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Novel diterpenoids from the Indian Ocean soft coral *Sarcophyton elegans*†

A. S. R. Anjaneyulu[a]*, P. Mangala Gowri[a], M. J. R. V. Venugopal[a], P. Sarada[a], M. V. R. Krishna Murthy[a], G. V. Rao[a], P. S. N. Murthy[b], C. V. Rao[b] and G. Kumar[b]

[a]Department of Organic Chemistry, Andhra University, Visakhapatnam-530 003, India

[b]Central Tobacco Research Institute, Rajahmundry, India

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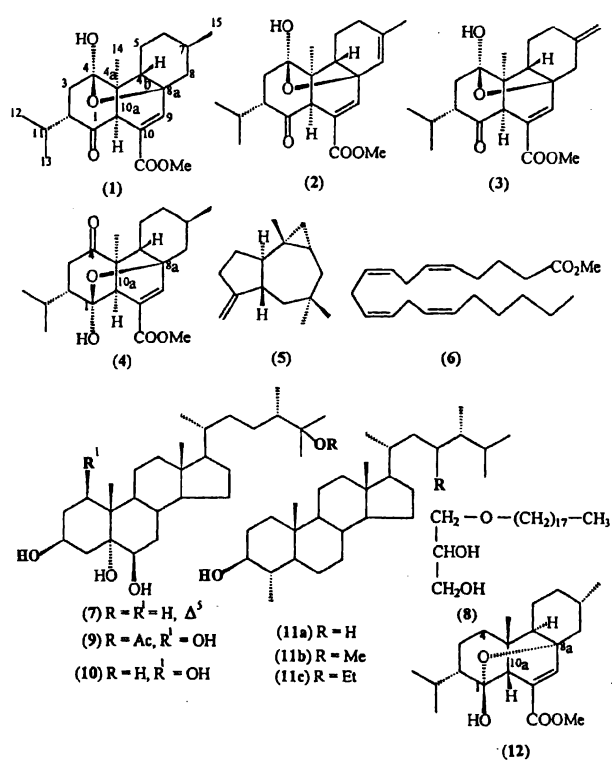
Chemical examination of the soft coral species *Sarcophyton elegans* collected from the Havelock Island of the Andaman and Nicobar group of Islands of the Indian Ocean resulted in the isolation of four novel diterpenoids, sarcophytin (1), 7-dehydrosarcophytin (2), $\Delta^{7(15)}$-dehydrosarcophytin (3) and isosarcophytin (4) along with some known compounds. The structures of the new diterpenoids were established by the help of their physical and spectral data including X-ray analysis on sarcophytin (1). The known compounds are $\Delta^{9(15)}$-africanene (5), methyl arachidonate (6), (24*S*)-24-methylcholestane-3β,25-diol (7), batyl alcohol (8), (24*S*)-24-methylcholestane-1β,3β,5α,6β,25-pentol (10), its 25-monoacetate (9), three monohydroxy sterols 4α,24-dimethylcholestan-3β-ol (11a), 4α,23ξ,24ξ-trimethylcholestan-3β-ol (11b) and 23ξ-ethyl-4α,24ξ-dimethylcholestan-3β-ol (11c) as a mixture.

The soft coral species of *Sarcophyton* genus occur abundantly in the coral reefs of the world over. Around 25 species of this have been chemically examined and their chemical constituents reviewed recently[1]. In our continuing interest on the bioactive secondary metabolites of the soft corals of the Indian Ocean[2,3], we have examined *Sarcophyton elegans* collected from the Havelock (12°19′N, 93°48′E) Island of the Andaman and Nicobar group of islands. Chemical examination of this species occurring in different regions of the Pacific Ocean has been reported earlier to yield polyhydroxy steroids[4–7] and cembranoid diterpenoids[8]. But to our surprise only modified cembranoid diterpenoids were found in the Indian species.



Results and Discussion

We now report from the Indian species, the isolation of four novel tetracyclic diterpenoids of perhydrophenanthrene skeleton, namely, sarcophytin (**1**), 7-dehydrosarcophytin (**2**), $\Delta^{7(15)}$-dehydrosarcophytin (**3**) and isosarcophytin (**4**) along with a number of steroids and others. The structures of the new diterpenoids were established by the help of their physical and spectral (UV, IR, ^{1}H, ^{13}C NMR, 2D ^{1}H-^{1}H, ^{13}C-^{1}H COSY, NOESY and mass) data including X-ray analysis on one of them, sarcophytin (**1**). While preliminary reports on three of these derivatives sarcophytin (**1**)[2], 7-dehydrosarcophytin (**2**)[9] and $\Delta^{7(15)}$-dehydrosarcophytin (**3**)[10] have been published, this report describes the full details of the chemical examination of a fourth member of this series, isosarcophytin (**4**) along with isolation and characterisation of several known compounds like $\Delta^{9(15)}$-africanene (**5**)[11,12], methyl arachidonate (**6**)[13], (24*S*)-24-methylcholestane-3β,25-diol (**7**)[14], batyl alcohol (**8**)[15], (24*S*)-24-methyl-1β, 3β, 5α, 6β, 25-pentol (**10**)[16], its 25-monoacetate (**9**)[16] and a mixture of monohydroxy sterols which was analysed by the GC/MS analysis on the acetate mixture to consist of 4α,24-dimethylcholestan-3β-ol (**11a**)[17], 4a,23ξ,24ξ-trimethylcholestan-3β-ol (**11b**)[18] and 23ξ-ethyl-4α,24ξ-dimethylcholestan-3β-ol (**11c**)[19]. The characterisation of the

†Dedicated to Professor D. Nasipuri in commemoration of his 75th. birth anniversary.

known compounds was done by comparison of their physical and spectral data (UV, IR, ^1H, ^{13}C NMR and mass) with the literature values of the respective compounds and by direct comparison with authentic samples wherever possible.

