Triterpenoid and Steroid from the Rind of *Chisocheton macrophyllus* (Meliaceae)

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Abstract. Triterpenoids form numerous and structurally diverse family of secondary metabolites derived from C₅ isoprene units joined in a head-to-tail fashion. Among the terpenoids, triterpenoids emerge as a unique and large group of phytochemicals. There is secondary metabolites steroid, which is modified triterpenoids containing tetracyclic ring system but losing three methyl at C-4 and C-14. Triterpenoids and steroids can be obtained in the family Meliaceae. The *Chisocheton* genus which is part of the Meliaceae family is known for secondary metabolites, such as terpenoids, phenolics, and steroids. The purpose of this research is to isolate and to elucidate triterpenoids and steroid compounds from rind of *Chisocheton macrophyllus*. Stigmastane-type steroid Stigmast-22-en-3β,5α-ol (1) and pentacyclic triterpenoid moronic acid (2), were isolated from the n-hexane extract of the rind of *C. macrophyllus* using column chromatography. The chemical structures of compounds 1-2, were identified on the basis of spectroscopic evidence and by comparison with those spectra previously.

1. Introduction

Terpenoids are a large and structurally diverse family of natural compounds derived from C₅ isoprene units. Isoprene was known as a fundamental building block to form terpenoids. Dimethylallyl pyrophosphate (DMAPP) and isopentenyl diphosphate (IPP) identified as active isoprene units derived by mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathway. Terpenoids structure joined in a head-to-tail fashion, classified as hemiterpenes (C₅), monoterpenes (C₁₀), sesquiterpenes (C₁₅), diterpenes (C₂₀), sesterterpenes (C₂₃), triterpenes (C₃₀), and tetraterpenes (C₄₀). Among the terpenoids, triterpenoids emerge as a unique and large group of phytochemicals. Triterpenoids were not formed by an extension of adding IPP but two molecules of farnesyl diphosphate (FPP) are joined in a tail-to-tail fashion resulting squalene skeleton. Squalene can fold into two different conformation resulting two different cations such as dammarenyl cation and proteosteryl cation. Proteosteryl cation undergoes further cyclizations and series of proton,methyl Wagner-Meerwein 1,2-shisfts until form a lanosterol skeletal structure. Lanosterol is a precursor for cholesterol and other steroids in animals. In plants, cycloartenol take a role as a precursor for steroids. Steroids can through modifications especially to the side-chain to help forming wide range of structurally diverse natural compound such as sterols, steroidal saponins, cardioactive glycosides, bile acids, corticosteroids, number of hormones,
and some hydrocarbons. All steroid classes and their metabolites play important roles in the physiology and biochemistry of living organisms.

During the course of our continuing search for novel pentacyclic triterpenoid and stigmastane-type steroid from the *Chisocheton* species, we isolated and described the pentacyclic triterpenoids, Betulonic acid from *C. macrophyllus* and stigmastane-type steroid, 7α-hydroxy-β-sitosterol, stigmasta-4,6-diene-3-one, stigmasterol, 7α-methoxy-3β-sitosterol, stigmast-5-en-3β-ol, and 7β-sitosterol-3-O-acetate from *C. tomentosus*, *C. macrophyllus*, and *C. cumingianus*.

In the further screening for pentacyclic triterpenoids and stigmastane-type steroids in *Chisocheton* species, we found that the methanolic extract of *C. macrophyllus* exhibited the presence of the triterpenoid and steroid based on the phytochemical test. *C. macrophyllus* is a higher plant and mainly distributed in Sumatera, Java, and Papua Island in Indonesia. Previous phytochemical studies reported the presence of limonoids, steroids, and triterpenoids from leaves, seed, and stembark of *C. macrophyllus*. Triterpenoids and steroids from the rind of *C. macrophyllus* is still unknown. We report herein the isolation and structure elucidation of pentacyclic triterpenoids and stigmastane-type steroids.

2. Experimental

2.1. General Experimental Procedure

Melting points were measured on a melting point M-565 apparatus. IR spectra were recorded by Perkin Elmer Spectrum 100 FT-IR Spectrometer in KBr. 1H- and 13C-NMR spectra of compound 1 and 2 were obtained with a JEOL 500 MHz spectrometer, using TMS as internal standard. Mass spectra were determined with Waters Q-TOF Xevo mass spectrometer instrument. Chromatographic separations were carried out on Merck silica gel 60 (70–230 mesh and 230–400 mesh). TLC glass plates were precoated with silica gel GF254 (Merck, 0.25 mm). Spots on the plates were detected under UV light (254 and 365 nm) and visualized by spraying with 10% sulfuric acid in ethanol, followed by heating.

