The Enantioselective Microbial Reduction of Methyl (±)-5-Chloro-2-oxobicyclo[2.2.1.]heptane-7-*anti*-carboxylate

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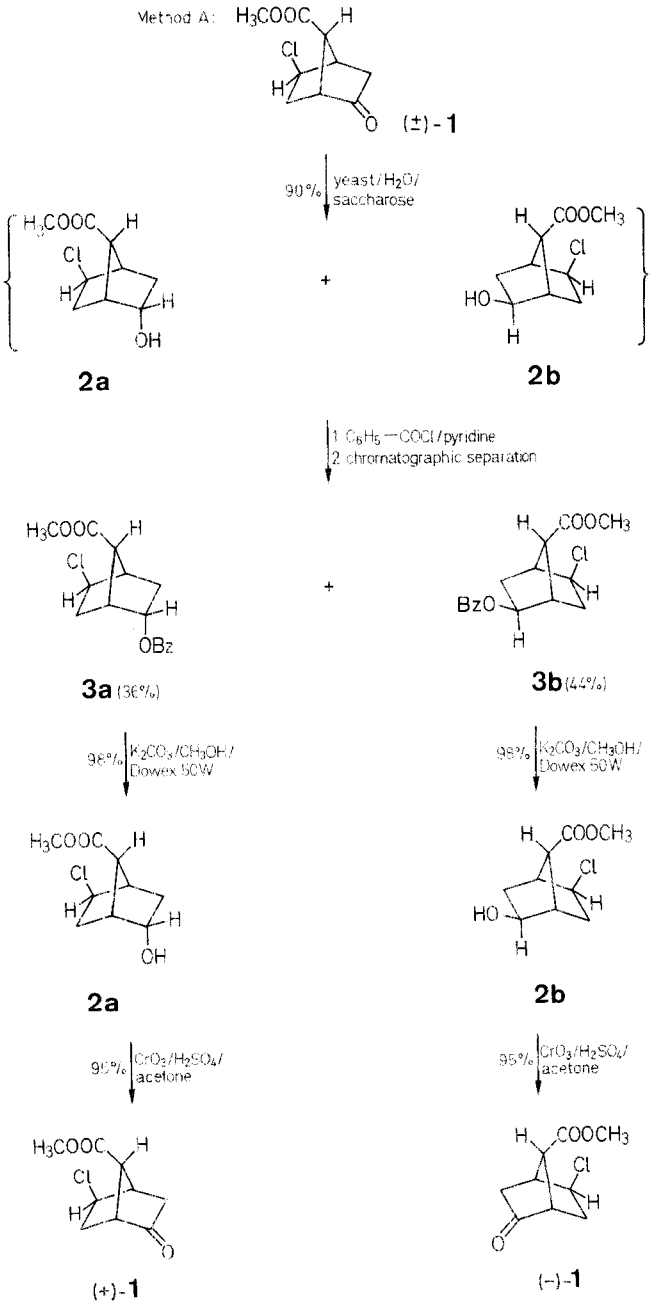
Methyl(+)- and (−)-5-chloro-2-oxobicyclo[2.2.1]heptane-7-*anti*-carboxylates, useful intermediates in prostaglandin synthesis, are prepared on a preparative scale from the racemic compound by two routes using enantioselective microbial reduction as a key-step.

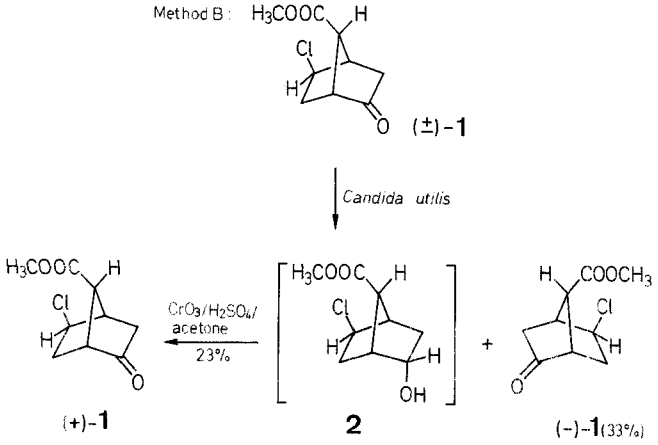
Substituted derivatives of bicyclo[2.2.1]heptane-2-one are useful intermediates in various syntheses of prostaglandins[1–4], the availability of optically active compounds of this type being of major importance. Several methods for the microbial synthesis of chiral compounds have been developed[5–8]. The known reduction of bicyclic ketones using baker's yeast has also been applied in the field of prostaglandins[9,10]. The bicyclo[2.2.1]heptane-2-one system has been studied with respect to stereoselective HLAD-catalyzed oxidoreductions[11,12] and the diamond lattice section of the active site of HLADH has been successfuly applied[13,14,15] in these reactions.

