

Two new terpenoid derivatives from a new soft coral species of the *Nephthea* genus of the Indian Ocean

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Received 10 August 1998; accepted 15 March 1999

Chemical examination of a new soft coral species of the *Nephthea* genus of the Indian Ocean furnishes a new sesquiterpenoid and a new diterpenoid along with some sesquiterpenes and steroids. The structure of the new sesquiterpenoid has been established as 1S, 3R, 4S, 5S, 7S-3,4-epoxy guaia- $\Delta^{10(12)}$ -ene **3** and that of a new monohydroxy diterpenoid as **4** by a study of their physical and spectral (^1H , ^{13}C , 2D NMR, IR, UV and mass) data. Other known compounds isolated are $\Delta^{9(15)}$ - africanene **1**, 9-aristolene **2**, the ubiquitous batyl alcohol **7**, two 4 α -methylsteroids, 4 α -methylcholest-24(28)-en-3 β -yl acetate **5** and 4 α , 23, 24-trimethylcholest-22-ene-3 β -yl acetate **6** two polyhydroxy steroids, 3 β , 7 β , 19-trihydroxyergosta-5, 24 (28)-diene **11** and 24-methylenecholestane-3 β , 5 α , 6 β -triol **12** in addition to three monohydroxysteroids, 24-methylcholesta-7, 25-diene-3 β -ol **8**, 24-ethylidenecholest-5-en-3 β -ol (Δ^5 -avenasterol, **9**) and 24-ethylcholest-5-en - 3 β -ol **10**. Besides the report of two new terpenoids, this is the first report on the presence of 4 α -methylsteroids in a *Nephthea* species.

A good number of soft corals of the *Nephthea* genus are known to occur in different sea waters and six identified and nine unidentified species of them have been chemically examined so far¹⁻²³. As in the case of soft corals of other genera, the chemical compounds isolated from this genus also comprise mainly diterpenoids, steroids, sesquiterpenoids and others in the order of predominance. In fact, the genus *Nephthea* is also considered as one of the most versatile in the field of terpene biosynthesis¹⁷.

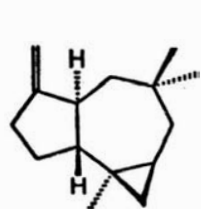
In our continuing interest on the bio-active principles of marine organisms and in particular of the soft corals of the Indian Ocean²⁴, we report herein the chemical examination of a new soft coral species of *Nephthea* genus collected from the Hutbay island of the Andaman and Nicobar group of islands of the Indian Ocean.

The initial methanolic extract of the organism after concentration to a small volume was reextracted into ethyl acetate. The residue from the ethyl acetate extract on subjecting to silica gel column chromatography furnished the nine pure compounds Np-01 to Np-07, Np-09 and Np-10 apart from a mixture of monohydroxysterols (500 mg) Np-08. Of the nine pure compounds isolated four were found to be terpenoids, four steroids and the ninth, Np-07 the ubiquitous batyl alcohol. Two of the four terpenoids were found to be new, one Np-03 **3**, a guaiane

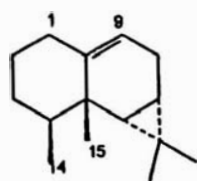
sesquiterpenoid and the other Np-04 **4**, a monohydroxyditerpen **1** and their structures have been established with the help of their physical and spectral (UV, IR, ^1H , ^{13}C NMR, 2DNMR, IR and mass) data.

The compounds Np-01 **1**, $\text{C}_{15}\text{H}_{24}$ and Np-02 **2**, $\text{C}_{15}\text{H}_{24}$, $M^+ 204$ (EIMS) were characterized as $\Delta^{9(15)}$ - africanene²⁵ and 9-aristolene^{26,27} by comparing their physical and spectral data with those reported in literature and the former also by direct comparison with an authentic sample^{24a}.

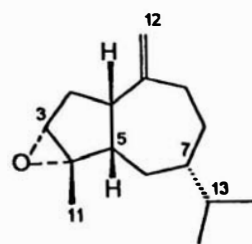
The compound Np-03 **3**, was found to be a new sesquiterpenoid and its molecular formula was assigned as $\text{C}_{15}\text{H}_{24}\text{O}$ by elemental analysis and its molecular ion $M^+ 220$ in its EI Mass spectrum. Its IR spectrum showed neither carbonyl nor hydroxy groups but indicated the presence of an epoxy group 1020 cm^{-1} thus accounting for the single oxygen atom. Besides, it showed exocyclic methylene absorption at 1640 and 885 cm^{-1} . No conjugation was noticed in its UV spectrum. Its ^1H NMR spectrum showed the presence of an epoxy proton ($\delta 2.9$ dd, $J=4$ and 10.5 Hz) and two exocyclic methylene protons ($\delta 4.89$ and 5.0 one proton each). The NMR spectrum further showed an isopropyl group ($\delta 1.03$, 6H , d, $J=6.6\text{ Hz}$ and 0.98 m, 1H) and one tertiary methyl connected to an oxygenated carbon as a singlet at $\delta 1.2$. Its ^{13}C NMR spectrum exhibited all the 15 carbons whose



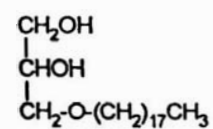
Np-01 (1)



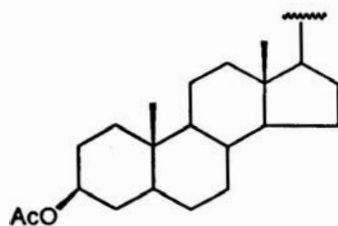
Np-02 (2)



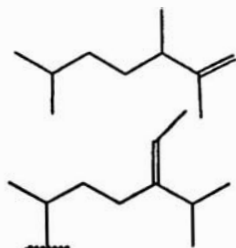
Np-03 (3, new)



Np-07(7)

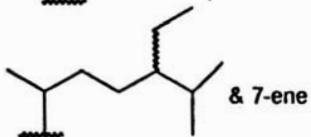


Np-08-a (8) R

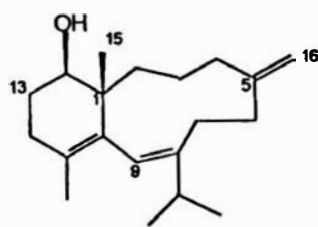


Np-08-b (9) R

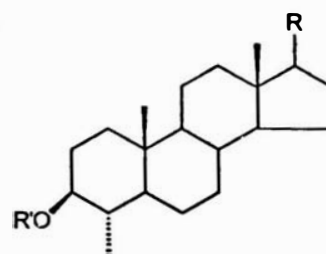
Np-08-c-(10) R



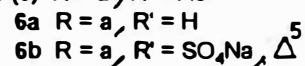
& 7-ene



Np-04 (4, new)



Np-06 (6) R = a, R' = Ac

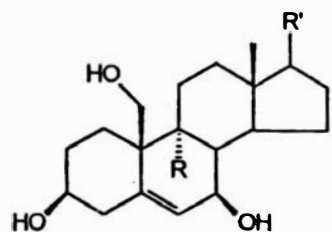


6a R = a, R' = H

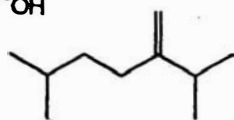
6b R = a, R' = SO₄Na, Δ⁵

Np-05 (5) R = b, R' = Ac

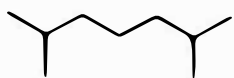
5a R = b, R' = H



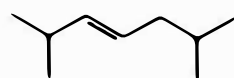
Np-09 (11) R = H, R' =



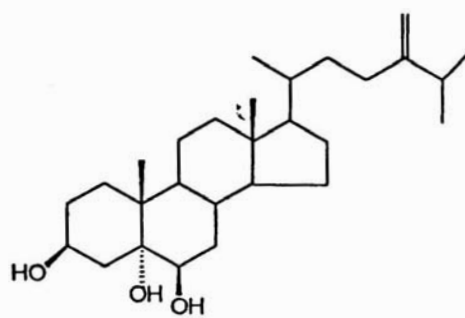
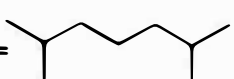
11a R = H, R' =



11b R = H, R' =



11c R = OH, R' =



Np-10 (12)

substitution pattern was revealed from its DEPT spectrum as three methyls, five methylenes, five methines and two quaternary carbons. The oxygenated carbons at δ 63.6 (d) and 59.7 (s) confirmed the presence of an epoxy group, the latter carbon in turn attached to a methyl group. The presence of an exocyclic methylene was evident from the carbons at δ 112.7 (t) and 156.7 (s).

