(m, 1 H), and 7.15–7.20 (m, 1 H); IR (neat) 3080, 3020, 2960, 2915, 1950, 1605, 1590, 1490, 1450, 1250, 850, and 760 cm^{-1}; UV (95% ethanol) 278 nm (ε 2100). Anal. Calcd for $C_{17}H_{24}OSi$: C, 74.94; H, 8.88. Found: C, 74.83; H, 8.83.

Direct Irradiation of 3,3-Dimethyl-2-phenyl-1-(trimethylsilyl)cyclopropene (31). A solution containing 200 mg of **31** in 200 mL of a 9:1 methanol–pyridine mixture was irradiated for 50 min with a 450-W Hanovia medium-pressure mercury arc lamp equipped with a Pyrex filter sleeve under an argon atmosphere. The solvent was removed under reduced pressure, and the crude residue was chromatographed on a silica gel column with hexane as the eluent. The major fraction contained 190 mg (95% yield) of a clear oil whose structure was assigned as (3-methyl-1-phenyl-1,2-butadienyl)trimethylsilane (**34**) on the basis of the following spectral properties: ^1H NMR ($CDCl_3$, 360 MHz) δ 0.21 (s, 9 H), 1.77 (s, 6 H), and 7.30 (br s, 5 H); IR (neat) 3060, 2940, 1940, 1605, 1495, 1450, 1360, 1250, 930, and 840 cm^{-1}; UV (95% ethanol) 248 nm (ε 10 500). Anal. Calcd for $C_{14}H_{20}Si$: C, 77.71; H, 9.32. Found: C, 77.84; H, 9.38.

A 200-mg sample of the above silane was dissolved in 10 mL of anhydrous tetrahydrofuran. To this mixture was added a 287-mg sample of tetrabutylammonium fluoride, and the mixture was allowed to stir for 15 min at 25 °C. The mixture was extracted with ether and washed with a saturated ammonium chloride solution. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to give 120 mg (89%) of a clear oil whose structure was assigned as 3-methyl-1-phenyl-1,2-butadiene (**35**) on the basis of the following spectroscopic properties: IR (neat) 3115, 3100, 3080, 3000, 2955, 1970, 1900, 1825, 1755, 1620, 1515, 1465, 1235, 760, and 720 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.35 (d, 6 H, J = 2.7 Hz), 6.02 (sept, 1 H, J = 2.7 Hz), and 7.25 (br s, 5 H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 20.31, 92.60, 99.14, 126.67, 128.51, 136.02, 141.27, and 203.17; UV (95% ethanol) 252 nm (ε 11 650).

Acknowledgment. We are grateful to the National Science Foundation for financial support.

Registry No. **4**, 56895-72-8; **5**, 119145-18-5; **6**, 119145-19-6; **7**, 119145-20-9; **8**, 119145-21-0; **9**, 119145-22-1; **13**, 119145-24-3; **14**, 119145-25-4; **16**, 119145-27-6; (*E*)-**17**, 119145-23-2; (*Z*)-**17**, 119145-28-7; **18**, 119145-29-8; **20**, 119145-30-1; **21**, 109900-77-8; **22** (n = 0), 109900-84-7; **22** (n = 1), 109900-85-8; **24** (R_2 = $CH_3C_6H_4$), 107696-98-0; **25** (R_2 = $(CH_2)_4Br$), 119145-26-5; **25** (R_2 = CH_3), 50902-98-2; **25** (R_2 = n-C_4H_9), 109900-82-5; **25** (R_2 = Ph), 50555-61-8; **25** (R_2 = t-C_4H_9), 109900-83-6; **26** (R_2 = n-C_4H_9), 109900-78-9; **26** (R_2 = t-C_4H_9), 109900-79-0; **26** (R_2 = PhCH=CH), 109900-81-4; **26** (R_2 = CH_3), 54599-15-4; **27**, 119145-31-2; **28**, 109900-86-9; **29**, 109900-87-0; **30**, 119145-32-3; **31**, 109900-80-3; **34**, 55967-10-7; **35**, 21020-31-5; $Br(CH_2)_3Ch{=}CH_2$, 1119-51-3; $Br(CH_2)_3C(CH_2){=}CH_2$, 41182-50-7; $Br(CH_2)_4Br$, 110-52-1; $CH_2{=}CHCHO$, 107-02-8; $(CH_3)_2C{=}N_2$, 2684-60-8; PhC≡CSO_2CH_3, 24378-05-0; PhC≡CSO_2Ph, 5324-64-1; $CH_2{=}CHBr$, 593-60-2; $CH_2{=}CHCH_2Li$, 3052-45-7; $CH_2{=}CHCH_2OC_6H_4$-*o*-Br, 60333-75-7; PhLi, 591-51-5; $CH_2{=}CH(CH_2)_2Li$, 14660-39-0; LiCH=CHPh, 4843-72-5; 3,3-dimethyl-5-(methylsulfonyl)-4-phenyl-3*H*-pyrazole, 119145-33-4; 3,3-dimethyl-4-phenyl-5-(phenylsulfonyl)-3*H*-pyrazole, 119145-34-5.

