DOI: 10.1002/ejoc.200500936

New α-Glucosidase Inhibitors and Antibacterial Compounds from *Myrtus communis* L.

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Keywords: *Myrtus communis* L. / Acylphloroglucinol / Myrtucommulone D, E and C / Antibacterial activity and α-glucosidase inhibition studies

Three new acylphloroglucinols – myrtucommulone D (**1**), myrtucommulone E (**2**), and myrtucommulone C (**3**) – and a new usnic acid derivative usnone A (**4**) have been isolated from *Myrtus communis* L., along with the known acylphloroglucinol myrtucommulone B (**5**), one sterol **8** and six triterpenes **10**–**15**, two flavonoids **6** and **9**, and 2,5-dihydroxy-4-methoxybenzophenone (cearoin; **7**). The structures of compounds **1**, **2**, **4**, and **5** were also unambiguously determined by single-crystal X-ray diffraction analysis. The compounds **1**–**3** and **5** were found to be more potent α-glucosidase inhibitors than the clinically used standard acarbose. Compound **3** exhibited the highest activity among all the acylphloroglucinols, with an IC_{50} (inhibition concentration) = 35.4 ± 1.15 μM. Compounds **1**, **2**, **4**, **7**, and **11**–**15** also exhibited antibacterial activities.

Introduction

Myrtus communis L. (Myrtaceae) commonly known in English as myrtle, is an evergreen shrub widely distributed in the Mediterranean region. Myrtle has been used as a folk medicine in several remedies.[1–5] Several studies have found strong antibacterial, anti-inflammatory, anti-hyperglycemic, and antioxidant activities in various extracts of this plant.[1,6–9] The characteristic constituents of this plant include monoterpenoids, flavonoids, triterpenoids,[10] and phloroglucinol-type compounds.[11–13] Recently α-glucosidase inhibition activity of aqueous extracts of myrtle has been reported.[14]

α-Glucosidase inhibitors are used in the management of non-insulin-dependent diabetes mellitus (NIDDM), acting by reversible inhibition of the gastrointestinal sucrase, glucoamylase, dextrinase, maltase, and isomaltase enzymes. These enzymes normally catalyze the conversion of dietary starch and sucrose into absorbable monosaccharides, and enzyme inhibition therefore delays and reduces the peak of postprandial blood glucose.[15] They have also been used as inhibitors of tumor metastasis, antiobesity drugs, fungistatic compounds, insect antifeedants, and antiviral and immune modulators.[16]

Here we report the isolation and structure determination of four new acylphloroglucinols: myrtucommulone D (**1**), myrtucommulone E (**2**), myrtucommulone C (**3**), and a new usnic acid derivative, usnone A (**4**). The structures of compounds **1**, **2**, **4**, and **5** were unambiguously deduced by single-crystal X-ray diffraction analysis. The known compounds myrtucommulone B (**5**),[11–13] tectochrysine (**6**),[17] 2,5-dihydroxy-4-methoxybenzophenone (cearoin; **7**),[18] β-sitosterol (**8**),[19] sideroxylin (**9**),[20] ursolic acid (**10**),[21] corosolic acid (**11**),[22] arjunolic acid (**12**),[23] erythrodiol (**13**),[24] oleanolic acid (**14**),[25] and betulin (**15**)[26] were also obtained. The compounds **6**–**15** were isolated from *Myrtus communis* L. for the first time. The new compounds **1**–**3**, as well as the known compound **5**, exhibited strong inhibition of α-glucosidase. Myrtucommulone C (**3**) was found to be the most potent α-glucosidase inhibitor of all compounds, with an IC_{50} = 35.4 ± 1.15 μM.

Results and Discussion

Myrtucommulone D (**1**) was isolated from the methanolic extract of *Myrtus communis* L. as yellow crystals. The compound **1** was assigned the formula $C_{38}H_{50}O_9$ on the basis of the ion peak at *m/z* 651 $[M + 1]^+$ by chemical ionization mass spectrometry (CI-MS) and X-ray and nuclear magnetic resonance (NMR) spectroscopic data.

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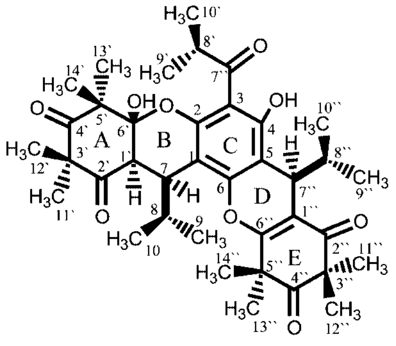
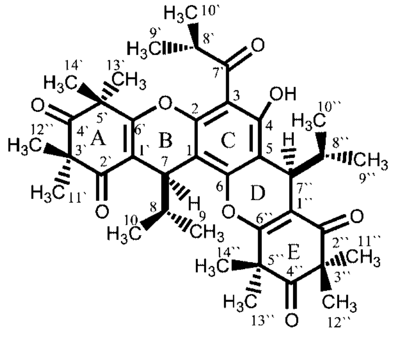
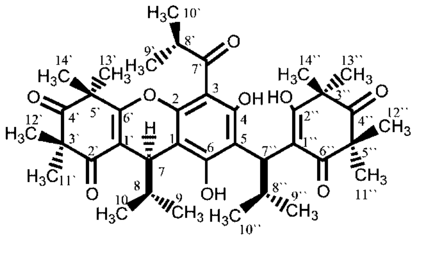


