

2-Pyranone-4,6-dicarboxylic acid as a source of green-plastics and anti-bacterial chemicals

Masami. Bito^{1a,2)}, Tsuyoshi Michinobu^{1a,b)}, Yoshihiro Katayama^{1c)}, Yuichiro Otsuka³⁾, Masaya Nakamura³⁾, Seiji Ohara³⁾, Eiji Masai⁴⁾ and Kiyotaka Shigehara^{1a,b,*)}

^{1a)} Graduate School of Eng., ^{1b)} Inst. of Symbiotic Sci. and Tech., ^{1c)} Graduate School of Bio-Appl. and Systems Eng., Tokyo Univ. of Agricul. and Technol., 2-24-16 Nakacho, Koganei, Tokyo 184-8588, Japan, Fax: +81-42-381-8175, e-mail: jun@cc.tuat.ac.jp

²⁾ Technical Development Dept. J-CHEMICAL,INC., Tokyo 104-0044, Japan

³⁾ Forestry and Forest Products Res. Inst. Tsukuba 305-8687, Japan

⁴⁾ Dept. Bioeng., Nagaoka Univ. Tech., Nagaoka 940-2188, Japan

* To whom correspondence should be addressed.

PDC, 2-pyranone-4,6-dicarboxylic acid, is the terminal chemical substance of lignin bio-degradation before streaming into the TCA cycle and has never appeared in organic chemical synthesis. Especially, its unique pseudo-aromatic ring with two -COOH is interesting because of possibilities to utilize PDC as the source of renewable and green plastics. During the course of a large scale production by using transformed bacterium bio-reactor, we found that PDC has exceptionally strong affinity for alkali metal ions and therefore common bacteria, such as *E. Coli O157*, *MRSA*, *Pseudomonas aeruginosa*, were prohibited to grow. Because PDC is short-lived in neutral - basic pH solution due to ring-opening hydrolysis, hydrolyzable copolyesters possessing PDC nuclei in the main chain were prepared from the corresponding low molecular weight analog, bis(2-hydroxyethyl) 2-pyranone-4,6-dicarboxylate.

Keywords: PDC, green plastics, anti-bacterial chemicals, biodegradable, hydrolyzable

