UV Absorbent, Marmesin, from the Bark of Thanakha, *Hesperethusa crenulata* L.

Se-Hwan Joo, Sang-Cheol Lee, and Seong-Ki Kim*
Department of Life Science, Chung-Ang University, Seoul 156-756, Korea

We used solvent extractions, SiO_2 column chromatographies, and HPLC to isolate from the bark of Thanakha (*Hesperethusa crenulata* L.) an active crystalline compound for absorbing UV-A radiation (320 to 380 nm). Analyses of low- and high-resolution FAB-MS revealed a compound, named marmesin, with a formula of $C_{14}H_{14}O_4$ and a molecular mass of 246. To determine its chemical structure, we conducted 300 MMz NMR analyses using various probes, ^1H, ^{13}C, and DEPT ^{13}C. Our NMR data showed a structure of 2,3-dihydro-2(1-hydroxy-1-methylethyl)-furanocoumarin. This active compound contains UV-absorbing chromophores, an aromatic ring, a double bond at C3-C4, and a carbonyl at C2. Its λ_{max} is 335 nm, indicating that marmesin could be commercially useful as a natural UV-A-filtering product.

Keywords: marmesin, Thanakha (*Hesperethusa crenulata* L.), UV-A-absorbent

Natural daylight comprises various wavelengths of radiation, including UV (ultraviolet) with its range of 200 to 380 nm. UV light can be further subdivided into UV-C (200 to 280 nm), UV-B (280 to 320 nm), and UV-A (320 to 380 nm) (Cockell, 1998). In general, UV-C is absorbed by the atmospheric ozone layer, whereas UV-B and UV-A can penetrate that ozone layer and reach the earth. Excessive exposure to UV-B and UV-A radiation can have deleterious effects on human beings, including sunburn, sun-damaged skin, cataracts, snow blindness, skin cancer, and immune system deficiencies (van der Leun, 1996; Duthie et al., 1999; Hawk, 1999; Hockwin et al., 1999). To provide protection from UV-B damage, numerous products have been developed by isolating active compounds from natural sources as well as through chemical synthesis. These are now used commercially as protectants or filters in many industries. However, only a limited number of compounds have been developed to guard against UV-A radiation. Therefore, the objective of our study was to identify potential UV A-filtering compound(s) from the bark of Thanakha (*H. crenulata* L.). Although powder obtained from this species has been traditionally used in UV-filtering cosmetics in Myanmar, its active ingredients have not been examined extensively.

We extracted bark tissue (600 g) with a mixture of methanol and chloroform (1:1, 5 L×3). After evaporation, 14 g of the extract was purified by silica gel chromatography eluted stepwise with chloroform containing 0, 1, 2, 3, 4, 5, 10, and 100% methanol. In TLC (Merck F254) developed with a 40:1 mixture of chloroform and methanol, those fractions eluted with 1% methanol showed strong fluorescent spots under UV illumination at 365 nm. Thereafter, the 3.7 g fractions were combined and subjected to a second silica gel chromatography, eluted with a 1:1 mixture of hexane and ethyl acetate. Under the same TLC analysis conditions, a spot at R_f 0.18 exhibited the strongest fluorescence. Fractions containing the active principle were re-combined and further purified via reversed-phase HPLC (Waters, μBondapak C18, 20×150 mm) by eluting them with 30% acetonitrile at a flow rate of 5 mL min^{-1}. The peak captured at 13.3 min was then dried and crystallized in methanol. From this, we were able to obtain needle-like crystals of the active compound (8 mg) that could be used in instrumental analyses to determine the structure of this compound.

Positive and negative low-resolution FAB-MS (JMS AX505WA MS spectrometer, Electron voltage: 70 eV, Matrix: meta-nitrobenzyl alcohol) revealed a prominent ion for $[M+H]^+$ and $[M-H]^-$ at *m/z* 247 and 245, respectively. This indicated that the molecular weight of the compound is 246. In positive high-resolution FAB-MS, an ion for $[M+H]^+$ detected at *m/z* 247.0965 had a molecular formula of $C_{14}H_{15}O_4$ (calculated molecular weight: 247.0971). Therefore, the molecular formula of the active compound was

*Corresponding author; fax +82-2-820-5210
e-mail skkimbio@cau.ac.kr

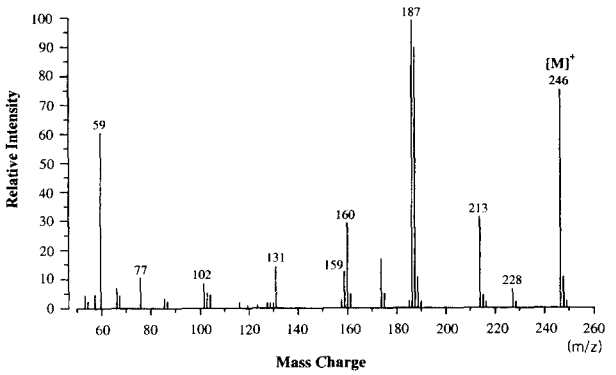

Figure 1. MS spectrum (JWS AX505 mass spectrometer, direct inlet, EI, 70 eV) of marmesin, a UV-A-absorbing compound from Thanakha bark.

determined to be $C_{14}H_{14}O_4$.

