Preparation of (−)-*cis*- and (−)-*trans*-2,4-Dimethylcyclohexan-1-ones for the Synthesis of Cycloheximides†

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Received February 5, 1987

Microbial reduction of (±)-2,4-dimethylcyclohex-2-en-1-one by *Beauveria sulfurescens* led mainly to two isomers of 2,4-dimethylcyclohexanols. By oxidation of these alcohols, chiral building blocks for cycloheximide synthesis were obtained, namely (−)-(2*R*,4*R*)-2,4-dimethylcyclohexan-1-one and (−)-(2*R*,4*S*)-2,4-dimethylcyclohexan-1-one.

Cycloheximide (actidione) **1** is an antibiotic that was first extracted in 1946 by Whiffen *et al.*[1] from a culture of *Streptomyces griseus*. It possesses interesting antitumoral,[2] antibiotic[3] and antifungal[4] properties, its absolute configuration being 2*S*,4*S*,6*S*,7*R*. Neoisocycloheximide **2** that was recently extracted from a culture of *Streptomyces* A 94[5] is a diasteroisomer of configuration 2*R*,4*S*,6*R*,7*S*. Another isomer, Naramycin B **3**, of configuration 2*S*,4*S*,6*S*,7*S* has been extracted from a culture of *Streptomyces naraensis*.[6]

All methods proposed so far for synthesizing cycloheximide have started from 2,4-dimethylcyclohexan-1-ones. In 1996, Johnson *et al.*[7] carried out the first synthesis of cycloheximide from (−)-*cis*-2,4-dimethylcyclohexan-1-one, which he had obtained by degradation of naturally-occurring cycloheximide in a basic medium.

The only reported synthesis of optically active 2,4-dimethylcyclohexan-1-one is that of Wolinski and Chan[8] in 1968. These authors obtained (−)-*cis*-2,4-dimethylcyclohexan-1-one from (+)-pulegone, but the purity of the product was low, and the method not well-suited to preparative work. In 1982, Oritani *et al.*[9] reported the first stereospecific synthesis of *cis*-2,4-dimethylcyclohexan-1-one. Starting from 2,4-dimethylcyclohexanol, the two enantiomers **8a** and **8b** were obtained *via* a microbiological hydrolysis. The main isomer of 2,4-dimethylcyclohexanol was isolated from a mixture of the diastereoisomers; its racemic acetate was then hydrolysed by *Bacillus subtilis* giving an alcohol, which, when oxidized, gave

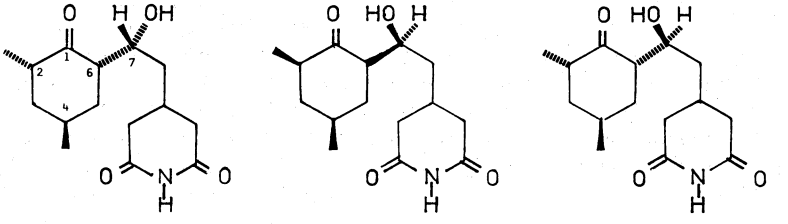

FIG. 1. Structure of Cycloheximide **1**, Neocycloheximide **2** and Naramycin B **3**.

† Use of Biological Systems for the Preparation of Chiral Synthons. Part II.

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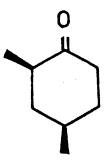
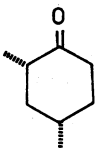


8a



8b

FIG. 2.

an enantiomer **8a** and an acetate. After hydrolysis and oxidation, this acetate gave enantiomer **8b**.

No stereospecific synthesis of the *trans* isomer of 2,4-dimethylcyclohexan-1-one has previously been reported. The only means of obtaining an optically active *trans* isomer has been by the thermal degradation of naturally-occurring cycloheximide.[10] This route was used in 1984 by Oritani *et al.*[11] for a synthesis of cycloheximide.[16]

We report here a method for stereospecifically synthesizing (−)-*cis*- and (−)-*trans*-2,4-dimethylcyclohexan-1-ones.

