mended as the most convenient general reagent for amination.

Experimental Section[6]

Ethyl *O*-(Mesitylenesulfonyl)acetohydroxamate (5a).—Mesitylenesulfonyl chloride (72 g) was added to a solution of ethyl acetohydroxamate[5] (4) (34 g) and triethylamine (33 g) in dimethylformamide (90 ml) in portions with stirring under ice cooling. After addition was complete, the reaction mixture was stirred for 20 min at 0° and then poured into ice water. A white precipitate was filtered and recrystallized from petroleum ether (bp 30–60°) to give colorless needles (83 g, 86%) of **5a**: mp 57–58°; ir (KCl) 1635, 1600, 1200, 1180, and 670 cm^{-1}; nmr ($CDCl_3$) τ 8.85 (3 H, t, J = 8 Hz), 8.02 (3 H, s), 7.75 (3 H, s), 7.42 (6 H, s), 6.15 (2 H, q, J = 8 Hz), and 3.08 (2 H, b s).

Anal. Calcd for $C_{13}H_{19}NO_4S$: C, 54.73; H, 6.71; N, 4.91. Found: C, 54.66; H, 6.44; N, 4.95.

Ethyl *O*-(2,4,6-Triisopropylbenzenesulfonyl)acetohydroxamate (5b).—Using the procedure described above for **5a**, **5b** was prepared from **4** (10.3 g) and 2,4,6-triisopropylbenzenesulfonyl chloride (30.3 g). Recrystallization from ethanol–water (20:1) gave colorless prisms (32 g, 87%) of **5b**: mp 75–76°; ir (KCl) 1630, 1600, 1350, 1195, and 670 cm^{-1}; nmr ($CDCl_3$) τ 8.88 (3 H, t, J = 6.8 Hz) 8.79 (18 H, d, J = 6.8 Hz), 8.00 (3 H, s), 7.13 (1 H, m), 6.09 (2 H, m), 5.83 (2 H, m), and 2.86 (2 H, s).

Anal. Calcd for $C_{19}H_{31}NO_4S$: C, 61.76; H, 8.46; N, 3.79. Found: C, 61.77; H, 8.34; N, 3.85.

Ethyl *O*-(*o*-Nitrobenzenesulfonyl)acetohydroxamate (5c).—Using the procedure described for **5a**, **5c** was prepared from **4** (1.0 g) and *o*-nitrobenzenesulfonyl chloride (2.1 g). Recrystallization from ligroin afforded white prisms (2.0 g, 73%) of **5c**: mp 79–80°; ir (KCl) 1615, 1530, 1380, 1325, and 1195 cm^{-1}; nmr ($CDCl_3$) τ 8.83 (3 H, t, J = 6.9 Hz), 7.96 (3 H, s), 6.09 (2 H, q, J = 6.9 Hz), and 2.28 (4 H, m).

Anal. Calcd for $C_{10}H_{12}N_2O_6S$: C, 41.67; H, 4.20; N, 9.72. Found: C, 41.68; H, 4.16; N, 9.81.

Ethyl *O*-Picrylacetohydroxamate (5e).—Utilizing the previously reported procedure for the preparation of ethyl *O*-2,4-dinitrophenylacetohydroxamate,[7] **5e** was prepared from **4** (6.2 g) and picryl chloride (15.0 g). Recrystallization from ethanol afforded pale yellow needles (15.3 g, 81%) of **5e**: mp 93.5–94.5°; ir (KCl) 1600, 1525, 1460, and 1340 cm^{-1}; nmr ($CDCl_3$) τ 8.75 (3 H, t, J = 6.9 Hz), 7.88 (3 H, s), 6.10 (2 H, q, J = 7.2 Hz), and 1.28 (2 H, s).

Anal. Calcd for $C_{10}H_{10}N_4O_8$: C, 38.22; H, 3.21; N, 17.83. Found: C, 38.34; H, 3.27; N, 17.55.

