Biosynthetic Studies of Marine Lipids. 14.[1] 24(28)-Dehydroaplysterol and Other Sponge Sterols from *Jaspis stellifera*

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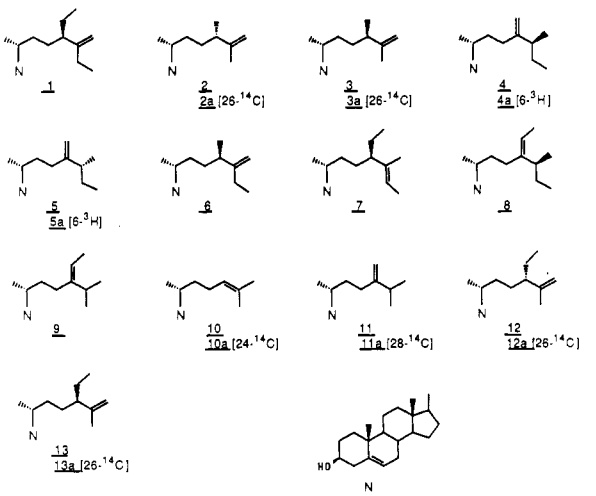
The biosynthesis of three marine sterols with unusual side chains was studied in the Australian sponge *Jaspis stellifera*. 24(*S*)-Methylcholesta-5,25-dien-3β-ol (codisterol, **2**) is favored over its 24(*R*)-epimer, epicodisterol (**3**), to the extent of 4:1 as a precursor of 24(28)-dehydroaplysterol (**4**), in spite of the absence of a chiral center at C-24 in **4**. Although biosynthetic pathways to stelliferasterol (**7**) and isostelliferasterol (**8**) could not be defined, the a priori most plausible triple bioalkylation sequences could be ruled out.

In contrast to extensive research on marine sterols since the early 1970s, resulting in the isolation and structure elucidation of a bewildering variety of new sterols with unprecedented side chains,[2,3] the biosynthesis of marine sterols with unusual side chain alkylation patterns has, with few exceptions,[4,5] been studied only very recently.[6] The reasons for this delay were largely of a technical nature: the difficulty of keeping a sponge alive during the lengthy incorporation experiments[7] as well as the unavailability of many potentially relevant precursors. These problems have now been largely solved in our laboratory,[7] and we are thus in a position to examine the biosynthetic pathways of unusual sterol side chains in marine organisms.

We have already gained considerable information on the biomethylation sequences.[8-13] Side-chain extension at C-26 was first demonstrated successfully in the Californian sponge *Aplysina fistularis*, where it was shown that the 24(*R*)-epimer, epicodisterol (**3**), but not its 24(*S*)-epimer, codisterol (**2**), is readily converted into 25(26)-dehydroaplysterol (**6**), thus demonstrating the expected retention of the C-24 stereochemistry during the chain extension (Scheme I).[7] In an examination of a triple biomethylation of the conventional cholesterol side chain, we demonstrated[12] that the operative biosynthetic sequence to strongylosterol (**1**) proceeds via codisterol (**2**) and 24(28)-dehydroaplysterol (**4**). Methylation of C-26 at the terminus of the side chain thus occurs prior to methylation of C-28 (Scheme II). Of particular relevance is the observation that the first biomethylation, at C-26, is strongly influenced by the C-24 stereochemistry (**2**), while the second side-chain extension, at C-28 (in **4** and **5**), is independent of the stereochemistry at C-25.

These observations prompted us to examine the biosynthesis of certain sterols in the sponge *Jaspis stellifera*, since the structures and all stereochemical features of three biosynthetically interesting candidates had been established in our laboratory.[14,15] These are 24(28)-dehydroaplysterol (**4**), a double-bond isomer of the earlier studied[7] 25(26)-dehydroaplysterol (**6**), and the two 24-ethylated sterols, stelliferasterol (**7**) and isostelliferasterol (**8**), which are double-bond isomers of strongylosterol (**1**). Since isofucosterol (**9**) also occurs in this sponge, it was examined as well.

