نواتج أيض نشطة بيولوجيا من مرجان البحر الاحمر سينيولاريا بوليذاكتيلا

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المستخلص: تم عزل و تعريف مركب تربين ثنائي، ساركتروكيلدايول (١) و كذلك مركب سيسكوتربين ينتمي الى مجموعة الافيكان، أفريكانين(٢) و كذلك مركبين ينتمون الى السيسكوتربينات من مجموعة الاروماندريين، ١٠ (١٤)-اروماندريين (٣) و البالوسترول (٤) من مرجان البحر الاحمر الرخو سينيولاريا بوليذاكتيلا. التركيب الكيميائي للمركبات جميعها تم باستخدام الطرق الطيفية (الرنين النوويالمغناطيسي، مطياف الكتلة، طيف الاشعة تحت الحمراء وطيف الاشعة فوق البنفسجية). تم تعيين نشاط هذه المركبات كمضادات لبعض انواع من البكتيريا.

The data obtained from Table 2 shows that 1 exhibits potent activity against *B. subtilis* and *P. aeruginosa*. While the 1, 2 and 4 show potent activity against *B. subtilis*.

Table 2. Diameter of inhibition zone in mm at concentration level of 1 mg/ml and Minimum inhibitory concentration (MIC) in mg/ml

Compound	<i>B. subtilis</i> MIC (mg/ml)	<i>P. aeruginosa</i> MIC (mg/ml)
1	17.9	18.2
	0.4	0.2
2	12.7	9.8
	0.6	-
3	17.9	10.0
	0.3	-
4	17.6	11.1
	0.5	-

Acknowledgement

The author thanks the marine biologist Mr. Kamal Al-Dahoudy, Faculty of Maritime, King Abdulaziz University for collection and identification of the sample.

References

- Abdel-Lateff, A., Alarif, W.M., Ayyad, S-E.N., Al-Lihaibi, S.S. and Basaif, S.A. (2014) New cytotoxic isoprenoid derivatives from the red sea soft coral *Sarcophyton glaucum*. *Natural product Research*, **29**: 24-30.
- Al-Lihaibi, S.S., Alarif, W.M., Abdel-Lateef, A., Ayyad, S-E. N., Abdel-Naim, A.B., El-Senduny, F.E. and Badria, F.A. (2014) Three new cembranoid-type diterpenes from Red Sea soft coral *Sarcophyton glaucum*: isolation and Antiproliferative activity against HepG2 cells, *European Journal Medicinal Chemistry*, **81**: 314-322.
- Barnes, R.D. (1968) Invertebrate zoology (2nd ed., pp. 101–113). Philadelphia: Saunders.
- Blunt, J.W., Brent, C.R., Munro, H.G., Peter, T. N. and Michele, R.P. (2003) Marine natural products. *Natural Product Reports*, **20**: 1–48.
- Cheer, C.J., Smith, D.H., Djerassi, C., Tursch, B., Braekman, I. C. and Daloz, D. (1976) Applications of artificial intelligence for chemical inference—XXI: Chemical studies of marine invertebrates—XVII. The computer-assisted identification of [4]-palustrol in the marine organism *Cespitularia* sp., aff. subviridis. *Tetrahedron*, **32**: 1807- 1810.
- Chen, W-T., Liu, H-L., Yao, L-G. and Guo, Y-w. (2015) 9,11-Secosteroids and polyhydroxylated steroids from two South China Sea soft corals *Sarcophyton trocheliophorum* and *Simularia flexibilis*. *Steroids*, **92**: 56-61.
- Gruickshank, R., Duguid, J. R., Marmion, B. P. and Swain, R. H. A. (1975) *Medicinal Microbiology*, Vol. 11. Churchill Livingstone, New York, p. 190.
- Kashman, Y., Bodner, M., Finer-Moore, J. S. and Clardy, J. (1980) $\Delta^{9(15)}$ -africanene a new sesquiterpene hydrocarbon from the soft coral *Simularia erecta*. *Experientia*, **36**: 891–892.
- Lakshmi, V. and Kumar, R. (2009) Metabolites from *Simularia* species. *Natural Product Research*, **23**, 801–850.
- Lei, L-F., Chen, M-F., Wang, T., He, X-X., Liu, B-X., Deng, Y., Chen, X-J., Li, Y-T., Guan, S-Y., Yao, J-H., Li, W., Ye, W-C., Zhang, D-M. and Zhang, C-X. (2014) Novel cytotoxic nine-membered macrocyclic polysulfur cembranoid lactones from the soft coral *Simularia* sp., *Tetrahedron*, **70**: 6851-6858.
- Reddy, G.B.S., Rao, D.V., Rao, C.B., Dhananjaya, N., Kuttan, R., and Babu, T.D. (1999) Isolation and structural determination of new sphingolipids and pharmacological activity of africanene and other metabolites from *Simularia leptoclados*. *Chemical & Pharmaceutical Bulletin*, **47**: 1214–1220.
- Scheuer, P.J. (1987) *Marine Natural Products, Chemical and Biological Perspective*. Terpenoids from Coelenterates, Academic Press, New York, pp 247 – 296.
- Warmers, U. and König, W.A. (1999) *Sesquiterpene constituents of the liverwort Calypogeia fissa*. *Phytochemistry*, **52**: 695–704.
- Zubair, M. S., Alarif, W. M., Al-Footy, Kh. O., Mohamed PH, Aly, M. M., Basaif, S. A., Al-Lihaibi, S. S. and Ayyad, S-E. N. (2016) New antimicrobial biscembranoid hydrocarbon and cembranoid diterpenes from the soft coral *Sarcophyton trocheliophorum*, *Turkish Journal of Chemistry*, **40** (3): 385-392.