***O*-Mesitylenesulfonylhydroxylamine (3a).**—To a solution of **5a** (75 g) in dioxane (50 ml) was added 70% perchloric acid (30 ml) with stirring at 0° over 10 min. The reaction mixture was poured into ice water to give a white solid, which was filtered and washed with water. Although the product (64 g) thus obtained contains 20% of water (by iodometry), it can be used simply by filtration of a methylene chloride solution to remove water separated. The solid was dissolved in ether and precipitated by the addition of petroleum ether to give white needles of **3a**: mp 93–94°; ir (KCl) 3340, 3250, 1600, 1350, 1190, 1180, and 780 cm^{-1}; acetone oxime mp 95–96° (lit.[2b] mp 95–96.5°).

***O*-(2,4,6-Triisopropylbenzenesulfonyl)hydroxylamine (3b).**—To a solution of **5b** (30 g) in dioxane (50 ml) was added 70% perchloric acid (30 ml) with stirring at 0° over 10 min. The reaction mixture was stirred for an additional 2 hr and poured into ice water. A white precipitate was treated as described for **3a** to give white crystals of **3b** (30 g, containing 31% of water): mp 137–138°; ir (KCl) 3340, 3260, 1600, 1350, 1200, 1190, and 665 cm^{-1}; acetone oxime mp 112–113° (from ethanol).

Anal. Calcd for $C_{15}H_{29}NO_3S$: C, 63.69; H, 8.61; N, 4.13. Found: C, 63.84; H, 8.84; N, 4.03.

***O*-(*o*-Nitrobenzenesulfonyl)hydroxylamine (3c).**—Using the same procedure described for **3a**, **3c** was prepared from **5c**[11] (3.0 g). Yellow crystals (0.35 g) of **3c** were obtained and characterized by its ir spectrum: ir (KCl) 3340, 3250, 1600, 1530, 1370, and 1200 cm^{-1}. Because this compound was found to decompose on exposure to air, no further investigation was carried out.

***O*-(2,4-Dinitrophenyl)hydroxylamine (3d).**—Using the same procedure described for **3a**, **3d** was prepared from **5d** (21.7 g). Recrystallization from ethanol gave pale yellow needles (12.5 g, 78%) of **3d**: mp 112–113° (lit.[2d] mp 112°); ir (KCl) 3325, 3250, 1600, 1510, and 1340 cm^{-1}; nmr ($CDCl_3$) τ 3.63 (2 H, b s, NH_2), 2.03 (1 H, d, J = 10 Hz), 1.62 (1 H, dd, J = 10 and 3 Hz), and 1.26 (1 H, d, J = 3 Hz).

***O*-Picrylhydroxylamine (1e).**—Using the same procedure described for **3a**, **3e** was prepared from **5e** (1.45 g). Recrystallization from chloroform gave yellow prisms of **3e** (0.7 g, 62%): mp 98–100° dec; ir (KCl) 3300, 3250, 1610, 1530, and 1350 cm^{-1}.

Anal. Calcd for $C_6H_4N_4O_7$: C, 29.52; H, 1.65; N, 22.95. Found: C, 29.56; H, 1.79; N, 23.08.

The acetone oxime had mp 122–123° (from ethanol).

Anal. Calcd for $C_9H_8N_4O_7$: C, 38.03; H, 2.84; N, 19.72. Found: C, 38.03; H, 2.89; N, 19.93.

Reactions of 3a,b,d,e,f with Nucleophiles. General Procedure.—To a stirred solution of substrate (tri-*n*-butylamine, pyridine, diphenyl sulfide, diphenyl sulfoxide, and triphenylphosphine) (1 mmol) in methylene chloride (5 ml) was added a solution of **3** (1 mmol) in methylene chloride (5 ml) at 0°. After the reaction mixture was allowed to stand at room temperature for 10 min, ether or petroleum ether was added to precipitate the product. In some cases this procedure was modified (see the footnotes of Table I). The results are summarized in Tables I and II.

