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Determination of the Structure for Polysubstituted Flavonoid and 6-C-Glucosyl Flavonoids using ^{13}C-^{1}H Long Range Couplings

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A flavone glycoside was isolated from the leaves of *Betula platyphylla* var. *latifolia* and chracterized as 4',6-Dimethoxy-5-hydroxyflavone-7-O-β-D-glucoside(pectolinarigenin-7-O-β-D-glucopyranoside) by method of chemical and NMR spectral analysis. ^{13}C-^{1}H long range coupling was confirmative for determination of its substituted position. In connection with this study, 6-C-Glucosylnalingenin and 6-C-Glucosylaromadendrin were confirmed its structures using this technique.

Key words : *Betula platyphylla* var. *latifolia*, Betulaceae, Flavonoid, Pectolinarigenin-7-O-β-D-glucopyranoside, 6-C-Glucosylnalingenin and 6-C-Glucosyaromadendrin, ^{13}C-^{1}H long range coupling

INTRODUCTION

Previous work on the flavonoid from *Betula platyphylla* var. *latifolia* (Betulaceae) had established 6-C-glucosylflavanone and dihydroflavonol along with some flavonoid O-glycosides (Lee, 1994).

Further studies of this plant has led to isolation and structural elucidation of one flavone glycoside pectolinarigenin-7-O-β-D-glucopyranoside (4',6-Dimethoxy-5-hydroxyflavone-7-O-β-D-glucoside.

In the polysubstituted flavonoid or C-glycosylflavonoid, especially C-6 substitution, determination of the substituted position was incomplete by conventional chemical and NMR study like anomalous $AlCl_3$ induced UV shift and up and down shift of NMR spectra. In such a case, ^{13}C-^{1}H long range coupling was confirmative for determination of its substituted position.

Here, the author describe the structural elucidation of a polysubstituted flavone (4',6-Dimethoxy-5-hydroxyflavone-7-O-β-D-glucoside) and the structural confirmation of 6-C-glucosylnalingenin and 6-C-glucosylaromadendrin using ^{13}C-^{1}H long range coupling method.

MATERIALS AND METHOD

General Experiment Procedures

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NMR spectra were measured with either a Bruker AM-300 or a Varian UNITY-500 Spectrometer. Positive FAB-MS was measured 10 Kv with NBA as a solvent. Details of chromatographic conditions used in this work are similar to those described in previous work (Lee, 1994).

Isolation of pectolinarigenin-7-O-β-D-glucopyranoside (1)

Fresh leaves of *Betula platyphylla* var. *latifolia* (5.5 Kg) were extracted with 80% aq Me_2CO at room temp. After removal of Me_2CO in vacuo, the aq. solution was filtered. The filtrate was concentrated and then applied to Sephadex LH-20 column. Elution with H_2O containing increasing proportion of MeOH afforded 3 frs, I (150 g), II (225 g) and III (320 g). Repeated CC of fr.II on MCI-gel CHP 20P with an H_2O-MeOH gradient system furnished pectolinarigenin-7-O-β-D-glucopyranoside (**1**, 200 mg).

Pectolinarigenin-7-O-β-D-glucopyranoside (**1**) -Yellow amorphous powder. $FeCl_3$: green, $[\alpha]_D^{25}$-121° (Me_2CO: c 0.1), Positive FAB-MS *m/z* 477$[M+H]^+$, 315$[M+H-glc]^+$; ^{1}H-NMR(DMSO-d_6) δ: 3.79 (3H, s, 7-OCH_3), 3.86 (3H, s, 4'-OCH_3), 5.14 (IH, d, J=5.7 Hz, anomeric H), 6.94 (IH, s, H-3), 7.04 (IH, s, H-8), 7.12 (2H in total, d, J=9 Hz, H-3' and H-5'), 8.06 (2H in total, d, J=9 Hz, H-2' and H-6'), 12.91 (1H, s, 5-OH). ^{13}C-NMR (DMSO-d_6) δ: 55.5 (4'-OCH_3), 60.2 (7-OCH_3), 60.5 (glc-6), 69.5 (glc-4), 73.1 (glc-2), 76.6 (glc-5), 77.2 (glc-3),

