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(12) Coop Student from Antioch College, Yellow Springs, Ohio, April–June 1974.

James K. Coward,* Roger Lok,[11] Orinne Takagi[12]
Department of Pharmacology
Yale University School of Medicine
New Haven, Connecticut 06510
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Dinosterol, the Major Sterol with a Unique Side Chain in the Toxic Dinoflagellate, *Gonyaulax tamarensis*[1]

Sir:

Phytoplankton constitute the basis of the food chain in the marine life, and their chemical constituents are of particular interest in regard to peculiar compounds often found in marine organisms.[2]

In search for possible sources of unusual marine sterols, the toxic dinoflagellate, *Gonyaulax tamarensis*, which is causing serious problems on the North Atlantic coasts, was investigated.

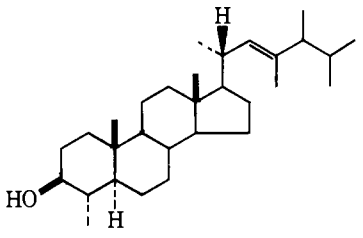
The chloroform extract of unialgal cultured *G. tamarensis* cells[3] afforded a sterol fraction which essentially consisted of cholesterol and a new C_{30} sterol (named dinosterol) in a ratio of 2:3 (GLC analysis).[4] Dinosterol (I) was purified by high speed liquid chromatography and recrystallized from $CHCl_3$–MeOH to needles (yield ca. 6 mg from 370 × 10^6 cells), mp 220–222°, $[\alpha]_D$ ±5° (*c* 0.6, $CHCl_3$), $C_{30}H_{52}O$ (calcd *m/e* 428.4041; found *m/e* 428.4054).

The mass spectrum pattern of I, *m/e* 316 (88%), 287 (100%), 271 (64%) was reminiscent of that of gorgosterol,[5,6] but the absence of a cyclopropane structure was obvious from the 100-MHz ^1H NMR spectrum which showed seven alkyl linked methyl signals (δ 0.70 (3 H, s), 0.80 (3 H, d, *J* = 7 Hz), 0.84 (3 H, s), 0.85 (3 H, d, *J* = 7 Hz), 0.94 (6 H, d, *J* = 6.5 Hz, isopropyl), 0.95 (3 H, d, *J* = 6 Hz)), an olefinic proton signal (δ 4.87 (1 H, q, *J* = 1.2, 10 Hz)), and a proton signal due to a secondary alcohol (δ 3.10 (1 H, m)). Decoupling study showed the presence of a partial structure -CHCH=C(CH_3)—which seemed to be located in the side chain leaving a few possibilities.

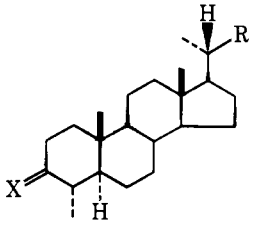
Jones oxidation of I afforded a ketone (V), mp 193–195°, whose positive CD curve, nm (Δε) in dioxane, 309 (+0.52), 298 (+0.97), 290 (+1.07), implicated the structure of 4α-methyl-5α-3-one.[7] Indeed 4α-methyl-5α-stigmast-22-en-3-one (III), mp 164–166°, which was synthesized for comparison by methylation and Birch reduction of stigmast-4,22-dien-3-one, showed a superimposable CD curve, nm (Δε) in dioxane: 310 (0.47), 297 (0.97), 291 (1.05). Moreover, the mass spectra of both II and III were found to show almost an identical fragmentation pattern, *m/e* 426 (M^+, 100%), 383, 328, 314, 287, and 285, strongly suggesting that the double bond in the side chain is located at the 22 position (consequently two methyl groups at the 23 and 24 position). The final proof of the structure was accomplished by the ozonolysis of I followed by $NaBH_4$ reduction of the ozonide to the diol (IV), mp 203–205°, *m/e* 348 (M^+, 100%), 333, 330, 262, 248, 247, and 229, which was unequivocally prepared by the ozonolysis and $NaBH_4$ reduction of III.

Since $NaBH_4$ reduction of 4α-methyl-5α-3-one is known to give the corresponding 3β-ol preferentially,[8] the structure of dinosterol is established as 4α, 23,24ξ-trimethyl-5α-cholest-22-en-3β-ol.

The existence of an unusual methyl group at the C-23 in I seems to be very significant, since the analogy can be only found in gorgosterol, acanthasterol,[9] and demethylgorgosterol[10] whose origins have been the subject of discussion.[11]



I



II, X = O; R = -CH=C(CH_3)CH(CH_3)CH(CH_3)$_2$
III, X = O; R = -CH=CHCH(C_2H_5)CH(CH_3)$_2$
IV, X =  ; R = -CH_2OH

Although the configuration at C-24 and the geometry with the 22-double bond in I are still unknown, I seems to be closely related to the above mentioned sterols. 4α-Methylsterols are intermedicates in sterol biosynthesis and known to be accumulated under anaerobic conditions.[7a,12] It might be significant to note that the mass spectroscopic analysis of sterols from anaerobically kept gorgonian associated zoxanthella was reported to give a molecular ion *m/e* 428 assigned to "dihydrogorgosterol."[11]

Investigation of the sterols in other dinoflagellates is now under way.

