

**FLAVONES FROM *MARCHANTIA CONVOLUTA*: ISOLATION OF
APIGENIN – 7 – O-β-D-GLUCURONIDE AND
5-HYDROXYL-7-METHOXYL-2-METHYLCHROMONE**

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ABSTRACT

Dried leaves of *Marchantia convoluta* are largely used as hepatoprotectives, and to treat tumefaction of skins in China. Two flavones were isolated from the leaves of *Marchantia convoluta* by silica gel column and preparative high performance liquid chromatography (PHPLC). These compounds were identified through spectral analysis (IR, UV, ¹HNMR, ¹³CNMR, MS) as 5-hydroxyl-7- methoxyl-2- methylchromone and a flavone glycoside, Apigenin-7-O-β-D-glucuronide.

Keywords: *Marchantia convoluta*, apigenin-7-O-β-D-glucuronide, spectral analysis, flavones,

INTRODUCTION

Marchantiaceae plants are well-known traditional Chinese medicinal herbs, extensively used to treat tumefaction of skins, protect the liver, treat hepatitis and as an antipyretic in the countryside (1-3). There are a large number of Marchantiaceae plants in Guangxi Zhuang Autonomous District such as *Marchantia polymorpha*, *Marchantia convoluta* and *Marchantia paleacea*. These species occur together and are usually not easily distinguishable due to physical

similarities arising from similarities in genetic make up.

Marchantia convoluta is presently only found in China (4). Compared to *Marchantia polymorpha*, *Marchantia convoluta* is quite rare. Several chemical constituents of *Marchantia convoluta* have been identified and include mainly flavonols, terpenoids, and steroids (5-8). The flavonoids of *Marchantia convoluta* mainly consist of quercetin, luteolin, apigenin and their O- and C-glycosides (6).

The dried leaves of *Marchantia convoluta* are largely used to protect livers, and to treat tumefaction of skins in China. High dosage of flavonoids from *Marchantia convoluta* (20 and 40 μg/ml) could significantly reduce the activity of

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ALT and AST in the serum of mice with acute hepatic injury caused by carbon tetrachloride (CCl₄) as well as increase the contents of TP and ALP and inhibit the auricle tympanites of mice caused by dimethylbenzene. Flavonoids from *Marchantia convoluta* have also been found to inhibit colibacillus, tyhoid bacillus, *Staphylococcus aureus*, bacillus enteritidis, hemolytic streptococci type B and *Diplococcus pneumoniae*, and possess distinct antibiosis, anti-inflammatory action and diuresis in mice (1). Extracts from *Marchantia convoluta* have also been reported to inhibit the human liver and lung cancer cell lines (2).

In this present study, the flavonoidal constituents of the *n*-butanol fraction of the ethanolic extract of *Marchantia convoluta* were investigated.

EXPERIMENTAL

Plant material

The leaves of *Marchantia convoluta* were collected in Shangling City of Guangxi Zhuang Autonomous District of China in August 2004. The specimen (No 20041364) was identified by Zhou Zi-jing at Biology Department of Guangxi Chinese Medical University, where a Voucher specimen has also been deposited. The leaves, after washing with water and air-drying for several days, were powdered.

Extraction and isolation

A 584 g quantity of the powdered leaves from above was extracted

with 80% ethanol for 1 hour at 70 °C. The solvent was distilled off under reduced pressure to dryness. The residue was successively treated with petroleum ether, ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH.) The *n*-BuOH fraction was chromatographed on silica gel column and preparative high performance liquid chromatograph (PHPLC). Pure fractions - 1 (305 mg) and 2 (87 mg) were obtained. Three other pure fractions also obtained from the extract (Fractions 3 – 5) had earlier been described (6).

Spectral analysis

Infra red (IR) spectra were carried out on a Bruker Vector 22 spectrophotometer in KBr pellets. Ultraviolet (UV) spectra were recorded with a Shimadzu UV-2501PC spectrophotometer. ¹HNMR and ¹³CNMR spectra were recorded in DMSO-d₆ using a JEOL A-500 spectrometer with TMS as an internal stand. FAB mass spectra were recorded with a JEOL HX-110 spectrometer.

RESULTS AND DISCUSSION

Spectral analysis

The *n*-butanol soluble portion of the ethanolic extract of the leaves of *Marchantia convoluta* was chromatographed on silica gel to yield fractions 1 and 2.

Fraction 1 was obtained as a yellow powder. The UV spectra showed absorption bands at 267 and 336 nm. The absorption band of 336 nm shifted to 382 nm by addition of NaOMe indicating the presence of

free hydroxyl groups at C-4. The absorption band of 336 nm shifted to 382 nm and other three bands 340 (a) 299 (b) and 276 nm (c) indicating the presence of free hydroxyl groups at C-5. The absorption band of 267 nm did not shift upon addition of NaOAc indicating the presence of *O*-glycoside at C-7 (9).

