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Rare Aromadendrane Diterpenoids From a New Soft Coral Species of *Sinularia Genus* of the Indian Ocean

Ammanamanchi S. R. Anjaneyulu, Moturu V. R. Krishnamurthy and Gottumukkala V. Rao

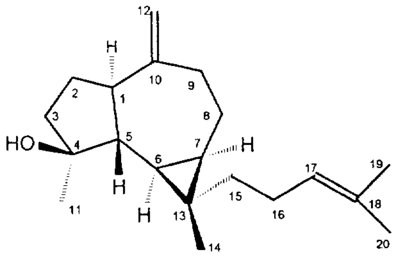
Department of Organic Chemistry, School of Chemistry, Andhra University,
Visakhapatnam - 530 003, India.

Abstract: Chemical examination of a new soft coral species of *Sinularia genus* of the Indian Ocean resulted in the isolation of 4 new and rare aromadendrane type of diterpenoids, in addition to the known $\Delta^{9(15)}$ - **africanene, batyl alcohol** and three **polyhydroxy steroids**. The structures of new diterpenoids have been established by a study of their spectral data. The diterpenoids of this class have now been reported for the first time from a marine organism, while they were reported only once from a terrestrial source. The crude extract of the organism has showed moderate larvicidal activitiy. © 1997 Elsevier Science Ltd.

In our continuing interest on the bioactive secondary metabolites of the soft corals of the Indian ocean[1-6] we have undertaken the chemical examination of a new soft coral species of *Sinularia genus* collected from Mandapam (9°16'N, 79° 12'E) coast of the Indian Ocean and the results are reported here.

The organisms were cut into small pieces and repeatedly percolated with cold methanol. The residue from the methanolic extract was fractionated into ethylacetate. The combined ethyl acetate fractions on evaporation left a residue which on chromatography on a column of silica gel furnished 9 pure compounds 1 to 9. Of those, compounds **5** and **6** were identified as the commonly occurring sesquiterpene, $\Delta^{9(15)}$ – africanene (**5**) and batylalcohol (**6**) respectively. Compounds **7**,**8** and **9** are polyhydroxy steroids, the last being a ring C seco derivative. Compounds **1** to **4** have been characterised as new and novel diterpenoids having aromadendrane skeleton. This is the first report of the occurrence of this class of diterpenoids from a marine organism while they were isolated only once from terristrial source, *Cneorum tricoccon* and *Neochemaelea pulverulenta*.[7]

Compound (**1**) colourless oil $[\alpha]_D^{25}$-25.9° (c 1.5, $CHCl_3$), $C_{20}H_{32}O$, M^+ 288 was readily recognised as a monohydroxy diterpene alcohol (OH, 3400 cm^{-1} in IR and no conjugation in U.V) from its molecular formula and its ^1H NMR spectrum. The NMR spectrum revealed the presence of an isopropylidene group and a trisubstituted olefinic proton [δ 1.6 (s, 3H), 1.7 (s, 3H), 5.1 (brt, 1H)] one tertiary methyl at δ 1.05 (s, 3H), a second tertiary methyl connected to an oxygenated carbon at δ 1.30 (s, 3H) and an exocyclic methylene appearing as a doublet centered at δ 4.7. The spectrum further revealed the presence of two cyclopropyl protons at δ 0.5 (dd, 11, 8Hz, 1H) and



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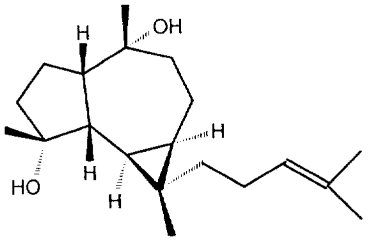

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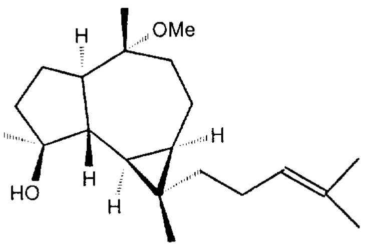

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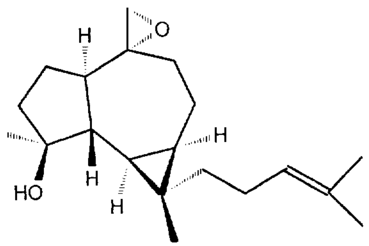

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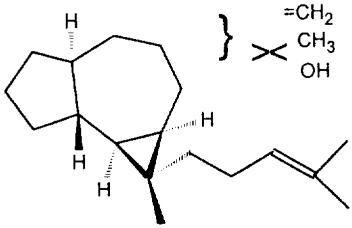

