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Metabolites of *Penicillium italicum* WEHMER: Isolation and Structures of New Metabolites Including Naturally Occurring 4-Ylidene-acyltetronic Acids, Italicinic Acid and Italicic Acid

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Four new metabolites, italicic acid, dihydro-italicic acid, methyl italicate and italicinic acid, were isolated from culture medium of *Penicillium italicum* WEHMER. The structures, possessing a 4-ylidene-acyltetronic acid unit, and their biosynthesis were elucidated. Two other new metabolites possessing a chromone skeleton were also isolated and their structures were established as 6,7-dihydroxy-3-(1′-hydroxy-3′-butanoyl)chromone-5-carboxylic acid and its 1′,2′-dehydrate.

Keywords *Penicillium italicum*; italicic acid; italicinic acid; 4-ylidene-acyltetronic acid; biosynthesis; chromone

Penicillium italicum is a phytotoxic fungus which causes the blue-mold rot of fruits (especially satsuma-mandarin var. unshu), and it has been reported to produce deoxybrevianamide E and 12,13-dehydro-deoxybrevianamide E.[1)]

The fungus was cultivated stationarily in malt extract medium. From the mycelium, xanthocillin-X[2)] and formylxanthocillin-X[3)] were obtained. From the culture medium, several new compounds, PI-1 (named italicinic acid) (**1**), mp 151—153 °C, PI-2 (named italicic acid) (**2**), mp 158—160 °C, methyl ester of PI-2 (**3**), mp 136 °C, dihydro PI-2 (**4**), mp 140—141 °C, and two chromone derivatives of PI-3 (**5**), mp 185—187 °C, and PI-4 (**6**), mp 245 °C, were isolated together with 4-methoxy-6-*n*-propenyl-2-pyrone,[4)] deoxybrevianamide E, 12,13-dehydro-deoxybrevianamide E, dehydrofulvic acid[5,6)] and 5-hydroxymethyl-2-furic acid[7)] (see Experimental).

Structure of PI-1 (1; Italicinic Acid) PI-1 (**1**), an optically active metabolite, is a major metabolite of this fungus. It possesses the molecular formula $C_{15}H_{18}O_6$ and gives a positive ferric chloride test. The infrared (IR) spectrum shows absorptions at 1790 (5-membered lactone), 1690 (carboxylic acid), 1675 (ketone), 1630 and 1576 cm^{-1}. The proton nuclear magnetic resonance (^1H-NMR) spectrum suggests the presence of the partial structure illustrated in Chart 1 in the molecule. Proton H_a (δ 7.07, d) is spin-coupled ($J=16$ Hz; *trans*) to proton H_b (δ 7.47, dd). H_b is further coupled ($J=7.5$ Hz) to H_c (δ 2.61) and the latter is further coupled to both methyl (δ 1.15, d) and methylene protons. Signals of one methine carbon (δ 37.8), three methylene carbons (δ 22.3, 29.1 and 35.2) and two methyl carbons (δ 13.4 and 18.6) in the ^{13}C-NMR spectrum confirmed the presence of a n-C_4H_9–$CH(CH_3)$– chain.

The residue $_{-\mathrm{O}}{>}\mathrm{C}=\mathrm{CH{-}COOH}$ was also indicated by the presence of signals of vinyl (δ 5.84, s) and hydroxyl (δ 6.51, br s) protons in the ^1H-NMR spectrum and signals at δ 98.4, 150.8 and 164.7 in the ^{13}C-NMR spectrum. In the tetrahydro derivative (**7**), $C_{15}H_{22}O_6$, which was obtained by hydrogenation of PI-1 at the ethylene and carboxylmethylidene double bonds with palladium–carbon (Pd–C) catalyst in benzene, the above vinylic signal was replaced by the diagnostic ABX pattern at δ 2.68 (dd, $J=6$, 18 Hz), 2.88 (dd, $J=4.5$, 18 Hz) and 4.83 (dd, $J=4.5$, 6.0 Hz) in the ^1H-NMR spectrum and by the signals at δ 34.4 (CH_2), 77.3 (CH), 169.8 (COOH) in the ^{13}C-NMR spectrum. Further, the chemical shift change of C-4 from δ 150.8 in **1** to δ 77.3 in **7** in the ^{13}C-NMR spectrum suggested that this carbon atom was bound to an oxygen atom.

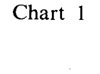


Chart 1

The presence of the 2-acyltetronic acid unit in PI-1 was supposed on the basis of the following evidence. Tetrahydro PI-1 (**7**) shows spectral properties characteristic of a 2-acyltetronic acid chromophore, *viz.* ultraviolet (UV) maxima at 233 and 268 nm (*cf.* carlosic acid,[8)] 232 and 267 nm). Its IR absorptions and the ^{13}C-NMR spectral signals (Table I) of the acyltetronic acid moiety of **7** are also closely similar to those of carlosic acid. Further, when catalytic hydrogenation of PI-1 was performed on Pd–C in methanol, hydrogenolysis also occurred to yield a deoxytetrahydro derivative (**8**), $C_{15}H_{24}O_5$, mp 178—180 °C (dec.), which showed spectral properties characteristic of a 2-alkyltetronic acid chromophore (UV max. 231 nm; *cf.* 2-ethyltetronic acid,[9)] 233 nm). It is known that the acyl carbonyl group in 2-acyltetronic acid is reduced to methylene by catalytic hydrogenation, while the double bond in the lactone ring is not saturated.[10)]

Treatment of **7** with bromine in 50% aqueous acetic acid[10)] afforded 2-bromo-4-carboxymethyl-tetronic acid (**9**), $C_6H_5BrO_5$, mp 197—199 °C (lit.[11)] mp 198—199 °C) by extrusion of the acyl side chain.

Reduction of PI-1 with sodium borohydride ($NaBH_4$) yielded a 2-alkyl-4-carboxymethylidene tetronic acid derivative (**10**), $C_{15}H_{22}O_5$, mp 157—158 °C. Its UV (264 and 295 nm) and IR (1720, 1700 (sh) and 1630 cm^{-1}) spectra were similar to those of multicolic acid,[12)] a metabolite

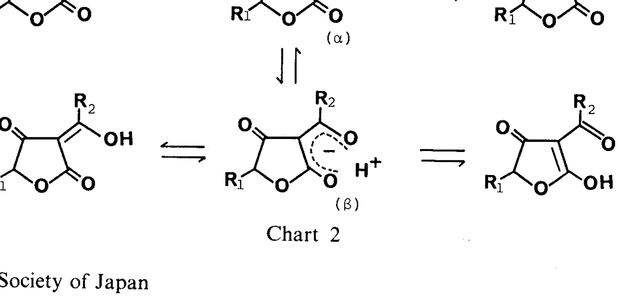


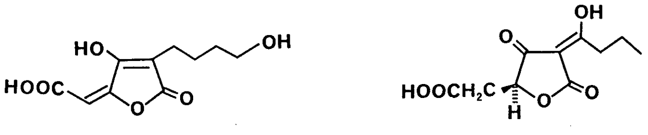
Chart 2

from *P. multicolor* (UV, 263 and 295 nm; IR, 1785, 1700 and 1640 cm^{-1}).

