mmol), treated with MeLi as described in the synthesis of 1, afforded 4 (500 mg, 90% yield): ^{13}C NMR (CD_3COCD_3) δ 26.86 (Me), 70.78, 72.64, 74.19, 75.63, 76.18, 76.35, 80.35, 84.75, 85.50; 98.38 (C-2). Anal. Calcd for $C_{35}H_{38}O_6$: C, 75.79; H, 6.91. Found: C, 75.67; H, 7.08.

2,5-Anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-D-*arabino*-hex-1-enitol (3). The crude reaction mixture formed by the combination of titanocene dichloride (1.25 g, 5 mmol) and Me_3Al (5 mL, 2 M in toluene),[7] was added after 72 h at −60 °C to a stirred solution of 2,3,5-tri-*O*-benzyl-D-arabinono-1,4-lactone[11] (2.0 g, 4.8 mmol) in a mixture of dry THF (3 mL), dry toluene (7 mL), and two drops of dry pyridine. The reaction was monitored by TLC (8:2) and after 2 h warmed to −20 °C, stirred 1.5 more hours, and quenched by slow addition of aqueous NaOH (1 mL, 4 N). Dilution with Et_2O (200 mL), filtration on Celite, and gravity chromatography (8:2) afforded 3 (1.41 g, 71% yield): mp 49–50 °C (from Et_2O–hexane); $[\alpha]_D$ +19.6° (*c* 1.5, $CHCl_3$)(lit.[12] oil, $[\alpha]_D$ +15.9°); ^{1}H NMR δ 3.60 (2 H, m, H-5a and H-5b), 4.05 (1 H, t, *J* = 3 Hz, H-4), 4.17 (2 H, broad s, H-1a and H-1b), 4.37 (1 H, *J* = 3 Hz, H-3), 4.41 (1 H, m, H-5), 4.51 (1 H, d, *J* = 12 Hz, OCHPh), 4.55 (4 H, s, OCH_2Ph), 4.65 (1 H, d, *J* = 13 Hz, OCHPh), 7.3 (15 H, m, PhH); ^{13}C NMR δ 69.93, 71.00, 71.92, and 73.53 (CH_2O); 81.85, 82.39, and 83.67 (C-3, C-4, and C-5); 85.79 (C-1); 160.15 (C-2). Anal. Calcd for $C_{27}H_{28}O_4$: C, 77.86; H, 6.77. Found: C, 77.72, H, 6.43. FAB MS *m/e* 417.

7,10-Anhydro-6-deoxy-7-*C*-methyl-1,3,4,8,9,11-hexa-*O*-benzyl-D-*gluco*-D-*lyxo*-undec-5-ulofuranose (2). Method A. To a stirred solution of 1 (700 mg, 1.6 mmol) in dry MeCN (25 mL) was added two drops of BF_3·OEt_2 at 0 °C, and the reaction was monitored by TLC (7:3). After 30 min, addition of water (100 mL) and usual workup afforded 2 (637 mg, 93% yield) as a mixture of two anomers.

Method B. To a stirred solution of 3 (580 mg, 1.4 mmol) in dry MeCN (50 mL) was added two drops of BF_3·OEt_2 at 0 °C. The reaction was immediate. Addition of water and usual workup afforded 2 (570 mg, 96% yield) as a 1:1 mixture of two anomers.

The anomers were separated by flash chromatography (8:2).

2β (higher R_f anomer): oil; $[\alpha]_D$ −32.9° (*c* 1, $CHCl_3$); ^{1}H NMR δ 1.53 (3 H, s, Me), 2.11 (1 H, d, *J* = 14 Hz, H-6a), 2.99 (1 H, d, *J* = 14 Hz, H-6b), 3.51 (2 H, d, *J* = 6 Hz, H-1 or H-11), 3.58 (2 H, m, H-1 or H-11), 3.92 (1 H, d, *J* = 4 Hz), 4.00 (1 H, d, *J* = 6 Hz, H-4, 6% NOE with H-6b), 4.10 (2 H, m), 4.26 (1 H, d, *J* = 2.5 Hz, H-8, 10% NOE with Me), 4.37 (1 H, d, *J* = 12 Hz, OCHPh), 4.22–4.80 (13 H, m), 7.4 (30 H, m, PhH); ^{13}C NMR δ 23.03 (Me), 49.01 (C-8), 71.20, 71.60, 71.86, 72.36, 72.66, 72.75, 72.12, 73.49 (CH_2O); 80.29, 84.26, 84.58, 84.82, 85.23, 91.00 (CHO); 90.58 (C-7), 113.45 (C-5); EI MS *m/e* 832 (M − H_2O). Anal. Calcd for $C_{54}H_{58}O_9$: C, 76.21; H, 6.87. Found: C, 75.81; H, 7.01.

