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Furo[3,2-*h*]isochroman, furo[3,2-*h*]isoquinoline, isochroman, phenol, pyranone, and pyrone derivatives from the sea fan-derived fungus *Penicillium* sp. PSU-F40

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ABSTRACT

Nine new fungal metabolites, penicisochromans A–E, penicipyrone, penicipyranone, peniciphenol, and penicisoquinoline, were isolated from the sea fan-derived fungus *Penicillium* sp. PSU-F40 together with five known compounds. Their structures were determined by spectroscopic analysis. Their antibacterial activity against the standard *Staphylococcus aureus* ATCC 25923 and methicillin-resistant *S. aureus* was evaluated.

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1. Introduction

The fungi in the genus *Penicillium* have produced various bioactive compounds, such as cytotoxic leptosphaerone,[1] antioxidant terrestrols,[2] antifungal citrinin,[3] antibacterial rugulotrosins,[4] and insecticidal decaturin.[5] In our ongoing search for antibacterial metabolites against *Staphylococcus aureus* ATCC 25923 (SA) and a clinical isolate of methicillin-resistant *S. aureus* (MRSA) from the marine-derived fungi, the fungus *Penicillium* PSU-F40 was isolated from a gorgonian sea fan of the genus *Annella*. Investigation of its culture broth led to the isolation of three new furo[3,2-*h*]isochromans, penicisochromans A–C (**1**–**3**), two isochromans, penicisochromans D and E (**4** and **5**), one new pyrone, penicipyrone (**6**), one new pyranone, penicipyranone (**7**), one new phenol, peniciphenol (**8**), and one new furo[3,2-*h*]isoquinoline, penicisoquinoline (**9**), along with two known furo[3,2-*h*]isoquinolines, TMC-120B (**10**)[6] and TMC-120C (**11**),[6] and one known ketophenol, 1-(2,4-dihydroxy-6-methylphenyl)-3-methyl-1-butanone (**12**).[7] Furthermore, two known metabolites, 2-(2-methoxybenzoly)pyrrole (**13**)[8] and nicotinic acid (**14**),[9] were isolated from the mycelia extract. Their antibacterial activity against SA and MRSA were examined.

2. Results and discussion

All compounds (**1**–**14**) (Fig. 1) were purified using chromatographic techniques and their structures were elucidated by spectroscopic data (IR, UV, NMR, and MS). For known compounds, the structures were confirmed by comparison of the ^{1}H and ^{13}C NMR data with those previously reported. The relative configuration was assigned on the basis of NOEDIFF data. No attempts were made to identify the absolute configuration of the new metabolites due to the minute amounts.

Penicisochroman A (**1**) was obtained as a colorless gum. The molecular formula $C_{16}H_{18}O_4$ was assigned by HREIMS of $M^{+}-CH_3OH$ and the presence of 16 carbon resonances for 16 carbons in the ^{13}C NMR spectrum. The UV spectrum displayed absorption bands for an aromatic chromophore at λ_{max} 213, 253, 269, and 324 nm. The IR spectrum showed absorption bands for conjugated ketone carbonyl and double bond functional groups at 1695 and 1647 cm^{-1}, respectively. The ^{1}H NMR spectrum (Table 1) consisted of signals for two *ortho*-coupled aromatic protons [δ 7.46 (1H, d, J=7.8 Hz) and 6.77 (1H, d, J=7.8 Hz)], two sets of nonequivalent methylene protons [δ 4.86/4.66 (each 1H, d, J=16.2 Hz) and 2.94/2.85 (each 1H, d, J=16.2 Hz,)], one methoxyl group (δ 3.27, 3H, s) and three methyl groups [δ 2.29 (3H, s), 2.02 (3H, s), and 1.46 (3H, s)]. The ^{13}C NMR and DEPT 135 spectra (Table 1) showed one conjugated ketone carbonyl (δ 183.4), six aromatic quaternary (δ 160.4, 145.3, 140.7, 131.5, 121.2, and 118.3), two aromatic methine (δ

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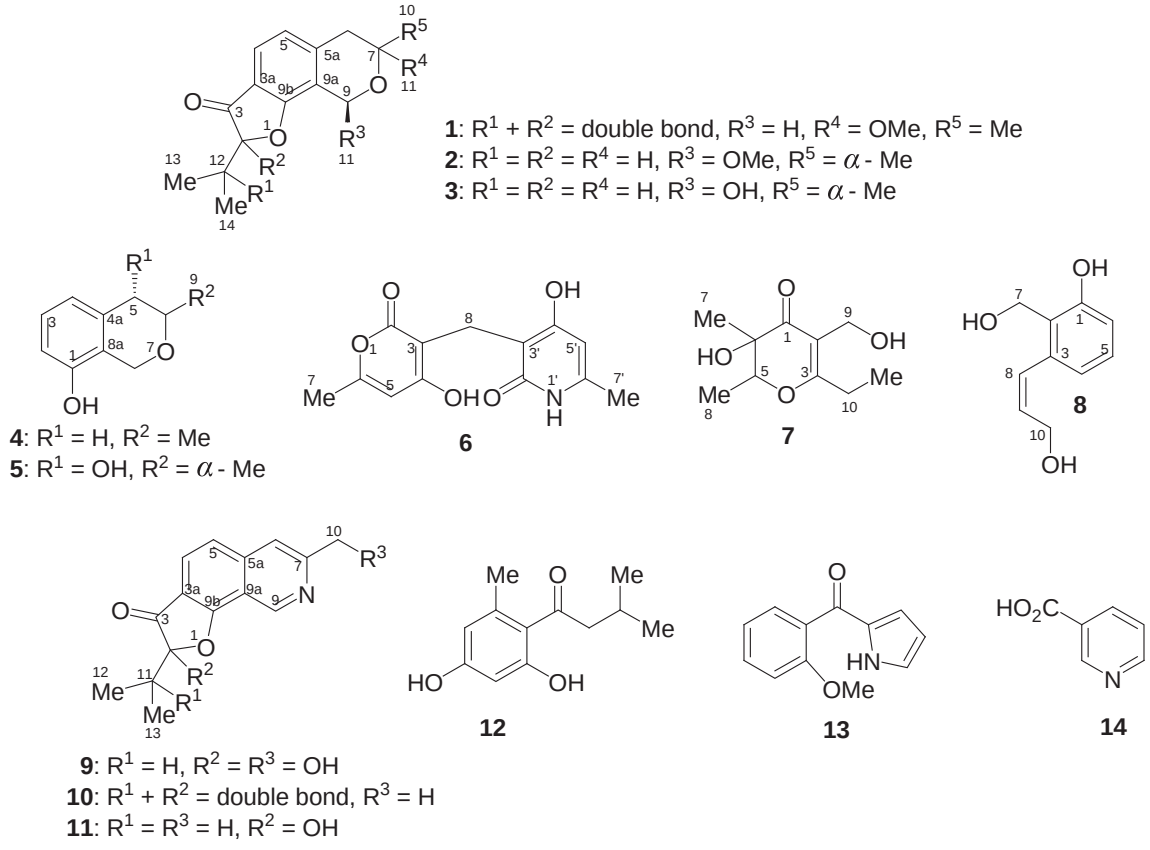


