# STRUCTURES OF KS-501 AND KS-502, THE NEW INHIBITORS OF $Ca^{2+}$ AND CALMODULIN-DEPENDENT CYCLIC NUCLEOTIDE PHOSPHODIESTERASE

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The structures of KS-501 and KS-502, new inhibitors of $Ca^{2+}$ and calmodulin-dependent cyclic nucleotide phosphodiesterase, were determined to be 2-(β-D-galactofuranosyloxy)-6-heptyl-4-hydroxybenzoic acid 3-heptyl-5-hydroxyphenyl ester and 2-(β-D-galactofuranosyloxy)-6-heptyl-4-hydroxybenzoic acid 4-carboxy-3-heptyl-5-hydroxyphenyl ester, respectively, on the basis of chemical and physico-chemical evidences.

KS-501 and KS-502, isolated from the culture broth of *Sporothrix* sp. KAC-1985, are novel and potent inhibitors of $Ca^{2+}$ and calmodulin-dependent cyclic nucleotide phosphodiesterase. The fermentation, isolation and biological properties of KS-501 and KS-502 have been reported by NAKANISHI *et al.*[1] We wish to describe the structure determination of these compounds in this paper.

The physico-chemical properties of KS-501 and KS-502, summarized in Table 1, are very similar, implying that they are closely related compounds.

## Structure of KS-501

KS-501 (**1**) was obtained as a colorless powder. SI-MS of **1** showed a pseudomolecular ion $(M+H)^+$ at *m/z* 605 and the molecular formula of **1** was determined to be $C_{33}H_{48}O_{10}$ by HR negative ion FAB-MS. KS-501 showed the existence of hydroxy (3400 $cm^{-1}$) and ester group (1720 $cm^{-1}$) in IR spectrum and conjugated aromatic system ($\lambda_{max}$ nm 254 and 278) in its UV spectrum.

The $^1H$ NMR spectrum of **1** exhibited six methyl and twentyfour methylene protons, two sets of *meta*-coupled aromatic protons (two and three protons, respectively), and five methine and one downfield methylene proton signals which connected sequentially with coupling. The $^{13}C$ NMR spectrum of **1** showed one ester carbonyl carbon signal, twelve methylene and two methyl carbons, twelve aromatic carbons, and five methine and one methylene carbons substituted by hetero atoms. From these spectral data, it was apparent that **1** contained 1,3,5-tri-substituted and 1,2,3,5-tetra-substituted benzene moieties, two *n*-heptyl groups, one hexose, and one ester functional group.

Treatment of **1** with hydrogen chloride in methanol at room temperature gave aglycone **3** and sugar methyl glycoside **4** (Fig. 1). Compound **4** was *p*-bromobenzoylated to afford methyl 2,3,4,6-tetra-*O*-*p*-bromobenzoyl-α-D-galactopyranoside (**5**) which was identical with an authentic sample[2] by comparison of their $^1H$ NMR spectrum and specific rotation. Aglycone **3** showed a molecular ion peak $(M)^+$ at *m/z* 442 in its EI-MS spectrum.

Methanolysis of **3** under refluxing temperature gave two compounds (**6** and **7**). The molecular formula of **6** was determined to be $C_{13}H_{20}O_2$ by HREI-MS. In the $^1H$ NMR of **6**, three *meta*-coupled aromatic protons (two of them were equivalent), two equivalent phenolic hydroxy protons and one *n*-heptyl group

Table 1. Physico-chemical properties of KS-501 (**1**) and KS-502 (**2**).

