde, the alcohol produced being relatively easily hydrolysed.¹² Thus, for example, compound (II) was very readily hydrolysed.

The above effect has previously resulted in poor yields of D-erythrose from periodate-oxidised starch,¹³ and in the case of compound (VI) also a very poor yield of 4-O-methyl-D-threose (IX) (13.7% after chromatography) was obtained and characterised as the



phenylosazone. Degradation by lime-water in accordance with the expectations derived from the similar case of 4-O-methyl-D-glucose¹ yielded $\alpha\beta$ -dihydroxy- α -methylpropionic acid (XII). The acid was not resolved, but consumed two mols. of periodate in accordance with structure (XII) and was characterised as its brucine salt.

EXPERIMENTAL

The following were used in paper chromatography with Whatman No. 1 paper at 25°: solvent (a), butan-1-ol-ethanol-water¹⁴ (4:1:1:1.9); (b) ethyl acetate-acetic acid-water¹⁵ (10:1.3:1); (c) butan-1-ol-pyridine-benzene-water (4:2:1:1); spray (a), sodium metaperiodate, potassium permanganate;¹⁶ (b) silver nitrate, sodium hydroxide;¹⁷ (c) bromophenol-blue;¹⁸ (d) hydroxylamine, ferric chloride.¹⁹ For 2:3:4:6-tetra-O-methyl-D-glucose $R_G = 1.00$, and for lactic acid $R_L = 1.00$.

Preparation of 3-O-Methyl-L-glycerotetrolulose.—3-O-Methyl-1:2:4:5-di-O-isopropylidene-D-fructose was prepared from the appropriate di-O-isopropylidene-D-fructose with dimethyl sulphate and sodium hydroxide in dioxan²⁰ and had the properties recorded by Irvine and Scott.²¹

Partial Hydrolysis of 3-O-Methyl-1:2:4:5-di-O-isopropylidene-D-fructose.—When the

⁸ Hurd, Baker, Holysz, and Saunders, *J. Org. Chem.*, 1953, **18**, 186; Maclay, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1939, **61**, 1660.

⁹ Cf. Barry and Mitchell, *J.*, 1953, 3631.

¹⁰ Haworth, *Ber.*, 1932, **65**, 50.

¹¹ Abdel-Akher, Hamilton, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1952, **74**, 4970.

¹² See, e.g., Smith and Van Cleve, *ibid.*, 1955, **77**, 3091.

¹³ Overend, Stacey, and Wiggins, *J.*, 1949, 1358; Jackson and Hudson, *J. Amer. Chem. Soc.*, 1937, **59**, 2049.

¹⁴ Hough, Jones, and Wadman, *J.*, 1950, 1702.

¹⁵ Moilanen and Richtzenhain, *Acta Chem. Scand.*, 1954, **8**, 704.

¹⁶ Lemieux and Bauer, *Analyt. Chem.*, 1954, **26**, 920.

¹⁷ Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444.

¹⁸ Brown, *Biochem. J.*, 1950, **47**, 598.

¹⁹ Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.

²⁰ Cf. Lindberg and Wickberg, *Acta Chem. Scand.*, 1954, **8**, 569.

²¹ Irvine and Scott, *J.*, 1913, **103**, 564.

diisopropylidene derivative (30.5 g.) in 80% acetic acid (200 ml.) was kept at 25°, the specific rotation varied as follows :

Time (hr.)	0.1	1.0	2.0	3.0	4.3	5.0	6.0
$[\alpha]_D^{25}$	-125°	-123.5°	-121.5°	-120°	118° -	-117.5°	-117°

After 6 hr. the solution was evaporated to dryness under reduced pressure, and the residue diluted with water (200 ml.). After filtration from unchanged starting material (8.2 g., 27%), the aqueous solution was left at room temperature for 20 hr. over Amberlite resin IRA-400(OH) (10 g.) to remove traces of acid and any 3-O-methyl-D-fructose. The filtered solution was next extracted continuously with light petroleum (b. p. 40—60°) for 3 hr., then evaporated to yield 3-O-methyl-1 : 2-O-isopropylidene-D-fructose as a colourless syrup (19.03 g., 72.5%), having, when dried at 50°/0.01 mm. over P₂O₅, $[\alpha]_D^{25}$ -126° (*c* 5 in EtOH) (Found : C, 50.9; H, 7.9; OMe, 13.9. C₁₆H₁₈O₆ requires C, 51.25; H, 7.75; OMe, 13.2%).