Registry No.—**3a**, 36016-40-7; **3b**, 38202-21-0; **3c**, 38202-22-1; **3d**, 17508-17-7; **3e**, 38100-34-4; **3f**, 37477-17-1; **4**, 10576-12-2; **5a**, 38202-27-6; **5b**, 38202-28-7; **5c**, 38202-29-8; **5e**, 38202-30-1; **7b**, 38202-31-2; **7d**, 38202-32-3; **7f**, 38202-33-4; **8b**, 38202-34-5; **8d**, 38202-35-6; **9b**, 38229-23-1; **9b**, 38202-36-7; **9e**, 38215-55-3; **11a**, 38215-56-4; **11b**, 38309-16-9; **11d**, 38229-24-2; $(n\text{-Bu})_3N$, 102-82-9; pyridine, 110-86-1; Ph_2S, 139-66-2; $Ph_2S(O)$, 945-51-7; Ph_3P, 603-35-0; triethylamine, 121-44-8; 2,4,6-triisopropylbenzenesulfonyl chloride, 6553-96-4; *o*-nitrobenzenesulfonyl chloride, 1694-92-4; picryl chloride, 88-88-0; *O*-picryl acetone oxime, 13194-03-1; *O*-(2,4,6-triisopropylbenzenesulfonyl) acetone oxime, 38215-59-7.

(6) Melting points are uncorrected. Nmr spectra were recorded on a Hitachi R-20 spectrometer using TMS as an internal standard. Infrared spectra were recorded on a Hitachi EPI-G2 instrument.

(7) A. O. Ilvespää and Q. Marxer, *Helv. Chim. Acta*, **46**, 2009 (1963).

Synthesis of Some 5-Carboxy-5-hydroxymethyl-1,3-dioxanes

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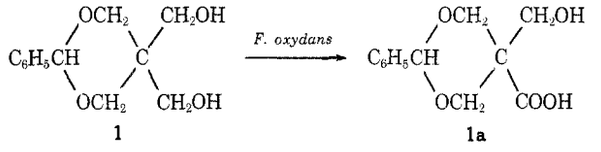
Oxidation of pentaerythritol with a mutant strain of *Flavobacterium oxydans* produces tris(hydroxymethyl)acetic acid, whereas the parent strain completely degrades pentaerythritol.[1]

Recent studies on the limits of oxidizing capability of the bacterium indicated that freeze-dried cells of the parent and mutant strains oxidized 2-phenyl-5,5-bis(hydroxymethyl)-1,3-dioxane (**1**) to 2-phenyl-5-carboxy-5-hydroxymethyl-1,3-dioxane (**1a**).[2]



1 **1a**

(1) C. T. Goodhue and J. R. Schaeffer, *Biotechnol. Bioeng.*, **11**, 1173 (1969).

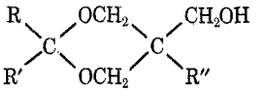
(2) The conversion of **1** to **1a** was first observed during an unpublished investigation carried out in collaboration with Dr. C. T. Goodhue, of thees laboratories.

This observation suggested the possibility of an alternative synthesis of tris(hydroxymethyl)acetic acid by oxidation of a derivative of pentaerythritol with the parent strain of the bacterium. We undertook the synthesis of several acetal and ketal derivatives of tris(hydroxymethyl)acetic acid from the corresponding derivatives of pentaerythritol with freeze-dried cells of the parent strain as the oxidizing agent in order to examine the feasibility of this synthesis.[3]

In connection with this study we wish to report that with freeze-dried parent cells, in addition to the oxidation of **1** to **1a**, 2-isopropyl-5,5-bis(hydroxymethyl)-1,3-dioxane (**2**), 3,3-bis(hydroxymethyl)-1,5-dioxaspiro[5.5]undecane (**3**), and 2,2-dimethyl-5,5-bis(hydroxymethyl)-1,3-dioxane (**4**) were oxidized to the corresponding monocarboxylic acids, which were identified as their trimethylsilyl derivatives by a combination of gas chromatography and mass spectrometry (Table I).

TABLE I

MASS SPECTRA OF TRIMETHYLSILYLATED OXIDATION PRODUCTS AND AUTHENTIC SAMPLES



Substituents	Peak temp on glc, °C	Peaks in mass spectrum of oxidation product and authentic sample	
1a, R = C_6H_5; R′ = H; R″ = COOH	245	382	261
		381	231
		367	147
		297	105
			103
2a, R = $(CH_3)_2CH$; R′ = H; R″ = COOH	201	333	147
		305	103
		233	75
		231	73
3a, R = R′ = $(CH_2)_5$; R″ = COOH	190	319	75
		247	73
		159	59
			43
4a, R = R′ = CH_3; R″ = COOH	232	374	
		331	
		73	
4b, R = R′ = CH_3; R″ = H[a]	152	203	
		103	
		73	

[a] Authentic sample was not available. Molecular weight and cracking pattern are consistent with **4b**.

