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Determination of Isoprenyl and Lavandulyl Positions of Flavonoids from *Sophora flavescens* by NMR Experiment

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All fifteen flavonoids (**1~15**) have been isolated from the roots of *Sophora flavescens* (Leguminosae) as active principles of the cytotoxic property toward human tumor cell lines such as A549, SK-OV-3, SK-Mel-2, XF498 and HCT15, *in vitro*. By means of spectral analyses, particularly by the aid of various two dimensional NMR experiments, all ^{1}H-NMR and ^{13}C-NMR signals of **1~15** were completely assigned, and thus the structures of **1~15** were established unambiguously.

Key words : *Sophora flavescens*, NMR, kushenol, COLOC, cytotoxicity, antitumor

INTRODUCTION

The flavonoids in *Sophora flavescens* (Leguminosae) are particularly called as kushen flavonoids or kushenols because they are exclusively abundunt in the species (Kushen is the name of the species in Chinese), which are members of the flavanone or flavonol (dihydroflavonol) with isopentenyl or lavandulyl side chains. Although lots of kushenols had been isolated and characterized so far from this species, in some of them, the partial structures, *i.e.*, the points of attachment of the isopentenyl or lavandulyl side chains on the flavone skeleton were remained partially unknown (Wu *et al.*, 1985). However, by the present study exploiting various HETCOR NMR experiments on **1~15**, all proton and carbon signals of **1~15** and the correlations between them were completely assigned, which enabled us to clarify the attachment point of side chains and also to establish the whole structures of **1~15**. In this paper, we report briefly the isolation of **1~15** from the roots of *S. flavescens* as well as the detailed ^{1}H and ^{13}C NMR signals of them assigned by various HETCOR NMR experiments.

MATERIALS AND METHODS

General

All NMR spectra were obtained on a Bruker AM-300 and Bruker AMX-500 spectrometer. High and low resolution MS were taken with a direct inlet and recorded with a JMS-DX303 mass spectrometer (JEOL). The optical rotations were determined on Autopol III automatic polarimeter. CD spectra were recorded on JASCO J-720 spectropolarimeter.

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Extraction and Isolation

The dried and chopped roots (3 Kg) of *S. flavescens* were extracted with MeOH at room temperature. Concentration of the solvents afforded a MeOH extract of ca. 240 g, which was suspended in H_2O and partitioned with CH_2Cl_2 (35 g) and EtOAc (50 g), successively. The CH_2Cl_2 soluble fraction was divided into six fractions (F1~F6) by silica gel column chromatography by way of gradient elution with a mixture of CH_2Cl_2 and MeOH. Among the resultant six fractions, only the F4 and F5 exhibited a significant cytotoxicity (ED_{50} values against A549 were *ca.* 15 μg/ml and 20 μg/ml), which were further divided into several subfractions followed by monitering the cytotoxicity. This procedure, the fractionation followed by cytotoxicity estimation was repeated until the isolation of nine pure active components **1~9** were achieved from F4 and F5, *i.e.*, **1** (*formononetin*, 72 mg), **2** (*kushenol E*, 55 mg), **3** (*kushenol B*, 250 mg), **4** (*sophoraflavanone G*, 480 mg), **5** (*kushenol L*, 30 mg), **6** (*kushenol M*, 500 mg), **7** (*kuraridin*, 110 mg), **8** (*kurarinone*, 120 mg), **9** (*kushenol N*, 20 mg), by the decreasing Rf order. The EtOAc fraction was also subjected to the same treatment as for the CH_2Cl_2 solu-

ble fraction to yield six active principles, **10~15**, *i.e.*, **10** (*kosamol A*, 85 mg), **11** (*norkurarinol*, 28 mg), **12** (*kurarinol*, 500 mg), **13** (*kushenol* H, 110 mg), **14** (*kushenol K*, 100 mg) and **15** (*trifolirhizin*, 210 mg) by the deceasing Rf order. These compounds **1~15** were identified by physicochemical and spectrospoic analyses such as m.p., NMR, MS and CD and were identical to previously published data (Shibata *et al.*, 1963, Wu *et al.*, 1985, Iinuma *et al.*, 1990, Ryu *et al.*, 1996).

1, *formononetin*, $C_{16}H_{12}O_4$ (M.W. 268) colorless neddles; mp 257-259°.