From these results, the structure of PI-1 (italicinic acid) is proposed as **1** (Chart 4). The chemical shift values of PI-1 and its derivatives in the ^{13}C-NMR spectra are shown in Table I. Tetronic acids are usually shown as having a structure possessing an enolic hydroxy group at C-3,[13] and 2-acyltetronic acids were reported to exist predominantly in α form[14,15] in the tautomeric equilibria (Chart 2).

The geometry of *E*- and *Z*-isomers of carboxymethylidene is easily distinguishable by NMR spectral studies, since the vinyl proton of the carboxymethylidene group in the *E*-isomer is more deshielded than that of the *Z*-isomer by virtue of its *cis*-relationship to the butenolide oxygen. For example, the signal of the vinyl proton in *Z*-multicolanic acid was observed at δ 5.65 in the ^{1}H-NMR spectrum (δ 94.5 in the ^{13}C-NMR spectrum), whereas that of the *E*-isomer was at δ_H 6.02 (δ_C 100.2).[16] The vinyl proton signal and carbon signal in the reduced derivative (**10**) appeared at δ_H 5.64 and δ_C 94.6, which corresponded well with those of the *Z*-isomer of multicolanic acid. Further, **10** did not give a bis-lactone on dehydration with *N*,*N*′-dicyclohexylcarbodiimide (DCC), which could only arise from the *E*-isomer.[17] These observations suggest the *Z*-stereochemistry at the exocyclic double bond of C-4.

Structure of PI-2 (2; Italicic Acid) The molecular formula shows that PI-2 (**2**), $C_{15}H_{16}O_6$, contains two less hydrogen atoms than PI-1 (**1**). PI-2 was negative in the ferric chloride test. The specific rotation of PI-2 changed to a constant value on prolonged standing, which suggests the occurrence of isomerization in solution. In the UV spectrum, PI-2 has almost the same maximum absorption (334 nm) as PI-1 (332 nm) and also shows very similar properties in the IR and NMR spectra, except that some signals in the NMR spectrum of PI-2 appear in duplicate. From the spectral properties, the structure of PI-2 was supposed to contain the same components, such as –CH=CH–, >C=CH–COOH, *n*-C_4H_9 side chain, methyl and five membered lactone (IR, 1800 cm^{-1}) moieties, as PI-1. Among them, the signal of methyl protons appears as a singlet (δ 1.73) and the methine signal coupled to the methyl signal in PI-1 is replaced by a quaternary carbon (δ 103.4 or 102.8) carrying an oxygen atom. Further, each proton of the –CH=CH– group appears as doublet and its coupling constant (J=6.0 Hz; *cis* relationship) suggests that this group is located in a ring.



multicolic acid

carlosic acid (3-keto form)

Chart 3

On methylation with methanol and DCC, PI-2 afforded a methyl ester, mp 135—136 °C, which was identical with another new metabolite (**3**) isolated from this fungus.

Catalytic hydrogenation of PI-2 on Pd–C in benzene proceeded successively at the ethylene and carboxymethylidene double bonds to afford dihydro (**4**) and tetrahydro (**11**) derivatives. The maximum UV absorptions at 295 nm in **4** and 277 nm in **11** suggest that these double bonds are both conjugated to the main chromophore of PI-2. The UV, IR and NMR spectra of **11** are very similar to those of carlic acid[18,19] (a metabolite of *P. charlesii*), carolic acid[18,20] (*P. charlesii*), dehydrocarolic acid[21] (*P. cinerascens*), and terrestric acid[22] (*P. terrestre*), which each have a furfurylidene-2,4-oxolanedion skeleton.[23] In these meta-

TABLE I. ^{13}C-NMR Data for PI-1 and Its Derivatives[a)]

Carbon	PI-1 (**1**)[b)]	**7**[b)]	**8**[c)]	**10**[c)]	Carlosic acid[d)]	Multicolanic acid[e)] *E*-Isomer	Multicolanic acid[e)] *Z*-Isomer[f)]
1	165.2	170.7	173.9	170.2	171.3	172.6	169.0
2	95.5	97.8	102.8	105.1	98.6	107.8	106.7
3	184.5	193.3	174.4	163.5	193.2	161.7	162.1
4	150.8	77.3	74.8	153.4	78.0	159.5	153.4
5	98.4	34.4	38.2	94.6	34.9	100.2	94.5
6	164.7	169.8	172.3	163.5	170.4	167.8	164.6
1′	179.6	195.9	24.1	22.7	196.4	21.4	21.4
2′	118.6	35.3	26.5	25.0	35.2	26.9	27.5
3′	162.6	31.8[g)]	37.8[g)]	36.4	18.8	22.3	22.3
4′	37.8	31.6[g)]	37.9[g)]	36.4	13.1	31.4	31.5
5′	35.2	30.6[g)]	33.7	32.4		13.5	13.5
6′	29.1	28.2	30.4	29.0			
7′	22.3	21.9	22.6	21.5			
8′	13.4	13.0	14.8	13.7			
9′	18.6	18.2	20.4	19.2			

a) Assignments are based on comparison with literature values and the results of ^{13}C-acetate incorporation experiments. *b*) Solvent, $CDCl_3$–MeOH-d_4. *c*) Solvent, acetone-d_6. *d*) Carlosic acid was a gift from Dr. Boll; solvent, $CDCl_3$. *e*) Reference 15; solvent, DMSO-d_6. *f*) Stereochemistry of carboxymethylidene bond. *g*) Assignments may be interchanged in each column.

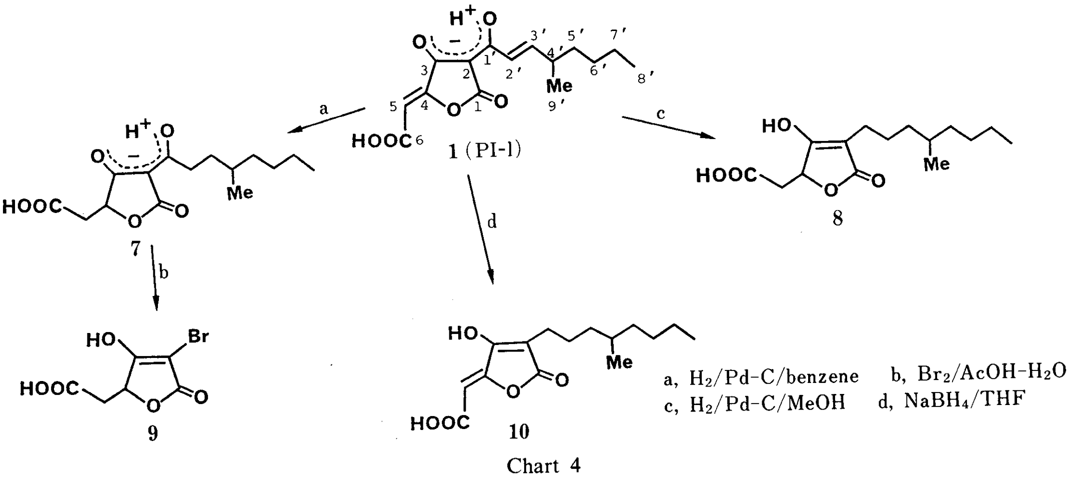
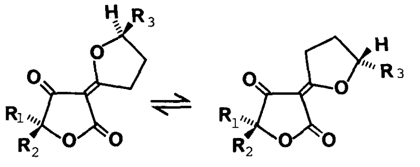