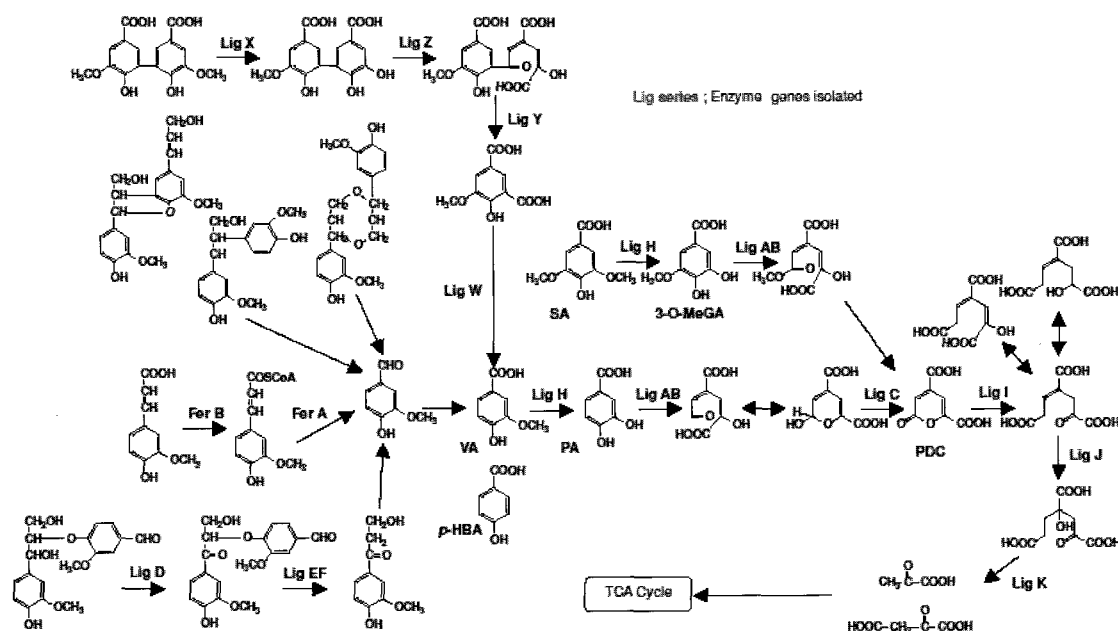


Figure 1 Lignin metabolism pathway in *Shingomonas paucimobilis* SYK-6

Introduction

Utilization of biomass in valuable fuels and industrial products has attracted much attention because the society requires the change from nonrenewable carbon resources to renewable bioresources. Lignin is one of the most abundant natural carbon resources, existing in trees at 15-30% by weight. However, an advanced system of utilizing it as biomass has not been established because the highly networked three-dimensional structures¹⁾ make it difficult to transform into well-defined versatile substances by conventional organic chemistry. In Fig.1 PDC, 2-pyranone-4,6-dicarboxylic acid, is the terminal chemical substance of lignin bio-degradation before streaming into the

TCA cycle and has never appeared in chemical synthesis²⁾. The metabolic conversion of lignin into stable and functional intermediates has been extensively investigated, and we recently succeeded in stably producing 2-pyranone-4,6-dicarboxylic acid (PDC) from protocatechuic acid on a kg scale by transformed bacterium.³⁾

The unique structure of PDC molecule may not only give the metal ion-capturing functionalities by the concerted coordination of carboxylate, carbonyl and etheric oxygen atoms as seen in macrocyclic anti-bacterial chemicals⁴⁾, but also open the possibility to prepare bio-degradable green plastics with certain rigidity derived from the pseudo-aromatic pyranone ring. In this paper, we report the

exceptionally strong complex formation between PDC and alkali metal or silver ions, the anti-bacterial activities of PDC complexes and esters, and the synthesis of bio-degradable PDC copolyesters.

Experimental Section

General. All NMR spectra were recorded on a JEOL Alpha400 with tetramethylsilane as an internal standard. Gel permeation chromatography was measured on a TOSOH TSKgel GMH-M using chloroform as an eluent after calibration with standard polystyrenes. Differential scanning calorimetry (DSC) measurements were carried out on a Rigaku Thermo plus DSC8230 at a scanning rate of 20 °C min⁻¹. X-ray diffraction measurements were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Cu-K α radiation. Elemental analysis was afforded by a Yanaco MT-5 analyzer.

Materials. All reagents were purchased from Wako, Tokyo Kasei, and Aldrich and were used without further purification. Solvents were purified by distillation under nitrogen after drying over suitable drying agents. PDC was prepared from protocatechuate as reported earlier.³⁾

Synthesis. PDC salts. Each 10 ml of saturated aqueous PDC solution (about 3.6 wt %) and 2 wt % aqueous alkali metal (Li, Na, K and Rb) chloride or silver nitrate solution was mixed. The resulting precipitate was collected and recrystallized from hot water (60 °C) with the descending temperature gradient of 0.5 °C day⁻¹. The yield was about 80 % in every case. The composition was estimated from the results of elemental analysis, the thermogravimetric weight loss of aquating water molecule (occurs at 110 – 130 °C) and the X-ray diffraction analysis. The solubility at 20 °C in water was determined to be, PDC: 182 mM, PDC-Li⁺ complex: 51, PDC-Na⁺: 9.1, PDC-K⁺: 6.7, PDC-Rb⁺: 7.4, PDC-Ag⁺: 5.1.

Bis(2-hydroxyethyl) 2-pyranone-4,6-dicarboxylate [BHPDC]. PDC (21.1 g, 110 mmol) was dissolved in 1,2-ethanediol (100 ml) at 120 °C. Conc.-HCl (10 drops) was added and the mixture was gradually evacuated to 115 mmHg and reacted for 5 h with removing water vapor. After cooled to -5 °C, the white short needles were collected and recrystallized from ethanol, yielding 16.2 g (52 %) of desired product. $T_m = 128.0 - 129.5$ °C. ¹H-NMR, δ (ppm, DMSO-d₆): 3.30 – 3.36 (t, 4H), 3.67 – 3.71 (t, 4H), 4.29 (s, 1H), 4.33 (s, 1H), 7.21 (s, 1H), 7.39 (s, 1H); IR (KBr): $\nu = 3430, 3087, 2963, 1714, 1635, 1558, 1452, 1402, 1375, 1333, 1281, 1185, 1118, 1069, 1016, 1002, 948, 910, 884, 841, 781, 765, 734, 715, 616, 554, 503$ cm⁻¹.

