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ANTIOXIDANT METABOLITES FROM THE TUNICATE *AMAROUCIUM MULTIPLICATUM*

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ABSTRACT.—The antioxidant activity-directed analysis of the extract of the colonial tunicate *Amaroucium multiplicatum* enabled isolation of one novel chromene **2** and two novel hydroquinones **4** and **5**, as well as previously reported compounds, as active compounds. Their structures were determined by spectroscopic data (^{1}H nmr, ^{13}C nmr, ms, uv, and ir) and confirmed by chemical synthesis. The isolated compounds were more potent than two standard antioxidants on the inhibitory effects on lipid peroxide formation in rat liver microsomes and on soybean 15-lipoxygenase.

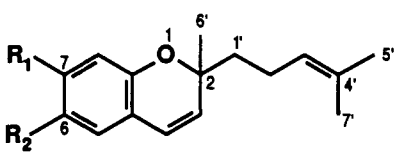
Excessive formation and accumulation of lipid peroxides may contribute to such cardiovascular diseases as arteriosclerosis, hypertension, and cardiac insufficiency. Recently, it has been reported (1,2) that probucol, a drug currently in clinical use for treatment of arteriosclerosis, prevented the progression of arteriosclerosis in Watanabe heritable hyperlipidemic (WHHL) rabbits, an animal model for familial hypercholesterolemia, without significant reduction of the plasma cholesterol levels. These reports suggest that the mechanism of action of probucol is due to its antioxidant properties.

In order to isolate antioxidant compounds as candidates or synthetic models for development of drugs applied to treatment of the diseases mentioned above, we have systematically undertaken pharmacological screenings of the extracts of marine organisms. The extracts were screened for inhibitory effects on lipid peroxide formation in rat liver microsomes (3) and on soybean 15-lipoxygenase (4). The extracts of about 100 marine organisms were tested to find several active ones (5).

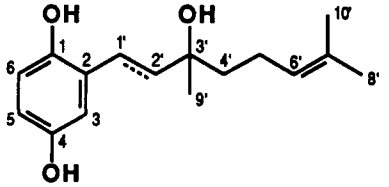
Here we report the isolation, characterization, synthesis, and antioxidant activities of the active principles (6,7) from the colonial tunicate, *Amaroucium multiplicatum* (Sluiter) Polyclinidae, which can be found, associated with other organisms attached to each other, not only on rocks, but also particularly on such artificial structures as banks, bridges, and aquaculture facilities in calm bays of central to southern Japan.

RESULTS AND DISCUSSION

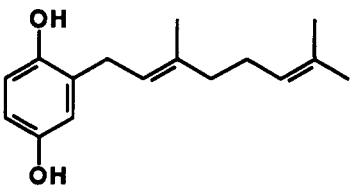
A. multiplicatum (wet wt 14 kg) was extracted with MeOH. The extract was frac-



