Synthesis of 2-*O*-(4-Coumaroyl)-3-(4-hydroxyphenyl)lactic Acid, an Important Intermediate of Rosmarinic Acid Biosynthesis

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A simple method to synthesize (±)-2-*O*-(4-coumaroyl)-3-(4-hydroxyphenyl)lactic acid (1), a key intermediate in rosmarinic acid biosynthesis in higher plant cells, was established by condensation of protected 4-coumaric acid and (±)-3-(4-hydroxyphenyl)lactic acid followed by deprotection. A stable supply of 1 thus attained will lead to biochemical and molecular biological characterization of later steps of rosmarinic acid biosynthesis.

Key words rosmarinic acid; 4-coumaroyl-4-hydroxyphenyl lactic acid; synthesis; biosynthetic precursor

Rosmarinic acid (2-*O*-caffeoyl-3-(3,4-dihydroxyphenyl)-lactic acid: RA) is a common 4-hydroxycinnammoyl ester distributing in species belonging to the families Boraginaceae and Lamiaceae. RA exhibits a potent antioxidative activity and has attracted attention on its pharmacological activities including inhibition of low density lipoprotein (LDL) oxidation[1] and proliferation of cultured murine mesangical cells.[2] 2-*O*-(4-Coumaroyl)-3-(4-hydroxyphenyl)lactic acid (**1**) is a first intermediate specific to RA biosynthesis in plant cells. Compound **1** is biosynthesized by condensation of 4-coumaroyl CoA formed from phenylalanine with tyrosine-derived 3-(4-hydroxyphenyl)lactic acid (Fig. 1). This step is catalyzed by RA synthase (RS).[3] Then, **1** is converted to RA through two consecutive hydroxylation reactions catalyzed by cytochrome P450s.[4] Thus, **1** plays a central role in RA biosynthesis. Biochemical and molecular biological investigations on RA biosynthesis have been vigorously carried out using plant cell cultures. Although enzymatic and molecular regulation of earlier steps of RA biosynthesis, *i.e.* the general phenylpropanoid pathway leading to 4-coumaroyl CoA and the tyrosine-derived pathway leading to 3-(4-hydroxyphenyl)-lactic acid have been substantially characterized,[5,6] later steps of RA biosynthesis remain to be characterized. A stable supply of **1** is highly desirable for biochemical and molecular biological investigation on the later steps of RA biosynthesis, because **1** is a product of RA synthase and a substrate of the cytochrome P450-dependent monooxygenase. In spite of such importance of **1**, there is no report describing the synthesis of the analogues of 2-*O*-cinnamoylphenyllactate including **1**. Thus, we established a chemical synthesis of **1** by condensing 4-coumaric acid with (±)-3-(4-hydroxyphenyl)-lactic acid.

Chemistry

The synthetic course for (±)-2-*O*-(4-coumaroyl)-3-(4-hydroxyphenyl)lactic acid (**1**) was shown in Chart 1. The carboxy group of (±)-3-(4-hydroxyphenyl)lactic acid was protected as its *tert*-butyl ester by condensation with *N*,*N*′-diiso-