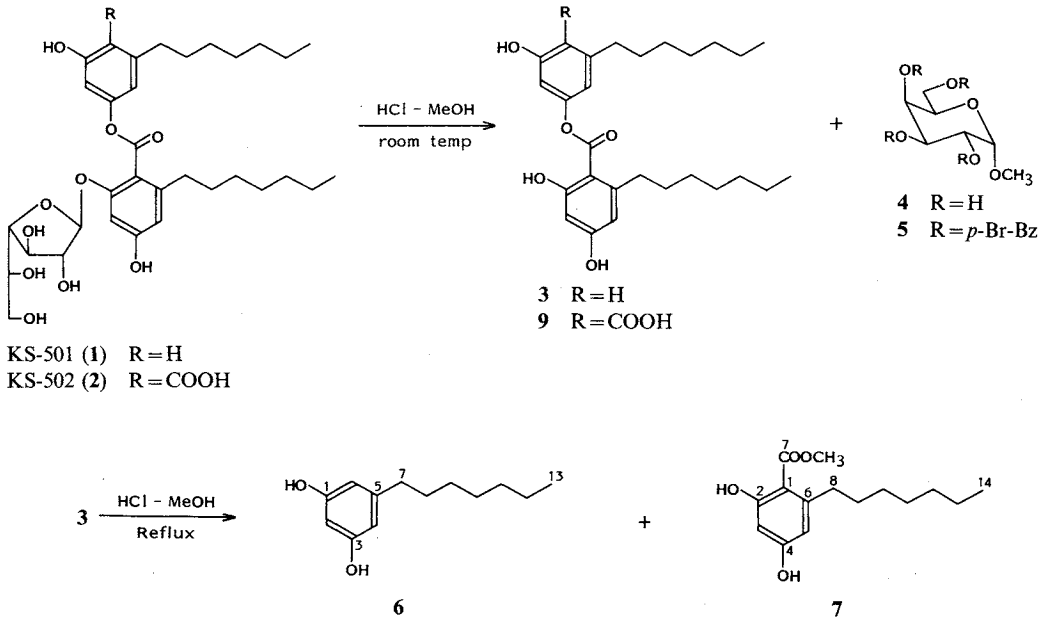
| | **1** | **2** |
|---|---|---|
| Appearance | Colorless powder | Colorless powder |
| Molecular formula | $C_{33}H_{48}O_{10}$ | $C_{34}H_{48}O_{12}$ |
| HRFAB-MS (negative mode) | | |
| Calcd: | 603.3170 ($C_{33}H_{47}O_{10}$) | 647.3068 ($C_{34}H_{47}O_{12}$) |
| Found: | 603.3169 | 647.3083 |
| $[\alpha]_D^{23}$ (*c* 0.3, MeOH) | −53° | −45° |
| UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$) | 207 (35,000), 254 (6,600), 278 (5,000) | 210 (56,000), 245 (12,000), 290 (6,500) |
| IR (KBr) $cm^{-1}$ | 3400, 1720, 1585, 1064 | 3450, 1724, 1595, 1057 |
| $^1H$ NMR $\delta$ (400 MHz, $CD_3OD$) | 6.58 (1H, d, $J$=2.1 Hz, 5′-H), 6.55～6.50 (3H, m, 2, 4, 6-H), 6.38 (1H, d, $J$=2.1 Hz, 5′-H), 5.55 (1H, d, $J$=1.8 Hz, 1″-H), 4.28 (1H, dd, $J$=3.9, 1.8 Hz, 2″-H), *ca.* 4.1 (2H, 3″, 4″-H), 3.76 (1H, br t, $J$=6.4 Hz, 5″-H), 3.65, 3.61 (2H, AB in ABX, 6″-$H_2$), 2.65 (2H, br dd, 8′-$H_2$), 2.56 (2H, br dd, 7-$H_2$), *ca.* 1.6 (4H, m), 1.2～1.4 (16H), 0.90 (3H, t, $J$=6.9 Hz, 13-$H_3$[a]), 0.88 (3H, t, $J$=7.1 Hz, 14′-$H_3$[a]) | 6.60 (1H, d, $J$=2.3 Hz, 3-H[b]), 6.58 (1H, d, $J$=2.1 Hz, 3′-H), 6.51 (1H, d, $J$=2.3 Hz, 5-H[b]), 6.38 (1H, d, $J$=2.1 Hz, 5′-H), 5.54 (1H, d, $J$=1.8 Hz, 1″-H), 4.27 (1H, br dd, 2″-H), *ca.* 4.1 (2H, 3″, 4″-H), 3.76 (1H, br t, $J$=5.9 Hz, 5″-H), 3.63, 3.61 (2H, AB in ABX, 6″-$H_2$), 3.09 (2H, br dd, 8-$H_2$), 2.65 (2H, br dd, 8′-$H_2$), *ca.* 1.6 (4H, m), 1.2～1.4 (16H), 0.88 (3H, t, $J$=6.6 Hz, 14-$H_3$[c]), 0.87 (3H, t, $J$=6.7 Hz, 14′-$H_3$[c]) |
| $^{13}C$ NMR $\delta$ (100 MHz, $CD_3OD$) | 168.8 (C-7′), 161.0 (C-4′), 159.3 (C-3), 157.2 (C-2′), 153.1 (C-1), 146.6 (C-5), 144.6 (C-6′), 116.2 (C-1′), 114.0 (C-6[d]), 113.7 (C-4[d]), 111.1 (C-5′), 108.3 (C-1″), 107.5 (C-2), 101.9 (C-3′), 85.4 (C-4″), 83.5 (C-2″), 78.5 (C-3″), 72.1 (C-5″), 64.4 (C-6″), 36.8 (C-7), 34.9 (C-8′), 33.0 (C-11, 12′), 32.6, 32.4 (C-8, 9′), 30.6, 30.3 (C-9, 10′), 30.3 (C-10, 11′), 23.7 (C-12, 13′), 14.4 (C-13, 14′) | 176.6 (C-7), 168.2 (C-7′), 164.3 (C-2), 161.2 (C-4′), 157.3 (C-2′), 154.6 (C-4), 149.6 (C-6), 144.8 (C-6′), 116.0 (C-1′[f]), 115.7 (C-5), 115.6 (C-1[f]), 111.1 (C-5′), 108.6 (C-3), 108.4 (C-1″), 102.0 (C-3′), 85.5 (C-4″), 83.5 (C-2″), 78.6 (C-3″), 72.2 (C-5″), 64.4 (C-6″), 36.5 (C-8), 34.9 (C-8′), 33.2 (C-9[e]), 33.1, 33.0 (C-12, 2), 32.6 (C-9′[e]), 31.0, 30.7 (C-10, 10′), 30.5, 30.3 (C-11, 11′), 23.74, 23.70 (C-13, 13′), 14.46, 14.43 (C-14, 14′) |