Biosynthetic Studies of Marine Lipids. 20.[1] Sequence of Double-Bond Introduction in the Sponge Sterol 24β-Methylcholesta-5,7,22,25-tetraen-3β-ol

Varghese John,[†] Ivan L. Stoilov,[†] Carl Djerassi,*[,†] Peter Karuso,[‡] Anthony Poiner,[‡] and Paul J. Scheuer[‡]

Department of Chemistry, Stanford University, Stanford, California 94305, and the Department of Chemistry, University of Hawaii at Manoa, 2545 The Mall, Honolulu, Hawaii 96822

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The biosynthesis of $\Delta^{5,7}$-sterols in *Ciocalypta* sp. was studied with radiolabeled Δ^5-sterol precursors. Of particular interest was the role of the tetraene, 24β-methylcholesta-5,7,22,25-tetraen-3β-ol, in the biosynthetic sequence. By examining C-24 epimeric pairs, stereospecific conversion of codisterol (but not epicodisterol) into the tetraene and ergosterol was demonstrated, which served also to establish the stereochemistry at C-24 of the natural sponge sterol. These biosynthetic steps are attributed to the sponge because of the absence of symbionts such as fungi and algae as shown by electron microscopy.

Introduction

A variety of unconventional sterols with unusual side chains and nuclei has been isolated from sponges.[2-4] Among these are the $\Delta^{5,7}$-sterols (**D**), encountered as major sterol components in some of these animals.[5] This particular nucleus, commonly found in sterols from yeast (fungi) and other terrestrial organisms, performs a role as pro-vitamin D.[6] In sponges these sterols possess 24-alkyl substituents typical of plant (algal) sterols and almost certainly function as components of the cell membranes.[7]

Three different $\Delta^{5,7}$-sterols comprise the total sterol mixture in the Australian sponge *Pseudaxinyssa* sp. Ergosterol (**4D**), which is the major component of the sterol mixture, was shown[5] to be formed from labeled codisterol (**7N**) through the intermediate tetraene (**1D**) (Chart I). This intermediate was not isolated or detected in *Pseudaxinyssa* sp., probably due to its rapid conversion to

[†] Stanford University.
[‡] University of Hawaii.

(1) For the preceding paper in this series, see: Malik, S.; Kerr, R. G.; Djerassi, C. *J. Am. Chem. Soc.* **1988**, *110*, 6895.
(2) Goad, L. J. In *Marine Products*; Scheuer, P. J. *Ed.*; Academic Press: New York, 1978; Vol. II, Chapter 2.
(3) Djerassi, C. *Pure Appl. Chem.* **1981**, *53*, 873.
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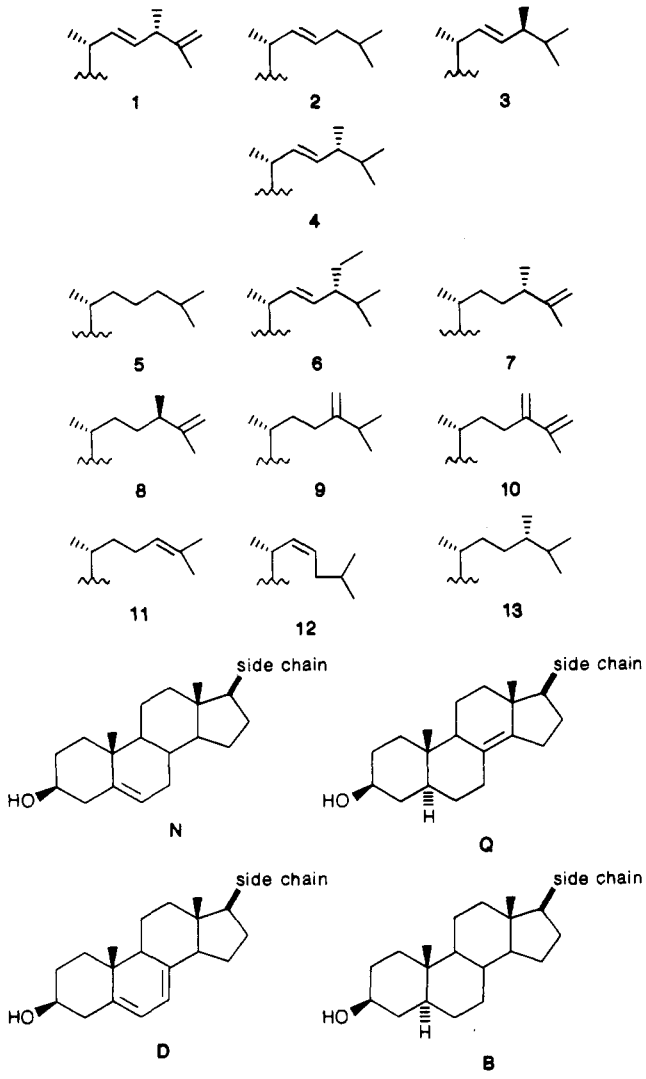
(5) Stoilov, I. L.; Moreau, M. B.; Thompson, J. E.; Djerassi, C. *Tetrahedron* **1987**, *43*, 2213 and references therein.
(6) (a) For general references, see: Fieser, L. F.; Fieser, M. *Steroids*; Reinhold Publishing: New York, 1959; p 90. Shoppee, C. W. *Chemistry of the Steroids*; Butterworth: London, 1968; p 62. Heftman, E. *Steroids Biochemistry*; Academic Press: New York, 1970; p 31. (b) Wilbur, K. In *Chemical Zoology*; Florkin, M., Scheer, B. T., Eds.; Academic Press: New York, 1972; Vol. 7, p 103.
(7) Lawson, M. P.; Stoilov, I. L.; Thompson, J. E.; Djerassi, C. *Lipids* **1988**, *23*, 750.

