Novel Epoxy Steroids from the Indian Ocean Soft Coral *Sarcophyton crassocaule*

Ammanamanchi S. R. Anjaneyulu,* Moturu V. R. Krishna Murthy, and Ponnapalli M. Gowri

School of Chemistry, Andhra University, Visakhapatnam-530 003, India

Received May 3, 1999

A detailed further examination of the Indian Ocean soft coral *Sarcophyton crassocaule* resulted in the isolation of altogether 17 compounds of which two (**1** and **2**) are novel 17β,20β-epoxy steroids and one is a new dihydroxygorgost-5-en (**3**). The other compounds include the four hippurin steroids (**4**−**7**) reported earlier, and some known derivatives such as methyl arachidonate, batyl alcohol, a mixture of monohydroxy sterols, 3β-hydroxypregn-5-en-20-one, two prostaglandin derivatives (PGB_2 acid and its methyl ester), and 9-oxo-9,11-secogorgost-5-ene-3β,11-diol (**8**). The structure of new dihydroxygorgostene derivative was established as gorgost-5-ene-3β,11α-diol (**3**), while the structures of the novel epoxy steroids were established as 17β,20β-epoxy-23,24-dimethylcholest-5-ene-3β,22-diol (**2**) and its 3β,22-diacetate (**1**), respectively.

Soft corals (family Alcyoniidae) are known to produce a variety of polyhydroxy steroids, sesquiterpenoids, diterpenoids, and even tetraterpenoids.[1] The corals of the genera *Sinularia*,[2] *Lobophytum*,[3] *Sarcophyton*,[4] and *Nephthea*[5] are the most widespread. Many have been chemically examined and their chemical constituents reviewed according to genus.[2−5] Nearly 30 species of the genus *Sarcophyton* occurring in different areas have been examined so far.

In our continuing interest in the bioactive secondary metabolites of the soft corals of the Indian Ocean[6a−e] we have recently examined the species *Sarcophyton crassocaule* (family Alcyoniidae) and reported the isolation of four new hippurin steroids (**4**−**7**) from its ethyl acetate extract.[7] A detailed further examination of the same extract resulted in the isolation of 17 compounds total, which include two novel 17,20-epoxy steroids, 17β,20β-epoxy-23,24-dimethylcholest-5-ene-3β,22-diol (**2**) and its 3β,22-diacetate (**1**), a new dihydroxygorgostene, gorgost-5-ene-3β,11α-diol (**3**), and some known derivatives that were characterized by a comparative study of their physical and spectral characteristics with the data described in the literature and by direct comparison with authentic samples wherever possible. These are methyl arachidonate;[8] batyl alcohol;[6b] a mixture of monohydroxy sterols, which was analyzed by GC−MS analysis on the acetate mixture to consist of four sterols, namely, 24-methylcholest-5-en-3β-ol (campesterol),[9] (24*Z*)-ethylidenecholest-5-en-3β-ol (fucosterol),[9] (24*E*)-ethylidenecholest-5-en-3β-ol (Δ^5- avenasterol),[9] and 24-propylidenecholest-5-en-3β-ol[10] with the help of eight-peak mass index;[11] 3β-hydroxypregn-5-en-20-one,[12a,b] four hippurin steroids (**4**−**7**), reported earlier from the same species;[7] two prostaglandin derivatives, PGB_2 acid[13,14] and its methyl ester,[14,15] and a secogorgostane derivative, 9-oxo-9,11-secogorgost-5-ene-3β,11α-diol (**8**).[16] The details of characterization and structure elucidation of the compounds are presented here.

This species collected from the Australian coast was earlier examined by Bowden et al.[17] and reported to contain five cembranoid diterpenoids of which sarcophine and sarcophytoxide released into the seawater were regarded as allelochemicals.[18] It is surprising that although a host of new and known steroids was isolated from the Indian species, no diterpenoids could be found.

Compounds **1** and **2** were recognized as closely related steroid derivatives by their characteristic display of colors (pink→blue→green) in the Lieberman−Burchard test and ^{1}H NMR spectral data. The molecular formula of compound **1** was fixed as $C_{33}H_{52}O_5$ by elemental analysis and mass spectral data. The molecule did not exhibit the molecular ion but showed a peak at *m*/*z* 468 corresponding to $[M - 60]^+$ in its EIMS. Its CIMS, however, showed the ion *m*/*z* 470 corresponding to $[M + 2H - 60]^+$ implying the capture of two hydrogens by the molecule in chemical ionization.

The IR spectrum of **1** showed, for the acetate functionality (1750, 1230 cm^{-1}), trisubstituted olefinic absorption (810, 1670 cm^{-1}) and strong absorption for an epoxy group (760, 970, 1250 cm^{-1})[19a] but no hydroxylic absorption. No UV absorption was shown. Its ^{1}H NMR spectrum indicated the presence of two secondary acetoxyl groups (δ 2.0 and 2.05 each s, 6H) with the corresponding α-methine protons at δ 4.6 (m, 1H) and 4.8 (d, J = 11 Hz, 1H). No epoxy proton could be noticed, indicating its tetrasubstituted nature. Its ^{1}H NMR spectrum further showed the presence of characteristic 18-H_3 δ 0.9 and 19-H_3 δ 1.01 of the steroid nucleus in addition to a methyl connected to an oxygenated carbon at δ 1.45 and four secondary methyls (see Table 1). The characteristic 6-H of steroid 5,6-enes was noticed at δ 5.4 as a doublet (J = 6 Hz). The molecular formula accounts for eight degrees of unsaturation, which were explained, two in acetoxy groups, four in the steroid nucleus, one in a 5,6 double bond, and another in an epoxy group. To arrive at the structure of the molecule the following functionalities need to be located: two acetoxyls, an epoxy group, and five methyls, including one connected to the oxygenated carbon. If one of the acetoxyls is taken at the ubiquitous 3β-position, the second one has to be located in the nucleus or in the side chain and so also in the epoxy group.

In its CIMS (Chart 1) the ion *m*/*z* 215 (38.0) happened to be the most intense peak after the base peak. This ion, accounting for $C_{12}H_{23}O_3$, suggests its origin from a C-10 side chain bearing the acetoxyl group. The corresponding ion in its EIMS $C_{12}H_{21}O_3$ was shown at *m*/*z* 213 (13.7). The above mass data not only confirmed the presence of an acetoxyl group in the side chain, though its exact position remains to be seen, but also indicated the possible presence

* To whom correspondence should be addressed. Tel.: (0891) 530284. Fax: (0891) 555547/570365. E-mail: pandarth@md3.vsnl.net.in.

