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Synthesis of 3β,29-Dihydroxystigmasta-5,24(28)(*E*)-dien-7-one

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The synthesis of 3β,29-dihydroxystigmasta-5,24(28)(*E*)-dien-7-one from 3β-acetoxy-22,23-dinorcholenaldehyde has been achieved in an overall yield of 7%. Wittig reaction of the aldehyde and diethyl (3-methyl-2-oxobutyl)phosphonate anion gave 3β-acetoxycholesta-5,22-dien-24-one which was hydrogenated selectively, and the saturated ketone was allowed to react with the anion of diethyl (cyanomethyl)phosphonate. The resulting nitrile was reduced via the aldehyde to 29-hydroxyfucosterol by using DIBAL-H. Acetylation of this diol and oxidation with chromium trioxide–3,5-dimethylpyrazole afforded the 7-ketone. Mild hydrolysis of the acetate groups completed the synthesis.

We recently reported the identification of 3β,11α,15β,29-tetrahydroxystigmasta-5,24(28)(*E*)-dien-7-one, 3β-isobutyrate ("dehydrooogoniol-1"), a female-activating hormone of the aquatic fungus *Achlya*.[1] The substance appears to be the most active of the oogoniols so far isolated, and we have undertaken synthetic studies to confirm the structure and to develop a method for securing adequate quantities of the steroid for further biological investigation. The model steroid 3β,29-dihydroxystigmasta-5,24(28)(*E*)-dien-7-one (29-hydroxy-7-oxofucosterol) was selected as our first synthetic target for two reasons. First, we felt that it would be readily accessible from 3β-acetoxy-22,23-dinorcholenaldehyde by a series of straightforward reactions. These could later be applied to the synthesis of dehydrooogoniol-1 itself by starting from a suitably functionalized aldehyde. Second, we were interested in determining if the model steroid which lacks the hydroxyl groups at C-11 and C-15 in dehydrooogoniol-1 would show any biological activity. This paper describes the synthesis of the model steroid (**12**) from the dinorcholenaldehyde (**1**).

Conversion of the dinorcholenaldehyde (**1**) to 3β-acetoxycholesta-5,22(*E*)-dien-24-one (**2**) was reported earlier.[2,3] While the yield obtained in the Wittig reaction was high, the conditions were rather severe (reactants in Me_2SO heated at 95 °C for 65 h). We have found that by using the more reactive phosphonate anion rather than phosphorane, a high yield of dienone **2** can be obtained by refluxing for 1.5 h in tetrahydrofuran. Catalytic hydrogenation of dienone **2** with 10% Pd on $BaSO_4$ afforded 24-oxocholesterol acetate (**3**) in 93% yield.

A number of ways were available for completing the fucosterol skeleton. Grignard reaction of vinylmagnesium bromide and ketone **3** gave a mixture of saringosterol and its acetate **4**. Acetylation of the mixture with acetic anhydride–pyridine gave a quantitative yield of **4**. Treatment of acetate **4** with a large excess of pyridinium chlorochromate converted the tertiary allylic alcohol moiety to the unsaturated aldehyde **5** in only moderate yield. An alternative route involved reaction of ketone **3** with the anion of diethyl (cyanomethyl)phosphonate which yielded the unsaturated nitrile as a mixture of isomers (ratio of 3:1 *E*/*Z*). The isomers could be distinguished by the signal for the C-28 vinyl proton which occurred at δ 5.01 in the *Z* isomer and at δ 5.08 in the *E* isomer. The signal for the C-25 methine proton in the *Z* isomer occurred at δ ~3.1 while that for the *E* isomer overlapped other signals at δ ~2.3.[4] The isomers had different retention times on gas chromatography. The mixture of nitriles (**6**) was treated with diisobutylaluminum hydride (DIBAL-H) followed by dilute acid to give, after reacetylation, the corresponding unsaturated aldehydes in high yield. These could be separated by chromatography.

Reduction of aldehyde **5** (mixture of isomers) with sodium borohydride furnished the 29-hydroxy derivative (**7**) together with some of the saturated alcohol resulting from 1,4-reduction. A better yield of alcohol **7** was obtained by reducing aldehyde **5** with DIBAL-H, and there was no evidence of 1,4-reduction.

Attempts were made to introduce the carbonyl function at position C-7 in the diol **7** by photooxygenation to the 5α-hydroperoxide followed by rearrangement to the 7α-hydroperoxide and loss of water. This method had been used successfully in the synthesis of antheridiol and deoxyantheridiol.[2] However, in the case of the diol **7** a complex mixture was formed because the side-chain double bond was also attacked by singlet oxygen. It has been reported that the presence of a hydroxyl group at an allylic position retards photooxygenation of the double bond, and conversion of the alcohol to an acetate or benzoate completely suppresses such a reaction.[5] The deactivation was attributed to electron withdrawal by the allylic substituent. The C-24(28) double bond in the diol **7** and in the corresponding diacetate was, however, very susceptible to attack by singlet oxygen, and a mixture of products was obtained in each caase.