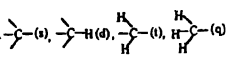
A survey of literature revealed that only four tetracyclic diterpenoid derivatives have been reported so far and that too only from soft coral species. Three of these are from the species of *Sinularia* genus, mandapamate from *Sinularia dissecta*, isomandapamate from *Sinularia maxima* and the third from *Sinularia dissecta* while the fourth, chatancin (**12**), is a PAF antagonist from a soft coral species of *Sarcophyton* genus. The structural features, spectral characteristics of sarcophytin find greater similarity with those of chatancin (**12**) (Tables 1 and 2), except for the difference that sarcophytin has an additional keto functionality. A similar structure of chatancin with a perhydrophenanthrene skeleton, was therefore, assumed for sarcophytin also in which the nature as well as the position of the cyclic hemiketal ring and the other functional groups carbonyl, an α,β-unsaturated ester need to be fixed. The main difference between chatancin and sarcophytin however lies in the ^{13}C chemical shift of hemiketalic carbon which appeared at δ 99.9 in the former, and at δ 105.6 in the latter, suggesting that the hemiketalic ring is a six-membered tetrahydropyran in **12** while the same in sarcophytin (**1**) is tetrahydrofuran reminiscent of the values of anomeric carbon in pyranose and furanose forms of the sugar series respectively[20]. The five-membered tetrahydrofuran hemiketalic link demands the linkage of C-4 and C-8a carbons as in sarcophytin and unlike in chatancin (**12**) where C-1 and C-8a are linked. The location of hemiketal link at C-4 was also supported by the highly deshielded ^{13}C value of 4a (δ 57.6) in **1** compared to that C-4 (δ 37.3) in chatancin. If the α,β-unsaturated ester functionality in sarcophytin (**1**) is assumed at the same position as in chatancin, the additional carbonyl group in

Table 1. ^1H NMR spectral data (δ) of chatancin (**12**), sarcophytin (**1**), isosarcophytin (**4**), 7-dehydrosarcophytin (**2**) and $\Delta^{7(15)}$-dehydrosarcophytin (**3**)

Assignment	Chatancin (CD_3OD)	Sarcophytin (200 MHz)	Isosarcophytin (90 MHz)	7-Dehydro-sarcophytin (200 MHz)	$\Delta^{7(15)}$-Dehydro sarcophytin (90 MHz)
2-H	1.37 (ddd, 13.1, 4.0, 2.4)	2.10 (m)	2.10 (m)	2.05 (m)	2.05 (m)
3-Ha	1.48 (m)	2.35 (m)	2.32 (m)	2.45 (m)	2.35 (m)
3-Hb	1.61 (qd, 13.1, 4.3)	1.85 (m)	1.90 (m)	1.85 (m)	1.83 (m)
4-Ha	1.27 (t, d, 13.1, 4.3)				
4-Hb	1.57 (m)				
4b-H	1.40 (ddd, 13.0, 3)	2.45 (m)	2.5 (m)	2.45 (m)	2.45 (m)
5-Ha	0.71 (qd, 13, 3)			1.64 (m)	1.58 (m)
		1.45 (m)	1.50 (m)		
5-Hb	1.54 (qd, 13, 3)				
6-Ha	0.91 (m)			2.4 (m) &	1.70 (m)
		1.66 (m)	1.65 (m)	1.8 (m)	
6-Hb	0.69 (brd, 13.0)				
7-H	1.66 (m)	0.98 (m)	1.02 (s)	–	–
8-Ha	1.19 (t, 12.5)			5.36 (m)	2.05 (m)
		2.10 (m)	2.1 (m)		
8-Hb	2.04 (ddd, 12.5, 3.5, 2)				
9-H	7.20(d, 2)	7.10 (s)	7.35 (brs)	6.98 (s)	7.0 (s)
10a-H	2.65 (d, 2)	2.98 (d, 2)	3.2 (d, 3)	3.11 (s)	2.98 (d, 2)
11-H	2.34 (sep×d, 6.7,2.4)	1.98 (m)	2.2 (m)	1.90 (m)	2.0 (m)
12-H_3	0.920 (d, 6.7)	0.84 (d, 6)	0.81 (s)	0.81 (d, 6.3)	0.80 (d, 6)
13-H_3	0.924 (d, 6.7)	0.84 (d, 6)	0.85 (s)	0.83 (d, 6.3)	0.85 (d, 6)
14-H_3	0.70 (s)	1.10 (s)	0.90 (s)	1.75 (s)	1.10 (s)
15-H_3	1.0 (d, 6.2)	1.06 (d, 5 5)	0.98 (s)	1.75 (s)	4.9 (d, 3)
17-H_3	3.76 (s)	3.73 (s)	3.80 (s)	3.71 (s)	3.70 (s)
				3.4 (s, OH)	

Table 2. ^{13}C NMR spectral data (δ) of chatancin (**12**), sarcophytin (**1**), isosarcophytin (**4**), 7-dehydrosarcophytin (**2**) and $\Delta^{7(15)}$-dehydrosarcophytin (**3**)

