

Biotransformation of Cycloalkanediones by *Caragana chamlagu*

Wen Chai, Hiroshi Sakamaki,† Susumu Kitanaka,†† and C. Akira Horiuchi*

Department of Chemistry, Rikkyo (St. Paul's) University, Nishi-Ikebukuro, Toshima-ku, Tokyo 171-8501

†College of Science and Technology, Nihon University, 7-24-1 Funabashi 274-8501

††College of Pharmacy, Nihon University, 7-7 Funabashi 274-8501

(Received July 31, 2002)

The biotransformation of alkylcycloalkanediones using suspension plant cultured-cells of *Caragana chamlagu* gave oxo carboxylic acids by oxidative cleavage. 5,6-Dioxoheptanoic acid was obtained in high yield (95%) in a short time (7 h) from 2-methyl-1,3-cyclohexanedione. However, 1,2- and 1,4-cycloalkanediones were reduced stereoselectively and *trans*-1,2-cyclohexanediol and *trans*-1,4-cyclohexanediol were obtained, respectively. The mechanism of the oxidative cleavage of alkylcycloalkanediones is also discussed.

Oxo carboxylic acids are important as intermediates in organic synthesis. The oxidative cleavage of ketones has found important applications in industrial syntheses,[1] such as the oxidation of 1,2-cyclohexanedione to adipic acid.[2] There are many syntheses of oxo carboxylic acids and esters, involving regiospecific oxidative ring cleavage of cycloalkanones with heteropolyacid and oxygen gas;[3] ring-opening oxygenation on treatment with lead(IV) acetate,[4] sodium periodate,[5] perbenzoic acid,[6] and oxovanadium(V)[7] in alcohol under oxygen; and electrochemical oxidative cleavage of α-substituted cycloalkanones.[8] Recently, we have reported that the reaction of α-alkylcycloalkanones with iodine-cerium(IV) salts,[9] and cerium(IV) salts[10] in alcohol yields the corresponding oxoalkanoic esters and ω,ω-dialkoxyalkanoic esters. However, most of these processes suffer from the disadvantages of the use of toxic reagents and heavy metals. More recently, from the viewpoint of "green chemistry", we have reported that the reaction of α-iodocycloalkanones with a high-pressure mercury lamp in alcohols containing a small amount of water yields the corresponding ω,ω-dialkoxyalkanoic esters.[11]

On the other hand, biotransformation of a synthetic substrate into more useful substances by plant cultured-cells is an important reaction in synthetic chemistry.[12] However, the hitherto known biotransformation of carbonyl compounds by plant cultured-cells is only the NADH-dependent reduction of the C-C double bond of α,β-unsaturated ketones.[13] There is only very little information on the biotransformation of diketones by plant cultured-cells. During the course of our studies, we have investigated the reduction of (+)- and (−)-camphorquinones with plant cultured-cells to give the corresponding α-keto alcohols.[14] Also, we have reported that in the biotransformation of 3,6-dialkylcyclohexane-1,2-diones by plant cultured-cells of *Marchantia polymorpha*, regioselective oxidative cleavage of the C–C bond occurred to give the corresponding oxo carboxylic acids decreased by one carbon.[15] However, all of these reactions required plant cultured-cells in large quantities. Usually, 30 g plant cultured-cells are required to transform 40 mg of substrates. Recently, we have tried to obtain useful products from the biotransformation using plant cultured-cells of *Caragana chamlagu* LAMARCK (*Leguminosae*) (also called *Caragana sinica*). This is a medicinal plant native to China. The dried roots of *C. chamlagu* have been used in Korea and China as a folk medicine effective against neuralgia, rheumatism and arthritis. This plant has yielded the anti-inflammatory principle, (+)-α-viniferine, and some oligomeric stilbenes, and then the calluses have been prepared to study biosynthesis of these compounds.[16] We first reported the biotransformation of thujopsene using a small quantity of plant cultured-cells of *C. chamlagu*;[17] i.e., only 2 g of cultured-cells could transform 60 mg of thujopsene. It was found that cultured suspension cells of *C. chamlagu* have high ability to oxidize thjuopsene regio- and stereoselectively; mayurone was obtained in good yields. In this paper, we report that the biotransformation of cycloalkanediones (**1a**–**1g**) by *C. chamlagu* yields the corresponding oxo carboxylic acids and alcohols (**2a**–**2g**).

Results and Discussion

The biotransformation of alkylcycloalkanediones (**1a**–**1d**) using *C. chamlagu* in aqueous solution at room temperature in the dark gave the products **2a**–**2d**. In the reaction mixtures of diosphenol (**1a**), two compounds **2a**, (44%) and **3a** (35%), were isolated. Compound **2a** showed absorptions at 1717 (O=C–OH), and 1707 cm^{-1} (C=O) in its IR spectrum. The CI-MS spectrum of **2a** showed an $[M + H]^+$ peak at *m*/*z* 173. The ^1H NMR spectrum showed two doublets at δ 1.21 (3H, J = 7.0 Hz, $CHCH_3$), and 1.10 (6H, J = 7.0 Hz, $CH(CH_3)_2$). The ^{13}C NMR spectra exhibited signals at 214.3 ppm and 182.4 ppm, which were assigned to a common carbonyl carbon and a carbon of carboxylic acid, respectively. Therefore, compound **2a** was identified to be 2,6-dimethyl-5-oxoheptanoic acid. The IR spectrum of **3a** showed absorption at 1708 cm^{-1} (C=O). This product **3a** was identified to be 6-methyl-2,5-heptanedione based on the presence of a singlet in the ^1H NMR spectrum at δ