MATERIALS AND METHODS

Analytical gas phase chromatography was performed with an Intersmat IGC 12 M instrument using a catharometric detector. The 3-m long stainless steel colum was filled with 20% Carbowax 20 M on Chromosorb W, the carrier gas was hydrogen (1bar pressure) and the oven temperature was 120°C.

Preparative gas chromatography was performed with a Varian–Aerograph 90-P using a catharometric detector. The 6-m long aluminium column was fitted with 20% Carbowax 20 M on Chromosorb W, the carrier gas being hydrogen at a pressure of 2 bars.

Optical rotations were determined with a Perkin-Elmer 141 polarimeter at 25°C for the mercury J-line (λ = 578 nm).

NMR spectra in $CDCl_3$ with TMS as an internal standard were recorded on a JEOL CX 60 instrument at 60 MHz for ^1H, and on a BRUKER 200 instrument at 50.327 MHz for ^{13}C. For optical purities, ^1H NMR spectra were recorded on a BRUKER 300 MSL at 300 MHz with $CDCl_3$ as the standard.

(±)-*2,4-Dimethylcyclohex-2-en-1-one* (**10a**+**10b**). 2,4-dimethylphenol was reduced in an ethanol solution with Raney nickel, and the mixture of isomers of 2,4-dimethylcyclohexanol (**4** to **7**) were oxidized in an acetone solution with CrO_3 in aqueous sulfuric acid, according to the reported method.[9]

Chlorination of the saturated ketone was carried out according to Warnhoff *et al.*[13]: 25 g of 2,4-dimethylcyclohexan-1-one in 100 ml of CCl_4 was placed in a 500-ml round-bottomed flask equipped with a mechanical stirrer, a condenser and a tap funnel. A solution of 18 ml of sulfuryl chloride in 30 ml of CCl_4 was added over 1 hour with shaking. The flask was cooled in a water bath, and the mixture was stirred for a further 2 hours. The resulting yellow solution was washed 3 times with 30 ml of water and then twice with 20 ml of saturated $NaHCO_3$ solution. After drying and evaporating the solvent, the residue was distilled, dehydrochlorination of the chlorinated derivative occurring *in situ* during this distillation. A strong discharge of hydrogen chloride was produced at an early stage of heating, and the distillation of (±) 2,4-dimethylcyclohex-2-en-1-one was then performed (18.5 g yield, 75%), bp 73 ~ 75°C (12 mmHg). 60 MHz NMR δ_H ($CDCl_3$): 6.60 to 6.75 (1H, m); 2.80 to 1.40 (5H, m); 1.78 (3H, s); 1.15 (3H, d, J = 6.7 Hz). *Anal.* Found: C, 77,25: H, 9.8. Calcd. for $C_8H_{12}O$: C,77.37; H, 9.74%.

Microbiological reduction of (±)-2,4-dimethylcyclohex-2-en-1-one. Beauveria sulfurescens (ATCC7159) was grown as described previously.[12] A solution of 1.7 g of (±)-2,4-dimethylcyclohex-2-en-1-one in 3 ml of DMSO was added to 1.7 l of a 24 hr-old culture of *B. sulfurescens*, areation being maintained at 10 ml/l/min. After 48 hr, the mixture was filtered and the filtrate saturated with $(NH_4)_2SO_4$, before being extracted 4 times with ether. After drying and evaporating the solvent, the residue was analyzed by gas chromatography. It consisted of 10% 2,4-dimethylcyclohexan-1-one (**8a** + **9a**), 40% 2,4-dimethylcyclohexanol **4a** and 50% 2,4-dimethylcyclohexanol **7a**. These were separated and purified on a Merck silicagel column with pentane–ether (90 : 10 v/v) as the eluent. *Rf*: **8a** + **9a**, 0.8; **4a**, 0.4; **7a**, 0.2.

