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中国南海紫柳珊瑚中过氧化甾醇类化学成分的研究

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[摘要] **目的** 对采自中国南海的紫柳珊瑚(*Muriceopsis flavida*)的生物活性成分进行研究。**方法** 应用硅胶柱色谱、Sephadex LH-20 凝胶柱色谱、反相高效液相色谱(RP-HPLC)等分离手段对紫柳珊瑚的乙醚提取物进行分离纯化,应用核磁共振(NMR)、质谱(MS)等波谱手段对得到的化合物进行结构鉴定,采用琼脂扩散试验法对分离得到的化合物进行体外抗微生物活性测试。**结果** 从紫柳珊瑚乙醚提取物中分离得到5种过氧化甾醇,分别鉴定为:(22E,24S)-5 α ,8 α -过氧化麦角甾-6,22-二烯-3 β -醇(1)、(22E,24R)-5 α ,8 α -过氧化麦角甾-6,22-二烯-3 β -醇(2)、(24R)-5 α ,8 α -过氧化胆甾-24-乙基-6-烯-3 β -醇(3)、(22E)-5 α ,8 α -过氧化胆甾-6,22-二烯-3 β -醇(4)、5 α ,8 α -过氧化胆甾-6-烯-3 β -醇(5)。体外抗微生物活性测试表明这5种化合物均有不同程度的抗微生物活性。**结论** 首次从中国南海紫柳珊瑚中得到5种过氧化甾醇,其中化合物2对微藻显示强烈的生长抑制活性,具有进一步研究的价值。

[关键词] 紫柳珊瑚;过氧化甾醇;抗菌活性;结构鉴定

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5 α ,8 α -epidioxy sterol components in gorgonian *Muriceopsis flavida* collected from the South China Sea

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[Abstract] **Objective** To investigate the bioactive chemical constituents of gorgonian *Muriceopsis flavida* collected from the South China Sea. **Methods** The Et₂O extract of the sample was purified by repeated column chromatographies on silica gel, Sephadex LH-20, and RP-HPLC. The structures of the obtained compounds were elucidated based on detailed spectroscopic analysis using mass spectrometry (MS) and nuclear magnetic resonance (NMR). The *in vitro* antimicrobial activities of the identified compounds were assessed by an agar diffusion test. **Results** Five 5 α , 8 α -epidioxy sterols were isolated from the Et₂O extract (1-5), and their structures were determined as: (22E, 24S)-5 α , 8 α -epidioxy-24-methyl-cholesta-6, 22-dien-3 β -ol (1), (22E, 24R)-5 α , 8 α -epidioxy-24-methyl-cholesta-6, 22-dien-3 β -ol (2), (24R)-5 α , 8 α -epidioxy-24-ethyl-cholesta-6-en-3 β -ol (3), (22E)-5 α , 8 α -epidioxy-cholest-6, 22-dien-3 β -ol (4), and 5 α , 8 α -epidioxy-cholest-6-en-3 β -ol (5). These compounds showed different degrees of antimicrobial activities in bioassay *in vitro*. **Conclusion** The five compounds have been identified for the first time from the gorgonian *Muriceopsis flavida*. Compound 2 shows a strong anti-algae effect and is worth further studying.

[Key words] *Muriceopsis flavida*; epidioxy sterols; antibacterial activity; structural elucidation

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珊瑚系低等腔肠门动物,全球共有6 100多种,生长在潮间带到深达4 000多米的海洋中,广泛分布于亚热带到两极的世界各地海域。柳珊瑚俗称海扇或海鞭,属珊瑚纲八放珊瑚亚纲动物,该亚纲可进一步分为2个亚目和11个科。我国海洋中生活有8科78种柳珊瑚,主要分布于广东、海南沿海海域^[1-3]。

国际上对柳珊瑚的研究始于20世纪60年代,从中发现了许多结构新颖且具有较好活性的化合物^[1]。甾醇化合物是生物膜的重要组成部分,同时也是合成激素的前体化合物,具有抗肿瘤、抗炎、细胞毒、抗菌等多种生物学活性,广泛分布于珊瑚体内^[4-5]。

为开发利用我国的海洋生物资源,研究海洋生

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物的生态活性物质,寻找具有生物活性及药用前景的海洋天然产物,我们课题组多年来一直对我国南海柳珊瑚的化学成分进行系统研究,并发现了一系列结构各异的甾体成分^[6-9]。紫柳珊瑚(*Muriceopsis flavida*)是柳珊瑚目(Gorgonacea)丛柳珊瑚科(Plaxauridae)动物,文献检索发现仅有1篇关于该种柳珊瑚化学成分的研究报道,从中分离得到一系列4-甲基化甾醇^[10]。