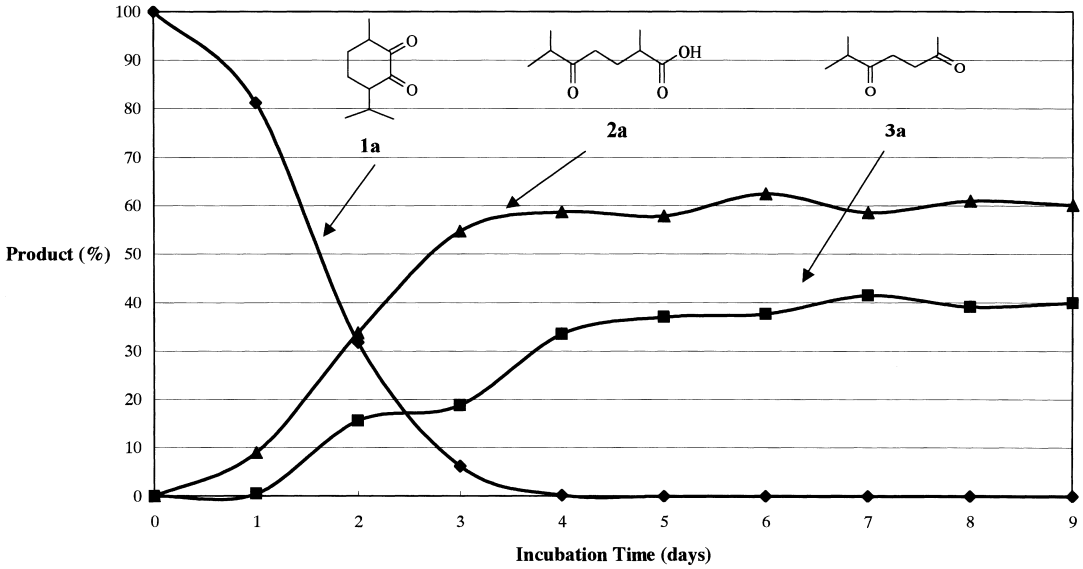
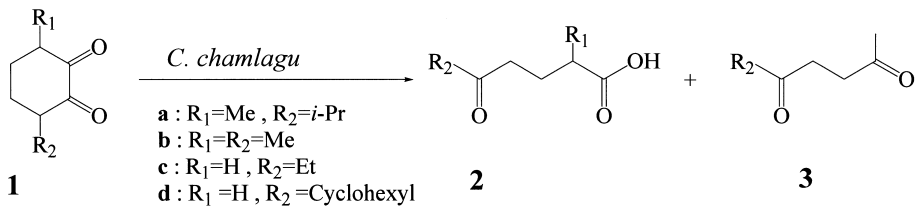


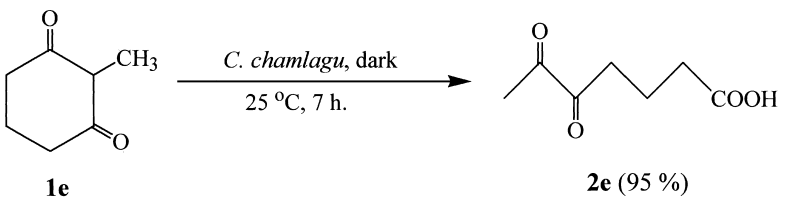
Fig. 1. Distribution of reactive products of diosphenol (**1a**) using *C. chamlagu*.

Table 1. Biotransformation of 3,6-Dialkyl-1,2-cyclohexanediones by Cultured-Cells of *C. chamlagu*



Run	Substrate	Time/d	Yield/%[a)]
1	**1a**	4	**2a** (44), **3a** (35)
2	**1b**	8	**2b** (67)
3	**1c**	5	**2c** (40)
4	**1d**	14	**2d** (55)

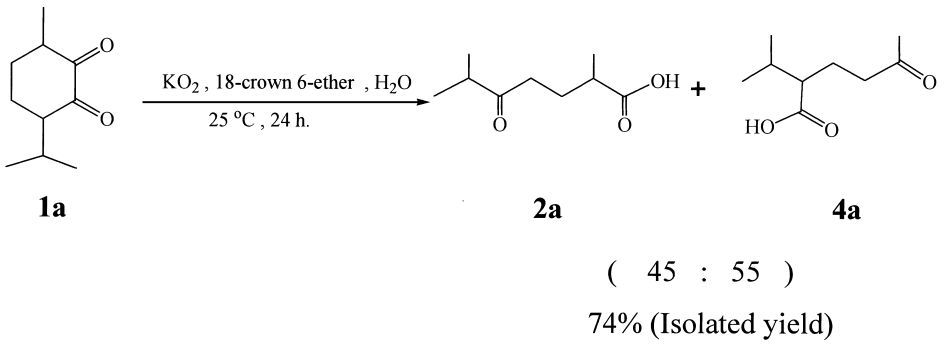
Reaction condition: Substrate (80 mg), *C. chamlagu* (2 g), and culture medium (100 mL) were employed at 25 °C in the dark. a) Isolated yield.