We describe here the reduction of methyl (±)-5-chloro-2-oxobicyclo[2.2.1]heptane-7-*anti*-carboxylic [(±)-**1**; readily available from norbornadiene[4]] with baker's yeast (Method A) and by an optimized procedure using *Candida utilis* (Method B).

The reduction of racemic **1** with baker's yeast under usual laboratory conditions gave a mixture of the isomeric hydroxyesters **2a** and **2b**. These hydroxy compounds were converted into a mixture of the diastereoisomeric benzoates **3a** and **3b** which could be separated by flash chromatography. Cleavage of the isolated benzoates **3a** and **3b** was achieved by treatment with methanolic potassium benzoate at ambient temperature and the resultant pure hydroxy compounds **2a** and **2b** were oxidized with chromium(VI) oxide/sulfuric acid in acetone (Jones oxidation) to give the pure compounds (+)-**1** and (−)-**1**, respectively (Method A).



In order to improve the somewhat laborious procedure of Method A, we have optimized the reduction using fermentation procedures under carefuly controlled conditions and various microorganisms**. The best results were obtained using a strain of *Candida utilis* (CCY 29-38-18) and stopping the reaction at 52–53% conversion of racemic **1**. Separation of the mixture of **2** and (−)-**1** thus obtained is easy. The isolated crude compound **2** is oxidized with Jones' reagent to give the pure compound (+)-**1** in 23% overall yield (Method B). The "unnatural" compound (−)-**1** is isolated in 33% yield. Comparable results are obtained when the reaction is performed on a 50 l fermentation scale.



The structures of compounds **2a, 2b, 3a,** and **3b** were elucidated by N.M.R.-spectral data (Tables 1 and 2). A discussion of the ^{1}H- and ^{13}C-N.M.R. spectra will be published separately.

Table 1. ^{1}H-N.M.R. ($CDCl_3$/TMS_{int}) Chemical Shifts of Compounds **2a, 2b, 3a,** and **3b**

Proton	δ [ppm]			
	2a	**2b**	**3a**	**3b**
1-H	2.83 br. d	2.78 m	2.94 br. d	3.08 tt
2-H	3.83 ddt	4.19 m	4.80 ddd	5.13 dddd
3-*endo*-H	1.87 ddd	2.21 ddd	1.94 ddd	2.44 ddd
3-*exo*-H	2.29 dddd	0.94 dd	1.85 dddd	1.21 dd
4-H	2.57 dp	2.71 tt	2.91 dp	2.91 br. d
5-H	3.77 br. dt	4.03 ddd	3.95 dd	4.09 ddd
6-*endo*-H	1.70 br. dd	2.77 ddd	2.08 ddd	2.75 ddd
6-*exo*-H	1.56 dddd	2.20 ddt	2.43 dddd	2.36 ddt
7-H	3.05 p	2.54 p	3.04 p	2.68 p
OCH_3	3.69 s	3.68 s	3.71 s	3.72 s
OH	1.70 br. s	1.57 br. d	–	–
O—CO—C_6H_5	–	–	7.40–7.48 m	7.42–7.50 m (2H)
			7.53–7.61 m	7.54–7.62 m (1H)
			7.97–8.02 m	7.98–8.04 m (2H)

Method A:

Mixture of Methyl (2*S*,5*R*,7*S*)- and (2*S*,5*S*,7*R*)-5-Chloro-2-hydroxybicyclo[2.2.1]heptane-7-carboxylates (2a + 2b):

Commercial baker's yeast (20 g) is suspended in water (200 ml), sacharose (20 g) is added, and mixture is stirred at 30–32 °C for 45 min. Racemic methyl 5-chloro-2-oxobicyclo[2.2.1]heptane-7-*anti*-carboxylate (**1**; 2.0 g, 0.01 mol) is added in one portion and the mixture is stirred at 28 °C for 2–3 days. The progress of reaction is monitored by T.L.C. (silicagel G; benzene/ethyl acetate/dioxan 90/3/7). The cells are separated by centrifugation or filtration through Celite®, and are washed with water (100 ml). The oil left after evaporation in vacuo is taken up in 1,2-dichloroethane (90 ml) and this solution is dried with sodium sulfate, filtered, and evaporated. Th crude product (2.4 g) is distilled in vacuo to give a mixture of 2a and 2b; yield: 1.8 g, 90%); b.p. 105–7 °C/31 Pa.