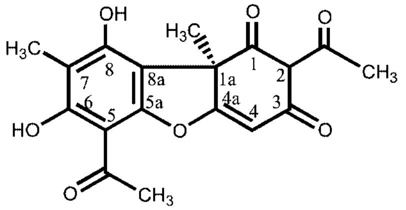
Myrtucommulone-D (**1**)



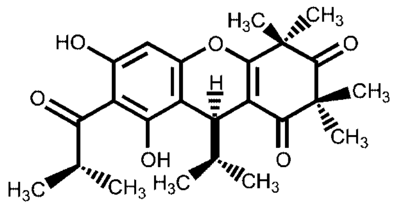
Myrtucommulone-E (**2**)



Myrtucommulone-C (**3**)



Usnone-A (**4**)



Myrtucommulone-B (**5**)

The ^{1}H NMR spectrum showed the presence of two isopropyl and one isobutylidene moieties and eight methyl groups bound to non-protonated aliphatic carbons. The ^{13}C NMR spectrum [Broad Band decoupling (BB), Distortionless Enhancement Polarization Transfer (DEPT)] (Table 1) showed 38 signals, including 14 methyls, 6 methines, and 18 quaternary carbons. In the hetero multiple bond connectivities (HMBC) spectrum, the C-1′ methine proton (δ = 3.67 ppm) showed correlations with C-2′ (δ = 204.9 ppm), C-6′ (δ = 100.1 ppm), and C-7 (δ = 28.9 ppm). The C-7 methine proton showed correlations with C-1 (δ = 106.7 ppm), C-1′ (δ = 45.7 ppm), C-8 (δ = 32.2 ppm), C-2′ (δ = 204.9 ppm), C-2 (δ = 150.6 ppm), and C-6′ (δ = 100.1 ppm). The C-9′ methyl (δ = 1.20 ppm) and C-8′ methine (δ = 3.89 ppm) protons showed correlations with the exocyclic carbonyl carbon (C-7′; δ = 210.2 ppm). Similarly the C-11′ (δ = 1.35 ppm) and C-12′ (δ = 1.29 ppm) methyl protons exhibited interactions with C-2′ (δ = 204.9 ppm), and C-4′ (δ = 211.9 ppm); furthermore, the C-13′ (δ = 1.56 ppm) and C-14′ (δ = 1.40 ppm) methyl protons showed HMBC interactions with C-4′ (δ = 211.9 ppm), C-6′ (δ = 100.1 ppm), and C-5′ (δ = 54.9 ppm).

Finally the structure and relative stereochemistry of myrtucommulone D (**1**) were unambiguously deduced by a single-crystal X-ray diffraction analysis (Figure 1). Compound **1** was obtained as a colorless block crystal (0.97 × 0.56 × 0.53 mm) and its X-ray analysis showed normal bond lengths and bond angles.[27] The pentacyclic benzopyranoxanthene system of **1** is nearly planar, with rings A and E slightly deviating from the plane of rings B, C, and D. The two isopropyl groups at C-7 and C-7′′ were oriented in the same direction, but opposite to the direction of the C-6′ hydroxy group. Rings A and B are transfused with each other and the hydroxy group at C-6′ is twisted towards the plane of the C-2′ carbon, the C-2′–C-1′–C6′–O9 torsion angle being 50.78° (17).

Myrtucommulone E (**2**) was isolated from the methanolic extract of *Myrtus communis* L., as yellow crystals. Compound **2** had molecular formula $C_{38}H_{48}O_8$, as derived from the ion peak at *m*/*z* 633 $[M + 1]^+$ by CI-MS and X-ray and NMR spectroscopic data. The NMR spectroscopic data for compound **2** showed a distinct resemblance to those for compound **1**, except for the absence of the C-1′ methine proton in the ^{1}H NMR spectrum of compound **2**

Table 1. ^1H and ^{13}C NMR spectroscopic data for **1** and **2** in $CDCl_3$ (400 MHz for ^1H and 100 MHz for ^{13}C).[a]

