TERPENOIDS AND PHENOLICS FROM MACROSOLEN TRICOLOR

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TÓM TẮT

TERPENOID VÀ PHENOLIC TỪ CÂY ĐẠI CÁN TAM SẮC MACROSOLEN TRICOLOR

Ba hợp chất terpenoid gồm 2 triterpenoid, acid ursolic (1), acid 1-oxooleanolic (2) và 1 sesquiterpenoid, bisacurone A (3), cùng với hỗn hợp 2 phenolic, (1E,4E)-1,5-bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (4A) và (+)-pinoresinol (4B) được phân lập từ cao n-hexane toàn cây Đại cán tam sắc. Cấu trúc của hợp chất được xác định bằng các phương pháp phổ hiện đại NMR (1D & 2D), HRMS và so sánh với tài liệu tham khảo. Đây là lần đầu tiên các hợp chất (2-4) được xác định từ chi Macrosolen và hợp chất 1 được công bố từ loài này.

Từ khóa: *Macrosolen tricolor, phenolic, sesquiterpenoid.*

1. INTRODUCTION

Macrosolen tricolor is used in traditional Vietnamese medicines to treat cough, diarrhea, bloating, broken bones, rheumatism, diuretic and laxative effects[2,3]. Our previous papers, four diarylalkanoids, six phenolics, four flavonoids and two steroids were reported from this species[5-7], this paper details the isolation and structural elucidation of three terpenoids and a mixture of two phenolics (**1**-**4**) from *M. tricolor*.

2. EXPERIMENTAL

2.1. Plant material

The whole plants of *M. tricolor* were collected in Ba Ria-Vung Tau province, and further specified by Dr. Van Son Dang, Institute of Tropical Biology.

2.2. Methods

The HR-ESI-MS (high resolution electrospray ionization mass spectrometry) was measured on Bruker MicrQTOF-QII spectrometer (500 MHz). The 1D and 2D NMR spectrum of all

compounds were recorded on a Bruker AM500 FT-NMR spectrometer using TMS (tetramethylsilane) or residual solvent signal (DMSO-d_6, δ_H 2.50, δ_C 39.52 ppm) as internal standard. Column chromatography (CC) was carried out by silica gel normal-phase (230-400 mesh). Analytical TLC was carried out on silica gel plates (Kieselgel 60 F254). Compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3-5 min.

2.3. Extraction and isolation

The dried whole plants of *M. tricolor* (4.3 kg) were extracted with EtOH 96% at room temperature for three times to make the crude extract. The EtOH extract (1100 g) was fractionated by LLE (liquid-liquid extraction) technique to give *n*-hexane and EtOAc extracts. The EtOAc extract (106 g) was separated on a silica gel column and eluted with *n*-hexane-EtOAc-MeOH (50-75-0→0-90-10, v/v/v) to give four fractions (MTE.I-IV). Fraction MTE.II (5.2 g) was chromatographed on silica gel column eluting with *n*-hexane-ethyl acetate (50:50→10:90, v/v) to deliver eight sub-fractions (MTE.II.1-8), respectively. Sub-fraction MTE.II.5 (0.6 g) was isolated on silica gel chromatography column with solvents of $CHCl_3$-MeOH (99:1, v/v) to give **1** (6 mg), and **2** (4 mg). Sub-fraction MTE.II.7 (1.2 g) was further purified on normal-phase column with solvent system of $CHCl_3$-MeOH (99:1, v/v) to give **3** (7 mg), and **4** (3.5 mg).

