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海洋真菌 *Aspergillus fumigatus* 次生代谢产物研究

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摘要:对来源于腔节藻属海藻 *Coelarthurm boergesenii*(采自中国南海西沙群岛)的一株海洋内生真菌 *Aspergillus fumigatus* 的发酵液进行化学成分的分离及鉴定。采用硅胶柱色谱、凝胶柱色谱和高压液相色谱等分离手段,从乙酸乙酯萃取部分中分离得到 16 个次级代谢产物(**1~16**),经核磁共振、质谱等现代波谱技术鉴定了它们的结构,分别为 gliotoxin(**1**)、bis (dethio) bis (methylsulfanyl) gliotoxin(**2**)、fumiquinazoline C(**3**)、(-)-chaetomium(**4**)、verruculogen(**5**)、fumitremorgin C(**6**)、brevianamide F(**7**)、fumigaclavine C(**8**)、decanedioic acid,2-methylene(**9**)、decanedioic acid,2-methylene-,1-methyl ester(**10**)、2-undecenedioic acid(**11**)、2-undecenedioic acid,1-methyl ester(**12**)、helvolic acid(**13**)、di-2-ethyl hexyl phthalate(**14**)、physcion(**15**)、emodin(**16**),其中化合物**9,10**和**12**为新天然产物。

关键词:海洋真菌;内生真菌;*Aspergillus fumigatus*;代谢产物;生物碱

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Secondary Metabolites of Marine Fungus *Aspergillus fumigatus*

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Abstract: In order to find new, potentially biologically active compounds from marine microorganisms, we investigated the metabolites of an endophytic fungus named *Aspergillus fumigatus*, which was separated from *Coelarthurm boergesenii* (collected from Paracel Islands in South China Sea). Accordingly, 16 compounds were isolated from the EtOAc-soluble portion of the fermentation broth of *Aspergillus fumigatus*. On the basis of extensive spectroscopic analyses (1D and 2D NMR, HR-ESI-MS) and by comparison with the spectroscopic data in the published literature, they were determined as gliotoxin (**1**), bis (dethio) bis (methylsulfanyl) gliotoxin (**2**), fumiquinazoline C (**3**), (-)-chaetomium (**4**), verruculogen (**5**), fumitremorgin C (**6**), brevianamide F (**7**), fumigaclavine C (**8**), decanedioic acid,2-methylene (**9**),2-methylene-,1-methyl ester (**10**),2-undecenedioic acid (**11**),2-undecenedioic acid,1-methyl ester (**12**),decanedioic acid, helvolic acid (**13**), and di-2-ethyl hexyl phthalate (**14**), physcion (**15**), emodin (**16**), respectively. Among them, compounds **9,10** and **12** are new natural products.

Key words: thalassiomycetes; endophytic fungi; *Aspergillus fumigatus*; metabolites; alkaloids

海洋微生物在海洋中广泛分布,因其所处的高盐、高压及低营养的特殊生态环境而进化出了独特的代谢机制,可产生与陆地微生物不同的次级代谢产物,是海洋天然产物的重要来源;其中海洋真菌以次级代谢产物量大、生物活性物质种类丰富等优点成为了海洋天然产物研究的重要资源^[1]。本实验对来源于腔节藻属海藻 *Coelarthurm boergesenii* 的内

生真菌 *Aspergillus fumigatus* 的乙酸乙酯萃取部分进行了化学成分研究,从中得到了 16 个化合物,采用 1D 和 2D NMR、HR-ESI-MS 等波谱数据分析以及与文献对照等方法,鉴定了这些化合物的结构。所得到的化合物中,化合物**9,10**和**12**为新天然产物。

1 仪器与试剂

Thermo LTQ-Orbitrap XL 质谱仪;Bruker-AM400 型核磁共振仪,TMS 为内标;硅胶(200~300 目,青岛海洋化工厂);Waters 2545 高效液相色谱

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仪(SunFire C₁₈制备柱,10×250 mm,5 μm)和Waters 2695高效液相色谱仪(SunFire C₁₈分析柱,2.1×150 mm,3.5 μm)。有机溶剂均为分析纯,购自上海国药化学试剂公司。

2 菌种与发酵培养

菌株于2014年4月从采自中国南海西沙群岛的腔节藻属(*Coelarthurum*)海藻中分离得到,由湖北省农业科学院方伟副研究员鉴定为*Aspergillus fumigatus*,菌株保存在湖北省农业科学院。发酵培养基为ISP2半固体培养基,配方为酵母膏3 g/L,麦芽汁3 g/L,蛋白胨5 g/L,琼脂2 g/L,PH6.5。共接种半固体培养基20 L,室温培养15 d,分别收集菌丝体和发酵液。