0022-3263/89/1954-1642$01.50/0

Chart I



ergosterol by the 25-hydrogenase. This unusual intermediate is similar to the 22,25-diene, which was demonstrated to be an intermediate in the biosynthesis of poriferasterol (**6N**)[8] in the alga *Trebouxia*.

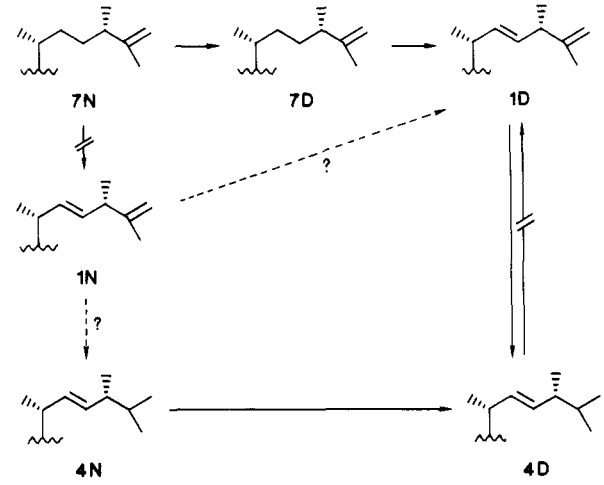
We have now found that the Hawaiian sponge *Ciocalypta* sp. contains only $\Delta^{5,7}$-sterols, notably the abovementioned 24-methylcholesta-5,7,22,25-tetraen-3β-ol (**1D**), thus offering the opportunity to examine the order in which the various double bonds are biosynthetically introduced in the tetraene. Incorporation studies with a number of radiolabeled sterol precursors (Tables I and II) have now permitted the elucidation of the principal biosynthetic paths and of the stereochemistry of the 24-methyl group in **1D**.

Results and Discussion

The results of the incorporation studies with radiolabeled precursors, assumed to be intermediates in the biosynthesis of the $\Delta^{5,7}$-sterols of *Ciocalypta* sp., are summarized in Table I. Each sterol precursor was either effectively incorporated into the $\Delta^{5,7}$-sterol mixture or recovered largely unchanged, depending on the precursor fitness in the biosynthetic transformation. The sponge sterols were purified by HPLC with a normal-phase column, which separates any Δ^5 components from the $\Delta^{5,7}$-dienes. While the sponge does not contain any detectable

(8) Wilkomirski, B.; Goad, L. J. *Phytochemistry* **1983**, *22*, 929.

Scheme I



amounts of Δ^5-sterols, all the precursors used in this study had a Δ^5-nucleus, thus necessitating such an initial purification. Previously,[5] adducts of the sterol nucleus with 4-phenyl-1,2,4-triazoline-3,5-dione[9] were used for the separation in *Pseudaxinyssa* sp. Further analysis of the $\Delta^{5,7}$ mixture on a reverse Altex column resulted in the isolation of six sterols (**1D–6D**) with different side chains (see Table III). As shown in Table III, striking quantitative variations in the sterol mixture were encountered in different samples that appeared to be taxonomically identical.

