5-Hexacosyn-1-ol (19). To an ice-cold solution of 5-hexyn-1-ol (1.98 g, 20 mmol) in dry HMPA (20 mL) was added *n*-butyllithium (1.6 M solution in hexane, 27.4 mL, 44 mmol) dropwise under nitrogen. After stirring of the mixture for 5 min, a solution of 1-bromoeicosane (7.94 g, 22 mmol) in dry HMPA (35 mL) was added dropwise over a period of 1 h. Stirring continued for an additional 1.5 h while warming up to 25 °C. The mixture was then diluted with water (200 mL) and extracted with $CHCl_3$ (3 × 200 mL). The extract was washed with water, dried ($MgSO_4$), and evaporated in vacuo. The residue was chromatographed on silica gel with ether/hexane (1:9) as eluent to yield the product, [19] (6.48 g, 86% yield): mp 65–66 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 3.656 (2 H, t, C-1, J = 6.2 Hz), 2.185 (2 H, tt, C-4, J = 6.6, 2.5 Hz), 2.119 (2 H, tt, C-7, J = 6.9, 2.5 Hz), 1.66 (2 H, m, C-2), 1.55 (2 H, m, C-3), 1.459 (2 H, quintet, C-8, J = 7.3 Hz), 0.869 (3 H, t, C-26, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 80.70 and 79.65 (C-6 and C-5), 62.48 (C-1), 32.03 and 31.95 (C-24 and C-2), 25.48 (C-3), 22.80 (C-25), 18.86 and 18.65 (C-4 and C-7), 14.25 (C-26), 29.79, 29.46, 29.26, 29.01 (overlapping remaining signals); MS (70 eV), m/z (relative intensity) 378 (M^+, 2), 349 (2), 335 (2), 334 (3), 313 (1), 292 (3), 153 (4), 139 (3), 135 (9), 111 (39), 94 (53), 81 (49), 68 (100), 57 (32), 43 (27); exact mass calcd for $C_{26}H_{50}O$ 378.3849, found 378.3837.

Methyl 5-Hexacosynoate (20). An ice-cold suspension of 5-hexacosyn-1-ol (4.86 g, 12.86 mmol) in acetone (250 mL) was titrated by dropwise addition of Jones reagent until the brown color persisted (ca. 15 mL). The mixture was then diluted with water (500 mL) and extracted with ether (3 × 300 mL). The ether extract was dried ($MgSO_4$) and evaporated in vacuo. The resulting acid was directly converted to the methyl ester by refluxing for 3 h with methanol (250 mL) containing a catalytic amount of *p*-toluenesulfonic acid (240 mg). The solvent was then evaporated in vacuo and the residue was chromatographed over silica gel. Elution with ether/hexane, 5:95, afforded pure ester **20** (3.87 g, 74%): mp 45–46 °C; GC, ecl = 26.157; ^1H NMR (400 MHz, $CDCl_3$) δ 3.667 (3 H, s, OCH_3), 2.433 (2 H, t, C-2, J = 7.7 Hz), 2.212 (2 H, tt, C-4, J = 7.1, 2.5 Hz), 2.119 (2 H, tt, C-7, J = 6.9, 2.5 Hz), 1.794 (2 H, quintent, C-3, J = 7.4 Hz), 0.873 (3 H, t, C-26, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.51 (C-1), 81.28 and 78.65 (C-6 and C-5), 51.52 (OCH_3), 32.98 and 32.03 (C-2 and C-24), 24.40 (C-3), 22.80 (C-25), 18.85 and 18.37 (C-7 and C-4), 14.23 (C-26), 29.80, 29.47, 29.26, 29.20, 29.00 (remaining carbons); MS (70 eV), m/z (relative intensity) 406 (M^+ 2), 375 (M^+ - 31 (OCH_3), 2), 330 (1), 181 (1), 150 (8), 140 (100), 121 (8), 108 (13), 98 (18), 83 (10), 81 (35), 80 (69), 79 (22), 67 (25), 57 (25), 55 (34), 43 (72); exact mass calcd for $C_{27}H_{50}O_2$ 406.3798, found 406.3792.

