Enantiotopically Selective Oxidation of α,ω-Diols with the Enzyme Systems of Microorganisms

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Gluconobacter were found to be capable of oxidizing prochiral diols such as 2-substituted propane-1,3-diols **1** and 3-substituted pentane-1,5-diols **4** with distinction of *pro-R* and *pro-S* sites of the molecules, in that (−)-(*R*)-α-substituted β-hydroxypropionic acids **2** and (+)-(3*S*)-3-substituted δ-valerolactones **5** were obtained, respectively. Oxidation of 3-methylpentane-1,3,5-triol **11** afforded unnatural (+)-(*S*)-mevalonolactone **12**. The steric bulkiness of the substituents on the prochiral center and the distance from the hydroxy group greatly affected the rate and the enantioselectivity of the reaction.

One of the advantages of utilizing enzyme systems in organic transformations is the fact that they are "chiral catalysts". Thus, the application of these systems provides a way to obtain optically active compounds starting from prochiral molecules.[1] Recent developments in asymmetric synthesis, especially in reductive and carbon–carbon bond-forming reactions, are remarkable. On the other hand, successes in asymmetric oxidation are relatively rare,[2] probably due to the difficulties in developing superior catalysts and reagents. Accordingly, enantioselective oxidation mediated by enzyme systems are of particular importance in constructing chiral molecules. The ability to effect stereoselective transformations of enantiotopic groups attached to a prochiral center has been well documented by the series of outstanding studies by Jones' group[3] and others.[4] In those studies, horse liver alcohol dehydrogenase (HLADH) was used as catalyst, which has the capacity to realize the preparative-scale oxidation of hydroxymethyl groups by distinguishing the *pro-R* and *pro-S* sites of the molecules. The main disadvantage of this system is the fact that expensive coenzyme NAD^+ is essential. Although methods for recycling a catalytic amount of NAD^+ during the oxidation reaction have been developed,[5] it is preferable to carry out the reaction without any additives. On the basis of this concept, we have sought microorganisms capable of oxidizing prochiral α,ω-diols with distinction of *pro-R* and *pro-S* sites.[6]

Results

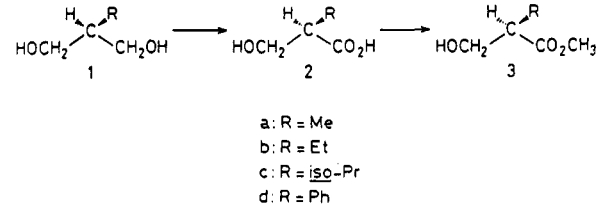
Gluconobacter and *Acetobacter* have been well-known to oxidize a variety of primary and secondary alcohols.[7] Thus, various strains of *Gluconobacter* were examined by using 2-methylpropane-1,3-diol (**1a**) as the simplest substrate that has α,ω-diol functions with a prochiral center.

Oxidation of 2-Substituted Propane-1,3-diols 1 with *Gluconobacters*. Among 16 strains of *Gluconobacter* and *Acetobacter*, 9 strains of *Acetobacter*, *Gluconobacter roseus* IAM 1841 was found to produce β-hydroxyisobutyric acid (**2a**) in the highest yield. The bacterium was grown in a nutrient medium and collected by centrifugation. The cells were suspended in acetate buffer and incubated with 2-methylpropane-1,3-diol (**1a**) on a rotary shaker at 30 °C. The yield of β-hydroxyisobutyric acid (**2a**) exceeded 45% in 24 h and varied little after that time for an additional 48 h. The best yield (47% based on employed substrate) was obtained by 48-h incubation when the starting pH of the buffer was 5.0 and the concentration of diol **1a** was 0.5% v/v. The product **2a** was isolated and purified by column chromatography after derivatization to the methyl ester **3a** with diazomethane and identified by comparison of spectroscopic data with those of a racemic authentic specimen and elemental analysis.

Table I. Oxidation of Propane-1,3-diols to β-Hydroxypropionic Acids

compd	R	incubation, day	chem yield,[a] %	$[\alpha]^{25}_D$ of 3, deg (% ee)[b]
1a	Me	2	47	−22.5 (83), *R*
1b	Et	5	55 (52)	−0.33
1c	*i*-Pr	6	(10)	−0.39
1d	Ph	7	(2>)	

[a] The yields were determined by GLC using internal standards, after derivatization to the corresponding methyl esters **3**. In parentheses are cited the isolated yields by column chromatography on silica gel with ethyl acetate–dichloromethane (1:4 v/v) as the eluent. [b] Measured as methanol solution.