1 R_1=H, R_2=OH
2 R_1=OMe, R_2=OH
3 R_1=OH, R_2=OMe



4 C-1′–C-2′=single bond
5 C-1′–C-2′=*E* double bond



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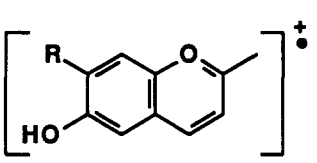
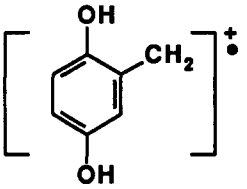
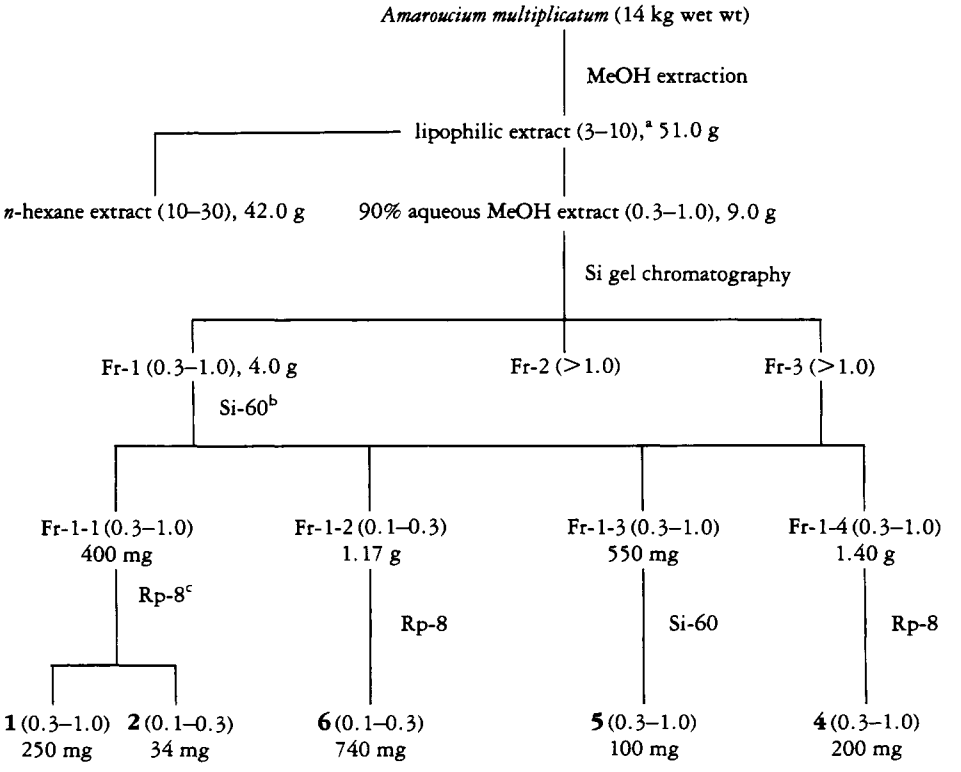


A R=OMe *m/z* 191.06877



B *m/z* 123.17097

tionated and purified by chromatography directed by the inhibitory effect on lipid peroxide formation in rat liver microsomes as shown in Scheme 1. From the least polar fraction (Fr-1-1), one of the most active compounds, compound **2**, was isolated. Compound **2**, optically inactive, has a molecular formula of $C_{17}H_{22}O_3$ (hrms $[M]^+$ *m/z* 274.13591). The uv absorption [λ max nm (ε) 276 (1000), 326 (3000)] is indicative of the presence of a 2H-1-benzopyran (chromene) skeleton, which is supported by the most abundant fragment ion (**A**, *m/z* 191.06877) in the mass spectrum. The ^{1}H-nmr spectrum of **2** indicates three methyl singlets, one of which is attached to the carbon (C-2) functionalized with an oxygen atom (δ 1.36); the other two are located on C-4′. A set of multiplets coupled to each other (δ 1.6–1.8 and 2.0–2.2) can be assigned to two adjacent methylene groups at C-1′ and C-2′, respectively. The olefinic proton (δ 5.09 bt,



SCHEME 1. Isolation procedure of chromenes and hydroquinones.

[a]Numbers in parentheses represent the concentration required for 50% inhibition of lipid peroxide formation in the rat liver microsomes (IC_{50} μg/ml).

[b]Si-60 = Merck normal phase Lobar cc.

[c]Rp-8 = Merck reversed-phase Lobar cc.

$J = 6.8$ Hz) couples not only to the methylene protons (H-2′) vicinally, but also to the olefinic methyls via long-range coupling. In addition to a methoxyl singlet, there was observed a pair of doublets (δ 5.45 and 6.25) assignable to two olefinic protons on the pyran ring and two isolated aromatic proton singlets (δ 6.38 and 6.56). These spectral features are compatible both with **2** and its positional isomer **3**. Unambiguous synthesis of **2** described later consequently revealed that **2** could be depicted as 6-hydroxy-7-methoxy-2-methyl-2-(4-methylpent-3-enyl)-2H-1-benzopyran.

Purification of the fraction Fr-1-4 by reversed-phase chromatography yielded an optically inactive compound, compound **4**, which has a molecular formula of $C_{16}H_{24}O_3$ (hrms $[M]^+$ *m/z* 264.17097). The uv absorption [λ max nm (ε) = 293 (3000)] and ms [**B**, base peak *m/z* 123.17097] spectra indicated the presence of a hydroquinone moiety. Comparison of the ^{1}H-nmr and ^{13}C-nmr spectra of **4** and geranylhydroquinone **6** isolated together clarified that **4** had a hydrated structure of the $\Delta^{2,3}$-double bond of **6** shown as 2-(3-hydroxy-3,7-dimethyloct-6-enyl)-1,4-benzenediol.