2α: oil; $[\alpha]_D$ +22.8° (*c* 1, $CHCl_3$); ^{1}H NMR δ 1.48 (3 H, s, Me), 2.18 (1 H, d, *J* = 15 Hz, H-6a), 2.40 (1 H, d, *J* = 15 Hz, H-6b), 3.43 (1 H, dd, *J* = 5 and 10.5 Hz, H-1a), 3.51 (1 H, dd, *J* = 5 and 10.5 Hz, H-1b), 3.63 (1 H, dd, *J* = 4.5 and 10 Hz, H-11a), 3.67 (1 H, dd, *J* = 6 and 10 Hz, H-11b), 3.87 (1 H, dd, *J* = 2.5 and 5 Hz, H-3), 3.96 (1 H, d, *J* = 2.5 Hz, H-4), 3.99 (1 H, dd, *J* = 2 and 6 Hz, H-9), 4.10 (1 H, q, *J* = 5 Hz, H-2), 4.19 (1 H, dt, *J* = 4.5, 6 and 6 Hz, H-10), 4.34 (1 H, d, *J* = 2 Hz, H-8, 27% NOE with Me), 4.35–4.75 (12 H, m, OCH_2Ph), 7.4 (30 H, PhH); ^{13}C NMR δ 23.66 (Me), 46.14 (C-6), 70.52, 71.66, 71.79, 72.09, 73.49 (CH_2O); 81.11, 82.49, 82.90, 86.92, 88.37, 94.21 (CHO); 89.04 (C-7), 116.97 (C-5); EI MS *m/e* 832 (M − H_2O). Anal. Found: C, 75.99; H, 6.74.

2,6-Anhydro-3,4,5,7-tetra-*O*-benzyl-1-deoxy-D-*gluco*-hept-1-enitol (5). The crude reaction mixture, formed by the combination of titanocene dichloride (1.25 g, 5 mmol) and Me_3Al (5 mL, 2 M in toluene), was added after 72 h at −60 °C to a stirred solution of 2,3,4,6-tetra-*O*-benzyl-D-glucono-1,5-lactone[12] (2.6 g, 4.9 mmol) in a mixture of dry THF (3 mL), dry toluene (7 mL), and two drops of dry pyridine. The reaction was monitored by TLC (8:2) and after 2 h warmed to −20 °C, stirred 1.5 more hours, and quenched by slow addition of aqueous NaOH (1 mL, 4 N). Dilution with Et_2O (200 mL), filtration on Celite, and gravity chromatography (8:2) afforded 5 (2.4 g, 92% yield): mp 65–68 °C (lit. mp 65–68 °C);[13] $[\alpha]_D$ +59.5° (*c* 1 $CHCl_3$); ^{1}H NMR, δ 3.66–3.85 (5 H, m), 3.96 (1 H, d, *J* = 9 Hz), 4.40–4.90 (10 H, m), 7.3 (20 H, PhH); ^{13}C NMR δ 68.72, 72.66, 73.40, 74.33, and 74.41 (CH_2O); 77.50, 78.43, 78.91, 84.57 (C-3, C-4, C-5, and C-6); 94.60 (C-1), 156.17 (C-2). Anal. Calcd for $C_{35}H_{36}O_5$: C, 78.33; H, 6.76. Found: C, 78.48; H, 6.55.

8,12-Anhydro-7-deoxy-8-*C*-methyl-1,3,4,5,9,10,11,13-octa-*O*-benzyl-D-*glycero*-D-*ido*-L-*gulo*-tridec-6-ulopyranose (6). 5 (156 mg, 0.28 mmol), treated as described in method B, afforded 6 (101 mg, 66% yield): mp 74–76 °C; $[\alpha]_D$ +49.5° (*c* 1, $CHCl_3$); ^{1}H NMR δ 1.51 (3 H, s, Me), 2.20 (1 H, d, *J* = 15 Hz, H-7a), 2.27 (1 H, d, *J* = 15 Hz, H-7b), 3.45–3.63 (3 H, m, H-3, H-9 and H-12), 3.65–3.75 (5 H, m, H-1a, H-1b, H-10, H-11, H-13a, and H-13b), 3.83 (1 H, m, H-2), 3.93 (1 H, d, *J* = 7 Hz, H-5), 4.32 (1 H, dd, *J* = 7 and 8.5 Hz, H-4), 4.47–5.00 (16 H, m, OCH_2Ph), 7.4 (40 H, PhH); ^{13}C NMR δ 29.36 (Me), 39.94 (C-7), 69.94, 70.31 (C-1 and C-13); 73.19 (C-2), 74.38 (C-12), 73.98, 74.19, 74.87, 75.64, 76.28 (OCH_2Ph); 77.32 (C-3), 78.54 (C-11), 82.80 (C-8), 83.63 (C-10), 84.04 (C-4), 85.01 (C-9), 87.77 (C-5), 110.60 (C-6). Anal. Calcd for $C_{70}H_{74}O_{11}$: C, 77.04; H, 6.83. Found: C, 76.89; H, 6.77.