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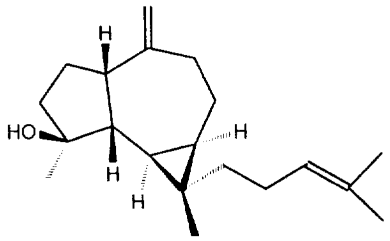

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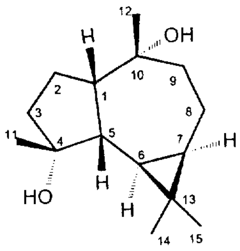

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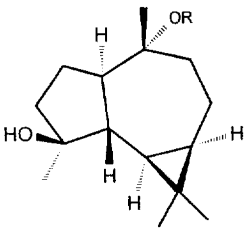

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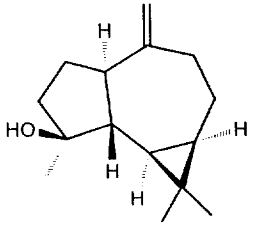
15 R=H



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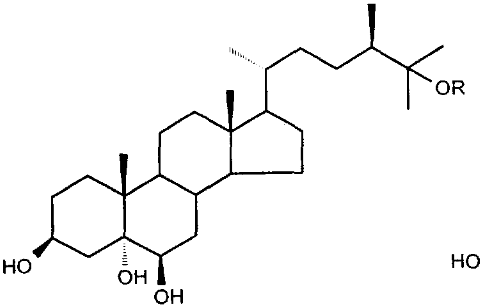

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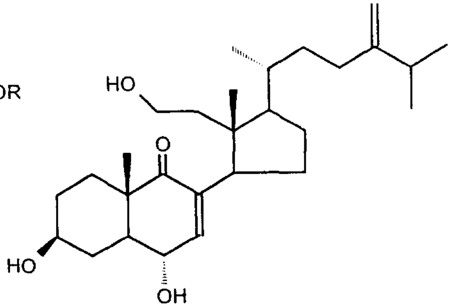
8 R=Ac



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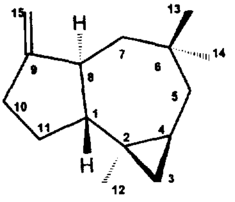

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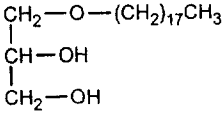

$$\begin{array}{l} CH_2-O-(CH_2)_{17}CH_3 \\ | \\ CH-OH \\ | \\ CH_2-OH \end{array}$$

6

at δ 0.7 (dt, 11,6 Hz, 1H) which are reminiscent of the cyclopropyl protons in an aromadendrane skeleton as noticed in a number of sesquiterpenoids [6,8-10]. The chemical shifts of these cyclopropyl protons (0.4 to 0.61) were, in fact, used to distinguish the aromandendrane skeleton and alloaromedendrane skeleton (one proton at higher field around at δ 0.02 and the other at lower field at δ 0.63)[8,9]. A tentative structure (**12**) with extension of an aromodendrane skeleton by an additional prenyl substituent at C-15 can be assumed for the molecule where in the position of exocyclic double bond can be located at C-10 and the tertiary methyl and hydroxyl at C-4 or vice versa .

Compound **1** has the same structural features as spathulenol (**14**)[11] except for-the additonal 5 carbon prenyl unit. Compound **1** showed all the 20 carbons in its ^{13}C NMR specturum whose substitution pattern was revealed by the DEPT spectrum. The chemical shifts of the respective carbons (Table-2) were assigned in comparision with aromadendrane sesquiterpenes and in particular, with spathulenol. The spectrum showed 4 .olefinic carbons, two of exocyclic methylene and two of a trisubstituted double bond. One oxygenated carbon was noticed at 81.1. Of the two tertiary methyls connected to a cyclopropane ring only one was noticed at 13.4 while the other appeared as a methylene at 39.1 (t) being substituted by a prenyl unit. The C_{14} and C_{15}

TABLE - 1

^{1}H NMR data of compounds 1, 2, 3, 4, 10, 11 & 13 in $CDCl_3$ TMS as reference, chemical shift (δ), J in brackets Hz