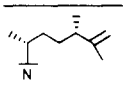
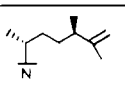
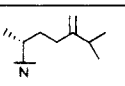
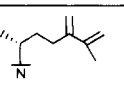
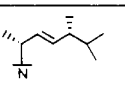
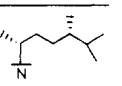
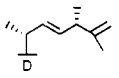
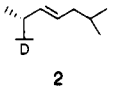
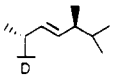
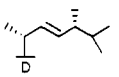
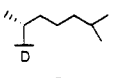
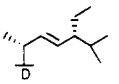
Our incorporation experiments clearly demonstrate the intermediacy of the tetraene (**1D**) in the biosynthesis of ergosterol. While ergosta-5,24(28)-dien-3β-ol (**9N**) was not incorporated into **1D**, its double bond isomer codisterol (**7N**) gave the best incorporation of all precursors tested. The transformation of codisterol (**7N**) into **1D** and to some extent into ergosterol (**4D**) demonstrated one of the principal biosynthetic pathways in this sponge (see Scheme I). Furthermore, stereospecificity was established in the conversion of codisterol (**7N**) into **1D**, since epicodisterol (**8N**) was not transformed into **1D** but was efficiently converted into ergosta-5,7,25(26)-trien-3β-ol (**8D**). On the basis of this experiment it was possible to assign the β-configuration to the C-24 methyl group of the tetraene **1D**.

The question of the sequence in which the second nuclear double bond and appropriate side chain modification occur in *Ciocalypta* sp. was addressed in the codisterol (**7N**) incorporation experiment by analysis of the radioactivity in the Δ^5-sterol fraction. The Δ^5-sterol fraction was analyzed by HPLC with a reverse Altex column and found to consist of a single component (>99%), which comigrated with cold codisterol. The transformation **7N** → **1N** (Scheme I) was not observed. These experimental results imply that in *Ciocalypta* sp. side chain modification does not precede nuclear modification, contrary to the de novo biosynthesis of similar $\Delta^{5,7}$-dienes in fungi.[10,11a]

The real intermediacy of the tetraene **1D** was demonstrated by the complete conversion of ergosta-5,22-dien-3β-ol (brassicasterol, **4N**) into ergosterol (**4D**). The lack of incorporation of radioactivity into **1D** eliminated the possibility of any conversion of **4D** into **1D** (see Scheme I). This experiment also demonstrated that introduction of the 22(23) unsaturation required the presence of the

(9) Barton, D. H. R.; Shioiri, T.; Widdowson, D. A. *J. Chem. Soc. C* **1971**, 1968.
(10) Pierce, A. M.; Pierce, H. D.; Unrau, A. M.; Oehlschlager, A. C.; Subden, R. E.; Renaud, R. L. *Can. J. Biochem.* **1979**, *57*, 112.
(11) (a) Goodwin, T. W. *Enzymes Biol. Membr.* **1985**, *2*, 205. (b) Knapp, F. F.; Goad, L. J.; Goodwin, T. W. *Phytochemistry* **1977**, *16*, 1683.

Table I. Incorporation Experiments in *Ciocalypta* Sp. Using 24-Methylated Precursors

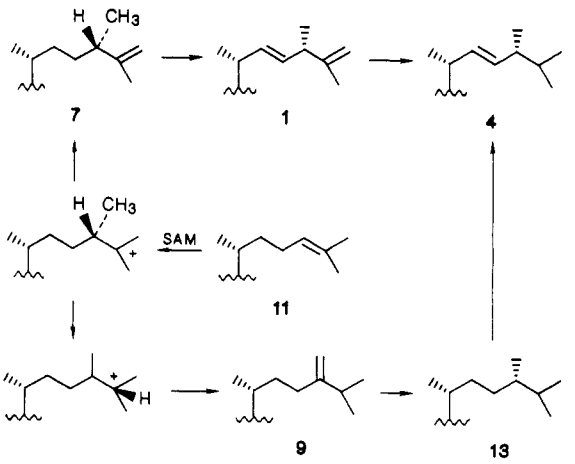
		precursor [label position]					
							
