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STRUCTURE ELUCIDATION AND NMR ASSIGNMENTS FOR TWO NEW QUINONES FROM FRUCTUS RHODOMYRTI OF *Rhodomyrtus tomentosa*

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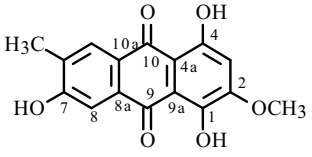
*Two new quinones, 1,4,7-trihydroxy-2-methoxy-6-methylanthracene-9,10-dione (**1**) and compound **2**, were isolated from fructus rhodomyrti of* Rhodomyrtus tomentosa *(Ari.) Hassk., which was collected from Guangdong Province. The structures were elucidated by 1D, 2D NMR, and HR-EI-MS spectroscopy methods. The cytotoxic activities of two compounds* in vitro *were tested. Compound **1** showed cytotoxicity against KB and KBv200 cell lines with IC_{50} of 17.1 and 19.5 μg/mL, and compound **2** with IC_{50} of 18.1 and 25.4 μg/mL, respectively.*

Keywords: NMR, fructus rhodomyrti, quinone, structure elucidation, cytotoxic activity.

Fructus rhodomyrti is the seed of *Rhodomyrtus tomentosa* (Ari.) Hassk., which is a traditional Chinese medicine material with antihepatitis property used in South China [1]. The flavonoid extracts from fructus rhodomyrti have antiradical and antioxidant activity as reported in [2]. It has been revealed that fructus rhodomyrti contains flavonoid glycosides, phenols, amino acids, organic acids, and other chemical constituents [1, 3], but there are few reports on the detailed chemical ingredients of fructus rhodomyrti. A systemic research of the components from fructus rhodomyrti has been carried out by us. Two new compounds, compound **1** and **2**, were isolated from the ethanol extracts of this traditional Chinese medicine. In the *in vitro* cytotoxic tests, compounds **1** and **2** showed cytotoxicity to KB and KBv200 cell lines with IC_{50} between 17.1 and 25.4 μg/mL. This paper reports the isolation, structural elucidation, and cytotoxic activities *in vitro* of the two new compounds.

Compound **1** has the molecular formula $C_{16}H_{12}O_6$, confirmed by HR-EI-MS. The ^1H NMR spectrum of **1** showed signals for two chelated phenolic hydroxyl signals at δ 13.37 and 13.39, and a hydroxyl group not H-bonded at δ 11.01. The ^{13}C NMR spectrum disclosed not only the presence of two ketone carbonyl groups (δ 184.9, 185.8) but signals for all sixteen carbons, including two methyl groups (δ 16.1, 56.5), three methines (δ 106.4, 129.5 110.9), and eleven quaternary carbon atoms (δ 105.5, 111.6, 124.2, 131.8, 133.6, 149.2, 157.5, 159.5, 162.0. 184.9, 185.8). The HMBC data show the overall structure of **1** (Table 1). The multiple correlations from 4-OH to C-3, 4, and 4a and OCH_3-12 to C-2 and 3 located the position of the two chelatted hydroxyls and the OCH_3. The correlations from H-5 to CH_3-11 and to C-5, 6, and 7, and H-8 to C-7,8a, and 9 located the position of CH_3-11 and OH-7. The structure of **1** was thus defined on the basis of the analysis of 1D and 2D NMR spectroscopic experiments.



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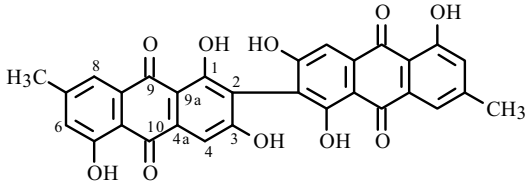

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TABLE 1. ^{1}H and ^{13}C NMR (500 and 125 MHz, δ, ppm, J/Hz) Spectral Data and HMBC Correlations of **1** and **2** (in DMSO-d_6)

C atom	**1**			**2**		
	δ_C	δ_H	HMBC (C to H)	δ_C	δ_H	HMBC
1	149.2 (C)	–	1-OH, H-3, CH_3-12	164.01 (C)	–	1-OH
2	157.5 (C)	–	1-OH, CH_3-12	123.57 (C)	–	H-4
3	106.4 (CH)	6.70 (s)	4-OH, CH_3-12	164.54 (C)	–	H-4
4	159.5 (C)	–	4-OH, H-3	107.04 (CH)	6.72 (1H, s)	–
4a	105.4 (C)	–	4-OH	131.17 (C)	–	–
5	129.5 (CH)	7.83 (s)	CH_3-11	160.89 (C)	–	H-6, 5-OH
6	131.8 (C)	–	H-5, 8, CH_3-11	123.13 (CH)	7.13 (1H, d, J = 1.5)	H-8, CH_3-11, 5-OH
7	162.0 (C)	–	H-5, 8, CH_3-11	148.01 (C)	–	CH_3-11
8	110.9 (CH)	7.46 (s)		120.24 (CH)	7.27 (1H, d, J = 1.0)	H-6, CH_3-11
8a	124.2 (C)	–	H-8	133.10 (C)	–	–
9	184.9 (C)	–	H-8	181.88 (C)	–	H-8
9a	111.4 (C)	–	1-OH	108.82 (C)	–	1-OH
10	185.8 (C)	–	H-5	189.41 (C)	–	H-4
10a	133.6 (C)	–	H-5	113.00 (C)	–	5-OH, H-6, 8
11	16.0 (CH_3)	2.20 (s)	–	21.32 (CH_3)	2.33 (3H, s)	H-6, 8
12	56.5 (CH_3)	3.92 (s)	–			
1-OH	–	13.39 (s)	–	–	12.76 (1H, s)	–
4-OH	–	13.37 (s)	–			
5-OH				–	12.02 (1H, s)	–
7-OH	–	11.01	–			