Methyl (5*Z*)-5-Hexacosenoate (21). A mixture of acetylenic ester **20** (100 mg, 0.24 mmol), Lindlar catalyst (30 mg), and quinoline (0.01 mL) in hexane (5 mL) was stirred under hydrogen at room temperature for 2 h. The catalyst was then filtered off and the solvent was removed under vacuum. Flash chromatography of the residue on silica gel using 0–2% ether/hexane as eluent afforded pure ester **21** (92 mg, 91%); GC, Ecl = 25.838; ^1H NMR (400 MHz, $CDCl_3$) δ 5.406 (1 H, dtt, C-5, J = 10.5, 7.1, 1.3 Hz), 5.311 (1 H, dtt, C-6, J = 10.5, 6.9, 1.3 Hz), 3.667 (3 H, s, OCH_3), 2.316 (2 H, t, C-2, J = 7.5 Hz), 2.069 (2 H, br q, C-4, J = 7.2 Hz), 1.998 (2 *H*, m, C-7, J = 7.0 Hz), 1.685 (2 H, quintet, C-3, J = 7.4 Hz), 0.879 (3 H, t, C-26, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.91 (C-1), 131.03 (C-6), 128.13 (C-5), 51.50 (OCH_3), 33.56 (C-2), 32.02 (C-24), 27.33 (C-7), 26.64 (C-4), 25.00 (C-3), 22.81 (C-25), 14.28 (C-26), 29.81, 29.68, 29.47 (overlapping signals, remaining carbons); MS (70 eV), m/z (relative intensity) 408 (M^+, 18), 378 (19), 376 (21), 359 (19), 347 (10), 334 (60), 292 (49), 278 (24), 250 (19), 222 (18), 166 (30), 165 (28), 152 (65), 141 (75), 137 (78), 127 (47), 124 (92), 112 (62), 109 (76), 97 (100), 83 (85), 75 (82), 68 (98), 59 (82), 53 (93).

Sodium (5*Z*)-[5,6-$^3H_2$]-5-Hexacosenoate. A mixture of acetylenic ester **20** (10.0 mg, 24.5 mmol), Lindlar catalyst (10 mg), and quinoline (0.01 mL) in heptane (1 mL) was exposed to a small amount of tritium-enriched hydrogen gas, generated externally by reaction of NaB^3H4 (ca. 30 mCi; specific activity 1.4 Ci/mol) with a saturated aqueous solution of $CoCl_2$ (0.2 mL). After 24 h, the reaction was completed by exosure to tritium-free hydrogen gas for 2 h. The catalyst was then filtered off and the mixture was chromatographed on silica gel with hexane as an eluent. Tritiated ester **21** obtained was directly hydrolyzed by 5% NaOH in aqueous ethanol to yield the product (700 μCi; specific activity 7.4 mCi/mmol).

Acknowledgment. Financial support was provided by the National Science Foundation (Grant No. DMB8606249) and the National Institutes of Health (Grant No. GM06840 and GM28352). We thank Prof. Robert Simoni of the Stanford Biology Department for the use of his scintillation counter and Annemarie Wegmann-Szente for mass spectral determinations.

Sterols in Marine Invertebrates. 60.[1] Isolation and Structure Elucidation of Four New Steroidal Cyclopropenes from the Sponge *Calyx podatypa*

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Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305. *Received May 2, 1988*

Abstract: Four new sterols (**4**, **8**, **9**, and **10**) with cyclopropene-containing side chains were isolated from the Caribbean sponge *Calyx podatypa*. Their structure elucidation was accomplished by ^1H NMR and mass spectrometry. The major sterol, **4**, was shown to be the 23-epimer of (23*R*)-23*H*-isocalysterol (**3**), previously isolated from the Mediterranean sponge *Calyx nicaeensis*. ^{13}C NMR spectra of cyclopropene-containing sterols were determined and signals were fully assigned. Trends in ^{13}C chemical shifts of the cyclopropene sterols are discussed. The biosynthetic implications suggest the operation of an unprecedented in vivo cyclopropene isomerization process.

The cyclopropene moiety is extraordinarily rare among natural products. Prior to the isolation of the sponge sterol calysterol (**1**),[2] the only known example was the fatty acid sterculic acid[3] and its congeners.[4,5] Since then, two more cyclopropenes (**2** and **3**) have been isolated in our laboratory.[6,7] As part of these studies we investigated the absolute configuration of these steroidal cyclopropenes as well as their photochemical and acid-catalyzed behavior.[8]

(1) For part 59, see: Lawson, M. P.; Stoilov, I. L.; Thompson, J. E.; Djerassi, C. *Lipids* **1988**, *23*, 750–754.
(2) Fattorusso, E.; Magno, S.; Mayol, L.; Santacroce, C.; Sica, D. *Tetrahedron* **1975**, *31*, 1715–1716.
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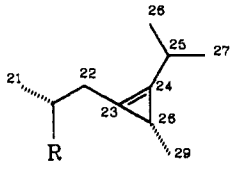
Chart I



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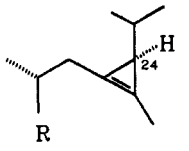

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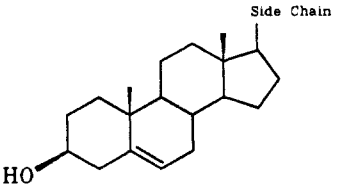
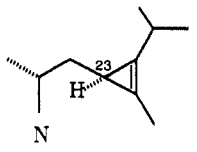