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(12) Prepared by PCC oxidation of commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose.

(13) (a) Csuk, R.; Glanzer, B. I. *Tetrahedron* **1991**, *47*, 1655. (b) RajanBabu, T. V.; Reddy, G. S. *J. Org. Chem.* **1986**, *51*, 5458.

Microbiological Transformations. 22. Microbiologically Mediated Baeyer–Villiger Reactions: A Unique Route to Several Bicyclic γ-Lactones in High Enantiomeric Purity

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Baeyer–Villiger-type oxidation reactions achieved using biocatalysts[1] are a new emerging type of bioconversion allowing for the one-step asymmetric synthesis of chiral lactones. These biocatalysts can be either purified enzymes[2] or whole-cell systems.[3,4] We have recently described a preliminary work showing an unexpected result from such a reaction. Using whole-cell cultures of *Acinetobacter* TD63, we have carried out the preparative-scale transformation of bicyclo[3.2.0]hept-2-en-6-one (**1a**) into the two regioisomeric lactones **2a** and **3a** with enantiomeric excesses (ee's) as high as 95%.[5] This reaction presents two interesting points. First, only a few examples of such high enantioselectivity have been reported for such reactions,[3,6] most substrates leading only to poor (if any) en-

(1) Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformations in Preparative Organic Chemistry: the Use of Isolated Enzymes and Whole Cell Systems in Synthesis*; Academic Press: London, 1989; pp 166–9.

(2) (a) Taschner, M. J.; Black, D. J. *J. Am. Chem. Soc.* **1988**, *110*, 6892. (b) Abril, O.; Ryerson, C. C.; Walsh, C.; Whitesides, G. M. *Bioorg. Chem.* **1989**, *17*, 41.

(3) (a) Ouazzani-Chahdi, J.; Buisson, D.; Azerad, R. *Tetrahedron Lett.* **1987**, *28*, 1109. (b) Levitt, M. S.; Newton, R. F.; Roberts, S. M.; Willetts, A. J. *J. Chem. Soc., Chem. Commun.* **1990**, 619.

(4) (a) Alphand, V.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1990**, *55*, 347. (b) Alphand, V.; Archelas, A.; Furstoss, R. *Biocatalysis* **1990**, *3*, 73. (c) Alphand, V. Thèse de Doctorat, Université Aix-Marseille II, 1990.

(5) Alphand, V.; Archelas, A.; Furstoss, R. *Tetrahedron Lett.* **1989**, *30*, 3663.

0022-3263/92/1957-1306$03.00/0

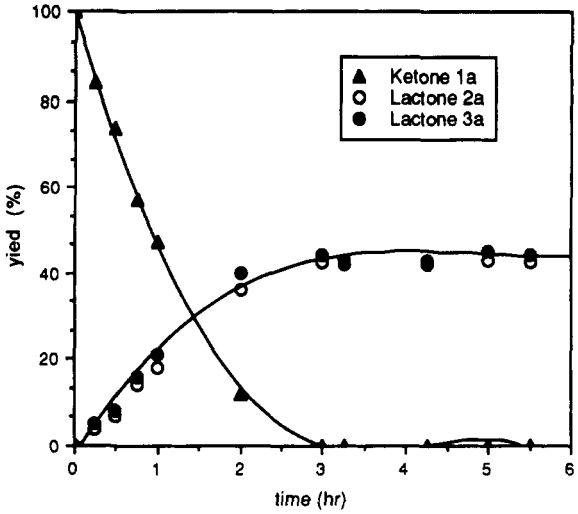


Figure 1. Time course of the bioconversion of ketone **1a** by *Acinetobacter* NCIB 9871; ▲, percentage of remaining ketone **1a** (%); ○, yield of lactone **2a** (%); ●, yield of lactone **3a** (%).

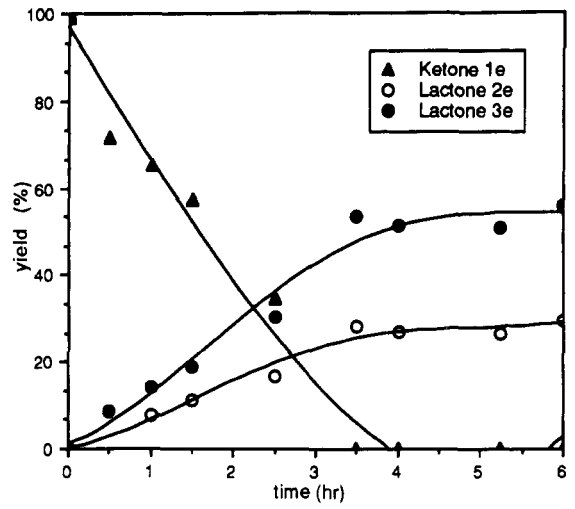


Figure 2. Time course of the bioconversion of ketone **1e** by *Acinetobacter* NCIB 9871; ▲, percentage of remaining ketone **1e** (%); ○, yield of lactone **2e** (%); ●, yield of lactone **3e** (%).

antiomeric purity for the formed lactones.[2b,7,8] Moreover, it is the first example of a reaction exhibiting such a divergent regioselectivity dependent upon the enantiomer of the substrate used.