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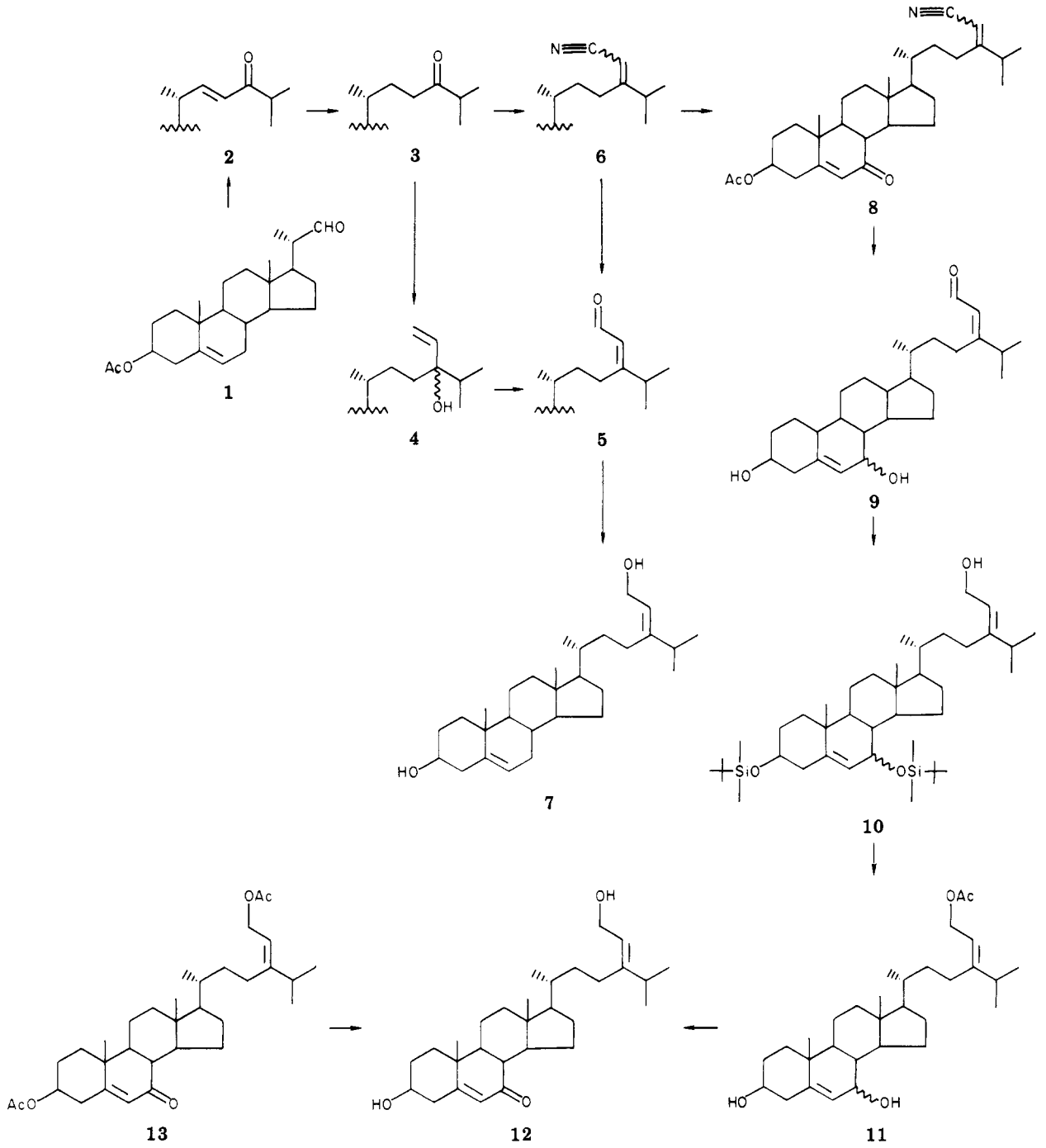
Scheme I



The reactivity of the C-24(28) double bond in 29-hydroxyfucosterol toward singlet oxygen is consistent with the known high reactivity of the corresponding double bond in fucosterol itself. The latter compound undergoes autoxidation on exposure to air giving, presumably, the hydroperoxide which decomposes to saringosterol on attempted isolation.[6]

When the nitrile **6**, which possesses a C-24(28) double bond of low nucleophilicity, was subjected to the photooxygenation–rearrangement sequence, the 7-oxo derivative **8** could be isolated in ~50% yield. Treatment of this compound with DIBAL-H followed by dilute acid yielded the diol–aldehyde **9**. Protection of the alcohol groups by forming the bis(*tert*-butyldimethylsilyl) ether and further reduction with DIBAL-H gave the 29-hydroxy steroid **10**. The bis(tetrahydropyranyl) ether of **9** could also be made readily, but deprotection with acid at a later stage gave low yields of **11**. Acetylation of alcohol **10** followed by removal of the *tert*-butyldimethylsilyl protecting groups with tetrabutyl ammonium fluoride gave diol **11**. Oxidation of the C-7 hydroxyl group with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and removal of the acetate protecting group yielded the target compound (**12**).

A more satisfactory way of introducing the C-7 carbonyl group was by acetylation of the diol **7** followed by oxidation with chromium trioxide–3,5-dimethylpyrazole in dichloromethane.[7] A 50% yield of the ketone **13** was obtained, and a substantial amount of starting compound was

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recovered. There appeared to be no allylic oxidation on the side chain. Removal of the acetate protecting groups in **13** afforded 29-hydroxy-7-oxofucosterol (**12**). The overall yield of this compound from the aldehyde **1** via the diacetate of diol **7** was 7%.

