Biosynthesis of Three Cyclopropene-containing Sterols in the Sponge *Calyx niceaensis*

Christian Margot,[a] Cesar A. N. Catalan,[a] John R. Proudfoot,[a] Guido Sodano,[b] Donato Sica,[b] and Carl Djerassi*[a]

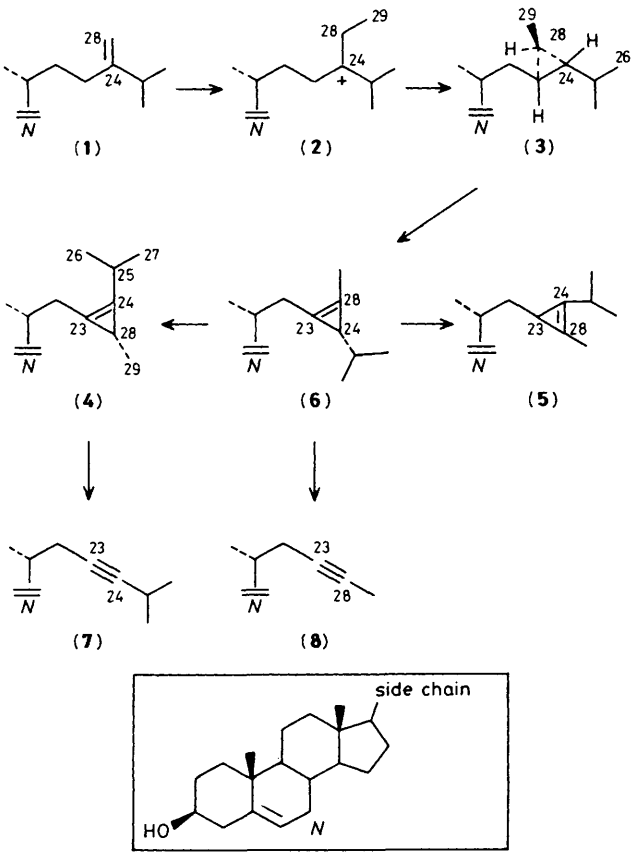
[a] *Department of Chemistry, Stanford University, Stanford, CA 94305, U.S.A.*
[b] *Istituto per la Chimica di Molecole di Interesse Biologico, Via Toiano 6, Arco Felice (Napoli), Italy*

As shown by a series of radiolabelling experiments in the sponge *Calyx niceaensis*, the cyclopropane sterol dihydrocalysterol (**3**), itself a metabolic product of 24-methylenecholesterol (**1**), undergoes formal *cis*-dehydrogenation to the cyclopropene 24*H*-isocalysterol (**6**).

The Mediterranean sponge *Calyx niceaensis* contains a variety of unusual side chain-bearing sterols. The cyclopropene sterol calysterol (**4**)[1] and its isomers 23*H*-isocalysterol (**5**)[2] and 24*H*-isocalysterol (**6**)[3] comprise up to 75% of the sterol fraction and are accompanied by the earlier predicted[4] (23*S*,24*S*,28*R*)-dihydrocalysterol (**3**).[2] The sponge is also a source of the only naturally occurring steroidal acetylenes, cholest-5-en-23-yn-3β-ol (**7**)[5] and 26,27-dinorcholest-5-en-23-yn-3β-ol (**8**).[5] Since labelled fucosterol, but not [1-^{14}C]acetate nor [*methyl*-^{14}C]methionine is incorporated[6] by the sponge into calysterol (**4**), it was concluded that the latter arose from metabolic transformation of dietary fucosterol.

In order to obtain additional information about the intermediate biosynthetic steps, we undertook incorporation experiments (for technique see ref. 7) which are summarized in Table 1. [28-^{14}C]-24-Methylenecholesterol (**1**)[7] as well as [26-^{3}H]-(23*S*,24*S*,28*R*)-dihydrocalysterol (**3**)† were very efficiently incorporated into the sterol fraction. Comparison of the specific activities (Table 1) permits the construction of the biosynthetic path outlined in Scheme 1 because the specific activity is expected to be inversely related to the number of intermediate steps from the fed precursor. Thus 24-methylenecholesterol (**1**) is transformed, probably *via* bioalkylation to give the cationic C_{29} intermediate (**2**), into the cyclopropane (**3**) (which we have postulated[8] to be a key intermediate in several sponge sterol biosyntheses), the next product being (24*S*)-24*H*-isocalysterol (**6**), the 'hottest' of the three cyclopropenes (**4**)—(**6**). Consideration of the recently established[8] stereochemistry of (23*S*,24*S*,28*R*)-dihydrocalysterol (**3**) implies that the formal dehydrogenation to (24*S*)=24*H*-isocalysterol (**6**) must be a *syn* process, which has

† The multi-step synthesis of [26-$^{3}H_1$]dihydrocalysterol will be reported in the full paper.



