Tetrahedron Letters No. 38, pp 3605 - 3608.
©Pergamon Press Ltd. 1979. Printed in Great Britain.

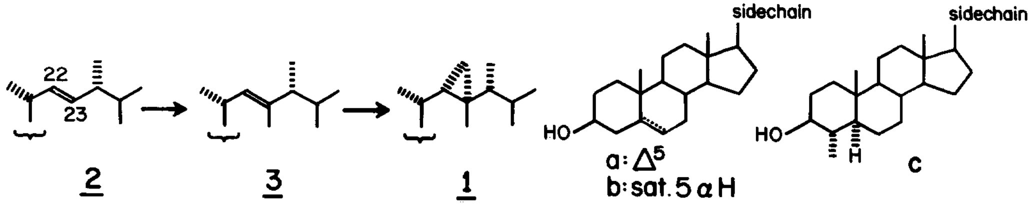
0040-4039/79/0915-3605$02.00/0

ISOLATION OF STEROLS WITH CYCLOPROPYL-CONTAINING SIDE CHAINS FROM THE CULTURED MARINE ALGA PERIDINIUM FOLIACEUM

Nancy W. Withers,[a,b] W. C. M. C. Kokke,[a,b]
Michel Rohmer,[b] W. H. Fenical,[*a] and Carl Djerassi[*b]
Joint Contribution from (a) Institute of Marine Resources, Scripps Institution
of Oceanography, La Jolla, California 92093, and
(b) Department of Chemistry, Stanford University, Stanford, California 94305

Abstract: The sterol fraction of the cultured dinoflagellate Peridinium foliaceum contains in addition to cholesterol, 24-methylcholesterol, 4,24-dimethylcholestanol and dinosterol (3c) small amounts of the cyclopropyl-containing sterols gorgosterol (1a) and 5α-gorgostanol (1b) as well as 4α-methylgorgostanol (1c) and E-4α,22ξ,23ξ (or 24ξ)-trimethylcholest-17(20)-en-3β-ol (6c or 7c). The biosynthetic implications of the first isolation of such sterols from a free-living alga are emphasized.

Gorgosterol (1a)[1] was the first of several naturally occurring marine sterols[2] in which the presence of a cyclopropyl-containing side chain was established.[3] This unique feature, coupled with the fact that no terrestrial sterols are known which are alkylated at positions 22 and/or 23 raises hitherto unresolved questions concerning the biological role and biosynthetic origin of such marine sterols. We had originally postulated[3] that gorgosterol (1a) may arise by bioalkylation of brassicasterol (2a) via a Δ^{22}-23,24-dimethyl precursor (3).



The subsequent isolation of 23,24-dimethylcholesta-5,22-dien-3β-ol (3a)[4] and of dinosterol (3c)[5] offered indirect support for our hypothesis. However, no experimental evidence has been presented so far about the biosynthesis of the cyclopropane ring, because gorgosterol has only been encountered in coelenterates containing dinoflagellate symbionts (zooxanthellae), and while the latter have been shown[6] to contain gorgosterol (1a), neither we nor anyone else have been able to detect gorgosterol in cultured zooxanthellae isolated from hosts known to contain 1a. The isolation of dinosterol (3c)[5] and its 24-demethyl analog[7] from dinoflagellates (occurring in nature as free-living phytoplankton or as symbiotic zooxanthellae) is important, because these algae can be cultivated and thus are potential experimental substrates for bio-incorporation studies.[8] However, it is very unlikely that dinosterol (3c) or any other sterol with a saturated 4α-methyl nucleus (c) can be a precursor of Δ^5-3β-alcohols (a). Furthermore, it should be noted that *until now no sterol with a cyclopropane-containing side chain has been found in a free-living alga*. We now report such an example which may represent the key to eventual incorporation studies dealing with the biosynthesis of the cyclopropane ring. That such a ring may not be a metabolic end product, but rather an intermediate in bioalkylation has been suggested recently.[9]

Two "red tide" phytoplankton species, *Peridinium foliaceum* Stein and *P. balticum* Lemmerman, each represent a heterotrophic dinoflagellate harboring a photosynthetic alga of non-dinoflagellate origin as an intracellular symbiont.[10] An investigation of the lipid composition of *P. foliaceum* (UTEX 1688) was therefore initiated for a chemotaxonomic evaluation of this symbiotic association.[11] GC-MS analysis of the total free sterols demonstrated the presence of 35% cholesterol, 9% 24ξ-methylcholesterol and 33% of a mixture of dinosterol (3c) and 4ξ,24ξ-dimethylcholestanol. Separation of the 4-demethyl sterols by reverse phase HPLC[12] afforded 5α-gorgostanol (1b) (0.4% of free sterol fraction), which was identified by GC mobility, mass and 360 MHz NMR spectral comparison with authentic material,[1] as well as a trace of gorgosterol (1a). Finally, two new sterols were obtained with relative GC retention times (3% SP2250, 260°; cholesterol = 1.00) of 1.77 (6%; M^+428) and 2.67 (14%; M^+ 442).[13]

