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Coumarins and γ-pyrone derivatives from *Prangos pabularia:* antibacterial activity and inhibition of cytokine release

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Abstract

The *n*-hexane and ethyl acetate extracts of the stems and the ethyl acetate extracts of roots from *Prangos pabularia* afforded an γ-pyrone derivative and furanocoumarin derivatives with three glucose and γ-pyrone (pabularin A, B and C), along with 26 previously known compounds (18 coumarins, six terpenoids and two glycosides). Their structures were established on the basis of spectroscopic studies. Of these, 16 coumarin derivatives isolated from *P. pabularia* were tested for antibacterial activity and inhibition of cytokine release. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Prangos pabularia*; Umbelliferae; Coumarins; γ-Pyrone derivatives; Antibacterial activity; Cytokine release

1. Introduction

Prangos pabularia belongs to the family Umbelliferae and is indigenous to Central Asia. It is a tall perennial herb, and its roots and fruits have medicinal value. This plant produces a large number of coumarins, and has been found to be relatively rich in secondary metabolic products. Earlier reports described the presence of some coumarins and terpenoids in this plant (Basa et al., 1971; Chatterjee et al., 1972). Extracts of the genus *Prangos* were reported to stop bleeding and heal scars when applied externally (Ulubelen et al., 1995). The coumarins that have been isolated thus far have only a slight antibiotic activity (Ulubelen et al., 1995). In the course of our studies on bioactive metabolites from this genus, we have isolated and determined the structures of seven monoterpenoids and four γ-pyrone derivatives from *P. tschimganica* (Shikishima et al., 2001). In this paper, we report the isolation and structure elucidation of 30 (**1**–**30**) compounds, including a new γ-pyrone derivative (**1**) and three new furanocoumarin derivatives with glucose and γ-pyrone, named pabularin A (**2**), B (**3**) and C (**4**), from the stems and roots of *P. pabularia* collected in Uzbekistan. The antibacterial activities and activities for the inhibition of cytokine release of some of the isolated compounds are also reported.

2. Results and discussion

The *n*-hexane- and ethyl acetate-solubles of *P. pabularia* were separated by a combination of silica gel and Toyo pearl column chromatographies as well as by normal and reversed-phase HPLC to give 30 compounds.

The molecular formula of **1** was determined to be $C_{14}H_{18}O_9$ on the basis of HR FABMS datum, and the UV and IR spectral data of **1** showed the presence of an α, β-unsaturated ketone (256 nm and 1731 cm^{-1}). The

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^{1}H NMR spectrum of **1** showed the presence of two singlet methyls [δ_H 2.31 and 1.92], a pair of methines [δ_H 7.91 and 6.35 (each 1H, *d*, J = 5.6 Hz)] and a sugar moiety. The ^{13}C NMR spectral data of **1** showed α, β, α′, β′-unsaturated ketone signals at δ_C 177.1 (C-4′), 164.4 (C-2′), 157.2 (C-6′), 143.5 (C-3′), and 117.3 (C-5′), as well as acetyl and β-glucopyranose resonances (Table 1). These data were very similar to those of maltol-β-D-glucopyranose (Tanaka et al., 1986), except for the presence of an acetyl group and downfield shift of glucose H-6″ in compound **1**. In the HMBC spectrum of **1**, the proton signals at δ_H 4.28 and 4.13 (H-6″ of glucose) were correlated with the carbon signal at δ_C 172.5 (acetyl carbonyl). Thus, **1** was elucidated to be maltol-(6-*O*-acetyl)-β-D-glucopyranoside.