3 提取与分离

向常温发酵2周的真菌*Aspergillus fumigatus*半固体发酵液(20 L)中加入等体积乙酸乙酯,摇床震荡提取2次,每次180 min,提取液浓缩得到乙酸乙酯部位浸膏12.7 g。经过硅胶柱层析(石油醚-乙酸乙酯1:0→0:1),利用TLC检测进行合并,得到Fr. 1

~14共14个部分。Fr. 5部分用HPLC(CH₃CN-H₂O 5:95→100:0)纯化得到化合物**16**(5.2 mg),Fr. 8部分用制备型HPLC(CH₃CN-H₂O 5:95→100:0)分离得到化合物**1**(21.6 mg)、**14**(11.4 mg)和**15**(23.4 mg)。Fr. 9部分用制备型HPLC(CH₃CN-H₂O 5:95→100:0)纯化得到化合物**3**(10.0 mg)、**13**(11.7 mg)和3个不纯的峰,后者经半制备型RP-HPLC(CH₃CN-H₂O 5:95→100:0)进一步纯化得到化合物**4**(3.5 mg)、**5**(3.3 mg)、**9**(26.4 mg);Fr. 10部分用制备型HPLC(CH₃CN-H₂O 5:95→100:0)分离,并经半制备型RP-HPLC(CH₃CN-H₂O 5:95→100:0)纯化得到化合物**2**(5.1 mg);Fr. 11部分用制备型HPLC(CH₃CN-H₂O 5:95→100:0)纯化得到化合物**6**(5.7 mg)和2个不纯的峰,后者经半制备型RP-HPLC(CH₃CN-H₂O 30:70→70:30)进一步纯化得到化合物**12**(7.2 mg)和**10**(10.3 mg);Fr. 12部分用制备型HPLC(CH₃CN-H₂O 5:95→100:0)分离,并经半制备型RP-HPLC(CH₃CN-H₂O 5:95→100:0)纯化得到化合物**7**(4.5 mg)、**8**(20.3 mg)、**11**(15.6 mg)。

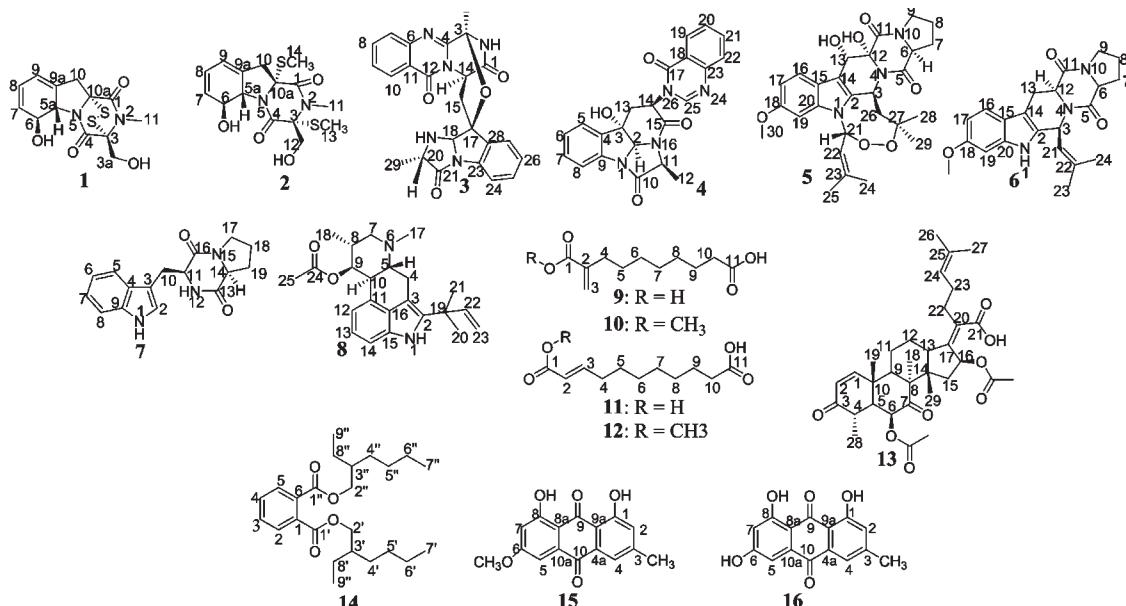


图1 化合物1~16的结构

Fig. 1 Chemical structures of compounds 1-16

4 结构鉴定

化合物1 白色针状结晶;¹H NMR(CDCl₃,400 MHz)δ:2.95(1H,d,J=18.1 Hz,H-10),3.20

(3H,s,CH₃-11),3.64(1H,dd,J=9.7,5.9 Hz,H-3a),3.74(1H,m,H-10),4.25(1H,dd,J=12.8,9.7 Hz,H-3a),4.42(1H,dd,J=12.8,5.9 Hz,H-5a),4.81(1H,s,H-6),5.78(1H,d,J=9.6 Hz,H-