NMR signals [a～e] and [f] are exchangeable.

were observed, which confirmed the structure of **6** to be 5-heptylresorcinol.[3] Compound **7**, molecular formula $C_{15}H_{22}O_4$ (obtained by HREI-MS), showed two *meta*-coupled aromatic protons, one methyl signal of a methoxycarbonyl group, one *n*-heptyl signal, and two phenolic hydroxy protons, one of which was hydrogen bonded, in its $^1H$ NMR, implying that **7** was a 2-hydroxybenzoate derivative. The location of the other two substituents, one hydroxy and one *n*-heptyl group, was determined to be the 4 and 6-positions, respectively, by a long-range selective decoupling (LSPD) experiment of **1**, where long range couplings ($^2J$=3 Hz) were observed between one aromatic proton ($\delta_H$ 6.58, 3′-H) and two oxygen-bonded carbons ($\delta_C$ 157.2, C-2′; 161.0, C-4′), and the other proton ($\delta_H$ 6.38, 5′-H) and one oxygen bonded carbon (C-4′). It also assisted in the location of the substituents on the benzoate ring of **7** that $^{13}C$ NMR chemical shifts of the hydroxy bonded carbon of **7** showed good agreement with that of calculated values for methyl 2,4-dihydroxy-6-heptylbenzoate based upon the additivity relationship.[4] Thus, the structure of aglycone, **3**, was deduced to be as shown in Fig. 1.

The bonding position of the sugar moiety was determined as follows. EI-MS of **1** gave a fragment ion *m/z* 396 corresponding to the glycosylated **7** ion cleaved at the ester bond (Fig. 2). A similar fragment ion was observed at *m/z* 411 in the EI-MS of the dimethyl ether of **1** (**8**) prepared from **1** by treatment with diazomethane (Fig. 3). These results suggested that the galactose moiety is bonded to the one of two hydroxy groups of the benzoate moiety. In the LSPD experiment of **1** (Fig. 2), long range coupling ($^3J$)

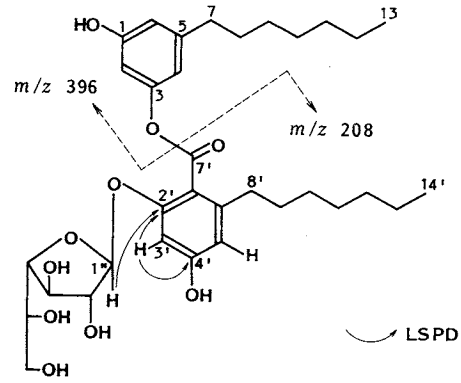
Fig. 1. Degradation studies of **1** and **2**.




between 1″-H ($\delta_H$ 5.55) and C-2′ was observed, confirming the glycosylation position at 2′. The bonding position of the galactose was also assisted by the observation of a NOE between 1″-H and 3′-H, and 4′-*O*-methyl and 3′-H and 5′-H in NOE experiment of diether **8** (Fig. 3).

The coupling constants ($J_{1,2}=1.8$ Hz and $J_{2,3}=3.9$ Hz) of the galactose moiety of **1** were differ from that of galactopyranoside. The galactose moiety was revealed to adopt a furanoside structure by comparison of the $^{13}C$ NMR chemical shifts of the galactose moiety of **1** with those of the four isomers of methyl galactoside.[5,6] As shown in Table 2, the chemical shifts of the sugar moiety of **1** exhibited a good agreement with those of methyl β-galactofuranoside.

Fig. 2. LSPD experiment and mass fragmentation of **1**.




Thus the structure of KS-501 (**1**) was determined to be as shown in Fig. 1.

## Structure of KS-502

The molecular formula of KS-502 (**2**) was demonstrated to be $C_{34}H_{48}O_{12}$ by HR-negative FAB, thus it is larger than **1** by $CO_2$ atoms. KS-502 appeared to have an additional carboxyl functional group substituted on an aromatic ring by comparison of its $^1H$ and $^{13}C$ NMR spectra with those of **1**.

