PHENOLIC COMPOUNDS
FROM *Aquilaria microcarpa* STEM BARK

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ABSTRACT

This paper reports a study of secondary metabolites contained in *Aquilaria microcarpa*, a species belonging to Thymelaceae. This species has not been investigated so far. *Aquilaria microcarpa* is one of *Aquilaria* species that grows in Indonesia. The sample plant used is taken from east Kalimantan. Extraction of stem bark is done using methanol. Two phenolic compounds, namely 5,3’,4’-trihydroxy-7-methoxyflavon or known as 3’-hydroxy genkwanin and 6-hydroxy-2-(2-phenylethyl)chromone are isolated and identified. The chemical structure of these compounds is determined based on spectroscopic data, as well as HR-ESI-MS and NMR spectra. Both compounds were reported previously but they were extracted from another species of *Aquilaria*.

Keywords: *Aquilaria microcarpa*, Thymelaeaceae, chromone, flavonoid.

INTRODUCTION

*Aquilaria* is a genus belonging to Thymelaeaceae family. The species of *Aquilaria* are widely distributed in Asia. Certain trees of *Aquilaria* species produce the fragrant resinous wood, known in the different regions as agarwood, eaglewood, gaharu, kanankoh, jinkoh, chen xiang or tram. Other people also call it aloeswood or agalloch [1, 2]. The *Aquilaria* genus is rich in a variety of different classes of natural products, especially sesquiterpenes and chromones. Flavonoid, benzophenone, diterpenoid, triterpenoid and lignin compounds are also present.

Agarwood preparations are used in Kampo medicine in Japan because of their sedative, analgesic or digestive properties [3]. *Aquilaria* leaves are applied in China topically to treat injuries such as fractures and bruises [4], while in Korea agarwood is used for the treatment of cough, asthma, and as a sedative among others [5]. In Saudi Arabia and other Arabic countries, the wood of *Aquilaria* trees is used as incense at important religious occasions [6, 7].

Some species of *Aquilaria* which are widely studied are *A. sinensis*, *A. malaccensis*, *A. crassna* and *A. agallocha*, whereas *A. beccariana*, *A. hirta*, *A. cumingiana*, *A. filaria* dan *A. microcarpa* are *Aquilaria* species that grow in Indonesia and are not studied so far.

This paper reports research results referring to *A. microcarpa* as one of these species. Two phenolic compounds are isolated from stem bark of *A. microcarpa*. They are identified as 5,3’,4’-trihydroxy-7-methoxyflavon or known as 3’-hydroxy genkwanin (1) and 6-hydroxy-2-(2-phenylethyl)chromone (2). Their structure of is determined spectroscopically. It is presented in Fig. 1.

EXPERIMENTAL

Non infected stem bark of *A. microcarpa* obtained from Bukit Bangkirai forest conservation, Samboja, Samarinda, Kalimantan Timur was used.

Methanol, *n*-hexane, ethyl acetate, diisopropyl ether, chloroform, cerium sulfate, acetone, silica gel 60 GF254 (Merck), silica gel 60 PF254 (Merck), silica gel 60 GF254 0.25 mm (Merck) were the chemical reagents applied.

A rotary vacuum evaporator, column and radial
chromatography were used in the course of the experimental procedure applied. The latter was as follows. 1.69 kg of stem bark was cut up and then subjected to extraction with n-hexane. The residue was separated from the filtrate and then macerated again using methanol. The extract methanol was fractionated with ethyl acetate. The ethyl acetate fraction was then separated using a column chromatography on silica gel. The fractions of interest were subsequently separated again using several methods of chromatography until a pure product was obtained. Compound 1 was obtained after a double separation using gravity column chromatography followed by a double separation using radial chromatography.

Meanwhile, compound 2 was obtained after a double separation using flash chromatography and a single separation using radial chromatography. A mixture of hexane - ethyl acetate whose polarity was gradually increased was the eluent used in those separations. Compounds 1 and 2 were obtained as much as 3 mg each.

The structure of each compound was elucidated using UV-Vis spectroscopy, HRESI-MS and NMR. UV-Vis Shimadzu 1800, HR-ESI-MS Waters LCT XE ESI-TOF (electrospray ionization-time of flight), and NMR JEOL ECA 400 (1H-NMR, 400 MHz) and (13C-NMR, 100 MHz) were used.

**RESULTS AND DISCUSSION**

5,3',4'-Trihydroxy-7-methoxyflavon (1) is a pale yellow solid; \( \lambda_{\text{max}} \) (MeOH) nm (log e): 278 (4.09), 287 sh (3.97), and 326 (3.97); HRESI-MS: \([M-H]^{-}\) at m/z 301.0718 in accordance with the molecular formula \( \text{C}_{16}\text{H}_{13}\text{O}_{6} \); the NMR data is showed in Table 1.