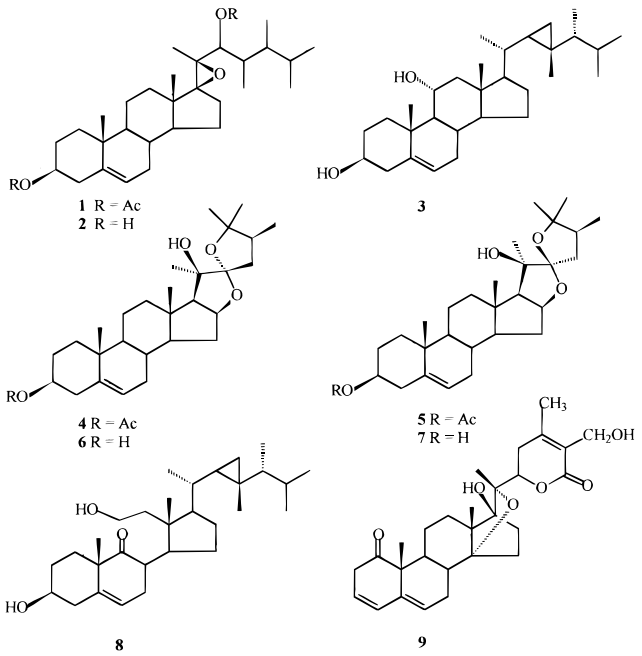
10.1021/np990205p CCC: $19.00
Published on Web 11/19/1999

Table 1. ^{1}H and ^{13}C NMR Spectral Data of Epoxy Sterols **1** and **2** and 24-Methylcholesterol (**A**) in $CDCl_3$

	δ_C (22.5 MHz)			δ_H	
position	1	2	A[49]	1 (400 MHz)	2 (200 MHz)
1	36.4	36.8	37.3		
2	31.6	32.1	31.8		
3	73.8	71.6	71.9	4.6 (m)	3.5 (m)
4	38.0	42.2	42.4	2.3 (m)	2.2 (m)
5	139.8	141.0	140.8		
6	122.1	121.1	121.7	5.4 (d, 6)	5.3 (d, 6)
7	31.1	32.3	32.0		
8	31.9	31.6	32.0		
9	49.7	49.7	50.2		
10	36.6	36.5	36.6		
11	21.1	21.2	21.2		
12	36.9	37.2	39.9		
13	43.4	43.7	42.4		
14	55.4	55.1	56.9		
15	23.6	23.7	24.4		
16	36.2	36.2	28.2		
17	79.0	79.8	56.1		
18	13.1	13.1	11.9	0.9 (s)	0.9 (s)
19	19.2	19.3	19.4	1.01 (s)	1.01 (s)
20	66.9	69.9	36.3		
21	17.2	15.8	19.0	1.45 (s)	1.33 (s)
22	77.8	76.5	33.8	4.8 (d, 11)	3.1 (d, 11)
23	27.7	31.9	30.7		
24	38.6	39.0	39.2		
25	32.3	30.9	31.5		
26	20.8	20.7	17.7	0.92 (d, 6)	0.93 (d, 6)
27	20.9	21.8	20.6	0.94 (d, 6)	0.92 (d, 6)
28	10.3	10.3	15.5	0.76 (d, 6)	0.79 (d, 6)
29	15.2	15.2		0.87 (d, 6)	0.84 (d, 6)
$CO\underline{C}H_3$	21.3			2.0 (s)	
$CO\underline{CH_3}$	21.8			2.05 (s)	
$\underline{CO}CH_3$	170.2				

of an epoxy group in the side chain. The presence of only one methyl connected to an oxygenated carbon and the tetrasubstituted nature of the epoxide suggest its possible location at C-17−C-20. With respect to the side chain, it can be 23,24- or 22,23- or 22,24-dimethylcholestane, in the absence of an ethyl group, with the secondary acetoxyl group consequently to be located at C-25, C-24, or C-23, respectively. The secondary acetoxyl group in the side chain was located at C-22 in preference to the other possibilities by considering the doublet (J = 11 Hz) nature of the α-acetoxy proton, which in any other position would have appeared as a double doublet or a triplet. This incidentally fixed the side chain to have the 23,24-dimethylcholestane skeleton. The appearance of an intense mass peak at m/z 298 (71.7) in its EIMS corresponding to the ion [M − 2 AcOH − SC]$^+$ cleaving between C-20 and C-22, strongly supported the presence of an epoxy group, α or β, at C-17−C-20. The mass spectral fragmentation does not, however, unequivocally exclude the remote possibility of an oxetane ring or a tetrahydrofuran linked to C-20 and the nucleus.