The Si gel chromatography of fraction Fr-1-3 gave an optically inactive compound, compound **5**, of molecular formula $C_{16}H_{22}O_3$. Compound **5** could be depicted as 2-(2*E*)-(3-hydroxy-3,7-dimethyloct-2,6-dienyl)-1,4-benzenediol, based on the trans olefinic proton signals [δ 6.07 (d, $J = 17.0$ Hz) and 6.84 (d, $J = 17.0$ Hz)] in the ^{1}H-nmr spectrum and the cyclization to the corresponding chromene **1** on standing at room temperature. Together with these three new compounds, **2**, **4**, and **5**, two known compounds, **1** and **6**, were obtained and identified (8–10) by spectral data and unambiguous syntheses.

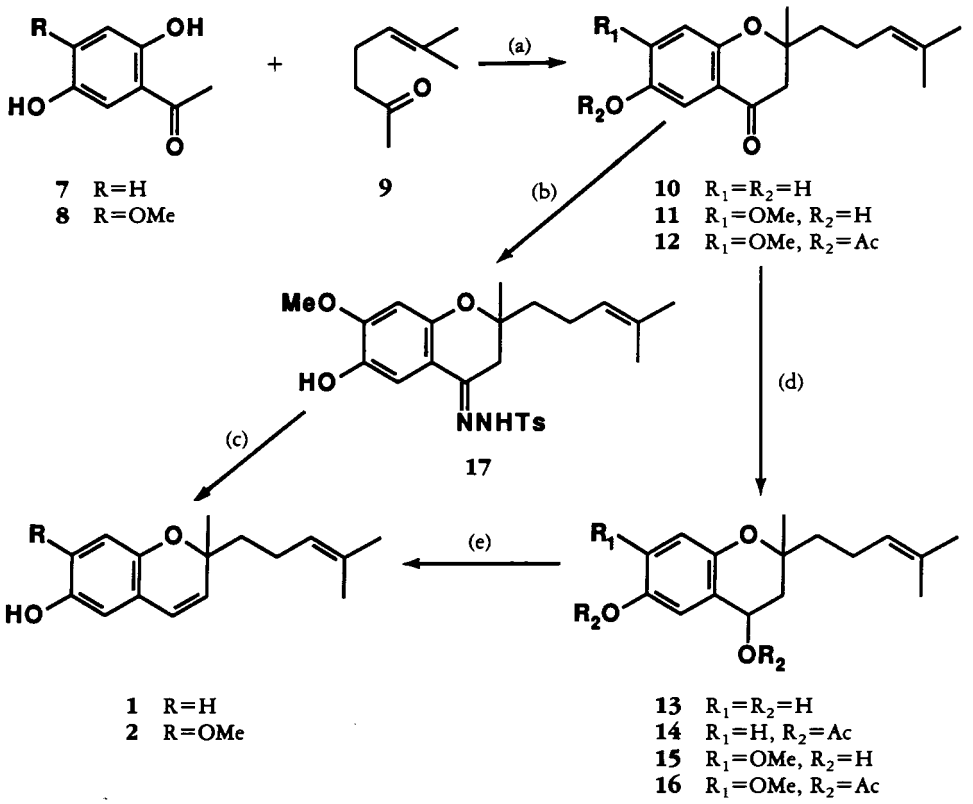
2,5-Dihydroxyacetophenones **7** and **8** and 2-methyl-2-hepten-6-one **9** were condensed to 4-chromanones **10** and **11** in the presence of pyrrolidine (11). The chromanones **10** and **11** were smoothly reduced to a diastereomeric mixture of corresponding 4-chromanols **13** and **15** on treatment with $LiAlH_4$ in dry THF. On heating in DMSO at 180°, the corresponding acetates **14** and **16** were converted to the desired chromene **1** and **2**, followed by alkaline hydrolysis. The Bamford-Stevens reaction (12) of the tosylhydrazone **17**, obtained by condensation of **12** with tosylhydrazine, gave **2** in the presence of Na in ethylene glycol at 120° (Scheme 2). The hydroxy and methoxy groups must therefore be situated on C-6 and C-7, respectively.