2,4-Dimethylcyclohexan-1-one (**8a**+**9a**, 0.120 g). t_R: 8 min, 30 sec. $[\alpha]_J^{25}$ −1° (c = 0.05, $CHCl_3$). 60 MHz NMR δ_H ($CDCl_3$): 2.7 ~ 1.5 (8H, s); 0.98 (6H, d, J = 6 Hz).

(+)-(*1S,2R,4R*)-*2,4-Dimethylcyclohexanol* (**4a**, 0.420 g). t_R: 11 min, 30 sec. $[\alpha]_J^{25}$ +30° (c = 0.21, $CHCl_3$). 300 MHz NMR δ_H ($CDCl_3$): 3.8 ~ 3.75 (1H, m); 1.9 ~ 1.8 (2H, m); 1.6 ~ 1.0 (6H, m); 0.95 (3H, d, J = 7 Hz); 0.90 (3H, d, J = 6 Hz). 50.327 MHz NMR δ_C ($CDCl_3$): 70 (C1), 36.9 (C3), 36.3 (C2), 33.4 (C6), 32.5 (C4), 28.1 (C5), 22.6 (Me-2), 18.5 (Me-4). *Anal.* Found: C, 74.85; H, 12.70. Calcd. for $C_8H_{16}O$: C, 74.94; H, 12.58%. Optical purity: (±)-**4** was obtained from a mixture of chemically-prepared diastereoisomers by separating on a Merck silicagel column with pentane–ether (90 : 10 v/v) as the eluent (Rf = 0.4). 300 MHz NMR δ_H ($CDCl_3$):

(±)-**4** (4 mg) and tris(3-(trifluoromethylhydroxymethylene)-*d*-camphorato)europium(III) (15 mg) in 0.3 ml $CDCl_3$. Methyl 2: 2.20 and 2.30. Proton 1: 6.45

and 6.52.

4a (4 mg) and tris(3-(trifluoromethylhydroxymethylene)-*d*-camphorato)europium(III) (13 mg) in 0.3 ml $CDCl_3$. Methyl 2: 2.15. Proton 1: 6.81.

(−)-(*1S,2R,4S*)-*2,4-Dimethylcyclohexanol* (**7a**, 0.640 g). t_R: 18 min. $[\alpha]_J^{25} -31°$ ($c=0.18$, $CHCl_3$). 300 MHz NMR δ_H ($CDCl_3$): 3.76 ~ 3.68 (1H, m); 2.08 ~ 1.98 (1H, m); 1.75 ~ 0.98 (7H, m); 0.95 (3H, d, $J=7$ Hz); 0.86 (3H, d, $J=6$ Hz). 50.327 MHz NMR δ_C ($CDCl_3$): 72.4 (C1), 39.1 (C3), 33.6 (C2), 32.2 (C5), 28.9 (C6), 25.6 (C4), 21.3 (Me-2), 12.5 (Me-4). *Anal.* Found: C, 75.06; H, 12.45. Calcd. for $C_8H_{16}O$: C, 74.94; H, 12.58%. Optical purity: (±)-**7** was obtained from a mixture of chemically-prepared diastereoisomers by preparative gas chromatography at an oven temperature of 160°C, t_R: 13 min, 300 MHz NMR δ_H ($CDCl_3$).

(±) **7** (4 mg) and tris(3-(trifluoromethylhydroxymethylene)-*d*-camphorato)europium(III) (15 mg) in 0.4 ml $CDCl_3$. Methyl 2: 2.58 and 2.69.

7a (3.5 mg) and tris(3-(trifluoromethylhydroxymethylene)-*d*-camphorato)europium(III) (12 mg) in 0.4 ml $CDCl_3$. Methyl 2: 2.63. Total yield of recovered products: 70%.