The molecular formula requires four degrees of unsaturation of which only one was accounted for by the exocyclic methylene suggesting the molecule to be tricyclic including that of an epoxy group. A comparison of ^1H and ^{13}C NMR spectral data of **3** with those of different groups of sesquiterpenoids revealed that the compound might be a guaiane derivative for which a tentative structure **A** could be proposed. The spectral data of **3** were compared with those of three closely related guaiane sesquiterpenoids **13**, **14** and **15**. In fact, two of these **13** and **14** are enantiomers isolated from the soft coral species *N. chabroli*¹⁰ and a *Xenia*²⁸ species, respectively while the third **15** from the plant *Alisma rhizomes*²⁹. These three literature compounds are in fact, isomers differing in stereochemistry at the carbons C-4 and C-5. Compound **3** has altogether five chiral centers, two of these, C-3 and C-7 being additional to the three centers C-1, C-4 and C-5 present in the above three compounds. The ^{13}C values of C-1 and C-5 of literature compounds agreed closely with the corresponding values of **3** at the ring junction. Curiously, the values of these two carbons did not differ very much in the compounds with either *cis* or *trans*-junction. However, the respective ring junction stereochemistry in these compounds was unequivocally established by conversion into derivatives of established stereochemistry²⁹.

A close examination of ^1H - ^1H COSY and NOESY spectra of Np-03 revealed some important, though not all, connectivities (see Figure 1) to establish the relative stereochemistry of the molecule. In the ^1H NMR spectrum, the ring junction 1-H appeared as a quartet with a coupling constant of 9 Hz, which

implied that it has the same coupling with both the methylene protons at C-2 as well as other ring junction proton at C-5 which appeared at δ 1.68 as dd, $J=9, 13$ Hz. This coupling constant suggested *cis* ring junction between the 5- and 7- membered rings. These 1-H and 5-H protons showed COSY and NOESY connectivities in support of their *cis* stereochemistry. The methyl group at C-4 (11-H₃) showed NOESY connectivity with 1-H suggesting their *cis* stereochemistry and incidentally, that of epoxide as α . Similarly, the 3-H connected to the epoxy carbon at 2.9 showed NOESY connectivity with 1-H at 2.65 as well as 5-H at 1.68 establishing their mutual *cis* relationship. One of the exocyclic methylene protons appearing at 4.85 showed NOESY connectivity to the ring junction 1-H and the other exocyclic methylene proton appearing at 4.9 showed NOESY connectivity to one of the C-9 methylene protons (α -H) at 0.98. The other proton at C-9 (β -H) appearing at 2.3 showed NOESY connectivity to 7-H appearing at 1.38 suggesting thereby that the 7-H is β axial with the isopropyl group being α . The 7- β H showed NOESY connectivity with the 5-H appearing at 1.68.

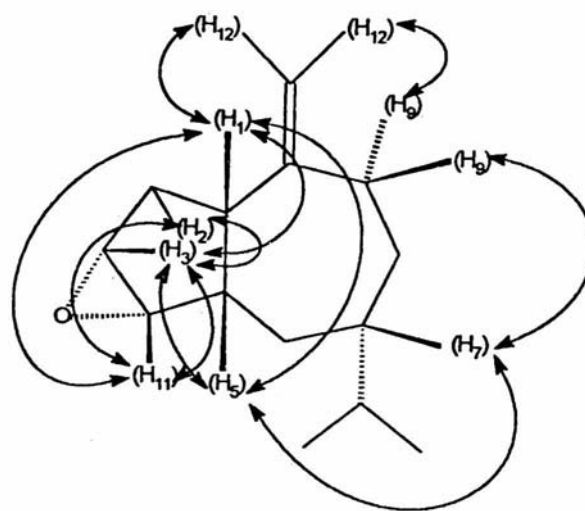


Figure 1 – NOESY connectivities of Np-03(3)

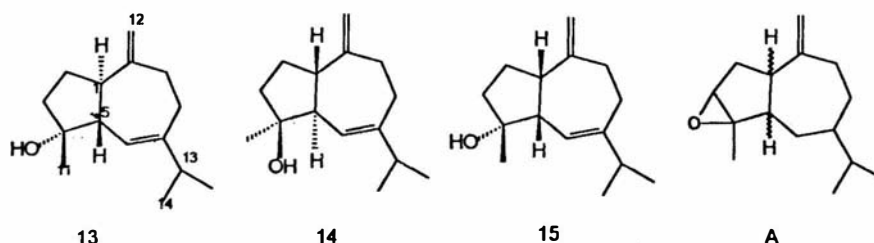


Table I—Comparative ^1H , ^{13}C and 2D NMR Spectral Data of Np-03, 13, 14 and 15
(Solvent CDCl_3 , chemical shift in δ , ppm; J in brackets in Hz and TMS as reference).

Position	Np-03 (3)			Literature compounds			Np-03 2D NMR CDCl_3 , 90 MHz	
	^{13}C NMR 22.5 MHz	^1H NMR 300 MHz	^{13}C - ^1H COSY Assignment	14^{24} 22.5 MHz	15^{25} 100 MHz	13^{15}	NOESY	
1	48.7 d	2.65 q, (9) 1H	1-C-H	47.1	47.3	47.2	Chemical Shift	Assignment
2	39.2 t	2.0 and 2.2 m, 2H	2-C-H ₂	24.8	25.3	24.2		
3	63.6 d	2.9 dd, (4, 11) 1H	3-C-H	40.0	40.6	40.2	2.65 and 1.2	H(1)-H ₃ (11)
4	59.7 s	-	-	40.0	40.6	40.2	2.65 and 1.68	H(1)-H(5)
5	50.9 d	1.68 dd, (9, 13) 1H	5-C-H	54.8	55.4	54.9	2.9 and 1.2	H(3)-H ₃ (11)
6	39.8 t	1.5 and 1.7 m, 2H	6-C-H ₂	121.6	123.2	121.4	2.9 and 2.2	H(3)-H(2)
7	30.1 d	1.38 m, 1H	7-C-H	149.1	148.6	149.5	2.9 and 2.65	H(3)-H(1)
8	27.2 t	1.68 m, 2H	8-C-H ₂	37.0	37.5	37.4	2.9 and 1.68	H(3)-H(5)
9	2.98 t	2.3 and 0.98, 2H	9-C-H ₂	21.9	30.3	30.0	2.65 and 5.0	H(1)-H ₁ (12)
10	151.8 s	-	-	153.7	154.3	153.7	0.98 and 4.9	H(9)-H ₂ (12)
11	16.9 q	1.2 s, 3H	11-C-H ₃	24.8	24.6	24.8	1.2 and 2.2	H ₃ (11)-H(2)
12	112.7 t	4.9 and 5.0 each s	12-C-H ₂	106.4	106.6	106.4	1.38 and 2.3	H(7)-H(9)
13	29.8 d	0.98 m, 1H	13-C-H	37.4	37.6	37.0	1.38 and 1.68	H(7)-H(5)
14	21.6 q	1.03, d 6H (6.6)	14-C-H ₃	28.2	21.4	21.5	^1H - ^1H COSY	
15	21.6 q	1.03, d	15-C-H ₃	21.4	21.7	21.3	2.3 and 4.9	H(9)-H ₂ (12)
							2.9 and 2.2	H(3)-H(2)
							2.65 and 1.68	H(1)-H(5)

The foregoing observations established the relative stereochemistry of the new sesquiterpenoid Np-03 as 1S,3R,4S,5S,7S-3,4-epoxyguaia- $\Delta^{10(12)}$ -ene **3**. The mass spectral fragmentation (**Chart 1**) of Np-03 observed in the expected lines also supported its structure.

The compound Np-04 was recognised as a new monohydroxy diterpenoid by a close examination of its physical and spectral (^1H and ^{13}C NMR) characteristics. Its molecular formula was assigned as $\text{C}_{22}\text{H}_{32}\text{O}$ by elemental analysis and molecular ion M^+ 288 in its EIMS spectrum. Its IR spectrum showed bands for hydroxylic absorption (3450cm^{-1}), exocyclic methylene ($1640, 885\text{cm}^{-1}$) and olefinic absorption (1600cm^{-1}). Its UV absorption 240 nm showed the presence of conjugated double bonds in the absence of a carbonyl group.

