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Note

A new scalarane sesterterpene from a marine sponge *Hyatella cribrifomis* Hyatt of the Indian ocean

M M Krishna Kumar, N Krishna, P Muralidhar
B S Sastry & D Venkata Rao*

University College of Pharmaceutical Sciences,
Andhra University, Visakhapatnam 530 003, India

E mail: dvraodod@rediffmail.com

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A new scalarane sesterterpene, 24-*β*-methoxyscalarolide **4**, along with known scalaranes- 12-*epi*-deoxoscalarin **1**, 12-*epi*-12-deacetyl-25-deoxyscalarin **2** and scalarolide **3** has been isolated from a marine sponge, *Hyatella cribriformis*, of the Indian ocean. The structure of compound **4** has been determined on the basis of ^{1}H and ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY and EIMS experiments.

Keywords: *Hyatella cribriformis*, marine sponge, scalarane sesterterpenes.

Scalarane-based metabolites occur in marine sponges belonging to the families Thorectidae and Spongiidae (order Dictyoceratida)[1,2] and in molluscs feeding on them [3-5]. Sponges of the family Spongiidae have been studied extensively and have given rise to a great array of sesterterpenoids. Scalaranes have been reported to exhibit a wide spectrum of biological activities including cytotoxicity[1,2,6], ichthyotoxicity[7,8], anti-inflammatory[9], antimicrobial[10], anti-HIV[11], antithrombocyte, and vasodilatory properties[12].

Results and Discussion

The compounds **1, 2** and **3** were identified as 12-*epi*-deoxoscalarin[13,14], 12-*epi*-12-deacetyl-25-deoxy-scalarin[15,16] and scalarolide[18] respectively by comparison of their NMR and EIMS spectral data with those reported earlier. All these compounds are new to this species.

Compound **4** was obtained as colourless needles from hexane-acetone, m.p. 192°C; $[\alpha]_D^{25}$ + 48.9° (*c* = 0.15 in $CHCl_3$). The molecular formula, $C_{26}H_{40}O_4$, was established by EIMS (*m/z* 416, M^+) and elemental analysis.

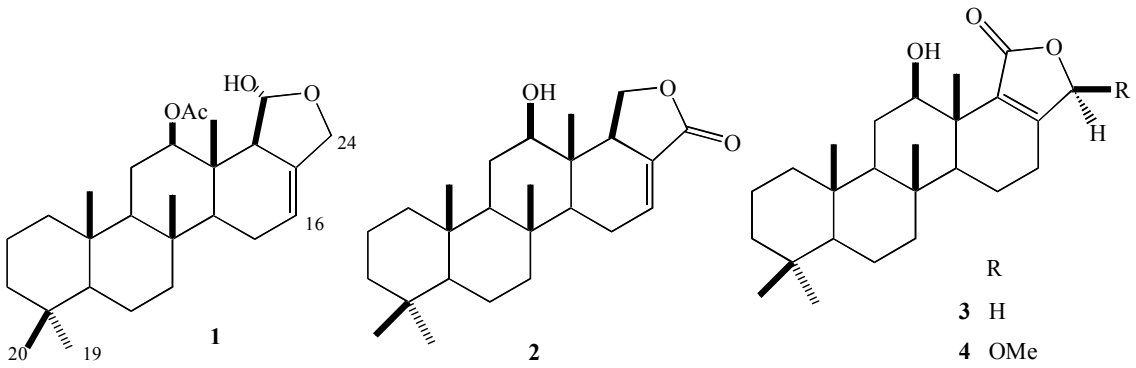
The UV spectrum showed absorption at λ_{max} 216 (ε12000) indicating the presence of an αβ unsaturated carbonyl system. The IR spectrum showed a broad peak at 3400 cm^{-1} due to hydroxyl and another peak at 1715 cm^{-1} for a lactone carbonyl.

The ^{1}H NMR spectrum (**Table I**) revealed resonances for five methyl groups (δ 0.80, 0.84, 0.85, 0.89 and 1.17), one methoxyl (δ 3.60) and two oxygenated methines (δ 5.79 and 3.55). The ^{13}C NMR data (**Table I**). and DEPT spectra showed resonances for 26 carbons including six methyls, ten methylenes, five methines and five quaternary carbons including two sp^2 carbons at δ 163.6, 126.8 and a carbonyl at δ 172.9. Three oxygenated carbon signals (δ 57.8, 76.1 and 104.4) were observed, with the last one belonging to a hemiacetal moiety.

