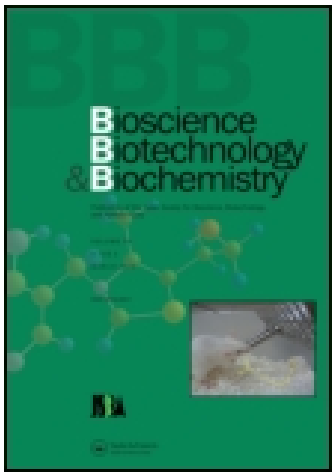

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Isolation and Synthesis of a New Bio-antimutagen, Petasiphenol, from Scapes of *Petasites japonicum*

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A new bio-antimutagen, petasiphenol [3-(3,4-dihydroxyphenyl)-2-oxopropyl caffeate] (1) was isolated from scapes of *Petasites japonicum* (AD_{50} = 95 μg/ml against UV-induced mutagenic *E. coli* WP2 B/r *Trp*$^-$. Petasiphenol (1) and its isomer (2) were synthesized. The activity of 1 was observed in the presence of soybean oil (glyceride), although the isomer (2) did not show any activity in doses up to 300 μg/ml.

Naturally occurring and synthetic compounds with bio-antimutagenic effects are known.[1-12] We have searched for compounds possessing such effects from plants and found a new compound, named petasiphenol [3-(3,4-dihydroxyphenyl)-2-oxopropyl caffeate] (**1**), from *Petasites japonicum* (Fuki in Japanese). We describe here the isolation, syntheses of petasiphenol (**1**) and its isomer (**2**), and their biological activity.

Commercial scapes of *Petasites japonicum* was extracted with acetone. The condensed mixture was reextracted with ethyl acetate, and the neutral fraction of the organic layer was collected. The extract was separated by silica gel TLC into four fractions by their striped pattern. By the paper disk method, an active compound was found to be in the lowest fraction, which showed faint fluorescence under a UV lamp. The active compound (petasiphenol (**1**), 0.024% of scapes) contained 3% (up to 10%) of a monoglyceride of a fatty acid (pentaethenoid) from its NMR spectrum (δ 3.69 ppm, 2H, dd, J=11.2 & 5.4 Hz, CH_2OH; 3.99, 1H, q, J=5.4 Hz, CHOH; 4.20, 2H, d, J=5.4 Hz, CH_2OCO; 5.2—5.45, 10H, m, =CH).

Since further purification could not be achieved, the impure compound was acetylated (pyridine + Ac_2O, 70°C), and the pure pentaacetate (**3**) of petasiphenol (**1**) could be isolated (18.7% yield) by TLC purification.

A high-resolution MS analysis of **3** showed the molecular formula $C_{28}H_{26}O_{12}$, so that the molecular formula for petasiphenol (**1**) was determined to be $C_{18}H_{16}O_7$.

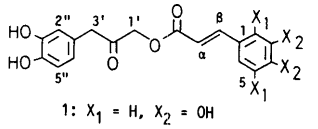
From the ^{1}H NMR spectrum, petasiphenol (**1**) was found to have two mono-substituted catechol moieties [δ 6.60 (J=8.1 & 2 Hz), 6.81 (J=2 Hz), and 6.86 (J=8.1 Hz) and 6.88 (J=8 Hz), 6.94 (J=8 & 1.8 Hz), and 7.12 (1.8 Hz)], a trans double bond (δ 6.25, J=15.9 Hz and 7.36 J=15.9 Hz) and two methylenes as a singlet (δ 3.61 and 4.77). From the ^{13}C-NMR spectrum, the presence of an ester (δ 166.66) and of a ketone (δ 202.60) was indicated. From the CH-correlation and COLOC spectra, the connectivity between H and C could be elucidated as $C_{2'}O$–$C_{1'}\underline{H}_2$–O–CO; CO–$C_\alpha\underline{H}$=C_β–C_1; $C_2\underline{H}$–C_1–C_6; $C_2\underline{H}$–C_3; $C_6\underline{H}$–C_5–C_4; $C_{3'}\underline{H}$–$C_{1''}$–$C_{2''}$(–$C_{6''}$); and $C_{5''}\underline{H}$–$C_{4''}$.

From these facts, petasiphenol (**1**) was determined to be 3-(3,4-dihydroxyphenyl)-2-oxopropyl caffeate (**1**). The bio-antimutagenic activity (AD_{50}[4]) of **1** containing 9% equimol. of the monoglyceride was 95 μg/ml against UV-induced mutagenic *E. coli* WP2 B/r *Trp*$^-$.