Although **4** was oxidized to **4a**, both compounds gradually disappeared from the oxidation medium over a 7-day period. During the same period of incubation, a new compound, 2,2-dimethyl-5-hydroxymethyl-1,3-dioxane (**4b**), was formed and accumulated as the only final product.

Synthesis of the dioxanes with freeze-dried mutant cells was also investigated. Higher yields of monocarboxylic acids were obtained with mutant cells than with parent cells (Table II). With mutant cells ketals **3** and **4** were converted to tris(hydroxymethyl)acetic acid in addition to **3a** and **4a**.

(3) Acetals and ketals of pentaerythritol were examined because of their ease of synthesis and hydrolysis.

Decarboxylation product **4b** was not produced when **4** was oxidized with mutant cells.

Addition of calcium carbonate to the incubation media improved the yields of monocarboxylic acids in all cases studied (Table II); however, a slight decrease in the yield of **4b** was observed. Suppression of tris(hydroxymethyl)acetic acid production from **3** and **4** occurred with mutant cells in the presence of carbonate.

In one case, that of oxidation of **2** to **2a**, the product was isolated from a scaled-up preparation with parent cells. The yield of pure acid was 15.5%.

The variation observed in the yields of the different acids (Table II) is due to a combination of enzymatic and hydrolytic stability[4] effects.

TABLE II

YIELDS OF ACETALS AND KETALS OF TRIS(HYDROXYMETHYL)ACETIC ACID

	Per cent yield (68 hr)			
	Parent cells		Mutant cells	
Oxidation product	With $CaCO_3$	Without $CaCO_3$	With $CaCO_3$	Without $CaCO_3$
1a	41	25[a]	87	54[b]
2a	37	29[a]	78	67[a]
3a	79	36[b]	91	37
4a	35	29	91	48

[a] 92 hr. [b] 164 hr.

Enzymatic effects are evidenced by the generally lower yields of acids obtained with parent cells compared with those produced by mutant cells. Accumulation of **4b** probably occurred through decarboxylation of the normal oxidation product **4a**. It is reasonable to propose that decarboxylation of **4a** resulted from some unique effect of **4** or **4a** on the enzymes in the parent organism or from differences in the enzymes in the two organisms.

Evidence of hydrolytic stability effects on product yield can be found in the observation that the ketals appear less stable than the acetals. Lower yields of products and substantial amounts of tris(hydroxymethyl)acetic acid were observed in the oxidation mixtures of **3** and **4** with mutant cells, whereas higher yields of products accompanied by trace amounts of tris(hydroxymethyl)acetic acid were observed with **1** and **2** under the same conditions.

The general yield improvement obtained with carbonate may be due to greater acetal and ketal stability at a higher pH. When carbonate was present in the media, the pH ranged from 7.4 to 8.2. In the absence of carbonate, incubation media with parent cells had a pH between 5.2 and 6.6 and with mutant cells between 6.5 and 6.8. It should be pointed out, however, that carbonate also maintains the pH of the medium at a level where the oxidizing enzymes appear to work more efficiently.[1]

The utility of this synthesis as a method of preparation of 5-carboxy-5-hydroxymethyl-1,3-dioxanes is limited to those acetal and ketal derivatives of pentaerythritol and tris(hydroxymethyl)acetic acid that are stable enough to survive both the oxidation and method of isolation.

(4) E. Berlow, R. H. Barth, and J. E. Snow, "The Pentaerythritols," Reinhold, New York, N. Y., 1958, p 143.

Experimental Section[5]

2-Isopropyl-5,5-bis(hydroxymethyl)-1,3-dioxane (2).—Isobutyraldehyde (26.5 g, 0.38 mol) and 23 ml of concentrated hydrochloric acid were added to a stirred solution of 50 g (0.37 mol) of pentaerythritol in 2.9 l. of water. After the mixture had been stirred for 24 hr at room temperature, insoluble solids were removed and the pH was adjusted to 8 with solid sodium carbonate. Water was evaporated and the solid residue was extracted with 800 ml of boiling *p*-xylene. After filtration, the extract stood overnight at 5°. The product weighed 25.3 g (36.4%), mp 97–99°.