The methyl ester **3a** showed the specific rotation of $[\alpha]^{25}_D$ −22.5 °C (*c* 1.80, MeOH, 83% ee)[8] and its absolute configuration was determined to be *R* by correlating the sign of the specific rotation to the results of Retey[9] and Sprecher.[10] Optically active β-hydroxybutyrate is expected

(1) Jones, J. B.; Sih, C. J.; Perlman, D. "Application of Biological Systems in Organic Chemistry"; Wiley: New York, 1976.
(2) (a) Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974. (b) Hentges, S. G.; Sharpless, K. B. *Ibid.*, **1980**, *102*, 4263. (c) Wynberg, H.; Marsman, B. *J. Org. Chem.* **1980**, *45*, 158. (d) Jew, S.-S.; Terashima, S.; Koga, K. *Chem. Pharm. Bull.* **1979**, *27*, 2351. (e) Terashima, S.; Hayashi, M.; Koga, K. *Tetrahedron Lett.* **1980**, *21*, 2733.
(3) (a) Jakovac, I. J.; Ng, G.; Lok, K. P.; Jones, J. B. *J. Chem. Soc., Chem. Commun.* **1980**, 515. (b) Jones, J. B.; Lok, K. P. *Can. J. Chem.* **1978**, *57*, 1025. (c) Goodbrand, H. B.; Jones, J. B. *J. Chem. Soc., Chem. Commun.* **1977**, 469. (d) Irwin, A. J.; Jones, J. B. *J. Am. Chem. Soc.* **1977**, *99*, 556 and references cited therein.
(4) (a) Hadorn, B.; Leuthardt, F.; Menard, E.; Vischer, D. *Helv. Chem. Acta* **1963**, *46*, 2003. (b) Bally, C.; Leuthardt, F. *Ibid.* **1970**, *53*, 732.
(5) (a) Jones, J. B.; Sneddon, D. W.; Higgins, W.; Lewis, A. J. *J. Chem. Soc., Chem. Commun.*, **1972**, 856. (b) Jones, J. B.; Taylor, K. E. *Ibid.* **1973**, 205. (c) Jones, J. B.; Taylor, K. E. *Can. J. Chem.* **1976**, *54*, 2969.
(6) Ohta, H.; Tetsukawa, H. *Chem. Lett.* **1979**, 1379.
(7) Ley, J. D.; Kersters, K. *Bacteriol. Rev.* **1964**, *28*, 164.
(8) The optical purity was calculated based on the value of $[\alpha]^{25}_D$ +27.0 (*c* 4.06, MeOH) reported for L-(+)-methyl β-hydroxyisobutyrate: Goodhue, C. T.; Schaeffer, J. R. *Biotech. Bioeng.* **1971**, *13*, 203.
(9) Retey, J.; Lynen, F. *Biochem. Biophys. Res. Commun.* **1964**, *16*, 358.

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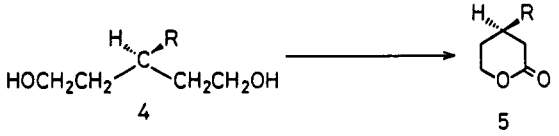
Table II. Oxidation of Pentane-1,5-diols to δ-Valerolactones

compd	R	incubation, day	chem yield,[a] %	$[\alpha]_D$, deg[b]	opt yield[d] (% ee)
4a	Me	2	57	+15.6	57, *R*
4b	*i*-Pr	7	29	+26.1	34, *R*
4c	Ph	7	4		

[a] Isolated yield by silica gel column chromatography. [b] Measured as chloroform solution at 27 °C. [c] Measured as ethanol solution at 20 °C. [d] Calculated based on the reported values; see ref 3d.

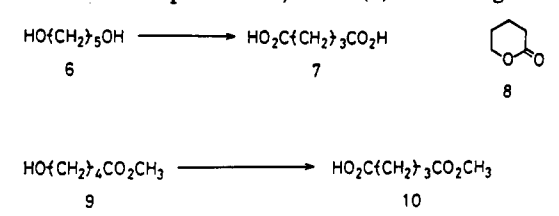
to be a useful building block for the synthesis of chiral molecules because it has two different functional groups from which carbon–carbon bond elongation is possible.[11] As can be seen from Table I, diols **1b** and **1c** were also oxidized to the corresponding β-hydroxy acid **2b** and **2c**. 2-Phenylpropane-1,3-diol (**1d**) was little affected by the bacterium.

Oxidation of 3-Substituted Pentane-1,5-diols 4 with *Gluconobacter roseus*. It is an interesting problem to examine how the distance between the reaction site and the prochiral center affects the stereochemical selectivity and the yield. Thus, *Gluconobacter roseus* mediated oxidation was applied to 3-substituted pentane-1,5-diols **4**.

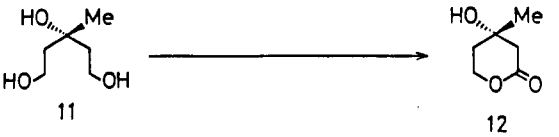


The reaction was carried out under the same conditions. The results are summarized in Table II. Oxidation of 3-methylpentane-1,5-diol (**4a**) gave (+)-(3*R*)-3-methyl-δ-valerolactone (**5a**) in 57% yield; $[\alpha]^{27}_D$ +15.7° (*c* 3.16, $CHCl_3$). The value of specific rotation indicates that the lactone **5a** exhibits the *R* absolute configuration of 57% optical purity.[3d] Isopropyl-substituted pentane-1,5-diol **4b** was oxdized more smoothly than propane-1,3-diol derivative **1c**, resulting in the formation of (+)-(3*R*)-3-isopropyl-δ-valerolactone (**5b**) in 29% yield. Pentane-1,5-diol bearing a phenyl group at the C-3 position was resistant to oxidation as in the case of 2-phenylpropane-1,3-diol **1d**.