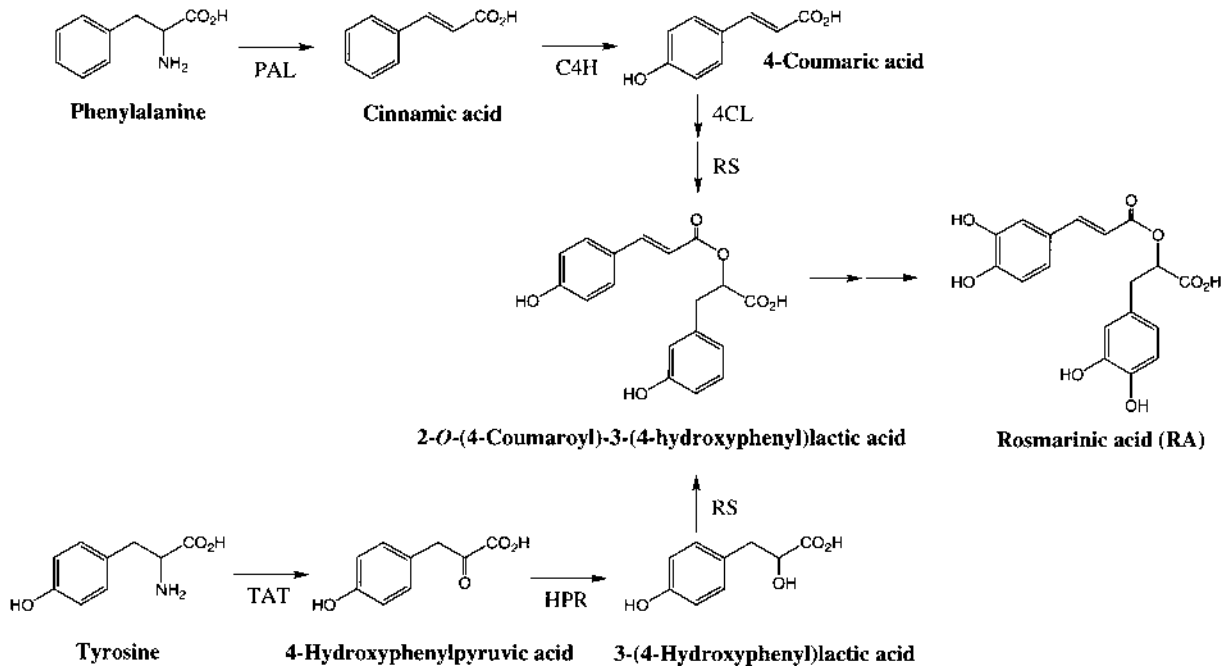

Fig. 1. A Biosynthetic Pathway Leading to RA

PAL, phenylalanine ammonia-lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumaric acid CoA ligase; TAT, tyrosine aminotransferase; HPR, hydroxyphenylpyruvate reductase.

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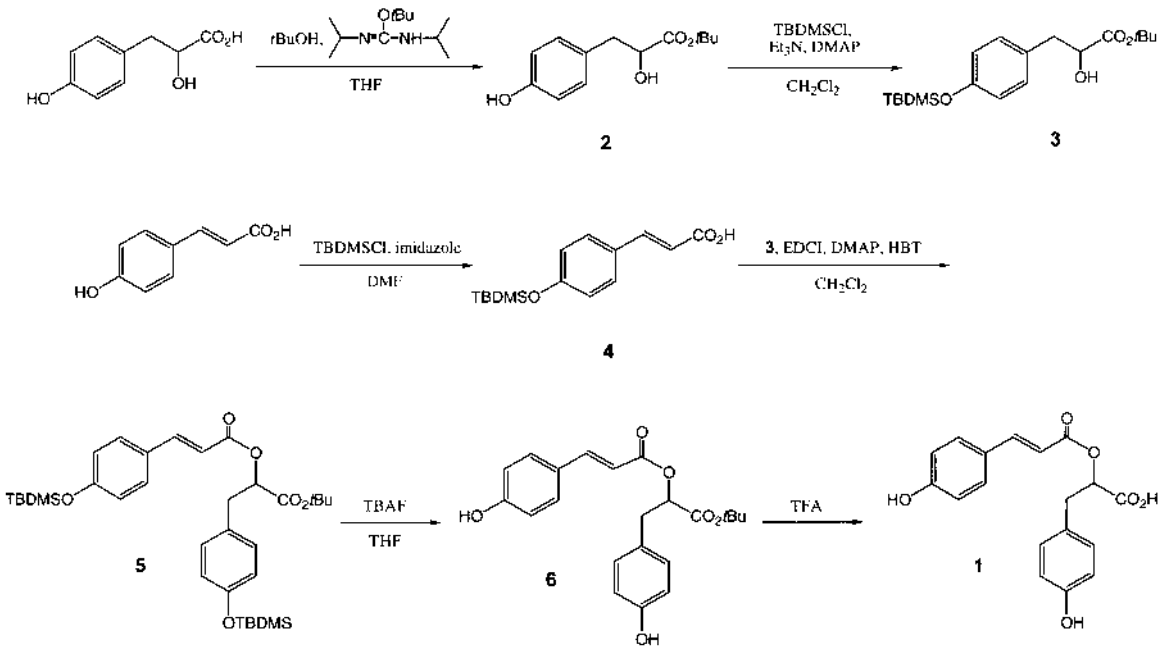