The terpene hydroquinones have been isolated from a broad range of phyla of marine organisms including algae (13–18), tunicates (8,9,19), octocorals (20–23), sponges (24–26), and molluscs (27). Interestingly, Targett and Keeran (8) have reported that some terpene hydroquinones possess cancer-protective activities, and most cancer-protective agents seem to have antioxidant activities. The antioxidant activities of **1**, **2**, and **4–6** are summarized in Table 1. They are more potent than the standards, α-tocopherol acetate and 2,6-di-*tert*-butyl-*p*-cresol in the two assay systems. In vivo tests are underway in an attempt to elucidate the relationship between antioxidant activity with hypolipidemic and cancer-protective activities.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured on a Yanagimoto hot stage apparatus and were uncorrected. The ^{1}H- and ^{13}C-nmr spectra were recorded on a JEOL JNM-GX-270 and a Varian EM-360 with TMS as internal standard in $CDCl_3$ (unless otherwise stated), and assignments with same superscript may be interchanged. The ir spectra were recorded on a Nicole 5SXC. The uv spectra were recorded on a Cary 118C in EtOH. The mass spectra were recorded on JEOL JMS-D300.

TUNICATE COLLECTION.—*A. multiplicatum* was collected at floats and underwater ropes of pearl aquaculture stations in Uranouchi Bay, Kochi, Japan, in 1985 and 1986 and was authenticated by Prof. M. Nakauchi, Department of Biology, Kochi University. The voucher specimen is deposited in our laboratories at Kochi University.



SCHEME 2. Reaction sequence for the synthesis of chromenes. The reaction conditions were as follows: (a) pyrrolidine/toluene; (b) 60°/H_2NNTS/HOAc; (c) 120°/$NaOCH_2CH_2OH$; (d) $LiAlH_4$/THF; (e) 180°/DMSO, 5% NaOH.

EXTRACTION AND ISOLATION.—A frozen specimen (14 kg) of the tunicate was thrice extracted with MeOH (35 liters) at room temperature. The combined extract was concentrated to dryness under reduced pressure. The EtOAc solution of the residue was washed with H_2O and dried over Na_2SO_4. Removal of EtOAc gave a brown oil (53.0 g, IC_{50} 3–10 μg/ml), which was partitioned with *n*-hexane and 90% aqueous MeOH into an *n*-hexane fraction (42.0 g, IC_{50} 10–30 μg/ml) and a 90% aqueous MeOH fraction (9.0 g, IC_{50} 0.3–1.0 μg/ml). The 90% aqueous MeOH fraction was subjected to Si gel chromatography (90 g of SiO_2). Elution with 30% EtOAc/*n*-hexane gave an active fraction (Fr-1, 4.0 g, IC_{50} 0.3–1.0 μg/ml). The more polar fractions (Fr-2 and Fr-3) showed no activity.

Fr-1 was further fractionated to four fractions, Fr-1-1 (eluted with 10% EtOAc/*n*-hexane, 400 mg, IC_{50} 0.3–1.0 μg/ml), Fr-1-2 (20% EtOAc/*n*-hexane, 1.17 g, IC_{50} 0.1-0.3 μg/ml), Fr-1-3 (30% EtOAc/*n*-hexane, 550 mg, IC_{50} 0.3–1.0 μg/ml), and Fr-1-4 (40% EtOAc/*n*-hexane, 1.40 g, IC_{50} 0.3–1.0 μg/ml). The more polar fractions were inactive. Fr-1-1 was separated by reversed-phase chromatography

TABLE 1. Inhibitory Activities of Compounds **1**, **2**, **4**–**6** on Lipid Peroxide Formation and 15-Lipoxygenase.[a]

Compound	Lipid Peroxide (Rat Liver Microsome)	15-Lipoxygenase (Soybean)
1	0.3–1.0	0.2
2	0.1–0.3	0.5
4	0.3–1.0	3.0
5	0.3–1.0	—
6	0.1–0.3	0.2
α-Tocopherol acetate	>10.0	>10.0
2,6-Di-*tert*-butyl-*p*-cresol	0.3–1.0	>10.0

[a]Table entries are IC_{50} in μg/ml.

(Merck Rp-8 Lobar column eluted with 85% aqueous MeOH) to yield **1** as a colorless oil (250 mg, IC_{50} 0.3–1.0 μg/ml) and **2** as a colorless oil (34 mg, IC_{50} 0.1–0.3 μg/ml).

Fr-1-2 was further purified to a main compound, geranylhydroquinone **6**, as a colorless oil (740 mg, IC_{50} 0.1–0.3 μg/ml) by reversed-phase chromatography eluted with 80% aqueous MeOH. Elution with 30% EtOAc/*n*-hexane on Si gel chromatography (Merck Si-60 Lobar column) gave an unstable compound **5** as a colorless oil (100 mg, IC_{50} 0.3–1.0 μg/ml), and compound **4** as a colorless oil (200 mg, IC_{50} 0.3–1.0 μg/ml) was obtained by reversed-phase chromatography of Fr-1-4 eluted with 70% aqueous MeOH.