2, *kushenol E*, $C_{25}H_{28}O_6$ (M.W. 424) pale yellow amorphous powder; $[\alpha]_D$ -42° (c=0.1, MeOH); UV λ_{max} nm (MeOH): 250 (sh), 290, 316; m/z (rel. int.): 424 (M^+, 82), 406 (M^+-H_2O, 37), 364 (30), 363 (100), 307 (27), 295 (27); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 1.53, 1.58, 1.61 and 1.70 (each 3H, s, H-4b and H-5b), 2.62 (1H, dd, *J*=2.8, 17.2 Hz, H-3β), 3.12 (1H, dd, *J*=13.0, 17.2 Hz, H-3α), 3.15 (4H, m, H-1b), 5.07 (2H, m, H-2b), 5.52 (1H, dd, *J*=2.8, 13.0 Hz, H-2), 6.25 (1H, dd, *J*=2.2, 8.4 Hz, H-5'), 6.34 (1H, d, *J*=2.2 Hz, H-3') and 7.18 (1H, d, *J*=8.4 Hz, H-6'), 12.40 (1H, s, D_2O exchangeable, 5-OH).

3, *kushenol B*, $C_{30}H_{36}O_6$ (M.W. 492) pale yellow amorphous powder; $[\alpha]_D$ -38° (c=0.1, MeOH); UV λ_{max} nm (MeOH): 295, 333; m/z (rel. int.): 492 (M^+, 10), 370 (26), 369 (100), 351 (37), 295 (40), 233 (27): ^{1}H-NMR δ (300 MHz, DMSO-d_6): 1.43, 1.53, 1.59, 1.60 and 1.69 (each 3H, s, H-6a, H-7a, H-10a, H-4b and H-5b), 1.92 (2H, m, H-3a), 2.38 (1H, m, H-2a), 2.52 (2H, m, H-1a), 2.62 (1H, dd, *J*=2.5, 17.2 Hz, H-3β), 3.17 (1H, dd, *J*=13.2, 17.2 Hz, H-3α), 3.21 (2H, m, H-1b), 4.53 and 4.59 (each 1H, brs, H-9a), 4.85 (1H, m, H-4a), 5.07 (1H, m, H-2b), 5.52 (1H, dd, *J*=2.5, 13.2 Hz, H-2), 6.25 (1H, dd, *J*=2.2, 8.4 Hz, H-5'), 6.36 (1H, d, *J*=2.2 Hz, H-3') and 7.25 (1H, d, *J*=8.4 Hz, H-6'), 12.42 (1H, s, D_2O exchangeable, 5-OH).

4, *sophoraflavanone G*, $C_{25}H_{28}O_6$ (M.W. 424) pale yellow needles in $CHCl_3$/hexane; mp. 175°; UV λ_{max} nm (MeOH): 293, 340; m/z (rel. int.): 424 (M^+, 70), 406 (4), 301 (78), 283 (84), 165 (100); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 1.43, 1.53 and 1.57 (each 3H, s, H-6a, H-7a and H-10a), 1.90 (2H, m, H-3a), 2.38 (1H, m, H-2a), 2.42 (2H, m, H-1a), 2.61 (1H, dd, *J*=2.8, 17.2 Hz, H-3β), 3.08 (1H, dd, *J*=13.2, 17.2 Hz, H-3α), 4.46 and 4.53 (each 1H, brs, H-9a), 4.85 (1H, m, H-4a), 5.45 (1H, dd, *J*=2.8, 13.2 Hz, H-2), 5.92 (1H, s, H-6), 6.25 (1H, dd, *J*=2.2, 8.4 Hz, H-5'), 6.33 (1H, d, *J*=2.2 Hz, H-3') and 7.20 (1H, d, *J*=8.4 Hz, H-6'), 12.12 (1H, s, D_2O exchangeable, 5-OH).