Nonsubstituted pentane-1,5-diol (**6**) is also a good substrate for *G. roseus*, but interestingly the product was glutaric acid (**7**), which is the product as a result of diterminal oxidation of the starting material.[12] When diol **6** was incubated for 2 days at 30 °C in phosphate buffer at pH 5, **7** was obtained in 93% isolated yield. On the other hand, valerolactone **8** was not detected by TLC analysis. Oxidation carried out in the nutrient medium also afforded the dibasic acid **7** in an almost quantitative yield. The simplest reaction pathway that one would expect is that the smooth biological oxidation of intermediary valerolactone **8** generates the dibasic acid **7** as the final product. But, in fact, the incubation of valerolactone **8** (1% v/v) with the bacterium in the nutrient medium under the same conditions gave glutaric acid (**7**) in only 8% yield. The unidentified oily products that were separated from the organic extract of the broth were thought to be caused by the ring-opening polymerization of lactone **8**. On the other hand, open-chain hydroxy ester **9**, on treatment with cells of *G. roseus*, gave cleanly glutaric acid monoester **10** in 81% yield. Accordingly, the generation of free lactone **8** in the enzyme system has little possibility, though the detailed mechanism is not yet apparent now.



Oxidation of 3-Methylpentane-1,3,5-triol 11 with *Gluconobacter*. Among pentane-α,ω-diol derivatives, 3-methylpentane-1,3,5-triol (**11**) is a compound of note. If



enantiotopically selective oxidation of this molecule is realized, it will present a synthesis of mevalonolactone **12**, the *R* form of which is well-known as the starting material of isoprenoid biosynthesis.[13] Further, it is also interesting to determine how the hydroxyl group at C-3 affects the enantioselectivity of the enzyme system.

A number of *Gluconobacter* were found to be capable of oxidizing triol **11** to unnatural (*S*)-**12**.[14] As can be seen from Table III, *G. scleroideus* IAM 1842 showed the highest selectivity. *G. roseus* gave poorer results than in the case of oxidation of 3-methylpentane-1,5-diol (**4a**). Obviously, the introduction of a hydroxyl group at C-3 affects the stereochemical selectivity of enzyme-catalyzed oxidation.

The reaction pathway of the formation of (*S*)-**12** can be interpreted in mainly two ways. (a) *Gluconobacter* oxidize preferentially the *pro-S* hydroxyethyl group attached to the prochiral center in **11**, thereby affording (*S*)-**12**. (b) The microbes do not distinguish the *pro-S* and *pro-R* site of the substrate **11**, resulting in the formation of *dl*-mevalonolactone. The organisms then selectively consume the natural *R* form of the lactone **12**, leaving behind the *S* enantiomer, which is isolated. We prefer the first mechanism on the following basis. The prolonged incubation (7 days) of *Gluconobacter scleroideus* with triol **11** gave (*S*)-mevalonolactone in 34% chemical yield. Its optical purity was 66% ee, which is not much different from that of **12** obtained by 3 days of incubation (Table III, columns 1 and 2). If pathway b is operating, the optical yield of **12** must become higher with longer period of cultivation. Thus, the selective consumption of the (*R*)-**12** can be regarded as the minor path, if any. More directly, we tested the ability of the enzyme system of *G. scleroideus* to differentiate two enantiomers of mevalonolactone. Exposure of *dl*-**12** to the organism in the medium for 3 days resulted in the recovery of racemic lactone **12**. This

(10) (a) Sprecher, M.; Clark, M. J.; Sprinson, D. B. *Biochem. Biophys. Res. Comm.* **1964**, *15*, 581. (b) Sprecher, M.; Sprinson, D. B. *J. Biol. Chem.* **1966**, *241*, 868. (c) Sprecher, M.; Clark, M. J.; Sprinson, D. B. *Ibid.* **1966**, *241*, 872.

(11) Branka, Q.; Fischli, A. *Helv. Chem. Acta* **1977**, *60*, 925.

(12) Pentane-1,5-diol (**6**) has already been demonstrated to be transformed into glutaric acid (**7**) in a high yield by *Gluconobacter suboxydans*: see ref 7.

(13) Cornforth, J. W.; Cornforth, R. H. "Natural Substances Formed Biologically from Mevalonic Acid"; Goodwin, T. W. Ed.; Academic Press: New York, 1970; p 5.

(14) (a) Eberle, M.; Arigoni, D. *Helv. Chem. Acta* **1960**, *43*, 1508. (b) Cornforth, J. W.; Cornforth, R. H.; Popjak, G. *Tetrahedron* **1962**, *18*, 1351.

