Magnesium and Ammonium–Potassium Lithospermates B, the Active Principles Having a Uremia-Preventive Effect from *Salvia miltiorrhiza*[1)]

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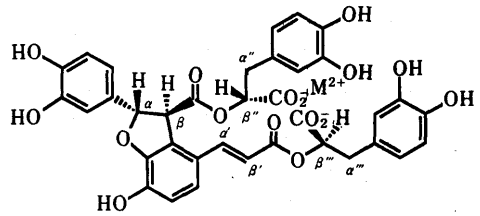
The active components which exhibit the improving effect on uremic symptoms have been isolated from Salviae miltiorrhizae Radix and characterized as magnesium lithospermate (1) and ammonium–potassium lithospermate (2). The stereostructure of lithospermic acid, which had remained unclarified, was also determined on the basis of chemical and spectroscopic data.

Keywords Salviae miltiorrhizae Radix; Labiatae; lithospermic acid B; magnesium lithospermate B; ammonium–potassium lithospermate B; uremic rat; uremia-preventive effect

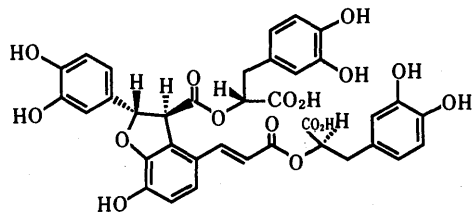
In the course of a search for the active principles which show improving effects on uremic symptoms in Salviae miltiorrhizae Radix, we previously reported the isolation of one of the active components from the aqueous extract.[2)] The active component, compound **1**, causes a significant decrease of urea nitogen, creatinine, methyl guanidine, and guanidinosuccinic acid levels in the blood of adenine diet-induced uremic rats, and shows remarkable improving effects on uremic symptoms of these rats. This paper deals with the chemical characterization of this compound and also the isolation of another active principle (compound **2**) from the aqueous extract of Salviae miltiorrhizae Radix.

Compounds **1** and **2** were major phenolic compounds isolated in 1.1 and 0.2% yields, respectively, by a combination of MCI gel CHP 20P and Sephadex LH-20 chromatographies. Despite their different elution patterns in Sephadex LH-20 chromatography, these compounds showed the same *Rf* value on silica gel and cellulose thin-layer chromatography (TLC) with acidic solvent systems. A dark green coloration with the ferric chloride reagent indicated the phenolic nature of these compounds. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of these compounds were almost indistinguishable from each other, and showed signals attributable to four carboxyl carbons, four aromatic rings, a double bond, and six aliphatic carbons. On methylation with diazomethane, compounds **1** and **2** gave the same product (**4**), whose electron-impact mass spectrum (EI-MS) exhibited the M^+ ion peak at *m/z* 844. These findings suggested that compounds **1** and **2** are tetramers of caffeic acid, being consistent with lithospermic acid B (**3**) previously isolated from an intravenous drip preparation of Salviae miltiorrhizae Radix.[3)] Comparison of the physical and spectral data revealed that the methylate (**4**) was identical with dimethyl nonamethyllithospermate.[4c)] The infrared (IR) spectra of compounds **1** and **2** were, however, obviously different from that of lithospermic acid.[4)] In addition, the fact that compounds **1** and **2** are less soluble in acetone and ethyl acetate and that their aqueous solutions are almost neutral (pH *ca.* 6) suggested that these compounds are salts of lithospermic acid. On treatment with cation exchange resin, or with dilute acids followed by extraction with ethyl acetate, compounds **1** and **2** afforded lithospermic acid B (**3**), as expected.

Negative fast atom bombardment mass spectroscopy (FAB-MS) provided useful information for the identification of the cation (Fig. 1). In the spectra, lithospermic acid B (**3**) exhibited an intense $(M-H)^-$ ion peak at *m/z* 717, whereas compound **1** showed a peak of a much higher mass number at *m/z* 739, and compound **2** at *m/z* 755. From these facts, it was deduced that compound **1** is the sodium or magnesium salt of lithospermic acid and that compound