Polymers. Poly(L-lactic acid) [PLA] of Mn=1.74·10⁵ with L-content > 98 % is the gift sample from Unitika Co. Ltd. Copolyesters with the repeating unit of oxethyleneoxy(2-pyranone-4,6-dicarbonyl) and lactate was prepared as follows. An equimolar mixture of PDC and BHPDC was further mixed with a given amount of L-lactic acid (see Scheme 1 and Table 3) and preheated to 140 °C to obtain homogeneous molten solution. In presence of methanesulfonic acid as a catalyst, the mixture was reacted for 6 h at 180 °C to promote oligomerization. The system was gradually evacuated to less than 2 mmHg and reacted for 4 h at 180 °C, then another 15 h at 120 °C. The products were purified by reprecipitation from chloroform/methanol twice. The yield (conversion) was more than 80 % in every feed of Table 3 and essentially stoichiometric except for mechanical losses. ¹H-NMR, δ (ppm, CDCl₃): 1.33-1.67, 4.20-4.24, 5.02-5.09, 7.10, 7.42; ¹³C-NMR, δ (ppm, CDCl₃): 17.05,

20.91, 63.38, 66.11, 69.27, 108.98, 124.02, 142.28, 148.79, 158.42, 162.18, 169.79, 174.54; IR (film): 2995, 2359, 1757, 1456, 1383, 1362, 1265, 1185, 1131, 1092, 1046, 869, 756 cm⁻¹.

Determination of MIC (minimum inhibitory concentration). According to the standardized method by Japan Society of Chemotherapy, MIC of *Pseudomonas aeruginosa*, *Staphylococcus aureus subsp. aureus* (MRSA), *Vibrio parahaemolyticus*, *Escherichia coli* O157:H7, *Trichophyton rubrum*, *Candida albicans*, *Cladosporium cladosporioides*, *Aspergillus niger* for PDC, BHPDC and PDC-Ag salt was determined at 35 ± 0.5 °C.

Evaluation of biodegradability of polymers. Each sample was pressed at r.t. (cellulose powder) or at 140 °C (PLLA and copolyesters) at 10 – 20 MPa into 2.0 mm ϕ × 3.0 mm pellets. According to ISO 14855-2 testing, the biodegradability of 1 g of pellets at 59.5 °C in an active compost obtained from Kanagawa Pref. recycle center was estimated by monitoring CO₂ evolved.

Results and Discussion

PDC is a strong dibasic acid with pK_{a1} and pK_{a2} of 1.13 and 2.52, respectively.⁵⁾ Although the solubility of PDC in water is as high as about 180 mM (ca. 3.6 wt %), the addition of alkali metal chloride solution decreased the solubility as shown in *Experimental* and caused instantaneous precipitation with evolving HCl.⁵⁾ This anomalous phenomenon corresponding to the equation as the case of sodium chloride for example, 2PDC + 4H₂O + NaCl → Na(OH)₂(PDC)(PDC)⁻ + HCl, was understood by the formation of poorly soluble triclinic complex crystals that had intermolecular coordination arrays between lactone carbonyl oxygen atoms and tetraaqua-sodium such as >C=O···Na⁺(OH₂)₄···O=C< and intermolecular hydrogen bonding between carboxylic acid and carboxylate anion such as -COOH···⁻OOC-.⁵⁾ Note that a sodium ion is eliminated from a carboxylate anion due to the strong coordination of 6 oxygen atoms. Similar crystals were found for other alkali metal ions and their crystal structure data were summarized in Table I, although some of them had no aquating water molecules depending on the size of the cation.

