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STRUCTURE DETERMINATION AND ABSOLUTE CONFIGURATION OF PISIFERDIOL, A NEW PHENOLIC DITERPENOID WITH A REARRANGED ABIETANE SKELETON from Chamaecyparis pisifera Endl

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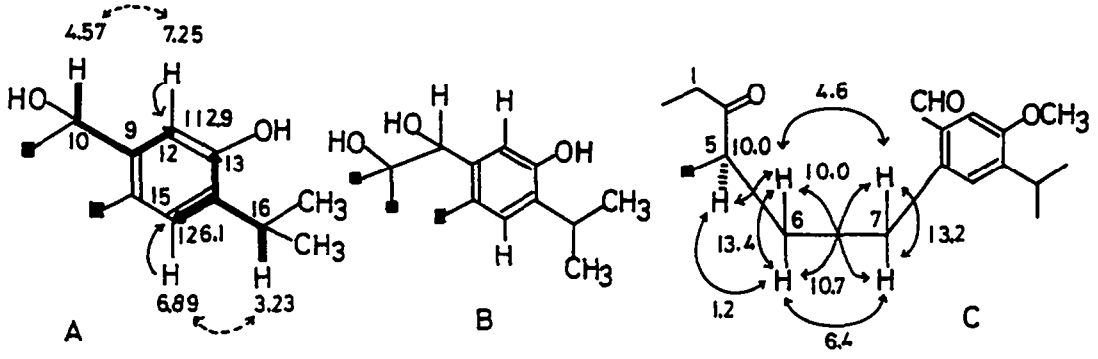
Abstract - Pisiferdiol 1, isolated from C. pisifera, was shown to be a new phenolic diterpenoid with a rearranged abietane skeleton. The structure was established by several degradation reactions and from spectral evidence including LSPD experiments and NOE difference spectra. The absolute configuration was determined by employing the dibenzoate exciton chirality method on the di-p-bromobenzoate 3.

In the course of our search for mite growth regulators in higher plants,[1,2] we isolated from C. pisifera a phenolic diterpenoid, pisiferdiol 1.[3] We now describe in full the determination of the structure and absolute configuration of pisiferdiol 1.

Pisiferdiol 1, had $[\alpha]_D^{27}$ +23.2° (c=0.12, MeOH), molecular formula $C_{20}H_{30}O_3$ (HRMS: M^+ obsd 318.3350, calcd 318.3332) and λmax nm (ε) : 217(7200) and 280 (2100). The IR spectrum of 1 showed OH absorptions (3550 and 3350 cm^{-1}). The three oxygens were evidently present as OH groups, since pisiferdiol gave a diacetate 2 which still showed OH absorption(3570 cm^{-1}). The phenolic nature of one OH group was indicated by the CO vibration at 1750 cm^{-1} in the acetate 2 and by the ^{13}C NMR spectrum of 1, which showed a signal at 152.5 ppm (s, aromatic C attached to O). The two remaining OH groups in 1 were clearly secondary(78.9 ppm, d) and tertiary(76.6 ppm, s), as shown in the ^{13}C NMR spectrum. The ^1H NMR spectrum of 1 showed the following groups: two para-aromatic protons(6.89 and 7.24 ppm, each s), two tertiary methyls(0.89 and 0.92 ppm, each s)(assigned to a gem-dimethyl, based on the observation of only one quaternary carbon(34.3 ppm, s) in the ^{13}C NMR spectrum), an isopropyl group [1.20 ppm(3H, d, J=7.0Hz), 1.22 ppm(3H, d, J=7.0Hz), 3.23 ppm(1H, sep, J=7.0Hz)], and a methine (4.57 ppm, s) attached to the OH-bearing carbon(it shifted to 6.03 ppm upon acetylation).