Scheme 1. Biotransformation of 2-methylcyclohexane-1,3-dione (**1e**) using *C. chamlagu*.

2.19 (3H, COCH_3). In order to investigate the time course of the reactions, alkylcycloalkanediones **1a**–**1d** using *C. chamlagu* were examined. As to diosphenol (**1a**), the substrate disappeared after 4 days incubation (Fig. 1). The results with other substrates (**1b**–**1d**) are summarized in Table 1. 3,6-Dimethyl-1,2-cyclohexanedione (**1b**) was transformed to 2-methyl-5-oxohexanoic acid (**2b**, 67%) after 8 days' incubation. 3-Ethyl-1,2-cyclohexanedione (**1c**) was converted to 5-oxoheptanoic acid (**2c**, 40%) as the major product after 5 days. Also, 3-cyclohexyl-1,2-cyclohexanedione (**1d**) was transformed to 5-cyclohexyl-5-oxopentanoic acid (**2d**, 55%) at the end of 14 days' incubation. On the basis of these results, it was found that the biotransformation of **1a**–**1d** is a biocatalytic oxidative cleavage of the alkylcycloalkanediones, which gave the oxo carboxylic acids **2a**–**2d** with decarbonylation.

As shown in Scheme 1, 2-methyl-1,3-cyclohexanedione (**1e**) disappeared after 7 hours incubation and compound **2e** (95%) was isolated. Its IR spectrum showed absorptions at 1717 (O=C–OH), and 1708 cm^{-1} (C=O). The CI-MS spectrum of **2e** showed an $[M + H]^+$ peak at *m/z* 159. The ^1H NMR spectrum showed one singlet at δ 2.34 (3H, COCH_3). The ^{13}C NMR spectra exhibited signals at 198.3 ppm, 197.2 ppm, and 178.8 ppm, which were assigned to two common carbonyl carbons and a carbon of carboxylic acid, respectively.



Scheme 2. Oxidation of diosphenol (**1a**) with potassium superoxide.

Therefore, compound **2e** was identified to be 5,6-dioxoheptanoic acid. In this case, the biocatalytic oxidation of β-diketone **1e** gave the oxo carboxylic acid **2e** without decarbonylation.

Therefore, the biotransformation of alkylcycloalkanediones using *C. chamlagu* is a new synthetic method to access oxo carboxylic acids, and the reaction mechanism is very interesting. On the other hand, the oxidative cleavage of some compounds, which have carbonyl, α-keto, esters, and carboxylic acids, with a superoxide radical anion from potassium superoxide (KO_2) was described.[18] Also, we have reported that in the biodegradation of bisphenol A by plant cultured-cells of *C. chamlagu*, 4-isopropenylphenol was obtained as an intermediate. In the case of degradation of bisphenol A with superoxide radical anion from potassium superoxide (KO_2), 4-isopropenylphenol was obtained.[19] The above results suggest that the superoxide radical anion participates in the biocatalytic oxidative cleavage for alkylcycloalkanediones using *C. chamlagu*.

In order to investigate the reaction mechanism of the biocatalytic oxidative cleavage for alkylcycloalkanediones using *C. chamlagu*, oxidation of diosphenol (**1a**) with the superoxide radical anion was carried out. In the experiment, powdered potassium superoxide was added to a suspension of diosphenol (**1a**) in H_2O containing 18-crown-6 ether with stirring at room temperature, and two products were obtained. One product was identified as 2,6-dimethyl-5-oxoheptanoic acid **2a** (33%) by its spectral data, which agreed with those of compound **2a**. Another product was revealed to be **4a** (41%) on the basis of the following spectral data. The CI-MS spectrum of **4a** showed the $[M + H]^+$ peaks at *m/z* 173. The IR spectrum showed carbonyl absorption and carboxylic acid absorption similar to **2a**. The ^{1}H NMR spectra exhibited the presence of methyl groups at δ 2.16 (3H, s, $COCH_3$) and 0.98 (6H, d, $C(CH_3)_2$), which agreed with the authentic data of 2-isopropyl-5-oxohexanoic acid.[20] Therefore, compound **4a** was identified as 2-isopropyl-5-oxohexanoic acid. The relative ratios of the products were determined on the basis of the ^{1}H NMR spectral peak areas of the proton of the isopropyl groups (Scheme 2).