3. RESULTS AND DISCUSSION

Compound **1**: ^{1}H-NMR ($CDCl_3$, δ_H ppm): 3.20 (1H, *dd*, *J* = 8.0, 8.0 Hz, H-3), 5.25 (1H, *brs*, H-12), 2.21 (1H, *d*, *J* = 11.0 Hz, H-18), 0.79 (3H, *s*, H-23), 0.99 (3H, *s*, H-24), 0.94 (3H, *s*, H-25), 0.83 (3H, *s*, H-26), 1.10 (3H, *s*, H-27), 0.87 (3H, *d*, *J* = 6.5 Hz, H-29), 0.93 (3H, *d*, *J* = 5.0 Hz, H-30). ^{13}C-NMR ($CDCl_3$, δ_C ppm): 39.1 (C-1), 27.0 (C-2), 78.9 (C-3), 39.6 (C-4), 55.3 (C-5), 18.3 (C-6), 33.1 (C-7), 39.6 (C-8), 47.6 (C-9), 38.7 (C-10), 23.3 (C-11), 125.6 (C-12), 138.2 (C-13), 42.1 (C-14), 28.1 (C-15), 24.2 (C-16), 47.8 (C-17), 52.9 (C-18), 38.9 (C-19), 39.1 (C-20), 33.1 (C-21), 36.8 (C-22), 15.4 (C-23), 28.0 (C-24), 15.3 (C-25), 16.8 (C-26), 23.4 (C-27), 180.8 (C-28), 16.9 (C-29) 20.9 (C-30).

Compound **2**: ^{1}H-NMR ($CDCl_3$, δ_H ppm): 3.03 (1H, *dd*, *J* = 12.0, 12.0 Hz, H-2a), 2.37 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-2b), 3.49 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-3), 5.30 (1H, *t-like*, *J* = 3.5 Hz, H-12), 2.82 (1H, *dd*, *J* = 13.5, 4.0 Hz, H-18), 1.05 (3H, *s*, H-23), 1.01 (3H, *s*, H-24), 1.29 (3H, *s*, H-25), 0.82 (3H, *s*, H-26), 1.17 (3H, *s*, H-27), 0.91 (3H, *d*, *J* = 6.5 Hz, H-29), 0.93 (3H, *d*, *J* = 7.0 Hz, H-30). ^{13}C-NMR ($CDCl_3$, δ_C ppm): 212.3 (C-1), 44.1 (C-2), 78.7 (C-3), 39.3 (C-4), 54.1 (C-5), 17.8 (C-6), 32.6 (C-7), 39.3 (C-8), 39.2 (C-9), 52.5 (C-10), 25.2 (C-11), 123.3 (C-12), 142.4 (C-13), 41.9 (C-14), 27.7 (C-15), 23.0 (C-16), 46.7 (C-17), 41.3 (C-18), 45.6 (C-19), 30.7 (C-20), 33.9 (C-21), 32.4 (C-22), 28.5 (C-23), 16.0 (C-24), 14.9 (C-25), 17.7 (C-26), 25.7 (C-27), 182.6 (C-28), 33.1 (C-29) 23.5 (C-30).

Compound **3**: ^{1}H-NMR (DMSO-d_6, δ_H ppm): 2.14-2.18 (1H, *m*, H-1), 5.45 (1H, *dd*, *J* = 10.0, 1.5 Hz, H-2), 5.44 (1H, *d*, *J* = 10.0 Hz, H-3), 3.49 (1H, *m*, H-5), 1.67 (1H, *ddd*, *J* = 12.0, 8.5, 2.5 Hz, H-6a), 1.47 (1H, *ddd*, *J* = 12.5, 11.5, 6.0 Hz, H-6b), 1.98-2.03 (1H, *m*, H-7), 2.45 (1H, *dd*, *J* = 15.5, 4.5 Hz, H-8a), 2.16 (1H, *dd*, *J* = 15.5, 9.0 Hz, H-8b), 6.14 (1H, *brs*, H-10), 2.05 (3H, *brs*, H-12), 1.85 (3H, *d*, *J* = 0.5 Hz, H-13), 0.78 (3H, *d*, *J* = 7.0 Hz, H-14), 1.09 (3H, *s*, H-15), 4.28 (1H, *s*, OH-4), 4.42 (1H, *d*, *J* = 4.5 Hz, OH-5). ^{13}C-NMR (DMSO-d_6, δ_C ppm): 35.7 (C-1), 129.7 (C-2), 133.7 (C-3), 68.7 (C-4), 71.8 (C-5), 28.2 (C-6), 32.6 (C-7), 48.2 (C-8), 200.1 (C-9), 124.0 (C-10), 153.7 (C-11), 20.2 (C-12), 27.0 (C-13), 16.7 (C-14), 24.9 (C-15).