Table I Crystal structure data of PDC complexes.
P=PDC, P' = PDC (1H⁺ released specimen), W=water

Ion	Formula	a, b, c (Å)			Space group
		$\alpha,$	$\beta,$	γ (°)	
Na	Na ₁ P ₁ P' ₁ W ₄	3.5841,	10.175,	24.081	P21/c
		90,	91.329,	90	
K	K ₁ P ₁	3.6362,	16.790,	12.376	P21/c
		90,	93.062,	90	
Ag	Ag ₁ P ₁ W ₁	5.1868,	8.7614,	10.231	P-1
		113.53,	94.673,	93.855	

The complex formation was also seen when mixed with silver nitrate solution. However, despite the fact that alkali metal ions are coordinated by the lactone carbonyl oxygens, silver ions strongly bind the carboxylic and carboxylate groups to yield specific PDC-Ag⁺ specimen that has very rare structure with Ag-Ag binary core akin to the binuclear silver complex of 4-fluorobenzoic acid.⁶⁾ In Fig. 2 (a) – (c), the ORTEP drawings of an asymmetric unit, two-dimensional layered structure and the Mercury⁷⁾ drawings of the projection along the *c* axis are shown. One can distinguish the chelating

coordination of two -COO- ions from the side directions of Ag-Ag core, whereas two carbonyl oxygen atoms of -COOH groups were attached to the axis of Ag-Ag core.

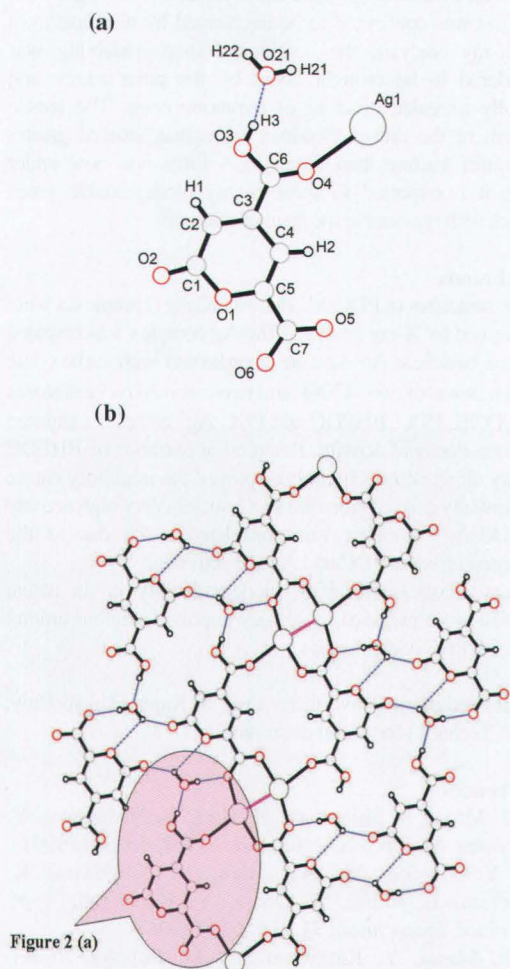


Figure 2 (a)

(c)

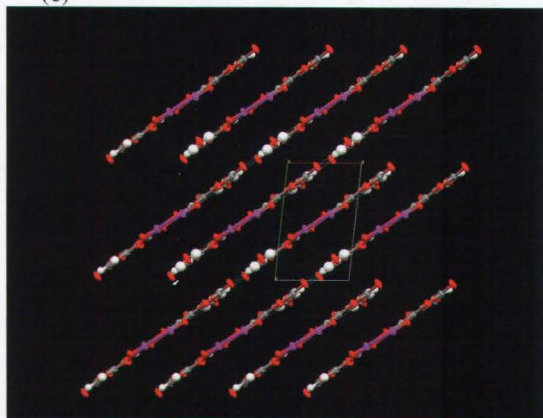


Figure 2 Crystal structure of PDC-Ag

Although PDC is decomposed very rapidly in nature into carbon dioxide and water by the TCA cycles of wild microbodies that can promote the degradation of lignin, similar pyranone derivatives were known to act as anti-bacterial chemicals against common strains such as *E. coli*.⁸⁾ MIC or the minimum inhibitory concentrations of PDC for the several strains were investigated and listed in

Table II. Relatively low MIC values were found for PDC; only by 15 min-contact with the PDC solution every strain was prohibited to grow and the colony forming units (cfu) per ml decreased to lower than 100 from the initial value of 4×10^6 . This inhibitory activity is not derived merely from the acidity of PDC. Although the pH value of phosphate buffer solution was lowered to about 3.5 by dissolving PDC in 0.25 wt % concentration, no apparent decrease of cfu was noticed in aqueous lower aliphatic acid solution of same pH.