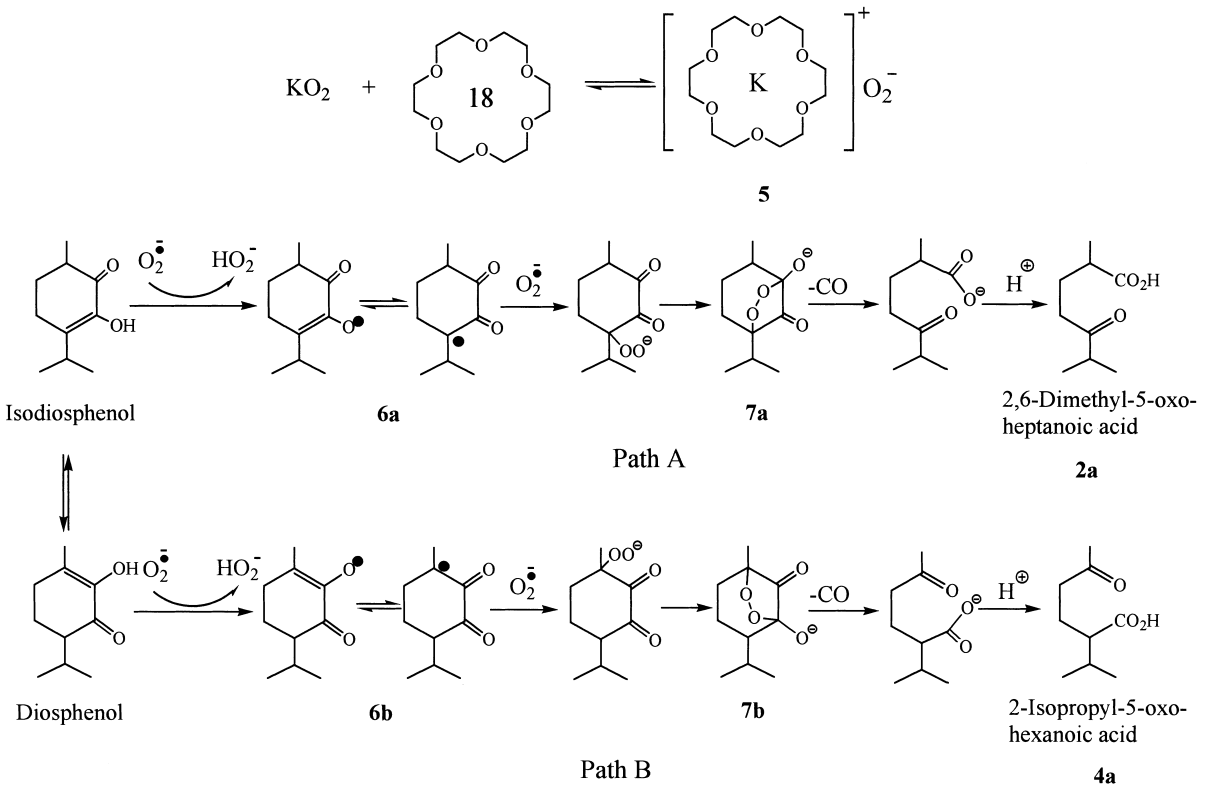
In order to explain the reaction with KO_2, we assumed the equilibria shown in Scheme 3. First, a superoxide radical anion is generated from the intermediate **5**. Then, the enol isomers of the diketone **1a** by an attack of the superoxide radical anion lead to the diosphenolyl radicals (**6a**, and **6b**). Continuously, the diosphenolyl radicals are attacked at the carbonyl group of β position; five-membered cyclic peroxy-anion intermediates (**7a**, and **7b**) forms via common peroxy-anions. Such a five-membered cyclic peroxy-anion is well known to be formed in the oxidative cleavage of α-diketones.[18c, 21] The products **2a** and **4a** were obtained by decarbonylation from path A or path B, respectively.

In the case of biotransformation of **1a** by *C. chamlagu*, 2-isopropyl-5-oxohexanoic acid (**4a**) was not obtained. It is considered that the mechanism of synthesis of **2a** resembled path A of Scheme 3. Hence, other alkylcycloalkanediones **1b–1d** are attacked by the superoxide radical anion to lead to five-membered cyclic peroxy-anions **8a**, and to yield **2b–2d** by decarbonylation, respectively (Scheme 4).

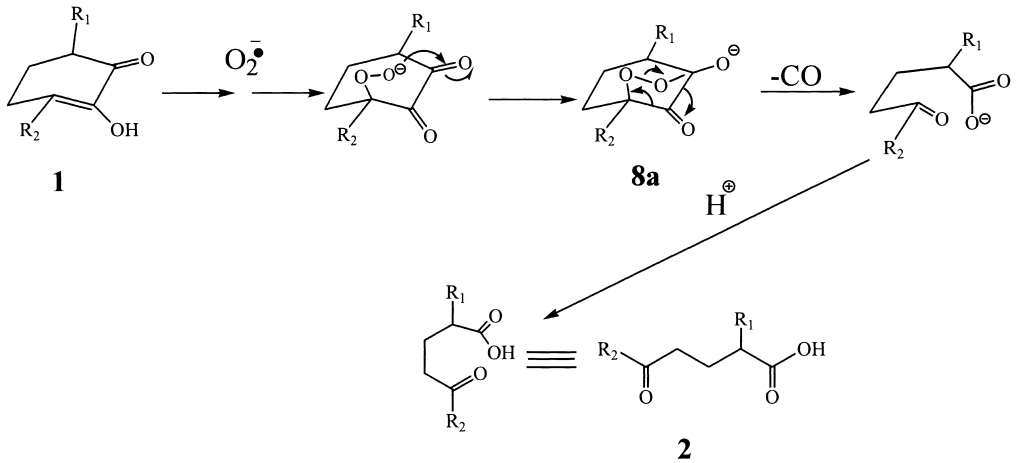
As is shown in Scheme 5, the enol isomer of 2-methyl-1,3-cyclohexanedione (**1e**) by attack of the superoxide radical anion leads to the peroxy-anion (**9a**). For all of the carbonyl groups situated at the α position of methyl group, **9a** can only form a four-membered cyclic peroxy-anion (**10a**). Thus, the oxidative cleavage of **1e** yielded 5,6-dioxoheptanoic acid (**2e**) without decarbonylation.