N



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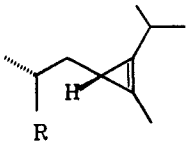

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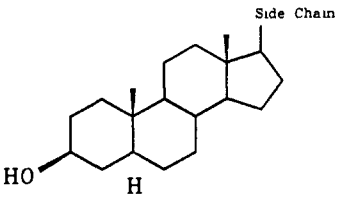
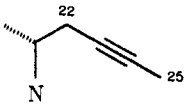
10 R = S



S



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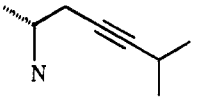

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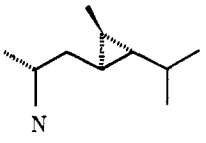

7

Table I. 400 MHz ^{1}H NMR Data for *Calyx* Sterols[a]

sterol	solvent	C-3	C-6	C-18	C-19	C-21	C-26[b]	C-27[b]	C-29	C-25
1[c]	$CDCl_3$	3.523 (m)	5.353 (m)	0.698 (s)	1.009 (s)	0.963 (d, 6.6)	1.103 (d, 6.8)	1.095 (d, 6.9)	0.997 (d, 4.6)	2.694 (t, sept, 1.3, 6.9)
2	$CDCl_3$	3.523 (m)	5.354 (m)	0.703 (s)	1.010 (s)	0.968 (d, 6.5)	0.792 (d, 6.8)	0.784 (d, 6.7)	2.022 (t, 1.7)	*f*
3	$CDCl_3$	3.521 (m)	5.349 (m)	0.689 (s)	1.007 (s)	1.004 (d, 6.5)	1.113 (d, 6.7)	1.098 (d, 6.8)	1.990 (d, 1.6)	2.654 (q, sept, 1.6, 6.9)
	C_6D_6	3.388 (m)	5.347 (m)	0.735 (s)	0.943 (s)	1.221 (d, 6.6)	1.154 (d, 6.9)	1.138 (d, 6.8)	1.957 (d, 1.6)	2.682 (q, sept, 1.6, 6.8)
4	$CDCl_3$	3.520 (m)	5.349 (m)	0.681 (s)	1.006 (s)	1.005 (d, 6.5)	1.114 (d, 6.9)	1.088 (d, 6.9)	2.009 (d, 1.6)	2.626 (q, sept, 1.6, 6.8)
	C_6D_6	3.412 (m)	5.351 (m)	0.726 (s)	0.949 (s)	1.225 (d, 6.5)	1.150 (d, 6.8)	1.131 (d, 6.9)	1.973 (d, 1.6)	2.663 (q, sept, 1.6, 6.9)
5	$CDCl_3$	3.524 (m)	5.347 (m)	0.680 (s)	1.004 (s)	1.061 (d, 6.6)				1.792 (t, 2.6)
6	$CDCl_3$	3.523 (m)	5.349 (m)	0.683 (s)	1.005 (s)	1.058 (d, 6.6)	1.143 (d, 6.9)	1.143 (d, 6.9)		2.526 (t, sept, 2.3, 6.8)
7[d]	$CDCl_3$	3.530 (m)	5.350 (m)	0.685 (s)	1.007 (s)	1.017 (d, 6.6)	0.928 (d, 6.8)	0.926 (d, 6.9)	0.988 (d, 6.1)	
8[e]	$CDCl_3$	3.597 (m)		0.666 (s)	0.801 (s)	0.945 (d, 6.5)	1.100 (d, 6.9)	1.092 (d, 6.9)	0.993 (d, 4.5)	2.690 (t, sept, 1.2, 6.8)
9	$CDCl_3$	3.583 (m)		0.668 (s)	0.800 (s)	0.947 (d, 6.6)	0.785 (d, 6.7)	0.778 (d, 6.7)	2.014 (t, 1.7)	*f*
10	$CDCl_3$	3.585 (m)		0.650 (s)	0.800 (s)	0.990 (d, 6.6)	1.112 (d, 6.8)	1.088 (d, 6.9)	2.007 (d, 1.6)	2.624 (q, sept, 1.5, 6.9)

[a] Chemical shifts expressed in ppm (δ); multiplicities and coupling constants (hertz) given in parentheses. [b] Assignments are interchangeable. [c] H-28: 1.326 (q, J = 4.6 Hz). [d] H-23 and H-28: 0.445 (m, 2 H); H-24: −1.270 (m, 1 H). [e] H-28: 1.320 (q, J = 4.5 Hz). [f] Overlapping with other peaks below 2.25 ppm.