Partial structure A surrounding the aromatic ring in 1 was deduced by {^{1}H- ^{1}H} long range selective decoupling(←----→) and {^{13}C-^{1}H} selective decoupling(⟶) experiments. The linkages of C-9 to C-10 and C-14 to C-16 were confirmed by long range selective proton decoupling (LSPD)[4] (⟹) experiments; irradiation of H-10 sharpened a C-12 signal and that of H-16 also sharpened both the C-13 and C-15 signals.



The presence of a vicinal diol group in 1 was established as a consequence of manganese dioxide oxidation of O-methylpisiferdiol 3, prepared from 1 by methylation(dimethyl sulfate). This gave a keto aldehyde 4, $C_{21}H_{30}O_3$, that showed a saturated ketone(1700 cm^{-1}) and a benzaldehyde moiety [1680 cm^{-1}, λmax nm(ε): 227(25400), 267 (12800) and 323(4800), 10.2 ppm(1H, s)]. The presence of a saturated ketone and a benzaldehyde moiety in 4 was further confirmed by sodium borohydride reduction of 4 into a diol 5, characterized by the presence of a secondary alcohol(4.15 ppm, 1H, brs) and a benzyl alcohol moiety [(4.59, 1H, d, J=17Hz) and 4.77(1H, d, J=17Hz)]. The ketoaldehyde 4, formed by the rather unusual oxidative cleavage of the vicinal diol in 3, was also obtained by the more normal periodate oxidation of 3. Thus, the partial structure A of 1 was extended to B. Extensive spin decoupling experiments in the ^{1}H NMR spectrum of 4 revealed the relationships between the benzylic methylene protons at C-7 and those at C-6 and C-5, as shown in partial structure C. The double doublet signal of a C-5 proton (2.19 ppm, J=10.0, 1.2 Hz) suggests that the C-4 and C-11 that are linked to C-5 do not bear hydrogens. Jones oxidation of 3 yielded a keto acid that was converted to a methyl ester 6, $C_{22}H_{32}O_4$ (M^+, m/z 360), νmax: 1705 and 1695 cm^{-1} and λmax nm(ε): 245(8900) and 297(3500). In the ^{1}H NMR spectrum of 6, three active hydrogens adjacent to a carbonyl group, C_1-H_2(2.20 - 2.40 ppm, m) and C_5-H(2.16 ppm, dd, J=11.5, 2.5Hz), were observed. In order to confirm this, 6 was treated with $NaHCO_3$ in D_2O. The partially deuterated product showed three M^+ peaks at m/z 361, 362 and 363, in addition to the undeuterated M^+(m/z 360), in the ^{1}H NMR spectrum of which a marked decrease in peak intensity was observed on the C-1 and C-5 protons. These oxidation and deuterium-labeling experiments made it possible to construct the seven membered ring structure with a vicinal diol group. Thus, the plane structure 1 was proposed for pisiferdiol.

The cis relationship of the vicinal diol group in 1 was obtained from the formation of an acetonide 7 from 3 upon treatment with acetone and p-toluenesulfonic acid. The relative stereochemistry of 1 was confirmed by the nuclear Overhauser effect(NOE) difference spectra[4] of 3(Fig. 1). The pseudoaxial relationships btween the three protons at C-5, C-7 and C-10 of 3 were shown by significant NOE enhancements as shown in Fig. 1. All other NOE enhancements observed in the spectra of 3 were well in accordance with the proposed relative stereochemistry.