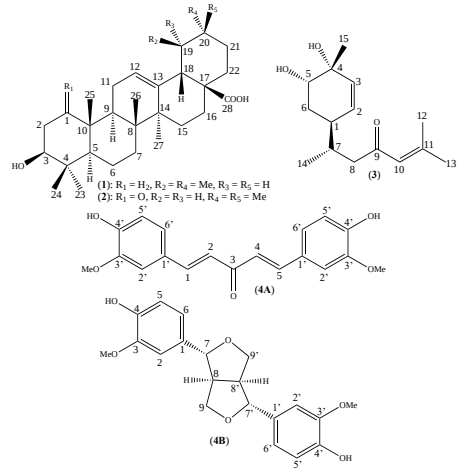

Figure 1. Chemical structures of 1-4

Compound **4A**: HR-ESI-MS: negative *m/z* 325.1086. ^{1}H-NMR (DMSO-d_6, δ_H ppm): 9.61 (2H, *s*, 2*OH-4'), 7.64 (2H, *d*, *J* = 15.5 Hz, H-1, H-5), 7.14 (2H, *d*, *J* = 16.0 Hz, H-2, H-4), 7.36 (2H, *d*, *J* = 1.5 Hz, 2*H-2'), 6.83 (2H, *d*, *J* = 8.0 Hz, 2*H-5'), 7.20 (2H, *dd*, *J* = 8.0, 2.0 Hz, 2*H-6'), 3.85 (6H, *s*, 2*OC$\underline{H}_3$). ^{13}C-NMR (DMSO-d_6, δ_C ppm): 142.6 (C-1), 123.0 (C-2), 187.9 (C-3), 123.0 (C-4), 142.6 (C-5), 126.3 (2*C-1'), 111.5 (2*C-2'), 147.9 (2*C-3'), 149.4 (2*C-4'), 115.6 (2*C-5'), 123.2 (2*C-6'), 55.7 (2*O$\underline{C}H_3$).

Compound **4B**: HR-ESI-MS: negative *m/z* 357.1355. ^{1}H-NMR (DMSO-d_6, δ_H ppm): 6.88 (2H, *d*, *J* = 1.5 Hz, H-2, H-2'), 6.73 (2H, *d*, *J* = 8.0 Hz, H-5, H-5'), 6.75 (2H, *dd*, *J* = 8.5, 2.0 Hz, H-6, H-6'), 4.60 (2H, *d*, *J* = 4.0 Hz, H-7, H-7'), 3.03 (2H, *m*, H-8, H-8'), 4.12 (2H, *dd*, *J* = 9.0, 7.0 Hz, H-9), 3.72 (2H, *dd*, *J* = 9.0, 3.0 Hz, H-9'), 3.76 (6H, *s*, 3-OC$\underline{H}_3$, 3'-OC$\underline{H}_3$). ^{13}C-NMR (DMSO-d_6, δ_C ppm): 132.2 (C-1, C-1'), 110.4 (C-2, C-2'), 147.5 (C-3, C-3'), 145.9 (C-4, C-4'), 115.1 (C-5, C-5'), 118.6 (C-6, C-6'), 85.1 (C-7, C-7'), 53.5 (C-8, C-8'), 70.9 (C-9, C-9'), 55.6 (3-O$\underline{C}H_3$, 3'-O$\underline{C}H_3$).