BHPDC gave low MIC similar to PDC, but its action was rather slow. It took almost 24 h to reach the inhibition at which BHPDC was spontaneously hydrolyzed to PDC with eliminating 1,2-ethanediol. In pure water its half lifetime was estimated from the pH change to be about 8 h, therefore its slow inhibitory action is coincident to the PDC regeneration. Since PDC is a short lived chemical in nature due to the existence of lignin-metabolizable microbodies as shown in Fig. 1, BHPDC is a useful substance as controlled-release anti-bacterial chemicals.

The PDC-Ag⁺ complex was expected to show stronger anti-bacterial activity. Due to the concerted anti-bacterial activity of PDC and silver ion, its MIC value was lowered to 0.025 or less, corresponding to 10 – 100 fold greater activity compared to simple silver ion.⁹⁾ Also, the storage lifetime of the common silver salts under ambient circumstances (air and illumination) is short due to discoloration by the photoreduction and subsequent basic silver oxide formation, no apparent change was found for the PDC-Ag⁺ complex. Such excellent longevity presumably given by the unique complex structure is another advantage.

Outbreak of recent biohazards by antibiotics-resistant bacteria such as O157, VRE (Vancomycin-resistant *E. coli*), MRSA and mutated *Salmonella* requires suitable anti-bacterial chemicals that do not induce mutation to wild bacteria and do not cause environmental damages. Therefore, PDC, BHPDC and the PDC-Ag⁺ complex, essentially derived from the naturally occurring molecule are good candidates as disinfection chemicals for dairy products.

Table II MIC values for several strains

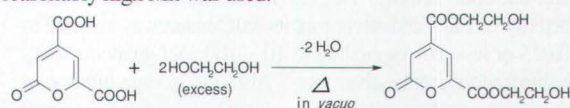
Name of Strain ¹⁾	MIC (wt %)		
	PDC	BHPDC	PDC-Ag ⁺
<i>Pseudomonas a.</i>	0.25	— ²⁾	—
<i>Staphylococcus a. s. a.</i>	0.25	1	<0.025
<i>Vibrio p.</i>	0.125	—	—
<i>E. coli</i> O157:H7	0.25	1	<0.025
<i>Trichophyton r.</i>	0.0625	—	—
<i>Candida a.</i>	1	—	—
<i>Cladosporium c.</i>	1	—	—
<i>Aspergillus n.</i>	1	—	—

1) see *Experimental*

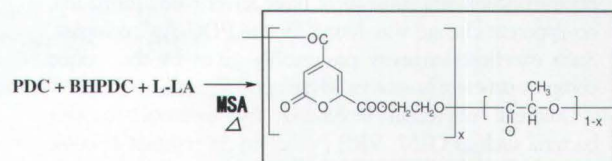
2) not tested

Hydrolyzable polyesters carrying 2-pyranone-4,6-dicarbonyl repeating units in the chain backbone are much interesting, because of their possible biodegradability and/or gradual regeneration of PDC nuclei along with the fragmentation. As shown in Fig.1, PDC accepts the ring-opening hydrolysis to yield a strongly hydrophilic specimen, 3-hydroxycarbonyl-5-oxo-hex-2-ene-dicarboxylic acid, as the preceding compound of oxalacetic acetic acid and pyruvic acid that are utilized in the TCA cycle, the biodegradation of such polyesters in an active compost, for example, might be the competitive reaction of the main-chain