The 360 MHz NMR spectrum (C_6D_6) of the MW 442 sterol demonstrated the presence of a 4α-methyl group (C-3 proton at δ 2.9 ($J_1 = J_2 = 10$Hz, $J_3 = 5$Hz) as a doubled triplet) and a cyclopropyl ring (cf. Table 1). The mass spectrum[14] strongly suggested a saturated 4-methyl sterol nucleus (c) and a gorgosterol side chain (1), because of the strong resemblance to 5α-gorgostanol (1b), except for 14 mass unit shifts of all diagnostic peaks. The complete structure and stereochemistry of this sterol was established as 4α-methyl-5α-gorgostanol (1c) by partial synthesis from Δ^4-gorgostenone[1] (m.p. 140-143°, obtained by Oppenauer oxidation of 1a

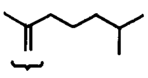
Table 1. 360 MHz NMR data (in C_6D_6; internal standard C_6H_6; shifts are δ values) for gorgosterol (1a), 5α-gorgostanol (1b) and 4α-methylgorgostanol (1c).[15]

	C-18	C-19	C-23 Me	High Field Multiplets	Methyl Doublets				
1a	0.685	0.953	0.923	-0.11 (1H,dd) J 6.0, 4.5 0.20 (2H,m) 0.50 (1H,dd) J 9.2, 4.5	0.902 J 8.1	---	1.018 J 6.7	1.026 J 6.9	1.138 J 6.4
1b	0.684	0.712	0.920	-0.11 (1H,dd) J 5.9, 4.3 0.22 (2H,m) 0.50 (1H,dd) J 9.0, 4.2	0.897 J 6.6	---	1.017 J 6.6	1.029 J 6.9	1.141 J 6.1
1c	0.694	0.752	0.921	-0.10 (1H,dd) J 5.6, 4.4 0.22 (2H,m) 0.51 (1H,dd) J 9.4, 4.4	0.898 J 6.6	1.002 J 6.1 C-4 Me	1.019 J 6.3	1.034 J 6.9	1.148 J 6.3

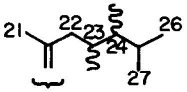
via methylation[16] to 4-methyl-Δ^4-gorgostenone (m.p. 191-192.5°, λ_{max}^{EtOH} 251 nm, ε 14000, M^+ 438), reduction[17] with Li/NH_3 to 4α-methyl-5α-gorgostanone (m.p. 205-206°, CD $[\theta]_{max}^{Diox}$ +3000° at 289 nm, M^+ 440) and lithium aluminum hydride reduction to 1c (m.p. 225.5-226°, m.p., mixture m.p., GC, MS and 360 MHz NMR identical with that of natural material).

The co-occurrence in isolable amounts in a cultured alga of a pair of sterols with the side chain patterns 3 and 1, but possessing identical nuclei (c) offers for the first time the opportunity of testing whether sterols of type 3 are indeed precursors for cyclopropyl ring introduction. It remains to be seen whether labeled one-carbon precursors (e.g., methionine) can be incorporated by P. foliaceum.

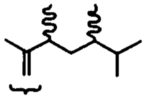
The most interesting feature of the 360 MHz NMR spectrum ($CDCl_3$) of the new sterol of MW 428 was the absence of the high field C-18 methyl singlet, since both C-18 and C-19 methyl signals appeared at δ 0.83 (resolved in C_6D_6 - cf. Table 2), typical of a $\Delta^{17(20)}$ sterol (4a).[18] The mass spectrum (m/e 287 due to loss of side chain + 2H) supports a sterol with an additional methyl in the nucleus and the base peak at m/e 315 (loss of part of the side chain by vinylic cleavage of the C-20 - C-22 bond) is typical of a $\Delta^{17(20)}$ double bond.[19] The absence of olefinic protons and the presence of a vinylic methyl NMR signal (δ 1.65) further support the presence of a $\Delta^{17(20)}$ olefin. The 360 MHz NMR spectrum (Table 2) displays 5 methyl doublets and since the mass spectrum indicates the presence of only one additional nuclear methyl group (4α-methyl according to C-3 proton NMR signal), four methyl groups must be in the side chain. Assuming the presence of the usual C-26 and C-27 methyl groups, only positions 22,23 or 24 are available for the remaining two secondary methyl substituents, with structure 5c being eliminated by decoupling experiments in C_6D_6 (via allylic protons absorbing in the region δ 1.66-2.29): irradiation at ∂ 1.92 collapsed one of the two methyl doublets at ∂ 0.831. Since the E configuration can be assigned on the basis of the C-21 methyl shift,[18] we feel justified in tentatively assigning either structure E-6c or E-7c to this new sterol. Either one of these structures represents a hitherto unprecedented side chain methylation pattern, which would be of major biosynthetic interest. Attempts to isolate enough of this sterol for definitive proof of structure and stereochemistry are now in progress.