For biological tests, 29-hydroxy-7-oxofucosterol was dissolved in acetone and the solution diluted with water to give a final solution containing ≤1% acetone. The highest concentration, 3.54 μg/mL, was inactive when the solution was added to a culture of *A. ambisexualis* 734 (♀). Oogoniol-1 at a concentration of 3.91 μg/mL was tested at the same time and found to be active. The latter compound had previously been shown to have about 1% of the activity of dehydrooogoniol-1. Thus further hydroxylation of the fucosterol skeleton (at C-11 and/or C-15) appears to be necessary for biological activity.

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Spectra were obtained on the following instruments: Varian EM-390, HR-220 (^{1}H NMR), Perkin-Elmer spectrophotometer 550 (UV), Beckman IR 18A-X (IR), LKB 9000 (mass spectra). The ionizing voltage for mass spectra was 70 eV. Infrared spectra were taken of KBr pellets. NMR spectra were taken in $CDCl_3$ with Me_4Si as an internal standard. Elemental analyses were performed by Pascher Laboratories, Bonn, West Germany.

Column chromatography was carried out with silica gel 60 (70–230 mesh or finer than 200 mesh, EM Laboratories, Elmsford, NY). Analytical thin-layer chromatography (TLC) was carried out on Merck 60 F-254 silica gel plates.

Dry tetrahydrofuran (THF) was prepared by heating at reflux over potassium in a circulating still. Benzene and toluene were each distilled from sodium.

Diethyl (3-Methyl-2-oxobutyl)phosphonate. 1-Bromo-3-methyl-2-butanone was prepared essentially by the method reported in the literature.[2] It is important to add the bromine in one portion to the stirred solution of 3-methyl-2-butanone in dry methanol at 0 °C in order to obtain a good yield. A mixture of the bromo ketone (21 g, 0.13 mmol) and triethyl phosphite (21 g, 0.13 mmol) was heated at 110 °C for 5 h with stirring and then distilled to give the phosphonate: bp 91–95 °C (0.65 mm) [lit.[8] bp 139–140 °C (11 mm)]; NMR δ 1.10 (d, J = 6.5 Hz, 6), 1.31 (t, J = 7.5 Hz, 6), 2.47 (septet, J = 6.5 Hz, 1), 3.13 (d, J = 22.5 Hz, 2), 4.13 (quintet, J = 7.5 Hz, 4).

3β-Acetoxycholesta-5,22(E)-dien-24-one (2). To a stirred solution of the phosphonate (2.98 g, 13.4 mmol) in 15 mL of dry THF in a flame-dried flask at room temperature (argon atmosphere) was added 565 mg (13.4 mmol) of 57% NaH/oil dispersion. After 15 min, the aldehyde **1** (1.0 g, 2.7 mmol) in 10 mL of dry THF was added in one portion. The mixture was stirred for 30 min and then refluxed for 90 min. Most of the solvent was removed at reduced pressure, and the residue was diluted with ether, washed with water, and dried ($MgSO_4$). Removal of the solvent and chromatography of the residue with hexane–ethyl acetate (5:1) gave the enone: **2**, 943 mg (2.1 mmol); mp 141–143 °C (after recrystallization from methanol) (lit.[3] mp 141.5–142.5 °C); NMR δ 0.72 (s, 3), 1.03 (s, 3), 1.11 (d, J = 7 Hz, 6), 2.01 (s, 3), 2.82 (septet, J = 7 Hz, 1), 4.3–4.8 (m, 1), 5.36 (m, 1), 6.03 (d, J = 16 Hz, 1), 6.70 (dd, J = 16, 9 Hz, 1).

3β-Acetoxycholest-5-en-24-one (3). A Parr hydrogenation bottle was charged with enone **2** (1.52 g, 3.5 mmol) and 10% Pd on $BaSO_4$ (400 mg) in 75 mL of ethyl acetate. Hydrogenation was performed at 55 psi of H_2 and room temperature for 4.5 h. The catalyst was separated by filtration through silica gel and the solvent removed from the filtrate, giving the ketone **3**: 1.4 g (3.2 mmol); mp 126–129 °C (after repeated recrystallization from methanol) (lit.[9] mp 128–129.5 °C); IR 1730, 1710 cm^{-1}; NMR δ 0.69 (s, 3), 1.02 (s, 3), 1.09 (d, J = 7 Hz, 6), 2.02 (s, 3), 2.67 (septet, J = 7 Hz, 1), 4.3–4.8 (m, 1), 5.33 (m, 1); MS m/z (relative intensity) 382 (M^+ – AcOH, 100), 367 (6), 342 (4), 339 (1), 312 (11), 297 (7), 255 (7).