Figure 1. Metabolites isolated from the sea fan-derived fungus *Penicillium* PSU-F40.

123.0 and 121.9), one dioxygenated quaternary (δ 97.4), two methylene (δ 57.8 and 39.3), one methoxy (δ 49.0), and three methyl (δ 23.0, 20.2, and 17.4) carbons. The HMBC correlations of the methyl protons, H_3-13 (δ 2.29) and H_3-14 (δ 2.02), with C-2 (δ 145.3), C-3 (δ 183.4), and C-12 (δ 131.5) as well as the chemical shifts of these carbons established a 2-oxy-3-methyl-2-butenoyl moiety. One of the *ortho*-coupled aromatic protons resonating at δ 7.46 was assigned as H-4 on the basis of its ^3J HMBC correlation with C-3 of the butenoyl unit. This conclusion was supported by its downfield appearance due to an anisotropic effect of the adjacent ketone carbonyl group. The other *ortho*-coupled proton (δ 6.77) was then attributed to H-5. The nonequivalent methylene protons, H_{ab}-6 (δ 2.94 and 2.85), gave HMBC cross peaks with C-5 (δ 123.0), C-5a (δ 140.7), C-7 (δ 97.4), and C-9a (δ 118.3) while the methoxy (δ 3.27) and the methyl (δ 1.46) protons showed cross peaks with the dioxygenated carbon, C-7. These results attached a 2-methoxy-2-oxypropyl unit at C-5a of the aromatic ring. In addition, the oxymethylene protons, H_{ab}-9 (δ 4.86 and 4.66), displayed the same correlations with C-5a, C-9a, and C-9b (δ 160.4), thus linking the oxymethylene unit at C-9a. An isochroman unit having the methoxyl and methyl groups at C-7 was established on the basis of the ^3J HMBC correlations of H_{ab}-9 with C-7. An ether linkage

Table 1
^{1}H and ^{13}C NMR data of penicisochromans A (**1**), B (**2**), and C (**3**)

Position	**1**		**2**		**3**	
	δ_H, mult, *J* in Hz	δ_C, mult.	δ_H, mult, *J* in Hz	δ_C, mult.	δ_H, mult, *J* in Hz	δ_C, mult.
2		145.3, qC	4.35, d, 4.0	90.2, CH	4.46, d, 3.5	91.0, CH
3		183.4, qC		202.0, qC		202.0, qC
3a		121.2, qC		120.1, qC		121.5, qC
4	7.46, d, 7.8	121.9, CH	7.43, d, 8.0	123.4, CH	7.46, d, 8.5	124.5, CH
5	6.77, d, 7.8	123.0, CH	6.72, d, 8.0	122.0, CH	6.76, d, 8.5	122.5, CH
5a		140.7, qC		145.4, qC		146.0, qC
6	a: 2.94, d, 16.2	39.3, CH_2	a: 2.68, dd, 17.0, 3.5	35.9, CH_2	a: 2.72, dd, 16.5, 2.5	36.9, CH_2
	b: 2.85, d, 16.2		b: 2.62, dd, 17.0, 11.0		b: 2.62, dd, 16.5, 10.0	
7		97.4, qC	4.25, m	62.6, CH	4.40, m	63.5, CH
9	a: 4.86, d, 16.2	57.8, CH_2	5.61, s	94.7, CH	6.18, s	88.8, CH
	b: 4.66, d, 16.2					
9a		118.3, qC		120.0, qC		121.0, qC
9b		160.4, qC		171.0, qC		172.5, qC
10	1.46, s	23.0, CH_3	1.32, d, 6,5	21.1, CH_3	1.33, d, 6.0	21.9, CH_3
11	3.27, s	49.0, CH_3	3.45, s	55.8, CH_3	2.29, m	32.0, CH
12		131.5, qC	2.28, m	31.1, CH	1.08, d, 6.5	19.5, CH_3
13	2.29, s	17.4, CH_3	0.80, d, 7.0	15.8, CH_3	0.81, d, 6.5	16.7, CH_3
14	2.02, s	20.2, CH_3	1.11, d, 7.0	19.0, CH_3		

between C-2 and C-9b was formed to construct a 3-oxobenzofuran unit according to the chemical shifts of C-2 and C-9b as well as the mass data. Therefore, the structure of penicisochroman A was established as depicted in **1**.