本研究运用常压凝胶柱色谱、硅胶柱色谱及HPLC等分离纯化技术,对紫柳珊瑚的化学成分进行了系统分离,得到5种过氧化甾醇化合物,采用NMR、MS等现代波谱技术对这些化合物进行分析鉴定,这些化合物的结构分别鉴定为:(22*E*,24*S*)-5 α ,8 α -过氧化麦角甾-6,22-二烯-3 β -醇(**1**)、(22*E*,24*R*)-5 α ,8 α -过氧化麦角甾-6,22-二烯-3 β -醇(**2**)、(24*R*)-5 α ,8 α -过氧化胆甾-24-乙基-6-烯-3 β -醇(**3**)、(22*E*)-5 α ,8 α -过氧化胆甾-6,22-二烯-3 β -醇(**4**)及5 α ,8 α -过氧化胆甾-6-烯-3 β -醇(**5**),结构式见图1。这些化合物均系首次从该属珊瑚中分离得到。

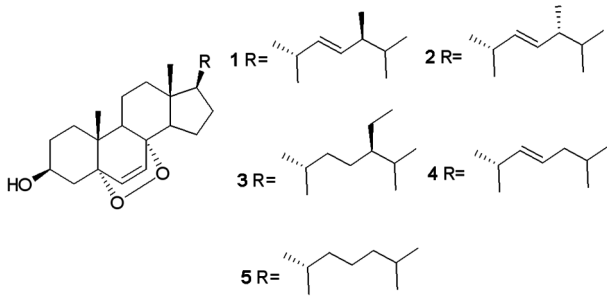


图1 化合物1~5的结构式
Fig 1 Structures of compound 1-5

1 材料和方法

1.1 样品 实验样品于2008年7月采自我国广西北海海域水下10 m处,立即冷冻备用。种属由中国科学院南海海洋研究所李秀保助理研究员鉴定,样品标本保存于第二军医大学药学院海洋药物研究中心,编号ZS-17。

1.2 主要仪器和试剂 NMR(Bruker Avance-500、Avance-400、Varian Inova-600);MAT-212质谱仪;XT5显微熔点测定仪。柱色谱硅胶(200~300目、400~600目)和TLC薄层板均由烟台芝罘黄务硅胶开发试验厂提供;Sephadex LH-20葡聚糖凝胶由Pharmacia公司提供;HPLC[Aglient 1100, RID检测器, Zorbax 300-C₁₈柱(250 mm×9.4 mm)],开放柱色谱所用试剂均为分析纯, HPLC所用试剂为色谱纯,均由中国医药集团上海化学试剂公司生产。

1.3 化合物的提取与分离 将湿质量1.4 kg的紫柳珊瑚样品切碎,用丙酮(acetone)超声提取,每次2 L,提取6次至无色,合并提取液并减压浓缩至干,得丙酮粗提物10.2 g,将粗提物用1 L蒸馏水混悬,依次用等体积乙醚和正丁醇萃取4次,合并萃取液并减压浓缩至干,分别得乙醚层浸膏7.3 g及正丁醇层浸膏1.8 g。乙醚层浸膏(7.3 g)经硅胶柱层析梯度洗脱(石油醚:乙酸乙酯99:1~纯乙酸乙酯),将其分成14个部分(Fr. 1~Fr. 14)。Fr. 6(500 mg)再经过Sephadex LH-20凝胶柱色谱(Et₂O:CHCl₃:MeOH=2:1:1)、正相硅胶柱色谱(400~600目硅胶、正己烷:丙酮=5:1洗脱)、RP-HPLC(Zorbax 300-C₁₈柱,流动相:95%甲醇-水;流速:1.5 ml/min;柱温:30℃)进一步纯化,分离得到化合物**1**~**5**:化合物**1**(2 mg)、化合物**2**(4 mg)、化合物**3**(5 mg)、化合物**4**(7 mg)、化合物**5**(3 mg)。