Saringosterol and Saringosterol 3β-Acetate (4). To magnesium turnings (0.2 g, 8.2 mmol) in 1 mL of dry THF in a flame-dried flask equipped with a dry ice–acetone condenser were added 3 drops of dibromoethane at room temperature. Then vinyl bromide (1.4 g, 13.1 mmol) in 4 mL of dry THF was added dropwise. The mixture was heated to 50 °C and stirred until all the magnesium metal dissolved. A portion of the solution (3.5 mL) was removed, and to the remainder (about 3.2 mmol) was added ketone **3** (185 mg, 0.42 mmol) in 2 mL of dry THF. The mixture was stirred at 50 °C for 1 h, and then the excess Grignard reagent was destroyed by the addition of a saturated NH_4Cl solution. The aqueous mixture was extracted thoroughly with ether. The combined extracts were dried ($MgSO_4$), and the solvent was removed to give a residue which was chromatographed with hexane–ethyl acetate (8:1), giving saringosterol 3β-acetate (**4**): 78 mg (0.17 mmol); mp 156–158 °C (after recrystallization from methanol) (lit. mp 160–161 °C,[10] 152–156 °C[11]); NMR δ 0.67 (s, 3), 0.85 (d, J = 7 Hz, 3), 0.88 (d, J = 7 Hz, 3), 1.00 (s, 3), 2.00 (s, 3), 4.63 (m, 1), 5.20 (ddd, J = 17, 11, 2 Hz, 2), 5.35 (m, 1), 5.82 (ddd, J = 17, 11, 2 Hz, 1). Also obtained was saringosterol: 50 mg (0.12 mmol); mp 156–159 °C (after recrystallization from methanol) (lit. 163–164 °C,[10] 156–159 °C[11]); NMR δ 0.66 (s, 3), 0.85 (d, J = 7 Hz, 3), 0.88 (d, J = 7 Hz, 3), 1.00 (s, 3), 3.52 (m, 1), 5.20 (ddd, J = 17, 11, 2 Hz, 2), 5.35 (m, 1), 5.82 (ddd, J = 17, 11, 2 Hz, 1).

A solution of sarinogsterol (1.3 g, 3.0 mmol) in 10 mL of dry pyridine and 1 mL of acetic anhydride was allowed to stand at 5 °C for 15 h and then at room temperature for 6 h. Water was added and the mixture extracted with ether. The combined extracts were washed with 10% HCl solution, water, saturated $NaHCO_3$ solution, and saturated NaCl solution and dried ($MgSO_4$). The solvents were removed, and benzene was added and then removed at 0.5 mmHg to give the monoacetate **4** (1.4 g, 3.0 mmol), identical with that obtained above.

3β-Hydroxystigmasta-5,24(28)-diene-29-nitrile 3β-Acetate (6). About 820 mg (19.4 mmol) of 57% NaH/oil dispersion was added to a solution of diethyl (cyanomethyl)phosphonate (5.92 g, 33.4 mmol) in 8 mL of dry THF (nitrogen atmosphere). After the mixture was stirred for 30 min, the temperature was lowered to −78 °C, and a solution of ketone **3** (1.23 g, 2.8 mmol) in 5 mL of dry THF was added. The mixture was allowed to warm to room temperature, stirred for 3 h, and refluxed for 5 h. Water was added, and the mixture was extracted with ether. The combined extracts were washed with water and saturated NaCl solution and dried ($MgSO_4$). Removal of the solvent and chromatography of the resultant yellow oil on silica gel (200 g) with hexane–ethyl acetate (4:1) gave nitrile **6** which was crystallized from methanol: 1.125 g (2.4 mmol); mp 106–108 °C (lit.[4] mp 108–109 °C); NMR δ 0.69 (s, 3), 1.02 (s, 3), 1.06 (d, J = 7.5 Hz, 6), 1.11 (d, J = 7 Hz, 3), 2.02 (s, 3), 4.3–4.9 (m, 1), 5.01 (s, (Z)-24(28) double bond, 0.2), 5.08 (s, (E)-24(28) double bond, 0.8), 5.37 (m, 1); MS m/z (relative intensity) 465 (M^+, 1), 405 (55), 390 (9), 284 (18), 255 (17), 215 (27), 108 (33), 81 (100).

29-Hydroxyfucosterol. (a) From Saringosterol 3β-Acetate (4). To a solution of the alcohol **4** (1.45 g, 3.1 mmol) in 60 mL of dry CH_2Cl_2 (nitrogen atmosphere) was added pyridinium chlorochromate (10.0 g, 46.4 mmol). The mixture was stirred at room temperature for 10 h, with the addition of pyridinium chlorochromate (2.0 g, 9.3 mmol) every 2 h. Ether was added, and the mixture was washed with 10% NaOH solution, 10% HCl solution, saturated $NaHCO_3$ solution, and saturated NaCl solution and dried ($MgSO_4$). The solvents were removed to give crude aldehyde **5** whose NMR spectrum indicated partial hydrolysis of the 3β-acetate (δ 3.3–3.7) and incomplete conversion of starting material, as indicated by the complexity of the δ 5.4–5.9 region. Aldehyde absorption occurred at δ 10.06.