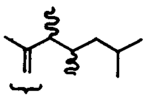
4



5



6



7

Table 2. 360 MHz NMR data ($CDCl_3$ and C_6D_6) of the new sterol of MW 428 (either E-6c or E-7c) (shifts are δ values; internal standard $CHCl_3$ or C_6H_6)

Solvent	C-3 H	C-4 Me	C-18 H	C-19 H	C-21 H	Other Methyl Doublets (Unassigned)			
$CDCl_3$	3.1 (m)	0.953 (d)	0.833	0.833	1.648	0.697	0.746	0.842	0.872
		J 6.2				J 5.8	J 6.9	J 6.2	J 6.6
C_6D_6	2.9 (m)	0.987 (d)	0.922	0.733	1.783	0.831 (6H)		0.892	0.936
		J 6.4				J 6.7		J 6.7	J 6.8

Acknowledgement. Financial support was provided by NSF grant No. OCE 76-81737 and NIH grant No. GM-06840. Use of the NMR/MS center at UCSD (NIH grant No. RR-708) and of the Stanford 360 MHz NMR facility (NSF grant No. GP-23633 and NIH grant No. RR-0711) is gratefully acknowledged, as is the help of Dr. Lois Durham.

References and Notes

1. R. L. Hale, J. Leclerq, B. Tursch, C. Djerassi, R. A. J. Gross, A. J. Weinheimer, K. C. Gupta and P. J. Scheuer, J. Am. Chem. Soc., 92, 2179 (1970).
2. F. J. Schmitz in Marine Natural Products (P. J. Scheuer, ed.), Academic Press, New York (1978), Vol. I, Chapter 5. L. J. Goad, ibid., Vol. II, Chapter 2.
3. N. C. Ling, R. L. Hale and C. Djerassi, J. Am. Chem. Soc., 92, 5281 (1970).
4. A. Kanazawa, S.-I. Teshima and T. Ando, Comp. Biochem. Physiol., 57B, 317 (1977).
5. Y. Shimizu, M. Alam and A. Kobayashi, J. Am. Chem. Soc., 98, 1059 (1976).
6. P. A. Steudler, F. J. Schmitz and L. S. Ciereszko, Comp. Biochem. Physiol., 56B, 385 (1977).
7. M. Alam, K. Schram and S. M. Ray, Tetrahedron Lett., 3517 (1978).
8. N. W. Withers, R. C. Tuttle, L. J. Goad and T. W. Goodwin, Phytochemistry, 18, 71 (1979) have actually been able to demonstrate the incorporation of labeled methionine into the side chain of dinosterol in *Crypthecodinium cohnii*.
9. C. Djerassi, N. Theobald, W. C. M. C. Kokke, C. S. Pak and R. M. K. Carlson, Pure Appl. Chem., in press.
10. R. N. Tomas and E. R. Cox, J. Phycol., 9, 304 (1975).
11. The wax ester, fatty acid, and pigment composition of this species are reported elsewhere: N. W. Withers and F. T. Haxo, Plant Sci. Lett., 5, 7 (1975) and N. W. Withers and J. C. Nevenzel, Lipids, 12, 989 (1977).
12. S. Popov, R. M. K. Carlson, A. Wegmann and C. Djerassi, Steroids, 28, 699 (1976).
13. The same sterols were encountered in *P. balticum*.
14. Mass spectral data of this sterol, as the TMS ether, were reported by N. W. Withers, Ph.D. dissertation, University of California, San Diego, 1976, p. 66.
15. Steudler *et al*. (ref. 6) reported on the basis of a mass spectrum that this compound exists in trace quantities in the zooxanthellae of *B. asbestinum*. Insufficient material was available for NMR measurements which would be required to establish the side chain stereochemistry and precise location of the nuclear methyl group. We have reinvestigated *B. asbestinum* and have isolated enough 1c to confirm its identity by 360 MHz NMR.
16. F. Knapp and G. J. Schroepfer, Chem. & Physics of Lipids, 17, 466 (1976).
17. D. G. Meakins and O. R. Rodig, J. Chem. Soc., 4679 (1959).
18. W. R. Nes, T. E. Varkey, D. R. Crump and M. Gut, J. Org. Chem., 41, 3429 (1976) report δ 0.86 for C-18 in the E isomer of 17(20)-dehydrocholesterol (4a); the C-21 methyl signal is reported at δ 1.68 for the E and δ 1.55 for the Z isomers.
19. Y. M. Sheikh and C. Djerassi, J. Org. Chem., 38, 3545 (1973).

(Received in USA 16 May 1979)