Its ^1H NMR spectrum, reminiscent of a diterpenoid skeleton, showed an isopropyl group (δ 1.03 and 1.10, 3H each, d, $J=7.0\text{Hz}$), a tertiary methyl at 0.99 (3H, s) and an olefinic methyl at δ 1.56 (3H, s) accounting for four of the five methyls required for a diterpenoid skeleton. In place of fifth methyl an exocyclic methylene was noticed with the methylene protons appearing at δ 4.90 and 5.05 one proton each as a singlet. In addition, one olefinic proton was noticed at 5.15 as a broad singlet and one carbinolic methine

proton at δ 3.90 1H, t, ($J=5.0\text{Hz}$) in support of the presence of a secondary hydroxyl in the molecule.

Its ^{13}C NMR spectrum showed all the 20 carbons whose substitution was revealed from its DEPT spectrum as four methyls, eight methylenes, three methines and five quaternary carbons. The carbon signals at 110.9 (t) and 158.3 (s) supported the presence of an exocyclic methylene. Four more olefinic carbons were noticed at 137.4 (s), 125.8 (d), 145.7 (s), and 134.6 (s) accounting for one tetra and one trisubstituted double bonds, consistent with the presence of an isolated olefinic proton in its ^1H NMR spectrum at 5.15. It might be possible that these two double bonds are conjugated to account for the UV absorption of the molecule. The carbon signal at δ 72.4 (d) accounted for the carbon bearing secondary hydroxyl. The molecular formula of Np-04, requires 5 degrees of unsaturation of which only 3 have been accounted for so far and all these in the double bonds to suggest the molecule to be bicyclic. Soft corals are known to elaborate a variety of diterpenoids although the 14 membered carbocyclic cembranoids are the most common. Even some of the bicyclic, tricyclic and tetra cyclic derivatives reported to occur are, in fact, derived from the cembranoid skeleton. For example, the bicyclic diterpenoid compounds **16** and **17** of caryophyllene skeleton isolated initially from

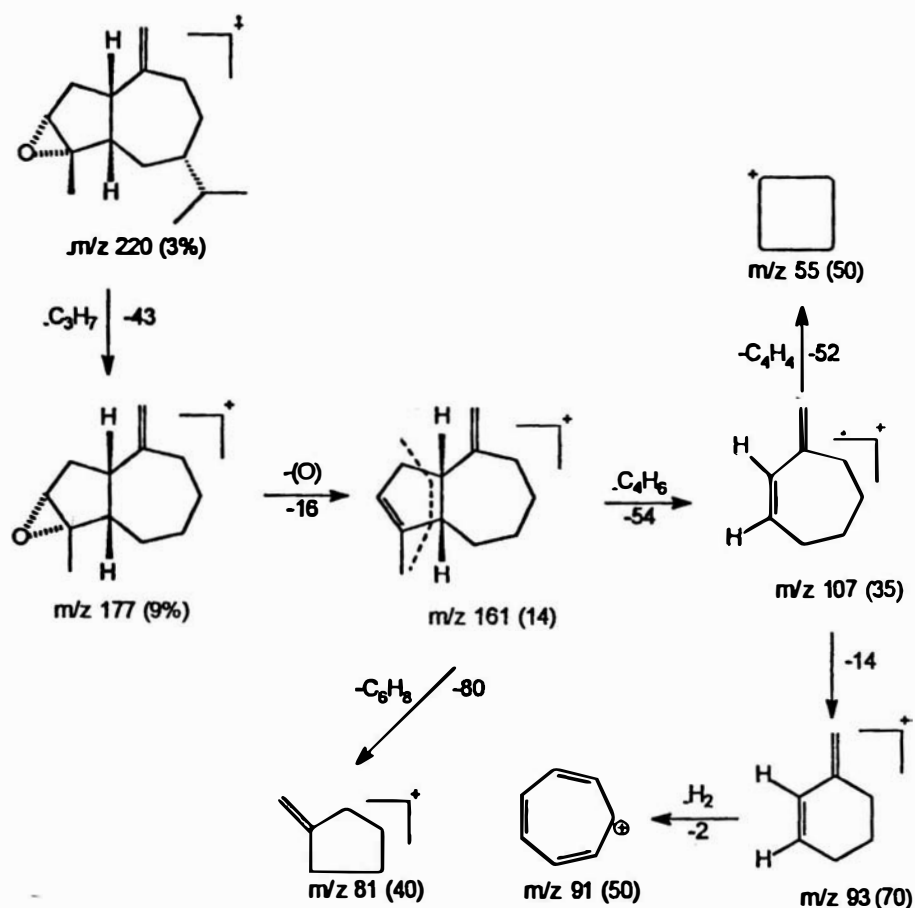
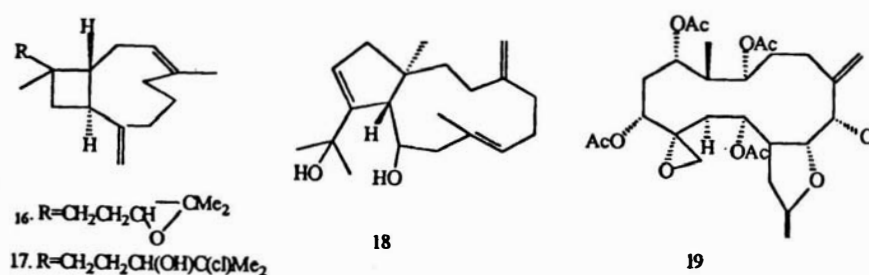
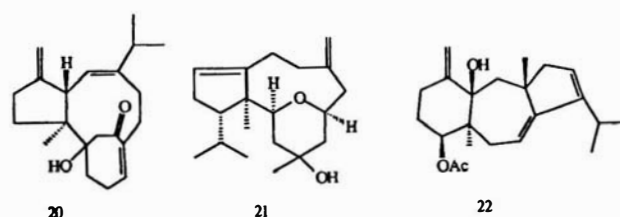


Chart 1

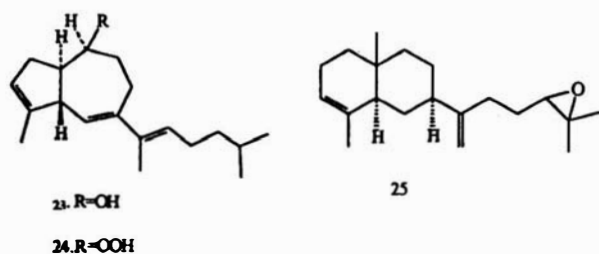


Xenia species³⁰ and later from *Nephthea chabroli*⁹ are derived from the cembranoid nucleus.

Of the other bicyclic diterpenoids, dollabellanes, like clavudiol-B **18** from *Clavularia viridis*³¹ from the South China Sea are derived from the cembranoid skeleton. Similarly, juncins like **19** from *Junceella juncea*³² of the Indian Ocean, sarsolide-A **20** from *Sarcophyton solidum*³³ of South China Sea possessing an additional lactone bridge and neodollabellane **21** possessing a pyran ring from *Lobophytum* species³⁴ of the Indian Ocean are tricyclic diterpenoids derived from the cembranoid skeleton. The above form only



representative examples while a host of others derived from cembranoids are known. The diterpenoids of cembranoid skeleton¹⁶ as well as those of caryophyllene skeleton⁹ have already been reported earlier from the species of *Nephthea* genus. In addition, the *Nephthea* species also elaborated the



diterpenoids having guaianane skeleton like **23** and **24**, from *Nephthea chabroli*⁷ or cadenane skeleton like **25** from a *Nephthea* species¹⁶

The new diterpenoid Np-04 contained a tertiary methyl group, not connected to an oxygenated carbon, obviously at the ring junction of a bicyclic system, either of a cadenane skeleton like **25** with eight carbon side chain or a dollabellane skeleton **18** with five and eleven membered rings fused together or a juncinoid **19** without the lactone, having six and ten membered rings. The tertiary methyl group might also exist *geminal* to an alkyl group as in caryophyllene type. The other bicyclic diterpenoid skeletons without a tertiary methyl group are ruled out for the molecule.