5, *kushenol L*, $C_{25}H_{28}O_7$ (M.W. 440) pale yellow amorphous powder; $[\alpha]_D$ +12° (c=0.1, MeOH); UV λ_{max} nm (MeOH): 288 (sh), 298, 343; m/z (rel. int.): 440(M^+, 23), 290 (20), 289 (32), 234 (40), 233 (100), 207 (42), 177 (29), 151 (28), 150 (39); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 1.38, 1.47, 1.52 and 1.61 (each 3H, s, H-4b and H-5b), 3.05 and 3.20 (each 2H, m, H-1b), 4.62 (1H, d, *J*=11.2 Hz, H-2), 5.01 (2H, m, H-2b), 5.22 (1H, d, *J*=11.2 Hz, H-3), 6.24 (1H, dd, *J*=2.2, 8.4 Hz, H-5'), 6.29 (1H, d, *J*=2.2 Hz, H-3') and 7.05 (1H, d, *J*=8.4 Hz, H-6'), 12.17 (1H, s, D_2O exchangeable, 5-OH).

6, *kushenol M*, $C_{30}H_{36}O_7$ (M.W. 508) pale yellow amorphous powder; $[\alpha]_D$ +18° (c=0.1, MeOH); UV λ_{max} nm (MeOH): 298, 350; IR ν_{max} (KBr) cm^{-1}: 3300, 1630, 1600; m/z (rel. int.): 508 (M^+, 15), 490 (5), 385 (82), 367 (33), 311 (35), 233 (100), 177 (67); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 1.41, 1.48, 1.50, 1.56 and 1.66 (each 3H, s, H-6a, H-7a, H-10a, H-4b and H-5b), 1.91 (2H, m, H-3a), 2.35 (1H, m, H-2a), 2.49 (2H, m, H-1a), 3.19 (2H, m, H-1b), 4.44 and 4.52 (each 1H, brs, H-9a), 4.62 (1H, d, *J*=11.3 Hz, H-3), 4.84 (1H, t, *J*=6.6 Hz, H-4a), 5.05 (1H, m, H-2b), 5.28 (1H, d, *J*=11.3 Hz, H-2), 6.29 (1H, dd, *J*=2.2, 8.4 Hz, H-5'), 6.35 (1H, d, *J*=2.2 Hz, H-3'), 7.16 (1H, d, *J*=8.4 Hz, H-6') and 12.16 (1H, s, D_2O exchangeable, 5-OH)

7, *kuraridin*, $C_{26}H_{30}O_6$ (M.W. 438) yellow amorphous powder, a *chalcone* of **8**; $[\alpha]_D$ +6° (c=0.1, MeOH); UV λ_{max} nm (MeOH): 390; m/z (rel. int.): 422 (M^+-16, 5), 329 (5), 299 (20), 179 (15); ^{1}H-NMR δ (500 MHz, DMSO-d_6): 1.49, 1.58 and 1.64 (each 3H, s, H-6a, 7a and 10a), 2.00 (2H, m, H-3a), 2.45 (1H, m, H-2a), 2.50 (2H, m, H-1a), 3.85 (3H, s, 5-OMe), 4.49 and 4.56 (each 1H, brs, H-9a), 4.95 (1H, m, H-4a), 6.03 (1H, s, H-6), 6.30 (1H, dd, *J*=2.2, 8.4 Hz, H-5'), 6.37 (1H, d, *J*=2.2 Hz, H-3') and 7.20 (1H, d, *J*=8.4 Hz, H-6'), 7.84 (1H, d, *J*=15.5 Hz, H-3), 7.94 (1H, d, *J*=15.5 Hz, H-2), 14.86 (1H, s, D_2O exchangeable, 9-OH).

8, *kurarinone*, $C_{26}H_{30}O_6$ (M.W. 438) pale yellow amorphous powder; $[\alpha]_D$ +12° (c=0.1, MeOH); UV λ_{max} nm (MeOH): 290; m/z (rel. int.): 438 (M^+, missing), 423 (12), 422 (22), 300 (30), 299 (100), 153 (47), 124 (49), 110 (55), 109 (44); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 1.41, 1.51 and 1.62. (each 3H, s, H-6a, H-7a and H-10a), 1.95 (2H, m, H-3a), 2.45 (1H, dd, *J*=2.5, 16.0 Hz, H-3β), 2.48 (1H, m, H-2a), 2.50 (2H, m, H-1a), 2.82 (1H, dd, *J*=13.2, 16.0 Hz, H-3α), 3.68 (3H, s, 5-OMe), 4.46 and 4.53 (each 1H, brs, H-9a), 4.85 (1H, m, H-4a), 5.42 (1H, dd, *J*=2.5, 13.2 Hz, H-2), 6.10 (1H, s, H-6), 6.23 (1H, dd, *J*=2.2, 8.4 Hz, H-5'), 6.31 (1H, d, *J*=2.2 Hz, H-3') and 7.20 (1H, d, *J*=8.4 Hz, H-6'). **8** was converted to the corresponding chalcone, **7** *kuraridin* by the treatment of NH_3.