C atom	Myrtucommulone D (**1**) ^{13}C (δ)	^1H NMR, δ (*J*, Hz)	Myrtucommulone E (**2**) ^{13}C (δ)	^1H NMR, δ (*J*, Hz)
1	106.7 (C)	–	103.2 (C)	–
2	150.6 (C)		151.0 (C)	
3	108.5 (C)		106.1 (C)	
4	161.4 (C)		160.9 (C)	
5	108.6 (C)		110.3 (C)	
6	153.5 (C)		153.3 (C)	
7	28.9 (CH)	4.18, dd, 3.6, 3.5	32.2 (CH)	4.38, d, 3.6
8	32.2 (CH)	2.35 m	35.4 (CH)	1.98 m
9	16.0 (CH_3)	0.66, d, 6.8	18.5 (CH_3)	0.81, d, 3.6
10	20.2 (CH_3)	0.89, d, 7.0	19.4 (CH_3)	0.94, d, 6.9
1′	45.7 (CH)	3.67, d, 5.9	111.9 (C)	
2′	204.9 (C)		197.6 (C)	
3′	56.2 (C)		56.2 (C)	
4′	211.9 (C)		211.5 (C)	
5′	54.9 (C)		47.2 (C)	
6′	100.1 (C)		167.2 (C)	
7′	210.2 (C)		209.5 (C)	
8′	39.9 (CH)	3.89, m	40.2 (CH)	3.83, m
9′	20.4 (CH_3)	1.20, d, 7.0	19.7 (CH_3)	0.83, d, 7.1
10′	17.9 (CH_3)	1.16, d, 6.6	17.7 (CH_3)	1.26, d, 6.0
11′	25.4 (CH_3)	1.57, s	23.8 (CH_3)	1.57, s
12′	24.2 (CH_3)	1.29, s	25.2 (CH_3)	1.29, s
13′	24.7 (CH_3)	1.56, s	25.5 (CH_3)	1.56, s
14′	19.2 (CH_3)	1.40, s	24.9 (CH_3)	1.40, s
1″	111.6 (C)		110.3 (C)	
2″	197.5 (C)		197.4 (C)	
3″	57.3 (C)		56.1 (C)	
4″	213.8 (C)		211.5 (C)	
5″	47.4 (C)		47.5 (C)	
6″	167.4 (C)		167.2 (C)	
7″	32.0 (CH)	4.37, d, 3.2	31.9 (CH)	4.40, d, 3.4
8″	34.2 (CH)	2.01, m	34.3 (CH)	1.95, m
9″	20.0 (CH_3)	0.88, d,	20.7 (CH_3)	1.26, d, 6.0
10″	18.3 (CH_3)	0.75, d,	18.5 (CH_3)	0.79, d,
11″	24.9 (CH_3)	1.47, s	24.9 (CH_3)	1.41, s
12″	22.1(CH_3)	1.32, s	23.9 (CH_3)	1.32, s
13″	25.3 (CH_3)	1.39, s	25.1 (CH_3)	1.77, s
14″	24.3 (CH_3)	1.62, s	25.0 (CH_3)	1.64, s

[a] The ^1H–^{13}C connectivities and ^{13}C multiplicities were deduced from HMQC and DEPT experiments.

and the presence of additional olefinic signals at δ = 111.9 and 167.2 ppm in the ^{13}C NMR spectrum (Table 2), indicating the presence of a double bond between C-1′ and C-6′ in compound **2**. The position of the olefinic bond between C-1′ and C-6′ was further confirmed by the HMBC spectrum, in which the C-7 methine proton (δ = 4.38 ppm) showed correlations with C-1 (δ = 103.2 ppm), C-1′ (δ = 111.9 ppm), C-8 (δ35.4), and C-2′ (δ = 197.5 ppm).

The structure of compound **2** was also unambiguously deduced by a single-crystal X-ray diffraction analysis (Figure 2). Compound **2** was obtained as colorless plate crystals (0.76×0.25×0.18 mm) and its X-ray analysis showed normal bond lengths and bond angles.[27] The pentacyclic benzopyranoxanthene system in **2** was also found to be a nearly planar moiety, in which rings A and E deviated slightly from the planes of rings B, C, and D. The two isopropyl groups at C7 and C7″ are oriented in the same direction.

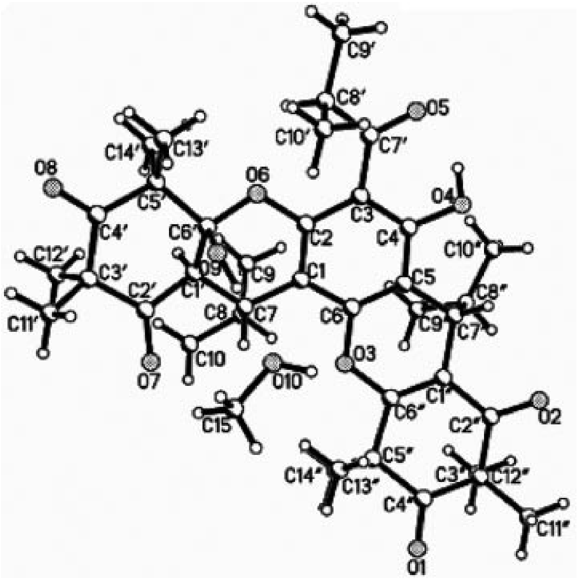


Figure 1. The structure of compound **1**, showing 50% probability displacement ellipsoids and the atom-numbering scheme.