1.4 抗菌活性试验 采用琼脂扩散试验法,以青霉素(penicillin)、链霉素(streptomycin)、酮康唑(ketoconazole)为阳性对照,溶剂丙酮为阴性对照,测定了化合物**1**~**5**对花药黑粉菌(*Microbotryum violaceum*)、灰霉菌(*Botrytis cinerea*)、壳针孢叶枯病菌(*Septoria tritici*)、大肠杆菌(*Escherichia coli*)、巨大芽孢杆菌(*Bacillus megaterium*)和小球藻(*Chlorella fusca*)的抑制活性。按抑菌圈直径大小评价其抗菌活性,平行实验3次,取平均值。

2 结果

2.1 化合物**1**的结构鉴定 白色针状结晶(25℃, CHCl₃), m. p. 178~180℃; $[\alpha]_D^{20} = -28^\circ$ (c 0.16, CHCl₃); ESI-MS (*m/z*): 429.33 ([M + H]⁺); ¹HNMR (400 MHz, CDCl₃): 3.96 (1H, tt, *J* = 11.3, 5.0 Hz, H-3), 6.24 (1H, d, *J* = 8.5 Hz, H-6), 6.50 (1H, d, *J* = 8.5 Hz, H-7), 0.82 (3H, s, H-18), 0.89 (3H, s, H-19), 0.99 (3H, d, *J* = 6.6 Hz, H-21), 5.13 (H, dd, *J* = 15.1, 7.7 Hz, H-22), 5.20 (1H, dd, *J* = 15.1, 7.7 Hz, H-23), 0.81 (3H, d, *J* = 6.9 Hz, H-26), 0.82 (3H, d, *J* = 7.0 Hz, H-27), 0.91 (3H, d, *J* = 6.8 Hz, H-28); ¹³CNMR (100 MHz, CDCl₃): 34.7 (t, C-1), 30.1 (t, C-2), 66.5 (d, C-3), 36.9 (t, C-4), 82.2 (s, C-5), 135.4 (d, C-6), 130.8 (d, C-7), 79.4 (s, C-8), 51.1 (d, C-9), 37.0 (s, C-10), 23.4 (t, C-11), 39.4 (t, C-12), 44.6 (s, C-13), 51.7 (d, C-14), 20.7 (t, C-15), 28.9 (t, C-16), 56.2 (d, C-17), 12.9 (q, C-18), 18.2 (q, C-19), 39.8 (d, C-20), 20.9 (q, C-21), 135.4 (d, C-22), 132.4 (d, C-23), 43.1 (d, C-24), 33.2 (d, C-

25), 19.7 (q, C-26), 20.1 (q, C-27), 18.0 (q, C-28)。¹H NMR 和 ¹³C NMR 数据与文献^[11-13]一致, 确定其结构为 (22*E*, 24*S*)-5 α , 8 α -过氧化麦角甾-6, 22-二烯-3 β -醇。

2.2 化合物 **2** 的结构鉴定 白色结晶 (25 $^{\circ}$ C, CHCl₃), m. p. 177~179 $^{\circ}$ C; $[\alpha]_{\text{D}}^{20} = -25^{\circ}$ (c 0.16, CHCl₃); ESI-MS (*m/z*): 429.33 ([M+H]⁺); ¹H NMR (500 MHz, CDCl₃): 3.97 (1H, tt, *J* = 11.0, 5.0 Hz, H-3), 6.24 (1H, d, *J* = 8.5 Hz, H-6), 6.50 (1H, d, *J* = 8.5 Hz, H-7), 0.82 (3H, s, H-18), 0.88 (3H, s, H-19), 1.00 (3H, d, *J* = 6.5 Hz, H-21), 5.15 (1H, dd, *J* = 15.1, 8.0 Hz, H-22), 5.22 (1H, dd, *J* = 15.1, 7.7 Hz, H-23), 0.81 (3H, d, *J* = 6.9 Hz, H-26), 0.82 (3H, d, *J* = 7.0 Hz, H-27), 0.91 (3H, d, *J* = 7.0 Hz, H-28); ¹³C NMR (125 MHz, CDCl₃): 34.7 (t, C-1), 30.1 (t, C-2), 66.5 (d, C-3), 36.9 (t, C-4), 82.2 (s, C-5), 135.4 (d, C-6), 130.7 (d, C-7), 79.4 (s, C-8), 51.1 (d, C-9), 37.0 (s, C-10), 23.4 (t, C-11), 39.4 (t, C-12), 44.6 (s, C-13), 51.7 (d, C-14), 20.6 (t, C-15), 28.6 (t, C-16), 56.2 (d, C-17), 12.9 (q, C-18), 18.2 (q, C-19), 39.7 (d, C-20), 20.9 (q, C-21), 135.2 (d, C-22), 132.3 (d, C-23), 42.8 (d, C-24), 33.1 (d, C-25), 19.9 (q, C-26), 19.6 (q, C-27), 17.6 (q, C-28)。¹H NMR 和 ¹³C NMR 数据与文献^[13-15]一致, 确定其结构为 (22*E*, 24*R*)-5 α , 8 α -过氧化麦角甾-6, 22-二烯-3 β -醇。