Chart 4

bolites, several of the signals in the ^{1}H- and ^{13}C-NMR spectra are doubled indicating the co-existence of *E*- and *Z*-isomers owing to the furfurylidene double bond. The isomerization of this group has been studied in detail and it was reported that the ^{13}C-NMR spectral chemical shift of C-1 (lactone carbonyl) in the *E*-isomer appears at about 3 ppm lower field than that of the *Z*-isomer.[23,24]

PI-2, its methyl ester (**3**) and tetrahydro derivative (**11**) also showed two sets of signals of approximately equal intensity in the NMR spectra. For example, in the ^{1}H-NMR spectrum of the freshly dissolved methyl ester of PI-2 in deuterium chloroform, a methine (C-5) signal is recognized at δ 5.92 together with a smaller signal at δ 5.94 (ratio; *ca.* 5:1). But, as time passes, the smaller signal increases in intensity at the expense of the δ 5.92 signal until the two signals become approximately equal in intensity. This isomerization proceeds more rapidly in a polar solvent such as methanol.

Catalytic reduction of PI-2 on Pd–C in methanol yielded an alkyltetronic acid derivative. It does not show the isomerization, which suggests the absence of the furfurylidene bond. The product was methylated and purified to give the *O*-dimethyl compound (**12**) in which the singlet signal of methyl protons (δ 1.24) in the ^{1}H-NMR spectrum also suggested a location on a quaternary carbon (δ_C 72.5) carrying a hydroxyl group. From a comparison of its spectral properties with those of other tetronic acid derivatives methylated at the lactone oxygen atom,[25] this compound seemed to possess a 1-methoxy-3-furanone skeleton. The NaBH$_4$-reduced derivative of PI-2 (**13**), $C_{15}H_{22}O_6$, also exhibited a singlet peak in the NMR spectrum and possessed the hydroxyalkyl side chain (quaternary carbon, δ 73.7) and the carboxymethylidene moiety. This compound (**13**) gave a mono-acetate (**14**) with acetic anhydride and pyridine. Terrestric acid was also reduced on Pd–C in methanol (or with NaBH$_4$) to cause a similar ring cleavage, which was confirmed by the formation of an alkyltetronic acid possessing a hydroxyl group on the methine (δ_H 3.80, δ_C 74.7) of the side chain.[26]



$R_1 = H, R_2 = CH_2COOH, R_3 = H$ carlic acid
$R_1 = Me, R_2 = R_3 = H$ carloic acid
$R_1 = Me, R_2 = H, R_3 = Et$ terrestric acid

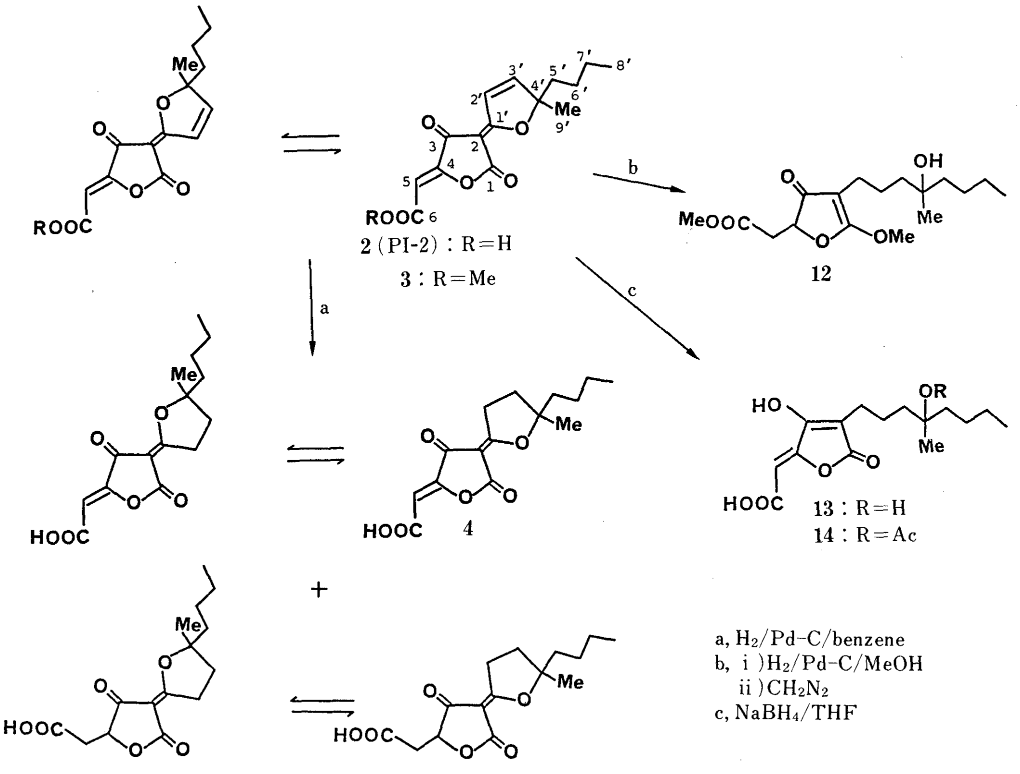
Chart 5

From these results, the structure of PI-2 (italicic acid) was proposed to be **2**.

The stereochemistry around the C-4 double bond was supposed to be *Z* from the chemical shift values of the vinyl proton (δ 5.55) and carbon (δ 94.2) of the NaBH$_4$-reduced compound (**13**) as in the case of PI-1. The sterochemistry of PI-1 and PI-2 will be discussed in detail in a future publication.

Biosynthesis of PI-1 and PI-2 Two different pathways in the biosynthesis of fungal tetronic acid have been established. One is a route *via* oxidative cleavage of an aromatic precursor originated from polyketide[12,27] (*e.g.* multicolic acid) and the other is a pathway *via* condensation of a poly-β-ketide with a C4-dicarboxylic acid (Krebs cycle component)[28] (*e.g.* carolic acid, carlosic acid).

