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Oxidative cleavage of the C–C bond of 3,6-dialkylcyclohexane-1,2-diones by cell suspension cultures of *Marchantia polymorpha*

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Abstract

Biotransformation of 3,6-dialkylcyclohexane-1,2-diones by cell suspension cultures of *Marchantia polymorpha* involves regioselective oxidative cleavage of the C–C bond to give the corresponding oxocarboxylic acids shortened by one carbon unit. In the case of cyclohexane-1,2-dione, adipic acid was obtained.

Keywords: *Marchantia polymorpha*; Leguminosae; Biotransformation; Oxidative cleavage of the C–C bond; Oxocarboxylic acids

1. Introduction

Previous synthetic studies (He et al., 1999; He and Horiuchi, 1999; Ji and Horiuchi, 2000) for producing oxocarboxylic acids and esters involve the use of metallic oxidizing agents to catalyze the regiospecific oxidative ring-opening of cyclohexanones. Some of these methods unfortunately are associated with the use of toxic reagents and heavy metals and the reaction products are racemic.

There has recently been growing interest in the ability of plant cultured cells to perform stereo- and regioselective biotransformations.This is in order to facilitate production of compounds that are useful in synthetic organic chemistry. Previous studies in our laboratory have successfully shown how cell cultures could be used, from the viewpoint of green chemistry, in catalyzing such biotransformations (Hirata et al., 1989; Hamada et al., 1991, 1993, 1997; Gotoh et al., 1994). The present study focuses on the use of *M. polymorpha* cell cultures to produce oxocarboxylic acids that are important intermediates in organic synthesis.

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2. Results and discussion

2.1. Products of biotransformation

The biotransformation of 3,6-dialkylcyclohexane-1,2-diones (**1a**–**1e**) by suspension cells of *M. polymorpha* is shown in Table 1 and Scheme 1. Compounds **1a**–**1d** gave oxocarboxylic acids **2a**–**2d**, respectively, whose structures were identified by HRMS, CIMS, EIMS, IR and NMR spectroscopic analysis as follows. The IR spectrum of **2a** showed a characteristic absorption of a carboxylic acid (1708 cm^{-1}). The ^1H NMR spectra showed the presence of a methyl group at $\delta = 1.21$ (3H, *d*) and of an isopropyl group at $\delta = 1.10$ (6H, *d*); and the ^{13}C NMR spectrum had signals at $\delta = 214.3$ and 182.4, which were assigned to the carbonyl and carboxylic acid groups, respectively. Therefore, compound **2a** was presumed to be 2,6-dimethyl-5-oxoheptanoic acid. Moreover, this structure was supported by the fragmentation patterns (*m*/*z* 154[M–H_2O]$^+$, 129[M–C_3H_7]$^+$, and 101[M–C_4H_7O]$^+$) of its GC–MS spectrum.

Compound **2b** had absorption at 1720 (C=O) and 1708 cm^{-1} (COOH) in the IR spectrum and the ^1H NMR spectrum displayed a singlet at $\delta = 2.16$ (3H) due to the $CH_3C{=}O$ and a doublet $\delta = 1.20$ (3H) due to the CH_3CH functionality; the ^{13}C NMR spectrum also exhibited signals at $\delta = 208.4$ and 182.1 assigned to the carbonyl group and carboxylic acid, respectively.

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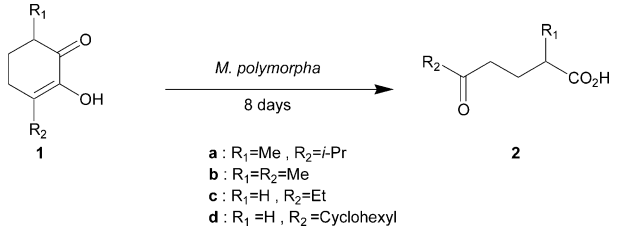
Accordingly, **2b** was presumed to be 2-methyl-5-oxohexanoic acid. The ^{1}H NMR spectra of compounds **2c** and **2d** both showed a triplet (3H, $J=7.3$ Hz) at $\delta=1.05$ due to the CH_3– for **2c**; and triplet (2H, 7.3 Hz) and (2H, $J=7.1$ Hz) at $\delta=2.35$ and $\delta=2.38$, due to the CH_2COOH, respectively. The C=O stretching band appeared at 1720 and 1712 cm^{-1} for **2c** and 1712 and 1698 cm^{-1} for **2d**, respectively. From these results, the structures of the oxo carboxylic acids were assigned to be 5-oxoheptanoic acid (**2c**) and 5-cyclohexyl-5-oxopentanoic acid (**2d**), respectively. These structures were also supported by analysis of the ^{13}C NMR spectra. Further, **2a** and **2b** were converted into methyl oxocarboxylates (**3a**,**3b**) using diazomethane. The IR spectra of compounds **3a** and **3b** showed the characteristic absorption of ester carbonyl groups (1736 cm^{-1}). The ^{1}H NMR spectra exhibited the presence of methyl ester groups at $\delta=3.68$ (3H, *s*, $COOCH_3$) for **3a** and $\delta=3.67$ (3H, *s*) for **3b**, and the ^{13}C NMR spectra displayed signals at $\delta=214.0$ and 176.7 for **3a** and at $\delta=208.1$ and 176.6 for **3b** which were assigned as the carbonyl carbon and ester carbonyl carbon functionalities, respectively. The structures of **3a** and **3b** were also supported by the MS analysis of the fragmentation pattern. Lastly, in the case of cyclohexane-1,2-dione (**1e**), adipic acid was obtained as the product (Table 1), whose structure was confirmed by the IR and ^{1}H and ^{13}C NMR spectra with an authentic sample.