Carbon no.	Chatancin (CD_3OD)	Sarcophytin (22.5 MHz)	Isosarcophytin (22.5 MHz)	7-Dehydro-sarcophytin (22.5 MHz)	$\Delta^{7(15)}$-Dehydrosarcophytin (22.5 MHz)
1	99.9	210.0 (s)	98.0 (s)	210.2 (s)	209.8 (s)
2	50.8	56.0 (d)	51.3 (d)	55.5 (d)	56.2 (d)
3	19.3	40.9 (t)	34.0 (t)	40.4 (t)	40.8 (t)
4	40.0	105.6 (s)	212.1 (s)	105.6 (s)	105.8 (s)
4a	37.3	51.9 (s)	49.0 (s)	50.9 (s)	51.9 (s)
4b	49.1	48.8 (d)	49.2 (d)	44.7 (d)	48.9 (d)
5	28.5	21.3 (t)	27.0 (t)	23.1 (t)	21.2 (t)
6	36.0	33.8 (t)	34.2 (t)	41.1 (t)	33.5 (t)
7	30.9	29.9 (d)	29.6 (d)	131.1 (s)	143.1 (s)
8	43.5	43.7 (t)	42.3 (t)	121.4 (d)	45.0 (t)
8a	77.1	79.2 (s)	76.1 (s)	77.6 (s)	78.9 (s)
9	144.5	146.0 (d)	143.4 (d)	145.9 (d)	145.2 (d)
10	137.0	130.9 (s)	134.5 (s)	131.7 (s)	130.8 (s)
10a	54.4	49.8 (d)	51.3 (d)	48.5 (d)	49.8 (s)
11	26.4	25.9 (d)	25.7 (d)	25.6 (d)	25.9 (d)
12	18.7	19.0 (q)	17.6 (q)	18.8 (q)	18.9 (q)
13	23.5	21.3 (q)	22.4 (q)	21.0 (q)	21.9 (q)
14	24.8	16.9 (q)	16.3 (q)	16.8 (q)	16.8 (q)
15	22.8	22.2 (q)	22.0 (q)	23.8 (q)	114.2 (t)
16	167.7	165.9 (s)	164.7 (s)	165.6 (s)	165.9 (s)
17	52.3	51.3 (q)	52.2 (q)	51.7 (q)	51.3 (q)

Substitution pattern derived from the DEPT spectrum 

sarcophytin can be located at C-1, C-3, C-5, C-6, or C-8. The appearance of C-10a carbon slightly deshielded at δ 49.8 indicated the location of carbonyl at C-1 in preference to the others.

The appearance of 10a-H at δ 2.98 as a narrow doublet $J = 2$ Hz, probable allylic coupling with 9-H also favours the location of carbonyl at C-1. The 2D-NMR spectral data (^1H-^1H COSY and NOESY) did not reveal the necessary and important correlations to derive the complete information regarding the relative stereochemistry of the respective chiral carbons C-2, C-4, C-4a, C-4b, C-7, C-8a and C-10a. The partial connectivities shown in the spectrum were recorded in the experimental. The structure of sarcophytin and its relative stereochemistry could, however, be obtained from X-ray analysis as depicted in **1**.

Sarcophytin (**1**) came as colourless feathery needles from methanol, m.p. 160–162°, $[\alpha]_D^{25}$ + 924.4° (*c* 0.5, $CHCl_3$). Its molecular formula was assigned as $C_{21}H_{28}O_5$ by elemental analysis and the M^+ 362 in its EI mass. The presence of hydroxylic (3520 cm^{-1}) and two carbonyl absorptions; an α,β-unsaturated ester (1708 cm^{-1}) and six-membered saturated cyclic ketone (1720 cm^{-1}) was noticed in its ^{13}C NMR spectrum. Its UV absorption at 219 nm indicated conjugation. It exhibited all the 21 carbon signals in its ^{13}C NMR spectrum which were analysed by the DEPT spectrum to consist of five methyls, four methylenes, six methines and six quaternary carbons. The chemical shifts of the respective carbons were assigned based on the connectivities noticed in its 2D-NMR (^{13}C-^1H COSY spectrum). The keto (δ 210.0) and α,β-unsaturated ester (COOMe δ 165.9 and COO*Me* δ 51.3) carbonyl carbons were evident from the ^{13}C and ^1H NMR spectra (COO*Me* δ 3.73). The ^{13}C NMR spectrum indicated the presence of a trisubstituted double bond (δ 146.0, d, 130.9, s) which was supported by the presence of the corresponding olefinic proton at δ 7.10 as a singlet assignable to a β proton of an α,β-unsaturated ester. The spectrum further showed two oxygenated carbons, one at δ 105.6 (s) assignable to a doubly oxygenated cyclic hemiketal carbon and the other at δ 79.2 as a tertiary carbon. All the five oxygens of the molecule could thus be accounted for, two in the ester group, one in the carbonyl, one in hydroxyl and the other in hemiketal. The molecular for-

mula requiring seven double bond equivalence, three of them being accounted for in the two carbonyl functionalities and a double bond, indicated its tetracyclic nature.

The ^{1}H NMR spectrum (Table 1) revealed its diterpeniod nature by showing five methyl groups, one as carbomethoxyl (δ 3.73, s), two in isopropyl group (0.84, d, J 6 Hz, 12 and 13-H_3), one as a secondary methyl (1.06, d, J 5.5 Hz, 15-H_3) and the remaining as a tertiary methyl (1.10, s, 14-H_3). The presence of six carbons, three in the form of isopropyl group, two as methyls and one in the form of carbomethoxyl, indicated it to be a tetracyclic diterpenoid derived from the basic fourteen-membered carbocyclic cembranoid skeleton with crosslinking.