Table 1. ^1H - and ^{13}C -NMR (at 600 and 150 MHz, resp., in CDCl_3) spectral data^a of **1** and **2**. Atom numbering as indicated in Fig.1

	1		2	
	$\delta(\text{C})$	$\delta(\text{H}), J \text{ Hz}$	$\delta(\text{C})$	$\delta(\text{H}) J \text{ Hz}$
1	45.6 (<i>d</i>)	1.34-1.36 (<i>m</i>)	52.7 (<i>d</i>)	1.32 (<i>m</i>)
2	72.0 (<i>d</i>)	4.62 (<i>dd</i> , 10.8; 4.8)	18.8 (<i>s</i>)	-
3	124.4 (<i>d</i>)	5.28 (<i>d</i> , 10.8)	23.3 (<i>t</i>)	0.54 (<i>ddd</i> , 12.0, 8.4, 3.6) 0.19 (<i>t</i> , 4.2)
4	141.2 (<i>s</i>)	-	22.0 (<i>d</i>)	0.49 (<i>m</i>)
5	42.5 (<i>t</i>)	2.75-2.80 (<i>dd</i> , 12.0, 10.8 H_a), 2.54-2.57 (<i>dd</i> , 12.0, 4.2 H_b)	43.2 (<i>t</i>)	1.81 (<i>m</i>) 1.11 (<i>m</i>)
6	128.2 (<i>d</i>)	5.84-5.89 (<i>dddd</i> , 15.6, 10.8, 4.2)	33.6 (<i>s</i>)	-
7	135.7 (<i>d</i>)	5.41 (<i>brd</i> , 15.6)	51.2 (<i>t</i>)	1.73 (<i>m</i>) 1.08 (<i>m</i>)
8	73.6 (<i>s</i>)	-	42.2 (<i>d</i>)	2.43 (<i>ddd</i> , 9, 6, 2.4)
9	39.9 (<i>t</i>)	1.54-1.56 (<i>m</i> , H_a), 1.86-1.88 (<i>m</i> , H_b)	158.3 (<i>s</i>)	-
10	22.8 (<i>t</i>)	1.74-1.76 (<i>m</i> , H_a), 1.26-1.30 (<i>m</i> , H_b)	33.6 (<i>t</i>)	2.41 (<i>ddd</i> , 8.4, 7.2, 1.2) 2.25 (<i>m</i>)
11	75.8.6 (<i>d</i>)	3.48 (<i>d</i> , 9.6)	27.7 (<i>t</i>)	1.70 (<i>m</i>), 1.57 (<i>m</i>)
12	74.9 (<i>s</i>)	-	24.1 (<i>q</i>)	1.05 (<i>s</i>)
13	18.6 (<i>t</i>)	1.62-1.64 (<i>m</i> , H_a), 1.35-1.36 (<i>m</i> , H_b)	20.5 (<i>q</i>)	0.98 (<i>s</i>)
14	34.1 (<i>t</i>)	2.28-2.32 (<i>m</i> , H_a), 1.20-1.22 (<i>m</i> , H_b)	33.9 (<i>d</i>)	0.90 (<i>s</i>)
15	29.4 (<i>d</i>)	1.20-1.22 (<i>m</i>)	106.3 (<i>t</i>)	4.86 (<i>q</i> , 1.2)-4.72 (<i>s</i>)
16	20.7 (<i>q</i>)	0.88 (<i>d</i> , 6.6)		
17	20.5 (<i>q</i>)	0.76 (<i>d</i> , 6.6)		
18	17.6 (<i>q</i>)	1.83 (<i>s</i>)		
19	29.3 (<i>q</i>)	1.33 (<i>s</i>)		
20	23.8 (<i>q</i>)	1.04 (<i>s</i>)		