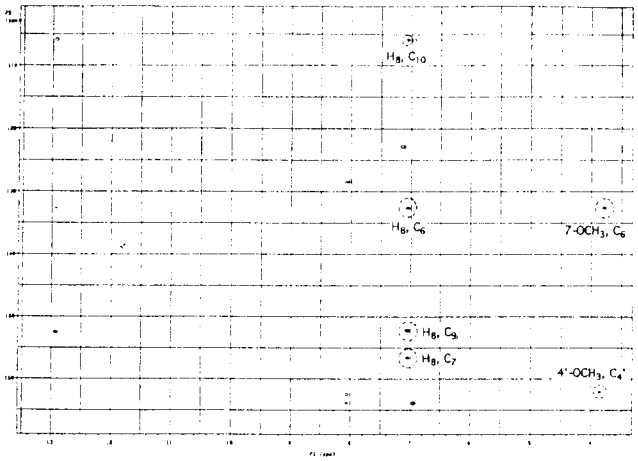


Fig. 1. ^{13}C-^{1}H long range HETEROCOSY Spectrum of pectolinarigenin-7-O-β-D-glucopyranoside (1) in DMSO-d_6.

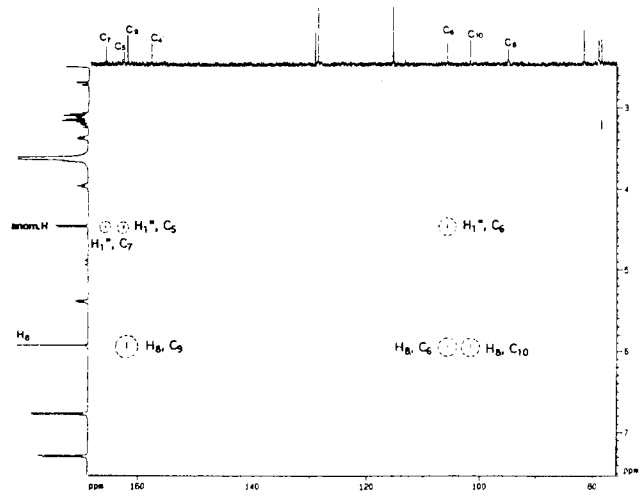
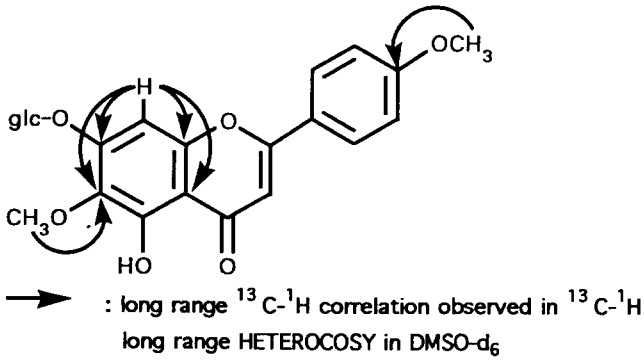
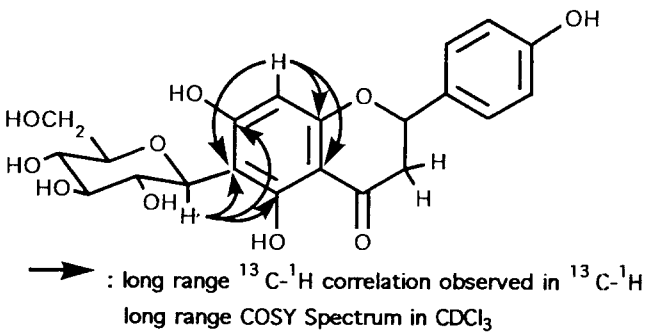


Fig. 2. ^{13}C-^{1}H long range COSY Spectrum of 6-C-glucosylnalingenin (2) in $CDCl_3$.