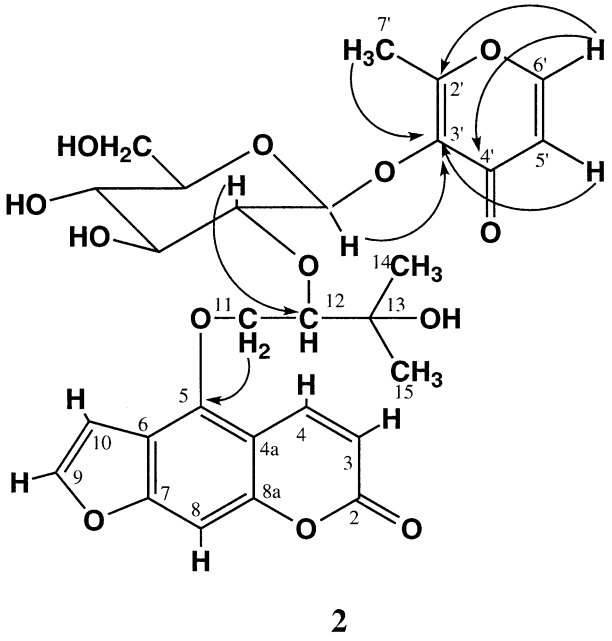
Compound **2** had the molecular formula $C_{28}H_{30}O_{13}$ based on its HR FABMS, and its IR spectrum showed the presence of hydroxyl groups (3421 cm^{-1}) and a ketone group (1725 cm^{-1}). The ^{1}H NMR spectrum of **2** revealed the presence of a furanocoumarin nucleus [δ_H 8.28 and 6.07 (each 1H, *d*, J = 9.8 Hz, H-4, 3), 7.66 and 7.11 (each 1H, *d*, J = 2.3 Hz, H-9, 10) and 7.08 (1H,*s*, H-8)], a 2-methylbutane unit [δ_H 1.25 and 1.22 (each 3H, *s*, H_3-14 and H_3-15), 3.79 (1H, *dd*, J = 13.6, 8.4 Hz, H-12), 4.77 and 4.27 (each 1H, *m* and *dd*, J = 10.3, 8.4 Hz, H_2-11)] (Harkar et al., 1984), a 3-methyl-γ-pyrone unit and a sugar moiety. The ^{13}C NMR spectrum of **2** showed 28 carbon atoms, consisting of 11 carbon atoms of furanocoumarin, 5 carbon atoms of the 2-methylbutane unit, 6 carbon atoms of 3-methyl-γ-pyrone and 6 carbon atoms of glucopyranose (Table 1). The ^{13}C NMR spectral data of compound **2** were similar to those of **1** except for the furanocoumarin and 2-methylbutane unit in **2** and the acetyl group in **1**. Therefore, **2** was thought to be a prenylated furanocoumarin derivative of compound **1**, without the acetyl group. The ^{1}H and ^{13}C NMR spectroscopic data of the prenylated furanocoumarin moiety in **2** were very similar to those of known oxypeucedanin hydrate (**5**) (Chatterjee et al., 1972). In the HMBC spectrum of **2**, the proton signals at δ_H 6.25 (H-5′), 4.68 (H-1 of glucose) and 2.20 (H-7′) were correlated with the carbon signal at δ_C 142.5 (C-3′) whereas the proton signal at δ_H 3.36 (H-2 of glucose) was correlated with the carbon signal at δ_C 77.1 (C-12) (Fig. 1). Furthermore, a ^{1}H–^{1}H COSY experiment showed correlations of H-11 with H-12, and of H-5′ with H-6′. Thus, pabularin A (**2**) was elucidated as illustrated in Fig. 1.

Compound **3**, which has the same molecular formula ($C_{28}H_{30}O_{13}$) as compound **2**, showed the presence of hydroxyl groups and a ketone group (3422 and 1719 cm^{-1}) in its IR spectrum and an α, β, α′, β′-unsaturated ketone (311 and 251 nm) in its UV spectrum. The ^{1}H and ^{13}C NMR spectral data of **3** were similar to those of