Incorporation patterns into PI-1 and PI-2 by the fungus fed with sodium[1-^{13}C], [2-^{13}C] or [1,2-$^{13}C_2$]acetate, are



a, H$_2$/Pd-C/benzene
b, i)H$_2$/Pd-C/MeOH
ii)CH$_2$N$_2$
c, NaBH$_4$/THF

Chart 6

TABLE II. ^{13}C-NMR Data for PI-2 and Its Derivatives[a]

Carbon	PI-2[b] (2)		PI-2 methyl ester[c] (3)		Dihydro PI-2[b] (4)		**11**[c]		**12**[c]	**13**[d]	Carlic acid[f]	
	E	*Z*[e]	*E*	*Z*	*E*	*Z*	*E*	*Z*			*E*	*Z*
1	166.4	165.9	166.0	164.1	166.1	165.7	173.7	170.7	180.8	163.5	172.63	169.97
2	90.9	90.3	92.1	91.6	92.1	91.6	100.9	100.3	93.1	105.0	97.00	96.42
3	180.6	181.5	180.4	182.3	188.4	189.2	192.4	197.2	196.5	169.2	196.15	198.82
4	152.8	152.6	152.9	152.6	152.5	152.4	94.5	94.9	82.0	153.5	80.11	80.50
5	94.9	94.9	95.5	95.5	94.8	94.5	35.9	35.9	35.8	94.2	36.32	36.32
6	160.9	160.9	163.3	163.3	162.1	162.1	167.1	167.1	169.9	164.2	172.63	172.63
1′	178.8	179.8	180.2	181.4	181.6	182.4	186.1	186.8	19.8	21.3	189.53	190.43
2′	121.5	121.4	123.1	123.1	35.6	35.3	34.4	34.1	26.7[g]	26.5[g]	34.76	34.50
3′	162.9	162.9	162.2	162.2	32.1	31.8	31.6	31.6	41.6	42.4	22.42	22.42
4′	103.4	102.8	103.6	103.0	102.1	101.5	78.2	78.2	72.5	73.7	79.85	79.33
5′	37.5	37.5	38.0	37.5	40.1	40.1	39.8	39.8	40.9	38.2		
6′	26.4	26.4	26.0	26.0	26.9	26.9	26.1	26.1	26.0[g]	26.2[g]		
7′	22.7	22.7	22.5	22.5	23.3	23.3	22.4	22.4	22.5	22.0		
8′	13.3	13.3	13.7	13.7	13.5	13.5	13.7	13.7	14.0	13.7		
9′	23.0	23.0	23.1	23.1	24.9	24.9	25.0	25.0	23.2	23.3		
Me	51.9	51.9							52.1 56.2			

a) Assignments are based on comparison with literature values and the results of ^{13}C-acetate incorporation experiments. *b*) Solvent, $CDCl_3$–MeOH-d_4. *c*) Solvent, $CDCl_3$. *d*) Solvent, acetone-d_6. *e*) Stereochemistry of furfurylidene bond. *f*) Reference 22; solvent, MeOH-d_4. *g*) Assignments may be interchanged in each column.

TABLE III. ^{13}C-NMR Data for ^{13}C-Enriched PI-1 and PI-2[a]

Carbon	PI-1			PI-2		
	[2-^{13}C]AcONa	[1-^{13}C]AcONa	[1,2-$^{13}C_2$]AcONa (Hz)[b]	[2-^{13}C]AcONa	[1-^{13}C]AcONa	[1,2-$^{13}C_2$]AcONa (Hz)[b]
1	0.98	14.8	76.5	n.d.[c]	5.0	76.5
2	10.2	0.95	76.5	1.7	n.d.	76.5
3	2.3	4.0		n.d.	n.d.	
4	1.6	0.85		1.2	n.d.	
5	4.3	0.82		1.3	n.d.	
6	2.0	4.1		1.4	n.d.	
1′	0.75	5.3	48.5	0.60	12.4	48.5
2′	6.7	0.81	48.5	6.5	0.64	48.5
3′	0.80	18.8	40.5	0.62	17.1	39.7
4′	11.1	1.0	40.5	4.8	1.0	39.7
5′	1.8	15.2	35.5	1.2	28.3	35.5
6′	15.5	1.5	35.5	10.0	1.1	35.5
7′	0.95	27.1	33.5	0.75	21.1	33.5
8′	12.6	1.0	33.5	4.7	0.80	33.5
9′	1.0	1.0		1.0	1.0	

a) Relative intensity values of enriched carbons were normalized with respect to the 9′-methyl signal. *b*) ^{13}C–^{13}C coupling constants. *c*) n.d., signal was not detected.

summarized in Table III. The ^{13}C-NMR spectra of PI-1 and PI-2 enriched by feeding of sodium[1-^{13}C]acetate to cultures of *P. italicum* both exhibited increases of intensity at alternate carbons 1, 1′, 3′, 5′ and 7′, while the intensity of signals at carbons 3 and 6 were slightly enhanced. On the other hand, the spectra obtained by incorporation of sodium[2-^{13}C]acetate showed high enrichment at alternate carbons 2, 2′, 4′, 6′ and 8′ with some incorporation at carbons 3, 4, 5 and 6. The intensity of the 9′-methyl carbon was not affected in either feeding experiment. Further, the ^{13}C-NMR spectrum of PI-2 prepared by feeding sodium[1,2-$^{13}C_2$]acetate showed ^{13}C–^{13}C couplings between the carbon atoms of C(1)–C(2), C(1′)–C(2′), C(3′)–C(4′), C(5′)–C(6′) and C(7′)–C(8′). These results indicate that the acyl side chain and C-1, C-2 of the lactone ring are derived from pentaketide.

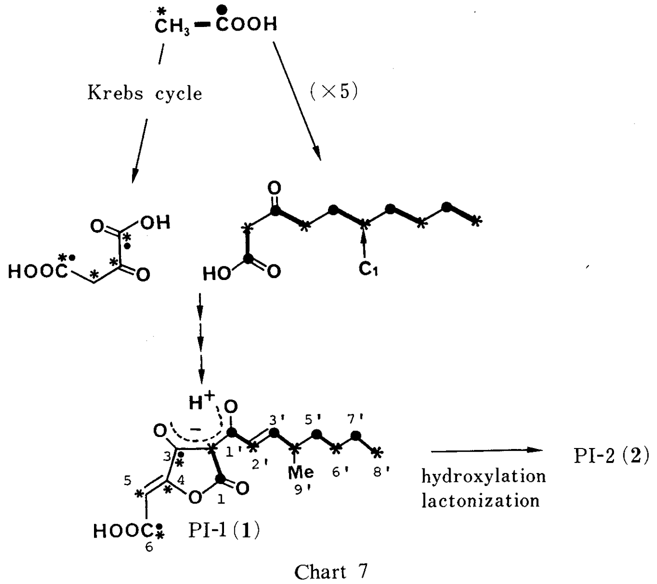
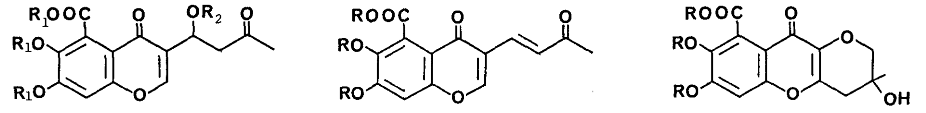


Chart 7

The above results, together with the random and low ^{13}C-incorporation at four other carbons (3, 4, 5 and 6), indicate that PI-1 and PI-2 are both biosynthesized by



5 (PI-3): $R_1=R_2=H$
15: $R_1=Me$, $R_2=H$
16: $R_1=R_2=Me$

6 (PI-4): $R=H$
17: $R=Me$

$R=H$ fulvic acid
$R=Me$ methyl *O*-dimethyl fulvate

Chart 8

condensation of a C4 acid (Krebs cycle component; probably oxaloacetate) with a pentaketide, similarity to carloic acid.

