Registry No. **1a**, 14417-01-7; **1b**, 5183-78-8; **1c**, 20383-28-2; **1d**, 1696-17-9; **1e**, 613-93-4; **1f**, 103-83-3; **2**, 19312-06-2; **3a**, 67448-06-0; **3b**, 13440-22-7; **3c**, 6641-72-1; **3d**, 2728-04-3; **3e**, 2170-09-4; **3f**, 4525-48-8; **4**, 71885-44-4.

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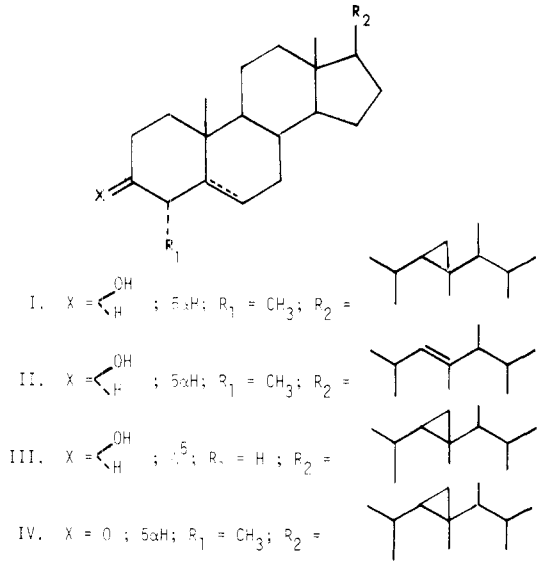
Dinoflagellate Sterols. 2. Isolation and Structure of 4-Methylgorgostanol from the Dinoflagellate *Glenodinium foliaceum*[1]

Summary: The dinoflagellate *Gonyaulax foliaceum* was found to contain four sterols, which were isolated and identified as cholesterol, 24-demethyldinosterol, dinosterol, and 4-methylgorostanol.

Sir: Dinoflagellates are unicellular organisms which along with diatoms and coccolithophores constitute what is commonly known as phytoplankton. Phytoplankton is the foundation for the food chain in the seas. Their distribution controls the whole pattern of life in the seas and is of great economic importance.

In our search of the origin of unusual sterols that have been isolated from marine invertebrates, especially from sponges, the dinoflagellate *G. foliaceum* was investigated for its sterol compositions.

The chloroform extract of the unialgal cultures of the *G. foliaceum*[2] gave a residue which after saponification afforded a sterol fraction consisting of four major sterols [cholesterol, 24-demethyldinosterol, dinosterol, and a new C_{31} sterol identified as 4-methylgorgostanol (1)] in the ratio



of 20:17:36:27 (GLC).[3] Sterol I was purified by high performance chromatography followed by preparative GLC,[4] and finally recrystallized by $CHCl_3$/MeOH to needles: mp 224–225 °C; $[\alpha]_D$ +6° (*c* 0.12, $CHCl_3$); $C_{31}H_{54}O$ (calcd *m/e* 442.417, found *m/e* 442.418).