7), 5.94 (1H, dd, $J = 9.3, 4.7$ Hz, H-8), 5.99 (1H, m, H-9); ^{13}C NMR (CDCl_3 , 100 MHz) δ : 27.6 (C-11), 36.7 (C-10), 60.8 (C-3a), 70.0 (C-5a), 73.3 (C-10a), 75.7 (C-6), 77.3 (C-3), 120.4 (C-9), 123.5 (C-8), 130.2 (C-7), 130.9 (C-9a), 165.5 (C-4), 166.2 (C-1)。以上氢谱和碳谱数据与文献^[2]报道的化合物 gliotoxin 一致。

化合物 2 黄色油状固体; ^1H NMR (CD_3OD , 400 MHz) δ : 2.25 (3H, s, H-13), 2.27 (3H, s, H-14), 2.95 (1H, d, $J = 15.7$ Hz, H-10), 3.12 (3H, s, H-11), 3.12 (1H, d, $J = 15.6$ Hz, H-10), 3.87 (1H, d, $J = 11.5$ Hz, H-12), 4.26 (1H, d, $J = 11.5$ Hz, H-12), 4.85 (1H, m, H-6), 4.93 (1H, m, H-5a), 5.68 (1H, d, $J = 9.7$ Hz, H-7), 5.94 (1H, m, H-8), 5.99 (1H, m, H-9); ^{13}C NMR (CD_3OD , 100 MHz) δ : 13.6 (C-14), 15.2 (C-13), 29.1 (C-11), 39.7 (C-10), 64.6 (C-12), 70.5 (C-5a), 73.1 (C-10a), 74.3 (C-3), 75.7 (C-6), 120.8 (C-9), 124.8 (C-8), 130.7 (C-7), 134.0 (C-9a), 167.7 (C-1), 168.4 (C-4)。以上氢谱和碳谱数据与文献^[3]报道的化合物 bis (dethio)bis (methylsulfanyl)gliotoxin 一致。

化合物 3 浅黄色粉末; ^1H NMR (CDCl_3 , 400 MHz) δ : 1.02 (1H, d, $J = 7.7$ Hz, H-19), 1.07 (3H, d, $J = 6.8$ Hz, H-29), 2.06 (3H, s, H-16), 2.14 (1H, d, $J = 15.1$ Hz, H-15b), 2.98 (1H, dd, $J = 15.1, 7.4$ Hz, H-15a), 3.71 (1H, m, H-20), 5.34 (1H, d, $J = 6.9$ Hz, H-18), 5.73 (1H, d, $J = 7.7$ Hz, H-14), 7.20 (1H, t, $J = 7.7$ Hz, H-26), 7.31 (1H, dd, $J = 7.7, 1.2$ Hz, H-25), 7.35 (1H, dd, $J = 6.4, 4.5$ Hz, H-27), 7.46 (1H, d, $J = 7.8$ Hz, H-24), 7.61 (1H, m, H-9), 7.80 (1H, d, $J = 7.1$ Hz, H-7), 7.85 (1H, m, H-8), 8.36 (1H, dd, $J = 8.2, 1.3$ Hz, H-10); ^{13}C NMR (CDCl_3 , 100 MHz) δ : 18.9 (C-29), 24.8 (C-16), 31.6 (C-15), 51.6 (C-14), 58.8 (C-20), 84.3 (C-3), 87.2 (C-17), 87.3 (C-18), 115.6 (C-24), 121.6 (C-11), 125.0 (C-27), 126.3 (C-26), 127.2 (C-10), 128.6 (C-7), 128.8 (C-9), 130.4 (C-25), 135.1 (C-8), 135.9 (C-23), 138.5 (C-28), 146.5 (C-6), 150.5 (C-4), 159.6 (C-12), 170.5 (C-1), 171.0 (C-21)。以上氢谱和碳谱数据与文献^[4]报道的化合物 fumiquinazoline C 一致。

化合物 4 白色粉末; ^1H NMR (CDCl_3 , 400 MHz) δ : 1.75 (3H, d, $J = 7.0$ Hz, H-12), 2.64 (1H,