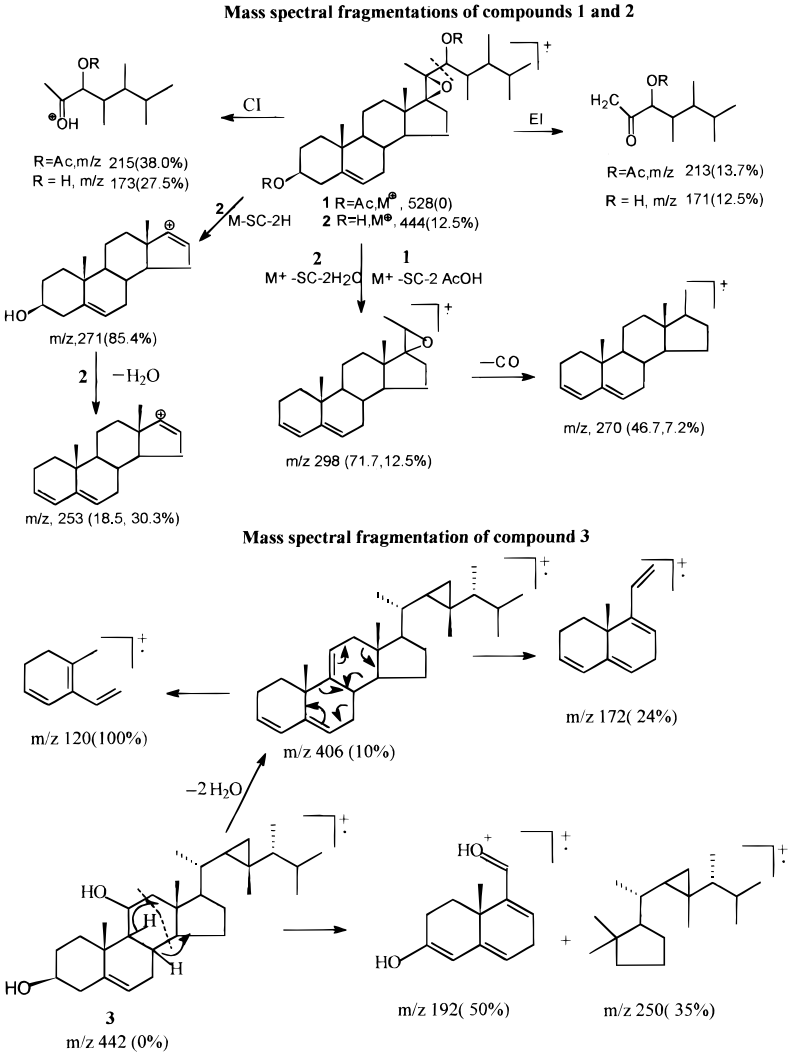
The structure of the compound was fully supported by its ^{13}C NMR spectral data discussed below. It showed all the 33 carbons whose substitution pattern was revealed from its DEPT spectrum as nine methyls, eight methylenes, nine methines, and seven quaternary carbons. The ^{13}C values of the respective carbons were assigned based on the partial and important ^{1}H−^{1}H and ^{13}C−^{1}H COSY correlations noticed in its 2D NMR spectra, which were finally compared with the corresponding values of 24-methylcholesterol (Table 1). The characteristic 5,6-olefinic carbons were found at (δ 139.8 and 122.1) and the acetyl carbons both at δ 170.2. Four oxygenated carbons were seen in the spectrum, two for the acetoxy bearing (δ 73.8, d; 77.8, d) and two connected to the epoxy group (δ 79.0, s; 66.9, s). The lower δ values, in particular the latter (66.9) for an oxygenated carbon, are closer to the values of an oxirane ring carbon rather than those of an oxetane or a tetrahydrofuran ring.[20] For example, in coagulin (**9**), a withanolide with a 14−20 tetrahydrofuran ring, the C-14 appeared at δ 88.9[21] and the C-20 at 80.9, at much higher δ values than those noticed in compound **1**, to rule out the possibility of a tetrahydrofuran ring in it. Similarly, even in the basic oxetane ring the α-methylene carbon appears around δ 72.6,[21] and a quaternary carbon like C-20 being part of an oxetane ring would have resonated at much lower field with higher δ values. On both these grounds the presence of the 17,20-epoxy ring (oxirane) was taken to be present in compound **1**, ruling out a tetrahydrofuran or an oxetane ring. Further, when compound **1** was treated with triphenylphosphine for 3 h at 200 °C a mixture (1:1) of **1** and the corresponding $\Delta^{17(20)}$- olefinic compound was obtained [as judged from the ^{1}H NMR spectrum of the mixture, which showed a methyl singlet at δ 1.6 for the olefinic 21-H_3 in place of 21-H_3 attached to an oxygenated carbon in **1** at δ 1.45]. This lent further support to the proposed structure. Neither could the reaction be pushed to completion by prolonged heating nor could the olefinic $\Delta^{17(20)}$ product be separated from the mixture.



In the absence of isomeric 17,20-epoxy derivatives for comparison and in the absence of further chemical evidence, which could not be obtained for want of material, it is difficult to establish the stereochemistry of the epoxy ring conclusively. However, the values of C-17 carbon in the diastereomeric 16,17-epoxy derivatives are available for comparison. In petuniasterone L (**10**) with a 16β,17β-epoxy ring the C-17 appeared at δ 75.0, while in petunisasterone R (**11**) with a 16α,17α-epoxy ring it appeared at δ 70.6.[22] The value of C-17 δ 79.0 in compound **1** is much higher than the values of C-17 in the above compounds, but nearer to the one in the former, to suggest that compound **1** might be a 17β,20β-epoxy derivative, to derive its structure as 17β,20β-epoxy-23,24-dimethylcholest-5-ene-3β,22-diol-3β,-22-di acetate (**1**).

Compound **2** analyzed for $C_{29}H_{48}O_3$, which was supported by its CIMS [M + H]$^+$ (445). Its molecular ion, however, could not be seen in its EIMS. It showed hydroxylic (3500

Chart 1



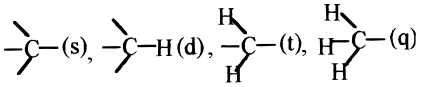
cm^{-1}), trisubstituted olefinic (795, 1675 cm^{-1}), and epoxy absorptions (1250, 970, and 760 cm^{-1})[19a] in its IR spectrum. The absence of acetate absorption in its IR spectrum as well as its molecular formula suggested it to be a dihydroxy compound, while compound **1** might be its diacetate. Its ^{1}H NMR spectrum (Table 1) was very similar to that of compound **1**, except that the acetoxymethine protons in **1** moved upfield to account for the corresponding carbinolic methine protons (δ 4.6→3.4 and 4.8→3.2). Further, compound **2** on acetylation with Py−Ac_2O gave a diacetate identical in every respect with that of compound **1** showing their relation so as to derive the structure of compound **2** as 17β,20β-epoxy-23,24-dimethylcholest-5-ene-3β,22-diol (**2**).

The structure of the compound was further supported by its mass spectral fragmentation as shown in Chart 1. The ion *m*/*z* 271 (85.4) corresponding to $[M - SC - 2H]^+$ is very prominent in the spectrum, followed by the ion *m*/*z* 253 (30.3), $[M - SC - 2H - H_2O]^+$. The ion *m*/*z* 173 (27.5) in its CIMS to the ion *m*/*z* 215 (38.0) in compound **1** accounted for the side chain fragment from $[M + 2H]^+$. Further, the ions *m*/*z* 298 and 270 formed by cleavage between the C-22,C-23 carbons support the presence of 17,20-epoxy group. The other fragmentations shown in the structure are consistent with the structure proposed. Its ^{13}C NMR spectrum showed all the 29 carbons whose substitution pattern was derived from its DEPT spectrum, and the chemical shift values of the respective carbons were assigned by a comparative study. The olefinic carbons C-5 and C-6 came at δ 140.8 (s) and 121.7 (d), while the four oxygenated carbons appeared at δ 71.6 (d), 76.5 (d), 79.8 (s), and 69.9 (s). The former two (C-3 and C-22) bearing hydroxyls, as expected, moved upfield when compared to the corresponding carbons δ 73.8 and 77.8 in its acetate, that is, compound **1**. The remaining two values correspond to the carbons bearing the epoxy group. It might be noted that one of these (C-17, δ 79.8) remained close to the corresponding value δ 79.0 in compound **1**, while the value of C-20 is shifted upfield in **1** to δ 66.9 from 69.9 in **2**, an effect expected after acetylation of the hydroxyl.[19b]