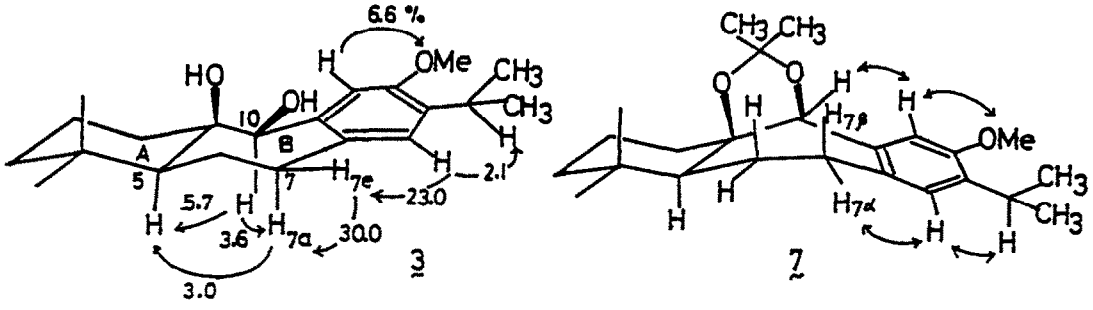
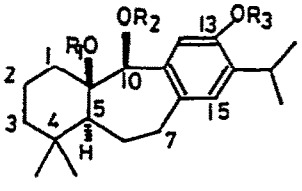
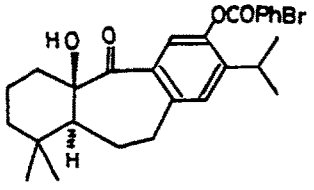


Fig.1 The NOEs in the NOE difference spectra of 3 and 7(360 MHz ^{1}H NMR) The NOEs observed are denoted by arrows.



1. R_1=R_2=R_3=H
2. R_1=H, R_2=R_3=Ac
3. R_1=R_2=H, R_3=Me
7. R_1=R_2=-$C(CH_3)_2$-, R_3=Me
8. R_1=H, R_2=R_3=COPhBr
9. R_1=R_2=H, R_3=COPhBr



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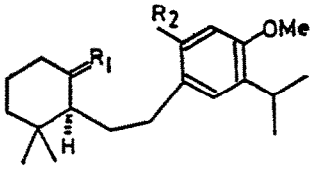
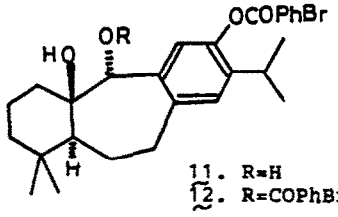


4. R_1=O, R_2=CHO
5. R_1=OH,H, R_2=CH_2OH
6. R_1=O, R_2=CO_2Me



11. R=H
12. R=COPhBr

Fig. 2 Pisiferdiol and Derivatives

The absolute configuration of 1 was finally determined by employing the dibenzoate exciton chirality method[6] on the di-p-bromobenzoate 12, which was synthesized as follows: The p-bromobenzoate 9 was oxidized with DMSO-Ac_2O to give a 10-ketonic product 10 that, upon sodium borohydride reduction, gave a trans-diol 11 as a sole product. 11 was converted to 12 by treatment with p-bromobenzoyl chloride and dimethylaminopyridine in pyridine.

The ring B in 12 has chair-like conformation similar to that of 3, because the coupling constants of the C-7 benzylic methylene protons [H_{7a}(3.18 ppm, ddd, J=13.4, 10.0, 1.0Hz) and H_{7e}(2.81 ppm, ddd, J=13.6, 6.7, 2.0)] were almost identical with those of 3 [H_{7a}(2.62 ppm, ddd, J=14.3, 10.9, 1.0) and H_{7e}(2.79 ppm, ddd, J=14.0, 7.2, 1.5)], but differ from those of the acetonide 7 that has boat-like conformation on the ring B [$H_{7\beta}$(3.56 ppm, ddd, J=14.9, 10.0, 8.0) and $H_{7\alpha}$(2.49 ppm, ddd, J=14.2, 10.9, 2.9)], as shown in the NOE difference spectrum of 7 (Fig. 1). The CD spectrum of 12 gave split-type Cotton effects at 249 nm($\Delta\varepsilon$ +8.15) and 225 nm($\Delta\varepsilon$ -4.07) in MeOH. The positive sign of the first Cotton effect indicates that two benzoate chromophores are twist in a clockwise sense. In comparison, the CD spectrum of the di-p-bromobenzoate 8 was measured, showing only a Cotton effect at 250 nm ($\Delta\varepsilon$ -11.04). The latter observation may be ascribed to the secondary 10-p-bromobenzoyloxy group in 8 being situated almost in the same plane as that of the benzene ring. Thus, the absolute configuration of pisiferdiol was established as 1.