In addition, the ^{13}C NMR spectrum showed the presence of three methines at δ 58.5, 56.6 and 55.4 charcteristic of normal scalaranes suggesting the presence of scalarane framework in compound **4** (ref. 17). The degree of unsaturation in combination with the presence of one carbonyl group and a double bond suggested the compound to be a pentacyclic sesterterpenoid. A comparison with metabolites in the literature revealed structural similarities with the scalarane type sesterterpenoids. The spectroscopic data for rings A-D showed very close resemblance to those of scalarolide **3** (ref.18), except for the absence of signals for the 24-methylene and appearance of those for an acetal carbon and a methoxy group. Moreover, the characteristic fragment ions at *m/z* 205 and 191 in the mass spectrum of **4**, ascribed to fragmentation across the C ring usually found in the scalarane derivatives, supported the absence of substituents on the A and B rings[13, 19].

Comparison of the signal observed for H-12 (δ 3.55, dd, *J* = 4.1, 10 Hz) in the ^{1}H-NMR spectrum of **4** with that of the same proton for scalarolide (**3**) (δ 3.65, dd, *J* = 4.2, 11 Hz)[18] showed that in each case the C-12 substituent was a hydroxyl group and with identical stereochemistry. The presence of a tetra substituted double bond and a methoxy group suggested that structural alterations existed on the E ring. Connectivities about the D and E ring system were deduced by COSY and are listed in **Table I**.

NOESY spectrum of **4** showed long range coupling between the protons resonating at δ 5.79 (H-24, brs) and δ 2.38 (H_α-16, m). Further, clear long range



coupling was observed between the methoxy protons at δ 3.60 (s) and a proton at 2.17 (H_β-16, m). This is possible only if the lactone carbonyl is at C-25 and the methoxy group on C-24 with axial orientation. The down field shift observed in the C-23 methyl carbon further supported the placement of a lactone carbonyl on C-25, same as scalarolide. Thus compound **4** is 24-*β*-methoxy-scalarolide, a new addition to the class of scalarane sesterterpenes.

Experimental Section

General Procedure: ^{1}H and ^{13}C NMR spectra are recorded on a Bruker DRX spectrometer operating at 300 MHz and 75 MHz respectively, the chemical shift values reported in ppm units and coupling constants in Hz. EIMS Spectra are recorded on a JEOL-JMS-D-300 at 70 eV. Optical rotations are taken on a JASCO DIP-370 polarimeter. Elemental analysis was carried out on a Carlo Erba 1108 analyzer. IR spectra were recorded on a Perkin-Elmer 881 instrument. Melting points were recorded on a Boitus melting point apparatus and are uncorrected. Silica gel column chromatography was carried out using silica gel (finer than 200#, ACME), TLC was carried out on silica gel G (ACME) thin layers and MPLC is performed on a Buchi B-688 MPLC system.

Collection and Isolation

The sponge *Hyatella cribriformis* was collected from Kanyakumari coast (8° 04′ N, 77° 36′ E) during March 2001. A voucher specimen (No. AU2-188) was deposited in the Dept. of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India. The sponge (*ca*.1kg, after extraction) was soaked in methanol immediately after collection and brought to the laboratory for further processing. It was extracted with MeOH eight times and the extracts concentrated under reduced pressure. The combined methanolic concentrate was fractionated with ethyl acetate. The ethyl acetate fraction on flash chromatography over a silica gel column using solvents of increasing polarity starting with hexane through ethyl acetate to methanol yielded 6 fractions. Fraction 2 (15% ethyl acetate in hexane) on further purification on silica gel columns yielded two known compounds 12-*epi*-deoxoscalarin (45 mg, **1**) and 12-*epi*-12-deacetyl-25-deoxyscalarin (38 mg, **2**). Fraction 3 (25% ethyl acetate in hexane) on recrystallisation from methanol yielded another known compound scalarolide (65 mg, **3**). Fraction 4 was highly contaminated with colouring matter, which on repeated column chromatography over MPLC followed by crystallization yielded the compound **4** (12 mg) as colourless needles.