The following eight labeled precursors were incorporated separately into each sponge specimen by using the earlier described incorporation procedures:[7,23] [28-^{14}C]-24-methylenecholesterol (**11a**),[7] [24-^{14}C]desmosterol (**10a**),[16] [26-^{14}C]codisterol (**2a**),[7] [26-^{14}C]epicodisterol (**3a**),[7] [26-^{14}C]clerosterol (**12a**),[12] [26-^{14}C]epiclerosterol (**13a**),[12] [6-^{3}H]-24(28)-dehydroaplysterol (**4a**),[12] and [6-^{3}H]-24(28)-



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(1) For preceding paper, see: Margot, C.; Catalan, C. A. N.; Proudfoot, J. R.; Djerassi, C. *J. Chem. Soc., Chem. Commun.* **1987**, 1441-1442.
(2) Djerassi, C. *Pure Appl. Chem.* **1981**, *53*, 873-890.
(3) Ikekawa, N. In *Sterols and Bile Acids*; Danielsson, H., Sjovall, J., Eds.; Elsevier: Amsterdam, 1985; pp 199-230.
(4) Minale, L.; Riccio, R.; Scalona, O.; Sodano, G.; Fattorusso, E.; Magno, S.; Mayol, L.; Santacroce, C. *Experientia* **1977**, *33*, 1550-1552.
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(7) Catalan, C. A. N.; Thompson, J. E.; Kokke, W. C. M. C.; Djerassi, C. *Tetrahedron* **1985**, *41*, 1073-1084.
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(9) Proudfoot, J. R.; Catalan, C. A. N.; Djerassi, C. *Tetrahedron Lett.* **1986**, *27*, 423-426.
(10) Stoilov, I. L.; Thompson, J. E.; Djerassi, C. *Tetrahedron* **1986**, *42*, 4147-4155.
(11) Stoilov, I. L.; Back, T. G.; Thompson, J. E.; Djerassi, C. *Tetrahedron* **1986**, *42*, 4156-4160.
(12) Stoilov, I. L.; Thompson, J. E.; Cho, J.-H.; Djerassi, C. *J. Am. Chem. Soc.* **1986**, *108*, 8235-8241.
(13) Stoilov, I. L.; Thompson, J. E.; Djerassi, C. *Tetrahedron Lett.* **1986**, *27*, 4821-4824.
(14) Theobald, N.; Wells, R. J.; Djerassi, C. *J. Am. Chem. Soc.* **1978**, *100*, 7677-7684.
(15) Cho, J.-H.; Djerassi, C. *J. Org. Chem.* **1987**, *52*, 4517-4521.
(16) Luo, F.-T.; Djerassi, C., in preparation.
(17) Goad, L. J.; Knapp, F. F.; Lenton, J. R.; Goodwin, T. W. *Lipids* **1974**, *9*, 582.
(18) Largeau, C.; Goad, L. J.; Goodwin, T. W. *Phytochemistry* **1977**, *16*, 1925-1930.
(19) The sterol mixture of *J. stellifera* contains 2.3% of codisterol (**2**); the presence of trace amounts of epicodisterol (**3**) is not ruled out, because both codisterol (**2**) and epicodisterol (**3**) have the same retention time in reverse HPLC. The total radioactivity associated with 24-methylenecholesterol (**11**) and codisterol (**2**) was 5%.
(20) Djerassi, C.; Theobald, N.; Kokke, W. C. M. C.; Pak, C. S.; Carlson, R. M. K. *Pure Appl. Chem.* **1979**, *51*, 1815-1828.

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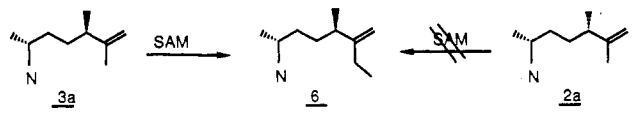
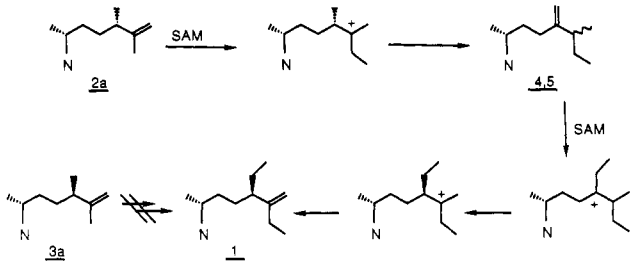
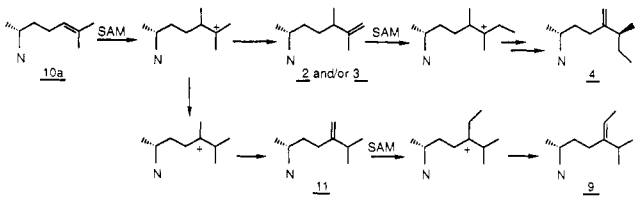
Scheme I