9, *kushenol N*, $C_{26}H_{30}O_7$ (M.W. 454) pale yellow amorphous powder; UV λ_{max} nm (MeOH); 290, 331; m/z (rel. int.): 436 (M^+-H_2O, 20), 315 (8), 314 (35), 313 (100), 179 (13), 153 (12); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 1.41, 1.54 and 1.60. (each 3H, s, H-6a, H-7a and H-10a), 1.95 (2H, m, H-3a), 2.45 (1H, m, H-2a), 2.50 (2H, m, H-1a), 3.73 (3H, s, 5-OMe), 3.76 (1H, s, H-3), 4.52 and 4.59 (each 1H, brs, H-9a), 4.85 (1H, m, H-4a), 5.38 (1H, d, *J*=1.5 Hz, H-2), 6.13 (1H, s, H-6), 6.24 (1H, dd, *J*=2.2, 8.4 Hz, H-5'), 6.31 (1H,

d, *J*=2.2 Hz, H-3') and 7.29 (1H, d, *J*=8.4 Hz, H-6').

10, *kosamol A*, $C_{30}H_{38}O_8$ (M.W. 526) pale yellow amorphous powder; $[\alpha]_D$ +36° (c=1.0, MeOH); IR ν_{max} (KBr) cm^{-1}: 3300, 1630, 1600, 1450; UV λ_{max} nm (MeOH): 298, 350; CD (c=2.2×10^{-4}, MeOH) θ (nm): +880 (320) (positive maximum), -1995 (295) (negative maximum); HRMS: 526.2563 (calculated 526.2567); LRMS (rel. int.): 526(M^+, 10), 508 (M^+-H_2O, 40), 490 (20), 453 (20), 385 (100), 367 (80), 357 (60), 311 (70), 233 (95), 177 (90); ^{1}H-NMR δ (300 MHz, DMSO-d_6): 1.15 (6H, s, H-4b and H-5b), 1.45, 1.52 and 1.53 (each 3H, s, H-6a, H-7a and H-10a), 1.52 (1H, m, H-2b), 1.92 (2H, m, H-3a), 2.36 (1H, m, H-2a), 2.55 (2H, m, H-1a), 2.64 (2H, m, H-1b), 4.44 and 4.52 (each 1H, brs, H-9a), 4.62 (1H, d, *J*=11.3 Hz, H-3), 4.86 (1H, t, *J*=6.6 Hz, H-4a), 5.26 (1H, d, *J*=11.3 Hz, H-2), 6.23 (1H, dd, *J*=2.2, 8.4 Hz, H-5'), 6.32 (1H, d, *J*=2.2 Hz, H-3'), 7.13 (1H, d, *J*=8.4 Hz, H-6') and 12.17 (1H, s, D_2O exchangeable, 5-OH). COLOC: Fig. 2.

11, *norkurarinol*, $C_{25}H_{30}O_7$ (M.W. 442) colorless amorphous powder; UV λ_{max} nm (MeOH): 294, 320 (sh); m/z (rel. int.): 442 (M^+, 5), 406 (10), 301 (95),424 (10), 283 (100), 165 (60). ^{1}H-NMR δ (300 MHz, DMSO-d_6): 0.95 (6H, s, H-6a and H-7a), 1.09 and 1.18 (each 1H, m, H-4a), 1.28 (2H, m, H-3a), 1.57 (3H, s, H-10a), 2.33 (1H, m, H-2a), 2.48 (2H, m, H-1a), 2.52 (1H, dd, *J*=2.2, 16.0 Hz, H-3β), 3.22 (1H, dd, *J*=13.2, 16.0 Hz, H-3α), 3.92 (1H, brs, D_2O exchangeable, 5a-OH), 4.45 and 4.56 (each 1H, brs, H-9a), 5.45 (1H, dd, *J*=2.2, 13.2 Hz, H-2), 5.92 (1H, s, H-6), 6.25 (1H, dd, *J*=1.8, 8.3 Hz, H-5'), 6.38 (1H, d, *J*=1.8 Hz, H-3') and 7.23 (1H, d, *J*=8.3 Hz, H-6'), 12.12 (1H, s, D_2O exchangeable, 5-OH).