Several steroid derivatives, in particular withanolides and physalins, are known to occur with epoxy groups at various positions such as 5,6;[23−26] 6,7;[27] 9,11;[28] 8,14;[29] 14,15;[30] 16,17;[22,31] 20,21,[32] and 22,23[33] and with an oxygen bridge between 14,20[21] and 4,19.[34] But to our knowledge there are no 17,20- epoxy steroids reported so far either from terrestrial or marine sources, and this forms their first report from the soft coral *Sarcophyton crassocaule*. However, it is not improbable for them to occur inasmuch as several 17-hydroxy steroids are noticed in marine organisms,[35] which, on dehydration, could give the $\Delta^{17\,(20)}$ compounds that could subsequently epoxidize. In this regard it is interesting to note the recent report on the co-occurrence of 17-hydroxy and $\Delta^{17\,(20)}$ steroids, latespinosides A−D from the Japanese sea star *Asteropecten latespinosus*.[36]

Table 2. Comparative ^{1}H and ^{13}C NMR Spectral Data of **3** and **13** (solvent $CDCl_3$, J in Hz, δ and TMS standard)[a]

	^{1}H NMR			^{13}C NMR		
assignment	**3** (400 MHz)	**13**[43]	carbon no.	**3** (22.5 MHz)	**13**[43]	multiplicity
22-H	0.25, 1H, t, 7	0.43, 1H, ddd, 9,9,6	1,	31.1	31.7	t
24-H	0.15, 1H, q, 5.0	0.32, 1H, dq, 8.5, 7.0	2	32.0	32.7	t
29-H_1	−0.14, 1H, dd, 6,5	0.04, 1H, dd, 5.5, 4.5	3	71.0	70.9	d
29-H_2	0.45, dd, 5, 7.0	0.59, 1H, dd, 9, 4.5	4	43.3	44.4	t
18-H_3	0.69, s, 3H	0.71, s, 3H	5	139.2	140.5	s
19-H_3	1.25, s, 3H	1.23, s, 3H	6	121.6	121.5	d
21-H_3	0.87, d, 7.0, 3H	0.88, d, 6.5	7	27.0	27.6	t
26-H_3	0.92, d, 7.0, 3H	0.96, d, 6.5	8	35.2	35.5	d
27-H_3	0.96, d, 7.0, 3H	0.97, d, 6.5	9	57.6	75.9*	d/s*
28-H_3	0.97, d, 7.0, 3H	0.91, s, 3H	10	43.0	43.9	s
30-H_3	1.02, s, 3H	1.05, s, 3H	11	69.3	69.7	d
3-H	3.5, m, $W_{1/2}$ 30 Hz	3.48, m, $W_{1/2}$ 30 Hz	12	46.9	46.5	t
6-H	5.45, d, 1H	5.45, 1H, m	13	42.8	43.2	s
11-H	4.15, br s, 1H	4.12, dd, 11.5, 5	14	49.4	49.6	d
12-H	2.10, m, 1H	1.99, 1H, dd, 12, 5	15	24.1	24.5	t
21-H_1		3.61, 1H, dd, 10.5, 2.0	16	28.3	28.6	t
21-H_2		3.86, 1H, dd, 10.5, 2.0	17	50.8	51.6	d
			18	12.1	13.0	q
			19	22.1	22.2	q
			20	34.5	42.4	d
			21	21.1	64.9*	q/t*
			22	27.8	27.9	d
			23	25.8	25.5	s
			24	49.5	51.0	d
			25	31.5	32.2	d
			26	21.4	21.6	q
			27	21.9	22.4	q
			28	15.4	15.6	q
			29	21.2	21.1	t
			30	14.3	14.4	q

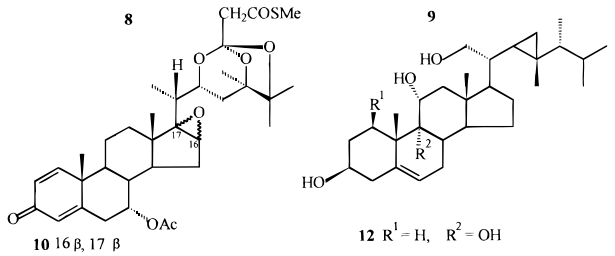
[a] Substitution pattern derived from the DEPT spectrum 

Compound **3** analyzed for $C_{30}H_{50}O_2$ from elemental analysis and the mass ion m/z 424 $[M - 18]^+$ in its EIMS. It was found to be a steroid derivative from its positive color reaction (pink→blue→green) in the Lieberman−Burchard test and from its ^{1}H NMR spectrum. Its IR spectrum showed the presence of broad hydroxylic absorption between 3450 and 3390 cm^{-1}, and the UV spectrum showed no conjugation.

The ^{1}H NMR spectrum (Table 2) is reminiscent of a steroid nucleus and, in particular, of a gorgostane derivative, such as compound **8**. It showed three tertiary methyls at δ 0.69, 1.25, and 1.02 as singlets and four secondary methyls at δ 0.87, 0.92, 0.96, and 0.97, each as a doublet (J = 7 Hz). The four protons appearing around the TMS signal at δ −0.14 1H, dd, J = 6, 5 Hz; 0.15, 1H, q, J = 5.0 Hz; 0.25, 1H, t, J = 7 Hz, and 0.45 dd, 1H, J = 5, 7 Hz are characteristic of a gorgostane skeleton, as in gorgosterol[37,38] and also in compound **8**. The characteristic 6-H of 5,6-en was noticed at δ 5.45 as a broad singlet. It showed two carbinolic methine protons as multiplets at δ 3.5 (m) and 4.15 (m), suggesting the presence of both the oxygens in the molecule as secondary hydroxyls. The former represents the characteristic carbinolic 3α-H as a mutiplet with a large half width ($W_{1/2}$ = 30 Hz) indicative of axial−axial coupling on either side. The molecular formula requires six double-bond equivalents, all of which could be explained in a gorgost-5-en skeleton, to suggest a dihydroxy gorgostane skeleton for compound **3**. With one of the hydroxyls being present at the ubiquitous 3β position, the position of second hydroxyl needs to be located. A review of literature revealed that no dihydroxy gorgostane has been reported in the literature while trihydroxy, tetrahydroxy, and even pentahydroxy gorgostanes are known. The gorgosterol side chain cannot accommodate a secondary hydroxyl, and therefore it must be located in one of the four rings of the basic skeleton, say, for example, at C-1, -2, -4, -7, -11, -12, -15, or -16. This hydroxyl could be placed at the 11α position by a discussion based on its ^{1}H NMR, ^{13}C NMR, and mass spectral fragmentation as described below.