The sugar moiety of **2**, obtained by methanolysis at room temperature, was determined to be D-galactose by comparison of the physico-chemical data of its *p*-bromobenzoate with an authentic sample. The other methanolysis product **9** showed two sets of aromatic protons *meta*-coupled to each other in its

Fig. 3. NOE experiment and mass fragmentation of **8**.

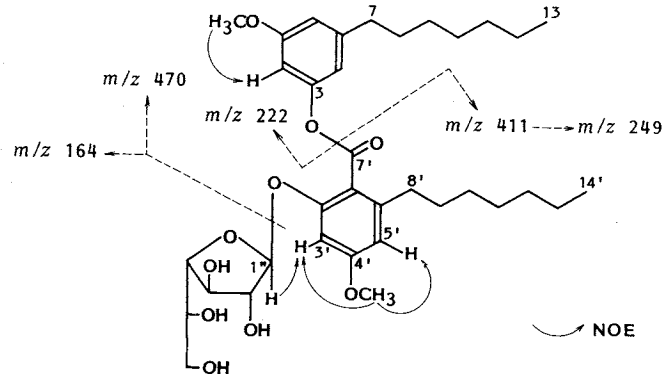
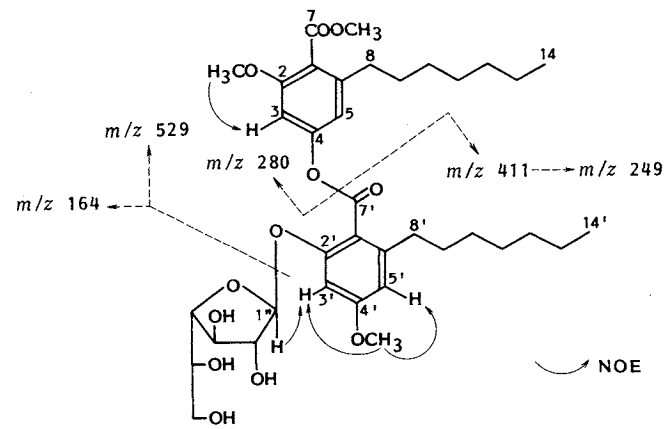



Table 2. Comparison of $^{13}C$ NMR data of KS-501 sugar moiety with those of methyl D-galactosides.

| | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 |
|---|---|---|---|---|---|---|
| Methyl D-galactoside | | | | | | |
| Py-α[a] | 100.5 | 69.4 | 70.6 | 70.4 | 71.8 | 62.3 |
| Py-β | 104.9 | 71.8 | 73.9 | 69.8 | 76.2 | 62.1 |
| Fu-α | 103.8 | 78.2 | 76.2 | 83.1 | 74.5 | 64.1 |
| Fu-β | 109.9 | 81.3 | 78.4 | 84.7 | 71.7 | 63.6 |
| KS-501 sugar moiety | 108.3 | 83.5 | 78.5 | 85.4 | 72.1 | 64.4 |

[a] Ring size and anomeric configuration: Py, pyranoside; Fu, furanoside.

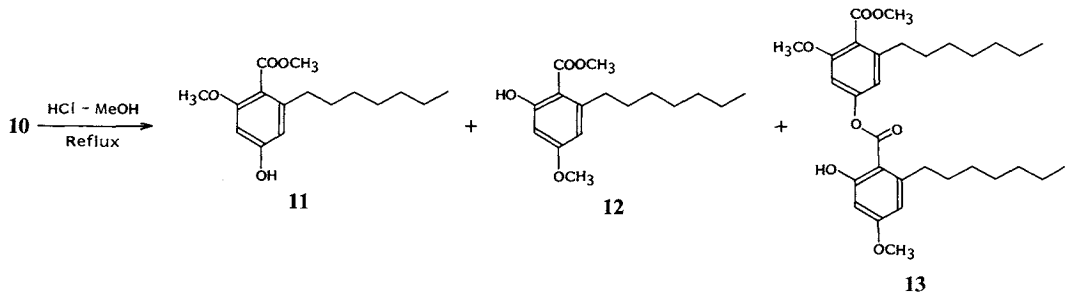
Fig. 4. NOE experiment and mass fragmentation of **10**.




$^1H$ NMR spectrum. Methanolysis of **9** under more vigorous conditions gave mainly product **7** in high yield accompanied with a small amount of its decarboxylation product **6**. This implies that **9** composed of two molecules of **7**.

