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Isolation and Synthesis of Tanyolides A and B, Metabolites of the Nudibranch *Sclerodoris tanya.*

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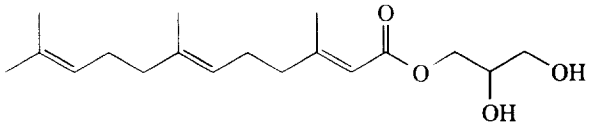
Abstract: The nudibranch *Sclerodoris tanya*, which was collected intertidally at La Jolla, contains two sesquiterpene glyceride esters, tanyolides A (**4**) and B (**5**). The structures of the tanyolides were elucidated by interpretation of spectral data. The absolute configuration of tanyolide B (**5**) was determined by synthesis of both enantiomers and the structure of tanyolide A (**4**) was also confirmed by synthesis. The tanyolides, which are located primarily in the dorsal mantle, deter feeding by two ecologically-relevant predatory fish.

Sclerodoris tanya is a relatively uncommon nudibranch from the intertidal zone in Southern California.[1,2] Several *Archidoris* species, to which the genus *Sclerodoris* is closely related, have been reported[3] to contain sesquiterpene and diterpene glycerides such as glyceryl farnesoate (**1**) from *A. ohdneri*[4] and the diacylglycerides **2** and **3** from *A. montereyensis*.[5] Feeding experiments using radiolabelled mevalonic acid revealed that *Archidoris* spp. were capable of *de novo* biosynthesis of the terpenoid portions of the sesquiterpene and diterpene glycerides.[6] In the majority of the sesquiterpene and diterpene glycerides, the terpenoid residue is linked to the 1-position of glycerol.[7] In *Sclerodoris tanya*, the two sesquiterpene glycerides, tanyolides A (**4**) and B (**5**), have the sesquiterpene residue linked to the 2-position of glycerol. The structures of tanyolides A (**4**) and B (**5**) were confirmed by synthesis.



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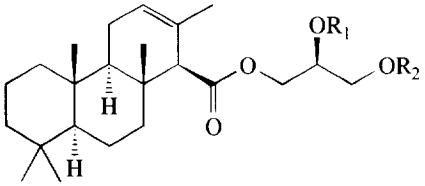
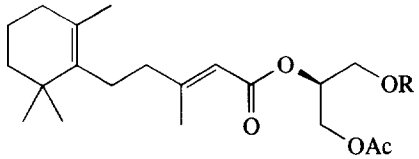


2 R_1 = H, R_2 = Ac
3 R_1 = Ac, R_2 = H



4 R = Ac
5 R = H

Three specimens of *Sclerodoris tanya* were collected at Casa Reef, La Jolla, in August 1993 and were held in aquaria for several weeks during an unsuccessful attempt to find a sponge on which they would feed. The specimens were individually extracted with acetone then methanol and the combined extracts for each specimen were partitioned between ethyl acetate and water. The organic extracts were chromatographed on silica using an elution gradient from hexane to ethyl acetate and selected fractions were separated by HPLC on silica to obtain tanyolides A (**4**) and B (**5**). An additional four specimens, collected in July 1994, were extracted immediately in a similar manner to obtain the same compounds in higher yields.

Tanyolide A (**4**), $[\alpha]_D = 0°$, was obtained as a colorless oil. The molecular formula, $C_{22}H_{34}O_6$, was obtained by high resolution mass spectrometry and confirmed by analysis of the ^{13}C NMR data (Table 1), which revealed that the six oxygen atoms were associated with three ester groups. The infrared band at 1740 cm^{-1} supported that assignment. The ^1H NMR spectrum (Table 1) contained signals at δ 5.28 (pentet, 1H, J = 5 Hz), 4.27 (dd, 2H, J = 12, 5 Hz), 4.19 (dd, 2H, J = 12, 5 Hz), and 2.06 (s, 6H) that were indicative of a symmetrical 1,3-diacetyl glyceride, having a sesquiterpene ester at the 2-position. The UV absorption at 241 nm was assigned to a β,β-disubstituted α,β-unsaturated ester. The ^{13}C NMR spectrum contained signals at δ 165.7, 114.2, and 162.7 that were appropriate for the unsaturated ester; two additional signals at δ 128.0 and 136.0 were assigned to a tetrasubstituted olefin. Comparison of the ^1H and ^{13}C NMR data (Table 1) with compounds having a 2,6,6-trimethylcyclohexene ring system[8] indicated that the sesquiterpene moiety was monocyclofarnesoic acid. The chemical shift of the vinyl methyl signal at δ 19.2 allowed assignment of the (2*E*) geometry. Tanyolide A is therefore 1',3'-diacetyl-2'-(2*E*)-monocyclofarnesoylglyceride (**4**), which is the first triacylglyceride reported from a nudibranch.