Table 1
^{13}C NMR spectral data of compounds **1–4**

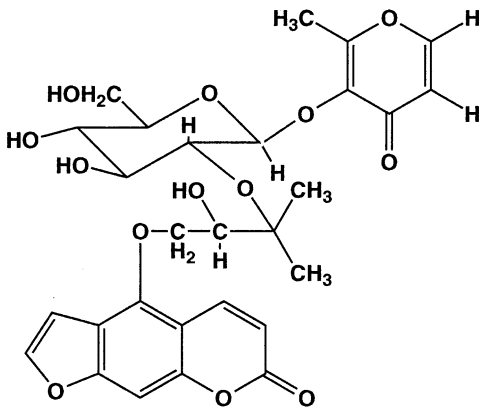
	1	2	3	4
Aglycone				
C-2	–	164.7	163.7	162.9
C-3	–	112.7	112.6	114.8
C-4	–	142.1	142.3	146.8
C-4a	–	109.1	107.9	118.0
C-5	–	150.3	151.1	115.0
C-6	–	116.1	115.0	128.1
C-7	–	159.6	160.0	149.2
C-8	–	94.8	94.2	132.5
C-8a	–	146.8	146.6	150.9
C-9	–	153.8	153.8	148.7
C-10	–	106.3	106.7	108.0
C-11	–	75.4	76.2	75.6
C-12	–	77.1	78.2	76.4
C-13	–	79.7	79.8	77.8
C-14	–	24.2	24.2	22.9
C-15	–	23.0	23.9	20.8
C-2′	164.4	163.2	163.4	164.9
C-3′	143.5	142.5	141.8	143.3
C-4′	177.1	176.5	175.9	177.8
C-5′	117.3	117.5	117.5	117.2
C-6′	157.2	157.0	156.3	157.1
C-7′	15.6	15.8	15.7	15.8
β-D-Glucose				
C-1″	105.0	103.1	102.3	105.0
C-2″	75.9	76.1	75.8	76.3
C-3″	77.8	78.1	77.9	77.7
C-4″	71.3	71.5	71.5	71.3
C-5″	77.8	77.7	77.7	77.4
C-6″	64.4	62.6	62.5	62.2
6″-OAc	172.5	–	–	–
	20.7	–	–	–

Measured in CD_3OD.

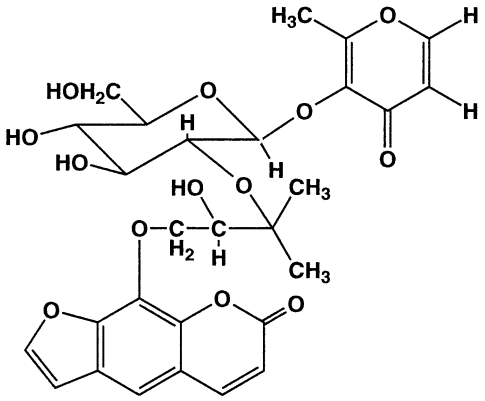


2

Fig. 1. Important ^{1}H–^{13}C HMBC correlations of compound **2**.



3



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2. In the HMBC spectrum, the proton signal at δ_H 3.48 (H-2 of glucose) correlated with the quaternary carbon signal at δ_C 79.8 (C-13). Thus, the difference between compounds **2** and **3** appears to result from the different connection of glucose at C-12 and C-13. Based on the results, the structure of pabularin B (**3**) was deduced as shown.

Compound **4** had the same molecular formula ($C_{28}H_{30}O_{13}$) as compounds **2** and **3**. The ^{13}C NMR spectral data of **4** were similar to those of compounds **2** and **3** except for the signals due to a furanocoumarin nucleus [**2**: δ_C 150.3 (C-5), 94.8 (C-8),**3**: δ_C 151.1 (C-5), 94.2 (C-8), **4**: δ_C 115.0 (C-5), 132.5 (C-8)]. The ^1H NMR spectral data of **4** were also similar to those of **2** except for a methine signal [**2**: δ_H 7.08 (1H, *s*, H-8), **4**: δ_H 7.48 (1H, *s*, H-5)]. Based on these results, **4** was considered to be an 8-substituted furanocoumarin derivative. In the HMBC spectrum of **4**, the proton signals at δ_H 6.25 (H-5′), 4.68 (H-1 of glucose) and 2.23 (H-7′) were correlated with the carbon signal at δ_C 143.3 (C-3′). The proton resonance at δ_H 3.25 (H-2 of glucose) was correlated with the carbon signal at δ_C 77.8 (C-13). Based on these observations, the furanocoumarin nucleus was located at C-5 and C-8 in **3** and **4**, respectively.