Table 2. ^1H and ^{13}C NMR spectroscopic data for (**3**) in $CDCl_3$ (400 MHz for ^1H and 100 MHz for ^{13}C).[a]

C atom	Myrtucommulone C (**3**) ^{13}C (δ)	^1H, δ (*J*, Hz)	C atom	^{13}C (δ)	^1H, δ (*J*, Hz)
1	103.0 (C)	–	10′	17.7 (CH_3)	1.16, d, 6.0
2	153.2 (C)		11′	23.8 (CH_3)	1.35, s
3	106.2 (C)		12′	25.2 (CH_3)	1.41, s
4	160.9 (C)	13.25 (OH)	13′	31.8 (CH_3)	1.60, s
5	110.3 (C)		14′	24.9 (CH_3)	1.40, s
6	151.0 (C)		1″	111.7 (C)	
7	32.2 (CH)	4.35, d, 3.4	2″	167.6 (C)	
8	34.3 (CH)	1.9 m	3″	47.5 (C)	
9	18.4 (CH_3)	0.76, br. s	4″	211.8 (C)	
10	19.4 (CH_3)	0.79, br. s	5″	56.2 (C)	
1′	111.9 (C)		6″	197.6 (C)	
2′	197.4 (C)		7″	31.9 (CH)	4.38, d, 3.4
3′	56.7 (C)		8″	35.2 (CH)	2.01, m
4′	211.5 (C)		9″	19.7 (CH_3)	0.88, d, 6.1
5′	47.1 (C)		10″	18.5 (CH_3)	0.75, d,
6′	167.2 (C)		11″	25.3 (CH_3)	1.47, s
7′	209.5 (C)		12″	25.1 (CH_3)	1.32, s
8′	40.2 (CH)	3.9, m	13″	24.0 (CH_3)	1.39, s
9′	20.7 (CH_3)	1.24, d, 6.0	14″	25.4 (CH_3)	1.62, s

[a] The ^1H–^{13}C connectivities and ^{13}C multiplicities were deduced from HMQC and DEPT experiments.

Myrtucommulone C (**3**) was also isolated from the methanolic extract of *Myrtus communis* L., as an amorphous powder. Compound **3** was assigned the formula $C_{38}H_{50}O_9$ on the bases of an ion peak at *m*/*z* 651 $[M + 1]^+$ by fast atomic bombardment mass spectrometry (FABMS) and NMR spectroscopic data. The electron ionization mass spectrum (EI-MS) of compound **3** exhibited a base peak at *m*/*z* 589, resulting from the loss of H_2O and $–C_3H_7$ groups from $[M]^+$. The IR spectrum shows absorption bands at 3500 (OH), 2968 (aromatic CH), 1717 (saturated –C=O), 1628 (aryl), and 1580 (enolic 1,3-diketone system). The NMR spectroscopic data for compound **3** indicated its resemblance to compound **2** except for the absence of the C-

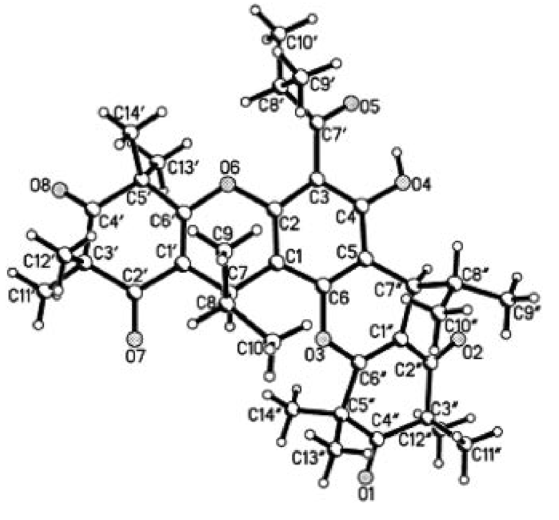


Figure 2. The structure of **2**, showing 50% probability displacement ellipsoids and the atom-numbering scheme.

6 and C-2′′ ether linkage. Its ^{13}C NMR spectrum (BB, DEPT; Table 2) showed 38 signals, including 14 methyls, 5 methines, and 19 quaternary carbons. In the HMBC spectrum (Figure 3), the C-7 methine proton (δ = 4.35 ppm) showed correlations with C-1 (δ = 103.0 ppm), C-1′ (δ = 111.9 ppm), C-8 (δ = 34.3 ppm), and C-2′ (δ = 197.4 ppm). The presence of the OH group at C-4 (δ = 160.9 ppm) was further confirmed by the HMBC correlations of the hydroxy proton (δ = 12.63 ppm) with the exocyclic carbonyl C-7′ (δ = 209.5 ppm), C-3 (δ = 106.2 ppm), C-2 (δ = 153.2 ppm), C-4 (δ = 160.9 ppm), and C-5 (δ = 110.3 ppm).

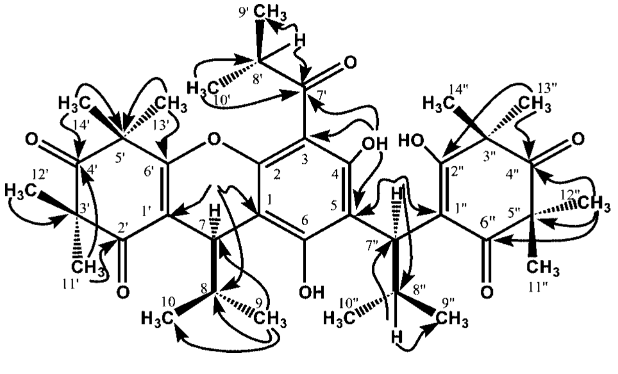


Figure 3. Key HMBC interactions in compound **3**.