Its ^{13}C NMR spectrum (Table 2) showed all the 30 carbons whose substitution pattern was derived from its DEPT spectrum. The chemical shifts of the respective carbons were assigned by a comparative study of the data available for gorgosterol and other related compounds. The carbons at δ 139.2 (s) and 121.6 (d) accounted for a 5,6-double bond as in the related steroid 5,6-ens.[39,40] The two oxygenated carbons appeared at δ 71.0 and 69.3. The value at δ 71.0 could be taken for C-3 with a β-OH, in the absence of a vicinal hydroxyl, as in andaman sterol (**12**).[41] Further, in the presence of a vicinal hydroxyl at C-2, both the carbons (C-2 and C-3) would have appeared at ca. δ 68.0, as in 2β,3β-dihydroxy steroids.[42] If the second hydroxyl was to be located at C-1, the C-1 would have come around δ 76.0, as in **13**,[43] ruling out its location at C-1. The presence of a second hydroxyl at C-12, C-15, or C-16 was ruled out, in which case the respective carbons with hydroxyls would have appeared at higher δ values, δ 78 for C-12,[44] δ 71.3 for C-15[45] or C-16.[46] Of the remaining positions, the presence of hydroxyl at C-7 or C-4 was eliminated, in which case the allylic carbinolic H-7 or H-4 would have appeared downfield at ca. δ 5.0 as a broad singlet or a narrow doublet. The value of C-11 with 11α hydroxyl as in compound **13** is δ 66.7, a value closer to that in compound **3** (δ 69.3), suggesting the presence of a hydroxyl at C-11. It may be noted that C-11 in both 11α- or 11β-hydroxy pregnene derivatives, in the absence of a hydroxyl either

at C-1 or C-9, appeared at δ 69.2 and 69.4 to favor the location of second hydroxyl in compound **3** at C-11,[47] although its configuration cannot be decided in view of the close chemical shift of C-11 in both the epimers. However, the proton chemical shift of 11β-hydrogen in 11α-hydroxy compounds[43] came around δ 4.15, which is very close to the chemical shift 4.12 of the proton in compound **3** in support of 11α-hydroxyl. In further support, the 11β-H appeared as a broad multiplet with a large half width ($W_{1/2}$ = 22 Hz), indicative of axial−axial coupling by the neighboring 11α- and 12α-hydrogens. The slight deshielded chemical shift of 19-H_3 in compound **3** at 1.25, in the absence of a hydroxyl at C-1, also supported the presence of a hydroxyl at C-11.



10 16β, 17 β
11 16α, 17α

12 R^1 = H, R^2 = OH

13 R^1 = OH, R^2 = H

The structure also was supported by its mass spectral fragmentation (Chart 1). Its molecular ion could not be noticed although the ion *m*/*z* 406 represented $[M - 2H_2O]^+$. The ion *m*/*z* 120 formed as the base peak as a result of retro-Diels−Alder fragmentation through a 5,6 double bond, after initial loss of water molecules. The base peak not only confirmed the presence of the 5,6 double bond but also the absence of a second hydroxyl in the rings A and B. The other intense peaks noticed were at *m*/*z* 192 and 250, which might be formed due to the cleavage of ring C between C-11 and C-12 and C-8 and C-14, with transfer of two hydrogens to the latter part as depicted (Chart 1) in support of the location of hydroxyl at C-11. Another prominent ion was noticed at *m*/*z* 172, which might have formed by retro-Diels−Alder fragmentation through the C-$\Delta^{9(11)}$ double bond in ring C. From the foregoing discussion, the structure of new dihydroxy gorgostene was derived as gorgost-5-ene-3β,11α-diol (**3**).

Compounds (**4**−**7**) were found to be hippurin steroids reported from the same species. Compounds **6** and **7** are the C-22 epimeric compounds, (22*S*,24*S*)-24-methyl-22,25-epoxyfurost-5-ene-3β,20β-diol and (22*R*,24*S*)-24-methyl-22,-25-epoxyfurost-5-ene-3β, 20β-diol, respectively, while the compounds **4** and **5** are their respective 3β-acetates. These hippurin steroids resemble those isolated from the plant sources with 3β-hydroxyl, unlike the hippurin steroids isolated from the marine sources with 3α-configuration.[7]

The monohydroxy sterol mixture was obtained as colorless needles from chloroform−methanol and formed an acetyl derivative with Ac_2O−pyridine. The acetate showed several spots on 20% silver nitrate-impregnated Si gel TLC plates. The individual sterols were identified as 24-methylcholest-5-en-3β-ol (campesterol),[9] (24*Z*)-ethylidenecholest-5-en-3β-ol (fucosterol),[9] (24*E*)-ethylidenecholest-5-en-3β-ol (Δ^5-avenasterol),[9] and 24-propylidene cholest-5-en-3β-ol[10] by GC−MS analysis of the acetate mixture with an eight-peak mass index.[11]

The prostaglandin derivatives, PG B_2 acid (5*Z*,13*E*,15*S*)-15α-hydroxy-9-oxoprosta-5,8(12),13- trien-1-oic acid[13,14] and its methyl ester, were characterized by comparison of their spectral data with the literature values. PG B_2-acid was isolated previously from a mollusk *Cymphona gibbosum*[13] and more recently from soft coral *Sarcophyton ehrenbergi*,[14] while the methyl ester was earlier reported from the soft corals *Sarcophyton ehrenbergi*[14] and *Lobophytum carnatum*.[15]

Compound **8** analyzed for $C_{30}H_{50}O_3$, *m*/*z* 440 $[M - H_2O]^+$, and was recognized as a steroid derivative from its positive color reaction (pink→blue→green) in the Lieberman−Burchard test and more closely as a gorgostane derivative from its ^{1}H NMR spectrum. It was finally characterized as the 9,11-secogorgostane derivative having the structure 9-oxo-9,11-secogorgost-5-ene-3β,11-diol (**8**) by comparing all its spectral characteristics with those reported in the literature.[16,48]

Experimental Section

General Experimental Procedures. Elemental analyses were determined on a Carlo Erba-1108 instrument. UV spectra were recorded on a Milton Roy 1201 spectrophotometer. IR spectra were recorded on a Perkin−Elmer 840 spectrophotometer. ^{1}H 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 JEOL JNM EX-90 spectrometer at 22.5 MHz using $CDCl_3$ as solvent and TMS as internal reference. MS were obtained on a JEOL JMS-300 spectrometer. 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.