12, *kurarinol*, $C_{26}H_{32}O_7$ (M.W. 456) colorless needles in Hexane/EtOAc; $[\alpha]_D$ -48° (c=1.0, MeOH); UV λ_{max} nm (MeOH): 285, 325 (sh); FABMS (rel. int.): 457 (M^++1, 40), 315 (40), 307 (70), 289 (50), 179 (70), 155 (100), 138 (99); ^{1}H-NMR δ (500 MHz, DMSO-d_6): 0.96 (6H, s, H-6a and 7a), 1.09 and 1.18 (each 1H, m, H-4a), 1.28 (2H, m, H-3a), 1.57 (3H, s, H-10a), 2. 33 (1H, m, H-2a), 2.50 (2H, m, H-1a), 2.52 (1H, dd, *J*=2.2, 16.0 Hz, H-3β), 2.82 (1H, dd, *J*=13.2, 16.0 Hz, H-3α), 3.70 (3H, s, 5-OMe), 4.03 (1H, brs, D_2O exchangeable, 5a-OH), 4.49 and 4.56 (each 1H, brs, H-9a), 5.45 (1H, dd, *J*=2.2, 13.2 Hz, H-2), 6.13 (1H, s, H-6), 6.28 (1H, dd, *J*=1.8, 8.3 Hz, H-5'), 6.36 (1H, d, *J*=1.8 Hz, H-3') and 7.23 (1H, d, *J*=8.3 Hz, H-6').

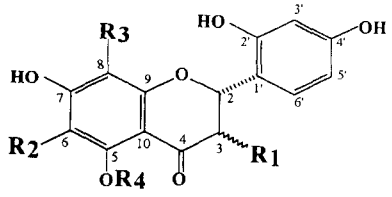
13, *kushenol H*, $C_{26}H_{32}O_8$ (M.W. 472) pale yellow amorphous powder; $[\alpha]_D$ +15° (c=1.0, MeOH); UV λ_{max} nm (MeOH): 288, 325; m/z (rel. int.): 454 (M^+-H_2 O, 10), 436 (10), 313 (100), 285 (15), 179 (20), 153 (60), 123 (10); ^{1}H-NMR δ (500 MHz, DMSO-d_6): 0.97 (6H, s, H-6a and H-7a), 1.09 and 1.14 (each 1H, m, H-4a), 1.25 (2H, m, H-3a), 1.49 (3H, s, H-10a), 2.28 (1H, m, H-2a), 2.40 (2H, m, H-1a), 3.71 (3H, s, 5-OMe), 3.97 (1H, brs, D_2O exchangeable, 5a-OH), 4.36 (1H, dd, *J*=1.0, 11.0 Hz, H-3), 4.41 and 4.48 (each 1H, brs, H-9a), 5.21 (1H, d, *J*=11.0 Hz, H-2), 6.12 (1H, s, H-6), 6.23 (1H, dd, *J*=2.0, 8.4 Hz, H-5'), 6.31 (1H, d, *J*=2.0 Hz, H-3') and 7.12 (1H, d, *J*=8.4 Hz, H-6').

14, *kushenol K*, $C_{26}H_{32}O_8$ (M.W. 472) pale yellow amorphous powder; $[\alpha]_D$ -81° (c=1.0, MeOH); UV λ_{max} nm (MeOH): 290, 325; m/z (rel. int.): 454 (M^+-H_2O, 10), 331 (5), 313 (100), 285 (10), 179 (30), 153 (40), 123 (10). ^{1}H-NMR δ (500 MHz, DMSO-d_6): 0.94 (6H, s, H-6a and H-7a), 1.05 and 1.16 (each 1H, m, H-4a), 1.26 (2H, m, H-3a), 1.60 (3H, s, H-10a), 2.32 (1H, m, H-2a), 2.53 (2H, m, H-1a), 3.71 (3H, s, 5-OMe), 3.82 (1H, brs, H-3), 3.95 (1H, brs, D_2O exchangeable, 5a-OH), 4.54 and 4.59 (each 1H, brs, H-9a), 5.37 (1H, d, *J*=1.5 Hz, H-2), 6.13 (1H, s, H-6), 6.24 (1H, dd, *J*= 2.2, 8.4 Hz, H-5'), 6.31 (1H, d, *J*=2.2 Hz, H-3') and 7.29 (1H, d, *J*=8.4 Hz, H-6').