2.2 Plant Materials

The rind of *C. macrophyllus* was collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia in August 2018. The plant was identified by Center for Plant Conservation Botanic Gardens, Bogor, Indonesia.

2.3 Extraction and Isolation

Powder of dried rind of *C. macrophyllus* (3.2 kg) was extracted with methanol at room temperature, filtered, and concentrated. The methanol extract (335.1 g) was resuspended in a mixture of water and methanol, then extracted with n-hexane, ethyl acetate, and n-butanol successively. Each extract was evaporated to yield the n-hexane extract (156.2 g, 5.4%), ethyl acetate extract (62.8 g, 2.2%), and n-butanol extract (40.2, 1.3%). The n-hexane extract (156.2 g) was separated by vacuum liquid chromatography on silica gel 60 using a gradient n-hexane and ethyl acetate to give fourteen subfractions (A–N). Subfraction E (51.0 g) was subjected to silica gel column chromatography using gradient elution of n-hexane-ethyl acetate to afford eight subfractions (E1–E8). Subfraction E5 (11.1 g) was further separated by column chromatography on silica gel using gradient elution of n-hexane-chloroform-ethyl acetate to afford six subfractions (E5a–E5f). Subfraction E5b (2.9 g) was separated by column chromatography on silica gel using gradient of water-methanol to afford compound 2 (31.2 mg). Subfraction K (11.3 g) was further separated by column chromatography on silica gel using gradient...
elution of n-hexane-ethyl acetate to afford seven subfractions (K1-K7). Subfraction K4 (4.0 g) was separated by column chromatography on ODS using gradient of water- methanol to afford compound 2 (12.2 mg).

2.3.1 Stigmast-22-en-3β-ol (1). White crystal; m.p. 160-162°C; IR (KBr) \text{max} 3377, 2929, 1466, and 1049 cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \(\delta_{\text{H}}\) 1.02 (1H, m, H-1a), 1.74 (1H, m, H-1b), 1.45 (1H, m, H-2a), 1.83 (1H, m, H-2b), 3.44 (1H, td, \(J=4.5, 10.6\) Hz, H-3), 0.94 (1H, m, 1H), 1.17 (2H, m, H-6), 1.45 (1H, m, H-7a), 1.83 (1H, m, H-7b), 1.45 (1H, m, H-8), 1.03 (1H, m, H-9), 1.28 (1H, m, H-11a), 1.31 (1H, m, H-11b), 1.15 (1H, m, H-12a), 1.98 (1H, m, H-12b), 1.07 (1H, m, H-14), 1.08 (1H, m, H-15a), 1.59 (1H, m, H-15b), 1.27 (1H, m, H-16a), 1.73 (1H, m, H-16b), 1.13 (1H, m, H-17), 0.69 (3H, s, Me-18), 0.98 (3H, s, Me-19), 1.98 (1H, m, H-20), 1.02 (3H, d, \(J=6.5\) Hz, Me-21), 5.16 (1H, dd, \(J=8.6, 15.1\) Hz, H-22), 5.02 (1H, dd, \(J=8.6, 15.1\) Hz, H-23), 1.54 (1H, m, H-24), 1.44 (1H, m, H-25), 0.86 (3H, m, Me-26), 0.85 (3H, m, Me-27), 1.17 (2H, m, H-28), 0.83 (3H, m, Me-29); \(^{13}\)C-NMR (CDCl\(_3\), 125 MHz) see Table 1; HR-TOFMS \(m/z\) 415.3951 [M+H]+, (calculated for C\(_{29}\)H\(_{51}\)O, \(m/z\) 415.3940).