Animal Material. The soft coral *S. crassocaule* is abundantly available at Rutland Island (12°09′ N,93°48′ E) of the Andaman and Nicobar group of islands of the Indian Ocean whence it was collected in March 1994. The soft coral was identified by Dr. (Mrs.) V. Jayasree, NIO, Goa, and Dr. P. A. Thomas, Vizinijam Research Center, CMFRI, Trivendrum, and the voucher specimens were preserved at the above museums and the School of Chemistry, Andhra University, Visakhapatnam, as AU1−120.

Extraction and Isolation. Slices of the soft coral (5.0 kg dry wt) were soaked in MeOH (10 L) at room temperature. The alcoholic extract was distilled at room temperature under reduced pressure. This process was repeated until the residue was colorless (eight times). The combined methanolic extract was concentrated under reduced pressure, and the concentrate was extracted with ethyl acetate. The ethyl-acetate solution was distilled under reduced pressure to leave a gummy residue (90 g). The weight of the organism after extraction was 4.0 kg. The crude extract of the organism showed moderate larvicidal activity, LC_{50} ± 5.74 mg L^{-1}. A part of the above residue (35 g) was chromatographed over a column (65 mm × 120 cm) of Si gel (350 g, 100−200 mesh Acme) using eluents of increasing polarity starting from hexane (60−80 °C) through ethyl acetate to methanol. Passing over Si gel columns or recrystallization from appropriate solvents further purified the selected fractions to yield 13 pure compounds apart from a mixture of monohydroxy sterols.

5,8,11,14-Eicosatetraenoic acid (methyl arachidonate): pale yellow oil; 1.65 g (0.033%); IR ($CHCl_3$) ν_{max} 3010, 1725, 1606, 1045, 865 cm^{-1}; physical and spectral (IR and ^{1}H NMR) data of the compound agreed with those of methyl arachidonate.[8]

3-Octadecyloxy-1,2-propanediol (batyl alcohol): colorless flakes (methanol) 1.24 g (0.0248%); mp 69−71 °C; $[\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.[6b]

3β-Hydroxypregn-5-en-20-one: colorless needles (hexane−acetone) 75 mg (0.0015%); mp 148−150° C; $[\alpha]^{25}_D$ +2.5° (*c* 1.5, $CHCl_3$). A comparison of these data with those of 3β-hydroxypregn-5-en-20-one proved its identity.[12a] This compound was isolated earlier from a *Lobophytum*[12a] species and

a *Scelerophytum*[12b] species, but this is its first report from a *Sarcoplyton* species.

17β,20β-Epoxy-23,24-dimethylcholest-5-ene-3β,22-diol-3β,22-diacetate (1): colorless oil, 35 mg (0.0007%); $[\alpha]^{25}_D$ −38.0° (*c* 0.002, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1750, 1670, 1250, 1230, 1080, 810, 760 cm^{-1}; UV ($CHCl_3$) λ_{max} no absorption above 200 nm; ^1H and ^{13}C NMR spectral data, see Table 1; EIMS *m/z* 528 $[M]^+$ (0), 510 (11.0), 215 (38.0), 213 (13.7), 369 (1.5); 255 (21), 253 (18.5), 159 (17.6), 270 (46.7), 298 (71.7), 105 (25.0); *anal*. C 75.1%, H 9.7%, calcd for $C_{33}H_{52}O_5$, C 75.0%, H 9.8%.

Reaction of Compound 1 with Triphenyl Phosphine. A mixture of compound **1** (0.067 mol), hydroquinone (0.27 mol), and triphenyl phosphine (0.09 mol) was heated on an oil bath at 200 °C under vacuum. The reaction mixture was poured into ice-cold water (15 mL), and the resultant solution was extracted with ethyl acetate (3 × 10 mL). Evaporation of the organic layer afforded a colorless oil (18 mg). The reaction did not proceed further even after prolonged heating. The product was found to be a mixture (1:1) of **1** and the corresponding 17,20-en from its ^1H NMR spectrum (δ, 90 MHz, $CDCl_3$, 21-H_3 appeared at δ 1.45 as in **1** and at δ 1.6 in the product 17,-20-en, being a methyl on double bond). This mixture could not be separated.

17β,20β-epoxy-23,24-dimethylcholest-5-ene-3β,22-diol (2): colorless needles, 30 mg (0.0006%); $[\alpha]^{25}_D$ −31.5° (*c* 0.003, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3500, 1675, 1230, 1080, 960, 795 cm^{-1}; UV ($CHCl_3$) λ_{max} no absorption above 200 nm; ^1H and ^{13}C NMR spectral data, see Tables 1 and 2; CIMS *m/z* 444 $[M]^+$ (12.5), 298 (12.5), 270 (7.2), 171 (12.5), 173 (27.5), 285 (3.3), 155 (40.4), 327 (4.2), 255 (22.6); 253 (30.3); 159 (31.9), 105 (38.0); *anal*. C 78.4%, H 10.7%, calcd for $C_{29}H_{48}O_3$, C 78.3%, H 10.8%.

Gorgost-5-en-3β,11α-diol (3): colorless needles (chloroform−methanol) 30 mg (0.0006%); mp 180−182° C; $[\alpha]^{25}_D$ + 36.0° (*c* 0.08,$CHCl_3$); IR ($CHCl_3$) ν_{max} 3450, 3390, 1050 cm^{-1}; UV ($CHCl_3$) λ_{max} no absorption above 200 nm; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 442 $[M]^+$ (0), 406 (10), 250 (35), 192 (50), 173 (23), 172 (24), 120 (18); *anal*. C 81.3%, H 11.4% calcd for $C_{30}H_{50}O_2$, C 81.4%, H 11.3%.

(22*S*,24*S*)-24-Methyl-22,25-epoxyfurost-5-ene-3β,20β-diol-3β-acetate (4): colorless flakes (chloroform−methanol) 30 mg (0.0006%); mp 180−184 °C, $[\alpha]^{25}_D$ −74.4° (*c* 0.002, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3469, 3300, 1736, 1465, 1376, 1239, 1229, 971, 921, 863 cm^{-1}; UV ($CHCl_3$) λ_{max} no absorption above 200 nm; ^1H and ^{13}C NMR, see Anjaneyulu et al.[7].