Acetic anhydride in pyridine yielded a crystalline diacetate (96%), having, after recrystallisation from ethyl acetate-light petroleum, m. p. 97—98°, $[\alpha]_D^{21}$ -119° (*c* 1 in EtOH) (Found : C, 52.5; H, 6.95. C₁₄H₂₂O₈ requires C, 52.8; H, 7.0%).

Periodate Oxidation of 3-O-methyl-1 : 2-O-isopropylidene-D-fructose.—(a) Consumption of periodate by 3-O-methyl-1 : 2-O-isopropylidene-D-fructose (0.1290 g.) in 0.02M-sodium metaperiodate (50 ml.), measured by reaction with acidic potassium iodide and titration with sodium thiosulphate, was 0.70 (10 min.), 0.88 (1 hr.), 0.955 (2 hr.) mole/mole.

(b) An aqueous solution (100 ml.) of 3-O-methyl-1 : 2-O-isopropylidene-D-fructose (5.25 g.) and sodium metaperiodate (5.3 g.) was kept at room temperature 1 hr., then evaporated to dryness under reduced pressure. The residue was extracted several times with boiling dry ether, and the extracts on evaporation yielded a colourless, syrupy dialdehyde (5.10 g.). Part of this material (0.160 g.) was heated under reflux for 20 hr. with 2 : 4-dinitrophenylhydrazine (0.26 g.) in ethanol (50 ml.); addition of water then precipitated a bis-2 : 4-dinitrophenylhydrazone, which recrystallised from benzene-light petroleum as yellow needles, m. p. 183—185° (Found : C, 44.3; H, 4.2; N, 19.1. C₂₂H₂₄O₁₂N₈ requires C, 44.6; H, 4.1; N, 18.9%).

3-O-Methyl-L-glycerotetrolulose.—The above dialdehyde (4.90 g.) was heated in dry ether (100 ml.) with lithium aluminium hydride (1 g.) under reflux for 15 min. Methanol (10 ml.) was next added, dropwise, followed by water (50 ml.), and organic solvents were removed by distillation. Dilute sulphuric acid was added to pH 9 and the filtered solution evaporated to dryness. The residue was extracted with boiling acetone-ether (2 : 1; 5 × 50 ml.) and on evaporation the extracts yielded a syrupy diol (4.98 g.), acetylation and toluene-*p*-sulphonylation of which gave amorphous products.

The diol (4.44 g.) was hydrolysed by 0.05N-sulphuric acid (50 ml.) at 100° for 1 hr., and the solution neutralised with Amberlite resin IR-4B and evaporated to a colourless mobile syrup (3.69 g.). Paper chromatography in solvent a, with spray a, showed two spots (*R*_G 0.73, 0.67) corresponding to ethylene glycol and the supposed 3-O-methyl-L-glycerotetrolulose respectively. A solution of the syrup in water (40 ml.) was transferred to a carbon-Celite column (80 × 4 cm.) and a linear gradient elution²⁰ carried out from water (4 l.) to 20% ethanol (4 l.). Ethylene glycol was first eluted, followed by pure 3-O-methyl-L-glycerotetrolulose, which was obtained as a colourless syrup (1.50 g.) by evaporation of the relevant solutions after de-ionisation with mixed Amberlite resins IR-120(H) and IR-4B. When dried at 50°/0.1 mm. over P₂O₅ the tetrolulose had $[\alpha]_D^{19}$ -17.7° (*c* 5 in H₂O) (Found : OMe, 22.2. C₅H₁₀O₄ requires OMe, 23.1%).