Assignment	1^a	2^b	3^b	4^b	10^{a*}	11^{a*}	13^c
6-H	0.5(dd,11,8)	0.1(m)	0.4(m)	0.4(m)	0.02(dd,11,7.5)	0.42(dd 11,8)	--
7-H	0.7(dt,11,6)	$0.65(m)^\#$	$0.65(m)^\#$	$0.65(m)^\#$	0.62(ddd, 13,8,5)	0.61(ddd, 13,8,5)	--
11-H_3	1.3(s)	1.25(s)	1.15(s)	1.12(s)	1.21(s)	1.13(s)	1.30(s)
12-H_3	$4.7(t,10)^+$	1.38(s)	1.25(s)	$3.4(q,13)^+$	1.35(s)	1.24(s)	$4.70(s)^+$
14-H_3	1.05(s)	1.10(s)	1.05(s)	1.01(s)	1.05(s)	1.04(s)	1.03(s)
15-H_3	--	--	--	--	1.06(s)	1.06(s)	--
19-H_3	1.6(s)	1.6(s)	1.58(s)	1.6(s)	--	--	1.61(s)
20-H_3	1.7(s)	1.7(s)	1.70(s)	1.68(s)	--	--	1.70(s)
1-H	2.45(dd,6,13)	2.5(m)	--	--	2.50(m)	--	--
17-H	5.1 (br t)	5.1(br t, 7.2)	5.13(t,7)	5.08(t,8)	--		5.11(t, 7)
OMe			3.20(s)			3.14(s)	

a : Spectra were recorded at 400 MHz, b : Spectra were recorded at 90 MHz, c : From reference 7, * : From reference 6
+ : methylene protons, # : not resolved in 90 MHz spectrum .

carbons of spathulenol appeared at δ 16.4 and δ 28.7. In **1** it was the lower field C_{15}-H_3 (28.7) that was missing showing that carbon-15 of **1** is substituted by a prenyl unit. The C-13 values of C-6, C-7, C-11, C-10 and C-12 of compound **1** agreed perfectly with the corresponding values of spathulenol, suggesting the location of exocyclic methylene at C-10 and the methyl and tertiary hydroxyl at C-4 . The chemical shift of C-6 at 29.1 in **1** as in spathulenol (**14**) and other related sesquiterpene alcohols[6,8,9] indicated the stereochemistry at C_4 with β-hydroxyl and α-methyl. The structure of compound **1** could thus be established as 4β -hydroxy-15 - (3 -methyl- 2- butenyl) aromadendr - $\Delta^{10(12)}$-en (**1**).

The 2D NMR (^1H-^1H COSY, ^{13}C-^1H COSY, NOESY) spectra of compound (**1**) could be obtained on 90 MHz instrument. Only a little information could be derived from the spectra as most of the protons appeared between δ 1.0 to δ 2.45 and that too remained unassigned being submerged under the intense methyl signals . In ^1H-^1H COSY a clear connectivity was noticed between the trisubstituted olefinic proton C-17H and the methyls on the double bond C-19H_3 and C-20H_3. In ^{13}C-^1H COSY the C-12 carbon showed connectivity with the exocyclic methylene protons at 4.7. Besides the carbons C-11, C-14, C-19 and C-20 showed connectivitites to the respective protons attached. Similarly in the NOESY spectrum connectivity could be noticed between the C-17H and C-19H_3 and C-20H_3.

A search in literature revealed that diterpenoids of this class have not been isolated from any marine organism so far, while a group of 5 closely related diterpenoids under the name *cneorubines* were reported only once from terristrial plants *Cneorum tricoccon* and *Neochamaelea pulverulenta*[7]. The physical and spectral characteristics of one of these, cneorubin-X(**13**) were found to agree largely with those of compound **1** but for minor differences in ^{13}C Values. The basic difference between cneorubin-X and compound **1** was, however , noticed with respect to the configaration at C-1. The stereochemistry of **13** was correlated with that of alloaromadendrane series by using extensive degradative studies and CD, ORD data[7]. The chemical shifts of the diagnostic cyclopropyl protons of **13** were not, however, recorded to make a comparison. Besides, there are some discrepancies in ^{13}C data of cneorubin-X. The C-11 was reported at 29.3 (erroneously as doublet) while this carbon was reported at ≈ 25.0 in all the sesquiterpenoid derivatives irrespective of aromadendrane or alloaromadendrane series and also irrespective of the stereochemistry at C-4.[6,8,9] So also the C-6 was reported at 25.3, a value diagnostic of C_4 stereochemistry with α-hydroxyl and β-methyl[8]. But opposite stereochemistry was derived at C_4 in **13.** Since the chemical shifts of cyclopropyl protons of compond **1** and its ^{13}C NMR data clearly showed that it belongs to aromadendrane series it is C_1-epimer of cneorubin-X (**13**). The structure was also supported by its mass fragmentation as reported[7].