^a All assignments are based on 1D and 2D recordings (HMBC, HSQC, COSY)

Compounds $\Delta^{9(15)}$ -africanene (**2**), 10(14)-aromadendrene (**3**) and palustrol (**4**), were identified by comparison of their spectral data with those in the literature (Kashman *et al.*, 1980; Warmers and König, 1999; Cheer *et al.*, 1976).

Biological activity of the compounds **1-4**, were screened for their antibacterial activity by disk-diffusion technique (Gruickshank *et al.*,

1975) against Gram-positive (*Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonas. aeruginosa*), at 1 mg/ml concentration. The inhibited zone diameters were measured and recorded in Table 2. Furacin was used as a standard compound. The minimal inhibitory concentration (MIC) of the strongly active compounds were also measured and listed in Table 2.

18.7 (CH₃, C-14), 17.7 (CH₃, C-15), 15.6 (CH₃, C-12).

Results and Discussion

The CH₂Cl₂-soluble material of the CHCl₃:MeOH extract of the soft bodied organism *Simularia polydactyla* was subjected to successive column and PTLC chromatographic purification processes to obtain **1** (3.5 mg, 0.0044%), **2** (3.6 mg, 0.0046%), **3** (2.8 mg, 0.0035%) and **4** (5.5 mg, 0.0069%).

Compound **1** was obtained as gummy material. **1** was analysed for C₂₀H₃₄O₃ on the basis of HREIMS at m/z 322.2500 (M)⁺ and ¹³C NMR spectra, implying four degrees of unsaturation. The ¹³C (*cf. exp.*) and DEPT NMR experiments allowed the determination of 20 resonances attributable to five Me carbons (δ_C 17.6, 20.5, 20.7, 23.8, and 29.3), five sp³ CH₂ carbons (42.5, 39.9, 22.8, 34.1, and 18.6), four sp³ CH carbons including two oxygen-bearing (72.0, and 75.8), three sp² CH (124.4, 128.2, and 135.7) and three quaternary C-atoms including two O-bearing (73.6 and 74.9). The ¹H NMR and COSY NMR of **1** displayed three Me singlet signals (δ_H 1.83, 1.33, 1.04), one ⁱPr [δ_H 1.22 (m, 1H), 0.88 (d, J = 6.6 Hz, 3H) and 0.76 (d, J = 6.6 Hz, 3H)], three olefinic protons [δ_H 5.28 (d, J = 10.8 Hz), 5.85 (ddd, J = 15.6, 10.8, 4.2 Hz) and 5.41 (br d, J = 15.6 Hz)] and two O-bearing CH protons [δ_H 4.62 (dd, J = 10.8, 4.8 Hz), 3.48 (d, J = 9.6 Hz)]. The NMR data of **1** displayed great similarity to those of known sarcotrocheliol (**5**) (Al-Lihaibi *et al.*, 2014).