1 : $M^{2+}=Mg^{2+}$
2 : $M^{2+}=NH_4^+ + K^+$



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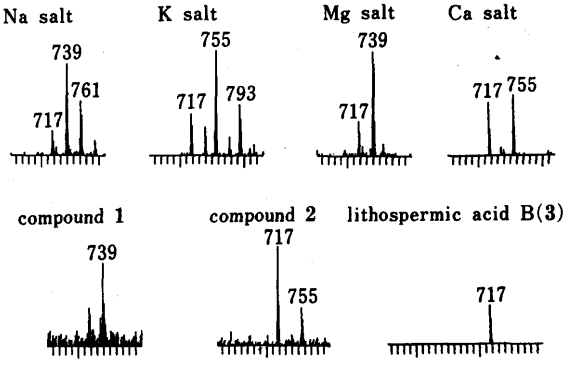

Fig. 1. Negative FAB-MS of Compounds **1** and **2**, and Various Salts of Lithospermic Acid B

2 is the potassium or calcium salt. The cationic metals were unambiguously identified by energy dispersing X-ray analyses, that is, X-ray signals specific for magnesium and potassium ions were observed in the spectra of compounds **1** and **2**, respectively (Fig. 2). Furthermore, the magnesium salt prepared by treatment of lithospermic acid B with magnesium hydroxide gave an IR spectrum and negative FAB-MS identical with those of compound **1**. However, the negative FAB-MS of the potassium salt prepared in a similar manner differed from that of compound **2**, exhibiting the ion peak at *m/z* 793 (Fig. 1), which corresponds to a di-potassium salt. This discrepancy is considered to arise from the presence of another cation in compound **2**. In fact, the IR spectrum of **2** showed absorption bands at 3200 and 1400 cm^{-1}, suggestive of the presence of an ammonium ion.

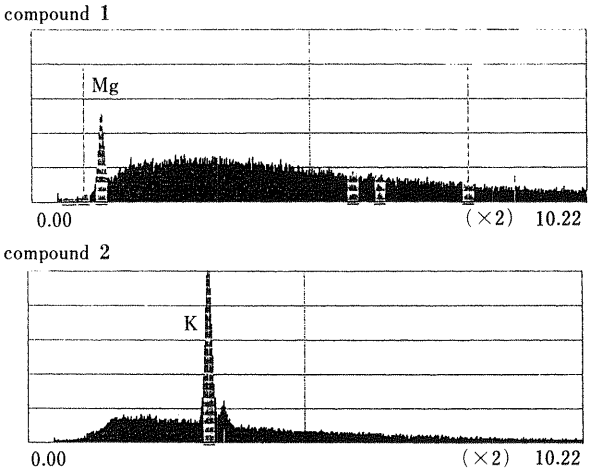

Fig. 2. Energy-Dispersive X-Ray Analyses of Compounds **1** and **2**

Moreover, the result of elemental analysis indicated the presence of a nitrogen atom in the molecule, and was consistent with the ratio of *ca.* 1:3 of potassium and ammonium salts. Final structural confirmation could be achieved by preparation of a mixed potassium–ammonium salt (*ca.* 1:5 by elemental analysis), whose IR spectrum coincided with that of compound **2**.

Consequently, compounds **1** and **2** were determined to be magnesium and ammonium–potassium salts of lithospermic acid B, respectively.

Since the stereochemistry of lithospermic acid B (**3**) had remained unclear, an attempt was made to establish the absolute structure as follows. When methanolyzed in an alkaline medium, dimethyl heptamethyl lithospermate B (**4**) afforded two major products (**5** and **6**). By comparison of the spectral data and specific optical rotation, the methanolysate (**5**) was identified as methyl 3-(3′,4′-dimethoxyphenyl)-(*R*)-lactate.[5] On the other hand, the ^{1}H-NMR spectrum of **6** showed two *trans*-olefinic proton signals (δ 6.28 and 7.71, each d, J=16.1 Hz), five aromatic proton signals, and a pair of mutually coupled aliphatic proton signals (δ 6.03 and 4.48, each d, J=5.6 Hz), along with five methoxyl signals. These observations clearly indicated that **6** possesses a dihydrobenzofuran moiety.

