of Point Loma Nazarene College, San Diego, CA, for the use of the Nicolet 710 FTIR instrument. High-resolution FAB mass spectra were obtained from Dr. Terry Lee, Beckman Research Institute of the City of Hope, Duarte, CA.

Biosynthetic Studies of Marine Lipids. 32.[1] The Missing Step in Sterol Cyclopropyl Biosynthesis: Enzymatic Desaturation of 24(*S*)-Ethylcholesterol

José-Luis Giner, Christopher J. Silva, and Carl Djerassi*

Department of Chemistry, Stanford University
Stanford, California 94305

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Marine sponges of the order Haplosclerida[2] contain many examples of the most unusual marine sterols: those containing cyclopropanes and cyclopropenes in the side chain (**1**–**4**).[3] In addition, the unusual acyclic side chain sterols, ficisterol (**5**)[4] and 26(29)-dehydroaplysterol (**6**),[5] which can formally be considered to arise through acid-catalyzed ring opening of the cyclopropyl sterols,[6] cooccur in these same sponges. Recently, through the degradation of biosynthetically radiolabeled petrosterol (**1**),[7] we demonstrated evidence for a unified biosynthetic scheme, involving a highly stereospecific rearrangement (Figure 1).[8] The source of the protonated cyclopropane[9] intermediate (**7**) has, until now, been unclear.

In feeding experiments with [3-^{3}H]dihydrocalysterol (**2**), no conversion to petrosterol (**1**) was detected, thereby ruling out direct enzymatic protonation.[7a] It was thought that *S*-adenosylmethionine (SAM) methylation of 24-methylenecholesterol (**8**, Figure 2), followed by isomerization of the tertiary carbonium ion (**9**) to the requisite secondary carbonium ion at C-23 (**10**), might be the enzymatic step leading to the cyclopropyl sterols (**1**–**4**).[7,8] However, when this was tested experimentally with [^{3}H]SAM in a cell-free extract of *Cribrochalina vasculum*, we found that 24-methylenecholesterol (**8**) was converted only to clerosterol (**11**, Figure 3).[10]

The finding that clerosterol (**11**) is the product of 24-methylenecholesterol (**8**) biomethylation in cyclopropyl sterol producing sponge suggested to us the intermediacy of the a priori unlikely clionasterol (**12**) in the biosynthesis of the cyclopropyl sterols (Figure 3). The isolation of a sterol bearing a 23-hydroxy side chain (**13**, R = Me) in a yeast mutant incapable of desaturation at the 22-position[11] suggests that the cyctochrome P-450

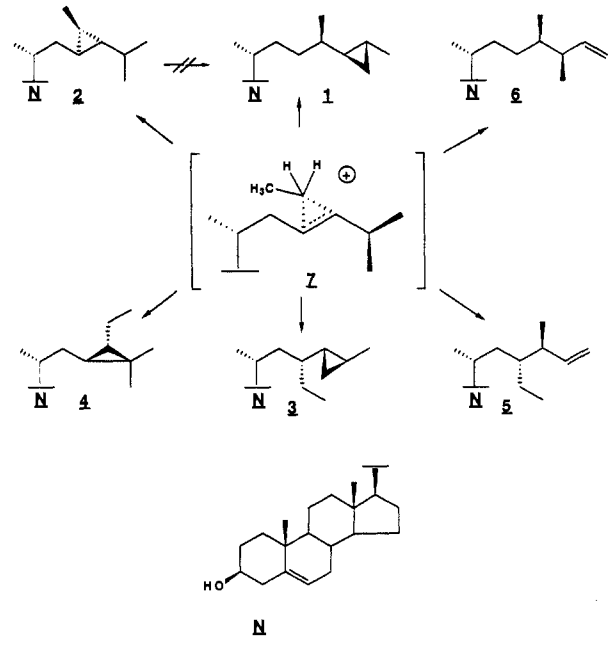

Figure 1. Unified biosynthetic pathway based on the intermediacy of the protonated cyclopropane **7**.[8]

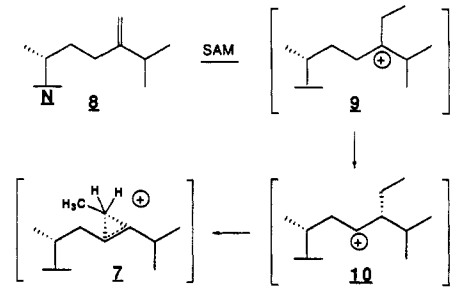

Figure 2. Possible route to the protonated cyclopropane intermediate **7**.