Table III. Oxidation of 3-Methylpentane-1,3,5-triol to Mevalonolactone

microorganism[a]	cultivation, day	chem[b] yield, %	$[\alpha]^{20}_D$[c]	opt[d] purity (% ee)
G. scleroideus IAM 1842	3	32[e]	+18.14	79, *S*
	7	34	+15.11	66, *S*
G. suboxydans IFO 3172	3	24	+8.52	37, *S*
G. roseus IAM 1841	3	27	+5.06	25, *S*
G. gluconicus IFO 1815	3	21	+7.28	32, *S*
G. albidus IFO 3250	3	17	+12.30	54, *S*
G. nonoxygluconicus IFO 3275	5	18	+9.29	41, *S*
G. nonoxygluconicus IFO 3276	5	20	+12.72	56, *S*

[a] Gluconobacter were used in all cases. [b] Unless otherwise stated, yields were determined by GLC using octane-1,8-diol as the internal standard. [c] Measured as ethanol solution. [d] Calculated based on the value of $[\alpha]^{20}_D$ −23.0° reported for (−)-(*R*)-mevalonolactone.[14b] [e] Isolated yield by column chromatography.

fact strongly supports the enantiotopically selective oxidation of *pro-S* hydroxyethyl group of **11** by the organism.

Sih et al. have also obtained (*S*)-**12** by oxidation of 3-methylpentane-1,3,5-triol (**11**) by *Flavobacterium oxydans*.[15] In this case, oxidation of both *pro-R* and *pro-S* hydroxyethyl groups of **11** followed by selective decomposition of (*R*)-mevalonolactone was suggested. Horse liver alcohol dehydrogenase has been also demonstrated to be capable of catalyzing the oxidation of **11** to chiral **12** by Jones et al.,[3d] but the yield was lower.

Discussion

The results described so far demonstrate that the enzyme system of *Gluconobacter* species is capable of distinguishing *pro-R* and *pro-S* sites of some α,ω-diols with a prochiral center. The substituents on the prochiral center seriously affected the rate of oxidation and optical yields. Because the enzyme system was not isolated and purified in our experiments, the rate of reaction might be discussed by taking into consideration the permeability of the substrates through the cell membrane. However, the substituent influences the rate and enantioselectivity in the same manner, i.e., the bulkier the substituent the lower the rate and optical yield in both the propane series and the pentane series. Accordingly, it seems likely that the substituents affect the interaction of the substrates with enzyme system rather than the membrane permeation.

Although the exact optical purities of ethyl- and isopropyl-substitued β-hydroxypropionic acids (**2b** and **2c**) are not known at present, they are most likely nearly racemic, judging from the values of the specific rotation of methyl esters **3a–c** as shown in Table I. 2-Methylpropane-1,3-diol (**1a**) appears to just fit the binding site of the enzyme system, and the substrate is fixed so as to give a high optical yield. On the other hand, substrates with ethyl and bulkier substituents appear to be too large to be arranged in an advantageous manner as to give high enantioselectivities.

A similar tendency was observed in the reaction of pentane-1,5-diols **4**. The *pro-R* sites of the substrates were selectively oxidized similarly to propane-1,3-diol derivatives **1**. The decrease in reactivity and optical purity of the product with increasing bulkiness of the alkyl group at the prochiral center (methyl to isopropyl) is less remarkable in the case of pentane-1,5-diols **4** than in the case of propane-1,3-diols **1**. The larger distance by one methylene group between the prochiral center and hydroxyl group reduces the effect of steric bulk. The lower optical yield of lactone **5a** compared with that of hydroxy acid **2a** is also understandable in analogy with the above interpretation.

The tendency that the increasing bulkiness of the C-3 substituent lowers the optical yield of lactone **5** has also been observed in the case of horse liver alcohol dehydrogenase catalyzed oxidation of diol **4**, although the enantioselectivity is opposite, resulting in the formation of (−)-(3*S*)-3-substituted δ-valerolactone **5**.[3d]

It is worthwhile to mention the oxidation of 3-methylpentane-1,3,5-triol (**11**). In this specific case, the enzyme systems of *Gluconobacter* and horse liver alcohol dehydrogenase[3d] exhibited the same enantioselectivity, in that both produced the unnatural (+)-(*S*)-**12**. No simple explanation for these results can be offered at present, but the diamond lattice[16] section analyses[3d,17] seems not to be applicable to the enzyme system of *Gluconobacter* species.

Experimental Section

General Procedure. ^{1}H NMR spectra were determined with a Varian EM 390 spectrometer. IR spectra were taken on a Hitachi 285 spectrophotometer. Mass spectra were obtained on a Hitachi RMU-6MG instrument at 100 °C and 70 eV. TLC analyses were carried out on plates spread with 0.25-mm layers of silica gel G (E. Merck, Darmstadt). For column chromatography, silica gel of 100–200 mesh was used (Wako Pure Chemical Ind., Osaka).

Medium for Cultivation of *Gluconobacter*. The composition of the medium was as follows: yeast extract, 5 g; peptone, 3 g; mannitol, 25 g in 1 L of distilled water. For the plate, 1.5% of agar was added.