		7	**8**	**9**	**10**	**4**	**13**
		[26-^{14}C]	[28-^{3}H]	[6-^{3}H]	[26-^{14}C]	[3-^{3}H]	[3-^{3}H]
sponge,[a] freeze dry weight	(g)	6.5	7.0	4.1	6.1	6.1	5.4
total activity fed	(μCi)	20	20	20	20	20	20
total free sterols	(mg)[b]	25	20	30	20	15	22
	(dpm)[c]	1 464 000	1 076 190	3 056 650	1 143 990	2 871 740	1 298 307
	(dpm/mg)[d]	58 560	53 809	101 888	57 199	191 449	59 014
Δ^5-sterol[e]	(dpm)	400 000	300 000	646 654	95 000	400 000	97 000
$\Delta^{5,7}$-sterol[f]	(mg)	24	18	19	18	13	19
	(dpm)	1 010 000	689 479	2 021 152	1 303 159	2 100 000	1 098 306
	(dpm/mg)	42 083	38 304	10 638	72 397	161 538	57 806
 **1**	(mg)	20.4	14.4	15	14.9	11.1	16.2
	(dpm)	700 483	cold	cold	cold	cold	cold
	(dpm/mg)	34 337					
 **2**	(mg)	1.9	1.8	2.1	1.3	1.0	1.5
	(dpm)	cold	408 201[g]	cold	cold	cold	cold
	(dpm/mg)		226 778				
 **3**	(mg)	0.5	0.5	0.6	0.4	0.3	0.4
	(dpm)	cold	cold	cold	cold	cold	cold
	(dpm/mg)						
 **4**	(mg)	0.5	0.5	0.6	0.5	0.3	0.4
	(dpm)	15 000	cold	67 350	cold	2 077 672	50 000
	(dpm/mg)	8000		112 250		6 925 573	125 000
 **5**	(mg)	0.3	0.2	0.3	0.3	0.2	0.2
	(dpm)	cold	cold	1 300 000[h]	950 000[h]	cold	1 040 000[h]
	(dpm/mg)			4 333 333	3 166 667		5 200 000
 **6**	(mg)	0.5	0.5	0.6	0.5	0.3	0.4
	(dpm)	cold	cold	cold	cold	cold	cold
	(dpm/mg)						

[a] All incubations were done for a period of about 30 days. [b] Weight of sterols. [c] Total radioactivity ("cold" radioactivity not significantly above background). [d] Specific radioactivity. [e] Purification of the Δ^5 from the $\Delta^{5,7}$-diene was done by HPLC on a normal-phase column. [f] HPLC separation of the $\Delta^{5,7}$-diene fraction on a reverse-phase column gave six sterols, **1D**–**6D**. [g] The radioactivity is solely associated with **8D**, which comigrates with **2D**. [h] The radioactivity is solely associated with **13D**, which comigrates with **5D**.

25(26) double bond. Although ergosta-5,24(28)-dien-3β-ol (**9N**) was not biosynthetically transformed to **1D**, it was efficiently converted into the 24-methyl 5,7-diene (**13D**) by dehydrogenation at position C-7 and reduction of the 24(28)-double bond. A similar process was seen in the incorporation of ergosta-5,24(28),25(26)-trien-3β-ol (**10N**). The expected conversion to ergosterol (**4D**) by dehydrogenation at C-22 was also demonstrated. The lack of incorporation of any radiolabeled 24-methylenecholesterol (**9N**) into the 24-ethyl homologue **6D** demonstrated that the biosynthetic C-28 alkylation sequence, elucidated previously in plants,[11b] was absent in *Ciocalypta* sp. Experiments with 24-methylcholesterol (**13N**) documented the efficient incorporation into the corresponding 5,7-diene (**13D**) and further conversion to ergosterol (**4D**).

A difficulty encountered in the experiments with ergosta-5,24(28)-dien-3β-ol (**9N**) and 24-methylcholesterol (**13N**) was the comigration of the 24-methyl 5,7-diene (**13D**) and the cholesta-5,7-diene (**5D**) during HPLC analysis on a reverse Altex column. Catalytic hydrogenation (see the Experimental Section) of the mixture of **13D** and **5D** with palladium on carbon produced a separable mixture of ergost-8(14)-en-3β-ol (**13Q**) and cholest-8-(14)-en-3β-ol (**5Q**). This method also solved a similar problem in the experiment with epicodisterol (**8N**) where the 24-methyl-5,7-diene (**8D**) and cholesta-5,7,22-trien-3β-ol (**2D**) also comigration during HPLC purification. Catalytic hydrogenation of these two sterols resulted in a mixture of ergosta-8(14)-en-3β-ol (**13Q**) and cholesta-8-

Scheme II



(14)-en-3β-ol (**5Q**), which were then separated readily by HPLC.

The results of these experiments demonstrate the existence of two parallel pathways (Scheme II) in *Ciocalypta* for the biosynthesis of ergosterol (**4D**) via the two key intermediates codisterol (**7N**) and ergosta-5,24(28)-dien-3β-ol (**9N**). A priori, these two intermediates could be derived by biosynthesis from desmosterol (**11N**) via C-24 SAM alkylation.[12,13] However, desmosterol was poorly incorporated (Table II) into the tetraene (**1D**) and ergosterol (**4D**). Therefore it is unlikely to be the precursor of the key intermediates **7N** and **9N**.