Scheme 1. Structure of pectolinarigenin-7-O-β-D-glucopyranoside (1).



Scheme 2. Structure of 6-C-glucosylnalingenin (2).

94.3 (C-8), 100.1 (glc-1), 103.2 (C-3), 105.7 (C-10), 114.5 (C-3′ and C-5′), 122.6 (C-1′), 128.2 (C-2′ and C-6′), 132.4 (C-6), 152.1 (C-9), 152.3 (C-5), 156.4 (C-7), 162.4 (C-4′), 163.7 (C-2), 182.3 (C-4).

Acid hydrolysis of **1**. a solution of **1** (20 mg) in 5 ml of 2N HCl-MeOH (1 : 1) on a steam bath for 90 minutes. Evaporate to dryness on a rotary evaporator and added H_2O then extract several times with EtOAc (Markham *et al.*, 1982). The H_2O layer was neutralized by Ag_2CO_3 and TLC was conducted for the identification of sugar as a glucose. The EtOAc layer was chromatographed over Sephadex LH 20(80% EtOH) to give aglycone of **1** as a yellow powder. EIMS *m/z*: 318 $[M]^+$. ^{1}H-NMR(DMSO-d_6): δ3.70 (3H, s, 4′-OCH_3), 3.78 (3H, s, 7-OCH_3), 6.59 (1H, s, H-8), 6.67 (1H, s, H-3), 7.04 (2H, d, J=7.2 Hz, H-3′ and H-5′), 7.92 (2H, d, J=7.2 Hz, H-2′ and H-6′), 13.03 (1H, s, 5-OH).

RESULTS AND DISSCUSION

Compound **1** was obtained as yellow amorphous powder and gave a green color in the ferric chloride. The ^{1}H-NMR spectrum of **1** exhibited two methoxyl protons at δ 3.70, 3.78 and a sugar moiety at δ 5.14(d, J=5.7Hz) with β-configuration.

The ^{1}H-NMR spectrum of **1** also showed typical four-protons of two doublets (each J=9 Hz) at δ7.12 and 8.06 suggested A_2B_2 pattern of B-ring, a broad singlet at δ12.91 for 5-OH and two singlets for one proton each at 6.94 and 7.04 suggested a 5,6,7- or 5,6,8-oxygenated A-ring as a flavone skeleton.

To solve substituted position of A-ring, ^{13}C-^{1}H long range coupling was studied (Fig. 1). In the spectrum, the long range coupling between H-8 and C-6, C-7, C-9, and C-10 were observed and another long range coupling were observed between C-6 and 6-OCH_3 and between C-4′ and 4′-OCH_3. The above data has led to confirm the structure of **1** (Scheme 1). Acid hydrolyis of **1** afforded 5,7-dihydroxy-4′,6-dimethoxyflavone (pectolinarigenin) and glucose as sugar.

Thus compound **1** was identified as 4′,6-dimethoxy-5-hydroxyflavone 7-O-β-D-glucoside(pectolinarigenin-7-O-β-D-glucopyranoside, Lee *et al.*, 1994).

In connection with this study, the author used this

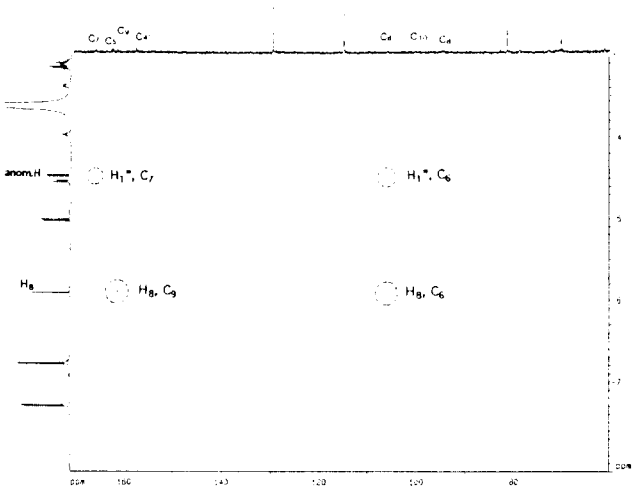
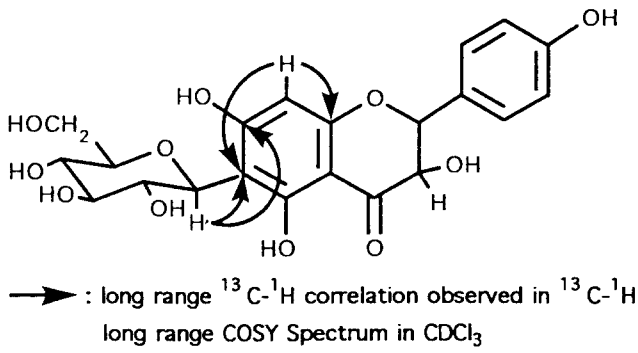