Compound **2** also came as an oil $[\alpha]_D^{25}$ - 19.6 (c 0.7, $CHCl_3$). Its molecular formula was assigned as $C_{20}H_{34}O_2$ by elemental analysis and mass ion 288 (M^+- H_2O). It was also found to be a diterpene alcohol (3310 cm^{-1}) related to compound **1.** It showed no absorption in UV spectrum. Its ^1H NMR spectrum showed the cyclopropyl protons at δ 0.1 (m, 1H) and 0.65 (m,1H) indicating that it belongs to alloaromadendrane series[8]. The NMR spectrum also revealed the

TABLE-2

^{13}C NMR data of compounds 1,2,3,4,10,11,13 and 14 in $CDCl_3$ in ppm.

Carbon No	1^a	2^a	3^a	4^a	10^b	11^b	13^c	14^d	Multiplicity
1.	53.0	54.0	48.3	51.6	53.9	48.2	53.7	53.6	d
2.	26.8	25.6	24.1	23.8	25.5	24.1	26.8	26.8	t
3.	43.2	37.6	41.0	40.7	37.5	41.1	43.4	41.9	t
4.	81.1	82.2	80.3	80.1	82.1	80.2	80.9	81.0	s
5.	53.9	47.3	47.6	47.3	47.7	48.2	53.1	54.4	d
6.	29.1	25.3	29.7	29.4	25.2	28.2	25.3	30.1	d
7.	26.3	27.6	27.0	26.9	28.8	26.7	24.9	27.6	d
8.	24.9	18.8	19.7	19.5	18.8	19.7	26.0	24.9	t
9.	41.7	38.0	37.5	38.0	38.0	41.1	41.7	39.0	t
10.	153.6	74.3	79.1	78.8	74.4	78.9	153.3	153.4	s
11.	25.9	25.2	26.1	25.1	25.4	24.4	29.3	26.1	q
12.	106.7*	32.1	23.7	55.6*	32.1	23.6	106.3*	106.4*	*t/q
13.	24.4	22.7	23.3	23.1	18.8	19.6	24.3	20.3	s
14.	13.4	13.5	13.7	13.5	16.2	16.4	13.7	16.4	q
15.	39.1	43.2	43.1	42.9	28.6	28.5	38.9	28.7	t
16.	25.6	25.8	25.7	25.9			25.7		t
17.	124.9	124.9	125.0	124.9			124.9		d
18.	131.2	131.0	131.2	130.8			130.8		s
19.	25.7	25.4	25.4	25.5			26.4		q
20.	17.6	17.5	17.7	17.3			17.5		q
			51.3			52.2			q

a : Spectra recorded at 22.5 MHz, b : Spectra recorded at 100 MHz from ref.6, c : from ref. 7, d : from ref. 8.

presence of an isopropylidene group and one tertiary methyl as in compund **1** but two methyls connected to oxygenated carbons instead of one in the latter (Table 1), suggesting the presence of hydroxyls both at C-4 and C-10.

Compound **2** showed all the 20 carbons in its ^{13}C NMR spectrum whose substitution pattern was derived from the DEPT spectrum. The trisubstituted olefinic carbons appeared at δ 124.9 and δ 131, the two oxygenated carbons at 82.2 and 74.3 as noticed in 4α, 10α-dihydroxy alloaromadendrane[6] (**10**) (cf Table-2). The ^{13}C values of compound **2** from C-1 to C-12 closely agreed with the values of **10** showing their structural as well as stereochemical identity in the tricyclic system. Here again, it was clear that the additonal prenyl unit was substituted to carbon-15 and not to 14 as in compound **1**. It is pertinent to note that the ^{13}C values of C-6(25.3) and C-12(32.1) in Compound **2** as in the diol **10** are diagnostic of the stereochemistry at C_4 with α-hydroxyl and C_{10} also with α-hydroxyl[8]. Compound **2** thus is new to literature and can be considered as 4α, 10α-dihydroxy-15-(3-methyl-2-butenyl) alloaromadendrane (**2**). The structure was also supported by mass fragmentation.

Compound **3** came as an oil . Its molecular formula was assigned as $C_{21}H_{36}O_2$ by elemental analysis and mass ion m/z 288 (M^+-CH_3OH). It was also found to be a dihydroxy diterpene related to compound **2** but a monomethyl ether. Its IR absorption showed for hydroxyl (3420 cm^{-1}) and a trisubstituted olefin (910 cm^{-1}) but no conjugation was noticed in its UV spectrum. In its ^1H NMR spectrum it showed the cyclopropyl protons at δ 0.40 (m, 1H) and at 0.65 (m 1H) relating it to aromadendrane series[8]. In addition to isopropylidene methyls at δ 1.58 (s, 3H) and 1.70 (s, 3H) and one tertiary methyl at δ 1.05 (s, 3H), it showed two more tertiary methyls at δ 1.15, and 1.25 connected to oxygenated carbons as in compard 2. An aliphatic methoxyl was noticed at δ 3.20 (s,3H).

