Compounds 7-Hydroxy-6-Metoxic Cytotoxic Cytotoxic Stem Leather From Chisochetonmacrophyllus (Meliaceae) Bark

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Abstract

A phenyl propanoid compound, 7-hydroxy-6-methoxycoumarin has been isolated from the bark of *Chisocheton macrophyllus* (Meliaceae). The chemical structure of isolated compound was identified on the basis of spectroscopic data including UV, IR, 1D-NMR, 2D-NMR and mass along with comparison with those spectral data previously reported. 7-hydroxy-6-methoxycoumarin compound showed cytotoxic effect against P-388 murine leukemia cell with IC_{50} value of 16.5 mg/mL. 7-hydroxy-6-methoxycoumarin compound was reported for the first time from genus of *Chisocheton*.

Key words: Meliaceae, *Chisocheton macrophyllus*, pheyepropanoid, coumarin, scopoletin.

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A. INTRODUCTION

Chisocheton is a genus of the Meliaceae family. This plant is widely known in tropical and subtropical areas such as in Indo-China, Papua New Guinea, South China, Thailand, Malaysia, Nepal, India, Bhutan and Myanmar (Vossen and Umali, 2002). Some of these plant species have been used traditionally as laxatives, medicinal and cosmetic ingredients and are widely used as poison in fish (Lim, 2008).

The content of compounds in the Chisocheton genus has been widely reported to have very beneficial activities both in the health and agriculture fields, including as an antimalarial, antimicrobial, cytotoxic (Maneerat et al., 2008; Phongmaykin et al., 2008; Mohamad et al., 2009)), cytotoxic (Wong et al., 2011; Mohamad et al., 2008; Awang et al., 2007), anti-tumor (Yang et al., 2009) and anti-inflammatory (Yang et al., 2011).

Inada et al., (1993) reported that from the leaves of Chisocheton macrophyllus (Meliaceae), a triterpenoid compound that has antitumor activity was found, namely 24-hydroxidamara -20,25-dien-3-on, and 3 known triterpenoid compounds, namely moronic acid. , oleanolic acid and betulonic acid. In our ongoing investigation of bioactive compounds from the Chisocheton plant, the ethyl acetate extract of C. macrophyllus stem bark demonstrated cytotoxic activity against murine leukemia P-388 cells. In this paper we will describe the isolation and structural identification of phenyl propanoneoid, 7-hydroxy-6-methoxy coumarin (scopoletin) which has cytotoxic activity against murine leukemia P-388 cells.

B. METHOD

General. Melting points were measured using a Fisher-John melting point apparatus (uncorrected). IR spectra were obtained from the FTIR spectrophotometer One Perkin Elmer spectrum at KBr. The 1H- and 13C-NMR spectra were obtained by a JEOL JNM ECA-500 spectrometer. MS spectra were obtained with the Mariner Biospectrometry-Finnigan instrument. Chromatographic separation was carried out on silica gel G60, silica gel (70-230 and 200-400 mesh, Merck). TLC plate was filled with silica gel GF254 (Merck, 0.25 mm) and detection was carried out by staining 10% H_2SO_4 in ethanol followed by heating.

Plant material. Bark of C. macrophyllus from the Metro Manila Government Laboratory.

Extraction and Isolation. The bark of C. macro-phyllus (3.1 kg) was crushed and extracted using a maceration technique (solid-liquid extraction) with n-hexane, ethylacetate, and methanol solvents at room temperature. Ethylacetate macerate was concentrated using a rotary evaporator to obtain ethyl acetate extract (53.5 g), then separated by vacuum liquid chromatography technique with silica gel as a stationary phase and a mobile phase of a solvent mixture in the form of n-hexane, ethylacetate, methanol; 10%, graded to obtain 9 fractions (A-I). The chemical content of the D fraction was then separated by using a vacuum liquid chromatography technique to obtain 9 combined fractions (D1-D9). The D5 and D6 fractions were then separated by column chromatography technique with the G60 silica gel stationary phase (70-230 mesh) and the mobile phase in the form of a fixed solvent ratio of chloroform: methanol (9.75: 0.25) to produce isolate D5-6 which was then recrystallized. at room temperature using n-hexane solvent and a pure solid (31 mg) was obtained. All separation steps were monitored by thin layer chromatography under UV light at wavelengths of 254 nm and 365 nm. The structure of the pure isolate D5-6 was elucidated using UV, IR, 1D-NMR, 2D-NMR spectroscopy methods.

C. RESULT AND DISCUSSION

Scopoletin obtained as a dark yellow (orange) tanamorph, HR-ESI-TOFMS shows a [M + H] + peak at m / z 193.0448 which corresponds to the molecular formula $C_{10}H_8O_4$. UV spectra in methanol solvent showed five peaks at the wavelengths of 345, 295, 253, 226 and 204 nm. The spectra in the shifting reagent (MeOH + NaOH) showed a bathochromic shift in the band 1 (345 nm) to 391 nm, band 3 (253 nm) to 274 nm, and band 5 (204 nm) to 212 nm. This batochromic shift indicates the presence of a free -OH group which can extend the conjugation of a number of double bonds.