Scheme II



Scheme III



dehydroepiaplysterol (**5a**).[12]

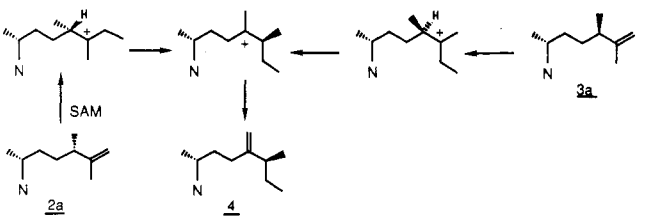
Results and Discussion

The results of the incorporations for each radiolabeled precursor in *J. stellifera* are summarized in Table I. Each sterol precursor tested was either transformed, to a substantial extent, into one of the sponge sterols or recovered unchanged. The latter observation was important, because it demonstrated that the labeled substrates were taken up by the sponge. Therefore, unsuccessful transformations were significant in that they indicated the absence of appropriate enzyme systems for the particular biosynthetic steps under consideration.

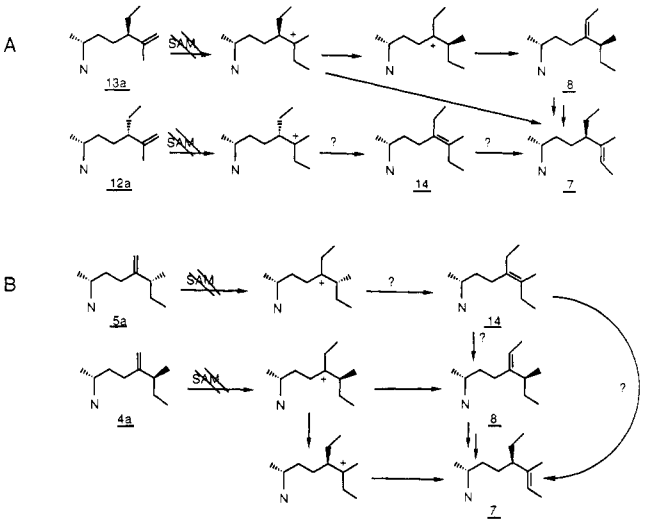
Incorporation of [24-^{14}C]Desmosterol (10a) and [28-^{14}C]-24-Methylenecholesterol (11a). Since desmosterol (**10**) serves as the precursor for the biosynthesis of sterol side chains such as 24-methylenecholesterol (**11**), codisterol (**2**), and epicodisterol (**3**) in plants[17,18] and marine organisms,[10,12,13] [24-^{14}C]desmosterol (**10a**) was selected first in order to establish whether these products of the initial bioalkylation (Scheme III) are used by *J. stellifera* for further alkylations. In the [24-^{14}C]desmosterol (**10a**) incorporation experiment (experiment 1 in Table I), some radioactivity indeed accumulated in 24-methylenecholesterol (**11**) and codisterol (**2**).[19] Therefore, successful incorporation of [24-^{14}C]desmosterol (**10a**) into 24(28)-dehydroaplysterol (**4**) and isofucosterol (**9**) can be rationalized as shown in Scheme III. The conventional path[10,18] to isofucosterol (**9**), outlined in Scheme III, was further confirmed by efficient incorporation (experiment 2 in Table I) of [28-^{14}C]methylenecholesterol (**11a**) into isofucosterol (**9**).