(−)-(*2R,4R*)-*2,4-Dimethylcyclohexan-1-one* (**8a**). A solution of 0.5 g of **4a** in 5 ml of CH_2Cl_2 was rapidly added with stirring to a suspension of 1.5 g of pyridiniumchlorochromate in 10 ml of CH_2Cl_2 at room temperature. After 1 hour of shaking, a black precipitate was formed. Ether was added to the mixture, the supernatant was decanted and the precipitate washed several times with ether. The etheral solution was filtered through a 50 : 50 mixture of silicagel and magnesium sulfate, and the solvent evaporated (0.45 g, 92% yield). $[\alpha]_J^{25} -5°$ ($c=0.15$, $CHCl_3$). 60 MHz NMR δ_H ($CDCl_3$): 2.8 ~ 1.7 (8H, m); 1.02 (6H, d, $J=6$ Hz). *Anal.* Found: C, 76.30; H, 10.98. Calcd. for $C_8H_{14}O$: C, 76.14; H, 11.18%.

(−)-(*2R,4S*)-*2,4-dimethylcyclohexan-1-one* (**9a**). 0.6 g of alcohol **7a** was oxidized by the same procedure (0.55 g, 94% yield). $[\alpha]_J^{25} -60.5°$ ($c=0.08$, $CHCl_3$). 60 MHz NMR δ_H ($CDCl_3$): 2.7 ~ 1.6 (8H, m), 1.02 (6H, d, $J=6$H). *Anal.* Found: C, 76.05; H, 11.30. Calcd. for $C_8H_{14}O$: C, 76.14; H, 11.18%.

RESULTS AND DISCUSSION

In the course of earlier work,[12] we showed that the mould *Beauveria sulfurescens* (ATCC 7159) under conditions of low aeration reduced the double bond of 2-methylcyclohex-2-en-1-ones with very high stereoselectivity, producing an asymmetrical 2-carbon with an *R* configuration.

The action of *B. sulfurescens* on (±) 2,4-dimethylcyclohex-2-en-1-one was investigated, the starting material being prepared from 2,4-dimethyl-phenol. The various reactions involved have been described elsewhere (see EXPERIMENTAL). However, during the distillation of chlorinated 2,4-dimethylcyclohexan-1-one, a spontaneous loss of HCl occurred, giving racemic 2,4-dimethylcyclohex-2-en-1-one (**10a** + **10b**) directly (Fig. 3).

The general bioconversion conditions for *B. sulfurescens* have already been described.[12] After the usual work-up, the residue was analyzed by gas-phase chromatography (GPC). After 48 hr the unsaturated ketone had completely disappeared. Three different reaction products were formed, the product with the shortest retention time accounting for 10% of the mixture. Comparison with an authentic sample showed this to be a mixture of the *cis* and *trans* isomers of 2,4-dimethylcyclohexan-

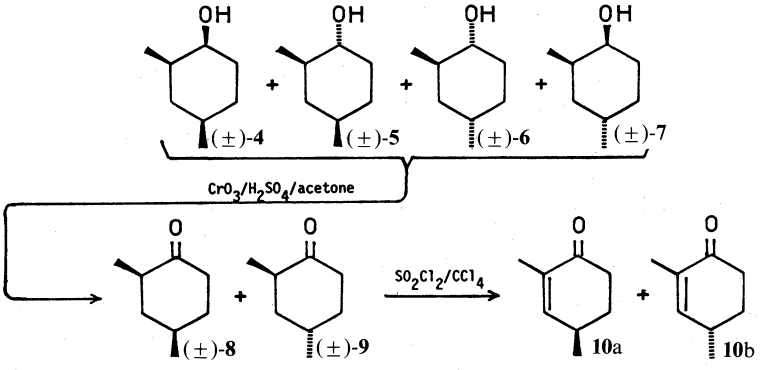


FIG. 3. Synthesis of (±)-2,4-Dimethylcyclohex-2-en-1-ones (**10a** + **10b**) from 2,4-Dimethylcyclohexanols.