Pisiferdiol is presumably biosynthesized from an abietane-type pisiferal that has been isolated from *C. pisifera*[7] through a skeletal rearrangement 9(10 → 20): a 1,2-migration of C_9-C_{10} bond to the C_{20} aldehyde group and subsequent introduction of a hydroxy group at C_{10}. Pisiferdiol is a new product belonging to the novel diterpenoids with the 9(10→20)-abeo-abietane skeleton, of which barbatusol,[8] icetexone and romologarzone,[9] and nilgherron A and B[10] had been isolated from Labiate plants.

Experimental

Optical rotations were measured with a JASCO DIP-4 polarimeter and CD spectra with a JASCO J-40 instrument. IR spectra were recorded in $CHCl_3$ solution with a JASCO A-3 spectrometer, UV spectra in MeOH solution with a JASCO UVIDEC-505 spectrophotometer, ^1H NMR spectra with a JEOL JNM-FX 200 (199.6 MHz), ^{13}C NMR spectra with a JEOL JNM-FX 200(50.3 MHz) Fourier transform spectrometer(tetramethylsilane as internal standard). Low resolution mass spectra(MS) were recorded with a JEOL JMS-D100 spectrometer, and high resolution mass spectra(HRMS) with a JEOL JMS-01SG2 spectrometer. A Bruker MW 360wb(360 MHz)Fourier transform spectrometer was used for NOE difference spectra. Kieselgel 60PF (Merck) self made plates were used for analytical and preparative TLC. Silica gel(Fuji BW-200) was used for column chromatography.

Isolation of pisiferdiol 1. C. pisifera Endl was collected at the surroundings of Inabu-cho, Aichi prefecture in July 1982. EtOAc soluble weak acidic fraction(67 g) was obtained from the MeOH extract of C. pisifera (10 kg). The fraction(10 g) was chromatographed on a slica gel (500 g) column with n-hexane-ethyl acetate mixture with stepwise increases in the ratio of the latter. Known diterpenoids, pisiferic acid(4.4 g) and O-methylpisiferic acid(170 mg) were obtained from the eluate of 15% EtOAc in n-hexane, and pisiferdiol 1 (350 mg) was eluted with 30% EtOAc in n-hexane. Recrystallization of the crude material from n-hexane gave pure 1 as colorless needles, mp 134-135°C, $[\alpha]_D^{27}$ +23.2°(c =0.12, MeOH), ν max: 3550, 2960, 2870, 1620, 1420 and 1370 cm^{-1}. λmax nm(ε) : 217(7200) and 280(2900). ^1H NMR(δ, $CDCl_3$): 0.89(3H, s), 0.92(3H, s), 1.20(3H, d, J=7.0Hz), 1.22(3H, d, J=7.0Hz), 3.23(1H, sep, J=7.0 Hz), 4.57(1H, s), 6.89(1H, s) and 7.24(1H, s). ^{13}C NMR(δ, $CDCl_3$): 18.4(t), 21.9(q), 22.4(q), 23.1(q), 24.5(t), 26.5 (d), 32.5(q), 34.1(t), 34.5(s), 34.9(t), 42.5(t), 56.2(d), 76.6(s) , 78.9(d), 112.98d), 126.1(d), 132.2(s), 133.6(s), 135.6(s) and 152.6(s). HRMS (Found: 318.2177. Calcd for $C_{20}H_{30}O_3$: 318.2195). MS[m/z(%)]: 318(M^+,10), 301 (100), 285(30), 271(29), 215(35), 192(69), 162(60), 111(45) and 70(62).