On reduction with lithium aluminum hydride, the methylate (**4**) afforded, among others, three major products (**7**—**9**). The most polar product (**7**) was readily characterized as 3-(3′,4′-dimethoxyphenyl)propane-1,2-diol on the basis of ^{1}H-NMR and EI-MS analyses. On the other hand, the products **8** and **9** showed the same *Rf* value on TLC and could not be separated. The ^{1}H–^{1}H homonuclear shift correlation (COSY) spectral analysis of this mxture indicated the compounds to possess a similar hydroxymethyl

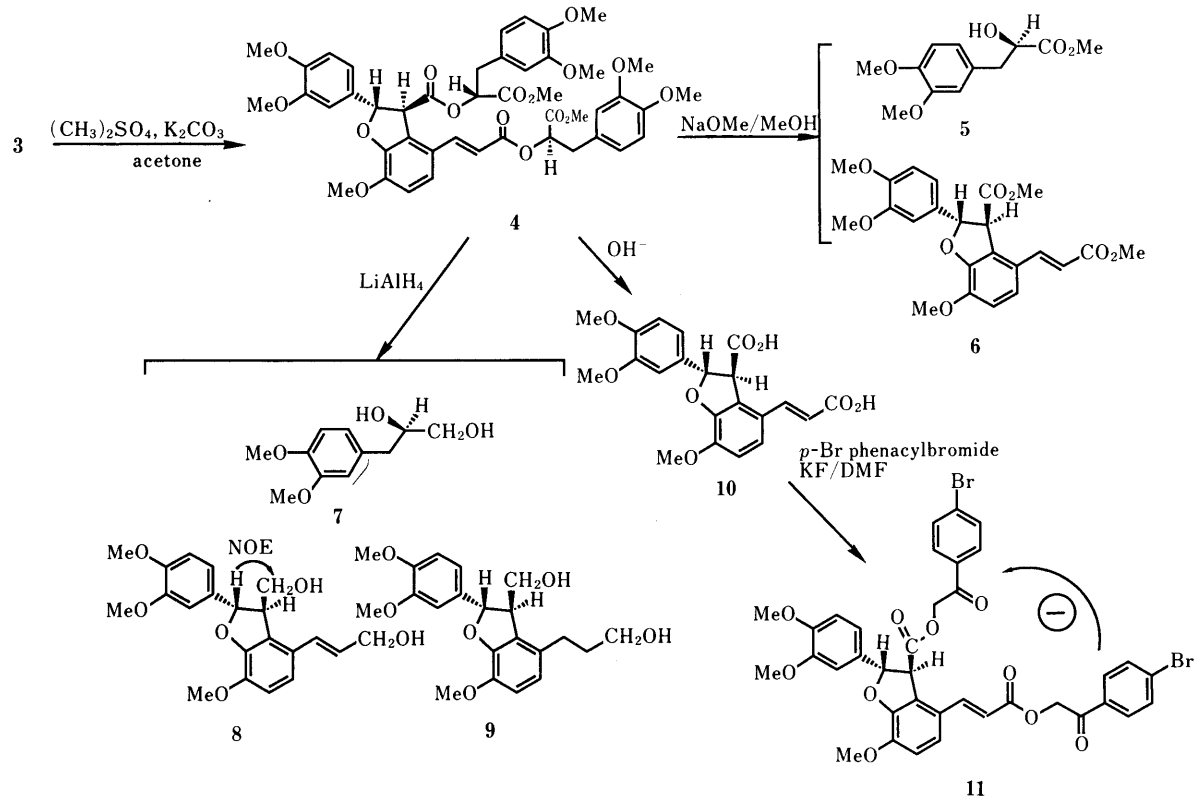

Chart 1

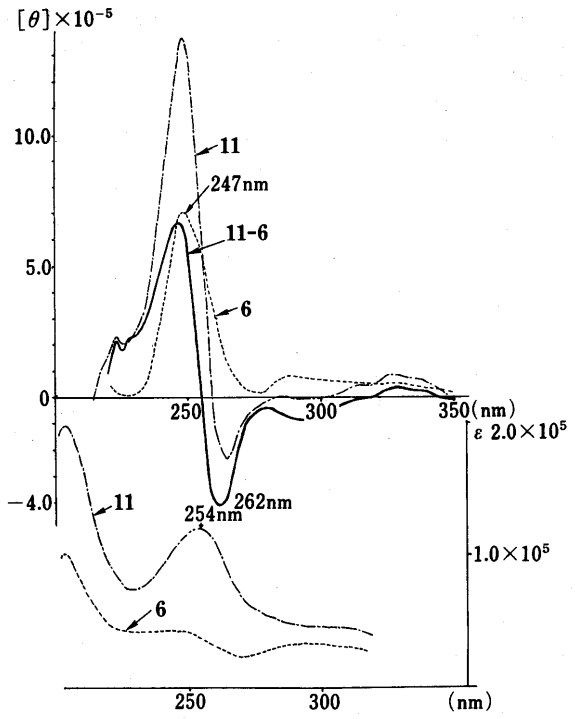

Fig. 3. UV, CD and CD Difference Spectra of **6** and **11**

TABLE I. ^{1}H-NMR Data for Dihydrobenzofuran Protons in Compounds **1** and **2**, and Various Salts of Lithospermic Acid B (**3**)

	H-α ppm (Hz)	H-β ppm
Compound **1**[a]	5.62 (4.0)	4.49
Mg salt[a]	5.44 (3.9)	4.56
Mg salt[b]	5.73 (4.4)	4.50
Ca salt[a]	5.53 (4.2)	4.56
Compound **2**[a]	5.82 (5.0)	4.56
K salt[a]	5.87 (5.4)	4.42
K salt[b]	6.05 (5.4)	—
Na salt[a]	5.78 (5.1)	4.45
K–NH_4 salt[a]	5.79 (4.9)	4.45
Lithospermic acid[c]	5.88 (4.6)	4.47

a) Measured in acetone-d_6 containing *ca.* 20% D_2O. *b*) Measured in acetone-d_6 containing *ca.* 30% D_2O. *c*) Measured in acetone-d_6.

group attached to a dihydrobenzofuran moiety, and to be closely related, differing only in the presence or absence of a double bond. In the two-dimensional nuclear Overhauser effect (NOESY) spectrum, the appearance of a cross peak between the hydroxymethyl protons and a benzylic α-proton (Chart 1), indicated that the dihydrobenzofuran ring possesses *trans*-configuration.