Its ^{13}C NMR spectrum showed all the 21-carbons whose chemical shifts were assigned with the help of DEPT spectrum and comparative data with closely related compounds. It cannot be considered as a monomethyl ether of compound **2** as it belongs to aromadendrane series while the latter to that of alloaromadendrane. The location of methoxyl at C-10 in preference to C-4 as well as the stereochemistry at C-4 and C-10 were decided by comparing the carbon chemical shifts of **3** with those of 4-β-hydroxy-10α-methoxyaromadendrane (**11**) isolated earlier from these laboratories from another soft coral *Sinularia maxima*[6]. The values of C-1 to C-12 in compound **3**, agreed closely with those of the corresponding values of compound **11**. The appearence of C-12 methyl carbon at 23.7 as in **11** suggested that the methyl at C_{10} is β-axial and the methoxyl α-equatorial. Similarly, the appearence of C-6 carbon at 29.7 as in **11** suggested β-hydroxyl at C-4 with α-methyl. Thus the structure of compound 3 could be establshed as 4β-hydroxy-10α-methoxy-15-(3-methyl -2-butenyl)-aromandendrane (**3**). The structure was supported by the expected mass fragmentation.

Compound **4** also came as an oil $[\alpha]_D^{25}$ - 42.5°, (c 1.0, $CHCl_3$). Its molecular formula was fixed as $C_{20}H_{32}O_2$ from elemental analysis and mass ion m/z 286 (M^+-H_2O). This was also recognised as a new diterpenoid similar to the above compounds. It exhibited the hydroxylic absorption (3410 Cm^{-1}) an epoxide (1060 cm^{-1}) and a trisubstituted olefinic group (920 Cm^{-1}) in its IR spectrum, but no conjugation in its UV spectrum. From the cyclopropyl protons at δ 0.4 (m, 1H)and at δ 0.6 (1H, m) in its ^{1}H NMR spectrum it was also recognised as an aromadendrane derivative.

It showed two methyls of an isopropylidene group at δ 1.6 and 1.8, one tertiary methyl at δ 1.01 and another tertiary methyl at δ 1.21 connected to an oxygenated carbon. In place of a second tertiary methyl also connected to oxygenated carbon in compounds **2** and **3** it showed a two proton methylene quartet at δ 3.4 (ABq, 13, 2H) suggesting the presence of an exocyclic epoxide connected either to 10 and 12 carbons or 4 and 11 carbons. The presence of an epoxide was also inferred from its ^{13}C NMR spectrum where it showed 3 oxygenated carbons for only two oxygens of the molecule. The epoxy group was located between C-10 and C-12 carbons since the C-11 methyl carbon appeared at δ 25.1 as noticed in compounds **3**,**2** and **1**. The appearance of C-6 carbon at δ 29.4 as in compound **3** suggested β-hydroxyl and α-methyl stereochemistry at C-4. The chemical shift of C-1 in compound **4** appeared deshielded by about 5 ppm compared to that in 4β, 10α-dihydroxy aromadendrane[6](15) and this was taken to be the effect of C-10, C-12 α-epoxy group on C-1α proton and consequently on the C-1 chemical shift. The structure of compound **4** could thus be assigned as 4β-hydroxy-10,12-α-epoxy-15-(3-methyl-2butenyl) aromadendrane (**4**). The structure was also supported by its mass fragmentation as noticed for the above related compounds.

Thus compounds **1** to **4** are new members of this rare group of diterpenoids having aromadendrane or alloaromadendrane basic skeletons and so regarded as their 15-prenylsubstituted derivatives.

Compound **5**, oil, $C_{15}H_{24}$ $[\alpha]_D^{25}$ + 86° (c 1.1, $CHCl_3$) was identified as the sesquiterpene,$\Delta^{9(15)}$ - africanene (**5**) by comparison of its physical and spectral data[12,13,14] and with an authentic sample[12]. Compound **6** m.p. 69-71°C $[\alpha]_D^{25}$ + 2.6° (C 1.0 $CHCl_3$) was identified as the commonly occurring batyl alcohol (**6**)[15,16] by direct comparision with an authentic sample[16].

Compound 7 m.p. 260-62°C $[\alpha]_D^{25}$ - 22° (c 0.9, MeOH) analysed for $C_{28}H_{50}O_4$ and was recognised as a tetrahydroxy steroid from its IR, ^{1}H and ^{13}C NMR spectra. A comparison of these data with those of (24S)-24-Methyl-3β, 5α, 6β, 25-tetrahydroxychloestane (7)[17,18,19] as well as a direct comparison with an authentic sample [19] proved their identity.