Penicisochroman B (**2**) was obtained as a colorless gum. The molecular formula $C_{16}H_{20}O_4$ from HREIMS suggested that **2** was a dihydro analogue of **1**. This conclusion was supported by the replacement of signals for two olefinic carbons, C-2 (δ 145.3) and C-12 (δ 131.5), in **1**, with those for two methine carbons (δ 90.2 and 31.1) in **2** (Table 1). The presence of the saturated ketone carbonyl functionality was further confirmed by the appearance of the ketone carbonyl carbon in **2** at δ 202.0, which resonated at much lower field than that observed in **1**. Furthermore, the dioxyquaternary carbon, C-7, and the oxymethylene carbon, C-9, in **1** were replaced by one oxymethine carbon (δ 62.6) and one dioxymethine carbon (δ 94.7) in **2**. These data were in agreement with the ^{1}H NMR data of which signals for the nonequivalent oxymethylene protons (H_{ab}-9) in **1** were replaced by those for one dioxymethine proton (δ 5.61, 1H, s, H-9) and one oxymethine proton (δ 4.25, 1H, m, H-7). In the ^{1}H–^{1}H COSY spectrum, H-7 showed cross peaks with H_{ab}-6 and the methyl protons, H_3-10 (δ 1.32, d, J=6.5 Hz), indicating the presence of a [$-CH_2CH(O-)CH_3$] unit. The methoxy protons, H_3-11 (δ 3.45), gave a ^3J HMBC cross peak with C-9 (δ 94.7) while H-9 (δ 5.61) displayed the same correlation with C-7 (δ 62.6). Consequently, an isochroman moiety having the methyl and the methoxyl groups at C-7 and C-9, respectively, was established. As H_b-6 was coupled with H-7 with a large coupling constant of 11.0 Hz, both of them were located at pseudoaxial position. Irradiation of H_3-11 (9-OMe) in the NOEDIFF experiment enhanced signal intensity of H-7, indicating *trans* relationship between pseudoequatorial H_3-10 (7-Me) and pseudoaxial 9-OMe. However, the NOEDIFF data were inadequate to identify the relative configuration at C-2. Therefore, penicisochroman B had the structure **2**.

Penicisochroman C (**3**) with the molecular formula $C_{15}H_{18}O_4$ from HREIMS was obtained as a colorless gum. Its UV spectrum was similar to that of **2** while the IR spectrum displayed an additional absorption band for a hydroxyl group at 3409 cm^{-1}. The ^{1}H NMR spectrum of **3** (Table 1) was similar to that of **2** except for the absence of the methoxyl signal. These data together with mass information revealed the replacement of the methoxyl group in **2** with a hydroxyl group in **3**. The DEPT spectrum showed one dioxygenated carbon (δ 88.8) and three methyl carbons (δ 21.9, 19.5, and 16.7), supporting the above conclusion. The location of 7-Me was at pseudoequatorial, identical to that in **2**, on the basis of a large coupling constant of 10.0 Hz between H_b-6 and H-7. Furthermore, compounds **2** and **3** gave almost identical optical rotation, indicating they would have the same absolute configuration. These data revealed that 9-OMe in **2** was replaced by a hydroxyl group in **3**. Therefore, penicisochroman C (**3**) was assigned as a 9-hydroxy derivative of **2**.

Penicisochroman D (**4**), a colorless gum, has the molecular formula $C_{10}H_{12}O_2$ established by HREIMS. The UV spectrum exhibited absorption bands at λ_{max} 251, 277, and 284 nm for a benzene chromophore. The IR spectrum showed an absorption band at 3351 cm^{-1} for a hydroxyl group. The ^{1}H NMR (Table 2) and ^{1}H–^{1}H COSY spectra displayed signals for three aromatic protons of a 1,2,3-trisubstitued benzene [δ 7.11 (1H, t, J=7.8 Hz), 6.74 (1H, d, J=7.8 Hz), and 6.71 (1H, d, J=7.8 Hz)], the 1-substituted 2-oxypropyl unit [δ 3.92 (1H, d br q, J=10.0 and 6.0 Hz), 2.75 (1H, dd, J=12.5 and 2.4 Hz), 2.74 (1H, dd, J=12.5 and 10.0 Hz), and 1.24 (3H, d, J=6.0 Hz)] and one set of nonequivalent oxymethylene protons [δ 4.85 and 4.79 (each 1H, d, J=13.2 Hz)]. The aromatic protons resonating at δ 6.74, 7.11, and 6.71 were attributed to H-2, H-3, and H-4, respectively. The nonequivalent methylene protons, H_{ab}-5 (δ 2.75 and 2.74), of the oxypropyl unit showed the HMBC correlations with C-4 (δ 122.5), C-4a (δ 137.7), and C-8a (δ 124.9), linking above unit at C-4a of the aromatic ring. Irradiation of H_{ab}-5 enhanced signal intensity of H-4, supporting the assignment. The remaining oxymethylene group was attached at C-8a as H_{ab}-8 (δ 4.85 and 4.79) were correlated with C-1 (δ 156.2), C-4a, and C-8a in the HMBC spectrum. These results together with the HMBC correlations of H_{ab}-8 with C-6 (δ 69.1) established an isochroman skeleton. The substituent at C-1 was a hydroxyl group due to the chemical shift of C-1. Therefore, penicisochroman D was determined as **4**. It is worth to note that the oxymethine proton, H-6 (δ 3.92, d br q, J=10.0 and 6.0 Hz), was located at psuedoaxial position due to a coupling constant of 10.0 Hz between H_b-5 and H-6.