Compound **1** was given as a white amorphous powder. The ^{1}H-NMR data of **1** displayed one olefinic proton at δ_H 5.25 (*brs*, H-12), one oxymethine proton at δ_H 3.20 (*dd*, 8.0, 8.0, H-3), five tertiary methyl and two secondary methyl groups at δ_H 0.93 (*d*, 5.0, H-30), 0.87 (*d*, 6.0, H-29). The ^{13}C-NMR spectrum of **1** detailed thirty carbons including one carboxylic carbon at δ_C 180.1 (C-28), one olefinic quaternary carbon at δ_C 138.2 (C-13), one olefinic methine carbon at δ_C 125.6 (C-12), one oxymethine carbon at δ_C 78.9 (C-3), four quaternary carbons, five methine carbons, nine methylene carbons, and seven methyl carbons, were classified triterpenoid bearing one hydroxyl group. The HMBC spectrum of **1** showed correlations between methyl protons at δ_H 0.79 (H-23), 0.99 (H-24) and oxymethine carbon at δ_C 78.9 (C-3), between methine proton at δ_H 2.21 (H-18) and olefinic carbons at δ_C 138.2 (C-13), 125.6 (C-12). Hence, the structure of **1** was evidenced as ursolic acid[11].

Compound **2** was obtained as a white amorphous powder. The ^{1}H and ^{13}C-NMR data of **2** evinced a triterpene acid similar **1**, except for the disappearance of two secondary methyl groups at δ_C 16.9 (C-29)/δ_H 0.87 (*d*, 6.0), 20.9 (C-30)/δ_H 0.93 (*d*, 5.0) in **1**, and the appearance of two tertiary methyl groups at δ_C 33.1 (C-29)/δ_H 0.91 (*s*), 23.5 (C-30)/δ_H 0.93 (*s*), together with upfield and downfield of two olefinic carbons at δ_C 123.3 (C-12), 142.4 (C-13), respectively, in **2 (**δ_C 125.6 (C-12), 138.2 (C-13) in 1**)**, which were concentrated the aglycon of **2** was to be oleanolic acid. Furthermore, the ^{13}C-NMR data of **2** exhibited one carbonyl carbon at δ_C 212.3 (C-1). On the other hand, methyl protons at δ_H 1.29 (*s*, H-25) and methylene protons at δ_H 3.03 (*dd*, 12.0, 12.0, H-2a), 2.37 (*dd*, 12.0, 5.0, H-2b) correlated with carbon at δ_C 212.3 (C-1) in HMBC, were clarified an oxo funtion was atttached to be C-1. Therefore, the structure of **2** was assigned as 1-oxooleanolic acid[9].

Compound **3** was yielded as a white amorphous powder. The molecular formula was established as $C_{15}H_{24}O_3$ by HR-ESI-MS data ($[M+Na]^+$ (+) *m/z* 275.1646, calcd. 275.1623). The ^{1}H-NMR spectra of **3** exhibited two hydroxyl protons at δ_H 4.28 (*s*, OH-4), 4.22 (*d*, 4.5, OH-5), two *meta*-coupled protons at δ_H 5.45 (*dd*, 10.0, 1.5, H-2), 5.44 (*d*, 10.0, H-3), one olefinic proton at δ_H 6.14 (*brs*, H-10), one oxymethine proton at δ_H 3.49 (*m*, H-5), four methyl groups including one tertiary, one secondary and two olefinic methyl moeties at δ_H 1.09 (*s*, H-15), 0.78 (*d*, 7.0, H-14), 2.05 (*brs*, H-12) and 1.85 (*d*, 2.0, H-13), respectively. The ^{13}C-NMR of **3** exposed fifteen carbons comprising one carbonyl carbon at δ_C 200.1 (C-9), four olefinic carbons at δ_C 129.7 (C-2), 133.7 (C-3), 124.0 (C-10), 153.7 (C-11), one oxygenated quaternary carbon at δ_C 68.7 (C-4), one oxymethine carbon at δ_C 71.8 (C-5), and four methyl carbons at δ_C 20.2 (C-12), 27.0 (C-13), 16.7 (C-14), 24.9 (C-15). These data were testified