Acetylation of pisiferdiol. To a cooled solution of pisiferdiol (9.5 mg) in pyridine(0.4 ml) was added 0.4 ml of acetic anhydride. The reaction mixture was kept at room temperature overnight, poured into ice-water, and extracted with EtOAc. The organic layer was washed with 5% HCl, 10% $NaHCO_3$, water and brine, dried(Na_2SO_4), and evaporated in vacuo. The product was purified by preparative TLC with 30% EtOAc in n-hexane to give 11 mg of diacetate 2. Compound 2: uncrystallized colorless solid, λmax nm(ε) : 215(11600), 265(1300) and 273(1000). ν max: 3570, 2950, 2850, 1750, 1735, 1500, 1370 and 1240 cm^{-1}. ^1H NMR(δ, $CDCl_3$): 0.87(3H, s), 0.95(3H, s), 1.17(3H, d, J=7.0Hz), 1.22(3H, d, J=7.0Hz), 2.20(3H, s), 2.30(3H, s), 2.95(1H, sep, J=7.0Hz), 6.03(1H, s), 7.01 (1H, s) and 7.04(1H, s). HRMS (Found: 402.2411. Calcd for $C_{24}H_{34}O_5$: 402.2406). MS[m/z(%)]: 402 (M^+, 13), 359(13), 343(100), 328(33), 301(100), 234(19), 204(19) and 162(28).

Methylation of pisiferdiol. Pisiferdiol(63 mg) in 8 ml of EtOH was refluxed in the presence of 22 μl of dimethylsulfate and 35 μl of 25% NaOH for 2 hr. The reaction mixture was poured into ice-water, and extracted with EtOAc. The organic layer was washed with water and brine, dried(Na_2SO_4), and evaporated in vacuo. The residue was purified by HPLC(LiChrosorb Si-60; 8 x 300 mm, 30% EtOAc in n-hexane, 1.5 ml/min) to give 36.5 mg of pure O-methylpisiferdiol 3 as an oil. Compound 3: λ[max nm(ε)] : 221(8600) and 278(3000). ν max: 3600, 2950, 2870, 1620, 1500, 1460 and 1260 cm^{-1}. ^1H NMR(δ, $CDCl_3$): 0.88 (3H, s), 0.93(3H, s), 1.18(3H, d, J=7.0Hz), 1.22(3H, d, J=7.0Hz), 2.62(1H, ddd, J=14.3, 10.9, 1.0Hz), 2.79(1H, ddd, J=14.0, 7.2, 1.5 Hz), 3.27(1H, sep, J=7.0Hz), 4.74(1H, s), 6.91(1H, s) and 7.12(1H, s). HRMS(Found: 332.2367. Calcd for $C_{21}H_{32}O_3$: 332.2382). MS[m/z(%)]: 332(M^+, 10), 314(100), 299(21), 206(63), 191(33), 176(35), 110(26) and 70(26).

Manganese dioxide oxidation of 3. Compound 3(11.5 mg) in 5 ml of $CHCl_3$ was stirred with 120 mg of MnO_2 for 1hr. The reaction mixture was passed through a short silica gel column for removing the inorganic materials and concentrated in vacuo. The residue was purified by HPLC(LiChrosorb Si-60; 8 x 300mm, 20% EtOAc in n-hexane, 1.5 ml/min) to give 11 mg of 4 as an oil. Compound 4: λ max nm(ε) : 227(25400), 267(12800) and 323(4800). ν max: 2950, 2870, 1700, 1680, 1600, 1500, 1460, 1400, 1250, 1180 and 1060 cm^{-1}. ^1H NMR(δ, $CDCl_3$): 0.73(3H, s), 1.01 (3H, s), 1.23(6H, J=7.0Hz), 2.19(1H, dd, J=10.0, 1.2Hz), 2.71(1H, ddd, J=13.2, 10.0, 6.4Hz), 3.00(1H, ddd, J=13.2, 10.8, 4.6Hz), 3.34 (1H, sep, J=7.0Hz), 3.87(3H, s), 7.07(1H, s) and 7.31(1H, s). HRMS (Found: 330.2190. Calcd for $C_{21}H_{30}$ O_3: 330.2195). MS[m/z(%)]: 330 (M^+, 10), 204(100), 176(52), 161(30) and 111(42).