Table 2
^{1}H and ^{13}C NMR data of penicisochromans D (**4**) and E (**5**)

Position	**4**		**5**	
	δ_H, mult, J in Hz	δ_C, mult.	δ_H, mult, J in Hz	δ_C, mult.
1		156.2, qC		150.4, qC
2	6.74, d, 7.8	115.0, CH	6.60, d, 8.0	114.6, CH
3	7.11, t, 7.8	129.0, CH	7.07, t, 8.0	129.3, CH
4	6.71, d, 7.8	122.5, CH	6.73, d, 8.0	114.0, CH
4a		137.7, qC		140.9, qC
5	a: 2.75, dd, 12.5, 2.4 b: 2.74, dd, 12.5, 10.0	41.8, CH_2	4.95, d, 2.0	88.5, CH
6	3.92, d br q, 10.0, 6.0	69.1, CH	3.85, qd, 6.5, 2.0	70.0, CH
8	a: 4.85, d, 13.2 b: 4.79, d, 13.2	58.5, CH_2	a: 5.06, d, 12.0 b: 5.01, d, 12.0	71.1, CH_2
8a		124.9, qC		126.1, qC
9	1.24, d, 6.0	23.4, CH_3	1.26, d, 6.5	18.6, CH_3

Penicisochroman E (**5**) was isolated as a colorless gum whose molecular formula was determined by HREIMS as $C_{10}H_{12}O_3$, with 16 mass unit higher than **4**. Its UV and IR spectra were almost identical to those of **4**. Its ^{1}H NMR spectrum (Table 2) was similar to that of **4** except for the replacement of signals for H_{ab}-5 in **4** with signal of a hydroxymethine proton (δ 4.95, 1H, d, J=2.0 Hz) in **5**. This was consistent with the molecular formula and the presence of two oxymethine carbons (δ 88.5 and 70.0) in the ^{13}C NMR and DEPT spectra. These data implied that **5** was a 5-hydroxy derivative of **4**. A small coupling constant between H-5 and H-6 in **5** indicated the replacement of H_b-5 in **4** with a hydroxyl group in **5**. Therefore, penicisochroman E (**5**) was determined as a 5-hydroxy derivative of **4**.

The molecular formula of penicipyrone (**6**) was established by analysis of its HREIMS as $C_{13}H_{13}NO_5$. The UV spectrum displayed characteristic absorption bands for a pyrone chromophore[10] at λ_{max} 224, 247, and 361 nm. The IR spectrum showed absorption bands at 3399 and 1683 cm^{-1} for hydroxyl and conjugated carbonyl groups, respectively. The ^{1}H NMR spectrum showed two olefinic protons of trisubstituted double bonds [δ 6.02 and 5.94 (each 1H, s)], one set of nonequivalent methylene protons [δ 3.64 and 3.54 (each 1H, d, J=9.6 Hz)], and two methyl groups [δ 2.30 (3H, s) and 2.22 (3H, s)]. The ^{13}C NMR and DEPT spectra exhibited one pyridinone carbonyl (δ 169.9),[11] one pyrone carbonyl (δ 166.5),[10] six quaternary (δ 169.1, 166.9, 160.7, 143.1, 108.2, and 102.4), two methine (δ 103.1 and 102.8), one methylene (δ 18.3), and two methyl (δ 19.7 and 19.0) carbons. The olefinic proton at δ 5.94 was attributed to H-5 of the pyrone moiety on the basis of its chemical shift as well as its HMBC cross peaks with C-3 (δ 102.4), C-4 (δ 169.1), and C-6 (δ 160.7). The methyl protons, H_3-7 (δ 2.22), gave the same correlations with C-5 (δ 102.8) and C-6. In addition, the methylene protons, H_{ab}-8 (δ 3.64 and 3.54), gave the HMBC cross peaks with C-2 (δ 166.5) and C-3. These results together with the chemical shift of C-4 indicated the attachment of the methylene group, a hydroxyl group and the methyl group at C-3, C-4, and C-6 of the pyrone ring, respectively. The chemical shifts of the remaining carbons indicated the presence of a pyridinone unit.[11] The olefinic proton at δ 6.02 was assigned as H-5′ due to its HMBC correlations with C-3′ (δ 108.2),

C-4′ (δ 166.9), and C-6′ (δ 143.1). The methyl protons, H_3-7′ (δ 2.30), gave the HMBC correlations with C-5′ and C-6′. These data revealed that the pyridinone unit carried hydroxyl and methyl groups at C-4′ and C-6′, respectively. The HMBC correlations from H_{ab}-8 of the pyrone moiety to C-2′ and C-3′ of the pyridinone established the methylene linkage between C-3 of the pyrone and C-3′ of the pyridinone. Consequently, penicipyrone was assigned as **6**. The appearance of the methylene protons (H_{ab}-8) as nonequivalent protons in the ^{1}H NMR spectrum might be due to the formation of H-bond between either the amino nitrogen or carbonyl oxygen of the pyridinone moiety and the hydroxy hydrogen of the pyrone unit, which would prevent bond rotation.