2.3 化合物 **3** 的结构鉴定 白色粉末, m. p. 145~147 $^{\circ}$ C; $[\alpha]_{\text{D}}^{20} = 0.0^{\circ}$ (c 0.16, CHCl₃); ESI-MS (*m/z*): 443.5 ([M+H]⁺); ¹H NMR (500 MHz, CDCl₃): 3.97 (1H, tt, *J* = 11.0, 5.3 Hz, H-3), 6.23 (1H, d, *J* = 8.5 Hz, H-6), 6.51 (1H, d, *J* = 8.5 Hz, H-7), 0.80 (3H, s, H-18), 0.88 (3H, s, H-19), 0.91 (3H, d, *J* = 6.5 Hz, H-21), 0.83 (3H, d, *J* = 6.7 Hz, H-26), 0.82 (3H, d, *J* = 7.0 Hz, H-27), 0.85 (3H, t, *J* = 7.1 Hz, H-29); ¹³C NMR (125 MHz, CDCl₃): 34.7 (t, C-1), 30.1 (t, C-2), 66.5 (d, C-3), 36.9 (t, C-4), 82.2 (s, C-5), 135.4 (d, C-6), 130.8 (d, C-7), 79.5 (s, C-8), 51.1 (d, C-9), 37.0 (s, C-10), 23.4 (t, C-11), 39.4 (t, C-12), 44.7 (s, C-13), 51.6 (d, C-14), 20.6 (t, C-15), 28.3 (t, C-16), 56.3 (d, C-17), 12.6 (q, C-18), 18.2 (q, C-19), 35.6 (d, C-20), 18.6 (q, C-21), 33.7 (t, C-22), 26.1 (t, C-23), 45.8 (d, C-24), 29.2 (d, C-25), 19.8 (q, C-26), 19.0 (q, C-27), 23.0 (t, C-28), 12.0 (q, C-29)。根据 ¹H NMR 和 ¹³C NMR 数据并结合文

献^[11-13], 得出化合物 **3** 的结构为 (24*R*)-5 α , 8 α -过氧化胆甾-24-乙基-6-烯-3 β -醇。

2.4 化合物 **4** 的结构鉴定 白色粉末, m. p. 168~170 $^{\circ}$ C; $[\alpha]_{\text{D}}^{20} = 12^{\circ}$ (c 0.13, CHCl₃); ESI-MS (*m/z*): 413.3 ([M+H]⁺); ¹H NMR (600 MHz, CDCl₃): 3.98 (1H, tt, *J* = 10.8, 5.8 Hz, H-3), 6.25 (1H, d, *J* = 8.4 Hz, H-6), 6.51 (1H, d, *J* = 8.4 Hz, H-7), 0.82 (3H, s, H-18), 0.89 (3H, s, H-19), 0.96 (3H, d, *J* = 6.6 Hz, H-21), 5.16 (1H, dd, *J* = 15.1, 8.0 Hz, H-22), 5.28 (1H, dd, *J* = 15.1, 8.1 Hz, H-23), 0.85 (3H, d, *J* = 6.6 Hz, H-26), 0.86 (3H, d, *J* = 6.8 Hz, H-27); ¹³C NMR (125 MHz, CDCl₃): 34.6 (t, C-1), 30.1 (t, C-2), 66.5 (d, C-3), 36.9 (t, C-4), 82.1 (s, C-5), 135.4 (d, C-6), 130.7 (d, C-7), 79.4 (s, C-8), 51.1 (d, C-9), 37.0 (s, C-10), 23.4 (t, C-11), 39.3 (t, C-12), 44.5 (s, C-13), 51.6 (d, C-14), 20.6 (t, C-15), 28.7 (t, C-16), 56.3 (d, C-17), 12.8 (q, C-18), 18.2 (q, C-19), 39.7 (d, C-20), 20.6 (q, C-21), 137.7 (d, C-22), 126.6 (d, C-23), 41.8 (t, C-24), 28.5 (d, C-25), 22.3 (q, C-26), 22.3 (q, C-27)。¹H NMR 数据与文献^[11]一致, 确定化合物 **4** 的结构为 (22*E*)-5 α , 8 α -过氧化胆甾-6, 22-二烯-3 β -醇。

