New Sesquiterpenes from *Pluchea arabica*

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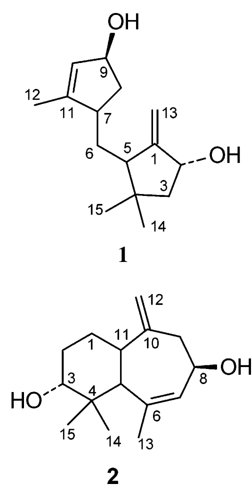
Two new sesquiterpenes, godotol A (**1**) and godotol **B** (**2**), were isolated from *Pluchea arabica*. Their structures were determined by analysis of NMR data. The absolute configurations of **1** and **2** were established by Mosher ester methodology. The godotols displayed weak activity against bacteria and the brine shrimp larvae. They were also inactive in the DPPH antioxidant assay.

Pluchea arabica Quiser and Lack. (syn. *P. laxa* Baker; *P. multiflora* O. Schwartz; and *Phagnalon arabicum* (Boiss.) Boiss) (Compositae), named *Gogot* in Jabbali, is found in Yemen and in the province of Dhofar in the Sultanate of Oman. The infusion of the aerial parts of the plant is used to treat ear infections and sores by the Dhofarians.[1] The juice expressed from the fresh twigs of *P. arabica* is rubbed on the body because of its pleasant aromatic smell. Previous chemical investigations of *Pluchea* plants have shown the presence of eudesmane sesquiterpenes,[2−10] monoterpenes, lignan glycosides, pentacyclic triterpenoids,[11] and flavanoids,[12,13] although the bioactive properties of the isolates are rarely reported. Investigation of the volatile oils of *P. dioscoridis* revealed the presence of oxygenated sesquiterpenes and sesquiterpene hydrocarbons that showed remarkable toxicity[14] to the larvae of *Culex pipiens* (LC_{50} 72 ppm).

In the course of our investigation of Omani medicinal plants for bioactive compounds, a combination of antimicrobial,[15,16] antioxidant,[17] and brine shrimp lethality tests[18] was used to guide the fractionation of the chloroform extract of the shoots of *P. arabica.* We report in this paper the isolation and bioactivity of godotol A (**1**) and godotol B (**2**), two new and structurally isomeric sesquiterpenes.



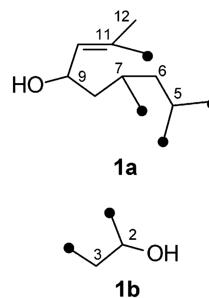
1

2

Compound **1** was obtained as an amorphous solid, $[\alpha]^{25}_{D}$ −125° (*c* 0.0048, CH_2Cl_2). Positive EIMS gave a molecular ion peak at *m/z* 236. HREIMS analysis suggested a molecular formula of $C_{15}H_{24}O_2$, which was consistent with the numbers of carbon and hydrogen estimated in the NMR spectra. The IR spectrum of **1** showed absorptions at 3399, 1645, and 814 cm^{-1}, indicating the presence of alcohol and olefin functional groups. In the UV, **1** absorbed at 230 nm. This implied the absence of a conjugated system. The prominence of the M-18 peak (*m/z* 218) in the EIMS spectra of **1** is indicative of the presence of a hydroxyl group. The NMR data (Table 1) revealed two allylic secondary alcohols, two nonconjugated di- and trisubstituted double bonds, a vinyl methyl, a geminal dimethyl, two methine, and three methylene groups. The molecular formula of **1** suggested a total of four unsaturation equivalents. The remaining two unsaturation equivalents must be due to ring forms. The tracking of correlations in the $^1H-^1H$ COSY and HSQC spectra permitted the establishment of two partial substructures, **1a** and **1b**, for compound **1**.