$C_9H_{13}ClO_3$	calc.	C 52.82	H 6.40
(204.6)	found	52.76	6.31

Table 2. Coupling Constants $J_{H,H}$ of Compounds **2a, 2b, 3a,** and **3b**

H_i, H_j	J [Hz]			
	2a	**2b**	**3a**	**3b**
1,2	1.0	3.6	0.9	4.3
1,4	1.2	1.5	1.2	1.4
$1,6_{endo}$	1.0	0.	1.6	0.
$1,6_{exo}$	4.8	4.8	5.0	4.3
1,7	1.4	1.3	1.4	1.4
$2,3_{endo}$	7.8	10.0	6.6	10.1
$2,3_{exo}$	3.2	3.6	2.9	3.4
2,7	1.4	1.3	0.	1.6
3_{endo}, 3_{exo}	14.7	13.8	14.6	14.3
3_{endo}, 4	0.	5.03	0.	5.1
3_{endo}, 7	1.4	0.	1.4	0.
3_{exo}, 4	5.0	0.	4.6	0.
3_{exo}, 5	0.	0.	1.2	0.
4,5	1.5	1.1	1.0	1.2
$4,6_{exo}$	1.2	1.0	1.1	1.3
4,7	1.4	1.3	1.4	1.4
$5,6_{endo}$	6.9	8.0	7.8	7.8
$5,6_{exo}$	2.5	3.4	3.2	3.6
6_{endo}, 6_{exo}	14.0	14.5	14.9	14.5
6_{endo}, 7	0.	1.6	0.	1.7
6_{exo}, 7	0.	0.	1.4	0.

Methyl (2*R*,5*R*,7*S*)-2-Benzoyloxy-5-chlorobicyclo[2.2.1]heptane-7-carboxylate (3a) and Methyl (2*S*,5*S*,7*R*)-2-Benzoyloxy-5-chlorobicyclo[2.2.1]heptane-7-carboxylate (3b):

The isomer mixture **2a** + **2b** (3.458 g, 16.9 mmol) is dissolved in pyridine (30 ml) and benzoyl chloride (6.0 g, 43 mmol) is added with stirring. The reaction is monitored by T.L.C. (silica gel; benzene/acetone 95/5). The mixture is left at ambient temperature overnight, then diluted with ether (250 ml). This solution is washed succesively with water (50 ml), hydrochloric acid (1/1; 2 × 50 ml), and water (2 × 50 ml), and is dried with sodium sulfate. The solvent is evaporated and the remaining crude oil is flash chromatographed (silica gel Merck H; benzene/acetone 99/1) to give the individual isomers **3a** and **3b**.

$C_{16}H_{17}ClO_4$	calc.	C 52.24	H 5.55
(308.8)	found for **3a**	52.20	5.48
	found for **3b**	52.31	5.49

Diastereoisomer **3a**; yield: 1.88 g (36%); m.p. 76.5–77.5 °C; $[\alpha]_D^{25}$: + 13.4° (c = 0.7, methanol).

Diastereoisomer **3b**; yield: 2.30 g (44%); m.p. 107–108 °C; $[\alpha]_D^{25}$: + 25.3° (c = 0.4, methanol).

Methyl (2*S*,5*R*,7*S*)-5-Chloro-2-hydroxybicyclo[2.2.1]heptane-7-carboxylate (2a) and Methyl (2*S*,5*S*,7*R*)-5-Chloro-2-hydroxybicyclo[2.2.1]heptane-7-carboxylate (2b):

The benzoate **3a** or **3b** (5.907 g, 18.5 mmol) is dissolved in methanol (150 ml), dry potassium carbonate (0.1 g) is added, and the mixture is stirred for 12 h. DOWEX 50W® (0.5 g) is then added with stirring. The mixture is filtered, and the ion-exchange resin thoroughly washed with methanol (2 × 50 ml). The combined organic phases are evaporated, the crude residue is triturated with heptane (100 ml),

and the product then crystallized from heptane (250 ml) to pure **2a** (3.43 g) or **2b**. An additional amount of **2a** (0.417 g) or **2b** is obtained by flash chromatography of the mother liquor.