In the IR spectral analysis, the peak at 3421 cm^{-1} , a broad band, means O-H stretching. The peaks at 2958 cm^{-1} , 2925 cm^{-1} and 2855 cm^{-1} showed the C-H bands stretching of saturated hydrocarbons, suggesting a glucuronide. The IR spectra also showed chromone carbonyl absorption at 1630 cm^{-1} and carbonyl absorption at 1736 cm^{-1} . 1736 cm^{-1} is a strong band showing the C=O stretching of -COOH in glucuronide.

The ^{13}C NMR spectra also showed two carbonyl carbon absorptions; one at 170.8 δ due to a chromone carbonyl carbon and another at 172.1 δ . The chemical shift assignment of the ^1H NMR and ^{13}C NMR spectral data supported the identity of the aglycone moiety as apigenin. The result of the FAB Mass spectra showed a molecular ion 446[M] $^+$. In the EI mass, 269 [M-glucuronic acid] $^+$ was given indicating that the sugar moiety was present as glucuronic acid (GA). Acid hydrolysis showed the presence of GA. In the ^1H NMR, an end proton was observed at δ 4.93(1H,d,J=7.2Hz) indicating that the structure of GA was β -D glucuronic acid. From the foregoing therefore, compound 1 was confirmed as apigenin 7-O- β -D-glucuronide. The flavones from Fractions 3 – 5 had earlier been shown to be apigenin, luteolin and

quercetin (6)

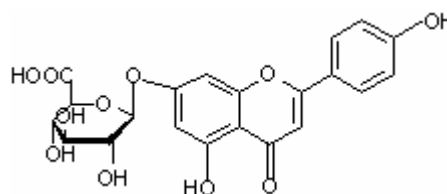


Fig. 1 Structure of Apigenin-7- β -D-glucuronide (Compound 1).

Details of the spectral data of the isolated compound 1 are given below:

A yellow powder. mp>300°C.
UV λ max(nm): 336, 296sh, 267(MeOH); 275, 323, 392 (NaOMe) 384, 346, 302, 276 (AlCl_3); 276, 299, 340, 382 (AlCl_3/HCl); 274, 300, 375 (NaOAc); 267, 302sh, 338 (NaOAc/ H_3BO_3).

IR (KBr) cm^{-1} : 3425, 2958, 2925, 2855, 1736, 1630, 1609, 1514, 1442, 1257, 1220, 1122, 1056, 1021.

FAB-MS: 446[M] $^+$, EI-MS: 269 [M-glu] $^+$, 178, 136, 121, 105, 91.

^1H -NMR (DMSO- d_6) δ : 6.46 (1H, s, H-3), 6.78 (1H, d, J=2.1Hz, H-6), 6.86 (1H, d, J=2.1Hz, H-8), 6.91(2H, d, J=8.2Hz, CH-3, 5), 7.92 (2H, d, J=8.2Hz, H-2, 6), 5.69 (1H, d, J=7.8Hz, H-4).

^{13}C -NMR (DMSO- d_6) δ : 163.5 (C-2), 104.5 (C-3), 172.1 (C-4), 161.9 (C-5), 95.8 (C-6), 163.8 (C-7), 96.2 C-8), 158.3 (C-9), 105.7 (C-10), 122.6 (C-1), 128.3 (C-2), 115.7 (C-3), 149.2 (C-4), 115.7 (C-5), 128.3 (C-6), 99.8 (C-1''), 74.7 (C-2''), 77.1 (C-3''), 73.1 (C-4''), 76.9 (C-5''), 170.8 (C-6'').

The spectral data of Compound 2 contained in Fraction 1 are as follows:

A yellow powder. Mp, 116-118°
C. UV λ max(nm): 230, 255, 285, 325sh (MeOH).

IR (KBr) cm^{-1} : 3423, 2956, 2925, 2854, 1706, 1625, 1590, 1511, 1384, 1140, 1079.

EI-MS: 206.6 [M]⁺, 178[M-CO]⁺, 149, 105, 91, 69, 43.

¹H-NMR (CDCl₃) δ : 2.35 (3H, s, 2-CH₃), 3.84 (3H, s, -OCH₃), 6.02 (1H, s, H-3), 6.32(1H, d, J=1.9Hz, H-8), 6.34 (1H, d, J=1.9Hz, H-6).

The above data led to the conclusion that the isolated Compound 2 is 5 – hydroxyl – 7 – methoxyl – 2 – methyl chromone.

CONCLUSION

The flavones apigenin 7-O- β -D-glucuronide and 5 – hydroxyl – 7 – methoxyl – 2 – methyl chromone have been isolated from the *n*-butanol fraction of the ethanolic extract of the plant *Marchantia convoluta*. These flavones were identified through spectral analysis (IR, UV, MS, ¹HCMR and ¹³NMR). Three other flavones (apigenin, luteolin and quercetin) had earlier been isolated from the same extract, by the authors.

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