Scheme 1. Biosynthesis of calysterol (**4**).

Table 1. Feeding experiments in *C. niceaensis*; (specific activities in disint. min^{-1} mg^{-1}).

Labelled precursor [nucleus]	Sterol[a] (3) (2%)	(4) (42%)	(5) (22%)	(6) (10%)	(7) (3.1%)	(8) (4.5%)
(1)[b] [28-^{14}C]	745 000	14 000	18 300	182 000	'cold'	13 900
(3)[c] [26-$^{3}H_1$]	989 000	14 000	19 000	167 000	58 000	'cold'

[a] The relative abundance of the sterol in the sponge sterol fraction is given in parentheses below each formula number. [b] 19% of total radioactivity recovered in sponge. [c] 20% of total radioactivity recovered in sponge.

also been demonstrated in the dehydrogenation of the cyclopropane precursor in the biosynthesis[9] of the cyclopropene fatty acid, sterculic acid. If dihydrocalysterol (**3**) is also a direct precursor of the other two calysterols (**4**) and (**5**), an *anti* elimination process would have to be advanced. Hence, we postulate (Scheme 1) that 24*H*-isocalysterol (**6**) is the product of the cyclopropane (**3**) by a formal dehydrogenation, with the two other cyclopropenes (**4**) and (**5**) then arising from isomerisation of (**6**). Finally, the end-product acetylenes (**7**) and (**8**) appear to originate by some type of retro-carbene addition,‡ a process that has also been accomplished photochemically in our laboratory.[10] As already suggested by the Italian group,[6] the acetylenes (**7**) and (**8**) are thus excluded as precursors of the cyclopropenes (**4**) and (**6**). We hope to conduct additional experiments with this rare sponge to shed further light on the reactions outlined in Scheme 1.

We thank the National Institutes of Health for financial support, and the Swiss National Science Foundation and the Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentina for postdoctoral fellowships.

Received, 5th May 1987; Com. 604

‡ *A priori*, it seems puzzling that the acetylene (**7**) has a higher radioactivity than its presumed precursor, calysterol (**4**). The simplest explanation for this apparent discrepancy is that the acetylene originates from an intermediate species in the isomerisation of 24*H*-isocalysterol (**6**) to calysterol (**4**). However, important differences in the relative rates of the successive transformations could conceivably also contribute to a discordance.

References

1 E. Fattorusso, S. Magno, L. Mayol, C. Santacroce, and D. Sica, *Tetrahedron*, 1975, **31**, 1715.
2 L. N. Li, H-T. Li, R. W. Lang, T. Itoh, D. Sica, and C. Djerassi, *J. Am. Chem. Soc.*, 1982, **104**, 6726.
3 T. Itoh, D. Sica, and C. Djerassi, *J. Org. Chem.*, 1983, **48**, 890.
4 C. Djerassi, N. Theobald, W. C. M. C. Kokke, C. S. Pak, and R. M. K. Carlson, *Pure Appl. Chem.*, 1979, **51**, 1815.
5 E. Steiner, C. Djerassi, E. Fattorusso, S. Magno, L. Mayol, C. Santacroce, and D. Sica, *Helv. Chim. Acta*, 1977, **60**, 475.
6 L. Minale, R. Riccio, O. Scalona, G. Sodano, E. Fattorusso, S. Magno, L. Mayol, and C. Santacroce, *Experientia*, 1977, **33**, 1550.
7 C. A. N. Catalan, J. E. Thompson, W. C. M. C. Kokke, and C. Djerassi, *Tetrahedron*, 1985, **41**, 1073.
8 J. R. Proudfoot and C. Djerassi, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1283.
9 I. Yano, L. J. Morris, B. W. Nichols, and A. T. James, *Lipids*, 1972, **7**, 35.
10 T. Itoh and C. Djerassi, *J. Am. Chem. Soc.*, 1983, **105**, 4407.