6-Hydroxy-2-(2-phenylethyl)chromone (2) is a pale yellow solid; \( \lambda_{\text{max}} \) (MeOH) nm (log ε) : 242 (4.33), 314 (3.58), and 369 (3.39); HRESI-MS: \([M-H]^{-}\) at m/z 265.0805 in accordance with the molecular formula \( \text{C}_{17}\text{H}_{14}\text{O}_{3} \); NMR data is shown in Table 1.

The 1H-NMR spectrum of the isolated flavonoid compounds 1 in CDCl3 shows two units of aromatic proton signals that refer to three aromatic proton signals of ABX system and two aromatic proton signals of AX system. The three aromatic proton signals of ABX system are attributed to the protons in the chemical shifts...
C-NMR spectrum of isolated compound shows fifteen and five monosubstituted aromatic proton signals. The referring to three ABX system aromatic proton signals appears also in compound 1 with one hydroxyl substituent. The proton signals of the other aromatic unit (five protons) appear as a multiplet at \( \delta \_H 7.27 \) ppm. The proton singlet signal at \( \delta \_H 6.04 \) ppm and the two proton multiplet signals at \( \delta \_H 3.08 \) and 2.96 show a characteristic chromosome class of compounds that have a hydroxy substituent at C-6 or C-7.

The \(^{13}\)C NMR spectrum analysis shows fifteen carbons that are perfectly separated. Based on HR - ESI - MS analysis there should be seventeen carbons. This suggests the existence of two symmetric carbons. Two symmetric carbons are located in ring B at C-2’ / 6’ and C-3’ / 5’. Fifteen carbon signals refer to six quaternary carbons (\( \delta \_C \) (ppm) 182.7; 168.9; 156.6; 151.2; 141.2; 124.6), seven methine carbons CH (\( \delta \_C \) (ppm) 109.7; 108.9; 120.2; 115.8; 129.2;127.1;129.3) and two methylene carbons CH\(_2\) (\( \delta \_C \) (ppm) 33.4 and 36.4). Four of these six quaternary carbon signals consist of three arylxary carbon signals (\( \delta \_C \) (ppm) 169.2; 156.6; 151.2) and one of carbonyl carbon signal (\( \delta \_C \) 182.7 ppm). This indicates that compound 2 has the structure of 2-(2-phenylethyl)chromone with one hydroxyl substituent. The \(^1\)H and \(^{13}\)C-NMR data of compound 2 is presented in Table 2. A correlation of one bond in the HMOC spectrum analysis of compound 1 shows seven correlations as presented in Table 2. The same correlation occurs also in compound 2.

The presence of a methoxy and hydroxyl groups in the structure of compound (1) is evidenced by the HMOC spectrum. It is confirmed that the two hydroxyl groups in ring B are positioned at 3’ and 4’. The position of the methoxy group and the remaining hydroxyl group in ring A has to be determined. The positions to be filled refer to numbers 4 and 7. The proton signal of the hydroxyl group (\( \delta \_H \) of 11.14 ppm) shows correlations with two quaternary carbon signals (\( \delta \_C \) of 163.6 ppm and 102.0 ppm) and one methine carbon signal at \( \delta \_C \) of 101.0 ppm. It has been stated above that a deshielding proton signal at \( \delta \_H \) of 11.14 ppm is characteristic for the hydroxyl group at C-5 which is capable of forming a hydrogen bond with the carbonyl group at C-4.

The \(^1\)H NMR spectrum of isolated compound (2) in acetone-\( d_6 \) shows two units of aromatic proton signals referring to three ABX system aromatic proton signals and five monosubstituted aromatic proton signals. The three ABX system aromatic proton signals appear in the chemical shifts \( \delta \_H \) of 6.88 (d, \( J = 8.3 \) Hz), 7.24 (\( dd, J = 8.3 \) and \( J = 2.1 \) Hz), and 7.48 (d, \( J = 2.1 \) Hz). The proton signals of the other aromatic unit (five protons) appear as a multiplet at \( \delta \_H 7.27 \) ppm. The proton singlet signal at \( \delta \_H 6.04 \) ppm and the two proton multiplet signals at \( \delta \_H 3.08 \) and 2.96 show a characteristic chromosome class of compounds that have a hydroxy substituent at C-6 or C-7.

The \(^{13}\)C NMR spectrum analysis shows fifteen carbons that are perfectly separated. Based on HR - ESI - MS analysis there should be seventeen carbons. This suggests the existence of two symmetric carbons. Two symmetric carbons are located in ring B at C-2’ / 6’ and C-3’ / 5’. Fifteen carbon signals refer to six quaternary carbons (\( \delta \_C \) (ppm) 182.7; 168.9; 156.6; 151.2; 141.2; 124.6), seven methine carbons CH (\( \delta \_C \) (ppm) 109.7; 108.9; 120.2; 115.8; 129.2;127.1;129.3) and two methylene carbons CH\(_2\) (\( \delta \_C \) (ppm) 33.4 and 36.4). Four of these six quaternary carbon signals consist of three arylxary carbon signals (\( \delta \_C \) (ppm) 169.2; 156.6; 151.2) and one of carbonyl carbon signal (\( \delta \_C \) 182.7 ppm). This indicates that compound 2 has the structure of 2-(2-phenylethyl)chromone with one hydroxyl substituent. The \(^1\)H and \(^{13}\)C-NMR data of compound 2 is presented in Table 2. A correlation of one bond in the HMOC spectrum analysis of compound 1 shows seven correlations as presented in Table 2. The same correlation occurs also in compound 2.