Structures of PI-3 and PI-4 Metabolites PI-3 (**5**), $C_{14}H_{12}O_8$, and PI-4 (**6**), $C_{14}H_{10}O_7$, showed similar physical properties. The NMR spectra (δ_H 2.14; δ_C 30.2 (q), 206.7 (s)) suggested the presence of a methylketone group in PI-3. This assumption was confirmed by a positive reaction to iodoform reagent. Methylation of PI-3 with diazomethane yielded tri- (**15**) and tetramethyl (**16**) derivatives, in which the newly appeared the IR absorption at 1729 or 1746 cm^{-1}, respectively, suggests the presence of a carboxyl group in PI-3.

A dehydrate (**17**) was formed by treatment of trimethyl-PI-3 with acetic anhydride and pyridine. It was identical with trimethyl-PI-4 which was derived by methylation of PI-4 with ethereal diazomethane in the presence of methanol. In the ^{1}H-NMR spectrum, the dehydrate (**17**) showed signals of a CH=CH group (δ 7.25 and 7.45, each d, $J=$ 16 Hz) in place of the ABX-type signals in PI-3 [δ 2.56 ($J=$ 8, 16 Hz), 2.84 ($J=3.5$, 16 Hz) and 5.06 ($J=3.5$, 8 Hz)], which suggested the existence of an HO–CH–CH$_2$– moiety in PI-3. Oxidation of trimethyl-PI-3 (**15**) with $KMnO_4$ in acetone yielded 3,4-dimethoxy-6-hydroxy-2-methoxycarbonylbenzoic acid, mp 146—148 °C, which was identical with the oxidation product of methyl *O*-dimethylfulvate.[5)] Further, the UV spectra of PI-3 (**5**) and its trimethyl derivative (**15**) are very similar to those of fulvic acid and its trimethyl derivative,[29)] respectively.

From the above results, PI-3 was supposed to be a derivative of 5-carboxyl-6,7-dihydroxy-chromone with a side chain at C-3, which is the most suitable stucture to explain the chemical shift values of two ring protons at δ 6.91 (s, benzene ring) and 8.07 (s, pyrone ring) in the ^{1}H-NMR spectrum. The side chain moiety ($C_4H_7O_2$) contains one methylcarbonyl group and one hydroxyl group.

All three carbonyl bands in PI-3 (IR, 1684, 1664 and 1622 cm^{-1}) were shifted to higher fields by methylation; namely 1729, 1710 and 1636 cm^{-1} in **15**, and 1746 (ester), 1721 (ketone) and 1646 (pyrone) cm^{-1} in **16**. These results suggest that the hydroxyl group in the side chain is located a suitable the position to form several hydrogen bonds between the carbonyl groups of methylketone and pyrone ring. Thus, the structure of PI-3 was proposed to be 6,7-dihydroxy-3-(1′-hydroxy-3′-butanoyl)chromone-5-carboxylic acid (**5**) and subsequently, PI-4 (**6**) was determined to be the 1′,2′-dehydrate of PI-3.

As metabolites possessing a similar structure, polivione from *P. frequentans*[30)] and lapidosin from *P. lapidosum*[31)] are known.

Experimental

Melting points were determined on Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured with a JASCO A-102 spectrometer, mass spectra (MS) with a Hitachi M-80 mass spectrometer, UV spectra with a Hitachi 323 spectrometer in ethanol, and ^{1}H- and ^{13}C-NMR spectra with a JEOL FX-100 spectrometer using tetramethylsilane as an internal standard. Optical activities were measured with a JASCO DIP-181. High-performance liquid chromatography (HPLC) and preparative HPLC were performed on a Waters Associates ALC/GPC 201 D compact-type liquid chromatograph using a TSK-GEL ODS-80 TM column (column size; 15 cm × 4.6 mm; detector, SPD-M6A (Shimadzu)). For drying of organic solutions, anhydrous Na_2SO_4 was used.

Cultivation *P. italicum* was cultivated stationarily at 27 °C for 3 weeks in 30 Roux flasks each containing 200 ml of glucose (20 g), malt extract (purchased from Difco Co., 20 g), polypeptone (1 g) and tap water (1 l). The culture broth was separated from the mycelia by filtration.

Isolation of Metabolites The filtrate (6 l, pH *ca.* 3.5) was concentrated *in vacuo* to one-fourth of the original volume and extracted with ethyl acetate three times. The combined solution was evaporated *in vacuo* to dryness (AcOEt extract, 2.8 g). The aqueous solution was made more acidic (pH <2) with concentrated HCl and then extracted with ethyl acetate. After concentration, the organic layer was left to stand overnight. The crystallized PI-1 (**1**) was collected and recrystallized from benzene to afford colorless prisms (520 mg), mp 151—153 °C.

The AcOEt extract was washed with petroleum ether and the insoluble portion was extracted with chloroform. The chloroform extract was evaporated and the dark-red residue was separated by silica gel (Wako gel, C-200) column chromatography using a gradient solvent of ethyl acetate–chloroform. From the chloroform eluate, the methyl ester of PI-2 (**3**) (4 mg), mp 135—136 °C, was obtained. From the 3% ethyl acetate–chloroform eluate, 4-methyl-6-*n*-propenyl-2-pyrone as colorless needles (hexane–chloroform), mp 96—100 °C, (lit.[4)] mp 102—103 °C), deoxybrevianamide E as a colorless powder (3 mg), mp >300 °C, dehydrofulvic acid (5 mg) as a pale yellow powder (methanol), mp 246—250 °C (lit.[5,6)] mp 245—246 °C), and 12,13-dehydro-deoxybrevianamide E (2 mg) as a pale yellowish oily material were obtained, successively. A mixture containing PI-2 (**2**) and dihydro PI-2 (**4**) was isolated from the fraction eluted with 5% ethyl acetate–chloroform. From the 10% ethyl acetate–chloroform eluate, PI-3 (**5**) as colorless needles (ethyl acetate–benzene), mp 185—187 °C, (87 mg) and PI-4 (**6**) as pale yellowish fine crystals (ethyl acetate), mp 245 °C, (4 mg), were obtained, successively.

The materials still remaining on the column were washed out with methanol and the solvent was evaporated off. The dark red residue was rechromatographed on a silicic acid column (Mallinckrodt, silicar CC-4 special) using 10% ethyl acetate in benzene to give PI-1 (**1**) and 5-hydroxymethyl-2-furic acid, mp 168—170 °C (lit.[7)] mp 164—165 °C) (20 mg). The above mixture of PI-2 and dihydro PI-2 was further separated by HPLC on ODS-80TM with CH_3CN–H_2O (5:95, (v/v), flow rate 1 ml/min). The first fraction eluted at around 3 min was crystallized from ether–petroleum ether to give dihydro PI-2 (**4**) as colorless crystals, mp 140—142 °C (240 mg), and the second fraction eluted at 12 min was crystallized from ether–petroleum ether to afford PI-2 (**2**) as colorless prisms, mp 158—160 °C (240 mg).

The dried mycelia (19 g) were extracted with petroleum ether, ether and acetone, successively. Each extract was evaporated to dryness under reduced pressure. The residue of the ether extract was subjected to silica gel chromatography using 2% methanol–chloroform to give xanthicillin-X (154 mg) as pale yellowish prisms, mp 220 °C (lit.[2)] mp 220 °C) and deoxybrevianamide E. The acetone extract was washed with ethyl acetate and the insoluble residue was crystallized from methanol to give formylxanthocillin-X (3 mg) as yellow fine crystals, mp 240 °C (dec.) (lit.[3)] mp 240 °C).