12-*Epi*-deoxoscalarin 1: Colourless needles; m.p. 196°C; $[\alpha]_D^{25}$ +13.9 (*c*, 0.5 in $CHCl_3$). IR (KBr): 3530, 3470, 1730, 1383, 1390 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.89 (3H, s, 19-CH_3), 0.80 (3H, s, 20-CH_3), 0.84 (6H, s, 21 and 22-CH_3), 0.94 (3H, s, 23-CH_3), 1.55 (1H, br d, *J* = 11.8 , 11-βH), 1.87 (1H, dd, *J* = 11.2, 4.2, 11-αH), 2.05 (3H, s, -$OCOCH_3$), 2.16 (1H, m, 15-βH), δ2.23 (1H, m, 18-αH), 2.35 (1H, m, 15-αH), 4.14 (1H, d, *J* = 11.4 Hz, 24-βH), 4.31 (1H, d, *J* = 12.2 Hz, 21-H_a), 4.41 (1H, d, *J* = 11.4 Hz, 24-αH), 4.52 (1H, d, *J* = 12.2 Hz, 21-H_b), 4.69 (1H, dd, *J* = 11.2,3.4 Hz, 12-αH), 5.41 (1H, br d, *J* = 3.4 Hz, 25-βH), 5.47 (1H, br s, 16-H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 9.89 (C-23), 16.55 (C-21 and 22), 18.09 (C-2), 18.46 (C-6), 21.30 ($OCOCH_3$), 21.48 (C-20), 22.19 (C-15), 23.62 (C-11), 33.25 (C-4 and 19), 37.23 (C-8), 37.48 (C-10), 38.60 (C-13), 39.74 (C-1), 42.05 (C-7), 44.49 (C-3), 50.66 (C-18), 53.91 (C-14), 56.50 (C-5), 58.33 (C-9), 68.41 (C-24), 82.59 (C-12), 99.89 (C-25), 116.38 (C-16), 136.18 (C-17), 171.3 ($OCOCH_3$); EIMS: *m/z* 412 (M^+-H_2O), 370, (M^+-CH_3COOH)

Table I — ^{1}H, ^{13}C and ^{1}H-^{1}H-COSY data of scalarolide **3** and compound **4**

Position	Scalarolide **3**		Compound **4***		
	δ C, ppm (m)	δ H, ppm (m, *J* in Hz)	δ C, ppm (m)	δ H, ppm (m, *J* in Hz)	^{1}H-^{1}H COSY
1	39.7 (t)		39.8 (t)		
2	18.5 (t)		18.5 (t)		
3	42.1 (t)		41.9 (t)		
4	33.2 (s)		33.2 (s)		
5	56.6 (d)		56.6 (d)		
6	18.2 (t)		18.5 (t)		
7	41.7 (t)		41.4 (t)		
8	37.4 (t)		37.5 (t)		
9	58.0 (d)		58.5 (d)		
10	37.2 (t)		37.4 (t)		
11	25.7 (t)	1.51 (H_β-11, m)	26.9 (t)	1.51 (H_β-11, m)	H-12, H-10
		1.97 (H_α-11, br d)		2.07 (H_α-11, br d)	
12	75.6 (d)	3.65 (H_α-12, dd, *J* = 4.2, 11Hz)	76.1 (d)	3.55 (H_α -12, dd, *J* = 4.1, 10Hz)	2H-11
13	42.1 (s)		41.8 (s)		
14	55.2 (d)		55.4 (d)		
15	17.2 (t)	1.62 (H_β-15, m)	17.6 (t)	1.62 (H_β-15, m)	2H-16
		1.86 (H_α--15, m)		1.86 (H_α-15, m)	
16	25.3 (t)	2.29 (H_β-16, m)	21.2 (t)	2.17 (H_β-16, m)	2H-15, H_α-16
		2.42 (H_α-16, m)		2.38 (H_α-16, m)	2H-15, H_β -16, H_α-24
17	162.1 (s)		163.6 (s)		
18	135.8 (s)		126.8 (s)		
19	33.2 (q)	0.80 (3H, s)	33.2 (q)	0.80 (3H, s)	
20	21.3 (q)	0.84 (3H, s)	21.5 (q)	0.84 (3H, s)	
21	16.4 (q)	0.85 (3H, s)	16.1 (q)	0.85 (3H, s)	
22	16.8 (q)	0.89 (3H, s)	16.2 (q)	0.89 (3H, s)	
23	15.9 (q)	1.17 (3H, s)	14.6 (q)	1.17 (3H, s)	
24	72.1 (d)	4.68 (2H, br s)	104.4 (d)	5.79 (1H, brs)	
25	175.9 (s)		172.9 (s)		
24-OCH_3			57.8 (q)	3.60 (3H, s)	