Goad[14] summarized the possible origin of sterols in sponges as follows: de novo biosynthesis; dietary uptake with and without further modification by the sponges or their symbionts; and incorporation of sterols biosynthesized by symbiotic fungi or algae. Initially, conclusions about de novo biosynthesis of sterols in sponges were inconclusive.[2,15] However, recently we have demonstrated unequivocally that neither radiolabeled cycloartenol nor lanosterol are transformed into sponge sterols by *Ciocalypta* sp.[16] Hence, de novo biosynthesis of sterols does not occur in this sponge. Furthermore, neither fungi or algae were detected by electron microscopy; rather, like many other tropical, flattened sponges,[17] the sponge contained only cyanobacteria. The existence of biosynthetic pathways for sterols in these symbionts has been debated,[18,19] since few instances are known of low levels of sterols in these organisms.[20-22] Thus the origin of the biosynthetic intermediates **7N** and **9N** in *Ciocalypta* sp. remains unsolved. These intermediates are probably not of dietary origin since there is no known planktonic source for these sterols. Whether they are produced by some microorganisms known to occur in sponges[23] is not clear.

Sterol precursors without a 24-alkyl substituent, including desmosterol (**11N**), were poorly transformed into the $\Delta^{5,7}$-dienes (Table II). Radiolabeled cholesta-5,22-dien-3β-ol (**2N**) was recovered almost entirely (>90%) in the Δ^5-sterol fraction. Analysis of this fraction, using techniques described above, showed it to be composed of starting material. The rest of the radioactivity was recovered in the $\Delta^{5,7}$-sterol fraction and was found to comigrate with cholesta-5,7,22-trien-3β-ol (**2D**). Similar incorporation of radiolabeled cholesterol resulted in most of the radioactivity being recovered in the Δ^5-sterol fraction and the remainder found in cholesta-5,7-dien-3β-ol (**5D**). These results show that sterols with 24-alkyl groups (Table I) are greatly preferred by the 7-dehydrogenase in this sponge.

The ratio of the sponge sterols **1D** to **6D** for most of the sponges studied remained unchanged through two seasonal collections (Table III; A). However, in two sponges (Table III; B) ergosterol (**4D**), rather than the tetraene **1D** was the major component.

The dramatic change in the ratio between **1D** and **4D** in these two sponges can only be due to species variability or some mechanism of induction of the 25-hydrogenase, leading to enhanced conversion of **1D** into **4D**. As the sponges were all from the same local environment, varying ingestion of brassicasterol (**4N**) from a dietary source (with subsequent transformation to ergosterol) seems unlikely.

Whereas the sterols **2D**, **3D**, **5D**, and **6D** are naturally occurring components of the sponge (cf. Table III), they are not involved in the biosynthesis of either the tetraene (**1D**) or ergosterol (**4D**). Their relative amounts, however, were maintained at a constant level in this sponge. The sponge may possess a mechanism for maintaining this sterol ratio through selective uptake and modification of dietary sterols, which are expected to vary seasonally depending on the abundance of microorganisms. An example of such uptake and modification would be ingestion of poriferasterol (**6N**), a known planktonic constituent,[24] and subsequent introduction of the Δ^7-double bond producing **6D**. Thus, our research supports the hypothesis that the sterol composition of sponges is species-dependent, rather than a reflection of the sterol composition of the planktonic microorganisms filtered indiscriminately by sponges.

In summary, the sponge *Ciocalypta* sp. was shown to transform efficiently Δ^5-sterols with 24β-alkyl substituents into $\Delta^{5,7}$-sterols. Two parallel biosynthetic pathways for ergosterol were demonstrated in this sponge. The first pathway involved synthesis of ergosterol (**4D**) from codisterol (**7N**) via the intermediate tetraene **1D**. The other pathway from 24-methylenecholesterol (**9N**) proceeds via the 24-methyl 5,7-diene. These experiments also demonstrate the absence of C-24 and C-28 alkylation sequences typically seen in algae of the class Chlorococcales[25] or in other sponges.[5]

Experimental Section

General. The separation of the sterol mixture was done with Waters HPLC equipment (M6000 A and M45 pumps, U6K injector, R401 differential refractometers) with a Rheodyne model 7120 and a Valco Model CV-6-UHPa-N60 injector. Altex Ultrasil Si normal-phase columns (10 mm i.d. × 25 cm) were used to separate various sterol nuclei, and further purification was achieved with two Altex Ultraspheres ODS columns (10 mm i.d. × 25 cm) connected in series. The purity of HPLC fractions was checked by using a Carlo Erba Model 4160 gas chromatograph with FID (HP Ultra 2 capillary column, 0.32 i.d. × 25 m with 0.52 μm film thickness). Low-resolution mass spectra were recorded with a Ribermag R-10-10 quadrupole instrument in either DI or GC-MS mode; 400-MHz ^{1}H NMR spectra were obtained on a Varian XL-400 spectrometer. Radioactivity was determined with a Beckman LZ7500 liquid scintillation counter.