The absolute configuration of the dihydrobenzofuran moiety was determined in the following manner. Alkaline hydrolysis of the methylate (**4**) afforded the dicarboxylic acid (**10**), which was subsequently treated with *p*-bromophenacyl bromide in the presence of potassium fluoride[6] to give the bis-*p*-bromophenacyl ester (**11**). The circular dichroism (CD) spectra of **11** and the methanolysate (**6**) showed similar Cotton effects (Fig. 3), but the difference curve (Fig. 3) exhibited strong Cotton effects resulting from the interaction of the two *p*-bromophenacyl groups.[7] The observation of a negative Cotton effect at 262 nm and a positive one at 247 nm indicated compound **11** to have negative chirality as proposed by Harada and Nakanishi,[8] and thus to possess the *S* configuration at the β position of lithospermic acid B.

On the basis of these results, the stereostructure of lithospermic acid B, including the absolute configuration, was established to be represented by the formula (**3**).

It should be noted that in the ^{1}H-NMR spectra of various natural and synthetic salts of lithospermic acid B, the chemical shifts of signals due to the α- and β-protons in the dihydrobenzofuran moiety were variable, depending upon the amount of added D_2O in acetone-d_6 (Table I). Furthermore, the coupling constants of these signals were not constant. For example, the observed coupling constants in the di-sodium, di-potassium, and ammonium–potassium salts were 4.9—5.4 Hz, while smaller values ($J_{\alpha,\beta}$ = 3.9—4.4 Hz) were observed in the spectra of magnesium and calcium salts. In addition, the coupling constant in the magnesium salt became larger when the proportion of D_2O in acetone-d_6 was increased (3.9 Hz in 20% D_2O and 4.4 Hz in 30% D_2O). These findings suggest the occurrence of chelate effects between two carboxyl anions and alkaline earth metals, and of some conformational torsion caused by chelation.