Table 1. ^{13}C (50 MHz, $CDCl_3$) and ^1H NMR (500 MHz, $CDCl_3$) data for tanyolide A (4).

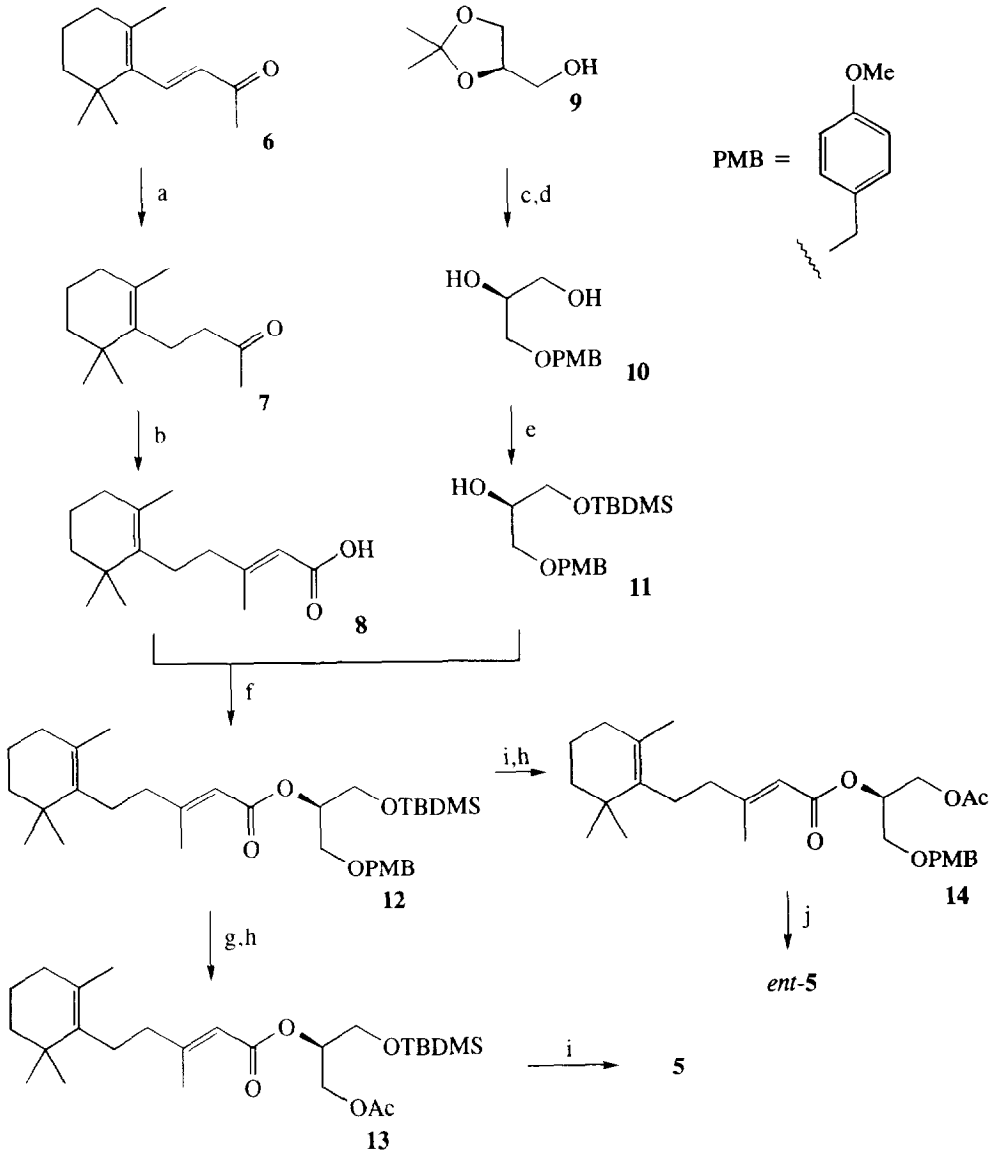
C#	δ_C	δ_H	(mult, *J*)	C#	δ_C	δ_H	(mult, *J*)
1	165.7			11	35.0		
2	114.2	5.69	(s, 1H)	12	19.2	2.19	(s, 3H)
3	162.7			13	19.8	1.59	(s, 3H)
4	41.6	2.18	(m, 2H)	14	28.6	0.98	(s, 3H)
5	27.0	2.12	(m, 2H)	15	28.6	0.98	(s, 3H)
6	136.0			1',3'	62.5	4.19	(dd, 2H, 12,5 Hz)
7	128.0					4.27	(dd, 2H, 12,5 Hz)
8	32.8	1.90	(t, 2H, 6 Hz)	2'	68.0	5.28	(pent, 1H, 5 Hz)
9	19.5	1.55	(m, 2H)	$COCH_3$	170.5		
10	39.8	1.40	(m, 2H)	$COCH_3$	20.7	2.06	(s, 6H)

Table 2. ^{13}C (50 MHz, $CDCl_3$) and ^1H NMR (500 MHz, $CDCl_3$) data for tanyolide B (5).