The location of the ester bond and the galactose moiety was determined using the methylation product of **2** (**10**) produced upon treatment with diazomethane (Fig. 4). The EI-MS of **10** showed fragment ions severed at the ester bond (*m/z* 280 and 411 which were assigned to the methylated benzoic acid moiety and the methylated glycosyl benzoic acid moiety, respectively). The $^1H$ NMR of **10** exhibited a NOE between

Fig. 5. Methanolysis of **10**.




the 4′-*O*-methyl and 3′-H and 5′-H, and 3′-H and 1″-H of galactose, suggesting that galactose is bonded to the 2′-position. Another NOE was observed between the 2-*O*-methyl and 3-H but not 5-H, which implied that the acyloxy group is at the 4-position.

The location of the ester bond was also demonstrated by the examination of the methanolysis products of **10**. If the acylated oxygen were located at the 2-position, a single methyl benzoate derivative should be obtained. Upon treatment of **10** with hydrogen chloride in methanol at reflux temperature two kinds of methyl benzoate derivatives (**11** and **12**) were formed in the ratio of 1 : 1, accompanied by an aglycone **13** where the ester bond still remained. This result suggested that the ester bond is located at 4-position. The structures of **11** and **12** were confirmed by $^1$H NMR and EI-MS. In the $^1$H NMR spectrum, both **11** and **12** showed one *n*-heptyl, two *O*-methyl, two aromatic and one hydroxy proton signals. However, **11** showed a free hydroxy proton at $\delta$ 9.70, whereas in the spectrum of **12** this appeared at rather low field ($\delta$ 10.24), suggesting a hydrogen bond involving the hydroxy group of **12**. In the EI-MS, **11** exhibited a fragment ion at *m/z* 249 $(M-CH_3O)^+$ and **12** showed a corresponding ion at *m/z* 248 $(M-CH_3OH)^+$. These facts implied that a hydroxy group of **12** is located at an adjacent position to the methoxycarbonyl group, and so the structures of **11** and **12** were determined to be as shown in Fig. 5.

The structure of the sugar moiety was confirmed to be $\beta$-galactofuranoside in a similar way as was done with **1**. Thus the structure of **2** was determined to be the 1-carboxyl derivative of **1**. Compound **2** is similar to the compounds named TPIs, disclosed in a Japanese Patent.[7] The difference in their structures is in the sugar moiety, that is the TPIs contain a $\beta$-D-glucopyranosyl or $\beta$-D-galactopyranosyl moiety instead of the $\beta$-D-galactofuranosyl moiety of **2**.

## Experimental

$^1$H and $^{13}$C NMR spectra were recorded on a Jeol JNM FX100 and Bruker AM400 and AM500 spectrometers with TMS (0 ppm) as an internal standard. The IR spectra were obtained using a Shimadzu IR-27G spectrometer. UV spectra were taken with a Hitachi 200-20 spectrometer. Mass spectra were measured on a Hitachi M-80B mass spectrometer. Optical rotation were measured with a Perkin-Elmer 141 polarimeter. MP's were taken with a Yanagimoto melting point apparatus and were uncorrected. TLC was performed on pre-coated plates, Merck Kieselgel $60F_{254}$ visualized by UV (254 nm) or spray of 1% $Ce(SO_4)_2$ - 10% $H_2SO_4$ and heating.

Methanolysis of KS-501 (**1**)

A solution of **1** (10 mg) in 5% HCl in MeOH (1 ml) was stirred for 6 hours at room temperature. The reaction mixture was diluted with EtOAc (50 ml) and washed with water and satd NaCl solution (each 50 ml). The organic layer was dried over magnesium sulfate and evaporated *in vacuo* to give a residue,

which was chromatographed on a silica gel column eluted by *n*-hexane - EtOAc (3 : 1) to afford **3** (6.6 mg). Concentration of the water fraction gave **4** (2.8 mg).

2,4-Dihydroxy-6-heptylbenzoic Acid 3-Heptyl-5-hydroxyphenyl Ester (**3**): Colorless powder. EI-MS *m/z* 442 $(M)^+$, 235, 208, 166, 137, 124; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$ 6.56 (1H, br t, 6-H), 6.47 (1H, br t, 4-H), 6.44 (1H, t, $J$=2.5 Hz, 2-H), 6.28 (1H, d, $J$=2.5 Hz, 3'-H), 6.22 (1H, d, $J$=2.5 Hz, 5'-H), 2.90 (2H, m, 8'-$H_2$), 2.56 (2H, br dd, 7-$H_2$), *ca.* 1.6 (4H, m), 1.4~1.2 (16H, m, 8-$H_2$ to 12-$H_2$ and 9'-$H_2$ to 13'-$H_2$), 0.89 (3H, t, $J$=7.0 Hz), 0.84 (3H, t, $J$=6.9 Hz, 13-$H_3$ and 14'-$H_3$); $^{13}C$ NMR (100 MHz, $CD_3OD$) $\delta$ 171.2 (C-7'), 166.0 (C-2'), 164.2 (C-4'), 159.4 (C-3), 152.3 (C-1), 149.4 (C-6'), 146.7 (C-5), 114.2 (C-6), 113.6 (C-4), 112.2 (C-5'), 107.4 (C-2), 105.5 (C-1'), 102.0 (C-3'), 37.8, 36.8, 33.5, 33.0, 32.3, 30.9, 30.3, 30.24, 30.21, 23.7, 14.4 (*n*-heptyl × 2).