From the calyxes of the plant, petasiphenol (**1**) was also detected by a similar extraction and TLC analysis.

Petasiphenol (**1**) was then synthesized. The epoxide (**4**) was transformed into a bromohydrin (**5**), and then into an ester (**6**) by esterification with an acid salt (**7**).

Swern oxidation[13] of ester **6** to ketone **8** and the subsequent deprotection of 8 gave pure petasiphenol (**1**), which was identified as natural petasiphenol (**1**) from its NMR spectra.



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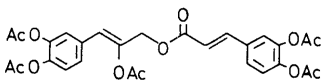
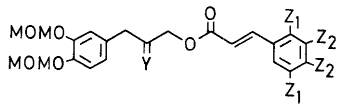


3

4 5

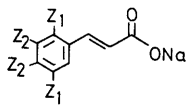


6: Y = H, OH; Z_1 = H; Z_2 = OMOM (methoxymethyl)

8: Y = O; Z_1 = H; Z_2 = OMOM

10: Y = H, OH; Z_1 = OMOM; Z_2 = H

11: Y = O; Z_1 = OMOM; Z_2 = H



7: Z_1 = H; Z_2 = OMOM

9: Z_1 = OMOM; Z_2 = H

The isomer (**2**) of petasiphenol (**1**) was synthesized from bromohydrin **5** and a protected 2,5-dihydroxycinnamic acid sodium salt (**9**) in a similar way to that just described, which gave a hydroxyester (**10**). Swern oxidation of **10** gave a ketone (**11**), and deprotection of **11** with 2 N AcOH yielded **2**.

Although the synthetic petasiphenol (**1**) did not show bio-antimutagenic activity and microbicidal activity in doses up to 170 μg/ml, it was active (AD_{50} = 100 μg/ml) in the presence of 30% equimol. of soybean oil. The soybean oil itself did not show any activity.

The isomer (**2**) did not show favorable activity in doses up to 300 μg/ml against UV-induced mutagenic *E. coli* WP2 B/r *Trp*$^-$, either in the presence or absence of the oil (30% equimol.). These facts suggest that the catechol moiety on a caffeic acid contributed to the activity.

Experimental

IR spectra were recorded with a JASCO IRA-1 spectrometer. NMR spectra were measured with a Bruker AC 250 spectrometer (^{1}H at 250.13 MHz; ^{13}C at 62.89 MHz), using Bruker's pulse program and tetramethylsilane as an internal standard. Precise MS spectra were measured with a Hitachi M-80/80B spectrometer, using a Hitachi data processing system.

Isolation of petasiphenol (**1**). Fresh scapes (300 g) of *Petasites japonicum* were soaked in acetone (500 ml) for a week. The scapes were filtered off and the solution was condensed, before the residual oily material was dissolved in EtOAc. The organic layer was sequentially washed with 2 N HCl, sat. $NaHCO_3$ and water, and dried over $MgSO_4$. Evaporation of the solvent gave an oily product (2.5 g, 0.8%), which was separated by preparative TLC (PLC) (hexane–EtOAc = 2 : 3, 3 developments) into 4 fractions. Each fraction was extracted with a mixed solvent of EtOAc + EtOH (9 : 1). From a qualitative bioassay[4,11] using UV-induced mutagenic *E. coli* WP2 B/r *Trp*$^-$, the lowest fraction showed bio-antimutagenic activity. The band was collected and extracted with EtOAc + EtOH (9 : 1). Evaporation of the solvent gave petasiphenol (**1**, 18 mg, 3% yield of the extracts (600 mg), 0.024% of scapes). By an NMR analysis, the fraction contained 3—10% equimol. of a monoglyceride (the pentaethenoid of a fatty acid) through several experiments. The second purification of the fraction (40 mg) by silica gel or ODS TLC gave a low yield of the impure phenol (**1**, 0.5—3 mg) due to absorption. By a quantitative bioassay,[4,11] petasiphenol (**1**) containing 9% equimol. of a glyceride showed bio-antimutagenic activity (AD_{50} = 95 μg/ml). IR ν_{max} (film) cm^{-1}: 3400 (OH), 1710 (CO & ester), 1630 (C=C), 1600 (phenyl). NMR ($CDCl_3$ + pyr-d_5) $\delta_H[\delta_C]$: 3.61 (2H, br. s, 3′)[46.18], 4.77 (2H, br. s, 1′)[67.30], 6.25 (1H, d, J = 15.9 Hz, α)[113.16], 6.60 (1H, dd, J = 8.1 & 2.0 Hz, 6″)[120.87], 6.81 (1H, d, J = 2 Hz, 2″)[116.32], 6.86 (1H, d, J = 8.1 Hz, 5″)[115.52], 6.88 (1H, d, J = 8 Hz, 5)[115.39], 6.94 (1H, dd, J = 8 & 1.8 Hz, 6)[122.02], 7.12 (1H, d, J = 1.8 Hz, 2)[114.32], 7.36 (1H, d, J = 15.9 Hz, β)[146.78], [124.33, 1″], [126.30, 1], [144.82, 3″], [145.71, 4″], [145.86, 4], [148.79, 3], [166.66, COO], [202.60, 2′].

