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ISOLATION AND CHARACTERIZATION OF THREE NATURAL COMPOUNDS FROM THE STEM BARK OF *Cassia grandis* L.F

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ABSTRACT

*With three previous reports, chemical investigation of the stem bark of Cassia grandis L.f has detected 1 anthraquinone, 3 sterols, 3 triterpenes and 2 esters of glycerol and fatty acid. In this report, three known natural compounds named n-heptacosan-1-ol (**1**), bis(2,3-dihydroxypropyl)-tetracosandioate (**2**) and moracin B (**3**) were firstly isolated from ethyl acetate extracts of the stem bark of Cassia grandis L.f. Their structures were elucidated by modern spectra including ESI-MS, 1D-NMR, 2D-NMR and by comparison with published data.*

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1 INTRODUCTION

We have recently reported our initial results about the chemical compositions of *Cassia grandis* L.f (Luan *et al*., 2013). More than 30 natural compounds has been successfully identified form this plant. In this paper we continue to present the isolation of three natural compounds which were detected for the first time in the stem bark of *Cassia grandis* L.f.

2 EXPERIMENTAL

2.1 Plant material

The stem bark of *Cassia grandis* L.f were collected in Vinh Long province in January, 2013. Voucher specimens have been identified by Dang Minh Quan, Can Tho University. After cleaning, poor quality sections of the stem bark were removed. Good material was dried at 50°C in order to reduce humidity to 0-2%, followed by crushing into fine powder.

2.2 General experimental procedures

2.2.1 Extraction and purification

Solid-liquid, liquid-liquid extraction were used with solvents as ethanol 96%, *n*-hexane, EtOAc, BuOH, MeOH. Solvent evaporating was applied by Buchi R-210 rotary evaporator system.

Thin layer chromatography (TLC) was carried out on pre-coasted silica gel $60F_{254}$ (0.25 mm) aluminum sheet (Merck, Germany) and compounds were detected under UV (254/365 nm) fluorescence or spraying 10% H_2SO_4 solution in EtOH, followed by heating at 105 °C for 1-2 min on electric stove.

For common phase column chromatography (CP-CC), silica gel 60 (0.040-0.063 mm, Merck, Germany) with increasing polarity solvent systems including *n*-hexane (H), EtOAc (E), $CHCl_3$ (C), MeOH (M) and H_2O (W) were used.

Compounds were purified by re-crystallization in pure solvents.

2.2.2 Structural elucidation and identification

Melting point (mp.) was recorded on a melting point meter (Electrothermal 9100-UK), using with capillary, uncorrected. ^{1}H-NMR, ^{13}C-NMR, DEPT, HSQC, HMBC, COSY spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker, USA). Mass spectrum (MS) was recorded on mass spectrometer (HP 1100 series, LC/MSD Trap, Agilent, USA). These used equipment are available at Vietnam Academy of Science and Technology.

2.3 Extraction and isolation

The dried powdered stem bark (17.5 kg) was exhausted extracted with ethanol 96% to gain ethanol extract (1.0 kg). The ethanol extract was then consecutively distributed into *n*-hexane, ethyl acetate and methanol.

The ethyl acetate extracts (BE, 145 g) was subjected to CP-CC with H:E solvent systems (radient, 0 to 100% E) as eluent to give 9 fractions (BE1-BE9). The fraction BE2 (H:E 9:1, 15.6 g) was taken CP-CC with H:E (radient, 95:5 to 9:1) as eluent to obtain 6 fractions (BE21-BE26). The fraction BE25 (H:E 9:1, 0.92 g) was re-crystallized in *n*-hexane to obtain compound **1** (7.2 mg).

The fraction BE3 (H:E 8:2, 15.4 g) was continued to CP-CC (H:E 8:2) to afford 7 fractions (BE31-BE37). The fraction BE32 (502 mg) was continued to CP-CC (H:E 8:2) to give 3 fractions (BE321-BE323). The fraction BE322 (24.7 mg) was re-crystallized in EtOAc to produce compound **2** (10.1 mg).

The fraction BE6 (H:E 6:4, 22.1 g) was continued to CP-CC (C:M, radient, 95:5 to 8:2) to afford 8 fractions (BE61-BE68). The fraction BE63 (C:M 9:1, 804 mg) was continued to CP-CC (C:M 9:1) to give 5 fractions (BE631-BE635). The fraction BE634 (20.7 mg) was re-crystallized in EtOAc to yield compound **3** (8.5 mg).