A close examination of the above possible skeletons was made keeping in view of the various carbon multiplicities of the molecule Np-04 and its mass spectral fragmentation. Its mass spectrum showed the molecular ion M^+ , at m/z 288 (8%) followed by the ions at m/z 270 (27%, $M^+ - H_2O$), 227 (40%, $M^+ - H_2O - \text{isopropyl}$), followed by several other peaks, m/z 149 being the base peak. The appearance of an intense peak by the loss of 43 amu, that is, an isopropyl group immediately after the loss of water molecule from the molecular ion, suggested the presence of an isopropyl group directly linked to one of the ring carbons and not at the end of a long side chain. The skeleton chosen for the molecule should also account for the base peak m/z 149 that is $C_{11}H_{17}$. At the same time it should also include five quaternary carbons, one exocyclic methylene and a conjugated diene system with only one olefinic proton. The cadenane skeleton is ruled out as the five quaternary carbons and other functionalities could not be accommodated.

All these data would fit into a caryophyllene skeleton as depicted by **16**, **17**. This structure also explains the formation of ion m/z 149 as the base peak although the formation of ion $[M^+ - H_2O - \text{isopropyl}]$ as a prominent (40%) peak stands against it. Another strong reason against this structure was noticed by the observation that the isopropyl proton in the molecule appeared very much deshielded at 2.85 which is very unusual. This proton must be allylic in nature and even so, a value around 2.3 was observed for such a proton normally. A value of 2.85 was noticed for this proton in compounds like **22**³⁵ where further deshielding was noticed due to olefinic bond. The proton appearing at 2.85 as multiplet in the molecule thus belongs to an isopropyl proton which was clearly revealed in its $^1H-^1H$ COSY, $^{13}C-^1H$ COSY and NOESY spectrum as well.

In view of the foregoing inadequacies coming in the way of a caryophyllene skeleton for the molecule, a juncin type bicyclic system was chosen to accommodate all the structural features of the molecule. This system is, in fact, derived from the fourteen membered cembranoid skeleton by a carbon-carbon bond between C-3 and C-8 carbons, the numbering of which changes in juncin skeleton to C-1 and C-10 and the latter numbering is chosen to describe the compound. The position of the conjugated double bonds appeared to be more or less fixed at 8, 9 and 10, 11 as any variation would reduce the quaternary carbons and also increase the olefinic protons. The secondary hydroxyl could be located at C-14 or C-2, while other positions are ruled out, to account for the deshielded neighbouring quaternary carbon 50.1 (s). The hydroxyl was, however, preferred at C-14 as location of it at C-2 would have given a more unsaturated fragment $C_{11}H_{15}$ as the base peak. The chemical shifts assigned to various carbons were very well supported by all the $^{13}C-^1H$ connectivities (**Table II**) noticed in the spectrum and are consistent with the comparative literature values. The $^1H-^1H$ COSY, NOESY spectra revealed only a few, but important connectivities.

The absence of NOESY connectivity between the carbinolic methine proton with the ring junction

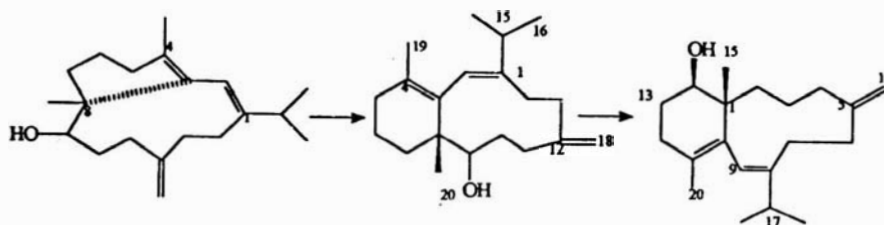


Table II—Comparative ^1H and ^{13}C NMR spectral data of compounds Np-04 and 21
(Chemical shifts in δ , ppm; J in brackets in Hz and TMS as reference)

Position	^{13}C NMR 22.5 MHz	Np-04 (4)		21 ⁹	Np-04 (4) 2DNMR (90 MHz)	
		^1H NMR 90 MHz	^{13}C - ^1H COSY assignment		NOESY	
1	50.1 s	-	-	52.3		
2	36.9 t	2.32 m	2-C-H ₂	34.7	Chemical Shift	Assignment
3	21.9 t	1.05 m	3-C-H ₂	23.8	3.9 and 1.48	H(14)-H(13)
4	30.3 t	1.3, 2.1 m	4-C-H ₂	135.4	2.3 and 1.48	H(12)-H(13)
5	158.3 s	-	-	124.5	2.85 and 1.03	H(17)-H ₃ (18)
6	27.5 t	2.15 m	6-C-H ₂	39.0	2.85 and 1.10	H(17)-H ₃ (19)
7	40.6 t	2.13 m	7-C-H ₂	39.8	5.05 and 1.3	H(16)-H ₂ (4)
8	137.4 s	-	-	154.6		
9	125.8 d	5.15 br s	9-C-H	48.4	^1H - ^1H COSY	
10	145.7 s	-	-	40.4	Chemical Shift	Assignment
11	134.6 s	-	-	35.8	3.9 and 1.48	H(14)-H(13)
12	35.3 t	2.3 m	12-C-H ₂	28.4	2.85 and 1.03	H(17)-H(18)
13	45.2 t	1.48, 2.3 m m	13-C-H ₂	29.9		
14	72.4 d	3.9 t (5)	14-C-H	64.7		
15	30.4 q	0.99 s	15-C-H ₃	58.3		
16	110.9 t	4.9, 5.05 s	16-C-H ₂	19.8		
17	27.2 d	2.85 m	17-C-H	18.5		
18	20.9 q	1.03 d (7.0)	18-C-H ₃	24.9		
19	21.3 q	1.10 d (7.0)	19-C-H ₃	112.0		
20	15.6 q	1.56 s	20-C-H ₃	16.3		

methyl protons indicated their *trans* orientation. With the methyl group taken to be β , the H-14 becomes α and consequently the 14-OH β -oriented. The structure **4** proposed for the molecule appears to be justified on the basis of biogenetic grounds and also on the basis of satisfactory accommodation of all its physical and spectral characteristics. The structure was also supported by its mass fragmentation (**Chart 2**).

The residue from hexane-ethyl acetate (9:1) fractions of the main column was found to be a mixture of two monohydroxy-4-methylsterols, which were separated as their acetates Np-05 **5** and Np-06 **6**.

Np-06 **6** C₃₂H₅₄O₂, m/z 410, M⁺-AcOH from its CIMS, in its IR spectrum exhibited acetate

absorptions (1725 and 1240 cm⁻¹) but no absorption for hydroxyl. No conjugation was noticed in its UV spectrum. Its ^1H NMR spectrum (**Table III**, 300 MHz), reminiscent of a steroid nucleus, showed characteristic 18-H₃ and 19-H₃ at 0.68 and 0.89 and five secondary methyls between δ 0.8 and 1.01 besides an olefinic methyl at 1.52. The acetyl group was evident from the peak at 2.01. In the olefinic region it showed one proton at 4.9, ($J = 9.0$ Hz) as a doublet. The one-proton signal appearing at 4.36 as dt, ($J = 5, 9$ Hz) is reminiscent of 3 α hydrogen over 3 β -acetoxy group as found in 4 α -methyl sterols³⁶⁻³⁸. That it is a 4-methyl steroid was very well supported by the intense ion (17%) at m/z 271^{37,39} and the ion m/z 203 forming