The following known compounds were identified by comparison with literature data: oxypeucedanin hydrate (**5**) (Harkar et al., 1984), oxypeucedanin methanolate (**6**) (Atkkinson et al., 1974; Mendez and Castro-Poceiro, 1983), isoimperatorin (**7**) (Muller et al., 1995), oxypeucedanin (**8**) (Harkar et al., 1984), oxypeucedanin hydrate 2′-*O*-monoacetate (**9**) (Harkar et al., 1984), oxypeucedanin hydrate 3′-*O*-β-D-glucopyranoside (**10**) (Koul et al., 1979), heraclenol (**11**) (Harkar et al., 1984), heraclenol 3′-Me ester (**12**) (Bandopadhyay and Seshadri, 1970), imperatorin (**13**) (Harkar et al., 1984), isogospherol (**14**) (Abyshev, 1974), heraclenol 3′-*O*-β-D-glucopyranoside (**15**) (Thastrup and Lemmich, 1983), ulopterol (**16**) (Lemmich and Havelundt, 1978), tamarin (**17**) (Ito et al., 1991), auraptenol (**18**) and osthol (**19**) (Zhou et al., 2000), paniculal (**20**) (Imai et al., 1987), majurin (**21**) (Abu-Mustafa et al., 1971), rivurobirin E (**22**) (Taniguchi et al., 1999), loliolide (**23**) (Ravi et al., 1982), 2,3,4-trimethylbenzylalcohol-*O*-β-D-glucopyranoside (**24**) (Shikishima et al., 2001), 1, 1, 5-trimethyl-2-hydroxymethyl-(2,5)-cyclohexadien-(4)-one-*O*-β-D-glucopyranoside (**25**) (Shikishima et al., 2001), 1, 1, 5-trimethyl-2-hydroxymethyl-(5)-cyclohexene-(4)-one-*O*-β-D-glucopyranoside (**26**) (Shikishima et al., 2001), spathulenol (**27**) (Juell et al., 1976), kauranol (**28**) (Fraga et al., 1987), β-sitosterol-β-D-glucopyranoside (**29**), 1-*O*-isopropyl-β-D-glucopyranoside (**30**) (Du et al., 1998).

Oxypeucedanin (**8**; 1.03%), imperatorin (**13**; 0.66%) and osthol (**19**; 0.89%) were isolated from a large amount of roots (1.20 kg). This is the first time that known compounds **6**, **9**, **12**, **14**, and **16**–**30** have been isolated from *P. pabularia*. *P. pabularia* is thus a rich source of coumarin derivatives and terpenoids.

Antibacterial activities of eight coumarin derivatives from *P. platychloena* were reported (Ulubelen et al., 1995), oxypeucedanin (**8**) and imperiatorin (**13**) showed slight activity against *Escherichia coli* and *Candida albicans*. We also checked the antibacterial activities of isolated compounds. Sixteen coumarin derivatives (**6**–**17** and **19**–**22**) isolated from the stems and roots of *P. pabularia* were screened for antibacterial activity by the disc-diffusion test for methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *E. coli* and *Pseudomonas aeruginosa* (Sato et al., 1997). As a result, four compounds (**6**, **8**, **13** and **19**) had antibacterial activity by the disc-diffusion test. These four compounds were then tested to determine their minimum inhibitory concentration (MIC) values against *S. aureus* (MSSA, MRSA), *E. coli* and *P. aeruginosa* (Table 2). However, tested compounds showed weak activities, whereas osthol (**19**) showed significant activity

Table 2
Antibacterial activity of compounds **6**, **8**, **13**, **19** against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*

Organism	Minimum inhibitory concentration (μg/ml)			
	6	**8**	**13**	**19**
Staphylococcus aureus No. 0006[a]	250	125	>250	>250
S. aureus No. 0007[a]	250	125	>250	>250
S. aureus No. 0008[a]	250	125	>250	>250
S. aureus No. 0010[a]	250	62.5	62.5	31.25
Escherichia coli NIHJ JC-2	>250	>250	>250	>250
Pseudomonas aeruginosa NCTC 10490	250	>250	62.5	31.25

[a] Methicillin-resistant *Staphylococcus aureus*.

against *S. aureus* (MRSA) and *P. aeruginosa* compared to other compounds.

Cytokines have various biological activities and are thought to be necessary for maintaining the homeostasis of the human body. In a continuing study of immunosuppressive active compounds from natural source, we have reported previously on the activity of triterpenoids (Yesilada et al., 2001). As a part of our work in search for immunosuppressive active compounds, we tested the inhibition of cytokine (IL-2, IL-4, IL-1β and TNF-α) release by the isolated compounds; the results are shown in Table 3. Twelve compounds (**5, 8, 11–14, 16, 17, 19, 21, 26**, and **30**) inhibited production of IL-2, IL-4, IL-1β and TNF-α. Ten of these compounds significantly affected the production of TNF-α.