3 was a bisaboladien-type sesquiterpenoid substituented one oxo and two hydroxyl functions. The HMBC spectrum of **3** revealed correlations between protons at δ_H 2.05 (H-12), 1.85 (H-13) and carbon at δ_C 124.0 (C-10), between protons at δ_H 1.09 (H-15) and carbon at δ_C 133.7 (C-3), which were disclosed a bisabola-2,10-diene skeleton. More, proton at δ_H 1.09 (H-15) correlated with carbons at δ_C 68.7 (C-4), 71.8 (C-5) in HMBC were comminicated that two hydroxyl groups were linked to be carbons C-4 and C-5. On the other hands, the larger coupling constant (J = 11.5 Hz) of proton H-6 (δ_H 1.47) in **3**, verified the stereochemistry of this proton as axial (α-type)[12,17], which was close in space to proton H-1 and OH-5 (Fig. 2). Whereas, the β-type protons (H-15) were correlated with proton H-5 in NOESY (Fig. 2). So, protons H-5, H-15 were β-type and two hydroxyl groups of these carbons C-4 and C-5 were α-type. Furthermore, the chemical shifts of carbons (C-1→C-6) of **3** were fairly close to those previously reported for bisacurone A, which were distinguished between its stereoisomers[12,13,17]. Thus, the structure of **3** was declared as bisacurone A[12,13].

Compound **4** was obtained as a yellow oil. The structure of **4** was was elucidated as a mixture of two phenolics, (1*E*,4*E*)-1,5-bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (**4A**) and (+)-pinoresinol (**4B**). Consequently, **4** was named **4A** and **4B** to determine clearly every compound. The molecular formula of **4A** was identified as $C_{19}H_{18}O_5$ by HR-ESI-MS data ($[M-H]^-$ (-) *m/z* 325.1086, calcd. 325.1076). The ^{1}H-NMR spectra of **4A** detailed two hydroxyl protons at δ_H 9.61 (2H, *s*, 2*OH-4'), four *trans*-coupled protons at δ_H 7.64 (2H, *d*, 15.5, H-1, H-5), 7.14 (2H, *d*, 16.0, H-2, H-4), six oxymethyl protons at δ_H 3.85 (3H, *s*, OC$\underline{H}_3$) and six ABX-type aromatic protons at δ_H 7.36 (2H, *d*, 1.5, 2*H-2'), 6.83 (2H, *d*, 8.0, 2*H-5'), 7.20 (2H, *dd*, 8.0, 2.0, 2*H-6') indicating of two 1,3,4-trisubstituted benzene systems. The ^{13}C-NMR of **4A** displayed nineteen carbons including one carbonyl carbon at δ_C 187.9 (C-3), four oxygenated quaternary aromatic carbons at δ_C 147.9 (2*C-3'), 149.4 (2*C-4'), two quaternary aromatic carbons, ten methine aromatic carbons and two oxymethyl carbons at δ_C 55.7 (2*O$\underline{C}H_3$) were confirmed a 1,5-diarylpenta-1,4-dien-3-on framework bearing two methoxy groups. The HMBC spectrum of **4A** disclosed correlations between protons at δ_H 6.83 (H-5'), 7.36 (H-2'), 3.85 (OC$\underline{H}_3$) and carbon at δ_C 147.9 (C-3'), between protons at δ_H 9.61 (OH-4') and carbon at δ_C 147.9 (C-3'), 115.6 (C-5'), which were justified two methoxy and two hydroxyl moieties attached to be C-3' and C-4', respectively. On the other hands, the configuration for C-1 and C-4 of **4A** were distinguished to be *E* by the large *J* coupling contants (15.5, 16.0 Hz, respectively) of protons H-1 & H-5 (δ_H 7.64), H-2 & H-4 (δ_H 7.14). Thence, the structure of **4A** was verified as (1*E*,4*E*)-1,5-bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one[10].