Chart 1

propyl-*O*-*tert*-butylisourea[7] to give **2**. Then the phenolic OH group was silylated by *tert*-butyldimethylchlorosilane (TBDMSCl) to give a monosilylated compound (**3**). In the ^{1}H-NMR spectrum of **3**, the singlet methyl signal due to Me–Si appeared at 0.17 ppm. Irradiation of this signal in the nuclear Overhauser effect (NOE) experiment induced 5.1% NOE on the aromatic proton signal at 6.75 ppm. Thus, we deduced that the TBDMS group was substituted on the phenolic OH. After protection of OH group of 4-coumaric acid as TBDMS ether, the resulting carboxylic acid (**4**) was condensed with **3** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI), 4-dimethylaminopyridine (DMAP) and 1-hydroxybenzotriazole (HBT) to give an ester (**5**). The ^{1}H-NMR spectrum of **5** showed the methyne signal at 5.20 ppm, downfield relative to that of **3**. The FAB-MS spectrum also supported the structure. The TBDMS groups of **5** were then cleaved in the presence of tetrabutylammonium fluoride (TBAF) to yield **6**. The ^{1}H-NMR spectrum of **6** showed no methyl signals due to TBDMS group. Finally, **6** was treated with trifluoroacetic acid (TFA) to give the desired ester (**1**). The ^{1}H-NMR spectrum indicated the presence of two sets of *para*-substituted phenyl group, a *trans*-olefin and a CH–CH_2 system. The high-resolution (HR)-FAB-MS spectrum also supported the structure of **1**.

As described above, (±)-2-*O*-(4-coumaroyl)-3-(4-hydroxyphenyl)lactic acid (**1**) was simply prepared from 4-coumaric acid and (±)-3-(4-hydroxyphenyl)lactic acid. To our knowledge, this is a first report about the preparation of a simple but important phenyllactic acid possessing a cinnamate substituent on the OH at the α position of the carboxy group. Compound **1** would be useful for characterization of RA biosynthesis.

Experimental

General The electron impact (EI) and FAB-MS and HR-EI and FAB-MS were measured with a JEOL JMS DX-505 or SX-102 mass spectrometer. The IR spectra were recorded on a Shimadzu FTIR-8100 spectrometer. The ^{1}H-NMR spectra were measured with a JEOL JNM Lambda 400 (400 MHz) spectrometer. The following abbreviations are used: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet.

(±)-*tert*-Butyl 3-(4-Hydroxyphenyl)lactate (2) *N*,*N*′-Diisopropyl-*O*-*tert*-butylisourea (150 μl, 8.37 mmol) was added to a suspension of (±)-3-(4-hydroxyphenyl)lactic acid (296 mg, 1.62 mmol) in tetrahydrofuran (THF) (3.0 ml) and *t*-BuOH (2.5 ml) at 0 °C and stirred at room temperature for 14 h. The reaction mixture was diluted with AcOEt and washed with 10% aqueous citric acid, 10% aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo.* The residue was chromatographed on SiO_2 (hexane–AcOEt, 10 : 1—2 : 1) to give **2** (352 mg, 91%) as colorless oil. IR (neat, cm^{-1}): 3470, 3319 (OH), 1713 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s, O–C(CH_3)$_3$), 2.84 (1H, dd, J=6.3, 14.1 Hz, C$\underline{H}_a$H$_b$-Ph), 2.88 (1H, dd, J=4.6, 14.1 Hz, CH$_a\underline{H}_b$-Ph), 4.27—4.31 (1H, m, OC$\underline{H}$<), 6.68, 7.06 (each 2H, both d, J=8.3 Hz, Ar-H). HR-EI-MS; *m*/*z*: 238.1208 [M$^+$] (Calcd for $C_{13}H_{18}O_4$: 238.1205).