Properties of PI-1 (1: Italicinic Acid) *Anal.* Calcd for $C_{15}H_{18}O_6$: C, 61.21; H, 6.17. Found: C, 60.95; H, 6.26. CI-MS *m/z*: 295 (M^++1), 277, 250, 234, 193, 167, 139, 97. EI-MS *m/z*: 276 (M^+-H_2O), 250, 165, 139, 69,

55. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3060, 2900, 1790, 1690 (sh), 1675, 1630, 1576. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 278, 333. ^{1}H-NMR (acetone-d_6) δ: 0.90 (3H, t, $J=6.0$ Hz), 1.15 (3H, d, $J=6.8$ Hz), 1.20—2.68 (6H, m), 2.61 (1H, m), 5.84 (1H, s), 6.51 (2H, br s), 7.07 (1H, d, $J=16$ Hz), 7.47 (1H, dd, $J=16$, 7.5 Hz). $[\alpha]_D$ $-9.2°$ ($c=0.89$, CHCl$_3$).

Properties of PI-2 (2; Italicic Acid) *Anal.* Calcd for $C_{15}H_{16}O_6$: C, 61.64; H, 5.52. Found: C, 61.44; H, 5.68. EI-MS *m/z*: 292 (M$^+$), 250, 236, 135, 94, 55. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 2920, 1800, 1690 (br), 1660. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 232 (sh), 240, 263 (sh), 325 (sh), 334, 350 (sh). ^{1}H-NMR (CDCl$_3$–MeOH-d_4 (3 : 1, v/v)) δ: 1.05 (3H, t, $J=6.5$ Hz), 1.30—1.66 (4H, m), 1.73 (3H, s), 1.76—2.02 (2H, m), 5.84, 5.83 (each 0.5 H, s), 7.66, 7.76, 7.94, 7.95 (each 0.5 H, d, $J=6$ Hz). $[\alpha]_D$ $+99°$ ($c=0.138$, MeOH); $+57°$ (after 5 min), $+88°$ (after 24 h) ($c=0.129$, CHCl$_3$).

Properties of Methyl Ester of PI-2 (3) *Anal.* Calcd for $C_{16}H_{18}O_6$: C, 62.74; H, 5.92. Found: C, 62.60; H, 5.82. EI-MS *m/z*: 306 (M$^+$), 275, 250, 135, 94. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1795, 1700, 1665. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 231 (sh), 239, 245 (sh), 263, 326 (sh), 335, 350 (sh). ^{1}H-NMR (CDCl$_3$) δ: 0.98 (3H, t, $J=5.8$ Hz), 1.16—1.60 (4H, m), 1.68 (3H, s), 1.88—2.15 (2H, m), 3.86 (3H, s), 5.92 (0.5H, s), 5.94 (0.5H, s), 7.49, 7.58 (each 0.5H, d, $J=6.0$ Hz), 7.58, 7.62 (each 0.5H, d, $J=6.0$ Hz). $[\alpha]_D$ $+70.9°$ (after 5 min), $+130.9°$ (after 24 h) ($c=0.11$, CHCl$_3$).

Properties of Dihydro PI-2 (4) *Anal.* Calcd for $C_{15}H_{18}O_6$: C, 61.21; H, 6.17. Found: C, 61.08; H, 5.98. EI-MS *m/z*: 294 (M$^+$). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3450, 1815, 1695, 1660 (sh), 1570. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 245 (sh), 295. ^{1}H-NMR (CDCl$_3$–MeOH-d_4 (3 : 1, v/v)) δ: 0.81 (3H, t, $J=6$ Hz), 1.03—1.35 (4H, m), 1.45 (3H, s), 1.60—1.95 (2H, m), 2.03, 2.08, 3.42, 3.46 (each 1H, t, $J=8$ Hz), 5.72, 5.74 (each 0.5H, s).

Hydrogenation of PI-1 (1) over Pd–C in Benzene PI-1 (100 mg) was hydrogenated with 5% Pd–C (20 mg) in benzene (10 ml) at room temperature. The product was purified by preparative thin layer chromatography (PLC) (chloroform–acetic acid (95 : 5, v/v)) and recrystallized from benzene–hexane to give tetrahydro PI-1 (**7**) as fine colorless crystals (quantitative yield), mp 101—103 °C. *Anal.* Calcd for $C_{15}H_{22}O_6$: C, 60.39; H, 7.43. Found: C, 60.43; H, 7.47. EI-MS *m/z*: 298 (M$^+$), 280, 213, 200, 195, 182, 177, 164, 97, 84, 55, 43. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1750, 1700, 1660, 1604. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 233, 268. ^{1}H-NMR (CDCl$_3$–MeOH-d_4 (10 : 1, v/v)) δ: 0.80 (3H, t, $J=5.6$ Hz), 0.84 (3H, d, $J=6.3$ Hz), 0.98—1.80 (9H, m), 2.68 (1H, dd, $J=6$, 18 Hz), 2.80 (1H, t, $J=7.5$ Hz), 2.88 (1H, dd, $J=4.5$, 18 Hz), 4.83 (1H, dd, $J=6$, 4.5 Hz).

Hydrogenation-Hydrogenolysis of PI-1 (1) over Pd–C in Methanol PI-1 (50 mg) was dissolved in methanol (5 ml) and the hydrogenation was carried out for 24 h. The product was purified by PLC (chloroform–ethyl acetate–acetic acid (85 : 10 : 5, v/v)) and crystallized from benzene–hexane to give deoxy-tetrahydro PI-1 (**8**) as fine colorless crystals (35 mg), mp 178—180 °C (dec.). *Anal.* Calcd for $C_{15}H_{24}O_5$: C, 63.09; H, 8.68. Found: 63.38; H, 8.45. EI-MS *m/z*: 284 (M$^+$), 199, 159, 69, 43. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: *ca.* 3200—2900, 1690, 1655. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 231. ^{1}H-NMR (acetone-d_6) δ: 0.88 (3H, d, $J=6.5$ Hz), 0.92 (3H, t, $J=5.6$ Hz), 1.10—1.68 (11H, m), 2.18 (2H, t, $J=7.0$ Hz), 2.64 (1H, dd, $J=16.5$, 7.5 Hz), 3.05 (1H, dd, $J=16.5$, 5.0 Hz), 5.08 (1H, dd, $J=7.5$, 5.0 Hz).

Bromination of Tetrahydro-PI-1 (7) Bromine (0.015 ml) in 50% aqueous acetic acid (1.5 ml) was added to a solution of tetrahydro-PI-1 (45 mg) in 50% aqueous acetic acid (2 ml). The solution was swirled for 2 h, the residue was poured into water and extracted with ethyl acetate. The organic layer was evaporated to dryness and then washed with chloroform. The residue was crystallized from benzene–ether to give 2-bromo-4-carboxymethyl-tetronic acid (**9**) as colorless crystals (10 mg), mp 197—199 °C (lit.[11] mp 198—199 °C). *Anal.* Calcd for $C_6H_5BrO_5$: C, 30.41; H, 2.13. Found: C, 30.49; H, 2.29. EI-MS *m/z*: 238 (M$^+$), 236, 122, 120, 71, 69. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1710 (sh), 1698, 1637. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 259. ^{1}H-NMR (acetone-d_6) δ: 2.80 (1H, dd, $J=8.0$, 16.5 Hz), 3.14 (1H, dd, $J=16.5$, 4.5 Hz), 5.25 (1H, dd, $J=8.0$, 4.5 Hz). $[\alpha]_D$ $+1.7°$ ($c=0.29$, MeOH).