The syrupy tetrolulose (0.40 g.) was heated with 15% acetic acid (10 ml.) containing phenylhydrazine (1.0 ml.) at 70° for 1 hr. 3-O-Methyl-L-glycerotetrosazone rapidly separated as a yellow powder (0.94 g.), and when twice recrystallised from aqueous ethanol had m. p. 157—158° (Found : C, 65.4; H, 6.2; N, 17.7. C₁₇H₂₀O₂N₄ requires C, 65.3; H, 6.5; N, 17.95%).

Action of Lime-water on 3-O-Methyl-L-glycerotetrolulose.—(a) *Quantitative.* The alkalinity of a solution of the tetrolulose (0.0940 g.) in oxygen-free 0.040N-lime-water (50 ml.) at 25° fell to a constant value, which after 94 hr. corresponded to the formation of 0.96 equiv. of acid. Paper chromatography in solvent a, with spray a, indicated disappearance of the original tetrolulose (*R*_G 0.67), intermediate formation and disappearance of a supposed 3-O-methyltetrose (*R*_G 0.62), and rapid formation and persistence of a small amount of an unidentified substance (*R*_G 0.44).

(b) *Qualitative.* A solution of 3-O-methyl-L-glycerotetrolulose (0.64 g.) in oxygen-free 0.04N-lime-water (200 ml.) was kept at room temperature overnight, then saturated with carbon dioxide and concentrated to ca. 50 ml. After filtration from calcium carbonate the solution

was evaporated to dryness. The pale yellow, solid residue was extracted with boiling acetone, leaving the mixed calcium salts (0.57 g.) as a yellowish powder. Paper chromatography in solvent b of the salts, after treatment with Amberlite resin IR-120(H), indicated the presence of $\alpha\gamma$ -dihydroxybutyric acids (R_L 0.66; sprays c and a), the corresponding lactones (R_L 0.98, sprays d and a), and lactic acid (R_L 1.00, sprays c, a). The salts also gave qualitative tests for formic²² and lactic acid.²³ Traces of an unidentified acid (R_L 0.73) and lactone (R_L 0.86) were also observed.

The consumption of periodate by the acids from the action of Amberlite resin IR-120(H) on the calcium salts (0.0251 g.) in 0.005M-sodium metaperiodate (50 ml.) containing 0.1N-sodium hydroxide (1 ml.), was measured by reaction with acidic potassium iodide and titration with sodium thiosulphate. The consumption was: 0.40 (1 hr.), 0.66 (18 hr.), 0.78 (70 hr.), 0.84 (165 hr.) mole/mole.

An aqueous solution of the calcium salts (0.251 g.) was shaken with excess of Amberlite resin IR-120(H) (2 g.) and filtered, and filtrate and washings were diluted to ca. 50 ml. and evaporated to dryness under reduced pressure, the distillate being collected at -10° . More water (5 ml.) was added and the whole again distilled to dryness, and this was repeated six times. The combined distillates when titrated with 0.025N-sodium hydroxide corresponded to 11.2% of the total acidity and gave qualitative tests for formic acid.²² The syrupy residue was dried over P_2O_5 and then repeatedly extracted with 2:1 ether-light petroleum (b. p. 40–60°) at the b. p. with decantation. The extracts on evaporation yielded a colourless oil (0.015 g.) corresponding on the paper chromatogram to lactic acid, and yielding 4-bromophenacyl lactate, m. p. and mixed m. p. 110–112°.

The residue after ether-extraction was dissolved in ethanol (10 ml.) and benzene (10 ml.), aniline (0.2 ml.) was added, and the solution concentrated to a thin syrup on the boiling-water bath. The residue was dried over P_2O_5 , then dissolved in benzene (10 ml.) and chloroform (5 ml.) and transferred to a bentonite-keiselguhr column (30 \times 1.5 cm.).²⁴ The column was eluted with chloroform followed by chloroform-ethanol (19:1), and evaporation of the latter eluate yielded a few colourless prisms which, when recrystallised from acetone-ether at -20° , had m. p. 114–116° alone or in admixture with (\pm)- $\alpha\gamma$ -dihydroxybutyranilide (kindly supplied by Dr. J. W. Green of the Institute of Paper Chemistry, Wisconsin, U.S.A.).