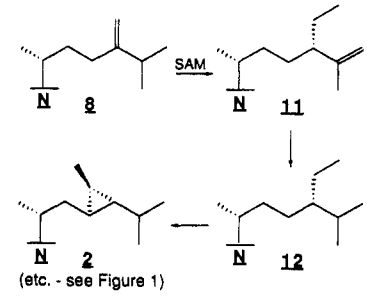

Figure 3. Biosynthetic pathway to sponge cyclopropyl sterols.

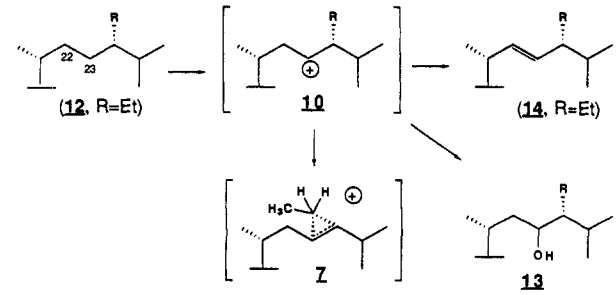

Figure 4. Products of the Δ^{22}-desaturase.

Δ^{22}-desaturase[12] initiates the formation of the double bond by deprotonation at C-23 (Figure 4). The secondary carbonium ion (**10**) thus formed can eliminate a proton directly from C-22 to produce the Δ^{22} double bond (e.g., **14**), rearrange via the pro-

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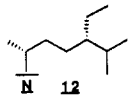
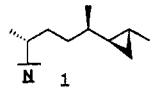
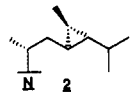
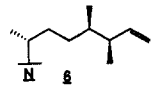
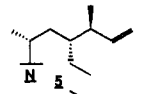
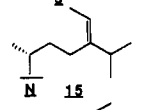
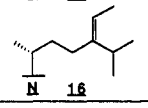
Table I. Results of Feeding Experiment with *P. ficiformis* (8.8×10^7 dpm [3-^{3}H]Clionasterol (**12**))

sterols	recovered radioact., dpm (% recovered)	sp act., dpm/mg
 **12**	7.4×10^6 (8.4%)	2.8×10^6
 **1**	4.4×10^6 (5.0%)	2.3×10^5
 **2**	6.0×10^5 (0.7%)	2.2×10^5
 **6**	7.1×10^4 (0.08%)	2.5×10^5
 **5**	2.0×10^4 (0.02%)	(not determined)
 **15**	1.0×10^4 (0.01%)	2.5×10^4
 **16**	0	0

tonated cyclopropane (**7**) to produce (Figure 1) the sponge sterols, or, in the yeast mutant, capture a hydroxyl group to yield the 23-hydroxy sterol (**13**).

When [3-^{3}H]clionasterol (**12**) was fed to *Petrosia ficiformis*, ca. 40% of the recovered radioactivity was found by reverse-phase HPLC in the cyclopropyl sterols, petrosterol (**1**) and dihydrocalysterol (**2**, Table I).[13] The remainder was in recovered starting material. The radiochemical purity of petrosterol (**1**) was checked by reverse-phase HPLC using a second solvent system and by hydrogenation to petrostanol;[6b] all of the radioactivity coeluted with the reduced sterol on HPLC. The purity of the other radioactive products was checked in the same way. Upon closer examination, small amounts of radioactivity were found to coincide with certain minor sterols present in the sponge, ficisterol (**5**) and 26(29)-dehydroaplysterol (**6**). These have previously been proposed to arise via the protonated cyclopropane intermediate **7** (Figure 1).[8] Fucosterol (**15**), but not isofucosterol (**16**), was also found to contain radioactivity. The specific activities of petrosterol (**1**), dihydrocalysterol (**2**), 26(29)-dehydroaplysterol (**6**), and ficisterol (**5**) were all approximately equal (Table I), strongly suggesting that these all arise from the partitioning of a common enzymatic reaction intermediate. The specific activity of fucosterol (**15**) was lower. This is consistent with either dilution by dietary sterol, since fucosterol (**15**) is a common sterol in the marine environment, or with the existence of a different pathway.

