A Triterpene from *Rosa sp.*

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The dichloromethane extract of red rose petals afforded 2-oxopomolic acid (**1**) and sitosterol by silica gel chromatography. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy. Antimicrobial tests on **1** indicated that it is active against the fungi *C. albicans* and *T. mentagrophytes*. It was also found to be slightly active against the bacteria *E. coli, P. aeruginosa,* and *S. aureus*. It was inactive against *B. subtilis* and *A. niger*.

Keywords: Rosa sp., *Rosaceae, 2-oxopomolic acid*

1. INTRODUCTION

Red rose is an ornamental plant commonly found in flower shops throughout Metro Manila. The large variety of red roses is usually grown in the Mountain Province where the cool climate is conducive for its growth. Literature search revealed that several studies have been conducted on the chemical constituents of the genus *Rosa*. *Rosa multiflora* which afforded fupenzic acid, 2-oxo-pomolic acid, euscaphic acid, 2α, 3α, 19α, 24-tetrahydroxyolean-12-en-28-oic acid, kaji-ichigoside F1, rosamultin, and niga-ichigoside F2 (Li, *et al.*, 2002). Pomolic acid, isolated from *R.woodsii* and *H.capitata*, was identified as an anti-HIV agent (EC50 1.4μg/mL, T.I. 16.6) (Neto, *et al.*, 2000). A 2-Oxopomolic acid indicated radical scavenging activities similar to α-tocopherol (D'Abrosca, *et al.*, 2005).

The study reports the isolation and structure elucidation by 1D and 2D NMR spectroscopy of 2-oxopomolic acid (**1**) and sitosterol from red rose petals.

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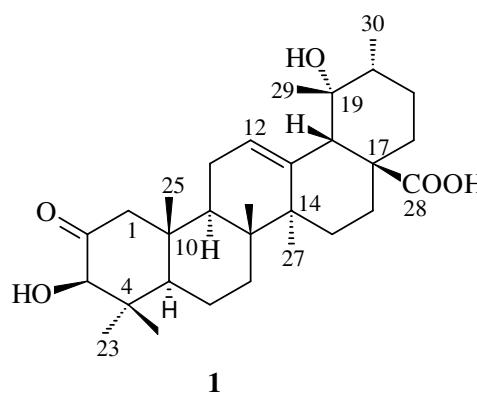
2. RESULTS AND DISCUSSION

The dichloromethane extract of the air-dried red rose petals afforded **1** and sitosterol by silica gel chromatography. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy as follows.

The ¹³C NMR spectral data of **1** (Table 1) gave resonances for thirty carbons with the following

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Table 1
400 MHz ^{1}H NMR and 100MHz ^{13}C NMR, HMBC and NOESY correlations of 1

| Position	δ_C	δ_H* mult. (J Hz)	HMBC Correlations	NOESY Correlations
1	52.9	2.47 (9.3), 2.12 (9.3)		
2	211.0		H_2-1, H-3	
3	82.9	3.91 (s)		H-1, H-5, H_3-24
4	45.7			
5	54.4	1.45	H_3-23, H_3-24	H-3, H-6, H-7, H-9
6	18.6	1.65, 1.80		
7	32.4	1.40, 1.60		
8	40.4			
9	47.2	1.95	H_3-26	H-5, H_3-27
10	43.7			
11	23.6	1.95		
12	128.4	5.35 (br s)	H-18	H-18, H_3-30
13	138.2		H-18, H-27	
14	41.3		H_3-26	
15	29.7	1.09, 1.20		
16	25.3	1.60, 2.55		
17	47.7			
18	52.9	2.56		
19	73.1		H-18, H_3-29, H_3-30	
20	41.1	1.40		
21	25.9	1.10, 1.74		
22	37.4	1.65, 1.75		
23	16.5	0.69 (Me, s)		H-24
24	29.4	1.19 (Me, s)		H-3, H-5
25	16.4	0.88 (Me, s)		H_3-23, H_3-26
26	16.1	0.74 (Me, s)		H_3-25
27	24.3	1.32 (Me, s)		H-9
28	183.0		H-18	
29	27.4	1.21 (Me, s)		H_3-30
30	16.1	0.95 (Me, d, 6.4)		H-12, H_3-29

*multiplet, unless otherwise indicated.