NMR signals due to impurities were δ_H 3.69 (2H, dd, J = 11 & 5.4 Hz, CH_2OH), 3.99 (1H, q, J = 5.4 Hz, CHOH), 4.20 (2H, d, J = 5.4 Hz, CH_2OCO), 5.2—5.45 (10H, m, =CH). Other signals at δ_H 0.8—1.2 (26H, m), 1.26 (50H, br. s), 1.6 (16H), 2.2—2.4 (9H) and 2.8 (6H, m) were also observed. δ_C 20.62, 20.67, 29.30, and 29.70 also showed as impurities. The signals at δ_C 20.62 and 20.67 were lacking in some cases, and the other signals were of low intensity.

When petasiphenol (**1**, 22.3 mg) was acetylated with pyridine (0.5 ml) and acetic anhydride (0.5 ml) at 70°C for 14 h, the pentaacetate (**3**) was obtained after silica gel purification (hexane–EtOAc = 4 : 3, 2 developments). IR ν_{max} (film cm^{-1}: 3060, 1620, 1600 (phenyl), 1770, 1730, 1210 (ester), 1650 (C=C). NMR ($CDCl_3$) $\delta_H[\delta_C]$: 2.23, 2.28, 2.29, 2.30, 2.31 (3H each, s, OAc)[20.66, 20.70, Me; 167.96, 168.03, 168.06, 168.14, 168.44, COO], 4.92 (2H, s, 1′)[63.90], 6.29 (1H, s, 3′)[119.01], 6.41 (1H, d, J = 16 Hz, α)[118.53], 7.15 (1H, J = 8.4 Hz, 5″)[123.36], 7.23 (1H, d, J = 8.4 Hz, 5)[123.99], 7.28 (1H, dd, J = 8.4 & 2 Hz, 6″)[126.92], 7.34 (1H, d, J = 2 Hz, 2″)[123.68], 7.37 (1H, d, J = 2 Hz, 2)[122.86], 7.42 (1H, dd, J = 8.4 & 2 Hz, 6)[126.53], 7.67 (1H, d, J = 16 Hz, β)[143.75], [141.52, 141.97, 142.48, 144.21, 3, 3″, 4, 4″], [165.91, COO]. HRMS m/z (M^+): Calcd. for $C_{28}H_{26}O_{12}$: 554.1422. Found: 554.1413.

Di(methoxymethyl) ether (**4**) *of 4-(2,3-epoxypropyl)-catechol.* The 4-allylcatechol derivative (800 mg, prepared from the diMOM of 3,4-dihydroxyphenyl magnesium bromide and allyl bromide at 55°C for 1.5 h) was oxidized with mCPBA (1.1 g) in CH_2Cl_2 (30 ml) for 24 h. After the usual work up, the pure epoxide (**4**) was obtained with PLC (hexane–EtOAc = 2 : 1) from the lower fraction (317 mg, 37.1% yield). IR ν_{max} (film) cm^{-1}: 3060, 1620, 1600 (phenyl), 1000 (C–O). NMR ($CDCl_3$) $\delta_H[\delta_C]$: 2.54 (1H, q, J = 5 & 2.7 Hz, 3′)[46.87], 2.8 (3H, m, 1′ & 3′)[38.23], 3.13 (1H, m, 2′)[52.49], 3.15, 3.52 (3H, s, OCH_3)[56.14, 56.23], 5.21, 5.22 (2H, s, OCH_2O)[95.52, 95.55], 6.85 (1H, dd, J = 8.3 & 2 Hz, 5)[122.90], 7.05 (1H, d, J = 2 Hz, 3)[117.61], 7.10 (1H, d, J = 8.3 Hz, 6)[116.98], [131.68, 4], [146.04, 2], [147.27, 1]. HRMS m/z (M^+): Calcd. for $C_{13}H_{18}O_5$: 254.1056. Found: 254.1104.