C#	δ_C	δ_H	(mult, *J*)	HMBC
1	166.2			
2	114.2	5.72	(s, 1H)	C-4, C-12
3	162.9			
4	41.6	2.18	(m, 2H)	C-2, C-3, C-5
5	27.0	2.12	(m, 2H)	C-4, C-6, C-7
6	136.0			
7	128.0			
8	32.8	1.40	(t, 2H, 6 Hz)	C-6, C-7, C-9, C-10
9	19.5	1.55	(m, 2H)	
10	39.8	1.40	(m, 2H)	C-6, C-8, C-9, C-14,15
11	35.0			
12	19.2	2.19	(s, 3H)	C-2, C-3, C-4
13	19.8	1.59	(s, 3H)	C-6, C-7, C-8
14	28.6	0.98	(s, 3H)	C-6, C-10, C-11, C-15
15	28.6	0.98	(s, 3H)	C-6, C-10, C-11, C-14
1′	62.5	4.30	(dd, 1H, 12, 5 Hz)	C-2′, C-3′, $COCH_3$
		4.26	(dd, 1H, 12, 5 Hz)	C-2′, C-3′, $COCH_3$
2′	71.4	5.09	(m, 1H, 5 Hz)	C-1
3′	61.8	3.77	(ddd, 1H, 12, 5, 2.5 Hz)	C-1′, C-2′
		3.73	(ddd, 1H, 12, 5, 2.5 Hz)	C-1′, C-2′
$COCH_3$	171.0			
$COCH_3$	20.8	2.07	(s, 3H)	$COCH_3$

Tanyolide B (**5**), $[\alpha]_D$ = -3.0° (*c* 0.1, $CHCl_3$), was isolated as a colorless oil. The molecular formula, $C_{20}H_{32}O_5$, and the NMR data all implied that tanyolide B differs from tanyolide A by the absence of an acetate group. Comparison of the NMR data of **5** (Table 2) with those of **4** (Table 1) indicated that the sesquiterpene moiety was the same in both compounds. The NMR data clearly shows that the glyceride is asymmetrically substituted in **5**. The substitution pattern was determined from the HMBC data which showed that the acetate group was attached at the *sn*-1 position of glycerol with the monocyclofarnesoate group at the *sn*-2 position. Since there was insufficient material to both determine the absolute configuration at C-2′ by degradation and to perform feeding inhibition assays, we decided to carry out a synthesis of **5** that was capable of producing both optical enantiomers.

Monocyclofarnesoic acid (**8**), which has been synthesized previously,[9] was prepared in a straightforward manner. Reduction of β-ionone with lithium aluminum hydride-cuprous iodide in THF at



Scheme 1. a. $LiAlH_4$-CuI/THF. b. $(EtO)_2P(O)CH_2COOEt$-NaH/THF; KOH/EtOH-H_2O. c. PMBCl, KO*t*-Bu/THF, 0°. d. HCl/MeOH. e. TBDMSCl, NEt_3, DMAP/CH_2Cl_2, 0°. f. DCC, DMAP/CH_2Cl_2, 10 days. g. DDQ/CH_2Cl_2-H_2O. h. Ac_2O, NEt_3, DMAP/CH_2Cl_2. i. Dowex AG50W-X8 (H^+)/MeOH, 3-5 days. j. TFA, anisole/CH_2Cl_2, 0°.

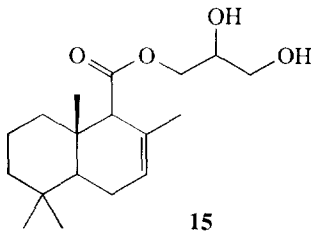
0 °C, according to the method of Negishi *et al.*,[10] gave dihydro-β-ionone (**7**). Reaction of **7** with triethyl phosphonoacetate and sodium hydride in THF at 25 °C, followed by hydrolysis of the product using potassium hydroxide in refluxing aqueous ethanol, according to the procedure of Schmidt *et al.*,[11] gave (2*E*)-monocyclofarnesoic acid (**8**) in 20% overall yield.