d, $J = 12.8$ Hz, H-13a), 2.80 (1H, m, H-13b), 4.44 (1H, q, $J = 7.0$ Hz, H-11), 5.52 (1H, s, H-2), 7.22 (1H, td, $J = 7.4, 1.2$ Hz, H-6), 7.39 (1H, td, $J = 7.6, 1.3$ Hz, H-7), 7.49 (1H, d, $J = 7.8$ Hz, H-5), 7.43 (1H, d, $J = 7.8$ Hz, H-8), 7.63 (1H, t, $J = 7.6$ Hz, H-20), 7.69 (1H, d, $J = 8.1$ Hz, H-22), 7.88 (1H, td, $J = 7.2, 1.4$ Hz, H-21), 8.22 (1H, m, H-19); ^{13}C NMR (CDCl_3 , 100 MHz) δ : 14.7 (C-12), 38.3 (C-13), 50.9 (C-14), 60.5 (C-11), 77.8 (C-3), 83.4 (C-2), 115.9 (C-8), 121.8 (C-18), 124.3 (C-5), 126.3 (C-6), 127.1 (C-19), 127.8 (C-22), 127.6 (C-20), 131.3 (C-7), 134.9 (C-21), 134.6 (C-4), 139.3 (C-9), 147.2 (C-23), 147.7 (C-25), 161.2 (C-15), 166.3 (C-17), 171.7 (C-10)。以上氢谱和碳谱数据与文献^[5]报道的化合物 (-)-chaetomium 一致。

化合物 5 无色晶体; ^1H NMR (CDCl_3 , 400 MHz) δ : 1.01 (3H, s, H-28), 1.65 ~ 2.55 (2H, m, H-7), 1.65 ~ 2.55 (2H, m, H-8), 1.65 ~ 2.55 (2H, m, H-26), 1.72 (3H, s, H-29), 1.74 (3H, s, H-24), 2.00 (3H, s, H-25), 3.65 (2H, dd, $J = 8.7, 4.7$ Hz, H-9), 3.85 (3H, s, H-30), 4.03 (1H, brs, OH-12), 4.48 (1H, dd, $J = 9.5, 7.3$ Hz, H-6), 4.77 (1H, brs, OH-13), 5.05 (1H, d, $J = 8.0$ Hz, H-22), 5.66 (1H, s, H-13), 6.05 (1H, d, $J = 10.1$ Hz, H-3), 6.60 (1H, d, $J = 2.2$ Hz, H-19), 6.64 (1H, d, $J = 8.1$ Hz, H-21), 6.83 (1H, dd, $J = 8.8, 2.2$ Hz, H-17), 7.90 (1H, d, $J = 8.7$ Hz, H-16); ^{13}C NMR (CDCl_3 , 100 MHz) δ : 18.8 (C-24), 22.6 (C-8), 24.2 (C-25), 25.6 (C-29), 27.1 (C-28), 29.0 (C-7), 45.3 (C-26), 48.9 (C-3), 51.2 (C-9), 55.8 (C-30), 58.7 (C-6), 68.7 (C-13), 82.1 (C-27), 82.5 (C-12), 85.8 (C-21), 94.0 (C-19), 105.5 (C-14), 109.4 (C-17), 118.5 (C-22), 121.0 (C-15), 121.6 (C-16), 131.6 (C-2), 136.2 (C-20), 143.1 (C-23), 156.4 (C-18), 166.2 (C-5), 170.7 (C-11)。以上氢谱和碳谱数据与文献^[6]报道的化合物 verruculogen 一致。

化合物 6 淡黄色粉末; ^1H NMR (CD_3OD , 400 MHz) δ : 1.65 (3H, s, H-23), 1.96 (1H, m, H-8), 2.00 (3H, s, H-24), 2.06 (1H, m, H-8), 2.33 (2H, m, H-7), 2.99 (1H, dd, $J = 15.6, 11.8$ Hz, H-13b), 3.43 (1H, dd, $J = 15.7, 5.0$ Hz, H-13a), 3.58 (2H,

$m, H-9$), 3.80 (3H, s, OCH₃-18), 4.25 (1H, d, $J = 7.8$ Hz, H-6), 4.29 (1H, m, H-12), 6.02 (1H, d, $J = 9.5$ Hz, H-3), 6.71 (1H, m, H-17), 6.87 (1H, d, $J = 1.7$ Hz, H-19), 7.37 (1H, d, $J = 8.6$ Hz, H-16); ¹³C NMR (CD₃OD, 100 MHz) δ : 18.3 (C-24), 22.8 (C-13), 23.9 (C-8), 25.9 (C-23), 29.5 (C-7), 46.4 (C-9), 52.4 (C-3), 56.0 (18-OCH₃), 57.9 (C-12), 60.4 (C-6), 96.0 (C-19), 106.1 (C-14), 110.1 (C-17), 119.2 (C-16), 122.0 (C-15), 125.2 (C-21), 133.6 (C-2), 135.1 (C-22), 138.9 (C-20), 157.6 (C-18), 168.1 (C-11), 171.6 (C-5)。以上氢谱和碳谱数据与文献^[7]报道的化合物 fumigaclavine C 一致。