The main difference were signals at δ_C 128.2 and 135.7, suggesting the presence of a

disubstituted double bond system in **1**, instead of a trisubstituted double bond system in **5**, and a signal at 73.6, indicating an extra oxygenated quaternary carbon. The ¹³C NMR signals at δ_C 124.4 and 141.2 indicated a non-conjugated trisubstituted double bond belongs to C-3-C-4 based on the HMBC correlation with Me-18, also ¹³C NMR signals at δ_C 128.2 and 135.7 were assigned as non-conjugated disubstituted double bonds belongs to C-6-C-7 by HMBC correlation between Me-19 and C-7. Moreover, ¹H-¹H COSY spectral analysis established three partial structures of consecutive proton spin systems from H-5 to H-7 through H-6; from H-9 to H-11 through H-10, and finally from H-3 to H-13 through H-2, H-1 (H-15), H-14 and H-13. These data, together with the HMBC correlations, from H-16 to C-15, C-17, and C-1; from H-18 to C-3, C-4, and C-5; from H-19 to C-7, C-8, and C-9 and from H-20 to C-12, C-11 and C-13 have established the connectivity within the 14-membered ring (Figure 2).

Figure 2. Selected ¹H-¹H COSY (—) and HMB (---) correlation of compound **1**

Analysis of all the NMR spectroscopic data confirmed **1** to be a cembranoid derivative, **1** was previously isolated by Zubair *et al.* 2016 but from the genus sarcophyton and given the name sarcotrocheldiol A

and further purified by preparative TLC of glass supported silica gel plates (20 cm x 20 cm) of 250 μ m thickness.

The fraction A eluted with 100% *n*-hexane (19:1, 23.00 mg) was purified by preparative TLC using solvent system (100%) *n*-hexane. The band with R_f 0.95 (violet color with sulfuric acid - methanol) was taken to give compound **3** as colorless oil (3.5 mg). The fraction B eluted with *n*-hexane:ethyl acetate (19:1, 15.00 mg) was purified by preparative TLC using the solvent system *n*-hexane:ethyl acetate (19:1), the band with R_f = 0.68 (orange-brown color with sulfuric acid-methanol) was taken to give colorless oil (3.6 mg) compound (**2**). The fraction C eluted with *n*-hexane:ethyl acetate (4:1, 30.00 mg) was purified by preparative TLC using the solvent system *n*-hexane:ethyl acetate (7:3). The band with R_f = 0.56 (violet color with sulfuric acid-methanol) was taken to give colorless oil (5.50 mg) of compound **4**.

The fraction D eluted with CH_2Cl_2 : MeOH (9:1, 35.00 mg) was purified by preparative TLC using the solvent system *n*-hexane: EtOAc (1:4) to give two bands. The first band with R_f 0.38 (brown color with H_2SO_4 -MeOH) was taken to give colorless oil (3.5 mg) of compound **1**.

Sinulardiol (**1**): (3E, 6E)-1-isopropyl-4,8,13-trimethyl-15-oxa-bicyclo [10.2.1] pentadeca-3,6-dien-8,14-diol; Colorless oil; IR λ_{max} (film) cm^{-1} : 3423 (O-H), 3180 (O-H), 2937 (C-H), 1645 (C=C), 1378, 1221, 1045; ^{13}C NMR (CDCl_3 , 150 MHz) and ^1H NMR (CDCl_3 , 600 MHz) spectroscopic data (Table 1); LCESIMS (negative mode) m/z 321.48 [M-H] $^-$ (Calculated m/z 322.48 for $\text{C}_{20}\text{H}_{34}\text{O}_2$).