Table 3
Inhibition of cytokine release by compounds **5, 8, 11–14, 16, 17, 19, 21, 26, 30** and prednisolone

Compounds	Inhibition %			
	IL-2	IL-4	IL-1β	TNF-α
5	75.4	–	77.6	78.4
8	48.8	17.1	17.7	52.8
11	–	2.6	17.3	66.7
12	35.2	–	9.7	58.3
13	31.7	50.7	50.6	58.9
14	28.2	15.5	31.2	71.1
16	55.6	22.0	–	32.4
17	3.7	30.4	–	60.3
19	38.3	49.3	31.6	51.1
21	–	–	–	55.8
26	55.6	19.6	20.4	13.6
32	81.7	12.3	59.6	52.6
Prednisolone	65.2	75.9	67.7	52.3

The compounds were tested at 10.0 μg/ml and prednisolone (positive control) at 0.3 μg/ml.

3. Experimental

3.1. General

^{1}H NMR 400 MHz, ^{13}C NMR 100 MHz with TMS as an internal standard; MS: Jeol JMSDX-303 and JMS SX102A instruments; CC: silica gel, Sephadex LH-20 (Pharmacia), Toyo Pearl HW-40 (Tosoh); HPLC: GPC (Asahipak GS-310 2G, MeOH; Shodex H-2001, 2002, $CHCl_3$), silica gel (Si60, Hibar TR250–25, Merck), ODS (RP-18, Hibar RT250–25, Merck).

3.2. Plant material

The stems and roots of *Prangos pabularia* were collected in June 1998 from the south part of Angren, Uzbekistan. Herbarium specimens (EMS-4055) were deposited in the herbarium of the Institute of Botany and Botanical Garden, Academy of Sciences, Uzbekistan; the plant was identified by Dr. Olimjon K. Kodzhimatov.

3.3. Extraction and fractionation

The stems and roots of *P. pabularia* (1.2 kg) were extracted successively with *n*-hexane, EtOAc, and MeOH in a soxhlet apparatus. The EtOAc extracts of stems were concentrated in vacuo to give a residue (18 g), which was subjected to silica gel column chromatography, and eluted with solvents of increasing polarity (*n*-hexane–AcOEt) to give 10 frs (1–10). Fr. 9 (1.7 g) was applied to a silica gel column, and eluted with $CHCl_3$–MeOH (9:1 to 1:1) to give 7 frs (9.1–9.7). Fr. 9.5 (249 mg) was separated using GPC (MeOH) and HPLC (ODS, MeOH–1H_2O, 8:2) to give 4 frs (9.5.1–9.5.4). Fr. 9.5.4 (23 mg) was purified by HPLC (ODS, MeOH–H_2O, 6:4) to give compounds **2** (6 mg) and **3** (3 mg). Fr. 9.5.3 (17 mg) was further purified by HPLC (ODS, MeOH–H_2O, 1:1) to afford compound **4** (2 mg). Fr. 8 (2.97 g) was applied to a silica gel column and eluted with $CHCl_3$–MeOH (9:1–1:1) to give 7 frs (8.1–8.7). Fr. 8.5 (251 mg) was separated by HPLC (ODS, MeOH–H_2O, 8:2), to give 6 frs (8.5.1–8.5.6). Fr. 8.5.1 (80 mg) was purified by HPLC (ODS, MeOH–H_2O, 1:1), to afford compound **1** (10 mg). Fr. 4 (2.15 g) gave **12** (16 mg), **23** (6 mg) and **28** (6 mg), whereas fr. 6 (1.39 g) gave **22** (31 mg), fr. 7 (591 mg) gave **29** (103 mg), fr. 8 (2.97 g) gave **24** (46 mg), and fr. 9 (1.71 g) gave **10** (9 mg), **15** (7 mg), **25** (6 mg), **26** (19 mg) and **30** (9 mg). The *n*-hexane

extracts of stems were concentrated *in vacuo* to give residue (28g) which was separated by silica gel, Toyo Pearl HW-40 and HPLC (GPC and Si60) respectively, to afford **5** (54 mg), **6** (5 mg), **7** (15 mg), **11** (213 mg), **14** (7 mg), **16** (12 mg), **17** (7 mg), **18** (11 mg), **20** (1.8 mg), **21** (63 mg) and **27** (8 mg). The EtOAc extracts of roots were concentrated in vacuo to give a residue (103 g) that was subjected to a silica gel and HPLC (GPC and Si60) to give **8** (12.3 g), **9** (15 mg), **13** (7.9 g) and **19** (10.7 g).