In the biotransformation of **1a,** 1-*p*-mentene-2,4-diol-3-one (**4**) was also isolated as an intermediate. Its structure was established as follows: the IR spectrum showed characteristic absorptions for hydroxyl group (3456 cm^{-1}), carbonyl (1674 cm^{-1}) and C–C double bond (1646 cm^{-1}) functionalities. The ^{1}H NMR spectra revealed the presence of two hydroxyl groups at $\delta=3.17$ [(C-4)–OH] and 5.73 [(C-2)–OH], and the ^{13}C NMR spectra had resonances corresponding to a carbonyl group at $\delta=197.9$ and a C–C double bond at $\delta=131.3$ (C-4) and 141.4 (C-2). Mass spectral analysis gave a *m*/*z* 184, presumably due to a molecular ion peak of $C_{10}H_{16}O_3$. It is thus assumed that oxidative cleavage of the C–C bond involves a related decarboxylation step.

Table 1
Biotransformation of 3,6-dialkylcyclohexane-1,2-diones using *M. polymorphia*



1

a : R_1=Me , R_2=*i*-Pr
b : R_1=R_2=Me
c : R_1=H , R_2=Et
d : R_1 =H , R_2 =Cyclohexyl

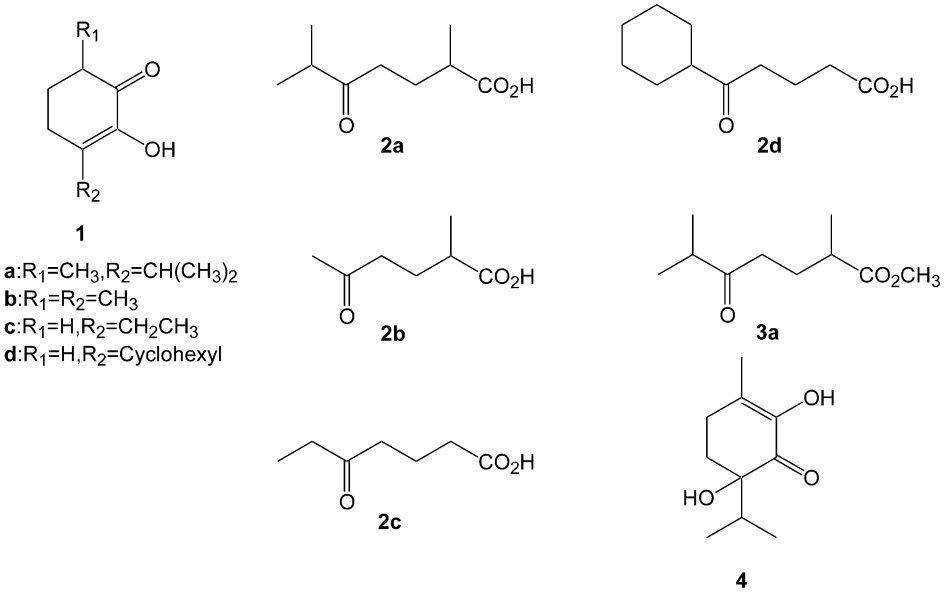
2

Run	Substrate (mg)	Product	Yield (%)[a]
1	**1a** (80)	**2a**	60
2	**1a** (20)	**2a**	92
3	**1b** (80)	**2b**	32
4	**1c** (80)	**2c**	52
5	**1d** (80)	**2d**	58
6	**1e** (80)	Adipic acid	10

[a] Isolated yield.