functionalities: a carbonyl of a ketone at δ 211.0, two carbinyl carbons at δ 82.9 and 73.1, and olefinic carbons at δ 128.4 and 138.2. The rest of the resonances belong to methyl, methine, and methylene carbons. This suggested a triterpene with the functionalities of a ketone, an olefin, and two alcoholic groups. The ^{1}H NMR spectral data of **1** (Table 1) indicated an olefinic proton at δ 5.35, a carbinyl proton at δ 3.91, six methyl singlets at δ 0.69, 0.74, 0.88, 1.19, 1.21, and 1.32, and a methyl doublet at 0.95. The geminal protons at δ 2.47 and 2.12 were assigned a to the carbonyl.

The COSY spectrum of **1** gave four isolated spin systems, which is as follows: H-5/H_2-6/H_2-7; H-9/H_2-11/H-12; H_2-15/H_2-16; H-20/H_2-21/H_2-22 (Figure 1).
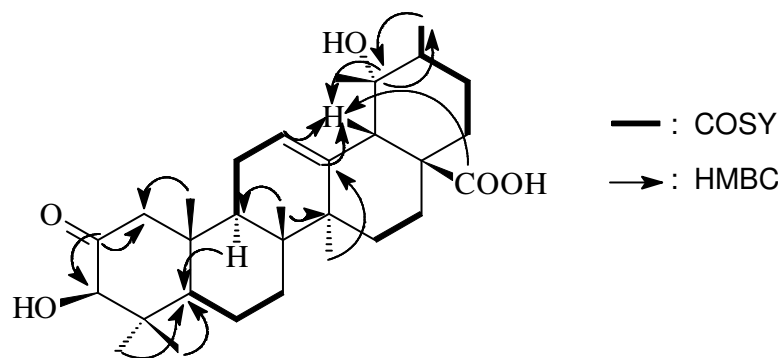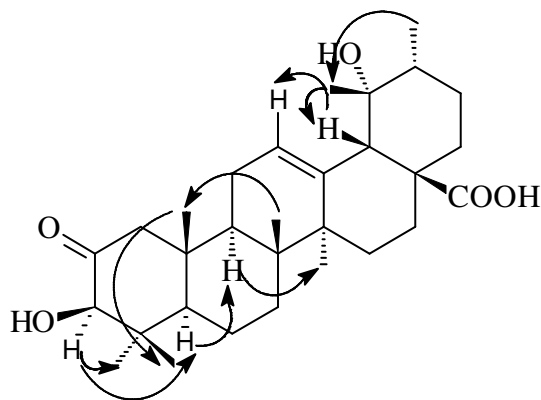
Figure 1
^{1}H-^{1}H COSY and Key ^{1}H-^{13}C Long-Range Correlations for 1

The ^{1}H and ^{13}C assignments of **1** (Table 1) were verified by HSQC and connectivity was verified by HMBC (Table 1 and Figure 1). The carbonyl was assigned to position 2, one of the carbinyl carbons was placed in position 3, and the α-methylene protons were in position 1 due to the long-range correlations between the carbonyl carbon at δ 211.0 (C-2), the methylene protons at δ 2.47 and 2.11 (H$_2$-1), and the carbinyl proton at δ 3.91 (H-3). The carboxylic acid was placed at position 28 on the basis of a long-range correlation between the carbonyl carbon at δ 183.0 (C-28) and the methine proton at δ 2.56 (H-18). This proton was, in turn, correlated long-range to the olefinic carbons at δ 138.2 (C-13) and 128.4 (C-12). Thus, the double bond was placed at C-12 and C-13. The second carbinyl was placed at C-19 due to the long-range correlation between the proton

at δ 2.56 (H-18) and the carbinyl carbon at δ 73.1 (C-19). All long-range correlations observed were consistent with the structure of **1**.

The relative stereochemistry **1** was deduced by NOESY as follows. The carbinyl proton at δ 3.91 (H-3) was close in space to the methyl protons at δ 0.69 (H$_3$-23) and the methine proton at δ 1.45 (H-5) which was, in turn, close to the proton at δ 1.95 (H-9), to which this was close to the methyl singlet at δ 1.32 (H$_3$-27). The methyl singlets at δ 1.19 (H$_3$-24), 0.88 (H$_3$-25), and 0.74 (H$_3$-26) were close to each other on the opposite face of **1**. The methyl singlet at δ 1.21 (H$_3$-29) was close to the methyl singlet at δ 0.95 (H$_3$-30), the methine proton at δ 2.56 (H-18), and the olefinic proton at δ 5.35 (H-12), indicating that they were close to each other in space.