15, trifolirhizin, $C_{22}H_{22}O_{10}$ (M.W. 446), colorless rods, mp 142°, $[\alpha]_D$ -185° (c=1.0, EtOH) (Shibata *et al.*, 1963).

RESULTS AND DISCUSSION

The species *Sophora flavescens* Aiton is a shrub in Leguminosae which is spread widely in northeast Asian countries. It is nowadays cultivated commonly and thus the roots of this species is available commercially in Korea as a generic name Kosam. It has been used as a folk medicine for antipyretic, analgesic, anthelmintic and stomachic uses in Korea. We



	R_1	R_2	R_3	R_4		R_1	R_2	R_3	R_4
2	-H, -H	-A	-A	-H	**9**	-H, -OH(α)	-H	-C	$-CH_3$
3	-H, -H	-A	-C	-H	**10**	-H, -OH(β)	-B	-C	-H
4	-H, -H	-H	-C	-H	**11**	-H, -H	-H	-D	-H
5	-H, -OH(β)	-A	-A	-H	**12**	-H, -H	-H	-D	$-CH_3$
6	-H, -OH(β)	-A	-C	-H	**13**	-H, -OH(α)	-H	-D	$-CH_3$
8	-H, -H	-H	-C	$-CH_3$	**14**	-H, -OH(β)	-H	-D	$-CH_3$

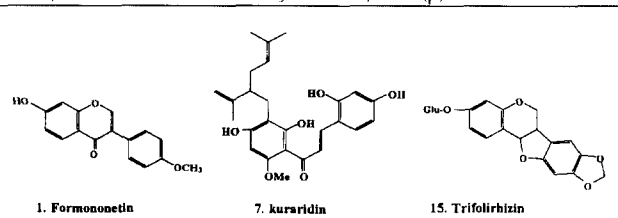


Fig. 1. Cytotoxic components from *Sophora flavescens*.

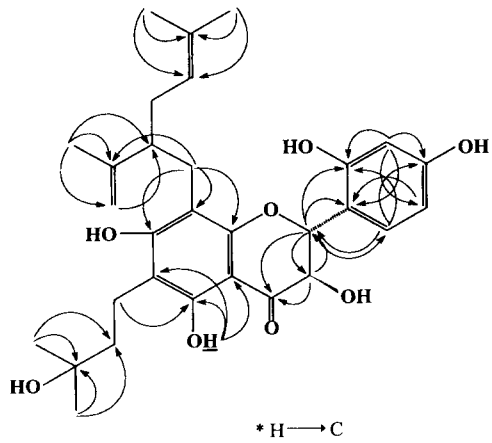


Fig. 2. Correlation of C-H hong range couplings of kosamol A (10) by COLOC experiment.

recently reported that the methanolic extracts of the roots of *S. flavescens* showed a moderate cytotoxicity against several cultured human tumor cell lines, A549, SK-OV-3, SK-Mel-2, XF498 and HCT15. (Ryu *et al.*, 1997). By the continuous investigation for active ingredients responsible for the cytotoxicity on tumor cells, all fifteen flavonoids (**1~15**) had been isolated (Ryu *et al.*, 1996). During the course of structure determination of **1~15**, we found that most of them (**2~14**) were comprised in kushenols and had been isolated previously from this species or from some other species in the Sophora genus and identified completely only except for the exact attachment point (C_6 or C_8) of some side chains A-D (Fig. 1). (Wu *et al.*, 1985). Therefore, Wu *et al.* postulated two possible structures of them, *i.e.*, one is as illustrated on Fig. 1 and the other is a inversion of R_2 (C_6) and R_3 (C_8). These sophisticated problems were clarified by the COLOC (Correlated spectroscopy for long range coupling) experiments to verify the exact attaching point of each side chains, which is based on the observation of the correlations between the protons (1a or 1b) in side chain and the adjacent carbons in flavonoid skeleton (C_5-C_{10}). We have already reported the structure determination of kushenol M (**6**) by this technique (Ryu *et al.*, 1995). The attaching point of two side chain B and C in kosamol A (10) was also established unambiguously as B on C_6 and C on C_8 by the COLOC experiment as shown in Fig. 2 (Ryu *et al.*, 1996). By the similar manner exploiting the COLOC

