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(25) The related dichloromethylcyclopentanecarboxylic acid (**10**), similarly derived from camphor, exhibited pK_a values of 6.25 in 50% EtOH and 5.30 in water (25 °C).[16]
(26) Evidently **6**, because of its high molecular weight and nonphenolic character, is not very polar and therefore is not readily neutralized in ethereal solution by treatment with aqueous $NaHCO_3$. The corresponding phenolic carboxylic acid (**3**) under the same conditions is more readily neutralized.

Identity of the Stereochemistry of Dinosterol and Gorgosterol Side Chain[1]

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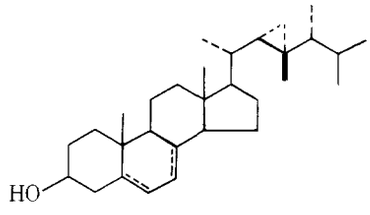
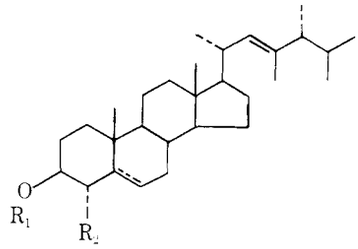
The structure of dinosterol, a peculiar sterol isolated from the dinoflagellate *Gonyaulax tamarensis*, was confirmed by x-ray crystallography. The proven identity of the stereochemistry of the side chain provided further support for the suspected close biogenetic relationship of dinosterol with gorgosterol and acanthasterol.

A difficult but intriguing problem in the study of marine animal constituents is the metabolite transfer inherent in the marine food chain and symbiosis. More often than not, it is difficult to determine whether an isolated compound is biosynthesized by the organism itself or is of dietary origin, either intact or partially transformed. A most intriguing example is gorgosterol **1** and its derivatives isolated from soft coral or gorgonians.[4] Their structures are unique not only for the presence of a cyclopropane ring but also for the unprecedented C-23 alkylated side chain. The abnormal side chain seems to be formed by methylation at C-24 followed by a second methylation at Δ^{22} and a third alkylation leading to the cyclopropane ring (Scheme I). In fact, Kanazawa et al.[5] isolated from a soft coral, *Sarcophyta elegans*, 23ξ,24ξ-dimethylcholesta-5,22-dien-3β-ol (**2**) which fits well into this scheme. A double bond isomer of gorgosterol, acanthasterol (**3**), was also isolated from the crown-of-thorns starfish, *Acanthaster planci*.[6] Since starfish are known to transform exogenous Δ^5 sterols to Δ^7 sterols,[7] it was immediately speculated that acanthasterol was of dietary origin. Indeed the crown-of-thorns starfish is known to feed on soft corals. As to the origin of gorgosterol in soft corals, Ciereszko et al.[8] already speculated that it might have come from symbiotic dinoflagellates, *Zooxanthellae*, which sometimes constitute a substantial part of the total body weight. The extract of the washed-out zooxanthellae was found to give a mass spectrum peak *m/e* 426 corresponding to gorgosterol. It was also noticed



1 Δ^5
3 Δ^7



2 Δ^5, R_1, R_2 = H
4 5α, R_1 = H; R_2 = CH_3
5 R_1 = *p*-I-C_6H_5CO; R_2 = CH_3

that the anaerobically kept zooxanthellae gave a *m/e* 428 peak of "dihydrogorgosterol".[8]

In view of the above-mentioned observation and the fact

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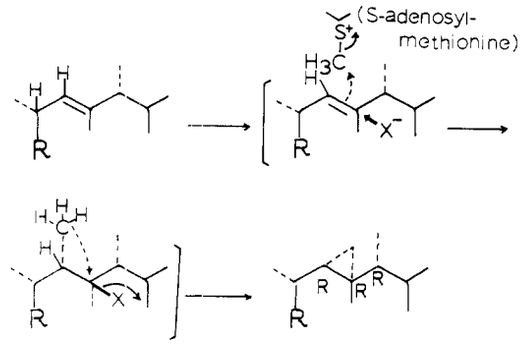
Scheme I



