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ISOLATION AND SYNTHESIS OF 23-METHYL-22-DEHYDROCHOLESTEROL - A MARINE STEROL OF BIOSYNTHETIC SIGNIFICANCE

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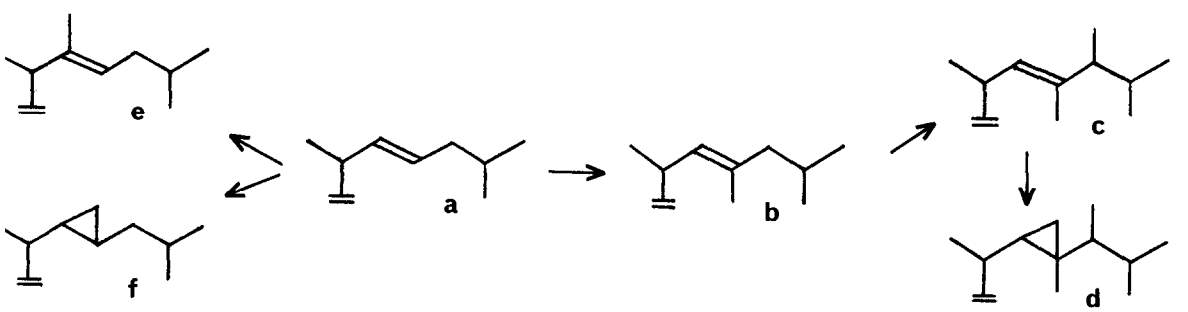
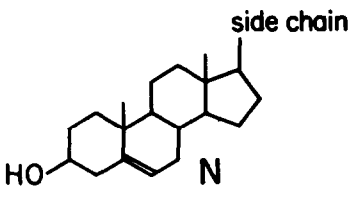
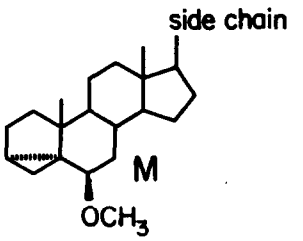
A new sterol, 23-methyl-22-dehydrocholesterol, and several closely related sterols, have been isolated from cultures of the dinoflagellate symbiont (zooxanthella) from the marine zoanthid Zoanthus sociatus. The structural features of these sterols suggest that direct bioalkylation of a Δ^{22} double bond may be a new biosynthetic route to novel sterol side chains.

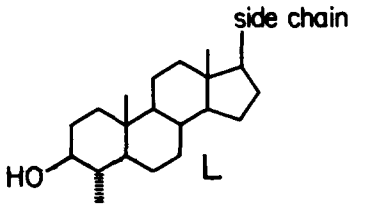
Marine sterols with alkylation at C-22, 23, 24, 26 and 27 are known,[1] and the most reasonable assumption is, that their biosynthesis proceeds via the well-documented methylation at C-24 of desmosterol (1), or an analog with the same side chain. However a fundamentally different alkylation path of the cholesterol side chain is also possible, namely direct bioalkylation of a Δ^{22} double bond.[1] Such a hypothesis is not unreasonable, because we, as well as other investigators, have found 22-dehydrocholesterol (2) in a variety of marine organisms. Direct bioalkylation of a Δ^{22} double bond (a) might give rise to a sterol with a methyl substituent in the 23-position (b); subsequent introduction of a Δ^{24} double bond, followed by further alkylation would then lead to a dinosterol (c) and gorgosterol (d) type side chain. Alternatively, alkylation of the Δ^{22} double bond might give rise to sterols with a 22-methyl (e) or a 22,23-methylene group (f); such sterols have not yet been isolated, but it has been shown that alkylation in the 23-position only does occur: 4α,23-dimethyl-22-dehydrocholestanol (5) is a known marine sterol,[2] although it is conceivable that it is the product of a C-28 bio-demethylation of dinosterol (7=c).

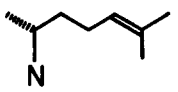


We wish to report the occurrence of the hitherto unknown 23-methyl-22-dehydrocholesterol (4) together with 5, and their suggested precursors (vide supra), viz. 4α-methyl-22E-dehydrocholestanol (3) and 22E-dehydrocholesterol (2), as well as dinosterol (7) (a possible product of further alkylation), as minor sterols[3] of the cultured zooxanthella[4] (dinoflagellate symbiont) from Zoanthus sociatus; the Δ^5-desmethyl analog 6 of dinosterol (7) was not found in this organism. Details of our sterol isolation work will be published in a comprehensive paper[5] on the sterol composition of several cultured zooxanthellae isolated from different invertebrate hosts. Here we describe the structure elucidation of the two new sterols (4 and 5)[6] which are of interest in connection with our proposed side chain alkylation path (8). The occurrence of sterols with unusual side chains (4,5,7) in an unicellular alga is significant because it opens the way to experiments on the biosynthetic generation of such side chains. Such experiments are planned. Hopefully the results will allow us to distinguish between the above outlined route (a→b), and the more conventional formation of 4 and 5 by biodemethylation (c→b) at C-24 of sterols with a dinosterol side chain (6, 7).