*3.3.1. Maltol-(6-O-acetyl)-β-D-glucopyranoside (**1**)*

$[\alpha]_D^{25}$ −31.9° (MeOH, *c* 0.27); UV λ_{max}^{MeOH} nm (log ε): 324 (3.0), 256 (3.8), 216 (3.8); IR ν_{max}^{KBr} cm^{-1}: 3361, 2923, 1731, 1074; HR FABMS: *m/z* 353.0851 $[M+Na]^+$, calc. for $C_{14}H_{18}O_9Na$, 353.0849; ^1H NMR (CD_3OD) δ: 7.91 (1H, *d*, *J* = 5.6 Hz, H-6′), 6.35 (1H, *d*, *J* = 5.6 Hz, H-5′), 4.73 (1H, *d*, *J* = 7.5 Hz, H-1 of glucose), 4.28 (1H, *dd*, *J* = 11.9, 2.3 Hz, H_2-6 of glucose), 4.13 (1H, *dd*, *J* = 11.9, 6.0 Hz, H_2-6 of glucose), 3.36–3.21 (3H, *m*, H-3, 4 and 5 of glucose), 3.29 (1H, *dd*, *J* = 9.2, 7.5, Hz, H-2 of glucose), 2.31 (3H, *s*, H_3-7′), 1.92 (3H, *s*, AcO); ^{13}C NMR spectral data: Table 1

*3.3.2. Pabularin A (**2**)*

$[\alpha]_D^{25}$ −166.7° (MeOH, *c* 0.09); UV λ_{max}^{MeOH} nm (log ε): 310 (4.2), 251 (4.5), 215 (4.6); IR ν_{max}^{KBr} cm^{-1}: 3421, 2923, 1725, 1646, 1074; HR FABMS: *m/z* 597.1606 $[M+Na]^+$, calc. for $C_{28}H_{30}O_{13}Na$, 597.1584; ^1H NMR (CD_3OD) δ: 8.28 (1H, *d*, *J* = 9.8 Hz, H-4), 7.85 (1H, *d*, *J* = 5.6 Hz, H-6′), 7.66 (1H, *d*, *J* = 2.3 Hz, H-9), 7.11 (1H, *d*, *J* = 2.3 Hz, H-10), 7.08 (1H, *s*, H-8), 6.25 (1H, *d*, *J* = 5.6 Hz, H-5′), 6.07 (1H, *d*, *J* = 9.8 Hz, H-3), 4.77 (1H, *m*, H-11), 4.68 (1H, *d*, *J* = 7.5 Hz, H-1 of glucose), 4.27 (1H, *dd*, *J* = 10.3, 8.4 Hz, H-11), 3.79 (1H, *dd*, *J* = 13.6, 8.4 Hz, H-12), 3.67 (1H, *dd*, *J* = 11.9, 2.3 Hz, H_2-6 of glucose), 3.49 (1H, *dd*, *J* = 11.9, 5.8 Hz, H_2-6 of glucose), 3.36 (1H, *dd J* = 9.6, 7.5 Hz, H-2 of glucose), 3.25–3.03 (3H, *m,* H-3, 4 and 5 of glucose), 2.20 (3H, *s*, H_3-7′), 1.25, 1.22 (each 3H, *s*, H_3-14, H_3-15); ^{13}C NMR spectral data: Table 1.