Table 1. ^{13}C and ^1H NMR Data of **1** in $CDCl_3$

position	δ_C	δ_H J (Hz)	HMBC correlations (J = 7 Hz)
1	151.9		
2	68.7	4.44 (obscd H-2)	C-13
3	46.7	2.56 d (12.0,)	C-1, C-13, C-5
		2.15 dd (14.0, 12.0)	C-1, C-5, C-13
4	32.5		
5	37.6	2.40 dd (9.0, 9.0)	C-13, C-7, C-3
6	41.5	1.76 m (obscd H-8)	C-14, C-15, C-8
		1.60 dd (12.0, 10.0)	C-14, C-15, C-1
7	53.3	1.94 m	C-12, C-5
8	33.5	1.74 m (obscd H-6)	C-10, C-11
		1.49 dd (13.0, 5.0)	C-11
9	69.3	4.42 m (obscd H-2)	C-12, C-7, C-11
10	130.6	5.22 d (8.3)	
11	137.9		
12	16.8	1.79 s	C-7, C-9
13	113.7	5.05 s	C-5, C-3, C-2
		4.99 s	C-5, C-3, C-2
14	29.7	1.04 s	C-15, C-6
15	23.2	0.96 s	C-14, C-6



1a

1b

In substructure **1a**, C-11 is bonded directly to C-7. An olefinic carbon and a *gem*-dimethyl carbon connected substructure **1a** to **1b**. In the $^1H-^1H$ COSY spectra, H-3a at δ 2.56 was coupled to H-3b at δ 2.15 (J = 12.0 Hz), but only H-3b was further coupled to H-2 at δ 4.44 (J = 12.0 and 14.0 Hz), respectively. The H-5 methine at δ 2.40 was

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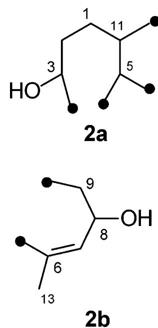
Table 2. ^{13}C and ^{1}H NMR Data of **2** in $CDCl_3$

position	δ_C	δ_H J (Hz)	HMBC correlations (J = 7 Hz)
1	41.6	1.77 m	
		1.52 m	
2	40.5	1.94−2.06 m	C-5, C-4
3	74.6	3.78 ddd (10.0, 7.0, 3.0)	C-15,
4	32.6		
5	52.0	1.36 dd (8.0, 4.0)	C-15, C-14, C-10, C-3
6	137.8		
7	129.8	5.59 d (10.0)	C-13, C-9
8	70.0	4.58 dt (10.0, 7.0)	C-6,
9	49.0	2.80 dd (12.0, 7.0)	C-12, C-11, C-7
		1.94−2.06 obsc	C-12, C-11
10	149.3		
11	48.1	2.23 m	C-12, C-9, C-2
12	112.7	4.95 s	C-11, C-9
		4.88 s	C-11, C-9
13	21.2	1.68 s	C-8, C-7
14	29.6	0.98 s	C-15, C-5
15	21.8	0.97 s	C-14

coupled to H-6a and H-6b protons of an AB system at δ 1.76 and 1.60 (J = 9.0 and 9.0 Hz). H-6a unlike H-6b was further coupled to the H-7 methine multiplet at δ 1.94 (J = 10 Hz). H-7 was also coupled to H-8a and H-8b, an AB system of protons resonating at δ 1.74 and 1.49, respectively. The oxymethine signal at δ 4.42 was coupled to H-8a at δ 1.74 and to H-10, a vinylic proton at δ 5.22. The lack of coupling between H-3b and H-2, H-8b and H-9, and H-6b and H-7 indicated that the vicinal protons of each pair are at right angles. The connectivity of the substructures was further established in the HMBC spectrum, which showed correlation of H-7 with C-12 and C-5, and H-5 with C-13 and C-3. The biogenetic origin of compound **1** is somewhat unusual inasmuch as its C-15 carbon skeleton appears to be derived from a combination of mevalonate and polyketide pathways.