7-Dehydrosarcophytin (**2**), m.p. 160–162°, $[\alpha]_D^{30}$+ 403.6° (c 0.5, $CHCl_3$) came as colourless needles from methanol and analysed for $C_{21}H_{28}O_5$ by elemental analysis which was supported by the low intense ion at m/z 330 corresponding to (M^+ –HCHO) in its EIMS. Its spectral (UV, IR, ^{1}H NMR) characteristics closely resembled those of sarcophytin, e.g. the presence of hydroxylic absorption (3410), two carbonyls, a six-membered saturated ketone (1714) and an α,β-unsaturated ester (1683 cm^{-1}) in its IR spectrum and UV absorption at 238 nm indicative of conjugation. Like sarcophytin, it exhibited a tertiary methyl (1 δ 1.11, s, 14-H_3), an isopropyl group (0.81, d, J 6.3 Hz, 12 and 13-H_3), a carbomethoxyl (3.71, s) and a β-proton of an α,β-unsaturated ester (6.98, s). Unlike in **1**, instead of a secondary methyl, a methyl on trisubstituted double bond was observed as a singlet at δ 1.75 with the corresponding olefinic proton at δ 5.36 as a clear singlet. The foregoing evidence suggested that this new diterpenoid is dehydrosarcophytin with the double bond at 6,7 on 7,8 position. If it were a 6-dehydroderivative, the trisubstituted olefinic 6-H should have appeared as a doublet by doublet, a triplet or at least as a doublet when one of the vicinal methylene protons at C-5 makes a dihedral angle of 90°. On the other hand, if it were a 7-dehydroderivative, the 8-H would have come as a singlet in the absence of vicinal protons. The appearance of this olefinic proton at δ 5.36 as a clear singlet, even in 300 MHz spectrum, was taken to support the structure of this dehydroderivative as 7-dehydrosarcophytin (**2**). Further, the same proton neither exhibited ^{1}H-^{1}H COSY nor NOESY connectivity with any other proton except for the allylic correlation with 15-H_3.

The ^{13}C NMR spectrum (Table 2) of 7-dehydrosarcophytin exhibited all the twentyone carbon signals, which were analysed by DEPT spectrum as five methyls, three methylenes, six methines and seven quaternary carbons. The chemical shifts of the respective carbons were assigned, based on the correlations noticed in its 2D-NMR (^{13}C-^{1}H COSY, experimental) spectrum. Evidence for the presence of an hemiketalic carbon at δ 105.6, another oxygenated carbon at 77.6 and six-membered ketocarbonyl at 210.2, ester carbonyl at 165.6 as in sarcophytin and four trisubstituted olefinic carbons at 145.9 (d), 131.7 (s), 131.1 (s) and 121.4 (d) was obtained from the ^{13}C NMR spectral data in support of the structure. Its NOESY spectrum, 10a-H exhilbited correlation with 14-H_3 indicating their *cis* relationship and to 9-H, showing allylic coupling. The same proton did not show any coupling with 2-H, showing their *trans* relationship. Similarly, the absence of the NOESY correlation between 14-H_3 and 4b-H again showed their *trans* relationship in support of overall relative stereochemistry as in sarcophytin.

$\Delta^{7(15)}$-Dehydrosarcophytin (**3**), came as colourless feathery needles from hexane-acetone, m.p. 162–163°, $[\alpha]_D^{30}$ + 126.17° (c 0.57, $CHCl_3$) and analysed for $C_{21}H_{28}O_5$, which was supported by its M^+ 360 in its EIMS. Its spectral (UV, IR, ^{1}H NMR) characteristics closely resembled those of sarcophytin (**1**) and 7-dehydrosarcophytin (**2**) and in particular with those of the latter, being isomeric with it. Like **1** and **2**, it exhibited ^{1}H NMR signals for a tertiary methyl (δ 1.10, s, 14-H_3), an isopropyl group (0.80, 0.85, each d, J 6 Hz), a carbomethoxyl (3.70, s) and a β-proton of an α,β-unsaturated ester (7.0, s). But unlike in sarcophytin (**1**) with a secondary methyl (δ 1.06, d, J 5.5 Hz, 15-H_3) and in 7-dehydrosarcophytin (**2**) with the same methyl attached to a double bond appearing as a singlet (δ 1.75), it exhibited an exocyclic methylene functionality. The appearance of exocyclic methylene functionality supported from its IR absorptions (1629, 899 cm^{-1})[21], ^{1}H NMR data (exocyclic methy-lene protons at δ 4.9, 2H, d, J 3 Hz) and ^{13}C NMR data (exocyclic methylene carbons δ 143.1, s, 114.2, t) suggested that this compound is, in fact, $\Delta^{7(15)}$-dehydrosarcophytin (**3**).

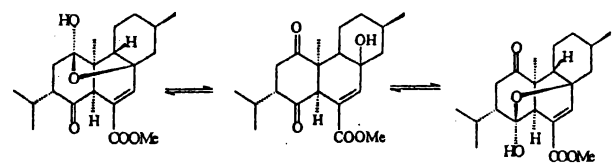
In its ^{13}C NMR spectrum (Table 2), the presence of cyclic hemiketal carbon at δ 105.8 (s), another oxygenated carbon at 79.8 (s), carbonyl carbons keto at 209.8 (s), ester at 165.9 and the trisubstituted olefinic carbons at 145.2, 130.8 as in compounds **1** and **2** supported their close relationship in their structures. In view of very close and comparable chemical shifts of .he remaining carbons and in particular with those of the chiral carbons C-2, C-4, C-4a, C-10a, C-4b and C-8a with those of **1** and **2**, the same relative stereochemistry was taken to be present in $\Delta^{7(15)}$-dehydrosarcophytin (**3**) as in **1** and **2**.