$\Delta^{9(15)}$ -africanene (**2**)

Colorless oil; ^{13}C NMR (CDCl_3 , 150 MHz) and ^1H NMR (CDCl_3 , 600 MHz) spectroscopic data (Table 1)

10(14)-aromadendrene (**3**)

Colorless oil, ^1H NMR (CDCl_3 , 600 MHz): δ = 2.66 (1H, m, H-1), 1.86 (1H, m, H_a -2), 1.70 (H, m, H_b -2), 1.75 (1H, m, H_a -3), 1.32 (H, m, H_b -3), 1.88 (1H, m, H-4), 2.07 (1H, m, H-5), 0.25 (1H, dd, J = 10.2, 9.0 Hz, H-6), 0.55 (1H, ddd, J = 10.8, 9.0, 6.0 Hz, H-7), 1.82 (1H, m, H_a -8), 1.22 (1H, m, H_b -8), 2.30 (2H, m, H_2 -9), 0.96 (3H, s, H_3 -12), 1.00 (3H, s, H_3 -13), 4.70 (1H, dd, J = 2.4, 1.8 Hz, H_a -14), 4.73 (1H, ddd, J = 2.4, 1.8, 1.2 Hz, H_b -14), 0.94 (3H, d, J = 7.2 Hz, H_3 -15); ^{13}C NMR (CDCl_3 , 150 MHz): δ = 152.6 (C, C-10), 109.7 (CH_2 , C-14), 50.8 (CH, C-1), 42.2 (CH, C-4), 37.8 (CH, C-5), 35.7 (CH_2 , C-9), 31.2 (CH_2 , C-3), 28.6 (CH_3 , C-13), 28.2 (CH, C-2), 24.8 (CH, C-7), 23.5 (CH, C-6), 22.3 (C, C-8), 17.2 (C, C-11), 16.4 (CH_3 , C-15), 15.9 (CH_3 , C-14).

Palustrol (**4**)

Colorless oil, ^1H NMR (CDCl_3 , 600 MHz): δ = 1.84 (1H, m, H_a -2), 1.37 (1H, m, H_b -2), 1.82 (2H, m, H_2 -3), 2.15 (1H, m, H-4), 1.60 (1H, dd, J = 10.2, 10.2 Hz, H-5), 0.51 (1H, dd, J = 10.8, 9 Hz, H-6), 0.68 (1H, ddd, J = 10.8, 9, 6.6 Hz, H-7), 1.88 (1H, m, H_a -8), 1.06 (1H, m, H_b -8), 1.47 (1H, m, H_a -9), 1.35 (1H, m, H_b -9), 1.27 (1H, m, H-10), 1.03 (3H, s, H_3 -12), 1.06 (3H, s, H_3 -13), 0.98 (3H, d, J = 6.6 Hz, H_3 -14), 0.92 (3H, d, J = 6.6 Hz, H_3 -15); ^{13}C NMR (CDCl_3 , 150 MHz): δ = 85.7 (CH, C-1), 47.2 (CH, C-5), 46.1 (CH, C-10), 38.9 (CH_2 , C-2), 35.0 (CH, C-4), 32.8 (CH_2 , C-3), 32.3 (CH_2 , C-9), 28.9 (CH_3 , C-13), 27.1 (CH, C-7), 24.5 (CH_2 , C-8), 22.0 (CH, C-6), 19.9 (C, C-11),

determination of the inhibition of the Ehrlich ascites tumor was made in case of africanene (Reddy *et al.*, 1999).

In the course of our continued program in investigation of the Red sea organisms screening for bioactive compounds, a specimen of the common soft coral along Jeddah's coast, *Sinularia polydactyla* was selected. The known cembranoid diterpene alcohol sarcotrocheldiol A (**1**), in addition to three known sesquiterpenoids, $\Delta^{9(15)}$ – africanene (**2**), 10(14)-aromadendrene (**3**) and palustrol (**4**), which were identified by comparison of their spectral data with those in literature (Kashman *et al.*, 1980; Warmers and König, 1999; Cheer *et al.*, 1976) (Figure 1). The antimicrobial activity of the previous metabolites was also evaluated.

Materials and Methods

General

Chromatographic material: Silica gel 60 for column chromatography (60-120 mesh LR), for thin layer chromatography TLC aluminum sheets 20 × 20 cm Silica gel 60 F₂₅₄ was used; for preparative, Pre-coated TLC glass plates SIL G-25 UV₂₅₄, 0.25 mm silica gel were used. Optical rotations were measured by ATAGO POLAX-L 2 polarimeter. Nuclear Magnetic Resonance spectra were recorded for 1D- and 2D-NMR: Bruker AVANCE III WM at 600 MHz and ¹³C-NMR at 150 MHz spectrometer. Chemical shifts are given in δ (ppm) relative to TMS as internal standard. Electron impact mass spectra were determined at 70 eV on a Kratos GCMS-25 instrument.