Compound **2** was isolated as a red powder, which had the molecular formula $C_{30}H_{18}O_{10}$ determined by HR-EI-MS. The NMR as well as HR-EI-MS indicated that **2** had the characteristic signals of the anthraquinone compounds, and the structure is symmetrical. The ^{1}H NMR spectrum of **2** showed two phenolic hydroxyl signals (δ 12.02 and 12.76); three aromatic proton signals at δ 6.70 (s), 7.27 (d), and 7.13 (d); and one methyl at δ 2.33 (s). The ^{13}C NMR spectrum disclosed not only the presence of two ketone carbonyl groups (δ 189.41 and 181.88) but signals for all 15 carbons, including one methyl group (δ 21.32), three methines (δ 107.04, 120.24 and 123.13), and 11 quaternary carbon atoms (δ 164.01, 123.57, 164.54, 160.89, 148.01, 181.88, 189.41, 131.17, 133.10, 113.00, and 108.82). The position of CH_3-11 was located by the correlation from CH_3-11 to C-6, 7, and 8 in the HMBC (Table 2); the correlations from H-4 to C-3, 4a, and 10 indicated the structure is joined by C-1 and C-1′. Taking all these spectroscopic data into account, we arrived at the overall structure of **2**.

Compounds **1** and **2** were tested for their inhibitory activities against the human nasopharyngeal epidermoid tumor KB cell and KBv200 lines. Compound **1** showed cytotoxicity toward KB and KBv200 cell lines with IC_{50} of 17.1 and 19.5 μg/mL, and compound **2** with IC_{50} of 18.1 and 25.4 μg/mL, respectively.

EXPERIMENTAL

General Experimental Procedures. Melting points were detected on a Fisher-Johns hot-stage apparatus and were uncorrected. The NMR data were recorded on a Varian INOVA-500 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Mass spectra were acquired on a VG-ZAB mass spectrometer. IR spectra were obtained on a Nicolet 5DX-FTIR spectrophotometer. Column chromatography was carried out on silica gel (200–300 mesh; Qingdao haiyang chemicals).

Plant Material. The seeds of *Rhodomyrtus tomentosa* (Ari.) Hassk. were collected from Guangdong Province, China, which was stored in the Affiliated Hospital of Guangdong Medicine College, Shenzhen Xixiang, Guangdong, P. R. China.

Extraction and Isolation. The dried and powdered fructus rhodomyrti of *Rhodomyrtus tomentosa* (Ari.) Hassk (3 kg) was exhaustively extracted with 95% EtOH at room temperature. The extract was concentrated under vacuum to yield 95 g of the crude product. This was suspended in a MeOH–H_2O (3:7 v/v) mixture and partitioned with hexane, $CHCl_3$, and EtOAc. The hexane fraction was then subjected to silica gel column chromatography and eluted with hexane, $CHCl_3$, and MeOH in increasing polarity gradient to give 100 fractions. The fractions were monitored by TLC and classified into 25 groups. Fractions

52–53 were purified by preparative TLC over silica gel using $CHCl_3$–MeOH (9:1) to afford anthraquinone **1** (21 mg), and fractions 71–75 were purified in the same way using hexane–EtOAc (1:4) to afford anthraquinone **2** (63 mg).

Compound 1: mp 240–242°C. IR (KBr, ν_{max}, cm^{-1}): 3374, 3086, 2923, 2853, 1728, 1615, 1570, 1470, 1435, 1410, 1370, 1271, 1213, 1154, 1106, 1079, 1038, 1013. HR-EI-MS *m/z* 300.0628 (calcd for $C_{16}H_{12}O_6$, 300.0620). For ^1H and ^{13}C NMR, see Table 1.

Compound 2: mp 209–211°C. EI-MS *m/z:* 538 $[M]^+$, 521, 504, 493, 469, 295, 269, 252, 220. HR-EI-MS *m/z* 538.0894 (calcd for $C_{30}H_{18}O_{10}$, 538.0894). UV ($CHCl_3$, λ_{max}, nm): 236 (ε 6558), 282 (ε 2668). For ^1H and ^{13}C NMR, see Table 1.

MTT Cytotoxicity Assay. The *in vitro* cytotoxic activity was determined by the MTT colorimetric method [4, 5]. Cells were harvested and seeded in 96-well plates at 3.0×10^3/well for KB and KBv200 in a final volume of 190 μL. After 24 h incubation, 10 μL cytotoxic agents or compound vehicles were added to each well. After 68 h, 10 μL MTT solution was added to each well. DMSO (100 μL) was added to each well 4 h later. The concentrations required to inhibit growth by 50% (IC_{50}) were calculated from the cytotoxicity curves (Bliss′s software).

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