The mass spectrum of the I[5] indicated the presence of a saturated steroid nucleus with a methyl group at C-4 (fragment ions *m/e* 289, 287, 271, 269, 247, and 229).[6,7,8] The intensities of the fragment ions [*m/e* 287 (100%), 271 (60%), 229 (27%)] were very close to that reported for dinosterol (II)[9] and 24-demethyldinosterol,[10] indicating the presence of dinosterol nucleus in the molecule. The presence of a methyl substituent at C-4 is also supported by the presence of the fragment ions at *m/e* 180, 179, and 125 corresponding $C_{12}H_{20}O$ and $C_{12}H_{19}O$ (ring A and B intact + CH_3) and $C_8H_{13}O$ (ring A + CH_3), respectively. The fragment ion at *m/e* 330 [77% ($C_{23}H_{30}O$ calcd 330.292, found 330.292)] corresponds to the cleavage of a cyclopropane ring in the side chain as in gorgosterol (III)[11] and 23-demethylgorgosterol.[12] The 100-MHz ^1H NMR spectrum of I has the characteristic absorption of a C-22,23 cyclopropane ring [δ −0.16 (1 H, d of d, J = 4 and 6 Hz), 0.06–0.3 (2 H, m), and 0.44 (1 H, d of d, J = 4 and 9 Hz)] as has been reported for gorgosterol[11] and acanthasterol.[13] The presence of eight alkyl-linked methyl signals [δ 0.63 (3 H, s), 0.70 (3 H, d, J = 6.5 Hz), 0.86 (3 H, s), 0.95 (3 H, s), 0.92 (3 H, d, J = 6.0 Hz), 1.05 (6 H, d, J = 6.3 Hz), 0.90 (3 H, d, J = 7 Hz)] was indicative of a molecule with additional methyl groups possibly at C-4, C-23, and C-24 (similar to those found in dinosterol). The presence of a methyl group at C-4 was confirmed by converting I into a ketone (mp 205–206 °C) by Jones oxidation. The CD curve of the oxidation product of I in dioxane (308, 298, and 289 nm) was similar to that reported for the ketone obtained from the oxidation of dinosterol.[9]

The ^1H NMR and mass spectral data of I implied the structure of 22,23-methylene-4α,23,24-trimethyl-5α-cholestan-3β-ol. The final proof of the structure of I was accomplished by the synthesis of 22,23-methylene-4α,23,24-trimethyl-5α-cholestan-3-one (IV), mp 205–206 °C, *m/e* 440 [(M^+, 4%), 328, 314, 287, 285, 269, 229], by methylation and Birch reduction of gorgost-4-en-3-one.[14] Compound IV and the oxidation poduct of I were found to be identical (GLC retention time, MS, and melting point).

The origin of unusual sterols isolated from marine invertebrates has been a subject of much discussion, since it is known that the sponges and possibly coelentrates are incapable of the de novo synthesis of sterols and carotenoids. It has been suggested that coelentrates and sponges may have acquired their sterols from dietary sources or from symbiotic microorganisms such as zooxanthellae.[15]

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(4) Dinosterol and 4-methylgorgostanol were separated from each other only by preparative GLC. Preparative GLC was done on a 1.4 m, 5% OV-17 column (i.d. 1 cm) equipped with a glass splitter (80:20): column temperature 295 °C, carrier gas 60 mL/min.
(5) Mass spectrum (rel intensity) (20 ev) *m/e* 442 (30), 427 (6), 339 (10), 371 (11), 353 (20), 330 (77), 300 (55), 287 (100), 271 (61), 229 (27), 149 (46), 180 (5), 179 (11), 125 (16).
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This assertion is supported by the fact that the 24-methylcholest-5,22-dien-3β-ol was found in a diatom (which forms the basis of the food chain in the marine environment) and also in a number of invertebrates.[16] Similarly the presence of peridinin, a carotenoid characteristic of dinoflagellates, in the sponge *Isis hippuris* has led to the suggestion that this carotenoid could have been derived from the food chain.[17] Recently it has been proposed that gorgosterol side chain (III) could have been derived from dinosterol (II) by a simple addition of a methylene group across the C-22,23 double bond.[18] The isolation of 4-methylgorgostanol (I) along with dinosterol and 24-demethyldinosterol from the dinoflagellate *G. foliaceum* supports the proposed mechanism[18] of the formation of gorgosterol side chain from dinosterol. Very recently Professor Djerassi's group has also isolated 4-methylgorgostanol from the dinoflagellate *Peridinium foliaceum*.[19]

We hope to resolve the biosynthetic scheme of 4-methylgorgostanol through studies currently in progress in our laboratory.