Penicipyranone (**7**) with the molecular formula $C_{10}H_{16}O_4$ from HREIMS was obtained as a colorless gum. The UV spectrum showed an absorption band at λ_{max} 269 nm. The IR spectrum displayed absorption bands at 3409 and 1687 cm^{-1} for hydroxyl and conjugated carbonyl functional groups, respectively. The ^{1}H NMR and ^{1}H–^{1}H COSY spectra revealed the presence of signals for one ethyl group [δ 2.45 (1H, dq, *J*=15.3 and 7.5 Hz), 2.40 (1H, dq, *J*=15.3 and 7.5 Hz) and 1.18 (3H, t, *J*=7.5 Hz)], one 1-oxyethyl group [(δ 4.52, 1H, q, *J*=6.6 Hz) and 1.31 (3H, d, *J*=6.6 Hz)], one hydroxymethyl group [δ 4.38 and 4.32 (each 1H, d, *J*=12.6 Hz)], and one methyl group (δ 1.40, 3H, s). The ^{13}C NMR and DEPT spectra displayed ten carbon resonances for one ketone carbonyl (δ 196.2), three quaternary (δ 175.6, 110.1, and 71.3), one methine (δ 82.0), two methylene (δ 56.3 and 25.6), and three methyl (δ 24.1, 12.3 and 11.4) carbons. The oxymethine proton of the 1-oxyethyl unit, H-5 (δ 4.52), gave the HMBC correlations with C-1 (δ 196.2), C-3 (δ 175.6), and C-6 (δ 71.3) while H_3-7 (δ 1.40) gave the same correlations with C-1, C-5 (δ 82.0), and C-6. In addition, the hydroxymethyl protons, H_{ab}-9 (δ 4.38 and 4.32), gave the HMBC cross peaks with C-1, C-2 (δ 110.1), and C-3. These results together with the chemical shifts of C-3, C-5, and C-6 established a dihydropyran-4-one unit with two methyl groups at C-5 and C-6, a hydroxyl group at C-6 and the hydroxymethyl substituent at C-2. Consequently, the remaining ethyl group was located at C-3. The HMBC correlations of the methylene protons, H_{ab}-10 (δ 2.45 and 2.40), of the ethyl group with C-2 and C-3 confirmed this assignment. The relative configuration between H-5 and H_3-7 was not assigned as either *cis* or *trans* relationship gave signal enhancement of H-5 upon irradiation of H_3-7. Therefore, penicipyranone (**7**) was determined as a new dihydropyran-4-one derivative.

Peniciphenol (**8**) with the molecular formula $C_{10}H_{12}O_3$ from HREIMS was obtained as a colorless gum. The UV spectrum displayed absorption bands at λ_{max} 250 and 289 nm for a benzene chromophore. The IR spectrum showed absorption bands at 3328 and 1638 cm^{-1} for hydroxyl and double bond functional groups, respectively. The ^{1}H NMR and ^{1}H–^{1}H COSY spectra showed characteristic signals for three aromatic protons of a 1,2,3-trisubstituted aromatic protons [δ 7.14 (1H, t, *J*=7.8 Hz), 6.81, and 6.59 (each 1H, d, *J*=7.8 Hz)], a (*Z*)-3-hydroxyl-1-propenyl unit [δ 6.64 (1H, d, *J*=11.4 Hz), 5.97 (1H, dt, *J*=11.4 and 6.9 Hz) and 4.07 (2H, dd, *J*=6.9 and 0.6 Hz)], and one hydroxymethyl group (δ 4.78, 2H, s). The aromatic protons resonating at δ 6.59, 7.14, and 6.81 were assigned as H-4, H-5, and H-6, respectively. The (*Z*)-3-hydroxyl-1-propenyl unit was located at C-3 (δ 136.3) based on the HMBC correlations of the olefinic proton, H-8 (δ 6.64), with C-3, and C-4 (δ 120.9). The hydroxymethyl protons, H_2-7 (δ 4.78), showed HMBC cross peaks with C-1 (δ 156.3), C-2 (δ 123.6), and C-3, thus connecting the hydroxymethyl group at C-2. The substituent at C-1 of the benzene ring was a hydroxyl group on the basis of its chemical shift. Signal enhancement of H_2-7 and H-4 upon irradiation of H-8 in the NOEDIFF spectrum supported the assigned location of these substituents. Therefore, peniciphenol had the structure **8**.

Penicisoquinoline (**9**) with the molecular formula $C_{15}H_{15}NO_4$ from HREIMS was obtained as a yellow gum. Its UV and IR spectra were almost identical to those of **11**. The ^{1}H NMR spectrum was similar to that of **11** except for the replacement of the downfield methyl signal in **11** with the signal of a hydroxymethyl group (δ 4.89, 2H, s). The presence of two methyl (δ 16.0 and 15.5) and one oxymethylene (δ 64.5) carbons in the ^{13}C NMR and DEPT spectra supported the ^{1}H NMR data. The HMBC correlations of the hydroxymethyl protons, H_2-10 (δ 4.89), with C-6 (δ 117.4), and C-7 (δ 159.3) together with signal enhancement of H_2-10 upon irradiation of H-6 (δ 7.79, 1H, s) established the linkage of the hydroxymethyl group at C-7. Consequently, penicisoquinoline (**9**) was identified as a 10-hydroxy derivative of **11**.

The isolated metabolites **1**, **4**, **6**–**12**, and **14** were tested for antibacterial activities against SA and MRSA. None of them were active at the concentration of 200 μg/mL. The remaining compounds were not evaluated for the antibacterial activity due to sample limitation.