The molecular formula of **4B** was determined as $C_{20}H_{22}O_6$ by HR-ESI-MS data ($[M-H]^-$ (-) *m/z* 357.1355, calcd. 357.1338). The ^{1}H-NMR spectra of **4B** disclosed two hydroxyl protons at δ_H 8.87 (2H, *s*, 2*OH-4'), six ABX-type aromatic protons at δ_H 6.88 (2H, *d*, 1.5, H-2, H-2'), 6.75 (2H, *dd*, 8.5, 2.0, H-6, H-6'), 6.73 (2H, *d*, 8.0, H-5, H-5') confirming of two 1,3,4-trisubstituted benzenes; two methine protons δ_H 3.03 (2H, *m*, H-8, H-8'), four oxymethylene protons at δ_H 4.12 (2H, *dd*, 9.0, 7.0, H-9a, H-9'a), 3.72 (2H, *dd*, 9.0, 3.0, H-9b, H-9'b), six oxymethyl protons at δ_H 3.76 (6H, *s*, 3-OC$\underline{H}_3$, 3'-OC$\underline{H}_3$). The ^{13}C-NMR of **4B** displayed twenty carbons comprising four oxygenated quaternary aromatic carbons at δ_C 147.5 (2*C-3'), 145.9 (2*C-4'), two quaternary aromatic carbons, six methine aromatic carbons, two oxymethine carbons, two oxymethylene carbons, two oxymethyl carbons and two methine carbons were specified a furofuran lignan bearing two methoxy groups. The HMBC spectrum of **4B** expressed correlations between protons at δ_H 6.73 (H-5'), 6.88 (H-2'), 3.76 (OC$\underline{H}_3$) and carbon at δ_C 147.5 (C-3'), between protons at δ_H 8.87 (OH-

4') and carbon at δ_C 147.9 (C-3'), 115.6 (C-5'), which were recognized two methoxy and two hydroxyl groups linked to be C-3' and C-4' of lignan skeleton, respectively. Moreover, the small *J* coupling values (4.5 Hz) of protons H-7/H-7' (δ_H 4.74) proved H-7/H-7' and H-8/H-8' were in a *trans* configuration. Hence, the structure of **4B** was evidenced as (+)-pinoresinol[4].

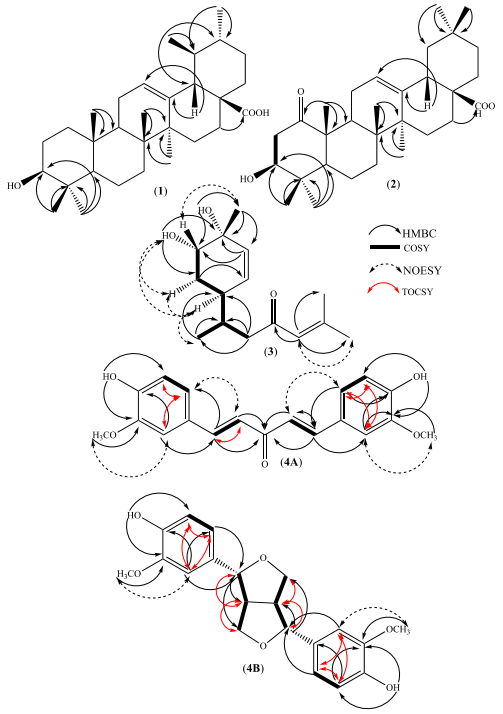

Figure 2. Selected HMBC, COSY, NOESY and TOCSY correlations of 1-4

4. CONCLUSION

From the *n*-hexane extract of the *Macrosolen tricolor* whole plants, three terpenoids comprising two triterpenoids, ursolic acid (**1**), 1-oxooleanolic acid (**2**) and one sesquiterpenoid, bisacurone A (**3**), together with a mixture of two phenolics, (1*E*,4*E*)-1,5-bis(4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (**4A**) and (+)-pinoresinol (**4B**) were detected. Their structures were elucidated by NMR, HRMS methods and further compared with those data of literatures. For the first time, all isolated compounds except **1** were detected from the *Macrosolen* genus, whiles, compound **1** was found from this species. Sesquiterpenoids were commonly reported as the main components of essential oils, such as *Cymbopogon citratus*[14], *Citrus maxima*[15], *Melaleuca sp.*[16],…However, it is the first time that the chemical structure of sesquiterpenoid was determined from the *Marosolen* genus.

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