化合物 7 黄色粉末; ¹H NMR (CD₃OD, 400 MHz) δ : 0.94 (1H, m, H-19b), 1.67 (1H, m, H-18b), 1.74 (1H, m, H-18a), 1.98 (1H, m, H-19a), 3.23 (1H, m, H-17b), 3.28 (1H, m, H-10b), 3.32 (1H, m, H-10a), 3.45 (1H, m, H-17a), 3.99 (1H, ddd, $J = 10.8, 6.6, 1.8$ Hz, H-14), 4.41 (1H, dt, $J = 4.8, 1.8$ Hz, H-11), 7.01 (1H, m, H-6), 7.09 (1H, s, H-2), 7.10 (1H, m, H-7), 7.33 (1H, d, $J = 8.1$ Hz, H-8), 7.58 (1H, d, $J = 7.9$ Hz, H-5); ¹³C NMR (CD₃OD, 100 MHz) δ : 22.5 (C-18), 29.1 (C-19), 29.2 (C-10), 46.0 (C-17), 57.2 (C-11), 60.1 (C-14), 109.5 (C-3), 112.3 (C-8), 119.8 (C-5), 119.9 (C-6), 122.6 (C-7), 125.6 (C-2), 128.7 (C-4), 138.0 (C-9), 167.5 (C-16), 170.7 (C-13)。以上氢谱和碳谱数据与文献^[8]报道的化合物 brevianamide F 一致。

化合物 8 无色晶体; ¹H NMR (CDCl₃, 400 MHz) δ : 1.35 (3H, d, $J = 7.4$ Hz, H-18), 1.50 (3H, s, H-20), 1.50 (3H, s, H-21), 1.87 (3H, s, H-25), 2.05 (1H, m, H-8), 2.64 (3H, s, H-17), 2.88 (1H, m, H-4a), 2.93 (1H, m, H-7a), 2.99 (1H, m, H-7b), 3.06 (1H, br d, $J = 12$ Hz, H-5), 3.55 (1H, d, $J = 10.4$ Hz, H-10), 3.55 (1H, d, $J = 10.4$ Hz, H-4b), 5.09 (1H, m, H-23a), 5.13 (1H, m, H-23b), 5.67 (1H, br s, H-9), 6.07 (1H, dd, $J = 17.6, 10.4$ Hz, H-22), 6.70 (1H, d, $J = 7.1$ Hz, H-12), 7.03 (1H, m, H-14), 7.09 (1H, br d, $J = 8.0$ Hz, H-13); ¹³C NMR (CDCl₃, 100 MHz) δ : 16.1 (C-18), 21.2 (C-25), 26.4 (C-4), 27.4 (C-21), 27.5 (C-20), 32.9 (C-8), 38.3 (C-19), 39.1 (C-10), 42.9 (C-

17), 56.8 (C-7), 61.8 (C-5), 70.6 (C-9), 104.7 (C-3), 108.2 (C-13), 112.0 (C-23), 112.9 (C-12), 122.3 (C-14), 127.7 (C-16), 127.7 (C-11), 132.3 (C-15), 137.3 (C-2), 145.7 (C-22), 170.7 (C-24)。以上氢谱和碳谱数据与文献^[9]报道的化合物 fumigaclavine C 一致。

化合物 9 无色油状物; HR-ESI-MS *m/z*: 213, 1200 [M-H]⁻; ¹H NMR 谱中有 2 个烯氢质子 δ 5.56 (1H, d, $J = 1.4$ Hz, H-3a) 和 6.11 (1H, d, $J = 1.5$ Hz, H-3b), 显示有一个末端烯键; 一组长链烷基 δ 1.34 (6H, m, H-5, 6, 7), 1.49 (2H, m, H-8), 1.61 (2H, m, H-9), 2.28 (2H, t, $J = 7.4$ Hz, H-4), 2.28 (2H, t, $J = 7.4$, H-10); ¹³C NMR 谱中可以看到 11 个碳, 包括 2 个羧基碳 δ 170.7 (C-1), 177.8 (C-11), 2 个末端烯键碳 δ 125.2 (C-3), 142.7 (C-2) 和 7 个亚甲基 δ 26.1 (C-9), 29.7 (C-8), 30.1 (C-5), 30.1 (C-6), 30.1 (C-7), 32.9 (C-4), 34.9 (C-10)。其 C-1 的化学位移与文献^[10]中 C-2 位没有末端烯键的化合物 decanedioic acid 相比向高场移动了, 证明末端烯键与羧基碳 (C-1) 相连。¹H-¹H COSY 谱显示 H-4/H-5, H-9/H-10 相关, 显示 C-4 与 C-5 相连, C-9 与 C-10 相连, 根据以上 1D、2D 谱数据, 推测该化合物与文献^[11]化合物 dimethyl 2-methylene-neoctanedioate 较为相似, 后者仅在碳链上少了两个 CH₂, 且碳链两端羧基均酯化成羧甲基, 并结合¹H-¹H COSY 谱图(图 2), 确定化合物 9 的结构与文献^[12]报道的化合物 decanedioic acid, 2-methylene 一致。该化合物系首次从自然界分离得到, 是一个新的天然产物, 其结构鉴定数据如下。