(*S*)-1-(*p*-Methoxyphenyl)methoxyl-2,3-propanediol (**10**), prepared from (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol (**9**) by the method of Manley *et al.*,[12] was treated with one equivalent of t-butyldimethylsilyl chloride in dichloromethane containing triethylamine and a catalytic amount of 4-N,N-dimethylaminopyridine (DMAP) at 0 °C to obtain a 70% yield of the secondary alcohol **11**. We chose to employ the TBDMS ether rather than the corresponding acetate, which would have resulted in a shorter synthesis, for two reasons; firstly, the acetate group might migrate under basic conditions from the 1- to the 2-position during the second acylation step and, secondly, we could prepare both (2′*S*)-tanyolide B (**5**) and (2′*R*)-tanyolide B (*ent*-**5**) by selective removal of the protecting groups.

Coupling of monocyclofarnesoic acid (**8**) to the secondary alcohol **11** proved more difficult than expected. Several methods resulted in isomerization of the conjugated double bond to the 3(12)-position. The most satisfactory method involved treatment of a mixture of **8** and **11** with excess dicyclohexylcarbodiimide (DCC) and DMAP in dry dichloromethane over a period of 10 days to obtain the ester **12** in 54% yield.[13] Removal of the *p*-methoxybenzyl group with DDQ in a 2-phase system of dichloromethane and water, followed by acetylation of the product gave the acetate **13** in 93% yield. The TBDMS ether was cleaved by methanolysis of **13** using Dowex AG50W-X8 (H^+) as catalyst to obtain a 62% yield of (2′*S*)-tanyolide B (**5**), which was identical in all respects including optical rotation to the natural product. Removal of the TBDMS group from ester **12** by methanolysis using Dowex AG50W-X8 (H^+) as catalyst followed by acetylation of the resulting alcohol gave the ester **14** from which the *p*-methoxybenzyl group was removed by treatment with TFA and anisole in dichloromethane[13] to obtain (2′*R*)-tanyolide B (*ent*-**5**). Despite small differences due to concentration and the presence of small quantities of a geometrical isomer in the synthetic products, comparison of the CD spectrum of the natural material with those of the (2′*R*) and (2′*S*) enantiomers of tanyolide B clearly indicated that the natural material was (2′*S*)-tanyolide B (**5**).

Tanyolide A (**4**) was synthesized, albeit in poor yield, by treatment of a mixture of 1,3-diacetoxy-3-propanol and monocyclofarnesoic acid (**8**) with excess DCC in dichloromethane containing DMAP. The synthetic materials were employed in assays to determine the biological activity of the metabolites.

Glyceride esters of terpenoic acids have been reported from species belonging to four nudibranch genera: *Archidoris*, *Austrodoris*, *Doris*, and *Sclerodoris*. Based on a limited number of biosynthetic studies and the absence of any known dietary source for such compounds, it has been concluded that members of this class of terpene glycerides are synthesized *de novo* by the nudibranchs.[6,14] Although a role for terpene glycerides in the chemical defense of the nudibranchs has been suggested, ecologically relevant bioactivity



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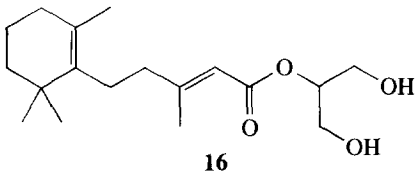

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data is lacking. The metabolites of *Archidoris tuberculata* and *Doris verrucosa* were shown to be ichthyotoxic to the freshwater fish *Gambusia affinis*, but this activity was not correlated to a role in defending the nudibranch against potential predators from the natural habitat.[7,15,16] In fact, studies on diacylglyceride **2** suggested that there was no correlation between ichthyotoxicity and fish feeding inhibition.[6,15] The only compound reported to inhibit feeding of a co-occurring predator, the tidepool sculpin *Oligocottus maculosus*, is the diol **15** from *Archidoris montereyensis*; compounds **1** and **2** were inactive in this assay.

Dissection of specimens of *S. tanya* into the dorsal mantle, the foot, the digestive gland and the viscera followed by extraction of each tissue and HPLC analysis of acetone extracts revealed that the tanyolides were located primarily in the dorsal mantle with slight traces in the foot and none in the digestive gland or viscera. This pattern of localization suggests *de novo* biosynthesis rather than dietary accumulation of the tanyolides.[17] When a specimen of *S. tanya* is poked, prodded, or injected, it immediately secretes mucous at and around the site of attack. The secretion washed from five specimens of *S. tanya* was extracted with acetone and the extract was purified by HPLC to obtain 1 mg of tanyolide B (**5**), proving that this metabolite is indeed released in a concentrated defensive secretion following molestation.