The furo[3,2-*h*]isoquinolines (**10**,**11**) have been isolated from the fungus *Aspergillus ustus* TC 1118[6] while this is the first report on the isolation of the corresponding isochromans (**1**–**3**). We proposed that all of the isolated isochromans (**1**–**5**) and furo[3,2-*h*]isoquinolines (**9**–**11**) would have a similar biosynthetic pathway. Penicisochromans A–E (**1**–**5**) would be derived from the same biosynthetic precursor, peniciphenol (**8**). Acid-catalysed intramolecular cyclization of the (*Z*)-3-hydroxyl-1-propenyl side chain of **8** would generate an isochroman ring as shown in Scheme 1. Dehydration and subsequent hydrogenation would produce **4** via an isochroman intermediate (**4a**). Penicisochromans B (**2**) and C (**3**) would be transformed from **4** via a 3-oxo-2-isopropylbenzofuran (**4b**) by acylation with a 3-methyl-2-butenoyl unit and subsequent cyclization. Oxidation of the isochroman unit of **4b** would yield compound **3**, which would be further methylated to the corresponding methyl ether **2**. Compound **1** would be obtained from **4a** by hydration and methylation to yield a ketal intermediate (**4c**), which would further undergo acylation, cyclization, and oxidation. Finally, penicisochroman E (**5**) would be derived from **8** via a different biosynthetic route, which would involve epoxidation, cyclization followed by dehydration and subsequent hydrogenation. In addition, the furo[3,2-*h*]isoquinolines (**9**–**11**) would be biosynthesized via the similar pathway as that for the furo[3,2-*h*]isochromans (**1**–**3**) using a 7-amino derivative of **8** as a biosynthetic precursor.

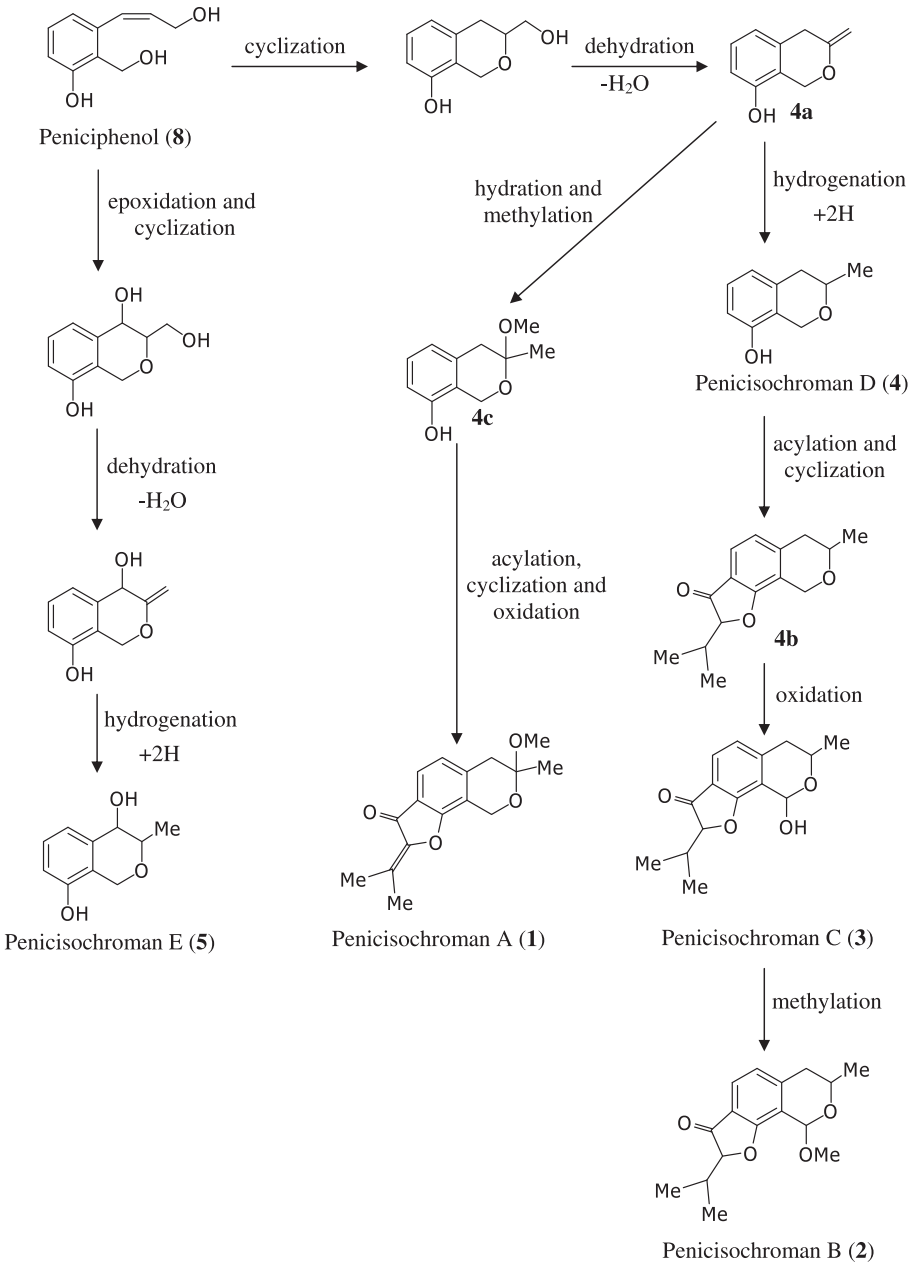
3. Experimental

3.1. General experimental procedures

Infrared spectra (IR) were recorded on a Perkin–Elmer 783 FTS 165 FT-IR spectrometer. Ultraviolet (UV) absorption spectra were measured in MeOH on a Shimadzu UV-160A spectrophotometer. Optical rotations were measured on a JASCO P-1020 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on 300 and 500 MHz Bruker FTNMR Ultra Shield spectrometer. Chemical shifts are expressed in δ (ppm) referring to the TMS peak. Mass spectra were measured on a MAT 95 XL mass spectrometer (Thermofinnigan). Thin-layer Chromatography (TLC) and precoated TLC (PTLC) were performed on silica gel GF_{256} (Merck). Column chromatography (CC) was carried out on silica gel (Merck) type 100 (70–230 Mesh ASTM) with a gradient system of MeOH–CH_2Cl_2, on Sephadex LH-20 with MeOH or on reverse phase silica gel C-18 with a gradient system of MeOH–H_2O, or otherwise stated.