Compound **8** m.p. 240-42°C $[\alpha]_D^{25}$ - 17.9° (c 1.5, MeOH) analysed for $C_{30}H_{52}O_5$. It was recognised as a tetrahydroxysterol mono acetate from its spectral characteristics, IR , ^{1}H, ^{13}C NMR which were found to be identical with those of **7** except that this is its 25-monoacetate. A comparison of the spectral characteristics of compound **8** with those of (24S)-24 - methyl - 3β, 5α. 6 β, 25 - tetrahydroxycholestane tetrol 25-mono acetate (**8**)[17,18,19] and a direct comparision with an authentic sample[19] proved their identity.

Compound **9** m.p. 160-63°C $[\alpha]_D^{25}$ + 27.5°C (c, 0.5, MeOH was also recognised as a polyhydroxy steroid from its IR, ^{1}H, ^{13}C NMR spectra (see experimental). In its ^{1}H NMR spectrum it exhibited an exocyclic methylene and an olefinic β-proton of an α, β -unsaturated carbonyl group. The conjugated carbonyl was also inferred from its UV absorption at 241 nm. Its ^{13}C NMR spectrum gave evidence for three oxygenated carbons, a carbonyl carbon and 4 olefinic carbons. From the double bond equivalence it was considered as a tricyclic seco-steroid. A comparision of the physical and spectral characteristics of this steroid with a ring C seco steroid, 9-oxo-9,11-seco-24-methylenechloest-7- ene-3β,6α,11-triol (**9**)[20] isolated recently from soft coral *Sinularia hirta*[21] and *Sinularia maxima*[22] and a direct comparision with an authentic sample[21,22] proved their identity.

The crude methanolic extract of the organism showed moderate larvicidal activity LD ± SE = 209.06 ± 7.11 mg/lit.

EXPERIMENTAL

^{1}H NMR spectra were measured on a Bruker 400 MHz and JEOL JNM EX-90 spectrometers. ^{13}C NMR spectra were measured on a JEOL JNM EX-90 Spectrometer at 22.5 MHz using $CDCl_3$ as solvent and tetramethylsilane as internal reference. Mass spectra were obtained on a JEOL JMS - 300 spectrometer UV spectra were recorded on a Milton Roy 1201 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 841 spectrophotometer. Melting points were determined on VEB-analytic dreder HMK hot plate and are uncorrected. Optical rotations were determined on a Perkin-Elmer model 141 polarimeter. Elemental analyses were determined on a Carlo Erba-1108 instrument.

Extraction and Isolation The soft coral species was collected in June 1993 at the Putti Island of the Mandapam coast in the Indian Ocean. It was described as a new species of *Sinularia genus* by Mr. Phil Alderslade, Curator, Museum and Art Gallery of the Northern Territory and the specimens were preserved at the above Museum as NTM C12399 and at the Museums of NIO, Goa and School of Chemistry, Andhra University as AU1-076. The crude extract of the organism showed moderate larvicidal activity. Slices of the soft coral (Ca . 8 Kg dry weight) were soaked in methanol (10 l) at room temperature and the extract was decanted carefully and concentrated under reduced pressure. This process was repeated six times. The concentrated extract was reextracted into ethyl acetate. The ethyl acetate extract was dried over anhydrous $MgSO_4$ and the residue (30 g) after removal of the solvent was chromatographed over a silicagel column (300 g : 100-200 mesh) using solvents of increasing polarity from hexane to ethyl acetate. The selected fractions were further purified by passing over small columns of silica gel impregnated with silver nitrate to yield 9 pure compounds.

Compound 1 : 4β-Hydroxy-15-(3-methyl-2-butenyl) aromadendr-$\Delta^{10(12)}$-en(1) colourless oil; 30 mg (0.000375%); $[\alpha]_D^{25}$ - 25.9° (c 1.5, $CHCl_3$); Found : C, 83.7; H, 11.2. Calc. for $C_{20}H_{32}O$: C, 83.3 ; H, 11.1% ; IR ($CHCl_3$) : 3400 (OH), 1680, 1620, 1480, 1220, 1070, 850 cm^{-1}; UV ($CHCl_3$) : No absorption above 200 nm ; ^{1}H and ^{13}C NMR data (See Tables 1 and 2); EIMS: M^+ 288 (18.8%), 270 (M^+-H_2O, 12.2%), 245 (30.4%), 201 (65.8%), 161 (71.1%), 159 (83.8%) 145 (100%), 119 (82.3%), 105(37%), 91(8.6%), 69 (82%) and 55 (57%).