¹H NMR (CD₃OD, 400 MHz) δ : 1.34 (6H, m, H-5, 6, 7), 1.49 (2H, m, H-8), 1.61 (2H, m, H-9), 2.28 (2H, t, $J = 7.4$ Hz, H-4), 2.28 (2H, t, $J = 7.4$, H-10), 5.56 (1H, d, $J = 1.4$ Hz, H-3a), 6.11 (1H, d, $J = 1.5$ Hz, H-3b); ¹³C NMR (CD₃OD, 100 MHz) δ : 26.1 (C-9), 29.7 (C-8), 30.1 (C-5), 30.1 (C-6), 30.1 (C-7), 32.9 (C-4), 34.9 (C-10), 125.2 (C-3), 142.7 (C-2), 170.7 (C-1), 177.8 (C-11)。

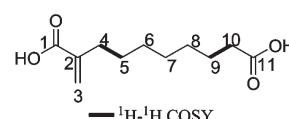


图 2 化合物 9 主要的¹H-¹H COSY 相关关系图

Fig. 2 Key HMBC correlations of compound 9

化合物 10 无色油状物; HR-ESI-MS m/z : 227.1349 [M-H]⁻; ¹H NMR 谱中有 2 个烯氢质子 δ 5.58 (1H, d, $J = 1.4$ Hz, H-3a), 6.11 (1H, d, $J = 1.5$ Hz, H-3b), 显示有 1 个末端烯键, 一个甲氧基 δ 3.74 (3H, s, CH₃O-1), 一组直链烷烃 δ 1.34 (6H, m, H-5, 6, 7), 1.46 (2H, m, H-8), 1.60 (2H, m, H-9), 2.28 (2H, t, $J = 7.4$ Hz, H-4), 2.28 (2H, t, $J = 7.4$, H-10); ¹³C NMR 谱中可以看到 12 个碳, 包括 1 个羧酸羰基 δ 177.8 (C-11), 1 个酯羰基 δ 169.3 (C-1), 2 个烯键碳 δ 125.4 (C-3), 142.7 (C-2), 1 个甲氧基 δ 52.3 (CH₃O-1) 和 7 个亚甲基 δ 26.1 (C-9), 29.6 (C-8), 30.1 (C-5), 30.1 (C-6), 30.1 (C-7), 32.9 (C-4), 35.0 (C-10)。HMBC 谱 H-4/C-1, H-3/C-1, H-3/C-4, CH₃O-1/C-1, H-9/C-11 的相关关系 (图 3) 显示甲氧基与 C-1 羰基相连, C-1 羰基与末端烯键 C-2 相连, C-2 与 C-4 相连, C-9 与 C-10 相连, C-10 与 C-11 羰基相连。其氢谱和碳谱数据与文献^[11] 化合物 dimethyl 2-methyleneoctanedioate 较为相似, 后者仅在碳链上少了两个 CH₂, 且末端羧基酯化成羧甲基, 经过 1D、2D NMR 解析, 且与化合物 9 数据比较, 化合物 10 仅是化合物 9 的 C-1 羰基酯化成羧甲基, 确定化合物 10 的结构为 decanedioic acid, 2-methylene-, 1-methyl ester, 经查证,, 该化合物是一个合成的商业化学原料, 系首次从自然界中分离得到, 并且首次提供氢谱碳谱数据, 是一个新天然产物, 其结构鉴定数据如下。

¹H NMR (CD₃OD, 400 MHz) δ : 1.34 (6H, m, H-5, 6, 7), 1.46 (2H, m, H-8), 1.60 (2H, m, H-9), 2.28 (2H, t, $J = 7.4$ Hz, H-4), 2.28 (2H, t, $J = 7.4$, H-10), 3.74 (3H, s, CH₃O-1), 5.58 (1H, d, $J = 1.4$ Hz, H-3a); ¹³C NMR (CD₃OD, 100 MHz) δ : 26.1 (C-9), 29.6 (C-8), 30.1 (C-5), 30.1 (C-6), 30.1 (C-7), 32.9 (C-4), 35.0 (C-10), 52.3 (CH₃O-1), 125.4 (C-3), 142.7 (C-2), 169.3 (C-1), 177.8 (C-11)。