hydrolysis and the ring-opening hydrolysis. If the cleavage of the main-chain ester linkages is the first occurring event, thus regenerated PDC may show certain inhibitory effect. In the present study, the copolyesters with L-lactic acid (LA) were prepared. Since PDC is a dibasic acid, the existence of equimolar amount of bis(*o*-hydroxyalkylene) 2-pyranone-4,6-dicarboxylate, or BHPDC as shown in Scheme 1, is necessary to maintain the stoichiometric balance of -COOH and -OH functionalities. The heat polymerization of molten mixture of PDC, BHPDC and LA in presence of methanesulfonic acid (MSA) as a catalyst gave copolyesters (see Scheme 2) soluble in chloroform or THF and with $T_d = 230 - 235$ °C, $T_m = 120 - 130$ °C, and $T_g = 55 - 65$ °C. As summarized in Table III, the number-average molecular mass, M_n , decreased with the increase of PDC unit (PDC+BHPDC). Prolonged polymerization period caused merely discoloration without the increment of M_n . Further attempts to polymerize at higher temperature resulted abrupt gelation when the PDC unit content was higher than the case of Run #5. For the subsequent study, the copolyester of Run #7 with reasonably high M_n was used.



Scheme 1 Synthesis PDC-diol [BHPDC]



Scheme 2 Synthesis of PDC Copolyester

Table III Synthesis of copolyesters

Run	Feed mol ratio ¹⁾		x ²⁾	Y (%)	M_n ($\times 10^{-3}$)	PD ³⁾
	PDC/BHPDC/LA					
1	1 / 1 / 10	0.17	88	1.2	2.7	
2	1 / 1 / 20	0.091	82	2.4	2.2	
3	1 / 1 / 30	0.063	87	3.9	2.7	
4	1 / 1 / 40	0.048	86	5.3	2.7	
5	1 / 1 / 50	0.038	88	11.9	1.2	
6	1 / 1 / 60	0.032	88	12.0	1.2	
7	1 / 1 / 80	0.024	88	23.3	1.4	

- [total monomer]=1.0g, [methanesulfonic acid]=3.0 mg.
- content of oxyethyleneoxy(2-pyranone-4,6- dicarbonyl) unit in copolyester, see Scheme 1.
- polydispersity; M_w/M_n , where M_w denotes weight-average molecular mass.

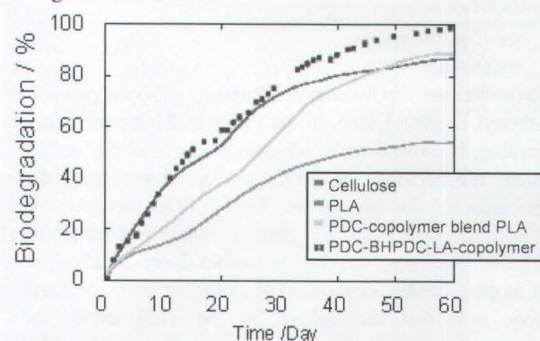


Figure 3 Biodegradability of polymers

Figure 3 illustrates the biodegradability of PDC-BHPDC-LA copolyester and 20 wt%-copolyester blended PLA. One can notice the biodegradability of PLA was improved by blending. Since the crystallinity of virgin PLA (ca. 6%) was confirmed to be unchanged by blending from the X-ray analysis, the accelerated biodegradability was considered to be brought about by the polar nature and spatially irregular structure of pyranone rings. The tensile strength of the drawn blendmer films that showed greater maximum fracture than virgin PLA films was now under study, it is expected to serve highly biodegradable green plastics with reasonable mechanical strength.

Conclusions

The structures of PDC- M^+ (M : Na, K, Ag) complexes were determined by X-ray analysis. The Ag complex was revealed to have binuclear Ag-Ag core coordinated with carboxylate oxygen atoms of two -COO⁻ and two carbonyl oxygen atoms of -COOH. PDC, BHPDC and PDC-Ag⁺ complex exhibited high anti-bacterial activity. Retarded appearance of BHPDC activity along with its hydrolysis proved the inhibitory nature is essentially derived from the PDC nuclei. Very high activity of PDC-Ag⁺ complex was considered to be due to the concerted effect of PDC and Ag-ion activities.

Green plastics with high biodegradability in an active compost were prepared by ternary copolymerization among PDC, BHPDC and LA.

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