$C_9H_{13}ClO_3$	calc.	C 52.82	H 6.40
(204.6)	found for **2a**	52.71	6.31
	found for **2b**	52.77	6.34

Isomer **2a**: yield: 3.85 g (98%); m.p. 89–91 °C; $[\alpha]_D^{25}$: +6.9° (c = 0.4, methanol).

Isomer **2b**; yield: 3.84 g (98%); m.p. 89–91 °C; $[\alpha]_D^{25}$: −20.9° (c = 0.4, methanol).

Methyl (+)- and (−)-5-Chloro-2-oxobicyclo[2.2.1]heptane-7-*anti*-carboxylate [(+)-1 or (−)-1, respectively]:

A 2.6 molar solution of chromium(VI) oxide in 8 molar sulfuric acid (4.5 ml) is added dropwise to a stirred solution of compound **2a** or **2b** (2.04 g, 10 mmol) in acetone (100 ml) and stirring is continued for 15 min. Excess oxidizing agent is then destroyed by the addition of 2-propanol (0.5 ml). The green precipitate is filtered off and washed with acetone (50 ml). The combined acetone solutions are evaporated in vacuo to give the pure product (+)-**1** or (−)-**1**, respectively.

Isomer (+)-**1**; yield: 1.91 g (95%); m.p. 105–106 °C (from water); $[\alpha]_D^{20}$: +31.9° (c = 0.5, methanol).

Isomer (−)-**1**; yield: 1.89 g (95%); m.p. 105–106 °C (from water); $[\alpha]_D^{20}$: −34° (c = 0.4, methanol).

Method B:

Methyl (+)- and (−)-5-Chloro-2-oxobicyclo[2.2.1]heptane-7-*anti*-carboxylate [(+)- 1 and (−)-1] by Partial Microbial Reduction:

Microorganism paste of *Candida utilis* (CCY 29-38-18; 100 g), water (2300 ml), and standard nutrient broth (60 ml) are placed in a 5000 ml fermentor. The pH value is adjusted to 4 with aqueous ammonia. During the fermentation, the ethanol content is held at 0.1% by the gradual addition of 96% ethanol. The suspension is aerated in such a manner that an oxygen saturation of 1.1% at 30 °C is maintained for 1 h. The racemic ester (±)-**1** (15 g, 74 mmol) is then added in one portion and the progress of the reaction is monitored by G.L.C. (SE-30). After ~ 3–4 h (consumption of 52–53% of **1**), the reaction is quenched by the addition of acetone (2000 ml). The microorganisms are and washed with acetone (100 ml) and the combined filtrate is evaporated in vacuo (50 °C). The remaining crude oil (22 g) is diluted with water (10 ml), and extracted with ethyl acetate (3 × 50 ml). The combined organic phases are evaporated in vacuo. The residual crude oily product [containing 50.1% (−)-**1** and 40.8% **2a**] is flash-chromatographed (silica gel; chloroform and chloroform/methanol 95/5) to give the crude product (−)-**1** [yield: 9 g; m.p. 75–80 °C; $[\alpha]_D^{20}$: −23.1° (c = 0.82, methanol)] and the crude product **2a** [yield: 7.6 g; $[\alpha]_D^{20}$: +4.24° (c = 0.82, methanol)]. The crude compound **2a** (7.6 g) is dissolved in acetone (80 ml) and a 2.6 molar solution of chromium(VI) oxide in 8 molar sulfuric acid (2 ml) is added dropwise, with stirring. Excess oxidizing agent is then destroyed by the addition of 2-propanol (3 ml). The green precipitate is filtered off and washed with acetone (80 ml). The combined acetone solutions are evaporated in vacuo to give the pure product (+)-**1** which is further purified by recrystallization from water (110 ml); yield: 3.50 g [23% based on (±)-**1**]; m.p. 105 °C; $[\alpha]_D^{20}$: +33.0° (c = 0.8, methanol).

The crude product (−)-**1** is purified by three recrystallizations from water; yield: 5 g [33%, based on (±)-**1**]; m.p. 105–106 °C; $[\alpha]_D^{20}$: −33.5° (c = 0.8, methanol).

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