Table I. ^{13}C-NMR chemical shifts (δ) of 2~14 in DMSO-d_6

C	2	3	4	5	6	7	8	9	10	11	12	13	14
2	73.8	73.9	73.9	77.8	77.8	138.7	73.6	76.4	77.6	73.9	73.8	77.0	76.5
3	41.3	41.8	41.5	70.4	70.9	122.8	44.4	71.2	70.7	42.0	44.5	71.5	71.3
4	197.6	197.6	197.2	198.8	199.2	192.0	189.1	189.4	199.0	197.1	189.5	191.0	189.5
5	158.2	158.4	161.2	158.0	158.4	165.3	162.5	162.1	158.4	161.1	159.8	159.4	160.2
6	107.7	107.7	95.2	108.0	108.0	90.6	92.5	92.4	108.9	95.1	92.7	92.4	92.4
7	161.5	162.1	164.8	162.0	162.3	162.6	162.1	161.8	162.6	164.8	162.4	162.5	162.2
8	107.2	106.7	106.5	107.2	106.8	108.0	107.0	106.6	106.7	106.5	107.4	107.0	106.9
9	158.5	158.8	160.8	158.6	158.7	161.1	159.6	160.2	158.3	160.8	162.8	162.0	162.0
10	101.8	101.8	101.6	100.5	100.7	104.5	104.4	101.9	100.4	101.6	104.6	102.4	102.5
1'	115.9	116.1	115.9	114.0	114.2	113.8	116.4	113.8	114.1	115.9	116.6	114.2	113.8
2'	155.7	155.5	155.5	157.2	157.3	159.0	155.3	154.7	157.2	155.5	155.4	157.0	154.8
3'	102.4	102.5	102.4	102.4	102.6	102.6	102.4	102.4	102.4	102.3	102.6	102.4	102.1
4'	158.4	158.6	158.4	158.5	158.5	160.4	158.2	157.8	158.5	158.3	158.3	158.4	157.9
5'	106.3	106.4	106.3	106.2	106.4	106.6	106.3	105.9	106.2	106.3	106.5	106.2	105.9
6'	127.8	127.7	127.6	129.4	129.6	130.3	127.3	129.2	129.6	127.6	127.5	129.3	129.3
1a	21.6	27.2	26.1	21.5	27.1	26.7	26.9	27.0	27.0	27.1	27.6	27.3	27.4
2a	122.8	46.6	46.4	122.9	46.6	46.2	46.4	46.3	46.5	46.6	46.9	46.5	46.6
3a	130.2	30.4	30.8	130.4	30.7	30.8	30.8	30.5	30.7	26.5	26.7	26.8	26.2
4a	25.6	123.5	123.4	25.6	123.6	123.4	123.5	123.4	123.5	41.5	41.7	41.6	41.5
5a	17.6	130.7	130.7	17.6	130.7	130.5	130.7	130.7	130.6	68.7	69.1	68.8	68.8
6a		25.6	25.5		25.7	25.6	25.5	25.5	25.6	29.4	29.6	29.5	29.5
7a		17.6	17.6		17.7	17.7	17.6	17.6	17.7	29.0	29.1	29.1	29.0
8a		147.9	147.8		147.9	147.8	148.0	148.0	147.8	147.9	148.4	148.0	148.4
9a		110.9	110.8		111.1	110.8	110.8	110.7	110.9	110.9	111.1	111.0	110.8
10a		18.9	18.6		18.9	18.5	18.6	18.7	18.6	18.0	18.2	17.9	18.3
1b	20.9	21.0		21.0	21.2				17.0				
2b	122.8	123.0		122.6	123.0				42.3				
3b	130.4	130.4		130.4	130.5				69.4				
4b	25.5	25.5		25.6	25.7				29.3				
5b	17.8	17.8		17.8	17.9				29.3				
-OCH_3						55.5	55.3	55.3			55.4	55.3	55.3

technique together with other HETCOR experiments, all attachment points of each side chains (A~D) in **2~14** were completely established as illustrated on Fig. 1. Besides, the detailed spectrocopic data including the ^{1}H-NMR (materials and methods) and ^{13}C-NMR signals of each components **2~14** were summarized on Table 1, which were established by various two dimentional NMR spectra (^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and COLOC) of them.

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