*p*-Bromobenzoylation of Methyl α-D-Galactopyranoside (**4**)

To a solution of **4** (1.5 mg) in pyridine (0.5 ml) *p*-bromobenzoyl chloride (20 mg) was added and stirred for 2 hours at 70°C. After the addition of water (0.1 ml), the reaction mixture was diluted with EtOAc (30 ml) and washed with a satd solution of sodium bicarbonate, water and a satd solution of NaCl (each 30 ml). The organic solution was dried over magnesium sulfate and evaporated *in vacuo* to give a residue which was purified by silica gel column chromatography with *n*-hexane - EtOAc (10 : 1 ~ 5 : 1) and preparative TLC with *n*-hexane - EtOAc (5 : 1) to afford **5** (2.4 mg).

Methyl 2,3,4,6-Tetra-*O*-*p*-bromobenzoyl-α-D-galactopyranoside (**5**): Colorless powder. $[\alpha]_D^{23}$ +141° (*c* 0.2, $CHCl_3$); $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 8.0~7.4 (16H, *p*-bromobenzoyl × 4), 5.96 (1H, br d, $J$=3.5 Hz, 4-H), 5.93 (1H, dd, $J$=10.6 and 3.5 Hz, 3-H), 5.62 (1H, dd, $J$=10.6 and 3.6 Hz, 2-H), 5.28 (1H, d, $J$=3.6 Hz, 1-H), *ca.* 4.6 (2H, ABq in ABX, 6-$H_2$), 4.37 (1H, X in ABX, 5-H), 3.48 (3H, s, 1-$OCH_3$).

Preparation of Authentic Methyl 2,3,4,6-Tetra-*O*-*p*-bromobenzoyl-α-D-galactopyranoside

A solution of D-galactose (350 mg) in 17% HCl in MeOH (6 ml) was heated under reflux for 30 minutes. The reaction mixture was evaporated under reduced pressure to give a residue. A 25-mg portion of the residue was dissolved in pyridine (2 ml) and *p*-bromobenzoyl chloride (130 mg) was added, followed by heating at 80°C for 2 hours. After the addition of water (0.2 ml), the reaction mixture was diluted with EtOAc (50 ml) and washed with satd $NaHCO_3$, water and satd NaCl (each 50 ml). The organic layer was dried over magnesium sulfate and evaporated *in vacuo*. The resultant residue was chromatographed on a silica gel column with *n*-hexane - EtOAc (10 : 1 ~ 3 : 1) to give the title compound (15 mg). $[\alpha]_D^{23}$ +141° (*c* 0.2, $CHCl_3$).

Methanolysis of 2,4-Dihydroxy-6-heptylbenzoic Acid 3-Heptyl-5-hydroxyphenyl Ester (**3**)

A solution of **3** (5 mg) in 17% HCl - MeOH (1 ml) was heated to reflux for 1 hour. The reaction mixture was evaporated and the residue was chromatographed on a silica gel column with $CHCl_3$ - MeOH (30 : 1) to give **6** (2.0 mg) and **7** (2.4 mg) in the order of elution.

5-Heptylresorcinol (**6**): Colorless powder. EI-MS *m/z* 208 $(M)^+$, 166, 137, 124; HR-MS Calcd for $C_{13}H_{22}O_2$: 208.1462, Found: 208.1457; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 6.24 (2H, d, $J$=2.2 Hz, 4-H and 6-H), 6.17 (1H, t, $J$=2.2 Hz, 2-H), *ca.* 4.8 (2H, br s, 1-OH and 3-OH), 2.48 (2H, m, 7-$H_2$), 1.57 (2H, m), 1.4~1.2 (8H, m, 8-$H_2$ to 12-$H_2$), 0.88 (3H, t, $J$=7.0 Hz, 13-$H_3$); $^{13}C$ NMR (25 MHz, $CDCl_3$) $\delta$ 159.3 (C-1 and C-3), 146.3 (C-5), 107.9 (C-4 and C-6), 101.1 (C-2), 37.0 (C-7), 32.4 (C-8), 30.3 (C-9 and C-10), 33.0 (C-11), 23.7 (C-12), 14.4 (C-13).