Isosarcophytin (**4**) crystallised from hexane-acetone as colourless needles, m.p. 163–164°, $[\alpha]_D^{25}$ –61.2° (c 0.25,

$CHCl_3$) and analysed for $C_{21}H_{30}O_5$, was supported by its molecular ion M^+ 362 in its EI mass spectrum. From its spectral characteristics, it was also found to be a new diterpenoid related to compounds **1**, **2** and **3**. Its ^1H NMR spectral data were found to be more or less identical to sarcophytin (**1**) to designate it as isosarcophytin. It showed the hydroxylic absorption at (3350), two carbonyls α,β-unsaturated ester (1690) and six-membered saturated ketone (1710), ether absorptions (1140, 1020 symmetric and asymmetric stretchings) and trisubstituted olefinic absorption (1620 cm^{-1}) as in sarcophytin (**1**). It also showed end absorption 222 nm in its UV spectrum in support of conjugation.

Its ^1H NMR spectrum (Table 1) resembling very close to that of sarcophytin (**1**), exhibited one tertiary methyl (δ 0.90, s, 14-H_3), an isopropyl (0.81, s, 12-H_3), 0.85 (s, 13-H_3) and 2.2 (m, 11-H) and one secondary methyl group (0.98, s, 15-H_3), a methyl ester (3.80, s) and a trisubstituted olefinic proton (7.35) as a broad singlet over an α,β-unsaturated ester. Except for minor deviations in the chemical shifts of the corresponding protons in sarcophytin (**1**) and isosarcophytin (**4**), are indistinguishable to show their close relationship. The characteristic difference between these two could, however, be noticed in their ^{13}C NMR spectra to indicate their isomeric nature. Like sarcophytin (**1**) it also exhibited all the 21 carbon signals whose multiplicities were revealed from the DEPT spectrum and the chemical shift assignments were made by comparison with those of sarcophytin. Isosarcophytin (**4**) exhibited the six-membered ketocarbonyl at δ 212.1 and the ester carbonyl at 164.7 as in sarcophytin (207.9 and 165.9). The essential difference between these two was noticed in the value of the hemiketal carbon, which appeared at δ 98.0 in **4** while the same appeared at 105.6 in sarcophytin. Thus the value of this carbon in **4** was found to be very close to the hemiketal carbon δ 99.9 noticed in chatancin (**12**) with a six-membered heterocyclic ring unlike in sarcophytin with a five-membered ring. It was, therefore, taken that isosarcophytin has a six-membered tetrahydropyran ring and isomeric to sarcophytin with a five-membered tetrahydrofuran ring. The ^1H and ^{13}C assignments based on the isomeric structure for isosarcophytin were found to be consistent. Since the ^{13}C values of sarcophytin and isosarcophytin are quite comparable (Table 2), the relative stereochemistry at the centres C-2, C-4, C-4b, C-7, C-8a and C-10a were taken to be similar to those of sarcophytin to consider its structure as isosarcophytin (**4**).

Since sarcophytin (**1**) and isosarcophytin (**4**) are isomeric hemiketal derivatives going through the intermediate keto alcohol (**A**), it might be argued that both could exist in equilibrium and one of these might be an artefact of the other. Although this idea may not be totally ruled out, it may be said to certain extent that sarcophytin and isosarcophytin do exist as such in the organism, for the fact that sarcophytin on methylation with methanolic HCl or PTS in methanol gave only sarcophytin methyl ether but not isosarcophytin methyl ether. This shows that the hemiketal ring in these is a little more stable than in anomeric hexoses or pentoses which exhibit mutarotation.



1 A 4

The mass spectral fragmentation of sarcophytin and the related compounds (**1-4**) showed a set pattern. The molecular ions are either weak as in **1**, **2** and **4**, or absent as in **3**. It appears that most of the fragments have arisen not from the hemiketal structure but from the isomeric keto alcohol structure, both of which can be in equilibrium, paricularly under the mass spectral conditions. Thus the base peak in sarcophytin (**1**) **B** *m/z* 221, might be taken to arise by the cleavage α- to the carbonyls and a methyl group. This base peak may in turn give rise to the ions **C** *m/z* 161, **D** 143 and **E** 119 (70%) and so also the ions in *m/z* 206 and **J** 189 (71.6%). A few other fragments shown in the Chart 1 for sarcophytin (**1**) are consistent with the structure. As indicated above, the mass fragmentations of other related compounds (**2-4**) were very similar to those of sarcophytin (**1**) and the prominent ions of the respective compounds are given in the experimental.