3.2. Fungal material

The marine-derived fungus *Penicillium* sp. PSU-F40 was isolated from the sea fan, collected near the Similan Islands, Phangnga Province, Thailand, in 2006. The sea fan was identified as *Annella* sp.



Scheme 1. Possible biosynthetic pathway of compounds **1**–**5** from compound **8**.

by C. Benzies and S. Plathong from the Coral Reef and Benthos Research Unit, Center for Biodiversity of Peninsular Thailand (CBIPT), Department of Biology, Prince of Songkla University, Thailand. The sample (voucher no. sea fan-Similan 1.4) was deposited at CBIPT while the fungus was deposited as PSU-F40 at the Department of Microbiology, Faculty of Science, Prince of Songkla University.

3.3. Fermentation, extraction, and isolation

The crude EtOAc extracts from the culture broth and mycelia were prepared using the same procedure as described earlier.[10] The crude extract from the broth (1.3 g, a dark brown gum) was fractionated by CC over Sephadex-LH 20 to give five fractions (A–E). Fraction B (325 mg) was purified by silica gel CC to afford three fractions (B1–B3). Fraction B3 (59.2 mg) was then separated by CC over reverse phase silica gel to afford four fractions. The second fraction (7.5 mg) contained **7** (2.3 mg) after separation by PTLC with 20% EtOAc–light petroleum. Compound **12** (2.4 mg) was obtained from the third fraction (5.8 mg) after purification by PTLC with 1% MeOH–CH_2Cl_2. Fraction C (396 mg) was separated by CC over silica gel to yield four fractions (C1–C4). Fraction C2 (8.2 mg) was further purified by PTLC using 50% CH_2Cl_2–light petroleum as a mobile phase to furnish **2** (1.6 mg) and **3** (1.0 mg). Fraction C4 (22.8 mg) was then separated by CC over reverse phase silica gel to afford **4** (3.5 mg). Fraction D (314 mg) was separated by silica gel CC to afford five fractions (D1–D5). Fraction D2 (106 mg) was then purified by CC over silica gel to give **1** (3.1 mg), **10** (9.7 mg), and **11** (3.4 mg). Compound **5** (2.0 mg) was obtained from the fraction D3 (33.5 mg) after purification by silica gel CC with a gradient of EtOAc–light petroleum followed by PTLC with 20% EtOAc–light petroleum. Fraction D4 (40.5 mg), upon CC over silica gel, afforded **8** (7.7 mg)

and **9** (3.5 mg). Fraction E (43.5 mg) was separated by silica gel CC to give three fractions. Compound **6** (3.4 mg) was obtained from the third fraction. The mycelial extract (540.0 mg, a brown gum) was separated by CC over Sepadex LH-20 to afford three fractions. The second fraction (92.6 mg) was further purified by silica gel CC followed by CC over reverse phase silica gel to furnish **13** (1.3 mg) and **14** (3.2 mg).

*3.3.1. Penicisochroman A (**1**).* Colorless gum; $[\alpha]_D^{25}$ +12 (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 213 (3.68), 253 (3.28), 269 (3.15), 324 (2.92) nm; IR (neat) ν_{max} 1695, 1647 cm^{-1}; HREIMS *m*/*z* $[M-CH_3OH]^+$ 242.0941 (calcd for $C_{15}H_{14}O_3$, 242.0943); ^1H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1.

*3.3.2. Penicisochroman B (**2**).* Colorless gum; $[\alpha]_D^{25}$ −57 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 240 (3.01), 256 (2.97), 307 (2.70) nm; IR (neat) ν_{max} 1700, 1650 cm^{-1}; HREIMS *m*/*z* $[M]^+$ 276.1374 (calcd for $C_{16}H_{20}O_4$, 276.1362); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1.

*3.3.3. Penicisochroman C (**3**).* Colorless gum; $[\alpha]_D^{25}$ −55 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 241 (3.04), 256 (2.99), 307 (2.73) nm; IR (neat) ν_{max} 3409, 1683, 1647 cm^{-1}; HREIMS *m*/*z* $[M]^+$ 262.1196 (calcd for $C_{15}H_{18}O_4$, 262.1205); ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1.

*3.3.4. Penicisochroman D (**4**).* Colorless gum; $[\alpha]_D^{25}$ −26 (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 251 (3.04), 277 (3.01), 284 (2.97) nm; IR (neat) ν_{max} 3351, 1657 cm^{-1}; HREIMS *m*/*z* $[M]^+$ 164.0836 (calcd for $C_{10}H_{12}O_2$, 164.0837); ^1H NMR ($CDCl_3$, 500 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

*3.3.5. Penicisochroman E (**5**).* Colorless gum; $[\alpha]_D^{25}$ −12 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 215 (3.35), 268 (2.63), 275 (2.61) nm; IR (neat) ν_{max} 3402, 1653 cm^{-1}; HREIMS *m*/*z* $[M]^+$ 180.0793 (calcd for $C_{10}H_{12}O_3$, 180.0786); ^1H NMR ($CDCl_3$, 500 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2.