(22*R*,24*S*)-24-Methyl-22,25-epoxyfurost-5-ene-3β,20β-diol-3β-acetate (5): colorless oil, 20 mg (0.0004%); $[\alpha]^{25}_D$ −61.5° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3434, 3250, 1740, 1454, 1372, 1238, 1056, 1017, 978, 918 cm^{-1}; UV ($CHCl_3$) λ_{max} no absorption above 200 nm; ^1H and ^{13}C NMR, see Anjaneyulu et al.[7]

(22*S*,24*S*)-24-Methyl-22,25-epoxyfurost-5-ene-3β,20β-diol (6): colorlesss needles (chloroform−methanol) 40 mg (0.0008%); mp 213−216 °C; $[\alpha]^{25}_D$ −38.0° (*c* 0.001, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3469, 3300, 1736, 1465, 1376, 1239, 1229, 971, 921, 863 cm^{-1}; UV ($CHCl_3$) λ_{max} no absorption above 200 nm; ^1H and ^{13} C NMR data, Anjaneyulu et al.[7]

(22*R*,24*S*)-24-Methyl-22,25-epoxyfurost-5-ene-3β,20β-diol (7): colorless prisms (chloroform−methanol) 20 mg (0.0004%); mp 198−200 °C; $[\alpha]^{25}_D$ −23.3° (*c* 0.002, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3430, 3440, 1730, 1440, 1370, 1235,1015 cm^{-1}; UV ($CHCl_3$) λ_{max} no absorption above 200 nm. ^1H and ^{13}C NMR data, see Anjaneyulu et al.[7]

Mixture of monohydroxy sterols: colorless needles (chloroform−methanol) 1.05 g (0.021%); mp 140−142 °C. It gave positive Lieberman−Burchard test with play of colors (pink→blue→green) characteristic of steroids and formed an acetyl derivative with Ac_2O−pyridine at room temperature. After usual work up, the acetyl derivative showed several spots on a 20% silver nitrate−Si gel TLC plate. Hence it was regarded as a mixture of monohydroxy sterols. The individual four sterols were identified by the GC−MS analysis of the acetate mixture as 24-methylcholest-5-en-3β-ol (campesterol),[9] (24*Z*)-ethylidene cholest-5-en-3β-ol (fucosterol),[9] (24*E*)-ethylidenecholest-5-en-3β-ol (Δ^5-avenasterol),[9] and 24-propylidene cholest-5-en-3β-ol.[10]

(5*Z*,13*E*,15*S*)-15-Hydroxy-9-oxoprosta-5,8(12),13-trien-1-oic acid: dark brown liquid, 180 mg (0.0036%); $[\alpha]^{25}_D$ 16.3° (*c* 0.024, $CHCl_3$); physical and spectral (IR, ^1H, ^{13}C NMR and MS) data of the compound agreed with those of PG B_2 acid.[13,14]

Methyl (5*Z*,13*E*,15*S*)-15α-hydroxy-9-oxo-prosta-5,8(12)-13-trien-1-oate: light brown liquid, 200 mg (0.004%); $[\alpha]^{25}_D$ 17.8° (*c* 0.02,$CHCl_3$); physical and spectral (IR, ^1H, ^{13}C NMR, and MS) data of the compound agreed with those of PG B_2 ester.[14,15]

9-Oxo-9,11-secogorgost-5-ene-3β,11α-diol (8): colorless flakes (chloroform−methanol), 35 mg (0.0007%); mp 170−73° C; $[\alpha]^{25}_D$ + 23.0° (*c* 0.8, $CHCl_3$); physical and spectral (IR, ^1H, ^{13}C NMR, and MS) data of the compound agreed with those of 9-oxo-9,11-seco gorgost-5-ene-3β,11α-diol.[16,48]

Acknowledgment. The authors thank the Department of Ocean Development, New Delhi, for financial assistance; to the Head, RSIC, Lucknow, for spectral data; to Dr. Mrs. V. Jayasree, Scientist, NIO, Goa, and Dr. P. A. Thomas, Senior Scientist, Vizinjam Research Centre, CMFRI, Trivendrum, for identification of the soft coral; and to the CSIR, New Delhi, for sanctioning Emeritus Scientist Scheme to the senior author (A.S.R.A.).

