

**MULBERROSIDE B, A NEW C-GLUCOSYLCOUMARIN FROM THE
CULTIVATED MULBERRY TREE (MORUS LHO KOIDZ.)**

Kazuhiro Hirakura, Isamu Saida, Toshio Fukai, and Taro Nomura*

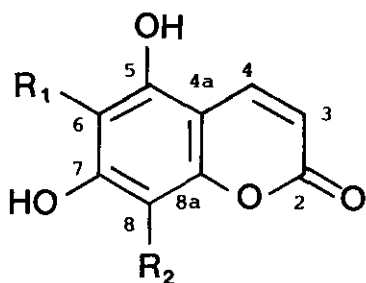
Faculty of Pharmaceutical Sciences, Toho University,
2-2-1, Miyama, Funabashi-shi, Chiba 274, Japan

Abstract ——— A new C-glucosylcoumarin, mulberroside B, was isolated from the acetone extract of the root bark of cultivated mulberry tree, and identified as 5,7-dihydroxycoumarin-6-C- β -glucopyranoside on the basis of spectral evidence.

Previously we reported the structure determination of a series of phenolic compounds isolated from the root bark of the cultivated mulberry tree (Japanese name "Rosō", a cultivated variety of Morus lhou Koidz.).¹⁻⁷ Further extensive fractionation of acetone extract of the root bark led to the isolation of a new phenolic glycoside, named mulberroside B (1). We herein report the characterization of this glycoside.

The dried root bark of the cultivated mulberry tree was extracted successively with hexane, benzene, and acetone. The acetone extract was fractionated sequentially by column chromatography on silica gel, and by gel permeation chromatography using Sephadex LH-20, resulting in the isolation of 1 ($4.6 \times 10^{-3}\%$ yield from the root bark).

Mulberroside B (1) was obtained as colorless needles, mp 220 - 222 °C, $[\alpha]_D^{18} + 55^\circ$ (c=0.26, pyridine), exhibiting negative ferric chloride reaction. The FD-MS of 1 showed the molecular ion peak at m/z 340, and the ^{13}C nmr spectrum indicated the presence of fifteen carbons. From these results, the molecular formula of 1 was suggested to be $\text{C}_{15}\text{H}_{16}\text{O}_9$. The compound (1) showed on a tlc plate a characteristic spot with blue fluorescence under uv light, and showed the following spectra: $\text{ir } \nu_{\text{max}}^{\text{KBr}} \text{ cm}^{-1}$: 3400 (br), 1720, 1710, 1630, and 1610; $\text{uv } \lambda_{\text{max}}^{\text{EtOH}} \text{ nm (log } \epsilon)$: 210 (4.17), 230 (sh 3.83), 251 (3.39), 260 (3.40), 330 (3.81), and 390 (sh 2.77). The uv spectrum was similar to that of 5,7-dihydroxycoumarin (2).⁸ These results suggested that 1 is one of the 5,7-dihydroxycoumarin



1 : R₁=β-glucosyl, R₂=H

1' : R₁=H, R₂=β-glucosyl

2 : R₁=R₂=H

Table 1. ¹³C nmr data of 1, 2, 3, and 4.

C	1	2	sugar C	1	3 ¹	4 ¹
2	154.0	156.6	G-1	74.5	73.9	72.9
3	107.9	107.3	G-2	72.1	71.4	70.4
4	139.0	140.0	G-3	77.6	78.8	78.8
4a	101.9	101.8	G-4	68.9	70.8	70.1
5	159.9	161.0*	G-5	80.5	81.4	81.3
6	108.2	98.6	G-6	59.8	61.5	61.3
7	159.9	162.9*				
8	93.8	93.3				
8a	154.4	157.5				

¹Data from reference 9.

*: Assignments may be interchanged.

Table 2. Selective decoupling data of ¹³C nmr of 2.