Biosynthesis of 24(28)-Dehydroaplysterol (4). Since 24(28)-dehydroaplysterol (**4**) lacks an asymmetric center at C-24, in contrast to its double-bond isomer 25(26)-dehydroaplysterol (**6**), both codisterol (**2**) and epicodisterol (**3**) are equally plausible precursor candidates for 24-(28)-dehydroaplysterol (**4**) (Scheme IV). In fact, codisterol (**2**) was favored over epicodisterol (**3**) to the extent of 4:1 (experiment 3 vs 4 in Table I). Furthermore, in the case of codisterol (**2a**) (experiment 3 in Table I), the major (81%) biosynthetic pathway leads to 24(28)-dehydroaplysterol (**4**), whereas in the case of epicodisterol (**3a**) (experiment 4 in Table I), only 20% of radiolabeled epicodisterol (**3a**) was incorporated into 24(28)-dehydroaplysterol (**4**). Therefore, the second biomethylation step at C-26 is largely, but not totally, dependent on the stereochemistry at C-24. Whether this is due to steric factors associated with the initial methyl transferase attack or the subsequent migration of the chiral C-24 proton to C-25 is still an open question.

Scheme IV



Scheme V

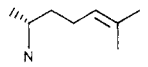
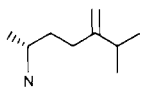
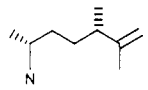
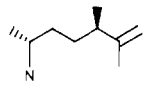
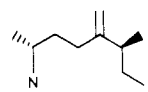
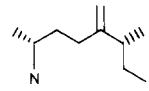
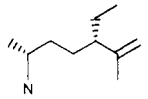
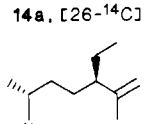


Stelliferasterol (7) and Isostelliferasterol (8). On the basis of biosynthetic precedents, two possible routes (A and B) to stelliferasterol (**7**) and isostelliferasterol (**8**) have been postulated (Scheme V).[2,14,20] By means of appropriately labeled sterols, we have now tested whether any of these hypothetical pathways is operative.

Even though clerosterol (**12**) or epiclerosterol (**13**) has not been encountered in *J. stellifera*, pathway A appears to be a plausible biosynthetic route by analogy to the demonstrated two successive "push–pull" mechanisms in the biosynthesis (Scheme II) of strongylosterol (**1**).[12] However, attempts to incorporate [26-^{14}C]clerosterol (**12a**) (experiment 7 in Table I) or [26-^{14}C]epiclerosterol (**13a**) (experiment 8 in Table I) into the sponge were not successful, although the total recovered yield of radioactivity (>36%) was the highest among all incorporation experiments performed in our laboratory. Therefore, **12** and **13** are efficiently taken up by the sponge, but are not metabolized further, and pathway A can definitely be ruled out.

(21) Knapp, F. F.; Goad, L. J.; Goodwin, T. W. *Phytochemistry* **1977**, *16*, 1683–1688 and references cited therein.
(22) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd ed.; Pergamon: New York, 1982.
(23) Carballeira, N.; Thompson, J. E.; Ayanoglu, E.; Djerassi, C. *J. Org. Chem.* **1986**, *51*, 2751–2756.

Table I. Results of Incorporation Experiments

expt no.	labeled precursor	total act. inc.,[b,c] μCi	recovered radioact. in free sterol, %	recovered radioact. in precursor, %	recovered radioact.:[a] total act., % sp. act. 4	11	10	9
1	 **10a**, [24-^{14}C]	16.7	3.4	22	7.0×10^5, 56%, 2.2×10^4	1.5×10^5, 12%, 8.7×10^4	6.2×10^3, 0.5%, 6.0×10^3	6.2×10^3, 0.5%, 1.0×10^3
2	 **13a**, [28-^{14}C]	20	22.0	1.5	0	8.0×10^6, 83%, 1.9×10^6	0	0
3	 **2a**, [26-^{14}C]	20	8.5	10	3.0×10^6, 81%, 1.6×10^5	0	2.6×10^4, 0.7%, 4.3×10^4	1.5×10^5, 3.9%, 4.0×10^4
4	 **3a**, [26-^{14}C]	20	19.3	50	1.7×10^6, 20%, 7.3×10^4	0	1.7×10^4, 0.2%, 2.3×10^4	5.9×10^4, 0.7%, 1.3×10^4
5	 **4a**, [6-^{3}H]	22	25.0	75	9.0×10^6, 75%, 1.1×10^5	0	2.4×10^5, 2.0%, 9.0×10^4	2.4×10^5, 2.0%, 1.5×10^4
6	 **5a**, [6-^{3}H]	24	19.0	79	7.9×10^6, 79%,[d] 1.1×10^5	0	1.0×10^5, 1.0%, 7.2×10^4	1.0×10^5, 1.0%, 1.2×10^4
7	 **14a**, [26-^{14}C]	17	41	99	0	0	0	0
8	 **15a**, [26-^{14}C]	22	36	99	0	0	0	0