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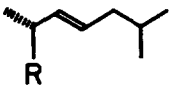
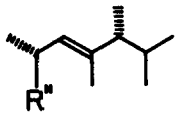
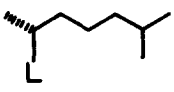


2 R=N
3 R=L

4 R'=N
5 R'=L



6 R"=N
7 R"=L



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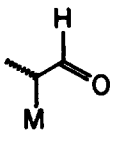

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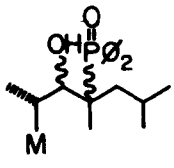

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23-Methyl-22-dehydrocholesterol (4): RRT 1.07; 1.1% free sterols. The mass spectrum of 4 displayed a prominent parent M^+ at 398, which was 63% of the m/z 69 and 125 base peaks. Other prominent peaks were m/z 255 (55%) and 300 (50%) (MS-9, 70eV). A Δ^5 sterol was indicated by both its mass spectral features (an M^+-18 peak as intense as the M^+-15 peak) and ^{1}H NMR characteristics (a 1H doublet at δ 5.35). ^{1}H NMR experiments further indicated the Δ^{22} olefin and C-23 methyl constellation. Specifically, irradiation at δ 2.3 (the C-20 methine) collapsed both an alkyl methyl doublet and an olefinic doublet at δ 4.88. Fortuitously, 4 had already been synthesized[8] as reference compound since the isolation of sterols with unusual alkylation patterns had been anticipated.[1] The protected aldehyde 9[9] was condensed with the anion of 2-(4-methylpentyl)-diphenylphosphinoxide[10] to yield the phosphinoxide 10. Sodium hydride elimination of diphenylphosphinic acid.[11] followed by deprotection gave 4 as an E/Z mixture. The predominant isomer[12] (m.p. 141-143°, $[\alpha]_D$ -36°, $CHCl_3$) and the natural product were identical by 360 MHz NMR comparisons; however we cannot assign a double bond configuration to 4 based upon existing information.

4α-Methyl-22E-dehydrocholestanol (3): RRT′ 1.06; 1% free sterols. The mass spectrum is similar to that of 22-dehydrocholestanol, but illustrates diagnostic skeletal fragments 14 mass units higher indicating the 4-methyl substituent. The 360 MHz ^{1}H NMR spectrum (cf. Table) of 3 confirmed that the additional methyl was 4α substituted by the predicted chemical shift of the C-3 proton at δ 3.1.[13] The shifts for the C-18 and C-19 methyls are consistent with the Zürcher values[14] for sterols with the normal (5a,14a) androstane skeleton. The configuration of the double bond was established as E by comparison of the chemical shifts of olefinic protons with those of 2 (22E) which occurs as 2.3% of the free sterols. In both sterols an identical eight line pattern is observed at δ 5.24 (360 MHz).

TABLE

360 MHz NMR data (shifts are the δ values in $CDCl_3$)

Sterol	C-3H	C-20H	C-22 and/or C-23H	C-4Me	C-18	C-19	C-21	C-23Me	C-26,27
4 (nat)	3.5(m)	2.3(m)	4.88(d) J 10.2	-	0.718	1.014	0.944(d) J 6.6	1.559(d) J 0.8	0.837(d) J 6.3 0.816(d) J 6.2
4 (syn) 85% E or Z (by NMR)	3.5(m)	2.3(m)	4.88(d) J 10	-	0.717	1.013	0.943(d) J 6.6	1.559(d) J 1.2	0.836(d) J 6.3 0.814(d) J 6.2
5	3.1(m)	2.3(m)	4.87(d) J 10.3	0.944(d) J 6.7	0.683	0.826	0.926(d) J 6.7	1.552(d) J 1.1	0.834(d) J 5.8 0.810(d) J 5.8
3	3.1(m)	-	5.24(m)	0.937(d) J 6.4	0.653	0.817	0.982(d) J 6.6	-	0.851(d) J 6.6 0.847(d) J 6.7
8	3.1(m)	-	-	0.946(d) J 6.3	0.645	0.823	0.895(d) J 6.7	-	0.863(d) J 6.6 0.859 J 6.5

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References and Notes

1. C. Djerassi, N. Theobald, W. C. M. C. Kokke, C. S. Pak and R. M. K. Carlson, Pure Appl. Chem., in press and references cited therein.
2. M. Alam, K. Schram and S. M. Ray, Tetrahedron Letters, 3517 (1978).
3. Main sterols: cholesterol (35.3%), 4α,24-dimethyl-22-dehydrocholestanol (28.3%), and 4α,24-dimethylcholestanol (24.4%).
4. This alga was isolated from Z. sociatus (phylum Cnidaria, Subclass Zoantharia, Order Zoanthidea). Cf. R. K. Trench, Helgoländer Wiss. Meeresunters., 26, 174 (1974).
5. N. W. Withers, W. C. M. C. Kokke, W. Fenical and C. Djerassi, to be published.
6. Another trace sterol (1.2%) was 4α-methylcholestanol (8).
7. Gas chromatographic conditions: 3% SP2250, 260°.
8. I. J. Massey and C. Djerassi, to be published.
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10. Prepared from ethyl-diphenylphosphinoxide by alkylation (n-BuLi, isopropylbromide).
11. L. Horner, H. Hoffmann and H. G. Wippel, Chem. Ber., 91, 61 (1958); L. Horner, H. Hoffmann, H.G. Wippel and G. Klahre, Chem. Ber., 92, 2499 (1959).
12. The only indication of the presence of the minor isomer were shoulders at the high field side of the peaks in the methyl region.
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14. R. F. Zurcher, Helv. Chim. Acta, 46, 2054 (1963).

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