Preparation of 2-Substituted Propane-1,3-diols 1. Reduction of α-substituted diethyl malonates with 1.5 mol equiv of lithium aluminum hydride in ether and ordinal workup gave the following 1,3-diols in yields of 80–90%.

2-Methylpropane-1,3-diol (1a): bp 115–116 °C (20 torr); IR (neat) 3350, 2960, 2940, 2870, 1460, 1380, 1030, 980 cm^{-1}; NMR ($CDCl_3$) δ 0.87 (d, 3), 1.90 (m, 1), 3.63 (m, 6).

2-Ethylpropane-1,3-diol (1b): bp 82 °C (1.2 torr); IR (neat) 3350, 2960, 1460, 1380, 1040 cm^{-1}; NMR ($CDCl_3$) δ 0.8–1.9 (m, 6), 3.20 (s, 2), 3.70 (m, 4).

2-Isopropylpropane-1,3-diol (1c): bp 100–101 °C (3.5 torr); IR (neat) 3370, 2960, 2800, 1460, 1380, 1050 cm^{-1}; NMR ($CDCl_3$) δ 0.90 (d, 6), 1.4–2.0 (m, 2), 3.80 (m, 6).

2-Phenylpropane-1,3-diol (1d): bp 119–121 °C (0.5 torr); IR (neat) 3300, 3030, 2940, 1050, 760, 700 cm^{-1}; NMR ($CDCl_3$) δ 3.07 (m, 3), 3.90 (d, 4), 7.72 (s, 5).

Preparation of 3-Substituted Pentane-1,5-diols 4. The following diols were prepared according to the literature procedures.

3-Isopropylpentane-1,5-diol (4b):[3d] bp 124–126 °C (2 torr); IR (neat) 3320, 2950, 1460, 1370, 1060 cm^{-1}; NMR ($CDCl_3$) δ 0.87 (d, 6), 1.4–1.7 (m, 6), 2.87 (s, 2), 3.70 (t, 4).

3-Phenylpentane-1,5-diol (4c):[18] bp 161–162 °C (3 torr); IR (neat) 3350, 2930, 1600, 1490, 1450, 1040, 760, 700 cm^{-1}; NMR ($CDCl_3$) δ 1.73 (s, 2), 1.7–2.1 (m, 5), 3.53 (t, 4), 7.3 (s, 5).

(15) Huang, F.-C.; Lee, L. F. H.; Mittal, R. S. D.; Ravikuman, P. R.; Chan, J. A.; Sih, C. J. *J. Am. Chem. Soc.* **1975**, *97*, 4144.

(16) Prelog, V. *Pure Appl. Chem.* *9*, 119.

(17) Irwin, A. J.; Jones, J. B. *J. Am. Chem. Soc.* **1976**, *98*, 8476.

(18) Altschul, R.; Bernstein, P.; Cohen, S. G. *J. Am. Chem. Soc.* **1956**, *78*, 5091.

Preparation of Methyl 5-Hydroxypentanoate (9). δ-Valerolactone (**8**) (8.0 g, 0.08 mol) was refluxed in a 2 N sodium methoxide solution in 80 mL of methanol for 3.5 h. A sequence of neutralization with aqueous ammonium chloride solution, extraction with ether, evaporation of the solvent, and distillation gave methyl 5-hydroxypentanoate as a colorless oil: yield 64%; bp 110 °C (14 torr); IR (neat) 3400, 2940, 2860, 1735, 1435, 1220, 1155, and 1050 cm^{-1}; NMR ($CDCl_3$) δ 1.5–1.8 (m, 4), 2.33 (t, 2), 2.53 (t, 1), 3.5–3.7 (m, 2), 3.66 (s, 3).

Preparation of 3-Methylpentane-1,3,5-triol (11). Ozonization of 4-hydroxy-4-methylhepta-1,6-diene followed by reduction of the ozonide with lithium aluminum hidride gave 3-methylpentane-1,3,5-triol (**11**):[19] yield 94%; bp 126–130 °C (0.3 torr); IR (neat) 3380, 2950, 1460, 1420, 1380, 1120, 1075, 1025 cm^{-1}; NMR (Me_2SO-d_6) δ 1.10 (s, 3), 1.60 (t, 4), 3.4–3.7 (m, 4), and 4.3–4.4 (m, 3).

3-Methylpentane-1,5-diol (**4a**), pentane-1,5-diol (**6**), δ-valerolactone (**8**) and *dl*-mevalonolactone were purchased from Tokyo Kasei Co. (Tokyo).