[a] Total radioactivity is in dpm. In addition, 6.2×10^4 dpm was found in the codisterol (2) fraction in experiment 2. The percentage of recovered radioactivity is based on the total recovered radioactivity in the free sterol. The specific radioactivity is in dpm/mg. [b] Specific activity of the tritium compounds is ≅ 300 mCi/mmol. [c] Specific activity of the ^{14}C compounds is 30–40 mCi/mmol. [d] The C-25 epimers of 24(28)-dehydroaplysterol 4 and 5 are inseparable. This is starting material.

Next, we examined pathway B, where side-chain elongation is assumed to proceed at C-28, followed by direct deprotonation to isostelliferasterol (8) or a 1,2-hydride shift to result in stelliferasterol (7). Stelliferasterol (7) might also be derived from isostelliferasterol (8) via double-bond migration involving a hydrogenation–dehydrogenation sequence.[21] Actually pathway B is the most plausible among the three postulated alternatives since modest incorporation of codisterol (2) and epicodisterol (3)—the progenitors of the 24(28)-dehydroaplysterols 4 and 5—into stelliferasterol (7) and isostelliferasterol (8) was observed (experiments 3 and 4 in Table I). Unexpectedly, incorporation of [6-^{3}H]-24(28)-dehydroaplysterol (**4a**) (experiment 5 in Table I) and its 25-epimer **5a** (experiment 6 in Table I) into stelliferasterol (7) and isostelliferasterol (8) was similar to that of [26-^{14}C]codisterol (**2a**) and [26-^{14}C]epicodisterol (**3a**). This strongly implies that neither 24(28)-dehydroaplysterol (4) nor its 25-epimer **5** are the immediate progenitors of these sterols (**9**, **10**), for if they were they would be expected to have much better incorporation than codisterol (2) or epicodisterol (3). This eliminates pathway B as a major route to stelliferasterol (7) and isostelliferasterol (8).

Experimental Section

General Methods. High-performance liquid chromatography (HPLC) was carried out on a Waters Associates HPLC system (M 6000 pump, U6K injector, R403 differential refractometer). For reverse-phase HPLC, two Altex Ultrasphere ODS 5-μm columns (25 cm × 10 mm i.d.) in series with methanol as the mobile phase (3 mL/min) were used for the fractionation of the sterol mixture and for purification of synthetic sterol intermediates. Retention times are relative to the retention time of cholesterol, coinjected in a separate run with a cold sterol mixture, with the point of injection, rather than the beginning of the solvent peak, used to calculate the relative retention time (RRT) in HPLC. The purity of HPLC fractions was checked by gas–liquid chro-

matography using a Hewlett-Packard Model 402 gas chromatograph with a flame-ionization detector (3% SP2250 column, 2 mm i.d. × 1.80 m, 260 °C). High-resolution mass spectra were recorded on an MS-30 instrument by a direct probe inlet system at the University of Minnesota. Low-resolution mass spectra were recorded on a Hewlett-Packard 5970 series mass spectrometer system with a 5890 GC apparatus for sample introduction and a Hewlett-Packard 9133 system for data acquisition. Fourier transform ^1H NMR spectra were recorded on a Nicolet Magnetic Corporation NMC-300 spectrometer equipped with a 1280 data system. All spectra were referenced to $CHCl_3$ (7.259 ppm). Commercial reagents and solvents were analytical grade or were purified by standard procedures[22] prior to use. Radioactivity was determined with a Beckman LZ7500 liquid scintillation counter through the courtesy of Prof. R. D. Simoni of the Biology Department at Stanford University.

Incorporation of Radiolabeled Precursors. *J. stellifera* (Carter, 1879) was obtained from 10 to 15 m at two reefs (John Brewer and Rib) located in the central section of the Australian Great Barrier Reef. Portions of sponges were attached underwater to PVC plastic plaques with nylon cable ties and were left for at least 1 week to allow reattachment to the plaques. Precursors were then incorporated into sponge transplants (June and October 1985) with methods modified after Catalan et al.[7] and Carballeira et al.[23] The precursors were transferred to 4-L glass containers containing 2–3 L of unfiltered seawater in 2 mL of ethanol. The contents were continuously aerated via a glass outlet and were maintained under dim natural light at ambient ocean surface temperature (approximately 25 °C). After 30 min of aeration, a single sponge transplant was placed in each aquarium for 6 to 12 h. The transplants were then returned to their original sites of collection for 30 days. Finally the sponges were frozen, lyophilized, and shipped to Stanford for analysis.