Usnone A (**4**), was isolated as yellow crystals from the ethyl acetate-soluble part of the methanolic extract of *Myrtus communis* L. High-resolution electron ionization mass spectrometry (HR-EIMS) analysis showed the [M]$^+$ signal at *m/z* 344.0851 ($C_{18}H_{16}O_7$ calcd. 344.0896) with eleven degrees of unsaturation. The ^{1}H NMR spectrum of **4** was distinctly similar to that of the known compound isousnic acid,[28] the main differences including the absence of an olefinic bond between the C-2 and C-3 positions and the presence of a carbonyl group instead of a hydroxy group at C-3 in compound **4**. The spectrum showed four singlets representing three protons each, resonating at δ = 1.73, 2.07, 2.63 and 2.65 ppm for C-1a, C-7 methyl, C-5 and C-2 methyls of acetyl groups. Two singlets of one proton resonating at δ = 13.28 and 10.99 ppm were assigned to the C-6 and C-8 hydroxy groups.

The ^{13}C NMR spectra (BB, DEPT; Table 3) showed the presence of eighteen carbon signals belonging to four methyl, two methine, and twelve quaternary carbons. The assignment of the carbon signals was done by comparison with spectra of related compounds and also inferred from the HMBC spectrum of usnone A (**4**; Figure 4)

Table 3. ^{1}H and ^{13}C NMR spectroscopic data for **4** in $CDCl_3$ (400 MHz for ^{1}H and 100 MHz for ^{13}C).[a]

C atom	Usnone A (**4**) ^{13}C (δ)	^{1}H NMR, δ (J, Hz)
1	198.0	–
2	98.3	4.30
3	191.6	–
4	105.2	5.95
5	101.5	–
6	163.8	–
7	109.2	–
8	157.4	–
1a	59.1	–
4a	179.3	–
5a	155.2	–
8a	103.8	–
9	201.7	–
10	27.9	2.63
11	200.3	–
12	31.2	2.65
13	32.1	1.73
14	7.5	2.07

[a] The ^{1}H–^{13}C connectivities and ^{13}C multiplicities were deduced from HMQC and DEPT experiments.

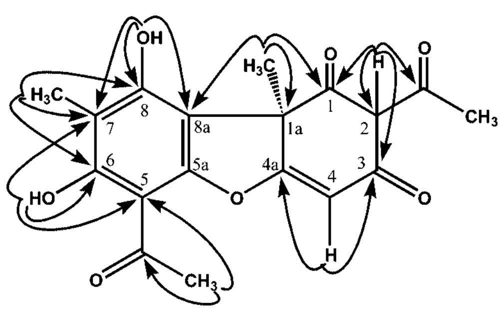


Figure 4. Key HMBC interactions in compound **4**.

The absolute structure of compound **4** was also unambiguously deduced from its single-crystal X-ray diffraction analysis (Figure 5). There are two crystallographically independent molecules in the asymmetric unit, and X-ray analysis showed normal bond lengths and bond angles in the compound,[27] the tricyclic dibenzofuran moiety being nearly planar, with a maximum deviation of 0.610(9) Å for C1a, an sp^3 carbon atom.

The structure of the known acylphloroglucinol myrtucommulone B (**5**) had previously been reported to have isomeric structures obtained by heating myrtucommulone A at 80.0 °C and it was also assumed to be an artifact of myrtucommulone A.[13] In the current study compound **5** was isolated from the hexane plant extract. The structure of **5** has

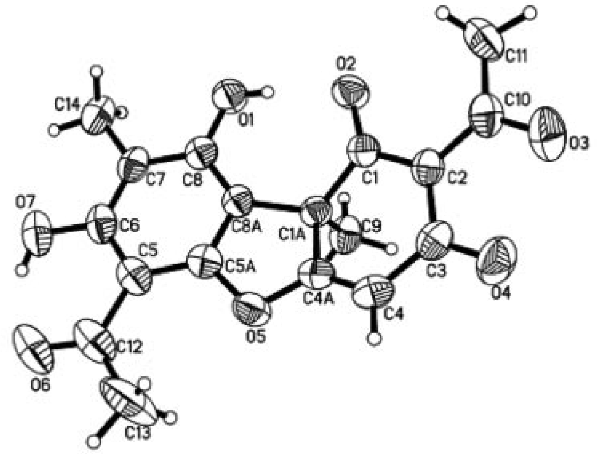


Figure 5. X-ray structure of compound **4**.

now been confirmed through X-ray diffraction studies (Figure 6).

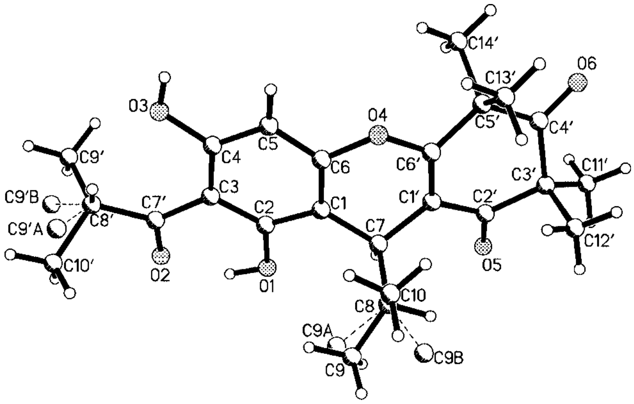


Figure 6. X-ray structure of compound **5**.