that dinoflagellates along with diatoms constitute the very basis of marine life, we have been investigating the steroidal components of unialgal cultures of dinoflagellates. In a previous communication[9] we reported the presence of dinosterol (**4**, $C_{30}H_{52}O$, mol wt 428) as the major sterol with a lesser amount of cholesterol in the toxic dinoflagellate, *Gonyaulax tamarensis*. The structure of dinosterol was determined as 4α,23,24ξ-trimethyl-5α-cholest-22-en-3β-ol by spectroscopic data and chemical correlation. The structure strongly implies that dinosterol is an intermediate to gorgosterol analogues or a compound just off the main metabolic stream. Significantly, the same sterol has been isolated from a soft coral, *Plexaura homomalla* and *Pseudoplexaura porosa*.[10]

To prove the close association of the dinoflagellate sterol with gorgosterol, it was felt to be very important to compare the stereochemistry of C-24 of both compounds and also to determine the geometry of C-22 double bond.

The *p*-iodobenzoate of dinosterol, **5**, crystallized from ethyl acetate as well formed rectangular solids, mp 217–221 °C. Preliminary x-ray photographs indicated orthorhombic symmetry and the systematic extinctions conformed to the common space group $P_{2_12_12_1}$. Accurate cell constants, determined by least-squares fitting of 15 high angle 2θ values, are $a = 8.081$ (2), $b = 10.658$ (3), and $c = 40.212$ (7) Å. All unique data with $\theta \leq 114°$ were collected using graphite monochromated Cu Kα (1.54178 Å) x rays. A total of 2738 reflections were measured and after correction for Lorentz–polarization and background corrections, 1889 (60%) were judged observed ($F_o \geq 3\sigma(F_o)$).

The iodine atom was located in the three-dimensional Patterson synthesis[11] and the remaining nonhydrogen atoms were located in a subsequent I-phased electron density synthesis. Hydrogen atoms were located and full-matrix least-squares refinement proceeded routinely. Correction for anomalous scattering from the iodine gave a conventional discrepancy index of 0.049 for the structure shown and 0.080 for its enantiomer.[12]

Figure 1 is a computer-generated perspective drawing of the final x-ray model less hydrogens. The configuration of the C-22 double bond is *E* and C-24 has the *R* absolute configuration. The molecular geometry agrees well with generally accepted values.[13]

The absolute configuration of gorgosterol was established as 22*R*, 23*R*, 24*R*.[4] Both dinosterol and gorgosterol have the same stereochemistry at C-24, i.e., brassicasterol type. The *E* form of the dinosterol double bond was anticipated considering the mechanism for the three-membered ring formation (Scheme I). The stereochemistry at C-20 was found to be the normal *R* configuration as assigned previously by the chemical correlation of dinosterol with stigmasterol.[9] Furthermore the projected figure shows that the side chain in this crystalline form is taking the right-handed rotamer position, the least energetic conformer as recently suggested by Nes et al.[14]

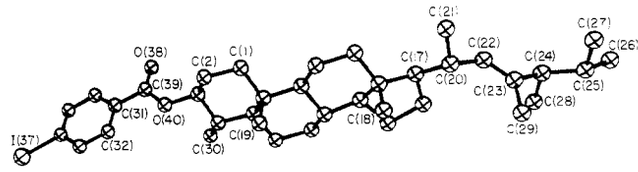


Figure 1. Computer generated perspective drawing of dinosterol *p*-iodobenzoate.

Our results strengthen the previous assertion that dinoflagellates are a primary source of novel sterols found in marine organisms. It is worth noting that the presence of a 4α-methyl group in dinosterol indicates that C-23 methylation in dinosterol may occur at an early stage.

Experimental Section

Low-resolution mass spectra were taken with a DuPont 21-490B model and high-resolution mass spectrum with a CEC 21-110B mass spectrometer. The ^1H NMR spectrum was measured on a Varian HA-100 spectrometer. Melting points were measured on a Fisher-Johns apparatus and were uncorrected. Gas liquid chromatography (GLC) was done with a Varian 1400 model equipped with a 6-ft column.