Of particular interest is the biosynthesis of these unique sterols and some preliminary investigations have been recorded[9,10] with the sponge *Calyx nicaeensis*. An additional bonus of these studies is the occurrence in the same sponge of the two acetylenes **5** and **6**, the only known naturally occurring steroidal acetylenes.[11] Thus the sponge *C. nicaeensis* offers two additional unusual substrates for biosynthetic scrutiny.

The relative rarity of *C. nicaeensis* in the Mediterranean Sea has made it difficult to perform the many incorporation experiments that need to be done with a variety of radiolabeled substrates that have been synthesized in our laboratory. Therefore we have searched for a more convenient source for such cyclopropenes and acetylenes. We report now our work on *Calyx podatypa* from the Bahamas, which has led to the isolation and structure elucidation of four new steroidal cyclopropenes in addition to some of the earlier known ones.[2,6,7]

Results

Fractionation (HPLC) of the total sterol mixture from the sponge *C. podatypa* revealed the presence (see Chart I) of one major sterol (ca. 80%) and at least 11 different minor ones (1–2% each).

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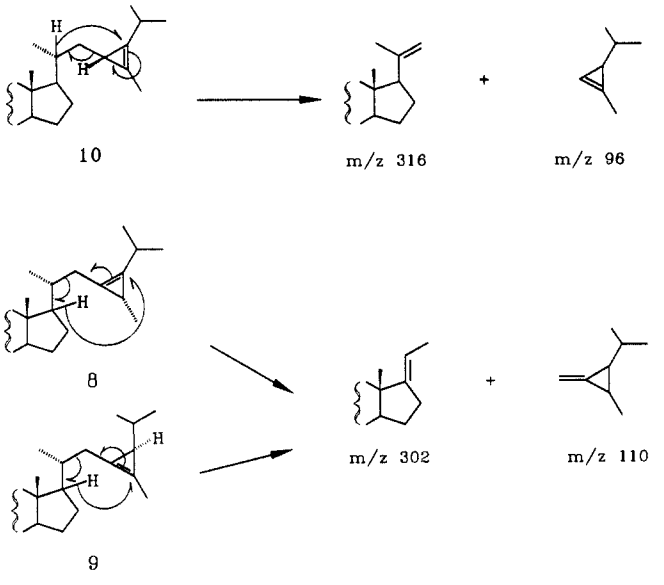
Scheme I



Calysterol (**1**),[2,6] 24*H*-isocalysterol (**2**),[7] dihydrocalysterol (**7**),[6,12] and the two acetylenic sterols, **5** and **6**,[11] were identified among the minor sterols by direct comparison of their 400-MHz ^{1}H NMR spectra (Table I) with those of authentic samples isolated from *C. nicaeensis*.

The major sterol exhibited an NMR spectrum (Table I) very similar to, but not identical with, that of (23*R*)-23*H*-isocalysterol (**3**).[6] It was identified as **4**—the 23-epimer of **3**—by comparison of its 400-MHz ^{1}H NMR chemical shifts, in both $CDCl_3$ and C_6D_6, with that of a sample obtained[8] by photochemical isomerization of **3**.

In addition, three new cyclopropene sterols (**8–10**) were also isolated in trace amounts from *C. podatypa*. They were identified as the 5α-stanols corresponding to the Δ^5-sterols **1**, **2**, and **4**, respectively. The 5α-dihydro-3β-hydroxystanol nucleus was evident from the absence of any olefinic signals in the ^{1}H NMR spectrum, as well as from the characteristic chemical shift of the C-3 and C-19 protons (around 3.580 and 0.800 ppm vs 3.520 and 1.007 ppm in Δ^5-sterols). The C-18 and C-21 methyl signals were consistently shifted upfield (by 0.030–0.035 and 0.015–0.021 ppm, respectively) compared to those of their Δ^5 counterparts. Other side-chain signals lay within ±0.008 ppm of the corresponding signals of Δ^5-sterols.

Of particular significance is the downfield shift of the C-29 methyl group in **10** (2.007 ppm) and **9** (2.014 ppm) vs that of **8** (0.993 ppm) being attached to an sp^2 carbon of the cyclopropene ring. Similarly, the C-25 resonance is shifted far downfield in **10** (2.624 ppm) and **8** (2.690 ppm) in contrast to **9** (Table I).

Homoallylic $^5J_{H,H}$ couplings were discernible in the spectra of **8–10** and were confirmed by two-dimensional COSY[13,14] experiments. Thus, the H-25 signal in **10** (as well as in **3** and **4**) appears as a 28-line pattern, being split by the C-26 and C-27 methyl groups into a septet (J = 6.9 Hz), each line of which is further split into a quartet (J = 1.5 Hz) by the C-29 methyl group. The latter, in turn, appeared as a doublet (J = 1.5 Hz).