Antibacterial Activity

Compounds **1** and **2** showed significant antibacterial activity against *Staphylococcus aureus*. Compound **4** showed significant activities against *Salmonella typhi* and *Pseudomonas aeruginosa*, while compounds **7**, **11**, **12**, **13**, **14**, and **15** showed significant activity against *Salmonella typhi* and *Pseudomonas aeruginosa* (Table 4).

α-Glucosidase Inhibition Studies

The phloroglucinol-type compounds **1**–**3** and **5** were found to be potent inhibitors of α-glucosidase, showing promising inhibitory activity in a dose-dependent manner. All compounds were more potent than the clinically used standard drug acarbose, widely prescribed to type II diabetic patients, and deoxynojirimycin, used as a standard inhibitor. Compound **3** exhibited the highest activity among all phloroglucinols tested (Table 5) against the enzyme.

Table 4. Antibacterial activities of compounds **1**, **2**, **4**, **7**, and **11**–**15**, relative to the standard drug imipenem. For details, see Exper. Part. Inhibition zones are given in mm. Abbreviations: EC = *Escherichia coli*, BS = *Bacillus subtilis*, SF = *Shigella flexneri*, SA = *Staphylococcus aureus*, PA = *Pseudomonas aeruginosa*, ST = *Salmonella typhi*.

Compound	EC	BS	SF	SA	PA	ST
Imipenem	30	33	27	33	24	25
1	–	15	12	24	–	15
2	10	15	10	–	13	17
4	11	16	15	15	17	17
7	–	12	–	15	16	18
11	–	15	15	–	17	17
12	9	15	13	15	12	16
13	16	17	–	11	12	19
14	–	15	10	–	16	16
15	–	–	15	–	18	16

Table 5. α-Glucosidase inhibitory activity.

Compound	Name	IC_{50} μM
1	myrtucommulone D	84.3 ± 3
2	myrtucommulone E	46.6 ± 0
3	myrtucommulone C	35.4 ± 1.15
5	myrtucommulone B	39.99 ± 1.00
Standard	deoxynojirimycin	425.6 ± 8.14
	acarbose	780 ± 0.028

Experimental Section

Equipment: Optical rotations were measured on a JASCO DIP 360 polarimeter, IR spectra were recorded on a JASCO 302-A spectrophotometer, and EI-MS and HREI-MS were recorded on Jeol JMS HX 110 with data system and on JMS-DA 500 mass spectrometers. The ^1H and ^{13}C NMR spectra were recorded on Bruker NMR spectrometers, operating at 500 and 400 MHz (100 and 125 MHz for ^{13}C). The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are given in Hz.

Chromatographic Conditions: Precoated aluminium sheets (silica gel G-60F-254, E. Merck) were used for thin-layer chromatography (TLC). Visualization of the TLC plates was achieved under UV at 254 and 366 nm and by spraying with ceric sulfate reagent. *n*-Hexane/ethyl acetate (7:2, 9.5:0.5) solvent systems were used.

Plant Material: The aerial parts (8 kg dry wt.) of *Myrtus communis* L. were collected from the village of Kabal in the Swat district, NWFP, Pakistan, at an elevation of 1800 m in May/June 2003 and were identified by Mr. Mahboob-ur-Rahman (Assistant Professor), Department of Botany, Govt Jahanzeb Post Graduate College, Saidu Sharif, Swat, NWFP, Pakistan. A voucher specimen (CM-03) was deposited in the herbarium of the botany department.

Extraction and Isolation: The freshly collected air-dried powdered plant material (8 kg) was crushed and extracted by maceration in 80% methanol for 10 days (3 × 50 L). The combined methanol extract was concentrated and the viscous extract was partitioned between *n*-hexane, ethyl acetate, and butanol. The ethyl acetate fraction (70 g) was fractionated by vacuum liquid chromatography (VLC) over silica gel (1.4 kg), and eluted with hexane and gradients of chloroform up to 100% and methanol up to 20%. As a result of this, five subfractions were obtained. Subfraction Fmc-3 (4–7, 100 mg), after repeated silica gel column chromatography and elution with ethyl acetate/hexane (20%), yielded usnone A (**4**), tectochrysine ($C_{16}H_{13}O_4$; **6**), and betulin ($C_{30}H_{50}O_2$; **15**). Similarly, subfraction Fmc-5, after further purification by silica gel column chromatography and elution with ethyl acetate/hexane (30%), yielded the compounds cearoin ($C_{14}H_{12}O_4$; **7**), sideroxyline ($C_{18}H_{16}O_5$; **9**), and oleanolic acid ($C_{30}H_{50}O_3$; **14**). The hexane-soluble fraction obtained from the crude methanolic extract was subjected to column chromatography with ethyl acetate/hexane (10%) as a mobile phase and yielded the compounds myrtucommulone D (**1**), myrtucommulone E (**2**), myrtucommulone C (**3**), and myrtucommulone B ($C_{24}H_{30}O_6$; **5**). After purification, myrtucommulones B, C, D and E were also detected in the crude hexane extract, which showed that they were not isolation artifacts. The methanol-soluble fraction was fractionated on a polyamide column, from subfractions 3 and 5, with acetone in chloroform (30%) as mobile phase, to provide erythrodiol ($C_{30}H_{50}O_2$; **13**), ursolic acid ($C_{30}H_{48}O_3$; **10**), corosolic acid ($C_{30}H_{48}O_4$; **11**), arjunolic acid ($C_{30}H_{48}O_5$; **12**), and *β*-sitosterol ($C_{29}H_{50}O$; **8**).