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Registry No. I, 71962-34-0; II, 58670-63-6; IV, 71912-00-0; cholesterol, 57-88-5; 24-demethyldinosterol, 71962-35-1; 3-oxo-4α-methyl-5α-gorgostane, 71912-00-0.

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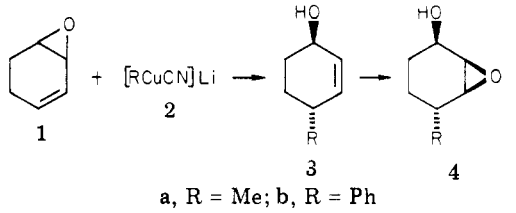
Stereospecific and Regiospecific Methodology for the Synthesis of Chiral Molecules

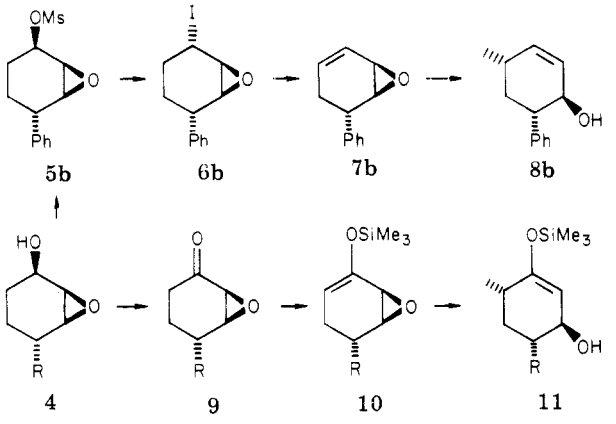
Summary: Sequential trans 1,4-openings of cyclohexene epoxides and hydroxyl directed epoxidations provide general methodology for the functionalization of five carbon atoms of a six carbon unit.

Sir: The formidable synthetic challenges associated with the total syntheses of macrolides and ionophores require efficient and general methods for the stereocontrolled introduction of substituents along a conformationally mobile backbone. Recent syntheses of the Prelog–Djerassi lactone by White[1] and Stork[2] as well as the macrolide total syntheses by Masamune[3] and Corey[4] have elegantly demonstrated the strategy of employing cyclic systems as precursors to chiral acyclic synthons. We wish to report the facile introduction of three chiral centers and the overall functionalization of five carbon atoms of a six-carbon unit. Our methodology is based on the stereospecific and regiospecific functionalization of the readily available 1,3-cyclohexadiene monoepoxide 1 and the subsequent oxidative cleavage of the final cyclohexene derivative. This approach relies on repetitive stereocontrolled 1,4 openings of cyclic epoxyalkenes[5] and hydroxyl-directed epoxidations.

Scheme I. Stereospecific Synthesis of Trans 4-Substituted *cis*-2,3-Epoxycyclohexanols



Scheme II. Stereospecific Synthesis of Trisubstituted Cyclohexenols



We have recently reported[5] a significant ligand effect in the reactions of mixed cyanoalkyl cuprates with 1,3-cycloheptadiene monoepoxide. Previous studies[6] of dimethylcopperlithium and epoxide 1 revealed that both 1,2 and 1,4 additions occurred as well as significant amounts of rearranged products. We have found that mixed cyanocuprates such as **2** add stereospecifically (100% trans) and regiospecifically (1,4 addition >95%) to epoxide 1 in ether at low temperatures (−78 to −40 °C) and in high yield (**3a**, R = Me, 95%; **3b**, R = Ph, 60%)[7] (Scheme I). Mixed cyanocuprates (**2**) are among the most stable organocopper reagents and can easily be prepared[8] on a large scale from

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(7) All reported yields are materials isolated from flash distillation (compounds **3a**, **4a**, **9a**, **10a**) or column chromatography (compounds **3b**, **4b**, **5**, **6**, **7**, **8**, **9b**, **10b**). Reaction conditions have not been optimized for each step. All of the compounds cited above have analyzed satisfactorily (elemental and spectroscopically).

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