To our knowledge, this is the first time a saturated alkyl group has been shown to be enzymatically converted to a cyclopropane. It is likely that the other unusual sterols (e.g., **3**, **4**) associated with the Haplosclerida also arise through the action of similar errant Δ^{22}-desaturases. The irony that these unique sponge sterols should result from the sponge's inability to form poriferasterol (**14a**, Figure 4) (Porifera = sponges) has not escaped us.

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(13) For general methods, see ref 7a.

Metallacycle Annelation: Reaction of a Metallacycle α-Substituent and a Vinylidene Ligand To Give a Bicyclic Metallalactone Complex

Joseph M. O'Connor,* Lin Pu, and Raj K. Chadha

Department of Chemistry (0506)
University of California at San Diego
La Jolla, California 92093-0506

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Metallacycle complexes are widely employed intermediates for an impressive array of cyclization chemistry.[1] Methodology for controlled elaboration of metallacycles will greatly expand the utility of this compound class. We recently reported that Ir(CR=CRCR=CR)$(PPh_3)_2(CH_3CN)_2^+BF_4^-$ (**1**; R = CO_2CH_3) undergoes reaction with methyl propiolate in the presence of $^{18}OH_2$ to give the carbonyl complex **2** and methyl acetate (Scheme I).[2] In order to explain the location of the ^{18}O label in the α-methoxycarbonyl metallacycle substituent of **2**, we proposed intramolecular transfer of the original α-methoxycarbonyl oxygen to an intermediate vinylidene ligand. This mechanistic hypothesis, as well as an earlier observation of a surprising metallacycle annelation of unknown mechanism,[3] indicated promise for the design and implementation of a new class of metallacycle annelation reaction. We now report synthetic and mechanistic results on such an annelation strategy, including the conversion of Ir(CR=CRCR=CR)$(PPh_3)_2(L)(CO)^+BF_4^-$ (R = CO_2CH_3, L = CH_3CN, OH_2)[2,4] (**3**-L) and phenylacetylene to a stable bicyclic metallalactone complex, **4**. Mechanistic insight into the formation of **4** is obtained from oxygen-18 and deuterium labeling studies as well as independent synthesis from a metallacycle–acetylide complex.

In wet chloroform solution the aquo complex Ir(CR=CRCR=CR)$(PPh_3)_2(OH_2)(CO)^+BF_4^-$ (**3**-OH_2, R = CO_2CH_3, 0.049 mmol, 9.3 mM) and phenylacetylene (20 μL, 0.182 mmol) generate the bicyclic metallalactone **4** in 91% yield (24 h at 23 °C).[5] The ^{1}H NMR spectrum ($CDCl_3$) of **4** establishes the presence of only three methyl groups [δ 3.70 (s, 3 H), 3.40 (s, 3 H), and 3.23 (s, 3 H)]. The observation of a single vinyl hydrogen resonance at δ 5.53 indicates a single alkene isomer of unknown configuration. In the ^{13}C NMR spectrum (126 MHz, $CDCl_3$), the carbon resonances of the exocyclic alkene are observed at 145.1 (td, $^2J_{CH}$ = 11.8 Hz, J_{CP} = 9.2 Hz, Ir*C*=CHPh) and 125.5 (br d, $^1J_{CH}$ = 152 Hz, IrC=*C*HPh) ppm. The lactone carbonyl carbon at 172.1 (s) ppm is distinguished from the methoxycarbonyl carbons by the absence of a $^3J_{CH}$ coupling. In the $^{31}P\{^1H\}$ NMR spectrum, a singlet at −10.0 ppm requires a plane of symmetry that bisects the phosphorus–phosphorus axis and contains the ring atoms.

In order to establish the mechanism for this new cyclization reaction, we carried out the reaction of **3**-CH_3CN and phenylacetylene in the presence of $^{18}OH_2$ at 50 °C ($CDCl_3$) to give **4**-O. A $^{13}C\{^1H\}$ NMR spectrum of the sample was taken, and **4** was then added. A $^{13}C\{^1H\}$ NMR spectrum (126 MHz, $CDCl_3$) of the ~1:2 mixture of **4**:**4**-O exhibited a 4.3-Hz upfield isotopic shift for a single resonance at 171.9 ppm [*C*(=O)OC(=CHPh)Ir, **4**].[6] Thus, the oxygen-18 from $^{18}OH_2$ was selectively incorporated

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