Experimental

M.ps. were determined on VEB-analytic Dreder HMK hot plate and are uncorrected. Preparative ODS (H) (20 mm × 250 mm) column, FCV 100 B fraction collector, C-R7A + integrator, SPD 10 A detector, SCL-10A system controller were used on a Schimadzu LC 8A for hplc. GC-MS were recorded on a Schimadzu QP-2000 at 70 eV. Elemental analyses were determined on a Carlo Erba – 1108 instrument. UV spectra were recorded on a Milton Roy 1201 spectrophotometer and IR spectra on a Perkin-Elmer 840 spectrophotometer. ^1H NMR spectra were measured on a Bruker 400 MHz or Gemini 200 MHz or JEOL JNM EX-90 spectrometers using $CDCl_3$ as solvent and tetramethylsilane as internal reference. ^{13}C nmr spectra were measured on a

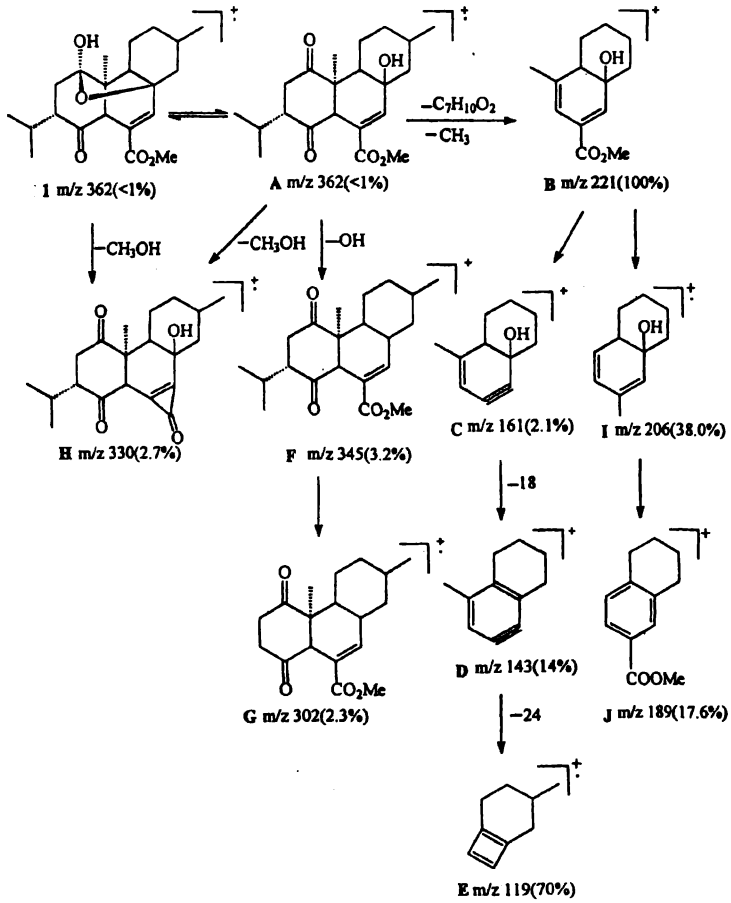


Chart 1

JEOL JNM EX-90 spectrometer at 22.5 MHz using $CDCl_3$ as solvent and TMS as internal reference. Mass spectra were obtained on a JEOL JMS – 300 spectrometer. Optical rotations were determined on a Rudolph Autopol III polarimeter. Silica gel 100–200 mesh (acme's), 230–400 mesh (Sigma) and silica gel G (Merck) were used for VLC, CC and TLC respectively.

The soft coral was collected from the Havelock Island of the Andaman and Nicobar group of Islands of the Indian Ocean, and identified[22] as *Sarcophyton elegans*. Neat voucher specimens containing all morphological details were deposited at the museum of National Institute of Oceanography, Goa and at the museum of Department of Organic Chemistry, Andhra University, Visakhapatnam, India, with registration No. AU1-111.

The soft coral was sliced into small pieces and the specimens were percolated with methanol (5×5 l). The aqueous methanolic concentrate (8 l) left over after concentrating the combined methanolic extract was extracted with ethyl acetate (8×500 ml). The combined ethyl acetate extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield a dark greenish residue (40 g). The dry weight of the organism after extraction was 3.2 kg.

A part of the above residue (20 g) from the ethyl acetate extract was subjected to vacuum liquid chromatography[23] over a column (85 cm×100 cm) of silica gel (200 g, 230–400 mesh) using gradient of solvent mixtures of hexane (b.p. 60–70°) through ethyl acetate-methanol. Fractions (each of 800 ml) were collected and monitored through silica gel tlc. The visualisation of the spots was carried under uv-light or iodine vapour or by spraying with 5% sulphuric acid in methanol and heating at 110°. The chromatographic details are recorded in Table 3. The fractions with identical spots were mixed together to form six fractions. Each fraction was further subjected to purification by passing through smaller columns of silica gel or by fractional crystallisation using appropriate solvent mixtures. A total of ten pure compounds were obtained along with a mixture of mono-hydroxysterols.

Fraction I : The pale yellow residue from this fraction on further purification by passing through a small column

Table 3. Chromatographic details of EtOAc extract of *Sarcophyton elegans*

Eluant*	Fractions	Residue (g)	Remarks	Compounds characterised
Hexane : EtOAc				
100 : 0	1–12	1.2	Fraction I	$\Delta^{9(15)}$-Africanene (**5**)
98 : 2	13–22	1.4	Fraction II	Methyl arachidonate (**6**)
95 : 5	23–34	3.4	Fraction III	Mixture of monohydroxysterols (**11**)
90 : 10	35–42	2.8	Fatty material	
85 : 15	43–54	1.9	Fraction IV	Novel tetracyclic diterpenoids sarcophytin (**1**), isosarcophytin (**4**), 7-dehydrosarcophytin (**2**) and $\Delta^{7(15)}$-dehydrosarcophytin (**3**)
80 : 20	55–62	1.3	Fraction V	(24*S*)-24-Methylcholestane-3β,25-diol (**7**) and batyl alcohol (**8**)
60 : 40	63–81	2.4	Green pigment	
40 : 60	82–93	2.2	Brown gum	
20 : 80	94–101	1.8	Fatty material	
0 : 100	102–110	3.4	Fraction VI	(24*S*)-24-Methylcholestane-1β,3β,5α,6β,25-pentol (**10**) and its 25-monoacetate (**9**)
EtOAc : MeOH				
9.5 : 0.5	111–115	2.5	Brown gum	

*The figures indicate volume by volume ratio of the mixture of solvents.