The above product was dissolved in 75 mL of absolute ethanol. To the solution was added $NaBH_4$ (800 mg, 21.1 mmol), and the

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mixture was stirred at room temperature for 30 min. Most of the ethanol was removed at reduced pressure. The residue was diluted with ether and washed with 10% NH_4Cl solution, saturated NaCl solution, and dried ($MgSO_4$). Removal of the solvent followed by chromatography of the residue on silica gel (40 g) with hexane–ethyl acetate (4:1) gave four products. (1) Saringosterol 3β-acetate (4); 161 mg (0.33 mmol). (2) 29-Hydroxyfucosterol 3β-acetate (acetate of 7): 283 mg (0.61 mmol); mp 123.5–126 °C (recrystallized from methanol); IR 3300, 1730, 1035 cm^{-1}; NMR δ 0.67 (s, 3), 1.00 (s, 3), 1.01 (d, J = 7 Hz, 6), 2.01 (s, 3), 4.13 (br d, J = 6 Hz, 2), 4.4–4.8 (m, 1), 5.33 (m, 2); MS m/z (relative intensity) 410 (M^+ – HOAc, 39), 392 (6), 385 (5), 342 (100), 314 (6), 296 (39), 255 (9), 253 (20). (3) Saringosterol, 111 mg (0.25 mmol). (4) 29-Hydroxyfucosterol (7): 235 mg (0.55 mmol); mp 138–141 °C (recrystallized from ethyl acetate); IR 3215, 1050 cm^{-1}; NMR δ 0.68 (s, 3), 0.98 (d, J = 7 Hz, 3), 1.00 (s, 3), 1.01 (d, J = 7.5 Hz, 6), 3.3–3.8 (m, 1), 4.15 (br d, J = 7.5 Hz, 2), 5.31 (m with br t, J = 7.5 Hz, 2); MS m/z (relative intensity) 428 (M^+, 15), 410 (19), 384 (16), 314 (100), 299 (36), 271 (67).

(b) From Nitrile 6. To a solution of nitrile 6 (100 mg, 0.22 mmol) in 4 mL of dry benzene was added 0.67 mL of 20% diisobutylaluminum hydride in hexane solution (0.89 mmol). The mixture was stirred at room temperature for 150 min and then cooled to 0 °C, and a mixture of 7% aqueous acetic acid–THF–methanol (1:1:1, 15 mL) was added dropwise over a period of 20 min while stirring vigorously. The mixture was then allowed to warm to room temperature, $CHCl_3$ (2 mL) was added, and stirring was continued overnight. Two clear phases formed. Water (20 mL) was added, and the product was extracted with $CHCl_3$ (3 × 20 mL). The combined extract was washed with $NaHCO_3$ solution and saturated NaCl solution and dried ($MgSO_4$). Removal of the solvent gave crystalline aldehyde (mixture of E and Z isomers): 95 mg (94%); mp 89–91 °C; NMR δ 0.69 (s, 3), 0.98 (s, 3), 1.11 (d, J = 7 Hz, 6), 1.15 (d, J = Hz, 3), 3.2–3.8 (m, 1), 5.34 (m, 1), 5.77 (d, J = 8.4 Hz, Z isomer, 0.25 H), 5.81 (d, J = 8.4 Hz, E isomer, 0.75 H), 9.97 (d, J = 8 Hz, E isomer, 0.75 H), 10.06 (d, J = 8 Hz, Z isomer, 0.25 H).

Acetylation of aldehyde with acetic anhydride–pyridine at room temperature overnight gave the crystalline acetate in almost quantitative yield; mp 117–121 °C. On TLC with hexane/ethyl acetate (4:1) the E isomer had R_f 0.33, and the Z isomer had R_f 0.30. Separation by preparative TLC gave pure E isomer (mp 124.5–125.5 °C) and pure Z isomer (mp 122.5–124 °C) in a ratio of about 3:1.

The above acetate (mixture of isomers, 30 mg, 0.06 mmol) was dissolved in dry toluene (1 mL), and to the cooled solution (0 °C) was added DIBAL-H (400 μL, 0.54 mol, 20% solution in hexane) under a nitrogen atmosphere. The solution was stirred for 1 h, water was added, and the mixture was extracted with chloroform (3 × 20 mL). The extract was dried ($MgSO_4$) and the solvent removed, leaving 29-hydroxy fucosterol, 20 mg (75%). Recrystallization from ethyl acetate gave pure E isomer.

The diacetate, prepared with acetic anhydride–pyridine, was recrystallized from methanol: mp 109–112 °C; NMR δ 0.68 (s, 3), 1.01 (s, 3), 1.01 (d, J = 6.5 Hz, 6), 2.01 (s, 3), 2.03 (s, 3), 4.58 (m, 3), 5.37 (m, 2); MS m/z (relative intensity) 512 (M^+, 5), 452 (1), 392 (100), 377 (4), 313 (5), 297 (11), 283 (5), 282 (5), 253 (37).

Photooxygenation of 29-Hydroxyfucosterol. (a) Oxygen was bubbled through a solution of diol 7 (17 mg, 0.04 mmol) and a trace of hematoporphyrin in 3 mL of dry pyridine which was being irradiated by two 15-W fluorescent lamps. The reaction was monitored by TLC. After 45 h there appeared to be no further change in the complex TLC pattern. The solution was diluted with ether and filtered through silica gel, and the ether was removed in a stream of N_2. To the remaining pyridine solution was added $Cu(OAc)_2 \cdot H_2O$ (13 mg, 0.07 mmol). After being stirred at room temperature for 7 h, the mixture was filtered through silica gel and completely eluted with ethyl acetate. Removal of the solvents followed by preparative TLC of the residue gave products whose NMR spectra showed a singlet at δ 5.7 expected for a 5-en-7-one system but no signals for the C-28 H or C-29 H of a 29-hydroxyfucosterol side chain. Similar results were obtained on photooxygenation of the diacetate of 29-hydroxyfucosterol.