Continuing our studies on microbiological Baeyer–Villiger reactions using whole-cell processes,[4] we wish now to report the biotransformations of various bicyclic ketones **1a–e** by two bacteria, *Acinetobacter* NCIB 9871[9] and *Acinetobacter* TD 63.[10] The expected products, lactones **2** and **3**, are important chiral synthons, particularly useful in the syntheses of prostaglandins[11] and nucleosides.[3b] Moreover, this method is particularly interesting as far as asymmetric synthesis is concerned since, to our best knowledge, this reaction has no counterpart in classical synthesis.

Results and Discussion

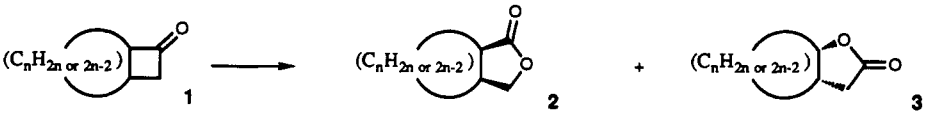
Using the bioconversion conditions described in the Experimental Section, ketones **1** were completely oxidized by both microorganisms to a mixture of the regioisomeric lactones **2** and **3**. The relative proportions and enantiomeric purities of these lactones depend primarily on the substrate used and, to a lesser extent, on the microorganism employed[12] (cf. Table I). However, in most cases, **2** and **3** are formed in approximately 1:1 ratios and with almost quantitative yields.[13]

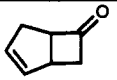
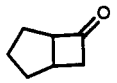
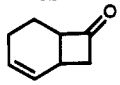
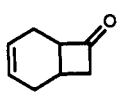
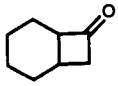
These results are quite surprising since, whereas lactones **3** arise from a "normal" Baeyer–Villiger-type oxygen insertion between the more substituted carbon atom and the carbonyl group of **1**, lactones **2** are formed with the chemically disfavored regiochemistry.[17] Furthermore, except for **3e**, all these lactones are obtained with excellent enantiomeric excesses (ee's >90% or even 95%). Interestingly, all lactones of one particular type are formed from the same enantiomer of the starting ketone. Thus, the enantiomer of **1** bearing a *S* configuration at the bridgehead carbon atom α to the carbonyl group leads to the "normal" lactones **3**, whereas the *R* configuration leads to the "abnormal" ones **2**. The only notable exception is observed for bicyclooctanone **1e**. Although **1e** gave also two lactones, the relative proportions **2e:3e** were 1:2 with both microorganisms. The ee's of **2e** were quite high (95%) but the ee's of **3e** were only moderate (50–61%). In this particular case, one of the enantiomers of ketone **1e** gives only one product, whereas the other enantiomer leads to two regioisomeric lactones.

Figures 1 and 2 show the time course of the bioconversion of ketones **1a** and **1e** by *Acinetobacter* NCIB 9871. Contrary to what we previously reported regarding the microbiological oxidation of α-substituted cyclopentanones,[4] NADPH present in the cells is sufficient to allow for complete ketone oxidation. In the conditions used, the substrate is the limiting reagent of the reaction which means that higher substrate concentrations could be employed. Moreover, the lactones were not further metabolized by the bacteria after their formation. It is interesting to note that the **2:3** ratio is constant and independent of time and the extent of conversion. In a recent paper concerning the oxidation of **1a** and **1c** by *Cylindrocarpon destructans*,[8] this ratio was reported to be dependent upon the conversion ratio. Also, it appears