$NaBH_4$ reduction of 4. To a cooled solution of 4(2.4 mg) in 0.5 ml of MeOH was added 2 mg of $NaBH_4$. The reaction mixture was stirred at room temperature for 1 hr, poured into ice-water, and extracted with EtOAc. The organic layer was washed with brine, dried(Na_2SO_4) and concentrated in vacuo. The product was purified by preparative TLC with 30% EtOAc in n-hexane to give 1.5 mg of 5 as an oil. Compound 5: λ max nm(ε) : 227(8200) and 278(2200). ν max: 3600, 2950, 2870, 1610, 1500, 1460, 1400 and 1260 cm^{-1}. ^1H NMR(δ, $CDCl_3$: 0.91(3H, s), 0.95(3H, s), 2.21(3H, d, J=7.0Hz), 2.22(3H, d, J=7.0Hz), 3.29 (1H, sep, J=7.0Hz), 4.15(1H, bs), 4.59(1H, d, J=13.0Hz), 4.77(1H, d, J=13.0Hz), 6.83(1H, s) and 7.04(1H, s). HRMS(Found: 334.2507. Calcd for $C_{21}H_{34}O_3$: 334.2507). MS[m/z(%)]: 334(M^+, 10), 316(34), 301(12), 273(100), 206(31), 190(80), 177(75), 123(60) and 69(62).

Preparation of methyl ester 6. To a cooled solution of 3 (2 mg) in 1 ml of acetone was added 20 l of Jones reagent. The reaction mixture was stirred at 0° C for 1 hr. The excess CrO_3 was destroyed by the addition of i-PrOH. The mixture was concentrated in vacuo, and the residue was diluted with water. It was then extracted with CH_2Cl_2. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue dissolved in 1 ml of 1% HCl in MeOH was refluxed for 2 hr. After removing MeOH, the residue was extracted with EtOAc. The organic layer was washed with brine, dired(Na_2SO_4), and concentrated in vacuo. The product was purified by HPLC(Develosil 60-5, 4 x 300 mm, 15% EtOAc in n-hexane, 1.2 ml/min) to give 1.5 mg of 6 as an oil. Compound 6: λmax nm(ε): 213(30800), 245(8900) and 297(3500). νmax: 2900, 2850, 1705, 1695, 1600, 1490, 1450, 1420 and 1200 cm^{-1}. ^1H NMR(δ, $CDCl_3$): 0.75(3H, s), 0.98(3H, s), 1.21(6H, d, J=7.0Hz), 2.16(1H, dd, J=11.5, 2.5Hz), 3.84(3H, s), 3.87(3H, s), 7.05(1H, s) and 7.32(1H, s). HRMS(Found: 360.2330. Calcd for $C_{22}H_{32}O_4$: 360.2327). MS[m/z(%)]: 360(M^+, 4), 235(21), 203(12), 191(12) and 186(12).

Deuterium labeling of methyl ester 6. A solution of 6 (1 mg) and $NaHCO_3$(1 mg) in D_2O(0.2 ml) and dioxane(1.5 ml) was refluxed for 1 hr. After evaporation of the solvent, the same treatment in D_2O(0.2 ml) and dioxane(1.5 ml) was repeated twice. The reaction mixture was then poured into 0.5 ml of D_2O, acidified to pH 2, and extracted with EtOAc. The organic layer was washed with brine, dried(Na_2SO_4), and concentrated in vacuo. The product was treated with ethereal diazomethane solution, and concentrated to give deuterated 6, MS(m/z): 363(M^+ + 3), 362(M^+ + 2), 361(M^+ + 1) and 360(M^+).