* Assignments were made with the help of DEPT, ^{1}H-^{1}H COSY and NOESY spectral analysis

12-*Epi*-12-deacetyl-25-deoxyscalarin 2: Colourless crystals, mp 298°C; $[\alpha]_D^{25}$ +39.4 (*c*, 0.5: $CHCl_3$). UV at λ_{max} 224 nm (ε 8500). IR($CHCl_3$)) ν_{max} 3510, 1710, 1385, 1390, 1185 and 1220 cm^{-1}; Found: C, 77.58%; H, 9.89%; O, 12.54% Calcd.: C, 77.66%; H, 9.91%; O, 12.42%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.80 (3H, s, 19-CH_3), 0.84 (3H, s, 20-CH_3), 0.88 (3H, s, 21-CH_3), 0.95 (3H, s, 23-CH_3), 1.55 (1H, br d, *J* = 11.8 Hz 11-βH), 1.87 (1H, dd, *J* = 11.2, 4.2 Hz, 11-αH), 3.43 (1H, m, 12-αH), 2.19 (1H, m, 15-βH), 2.37 (1H, dq, *J* = 3.9, 6, 14 Hz,15-αH), 6.85 (1H, d, *J* = 3.6 Hz, 16-H), 2.84 (1H, m, 18-H), 4.503 (1H, t, *J* = 9.6 Hz, 25-αH), 4.19 (1H, t, *J* = 9.6 Hz, 25-βH) ^{13}C NMR ($CDCl_3$, 100 MHz): δ 7.83 (C-23), 16.4 (C-21 and 22), 18.10 (C-6), 18.58 (C-2), 21.31 (C-20), 23.74 (C-11), 27.68 (C-15), 33.26 (C-4 and 19), 37.48 (C-7 and 10), 39.92 (C-8), 40.10 (C-1), 42.10 (C-3), 50.66 (C-18), 53.49 (C-14), 56.61 (C-5), 58.83 (C-9), 69.09 (C-25), 81.28 (C-12), 127.42 (C-17), 135.18 (C-16), 169.97 (C-24); EIMS: *m/z* 386 (M^+)

Scalarolide 3: Colourless needles; m.p. 318°C; $[\alpha]_D^{25}$ +24.9' (*c* 1.35, $CHCl_3$); UV (MeOH): 219 nm **(ε** 24500); IR (KBr): 3460,1715,1655 cm^{-1}; EIMS: *m/z* 386 (M^+); The ^{1}H, ^{13}C NMR and ^{1}H-^{1}H COSY spectral data of **3** are presented in **Table I**

24β- Methoxyscalarolide 4: Colourless needles; m.p.192°C; Elemental analysis C, 74.96; H, 9.71; O, 15.43 . $C_{26}H_{40}O_4$ requires: C, 74.93; H, 9.72; O, 15.45%. $[\alpha]_D^{27}$ + 48.9° (*c* = 0.15 in $CHCl_3$); UV ($CHCl_3$): λ_{max} 223 nm (ε 24,500); IR ($CHCl_3$): 3400, 1720, 1685, 1385 and 1390 cm^{-1}; EIMS (70 e.V.): *m/z* (%) 416 (M^+, 4), 398 (M^+- H_2O, 18), 258 (21), 235 (38), 217 (23), 191 (100), 151 (38), 137 (45), 123 (38); The ^1H, ^{13}C NMR and ^1H-^1H COSY spectral data are presented in **Table I**.

Acetylation of Compound **4:** 5 mg of the compound was treated with Ac_2O (0.5 mL) in pyridine (1 mL) at room temperature and was kept overnight. The excess reagents were removed *in vacuo* and the residue, on chromatography over small column of silica gel yielded monoacetyl derivative (**4a**, 3 mg, low melting solid).

12β-Acetoxy-24β-methoxyscalarolide 4a: IR ($CHCl_3$): 3510, 1755, 1722, 1690, 1385, 1390, 1220 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$: δ 0.80 (3H, s), 0.84 (3H, s), 0.85 (3H, s), 0.89 (3H, s), 1.17 (3H, s), 1.51 (1H, m), 1.99 (s, 3H), 2.07 (1H, br d), 2.17 (1H, m), 2.38 (1H, dd), 1.62 (1H, m), 1.86 (1H, m), 4.66 ((1H, dd, *J* = 11.2,3.4 Hz), 3.60 (3H, s), 5.79 (1H, d, *J* = 3 Hz); EIMS: *m/z* 398 (M^+- AcOH, 12)

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