In a similar manner, H-25 in **8** (as well as in **1**) gave rise to a 21-line pattern (a septet further split into triplets by H-22). The C-29 methyl group in **9** (as well as in **2**) appeared as a triplet (J = 1.7 Hz) again due to homoallylic coupling with H-22.

The high-resolution mass spectra of the stanols **8–10** showed weak molecular ions corresponding to $C_{29}H_{48}O$. A strong fragment peak at m/z 273 corresponded to the loss of the side chain together with two hydrogens, which is typical for sterols with an unsaturated side chain.[15] Other fragment peaks (m/z 255, 233, 215) were shifted by two mass units compared to the usual fragmentation pattern of the Δ^5 nucleus.[15,16] In addition, diagnostic peaks[6,7] at m/z 96 and 316 in the spectrum of **10** and at m/z 110 and 302 in the spectra of both **9** and **8** arose from the McLafferty rearrangements[15,17,18] shown in Scheme I. The base peak in the spectrum of **10** (m/z 95) was a result of allylic cleavage between C-22 and C-23. Similar cleavage between C-24 and C-25 in **9** and between C-28 and C-29 in **8** led to fragment ions of mass 369 and 397, respectively.

Table II. Carbon-13 NMR Data for *Calyx* Sterols[a]

	sterol						
carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**
C-1	37.24	37.24	37.26	37.24	37.19	37.22	37.25
C-2	31.65[c]	31.64	31.67	31.65	31.52	31.61	31.66
C-3	71.80	71.78	71.82	71.80	71.73	71.76	71.81
C-4	42.29	42.28	42.31	42.28	42.16	42.26	42.30
C-5	140.74	140.73	140.75	140.74	140.68	140.73	140.74
C-6	121.69	121.67	121.74	121.73	121.62	121.64	121.71
C-7	31.87	31.86	31.91	31.90	31.82	31.83	31.90
C-8	31.89	31.89	31.91	31.90	31.85	31.87	31.90
C-9	50.08	50.08	50.15	50.14	50.02	50.05	50.11
C-10	36.50	36.49	36.48	36.49	36.44	36.47	36.50
C-11	21.06	21.05	21.11	21.09	20.99	21.02	21.08
C-12	39.67	39.70	39.81	39.78	39.50	39.58	39.78
C-13	42.32	42.31	42.34	42.28	42.25	42.26	42.31
C-14	56.75	56.74	56.83	56.80	56.58	56.65	56.80
C-15	24.32	24.30	24.31	24.29	24.23	24.26	24.30
C-16	28.24	28.25	28.46	28.40	28.02	28.01	28.53
C-17	55.61	55.63	56.56	56.29	55.06	55.21	56.23
C-18	11.90	11.89	11.87	11.83	11.92	11.96	11.82
C-19	19.40	19.39	19.40	19.40	19.35	19.37	19.40
C-20	34.92	34.97	36.48	36.24	35.80	35.91	36.78
C-21	19.57	19.57	19.23	19.17	19.04	18.94	18.87
C-22	31.62[c]	32.91	43.53	43.15	25.88	25.84	34.86
C-23	114.75	115.30	18.99	18.32	77.93	77.97	19.49
C-24	123.14	27.69	122.67	122.74	76.27	87.10	35.04[d]
C-25	26.41	33.04	26.40	26.40	3.51	20.58	33.39[d]
C-26[b]	20.97	21.84	21.08	20.85		23.52	22.09
C-27[b]	21.17	21.92	21.24	21.12		23.52	22.19
C-28	21.55	111.73	112.23	111.46			15.35
C-29	14.15	11.49	10.67	10.53			13.45

[a] Carbon-13 chemical shifts expressed in ppm (δ), recorded at 100 MHz in $CDCl_3$. [b-d] Assignments interchangeable.

Carbon-13 NMR Spectra of *Calyx* Sterols. Given the unusual nature of the cyclopropene sterols isolated from *C. podatypa* and *C. nicaeensis*, it was of interest to determine their ^{13}C NMR chemical shifts since none have so far been reported for cyclopropene-containing sterols. The ^{13}C NMR spectra of the cyclopropenes **1–4**, as well as dihydrocalysterol (**7**) and the two acetylenic sterols, **5** and **6**, were determined and the signals were fully assigned (Table II). ^{13}C chemical shifts of the sterol nucleus were virtually invariable and could be easily recognized by comparison with the chemical shifts of cholesterol.[19] The signals of the side chain were assigned on the basis of their multiplicity as evident from the APT[20] or DEPT[21-23] spectra, as well as the internal consistency of the chemical shifts of closely related structural fragments (see below).