3β-Acetoxy-7-oxostigmasta-5,24(28)-diene-29-nitrile (8). A solution of nitrile 6 (850 mg, 1.82 mmol) in dry pyridine (60 mL) containing hematoporphyrin (40 mg) was irradiated with four 15-W fluorescent lamps for 72 h, during which time oxygen was bubbled through the solution. The reaction mixture was diluted with ether (70 mL), treated with charcoal (120 mg), and filtered through a short column of silica gel and Celite. The ether was removed in a stream of N_2, and $Cu(OAc)_2 \cdot H_2O$ (850 mg) was added to the remaining pyridine solution. The mixture was stirred for 48 h at 50 °C. After being diluted with ethyl acetate (250 mL), the solution was washed with dilute phosphoric acid and $NaHCO_3$ solution and dried ($MgSO_4$), and the solvent was removed. Chromatography of the residue with hexane–ethyl acetate 3.5:1 yielded the ketone 8: 512 mg (1.1 mmol); mp 125–129 °C. The product was recrystallized from methanol: mp 139–140 °C; IR 2210, 1722, 1660 cm^{-1}; UV (CH_3OH) λ_{max} 216 nm (ε 17 000); NMR δ 0.70 (s, 3), 1.07 (d, J = 7 Hz, 6), 1.10 (d, J = 8.5 Hz, 3), 1.22 (s, 3), 2.04 (s, 3), 4.46–4.94 (m, 1), 5.03 (s, Z isomer, 0.25 H), 5.10 (s, E isomer, 0.75 H), 5.69 (s, 1); MS m/z (relative intensity) 419.3185 (M^+ – CH_3COOH, 100), 405.3006 (8), 404.2919 (M^+ – CH_3COOH – CH_3, 8), 227.1435 (19), 195.1145 (4), 187.1130 (23), 174.1044 (54), 161.0977 (60).

3β,7ξ-Dihydroxystigmasta-5,24(28)-dien-29-aldehyde (9). To 150 mg (0.31 mmol) of keto nitrile 8 in a dry three-necked flask (N_2 atmosphere) was added 5 mL of dry toluene, the resulting solution was cooled to 0 °C, and 1.5 mL of DIBAL-H (20% solution in hexanes, 2.0 mmol) was added. The ice–water bath was removed, and the reaction mixture was stirred at room temperature for 2.5 h. The solution was cooled to 0 °C, and a mixture of 6 mL of 7% aqueous acetic acid, 5 mL of methanol, and 2.5 mL of THF was added dropwise over a period of 15 min with vigorous stirring. The ice–water bath was removed, and chloroform (2.5 mL) was added to dissolve the precipitate which had formed. Two clear phases resulted which were stirred vigorously overnight, water was added (30 mL), and the whole mixture was extracted with chloroform (2 × 60 mL). The extract was washed with water and dried ($MgSO_4$), and the solvent was removed, leaving the crude product which was chromatographed with chloroform–methanol (100:6), yielding 95 mg of 9: mp 66–76 °C; IR 3300, 1650 cm^{-1}; UV (CH_3OH) λ_{max} 239 nm (ε 14 000); NMR δ 0.69 (s, 3), 1.00, 1.07 (2 s, 19-H, two isomers), 1.09 (d, J = 8 Hz, 6), 1.18 (d, J = 7 Hz, 3), 3.3–3.8 (m, 1), 3.83 (m, 1), 5.28 (s, 6-H in 7β-OH isomer, 0.7 H), 5.57 (m, 6-H in 7α-OH isomer, 0.3 H), 5.78, (d, J = 9 Hz, 28-H in Z isomer, 0.2 H), 5.81 (d, J = 9 Hz, 28-H in E isomer, 0.8-H), 10.04 (t resulting from two superimposed d, 29-H, E + Z isomer, 1); MS m/z (relative intensity) 424 (M^+ – H_2O, 3), 406 (4), 269 (2), 219 (10), 211 (s), 161 (12).

3β,7ξ-Bis[(*tert*-butyldimethylsilyl)oxy]stigmasta-5,24-(28)-dien-29-ol (10). To a solution of diol 9 (720 mg, 1.6 mmol) in 8 mL of dry CH_2Cl_2 was added *tert*-butyldimethylsilyl chloride (1481 mg, 9.8 mmol), 4-(dimethylamino)pyridine (600 mg, 4.9 mmol), and triethylamine (1088 mg, 10.8 mmol). The solution was stirred at room temperature for 24 h, diluted with CH_2Cl_2 (120 mL), and washed with water (100 mL). The aqueous layer was extracted with CH_2Cl_2 (120 mL), the combined CH_2Cl_2 layers were washed with 10% NH_4Cl solution and brine and dried, ($MgSO_4$), and the solvent was removed. The crude product was chromatographed with hexane–ethyl acetate (5:1) to give the disilyl ether (800 mg) and the mono silyl ether (165 mg). The disilyl ether had the following: mp 47–55 °C; IR ($CHCl_3$) 1660 cm^{-1}; UV (CH_3CN) λ_{max} 239 nm (ε 10 500); NMR δ 0.05 (s, 12), 0.67 (s, 3), 0.87 (s, 18), 1.09 (d, J = 8 Hz, 6), 3.3–3.7 (m, 1), 3.95 (m, 1), 5.24 (s, 0.85), 5.48 (m, 0.15), 5.78 (d, J = 9 Hz, 0.2), 5.81 (d, J = 9 Hz, 0.8), 10.03 (t resulting from two superimposed d, 1); MS m/z (relative intensity) 613 (M^+ – t-Bu, 1), 538 (2), 264 (1), 251 (2), 219 (6), 211 (3), 75 (100).