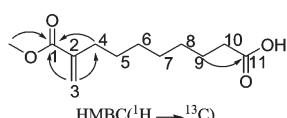


图 3 化合物 10 主要的 HMBC 相关关系图

Fig. 3 Key HMBC correlations of compound 10

化合物 11 无色油状物; ¹H NMR 谱有两个烯氢质子 δ 5.79 (1H, d, $J = 15.6$ Hz, H-2), 6.94 (1H,

dt, $J = 7.0, 15.5$ Hz, H-3), 显示有一个反式取代的烯键; 一组长链烷基 δ 1.35 (2H, m, H-5), 1.35 (2H, m, H-6), 1.35 (2H, m, H-7), 1.48 (2H, m, H-8), 1.60 (2H, m, H-9), 2.22 (2H, td, $J = 1.3, 8.2$ Hz, H-4), 2.28 (2H, t, $J = 7.4$ Hz, H-10); ¹³C NMR 谱中显示有 11 个碳信号, 包括 2 个羧基碳 δ 170.5 (C-1), 177.9 (C-11), 2 个烯键碳 δ 122.7 (C-2), 151.0 (C-3), 和 7 个亚甲基 δ 26.1 (C-9), 29.2 (C-8), 30.1 (C-5), 30.1 (C-6), 30.1 (C-7), 33.1 (C-4), 35.0 (C-10), 且烯键与羧基碳 (C-1) 相连。以上氢谱和碳谱数据与文献^[13] 中化合物 *trans*-2-decenedioic acid 非常相似, 前者仅在碳链中多一个 CH₂, 经 1D、2D NMR 分析, 化合物 11 结构与文献^[14] 中的 2-undecenedioic acid 一致。¹H NMR (CD₃OD, 400 MHz) δ : 1.35 (2H, m, H-5), 1.35 (2H, m, H-6), 1.35 (2H, m, H-7), 1.48 (2H, m, H-8), 1.60 (2H, m, H-9), 2.22 (2H, td, $J = 1.3, 8.2$ Hz, H-4), 2.28 (2H, t, $J = 7.4$ Hz, H-10), 5.79 (1H, d, $J = 15.6$ Hz, H-2), 6.94 (1H, dt, $J = 7.0, 15.5$ Hz, H-3); ¹³C NMR (CD₃OD, 100 MHz) δ : 26.1 (C-9), 29.2 (C-8), 30.1 (C-5), 30.1 (C-6), 30.1 (C-7), 33.1 (C-4), 35.0 (C-10), 122.7 (C-2), 151.0 (C-3), 170.5 (C-1), 177.9 (C-11)。

化合物 12 无色油状物; HR-ESI-MS m/z : 227.1362 [M-H]⁻; ¹H NMR 谱有 2 个烯氢质子 δ 5.84 (1H, d, $J = 15.7$ Hz, H-2), 6.97 (1H, dt, $J = 15.8, 6.9$ Hz, H-3), 1 个甲氧基 δ 3.71 (3H, s, CH₃O-1), 一组长链烷烃 δ 1.34 (2H, m, H-5), 1.34 (2H, m, H-6), 1.34 (2H, m, H-7), 1.47 (2H, m, H-8), 1.60 (2H, m, H-9), 2.28 (2H, m, H-4), 2.28 (2H, m, H-10); ¹³C NMR 显示有 12 个碳信号, 包括 1 个羧酸羰基 δ 178.0 (C-11), 1 个酯羰基 δ 168.9 (C-1), 2 个烯键碳 δ 121.8 (C-2), 151.3 (C-3), 1 个甲氧基 δ 51.9 (C-OCH₃), 和 7 个亚甲基 δ 26.1 (C-9), 29.1 (C-8), 30.0 (C-5), 30.1 (C-6), 30.1 (C-7), 33.1 (C-4), 35.0 (C-10); HMBC 谱中 (图 4) CH₃O-1/C-1, H-2/C-4, H-3/C-1, H-9/C-11 以及 ¹H-¹H COSY 谱中 (图 4) H-3/H4, H4/H-5, H-9/H-10 的相关关系显示甲氧基与 C-1 羰基相连, C-1 羰基与烯键相连, C-3 与 C-4 相连, C-4 与 C-5 相连, C-9 与 C-10 相连, C-10 与 C-11 相连。以上氢谱和碳谱数据与文献^[13] 中化合物 *trans*-2-decenedioic acid 非常相似, 前者仅在碳链中多一个 CH₂, 且 C-1

羧基酯化成羧甲基,经1D、2D NMR分析并与化合物**11**比较,化合物**12**仅是化合物**11**的C-1羧基酯化成羧甲基,确定化合物**12**的结构与文献^[15]中的2-undecenedioic acid,1-methyl ester一致。该化合物系首次从自然界分离得到,是一个新的天然产物,其结构鉴定数据如下。