COMPOUND **2**.—Ir ν max cm^{-1} (film) 3350, 1625, 1585, 1290; uv λ max nm (ε) 276 (1000), 326 (3000); ^{1}H nmr δ 1.36 (3H, s, C-6′), 1.58 (3H, s, H-7′), 1.66 (3H, s, H-5), 1.6–1.8 (2H, m, H-1′), 2.0–2.2 (2H, m, H-2′), 3.84 (3H, s, 7-OC*H*$_3$), 5.09 (1H, bt, J = 6.8 Hz, H-3′), 5.45 (1H, d, J = 9.8 Hz, H-3), 6.25 (1H, d, J = 9.8 Hz, H-4), 6.38 (1H, s, H-8), 6.56 (1H, s, H-5); ^{13}C nmr δ 17.6 (q, C-7′), 22.7 (t, C-2′), 25.6[a] (q, C-6′), 26.0[a] (q, C-5′), 40.9 (t, C-1′), 55.9 (q, 6-O*C*H$_3$), 78.0 (s, C-2), 100.0 (d, C-8), 111.6 (d, C-5), 113.8 (s, C-4a), 122.3 (d, C-4), 124.1 (d, C-3′), 127.4 (d, C-3), 131.4 (s, C-4′), 139.1 (s, C-6), 146.5 (2 × s, C-7, -8a) (values with the same superscript may be interchanged); eims *m/z* [M]$^+$ 274.15391 ($C_{17}H_{22}O_3$), 159, 231, 191.06877 (base peak, $C_{11}H_{11}O_3$), 176, 69.

COMPOUND **4**.—Ir ν max cm^{-1} (film) 3340, 1655, 1600, 1500; uv λ max nm (ε) 293 (3000); ^{1}H nmr δ 1.24 (3H, s, H-9′), 1.62 (3H, s, H-10′), 1.69 (3H, s, H-8′), 1.57 (2H, t, J = 7.5 Hz, H-4′), 1.76 (2H, t, J = 7.5 Hz, H-2′), 2.05 (2H, t, J = 7.5 Hz, H-5′), 2.65 (2H, t, J = 7.5 Hz, H-1′), 5.13 (1H, bt, J = 7.0 Hz, H-6′), 6.57 (1H, dd, J = 7.8, 3.4 Hz, H-5), 6.58 (1H, d, J = 3.4 Hz, H-3), 6.70 (1H, d, J = 7.8 Hz, H-6); ^{13}C nmr δ 17.7 (q, C-10′), 23.7 (t, C-5′), 25.9 (q, C-9′), 26.0 (t, C-1′), 27.0 (q, C-8′), 42.5[a] (t, C-2′), 42.7[a] (t, C-4′), 73.3 (s, C-3′), 113.8[b] (d, C-3), 116.5[b] (d, C-5), 117.3[b] (d, C-6), 125.7 (d, C-6′), 131.2[c] (s, C-2), 131.8[c] (s, C-7′), 148.9[d] (s, C-1), 150.9[d] (s, C-4) (values with same superscript may be interchanged); eims *m/z* [M]$^+$ 264.17097 ($C_{16}H_{24}O_3$), 246, 163, 161, 123.17097 (base peak, $C_7H_7O_2$).

COMPOUND **5**.—^{1}H nmr δ (Me_2CO-d_6) 1.33 (3H, s, H-9′), 1.60 (3H, s, H-10′), 1.4–1.8 (2H, m, H-2′, -4′), 1.8–2.3 (2H, m, H-1′, -5′), 5.10 (1H, bt, J = 6.8 Hz, H-6′), 6.10 (1H, d, J = 18.0 Hz, H-2′), 6.6–7.0 (3H, m, H-3, -5, -6), 6.97 (1H, d, J = 18.0 Hz, H-1′); ^{13}C nmr δ 17.6 (q, C-10′), 22.5 (t, C-5′), 23.2 (q, C-9′), 25.8 (q, C-8′), 40.9 (t, C-4′), 77.6 (s, C-3′), 112.9[a] (d, C-2), 115.8[a] (d, C-5), 117.0[a] (d, C-6), 125.1[b] (d, C-6′), 125.3[b] (d, C-2′), 125.4 (s, C-6′), 131.3 (s, C-7′), 134.7 (d, C-1′), 148.3[c] (s, C-1), 151.0[c] (s, C-4) (values with the same superscript may be interchanged); eims *m/z* 264, 244, 229, 201, 196, 161, 69.