2.2. Possible reaction mechanism

The reaction mechanism of diosphenol (**1a**) metabolism (Scheme 2) described by Nishimura and Mihara (1986), suggested that 2-(1-methylethyl)-5-oxohexanoic acid (**2a′**) could be produced. However, the biotransformation of **1a** produced **2a** rather than **2a′**. It is



Scheme 1. Substrates and biotransformation products.

thus considered that a hydroperoxy radical attacks at the C_4-position of **1a**–**1d** and then the C_4-peroxy ions of 3,6-dialkylcyclo-hexane-1,2-dione are formed. Continuously, the peroxy ion attacks at the C_1-carbonyl group and cleavage of the C_1–C_2 bond occurs according to Scheme 2, and the CO group is eliminated. This reaction mechanism is supported by the fact that 1-*p*-menthene-2,4-diol-3-one (**4**) is obtained as an intermediate. Moreover, the transformation of diosphenol (**1a**) under oxygen atmosphere without cell suspension cultures of *M. polymorpha* resulted in the recovery of the starting material. This result rules out the assumption that the cleavage of the C_1–C_2 bond is effected solely by oxygen.

3. Experimental

3.1. General

Melting points were determined on a Yanaco micro melting point apparatus. IR spectra were recorded on a Jasco FT-IR 230 spectrometer. ^1H and ^{13}C NMR spectra were measured on a Jeol GSX 400 spectrometer. Samples were dissolved in $CDCl_3$ with TMS as the internal standard. GC–MS (EI) analyses were performed on a Shimadzu GCMS-QP5050 with an ionizing energy of 70 eV. HRMS (EI) analyses were performed on a JMS-GC mateII/HP-6890 with an ionizing energy of 70 eV. CIMS (*iso*-butane reagent gas) were recorded on a Shimadzu GCMS-QP5050 with an ionizing energy of 300 eV. ^{13}C NMR spectroscopic assignments are reported in Table 2.

Compounds **1a**–**1d** were prepared according to Utaka et al. (1980). Compound **1e** (extra pure grade) of Tokyo Chemical Co. Ltd. was used without purification.

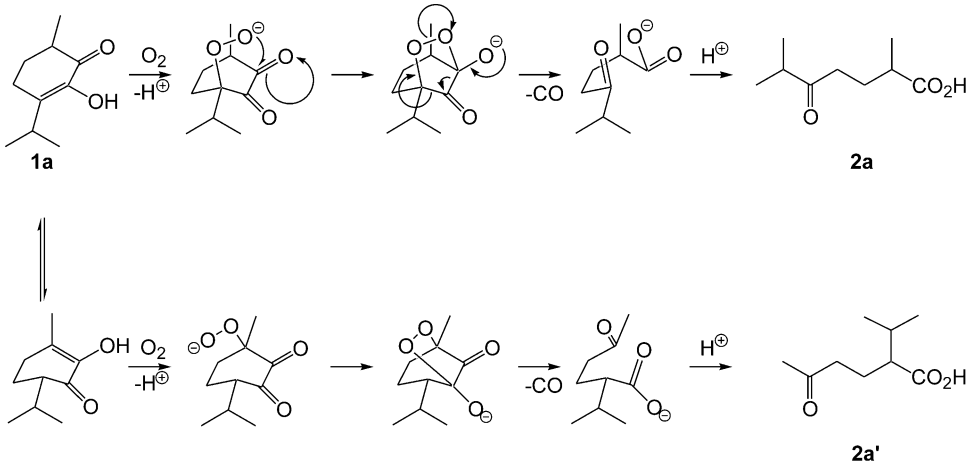
Table 2
^{13}C NMR chemical shift assignments for products (**2a**–**2d**, **3a**, **3b**)

	2a	2b	2c	2d	3a	3b
C-1	182.4	182.1	179.0	179.4	176.7	176.6
C-2	38.7	38.5	36.0	33.1	38.7	38.6
C-3	27.2	30.0	18.8	10.3	27.5	27.4
C-4	37.6	41.0	41.1	39.2	37.6	41.1
C-5	214.3	208.4	211.1	213.6	214.0	208.1
C-6	40.9	27.1	33.4	50.9	40.9	29.7
C-7	18.2	17.0	7.8	25.6	18.3	17.1
C-8	17.1		25.8	17.2		
C-9	18.2			28.5	18.2	

3.2. Incubation of substrates *1a–1e*

Cultured cells of *M. polymorpha* (60 g) were transplanted to MS (Murashige Skoog) medium (200 ml) containing 1 ppm of 2,4-dichlorophenoxyacetic acid and the suspension cells were then incubated under shaking (120 rpm) at 25 °C in the light (2000 l×) for 8 days. Using diosphenol (**1a**) (80 mg) as an example it was next added to suspension cells, which were incubated for 8 days. Cultured cells were removed by filtration, and the filtrate was extracted with EtOAc–Et_2O (1:1). The organic layer was washed with a satd. solution of NaCl, dried over anhydr. Na_2SO_4 and filtered. After the solvent was removed, the oxocarboxylic acid **2a** was obtained as a yellow oil. Then the oil was subjected on silica gel. Chromatography, elution of which with hexane–EtOAc (7:3) gave pure oxocarboxylic acid **2a** (see Table 1 for yields).