The chromatographic behaviors of the above-mentioned salts were quite different. Namely, the ammonium–potassium salt (compound **2**) was eluted very fast from a Sephadex LH-20 column with water, and its elution pattern was similar to those of the di-sodium and di-potassium salts. The magnesium salt (compound **1**) and calcium salt, on the other hand, were slightly adsorbed on the Sephadex LH-20 gel, and were eluted more slowly with water. The free acid, although not detected in the plant material, was not eluted from the Sephadex LH-20 column with water, and about 80% aqueous methanol was required for the recovery of the sample.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. FAB-MS and EI-MS were measured with JEOL DX-300 and JEOL D-300 spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken with JEOL PS-100 and JEOL FX-100 spectrometers, with tetramethylsilane as an internal standard, and chemical shift values are given in δ (ppm). ^{1}H–^{1}H COSY and NOESY spectra were obtained on a JEOL GX-270 spectrometer. CD spectra were measured with a JASCO J-20 apparatus. Energy-dispersive micro X-ray analysis was recorded by using a Hitachi S-50 scanning electron microscope at 20 kV with an EMAX-1770 energy-dispersive X-ray analyzer (Horiba Seisakusho Co., Ltd.) at 20 eV for 900 s. Column chromatography was performed using MCI-gel CHP-20P (75—150 μm, Mitsubishi Chemical Industries Ltd.), Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemicals), and Kieselgel 60 (70—230 mesh, Merck). TLC was conducted on precoated Kieselgel 60 F_{254} plates (0.20 mm thick, Merck) with benzene–ethyl formate–formic acid (1 : 7 : 1, for phenolics) and benzene–acetone (5 : 1 and 3 : 1, for methyl derivatives), and precoated cellulose F_{254} plates (Merck, 0.1 mm thick, Merck) with 2% acetic acid, and spots were detected by spraying 2% ethanolic ferric chloride or spraying 5% sulfuric acid followed by heating. The ratio of solvents is given in v/v in each case.

Isolation of Compounds 1 and 2 Commercially available Salviae miltiorrhizae Radix (1.0 kg) produced in China was extracted twice with water (1.5 l) at 80 °C. After removal of the insolubles by filtration, the filtrate was concentrated under reduced pressure (40 °C), and subjected to MCI-gel CHP-20P (7.5 cm i.d. × 35 cm) column chromatography. After washing of the column with water, elution with 50% aqueous methanol yielded polyphenols (62 g), which were chromatographed over Sephadex LH-20 (5.0 cm i.d. × 42 cm) with water containing increasing amounts of ethanol

to afford three fractions; frs. I (4.8 g), II (0.35 g), and III (5.9 g), and compound **1** (7.56 g). Fractions I and III were separately rechromatographed over a Sephadex LH-20 column using water as the eluent to furnish compound **2** (1.98 g) and a further crop of compound **1** (4.3 g), respectively.

Compound 1 (Magnesium Lithospermate B) A tan amorphous powder, $[\alpha]_D^{26}$ +147.7° (c=0.7, MeOH). *Anal.* Calcd for $C_{36}H_{28}MgO_{16}\cdot 4H_2O$: C, 53.19; H, 4.46. Found: C, 53.23; H, 4.32. Negative FAB-MS (Fig. 1). IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1602, 1520, 1410, 1290. ^{1}H-NMR (acetone-d_6+D_2O) δ: 7.52 (1H, d, J=16 Hz, H-α'), 7.15 (1H, d, J=8 Hz, H-6'), 6.44—6.90 (10H, m), 6.30 (1H, d, J=16 Hz, H-β'), 5.62 (1H, d, J=4 Hz, H-α), 5.00 (2H, m, H-β'', β'''), 4.49 (d, J=4 Hz, H-β), 2.70—3.30 (4H, m, H-α'', α'''). ^{13}C-NMR (acetone-d_6+D_2O) δ: 177.3, 176.6 (COO$^-$), 172.5 (–COO–), 169.2 (–COO–), 148.2, 145.9 (×3), 145.4, 144.5, 144.3 (×2), 143.2, 133.4, 130.7, 130.5, 126.5, 124.4, 121.5 (×3), 118.0, 117.6, 117.2, 116.9, 116.5, 116.2, 116.0 (×3), 113.3 (5'), 87.2 (α), 78.3, 78.1 (β'', β'''), 56.7 (β), 37.7 (α'', α''').