When assayed for predator deterrence according to the method of Pawlik and Fenical,[18] neither tanyolide A (**4**) nor B (**5**) showed any feeding inhibition activity, even at ten times the concentration found in *S. tanya*, and the diol **16**, a potential hydrolysis product, was also ineffective. However, when the assay method was modified by coating the pellets with the compounds to be tested rather than incorporating the compounds into the pellets, quite different results were obtained. The compounds were coated on pellets (120 mg) of a squid/alginic acid mixture (see experimental section) and pieces were fed to *Gibbonsia elegans* and *Paraclinus integrippinis*, two predatory fish that are known to feed on molluscs and are found in the same tidepools where the nudibranchs were collected.[19,20] The assay results using *G. elegans*, shown in Table 3, indicated that tanyolide A (**4**), (2′*S*)-tanyolide B (**5**), (2′*R*)-tanyolide B (*ent*-**5**), and monocyclofarnesoic acid (**8**) were all active at 1 mg/pellet. Identical results were obtained when the first three compounds were assayed at 1 mg/pellet using *P. integrippinis*. Since each *S. tanya* contains about 3

Table 3. Consumption by *Gibbonsia elegans* of food pellets coated with tanyolide A (4), (2′*S*)-tanyolide B (5), (2′*R*)-tanyolide B (*ent*-5), and monocyclofarnesoic acid (8) (pellets consumed/pellets offered).

Compound	Amount (mg)/pellet	Treated pellets consumed	Control pellets consumed
4	1	0/10	10/10
5	1	0/10	10/10
	0.67	2/10	10/10
ent-**5**	1	0/10	10/10
8	1	0/10	10/10

mg of tanyolide B (**5**), protection against predation by fish can be achieved using less than one third of the available defensive chemicals. The activity associated with monocyclofarnesoic acid (**8**) suggests that the terpene portion of the molecule is primarily responsible for feeding inhibition. Tanyolide B (**5**) has no antimicrobial or cytotoxic activity and fish that were fed relatively high concentrations suffered no deleterious effects. However, using a standard ichthyotoxicity assay,[21,22] both tanyolides A and B were toxic to freshwater guppies (*Poecilia reticulata*) at a concentration of 1 μg/mL, an activity that is comparable to that observed for other terpene glyceride esters.[16] These data indicate the importance of choosing an ecologically relevant assay:[23] an assay method designed to test the efficacy of compounds that are evenly distributed within the tissues of the producing organism cannot be used for testing the effects of nudibranch metabolites that are concentrated in a mucous exudate. Furthermore, it appears that the standard ichthyotoxicity assay using freshwater fish is not relevant for testing marine natural products since it probably measures the effects of chemicals on the surface of the gill tissue.[24]

EXPERIMENTAL SECTION

General: Ultraviolet and infrared spectra were recorded on Perkin-Elmer Lambda 3B and 1600 series spectrometers, respectively. ^{1}H NMR spectra were recorded on a Varian Unity 500 spectrometer (UCSD NMR Center). ^{13}C NMR spectra were recorded on a Bruker WP-200 SY spectrometer. Optical rotations were measured on Rudolph Research Autopol III polarimeter and CD spectra were obtained using a Jasco J-720 CD spectropolarimeter. Low resolution mass spectra were recorded on a Hewlett Packard 5988A spectrometer. High resolution mass spectra were measured on a VG ZAB mass spectrometer at the Regional Mass Spectrometry Facility, UC Riverside.

Collection, Extraction and Purification: Three specimens (1.44 g dry wt. total) of *Sclerodoris tanya* (Marcus, 1971) were collected in the intertidal zone at South Casa Reef, La Jolla, CA in August 1993. The specimens were maintained in aquaria for several weeks during an unsuccessful attempt to find their food source. Each specimen was individually extracted with acetone for 3 days, then with methanol overnight. For each specimen, the extracts were combined, the solvents evaporated and the resulting aqueous suspension diluted with water (30 mL) and extracted with ethyl acetate (3 x 50 mL). Each organic extract was dried over anhydrous magnesium sulfate and the solvent evaporated to obtain an oily residue, which was subjected to flash chromatography on silica gel using an elution gradient from hexane to ethyl acetate. The fraction eluting with 10% ethyl acetate in hexane was purified by HPLC on a Partisil column (20% ethyl acetate in hexane) to obtain tanyolide A (**4**, 0.4 mg total, 0.03% dry wt.). The fraction eluting with 20% ethyl acetate in hexane was purified by HPLC on an Optisil 10 column (500 x 9.4 mm) using 40% ethyl acetate in hexane as eluant to obtain tanyolide B (**5**, 2.0 mg total, 0.14% dry wt.).