The experimental procedure followed was similar to that reported in the literature (Fales et al., 1973). Oxocarboxylic acids (**2a**–**2d**) were treated with diazomethane in dry Et_2O. The ethereal solution was washed with a satd. soln. of NaCl, dried with anhydr. Na_2SO_4 and the solvent was removed in vacuo. The corre-



Scheme 2. Proposed reaction mechanism of biotransformation of diosphenol (**1a**) leading to two different products **2a** and **2a**′. (Mechanism for formation of **2a** is adapted from Nishimura and Mihara, 1986).

sponding methyl ester was obtained as colorless oil. In the case of substrate **1a**, 1-*p*-menthene- 2,4-diol-3-one (**4**) was also isolated as an intermediate. Then, cyclohexane-1,2-dione (**1e**) was transformed to adipic acid.

3.3. 2,6-Dimethyl-5-oxoheptanoic acid (*2a*)

Oil; HRMS (EI): *m/z* 172.1099 $[M]^+$ (20.2), 154.1017 $[M–H_2O]^+$ (100); CIMS *m/z* 173 $[M+H]^+$ (100), 155 $[(M+H)\text{-}\ H_2O]^+$ (96); EIMS *m/z* 154 $[M–H_2O]^+$ (2), 129 $[M\text{-}43]^+$ (15), 101 $[M–C_4H_7O]^+$ (32); IR (NaCl): ν_{max} cm^{-1} 1718, 1708; ^{13}C NMR ($CDCl_3$): δ 214.3, 182.4, 40.9, 38.7, 37.6, 27.2, 18.2, 17.1; ^1H NMR ($CDCl_3$): δ 2.61 (*m*, 1H, *w*/2 = 7.3 Hz, H-6), 2.54 (*m*, 1H, *w*/2 = 7.0 Hz, 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); [α] $[\alpha]_D^{26}$ −13.5° (*c* 19.4,$CHCl_3$).

3.4. Methyl 2,6-dimethyl-5-oxoheptanoate (*3a*)

Oil; CIMS *m/z* 187 $[M+H]^+$ (100); EIMS *m/z* 143 $[M–(CH_3)_2CH]^+$, 15 $[M–(CH_3)_2CHCO]^+$; IR (NaCl): ν_{max} cm^{-1} 1736, 1720, 1164; ^{13}C NMR ($CDCl_3$): δ 214.0, 176.7, 51.6, 40.9, 38.7, 37.6, 27.5, 18.3, 18.2, 17.2; ^1H NMR ($CDCl_3$): δ 3.68 (*s*, 3H, H-10), 2.60 (*m*, 1H, *w*/2 = 7.0 Hz, H-6), 2.49 (*m*, 1H, *w*/2 = 7.0 Hz, H-2), 2.47 (*m*, 2H, *w*/2 = 7.0 Hz, H-4), 2.81 (*m*, 2H, *w*/2 = 7.0 Hz, H-3), 1.16 (*d*, 3H, *J* = 7.0 Hz, H-8), 1.08 (*d*, 6H, *J* = 7.0 Hz, H-7 and H-9).

3.5. 2-Methyl-5-oxohexanoic acid (*2b*)

Oil; HRMS (EI): *m/z* 144.0784 $[M]^+$ (6.5), 126.0676 $[M–H_2O]^+$ (19.1), 115.0403 $[M–CHO]^+$ (100); 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); IR (NaCl): ν_{max} cm^{-1} 1720, 1708; ^{13}C NMR ($CDCl_3$): δ 208.4, 182.1, 41.0, 38.5, 30.0, 27.1, 17.0 ; ^1H NMR ($CDCl_3$):δ 2.53 (*m*, 1H, *w*/2 = 6.6 Hz, H-2), 2.16 (*s*, 3H, H-6), 1.20 (*d*, 3H, *J* = 7.0 Hz, H-7); $[\alpha]_D^{26}$ + 5.7° (*c* 2.47, $CHCl_3$).