Synthesis of Racemic Methyl β-Hydroxyisobutyrate (3a).[20] Paraformaldehyde (0.60 g, 20 mmol), methyl α-bromopropionate (4.18 g, 25 mmol), and zinc–copper couple prepared from metallic zinc (3 g, 46 mg atom) and cupric acetate dihydrate (0.3 g, 1.4 mmol)[21] were heated in refluxing benzene (20 mL) for 1 h. The reaction mixture was allowed to cool to room temperature, and 5 N sulfuric acid (20 mL) was added. Ether (100 mL) was added, and the organic layer was separated, washed with sodium bicarbonate solution and brine, and dried over anhydrous magnesium sulfate. Filtration followed by evaporation of the solvent gave a colorless oil. Distillation under reduced pressure gave methyl β-hydroxyisobutyrate (**3a**) in 21% yield (0.41 g, 3.47 mmol): bp 78 °C (21 torr); IR (neat) 3450, 2980, 2950, 2890, 1740, 1455, 1435, 1375, 1200, 1035, 980 cm^{-1}; NMR ($CDCl_3$) δ 1.18 (d, 3), 2.67 (sextet + s, 2), 3.72 (s + m, 5); mass spectrum (*m/e*) 15, 29, 31, 57, 59, 88.

Synthesis of Racemic 3-Substituted Lactones. 3-Isopropyl-δ-valerolactone (**5b**) and 3-phenyl-δ-valerolactone (**5c**) were prepared by reduction of the corresponding anhydrides[3d,18] with sodium borohydride[22] according to the known procedures.

3-Isopropyl-δ-valerolactone (5b): bp 96–103 °C (4 torr); IR (neat) 2950, 2870, 1740, 1400, 1180, 1140, 1080 cm^{-1}; NMR ($CDCl_3$) δ 0.93 (d, 6), 1.3–2.0 (m, 4), 2.2–2.8 (m, 2), 4.3 (m, 2).

3-Phenyl-δ-valerolactone (5c): bp 127–132 °C (1.5 torr); NMR ($CDCl_3$) δ 1.9–2.3 (m, 2), 2.5–3.3 (m, 3), 4.3–4.5 (m, 2), 7.3 (m, 5).

Microbial Oxidation of 2-Substituted Propane-1,3-diols 1. Oxidation of 2-methylpropane-1,3-diol (**1a**) is representative. The sterilized medium contained in a 500-mL Erlenmeyer flask was inoculated with a loopful of *Gluconobacter roseus* IAM 1841. Incubation was carried out on a rotary shaker at 30 °C for 2 days. The grown cells were collected by centrifugation, washed with sterilized acetate buffer of pH 5 and suspended in 50 mL of the same buffer contained in a 500-mL Erlenmeyer flask. To this flask was added 250 mg (2.78 mmol) of 2-methylpropane-1,3-diol (**1a**), and the whole was shaken on a rotary shaker at 30 °C for 2 days. The broth was acidified by 2 N hydrochloric acid and extracted with three 50-mL portions of ethyl acetate. An ethereal solution of excess diazomethane was added to the combined organic layer and dried over anhydrous sodium sulfate. Filtration and evaporation of the solvent under reduced pressure gave 162 mg of colorless oil. To this crude product 109 mg of lauryl alcohol was added as an internal standard and methyl β-hydroxyisobutyrate (**3a**) was determined by GLC: column BDS (butane-1,4-diol succinate), 130 °C; retention time for **3a**, 3.2 min, for lauryl alcohol, 15.8 min. No peak due to the starting material was found on the chromatogram. For the measurement of optical rotation, **3a** was isolated by a larger scale experiment according to the procedure described above and purified by column chromatography. The spectroscopic data were identical with those of racemic authentic sample. Anal. Calcd for $C_5H_{10}O_3$: C, 50.83; H, 8.53%. Found: C, 50.54; H, 8.66.

Oxidation of 2-ethylpropane-1,3-diol (**1b**), 2-isopropylpropane-1,3-diol (**1c**), and 2-phenylpropane-1,3-diol (**1d**) was carried out in a similar way by using 1.50 g of each substrate. After derivatization to the corresponding methyl esters, the molar ratios of the starting materials vs. products were determined by GLC (column BDS): **1b/3b**, 0:100 (oven 140 °C); **1c/3c**, 86:14 (150 °C); **1d/3d**, 95:5 (190 °C). The methylated products **3b–d** were isolated by column chromatography on silica gel. NMR spectroscopically pure fractions were collected and weighed.

Methyl α-ethyl-β-hydroxypropionate (3b): IR (neat) 3450, 2975, 2890, 1735, 1460, 1435, 1380, 1260, 1200, 1170, 1050 cm^{-1}; NMR ($CDCl_3$) δ 0.93 (t, 3), 1.57 (quint, 2), 2.37 (s, 1), 2.47 (quint, 1), 3.70 (s, 3), 3.73 (m, 2).

Methyl α-isopropyl-β-hydroxypropionate (3c): IR (neat) 3440, 2950, 2870, 1735, 1460, 1435, 1390, 1370, 1270, 1190, 1165, 1040 cm^{-1}; NMR ($CDCl_3$) δ 0.93 (d, d, 6), 1.98 (m, 1), 2.37 (m, 2), 3.69 (s, 3), 3.73 (m, 2).