(6) Carnell, A. J.; Roberts, S. M.; Sik, V.; Willetts, A. J. *J. Chem. Soc., Chem. Commun.* **1990**, 1438.
(7) Königsberger, K.; Alphand, V.; Furstoss, R.; Griengl, H. *Tetrahedron Lett.* **1991**, *32*, 499.
(8) Königsberger, K.; Braunegg, G.; Faber, K.; Griengl, H. *Biotechnol. Lett.* **1990**, *12*, 509.
(9) (a) Donoghue, N. A.; Norris, D. B.; Trudgill, P. W. *Eur. J. Biochem.* **1976**, *63*, 1750. (b) For review about this bacteria, see: Walsh, C. T.; Chen, Y. C. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 333.
(10) Hydrolase deficient mutant, see: Davey, J. F.; Trudgill, P. W. *Eur. J. Biochem.* **1977**, *74*, 115.
(11) (a) Bindra, J. S. *The Total Synthesis of Natural Products*; ApSimon, J., Ed.; Wiley-Interscience: New York, 1981; Vol. 4, p 353. (b) Newton, R. F.; Roberts, S. M. *Tetrahedron* **1980**, *36*, 2163.
(12) The results described here are quite different from those reported very recently by Roberts and Willetts concerning the biotransformation of some bicycloketones by *Acinetobacter* NCIB 9871.[6] However, it is difficult to suggest an explanation in absence of experimental data.
(13) Measured by gas chromatography.
(14) (a) Jakovac, I. J.; Goodbrand, H. B.; Lok, K. P.; Jones, J. B. *J. Am. Chem. Soc.* **1982**, *104*, 4659. (b) Nara, M.; Terashima, S.; Yamada, S. *Tetrahedron* **1980**, *36*, 3161. (c) Cotterill, I. C.; MacFarlane, E. L. A.; Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **1988**, *1*, 3387. (d) Kosugi, H.; Tagami, K.; Kanna, H.; Uda, H. *J. Chem. Soc., Perkin Trans. 1* **1989**, *5*, 935. (e) Pirkle, W. H.; Adams, P. E. *J. Org. Chem.* **1980**, *45*, 4111.
(15) This column was made by Dr. Lizzani-Cuvelier and Prof. R. Fellous (Laboratoire de Chimie des Arômes, Nice, France).
(16) (a) Hudlicky, T.; Reddy, D. B.; Govindan, S. V.; Kulp, T.; Still, B.; Sheth, J. P. *J. Org. Chem.* **1983**, *48*, 3422. (b) Grieco, P. A. *J. Org. Chem.* **1972**, *37*, 2363. (c) Irwin, A. J.; Jones, J. B. *J. Am. Chem. Soc.* **1977**, *99*, 1625. (d) Corey, J.; Petrzilka, M. *Tetrahedron Lett.* **1975**, 2537. (e) Marshall, J. A.; Ellison, R. H. *J. Org. Chem.* **1975**, *40*, 2070. (f) Iwasa, S.; Yamamoto, M.; Kohmoto, S.; Yamada, K. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1173.
(17) The only examples of chemical Baeyer–Villiger reactions exhibiting such a "abnormal" regioselectivity involve compounds with high steric or electronic strain. See: Krow, G. R. *Tetrahedron* **1881**, *37*, 2697. Noyori, R.; Sato, T.; Kobayashi, H. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2661. Chandrasekhar, S.; Roy, C. D. *Tetrahedron Lett.* **1987**, *28*, 6371.

Table I. Bioconversions of Ketones 1a–e by *Acinetobacter* NCIB 9871 and *Acinetobacter* TD63



ketones	micro-organism[a]	react time (h)	lactone 2 yld[b] (%)	lactone 2 [α]D (20 °C)	lactone 2 op[c] (%)	lactone 2 ee[d] (%)	lactone 2 abs conf	lactone 2 ref	lactone 3 yld[b] (%)	lactone 3 [α]D (20 °C)	lactone 3 op[c] (%)	lactone 3 ee[d] (%)	lactone 3 abs conf	lactone 3 ref
 1a	A	3	42 [33]	−67.9° c = 2.3 $CHCl_3$		>95	1*R*,5*S*	16a	44 [30]	−102.3° c = 1.2 MeOH	98	>95	1*S*,5*R*	16b
	B	5	46 [35]	−66.7° c = 0.9 $CHCl_3$		>95	1*R*,5*S*		46 [28]	−102.2° c = 1.0 MeOH	98	>95	1*S*,5*R*	
				−91.5°[e] c = 0.9 $CHCl_3$	94[e]	>95[e]								
 1b	A	3.5	31 [20]	−93.2° c = 2.3 $CHCl_3$	96	>95	1*R*,5*S*	14a	36 [19]	*f*		>95	1*S*,5*S*	16c
	B	5	33 [25]	−87.4 c = 1.7 $CHCl_3$	90	>95	1*R*,5*S*		35 [21]	*f*		[>95]	1*S*,5*S*	
 1c	A	3	37 [35]	−50.1°[e] c = 0.7 $CHCl_3$	100[e]	>95[e] >95	1*S*,6*R*	16d	43 [27]	−23.7° c = 1.0 Et_2O	95	>95[e] [>95][e]	1*R*,6*S*	14b 14c
	B	5	39 [37]	−43.4°[e] c = 2.1 $CHCl_3$	90[e]	>95[e] [>95]	1*S*,6*R*		40 [21]	−26.1° c = 1.1 Et_2O	100	>95[e] [>95][e]	1*R*,6*S*	
 1d	A	5	36 [22]	+69.7° c = 1.1 MeOH	98	>95 [>95]	1*S*,6*R*	14a	41 [27]	−49.5° c = 1.2 MeOH	90		1*S*,6*S*	14d 16e
										−37.6°[e] c = 1.3 MeOH	83[e]	86[e]		
	B	7	41 [26]	+65.5° c = 1.5 MeOH	92	>95 [>95]	1*S*,6*R*		43 [20]	−53.9° c = 1.0 MeOH	98		1*S*,6*S*	
										−42.1°[e] c = 1.1 MeOH	93[e]	92[e]		
 1e	A	4.5	28 [18]	−48.2° c = 1.0 $CHCl_3$	99	>95 [>95]	1*S*,6*R*	14a 16f	52 [30]	−24.5° c = 0.9 $CHCl_3$	61	60 [61]	1*S*,6*S*	14e
	B	8	30 [12]	−48.0° c = 1.0 $CHCl_3$	98	>95 [>95]	1*S*,6*R*		47 [24]	−21.8° c = 1.0 $CHCl_3$	54	50 [53]	1*S*,6*S*	