Reduction of PI-1 (1) with NaBH$_4$ A solution of PI-1 (70 mg) in anhydrous tetrahydrofuran (THF) (4 ml) was treated with NaBH$_4$. The mixture was stirred at room temperature for 1 d and then poured into ice water. The reacted solution was acidified with HCl and extracted with ethyl acetate. The extract was concentrated and the residue was separated by PLC (benzene–ethyl acetate–acetic acid (60 : 20 : 5, v/v)). The major product (*Rf* 0.6) was crystallized from ether–hexane to give deoxy-dihydro PI-1 (**10**) as a colorless solid (14 mg), mp 157—158 °C. *Anal.* Calcd for $C_{15}H_{22}O_5$: C, 63.81; H, 7.85. Found: C, 63.59; H, 7.75. EI-MS *m/z*: 238 (M$^+$ $-$CO$_2$), 154, 126, 69. CI-MS *m/z*: 283 (M$^+$ $+1$), 239, 179, 126. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: *ca.* 3400—2800, 1720, 1700 (sh), 1630. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 264, 295 (sh). ^{1}H-NMR (acetone-d_6) δ: 0.90 (3H, d, $J=6.5$ Hz), 0.94 (3H, t, $J=5.7$ Hz), 1.08—1.80 (11H, m), 2.36 (2H, t, $J=7.5$ Hz), 5.64 (1H, s).

Methylation of PI-2 (2) with MeOH/DCC DCC (40 mg), dry methanol (0.1 ml) and 4-dimethylaminopyridine (trace) were added to a solution of PI-2 (30 mg) in dry dichloromethane (2 ml). The mixture was stirred overnight at room temperature. The resulting precipitate was filtered off and the filtrate was evaporated to dryness. The residue was purified by PLC (chloroform–ethyl acetate (4 : 1, v/v)) and then crystallized from ether–hexane to give the methyl ester of PI-2 (**3**) (21 mg), mp 135—136 °C, as colorless needles.

Hydrogenation of PI-2 (2) over Pd–C in Benzene PI-2 (140 mg) was mixed with 5% Pd–C (50 mg) and benzene (30 ml) in a hydrogenation bottle. The mixture was stirred for 4 h at room temperature under a hydrogen atmosphere. Filtration and concentration of the reaction mixture afforded tetrahydro PI-2 (**11**) in quantitative yield as a colorless gum.

When hydrogenation was stopped at 1 h, a mixture of dihydro (**4**) and tetrahydro (**11**) derivatives was obtained. From the upper band on PLC (chloroform–ethyl acetate (4 : 1, v/v)), dihydro PI-2 (**3**) was obtained.

Tetrahydro-PI-2 (**11**): EI-MS *m/z*: 296 (M$^+$), 278, 200, 138, 96, 54. HI-MS *m/z*: $C_{15}H_{20}O_6$ required for 296.1257. Found: 296.1257. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1750, 1700, 1585. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 277. ^{1}H-NMR (acetone-d_6) δ: 0.96 (3H, t, $J=6.0$ Hz), 1.20—1.56 (4H, m), 1.56 (3H, s), 1.72—1.92 (2H, m), 2.12—2.52 (2H, m), 2.52—3.08 (2H, m), 3.40—3.64 (2H, m), 4.68—4.88 (1H, m). $[\alpha]_D$ $+180°$ ($c=0.2$, CHCl$_3$).

Hydrogenation of PI-2 (2) over Pd–C in MeOH The mixture of PI-2 (100 mg) and 5% Pd–C (50 mg) in methanol (10 ml) was stirred for 5 h at room temperature under a hydrogen atmosphere. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was dissolved in ether, then ethereal diazomethane solution was added and mixture was allowed to stand overnight. The product was purified by PLC (ethyl acetate–chloroform (1 : 1, v/v)) to give the dimethyl derivative (12) (110 mg) as an oily material. EI-MS *m/z*: 328 (M$^+$), 313, 271, 212, 199, 153, 113. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1745, *ca.* 1710—1670 (br), 1595. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 266. ^{1}H-NMR (CDCl$_3$) δ: 1.00 (3H, t, $J=6.0$ Hz), 1.24 (3H, s), 1.28—1.72 (11H, m), 2.12—2.28 (2H, m), 2.68 (1H, dd, $J=9.5$, 17.0 Hz), 3.12 (1H, dd, $J=17.0$, 3.5 Hz), 3.90, 4.16 (each 3H, s), 5.04 (1H, dd, $J=9.5$, 3.5 Hz). $[\alpha]_D$ $-8°$ ($c=0.105$, CHCl$_3$).

Reduction of PI-2 (2) with NaBH$_4$ PI-2 (50 mg) was dissolved in dry THF (5 ml), and NaBH$_4$ (50 mg) was added. The mixture was stirred overnight at room temperature and poured into ice water. After acidification, the reaction mixture was extracted with ethyl acetate and the extract was purified by PLC (chloroform–acetic acid (9 : 1, v/v)) to give **13** as colorless needles (32 mg), mp 128 °C, *Rf* 0.35. *Anal.* Calcd for $C_{15}H_{22}O_6$: C, 60.39; H, 7.43. Found: C, 60.49; H, 7.61. EI-MS *m/z*: 280 (M$^+$ $-$H$_2$O), 236, 197, 126, 111, 97, 69. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1770, 1708, 1650, 1640. UV (EtOH) nm: 263, 353. ^{1}H-NMR (acetone-d_6) δ: 0.90 (3H, t, $J=6.0$ Hz), 1.22 (3H, s), 1.20—1.80 (10H, m), 2.44 (2H, t, $J=6.5$ Hz), 5.55 (1H, s).

Acetylation of 13 **13** (50 mg) was reacted with acetic anhydride (1 ml) and pyridine (0.5 ml) at room temperature. Purification by PLC (chloroform–acetic acid (10 : 1, v/v)) gave an acetate (**14**) as a colorless gum. EI-MS *m/z*: 236, 126, 69, 55. CI-MS *m/z*: 281 (M$^+$ $+1-$CH$_3$CO$_2$H), 263, 237, 219. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1770 (br), 1710, 1650. ^{1}H-NMR (acetone-d_6) δ: 0.90 (3H, t, $J=6.0$ Hz), 1.16—1.84 (10H, m), 2.36 (2H, t, $J=6.5$ Hz), 2.48 (3H, s), 5.61 (1H, s). ^{13}C-NMR (acetone-d_6) δ: 14.8, 20.9, 22.3, 24.5, 25.0, 27.3, 27.7, 42.5, 43.0, 72.3, 95.4, 97.8, 152.6, 156.5, 164.5 (C × 2), 167.4, 168.0.