Compound 2 : 4α, 10α-Dihydroxy-15-(3-methyl-2-butenyl) alloaromadendrane(2) colourless oil; 15 mg (0.0001875%); $[\alpha]_D^{25}$ - 19.6° (c 0.7, $CHCl_3$); Found : C, 78.5; H, 11.1, Calc. for $C_{20}H_{34}O_2$; C, 78.4; H, 11.1% IR ($CHCl_3$) : 3310 (OH), 1470, 1360, 1090 and 920 cm^{-1} ; UV

(CHCl$_3$) :No absorption above 200 nm; ^{1}H and ^{13}C NMR data (See Tables 1 and 2); EIMS : M$^+$ 306 (0%), 288 (M$^+$-H$_2$O, 13.6%), 270(7.2%), 245(24.0%), 203(21.2%), 201(5.2%), 161(19.6%), 159(21.1%), 145(29.0%), 119(57.1%), 105(53.6%), 915(59%), 69(48%) and 55(53%).

Compound 3 : 4β-Hydroxy-10α- methoxy-15-(3-methyl-2-butenyl) aromadendrane (3) : colourless oil; 15 mg (0.0001875%); $[\alpha]_D^{25}$ - 23.7° (c 0.7, CHCl$_3$); Found : C, 78.5; H, 11.7, Calc. for C$_{21}$H$_{36}$O$_2$; C, 78.7; H, 11.2% IR (CHCl$_3$) : 3420 (OH), 1480, 1350, 1080 and 910 cm^{-1} ; UV (CHCl$_3$) :No absorption above 200 nm; ^{1}H and ^{13}C NMR data (See Tables 1 and 2); EIMS : M$^+$ 320 (0%), 288 (M$^+$-MeOH, 1.6%), 270(1.4%), 244(2.1%), 201(5.4%), 200(9.2%), 161(7.2%), 160(20,5%), 159(8,7%), 158(13.0%), 145(4.4%), 144(26.9%), 119(26.5%), 105(59%), 91(37%), 69(78%) and 55(85%).

Compound 4 : 4β-Hydroxy-10,12-α-epoxy-15-(3-methyl-2-butenyl) aromadendrane (4): colourless oil; 20 mg (0.00025%); $[\alpha]_D^{25}$ - 42.5° (c 1.0, CHCl$_3$); Found : C, 78.7; H, 10.9, Calc. for C$_{20}$H$_{32}$O$_2$; C, 78.9; H, 10.5%; IR (CHCl$_3$) : 3410 (OH), 1470, 1340 and 920 cm^{-1} ; UV (CHCl$_3$) :No absorption above 200 nm; ^{1}H and ^{13}C NMR data (See Tables 1 and 2); EIMS : M$^+$ 304 (0%), 286 (M$^+$-H$_2$O, 9%). 268(6.9%), 245(3.1%), 200(8.0%), 161(21.3%), 159(5.7%), 158(41.9%), 146(30.1%), 144(15.8%), 199(7.6%), 118(44.5%), 105(13.4%), 91(26.8%), 68(86%) and 55 (78%).

Compound 5 : $\Delta^{9(15)}$ - Africanene (5) [12,13,14] : colourless oil : 100 mg (0.00125%) $[\alpha]_D^{25}$ + 86° (c 1.1, CHCl$_3$); Found : C, 88.43; H, 11.57%, Calc. for C$_{15}$H$_{24}$; C, 88.23; H, 11.78% IR (CHCl$_3$) : 3070, 3020, 1650, 1384, 1020, 885 cm^{-1}; UV (CHCl$_3$) : No absorption above 200 nm. The physical and spectral characteristics of compound **5** agreed with those of africanene[12,13,14].

Compound 6 : 3-Octa decyloxy-1,2-propane diol (batyl alcohol) (6)[15,16] : colourless flakes from methanol 200 mg (0.0025% m.p 69-71° . $[\alpha]_D^{25}$ + 2.6° (c, 1.0 CHCl$_3$); Physical and spectral (IR, ^{1}H and ^{13}C NMR) data of the compound agreed with those of batyl alcohol[15,16].

Compound 7 : (24S)-24-Methylcholestane-3β,5α,6β, 25-tetrol (7)[17,18,19] :colourless needles from chloroform - methanol, 150 mg (0.001875%) m.p. 260 - 62° ; $[\alpha]_D^{25}$ - 22° (c 0.34, MeOH); Found: C, 74.6; H, 11.2. Calc. for C$_{28}$H$_{50}$O$_4$: C, 74.67; H, 11.11%; IR (KBr) : 3520 (br, OH), 2900, 1426, 1050 ad 920 cm^{-1}, UV:No conjugation above 200 nm.