Figure 2
Key NOESY Correlations for 1

Literature search revealed that **1** is 2-oxopomolic acid. The ^{13}C NMR spectral data of **1** and 2-oxopomolic acid β-D-glucopyranosyl ester (Zhong-Jian, *et al.,* 1993) were similar, except in the region where differences occur, e.g., glucose esterified to the carboxylic acid.

As part of our continuing search for possible antimicrobial compounds from Philippine medicinal plants, **1** was tested for its antimicrobial potential against seven microorganisms. Results of the study (Table 2) indicated that **1** is active against the fungi *C. albicans* and *T. mentagrophytes*, with an activity index of 0.3 and 0.2, respectively, at a concentration of 30 μg. It was also found to be slightly active against the bacteria *E. coli*, *P. aeruginosa,* and *S. aureus*, with an activity index of 0.1, 0.2, and 0.1, respectively, at the same concentration. It was inactive against *B. subtilis* and *A. niger*.

Table 2
Antimicrobial Test Results on 1

Organism	Sample(30 μg)	Clearing Zone (mm)			Antimicrobial Index (AI)
		Replicate 1	Replicate2	Replicate3	
E. coli	**1**	11	11	11	0.1
	Chloramphenicol	23			2.8
P. aeruginosa	**1**	12	12	12	0.2
	Chloramphenicol	14			1.3
S. aureus	**1**	11	11	11	0.1
	Chloramphenicol	25			3.2
B. subtilis	**1**	-	-	-	0
	Chloramphenicol	20			2.3
C. albicans	**1**	13	13	13	0.3
	Canesten, 0.2 g[a]	18			0.8
T. mentagrophytes	**1**	12	12	12	0.2
	Canesten, 0.2 g[a]	55			4.5
A. niger	**1**	-	-	-	0
	Canesten, 0.2 g[a]	23			1.3

[a] Contains 1% chlotrimazole.

2.1   General Experimental Procedures

NMR spectra were recorded on a Bruker Avance 400 in CDCl$_3$ at 400 MHz. for ^{1}H and 100 MHz for ^{13}C. Column chromatography was performed with silica gel 60 (70-230 mesh), while the TLC was performed with plastic-backed plates coated with silica gel F$_{254}$. The plates were visualized with vanillin-H$_2$SO$_4$ and warming. (?)

2.2   Sample Collection

Flowers of red roses were obtained from a flower shop in Cubao, Quezon City in February. It was identified as *Rosa sp*. at the Philippine National Museum and a voucher specimen, PNH No. 252635, was deposited at the Philippine National Museum. However, since the commercial red roses may be a cross breed of several varieties of roses, the species could not be identified.

2.3   Isolation

The air-dried flowers (1.2 kg) of *Rosa sp.* were ground in an osterizer, soaked in dichloromethane for three days, then filtered. The filtrate was concentrated under a vacuum to afford a crude extract (50 g) which was chromatographed in increasing proportions of acetone in dichloromethane at 10% increments. The dichloromethane and 10% acetone in dichloromethane fractions were rechromatographed (thrice) in 15% ethyl acetate in petroleum ether to afford sitosterol (20 mg). The 60-70% acetone in dichloromethane fractions were rechromatographed (five times) in diethyl ether:acetonitrile: dichloromethane (1.5:1.5:7) to afford **1** (15 mg).

2.4   Antimicrobial Tests

The microorganisms used were obtained from the University of the Philippines Culture Collection (UPCC). These were *Pseudomonas aeruginosa* (UPCC 1244), *Bacillus subtilis* (UPCC 1149), *Escherichia coli* (UPCC 1195), *Staphylococcus aureus* (UPCC 1143), *Candida albicans* (UPCC 2168), *Trichophyton mentagrophytes* (UPCC 4193) and *Aspergillus niger* (UPCC 3701). Compound **1** was dissolved in 95% ethanol. The antimicrobial assay reported in the literature was employed [5].

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