Anal. Calcd for $C_9H_{18}O_4$: C, 56.8; H, 9.5; mol wt, 190.2. Found: C, 56.7; H, 9.4; mol wt, 188.

3,3-Bis(hydroxymethyl)-1,5-dioxaspiro[5.5]undecane (3).—The compound was prepared in 3.5% yield by the procedure of Issidroides and Gulen,[5] mp 125–127°.

Anal. Calcd for $C_{11}H_{20}O_4$: C, 61.1; H, 9.3; mol wt, 216.2. Found: C, 61.1; H, 9.1; mol wt, 216.

2-Phenyl-5-carboxy-5-hydroxymethyl-1,3-dioxane (1a).—Dioxane **1a** was prepared according to a procedure published by Sulzbacher, *et al.*,[6] in 67.2% yield, mp 176–178°.

Anal. Calcd for $C_{12}H_{14}O_5$: C, 60.5; H, 6.0; mol wt, 238.2. Found: C, 60.5; H, 6.1; mol wt, 229.

2-Isopropyl-5-carboxy-5-hydroxymethyl-1,3-dioxane (2a).—The dioxane was prepared in 29% yield, mp 149–151°.

Anal. Calcd for $C_9H_{16}O_5$: C, 52.9; H, 7.9; mol wt, 204. Found: C, 52.9; H, 7.9; mol wt, 226.

3-Carboxy-3-hydroxymethyl-1,5-dioxaspiro[5.5]undecane (3a).—Compound **3a** was prepared in 47.3% yield, mp 132–134°.

Anal. Calcd for $C_{11}H_{18}O_5$: C, 57.4; H, 7.9; mol wt, 230.2. Found: C, 57.8; H, 7.9; mol wt, 247.

2,2-Dimethyl-5-carboxy-5-hydroxymethyl-1,3-dioxane (4a).—Compound **4a** was prepared in 79% yield with a 20:1 molar ratio of ketone to acid, mp 128–130°.

Anal. Calcd for $C_8H_{14}O_5$: C, 50.5; H, 7.4; mol wt, 190.2. Found: C, 50.5; H, 7.5; mol wt, 185.

Preparation of Cells.—Growth of the parent strain was carried out on a medium containing 10.0 g of pentaerythritol, 2.0 g of acetic acid, 10 g of ammonium sulfate, 1.0 g of dipotassium hydrogen phosphate, 1.0 g of yeast extract, and 10 ml of mineral salts solution in 1 l. of distilled water adjusted to pH 7 with potassium hydroxide prior to sterilization.

The medium used for growth of the mutant strain has been reported.[1]

Flask culturing was carried out at 30° in 2.8-l. Fernbach flasks fitted with gauze closures. The inoculum was prepared in 25-ml erlenmeyer flasks fitted with Morton closures. The flasks were inoculated under sterile conditions from slants of either the parent strain[7] or the mutant strain (ATCC No. 21,245) and shaken on a rotary shaker at 400 rpm for 72 hr. Fernbach flasks were shaken at 150 rpm for 72 hr, after addition of a 5% (v/v) inoculum. Cells were harvested by centrifugation with a Sorvall refrigerated centrifuge operated at 9000 rpm for 20 min at 5°, freeze-dried in 0.4% potassium phosphate buffer, and stored at 5°.

Analysis of Oxidation Mixtures.—Acetals and ketals were estimated as the trimethylsilyl derivatives by gas–liquid chromatography.[1] Trimethylsilyl derivatives were prepared directly from freeze-dried samples of the oxidation mixtures. Standard curves were prepared with the chemically synthesized compounds using *n*-octadecane as the internal standard, with the exception of compounds **3** and **3a**, in which case *n*-dodecane was used. The accuracy of the estimation was 10%.

Biooxidation Reactions.—The oxidations were performed in 125-ml erlenmeyer flasks fitted with Morton closures. Cells (200 mg) and compound (225 mg) were suspended in 25 ml of nonsterile phosphate buffer. The mixtures were incubated at 30° on a shaker operated at 400 rpm. One-milliliter samples were removed at 24-hr intervals over a period of 7 days and analyzed.

Yield of product was reported based on the highest concentration observed during the incubation.