An additional 4 specimens (1.45 g dry wt.) were collected at the same site in July 1994. The specimens were immediately extracted with acetone (2 x 50 mL) for 4 days. The extracts were combined and chromatographed as before to obtain tanyolide A (**4**, 0.8 mg, 0.06% dry wt.) and tanyolide B (**5**, 12 mg, 0.83% dry wt.).

Tanyolide A (4): colorless oil; UV ($CHCl_3$) 241 nm (ε 7330); IR ($CHCl_3$) 3020, 2930, 1740, 1645, 1460, 1440, 1370, 1230, 1135, 1050 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (50 MHz, $CDCl_3$) see Table 1; CIMS *m/z* (int., %) 393 (1), 335 (26), 219 (100), 191 (8), 137 (15); HRCIMS, obsd. *m/z* = 412.2679, $C_{22}H_{38}NO_6$ $[M+NH_4]^+$ requires *m/z* = 412.2699.

Tanyolide B (5): colorless oil; $[\alpha]_D$ = -3.0° (*c* 0.1, $CHCl_3$); CD λ 216 nm (Δε +4.0), 200 nm (Δε -2.5); UV ($CHCl_3$) 241 nm (ε 7140); IR ($CHCl_3$) 3600, 3530, 3020, 2935, 1730, 1645, 1460, 1440, 1370, 1225, 1140, 1050 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) see Table 2; ^{13}C NMR (50 MHz, $CDCl_3$) see Table 2; CIMS *m/z* (int., %) 335 (10), 292 (10), 219 (100), 191 (14), 137 (28), 117 (24); HRCIMS, obsd. m/z = 353.2309, $C_{20}H_{33}O_5$ $[M+H]^+$ requires *m/z* = 353.2328.

(*R*)-1-*t*-Butyldimethylsilyloxy-3-(*p*-methoxyphenyl)methoxyl-2-propanol (11): *t*-Butyldimethylsilyl chloride (1.11 g, 7.4 mmol) was added to a solution of (*S*)-1-(*p*-methoxyphenyl)methoxyl-2,3-propanediol (**10**, 7.4 mmol), triethylamine (4.12 mL, 29.6 mmol), and 4-N,N-dimethylaminopyridine (DMAP, 0.14 g, 1.1 mmol) in dry dichloromethane (40 mL) and the solution was stirred at 25 °C for 16 hr. The reaction mixture was purified by chromatography on silica, using 20% ethyl acetate in hexane as eluant, to obtain the TBDMS ether **11** (1.7 g, 70%), as a colorless oil: $[\alpha]_D$ = -3.5° (*c* 0.3, $CHCl_3$); UV ($CHCl_3$) 273 nm (ε 1850), 239 nm (ε 1880); IR ($CHCl_3$) 3620, 3010, 2960, 2930, 2860, 1710, 1610, 1515, 1470, 1360, 1265 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.25 (d, 2H, *J* = 8 Hz), 6.86 (d, 2H, *J* = 8 Hz), 4.46 (s, 2H), 3.84 (m, 1H), 3.79 (s, 3H), 3.61 (m, 2H), 3.47 (m, 2H), 0.87 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ

159.2, 130.1, 129.3, 113.7, 73.0, 70.6, 70.5, 64.0, 55.1, 25.8, 18.2, -0.1; HRCIMS, obsd. m/z = 325.1851, $C_{17}H_{29}O_4Si$ [M-H] requires *m/z* = 325.1851.