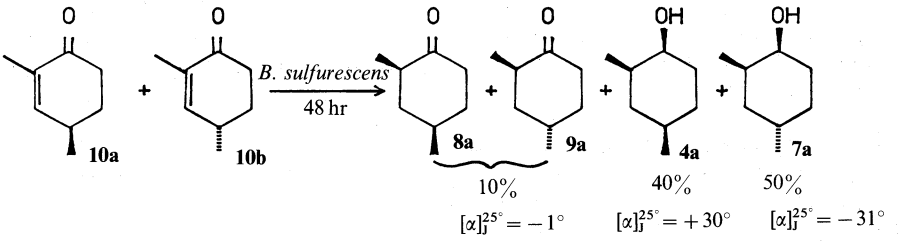


FIG. 4. Microbiological Reduction of (±)-2,4-Dimethylcyclohex-2-en-1-ones by *Beauveria sulfurescens*.

1-one, with the *cis* isomer predominating (Fig. 4).

Given the stereochemistry observed previously[12] for the reduction of the double bond of 2-methylcyclohex-2-en-1-one by *B. sulfurescens*, it could be assumed that the mixture that was obtained consisted of the isomers **8a** (predominant) and **9a**.

As the amount of saturated ketone formed was small and the isomers were difficult to separate, this would not be a good method for preparing 2,4-dimethylcyclohexan-1-ones directly. However, the two other products formed proved to be isomers of 2,4-dimethylcyclohexanol, which were purified by column chromatography and then oxidixed to give the corresponding isomers of 2,4-dimethylcyclohexan-1-one.

The structure of the two dimethylcyclohexanols was then determined.

Alcohol **4a**: A comparison between its GPC retention time and those of authentic samples of the four 2,4-dimethylcyclohexanols showed this to be identical with the isomer of the shortest retention time, having an axial hydroxyl and equatorial methyls. This was confirmed by comparing its ^{13}C NMR spectrum with data obtained by Grenier-Loustalot *et al.*[14] These authors had correlated the ^{13}C NMR spectra of numerous mono-and dimethylated cyclohexanols with their structure and retention time. In Table I, the parameters for the alcohol **4a** are compared with those of Grenier-Loustalot for the (±)-isomer **4.**

In order to determine the absolute configuration of **4a**, we carried out a stereoselective oxidation using the method of Corey *et al.*[15] (Fig. 5).

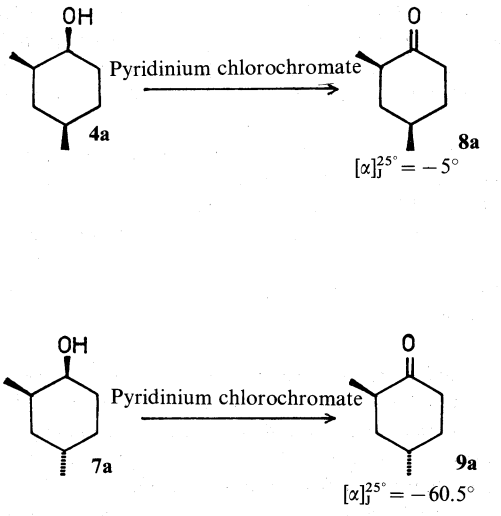


FIG. 5. Oxidation of **4a** and **7a** by Pyridinium Chlorochromate.

A *cis*-2,4-dimethylcyclohexan-1-one with negative optical rotation was obtained. Comparison of this value with those determined by Oritani *et al.*[9] for the two enantiomers of *cis*-2,4-dimethylcyclohexan-1-one showed **8a** to be of the configuration 2*R*,4*R* ($[\alpha]_D^{20} = -2.2°$[9]). This allowed the absolute configuration of **4a** to be assigned, since the hydroxyl was *cis* with respect to the 2-methyl (Fig. 3)

Thus, alcohol **4a** had the configuration 1*S*,2*R*,4*R*.