4-CHROMANONE **10**.—Pyrrolidine (6.12 g) was add to a solution of 2,5-dihydroxyacetophenone **7** (10.15 g) and 2-methyl-2-hepten-6-one **9** (11.12 g) in toluene (120 ml). The mixture was kept at room temperature overnight and refluxed for 6 h with azeotropic removal of H_2O formed. After the reaction was complete, the reaction mixture was washed with cold 6 N HCl and H_2O successively and then was dried over Na_2SO_4. Removal of the solvent under reduced pressure gave a red oil (20 g), which was subjected to Si gel chromatography (240 g). Elution with 10% EtOAc/*n*-hexane gave **10** (11.50 g, mp 88–88.5°) followed by recrystallization from *n*-hexane/EtOAc: ir ν max cm^{-1} ($CHCl_3$) 3300, 1665, 1615, 1450, 1370, 1300; uv λ max nm (ε): 226 (16700), 255 (6200), 360 (3500); ^{1}H nmr δ 1.40 (3H, s, H-6′), 1.57 (3H, s, H-7′), 1.66 (3H, s, H-5′), 1.6–1.9 (2H, m, H-1′), 2.0–2.2 (2H, m, H-2′), 2.72 (2H, ABq, J = 16.9 Hz, H-3), 5.06 (1H, bt, J = 7.0 Hz, H-3′), 6.07 (1H, s, 6-OH), 6.84 (1H, d, J = 8.4 Hz, H-8), 7.07 (1H, dd, J = 8.4, 2.9 Hz, H-7), 7.38 (1H, d, J = 2.9 Hz, H-5); ^{13}C nmr δ 17.5 (q, C-7′), 22.2 (t, C-2′), 23.8 (q, C-6′), 25.6 (q, C-5′), 39.6 (t, C-1′), 47.4 (t, C-3), 80.7 (s, C-2), 110.6 (d, C-8), 119.5 (d, C-7), 120.0 (s, C-4a), 123.1 (d, C-3′), 125.3 (d, C-5), 132.3 (s, C-4′), 149.9 (s, C-6), 154.1 (s, C-8a), 194.1 (s, C-4); eims *m/z* 260, 177, 137, 109, 69; elemental analysis calcd for $C_{16}H_{20}O_3$, C 73.85, H 7.69, found C 73.95, H 7.60.

COMPOUND **11**.—By the above method, 2,5-dihydroxy-4-methoxyacetophenone **8** (5.80 g) gave **11** (5.79 g, mp 103°) followed by recrystallization from *n*-hexane/EtOAc: ir ν max cm^{-1} ($CHCl_3$) 3550, 3300, 1670, 1625, 1500, 1290, 1270; uv λ max nm (ε) 239 (16000), 273 (9800), 346 (6000); ^{1}H nmr δ 1.40 (3H, s, H-6′), 1.58 (3H, s, H-7′), 1.67 (3H, s, H-5′), 1.6–1.9 (2H, m, H-1′), 2.0–2.2 (2H, m, H-2′), 2.58 (2H, ABq, J = 16.5 Hz, H-3), 3.92 (3H, s, 7-OMe), 5.08 (1H, bt, J = 7.0 Hz, H-3′), 5.25 (1H, s, 6-OH), 6.39 (1H, s, H-8), 7.33 (1H, s, H-5); ^{13}C nmr δ 17.6 (q, C-7′), 22.3 (t, C-2′), 23.8 (q, C-6′), 25.6 (q, C-5′), 39.4 (t, C-1′), 47.1 (t, C-3), 56.1 (q, 7-O*C*H$_3$), 81.2 (s, C-2), 98.9 (d, C-8), 109.9 (d, C-5), 113.2 (s, C-4a), 123.2 (d, C-3′), 132.0 (s, C-4′), 140.1 (s, C-6), 153.8[a] (s, C-7), 155.2[a] (s, C-8a), 191.1 (s, C-4) (values with the same superscript may be interchanged); eims *m/z* 290, 207, 167, 109, 69; elemental analysis calcd for $C_{17}H_{22}O_4$, C 70.35, H 7.59, found C 70.30, H 7.50.