As can be seen from Schemes 4 and 5, the alkylcycloalkanediones were oxidized via a process involving an attack of the superoxide radical anion with the tertiary carbon at the α position of the carbonyl group. Thus, the cycloalkanediones, which do not have a tertiary carbon at the α position of carbonyl, would not be transformed to oxocarboxylic acids by oxidative cleavage. In order to compare the reactivity of the cycloalkanediones without a tertiary carbon at the α position of carbonyl, the biotransformation of 1,2-cyclohexanedione (**1f**) and 1,4-cyclohexanedione (**1g**) was carried out. As can be seen from Table 2, the biotransformation of **1f** and **1g** using *C. chamlagu* gave reduced products, (+)-*trans*-1,2-cyclohexanediol (**2f**) and *trans*-1,4-cyclohexanediol (**2g**), respectively. The structures of **2f** and **2g** were identified from their spectral data, which agreed with those of the authentic samples.[22, 23] The reductions of **2f** and **2g** support the mechanism of the oxidation postulated.

These results show that alkylcycloalkanediones **1a–1e** are biotransformed to the corresponding oxo carboxylic acids **2a–2e**. 2-Methyl-1,3-cyclohexanedione (**1e**) was transformed to 5,6-dioxoheptanoic acid (**2e**) in high yield (95%) in short time (7 h). These experiments indicate that only 2 g of plant-cultured-cells of *C. chamlagu* are required to transform 80 mg of substrates **1a–1d**. In the case of *M. polymorpha*, 30 g plant-cultured-cells are required to transform 40 mg of substrates **1a–1d**. Thus, the reactivity of *C. chamlagu* was thirty times as



Scheme 3. The mechanism of oxidation by KO_2.



Scheme 4. Biotransformation of 3,6-dialkyl-1,2-cyclohexanediones (**1a–1d**) using *C. chamlagu*.

that of *M. polymorpha*. In conclusion, the present biocatalytic oxidation affords a new synthetic method that is more convenient and cleaner than the method used heretofore.

Experimental

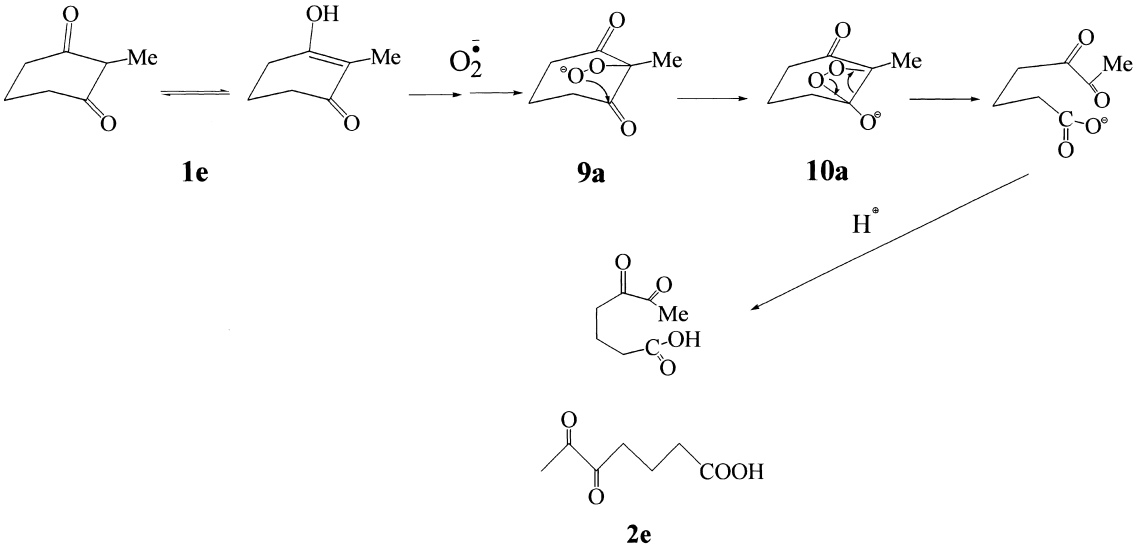
Analytical and Substrates. Melting points were determined on a Yanaco micro melting point apparatus. IR spectra were recorded on a Jasco FT-IR 230 spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a JEOL GSX 400 spectrometer. $CDCl_3$ with tetramethylsilane as the internal standard. GC-MS (EI) analyses were performed on a Shimadzu GCMS-QP5050 with an ionizing energy of 70 eV. CIMS (isobutane reagent gas) were recorded on a Shimadzu GCMS-QP5050 with an ionizing energy of 300 eV.

3,6-Dialkyl-1,2-cyclohexanediones (1a–1d). These compounds (**1a–1d**) were synthesized by the methods described in the literature.[24]

2-Methyl-1,3-cyclohexanedione (1e), 1,2-Cyclohexanedione (1f), 1,4-Cyclohexanedione (1g), 18-Crown 6-ether. These compounds were purchased from Tokyo Kasei Kogyo Co., Ltd..

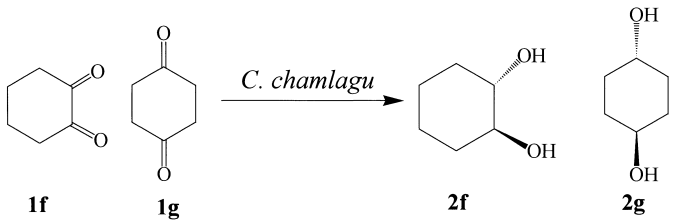
Potassium Superoxide. The compound was purchased from Kanto Chemicals Co., Ltd..