2.5 化合物 **5** 的结构鉴定 白色无定形粉末, m. p. 150~152 $^{\circ}$ C; $[\alpha]_{\text{D}}^{20} = -6.0^{\circ}$ (c 0.15, CHCl₃); ESI-MS (*m/z*): 417 ([M+H]⁺); ¹H NMR (600 MHz, CDCl₃): 3.97 (1H, tt, *J* = 11.6, 6.0 Hz, H-3), 6.25 (1H, d, *J* = 8.4 Hz, H-6), 6.51 (1H, d, *J* = 8.4 Hz, H-7), 0.82 (3H, s, H-18), 0.88 (3H, s, H-19), 0.90 (3H, d, *J* = 6.6 Hz, H-21), 0.86 (3H, d, *J* = 6.6 Hz, H-26), 0.87 (3H, d, *J* = 6.6 Hz, H-27); ¹³C NMR (125 MHz, CDCl₃): 34.7 (t, C-1), 30.1 (t, C-2), 66.5 (d, C-3), 37.0 (t, C-4), 82.2 (s, C-5), 135.4 (d, C-6), 130.8 (d, C-7), 79.4 (s, C-8), 51.1 (d, C-9), 37.0 (s, C-10), 23.4 (t, C-11), 39.4 (t, C-12), 44.7 (s, C-13), 51.6 (d, C-14), 20.6 (t, C-15), 28.3 (t, C-16), 56.4 (d, C-17), 12.6 (q, C-18), 18.2 (q, C-19), 35.2 (d, C-20), 18.6 (q, C-21), 36.0 (t, C-22), 23.8 (t, C-23), 39.4 (t, C-24), 28.1 (d, C-25), 22.5 (q, C-26), 22.8 (q, C-27)。结合文献^[11], 推断出化合物 **5** 的结构为 5 α , 8 α -过氧化胆甾-6-烯-3 β -醇。

2.6 抗菌活性测定结果 结果(表1)显示: 这些化合物对受试微生物的生长均有不同程度的抑制活性, 化合物 **4**、**5** 对巨大芽孢杆菌的抑制作用较强, 化合物 **2** 对小球藻的抑制活性最强。

表 1 琼脂扩散实验活性筛选

Tab 1 Agar diffusion assays for antibacterial, antifungal and antialgal activities

	(d/cm)					
	Fungus			Bacteria		Alga
	<i>Microbotryum violaceum</i>	<i>Botrytis cinerea</i>	<i>Septoria tritici</i>	<i>Escherichia coli</i>	<i>Bacillus megaterium</i>	<i>Chlorella fusca</i>
Compound 1	1.2	—	1.4	1.3	1.2	1.6
Compound 2	1.3	—	1.4	1.2	1.4	1.8
Compound 3	1.6	—	1.4	1.4	1.6	1.1
Compound 4	1.8	1.1	1.2	1.1	2.0	1.4
Compound 5	1.4	—	0.8	1.1	1.9	1.5
Penicillin	2.0	—	1.6	2.2	2.6	1.7
Streptomycin	1.8	—	1.2	1.8	1.2	1.3
Ketoconazole	3.0	3.5	3.0	1.8	1.7	1.0
Acetone	1.2	—	1.1	1.1	1.0	1.0

0.6 mg of the test or control substances dissolved in acetone were applied to a filter disc and sprayed with the respective test organism.

—: No activities were detected in agar diffusion assays

3 讨论

本研究对中国南海紫柳珊瑚的化学成分进行了初步研究,5种过氧化甾醇均为首次从该属珊瑚中分离得到。体外活性测试结果表明,化合物1~5对花药黑粉菌、灰霉菌、壳针孢叶枯病菌、大肠杆菌、巨大芽孢杆菌和小球藻均显示不同程度的抗生长抑制活性,提示这些化合物在紫柳珊瑚的化学防御过程中可能发挥重要作用。化合物4、5对巨大芽孢杆菌显示较强的生长抑制活性,化合物2对小球藻的生长抑制作用强于所有的对照药,具有进一步研究的价值。

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