3.6. Methyl 2-methyl-5-oxohexanoate (*3b*)

Oil;CIMS (*m/z*): 159 $[M+H]^+$ (100); EIMS (*m/z*): 143 $[M–CH_3]^+$ (2), 126 $[M–CH_3OH]^+$ (64), 115 $[M–CH_3CO]^+$ (16), 101 $[M–CH_3COCH_2]^+$ (51), 98 $[M–HCOOCH_3]^+$ (96), 88 $[M–CH_3COCH_2CH_2]^+$ (100); IR (NaCl): ν_{max} cm^{-1} 1736, 1720, 1164; ^{13}C NMR ($CDCl_3$): δ 208.1, 176.6, 50.8, 41.1, 38.6, 29.7, 27.4, 17.1; ^1H NMR ($CDCl_3$): δ 3.67 (*s*, 3H, H-8), 2.46 (*m*, 1H, *w*/2 = 7.0 Hz, H-2), 2.31 (*m*, 2H, *w*/2 = 7.0 Hz, H-4), 2.14 (*s*, 3H, H-6), 2.01 (*m*, 2H, *w*/2 = 7.0 Hz, H-3), 1.17 (*d*, 3H, *J* = 7.2 Hz, H-7).

3.7. 5-Oxoheptanoic acid (*2c*)

Oil; HRMS (EI): *m/z* 144.0784 $[M]^+$ (0.3), 126.0679 $[M–H_2O]^+$ (100), 98 $[M–H_2O–CO]^+$ (20.1); CIMS (*m/z*): 145 $[M+H]^+$ (71), 127 $[(M+H)–H_2O]^+$ (100); EIMS *m/z* 126 $[M–H_2O]^+$ (2), 98 $[M–H_2O–CO]^+$ (5), 74 $[M\text{-}H_2O\text{-}CO\text{-}C_2H_4]^+$ (5); IR (NaCl): ν_{max} cm^{-1} 1720, 1712; ^{13}C NMR ($CDCl_3$): δ 211.1, 179.0, 41.1, 36.0, 33.4, 18.8, 7.8; ^1H NMR ($CDCl_3$): δ 1.05 (*t*, 3H, *J* = 7.3 Hz, H-7), 1.89 (*q*, 2H, *J* = 7.3 Hz, H-3), 2.35 (*t*, 2H, *J* = 7.3 Hz, H-2), 2.43 (*q*, 2H, *J* = 7.3 Hz, H-6), 2.49 (*t*, 2H, J = 7.3 Hz, H-4), 7.8 (*s*, 1H, H-1).

3.8. 5-Cyclohexyl-5-oxopentanoic acid (*2d*)

Needles from EtOH; mp 56.9–58.1 °C; HRMS (EI): *m/z* 198.1260 $[M]^+$ (100), 180.1141 $[M–H_2O]^+$ (21.2); CIMS (*m/z*): 199 $[M+H]^+$ (100), 181 $[(M+H)–H_2O]^+$ (37); 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 $[M–C_6H_{11}COCH_2CH_3]^+$ (12); IR (KBr): ν_{max} cm^{-1} 1712, 1698; ^{13}C NMR ($CDCl_3$): δ 213.6, 179.4, 50.9, 39.2, 33.1, 28.5, 25.8, 25.6, 10.3; ^1H NMR ($CDCl_3$): 1.89 (*m*, 2H, *w*/2 = 7.1 Hz, H-3), 2.32 (*m*, 1H, *w*/2 = 7.1 Hz, H-6), 2.38 (*t*, 2H, *J* = 7.1 Hz, H-2), δ 2.54 (*t*, 2H, *J* = 7.1 Hz, H-4).

3.9. 1-p-Menthene-2,4-diol-3-one (*4*)

Needles from EtOH; mp 70–76 °C; CIMS (*m/z*): 185 $[M+H]^+$ (100); EIMS *m/z* 184 $[M]^+$ (8), 166 $[M–H_2O]^+$ (4), 141 $[M–(CH_3)_2CH]^+$ (34), 113 (54), 95 (16), 71 (34); IR (KBr): ν_{max} cm^{-1} 3456, 1674, 1646; ^{13}C NMR ($CDCl_3$): δ 197.9, 141.4, 131.3, 76.4, 32.1, 30.6, 27.3, 16.9, 16.0; ^1H NMR ($CDCl_3$): δ 0.75 (*d*, 3H, *J* = 7.0 Hz, H-9), 1.02 (*d*, 3H, *J* = 7.0 Hz, H-10), 1.90 (*s*, 3H, H-7), 1.97 (*m*, 2H, H-5α/β and H-8), 2.27 (*m*, 2H, H-5α/β and H-6α/β), 2.47 (*m*, 1H, H-6α/β).

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