δ _C	93.3	98.6	101.8	107.3	140.0	156.6	157.5	161.0	162.9
o.-r. ¹	d	d	s ²	d	d	s ³	s ²	s	s
irrad. at									
7.99 ppm					s				
6.26		s							
6.12	s								
5.97				s					

¹Off-resonance decoupling.

²The signal is medium.

³The signal is weak.

Abbreviations: s=singlet, d=doublet.

derivatives. This suggestion was further supported by examination of the ^1H nmr and ^{13}C nmr spectra of 1. The ^1H nmr spectrum (dmsO-d_6 , 400 MHz) of 1 showed the proton signals of a 6 or 8-substituted 5,7-dioxygenated coumarin moiety as follows: δ 6.04 (1H, d, $J = 9.5$ Hz, C-3-H), 6.25 (1H, s, C-6 or C-8-H), 8.02 (1H, d, $J = 9.5$, C-4-H). The spectrum also showed the signal of a sugar anomeric proton at δ 4.75 (1H, d, $J = 10$ Hz) and those of other sugar protons at δ 3.25 - 3.83 (10 H, m). The ^{13}C nmr spectrum indicated the presence of two aliphatic carbons ($-\text{CH}=\text{CH}-$), six aromatic carbons ($\text{CH} \times 1$, $\text{C} \times 2$, $\text{C}-\text{O} \times 3$), one carbonyl carbon, and six sugar carbons. The chemical shifts values of the sugar carbons were similar to those of the relevant carbon atoms of glucose moieties of model compounds, such as vitexin (3) and isoorientin (4)⁹ (Table 1). The acid hydrolysis of 1 proved unsuccessful with H_2SO_4 -EtOH (10% H_2SO_4 -EtOH=1:3, v/v) at 100 °C for 6 h, while the hydrolysis of 1 with ferric chloride^{10,11} gave an aglycone identified with 5,7-dihydroxycoumarin (2)¹² and led to arabinose¹¹ being identical with authentic samples as a TMSi derivative by GC. From the above results, mulberroside B (1) is a 5,7-dihydroxycoumarin 6 or 8-C-glucoside. The location of the sugar moiety in 1 was confirmed by the following spectral data. The ^1H nmr spectra of 2 were analysed as follows: (dmsO-d_6 , 270 MHz) δ 5.97 (1H, d, $J = 9.5$ Hz, C-3-H), 6.12 (1H, br d, $J = 2$, C-8-H), 6.26 (1H, d, $J = 2$, C-6-H), 7.99 (1H, br d, $J = 9.5$, C-4-H); (acetone- d_6 , 90 MHz) δ 6.20 (1H, dd, $J = 1$ and 2.5, C-8-H), 8.06 (1H, dd, $J = 1$ and 9.5, C-4-H). On the ^{13}C nmr spectrum (dmsO-d_6 , 67.8 MHz) of 2, the signals were assigned to the carbon atoms at C-3, 4, 6, and 8 positions by using off-resonance decoupling and selective decoupling techniques (Table 2). The ^{13}C nmr spectrum (dmsO-d_6 , 100.4 MHz) of 1 being compared with that of 2, the chemical shift values of the carbon atoms of coumarin moiety were similar to those of the relevant carbon atoms of 2 except that of carbon atom at C-6 position which was subjected to an additional effect⁹ (Table 1). The ^1H nmr coupling constant value of the anomeric proton ($J = 10$ Hz) showed the β -glucopyranose form of the sugar moiety.¹³ Accordingly, mulberroside B could be characterized as 5,7-dihydroxycoumarin 6-C- β -glucopyranoside. To the author's knowledge, mulberroside B is the second example of a coumarin C-glycoside found in nature. The first one, daurosides D, 5,7-dihydroxycoumarin 6 or 8-C- β -glucopyranoside (1 or 1'), was reported by Batsuren, et al., but the location of the sugar moiety could not be confirmed.^{14,15}

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