The disilyl ether (320 mg, 0.48 mmol) was treated with DIBAL-H (0.8 mL) in the same way as described for the reduction of the keto nitrile 8. The crude product on chromatography with benzene–ethyl acetate (1:3) yielded the alcohol 10: (225 mg, (0.34 mmol); amorphous solid; NMR δ 0.05 (12, s), 0.67 (s, 3), 0.87 (s, 18), 1.03 (d, J = 6 Hz, 6), 1.05 (s, 3), 3.3–3.7 (m, 1), 3.96 (m, 1), 4.15 (d, J = 8 Hz, 29-H, 2), 5.1–5.5 m, 6-H and 28-H,2); MS m/z (relative intensity) 672 (M^+, < 0.1), 615 (0.6), 541 (1.4), 523 (2.2).

3β,7β,29-Trihydroxystigmasta-5,24(28)-diene 29-Acetate (11). The alcohol 10 (210 mg) was converted to the corresponding acetate with acetic anhydride (0.6 mL) and pyridine (0.6 mL). The product was an oil: NMR δ 0.05 (s, 12), 0.68 (s, 3), 0.88 (s, 18), 1.00 (d, J = 7 Hz, 6), 1.03 (s, 3), 2.03 (s, 3), 3.3–3.7 (m, 1),

3.95 (s, 1), 4.58 (d, J = 8 Hz, 2), 5.1–5.5 (m, 6-H and 28-H, 2); MS m/z 654 (M^+ – AcOH and (*tert*-butyldimethylsilyl)oxy).

To the acetate (200 mg, 0.28 mmol) was added 1.5 mL of tetrabutylammonium fluoride solution in THF (1.5 mmol). The mixture was stirred for 5 h at room temperature, diluted with water (100 mL), and extracted with CH_2Cl_2 (2 × 120 mL). The extract was dried ($MgSO_4$) and the solvent removed, leaving the crude product which on chromatography with ethyl acetate–chloroform (2:1) gave the diol acetate 11: 100 mg; mp 100–102 °C. It consisted essentially of the 7β-hydroxy isomer only. On recrystallization from ethyl acetate the compound had the following: mp 102–103 °C; IR 3310–3100, 1730 cm^{-1}; NMR δ 0.69 (s, 3), 1.02 (d, J = 7 Hz, 6), 1.05 (s, 3), 2.03 (s, 3), 3.3–3.95 (m, 3-H and 7-H, 2), 4.56 (d, J = 7 Hz, 2), 5.27 (m, 6-H and 28-H, 2); MS m/z (relative intensity) 484 (M^+, 1), 468 (1), 450 (1), 426 (M^+ – AcOH, 3).

3β,29-Dihydroxystigmasta-5,24(28)(*E*)-dien-7-one (12). (a) 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (112 mg, 0.5 mmol) was added to a solution of the diol (11, 80 mg, 0.16 mmol) in 3 mL of dry benzene and the mixture was shaken at room temperature for 24 h. It was diluted with benzene and filtered through a short column of silica gel. The crude product was chromatographed with ethyl acetate–chloroform (1:2), yielding the enone: 63 mg (0.13 mmol); semicrystalline solid; IR 3300–3100, 1732, 1670 cm^{-1}; UV (CH_3OH) λ_{max} 233 nm (ϵ 10200); NMR δ 0.67 (s, 3), 1.00 (d, J = 6 Hz, 6), 1.18 (s, 3), 2.03 (s, 3), 3.4–3.9 (m, 1), 4.57 (d, J = 7 Hz, 2), 5.28 (t, J = 7 Hz, 1), 5.66 (s, 1); MS m/z (relative intensity) 484 (M^+, 4).

The enone (58 mg) was dissolved in THF (1 mL) and methanol (8 mL) and a 10% solution of K_2CO_3 (in 3:2 methanol–water, 0.35 mL) added. After the solution was stirred overnight, solid NH_4Cl was added, and the solvent was removed in a stream of N_2. The residue was chromatographed with chloroform–ethyl acetate (1:1) to give the diol 12: 50 mg; mp 170–180 °C; NMR ($CDCl_3$–CD_3OD) δ 0.67 (s, 3), 0.97 (d, J = 7 Hz, 3), 0.99 (d, J = 6 Hz, 6), 1.17 (s, 3), 4.11 (d, J = 6 Hz, 29-H of *E* isomer), 4.13 (d, J = 6 Hz, 29-H of *Z* isomer), 5.30 (t, J = 6 Hz), 5.64 (s, 1). The signals for 29-H in the *E* and *Z* isomers could be seen clearly in the expanded spectrum (5-ppm sweep width). The *Z* isomer was estimated to amount to less than 15% of the mixture.