^{1}H NMR (90 MHz,d$_5$-pyridine) : δ 0.73(s, 18-H$_3$), 1.03 (s, 28-H$_3$), 1.09 (s, 21-H$_3$), 1.35 (s, 26-H$_3$), 1.35 (s, 27-H$_3$), 1.65 (s, 19-H$_3$), 2.90 (t, 12.0,4-H), 4.1 (brs, 6-H), 4.88 (m, 3-H): ^{13}C NMR (22.5 MHz d$_5$-pyridine) : 32.6(t), 33.9(t), 67.5(d), 41.8(t), 75.9(s), 76.6(d), 34.9(t), 31.3(d), 46.8(d), 39.8(s), 20.9(t), 41.6(t), 43.7(s), 56.4(d), 24.6(t), 29.4(t), 56.4(d), 12.4(q), 17.8(q) 36.1(d), 19.4(q), 35.6(t) 27.9(t), 46.7(d), 72.6(s), 25.9(q) 28.2(q), 15.7(q), (C$_1$ to C$_{28}$ respectively)

Compound 8 : (24S)-24-Methylcholestane-3β,5α,6β,25-tetrol-25-monoacetate (8)[17,18,19] : colourless needles from chloroform-methanol 175 mg (0.00218%) m.p. 240-42°C $[\alpha]_D^{25}$ - 17.9° (c 1.5, MeOH) ; Found : C, 73.09; H, 10.56. Calc. for C$_{30}$H$_{52}$O$_5$: C, 73.17, H; 10.56; IR (KBr) : 3500 (br), 1720, 1250, 1120, 1030, 920 Cm^{-1}; UV No. characteristic absorption above 200 nm.

^1H NMR (90 MHz, d_5-pyridine); δ 0.74(s, 18-H_3), 0.91 (s, 28-H_3) 0.99 (s, 21-H_3), 1.49 (s, 26-H_3), 1.49 (s, 27-H_3), 1.64 (s, 19-H_3), 2.01 (s, $CO\underline{CH}_3$), 2.90 (t, 12, 4-H), 4.19 (brs, 6-H), 4.88 (m, 3-H); ^{13}C NMR (22.5 MHz $CDCl_3$): 32.6 (t), 33.9 (t), 67.5 (d), 41.8 (t), 75.9 (s), 76.6 (d), 34.9 (t), 31.3 (d), 46.8 (d), 39.8 (s), 20.9 (t), 41.6 (t), 43.7 (s), 56.4 (d), 24.6 (t), 29.4 (t), 56.4 (d), 12.4 (q), 17.8 (q), 36.1 (d), 19.4 (q), 35.6 (t), 27.9 (t), 42.4 (d), 85.5 (s), 23.1 (q), 23.5 (q), 15.7 (q), (C_1 to C_{28} respectively and 170.0, 22.3 for CO and CH_3).

Compound 9 : 9-Oxo--9,11-Seco-24-methylene-cholest-7-ene-3β, 6α,11-triol (9)[20,21,22] : colourless flakes from chloroform-methanol, 50 mg (0.000625%) 160 - 63°C $[\alpha]_D^{25}$ + 27.5° (c 0.5, MeOH). Found: C, 73.01; H, 10.3%: Calc. for $C_{28}H_{46}O_4$; C, 73.3 ; H, 10.3%; IR (KBr) 3620 and 3350 (-OH), 1670, 1650, 1520, 1280, 875 cm^{-1}. UV (MeOH) 241 nm.

^1H NMR (90 MHz, d_5-pyridine) δ 0.81 (s, 18-H_3), 1.05 (d, 6.5, 21-H_3), 1.06 (d, 6.5, 26-H_3), 1.09 (d, 6.5, 27-H_3), 1.24 (s, 19-H_3), 2.97 (br d, 4-H), 4.14 (m, 11-H_2), 4.20 (br d, 6-H), 3.9 (m, 3-H), 7.18 (d, 2, 7-H), 4.84 (br s, 28-H_2). ^{13}C NMR (22.5 MHz, d_5-pyridine): 32.7 (t), 31.9 (t), 69.7 (d), 34.6 (t), 50.5 (t), 68.8 (d), 149.2 (d), 136.2 (s), 204.8 (s), 46.4 (s), 58.2 (t), 42.3 (t), 45.2 (s), 42.8 (d), 26.2 (t), 22.4 (t), 50.0 (d), 16.1 (q), 17.3 (q), 34.6 (d), 19.1 (q), 34.6 (t), 31.3 (t), 156.6 (s), 33.9 (d), 21.8 (q), 22.3 (q), 106.8 (t).

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