The presence of a methoxy and hydroxyl groups in the structure of compound (1) is evidenced by the HMOC spectrum. It is confirmed that the two hydroxyl groups in ring B are positioned at 3’ and 4’. The position of the methoxy group and the remaining hydroxyl group in ring A has to be determined. The positions to be filled refer to numbers 5 and 7. The proton signal of the hydroxyl group (\( \delta \_H \) of 11.14 ppm) shows correlations with two quaternary carbon signals (\( \delta \_C \) of 163.6 ppm and 102.0 ppm) and one methine carbon signal at \( \delta \_C \) of 101.0 ppm. It has been stated above that a deshielding proton signal at \( \delta \_H \) of 11.14 ppm is characteristic for the hydroxyl group at C-5 which is capable of forming a hydrogen bond with the carbonyl group at C-4. Hence it follows that the signal at 163.6 ppm refers to C-5, that at 102.0 ppm
ppm refers to C-4a, while that at 101.0 ppm refers to C-6. The singlet proton signal of the methoxy group (δ_H of 3.67 ppm) gives one correlation with the quaternary carbon signal at δ_C of 166.7 ppm. The latter is a signal referring to C-7. The results of HMBC spectrum analysis of compound 1 are illustrated in Fig. 2.

HMBC analysis is required to verify the hydroxy group position (at C-6 or C-7) in compound 2 structure. A singlet proton signal at δ_H of 6.04 ppm (H-3) shows correlations with two quaternary carbon signals at δ_C of 169.1 ppm (C-2) and 124.6 ppm (C-4a) and one methine carbon at δ_C of 36.4 ppm (C-8'). Another correlation is revealed between the aromatic proton in ABX system and the two aryloxy carbons and one quaternary carbon. The proton considered appears as a doublet with a coupling constant of 2.1 Hz at δ_H of 7.48 ppm (H-5). The two aryloxy carbons mentioned above show signals at δ_C of 156.6 (C-6) ,151.2 (C-8a) ppm respectively, while the quaternary carbon appears at δ_C of 124.6 ppm (C-4a). A correlation is also observed between the doublet (J = 8.3 Hz) proton signal at δ_H of 6.88 ppm (H-8) with one aryloxy carbon at δ_C of 151.2 ppm (C-8a) and one methine carbon at δ_C of 120.2 (C-7).

The overall correlations data indicates that the hydroxyl group is attached at C-6. The results of HMBC spectrum analysis of compound 2 are shown in Fig. 2.

All data obtained is compared to that found in the literature. Compound 1 is reported by Qi [8] who succeeds isolating this compound from the leaves of A. sinensis. In that report, Qi mentions also that this 3’-hydroxy genkwanin has a significant inhibitory activity in respect to neutrophils respiratory burst stimulated by PMA with IC_{50} value 0.80 ± 0.13 µmol/l. Likewise, compound 2 is compared to the same compound isolated [9] from an ether extract of powdered agarwood from Kalimantan. In 1989 Yang [10] is the first to obtain this compound from the ether soluble fraction of an alcoholic extract of A. malaccensis agarwood chips [11] and from an EtOAc extract of Chinese agarwood induced by artificial holing from A. sinensis [12]. The latter reference mentions also that this compound exhibits acetylcholinesterase inhibitory activity with percentage of inhibition of 19.3 ±0.8 %. Bioactivity tests referring to compound 1 are not performed in the course of the present investigation because of the small quantity isolated. However an anticancer activity test against T47d cell line is carried out with the participation of compound 2. The result indicated that the IC_{50} is 2884.03 µg/mL. The same test is also carried out with the ethyl acetate fraction used for the isolation of compound 1 and compound 2. The result shows that this fraction has a weak activity with IC_{50} value of 26.48 ± 0.02 µg/mL.

CONCLUSIONS

A plant sample of Aquilaria microcarpa was used to isolate two compounds. Their structure was determined based on analyses, including UV, HR-ESI-MS and NMR. The compounds obtained were identified as 5,3’,4’-tri hydroxy-7-methoxyflavon or known as 3’-hydroxy genkwanin 1 and 6-hydroxy-2-(2-phenylethyl)chromon 2. They were isolated by other research groups using A. sinensis and A. malaccensis, which are different species of Aquilaria.

Acknowledgements

This work was financially supported by 2016 grant from the Ministry of Research and Higher Education. The authors are grateful to Bukit Bangkirai Forest Conservation, Samboja, Samarinda, Kalimantan Timur for the sample material provision.
REFERENCES