Compound 2 (Ammonium–Potassium Lithospermate B) A tan amorphous powder, $[\alpha]_D^{26}$ +116.6° (c=2.2, MeOH). *Anal.* Calcd for $(C_{36}H_{28}O_{16})_2K(NH_4)_3\cdot 3H_2O$: C, 54.72; H, 4.72; N, 2.67. Found: C, 54.72; H, 4.46; N, 2.32. Negative FAB-MS (Fig. 1). IR ν_{max}^{KBr} cm^{-1}: 3400, 3200, 1700, 1602, 1590, 1400. ^{1}H-NMR (acetone-d_6+D_2O) δ: 7.45 (1H, d, J=16 Hz, H-α'), 7.15 (1H, d, J=8 Hz, H-6'), 6.59—6.96 (10H, m), 6.40 (1H, dd, J=2, 8 Hz, H-6), 6.26 (1H, d, J=16 Hz, H-β'), 5.82 (1H, d, J=5 Hz, H-α), 5.00 (2H, m, H-β'', β'''), 4.56 (1H, d, J=5 Hz, H-β), 2.70—3.24 (4H, m, H-α'', α'''). ^{13}C-NMR (acetone-d_6+D_2O) δ: 176.9, 175.9 (COO$^-$), 172.5 (–COO–), 168.5 (–COO–), 148.3, 145.8, 145.3 (×3), 144.4, 144.2 (×2), 142.6, 133.3, 130.8, 130.4, 126.5, 124.3, 121.6 (×3), 118.0, 117.6, 117.5, 117.1, 116.8, 116.3 (×3), 113.7, 87.3 (α), 78.4, 78.8 (β'', β'''), 57.2 (β), 38.0 (α'', α''').

Lithospermic Acid B (3) Aqueous solutions of compounds **1** (16 mg) and **2** (22 mg) were separately treated with IR 120B resins (H^+ form) or with 0.5 N HCl. Extraction of the reaction mixture with ethyl acetate yielded lithospermic acid B (**3**) (13 mg from compound **1** and 19 mg from compound **2**), a white hygroscopic powder, $[\alpha]_D^{26}$ +146.3° (c=1.2, H_2O). *Anal.* Calcd for $C_{36}H_{30}O_{16}\cdot H_2O$: C, 58.70; H, 4.38. Found: C, 58.57; H, 4.69. Negative FAB-MS (Fig. 1). IR ν_{max}^{KBr} cm^{-1}: 3400, 1720, 1700, 1604, 1508, 1450, 1360, 1290. ^{1}H-NMR (acetone-d_6) δ: 7.64 (1H, d, J=15.9 Hz, H-α'), 7.26 (1H, d, J=8.5 Hz, H-6'), 6.44 (1H, dd, J=2, 8 Hz, H-6), 6.28 (1H, d, J=18.1 Hz, H-β'), 5.88 (1H, d, J=4.6 Hz, H-α), 5.20 (2H, m, H-β'', β'''), 4.47 (1H, d, J=4.6 Hz, H-β), 3.14 (2H, dd, J=4.9, 14.2 Hz, H-α'', α'''), 2.90 (2H, dd, J=8.3, 14.2 Hz, H-α'', α'''). ^{13}C-NMR (acetone-d_6) δ: 171.5, 171.2, 170.7, 166.6 (COO), 148.6, 146.3, 146.0, 145.6, 144.7 (×2), 144.4, 142.8, 133.3, 129.1, 128.6, 126.1, 124.6, 121.6 (×2), 118.2 (×2), 117.4, 117.2, 116.8, 116.1 (×3), 113.3, 87.5 (α), 74.8, 73.8 (β'', β'''), 57.1 (β), 37.4, 37.1 (α'', α''').