A further sample of the mixed calcium salts (0.250 g.) was dissolved in water (10 ml.) and treated with Amberlite resin IR-120(H). The solution was filtered, neutralised with zinc carbonate, boiled for a few minutes, again filtered, concentrated to ca. 2 ml., and kept at 0° overnight. A small amount of crystalline zinc lactate was then separated by filtration, the filtrate treated with Amberlite resin IR-120(H), and after removal of the resin, heated at 100° for 16 hr. with excess of brucine. The resulting solution was filtered, washed with chloroform, and evaporated to dryness leaving a pale yellow amorphous residue (0.83 g.) which was dissolved in ethanol and fractionally precipitated with ether. Recrystallisation of the earlier fractions from ethanol yielded brucine (\pm)- $\alpha\gamma$ -dihydroxybutyrate, m. p. 167–169° (Found: N, 5.3. Calc. for $C_{27}H_{34}O_8N_2$: N, 5.5%). Glattfeld and Sander⁴ report m. p. 169°.

Preparation of 4-O-Methyl-D-threose.—*Methyl 6-O-methyl-D-galactofuranoside.* A solution of 6-O-methyl-D-galactose (20.5 g.) in dry methanol (250 ml.) containing 1% of hydrogen chloride showed the following changes in optical rotation at room temperature: $[\alpha]_D^{19} +46^\circ$ (1 hr.); $+28^\circ$ (2.5 hr.); $+2^\circ$ (4.5 hr.); -11° (6 hr.); -18° (7 hr.); -20° (7.5 hr.). At this stage the solution was neutralised with silver carbonate, filtered, and evaporated to a syrup, which distilled almost completely at 160–170° (bath-temp.)/0.05 mm. *Methyl 6-O-methyl- $\alpha\beta$ -D-galactofuranoside* so obtained (17.6 g., 80%) had n_D^{17} 1.4810, $[\alpha]_D^{19} -23^\circ$ (*c* 2 in MeOH) (Found: OMe, 29.5. $C_8H_{16}O_6$ requires OMe, 29.8%).

The consumption of periodate by this product (0.1050 g.) in 0.02M-sodium metaperiodate (50 ml.) at room temperature, measured by reaction with acidic potassium iodide and titration with sodium thiosulphate, was 0.19 (0.1 hr.), 0.55 (1.0 hr.), 0.86 (3.0 hr.), 0.95 (5.0 hr.) mole/mole. In a similar large-scale reaction an aqueous solution (50 ml.) of methyl 6-O-methyl- $\alpha\beta$ -D-galactofuranoside (5.01 g.) was treated with a solution of sodium metaperiodate (7.7 g., 100 ml.), adjusted to pH 7 with sodium hydrogen carbonate solution, and kept at room temperature for 2 hr. The solution was then evaporated to dryness and the residue extracted with boiling

²² Hopton, *Analyt. Chim. Acta.*, 1953, **8**, 429.

²³ Eegriwe, *Z. analyt. Chem.*, 1933, **95**, 324.

²⁴ Elvidge and Whalley, *Chem. and Ind.*, 1955, 589.

ethanol-ether (1:1). Evaporation of the extracts yielded a colourless syrup which was redissolved in water; the solution was de-ionised with Amberlite resins IR-120(H) and IR-4B, and evaporated to a syrupy dialdehyde (4.70 g., 94%), $[\alpha]_D^{19} + 4^\circ$ (*c* 1 in H₂O), giving a single spot on a paper chromatogram (R_F 0.75, solvent *c*; R_F 0.77, solvent *a*).

4-O-Methyl-D-threose. The dialdehyde (10.0 g.) was steam-distilled with 0.5*N*-sulphuric acid (100 ml.) at constant volume for 1 hr. Milder treatments caused very little hydrolysis, while more drastic treatment resulted in extensive decomposition. The resulting solution was neutralised with barium carbonate, filtered, and evaporated to dryness, and an aqueous solution (75 ml.) of the residue (8.57 g.) added to a carbon-Celite column (45 × 5 cm.). Elution with aqueous ethanol whose concentration was increased by a linear gradient²⁰ from 0 to 50% in 12 l., yielded fractions: (i) Inorganic and organic salts (1.5 g.). (ii) Pure 4-*O*-methyl-*D*-threose (0.89 g.), R_G 0.80 in solvent *c* (see below). (iii) Mixtures of (ii) with the unchanged dialdehyde (1.9 g.). (iv) Unchanged dialdehyde (2.3 g.), R_G 0.94, solvent *c*.