Methyl α-phenyl-β-hydroxypropionate (3d): IR (neat) 3420, 3050, 3010, 2940, 2860, 1730, 1595, 1485, 1445, 1425, 1350, 1240, 1155, 1035, 840, 760, 730, 695 cm^{-1}; NMR ($CDCl_3$) δ 2.33 (br s, 1), 3.70 (s, 3), 3.7–3.9 (m, 3), 7.63 (br s, 5).

Microbial Oxidation of 3-Substituted Pentane-1,5-diols 4. *Gluconobacter roseus* IAM 1841 was grown in 300 mL of the medium by rotary shaking cultivation. The cells were collected by centrifugation and suspended in 6 mL of acetate buffer of pH 5. Then 1-mL portions of the cell suspension were added to six sterilized 500-mL Erlenmeyer flasks containing 50 mL of the same acetate buffer. To each flask 250 mg of 3-methylpentane-1,5-diol (**4a**) was added (total 1.50 g, 12.7 mmol), and the flasks were shaken for 2 days at 30 °C. After addition of 3 N hydrochloric acid and excess sodium chloride, the combined broth was extracted three times with 300 mL portions of ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate. Filtration followed by removal of the solvent under reduced pressure gave 1.8 g of brown oil. GLC analysis revealed that the molar ratio of diol **4a** vs. lactone **5a** was 17/83. Purification by column chromatography on silica gel (eluent hexane–ether 1:1 v/v) gave 746 mg of 3-methyl-δ-valerolactone (**5a**); yield 57% IR (neat) 2970, 1730, 1460, 1255, 1230, 1170, 980 cm^{-1}; NMR ($CDCl_3$) δ 1.05 (d, 3), 1.3–2.5 (m, 5), 4.1–4.4 (m, 2); $[\alpha]^{27}_D$ = +15.6° (*c* 3.16, $CHCl_3$), 57% ee.[3d]

Oxidation of 3-isopropylpentane-1,5-diol (4b) and 3-phenylpentane-1,5-diol (**1c**) was carried out in a similar manner as described above. The molar ratios of the recovered starting 1,5-diols vs. formed lactones in the organic extract of the broth were determined by GLC (column BDS, 190 °C): **4b/5b**, 50:50; **4c/5c**, 94:6. After purification by column chromatography on silica gel (eluent hexane–ether 1:1 v/v), lactones **5b** and **5c** were characterized by comparing the spectroscopic data with those of racemic authentic specimen.

Microbiol Oxidation of Pentane-1,5-diol (6). To a sterilized 500-mL Erlenmeyer flask 50 mL of the medium was added and autoclaved. The flask was allowed to cool to room temperature and inoculated with a loopul of *Gluconobacter roseus* IAM 1841. After shaking for 2 days, 0.25 mL (229 mg, 2.20 mmol) of pentane-1,5-diol (**6**) was added to the flask, and the incubation was continued for additional 2 days under the same conditions. The broth was extracted with three 50-mL portions of ethyl acetate. The combined organic layer was washed with brine and dried over anhydrous sodium sulfate. Filtration and evaporation of the solvent afforded glutaric acid (**7**) as white crystals: yield 281 mg (2.13 mmol), 97%; mp 95–97 °C (after single crystallization from ethyl acetate, nondepression when mixed with an authentic sample); IR (KBr disk) 3500–2500, 1700, 1440, 1415, 1310, 1270, 1210, 920 cm^{-1}.

Reaction of δ-Valerolactone (8) with *G. roseus*. To six 500-mL Erlenmeyer flasks containing grown cells of *G. roseus* in the medium 0.5-mL portions of δ-valerolactone **8** were added (total 3.0 mL, 2.98 g, 30.0 mmol), and the flasks were shaken for 2 days at 30 °C on a rotary shaker. The broth was combined and extracted with three 300-mL portions of ethyl acetate. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure to give 1.37 g of pale yellow oil, which was subjected to column chromatography on silica gel. Elution with a mixture of ether and hexane (2:1 v/v) gave glutaric acid (**7**) as white crystals, yield 297 mg (8%). Further development with the

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same solvent and ether resulted in the successive elution of pale yellow oil: total yield 757 mg; IR (neat) 3500–2500, 2950, 1730 (br), 1400, 1240, 1160, 1060 cm^{-1}; NMR ($CDCl_3$) δ 1.5–1.9 (m, 4), 2.2–2.5 (m, 2), 3.9–4.3 (m, 2).

Microbial Oxidation of Methyl 5-Hydroxypentanoate (9). *Gluconobacter roseus* IAM 1841 was grown in 100 mL of the medium contained in two 500-mL Erlenmeyer flasks. To each flask was added a 0.5-mL (502 mg, 3.94 mmol) portion of methyl 5-hydroxypentanoate (**9**), and they incubated on a rotary shaker for 4 days at 30 °C. After addition of hydrochloric acid to pH 3–4, the combined broth was extracted with three 100-mL portions of ethyl acetate. The organic layer was washed with brine and dried over anhydrous sodium sulfate. Filtration followed by evaporation of the solvent under reduced pressure gave glutaric acid monomethyl ester (**10**) as a pale yellow oil: yield 938 mg (81%); IR (neat) 3300–2500, 1740–1700, 1440, 1380, 1240, 1200, 1160, 1040 cm^{-1}; NMR ($CDCl_3$) δ 1.9–2.1 (m, 2), 2.4 (m, 4), 3.63 (s, 3).