Product Identification.—Oxidation products were identified by comparison of the mass spectra of trimethylsilylated products isolated by gas–liquid chromatography with those of trimethylsilylated authentic samples (Table I).

Isolation of 2a.—Compound **2a** was prepared by a 20-fold scale-up of the biooxidation procedure. The cells were removed by centrifugation at 9000 rpm for 20 min at 5°. Ion exchange of the clarified solution was carried out on a column of Dowex 1 × 8 resin (100 ml in formate form). Elution was made with 4 *N* formic acid. Fractions containing pure product were freeze-dried. The product weighed 0.84 g (15.5%), mp 154–156°. A mixture melting point with an authentic sample was not depressed.

Registry No.—**1**, 2425-41-4; **1a**, 37951-01-2; **2**, 37951-03-4; **2a**, 37951-04-5; **3**, 714-88-5; **3a**, 38165-52-5; **4**, 770-74-1; **4a**, 16837-15-3; isobutyraldehyde, 78-84-2; pentaerythritol, 115-77-5.

Acknowledgment.—We wish to thank Dr. C. T. Goodhue for helpful advice, Mr. H. A. Risley for several samples of mutant cells, Mr. D. P. Maier for interpretation of the mass spectra, and Mr. R. E. Sceusa for technical assistance.

(5) Melting points are uncorrected. Molecular weights were obtained in acetone by the ebulliometric method. All evaporations were carried out under reduced pressure. The drying agent was sodium sulfate. Compounds **1** and **4** were prepared by published procedures [C. H. Issidroides and R. Gulen in "Organic Syntheses," Collect. Vol. IV, Wiley, New York, N. Y., 1963, p 679; L. Orther and G. Freyss, *Justus Liebigs Ann. Chem.*, **484**, 131 (1930)]. Gas chromatography was carried out with an F & M Model 810 gas chromatograph equipped with a thermal conductivity detector. The column was stainless steel 6 ft × 0.125 in. o.d. packed with SE-30 on Chromosorb W. A 30°/min column temperature rise from 100 to 300° was employed. Mass spectra were determined on either a Hitachi Perkin-Elmer RMS-4 or a C. E. C. 21-110B mass spectrometer.

(6) M. Sulzbacher, E. Bergman, and E. R. Pariser, *J. Amer. Chem. Soc.*, **70**, 2828 (1948); dioxanes **2a**, **3a**, and **4a** were also prepared by this method.

(7) This strain is maintained by Dr. C. T. Goodhue, Research Laboratories at Kodak Park Division of Eastman Kodak Co., Rochester, N. Y. 14650.

A Regiospecific Synthesis of 4-Chloroalkylbenzenes

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Although 4-chloro-1,2-dimethylbenzene (**1**) has been prepared in several different ways, each route has disadvantages as a practical synthetic method. Direct chlorination in the presence of Lewis acids results in formation of approximately equal amounts of the 3- and 4-chloro isomers.[1,2] These have been separated only by sulfonation, formation and fractional crystallization of the barium salts of the sulfonic acids, conversion of the barium salts to the sodium salts, and then desulfonation.[1] A multistep synthesis terminating in a Sandmeyer reaction of 3,4-dimethylbenzenediazonium chloride produces pure **1**,[3] but the yields are poor since nitration of *o*-xylene in the first step gives more 3- than 4-nitro-1,2-dimethylbenzene.[4,5]

Similar difficulties occur in the synthesis of other 4-haloalkylbenzenes, although separation of isomers is often simpler than in the case of **1**.

This paper reports a regiospecific synthesis of 4-

(1) A. Krüger, *Chem. Ber.*, **18**, 1755 (1885).

(2) A. Claus and O. Bayer, *Justus Liebigs Ann. Chem.*, **274**, 305 (1893).

(3) D. R. Lyon, F. G. Mann, and G. H. Cookson, *J. Chem. Soc.*, 662 (1947).

(4) A. W. Crossley and N. Renouf, *ibid.*, 202 (1909).

(5) Formation of **1** by reaction of 3,4-dimethylbenzenesulfonic acid with cuprous chloride has been reported: P. S. Varma, N. B. Parekh, and V. K. Subramanium, *J. Indian Chem. Soc.*, **16**, 460 (1939). I have been unable to reproduce this work.