Recrystallization from ethyl acetate gave pure *E* isomer: 33 mg; mp 188–191 °C; the purity was confirmed by the 360-MHz NMR spectrum; IR 3450–3150, 2950, 1670, 1645, 1475, 1380, 1230, 1050 cm^{-1}; UV (CH_3OH) λ_{max} 234 nm (ϵ 11000); MS m/z (relative intensity) 442.3452 [M^+ (calcd 442.3435), 2], 329.2452 [M^+ – $C_7H_{13}O$ (fragment from allylic cleavage at C(22)–C(23), 9]. Anal. Calcd for $C_{29}H_{46}O_3$: C, 78.67; H, 10.48; O, 10.84. Found: C, 78.68; H, 10.25; O, 10.80.

(b) 3,5-Dimethylpyrazole (309 mg, 3.2 mmol) was added to chromium trioxide (322 mg, dried in vacuo at 100 °C overnight) suspended in dry methylene chloride (1.5 mL) at –15 °C. The mixture was stirred for 10 min, and 29-hydroxyfucosterol 3β,29-diacetate (110 mg, 0.2 mmol) was added in one portion. Stirring was continued for 5 h while the temperature was maintained between –10 and –20 °C. Sodium hydroxide solution (1 mL, 5 N) was next added, and after a further 1 h at 0 °C, methylene chloride was added, and the organic phase was separated, washed with dilute hydrochloric acid and water, and dried ($MgSO_4$). Removal of the solvent and chromatography of the residue with ethyl acetate–hexanes (1:4) gave the ketone 13 (60 mg) and unreacted 29-hydroxyfucosterol diacetate (40 mg). Ketone 13 was recrystallized from methanol: mp 151–153 °C; UV (CH_3OH) 235 nm (ϵ 12000); IR 2950, 1730, 1660, 1370, 1240, 1030 cm^{-1}; NMR δ 0.68 (s, 3), 0.98 (s, 3), 1.05 (s, 3), 1.19 (s, 3), 2.03 (s, 6), 4.58 (d, J = 7 Hz, 2), 5.30 (t, J = 7 Hz, 1), 5.66 (s, 1); MS m/z (relative intensity) 526 (M^+, 1), 466 (M^+ – AcOH, 2).

To a stirred solution of ketone 13 (10 mg, 0.02 mmol) in 0.3 mL of THF and 2 mL of methanol was added 10% K_2CO_3 solution (in 60% CH_3OH/40% H_2O, 0.1 mL). The reaction mixture was kept at room temperature for 20 h and then evaporated to dryness. Chloroform was added and the mixture filtered. On evaporation of the solvent, the filtrate yielded the product (12) which was recrystallized from ethyl acetate: mp 188–191 °C; 5 mg.

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Registry No. 1, 10211-88-8; (*E*)-2, 32230-64-1; 3, 20981-59-3; 4, 13254-10-9; (*E*)-5, 81256-53-3; (*Z*)-5, 81256-54-4; (*E*)-5 alcohol, 81256-55-5; (*Z*)-5 alcohol, 81256-56-6; (*E*)-6, 52065-17-5; (*Z*)-6, 81256-57-7; (*E*)-7, 81256-58-8; (*Z*)-7, 81256-59-9; (*E*)-7 acetate, 81256-60-2; (*E*)-7 diacetate, 81256-61-3; (*E*)-8, 81256-62-4; (*Z*)-8, 81256-63-5; (*E*)-9, 81256-64-6; (Z)-9, 81256-65-7; 9 diether, 81256-66-8; 10, 81256-67-9; 10 acetate, 81256-68-0; 11, 81256-69-1; 11 enone, 81256-70-4; (*E*)-12, 81256-71-5; (*Z*)-12, 81256-72-6; 13, 81256-73-7; diethyl (3-methyl-2-oxobutyl)phosphonate, 7751-67-9; vinyl bromide, 106-95-6; saringosterol, 6901-60-6.

Triophamine, a Unique Diacylguanidine from the Dorid Nudibranch *Triopha catalinae* (Cooper)

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Triophamine (1), a symmetrical diacylguanidine, has been isolated from skin extracts of the dorid nudibranch *Triopha catalinae*. The proposed structure of 1 was based on interpretation of the mass, IR, UV, ^1H NMR, and ^{13}C NMR spectra. It was verified by comparison with diacetylguanidine (3) and by base-catalyzed hydrolysis to guanidine and 2,4-diethyl-4-hexenoic acid (6).

Dorid nudibranchs are delicate, shell-less, and often strikingly colored marine molluscs that despite their conspicuousness and vulnerability have almost no known predators.[1] A number of dorids utilize chemical antifeedants obtained from their sponge diets as one component of their defensive arsenal. Several well-documented examples include albicanyl acetate[2] and furodysinin[2,3] from *Cadlina luteomarginata*, 9-isocyanopupukeanane from *Phyllidea varicosa*,[4] and nakafuran-8 and nakafuran-9 from *Hypselodoris godeffroyana* and *Chromodoris mari-*

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