Fig. 3. ^{13}C-^{1}H long range COSY Spectrum of 6-C-glucosylaromadendrin (3) in $CDCl_3$.



Scheme 3. Structure of 6-C-glucosylaronadendrin (3).

technique for confirmation of the structures of 6-C-glycosylflavanone (6-C-glucosylnalingenin, 2) and 6-C-glycosyl dihydroflavonol (6-C-glucosylaromadendrin, 3) which its chemcal shifts of ^{13}C-NMR spectra were reported at previous work in same plant.

The signal of C_6-H and C_8-H in nalingenin appear at about δ6.0 overlapping each other in ^{1}H-NMR spectrum, and the signal of C-6 and C-8 appear at δ95.9 and 95.0 (in DMSO-d_6) in ^{13}C-NMR spectrum (Harborne *et al.*, 1982). So determination of side chain located at C-6 or C-8 in 5,7-dihydroxyflavanone(nalingenin) was incomplete by comparing method with nalingenin in NMR Spectra. The same case was observed in 5,7-dihydroxydihydroflavonol(aromadendrin). In the ^{13}C-NMR signal of C-6 and C-8 appeared at δ97.46, and 96.40 (in CD_3OD). So the determination of position of side chain located at C-6 or C-8 in aromadondrin was ambiguous also.

In prievious work, the author determined the structure of each compound(6-C-glucosylnalingenin, **2** and 6-C-glucosyaromadendrin, **3**) tentatively by comparison with the ^{13}C-NMR spectrum of its aglycone in the literatures (Harbone *et al.*, 1980 and Shen *et al.*, 1985), and other spectral evidences (Lee, 1994).

And in this paper, using the ^{13}C-^{1}H long range coupling method, confirmation of its structures of 6-C-glucosylnalingenin (**2**) and 6-C-glucosylaromadendrin (**3**) were conducted. In the ^{13}C-^{1}H long range COSY spectrum of 6-C-glucosylnalingenin (**2**, Fig. 2), the long range couplings were observed between H-8 and C-10, C-9, and C-6 and another long range couplings were observed between glucose anomeric proton and C-5, C-6 and C-7.

And in the spectrum of 6-C-glucosylaromadendrin (**3**, Fig. 3), the long range couplings were observed between H-8 and C-6, and C-9 and another long range couplings were observed between anomeric proton and C-6 and C-7.

These findings confirmed the structures of **2** and **3** (Scheme 2 and 3) unambiguously. It is interesting that C-glucoside (**2**, **3**) showed clear long range ^{13}C-^{1}H correlation between its anomeric proton and other near carbons but in O-glucoside (**1**), the long range ^{13}C-^{1}H correlation between its anomeric proton and other near carbon was not observed.

Some researchers used long range selecctive proton decoupling (LSPD, shirataki *et al.*, 1985; Bruno *et al.*, 1985) method for the structural elucidation of some 6-C-prenylated flavonoids.

In conclusion, ^{13}C-^{1}H long range coupling method without LSPD also showed its usefulness in structure analysis of C-6 substituted 5,7-dihydroxylated flavanone and dihydroflavonol(espicially C-glucoside) and polysubstituted flavone.

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