[a] Microorganism A: *Acinetobacter* NCIB 9871. Microorganism B: *Acinetobacter* TD63. [b] Analytical yields (measured by GC); in brackets, yields of isolated products. [c] Lit.: (+)-**2b** $[\alpha]_D$ = +96.9° c = 1 $CHCl_3$;[14a] (−)-**3a** $[\alpha]_D$ = −104° c = 1.2 MeOH;[14b] (−)-**3c** $[\alpha]_D$ = −25° c = 6 Et_2O;[14c] (−)-**3d** $[\alpha]_D$ = −71° c = 0.9 MeOH[14a] and $[\alpha]_D$ = −55° c = 0.6 MeOH;[14d] (+)-**2e** $[\alpha]_D$ = +48.8° c = 0.5 $CHCl_3$;[14a] (−)-**3e** $[\alpha]_D$ = −40.3° c = 8 $CHCl_3$.[14e] [d] ee's measured by NMR spectroscopy using the shift reagent Eu(tfc)$_3$; ee's in brackets were measured by chiral gas–liquid chromatography using a capillary column coated with a modified cyclodextrine.[15] [e] Values obtained from hydrogenated compounds. [f] Lactone **3b**, even after purification by HPLC, is contaminated by a trace amount of lactone **2b**; thus, the optical purity could not be measured precisely. However, the − sign of the rotation of **3b** allows us to assign the absolute configuration as 1*S*,5*S*.[16c]

that both enantiomers of each starting ketone **1** are oxidized, a result clearly different from the one we have previously observed on a norbornanone derivative,[18] where one enantiomer was oxidized to a lactone, but the other one was reduced to the corresponding alcohol.[7]

At this point in our studies, it is difficult to determine whether one or two monooxygenases are involved in these biotransformations. The similarity of formation rates of each of the lactones might point to the presence of only one enzyme, and also to the fact that only one oxygenase was isolated from *Acinetobacter* NCIB 9871 by both Trudgill[9a] and Walsh.[9b] In this hypothesis, and according to the mechanism proposed by Walsh,[9b] a 4a-hydroperoxyflavine would be the oxygen transfer agent. The enantioselectivity of the reaction would be due to a different positioning of each peroxidic intermediates into the active site (cf. Figure 3). We suppose primarily that the attack of the hydroperoxiflavine should take place on the least hindered face of the ketones **1a–e**. On the other hand, *the migrating C–C bond of the peroxidic intermediate should be antiperiplanar to the peroxidic bond and to a nonbonded electron pair of the hydroxide group*, as suggested by Deslongchamps[19] for chemical Baeyer–Villiger oxidations. Thus, the cycloalkyl part of the enantiomer (*S*,*S*) of the ketone (the one leading to lactone **3**) could be accommodated in only one region of the active site (position 1). The position 2 would be never adopted due to some steric hindrance with the active site (dotted cube). In the

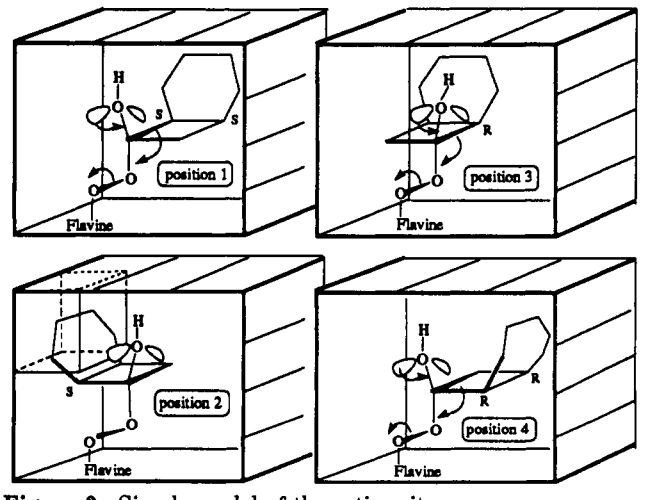


Figure 3. Simple model of the active site.