2.4 1D-NMR spectral data

***n*-Heptacosan-1-ol** (**1**): ESI-MS *m/z* 397.1 $[M+H]^+$; 395.1 $[M-H]^-$.

^{1}H-NMR (CD_3OD, 500 MHz, δ_H ppm, *J* Hz): 2.35 (2H, *t*, *J*=7.5, H-1); 1.63 (2H, *quintet*, *J*=7.5 and 7.7, H-2); 1.32 (2H, *overlap,* H-3); 1.29 (2H, *overlap*, H-26); 1.25 (44H, *overlap*, H-4 ÷ H-25); 0.88 (3H, *t*, *J*=7.0, H-27).

^{13}C-NMR (CD_3OD, 125 MHz, δ_C ppm): 63.8 (C-1); 33.9 (C-2); 31.9 (C-25); 29.6 (*overlap*, C-5 ÷ C-21); 29.5 (C-24); 29.4 (C-23); 29.3 (C-4); 29.1 (C-22); 24.7 (C-3); 22.7 (C-26); 14.1 (C-27).

bis(2,3-Dihydroxypropyl)-tetracosandioateb (2): ESI-MS *m/z* 569.28 $[M+Na]^+$.

^{1}H-NMR (DMSO-d_6, 500 MHz, δ_H ppm, *J* Hz): 4.60 (2H, *d*, *J*=5.0, 2'-OH and 2"-OH); 4.37 (2H, *t*, *J*=5.5, 3'-OH and 3"-OH); 4.05 (2H, *dd*, *J*=4.5 and 11.0, H-1'a and H-1"a); 3.93 (2H, *dd*, *J*=6.5 and 11.0, H-1'b and H-1"b); 3.65 (2H, *dd*, *J*=5.5 and 10.5, H-2' and H-2"); 3.36-3.38 (4H, *m*, H-3'ab and H-3"ab); 2.26-2.29 (4H, *m*, H-2ab and H-23ab); 1.54 (4H, *t*, *J*=7.0, H-3ab and H-22ab); 1.30 (4H, *overlap*, H-4ab and H-21ab); 1.25 (32H, *overlap*, H-5ab ÷ H-20ab).

^{13}C-NMR (DMSO-d_6, 125 MHz, δ_C ppm): 172.5 (C-1 and C-24); 69.2 (C-2' and C-2"); 65.2 (C-1' and C-1"); 62.5 (C-3' and C-3"); 28.6 (*overlap*, C-7 ÷ C-18); 28.5 (C-6 and C-19); 28.3 (C-5 and C-20); 28.1 (C-4 and C-21); 24.1 (C-3 and C-22).

Moracin B (**3**): ESI-MS *m/z* 285.08 $[M-H]^-$.

^{1}H-NMR (CD_3COCD_3-d_6, 500 MHz, δ_H ppm, *J* Hz): 8.54 (1H, *s*, 3'-OH); 7.74 (1H, *s*, 6-OH); 7.13 (1H, *s*, H-3); 7.09 (1H, *d*, *J*=1.5, H-4); 7.03 (1H, *d*, *J*=0.5, H-7); 6.93 (1H, *t*, *J*=2.0, H-6'); 6.90 (1H, *dd*, *J*=1.5 and 2.0, H-2'); 6.39 (1H, *t*, *J*=2.0, H-4'); 3.90 (3H, *s*, 5-OCH_3); 3.82 (3H, *s*, 5'-OCH_3).

^{13}C-NMR (CD_3COCD_3-d_6, 125 MHz, δ_C ppm): 162.4 (C-3'); 159.8 (C-5'); 155.4 (C-2); 150.8 (C-8); 146.8 (C-6); 146.3 (C-5); 133.5 (C-1'); 121.6 (C-9); 104.7 (C-6'); 103.3 (C-3); 103.0 (C-4); 102.1 (C-4'); 102.0 (C-2'); 98.5 (C-7); 56.8 (5-OCH_3); 55.6 (5'-OCH_3).

3 RESULTS AND DISCUSSION

3.1 Compound 1

Compound **1** was obtained as a white amorphous powder, mp. 81-82 °C. 1D-NMR spectra of compound **1** were rather simple. ^{1}H-NMR spectrum revealed only one *singlet* signal of proton methyl at δ_H 0.88 ppm, two protons of a hydroxymethylene group at δ_H 2.35 ppm, the remaining signals of protons of 25 methylene groups at δ_H 1.63, 1.32, 1.29 and 1.25 (*overlap*) ppm. ^{13}C-NMR and DEPT spectra exhibited one signal of methyl carbon at δ_C 14.1 ppm, one hydroxymethylene at δ_C 63.8 ppm and then signals of many methylene groups at δ_C 33.9, 31.9, 29.6 (*overlap*), 29.5, 29.4, 29.3, 29.1, 24.7 and 22.7 ppm. The typical signals of proton and carbon in 1D-NMR spectra showed that compound **1** could be a fatty alcohol. The ESI-MS spectrum showed two quasi-molecular ion peaks at *m/z* 397.1 $[M+H]^+$ and 395.1 $[M-H]^-$, confirming the $C_{27}H_{56}O$ molecular formula for compound **1**. Furthermore, compound **1** was a known natural compound confirmed directly
by comparison with spectral data from literature (Koay *et al*., 2013). Therefore, compound **1** was identified as *n*-heptacosan-1-ol (Fig. 1).