*3.3.6. Penicipyrone (**6**).* Colorless gum; UV (MeOH) λ_{max} (log ε) 224 (3.64), 247 (3.43), 361 (3.31) nm; IR (neat) ν_{max} 3399, 1683 cm^{-1}; HREIMS *m*/*z* $[M]^+$ 263.0795 (calcd for $C_{13}H_{13}NO_5$, 263.0794); ^1H NMR ($CDCl_3$+CD_3OD, 300 MHz) δ_H 6.02 (1H, s, H-5′), 5.94 (1H, s, H-5), 3.64 (1H, d, *J*=9.6 Hz, H_a-8), 3.54 (1H, d, *J*=9.6 Hz, H_b-8), 2.30 (3H, s, H-7′), 2.22 (3H, s, H-7); ^{13}C NMR ($CDCl_3$+CD_3OD, 75 MHz) δ_C 169.9 (C-2′), 169.1 (C-4), 166.9 (C-4′), 166.5 (C-2), 160.7 (C-6), 143.1 (C-6′), 108.2 (C-3′), 103.1 (C-5′), 102.8 (C-5), 102.4 (C-3), 18.3 (C-8), 19.7 (C-7), 19.0 (C-7′).

*3.3.7. Penicipyranone (**7**).* Colorless gum; $[\alpha]_D^{25}$ −135 (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 269 (3.67) nm; IR (neat) ν_{max} 3409, 1687 cm^{-1}; HREIMS *m*/*z* $[M]^+$ 200.1057 (calcd for $C_{10}H_{16}O_4$, 200.1049); ^1H NMR ($CDCl_3$, 300 MHz) δ_H 4.52 (1H, q, *J*=6.6 Hz, H-5), 4.38 (1H, d, *J*=12.6 Hz, H_a-9), 4.32 (1H, d, *J*=12.6 Hz, H_b-9), 2.45 (1H, dq, *J*=15.3, 7.5 Hz, H_a-10), 2.40 (1H, dq, *J*=15.3, 7.5 Hz, H_b-10), 1.40 (3H, s, H-7), 1.31 (3H, d, *J*=6.6 Hz, H-8), 1.18 (3H, t, *J*=7.5 Hz, H-11); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 196.2 (C-1), 175.6 (C-3), 110.1 (C-2), 82.0 (C-5), 71.3 (C-6), 56.3 (C-9), 25.6 (C-10), 24.1 (C-7), 12.3 (C-8), 11.4 (C-11).

*3.3.8. Peniciphenol (**8**).* Colorless gum; UV (MeOH) λ_{max} (log ε) 250 (2.49), 289 (2.19) nm; IR (neat) ν_{max} 3328, 1638 cm^{-1}; HREIMS *m*/*z* $[M]^+$ 180.0788 (calcd for $C_{10}H_{12}O_3$, 180.0786); ^1H NMR ($CDCl_3$+CD_3OD, 300 MHz) δ_H 7.14 (1H, t, *J*=7.8 Hz, H-5), 6.81 (1H, d, *J*=7.8 Hz, H-6), 6.64 (1H, d, *J*=11.4 Hz, H-8), 6.59 (1H, d, *J*=7.8 Hz, H-4), 5.97 (1H, dt, *J*=11.4, 6.9 Hz, H-9), 4.78 (2H, s, H-7), 4.07 (2H, dd, *J*=6.9, 0.6 Hz, H-10); ^{13}C NMR ($CDCl_3$+CD_3OD, 75 MHz) δ_C 156.3 (C-1), 136.3 (C-3), 131.9 (C-9), 130.0 (C-8), 128.5 (C-5), 123.6 (C-2), 120.9 (C-4), 115.3 (C-6), 59.1 (C-7), 58.6 (C-10).

*3.3.9. Penicisoquinoline (**9**).* Yellow gum; $[\alpha]_D^{25}$ −21 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 252 (3.69), 353 (3.19) nm; IR (neat) ν_{max} 3245, 1692, 1641 cm^{-1}; HREIMS *m*/*z* $[M]^+$ 273.1009 (calcd for $C_{15}H_{15}NO_4$, 273.1001); ^1H NMR ($CDCl_3$+CD_3OD, 300 MHz) δ_H 9.48 (1H, s, H-9), 7.79 (1H, s, H-6), 7.72 (1H, d, *J*=8.4 Hz, H-4), 7.36 (1H, d, *J*=8.4 Hz, H-5), 4.89 (2H, s, H-10), 2.36 (1H, m, H-11), 1.19 (3H, br s, H-13), 0.97 (3H, br s, H-12); ^{13}C NMR ($CDCl_3$+CD_3OD, 75 MHz) δ_C 199.0 (C-3), 171.9 (C-9b), 159.3 (C-7), 146.6 (C-9), 142.7 (C-5a), 124.5 (C-4), 120.3 (C-5), 117.4 (C-6), 115.9 (C-3a, C-9a), 110.1 (C-2), 64.5 (C-10), 34.2 (C-11), 16.0 (C-12), 15.5 (C-13).

3.4. Antibacterial activity testing

MICs were determined by the agar microdilution method.[12] The test substances were dissolved in DMSO (Merck, Germany). Serial twofold dilutions of the test substances were mixed with melted Mueller–Hinton agar (Difco) in the ratio of 1:100 in microtiter plates with flat-bottomed wells (Nunc, Germany). Final concentration of the test substances in agar ranged from 0.03 to 128 μg/mL. SA and MRSA were used as test strains. Inoculum suspensions (10 μL) were spotted on agar-filled wells. The inoculated plates were incubated at 35 °C for 18 h. MICs were recorded by reading the lowest substance concentration that inhibited visible growth. Vancomycin, a positive control drug, exhibited the MIC value of 1 μg/mL. Growth controls were performed on the agar containing DMSO.

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