Culture of *Gonyaulax tamarensis*. An isolated sample obtained at the height of the bloom at Ipswitch, Massachusetts, in September 1972, was used as the seed culture. Large-scale culture of the organism was done in 20-L carboys using a culture medium based upon Guillard F.[15]

Sea water collected at the Beaver Tail Point, Jamestown, R.I., was stored in a dark room at 12 °C for more than 2 weeks and was then filtered through a charcoal layer (Fisher Scientific, coconut charcoal). The filtered sea water was further passed through a Millipore filter (0.22 μm). The filtrate was added with the inorganic ingredients and autoclaved for 15 min. After cooling the organic ingredients were added through a Millipore filter (0.22 μm). The sterile culture medium (12 L) was inoculated with 1 L of the seed culture. The culture bottle was kept under fluorescent illumination at 12 °C without agitation.

After 4 weeks when the population of the organism reaches about 5000–10000/mL, the organism was collected by centrifugation with a Szent-Gyorgi-Blum's continuous system at 5 °C.

Extraction and Isolation of Dinosterol 4. (a) The reddish brown dinoflagellate cells (370×10^6) were digested with 5% ethanolic KOH solution for 3 h on a steam bath. The mixture was extracted with ether (3×50 mL). The ethereal extract was chromatographed on a silica gel 60 prepacked column (1.5×18 mm, E. Merck) using methanol–chloroform as eluting solvents. The dinosterol fraction (7.0 mg) was eluted just after phytol slightly overlapped with cholesterol. Rechromatography on the same column gave a chromatographically pure fraction (TLC and GLC). Recrystallization from MeOH gave needles: mp 220–222 °C; $[\alpha]_D$ ±5° (*c* 0.6, $CHCl_3$); $C_{30}H_{52}O$ (calcd. *m/e* 428.4041; found: *m/e* 428.4054), *m/e* 428 (26), 387 (15), 370 (10), 316 (65), 303 (24), 287 (100), 271 (67); ^1H NMR δ 0.70 (3 H, s), 0.90 (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), 0.95 (3 H, d, $J = 7$ Hz), 3.10 (1 H, m), 4.87 (1 H, q, $J = 1.2, 10$ Hz). Gas liquid chromatography relative retention time to cholesterol 1.59 (1% OV-17, 240 °C).

The cholesterol fraction (10.5 mg), mp 146–147 °C, gave a single peak in the GLC system. A specimen recrystallized from methanol showed the IR and mass spectrum identical with those of an authentic sample of cholesterol.

(b) The algal cells were extracted with chloroform. The chloroform extract was directly chromatographed with the chromatographic system described in (a) without prior saponification. Dinosterol and cholesterol were obtained in a same proportion (GLC) as isolated in procedure (a) indicating the absence of the preferentially esterified forms of a sterol in the organism.

Dinosterol *p*-Iodobenzoate (5). To a solution of 1 mg of dinosterol in pyridine (0.5 mL) was added 20 mg of *p*-iodobenzoyl chloride. After 2 days at room temperature, the mixture was diluted with ice–water and extracted with ether. After the usual procedure, a crystalline residue was recrystallized from $CHCl_3$ to needles: *mp* 217–221 °C; *m/e* 658 (17, M^+), 345 (51), 516 (69), 317 (21), 271 (100), 231 (38), 139 (81).

Registry No.—**1**, 29782-65-8; **4**, 58670-63-6; **5**, 65495-99-0; *p*-iodobenzoyl chloride, 1171-02-0.

Supplementary Material Available: A listing of fractional coordinates and temperature factors (Table I), bond lengths (Table II), and bond angles (Table III) of dinosterol (6 pages). Ordering information is given on any current masthead page.