In addition to the olefinic signals from the nucleus (C-5 and C-6), the spectra of **1–4** all show two olefinic signals belonging

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to the cyclopropene sp^2 carbons. These signals are shifted upfield from the normal range of conventional tetrasubstituted alkenes.[24] Their chemical shifts are in good agreement with that of cyclopropene itself and of methylcyclopropenes reported by Günther and Seel.[25] The exact chemical shifts depend on the effects of the attached substituents. The carbons bearing the isopropyl group (C-24 in **1**, **3**, and **4**) are most deshielded (appearing around 123.0 ppm), while the carbons bearing a methyl group (C-28 in **2**–**4**) are least deshielded (111.46–112.23 ppm). C-23 in **1** and **2** have intermediate chemical shift values (114.75–115.30 ppm).

Vinylic carbons attached to a cyclopropene ring were shown to be shifted upfield when compared to those attached to a cyclopropane ring.[25] The same trend is found in the present case of steroidal cyclopropenes. Thus, the C-29 methyl group resonates at a higher field in **3** (10.67 ppm), **4** (10.53 ppm), and **2** (11.49 ppm), in contrast to that of **1** (14.15 ppm) and **7** (13.45 ppm). In the same manner, C-25 is shifted upfield in **1** (26.41 ppm), **3** (26.40 ppm), and **4** (26.40 ppm) compared to that of **2** (33.04 ppm) or **7** (33.39 ppm). Also, C-22 in **1** (31.62 ppm) and **2** (32.91 ppm) is shielded in comparison to C-22 in **4** (43.15 ppm) or **3** (43.53 ppm). Such shielding is in contrast to that of vinylic carbons in conventional olefins[24] but in line with that of acetylenes, thus supporting the acetylenic character of the cyclopropene "double" bond.[25] The shielding of carbons attached to an sp center is evident in the chemical shift of the acetylenes **5** and **6**, in comparison with that of the cyclopropenes **1**–**4**; for example, C-25 in **5** (3.51 ppm) compared with C-29 in **2**–**4**, and C-25 in **6** (20.58 ppm) compared with C-25 in **1**, **3**, and **4**. The same trend is evident in the case of C-22 in **5** (25.88 ppm) and **6** (25.84 ppm) in comparison with that of **1** (31.62) and **2** (32.91 ppm).

It is interesting to compare the chemical shifts of the two stereoisomeric sterols **3** and **4**. As is evident from Table II, their chemical shift values are very close, the largest differences occurring at C-23 (0.67 ppm) and C-28 (0.77 ppm).

The ^{13}C NMR data for these *Calyx* sterols not only support their unusual structure, but also provide a background for future structure elucidation of related sterols.

Discussion

More than 200 new sterols with unprecedented structures have so far been isolated from marine organisms (for recent reviews see ref 26–28). Although cyclopropane-containing sterols are relatively common in sponges, the sponge *C. nicaeensis* is the only known source of both cyclopropene- and acetylene-containing sterols. The present finding that *C. podatypa* contains related cyclopropenes implies that cyclopropene sterols are probably a common denominator among *Calyx* species. Sterol analysis could thus be extremely valuable for the chemotaxonomy of such sponges.[29,30] In this connection, it is relevant to mention that *C. podatypa* has only recently been reclassified under the genus *Calyx*.[31] Formerly, it was described as *Pachypellina podatypa*.[32]

The major sterol in *C. podatypa* (**4**) was hitherto unknown as a naturally occurring sterol. It was, however, encountered during an earlier study of the photochemical isomerization of **3**.[8] Its stereochemistry had been confirmed by correlation with the dihydrocalystanols produced by catalytic hydrogenation.[8]

The origin of the isomeric cyclopropenes is of considerable interest. There exists a possibility that the different isomers are artifacts of the isolation procedure, resulting from photochemical or acid-catalyzed rearrangements. This possibility is, however, quite unlikely as a detailed study[8] showed the lack of stereospecificity in the photochemical or acid-catalyzed processes. The existence of only one stereoisomer (**4**) in *C. podatypa* and the opposite isomer (**3**) in *C. nicaeensis* suggests that they are produced by highly stereospecific (enzymatic) processes. It is important to note that the same isolation procedure was followed in the case of both sponges.

The biosynthesis of *Calyx* sterols was the target of early[9] as well as recent studies.[10] Feeding experiments in *C. nicaeensis* demonstrated that the cyclopropane dihydrocalysterol (**7**) is a central intermediate in the biosynthesis of the cyclopropenes **1**–**3**.[10] The acetylenes **5** and **6** seem to be produced from **2** and **1**, respectively, by a process that can formally be described as retro carbene addition.