Preparation of acetonide 7. A solution of 3 (6.6 mg) and 1.5 mg of p-TsOH in 3 ml of dry acetone was stirred at room temperature for 1 hr. To the reaction mixture was added excess amount of $NaHCO_3$ powder. It was concentrated in vacuo. The residue was extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried(Na_2SO_4), and concentrated in vacuo. The residue was purified by preparative TLC(15% EtOAc in n-hexane) to give 6.8 mg of 7 as an oil. Compound 7: λmax nm(ε): 228(8800) and 278(3000). νmax: 2950, 2870, 1500, 1460, 1420, 1380, 1260, 1200, 1040, and 900 cm^{-1}. ^1H NMR(δ, $CDCl_3$): 0.79(3H, s), 0.95(3H, s), 1.16(3H, d, J=7.0Hz), 1.23(3H, d, J=7.0Hz), 1.46(3H, s), 1.56(3H, s), 2.49(1H, ddd, J=14.2, 10.9, 2.7Hz), 3.27(1H, sep, J=7.0Hz), 3.56(1H, ddd, J=14.7, 10.0, 8.0Hz), 4.56(1H, s), 6.65(1H, s) and 6.91(1H, s). HRMS(Found: 372.2650. Calcd for $C_{24}H_{36}O_3$: 372.2664). MS[m/z(%)]: 372(M^+,50), 357(38), 314(100), 296(90), 204(20), 195(18), 183(20), 133(20) and 91(9).

p-Bromobenzoylation of 1. To a solution of 1 (10 mg) and 4-dimethylaminopyridine(100 mg) in 1 ml of dry pyridine was added p-bromobenzoyl chloride(42 mg). The reaction mixture was stirred at 40°C for 48 hr, poured into ice-water, and extracted with EtOAc. The organic layer was washed with 5% HCl, 10% $NaHCO_3$ and brine, dried(Na_2SO_4), and concentrated in vacuo. The residue was purified by preparative TLC(15% EtOAc in n-hexane) to give di-p-bromobenzoate 8 (16.4 mg, R_f=0.53) and 13-p-bromobenzoate 9 (2.4 mg, R_f=0.25). Di-p-bromobenzoate 8: λmax nm(ε): 243(44900), 272(6100) and 283(3200). νmax: 3600, 2950, 2850, 1720, 1590, 1270, 1250, 1100 and 1050 cm^{-1}. ^1H NMR(δ, $CDCl_3$): 0.90(3H, s), 0.96(3H, s), 1.18(3H, d, J=7.0Hz), 1.23(3H, d, J=7.0Hz), 6.29(1H, s), 7.11(1H, s), 7.12(1H, s), 7.60(2H, d, J=8.6Hz), 7.64(2H, d, J=8.6Hz), 8.01(2H, d, J=8.6Hz) and 8.02(2H, d, J=8.6Hz). CDλext: 250 nm(Δε-11.0). 13-p-bromobenzoate 9: λmax nm(ε): 245(20000), 275(3800) and 283(2000). νmax: 3600, 2950, 2850, 1730, 1590, 1260, 1100, 1070 and 1010 cm^{-1}. ^1H NMR(δ, $CDCl_3$): 0.90(3H, s), 0.95 (3H, s), 1.20(3H, d, J=7.0Hz), 1.23(3H, d, J=7.0Hz), 3.01(1H, sep, J=7.0Hz), 4.73(1H, s), 7.07(1H, s), 7.32(1H, s), 7.66(2H, d, J=8.6Hz) and 8.05(2H, d, J=8.6 Hz). MS[m/z(%)]: 502(M^+, 7), 485(35), 483(34), 470(14), 468(15), 347(14), 345(14) and 185(100).