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Studies in Protoberberine Alkaloids. 14. Use of a Mixture of Phosphorus Pentabromide and Phosphorus Pentoxide As a Cyclizing Reagent in Protoberberine Synthesis

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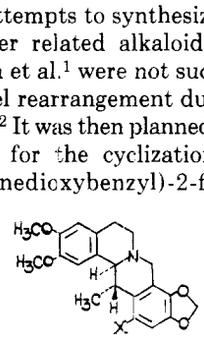
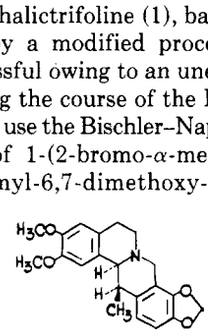
Cyclization of 1-(2-bromo-α-methyl-4,5-methylenedioxybenzyl)-2-formyl-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (**3**) with a mixture of PBr_5 and P_2O_5 (followed by reduction with $NaBH_4$ in MeOH) led to the formation of 13β-methyl-13aα*H*-tetrahydropseudoepiberberine (**7**), 4-bromo-13β-methyl-13aα*H*-tetrahydropseudoepiberberine (**17**), 13α-methyl-13aα*H*-tetrahydropseudoepiberberine (**8**), and 4-bromo-13α-methyl-13aα*H*-tetrahydropseudoepiberberine (**18**). Similarly the *N*-formyl derivative **4** gave 13β-methyl-13aα*H*-tetrahydropseudocoptisine (**9**), 13α-methyl-13aα*H*-tetrahydropseudocoptisine (**10**), 4-bromo-13α-methyl-13aα*H*-tetrahydropseudocoptisine (**19**), and 4-bromo-13α-methyl-13aα*H*-tetrahydropseudocoptisine (**20**).

Our attempts to synthesize thalictrifoline (**1**), base II (**2**), and other related alkaloids by a modified procedure of Shamma et al.[1] were not successful owing to an unexpected but novel rearrangement during the course of the Mannich reaction.[2] It was then planned to use the Bischler–Napieralsky reaction for the cyclization of 1-(2-bromo-α-methyl-4,5-methylenedioxybenzyl)-2-formyl-6,7-dimethoxy-1,2,3,4-



1 X = H
11 X = Br



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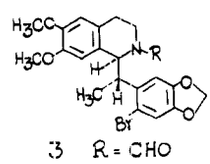
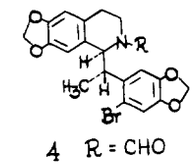
tetrahydroisoquinoline (**3**) and its bismethylenedioxy analogue **4** to get the required 13-methyltetrahydroprotoberberines.

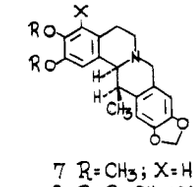
The tetrahydroisoquinolines **5** and **6** gave the corresponding *N*-formyl derivatives **3** and **4** when heated with formic acid and triethylamine. The *N*-formyl derivative **3** was then refluxed with freshly distilled phosphorus oxychloride in benzene and the quaternary salt formed was directly reduced with sodium borohydride in methanol. A mixture of two products was obtained the constituents of which were separated by chromatography and identified from their spectral, physical, and analytical data as the diastereoisomeric 13-methyltetrahydropseudoprotoberberines viz. 13β-methyl-13aα*H*-tetrahydropseudoepiberberine (**7**) and 13α-methyl-13aα*H*-tetrahydropseudoepiberberine (**8**). Similarly, when the *N*-formyl derivative **4** was cyclized using $POCl_3$, a mixture of two products was obtained and these were identified as 13β-methyl-13aα*H*-tetrahydropseudocoptisine (**9**) and 13α-methyl-13aα*H*-tetrahydropseudocoptisine (**10**). The structures of compounds **7**, **8**, **9**, and **10** were confirmed by comparison with authentic synthetic samples prepared as reported earlier.[1,3] Identical results were obtained when distilled $POBr_3$[4] was used for cyclization in the place of $POCl_3$.



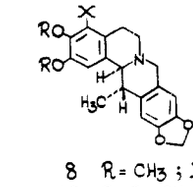
3 R = CHO
5 R = H



4 R = CHO
6 R = H



7 R = CH_3; X = H
9 R+R = CH_2; X = H
17 R = CH_3; X = Br
19 R+R = CH_2; X = Br



8 R = CH_3; X = H
10 R+R = CH_2; X = H
18 R = CH_3; X = Br
20 R+R = CH_2; X = Br

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