4-O-Methyl-D-threose was a colourless syrup, $[\alpha]_D^{18} + 3^\circ$ (*c* 2 in H₂O) (Found: OMe, 21.9. C₅H₁₀O₄ requires OMe, 23.1%), which readily yielded a *phenylosazone* (crude yield, 97%), recrystallising from aqueous ethanol as a yellow powder, m. p. 121–124° (Found: C, 65.7; H, 6.4; N, 18.1. C₁₇H₂₀O₂N₄ requires C, 65.3; H, 6.5; N, 17.95%).

Action of Lime-water on 4-O-Methyl-D-threose.—(a) *Quantitative.* Formation of acid in a solution of 4-*O*-methyl-*D*-threose (0.1057 g.) in oxygen-free 0.045*N*-lime-water (50 ml.) at 25° reached a constant value of 1.09 equiv./mole after 165 hr. The final lime-water solution, when treated with Amberlite resin IR-120(H) and examined by paper chromatography in solvent *b*, with sprays *a* and *c*, gave only a single spot of R_L 0.70.

(b) *Qualitative.* A solution of 4-*O*-methyl-*D*-threose (0.41 g.) in oxygen-free 0.04*N*-lime-water (250 ml.) was kept at 25° for 2 days, then saturated with carbon dioxide, concentrated to ca. 10 ml., filtered, diluted with ethanol (100 ml.), and kept at 0° overnight. The calcium salts which separated were washed with ethanol and dried to a pale yellow powder (0.30 g.) (Found: C, 34.0; H, 4.7. Calc. for C₄H₇O₄Ca_½: C, 34.5; H, 5.1%).

A solution of the calcium salts (0.15 g.) in water (10 ml.) was passed through Amberlite resin IR-120(H) and then heated on the boiling-water bath overnight with powdered brucine (0.5 g.). The solution was then cooled, filtered from excess of brucine, washed with chloroform, and evaporated to dryness. The residue crystallised on trituration with ethanol and recrystallised from the same solvent as needles, m. p. 194–195° $[\alpha]_D^{18} - 26^\circ$ (*c* 1 in H₂O) (Found: C, 62.7; H, 6.4; N, 5.2. Calc. for C₂₇H₃₄O₈N₂: C, 63.0; H, 6.6; N, 5.5%). Glattfeld and Sherman²⁵ recorded $[\alpha]_D$, *ca.* -27° , but no m. p. for racemic brucine $\alpha\beta$ -dihydroxy- α -methylpropionate.

A solution of a further sample of the calcium salts (0.0170 g.) after passage through a column of Amberlite resin IR-120(H) was treated with 0.05*M*-sodium metaperiodate (10 ml.) and 0.1*N*-sodium hydroxide (1 ml.) and then diluted to 50 ml. The consumption of periodate was determined in aliquot portions by reaction with acidic potassium iodide and titration with sodium thiosulphate as follows: 0.50 (0.8 hr.); 0.82 (17 hr.) [at this point *N*-sodium hydroxide (0.5 ml.) was added to the remaining 40 ml. (cf. ref. 26)]; 1.27 (22.5 hr.); 1.48 (41 hr.); 1.68 (65 hr.); 1.79 (137 hr.). In a separate experiment an attempt to identify the products of the oxidation resulted only in the isolation of formaldehyde as its 2:4-dinitrophenylhydrazone, m. p. and mixed m. p. 167–168°.

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²⁵ Glattfeld and Sherman, *J. Amer. Chem. Soc.*, 1925, **47**, 1742, and earlier references.

²⁶ Sprinson and Chargaff, *J. Biol. Chem.*, 1946, **164**, 433.