(18) We also carried out this reaction with racemic norbornanone (bicyclo[2.2.1]heptanone) and norbornenone (bicyclo[2.2.1]hept-5-en-2-one).[4c] Norbornanone led to two lactones: the racemic 2-oxabicyclo[3.2.1]octan-3-one and 3-oxabicyclo[3.2.1]octan-2-one in a 10:1 ratio for *Acinetobacter* NCIB 9871 and 19:1 for *Acinetobacter* TD63. Norbornenone gave only one racemic lactone, 2-oxabicyclo[3.3.0]oct-7-en-3-one.

(19) Deslonchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: Oxford, 1983; pp 313–314.

case of the enantiomer (*R,R*) (leading to lactones **2** and **3**), this portion of the molecule could adopt two orientations (position 3 and 4), although one (position 4) would be favored over the other because of some electronic interactions with the active site.[20]

Experimental Section

General Procedures and Materials. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$. FID gas chromatography (GC) analyses were performed using a capillary column (OV-1701, 25 m). Separations by flash chromatography were achieved with Merck silica gel, and separations by HPLC were carried out with a Si60 column (1-in. diameter) using hexane/ether (70/30–20 mL/min).

Acinetobacter NCIB 9871 was a generous gift from Prof. C. T. Walsh and *Acinetobacter* TD63 from Prof. P. W. Trudgill. Stock cultures were grown on nutrient agar at 30 °C, stored at 4 °C, and subcultured at monthly intervals.

Ketone **1a** was purchased from Merck. Catalytic reduction of **1a** (H_2, atmospheric pressure, 5% Pd/C, AcOEt) led to ketone **1b**. Ketones **1c–e** were obtained from the corresponding olefins by [2 + 2] cycloaddition of dichloroketene (generated in situ from trichloroacetyl chloride according to the procedure of Mehta and Rao),[21] followed by dechlorination (zinc and acetic acid).[22] The IR and ^1H NMR spectra of compounds **1c**,[23a] **1d**,[23b] and **1e**[23a] were identical with those previously reported.

Typical Biotransformation Experiment. A 1-L minimal mineral medium culture[4] with 2 g of *cis/trans*-1,2-cyclohexanediol (Prolabo) as only carbon source was used. Cells were grown for 15 h at 30 °C in a 2-L fermentor with vigorous aeration and stirring at 400 rpm. At the end of the growth period, the temperature was lowered to 25 °C[24] and pH was adjusted to 7.1. Additional cyclohexanediol (0.25 g) and, for *Acinetobacter* NCIB 9871, tetraethylpyrophosphate (200 mg) as a hydrolase inhibitor,[4] was added. After 45 min, 1 g of ketone dissolved in 5 mL of EtOH was added. The progress of the reaction was followed by periodic analysis of aliquots (1 mL) by capillary GC using tetradecane as an internal standard. After completion of the reaction (2–6 h), the biotransformation medium was acidified (pH 1) and extracted with dichloromethane (continuous extraction, 24 h). Products, which were all liquids, were purified by flash chromatography and/or bulb-to-bulb distillation.

The lactones **2a,b,d,e** and **3a–e** were identified by comparison of their IR and ^1H and ^{13}C NMR spectra with those already described in the literature (cf. Table I for references).

(1*S*,6*R*)-(−)-8-Oxabicyclo[4.3.0]non-2-en-7-one (2c):[16d] IR (neat) 1760 cm^{-1}; ^1H NMR δ 1.1–1.98 (m, 7 H), 2.20 (m, 1 H), 2.42 (m, 1 H), 2.63 (m, 1 H), 3.95 (d, 1 H), 4.20 (m, 1 H); ^{13}C NMR δ 186.5 (C=O), 130.7 (CH), 125.2 (CH), 72.0 (CH_2), 38.0 (CH), 35.4 (CH), 21.1 (CH_2), 19.8 (CH_2). Anal. Calcd for $C_6H_{10}O_3$: C, 69.54; H, 7.30. Found: C, 69.24; H, 7.42.

The assessments of enantiomeric excesses were made utilizing NMR spectroscopy in the presence of a shift reagent, $Eu(tfc)_3$, according to the method of Jakovac and Jones.[25] In some cases, the optical purities and ee's of unsaturated lactones were determined on the corresponding saturated compounds obtained by hydrogenation over a Pd/C catalyst. The absolute configurations of the lactones were determined on the basis of previously published results (cf. Table I for references).