Fraction numbers were given to only those fractions that showed prominent spots on TLC and from which pure compounds were obtained.

of silica gel using hexane as eluant furnished $\Delta^{9(15)}$-africanene (**6**) as a colourless oil (60 mg), $[\alpha]_D^{25}$ + 86° (*c* 1.1, $CHCl_3$), identified by comparison of its physical and spectral (IR, ^1H and ^{13}C NMR) data[11,12].

Fraction II : The yellow residue from this fraction showed a single spot with underlying fat streak on TLC. It was found to contain high percentage of fatty material which on purification by passing through a small column of silica gel using hexane-ethyl acetate (9.8 : 0.2) mixture furnished methyl arachidonate, pale yellow oil (25 mg), identified its UV, IR, ^1H NMR and mass data[13].

Fraction III : The residue from this fraction showed a pink spot on TLC which on purification by passing through a small column of silica gel using hexane-ethyl acetate (9.5 : 0.5) mixture as eluant and further crystallisation from chloroform-methanol furnished a mixture of monohydroxysterols as colourless needles (200 mg), mp.135–138°. Its positive (pink-blue-green) Lieberman-Burchard test and ^1H NMR showed its steroid nature. Its acetyl derivative (Ac_2O-Py) showed it to be a mixture of acetates on a 20% silver nitrate-Si gel TLC plate. The GC/MS analysis on the acetate mixture was found to consist of the acetates of three monohydroxysterols, namely, 4α,24-dimethylcholestan-3β-ol[17], [R_t (min) 3.10, major fragmentation peaks 398 (30), 340 (40), 271 (60), 255 (65), 229 (15), 213 (30), 159 (60), 55 (100), 43 (89)], 4α,23ξ24ξ-trimethylcholestan-3β-ol[18], [R_t (min) 4.03, major fragmentation peaks 412 (15), 314 (95), 271 (55), 255 (50), 229 (53), 213 (50), 161 (45), 159 (50), 55 (100), 43 (95)] and 23ξ-ethyl-4α,24ξ-dimethylcholestan-3β-ol[19], [R_t (min) 4.50, major fragmentation peaks 426 (25), 355 (10), 314 (55), 271 (80), 255 (45), 229 (40), 213 (38), 159 (55), 55 (98), 43 (100)] by the help of eight peak mass index[24].

Fraction IV : The green residue from this fraction showed a single bright yellow spot embedded in a green pigment on a TLC plate. However, it was found to be a mixture of three spots on 20% silver nitrate impregnated silica gel TLC plate. This mixture was purified by chromatography over silver nitrate impregnated column of silica gel using hexane-acetone mixture as eluant. The initial fractions on repeated crystallisation from hexane-acetone afforded sarcophytin (**1**)[2] as colourless feathery needles (60 mg), m.p. 162–163° and the mother liquor though showed a single spot on 20% silver nitrate impregnated silica TLC, was found to be a mixture by ^1H and ^{13}C NMR spectral data. This fraction was purified by HPLC using reverse phase technique on pre-

parative ODS (H) (20 mm×250 mm) column. The solvent was methanol-water 70 : 30 (v/v) pumped at a flow rate of 10 ml/min to afford pure isosarcophytin (**4**) as colourless needles (25 mg), m.p. 163–164°. Further elution of the column with hexane-acetone mixture gave a mixture which on repeated purification by passing over silver nitrate impregnated column of silica gel using hexane-acetone mixture as eluant afforded 7-dehydrosarcophytin (**2**) as colourless needles (80 mg), m.p. 160–162° and $\Delta^{7(15)}$-dehydrosarcophytin (**3**) as colourless feathery needles (20 mg), m.p. 160–162°.

Fraction V : The residue from this fraction showed two spots on TLC plate. It contained a lot of gummy material. This mixture was repeatedly chromatographed over small columns of silica gel using hexane-ethyl acetate mixture as eluant. The initial fractions on evaporation followed by crystallisation of the residue furnished (24*S*)-24-methylcholestane-3β,25-diol (**7**) as colourless needles (25 mg), m.p. 192–194°, $[\alpha]_D^{25}$ –50.7° (*c* 1.01, $CHCl_3$), identified by comparison of its physical and spectral (UV, IR, ^1H and ^{13}C NMR and mass) data[14] and batyl alcohol (**8**) as colourless flakes (150 mg), m.p. 69–71°, $[\alpha]_D^{25}$ +2.6° (*c* 1.1, $CHCl_3$), identified by comparison of its physical and spectral (IR, ^1H and ^{13}C NMR and mass) data[15].