Compound **2** was also obtained as an amorphous solid, $[\alpha]^{25}_D = -105°$ (c 0.0076, CH_2Cl_2). The IR spectrum showed absorptions at 3404, 1600, and 801 cm^{-1}. The UV absorption was at 230 nm, thus sharing similar functional characteristics with compound **1**. The molecular mass of compound **2** was determined as $C_{15}H_{24}O_2$ on the basis of HREIMS and NMR data. Judging from elemental composition, **2** was an isomer of **1**. Compound **2** also has four unsaturation equivalents and a total of two double bonds. The structure of **2** was determined by detailed analysis of its 1D and 2D NMR spectra. The HSQC and DEPT spectra allowed the assignment of all the proton and corresponding carbon signals (see Table 2). The $^{1}H-^{1}H$ COSY, HSQC, and HMBC data analyses revealed structural differences between **1** and **2**. The chemical shifts of the oxymethine protons at δ 3.78 and δ 4.58 suggested that one of the secondary alcohol groups in **2**, in contrast to **1**, is allylic. The tracking of correlations in the $^{1}H-^{1}H$ COSY and HSQC spectra led to two partial substructures, **2a** and **2b**.



In substructure **2a**, C-3 is connected to C-5 by a *gem*-dimethyl carbon. Substructure **2a** is bonded to **2b** through an alkene carbon, which connects C-11 and C-9, and a carbon−carbon bond that connects C-5 to C-6. Analysis of the $^{1}H-^{1}H$ COSY spectra of **2** revealed the connectivity between H-1, H-2, H-3, H-5, and H-11. The H-5 signal at δ 1.36 was coupled to H-11 at δ 2.23 (J = 8.0 Hz). H-11 was further coupled to H-1a and H-1b, an AB system of protons that was observed as multiplets at δ 1.77. H-2 was also observed as a multiplet at δ 1.94−2.06, and it was coupled to both H-3 at δ 3.78 and H-1b at δ 1.52. H-8 was coupled to H-9a at δ 2.8 (J = 7.0 Hz) and to H-9b of an AB system at δ 1.94−2.06. The multiplicity of H-9b is obscured by signal overlap with H-2, which was also observed at δ 1.94−2.06. The vinylic H-7 at δ 5.59 was coupled to the oxymethine H-8 at δ 4.58 (J = 10.0 Hz). The connectivity between the substructures was further established from the following HMBC spectral evidence. H-5 has cross-peaks with C-15, C-14, C-10, and C-3, and H-11 with C-12, C-9, and C-2.

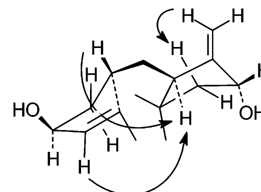


Figure 1. Major NOESY correlations for compound **1**.

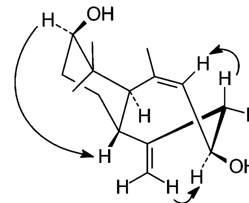


Figure 2. Major NOESY correlations for compound **2**.

With the help of a molecular model, the relative configurations of **1** and **2** were suggested by NOESY correlations shown in Figures 1 and 2. Correlations between H-5 and H-7, H-5 and H-10, and H-3a and H-13a were observed for compound **1** and between H-3 and H-11, H-7 and H-9, and H-8 and H-12 for compound **2**. The absolute configurations of the carbinols were determined as C-2 (R) and C-9 (S) for compound **1** and C-3 (R) and C-8 (R) for compound **2** by advanced Mosher ester methodology, based on $\Delta\delta_H = (\delta_S - \delta_R)$ values[19] of the protons on both sides of the carbinyl carbons (Table 3).