(±)-*tert*-Butyl 3-(4-*tert*-Butyldimethylsilyloxyphenyl)lactate (3) To a solution of **2** (98.1 mg, 0.41 mmol), DMAP (13.3 mg, 0.11 mmol) and triethylamine (Et_3N) (125 μl, 0.89 mmol) in THF (2.0 ml), a solution of TBDMSCl (94.9 mg, 0.62 mmol) in THF (1.0 ml) was added in dropwise at 0 °C. The mixture was stirred at room temperature for 14 h, diluted with AcOEt, washed with 5% aqueous citric acid, 5% aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo.* The residue was chromatographed on SiO_2 (hexane–AcOEt, 100 : 1—9 : 1) to give **3** (103 mg, 72% yield) as colorless oil. IR (neat, cm^{-1}): 3584 (OH), 1726 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.17 (6H, s, Si(CH_3)$_2$), 0.97 (9H, s, Si–C(CH_3)$_3$), 1.42 (9H, s, O–C(CH_3)$_3$), 2.88 (1H, dd, J=6.1, 14.1 Hz, C$\underline{H}_a$H$_b$-Ph), 3.00 (1H, dd, J=4.9, 14.1 Hz CH$_a\underline{H}_b$-Ph), 4.26—4.30 (1H, m, OC$\underline{H}$<), 6.75, 7.10 (each 2H, both d, J=8.3 Hz, Ar-H). HR-EI-MS; *m*/*z*: 352.2071 [M$^+$] (Calcd for $C_{19}H_{32}O_4Si$: 352.2070).

4-*O*-*tert*-Butyldimethylsilylcoumaric Acid (4) TBDMSCl (1074 mg, 7.12 mmol) was added to a solution of 4-coumaric acid (98.7 mg, 0.60 mmol) and imidazole (423 mg, 7.12 mmol) in *N*,*N*′-dimethylformamide (DMF, 1.5 ml) at 0 °C, and the mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with AcOEt and the solution was washed with 10% aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo.* The residue was chromatographed on SiO_2 (hexane–AcOEt, 9 : 1—1 : 1) to give **4** (164 mg, 98% yield) as amorphous powder. IR (neat, cm^{-1}): 1682 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.23 (6H, s, Si(CH_3)$_2$), 0.99 (9H, s, Si–C(CH_3)$_3$), 6.32 (1H, d, J=15.9 Hz, CO–C$\underline{H}$=CH), 6.86, 7.45 (each 2H, both d, J=8.5 Hz, Ar-H), 7.74 (1H, d, J=15.9 Hz, PhC$\underline{H}$=CH). HR-EI-MS; *m*/*z*: 278.1340 [M$^+$] (Calcd for $C_{15}H_{22}O_3Si$: 278.1339).

(±)-*tert*-Butyl 2-*O*-(4-*O*-*tert*-Butyldimethylsilylcoumaloyl)-3-(4-*tert*-butyl-dimethylsilyloxyphenyl)lactate (5) To a solution of **4** (164 mg, 0.588 mmol), HBT (82.6 mg, 0.611 mmol), EDCI (195 mg, 1.02 mmol) and DMAP (85.2 mg, 0.697 mmol) in CH_2Cl_2 (2.0 ml), a solution of **3** (185 mg,