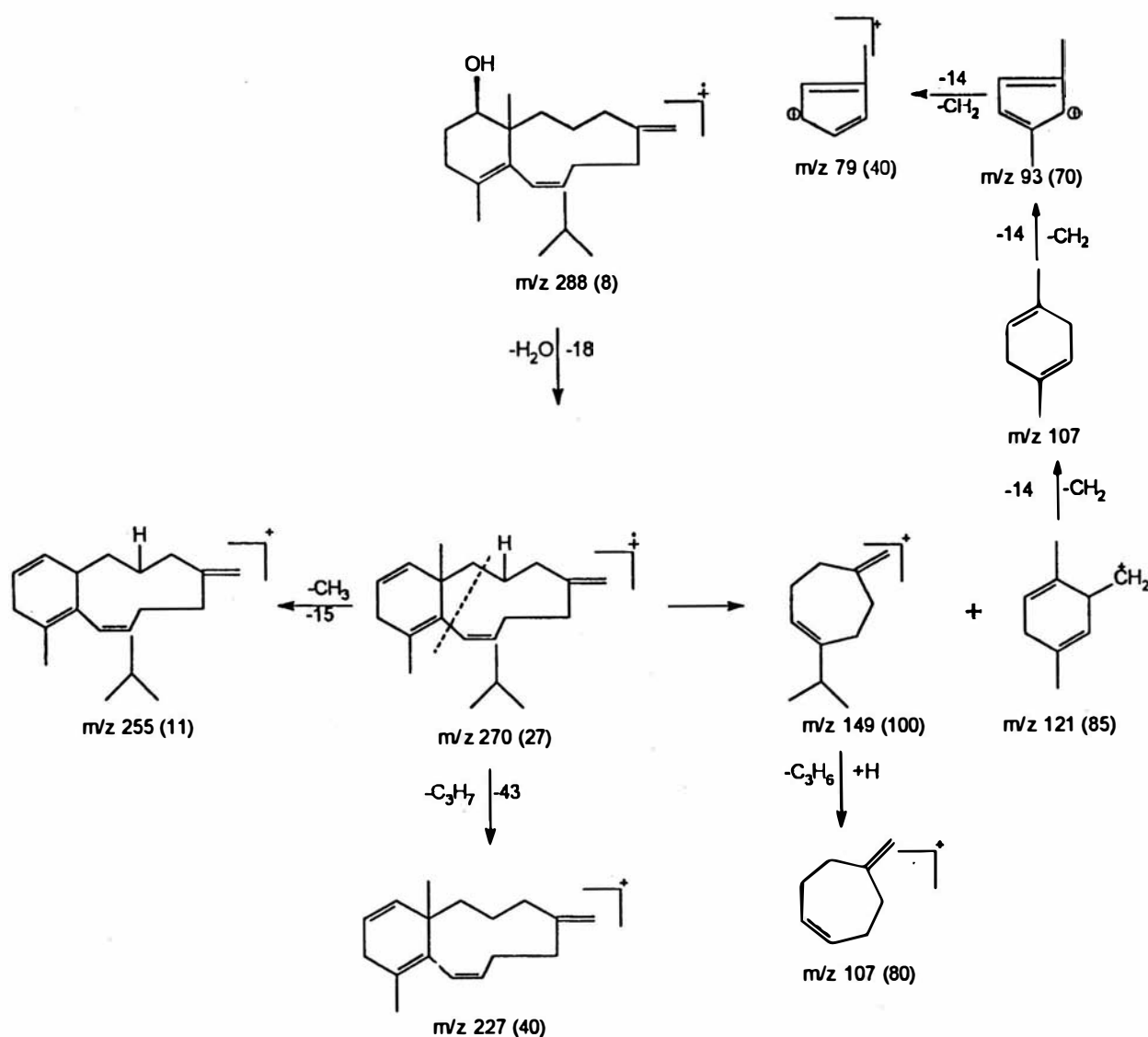


Chart 2

the base peak, derived by the loss of C_5H_8 by RDA fragmentation of ring-A as reported in literature³⁷. From this as well as the appearance of mass ion m/z 139 corresponding to the side chain $C_{10}H_{19}$ as a prominent peak suggested that the ten carbon side chain should contain the remaining four secondary methyls as well as the trisubstituted double bond.

Several alternative structures could be drawn up for this ten carbon side chain to accommodate all the above functional groups. A literature survey revealed that a large number of 4 α -methylsterols have been isolated from different marine flora and fauna. These include (a) gorgonians for example *Briareum asbestinum*³⁷, *Gorgonia mariae*³⁷; (b) Sea cucumber, *Psolus fabricii*³⁶ and (c) Soft coral, *Lobophytum* species⁴¹.

The physical and spectral (UV, IR, ^1H NMR) characteristics of Np-06 agreed very closely with those of dinosterol **6a**³⁷, except for the presence of an acetoxy group at C-3 in Np-06. Dinosterol was reported, first from a toxic Dinoflagellate *Gonoyaulax famerensis*⁴⁰ and subsequently from *Crypthecodinium cohnii*³⁹, *Glenodinium foliaceum*⁴² and so on. Its ^{13}C NMR spectrum was not published in literature for making a comparison with that of Np-06. However, the ^{13}C NMR spectral data of the sodium salt of 3-sulphate of 5-dehydrodinosterol **6b** has been published recently⁴³.

The ^{13}C NMR spectrum (**Table III**) of Np-06 could be obtained and the chemical shifts of the respective carbons assigned by a comparative study with the above compound **6b**. The carbon chemical shift

Table III — Comparative ^1H and ^{13}C NMR spectral data of compounds dinosterol **6a**, sodium salt of 5,6-dehydrodinosterol-3-sulphate **6b** and Np-06
(Solvent CDCl_3 , chemical shifts in δ , ppm; J in brackets in H_2 and TMS as reference)

Assignment	^1H NMR			Carbon No.	^{13}C NMR				
	6a ³⁷	6b ⁴³	Np-06 6 300 MHz		6b ⁴³ 1:1 CDCl_3 - CD_3OD	Np-06 6 22.5 MHz	Carbon No.	6b ⁴³ 1:1 CDCl_3 - CD_3OD	Np-06 6 22.5 MHz
18- H_3	0.68 s	0.74 s	0.68 s	1	37.8	36.1	17	57.5	56.5
19- H_3	0.83 s	1.04 s	0.89 s	2	31.3	30.8	18	12.5	12.4
21- H_3	0.91 d (6.5)	0.96 d (6.7)	0.93 d (6)	3	79.7	78.9	19	13.4	13.2
26- H_3	0.78 d (6.6)	0.8 d (6.6)	0.81 d (6)	4	40.3	40.0	20	32.1	34.9
27- H_3	0.83 d (6.6)	0.8 d (6.6)	0.85 d (6)	5	140.7	50.2	21	17.2	16.9
28- H_3	0.82 d (6.6)	0.96 d (6.7)	0.92 d (6)	6	123.0	29.2	22	135.8	135.2
29- H_3	1.49 s	1.53 s	1.52 s	7	32.5	32.1	23	132.4	131.8
30- H_3	0.95 d (6.1)	-	0.81 d (6)	8	35.1	35.9	24	50.9	51.1
3-H	3.1 m	4.23 m	4.36 dt (5,9)	9	50.9	54.5	25	28.0	27.2
20-H	-	2.4 m	2.3 m	10	37.1	34.5	26	21.6	21.7
22-H	4.87 d (9.6)	4.9 d (9.6)	4.9 d (9)	11	20.1	20.9	27	22.0	21.3
29-H	1.5 d (1.2)	-	-	12	39.7	36.6	28	19.6	20.6
COCH_3	-	-	2.01 s	13	42.8	42.4	29	20.3	21.2
				14	57.4	57.0	30	-	15.1
				15	24.8	24.1	CO	-	170.5
				16	28.3	27.9	CH_3	-	21.9

values of Np-06 are very close with those of the reference compound and in particular the carbons in the side chain in support of its characterisation as 4 α -23,24-trimethylcholest-22en-3 β -yl acetate (dinosteryl acetate, **6**). Its mass spectral fragmentation (**Chart 3**) further supported its structure.

Np-05, $\text{C}_{31}\text{H}_{52}\text{O}_2$, M^+ 456 in its, EIMS, showed the acetate ($1725, 1240\text{ cm}^{-1}$) and end methylene ($890, 1640\text{ cm}^{-1}$) absorptions in its IR spectrum but no conjugation in its UV spectrum. Its ^1H NMR spectrum, like that of Np-06, is reminiscent of a 4 α -methyl steroid nucleus where in the 3 α -H over the 3 β -acetate appeared at 4.36 as dt, $J = 5, 9\text{ Hz}$. It also showed the characteristic end methylene protons at 4.68 and 4.70 each as a singlet. Its mass spectral fragmentation was also found to be consistent with its 4 α -methyl steroid skeleton. Unlike Np-06 it has a 9 carbon side chain with the possible presence of 24(28) methylene group. In fact, its physical and spectral (UV, IR, ^1H NMR) characteristics agreed closely with those of 4 α -methyl-24-methylencholestane-3 β -ol **5a**³⁷, except for the difference that it is an acetate while the later is a hydroxy derivative. The mass spectral fragmentation (**Chart 3**) also supported its structure. This parent alcohol was isolated earlier from a Caribbean gorgonian³⁷ and more recently from a *Lobophytum* species⁴¹. The ^{13}C NMR data of either the parent sterol or its acetate have not been published