DMSO oxidation of 9. Compound 9 (2.6 mg) in dry DMSO(20 μl) and acetic anhydride(15 μl) was stirred at room temperature for 3 hr, poured into water, and extracted with EtOAc. The organic layer was washed with 10% $NaHCO_3$ and brine, dried(Na_2SO_4), and concentrated in vacuo. The residue was purified by preparative TLC(20% EtOAc in n-hexane) to give a ketone 10 (1.5 mg). Compound 10: λmax nm(ε): 247(17800), 272(3200) and 283(1800). νmax: 3600, 2950, 2870, 1740, 1680, 1600, 1260, 1200, 1170, 1070 and 1010 cm^{-1}. ^1H NMR(δ, $CDCl_3$): 0.92(3H, s), 1.07(3H, s), 1.22(3H, d, J=7.0Hz), 1.26(3H, d, J=7.0Hz), 7.07(1H, s), 7.12(1H, s), 7.67(2H, d, J=8.6Hz) and 8.05(2H, d, J=8.6Hz). HRMS(Found: 500.1369. Calcd for $C_{27}H_{31}O_4{}^{81}Br$: 500.1386). MS[m/z(%)]: 500(M^+, 6),498(7), 375(4), 373(5), 362(19), 360(29), 185(91), 183(100), 157(19), 155(17) and 101(30).

NaBH$_4$ reduction of 10. Compound 10(1.5 mg) and NaBH$_4$(1 mg) in 0.5 ml of MeOH was stirred at 0°C for 2 hr, poured into water, and extracted with EtOAc. The organic layer was washed with brine, dried(Na_2SO_4), and concentrated in vacuo. The residue was purified with preparative TLC to give 10-epimer of 9, 11(1.1 mg). Compound 11: λmax nm(ε) : 245(21000), 275(4000) and 283(2699). νmax: 3600, 2950, 2870, 1730, 1610, 1580, 1500, 1460, 1360, 1340, 1260, 1200, 1030 and 970 cm^{-1}. ^{1}H NMR(δ, $CDCl_3$): 0.93(3H, s), 0.98(3H, s), 1.19(3H, d, J=7.0Hz), 1.26(3H, d, J=7.0Hz), 5.30(1H, s), 6.97(1H, s), 7.09(1H, s), 7.66(2H, d, J=8.5Hz) and 8.05(2H, d, J=8.5Hz). MS[m/z(%)]: 502(M^+, 7), 500(8), 485(35), 483(34), 470(14), 468(15), 347(14), 345(14), 185(100) and 183(97).

p-Bromobenzoylation of 11. Compound 11(1 mg) was treated with p-bromobenzoyl chloride(4 mg) and 4-dimethylaminopyridine(20 mg) in 0.4 ml of pyridine at 80°C for 72 hr. The reaction mixture was poured into water, and extracted with EtOAc. The organic layer was washed with 5% HCl, 10% $NaHCO_3$ and brine, dried(Na_2SO_4) and concentrated in vacuo. The residue was purified by preparative TLC to give di-p-bromobenzoate 12. Compound 12: λmax nm(ε): 243(45000), 272(6400) and 283(3500). νmax: 3600, 2950, 2850, 1720, 1590, 1270, 1250, 1100 and 1050 cm^{-1}. ^{1}H NMR(δ, $CDCl_3$): 0.97(3H, s), 1.04(3H, s), 1.18(3H, d, J=7.0Hz), 1.21(3H, d, J=7.0Hz), 2.81(1H, ddd, J=13.6, 6.7, 2.0Hz), 2.99(1H, sep, J=7.0Hz), 3.18(1H, ddd, J=13.4, 10.0, 1.0Hz), 5.58(1H, brs), 7.07(1H, s), 7.21(1H, s), 7.60(2H, d, J=8.5Hz), 7.66 (2H, d, J=8.5Hz), 7.88(2H, d, J=8.5Hz) and 8.04(2H, J=8.5Hz). CD λext in MeOH: 249 nm(Δε+8.2) and 225(Δε-4.1).

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