Di(methoxymethyl) ether (**5**) of *4-(3-bromo-2-hydroxypropyl)-catechol.* The epoxide (**4**, 217 mg) in ether (15 ml) was stirred at −15—−20°C in a 1 N HBr solution (10 ml) containing LiBr (1 g) for 1.6 h. EtOAc was added, and the organic layer was washed with $NaHCO_3$ and water, and dried over $MgSO_4$. Evaporation of the solvent gave the pure bromohydrin (**5**, 286 mg, quantitative yield). Without LiBr, a glycol was obtained. Under these conditions, the reaction proceeded regioselectively. IR ν_{max} (film) cm^{-1}: 3500 (OH), 3060, 1620, 1600 (phenyl). NMR ($CDCl_3$) $\delta_H[\delta_C]$: 2.82 (2H, d, J = 6.5 Hz, 1′)[39.08], 3.38 (1H, dd, J = 7.9 & 6.1 Hz, 3′)[40.89, 3.4—3.5 (1H, m, 3′), 3.51, 3.52 (3H, s, Me)[56.17, 56.23], 3.98 (1H, m, 2′)[71.78], 5.21, 5.22 (2H each, s, OCH_2O)[95.47], 6.82 (1H, dd, J = 8.2 & 2 Hz, 5)[123.28], 7.04 (1H, d, J = 2 Hz, 3)[117.88], 7.09 (1H, d, J = 8.2 Hz, 6)[117.00], [131.44, 4], [146.15, 2], [147.30, 1]. HRMS m/z [$(M-H)^+$]: Calcd. for $C_{13}H_{18}O_5Br$: 333.0336. Found: 333.0278.

Formation of the ester (**6**) *from the bromohydrin* (**5**) *and the acid sodium salt* (**7**). The acid sodium salt (**7**, 330 mg) was added to a solution of the bromohydrin (**5**, 315 mg) in DMF (4 ml) and HMPA (2.5 ml), and the reaction mixture was heated to 85°C. After 30 min, the precipitates had disappeared, and the solution was kept at 85°C for 8 h. After cooling, water was added, and the mixture was extracted with EtOAc. The organic layer was sequentially washed with 2 N HCl, $NaHCO_3$ and water, and dried over $MgSO_4$. Evaporation of the solvent gave a pure oily product (**6**, 450 mg, 91.3% yield). IR ν_{max} (film) cm^{-1}: 3500 (OH), 3060, 1610, 1600 (phenyl), 1720 (COO), 1640 (C=C). NMR ($CDCl_3$) $\delta_H[\delta_C]$: 3.4—3.6 (4H, m, 1′ & 3′)[39.53, 67.71], 3.51 (9H, s, OCH_3), 3.52 (3H, s, OCH_3)[56.16, 56.22, 56.29, 56.33], 4.15 (1H, m, 2′)[70.85], 5.21, 5.23, 5.26, 5.27 (2H, s, OCH_2O)[95.10, 95.49 × 3], 6.39 (1H, d, J = 16 Hz, α)[115.60], 6.85 (1H, dd, J = 8.3 & 2 Hz, 6″)[123.30], 7.06 (1H, d, J = 2 Hz, 2″)[117.87], 7.10 (1H, d, J = 8.3 Hz, 5″)[116.97], 7.16 (2H, br. s, 5 & 6)[116.14, 123.63], 7.38 (1H, br. s, 2)[116.04], 7.65 (1H, d, J = 16 Hz, β)[145.03], [128.74, 1″], [131.74, 1], [146.06, 3″], [147.32, 4″], [147.42, 4], [149.30, 3], [167.24, COO]. Treatment of the epoxide (**4**) with caffeic acid did not give the ester, but a cyclic alcohol instead. HRMS m/z (M^+): Calcd. for $C_{26}H_{34}O_{11}$: 522.2117. Found: 522.2119.