Coupling of monocyclofarnesoic acid (8) with alcohol 11: A 1.0 M solution of dicyclohexyl-carbodiimide (DCC) in dichloromethane (0.1 mL, 0.1mmol) was added to a solution of monocyclofarnesoic acid (**8**, 20 mg, 0.08 mmol), which was prepared from β-ionone (**6**) using literature methods,[10,11] alcohol **11** (33 mg, 0.1 mmol), and DMAP (10 mg, 0.08 mmol) in dry dichloromethane (5 mL) and the solution was stirred at 25 °C for 4 days. Additional monocyclofarnesoic acid (**8**, 20 mg, 0.08 mmol) and DCC solution (0.1 mL, 0.1mmol) were added and stirring was continued for a further 6 days, when the alcohol was judged to be reacted, and the solvent was evaporated. The residue was chromatographed on silica using 10% ethyl acetate in hexane as eluant to obtain a fraction containing esters, which was further purified by HPLC on Optisil 10, using 10% ethyl acetate in hexane as eluant, to obtain the ester **12** (29 mg, 54%): colorless oil; $[\alpha]_D$ = -3.6° (*c* 0.3, $CHCl_3$); UV ($CHCl_3$) 240 nm (ε 7620); IR ($CHCl_3$) 3010, 2960, 2930, 2860, 1710, 1645, 1515, 1265 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.25 (d, 2H, *J* = 8 Hz), 6.86 (d, 2H, *J* = 8 Hz), 5.72 (br s, 1H), 5.06 (pent, 1 H, *J* = 5Hz), 4.48 (s, 2H), 3.79 (s, 3H), 3.75 (d, 2H, *J* = 5 Hz), 3.62 (d, 2H, *J* = 5 Hz), 2.19 (s, 3H), 2.12 (m, 2H), 1.91 (t, 2H, *J* = 6 Hz), 1.59 (s, 3H), 1.55 (m, 2H), 1.40 (m, 2H), 0.99 (s, 6H), 0.86 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.2, 161.0, 159.2, 136.2, 130.3, 129.3, 127.9, 115.1, 113.7, 72.9, 72.2, 68.1, 61.7, 55.2, 41.5, 39.8, 35.0, 32.7, 28.5, 27.0, 25.8, 19.8, 19.5, 19.0, 18.2, 0.0; HRCIMS, obsd. m/z = 545.3633, $C_{32}H_{53}O_5Si$ requires *m/z* = 545.3662.

Synthesis of Tanyolide B (5): 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 82 mg, 0.36 mmol) was added to a solution of ester **12** (98 mg, 0.18 mmol) in a biphasic mixture of dichloromethane (10 mL) and water (0.5 mL). After 1 hr., when the green color of the solution had changed to orange-brown, the reaction mixture was quenched with satd. sodium bicarbonate solution (20 mL.) and extracted with dichloromethane (3 x 30 mL). The combined organic extracts were washed with water (30 mL), dried over anhydrous magnesium sulfate, filtered, and the solvent evaporated to obtain an oily residue. The residue was dissolved in dry dichloromethane (15 mL) and triethylamine (68 μL, 0.45 mmol), DMAP (2.7 mg, 0.02 mmol), and acetic anhydride (23 μL, 0.25 mmol) were added to the resulting solution. After 30 min., the solvent was evaporated and the product was purified by chromatography on silica using an elution gradient from hexane to ethyl acetate to obtain the ester **13** (78 mg, 93%): oil; $[\alpha]_D$ = +10.5° (*c* 0.3, $CHCl_3$); UV ($CHCl_3$) 241 nm (ε 6190); IR ($CHCl_3$) 3010, 2960, 2930, 2860, 1735, 1645, 1265, 1225, 1145 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 5.67 (br s, 1H), 5.09 (pent, 1H, *J* = 5Hz), 4.31 (dd, 1H, *J* = 12, 5 Hz), 4.18 (dd, 1H, *J* = 12, 5 Hz), 3.74 (dd, 1H, *J* = 12, 5 Hz), 3.69 (dd, 1H, *J* = 12, 5 Hz), 2.17 (s, 3H), 2.13 (m, 4H), 2.04 (s, 3H), 1.88 (t, 2H, *J* = 6 Hz), 1.57 (s, 3H), 1.55 (m, 2H), 1.38 (m, 2H), 0.97 (s, 6H), 0.85 (s, 9H), 0.02 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.7, 165.9, 161.6, 136.1, 127.9, 114.7, 70.8,

62.9, 61.4, 41.5, 39.7, 35.0, 32.7, 28.5, 27.0, 25.7, 20.8, 19.8, 19.4, 19.0, 18.2, 0.0; HRCIMS, obsd. m/z = 467.3184 $[M+H]^+$, $C_{26}H_{47}O_5Si$ requires *m/z* = 467.3193.

Dowex AG50W-X8 (H^+) resin (100 mg) was added to a solution of the ester **13** (78 mg) in methanol (5 mL) and the mixture was stirred at 25 °C for 5 days. The resin was removed by filtration and the solvent was evaporated to give a residue that was purified by HPLC on Partisil, using 40% ethyl acetate in hexane as eluant, to obtain tanyolide B (**5**, 36 mg, 62%), identical in all respects, including CD spectrum, to the natural product, together with the diol **7** (8 mg, 14%): oil; ^1H NMR (200 MHz, $CDCl_3$) δ 5.73 (br s, 1H), 4.92 (pent, 1H, *J* = 5Hz), 3.82 (d, 4H, *J* = 5 Hz), 2.19 (s, 3H), 2.13 (m, 4H), 1.90 (t, 2H, *J* = 6 Hz), 1.58 (s, 3H), 1.55 (m, 2H), 1.38 (m, 2H), 0.97 (s, 6H).