¹H NMR (CD₃OD, 400 MHz) δ: 1.34 (2H, m, H-5), 1.34 (2H, m, H-6), 1.34 (2H, m, H-7), 1.47 (2H, m, H-8), 1.60 (2H, m, H-9), 2.28 (2H, m, H-4), 2.28 (2H, m, H-10), 3.71 (3H, s, CH₃O-1), 5.84 (1H, d, J = 15.7 Hz, H-2), 6.97 (1H, dt, J = 15.8, 6.9 Hz, H-3); ¹³C NMR (CD₃OD, 100 MHz) δ: 26.1 (C-9), 29.1 (C-8), 30.0 (C-5), 30.1 (C-6), 30.1 (C-7), 33.1 (C-4), 35.0 (C-10), 51.9 (C-OCH₃), 121.8 (C-2), 151.3 (C-3), 168.9 (C-1), 178.0 (C-11)。

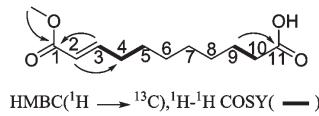


图4 化合物**12**主要的HMBC和¹H-¹H COSY相关关系图

Fig. 4 Key HMBC and ¹H-¹H COSY correlations of compound **12**

化合物13 无色针状晶体; ¹H NMR (CDCl₃, 400 MHz) δ: 0.94 (3H, s, H-18), 1.18 (3H, s, H-29), 1.28 (3H, d, J = 6.8 Hz, H-28), 1.45 (3H, s, H-19), 1.57 (1H, m, H-11a), 1.61 (3H, s, H-26), 1.69 (3H, s, H-27), 1.81 (1H, m, H-12a), 1.92 (1H, m, H-15a), 1.97 (3H, s, 16-OCOCH₃), 1.98 (1H, m, H-11b), 2.09 (1H, m, H-23), 2.11 (3H, s, 6-OCOCH₃), 2.14 (1H, m, H-23), 2.22 (1H, m, H-15b), 2.27 (1H, d, J = 12.2 Hz, H-5), 2.41 (1H, m, H-12b), 2.47 (2H, m, H-22), 2.57 (1H, m, H-13), 2.62 (1H, m, H-9), 2.77 (1H, dd, J = 12.3, 6.7 Hz, H-4), 5.11 (1H, m, H-24), 5.24 (1H, s, H-6), 5.86 (1H, d, J = 9.9 Hz, H-2), 5.91 (1H, d, J = 7.9 Hz, H-16), 7.30 (1H, d, J = 10.0 Hz, H-1)。以上氢谱数据与文献^[16]报道的化合物helvolic acid一致。

化合物14 无色油状物; ¹H NMR (CD₃OD, 400 MHz) δ: 0.92 (6H, m, H-7', 7''), 0.96 (6H, m, H₃-9', 9''), 1.27 ~ 1.47 (16H, m, H-4', 4'', 5', 5'', 6', 6'', 8', 8''), 1.69 (2H, m, H-3', 3''), 4.22 (4H, dd, J = 5.7, 1.9 Hz, H-2', 2''), 7.62 (2H, dd, J = 5.7,

3.3 Hz, H-3, 4), 7.72 (2H, dd, J = 5.8, 3.3 Hz, H-2, 5'); ¹³C NMR (CD₃OD, 100 MHz) δ: 11.4 (C-7', 7''), 14.4 (C-9', 9''), 24.0 (C-4', 4''), 25.0 (C-5', 5''), 30.1 (C-6', 6''), 31.6 (C-8', 8''), 40.2 (C-3', 3''), 69.1 (C-2', 2''), 129.9 (C-3, 4), 132.4 (C-1, 6, 2, 5), 169.4 (C-1', 1'')”。以上氢谱与碳谱数据与文献^[17]报道的化合物di-2-ethyl hexyl phthalate一致。

化合物15 黄色晶体; ESI-MS *m/z*: 285.0758 [M + H]⁺; ¹H NMR (CD₃OD, 400 MHz) δ: 2.42 (3H, s, CH₃), 3.97 (3H, s, OCH₃), 6.83 (1H, d, J = 2.4 Hz, H-7), 7.09 (1H, brs, H-2), 7.29 (1H, d, J = 2.4 Hz, H-5), 7.53 (1H, s, H-4)。以上氢谱和碳谱数据与文献^[18]报道的化合物physcione一致。

化合物16 黄色针晶; ¹H NMR (CD₃OD, 400 MHz) δ: 2.43 (3H, s, CH₃-3), 6.55 (1H, d, J = 2.4 Hz, H-4), 7.09 (1H, d, J = 1.6 Hz, H-5), 7.17 (1H, d, J = 2.4 Hz, H-2), 7.56 (1H, d, J = 1.6 Hz, H-7)。以上氢谱和碳谱数据与文献^[19]报道的化合物emodin一致。

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