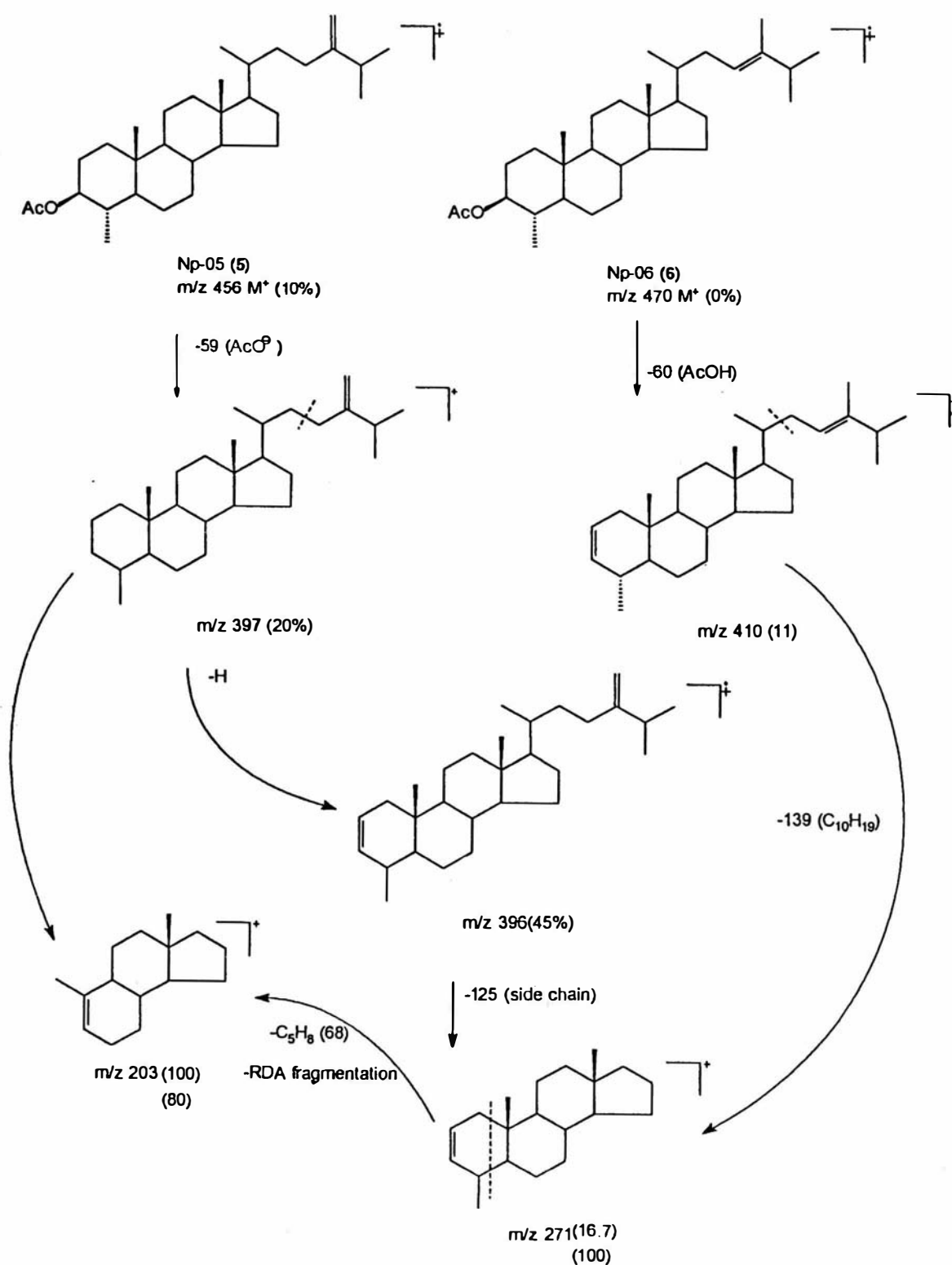
so far. The ^{13}C NMR spectrum of NP-05 could be obtained now and the chemical shifts of the respective carbons were assigned in comparison with the values of Np-06 (dinosterol acetate).

There is no report on the occurrence of 4 α -methylsterols from any species of *Nephthea* genus and this forms the first report of their occurrence in a *Nephthea* species.

Np-07 was identified as 3-octadecyloxy-1,2-propanediol⁴⁴ (batyl alcohol, **7**) by comparison of its physical and spectral characteristics as well as by direct comparison with an authentic sample^{24a}.

Np-08 was found to be a mixture of 24-methylcholesta-7, 25-diene-3 β -ol **8**¹⁵, 24-ethylidenecholest-5-en-3 β -ol⁴⁶ (Δ^5 -avenasterol, **9**) and 24-ethylcholest-5-en-3 β -ol⁴⁷ **10** by GC/MS analysis of its acetate.

The compounds Np-09 and Np-10 were characterised as trihydroxy steroids. NP-09, $\text{C}_{28}\text{H}_{46}\text{O}_3$, m/z 412, (M^+ -18), in its CIMS, showed the presence of hydroxylic (3400 cm^{-1}), olefinic and methylene absorptions ($1640, 885\text{ cm}^{-1}$) in its IR spectrum, but no conjugation in its UV spectrum. Its ^1H NMR spectra (90 MHz and 300MHz, d_5 -pyridine) suggested its steroid nature and showed three secondary methyls (1.025, 6H, d, $J=6.5\text{ Hz}$ and 1.0, 3H, d, $J=6.5\text{ Hz}$) and one tertiary methyl (0.8, 3H, s). The second tertiary methyl 19- H_3 of a steroid nucleus was conspicuously



missing indicating its possible presence as a hydroxy methyl. Consistent with this observation, the *geminal* protons of a hydroxymethyl group appeared around 3.95 in ^1H NMR ($CDCl_3$) spectrum. These protons appeared

clearly separated in the spectra taken in pyridine both on 90 and 300MHz instruments at 4.28, 3.96 each as a doublet ($J=12.0$ Hz). Two more protons appeared in the spectrum taken in pyridine (300MHz) at 4.14 and

Table IV — Comparative ^1H and ^{13}C NMR spectral data of compounds 4 α -methyl-24-methylencholestane-3 β -ol **5a**, Np-05 and Np-06(Solvent CDCl_3 , chemical shifts in δ , ppm, J in brackets in Hz and TMS as reference)

Assign- ment	^1H NMR			^{13}C NMR					
	Np-05 (5) 300 MHz	Np-06 (6) 300 MHz	(5a) ¹⁷	Carbon No.	Np-05 (5) 22.5 MHz	Np-06 (6) 22.5 MHz	Carbon No.	Np-05 (5) 22.5 MHz	Np-06 (6) 22.5 MHz
18-H ₃	0.65 s	0.68 s	0.65 s	1	35.8	36.1	17	56.1	56.5
19-H ₃	0.88 s	0.89 s	0.82 s	2	31.0	30.8	18	12.0	12.4
21-H ₃	0.96 d (6)	0.93 d (6)	0.93 d (6.2)	3	78.7	78.9	19	13.2	13.2
26-H ₃	0.98 d (6)	0.81 d (6)	1.02 d (6.3)	4	40.0	40.0	20	35.7	34.9
27-H ₃	1.02 d (6)	0.85 d (6)	1.02 d (6.3)	5	51.6	50.2	21	17.2	16.9
28- H ₃ /H ₂	4.68 s, 4.7 s	0.92 d (6)	4.65 s, 4.71 s	6	29.7	29.2	22	34.9	135.2
29-H ₃	-	1.52 s	-	7	32.1	32.1	23	34.7	131.8
30-H ₃	0.87 d (6)	0.81 d (6)	0.81 d (6)	8	33.8	35.1	24	156.4	51.1
3-H	4.36 dt (5, 9)	4.36 dt (5, 9)	3.1 m	9	54.4	54.5	25	27.1	27.2
25-H	2.2 m	-	2.3 m	10	36.5	34.5	26	21.8	21.7
COCH ₃	2.01 s	2.01 s	-	11	21.1	20.9	27	21.9	21.3
				12	36.1	36.6	28	106.4	20.6
				13	42.4	42.4	29	-	23.7
				14	56.4	57.0	30	15.1	15.1
				15	24.2	24.1	CO	170.4	170.4
				16	28.2	27.9	CH ₃	21.9	22.1

3.95 each as a doublet corresponding to two carbinolic methine protons over secondary hydroxyls. It is thus evident that it is a trihydroxy steroid with two secondary hydroxyls and one tertiary hydroxyl. The signal at 4.75 integrating for two protons accounted for a pair of exocyclic methylene protons. So also, the signal at 5.75 accounted for a trisubstituted olefinic proton, slightly deshielded to the possible presence of a hydroxyl in its allylic position. The same proton appeared very much deshielded around at δ 6.0 in the spectra taken in pyridine further supporting the presence of an allylic hydroxyl. A 7-hydroxy-5,6-ene or a 6-hydroxy-7-ene system might be present in the molecule taking the other secondary hydroxyl at the ubiquitous 3 β -position.