Cultivation of Suspension Cells of *C. chamlagu*. The callus tissues induced from the stem of *Caragana chamlagu* (Leguminosae) that were used in our previous study[17] were also used in this investigation. The callus tissues of *C. chamlagu* have been maintained for approximately 6 years. The callus tissues of *C. chamlagu* were transferred to freshly prepared MS medium[25] con-



Scheme 5. Biotransformation of 2-methyl-1,3-cyclohexanedione (**1e**) using *C. chamlagu*.

Table 2. Biotransformation of Cyclohexanediones (**1f**, **1g**) Using Cultured-Cells of *C. chamlagu*



Run	Substrate	Time/d	Product	Yield/%[a)]
1	**1f**	14	**2f**	37
2	**1g**	14	**2g**	53

Reaction condition: Substrate (40 mg), *C. chamlagu* (2 g), and culture medium (100 mL) were employed at 25 °C in the dark. a) Isolated yield.

taining 1 ppm of 2,4-dichlorophenoxyacetic acid as auxin and 3% sucrose, and then were grown with continuous shaking (110 rpm) for 5 days at 25 °C in the dark.

Incubation of Cycloalkanediones (1a–1g). The procedures are described in the case of substrate **1a** as an example. A part of the callus tissues (2 g) was transferred to 100 mL culture medium in a 300 mL Erlenmeyer flask and grown with continuous shaking for 5 days at 25 °C in the dark. The substrate **1a** (80 mg) was added to the suspension and the mixture was incubated at 25 °C on a rotary shaker (110 rpm) in the dark. After the incubation, the culture medium was separated by filtration. The filtrate was saturated with NaCl and extracted with EtOAc–Et_2O (1:1). The extract was purified by chromatography to give **2a** (44%), and **3a** (35%) as the major products. The products isolated were elucidated by their spectral data.

Reaction of Diosphenol (2-Hydroxy-1-*p*-menthen-3-one) (1a) with Superoxide Anion Radical [O_2^-]. Potassium superoxide (24.5 mmol), 18-crown 6-ether (2.8 mmol), diosphenol (**1a**, 2.0 mmol), and H_2O (60 mL) were treated at 25 °C for 24 h. The resulting mixture was extracted with ethyl acetate and purified by chromatography to give a mixture of ketoacids (**2a**, and **4a**, 75%). The mixture was identified by based on the comparison of their ^{1}H-NMR spectra with the reported data.[19] The ratios of the keto acids were calculated from the peak areas of ^{1}H NMR spectral data: δ 1.10 (d, C $(CH_3)_2$ for **2a**), 0.98 (d, C $(CH_3)_2$ for **4a**).

Spectral Data of Compounds. 2,6-Dimethyl-5-oxoheptanoic Acid (2a): Yellow oil; IR (NaCl) 1717 and 1707 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.61 (m, 1H, H-6), 2.54 (m, 1H, H-2), 1.21 (d, 3H, J =7.0 Hz, H-8), 1.10 (d, 6H, J = 7.0 Hz, H-7 and H-9); ^{13}C NMR ($CDCl_3$) δ 214.3, 182.4, 40.9, 38.7, 37.4, 27.2, 18.2, 17.1; CIMS m/z 173 $[M + H]^+$ (100), 155 $[(M + H) - H_2O]^+$ (96); EIMS m/z 154 $[M - H_2O]^+$ (2), 129 $[M - 43]^+$ (15), 101 $[M - C_4H_7O]^+$ (32); HRMS Found: m/z 172.1099 $[M]^+$ (20.2), 154.1017 $[M - H_2O]^+$ (100). Calcd for $C_9H_{16}O_3$: M, 172.1099. $[\alpha]_D^{26} = -21.2$ (c 1.8, $CHCl_3$)

2-Isopropyl-5-oxohexanoic Acid (4a): Yellow oil; IR (NaCl) 1718 and 1708 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.63 (m, 1H, H-2), 2.56 (m, 1H, H-7), 2.16 (s, 3H, H-6), 0.98 (d, 6H, J = 6.6Hz, H-8 and H-9); ^{13}C NMR ($CDCl_3$) δ 209.0, 181.1, 51.5, 41.5, 30.0, 22.8, 20.5, 19.8; CIMS m/z 173 $[M + H]^+$ (100), 155 $[(M + H) - H_2O]^+$ (86); HRMS Found: m/z 172.1099 $[M]^+$. Calcd for $C_9H_{16}O_3$: M, 172.1099. $[\alpha]_D^{27} = -7.4$ (c 3.8, $CHCl_3$)

6-Methyl-2,5-heptanedione (3a): Yellow oil; IR (NaCl) 1708 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.65 (m, 1H, H-2), 2.19 (s, 3H, H-7), 1.12 (d, 6H, J = 7.3 Hz, H-1 and H-8); ^{13}C NMR ($CDCl_3$) δ 213.3, 207.4, 40.8, 36.9, 33.8, 30.0, 18.3; CIMS m/z 143 $[M + H]^+$ (100), 125 $[(M + H) - H_2O]^+$ (92); HRMS Found: m/z 142.0996 $[M]^+$. Calcd for $C_9H_{16}O_3$: M, 142.0994.