0.524 mmol) in CH_2Cl_2 (2.0 ml) was added in dropwise at 0 °C. The mixture was stirred at room temperature for 40 h, quenched by addition of brine, and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo.* The residue was chromatographed on SiO_2 (hexane–AcOEt, 100 : 1) to give **5** (105 mg, 33% yield) as pale yellow oil. IR (neat, cm^{-1}): 1743, 1718 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.17 (6H, s, $Si(CH_3)_2$), 0.22 (6H, s, $Si(CH_3)_2$), 0.97 (9H, s, $Si–C(CH_3)_3$), 0.98 (9H, s, $Si–C(CH_3)_3$), 1.40 (9H, s, $O–C(CH_3)_3$), 3.10 (1H, dd, J=7.2, 14.3 Hz, $C\underline{H}_aH_b$-Ph), 3.12 (1H, dd, J=5.7, 14.3 Hz, $CH_a\underline{H}_b$-Ph), 5.19 (1H, dd, J=5.7, 7.2 Hz, OCH≺), 6.33 (1H, d, J=16.0 Hz, CO–C$\underline{H}$=CH), 6.77 (2H, d, J=8.5 Hz, Ar-H), 6.83 (2H, d, J=8.5 Hz, Ar-H), 7.13 (2H, d, J=8.5 Hz, Ar-H), 7.41 (2H, d, J=8.5 Hz, Ar-H), 7.64 (1H, d, J=16.0 Hz, PhC$\underline{H}$=CH). HR-EI-MS; *m*/*z*: 613.3409 [M^+] (Calcd for $C_{34}H_{53}O_6Si_2$: 613.3381).

(±)-*tert*-Butyl 2-*O*-(4-Coumaroyl)-3-(4-hydroxyphenyl)lactate (6) To a solution of **5** (105 mg, 0.172 mmol) in THF (2.0 ml), a solution of TBAF (120 mg, 0.380 mmol) in THF (1.0 ml) was added. The mixture was stirred at room temperature for 1 h, quenched by addition of brine, and extracted with AcOEt. The organic layer was dried over Na_2SO_4, and concentrated *in vacuo.* The residue was chromatographed on SiO_2 (hexane–AcOEt, 50 : 1—4 : 1) to give **6** (48.0 mg, 74% yield) as colorless oil. IR (neat, cm^{-1}): 3400 (OH), 1713 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s, $O–C(CH_3)_3$), 3.10 (2H, d, J=6.6 Hz, CH_2-Ph), 5.20 (1H, t, J=6.6 Hz, OCH≺), 6.23 (1H, d, J=15.9 Hz, CO–C$\underline{H}$=CH), 6.77 (2H, d, J=8.5 Hz, Ar-H), 6.80 (2H, d, J=8.5 Hz, Ar-H), 7.13 (2H, d, J=8.5 Hz, Ar-H), 7.33 (2H, d, J=8.5 Hz, Ar-H), 7.58 (1H, d, J=15.9 Hz, PhC$\underline{H}$=CH). FAB-MS; *m*/*z*: 385 [MH^+].

(±)-2-*O*-(4-Coumaroyl)-3-(4-hydroxyphenyl)lactic Acid (1) Compound **6** (40.0 mg, 0.102 mmol) was dissolved in TFA (1.5 ml) and stirred at room temperature for 2.5 h. The solvent was removed *in vacuo* to give **1** as colorless syrup (34.6 mg, 100% yield). IR (KBr, cm^{-1}): 3390 (OH), 1700 (C=O). ^{1}H-NMR (CD_3OD) δ: 3.06 (1H, dd, J=8.3, 14.4 Hz, $C\underline{H}_aH_b$-Ph), 3.14 (1H, dd, J=4.4, 14.4 Hz, $CH_a\underline{H}_b$-Ph), 5.20 (1H, dd, J=4.4, 8.3 Hz, OCH≺), 6.31 (1H, d, J=15.9 Hz, CO–C$\underline{H}$=CH), 6.72 (2H, d, J=8.5 Hz, Ar-H), 6.80 (2H, d, J=8.5 Hz, Ar-H), 7.11 (2H, d, J=8.5 Hz, Ar-H), 7.44 (2H, d, J=8.5 Hz, Ar-H), 7.60 (1H, d, J=15.9 Hz, PhC$\underline{H}$=CH). ^{13}C-NMR (CD_3OD) δ: 37.7 (CH_2), 74.6 (CH), 114.5 (CH), 116.2 (CH), 116.9 (CH), 127.1 (C), 128.5 (C), 131.3 (CH), 131.5 (CH), 147.3 (CH), 157.4 (C), 161.4 (C), 168.4 (C), 173.5 (C). HR-FAB-MS; *m*/*z*: 329.1030 [MH^+] (Calcd for $C_{18}H_{17}O_6$: 329.1025).

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