Synthesis of Labeled Precursors. The synthesis of labeled precursors has been described elsewhere: [26-^{14}C]codisterol,[26] [28-^{3}H]epicodisterol,[27a] [3-^{3}H]ergosta-5,24(28)-dien-3β-ol,[27a] [26-^{14}C]ergosta-5,24(28),25(26)-trien-3β-ol,[28] [3-^{3}H]ergosta-5,22(23)-dien-3β-ol,[27b] [3-^{3}H]ergosta-5-en-3β-ol,[27b] [3-^{3}H]-cholesta-5-en-3β-ol,[27b] [6-^{3}H]-(*E*)-cholesta-5,22(23)-dien-3β-ol,[27a] [6-^{3}H]-(*Z*)-cholesta-5,22(23)-dien-3β-ol,[27a] [3-^{3}H]desmosterol.[27b]

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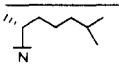
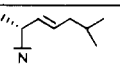
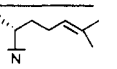
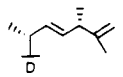
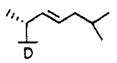
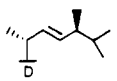
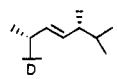
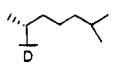
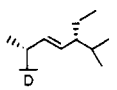
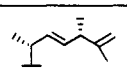
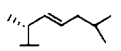
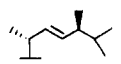
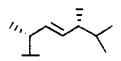
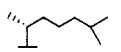
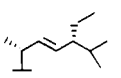
Table II. Incorporation Experiments in *Ciocalypta* Sp. with Precursors Lacking 24-Alkyl Substituents[a]

		precursor [label position]			
					
		5	**2**	**12**	**11**
		[3-^{3}H]	[6-^{3}H]	[6-^{3}H]	[3-^{3}H]
sponge,[a] freeze dry weight	(g)	6.0	7.0	5.0	5.1
total activity fed	(μCi)	20	20	20	20
total free sterols	(mg)[b]	20	10	12	16
	(dpm)[c]	2 846 254	549 600	162 000	1 003 906
	(dpm/mg)[d]	142 313	54 960	13 502	62 744
Δ^5-sterol[e]	(dpm)	2 704 334	533 073	150 653	809 540
$\Delta^{5,7}$-sterol[f]	(mg)	17	7	10	15
	(dpm)	100 860	35 000	cold	25 000
	(dpm/mg)	5933	5000		1667
 **1**	(mg)	14.5	6	8.1	12.8
	(dpm)	cold	cold	cold	22 000
	(dpm/mg)				1719
 **2**	(mg)	1.4	0.5	0.7	1.2
	(dpm)	cold	20 000	cold	cold
	(dpm/mg)		40 000		
 **3**	(mg)	0.3	0.2	0.3	0.3
	(dpm)	cold	cold	cold	cold
	(dpm/mg)				
 **4**	(mg)	0.3	0.2	0.2	0.3
	(dpm)	cold	cold	cold	cold
	(dpm/mg)				
 **5**	(mg)	0.2	0.1	0.2	0.2
	(dpm)	96 857	cold	cold	cold
	(dpm/mg)	484 285			
 **6**	(mg)	0.3	0.2	0.3	0.3
	(dpm)	cold	cold	cold	cold
	(dpm/mg)				

[a] For abbreviations, see footnotes in Table I.

Table III. Sterol Composition of *Ciocalypta* Sp.

no.	side chain	sample A[a] % composition	sample B[b] % composition
1D		85	5
2D		8	5
3D		2	4
4D		2	80
5D		1	3
6D		2	3

[a] The sponges come from two collections (July 1987 and February 1988). [b] This sample represents two sponges collected in February 1988.

Collection of Sponges for the Incorporation Experiments. The sponges were collected in July 1987 and February 1988 at depths of about 7 m at Pupukea on the north shore of Oahu or in small underwater caves off Magic island on the south shore of Oahu.

Incorporation Experiments. Specimens were collected and transplanted in situ onto plastic plaques. The precursors were then introduced into the specimens via 10–12-h aquarium incubations[26,29] and finally returned to their original collection site for approximately 1 month before collection[30] and analysis.

Extraction and Isolation of Free Sterols. Freeze-dried samples of the sponge were cut into small pieces (1 cm^3) and extracted with chloroform for a period of 48 h at 5 °C. The solvent was removed under reduced pressure, and the crude residue was chromatographed on a column of silica gel (50 g) with hexane/diethyl ether (1:1, v/v). Fractions (100 mL) were collected and checked by TLC for sterol content. The complete sterol mixture was first purified by HPLC on a normal-phase column with ethyl acetate/hexane (6% v/v) as the eluent. This gave base-line separation between Δ^5- and $\Delta^{5,7}$-sterols. Further purification of the $\Delta^{5,7}$ fraction was done on a reverse Altex column, yielding six sterols, **1D–6D**. Catalytic hydrogenations were done in HOAc/EtOAc (10% v/v) with a catalytic amount of (5%) palladium on activated carbon.