The racemic lactones **3a–e** were prepared by chemical Baeyer–Villiger oxidation (H_2O_2–AcOH) at 0 °C.[26] The racemic lactones **2b** and **2e** are obtained after reduction of the corresponding anhydrides using $NaBH_4$.[27]

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Registry No. **1a**, 62182-73-4; **1b**, 138124-04-6; **1c**, 52466-03-2; **1d**, 137917-81-8; **1e**, 109660-29-9; **2a**, 128946-78-1; **2b**, 121960-86-9; **2c**, 138124-05-7; **2d**, 88586-06-5; **2e**, 89395-29-9; **3a**, 43119-28-4; **3b**, 43119-29-5; **3c**, 43119-25-1; **3d**, 124094-64-0; **3e**, 74708-16-0.

(20) This hypothesis could explain the difference of behavior observed between the five-membered ring compounds **1a,b** and the six-membered ring compounds **1c,d**. **1a** and **1b** could not adopt the position 3, because these molecules, more concave than the six-membered ring, would be partially situated in the "forbidden zone" (dotted cube).

(21) Mehta, G.; Rao, K. S. *Synth. Commun.* **1985**, *15*, 991.

(22) Kertesz, D. J.; Kluge, A. F. *J. Org. Chem.* **1988**, *53*, 4962.

(23) (a) Ghosez, L.; Montaigne, R.; Roussel, A.; Vanlierde, H.; Mollet, P. *Tetrahedron* **1971**, *27*, 615. (b) Miller, R. D.; Dolce, D. L.; Merritt, V. Y. *J. Org. Chem.* **1976**, *41*, 1221.

(24) It is important to lower the temperature to 25 °C since, at 27.5 °C, ketone **1a** remained unchanged in this medium.

(25) Jakovac, I. J.; Jones, J. B. *J. Org. Chem.* **1979**, *44*, 2165.

(26) Corey, E. J.; Noyori, R. *Tetrahedron Lett.* **1970**, 311.

(27) Bailey, D. M.; Johnson, R. E. *J. Org. Chem.* **1970**, *35*, 3574.

Reactivity of (3-Chloro-2-methylenecycloalkyl)palladium Chloride Dimers: A Palladium-Mediated Ring Homologation–Functionalization Approach to 4-Aryltropones Related to Colchicine

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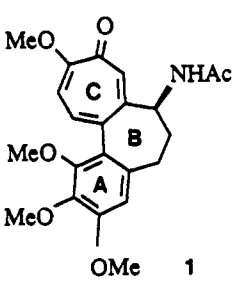
The tricyclic alkaloid colchicine (**1**) is the active principle of the toxic meadow saffron (*colchicum automnal*).[1] It has been used as a treatment for gout,[2] glaucoma,[3] and HIV-1 and -2.[4] The slow, irreversible 1:1 binding of colchicine to the tubulin protein inhibits in vivo microtubule formation.[5] There are two distinct binding sites which individually recognize the A and the C rings of colchicine.[6] Thus, the A–C linked molecule 2-methoxy-5-(2′,3′,4′-trimethoxyphenyl)tropone (**2**) binds rapidly and



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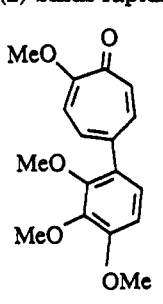

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(1) Eigsti, O. J.; Dustin, P., Jr. *Colchicine*; Iowa State College Press: Ames, IA, 1955; Capraro, H.-G.; Brossi, A. *The Alkaloids*; Brossi, A., Ed.; Academic Press: Orlando, FL, 1984; Vol. 23, p 1.

(2) Brossi, A.; Yeh, H. J. C.; Chrzanowska, M.; Wolff, J.; Hamel, E.; Lin, M. C.; Quinn, F.; Suffness, M.; Silverton, J. V. *Med. Res. Rev.* **1988**, *8*, 77.

(3) Williams, R. N.; Bhattacherjee, P. *Eur. J. Pharmacol.* **1982**, *77*, 17.

(4) Hall, W. W.; Read, S. E.; Lyons, M.; Zabriski, J. B. PCT INT. Appl. WO 89 12444; *Chem. Abstr.* **1990**, *112*, 21097z; *Chem. Eng. News* **1989**, June 26, 11.

(5) Bhattacharyya, B.; Wolff, J. *Biochemistry* **1976**, *15*, 2283–2288. Wilson, L. *Biochemistry* **1970**, *9*, 4999–5007.

(6) Wilson, L. *Ann. N.Y. Acad. Sci.* **1975**, *253*, 214. Cortese, F.; Bhattacharyya, B.; Wolff, J. *J. Biol. Chem.* **1977**, *252*, 1134–1140. Andreu, J. M.; Timasheff, S. N. *Biochemistry* **1982**, *21*, 534–543.

0022-3263/92/1957-1309$03.00/0