Synthesis of the enantiomer of Tanyolide B (*ent*-5): Dowex AG50W-X8 (H^+) resin was added to a solution of the ester **12** (60 mg, 0.11 mmol) in methanol (5 mL) and the mixture was stirred at 25 °C for 3 days. The resin was removed by filtration, the solvent evaporated, and the residue redissolved in dichloromethane and passed through a short column of silica gel to remove solids. Acetic anhydride (20 μL, 0.2 mmol) was added to a solution of the residue in dichloromethane (15 mL) containing triethylamine (30 μL, 0.2 mmol) and DMAP (1.3 mg, 0.01 mmol). After 1 hr., the solvents were evaporated and the residue was purified by HPLC on Partisil using 20% ethyl acetate in hexane as eluant to obtain ester **14** as a clear oil (47 mg, 92%): $[\alpha]_D$ = -2.0° (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) 253 nm (ε 7690), 239 nm (ε 8120); IR ($CHCl_3$) 3020, 2960, 2930, 2860, 1718, 1605, 1515, 1215, 1115 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.19 (d, 2H, *J* = 8 Hz), 6.81 (d, 2H, *J* = 8 Hz), 5.67 (br s, 1H), 5.19 (m, 1H), 4.42 (s, 2H), 4.21 (s, 2H) 3.77 (s, 3 H), 3.53 (d, 2H, *J* = 5 Hz), 2.19 (s, 3H), 2.18 (m, 2H), 2.12 (m, 2H), 2.04 (s, 3H), 1.91 (t, 2H, *J* = 6 Hz), 1.59 (s, 3H), 1.55 (m, 2H), 1.40 (m, 2H), 0.99 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.7, 165.9, 162.1, 159.2, 136.0, 129.8, 129.3 (2C), 127.9, 114.5, 113.7 (2C), 72.9, 69.2, 67.8, 63.1, 55.2, 41.5, 39.8, 35.0, 32.7, 28.5, 27.0, 20.8, 19.8, 19.5, 19.0; EIMS *m/z* (intensity) 333 (1), 291 (1), 217 (8), 191 (8), 137 (25), 121 (100).

A solution of TFA (69 μL, 0.9 mmol) in dichloromethane (1 mL) was added to a chilled solution of ester **14** (14 mg, 0.03 mmol) and anisole (97 μL, 0.9 mmol) in dichloromethane (2 mL) and the mixture was stirred at 0 °C for 1 hr. The solvents and excess reagents were removed under high vacuum and the residue was purified by HPLC on Partisil using 40% ethyl acetate in hexane as eluant to obtain the alcohol (ent-**5**, 8.5 mg, 87%), which had spectroscopic data identical to that of tanyolide B (**5**), except that the CD spectrum was essentially opposite in sign: CD λ 216 nm (Δε -2.6), 200 nm (Δε +2.6).

Synthesis of Tanyolide A (4): A 1.0 M solution of DCC in dichloromethane (0.57 mL, 0.57 mmol) was added to a solution of monocyclofarnesoic acid (**8**, 112 mg, 0.47 mmol), 1,3-diacetylglycerol (83 mg, 0.47 mmol), and DMAP (29 mg, 0.24 mmol) in dry dichloromethane (5 mL) and the solution was stirred at 25

°C for 5 hr. Additional monocyclofarnesoic acid (**8**, 90 mg, 0.38 mmol) and DCC solution (0.23 mL, 0.23 mmol) were added and stirring was continued for a further 16 hr., when the solvent was evaporated. The residue was chromatographed on silica gel, using 20% ethyl acetate in hexane as eluant, to obtain a fraction containing esters, which was further purified by HPLC on Partisil, using 20% ethyl acetate in hexane as eluant, to obtain tanyolide A (**4**, 68 mg, 37%), identical in all respects to the natural product.

Fish Feeding Assay: The method of Pawlik and Fenical[18] is modified to use coated pellets. Pellets (120 mg dry wt., 2 mL hydrated volume) of 5:1 squid-alginic acid were made to approximate the size of an individual *S. tanya*. Treatment pellets were coated with a known amount of compound in acetone (0.1 mL) and the solvent was evaporated; control pellets were treated with acetone only. Pairs of treated and control pellets were offered to 10 specimens of the predatory fish *Gibbonsia elegans* and *Paraclinis integrippinis* collected from the same tidepools as the nudibranchs. A negative feeding response was scored when the fish swallowed then spat out a pellet three consecutive times. Fish were fed untreated pellets before and after each assay to ensure that all were feeding normally.

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