The presence of the OH group was confirmed from the IR spectrum at 3425 cm-1, and the presence of the C-O ester and C = O lactone ester stretches at 1292 cm-1 and 1707.00 cm-1 which indicated the presence of a six-membered cyclic ester (lactone). In

addition, there is also an aromatic C = C stretch at 1566 cm-1 as well as C-H bending out of the plane of the aromatic ring in the area of 1141 cm-1.

The amount of carbon present in the structure of the compound can be determined from the 13C-NMR spectrum. It can be observed that there are ten carbon signals consisting of six aromatic carbons at δC (ppm): 103.3 (C-8), 108.1 (C-5), 111.2 (C-10), 145.4 (C -6), 151.0 (C-7), 150.0 (C-9), two carbonolyphenics at δC (ppm): 112.2 (C-4), 144.3 (C-3), and one carbonyl ester at δC (ppm): 162.7 (C-2) (Table 1). The presence of oxygenated sp3 carbon in this compound was observed at δC 56.3 ppm which strengthened the presence of a methoxy group in this compound. Based on MS, UV, IR and 13C-NMR data, it can be concluded that there is a bicyclic framework with a methoxy group as one of the substituents.

The type of carbon (methyl, methine, methylene or quaternary carbon) was determined by the 135o DEPT (Distortionless Enhancement by Polarization Transfer) experiment. The suspected bicyclic framework with a methoxy group as its substituent was confirmed by the 1H-NMR spectrum. It is known that there is a signal that shows the characteristics of methoxy and hydroxyl protons at δH 3.84 ppm (s, H-6) and 4.83 ppm (br.s, H-7), proto naromatic at δH ppm: 6.78 (s , H-8), 6.79 (s, H-5). The presence of a lactone ring is represented by olifenic protons at δH (ppm): 6.14 (d, J = 9.75 Hz, H-4) and olifenic protons that are less shielded at δH (ppm): 7.58 (d, J = 9.75 Hz, H-3), so it can be assumed that the chemical structure of the compound is scopoletin.

The existence of functional groups in the partial structure can be proven by using the HMBC correlation. The HMBC spectrum shows that the presence of carbonyl groups at C-3 can be proven by the presence of a correlation of H-3 (δH7,58) with a distance of four bonds (matchmaking long distance) to C-5 (δC 108.1), the correlation with the three-bond distance to C-9 (δC 150.0), and the correlation with the two-bond distance to C-2 (δC162.7). Furthermore, there is also a correlation between H-4 (δH 6.14) with a distance of three bonds to C-2 (δC 162.7) and a correlation with a distance of two bonds to C-10 (δC 111.2). Furthermore, the correlation H-5 (δH 6.79) with a distance of five bonds to C-10 (δC 111.2) and correlation with a distance of three bonds to C-7 (δC 151.0). There is also a correlation of H-7 (δH 6.78) with a distance of three bonds to C-9 (δC 150.0). The correlation between the methoxy proton (δH 3.84) and the distance of two bonds to C-6 (δC 145.4) proves that the position of the methoxy group is attached to the carbon with δC 145.4 ppm. While the hydroxyl group is attached to the carbon with δc 151.0 ppm.

Table 1. NMR data of scopoletin compounds

Postition C	1H-*NMR* δ_H (ppm), (GH,-mult, Hz)	13C-*NMR* δ_C (ppm)	*HMBC* (H◎C)	1H-1H *COSY*
2	-	162,7	-	-
3	7,58 (1H, d, 9,75)	144,3	5,9,2	H-4
4	6,14 (1H, d, 9,75)	112,2	2,10	H-3
5	6,79 (1H, s)	108,1	10,7	-
6	-	145,4	-	-
7	-	151,0	-	-
8	6,78 (1H, s)	103,3	9	-
9	-	150,0	-	-
10	-	111,2	-	-
$-OCH_3$	3,84 (1H,s)	56,3	6	-

D. CONCLUSION

Based on the results of the interpretation of UV, IR, MS, 1H-NMR, 13C-NMR, DEPT, HMQC, HMBC, 1H-1HCOSY spectra and comparative data, the isolate was a pale yellow amorphous solid as much as 31 mg isolated from 3.1 kg powdered stem bark of C. macrophyllus is defined as a phenyl propanoid compound, namely 7-hydroxy-6-methoxy coumarin (scopoletin). Sen-yawa phenyl propanoid, 7-hydroxy-6-methoxy coumarin has been reported for the first time from the C. macrophyllus plant.

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