Dimethyl Heptamethyllithospermate B (4) a) From Compounds **1** and **2**: Compound **1** (500 mg) was dissolved in 80% aqueous acetone and treated with ethereal diazomethane at room temperature for 5 h. After removal of the solvent under reduced pressure, the residue was applied to a Silica gel 60 column using benzene–acetone (9 : 1) as the eluent, to yield dimethyl heptamethyllithospermate B (**4**) (27 mg), a white powder, $[\alpha]_D^{26}$ +88.6° (c=0.8, $CHCl_3$). *Anal.* Calcd for $C_{45}H_{48}O_{16}$: C, 63.97; H, 5.73. Found: C, 63.86; H, 5.69. IR ν_{max}^{KBr} cm^{-1}: 2950, 2840, 1740, 1718, 1610, 1515, 1265. EI-MS m/z: 844 (M^+, 0.4), 602 (17), 380 (42), 240 (0.9), 222 (67), 151 (100). ^{1}H-NMR ($CDCl_3$) δ: 7.56 (1H, d, J=16.0 Hz, H-α'), 7.15 (1H, d, J=8.6 Hz, H-6'), 6.91—6.53 (10H, m), 6.20 (1H, d, J=16.0 Hz, H-β'), 6.02 (1H, d, J=5.9 Hz, H-α), 5.30—5.15 (2H, m, H-β'', β'''), 4.43 (1H, d, J=5.9 Hz, H-β), 3.95, 3.86, 3.84, 3.69 (27H in total, OMe), 3.16—2.96 (4H, H-α'', α'''). Methylation of compound **2** (300 mg) in a similar manner afforded **4** (20 mg).

b) From Lithospermic Acid B (**3**): A mixture of **3** (1.0 g), dimethylsulfate (4 ml) and anhydrous potassium carbonate (4 g) in dry acetone was refluxed for 5 h. After removal of the inorganic salts by filtration, the filtrate was concentrated to a syrup, which was applied to a column of silica gel. Elution with benzene–acetone (9 : 1) gave **4** (630 mg).

Methanolysis of 4 Compound **4** (39 mg) was treated with sodium methoxide (3 mg) in methanol (3 ml) and the mixture was allowed to stand for 5 h at room temperature. After neutralization with Amberlite IR-120B (H^+ form) resins, the solution was concentrated to dryness. The residue was chromatographed over silica gel (4 g) with benzene–acetone (95 : 5—93 : 7) to afford methanolysates **5** (16.5 mg), a colorless syrup, $[\alpha]_D^{26}$ −4.5° (c=1.6, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 6.87—6.69 (3H, m, H-2', 5', 6'), 4.52—4.35 (1H, m, H-2), 3.87, 3.86, 3.78 (each 3H, s, OMe), 3.10 (1H, dd, J=4.6, 13.6 Hz, H-3), 2.89 (1H, dd, J=6.3, 13.6 Hz, H-3), 2.71 (1H, d, J=6.1 Hz, OH), and **6** (13.2 mg), a colorless syrup, $[\alpha]_D^{26}$ +124.1° (c=0.6, $CHCl_3$). *Anal.* Calcd for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65. Found: C, 64.23; H, 5.91. EI-MS m/z: 428 (M^+, 69), 396 (30), 368 (30), 364 (35), 337 (100). ^{1}H-NMR ($CDCl_3$) δ: 7.71 (1H, d, J=16.1 Hz, H-β'), 7.22 (1H, d, J=9.3 Hz, H-6'), 6.98—6.77 (4H, m), 6.28 (1H, d, J=16.1 Hz, H-α'), 6.03 (1H, d, J=5.6 Hz, H-α), 4.48 (1H, d, J=5.6 Hz, H-β), 3.93, 3.87, 3.86, 3.80, 3.78 (each 3H, s, OMe). UV λ_{max}^{MeOH} (ε): 204 (102000), 260 (28800), 286 (31200), 298 (31500). CD (c=2.2×10^{-4}, MeOH): Fig. 3.