2.3.2 Moronic acid (2) White crystal; m.p. 108-109°C; IR (KBr) \text{max} 3233, 2946, 1708, and 1457 cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\), 500 MHz): \(\delta_{\text{H}}\) 1.98 (2H, m, H-1), 2.48 (2H, m, H-2), 1.37 (1H, m, H-5), 1.46 (2H, m, H-6), 1.48 (2H, m, H-7), 1.39 (1H, m, H-9), 1.33 (2H, m, H-11), 1.64 (2H, m, H-12), 2.26 (1H, d, \(J=10.9\) Hz, H-13), 1.65 (2H, m, H-15), 1.38 (2H, m, H-16), 5.19 (1H, s, H-19), 2.19 (2H, d, \(J=13.3\) Hz, H-21), 1.65 (2H, m, H-22), 1.04 (3H, s, Me-23). 1.09 (3H, s, Me-24), 0.96 (3H, s, Me-25), 1.06 (3H, s, Me-26), 0.80 (3H, s, Me-27), 1.01 (3H, s, Me-29), 0.99 (3H, s, Me-30); \(^{13}\)C-NMR (CDCl\(_3\), 125 MHz) see Table 1; HR-TOFMS \(m/z\) 454.3500 [M-H]-, (calculated for C\(_{30}\)H\(_{47}\)O\(_3\), \(m/z\) 455.3525).

3. Result and Discussion

The n-hexane extract of the rind of C. macrophyllus was subjected to column chromatography and recrystallization to afford stigmastane-type steroid stigmast-22-en-3β-ol (1) and pentacyclic triterpenoid moronic acid (2). Compounds 1 were isolated from this plant for the first time.

![Figure 1. Chemical structures of compounds 1-2](image-url)

Compound 1 was obtained as white crystal. Its molecular formula was established as C\(_{29}\)H\(_{50}\)O from a molecular ion peak at \(m/z\) 415.3951 ([M+H]+ calculated C\(_{29}\)H\(_{51}\)O, \(m/z\) 415.3940) in the HR-TOFMS, thus requiring five degrees of unsaturation. The IR spectrum showed the absorption bands due to hydroxyl group at 3377 cm\(^{-1}\) and olefinic group at 1466 cm\(^{-1}\).

The \(^1\)H-NMR spectrum showed presence singlet signals for two tertiary methyl groups at \(\delta_{\text{H}}\) 0.67 (3H, s, Me-18), 1.02 (3H, s, Me-19); three secondary methyl groups at \(\delta_{\text{H}}\) 0.92 (3H, m, Me-21); 0.85 (3H, m, Me-26); and 0.83 (3H, m, Me-27); one primary methyl group at \(\delta_{\text{H}}\) 0.83 (3H, m, Me-29); and that indicating the characteristic for steroid stigmastane\(^{15}\). An additional functionalities found included oxygenated proton at \(\delta_{\text{H}}\) 3.44 (1H, td, \(J=4.5, 10.6\) Hz, H-3) and two olefinic methines at \(\delta_{\text{H}}\)
5.16 (1H, dd, J=8.6, 15.1 Hz, H-22) and δH 5.02 (1H, dd, J=8.6, 15.1 Hz, H-23), which indicating for olefinic methine having trans position at C-22 and C-23.

A total twenty nine carbon resonances were observed in the 13C-NMR spectrum. These were assigned by DEPT and HSQC experiments to six methyls, eleven sp3 methylenes, seven sp3 methines, two quaternary carbons, two olefinic methines at δC 129.3 and 138.2, and one oxygenated methine carbons at δC 71.3. These functionalities accounted for one out of the total five degrees of unsaturation. The remaining four degrees of unsaturation were consistent with the molecule containing four rings which characteristic for stigmastane-type steroid structure.

The presence of stigmastane-type steroid structure in compound 1 was confirmed from HMBC experiment, (Figure 2). The HMBC spectra showed the correlation between proton of methyl groups Me-18, Me-19, Me-21, Me-26, Me-27, Me-29 with neighbor carbons supporting the presence of stigmastane-type steroid in 1.

In the HMBC spectrum, showed cross peaks olefinic methane from δH 5.02 (H-23) to another olefinic methine at δC 138.2 (C-25), 40.5 (C-20), and 51.2 (C-24) were indicated olefinic methine was attached at C-22 and C-23. Hidroxyl position in HMBC showed cross peaks δH 1.04 (H-1), 1.83 (H-2), and 2.02 (H-4) to hydroxyl methines δC 71.3(C-3) were indicated hydroxyl group attached at C-3.

The stereochemistry of compound 1 were determined based on NOESY spectrum. In the NOESY spectrum showed cross peaks from δH 1.03 (H-9) to 3.44 (H-3) were indicated that H-3 is β orientation consequently configuration for hydroxyl at C-3 is β orientation.