Alcohol **7a**: A comparison between retention times showed **7a** to be identical to the isomer with the longest retention time, with an equatorial hydroxyl, axial 2-methyl and equatorial 4-methyl. Comparing the ^{13}C NMR parameters of **7a** with those of Grenier-Loustalot *et al.* for the (±) isomer **7** confirmed this struc-

TABLE I. COMPARISON OF ^{13}C NMR SPECTRAL DATA FOR ISOMERS **4a** AND **7a** WITH VALUES DETERMINED BY GRENIER-LOUSTALOT *et al.*[14]

	Isomer **4a**	(±)-**4**	Isomer **7a**	(±)-**7**
C_1	70.0	69.4	72.4	72.2
C_2	36.3	37.0	33.6	34.2
C_3	36.9	37.3	39.1	39.7
C_4	32.5	33.0	25.6	25.9
C_5	28.1	28.5	32.2	32.9
C_6	33.4	33.9	28.9	29.0
Me-2	22.6	22.8	21.3	21.8
Me-4	18.5	19.0	12.5	12.6

ture (Table I).

Like **4a**, **7a** was oxidized by pyridinium-chlorochromate[15] to give **9a** ($[\alpha]_D^{25} = -60.5°$) (Fig. 5).

The optical rotation of the *trans* isomer obtained by the thermal degradation of naturally-occurring cycloheximide is $[\alpha]_D^{20} = +58.3°$.[11] Oritani assigned to it the configuration 2*S*,4*R*. Accordingly, **9a** must have had the configuration 2*R*,4*S*. In the alcohol **7a**, the hydroxyl was *cis* with respect to the 2-methyl (Fig. 3), the isomer (**7a**) therefore having the configuration 1*S*,2*R*,4*S*.

The structures of **4a** and **7a** are consistent with previous results obtained for the microbiological reduction of 2-methylcyclohex-2-en-1-ones with *B. sulfurescens*,[12] the absolute configuration of the hydroxyl-bearing carbon always being S and that of the 2-methyl-bearing carbon always being *R*.

Thus, the reduction of (±)-2,4-dimethylcyclohex-2-en-1-one with *B. sulfurescens* led mainly to the two isomers of 2,4-dimethylcyclohexanol (**4a** and **7a**), which were readily purified and gave quantitatively upon oxidation two isomers of 2,4-dimethylcyclohexan-1-one: (−)-(2*R*,4*R*)-2,4-dimethylcyclohexan-1-one **8a** and (−) (2*R*,4*S*) 2,4-dimethylcyclohexan-1-one **9a**.

All the compounds were optically pure, the purity of alcohols **4a** and **7a** having been checked by ^1H NMR, using an europium chiral shift reagent, by comparing their spectra with the spectra obtained for the corresponding racemates. The racemic alcohols could be extracted from the mixture of diastereoisomers by column chromatography ((±) **4**) and preparative gas chromatography ((±) **7**).

The ^1H NMR spectrum obtained for (±) **4** at 300 MHz in the presence of tris(3-(trifluoromethylhydroxymethylene)-*d*-camphorato)europium(III) showed the C-2 methyl signal split into two doublets, one for each enantiomer (see EXPERIMENTAL). Two broad singlets were obtained for the C-1 proton geminal to the hydroxyl group. Under the same conditions, the ^1H NMR spectrum of the alcohol **4a** showed only one doublet for C-2 and one singlet for the C-1 proton signal.

The ^1H NMR spectrum obtained for (±) **7** at 300 MHz with a chiral reagent showed two doublets for the C-2 methyl signal and only one singlet for the C-1 proton signal. Under the same conditions, the **7a** spectrum showed only one doublet for the C-2 methyl, the C-1 proton signal being unchanged.

These results indicate that the alcohols obtained by microbiological reduction were optically pure. The subsequent step for the oxidation of **4a** and **7a** according to Corey[15] was stereospecific, so that the corresponding ketones were, therefore, optically pure.

This preparation of **9a** is the first reported stereospecific synthesis of an optically active *trans*-2,4-dimethylcyclohexan-1-one.[16]

The results described here are yet another reminder of the usefulness of biological systems for preparing chiral molecules.

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