Myrtucommulone D (1): Yellowish crystals (27 mg); m.p. 138–140 °C. $[a]_{\mathrm{D}}^{30}$ = +375.0 (c = 0.8, $CHCl_3$). ^1H, ^{13}C NMR: see Table 1. IR ($CHCl_3$): $\tilde{\nu}_{max}$ = 3450 (OH), 2968 (aromatic CH), 1719 (saturated ketone), 1601 (aryl), 1659 (C=C), 1590 (enolic 1,3-diketone system), 1250–1383 (C–C) cm^{-1}. CI-MS m/z 651 $[M + 1]^+$.

Myrtucommulone E (2): Yellowish crystals (18 mg); m.p. 163–165 °C. $[a]_{\mathrm{D}}^{30}$ = –166.7 (c = 1.2, $CHCl_3$). ^1H, ^{13}C NMR: see Table 1. IR ($CHCl_3$): $\tilde{\nu}_{max}$ = 3422 (OH), 2968 (aromatic CH), 1719 (saturated ketone), 1601 (aryl), 1659 (C=C), 1590 (enolic 1,3-diketone system), 1250–1383 (C–C) cm^{-1}. CI-MS: m/z 633.7834 $[M + 1]^+$.

Myrtucommulone C (3): Yellow amorphous powder (15 mg). $[a]_{\mathrm{D}}^{30}$ = +13.0 (c = 1.5, $CHCl_3$). ^1H, ^{13}C NMR: see Table 2. IR ($CHCl_3$): $\tilde{\nu}_{max}$ = 3500 (OH), 2968 (aromatic CH), 1717 (saturated ketone), 1628 (aryl) and 1590 (enolic 1,3-diketone system) cm^{-1}. FAB-MS: m/z 651 $[M + 1]^+$

Usnone A (4): Yellow crystals (30 mg). $[a]_{\mathrm{D}}^{30}$ = +492.8 (c = 1.0, $CHCl_3$). ^1H, ^{13}C NMR: see Table 3. IR ($CHCl_3$): $\tilde{\nu}_{max}$ = 3640, 3700 (OH), 1687, 1626 ($CCOCH_3$) cm^{-1}; HR-EIMS: m/z 344.0896 ($C_{18}H_{16}O_7$ calcd. 344.0883).

Myrtucommulone B (5): Yellowish crystals (10 mg). $[a]_{\mathrm{D}}^{30}$ = +78.3 (c = 1.2, $CHCl_3$). IR ($CHCl_3$): $\tilde{\nu}_{max}$ = 3500 (OH), 2968 (aromatic CH), 1717 (saturated ketone), 1628 (aryl) and 1590 (enolic 1,3-diketone system) 3492 (OH), 1083 (simple ether bonds) cm^{-1}. HREI-MS: m/z 414.491 $C_{24}H_{32}O_7$ (calcd. 414.487).

X-ray Data for Myrtucommulone D (1): A slab-shaped yellow crystal of compound **1**, with dimension 0.97 × 0.56 × 0.53 mm, was selected for X-ray diffraction studies. $C_{38}H_{50}O_9$, Mr = 650.7984; monoclinic; a = 19.1180(9), b = 10.2680(5), and c = 20.1967(9) Å, V = 3834.3(3) $Å^3$, space group $P2(1)/n$, Z = 4, $D_{calcd.}$ = 1.183 $Mg{\cdot}m^{-3}$, $F(000)$ = 1472, Mo-$K\alpha$ (λ = 0.7107 Å). Intensity data for compound **1** were collected on a Siemens Smart CCD 1-K area-detector diffractometer.[29] Data reductions were performed with SAINT. The structure was solved by direct methods[30] and refined by full-matrix, least-squares on F^2 by use of the SHELXTL-PC package.[31] The intensity data within the θ range 2.09–25.00, were collected at 293(2) K. A total of 18816 reflections were recorded, of which 6743 reflections were observed on the basis of $I > 2\sigma(I)$. The final R and Rw were 0.0479 and 0.1365, respectively. The figure was plotted with the aid of ORTEP.[32]