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1999**, *16*, 155−198 and the earlier references in this series cited therein.
(2) Anjaneyulu, A. S. R.; Venkateswara Rao, G. *J. Sci. Ind. Res.* **1995**, *54*, 637−664.
(3) Anjaneyulu, A. S. R.; Venkateswara Rao, G. *Nat. Prod. Sci.* **1995**, *1*, 70−85.
(4) Anjaneyulu, A. S. R.; Venkateswara Rao, G. *J. Indian Chem. Soc.* **1997**, *74*, 272−278.
(5) Anjaneyulu, A. S. R.; Krishna Murthy, M. V. R. *Indian J. Chem*. (in press).
(6) (a) Anjaneyulu, A. S. R.; Sagar, K. S.; Venugopal, M. J. R. V. *Tetrahedron* **1995**, *51*, 10997−11010. (b) Anjaneyulu, A. S. R.; Krishna Murthy, M. V. R.; Rao, G. V. *Tetrahedron* **1997**, *53*, 9301−9312. (c) Anjaneyulu, A. S. R.; Venugopal, M. J. R. V.; Sarada, P.; Rao, G. V.; Clardy, J.; Lobkovsky, E. *Tetrahedron Lett.* **1998**, *39*, 135−138. (d) Anjaneyulu, A. S. R.; Sagar, K.S.; Rao, N. S. K. *Nat. Prod. Lett.* **1997**, *11*, 5 and the references therein. (e) Anjaneyulu, A. S. R.; Venugopal, M. J. R. V.; Sarada, P.; Clardy, J.; Lobkovsky, E. *Tetrahedron Lett.* **1998**, *39*, 139−142.
(7) Anjaneyulu, A. S. R.; Krishna Murthy, M. V. R.; Rao, N. S. K. *J. Chem. Res.* (S), **1997**, 450−451; *J. Chem. Res.* (M), **1997**, 2743 and the references therein.
(8) Anjaneyulu, A. S. R.; Rao, G. V. *Indian J. Chem.* **1996**, *35B*, 826−831.
(9) Knights, B. A.; Brooks, C. J. W. *Phytochemistry* **1969**, 8, 463.
(10) Rohmar, M.; Kokke, W. C. M. C.; Fenical, W.; Djerassi, C. *Steroids*. **1980**, *35*, 219.
(11) Eight Peak Index of Mass Spectra compiled and published by the spectrometry data centre, the University of Nottingham, U.K., **1983**, 3rd ed.
(12) (a) Anjaneyulu, V.; Hari Babu, B. *Indian J. Chem*. **1992**, *31B*, 708. (b) Venkata Rao, D.; Sudhakara Rao, T.; Rao, C. B. S. *Indian J. Chem*. **1990**, *35B*, 826.
(13) Ciereszko, L. S.; Schneider, W. P. *Bull. Mar. Sci.* **1987**, *41*, 634.
(14) Chandra Sekhar, V. Ph.D. Thesis, submitted to Andhra University, 1995.
(15) Latyshev, N. A.; Bezuglov, V. V.; Kogtev, L. S.; Hung, N. K.; Sadovskaya, V. L.; Rozynov, B. V.; Bergel'son, L. D. *Sov. J. Mar. Biol., Engl. Transl.* **1986**, *12*, 116.
(16) Enwall, E. L.; Van Der Helm, D.; Nan Hsu, I.; Pattabi Kiraman, T.; Schmitz, F. J.; Spraggins, R. L.; Weinheimer, A. J. *J. Chem. Soc., Chem. Commn.* **1972,** 215.
(17) Bowden, B. F.; Coll, J. C.; Mitchel, S. J. *Aust. J. Chem*. **1979**, *33*, 879.
(18) Coll, J. C.; Bowden, B. F.; Tapioas, D. M.; Dunlop, W. C. *J. Exp. Mar. Biol. Ecol.* **1982**, *60*, 293.
(19) (a) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 4th ed., 1981; p 116. (b) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds* 4th ed., 1981; p 266.
(20) Eliel, E. L.; Pietrusiewicz, K. M. In *Topics in Carbon*-13 *NMR Spectroscopy*, G. C. Levy, Ed. John Wiley & Sons: New York, 1979; Vol. 3, p 174.
(21) Atta-Ur-Rahman; Abbas, S.; Shahwar, D.; Jamal, S. A.; Choudary, M. I. *J. Nat. Prod*. **1993**, *56*, 1000.
(22) Elliger, C. A.; Waiss, A. C., Jr; Benson, M. *J. Nat. Prod*. **1992**, *55*, 129.
(23) Veleiro, S. A.; Oberti, J. C.; Burton, G. *Phytochemistry* **1992**, *31*, 935.
(24) Gottlieb, H. E.; Kirson, I. *Org. Magn. Reson.* **1981**, *16*, 20.
(25) Venkateswarlu, Y.; Rami Reddy, M. V.; Rama Rao, M. *J. Nat. Prod*. **1996**, *59*, 876.

(26) Iguchi, K.; Saitoh, S.; Yamada, Y. *Chem. Pharm. Bull.* **1989**, *37*, 2553.
(27) Evans, W. C.; Grout, R. J.; Mensh, M. L. K. *Phytochemistry* **1984**, *23*, 1717.
(28) Alam, M.; Sanduja, M.; Weinheimer, A. J. *Steroids* **1988**, *52*, 45.
(29) D'Auria, M. V.; Gomez, L. P.; Minale, L.; Riccio, R.; Debitus, C.; Levi, C. *J. Nat. Prod.* **1992**, *55*, 311.
(30) Atta-Ur-Rahman; Jamal, S. A.; Choudary, M. I. *Heterocycles* **1992**, *34*, 689.
(31) Shingu, K.; Yahara, S.; Okabe, H.; Nohra, T. *Chem. Pharm. Bull.* **1992**, *40*, 2448.
(32) Iguchi; K.; Fujita, M.; Nagaok, H.; Mitome, H.; Yamada, Y. *Tetrahedron Lett.* **1993**, *34*, 6277.
(33) De Simone, F.; Dini, A.; Finamore, E.; Minale, L.; Pizza, C.; Riccio, R.; Zollo, F. *J. Chem, Soc., Perkin Trans. 1* **1981**, 1855.
(34) Yada, H.; Sato, H.; Kaneko, S.; Akitami, I. *Tetrahedron Lett.* **1994**, *35*, 4393.
(35) D'Auria, M. V.; Minale, L.; Riccio, R. *Chem. Rev.* **1993**, *93*, 1839.
(36) Higuchi, R.; Fujita, M.; Matsumoto, S.; Yamada, K.; Miyamot, T.; Sasaki, T. *Liebigs Ann. Chem.* **1996**, 837.
(37) Hale, R. L.; Leclerq, J.; Tursch, B.; Djerassi, C., Gross, R.A.; Weinheimer, A. J.; Gupta, K.; Scheuer, P. J. *J. Am. Chem. Soc.* **1970**, *92*, 2179.
(38) Ling, N. C.; Hale, R. L.; Djerassi, C. *J. Am. Chem. Soc.* **1970**, *92*, 5281.
(39) Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479.
(40) Agrawal, P. K.; Jain, D. C.; Pathak, A. K. *Magn. Reson. Chem.* **1995**, *33*, 923.
(41) Kobayashi, M.; Kobayashi, K.; Ramana, K. V.; Rao, C. V. L.; Rao, D. V.; Rao, C. B. S. *J. Chem. Soc., Perkin Trans. 1* **1991**, *1*, 493.
(42) Hikino, H.; Okuyama, T.; konno, C.; Takemoto, T. *Chem. Pharm. Bull.* **1975**, *23*, 125.
(43) Kobayashi, M.; Krishna, M. M.; Anjaneyulu, V. *J. Chem. Res (S)* **1993**, 112−113.
(44) Kobayashi, J.; Ishida, K.; Haitoh, K.; Shigemori, H.; Mikami, Y.; Sasaki, T. *J. Nat. Prod.* **1993**, *56*, 1350−1355.
(45) Kobayashi, M.; Krishna, M. M.; Haribabu, B.; Anjaneyulu, V. *Chem. Pharm. Bull.* **1993**, *41*, 87−89.
(46) Kobayashi, M.; Krishna, M. M.; Anjaneyulu, V. *Chem. Pharm. Bull.* **1992**, *40*, 2845−2846.
(47) Blunt, J. W.; Stethers, J. B. unpublished results.
(48) Bonini, C.; Cooper, C. B.; Kazlauskas, R.; Wells, R. J.; Djerassi, C. *J. Org. Chem.* **1983,** *48*, 2108.
(49) Rubinstein, I.; Good, L. J.; Clauge, A. D.; Mulherin, J. *Phytochemistry* **1976**, *15*, 195.

NP990205P