In direct EI MS (70 eV), a molecular ion was also detected at *m/z* 246 (Fig. 1). Prominent ions due to fissions of hydroxyl and methyls at *m/z* 228[M-H_2O]$^+$, 213[M-H_2O-CH_3]$^+$, and 187[M-H-H_2O-2CH_3]$^+$ were also shown, suggesting that the structure contains a tertiary alcohol moiety. Moreover, the presence of an ion at *m/z* 77 indicated that an aromatic ring is present (Silverstein et al., 1991).

300 MHz ^{13}C DEPT NMR revealed that signals at δ 24.38 and 26.13 were for methyls; at 29.55 for a methylene; and at 91.32, 98.09, 112.45, 123.63, and 143.94 for methines (Table 1). Signals at δ 71.77, 112.97, 125.33, 155.94, 161.73, and 163.50 represented non-protonated carbons. Thus, the extra formula of HO_4 is thought to be a hydroxyl and three single oxygen groups, including ester(s) and/or carbonyl(s). Absorption at δ 71.77 indicated that the hydroxyl is attached to a non-protonated carbon. Two methyl signals from 300 MHz ^{1}H NMR were detected as singlets at δ 1.146 and 1.279, both of which are down-shifted by the hydroxyl. By this we were able to confirm that a hydroxy-methylethyl (i.e., a tertiary alcohol) group is present in the structure. Signals at δ 112.97 and 125.33 in ^{13}C NMR were assignable for two methines in an aromatic ring. Those shown at δ 6.632 and 7.129 were singlets in ^{1}H NMR, indicating that four carbons in the aromatic ring are substituted and the two methines are separated.

Table 1. ^{13}C and ^{1}H NMR (Varian Gemini 2000 spectrometer, 300 MHz) data ($CDCl_3$, δ, ppm from trimethylsilane) for marmesin.

	^{13}C	^{1}H
Ring structure		
C-2	161.73	-
C-3	112.45	7.501 d (*J*=9.3)
C-4	143.94	6.109 s (*J*=9.3)
C-4a	112.97	-
C-5	123.63	6.632 s
C-6	125.33	-
C-7	163.50	-
C-8	98.09	7.129 s
C-8a	155.94	-
C-2	91.32	4.651 dd (*J*=8.7, 8.4)
C-3	29.55	3.125 dd (*J*=8.7, 7.2)
Side chain		
C-1	71.77	-
C-2 or C-3	24.38 or 26.13	1.146 s or 1.279 s

The presence of olefinic carbons due to absorptions at δ 115.94 and 161.73 in ^{13}C NMR was detected as doublets (*J*=9.3 Hz) at δ 6.109 and 7.501 in ^{1}H NMR. Furthermore, chemical shifts of the olefin in both ^{13}C and ^{1}H NMR were identical to those derived from coumarin, a compound that has a lactone with a vicinal double bond in the ring structure (Charles and Jacqlynn, 1993). Because of the presence of an aromatic ring, we believe this compound is a coumarin derivative. The signal for a methylene at δ 3.125 (2H, dd, *J*=8.7, 7.2 Hz) in ^{1}H NMR was coupled with that for a methine at δ 4.651 (H, dd, *J*=8.7, 8.4 Hz), indicating that an additional CH_2-CH- structure is involved in the compound.

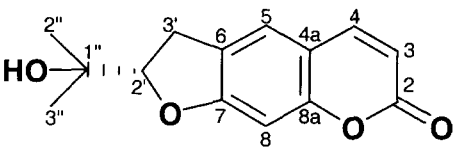

Figure 2. Structure of marmesin.

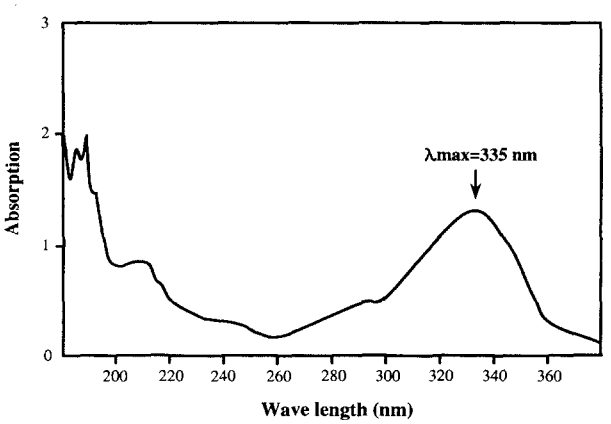

Figure 3. UV absorption spectrum (Beckman DU 650, in ethanol) of marmesin.

Thus, we assigned the final single oxygen as an ether. Taking these results altogether, we can characterize this active compound, named marmesin, as 2,3-dihydro-2-(1-hydroxy-1-methylethyl)-furanocoumarin (Fig. 2) (Murray et al., 1971; Elgamal et al., 1979).

Marmesin contains UV-absorbing chromophores, an aromatic ring, a double bond, and a carbonyl group in its structure. As expected, this compound absorbs a wide range of UV-A radiation, with λmax at 335 nm (Fig. 3). Because the powder from Thanakha bark has long been used as a cosmetic in Myanmar without causing toxicity, marmesin could be commercially useful as a UV A-blocking product. In addition, marmesin might serve in synthesizing even stronger UV A-filtering compounds through structural modifications.

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