Fraction VI : This fraction which showed close running blue spots on tlc plate, contained a lot of gum. It was repeatedly passed through small columns of silver nitrate impregnated silica gel and on crystallisation from methanol furnished (24*S*)-24-methylcholestane-1β,3β,5α,6β,25-pentol-25-monoacetate (**9**) as colourless crystals (300 mg), m.p. 264–266°, $[\alpha]_D^{25}$ –15.7° (*c* 1.5, MeOH), identified by comparison of its physical and spectral (IR, ^1H and ^{13}C NMR and mass) data[16] and (24*S*)-24-methylcholestane-1β,3β,5α,6β,25-pentol (**10**) as colourless flakes (85 mg), m.p. 268–270°, $[\alpha]_D^{25}$ –19.3° (*c* 0.6, MeOH), identified by comparison of its physical and spectral (IR, ^1H and ^{13}C NMR and mass) data[16].

Sarcophytin (**1**) : Colourless feathery needles (60 mg), m.p. 162–163° (Found : C, 69.8; H, 8.1. $C_{21}H_{30}O_5$ requires : C, 69.6; H, 8.3%); $[\alpha]_D^{25}$ +924.4° (*c* 0.5, $CHCl_3$); λ_{max} ($CHCl_3$) 219 nm; ν_{max} 3520, 1708, 1720, 1620, 1040, 1070 cm^{-1}, ^1H, ^{13}C NMR (Tables 1 and 2), ^1H-^1H COSY : 4b-H – 5-H, 5-H – 6-H, 6-H – 5-H, 7-H – 15-H_3, 9-H – 10a-H, 10a-H – 9-H, 11-H – 12-H_3, 11-H_3 – 13-H_3, 12-H_3 – 11-H, 13-H_3 – 11-H; ^1H*- ^{13}C COSY : 2-H – C-2, 3-H – C-3, 4b-H – C-4, 5-H – C-5, 6-H – C-6, 8-H – C-8, 9-H – C-9, 10a-H – C-10a, 11-H – C-11, 12-H_3 – C-12, 13-H_3 – C-13, 14-H_3 – C-14, 15-H_3 – C-15, 17-H_3 – C-17; NOESY : 7-H – 15-H_3, 9-H – 10a-H, 10a-H – 9-H, 2-H – 12-H_3, 2-H – 13-H_3; EI mass *m/z* (%) M^+ 362 (<1), 330 (2.7), 345 (3.2), 302 (2.3), 221 (100), 206 (38), 189 (17.6), 161 (2.1), 143 (14), 119 (70).

7-Dehydrosarcophytin (**2**) : Colourless needles (80 mg), m.p. 160–162° (Found : C, 70.2; H, 7.7. $C_{21}H_{28}O_5$ requires : C, 70.0; H, 7.8%); $[\alpha]_D^{25}$ +403.6° (*c* 0.5, $CHCl_3$); λ_{max} ($CHCl_3$) 238 nm; ν_{max} (nujol) 1714, 1683, 1450, 1366, 1277, 1245, 1205, 1109, 750 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); ^1H-^1H COSY : 2-H – 12-H_3, 13-H_3, 3-H – 2-H, 4b-H – 5-H, 5-H – 6-H, 6-H – 15-H_3, 8-H – 15-H_3, 9-H – 10a-H, 14-H_3, 12-H_3, 13-H_3 – 2-H, 13-H – 11-H, 14-H – 11-H; ^1H-^{13}C COSY : 2-H – C-2, 3-H – C-3, 4b-H – C-4, 5-H – C-5, 8-H – C-8, 9-H – C-9, 12-H_3 – C-12, 13-H_3 – C-13, 14-H_3 – C-14, 17-H_3 – C-17; NOESY : 2-H – 12-H_3, 13-H_3, 3-H, 3-H – 2-H, 6-H – 15-H_3, 9-H – 10a-H, 10a-H – 9-H, 14H_3, 14-H_3 – 10a-H, 15-H_3 – 6-H, 17-H_3 – 10a-H, 3-H; EI mass *m/z* (%) M^+ 360 (0), 330 (2.7), 300 (< 2), 302 (2.3), 284 (< 2), 219 (100), 204 (40.4), 189 (17.6), 187 (37.8), 161 (2.1), 160 (88.9), 144 (37.3), 143 (14), 119 (70).

$\Delta^{7(15)}$-Dehydrosarcophytin (**3**) : Colourless feathery needles (20 mg), m.p. 160–162° (Found : C, 70.1; H, 7.7. $C_{21}H_{28}O_5$ requires : C, 70.0; H, 7.8%); $[\alpha]_D^{25}$ +126.17° (*c* 0.57, $CHCl_3$); λ_{max} ($CHCl_3$) 233 nm; ν_{max} (nujol) 3434, 2980, 1720, 1705, 1629, 899 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); EI mass *m/z* (%) M^+ 360 (3.3), 340 (28.2), 324 (7.5), 308 (18.8), 281 (19.8), 265 (47.8), 237 (14.6), 216 (42), 156 (83.8), 140 (83.8), 90 (30), 237 (14.6), 114 (35).

Isosarcophytin (**4**) : Colourless needles (30 mg), m.p. 163–164° (Found : C, 69.7; H, 8.2. $C_{21}H_{30}O_5$ requires : C, 69.6; H, 8.3%); $[\alpha]_D^{25}$ – 61.2° (*c* 0.25, $CHCl_3$), λ_{max} ($CHCl_3$) 222 nm; ν_{max} (nujol) 3350, 1710, 1620, 1500, 1370, 1255, 1140, 1020, 940 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); EI mass *m/z* (%) M^+ 362 (<1), 344 (2.4), 221 (23.6), 219 (100), 204 (49.2), 187 (38.1), 162 (20.8), 159 (15.9), 144 (16.6), 142 (13.9), 118 (99.5).

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