*3.3.3. Pabularin B (**3**)*

$[\alpha]_D^{25}$ −211.4° (MeOH, *c* 0.035); UV λ_{max}^{MeOH} nm (log ε): 311 (4.5), 259 (4.8), 251 (4.8), 217 (4.9); IR ν_{max}^{KBr} cm^{-1}: 3422, 2924, 1719, 1623, 1457, 1384, 1258, 1198, 1077; HR FABMS: *m/z* 597.1610 $[M+Na]^+$, calc. for $C_{28}H_{30}O_{13}Na$, 597.1584; ^1H NMR (CD_3OD) δ: 8.26 (1H, *d*, *J* = 9.8 Hz, H-4), 7.70 (1H, *d*, *J* = 5.6 Hz, H-6′), 7.65 (1H, *d*, *J* = 1.8 Hz, H-9), 7.10 (1H, *br s*, H-10), 7.03 (1H, *s*, H-8), 6.15 (1H, *d*, *J* = 9.8 Hz, H-3), 6.02 (1H, *d*, *J* = 5.6 Hz, H-5′), 4.98 (1H, *d*, *J* = 7.6 Hz, H-1 of glucose), 4.85 (1H, *d*, *J* = 9.4 Hz, H_2-11), 4.39 (1H, *t*, *J* = 9.4 Hz, H_2-11), 3.92 (1H, *m*, H-12), 3.66 (1H, *dd*, *J* = 11.7, 2.3 Hz, H_2-6 of glucose), 3.52 (1H, *dd*, *J* = 11.7, 5.8 Hz, H_2-6 of glucose), 3.48 (1H, *dd*, *J* = 9.6, 7.6 Hz, H-2 of glucose), 3.28–3.06 (3H, *m*, H-3, 4 and 5 of glucose), 2.25 (3H, *s*, H_3-7′), 1.30 (3H, *s*, H_3-14), 1.28 (3H, *s*, H_3-15); ^{13}C NMR spectral data: Table 1.

*3.3.4. Pabularin C (**4**)*

$[\alpha]_D^{25}$ −33.3° (MeOH, *c* 0.09); UV λ_{max}^{MeOH} nm (log ε): 299 (4.1), 250 (4.5), 215 (4.6); IR ν_{max}^{KBr} cm^{-1}: 3421, 2923, 1646, 1091, 804; HR FABMS: *m/z* 597.1590 $[M+Na]^+$, calc. for $C_{28}H_{30}O_{13}Na$, 597.1584; ^1H NMR (CD_3OD) δ: 7.94 (1H, *d*, *J* = 9.6 Hz, H-4), 7.82 (1H, *d*, *J* = 2.2 Hz, H-9), 7.75 (1H, *d*, *J* = 5.6 Hz, H-6′), 7.48 (1H, *s*, H-5), 6.87 (1H, *d*, *J* = 2.2 Hz, H-10), 6.29 (1H, *d*, *J* = 9.6 Hz, H-3), 6.25 (1H, *d*, *J* = 5.6 Hz, H-5′), 4.68 (1H, *d*, *J* = 7.6 Hz, H-1 of glucose), 4.62 (1H, *dd*, *J* = 10.5, 2.6 Hz, H_2-11), 4.36 (1H, *dd*, *J* = 10.5, 8.2 Hz, H_2-11), 3.83 (1H, *dd*, *J* = 8.2, 2.6 Hz, H-12), 3.65 (1H, *dd*, *J* = 11.8, 5.5 Hz, H_2-6 of glucose), 3.51 (1H, *m*, H_2-6 of glucose), 3.28–3.21 (3H, *m*, H-3, 4 and 5 of glucose), 3.25 (1H, *m*, H-2 of glucose), 2.23 (3H, *s*, H_3-7′), 1.17 (3H, *s*, H_3-14), 1.12 (3H, *s*, H_3-15); ^{13}C NMR spectral data: Table 1.

3.4. Antibacterial activity

3.4.1. Preparation of bacterial cells

Methicillin resistant *S. aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA) strains (Sato et al., 1997) and other bacterial strains listed in Table 2 were obtained from laboratory stock cultures. After culturing all strains on Muller–Hinton agar (Difco, Detroit, MI), the cells were resuspended in Muller–Hinton broth (Difco) to give 10^8 colony-forming units/ml, and the resuspended cells were then incubated.

3.5. Determination of antibacterial activity

Disc-diffusion tests were performed with Whatman AA discs (6.0 mm) containing various concentrations of extracts and DMSO as a control. The discs were placed on Muller–Hinton agar inoculated with 10^5 colony-forming units/ml of MSSA, MRSA, *E coli* and *P. aeruginosa*. The zone of inhibition was determined after incubation for 24 h at 37 °C. The disc containing DMSO showed no zone of inhibition. To estimate the antibacterial activity of the purified isolates, the minimum inhibitory concentration (MIC) was determined according to the method described by the Japanese Society for Antimicrobial Chemotherapy using Muller–Hinton agar (Sato et al., 1997). Cell suspensions (1×10^6 colony-forming units/ml) of the examined bacteria were inoculated onto agar plates using a replicating device. Plates were read after 24 h of incubation at 37 °C.

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