Incorporation of ^{13}C-Labeled Sodium Acetate (a) Sodium [1-^{13}C]acetate (500 mg) was dissolved in water (9 ml) and 1 ml aliquots were fed to each of three Roux culture flasks on the sixth, eighth and tenth days. On the 14th day the three cultures were combined, and PI-1 (6 mg) and PI-2 (5 mg) were isolated. (b) Sodium [2-^{13}C]acetate (500 mg) was dissolved in water (15 ml) and fed to five culture flasks in the same way as described in (a). PI-1 (10 mg) and PI-2 (8 mg) were obtained, respectively. (c) Sodium [1,2-^{13}C$_2$]acetate (200 mg) was dissolved in water (15 ml) and diluted with non-labeled sodium acetate (300 mg). The solution was fed to five culture flasks as described in section (a). Yields of PI-1 and PI-2 were 5 and 20 mg, respectively.

Properties of PI-3 (5) *Anal.* Calcd for $C_{14}H_{12}O_8$: C, 54.55; H, 3.92. Found: C, 54.17; H, 3.94. MS *m/z*: 308 (M$^+$), 230, 203. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: (log ε): 226 (4.20), 319 (sh, 3.77), 344 (3.82), 355 (sh, 3.81), 372 (sh, 3.63). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3541, 3368, 3100—2800, 1684, 1664, 1622, 1554. ^{1}H-NMR (DMSO-d_6) δ: 2.14 (3H, s), 2.56 (1H, dd, $J=8.0$, 16 Hz), 2.84 (1H, dd, $J=3.5$, 16 Hz), 5.06 (1H, dd, $J=3.5$, 8.0 Hz), 6.91 (1H, s), 8.07 (1H, s), 9.46, 11.27 (each 1H, br s, exchangeable with D$_2$O). ^{13}C-NMR (DMSO-d_6) δ: 30.2 (q), 49.9 (t), 62.1 (d), 102.2 (d), 112.4 (s), 118.9 (s), 125.3 (s), 141.5 (s), 150.6 (s), 152.0 (s), 152.0 (d), 167.0 (s), 173.0 (s), 206.7 (s). $[\alpha]_D$ $-156°$ ($c=0.1$, THF).

Properties of PI-4 (6) *Anal.* Calcd for $C_{14}H_{10}O_7$: C, 57.94; H, 3.47.

Found: C, 58.01; H, 3.43. MS m/z: 290 (M^+), 229, 203. UV λ_{max}^{EtOH} nm: 291, 338. IR ν_{max}^{KBr} cm^{-1}: 1630, 1600. ^{1}H-NMR (DMSO-d_6) δ: 2.29 (3H, s), 6.94 (1H, s), 7.35 (2H, br s), 8.69 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 28.2, 103.1, 113.5, 118.1, 120.6, 129.3, 135.9, 142.6, 150.7, 153.0, 159.2, 168.2, 174.2, 198.9.

Methylation of PI-3 (5) Ethereal diazomethane was added to a solution of PI-3 (100 mg) in ether and the mixture was allowed to stand for 3 h. The solvent was removed and the residue was separated by PLC (chloroform–acetone (4 : 1, v/v)). From the fraction of *Rf* 0.6, the trimethyl derivative (**15**) was obtained as a colorless oil (93 mg), and from the fraction of *Rf* 0.8, the tetramethyl derivative (**16**) was isolated as colorless needles (cyclohexane), mp 152—154 °C (7 mg).

Trimethyl PI-3 (**15**): MS m/z: 350 (M^+), 289, 261. UV λ_{max}^{EtOH} nm: 220, 230, 250, 280, 312. IR ν_{max}^{KBr} cm^{-1}: 3346, 2949, 1729, 1710, 1636, 1600. ^{1}H-NMR (CDCl$_3$) δ: 2.13 (3H, s), 2.61 (1H, dd, $J=8.0$, 16 Hz), 3.23 (1H, dd, $J=4.0$, 16 Hz), 3.82, 3.91, 3.95 (each 3H, s), 5.02 (1H, dd, $J=4.0$, 8.0 Hz), 6.82, 7.86 (each 1H, s).

Tetramethyl-PI-3 (**16**): *Anal.* Calcd for $C_{18}H_{20}O_8$: C, 59.05; H, 5.58. Found: C, 59.33; H, 5.53. MS m/z: 364 (M^+), 349, 317, 289, 259. UV λ_{max}^{EtOH} nm: 219, 231, 251, 279, 306, 311. IR ν_{max}^{KBr} cm^{-1}: 2937, 1746, 1721, 1646, 1608. ^{1}H-NMR (CDCl$_3$) δ: 2.20 (3H, s), 2.66 (1H, dd, $J=9.4$, 17 Hz), 2.96 (1H, dd, $J=3.5$, 9.4 Hz), 3.77, 3.89, 3.99, 4.03 (each 3H, s), 4.84 (1H, dd, $J=3.5$, 9.4 Hz), 6.93, 7.87 (each 1H, s).

Dehydration of Trimethyl PI-3 (15) Acetic anhydride (0.5 ml) was added to a solution of trimethyl PI-3 (40 mg) in pyridine under ice cooling and the solution was allowed to stand overnight at room temperature. The reaction mixture was poured into ice water and kept standing for 3 h. The precipitate was collected, dried, and recrystallized from ethanol to give a dehydrate (**17**) as colorless needles, mp 216—218 °C (27 mg). *Anal.* Calcd for $C_{17}H_{16}O_7$: C, 61.33; H, 4.86. Found: C, 61.44; H, 4.85. MS m/z: 332 (M^+), 289, 257, 214. UV λ_{max}^{EtOH} nm (log ε): 221 (4.28), 281 (4.51). IR ν_{max}^{KBr} cm^{-1}: 2954, 1739, 1694, 1601. ^{1}H-NMR (CDCl$_3$) δ: 2.34 (3H, s), 3.91, 4.01, 4.07 (each 3H, s), 6.95 (1H, s), 7.25, 7.45 (each 1H, d, $J=16$ Hz), 8.07 (1H, s).

Oxidation of Trimethyl-PI-3 (15) with KMnO$_4$ A suspension of trimethyl-PI-3 (80 mg) and KMnO$_4$ (290 mg) in acetone (6.5 ml) was refluxed for 2 h. The reaction mixture was added to water (3 ml) and decolorized by passing SO$_2$ gas through the combined mixture. The clear solution obtained was extracted repeatedly with ether. The ether solution was washed with 10% NaHCO$_3$ and the combined aqueous solution was acidified and then extracted with ether. The ether extract was evaporated to dryness and the residue was crystallized from benzene to give 3,4-dimethoxy-6-hydroxy-2-methoxycarbonylbenzoic acid, mp 146—148 °C (lit.[5] mp 147—148 °C) as colorless needles (25 mg). *Anal.* Calcd for $C_{11}H_{12}O_7$: C, 51.23; H, 4.76. Found: C, 51.56; H, 4.72. MS m/z: 256 (M^+), 238, 195, 152. UV λ_{max}^{EtOH} nm: 250, 309. IR ν_{max}^{KBr} cm^{-1}: 3373, 2929, 1693, 1609. ^{1}H-NMR (CDCl$_3$) δ: 3.83 (3H, s), 3.96 (6H, s), 6.20 (1H, s), 10.85 (1H, br s).

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