Methyl 6-Heptyl-4-hydroxysalicilate (**7**): Colorless powder. EI-MS *m/z* 266 $(M)^+$, 234, 192, 163, 150; HR-MS Calcd for $C_{15}H_{22}O_4$: 266.1517, Found: 266.1522; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 11.66 (1H, s, 2-OH), 6.28 (1H, d, $J$=2.6 Hz, 5-H), 6.23 (1H, d, $J$=2.6 Hz, 3-H), 5.34 (1H, br s, 4-OH), 3.92 (3H, s, $COOCH_3$), 2.83 (2H, m, 8-$H_2$), 1.52 (2H, m), 1.4~1.2 (8H, m, 9-$H_2$ to 13-$H_2$), 0.89 (3H, t, $J$=7.0 Hz, 14-$H_3$); $^{13}C$ NMR (25 MHz, $CDCl_3$) $\delta$ 163.6 (C-7), 149.1 (C-6), 111.8 (C-3), 101.8 (C-5), 52.1 ($OCH_3$), 37.5 (C-8), 33.04, 32.98, 30.8, 30.3 (C-9 to C-12), 23.7 (C-13), 14.4 (C-14).

Methylation of KS-501 (**1**)

To a solution of **1** (5 mg) in MeOH (0.5 ml) diazomethane ether (1 ml) prepared from bis-(*N*-methyl-*N*-nitroso)terephthalamide (9 g) in ether (40 ml), was added and stirred for 1 hour at room

temperature. The reaction mixture was evaporated and the residue was chromatographed on a silica gel column with $CHCl_3$-MeOH (20:1) to give a colorless powder of **8** (4 mg). SI-MS *m/z* 633 $(M+H)^+$, 471, 411, 249; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$ 6.71 (1H, d, $J$=2.2 Hz, 3'-H), 6.7~6.6 (3H, m, 2-H, 4-H and 6-H), 6.52 (1H, d, $J$=2.2 Hz, 5'-H), 5.59 (1H, d, $J$=1.8 Hz, 1''-H), 4.28 (1H, dd, $J$=3.8 and 1.8 Hz, 2''-H), *ca.* 4.15 (2H, 3''-H and 4''-H), 3.82 (3H, s), 3.80 (3H, s, 3-$OCH_3$ and 4'-$OCH_3$), 3.75 (1H, br t, $J$=*ca.* 6 Hz, 5''-H), 3.63 and 3.60 (2H, ABq in ABX, $J$=11.2, 7.0 and 5.7 Hz, 6''-$H_2$), 2.69 (2H, dd, $J$=9.6 and 6.1 Hz, 8'-$H_2$), 2.61 (2H, dd, $J$=8.9 and 8.5 Hz, 7-$H_2$), 1.64 (4H, m), 1.4~1.2 (16H, m, 8-$H_2$ to 12-$H_2$ and 9'-$H_2$ to 13'-$H_2$), 0.89 (3H, t, $J$=6.9 Hz) 0.87 (3H, t, $J$=7.0 Hz, 13-$H_3$ and 14'-$H_3$).

Methanolysis of KS-502 (**2**)

A solution of **2** (5 mg) in 5% HCl-MeOH (0.5 ml) was stirred for 2 hours at room temperature. The reaction mixture was diluted with EtOAc (30 ml) and washed with water and satd NaCl (each 30 ml). The EtOAc solution was dried over magnesium sulfate and evaporated *in vacuo* to give a residue which was chromatographed on a silica gel column with $CHCl_3$-MeOH (5:1) giving **9** (2.8 mg). $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$ 6.62 (1H, d, $J$=2.2 Hz), 6.56 (1H, d, $J$=2.2 Hz), 6.28 (1H, d, $J$=2.4 Hz), 6.22 (1H, d, $J$=2.4 Hz, 3-H, 5-H, 3'-H and 5'-H), 2.96 (2H, br t, $J$=*ca.* 7.5 Hz), 2.88 (2H, dd, $J$=8.0 and 7.8 Hz, 8-$H_2$ and 8'-$H_2$), *ca.* 1.6 (4H, m), 1.4~1.2 (16H, m, 9-$H_2$ to 13-$H_2$ and 9'-$H_2$ to 13'-$H_2$), 0.89 (3H, t, $J$=6.9 Hz), 0.84 (3H, t, $J$=7.0 Hz, 14-$H_3$ and 14'-$H_3$).

The water layer was evaporated to driness to give a sugar portion (2.1 mg), which was *p*-bromobenzoylated and purified in a similar manner to that described above to afford **5** (1.6 mg). $[\alpha]_D^{23}$ +134° (*c* 0.1, $CHCl_3$).

Methanolysis of 2,4-Dihydroxy-6-heptylbenzoic Acid 4-Carboxy-3-heptyl-5-hydroxyphenyl Ester (**9**)

A solution of **9** (4 mg) in 17% HCl-MeOH (2 ml) was heated to reflux for 2 hours. The reaction mixture was evaporated and the residue was chromatographed on a silica gel column with $CHCl_3$-MeOH (30:1) to give methyl 6-heptyl-4-hydroxysalicilate (**7**) (2.0 mg) and 5-heptylresorcinol (**6**) (0.5 mg) in the order of elution.