A comparison of the NMR data of compound 1 with those of stigmasterol isolated from Sesbania grandiflora10, revealed that the structures of the two compounds are similar but different at C-5 and C-6 consequently compound 1 was identified as stigmast-22-ene-3β-ol.

Compound 2 was obtained as white crystal. Its molecular formula was established as C30H46O3 from a molecular ion peak at 455.3500 ([M+H]+ calculated C30H47O3 455.3525) in the HR-TOFMS, thus requiring eight degrees of unsaturation. The IR spectrum showed the absorption bands due to hydroxyl at 3233 cm⁻¹, ketone carbonyl groups at 1708 cm⁻¹, and alkene at 1457 cm⁻¹.

The 1H-NMR spectrum showed presence singlet signals for seven tertiary methyl groups at δH 0.80 (3H, s, Me-27), 0.96 (3H, s, Me-25), 0.99 (3H, s, Me-30), 1.01 (3H, s, Me-29), 1.04 (3H, s, Me-23), 1.06 (3H, s, Me-26), and 1.09 (3H, s, Me-24). An additional functionalities found included olefinik proton at δH 5.19 (1H, s, H-19).

Table 1. 13C-NMR data for compounds 1-2

| No | 1             | 2             |
|----|---------------|---------------|
| 1  |               |               |
| 2  | Moronic acid  |               |
| 3  |               |               |
| 4  |               |               |
| 5  |               |               |
| 6  |               |               |
| 7  |               |               |
| 8  |               |               |
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| 30 |               |               |

Figure 2. Key HMBC (→) and 1H-1H COSY (→→) correlations of 1-2.
A total thirty carbon resonances were observed in the $^{13}$C-NMR spectrum. These were assigned by DEPT and HSQC experiments to seven methyls, ten $sp^3$ methylenes, three $sp^3$ methines, six quaternary carbons, one quartenary $sp^2$ carbon at $\delta_C 136.6$, one olefinic carbons at $\delta_C 133.4$, one carboxyl group at $\delta_C 181.2$, and one ketone carbonyl at $\delta_C 218.2$. These functionalities accounted for three out of the total eight degrees of unsaturation. The remaining five degrees of unsaturation were consistent with the molecule containing oleanane-type pentacyclic triterpenoid structure.

The presence of pentacyclic triterpenoid structure in compound 2 was confirmed from HMBC and $^1$H-$^1$H-COSY experiments (Figure 2). The $^1$H-$^1$H-COSY spectrum showed the correlation proton of H-1/H-2, H-5/H-6/H-7, H-9/H-11/H-12/H-13, H-15/H-16, H-21/H-22, supporting the presence of pentacyclic triterpenoid in 2. The HMBC spectra showed the correlation between proton of methyl groups H-23, H-24, H-25, H-26, H-27, H-29, H-30 with neighbor carbons supporting the presence of oleanane-type pentacyclic triterpenoid in 2.

The HMBC spectrum of compound 2 showed cross peaks from H-2 at $\delta_H 2.48$ (2H, m, H-2) to carboxyl group at $\delta_C 218.3$ (C-3) and 39.8 (C-1) indicated presence carboxyl group attached at C-3. HMBC correlations from H-22 at $\delta_H 1.65$ (2H, m, H-22) to carboxylic acid group position showed cross peaks from H-22 at $\delta_H 181.2$ (C-28), 33.3 (C-16), and 47.9 (C-17) indicated presence carboxylic acid group attached at C-17. HMBC spectrum also showed cross peaks from olefinic methane proton H-19 at $\delta_H 5.19$ (1H, s, H-19) to carbon at $\delta_C 41.5$ (C-13), 47.9 (C-17), 30.3 (C-29), and 32.0 (C-20) indicated that oliefnic methine position at C-18 and C-19.

A comparison of the NMR data of compound 2 with those of moronic acid isolated from Phoradendron reichenbachianum, revealed that the structures of the two compounds are similar, consequently compound 2 was identified as moronic acid.
4. Conclusion

One steroid compounds, stigmast-22-ene-3β-ol (1) and one triterpenoid moronic acid (2) have been isolated from the n-hexane extract of the rind of Chisocheton macrophyllus A subsubsection.

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