The spray reagent is methanol- sulfuric acid. A freshly prepared solution was made by adding

concentrated sulfuric acid 50 ml to methanol 50 ml.

Figure 1. Compounds isolated from *Sinularia polydactyla*

The soft coral *Sinularia polydactyla* was collected in Dec. 2016, off the Saudi Arabia Red Sea Coast at Jeddah. Voucher sample (JAD 09080) was deposited at the Chemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

Extraction and Isolation

Coral material was washed with water and dried in the shade at room temperature. The dried material (80.00 g) was exhaustively extracted with equal volumes of CHCl₃/MeOH (2 x 6 L, 24 hours for each batch) at room temperature. The residue (20.25 g) was partitioned between CH₂Cl₂ and water, the organic layer was dried (8 g) chromatographed on NP (Merck, 60G) column chromatography employing *n*-hexane/ethyl acetate mixtures with increasing polarity. Fractions of ~50 ml were collected. TLC was carried out by employing silica-gel chromatoplates, using the appropriate solvent system and 50%-sulfuric acid in methanol as spraying reagent. Fractions containing a single compound were combined

Bioactive metabolites from the Red Sea soft coral *Sinularia polydactyla*

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Abstract: A cembranoid diterpene alcohol (1), along with the africane-type squiterpene $\Delta^{9(15)}$ -africanene (2), and two aromadendrane-type sesquiterpenoids; 10(14)-aromadendrene (3) and palustrol (4) have been isolated from the organic extract of the soft coral *Sinularia polydactyla*. Chemical structures were established on the basis of NMR, MS, IR spectroscopic analysis and comparison with literature. The antibacterial activity of all isolated compounds was evaluated.

Keywords: Red Sea, octacorallia, diterpenes, sesquiterpenes, antimicrobial

Introduction

Soft bodied corals are a group of colonial invertebrates [phylum Coelentrata (cnidaria), class Anthozoa, subclass octacorallia, orders Alcyonaceae] (Barnes, 1968). Members of the genus *Sinularia* are widely distributed in the tropical reef environment. Out of one hundred identified species of the genus *Sinularia*, only fourteen have been investigated for secondary metabolite contents (Blunt et al., 2003; Faulkner, 1986). Literature screening revealed the richness of the *Sinularia* species with natural products, such as, seco- and polyhydroxylated sterols, sesquiterpenoids, diterpenoids, and nitrogen containing compounds (Chen et al., 2015; Lei et al., 2014; Lakshmi and Kumar, 2009).

The *Sinularia* isolated metabolites were shown to possess an appreciated spectrum of biological activities, including cytotoxic, antifouling, antimicrobial, anti-inflammatory, histamine-release and inhibition activities (Lakshmi and Kumar, 2009). The genus

Sinularia is a prolific source of biologically active diterpenoids, especially those with cembrane carbon skeleton. Cembranoids are a 14-membered carbocyclic skeleton, festooned with carbon-carbon double bond(s) and methyl groups. This framework is easily exposed to oxidation, photochemical, rearrangement and enzymatic processes leading to the formation of cyclic ether, lactone, pyran, or furan moieties around the cembrane skeleton (Abdel-Lateff et al., 2014). Next to diterpenoids, sesquiterpenes come as second common terpenoids especially those with africane-type squiterpenoid, as major secondary metabolites from the genus *Sinularia*. The sesquiterpenoid, africanene (2) was tested for short term in vitro cytotoxic activity against the Ehrlich ascites carcinoma (EAC) and Dalton's lymphoma ascites (DLAT) tumor cells at 10 mg mL⁻¹ concentration, which caused the death of the EAC and DLAT cells to 100% and 92%, respectively. While in *in vivo* cytotoxic assay,