COMPOUND **12**.—Compound **11** (100 mg) was dissolved in Ac_2O (0.5 ml) and pyridine (0.5 ml).

The mixture was kept at room temperature for 1 h. Compound **12** (103 mg, mp 127–128°) was purified by recrystallization from *n*-hexane/EtOAc: ir ν max cm^{-1} ($CHCl_3$) 1760, 1675, 1620, 1580, 1500, 1450, 1370, 1280, 1160; uv λ max nm (ϵ) 232 (16800), 267 (12600), 318 (6500); ^1H nmr δ 1.42 (3H, s, H-6′), 1.56 (3H, s, H-7′), 1.67 (3H, s, H-5′), 1.6–1.9 (2H, m, H-1′), 2.0–2.2 (2H, m, H-2′), 2.29 (3H, s, 6-OAc), 2.66 (2H, ABq, J = 16.5 Hz, H-3), 3.85 (3H, s, 7-OCH_3), 5.08 (1H, bt, J = 7.0 Hz, H-3′), 6.44 (1H, s, H-8), 7.50 (1H, s, H-5); ^{13}C nmr δ 17.6 (q, C-7′), 20.4 (q, 6-$OCOCH_3$), 22.3 (q, C-2′), 23.9 (q, C-6′), 25.6 (q, C-5′), 39.4 (t, C-1′), 46.8 (t, C-3), 5.61 (q, 7-OCH_3), 81.8 (s, C-2), 100.9 (d, C-8), 113.0 (s, C-4a), 119.7 (d, C-5), 123.1 (d, C-3′), 132.1 (s, C-4′), 134.2 (s, C-6), 157.6[a] (s, C-7), 159.4[a] (s, C-8a), 168.8 (s, $OCOCH_3$), 190.2 (s, C-4), (values with the same superscript may be interchanged); eims *m/z* 332, 290, 207, 167, 109, 69; elemental analysis calcd for $C_{19}H_{24}O_5$, C 68.68, H 7.23, found C 68.79, H 7.16.

$NaBH_4$ REDUCTION OF **10**.—To a solution of $NaBH_4$ (1.20 g) in EtOH (30 ml) was added a solution of **10** (2.80 g) in EtOH (10 ml). The mixture was stirred at room temperature overnight. The reaction mixture was subjected to Si gel chromatography (60 g). Elution with 30% EtOAc/*n*-hexane gave an epimeric mixture of **13** (2.54 g), which was also prepared by $LiAlH_4$ reduction: ir ν max cm^{-1} ($CHCl_3$) 3350, 1620, 1495, 1450, 1380, 1200; uv λ max nm (ϵ) 229 (5800), 299 (3200); eims *m/z* 262, 244, 229, 201, 177, 161, 139, 138, 111, 69.

$LiAlH_4$ REDUCTION OF **12**.—To a solution of $LiAlH_4$ (0.50 g) in dry THF (40 ml) was dropwise added a solution of **12** (4.00 g) in dry THF (10 ml) at 0–5°. After careful treatment with HOAc at 0–5°, an EtOAc extract of the mixture gave an epimeric mixture of **14** (3.60 g): ir ν max cm^{-1} ($CHCl_3$) 3550, 1630, 1600, 1500, 1450, 1380, 1280, 1260; uv λ max nm (ϵ) 295 (5000); eims *m/z* 292, 274, 259, 231, 207, 191, 169, 168, 153, 109, 69.

ELIMINATION REACTION.—A solution of **14** (4.44 g) in DMSO (44 ml) was heated at 180° under N_2 for 40 min. *n*-Hexane and H_2O were then added to the reaction mixture. The *n*-hexane layer was washed with H_2O and dried over Na_2SO_4. Removal of the solvent under reduced pressure gave a light yellow oil (3.96 g), which was subjected to Si gel chromatography (120 g). Elution with 10% EtOAc/*n*-hexane gave a desired acetate (1.76 g) of **1** and the starting material **14** (2.06 g). The acetate of **1** was further purified by chromatography using 5% $AgNO_3$-impregnated SiO_2 (40 g). The eluent with 5% EtOAc/*n*-hexane was hydrolyzed with 5% NaOH to yield **1** (911 mg). By the above method, **2** (300 mg) was obtained from **15** (4.0 g).