Electron Microscopy. For *Ciocalypta* sp. intact surface (<0.5 mm) and internal tissue was fixed[31] and examined by electron microscopy (3000×) for the presence of microorganism symbionts.

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facility at Stanford University was made possible by NSF Grant CHE81-09064. We thank Prof. R. D. Simoni for the use of his liquid scintillation counter and Max Hoberg for electron microscopy. The University of Hawaii authors acknowledge financial support from the National Science Foundation and thank Dr. Rolf Herb for his assistance with the underwater experiments.

Registry No. **1D**, 119147-12-5; **2D**, 22643-62-5; **3D**, 57760-53-9; **4D**, 57-87-4; **5D**, 434-16-2; **6D**, 19432-13-4; **2N**, 34347-28-9; **4N**, 474-67-9; **5N**, 57-88-5; **7N**, 52936-69-3; **8N**, 71486-08-3; **9N**, 474-63-5; **10N**, 102607-76-1; **11N**, 313-04-2; **12N**, 26033-10-3; **13N**, 4651-51-8.

An Anionic 3 + 2 Cyclization–Elimination Route to Cyclopentenes

Peter Beak* and Douglas A. Burg

Department of Chemistry, University of Illinois, Urbana, Illinois 61801

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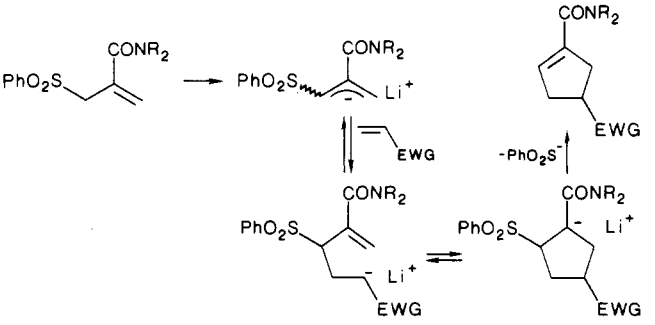
The formation and reactions of [1-(phenylsulfonyl)-2-(diisopropylcarbamoyl)allyl]lithium (**10**) are reported. When **10** is allowed to react with olefins bearing an electron-withdrawing group the 4-substituted cyclopent-1-enecarboxamides **11–19** are produced in 22–89% yields. Methyl-substituted analogues of **10**, the allyllithium reagents **23** and **25**, react in a similar manner to produce cyclopentenes that have methyl groups in the 2 or 5 positions. The corresponding [2-(dimethylcarbamoyl)allyl]lithium and [2-(phenylcarbamoyl)allyl]lithium reagents also react with electron-deficient olefins to produce the substituted cyclopentenes **28** and **30**, which can be hydrolyzed readily to the carboxylic acid **42**. The formation of the cyclopentenes occurs in a stepwise fashion by an initial highly regioselective addition to the electron-deficient olefin by the allyllithium reagent followed by a *5-Endo-Trig* cyclization and elimination of benzenesulfinate. The allyllithium **10** undergoes polydeuteration on reaction with methanol-*O*-*d* and acetone-d_6, alkylation with methyl iodide, and addition–dehydration on reaction with benzaldehyde.

Introduction

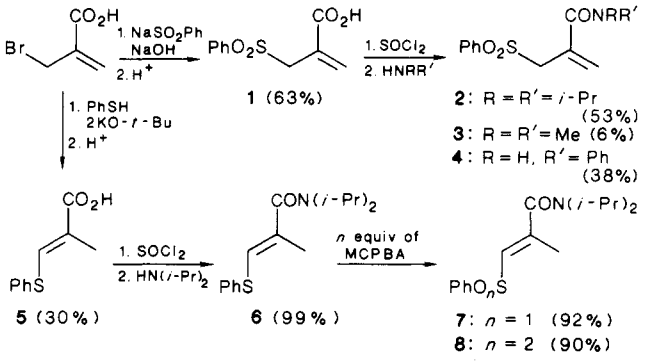
The development of methodology for the synthesis of five-membered carbocycles has been an active area of investigation in recent years and many ingenious approaches have emerged.[1] One of the most efficient methods of cyclopentane formation would be a regio- and stereospecific $4\pi s + 2\pi s$ cycloaddition between an allyl anion and an olefin. This reaction, termed the anionic 3 + 2 cycloaddition, was pioneered by Kauffman with early contributions from Böche and Ford.[2] We have reported cyclopentene ring formation by reaction between a [1-(phenylthio)-2-carbamoylallyl]lithium reagent and an acrylamide in a sequence that involves a formal anionic 3 + 2 cycloaddition as the key step.[3,4] We subsequently communicated the fact that the use of a phenylsulfonyl group at the β' position of the α,β-unsaturated amide overcomes the drawbacks of the phenylthio system in this synthetic

Scheme I[a]



[a] EWG = electron-withdrawing group.

Scheme II



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