2-Methyl-5-oxohexanoic Acid (2b): Yellow oil; IR (NaCl) 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.53 (m, 1H, H-2), 2.16 (s, 3H, H-6), 1.20 (d, 3H, J = 7.0 Hz, H-7); ^{13}C NMR ($CDCl_3$) δ 208.4, 182.1, 41.0, 38.5, 30.0, 27.1, 17.0; CIMS m/z 145 $[M + H]^+$ (100), 127 $[(M + H) - H_2O]^+$ (60); EIMS m/z 144 $[M]^+$ (1), 126 $[M - H_2O]^+$ (5), 115 $[M - CHO]^+$ (21), 87 $[M - CH_3COCH_2]^+$ (27), 57 $[M - CH_2(CH_3)CHCOOH]^+$ (100); HRMS Found: m/z 144.0784 $[M]^+$. Calcd for $C_7H_{12}O_3$: M, 144.0786. $[\alpha]_D^{27} = -6.0$ (c 5.0, $CHCl_3$)

5-Oxoheptanoic Acid (2c): Yellow oil; IR (NaCl) 1714 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (dd, 3H, J = 7.3 Hz, H-7); ^{13}C NMR ($CDCl_3$) δ 211.1, 179.0, 41.1, 36.0, 33.4, 18.8, 7.8; CIMS m/z 145 $[M + H]^+$ (71), 127 $[(M + H) - H_2O]^+$ (100); EIMS m/z 126 $[M - H_2O]^+$ (2), 98 $[M - H_2O\text{-}CO]^+$ (5), 74 $[M - H_2O - CO - C_2H_4]^+$ (5); HRMS (EI) m/z 144.0784 $[M]^+$. Calcd for $C_7H_{12}O_3$: M, 144.0786.

5-Cyclohexyl-5-oxopentanoic Acid (2d): Colorless needless from EtOH; mp 56.9–58.1 °C; IR (KBr) 1707 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (t, 2H, J = 7.1 Hz, H-4), 2.38 (t, 2H, J = 7.1 Hz, H-2), 2.32 (m, 1H, H-6), 1.89 (m, 2H, H-3); ^{13}C NMR ($CDCl_3$) δ 213.6, 179.4, 50.9, 39.2, 33.1, 28.5, 25.8, 25.6; EIMS m/z 115 $[M - C_6H_{11}]^+$ (28), 111 $[M - (CH_2)_3COOH]^+$ (13), 87 $[M - C_6H_{11}CO]^+$ (26), 83 $[C_6H_{11}]^+$ (81), 60 $[M - C_6H_{11}COCH_2\text{-}CH_3]^+$ (12); HRMS Found: m/z 198.1260 $[M]^+$. Calcd for $C_{11}H_{18}O_3$: M, 198.1256.

5,6-Dioxoheptanoic Acid (2e): Yellow oil; IR (NaCl) 1717 and 1708 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.84 (t, 2H, J = 7.3 Hz, H-4), 2.43 (t, 2H, J = 7.3 Hz, H-2), 2.34 (s, 3H, H-7), 1.93 (m, 2H, H-3); ^{13}C NMR ($CDCl_3$) δ 198.3, 197.2, 178.8, 34.7, 32.8, 23.6, 17.9; CIMS m/z 159 $[M + H]^+$ (55), 141 $[(M + H) - H_2O]^+$ (41); EIMS m/z 115 $[M - CH_3CO]^+$ (12), 87 $[M - CH_3CO - CO]^+$ (10); HRMS Found: m/z 158.0574 $[M]^+$. Calcd for $C_7H_{10}O_4$: M, 158.0579.

(+)-*trans*-1,2-Cyclohexanediol (2f):[22] Colorless needless from EtOH; mp 112.9–114.1 °C; IR (KBr) 3372 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.34 (m, 2H), 2.70 (brs, 2H), 1.99 (m, 2H), 1.69 (m, 2H), 1.25 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 75.84, 32.85, 24.32; CIMS m/z 117 $[M + H]^+$ (48), 99 $[(M + H) - H_2O]^+$ (100), 81 $[M - 2H_2O]^+$ (47); EIMS m/z 116 $[M]^+$ (8), 98 $[M - H_2O]^+$ (22), 83 $[M - H_2O - CH_3]^+$ (27); HRMS Found: m/z 116.0839 $[M]^+$ (6.5). Calcd for $C_6H_{12}O_2$: M, 116.0837; $[\alpha]_D^{29} = 30.6$ (c 0.8, H_2O).

***trans*-1,4-Cyclohexanediol (2g):**[23] Colorless plates from EtOH; mp 103.1–104.0 °C; IR (NaCl) 3355 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.20 (m, 2H), 3.80 (m, 2H), 1.73 (m, 4H), 1.67 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 68.02, 66.38, 37.16, 36.68, 33.71, 30.32; CIMS m/z 117 $[M + H]^+$ (18), 99 $[(M + H) - H_2O]^+$ (100); EIMS m/z 116 $[M]^+$ (1), 98 $[M - H_2O]^+$ (66), 83 $[M - H_2O - CH_3]^+$ (32), 69 $[M - H_2O - COH]^+$ (46); HRMS Found: m/z 116.0837 $[M]^+$. Calcd for $C_6H_{12}O_2$: M, 116.0837.

References

1 D. E. Danly and C. R. Campbell, "Kirk-Othmer Encyclopedia of Chemical Technology Vol. 1," ed by M. Grayson, Wiley, New York (1978).