The details of formation of the cyclopropenes **1**–**3** from dihydrocalysterol (**7**) are largely unknown. Taking the established[12] stereochemistry of **7** into consideration, only calysterol (**1**) and 24*H*-isocalysterol (**2**) can be produced directly by removal of two hydrogens from **7**. The transformation **7** → **1** requires a trans elimination of H-23 and H-24. In contrast, **7** → **2** represents a cis dehydrogenation process. The third cyclopropene (**3**) from *C. nicaeensis* cannot be produced directly from **7** since elimination of H-24 and H-28 would result in the 23*S* epimer **4** rather than the 23*R* epimer **3**. It is thus unlikely that all the cyclopropene sterols arise *directly* from dihydrocalysterol (**7**).

On the basis of the observation that, upon feeding *C. nicaeensis* radiolabeled **7**, the cyclopropene **2** was about 10 times as radioactive as either **1** or **3**, we suggested[10] that **2** is directly produced from **7** and subsequently isomerizes to **1** and **3**. The cis stereochemistry of the dehydrogenation of **7** is similar to the biosynthesis of the cyclopropene fatty acid sterculic acid.[33] The same mechanism is probably operative in the case of *C. podatypa*. However, here (23*S*)-23*H*-isocalysterol (**4**) is produced instead of the 23*R* isomer **3**.

The isomerization of **2** to **1**, **3**, or **4**, in both *Calyx* species can be explained by the following unified mechanism (Scheme II). A stereospecific protonation of **2** at C-28 from the *re* side results in the cyclopropyl cation **11**, which undergoes one of the following three processes: (a) loss of H-24 as a proton, giving calysterol (**1**); (b) hydride shift of H-28 to C-23, taking place on the same face of the cyclopropane ring, followed by loss of H-24 as a proton, giving (23*S*)-23*H*-isocalysterol (**4**); (c) similar hydride shift of H-24 to C-23, followed by proton loss from C-28, producing **3** (with stereochemistry at C-23 opposite to that of **4**).

Although the above mechanism is not the only possible one, it nicely explains the formation and stereochemistry of the different cyclopropenes, **1**–**4**, from a single central intermediate (**11**). We intend to perform feeding experiments with appropriately labeled cyclopropene precursors to verify the above biosynthetic scheme.

Experimental Section

General Methods. High-performance liquid chromatography (HPLC) was carried out on a Waters Associates HPLC system (M 6000 pump, UK6 injector, R403 differential refractometer) using two Altex Ultrasphere ODS 5-μm columns (25 cm × 10 mm, i.d.) connected in series. A mixture of acetonitrile–methanol–ethyl acetate (22:9:7) was used as the mobile phase at a flow rate of 3 mL/min. Relative retention times are given with reference to calysterol (= 1.00). Gas–liquid chromatographic analysis was performed on a Hewlett-Packard 402A gas chromatograph with a flame-ionization detector and a 25-m DB-5 capillary column.

^1H and ^{13}C NMR spectra were recorded on a Varian XL-400 spectrometer operating at 400 and 100 MHz, respectively. ^1H NMR spectra were referenced to residual solvent resonances ($CHCl_3$ at 7.260 ppm, C_6H_6 at 7.150 ppm). Small coupling constants were resolved by recording resolution-enhanced spectra using Varian's RESOLV subroutine. ^{13}C NMR spectra were referenced to the center peak of $CDCl_3$ at 77.00

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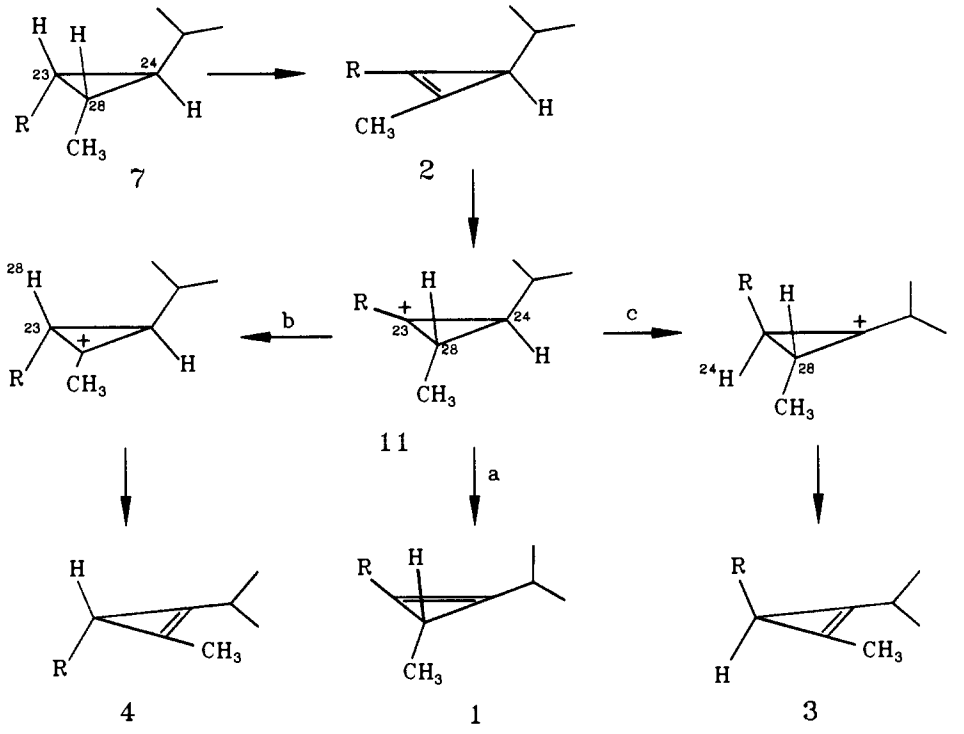
Scheme II