Hydrolysis of Glutaric Acid Monomethyl Ester (10) to Glutaric Acid (7). A mixture of methanol (15 mL), a 15% aqueous solution of sodium hydroxide (5 mL), and 535 mg (3.66 mmol) of glutaric acid monomethyl ester (**10**) was stirred at room temperature for 3 h. After removal of methanol under reduced pressure, 5 mL of water and concentrated hydrochloric acid were added to the reaction mixture. Extraction with a 20-mL portion of ethyl acetate for three times and a sequence of washing with brine, drying over anhydrous sodium sulfate, filtration, and evaporation of the solvent gave glutaric acid (**7**) as white crystals: mp 95–98 °C; yield 274 mg (54%).

Microbial Oxidation of 3-Methylpentane-1,3,5-triol (11). To six 500-mL Erlenmeyer flasks containing grown cells of *Gluconobacter scleroideus* IAM 1842 in 50 mL of the medium, 3 g of 3-methylpentane-1,3,5-triol (**11**) was added in equal protions. The flasks were shaken for 3 days at 30 °C. The broth was combined and extracted with three 300-mL portions of ethyl acetate. The combined organic layer was washed with brine and dried over anhydrous sodium sulfate. Filtation and evaporation of the solvent gave 1.33 g of colored oil. It was subjected to column chromatography on silica gel. Elution with ethyl acetate gave (+)-(*S*)-**12** as a colorless oil: yield 915 mg (32%); IR (neat) 3400, 2960, 2910, 1720, 1235, 1125, 1065, 1020, 930, 880, 800 cm^{-1}; NMR ($CDCl_3$) δ 1.37 (s, 3), 1.89 (m, 2), 2.55 (m, 2), 3.37 (br s, 1) and 4.2–4.8 (m, 2); $[\alpha]^{20}_D$ +18.14° (*c* 2.27, EtOH); mp of benzhydryl amide 97–98 °C (lit. mp 98–99 °C[14b]). Anal. Calcd for $C_{10}H_{23}NO_3$: C, 72.82; H, 7.40; N, 4.47%. Found: C, 72.85; H, 7.54; N, 4.25.

Registry No. **1a**, 2163-42-0; **1b**, 2612-29-5; **1c**, 2612-27-3; **1d**, 1570-95-2; (−)-(*R*)-**2a**, 1910-47-0; (±)-**3a**, 64809-29-6; (−)-(*R*)-**3a**, 72657-23-9; (±)-**3b**, 81444-76-0; (−)-**3b**, 72604-81-0; (±)-**3c**, 81444-77-1; (−)-**3c**, 72604-82-1; (±)-**3d**, 81444-78-2; **4a**, 4457-71-0; **4b**, 61898-54-2; **4c**, 829-27-6; (+)-(*R*)-**5a**, 61898-55-3; (±)-**5b**, 21754-22-3; (+)-(*R*)-**5b**, 37147-17-4; (±)-**5c**, 61949-75-5; **6**, 111-29-5; **7**, 110-94-1; **8**, 542-28-9; **9**, 14273-92-8; **10**, 1501-27-5; **11**, 7564-64-9; (+)-(*S*)-**12**, 19022-60-7.

Routes to Mitomycins. Application of Iminium Salts to the Synthesis of 7-Methoxymitosene

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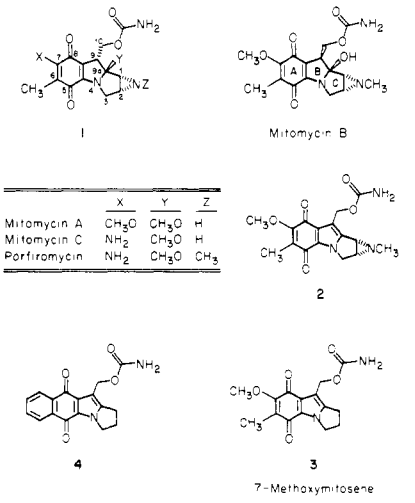
The synthesis of 7-methoxymitosene (**3**), a synthetic analogue of the mitomycins, is presented. Key steps include a regioselective addition of proline methyl ester to an unsymmetric benzoquinone, side-chain introduction through specific allylation, and an active methylene–iminium salt ring closure. Several methods for allylation of arenes and quinones were evaluated in building the pyrroloindole nucleus. The quinone function was incorporated in the educt, carried through as various hydroquinone derivatives, and regenerated in the very late stages by oxidative dealkylation.

The isolation, structure, chemistry, pharmacology, biosynthesis, and synthetic studies of the mitomycin antitumor antibiotics **1** and analogues have been thoroughly reviewed.[1] Further work in these areas has led to the recent isolation of new mitomycins[2] and to the preparation of mitomycin analogues with improved biological activity.[1b]

Chart I. Mitomycins and Mitosene Analogues



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