2 J. R. Lindsay Smith, D. I. Richards, C. B. Thomas, and M. Whittaker, *J. Chem. Soc., Perkin Trans. 2*, **1985**, 1677.

3 B. E. Ali, J.-M. Bregeault, J. Mercier, J. Martin, C. Martin, and O. Convert, *J. Chem. Soc., Chem. Commun.*, **1989**, 825.

4 a) E. Baer, *J. Am. Chem. Soc.*, **62**, 1597 (1940). b) E. Baer, *J. Am. Chem. Soc.*, **64**, 1416 (1942).

5 a) P. W. Clutterbuck and F. Reuter, *J. Chem. Soc. B*, **1935**, 1467. b) C. T. Buse, W. A. Kleschick, M. C. Pirrung, J. E. Sohn, and J. Lampe, *J. Org. Chem.*, **45**, 1066 (1980).

6 L. Knof, *Justus Liebigs Ann. Chem.*, **656**, 183 (1962).

7 T. Hirao, M. Mori, and Y. Ohshiro, *Bull. Chem. Soc. Jpn.*, **62**, 2399 (1989).

8 a) S. Torii, T. Inokuchi, and R. Oi, *J. Org. Chem.*, **47**, 47 (1982). b) M. Stoll and Rouve, *Helv. Chim. Acta*, **30**, 1822 (1947). c) T. Tsuji, *Bull. Chem. Soc. Jpn.*, **62**, 645 (1989).

9 L. He, M. Kanamori, and C. A. Horiuchi, *J. Chem. Res. (Synop.)*, **1999**, 122.

10 L. He and C. A. Horiuchi, *Bull. Chem. Soc. Jpn.*, **72**, 2515 (1999).

11 S.-J. Ji and C. A. Horiuchi, *Bull. Chem. Soc. Jpn.*, **73**, 1645 (2000).

12 a) T. Suga and T. Hirata, *Phytochemistry*, **29**, 2393 (1990). b) H. Hamada, H. Yasumune, Y. Fuchikami, T. Hirata, I. Sattler, H. J. Williams, and A. I. Scott, *Phytochemistry*, **44**, 615 (1997). c) T. Hirata and S. Izumi, *Plant Tissue Culture Lett.*, **10**, 215 (1993). d) S. Gotoh, M. Aoki, T. Iwaeda, S. Izumi, and T. Hirata, *Chem. Lett.*, **1994**, 1519. e) H. Hamada, H. Konishi, H. J. Williams, and A. I. Scott, *Phytochemistry*, **30**, 2269 (1991). f) H. Hamada, S. Naka, and H. Kurban, *Chem. Lett.*, **1993**, 2111.

13 a) T. Hirata, H. Hamada, T. Aoki, and T. Suga, *Phytochemistry*, **21**, 2209 (1982). b) K. Shimoda, T. Hirata, and Y. Noma, *Phytochemistry*, **49**, 49 (1998).

14 W. Chai, H. Hamada, J. Suhara, and C. A. Horiuchi, *Phytochemistry*, **57**, 669 (2001).

15 Y. Matsuura, W. Chai, E. Endoh, J. Suhara, H. Hamada, and C. A. Horiuchi, *Phytochemistry*, in press.

16 a) S. Kitanaka, T. Ikezawa, K. Yasukawa, S. Yamanouchi, M. Takido, H.K. Sung, and I.H. Kim, *Chem. Pharm. Bull.*, **38**, 432 (1990). b) S. Kitanaka, M. Takido, K. Mizoue, H. Kondo, and S. Nakaike, *Chem. Pharm. Bull.*, **44**, 565 (1996).

17 H. Sakamaki, S. Kitanaka, W. Chai, Y. Hayashida, Y. Takagi, and C. A. Horiuchi, *J. Nat. Prod.*, **64**, 630 (2001).

18 a) J. S. Filippo, Jr. Chuen-Ing Chern, and J. S. Valentine, *J. Org. Chem.*, **41**, 1077 (1976). b) R. A. Johnson and E. G. Nidy, *J. Org. Chem.*, **40**, 1680 (1975). c) E. Alvarez, C. Betaucor, R. Freire, A. Martin, and E. Suarez, *Tetrahedron Lett.*, **22**, 4335 (1981).

19 W. Chai, H. Sakamaki, S. Kitanaka, M. Saito, and C. A. Horiuchi, *Biosc. Biotech. Biochem.*, in press.

20 O. Nishimura, and S. Mihara, Flavors and Fragrances, a world perspective. Proceedings of the 10th international congress of essential oils, fragrances and flavors, Washington, DC, U. S. A., 375 (1986).

21 a) M. Utaka, S. Matsushita, H. Yamasaki, and A. Takeda, *Tetrahedron Lett.*, **21**, 1063 (1981). b) V. Rajananda, and S. B. Brown, *Tetrahedron Lett.*, **22**, 4331 (1981).

22 W. B. Smith, *J. Org. Chem.*, **44**, 1631 (1979).

23 K. Yasuaki, K. Yasushi, T. Yasutaka, T. Hiroo, and A. Yasuhiro, *J. Am. Chem. Soc.*, **113**, 1349 (1991).

24 M. Utaka, S. Matsushita, and A. Takeda, *Chem. Lett.*, **1980**, 779.

25 T. Murashige and F. Skoog, *Physiol. Plant.*, **15**, 473 (1962).