Its ^{13}C NMR spectrum showed all the 28 signals, which included four olefinic carbons two of an exocyclic methylene at 106.6 (t) and 156.8 (s) and two of a trisubstituted double bond at 139.3 (s) and 131.3 (d). Three oxygenated carbons were noticed at 62.8 (t), 71.2 (d) and 72.2 (d). A literature survey revealed that three trihydroxy derivatives (**11**, **11a**, **11b**) and one tetrahydroxy derivative **11c** have been isolated recently from the black coral *Antipathes subpinnata*⁴⁸ collected in the Bay of Naples. The

physical and spectral characteristics of Np-09 closely agreed with those of 3 β , 7 β , 19-trihydroxyergosta-5, 24 (28)-diene **11** showing their identity. In fact, this trihydroxy steroid was also isolated earlier from the soft corals *Litophyton viridis*⁴⁹ and a *Nephthea* species.^{3, 4, 12} Its mass spectral fragmentation was found to be consistent with its structure in showing the peaks at m/z 412 ($\text{M}^+ - \text{H}_2\text{O}$), 394 ($\text{M}^+ - 2\text{H}_2\text{O}$), 382 ($\text{M}^+ - \text{H}_2\text{O} - \text{CH}_2\text{O}$), 364 ($\text{M}^+ - 2\text{H}_2\text{O} - \text{CH}_2\text{O}$) and others.

Np-10, $\text{C}_{28}\text{H}_{48}\text{O}_3$, m/z 432, (M^+) in its EIMS also showed hydroxylic absorption (3400cm^{-1}) and olefinic and exocyclic methylene unsaturation (1650 and 890cm^{-1}) in its IR spectrum. Its ^1H NMR spectrum exhibited the normal tertiary methyls of steroid nucleus 18-H₃ at 0.75 and 19-H₃ at 1.66, the latter very much deshielded indicating the possible presence of one of the hydroxyls at 6 β -position. Besides, it showed 3 secondary methyls and one exocyclic methylene. The presence of exocyclic double bond was evident from its ^{13}C NMR spectrum showing signals at δ 106.5 (t) and 156.7 (s), the only unsaturation present in the molecule. The ^{13}C NMR spectrum also showed 3 oxygenated carbons at 67.4 (d), 76.0 (s) and 76.2 (d) suggesting the presence of two secondary hydroxyls and one tertiary hydroxyl in the molecule. With one of the hydroxyls located at the

ubiquitous 3β -position, the second hydroxyl could be located at 6β in view of the deshielded 19-H_3 as well as the appearance of deshielded $4\beta\text{-H}$ proton at 2.95 as found in 6β -hydroxy steroids⁵². Two carbinolic methine protons were noticed, one at 4.15 and the other at 5.05 corresponding to the $6\alpha\text{-H}$ and $3\alpha\text{-H}$ protons. The appearance of $3\alpha\text{-H}$ proton very much deshielded indicated the presence of 5α -hydroxyl. The foregoing information is consistent with the structure of Np-09 as 24-methylene cholestane- 3β , 5α , 6β -triol 12. Its physical and spectral data were found to be identical with the literature values of this sterol isolated from the soft corals *S. dissecta*⁵⁰ and an unidentified *sinularia* species⁵¹ and more recently from *Lobophytum catalai*⁵² from these laboratories. A direct comparison of the compound with an authentic sample proved their identity further⁵².

Experimental Section

General. Melting Points were determined on a VEB Analytik 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. IR spectra were recorded on a Perkin-Elmer-model-841 spectrophotometer and UV spectra on a Milton Roy spectronic 1201 spectrophotometer. ^1H NMR spectra were recorded on a Bruker DRX-300 MHz or JEOL JNM EX-90 instrument using CDCl_3 or pyridine- d_5 and TMS as internal standard. ^{13}C NMR spectra were recorded on a JEOL JNM EX-90 spectrometer and CIMS/EIMS were carried out on a Hitachi RMU-6U or JEOL JMS-300 at 70 eV mass spectrophotometer. Column chromatography was performed on silica gel of 100-200 mesh. All the spots were visualised by spraying 5% sulphuric acid in methanol.

Collection, extraction and isolation. The soft coral *Nephthea* species was collected from the Hutbay Island ($12^\circ 9' \text{N}$, $93^\circ 48' \text{E}$) of the Andaman and Nicobar group of islands of the Indian Ocean during 1994. This species was identified as new *Nephthea* sp. by Dr. Phil Alderslade, Curator, Museum and Art Gallery of the Northern Territory, Australia. The freshly collected species were washed thoroughly with fresh water and shade dried. The organism was sliced and percolated several times with methanol at room temperature for 2 days. The extract (8L) after percolation was evaporated at room temperature under reduced pressure. This process was repeatedly

done until the residue was colourless. The residue from the combined methanolic extract was fractionated into ethyl acetate and removal of solvent under reduced pressure left a gummy residue (30g). The weight of the organism was found to be 4.0 kgs after extraction. The ethyl acetate extract (30g) was chromatographed over silica gel (100-200 mesh; 300g) column using solvents of increasing polarity from hexane through ethyl acetate to methanol. The selected fractions were further purified by passage through a silica gel column or by recrystallisation to yield nine pure compounds Np-01 to Np-07, Np-09 and Np-10 along with a mixture of monohydroxy sterols Np-08.

Compound Np-01: $\Delta^9(15)$ – Africanene 1^{24a,25}.

Colourless oil, 120 mg; $[\alpha]_D^{25}$ -86.0° (c 1.1, chloroform); R_f 0.9 (pure hexane); UV (CHCl_3): no absorption above 200 nm; IR (CHCl_3): 1640 and 880 cm^{-1} .

Compound Np-02: 9-Aristolene 2^{26,27}. Colourless oil, 80 mg; $[\alpha]_D^{25}$ -80.9° (c 1.0, chloroform); R_f 0.8 (pure hexane); UV (CHCl_3): no absorption above 200 nm; IR (CHCl_3): 1640 and 1470 cm^{-1} ; ^1H NMR (chloroform, 400 MHz): 1.6 (m, 4-H), 0.6 (d, 9.0 Hz, 6H), 0.77 (t, 9.0 Hz, 7-H), 5.10 (brs, 9-H), 1.1 (s, 12 H_3), 1.04 (s, 13 H_3), 0.95 (d, 6.5 Hz, 14- H_3) and 1.10 (s, 15 H_3); ^{13}C NMR (Chloroform, 22.5 MHz): 27.2 (C-1, t), 31.5 (C-2, t), 17.9 (C-3, t), 21.8 (C-4, d), 33.1 (C-5, s), 36.8 (C-6, d), 37.9 (C-7, d), 29.5 (C-8, t), 118.4 (C-9, d), 141.5 (C-10, s), 16.0 (C-11, s), 21.4 (C-12, q), 19.3 (C-13, q), 15.7 (C-14, q) and 29.8 (C-15, q).

Compound Np-03: 1S, 3R, 4S, 5S, 7S-3,4-Epoxy guai- $\Delta^{10(12)}$ -3-ene 3. Colourless oil, 40 mg; $[\alpha]_D^{25}$ $+69.0^\circ$ (c 0.011, chloroform); R_f 0.85 (hexane-benzene 1:1). Anal. Calcd. for $\text{C}_{15}\text{H}_{24}\text{O}$: C, 81.8; H, 10.9%. Found: C, 81.2; H, 11.0%. IR (nujol): 1640, 1025 and 885 cm^{-1} ; UV (CHCl_3): no absorption above 200 nm; ^1H , ^{13}C and 2DNMR data (see Table I); EIMS m/z (%): 220(3), 177(9), 161(14), 107(35), 93(70), 91(50), 81(40) and 55(100).