Following standard protocols[16−18] compounds **1** and **2** were evaluated for biological activities and they both showed weak antibacterial activity, inhibiting the growth of *Staphylococcus aureus* at MIC values of 250 and 500 μg/mL, killed brine shrimp larvae at BST LC_{50} of 290 and 540 μg/mL, and inhibited 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals at less than 10% control.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. UV spectra were recorded using a UV−visible HP-8453 spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 341 polarimeter at 25 °C. IR spectra were recorded on a Nicolet FT-IR spectrometer using KBr disks. NMR spectra including $^{1}H-^{1}H$ COSY, HSQC, DEPT, HMBC, NOESY, and NOESY experiments were recorded on a Bruker Advance NMR spectrometer operating at 400.13 MHz for ^{1}H and 100.6 for ^{13}C in $CDCl_3$ with TMS as internal standard. HREIMS spectra were recorded with a JEOL JMS-SX102A (EIMS, 70 eV, gun high 3.0 kV). For chromatography, EM

Table 3. ^{1}H NMR Chemical Shift Data for Relevant Protons for the (*S*)- and (*R*)-MPTA Mosher Ester Derivatives of **1** and **2**

compound **1**					compound **2**				
MPTA config	position	δ_H	$\Delta\delta_H = \delta_S - \delta_R$	assigned config	MPTA config	position	δ_H	$\Delta\delta_H = \delta_S - \delta_R$	assigned config
S	H-13a	5.12	pos		*S*	H-2	1.94	pos	
R		5.11			*R*		1.81		
S	H-13b	5.17	pos						C-3*R*
R		5.15							
				C-2*R*	*S*	H-14	1.00	neg	
					R		1.01		
S	H-3a	2.73	neg		*S*	H-15	0.94	neg	
R		2.75			*R*		0.96		
S	H-3b	2.31	neg			H-12a	5.12	pos	
R		2.40					5.11		
S	H-8	1.57	neg			H-12b	5.09	pos	
R		1.65					5.08		
				C-9*S*	*S*	H-9a	3.58	neg	
					R		3.75		
S	H-10	5.19	neg		*S*	H-9b	2.93	pos	
R		5.35			*R*		2.87		
S	H-12	1.56	zero						C-8*R*
R		1.56							
S	H-14	1.08	zero		*S*	H-7	5.61	pos	
R		1.08			*R*		5.60		
S	H-15	1.07	pos		*S*	H-13	1.57	neg	
R		0.97			*R*		1.93		

Science silica gel 60 (70−230 mesh ASTM) was used. Whatman precoated silica gel (60A K6F) plates were used for TLC, with compounds visualized by UV lamp and spraying with 10% (v/v) H_2SO_4 or vanillin-H_2SO_4 followed by heating.

Plant Material. The shoots of *P. arabica* were collected from Sansevieria-Wadi, Taqah, Oman, in September 2001 (collection number MP 345), and identified by Dr. Shahina A. Ghazanfar, a plant taxonomist at the Royal Botanic Gardens Kew, Richmond Surrey, UK. A specimen of the material is preserved in the University Herbarium, in the Department of Biological Science, Sultan Qaboos University, Oman.

Extraction and Isolation. Dried twigs of *P. arabica* were powdered (375 g) and extracted with $CHCl_3$ (2 × 3 L) by maceration for one week. The extracts were concentrated in vacuo to give a residue (35.0 g). The plant material was further extracted with EtOH (2 × 3 L) by maceration to give a residue of 13.0 g.

The $CHCl_3$ extract (35 g) was chromatographed on silica gel (200 g) and eluted with 1.0 L of each of the following solvents: petroleum ether (1.73 g), $CHCl_3$ (14.57 g), $CHCl_3$−EtOAc (1:1) (11.17 g), EtOAc (1.5 g,), EtOAc−EtOH (1:1) (2.93 g), and EtOH (0.25 g). Weak larvicidal and antimicrobial activities were detected in fractions eluted with $CHCl_3$−EtOAc (1:1). A portion of fraction 3 (6 g), eluted with $CHCl_3$−EtOAc (1:1), was further chromatographed on silica gel (125 g), eluting with $CHCl_3$−EtOAc mixtures. $CHCl_3$−EtOAc (9.5:0.5) eluted compound **1** (amorphous solid, 150 mg, R_f = 0.68, $CHCl_3$), and $CHCl_3$−EtOAc (9.25:0.75) eluted compound **2** (amorphous solid, 300 mg, R_f = 0.62, $CHCl_3$). Compounds **1** and **2** inhibited the growth of *S. aureus* at MIC of 250 and 500 μg/mL and were also lethal to brine shrimp larvae at LC_{50} values of 290 and 540 μg/mL respectively.