ppm. APT[20] and DEPT[21-23] spectra were obtained with the pulse sequences supplied in the manufacturer's software.

Low-resolution mass spectra were recorded on a Hewlett-Packard 5970 series mass spectrometer system with a Model 5890A GC for sample introduction and a Hewlett-Packard 9133 system for data acquisition. High-resolution mass spectra were recorded on an AEI MS-30 instrument by a direct probe inlet system at the University of Minnesota mass spectrometry service laboratory. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

Isolation of *C. podatypa* Sterols. An air-dried sample (ca. 50 g) of *C. podatypa*, collected in the Bahamas near Rum Cay at a depth of 117 ft, was extracted four times with chloroform–methanol (3:1). The organic layer was concentrated under reduced pressure with minimal heating. The crude extract was fractionated on an open silica gel column (eluent, hexane–ether, 3:1). The sterol fractions (R_f = cholesterol by TLC) were combined and evaporated under reduced pressure. The total sterol mixture (40 mg) was fractionated by reverse-phase HPLC using a mixture of acetonitrile–methanol–ethyl acetate (22:9:7). Individual fractions were reinjected as many times as necessary to get pure sterols, with either the above solvent system or absolute methanol as eluent.

(23*S*)-23*H*-Isocalysterol (4): mp 112–113 °C; HPLC relative retention time = 1.05; ^{1}H NMR (Table I); ^{13}C NMR (Table II); mass spectrum, *m/z* (relative intensity) 410 (2), 395 (2), 367 (2), 314 (2), 300 (6), 283 (10), 271 (43), 267 (6), 253 (7), 241 (5), 231 (2), 230 (2), 215 (8), 213 (7), 201 (4), 159 (13), 133 (20), 147 (11), 109 (45), 107 (16), 96 (52), 95 (100); exact mass calcd for $C_{29}H_{46}O$ 410.3548, found 410.3546.

(23*S*)-23*H*-5α-Isocalystanol (10): HPLC relative retention time = 1.24; ^{1}H NMR (Table I); mass spectrum, *m/z* (relative intensity) 412 (3), 397 (3), 396 (2), 369 (2), 316 (2), 302 (6), 299 (3), 287 (6), 285 (15), 273 (45), 269 (6), 257 (7), 255 (7), 233 (2), 217 (6), 215 (5), 203 (7), 201 (4), 189 (7), 176 (5), 175 (9), 161 (16), 149 (16), 147 (19), 137 (19), 135 (18), 123 (11), 121 (22), 110 (23), 109 (44), 107 (24), 96 (62), 95 (100); exact mass calcd for $C_{29}H_{48}O$ 412.3705, found 412.3682.

5α-Calystanol (8): HPLC relative retention time = 1.19; ^{1}H NMR (Table I); mass spectrum, *m/z* (relative intensity) 412 (4), 398 (3), 397 (6), 369 (9), 302 (12), 287 (15), 285 (6), 273 (71), 269 (6), 257 (11), 255 (9), 233 (12), 215 (19), 177 (12), 176 (12), 175 (12), 161 (31), 149 (26), 147 (15), 137 (22), 135 (38), 121 (59), 110 (100), 109 (49), 107 (48), 96 (11), 95 (58); exact mass calcd for $C_{29}H_{48}O$ 412.3705, found 412.3694.

(24*S*)-24*H*-5α-Isocalystanol (9): HPLC relative retention time = 1.13; ^{1}H NMR (Table I); mass spectrum, *m/z* (relative intensity) 412 (16), 398 (9), 397 (7), 369 (25), 314 (30), 302 (44), 299 (10), 287 (19), 285 (14), 273 (100), 269 (8), 257 (17), 255 (11), 233 (15), 215 (28), 201 (15), 189 (15), 177 (10), 176 (9), 175 (17), 165 (14), 161 (29), 149 (30), 147 (29), 137 (40), 135 (28), 131 (37), 125 (22), 123 (22), 121 (37), 110 (48), 109 (46), 107 (43), 96 (16), 95 (76); exact mass calcd for $C_{29}H_{48}O$ 412.3705, found 412.3668.

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