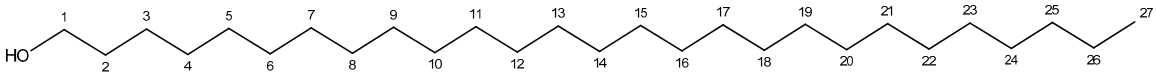

Fig. 1: Chemical structure of n-heptacosan-1-ol (1)

3.2 Compound 2

Compund **2** was isolated as a white amorphous powder, mp. 111-112 °C. ^{1}H-NMR appeared proton signals of two hydroxyl groups at δ_H 4.60 and 4.37 ppm (confirmed by HSQC spectrum), three of oxygenated methylenes at δ_H 4.05, 3.93 and 3.36-3.38 ppm, one of oxygenated methine at δ_H 3.65 ppm and signals of about 22 methylene groups at δ_H 2.28, 1.54, 1.30 and 1.25 (*overlap*) ppm. ^{13}C-NMR and DEPT spectra exhibited signals of one carbonyl carbon at δ_C 172.5 ppm, one of methine carbon at δ_C 69.2 ppm, two of hydroxymethylene groups at δ_C 65.2, 62.5 ppm and many methylene groups at δ_C 33.9, 28.6 (*overlap*), 28.3, 28.1, 24.1. These ^{1}H-NMR and ^{13}C-NMR spectra were typical of a fatty acid glyceryl ester, but the ^{1}H-NMR and ^{13}C-NMR spectra did not reveal any methyl signal, which suggested that compound **2** was a symmetric dibasic acid diester. The existence of hydroxymethine group (δ_H 3.65 ppm and δ_C 69.2 ppm) indicated that compound **2** was a 1-*O*-substituted glyceryl ester. Besides, the molecular formula of compound **2** was established as $C_{30}H_{58}O_8$ by ESI-MS (*m/z* 569.28 $[M+ Na]^+$). Thus, the fatty acid was deduced to be tetracosandioic. Assignments of all protons and carbons in compound **2** were also made by HSQC and HMBC spectra. Moreover, the 1D-NMR spectral data of compound **2** were similar to those given in the literature (Yang *et al*., 2009). Consequently, the structure of compound **2** was determined as bis(2,3-dihydoxypropyl)-tetracosandioate (Fig. 2).

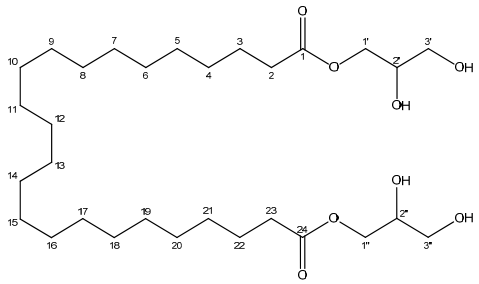

Fig. 2: Chemical structure of bis(2,3-dihydroxypropyl)-tetracosandioate (2)

3.3 Compound 3

Compound **3** was obtained as a yellow amorphous powder, mp. 184-185 °C, which produced a positive reaction to $FeCl_3$ reagent. The typical signals of proton and carbon in 1D-NMR showed that compound **3** could be a phenolic compound.

The ^{1}H-NMR spectrum of compound **3** showed two hydroxyl groups (which were confirmed by HSQC spectrum) at δ_H 8.54 and 7.74 ppm, five aromatic protons which were *m*-coupled *doublet* and *triplet* signals at δ_H 7.09, 7.03, 6.93, 6.90 and 6.39 ppm (each, J=1.5÷2.0 Hz); one *singlet* signal of other ring double-bonded methine group at δ_H 7.13 ppm; two methoxy groups at δ_H 3.90 and 3.82 ppm.