Reduction of 4 A mixture of **4** (50 mg) and lithium aluminum hydride (10 mg) in dry tetrahydrofuran (7 ml) was stirred at room temperature for 30 min and then under reflux for 30 min. After cooling, the excess reagent was decomposed by adding ethyl acetate (6 ml). The ethyl acetate solution was washed with water, dried over anhydrous sodium sulfate, and concentrated to dryness. The residue was applied to a column of silica gel (5 g) with benzene–acetone (17 : 3). Further separation by preparative TLC (benzene–acetone, 2 : 1) afforded **7** (11.3 mg), a colorless syrup, $[\alpha]_D^{26}$ +15.3° (c=1.1, $CHCl_3$). EI-MS m/z: 212 (M^+, 14), 194 (28), 176 (17), 151 (100). ^{1}H-NMR ($CDCl_3$) δ: 6.84—6.66 (3H, m), 4.00—3.82 (7H, OMe, H-2), 3.66 (1H, dd, J=11, 3 Hz, H-1), 3.50 (1H,dd, J=11, 6 Hz, H-1), 2.68 (2H, br d, J=7 Hz, H-3), and a mixture of **8** and **9** (11.8 mg), $[\alpha]_D^{26}$ +73.5° (c=0.7, $CHCl_3$). EI-MS m/z: 374 (M^+ of **9**, 49), 372 (M^+ of **8**, 12), 356 (71), 354 (59), 151 (100). ^{1}H-NMR ($CDCl_3$) δ: 7.05—6.70 (m, aromatic-H), 6.52 [d, J=16 Hz, H-α' (**8**)], 6.25 [1H, dt, J=16, 5 Hz, H-β (**8**)], 5.75 [d, J=5 Hz, H-α (**9**)], 5.70 [d, J=5 Hz, H-α (**8**)], 4.30 [br d, J=5 Hz, H-γ' (**8**)], 3.96 [m, H-γ (**8** and **9**)], 3.93, 3.90, 3.86, 3.85 (each s, OMe), 3.64 [m, H-γ' (**9**), H-β (**8**)], 2.66 [br t, J=8 Hz, H-α' (**9**)], 1.84 [m, H-β' (**9**)].

Bis-*p*-bromophenacyl Ester (11) A 3% aqueous NaOH solution (1.25 ml) was added dropwise to a solution of **4** (197 mg) in acetone–H_2O (4 : 1, 1 ml), and the mixture was stirred at room temperature for 30 min. After removal of the acetone under reduced pressure, followed by acidification with HCl, the solution was extracted with ether. The organic layer was dried over sodium sulfate and concentrated to dryness. The residue was dissolved in dimethylformamide (2 ml) and treated with *p*-bromophenacyl bromide (307 mg) and potassium fluoride (130 mg) at room temperature for 30 min. The reaction mixture was partitioned between water and ether, and the ether layer was washed five times with water, dried over sodium sulfate, and concentrated to a syrup. Silica gel column chromatography (30 g) with benzene–acetone (96 : 4) afforded the bis-*p*-bromophenacyl ester (**11**) (23 mg), colorless fine needles (MeOH), mp 169—170 °C. $[\alpha]_D^{26}$ +119.8° (c=0.21, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.86 (1H, d, J=16 Hz, H-α'), 7.81—7.54 (8H, m), 7.24 (1H, d, J=8 Hz, H-6'), 7.08—6.80 (4H, m, H-2, 6, 5, 5'), 6.40 (1H, d, J=16 Hz, H-β'), 6.25 (1H, d, J=6 Hz, H-α), 5.47, 5.44, 5.59, 5.36 (4H in total, $-CH_2-$), 4.66 (1H, d, J=6 Hz, H-β), 3.97, 3.90, 3.88 (each 3H, s, OMe). UV λ_{max}^{MeOH} (ε): 204 (199000), 254 (121000). CD (c=3.15×10^{-5}, MeOH): Fig. 3.

Preparation of Lithospermic Acid B Salts A solution of lithospermic acid B (72 mg) in water (0.5 ml) was treated with 0.1 N $Mg(OH)_2$ (1.0 ml), 0.1 N $Ca(OH)_2$ (1.0 ml), 0.1 N KOH (2.0 ml), 0.1 N NaOH (1.8 ml), or 0.1 N KOH (0.5 ml) and 0.1 N NH_4OH (1.5 ml). The mixture was directly applied to a Sephadex LH-20 column (2.3 cm i.d. × 25 cm) using water with increasing amounts of EtOH as the eluant. Dipotassium, di-sodium and ammonium–potassium salts were eluted with water, while magnesium and calcium salts were eluted with 20% EtOH.

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References and Notes

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3) There is some confusion in the use of the name of lithospermic acid. That is, Kelley *et al.* determined the structure of lithospermic acid to be a caffeic acid trimer, whereas Chen *et al.* later called compound **3** lithospermic acid, and more recently, Ikeshiro *et al.* have used the name of lithospermic acid B for compound **3**. *a*) J. S. Chen, W. H. Guh, H. J. Hwang, S. M. Yang, T. J. Shuen, J. C. Chen, Y. L.

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