Compound Np-04: Monohydroxy diterpenoid 4. Colourless oil, 40 mg; $[\alpha]_D^{25}$ -77.1° (c 0.014, chloroform); R_f 0.5 (hexane-benzene 1:1). Anal. Calcd. for $\text{C}_{20}\text{H}_{32}\text{O}$: C, 83.3; H, 11.1%. Found: C, 83.1; H, 11.4%; UV (CHCl_3): 240 nm; IR (nujol): 3450, 1640, 1600 and 885 cm^{-1} ; ^1H , ^{13}C and 2D NMR data (see Table II); EIMS m/z (%): 288(8), 270(27), 255(11), 149(100), 121(83), 227(40), 107(80), 93(70) and 79(40).

Acetylation of the sterols mixture. The residue from the main column fractions was found to be a mixture and left over night with $\text{Ac}_2\text{O}/\text{Py}$ (2mL each). Usual workup resulted in the isolation of the acetate mixture and on column chromatography it was separated into Np-05 and Np-06.

Compound Np-05: 4 α -Methylcholest-24(28)-en-3 β -yl acetate 5. Colourless needles, 100 mg; m.p. 160-63 °C; $[\alpha]_D^{25} + 30.4^\circ$ (c 0.009, chloroform); R_f 0.9 (pure benzene). Anal. Calcd. for $\text{C}_{31}\text{H}_{52}\text{O}_2$; C, 81.5; H, 11.4%. Found : C, 81.2; H, 11.7%. IR (nujol) : 1750, 1640, 1380 and 880 cm^{-1} ; UV (CHCl_3): no absorption above 200 nm; EIMS m/z (%): 456(10), 397(20), 396(45), 271(100) and 203(90); ^1H and ^{13}C data (see Table III).

Compound Np-06: 4 α , 23, 24-Trimethylcholest-22-en-3 β -yl acetate 6. Colourless needles, 125 mg, m.p. 190-93 °C; $[\alpha]_D^{25}$ (c 0.002, chloroform); R_f 0.8 (pure benzene). Anal. Calcd. for $\text{C}_{32}\text{H}_{54}\text{O}_2$: C, 81.5; H, 11.4; Found : C, 81.3; H, 11.6%. IR (nujol): 1720, 1640 and 1420 cm^{-1} ; UV (CHCl_3): no absorption above 200 nm. CIMS m/z (%): 410(11), 271(16), 273(32) and 203(100); ^1H and ^{13}C NMR data (see Table III).

Compound Np-07: 3-Octadecyloxy-1, 2-propanediol (batyl alcohol 7)^{24a,44}. Colourless pellets, 200 mg, m.p. 69-71 °C; $[\alpha]_D^{25} + 2.6$ (c 1.0, chloroform); R_f 0.75 (hexane-ethyl acetate 8:2); IR (CHCl_3): 3450, 2900, 1460, 1125, 1045, 870 and 710 cm^{-1} ; UV (CHCl_3): no absorption above 200 nm.

Np-08-monohydroxy sterols mixture. Colourless needles (500 mg). It gave +ve LB test with a play of colours characteristic of steroids. It was acetylated with $\text{Ac}_2\text{O}/\text{pyridine}$ at room temperature to give the acetate on usual work-up. The acetyl derivative showed several spots on 20% silver nitrate-silicagel TLC plate. The individual sterols were identified by

the GC/MS analysis of acetylated steroid mixture and the details are given in Table V.

Compound Np-09: 3 β , 7 β , 19-Trihydroxyergosta-5, 24 (28)-diene 11^{48,49}. Colourless flakes, 35 mg, mp 112-114 °C; $[\alpha]_D^{25} + 16.0$ (c 0.5, chloroform); R_f 0.5 (hexane-ethyl acetate 8:2); IR (CHCl_3): 3400, 1640 and 890 cm^{-1} ; UV (CHCl_3): no absorption above 200 nm; CIMS m/z (%): 430 (0), 412(20), 394(18), 376(40), 269(50), 251(35) and 44(100); ^1H NMR (pyridine, 300 MHz): 3.95 (m, 3-H), 5.75 (brs, 6-H), 4.14 (brs, 7-H), 3.96 and 4.25 (d, $J=12$ Hz, 19-H₂), 0.8 (s, 18-H₃), 1.0 (d, 6.5Hz, 21-H₃), 1.025 (d, 6.5 Hz, 26-H₃ and 27-H₃) and 4.75 (d, 28-H₂); ^{13}C NMR (pyridine, 22.5 MHz): 34.1 (C-1), 31.4 (C-2), 71.2 (C-3), 40.7 (C-4), 139.3 (C-5), 131.3 (C-6), 72.7 (C-7), 42.5 (C-8), 49.8 (C-9), 43.4 (C-10), 22.0 (C-11), 39.0 (C-12), 42.1 (C-13), 57.8 (C-14), 27.0 (C-15), 29.0 (C-16), 55.9 (C-17), 12.1 (C-18), 62.8 (C-19), 36.1 (C-20), 19.0 (C-21), 33.9 (C-22), 33.2 (C-23), 156.8 (C-24), 35.2 (C-25), 22.2 (C-26), 22.4 (C-27) and 106.6 (C-28).

Compound Np-09: 24-Methylenecholestane-3 β , 5 α , 6 β -triol (12)⁵⁰⁻⁵². Colourless needles, 30 mg, mp. 241-42 °C; $[\alpha]_D^{25} -4.0^\circ$ (c 0.25, pyridine); R_f 0.56 (hexane-ethyl acetate 8:2); IR (CHCl_3): 3409, 2940, 2860, 1660 and 1460 cm^{-1} ; UV (CHCl_3): no absorption above 200 nm; EIMS, (%) m/z : 432(60), 414(20), 381(15), 348(30), 330(50), 269(30), 81(80) and 55(100); ^1H NMR (pyridine, 90 MHz): 5.05 (brs, 3 α -H), 5.04 and 5.3 (each brs, 28-H₂), 4.15 (6 α -H) 2.95 (m, 4 β -H), 1.66 (19-H₃), 1.05 (s, 26-H₃), 1.05 (s, 27-H₃), 1.02(s, 21-H₃) and 0.75 (s, 18-H₃); ^{13}C NMR (d_5 -pyridine, 22.5 MHz): 33.2 (C-1), 32.3 (C-2), 67.4 (C-3), 42.6 (C-4), 76.0 (C-5), 76.2 (C-6), 35.6 (C-7), 31.3 (C-8), 45.8 (C-9), 39.1 (C-10), 21.9 (C-11), 40.0 (C-12), 43.0 (C-13), 56.5 (C-14), 24.5 (C-15), 28.5 (C-16), 56.4 (C-17), 12.3 (C-18), 17.1 (C-19), 35.9

Table V—GC/Mass profile of the acetylated steroid mixture

Code	Name (acetates of)	Rt (min)	RRt	Major fragmentation peaks	Ref
Np-08-a	24-Methylcholesta-7,25-diene-3 β -ol (8)	4.03	1.02	380(80), 255(34), 94(40), 69(63), 43(100)	45
Np-08-b	24E-Ethylidenecholest-5-ene-3 β -ol(Δ^5 -avenasterol,9)	4.56	1.15	394(70), 296(5), 281(10), 253(50), 55(100)	46
Np-08-c	24E-Ethylcholest-5-ene(10)	4.66	1.18	396(80), 381(24), 255(30), 213(25), 120 (12), 43 (100)	47

Instrument: Shimadzu GP-2000 at 70 eV.

GC column: ULBN HR-I equivalent to OV-1, fused silica.

Capillary: -0.25mmX50 M with film thickness 0.25micron.

(C-20), 18.9 (C-21), 35.0 (C-22), 31.1 (C-23), 156.7 (C-24), 34.0 (C-25), 22.0 (C-26), 21.7 (C-27) and 106.5 (C-28).

Acknowledgement

We are grateful to the Department of Ocean Development, New Delhi for financial support, the Coordinator, UGC COSIST and DRS programmes, School of Chemistry, Andhra University, the Head RSIC, Lucknow, India for providing spectral data, Dr (Mrs) V, Jaya Sree. NIO, Goa, India and to Dr Phil Alderslade, Curator, Museum and Art Gallery of the Northern Territory, Australia for the identification of the soft coral.

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