Oxidation of the ester (**6**). The hydroxyester (**6**, 378 mg) was added to DMSO complex [oxalyl chloride (0.14 ml) and DMSO (0.35 ml) in CH_2Cl_2 at −78°C for 10 min] at −78°C and stirred at −20°C for 30 min. After adding Et_3N (1 ml) at −78°C, the mixture was stirred for 20 min at room temperature. Extraction of the products with EtOAc and the usual work up gave a mixture of the ketone (**8**) and the starting material (**6**). Separation by PLC (hexane–EtOAc = 1 : 1, 4 developments) gave the ketone (**8**, 91 mg, 24% yield from the upper fraction) and the starting alcohol (**6**, 80 mg, 21.2% yield). IR ν_{max} (film) cm^{-1}: 3060, 1620, 1600 (phenyl), 1740—1720 (CO & COO), 1640 (C=C). NMR ($CDCl_3$) $\delta_H[\delta_C]$: 3.51 (9H, s, OCH_3), 3.53 (3H, s, OCH_3)[56.19, 56.24, 56.28, 56.34], 3.72 (2H, s, 3′)[45.89], 4.82 (2H, s, 1′)[67.52], 5.22, 5.23, 5.26, 5.27 (2H, s, OCH_2O)[95.10, 95.74 × 2, 95.52], 6.42 (1H, d, J = 15.9 Hz, α)[115.23], 6.83 (1H, dd, J = 8.3 & 2 Hz, 6″)[123.45], 7.05 (1H, d, J = 2 Hz, 2″)[117.88], 7.13 (1H, d, J = 8.3 Hz, 5″)[116.96], 7.16 (2H, br. s, 5 & 6)[116.11][123.76], 7.39 (1H, d, J = 1.8 Hz, 2)[115.71], 7.68 (1H, d, J = 15.9 Hz, β)[145.84], [127.00, 1″], [128.62, 1], [146.58, 3″], [147.41, 4″], [147.45, 4], [149.44, 3], [166.21, COO], [201.21, 2′]. MS m/z [$(M-C_2H_4O-CH_2O)^+$]: Calcd. for $C_{23}H_{26}O_9$: 446.1570. Found: 446.1575.

Deprotection of the ketoester (**8**). The ketoester (**8**, 41 mg) was dissolved in 70% EtOH (10 ml) containing *p*-TsOH (15 mg). After stirring at 65°C for 48 h, the solution was condensed and the products were extracted with EtOAc. The organic layer was washed with $NaHCO_3$ and water, and dried over $MgSO_4$. Evaporation of the solvent gave an oily product (29.2 mg, 89% yield, 96% purity), which was identified as petasiphenol (**1**) from its NMR spectra. With a quantitative bioassay, synthetic petasiphenol showed activity ($AD_{50} = 100\,\mu g/ml$) in the presence of soybean oil (30% equimol.), but did not show any activity in doses up to 170 μg/ml in the absence of the oil. HRMS *m/z* (M^+): Calcd. for $C_{18}H_{16}O_7$: 344.0895. Found: 344.0932.

Synthesis of the isomer (**2**). The isomeric ester (**10**) was obtained by the methods already described [bromohydrin (**5**, 413 mg) and sodium salt (**9**, 358 mg) in DMF (3 ml) and HMPA (1.5 ml) at 85°C for 8 h] (80.5% yield). The hydroxyester (**10**, 517 mg) gave the ketonic ester (**11**, 261 mg, 40.9% yield) by Swern oxidation. The protective groups were cleaved by heating the ketone (**11**, 215 mg) in 2 N acetic acid at 90°C for 8 h to give **2** (144 mg, quantitative yield). The isomeric ketoester (**2**) did not show any biological activity against UV-induced *E. coli* at doses up to 300 μg/ml, in either the presence or absence of soybean oil. IR ν_{max} (film) cm^{-1}: 3400 (OH), 1740 (CO), 1700, 1220 (COO), 1660 (C=C), 1640 (phenyl). NMR ($CDCl_3$ + pyr-d_5) $\delta_H[\delta_C]$: 3.82 (2H, s, 3′)[45.89], 5.10 (2H, s, 1′)[67.94], 6.83 (1H, dd, J=7.9 & 1.9 Hz, 6″)[121.21], 7.10 (1H, d, J=16.1 Hz, α)[118.00], 7.10 (1H, d, J=8.7 Hz, 3)[116.97], 7.16 (1H, dd, J=8.7 & 2.7 Hz, 4)[120.61], 7.19 (1H, d, J=7.9 Hz, 5″)[116.72], 7.27 [1H, d, J=1.9 Hz, 2″)[117.92], 7.54 (1H, d, J=2.7 Hz, 6)[115.25], 8.70 (1H, d, J=16.1 Hz, β)[142.61], [122.73, 1″], [125.16, 1′], [146.52, 4″], [147.49, 3″], [151.68, 2 & 5], [167.06, COO], [202.46, CO]. Isomer **2** decomposed during MS measurement. HRMS *m/z* (M^+) of ester **11**: Calcd. for $C_{26}H_{32}O_{11}$: 520.1942. Found: 520.1931.

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