Godotol A (1): amorphous solid ($CHCl_3$−EtOAc), mp 55.4−55.6 °C; $[\alpha]^{25}_D$ −125° (*c* 0.0048, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ϵ) 230 (4.01) nm; IR (KBr) ν_{max} 3399, 2922, 1645, 1449, 1261, 1096, 1026, 814 cm^{-1}; ^{13}C NMR ($CDCl_3$ 400.13 MHz) and ^{1}H NMR ($CDCl_3$ 100.6 MHz) data, see Table 1; EIMS *m/z* 236 (M^+, 29), 218 (M^+ − 18, 4), 180 (10), 150 (13), 147 (14), 137 (11), 135 (15), 123 (29), 121 (20), 109 (20), 107 (30), 100 (39), 99 (100), 98 (34), 95 (34), 93 (27), 91 (34), 82 (26), 81 (44), 79 (56), 71 (78), 69 (63); HREIMS *m/z* 236.1788 (calcd for $C_{15}H_{24}O_2$, 236.1776).

Godotol B (2): amorphous solid ($CHCl_3$−EtOAc), mp 36.9−37.8°C; $[\alpha]^{25}_D$ −105° (*c* 0.0076, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ϵ) 234 (3.76) nm; IR (KBr) ν_{max} 3404, 2930, 1610, 1455, 1266, 1085, 1014, 801 cm^{-1}; ^{13}C NMR ($CDCl_3$ 400.13 MHz) and ^{1}H NMR ($CDCl_3$ 100.6 MHz) data, see Table 1; EIMS *m/z* 236 (M^+, 10), 221 (3), 218 (M^+ − 18, 7), 189 (7), 175 (8), 165 (10), 149 (8), 136 (18), 124 (19), 123 (91), 119 (17), 110 (15), 109 (41), 107 (43), 105 (24), 100 (34), 99 (38), 98 (52), 95 (36), 93 (28), 91 (34), 82 (26), 81 (66), 79 (53), 77 (24), 71 (89), 69 (100), 67 (25); HREIMS *m/z* 236.1793 (calcd for $C_{15}H_{24}O_2$, 236.1776).

Mosher Esters. A mixture of either **1** or **2** (3.5 mg) and 10 millimolar equivalents of (*R*)- or (*S*)-MPTA chloride, 4-(dimethylamino)pyridine (1.6 mg), and 2 drops of pyridine, in 1 mL of CH_2Cl_2, was allowed to stand at room temperature in a capped vial. After 2 days, the reaction mixture was mixed with hexane (1 mL) and chromatographed over silica gel in a disposable pipet, eluting with a gradient of CH_2Cl_2−EtOAc mixtures. (See Table 3 for relevant protons of the (*S*)- and (*R*)-MTPA ester derivatives.)

Biological Assays. DPPH free radical scavenging activity[17] and brine shrimp lethality tests[18] were performed on extracts and compounds **1** and **2** according to standard protocols. Compounds **1** and **2** lack antioxidant activity, inhibiting DPPH radicals at less than 10%, and are weakly lethal to brine shrimp larvae with BST LC_{50} value of 290 μg/mL for **1** and 540 μg/mL for **2**, respectively. The antimicrobial activities of **1** and **2** were evaluated against *Escherischia coli* ATCC 9637, *S. aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, *Klebsiella pneumonia* ATCC 10031, and *Salmonella choleraesuis* ATCC 14028 using the agar-dilution-streak method.[15] MIC was determined by a broth dilution method according to the NCCLS.[16] The tested compounds were dissolved in DMSO, and graded concentrations were made in tryptone soy broth. Compounds **1** and **2** inhibited the growth of *S. aureus* at MIC values of 250 and 500 μg/mL. Neither compound was active against the other organisms at doses as high as 500 μg/mL.

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