X-rays Data for Myrtucommulone E (2): A plate-shaped colorless crystal of compound **2** with dimensions 0.76 × 0.25 × 0.18 mm was selected for X-ray diffraction studies. $C_{38}H_{48}O_8$, Mr = 632.76; monoclinic; a = 13.317(2), b = 15.201(2), c = 18.692(3) Å, V = 3596.3(9) $Å^3$, space group = $P21/c$, Z = 4, $D_{calcd.}$ = 1.169 $g{\cdot}cm^{-3}$, $F(000)$ = 1360.0, Mo-$K\alpha$ (λ = 0.7107 Å). Intensity data for compound **2** were collected on a Siemens Smart CCD 1-K area-detector diffractometer.[29] Data reductions were performed with SAINT. The structure was solved by direct methods[30] and refined by full-matrix, least-squares on F^2 by use of the SHELXTL-PC package.[31] The intensity data within the θ range 1.61–25.00 were collected at 293(2) K. A total of 17852 reflections were recorded, of which 6320 reflections were observed on the basis of $I > 2\sigma(I)$. The final R and Rw were 0.0566 and 0.1563, respectively. Figure 4 was plotted with the aid of ORTEP.[32]

X-ray Data for Usnone A (4): A plate-shaped yellow crystal of compound **4** with dimensions 0.35 × 0.19 × 0.06 mm was selected for X-ray diffraction studies. $C_{36}H_{30}O_{14}$, Mr = 686.60; orthorhombic; a = 8.0646(3), b = 19.0358(6), c = 20.3443(7) Å, V = 3123.18(19) $Å^3$, space group $P2(1)2(1)2(1)$, Z = 4, $D_{calcd.}$ = 1.460 $mg{\cdot}m^{-3}$, $F(000)$ = 1432, Mo-$K\alpha$ (λ 0.7107 Å). Intensity data for compound **4** were collected on a Siemens Smart CCD 1-K area-detector diffractometer.[29] Data reductions were performed with SAINT. The structure was solved by direct methods[30] and refined by full-matrix, least-squares on F^2 by use of the SHELXTL-PC package.[31] The intensity data within the θ range 2.00–26.00 were collected at 293(2) K. A total of 17166 reflections were recorded, of which 3464 reflections were observed on the basis of $I > 2\sigma(I)$. The final R and Rw were 0.0439 and 0.1129, respectively. The figure was plotted with the aid of ORTEP.[32]

X-ray Data for Myrtucommulone B (5): The X-ray experiment was conducted on a STOE Stadi-4, with use of Mo-K_α radiation (graphite monochromator). Crystals of **5** were grown in ethyl acetate in hexane (15%) solution. General crystallographic data and conditions of the X-ray experiment are given in Table 6.

Table 6. General crystallographic data and conditions for X-ray experiment with myrtucommulone B (**5**).

Molecular formula	$C_{24}H_{32}O_7$
Molecular weight	414.491
Temperature [K]	293
Space group	$P2_1/c$, Z = 4
a [Å]	9.123(2)
b [Å]	27.123(5)
c [Å]	9.329(2)
B	99.19(3)
V [$Å^3$]	2278.8(8)
P, r/cm^3	1.202
Absorption coefficient (Mo) [mm^{-1}]	0.086
Size of crystal [mm]	0.00 × 0.00 × 0.00
Range of angles [deg]	From 1.50 to 24.99
Common number of reflections	4011
Number of reflections [$I > 2\sigma(I)$]	2233
R factor [$I > 2\sigma(I)$]	R_1 = 0.0795, wR_2 = 0.1563
R factor (for all array)	R_1 = 0.1294, wR_2 = 0.1668
Goodness-of-fit on F^2	1.147
Largest diff. peak and hole	0.172 and –0.175 eA^{-3}

CCDC-278308, -278408, and -287867 (for **1**, **2**, and **4**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Antibacterial Activity: All of the isolated compounds were screened against strains of *Escherichia coli*, *Bacillus subtilis*, *Shigella flexneri*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. For antibacterial screening, 3 mg of sample was taken and dissolved in DMSO (3 mL). Molten nutrient agar (45 mL) was poured onto sterile petri plates, where it was allowed to solidify. Bacterial cultures were produced on these nutrient agar plates by dispensing 7 mL of sterile soft agar containing 100 µL of test organism culture. Wells were dug with a 6 mm sterile metallic borer at appropriate distances, 100 µL of sample was poured into each well, and the plates were incubated at 37 °C for 24 h. The results, in terms of *inhibition zones*, were noted. The drug *Imipenem*, a broad-spectrum *β*-lactam antibiotic, was used as a positive control. As a negative control, DMSO was used. The results of these experiments are summarized in Table 4.

Enzyme Inhibition Assay: α-Glucosidase (E.C.3.2.1.20) enzyme inhibition assay was performed by the slightly modified method of Matsui et al. α-Glucosidase from Saccharomyces species was purchased from Wako Pure Chemical Industries Ltd. (Wako 076–02841). The enzyme inhibition was measured spectrophotometrically at pH 6.9 and at 37 °C with *p*-nitrophenyl α-D-glucopyranoside (PNP-G; 0.7 mM) as a substrate and 500 mU/mL enzyme, in sodium phosphate buffer (50 mM) containing NaCl (100 mM). 1-Deoxynojirimycin (0.425 mM) and acarbose (0.78 mM) were used as positive control. The increment in absorption at 400 nm, due to the hydrolysis of PNP-G by α-glucosidase, was monitored continuously with a microplate spectrophotometer (Spectra Max Molecular Devices, USA).[33]

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Received: November 30, 2005