The ^{13}C-NMR and DEPT spectra of compound **3** appeared signals of total 16 carbons. In which, there were 5 aromatic carbons at δ_C 104.7, 103.0, 102.1, 102.0 and 98.5 ppm; five aromatic oxygenated quaternary carbons at δ_C 162.4, 159.8, 150.8, 146.8 and 146.3 ppm; two aromatic quaternary carbons at δ_C 133.5 and 133.6 ppm; two substituted methyl groups at δ_C 56.8 and 55.6 ppm (further confirmed by its HMBC spectrum); one double-bonded methine group at δ_C 103.3 ppm and one oxygenated quaternary carbon at δ_C 155.4 ppm of a heterocyclic ring.

The molecular formula of compound **3** was speculated to be $C_{16}H_{14}O_5$ (calc. for 286.08, ten degrees of unsaturation) on the basis of the ESI-MS (*m/z* 285.08 $[M-H]^-$) and above mentioned 1D-NMR data. The chemical structure of compound **3** contained two benzene rings (due to 12 aromatic carbons), with two methoxy substitued groups, it also indicated that the other ring (with two remaining carbons at δ_C 103.3 and 155.4 ppm) was oxygened pentaheterocycle. Therefore, compound **3** gave the characteristic spectra possessing pattern of a benzofuran derivative. Two carbons at δ_C 103.3 and 155.4 ppm forming >C=CH- that linked with each other proved that compound **3** was 2-phenyl-1-benzofuran derivative.

The leash of aromatic *m*-coupled protons at δ_H 6.90, 6.39 and 6.93 ppm indicated that three of them were depended on the phenyl ring (Figure 3) and were in turn assigned to the 2', 4', and 6' positions, then the two substituted groups would be attached at the 3' and 5'. The HSQC spectrum confirmed the assignments of C-2', C-4', and C-6' were in order of δ_C 102.0, 102.1 and 104.7 ppm. The HMBC spectrum exhibited signals at δ_H 6.90, 6.39 and 6.93 ppm correlated with two aromatic oxygenated quaternary carbons at δ_C 162.4, 159.8 ppm and aromatic quaternary carbon at δ_C 133.5 ppm proved that these carbons belonged to this phenyl moiety, too. The signal of hydroxyl proton at δ_H

8.54 ppm correlated with the carbon at δ_C 159.8, which represented for C-3'. So the carbon at δ_C 162.4 was C-5' by correlation between the methoxy proton at δ_H 3.82 ppm correlated with it, and of course, the carbon at δ_C 133.5 ppm was C-1'.

In the benzofuran aglycone, oxygenated quaternary carbon at δ_C 155.4 ppm was assigned to C-2 due to correlation between it and H-2' and H-6'. The *singlet* at δ_H 7.13 ppm was determined to be H-3 because two aromatic protons at δ_H 7.03, 7.09 ppm were *para*-coupled (each, J=0.5 Hz), and the carbon at δ_C 103.3 ppm to be C-3, confirmed by HSQC spectrum. Two para-coupling protons and two remaining substituted groups in a benzene ring indicated that these protons had to locate at positions 4 and 7. The proton at δ_H 7.09 was determined to be H-4 by its correlation with C-3 while the proton at δ_H 7.03 had no correlation with C-3, and so carbons at δ_C 103.0 and 98.5 ppm were also determined to be C-4 and C-7, respectively by correlations observed in HSQC spectrum.

The correlations between H-3, H-4, H-7 and the oxygenated quaternary carbon at δ_C 150.8 ppm confirmed that this carbon was C-8, and then the last quaternary carbon at δ_C 121.6 ppm was C-9. The hydroxyl proton at δ_H 7.74 correlated with C-7 and the oxygenated quaternary carbon at δ_C 146.8 ppm indicated that this carbon was C-6 and the substituted hydroxyl group linked to the aromatic ring at C-6. The last oxygenated quaternary carbon at δ_C 146.3 ppm was C-5, certainly.

Based on the above spectral data analysis and comparison with those given in the literature (Takasugi *et al*., 1978), the structure of compound **3** was suggested as Figure 3 and identified as moracin B.

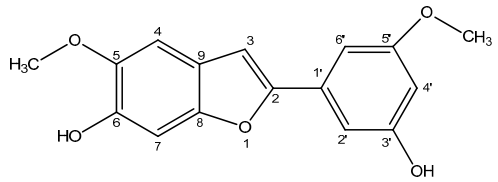

Fig. 3: Chemical structure of moracin B (3)

4 CONCLUSION

From ethyl acetate extracts of the stem bark of *Cassia grandis* L.f collected in Vinh Long province, three known natural compounds named *n*-heptacosan-1-ol, bis(2,3-dihydroxypropyl)-tetracosandioate and moracin B were isolated. This is the first time these compounds were detected from *Cassia grandis* L.f.

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