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Yuzuru Shimizu,* Maktoob Alam, Akio Kobayashi

Department of Pharmacognosy, College of Pharmacy
University of Rhode Island
Kingston, Rhode Island 02881

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Use of Kinetic Isotope Effects in Mechanism Studies. Anomalous Arrhenius Parameters in the Study of Elimination Reactions

Sir:

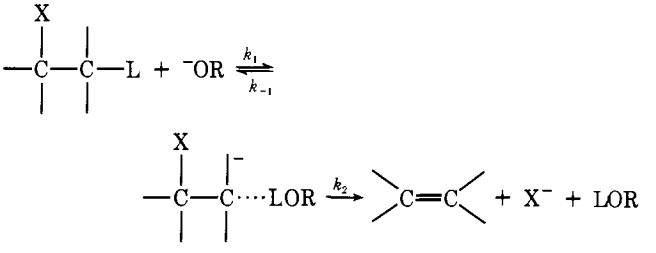
We have recently reported kinetic evidence that suggested the formation of carbanion intermediates during the ethanolic ethoxide dehydrohalogenation of $C_6H_5CHClCF_2Cl$ (III-*h*) even though the benzylic proton does not exchange with bulk solvent prior to elimination.[1] A kinetic study of the three isotopes of hydrogen was undertaken to find out if the low primary kinetic isotope effect, $k^H/k^D = 3.00$ at 0°, resulted from an internal return mechanism.[2] During the course of our studies we encountered anomalous Arrhenius parameters and we wish to report these unusual results at this time.

Primary kinetic isotope effects should arise from differences in zero point energies between C-L bonds in the ground state and transition state. In normal situations one would therefore expect rate differences between isotopically labeled compounds to come from differences in activation energies and that the ratio of Arrhenius preexponential factors, A^H/A^D, be 1.0 ± 0.4.[3] Bell has reviewed recent advances in the study of kinetic hydrogen isotope effects and lists anomalous effects on Arrhenius parameters.[4] Most cases cited gave values of A^H/A^D less than unity, and this has been interpreted as evidence for proton tunneling. In such cases $E_A^D - E_A^H$ is larger than $E_0^D - E_0^H$ and k^H/k^D is usually larger than predicted. For our systems, we find exactly the reverse situation (Tables I and II). The methanolic sodium methoxide dehydrohalogenation of $C_6H_5CLBrCF_2Br$ (II), III, *p*-$ClC_6H_4CLClCF_2Cl$ (IV), and $C_6H_5CLBrCH_2Br$ (V) shows anomalous behavior since $E_A^D \simeq E_A^H$ and $A^H/A^D > 2$. Compound III exhibits similar behavior in ethanolic sodium ethoxide. *p*-$ClC_6H_4CLBrCF_2Br$ (I) shows more normal behavior with $E_A^D - E_A^H = 0.5$ kcal mol^{-1} and the A^H/A^D value of 1.5 is just outside the limits of normal behavior. In all systems studied the k^H/k^D values are within the range of 2–5 at 25°.

Compound I is the most reactive and may mark the start of normal behavior, while V is the least reactive and still gives anomalous Arrhenius parameters. Included in Tables I and II are literature data for the ethanolic sodium ethoxide dehydrobromination of $C_6H_5CL_2CH_2Br$ (VII)[5] and $C_6H_5CL(CH_3)CH_2Br$ (VI).[6] These two compounds are less reactive than our systems and, although they also give anomalous Arrhenius parameters, they are of the usual type cited by Bell.

A major reason for the study of primary kinetic isotope effects is to gain insight into reaction pathways and to attempt to describe the nature of the transition state(s) during that reaction. There has been a renewed interest in mechanisms of elimination reactions,[7] and along with other investigators,[8,9] we are currently trying to find if there is a smooth transition between the concerted E2 and the two-step E1cb mechanisms. We feel that our isotope effect data are inconsistent with both the E2 pathway or with an E1cb mechanism where either the first or second step is clearly rate limiting. The anomalous Arrhenius parameters indicate that in our systems the E1cb mechanism may have a fine balance where neither step is clearly rate determining and that internal return (k_{-1}) can compete favorably with the forward reaction (k_2) in Scheme I. Additional evidence has been obtained for II and III to suggest that an intermediate carbanion is present along the reaction pathway. Hammett ρ values of 3.6 and 3.9 were calculated for substituted derivatives of II and III, respectively.[10] Sodium methoxide concentrations were varied from 0.2 to 2.1 N and the log of the corresponding pseudo-first-order rate constants were plotted against Streitwieser's H_M values,[11] to give good linear plots with slopes of 0.99 ± 0.03 (II) and 1.02 ± 0.02 (III).

Scheme I



Streitwieser[2] has shown that one of the simplest experimental methods of detecting internal return, when k_{-1} and k_2 are of comparable size, is to make use of the Swain-Schaad equation:[12]

$$k^H/k^T = (k^D/k^T)^y$$

For a simple proton transfer process, which is not complicated by internal return, $y = 3.26$[12] or 3.344,[2] depending on assumptions made in the derivation. Ethoxide dehydrochlorination of III and methoxide dehydrobromination of II require y values of 3.14 and 3.39 which suggest negligible internal return. Methoxide dehydrochlorination of III requires $y = 2.94$ which results in $a_H \sim 0.5$, $a_D \sim 0.1$, and a_T