Methylation of KS-502 (**2**)

To a solution of **2** (5 mg) in MeOH (0.5 ml) diazomethane ether (1 ml) prepared in a manner described above was added and stirred for 1 hour at room temperature. The reaction mixture was evaporated and the residue was chromatographed on a silica gel column with $CHCl_3$-MeOH (20:1) to give a colorless powder of **10** (4 mg). EI-MS *m/z* 529, 411, 326, 280, 249, 196; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$ 6.81 (1H, d, $J$=2.0 Hz, 3-H), 6.72 (1H, d, $J$=2.2 Hz, 3'-H), 6.71 (1H, d, $J$=2.0 Hz, 5-H), 6.53 (1H, d, $J$=2.2 Hz, 5'-H), 5.60 (1H, d, $J$=1.7 Hz, 1''-H), 4.27 (1H, dd, $J$=3.6 and 1.7 Hz, 2''-H), *ca.* 4.1 (2H, m, 3''-H and 4''-H), 3.86 (3H, s), 3.83 (3H, s), 3.82 (3H, s, each $OCH_3$), *ca.* 3.75 (1H, m, 5''-H), *ca.* 3.6 (2H, AB in ABX, 6''-$H_2$), 2.69 (2H, m, 8'-$H_2$), 2.56 (2H, m, 8-$H_2$), 1.7~1.5 (4H, m), 1.4~1.2 (16H, m, 9-$H_2$ to 13-$H_2$ and 9'-$H_2$ to 13'-$H_2$), 0.89 (3H, t, $J$=6.7 Hz), 0.87 (3H, t, $J$=7.1 Hz, 14-$H_3$ and 14'-$H_3$).

Methanolysis of 2,4'-Di-*O*-methyl KS-502 Methyl Ester (**10**)

A solution of **10** (8 mg) in 17% HCl-MeOH (0.5 ml) was heated to reflux for 4 hours. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in EtOAc (30 ml). EtOAc solution was washed with water and satd NaCl (each 30 ml), and dried over magnesium sulfate. The organic layer was evaporated and the residue was chromatographed on a silica gel column with *n*-hexane-EtOAc (10:1~3:1) to give **11** (1 mg), **12** (1 mg) and **13** (2 mg) in the order of elution.

Methyl 6-Heptyl-4-hydroxy-2-*O*-methylsalicilate (**11**): Colorless powder. EI-MS *m/z* 280 $(M)^+$, 248, 196, 177, 164; $^1H$ NMR (100 MHz, DMSO-$d_6$) $\delta$ 10.24 (1H, s, 2-OH), 6.29 (2H, s, 3-H and 5-H), 3.77 (3H, s), 3.72 (3H, s, each $OCH_3$), 1.6~1.1 (10H, m, 8-$H_2$ to 12-$H_2$), 0.86 (3H, t, $J$=7.0 Hz, 13-$H_3$).

Methyl 6-Heptyl-4-methoxysalicilate (**12**): Colorless powder. EI-MS *m/z* 280 $(M)^+$, 249, 196, 177; $^1H$ NMR (100 MHz, DMSO-$d_6$) $\delta$ 9.70 (1H, br s, 4-OH), 6.28 (1H, d, $J$=2.2 Hz), 6.22 (1H, d, $J$=2.2 Hz, 3-H and 5-H), 3.72 (3H, s), 3.67 (3H, s, each $OCH_3$), 1.6~1.1 (10H, m, 8-$H_2$ to 12-$H_2$), 0.85 (3H, t, $J$=7 Hz, 13-$H_3$).

6-Heptyl-4-methoxysalicilic Acid 3-Heptyl-5-methoxy-4-methoxycarbonylphenyl Ester (**13**): Colorless

powder. EI-MS *m/z* 496, 468, 280, 249, 196, 177; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 6.78 (1H, d, $J$=2.0 Hz, 3-H), 6.69 (1H, d, $J$=2.0 Hz, 5-H), 6.41 (1H, d, $J$=2.5 Hz, 3'-H), 6.38 (1H, d, $J$=2.5 Hz, 5'-H), 3.87 (3H, s), 3.823 (3H, s), 3.815 (3H, s, each $OCH_3$), 2.93 (2H, ABq, 8'-$H_2$), 2.57 (2H, ABq, 8-H), *ca.* 1.7 (2H, m), *ca.* 1.6 (2H, m), 1.5~1.2 (16H, m, 9-$H_2$ to 13-$H_2$ and 9'-$H_2$ to 13'-$H_2$), 0.89 (3H, t, $J$=7.1 Hz), 0.84 (3H, t, $J$=7.0 Hz, 14-$H_3$ and 14'-$H_3$).

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