Isolation and Purification of Sterols. The sterol fraction of the sponge samples from the incorporation experiments was obtained according to our standard procedure.[7]

Acknowledgment. Financial support was provided by NIH Grants GM-06840 and GM-28352. Use of the 300-MHz NMR spectrometers at Stanford University was made possible by NSF Grant CHE 81-09064. We thank Prof. R. D. Simoni for the use of his liquid scintillation counter and Dr. Peter Murphy, Libby Evans, and Jane Fromont for field assistance. This is contribution no. 369 from the Australian Institute of Marine Science.

Registry No. 2, 52936-69-3; 3, 71486-08-3; 4, 38636-50-9; 7, 69081-87-4; 8, 69081-88-5.

Stereoselective Formation of a Steroidal 20-Hydroxy-24-oic Lactone by a Novel Reaction of Dichloroketene with an Epoxy Olefin

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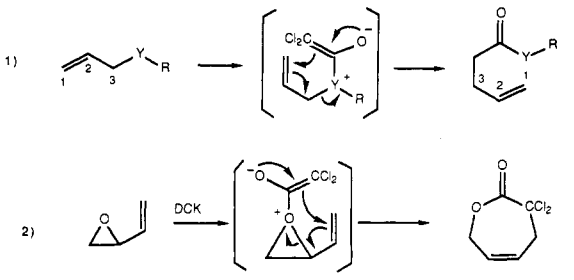
Reaction of dichloroketene (DCK) (generated from trichloroacetyl chloride and zinc dust) with the 20-methylene derivative of 16α,17α-epoxy-3β-acetoxy-5-pregnene (1) gave (20*R*)-3β-acetoxy-23,23-dichloro-20-hydroxy-5,16-choladien-24-oic lactone (4) in high yield. Zinc dust dechlorination of 4 gave lactone **6b**, which was converted to triol **9a** on treatment with lithium aluminum hydride. A single-crystal X-ray analysis of 5, a monodechlorinated derivative of 4, established the structure and C-20 configuration of all these five-membered lactones. The expected product from the reaction of DCK with 1 was a dichloro seven-membered lactone, 2, which is proposed as an intermediate in the formation of 4. On treatment with zinc chloride, 1 was rapidly transformed to an allylic chloro alcohol, 7, which also gave lactone 4 on subsequent reaction with DCK. Alkylation of 7 with dimethyl malonate produced a seven-membered lactone ester (11), analogous to the proposed intermediate 2. Treatment of 11 with zinc chloride induced a facile rearrangement to an isomeric five-membered lactone (12), which on hydrolysis and decarboxylation gave **6a**, identical in all respects with the hydrolysis product (3β-alcohol) from **6b**.

The chemistry of dichloroketene (DCK) and its use in synthesis have been studied and reviewed by Brady and co-workers.[1] This in situ generated reagent has been used chiefly in 2π + 2π cycloaddition reactions with olefins, and recent improvements in its preparation from di- or trichloroacetyl chloride[2] are such that DCK merits serious consideration as a convenient ketene equivalent despite the large mass loss due to discarded chlorine.

The scope of dichloroketene cycloadditions is limited in part by the tendency of allyl ethers and allyl sulfides to rearrange as shown in eq 1.[3] In a recently reported synthesis of (±)-lineatin,[4] this rearrangement was suppressed by substitution of 1,2-dimethoxyethane (glyme) for phosphorus oxychloride as a sequestering solvent in the reaction of zinc dust with trichloroacetyl chloride.



Our interest in dichloroketene lay in part on our expectation that it would react with conjugated epoxy olefins to form seven-membered lactones under mild conditions, as shown in eq 2. The substrate we chose to examine first in this respect was the 20-methylene derivative 1 of 16α,17α-epoxypregnenolone acetate. Dechlorination of the anticipated product (2) from reaction of 1 with DCK

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