TOSYLHYDRAZONE **17**.—A mixture of **12** (500 mg) and tosylhydrazine (780 mg) in HOAc (2 ml) was heated at 60° for 8 h. After the reaction was complete, removal of HOAc under reduced pressure gave an oily product, which was subjected to Si gel chromatography (30 g). Elution with 30% EtOAc/*n*-hexane gave a desired tosylhydrazone **17** (402 mg, mp 131–133°) followed by recrystallization from *n*-hexane/Et_2O: ir ν max cm^{-1} ($CHCl_3$) 3550, 3300, 3200, 1630, 1610, 1600, 1500, 1460, 1455, 1380, 1315, 1270, 1175, 1070; uv λ max nm (ϵ) 275 (12300), 334 (11400); ^1H nmr δ 1.29 (3H, s, H-6′), 1.55 (3H, s, H-7′), 1.60 (2H, m, H-1′), 1.65 (3H, s, H-5′), 2.03 (2H, m, H-2′), 2.42 (3H, s, 4″-CH_3), 2.50 (2H, ABq, J = 16.1 Hz, H-3), 3.85 (3H, s, 7-OCH_3), 5.01 (1H, bt, J = 7.0 Hz, H-3′), 5.20 (1H, s, 6-OH), 6.31 (1H, s, H-8), 7.31 (1H, s, -$NHSO_2$-), 7.33 (2H, d, J = 8.0 Hz, H-3″, -5″), 7.43 (1H, s, H-5), 7.90 (2H, d, J = 8.0 Hz, H-2″, 6″); ^{13}C nmr δ 17.5 (q, C-7′), 21.6 (q, 4″-CH_3), 22.2 (t, C-2′), 24.6 (q, C-6′), 25.6 (q, C-5′), 34.3 (t, C-3), 39.0 (t, C-1′), 55.9 (q, 7-OMe), 77.4 (s, C-2), 100.1 (d, C-8), 108.6 (d, C-5), 111.2 (s, C-4a), 123.3 (d, C-3′), 127.9 (2 × d, C-2″, -6″), 129.4 (2 × d, C-3″, -5″), 131.8 (s, C-4′), 135.1 (s, C-4″), 139.7[a] (s, C-1″), 143.9[a] (s, C-6), 148.8[b] (s, C-7), 149.4[b] (s, C-8a), 149.9[b] (s, C-4) (values with the same superscript may be interchanged); eims *m/z* 274, 259, 191, 156, 91; elemental analysis calcd for $C_{24}H_{30}N_2O_5S$, C 62.88, H 6.55, found C 62.73, H 6.51.

THE BAMFORD-STEVENS REACTION OF **17**.—The tosylhydrazone **17** (100 mg) was added to a solution prepared from Na (50 mg) and ethylene glycol (10 ml). The mixture was heated at 120° for 1 h. Removal of Et_2O gave an oily product (109 mg), which was subjected to Si gel chromatography (Merck Si-60 Lobar column). Elution with 10% EtOAc/*n*-hexane yielded **2** (15 mg).

LIPID PEROXIDE FORMATION INHIBITORY EFFECT.—To a solution (1.2 ml) of 0.1 M Tris HCl buffer (0.4 ml, pH 7.4) and 0.15 M KCl (0.8 ml) was added a DMSO solution (15 μl) of an inhibitor. This solution was treated with rat liver microsomes (300 μl) and preincubated at 37° for 5 min. Peroxidation was initiated by the addition of a solution (30 μl) of 0.5 mM $FeSO_4$ (15 μl) and 50 mM L-cysteine (15 μl). After incubation at 37° for 30 min, the peroxidation was stopped by the addition of ice-cold 10% trichloroacetic acid (3 ml). The supernatant fluid (1 ml) obtained by centrifugation at 2150*g* for 10 min was added to a 0.67% solution of thiobarbituric acid in 50% aqueous HOAc (3 ml). After the mixture was heated at 100° for 15 min, the optical density was measured at 535 nm.

LIPOXYGENASE INHIBITORY EFFECT.—To a sample chamber containing 0.1 M KP buffer (2.785 ml, pH 8.0) was added a solution of 30 mM $CaCl_2$ (100 μl). After standing at 25° for 3 min, the solution was mixed with KP buffer (100 μl) containing 0.25 mg/ml of soybean lipoxygenase and then with a DMSO solution (5 μl) of an inhibitor. After preincubation at 25° for 5 min, reaction was initiated by the addition of EtOH (10 μl) containing 50 mg/ml of arachidonic acid. Decrease in remaining oxygen content of the buffer solution was measured by the oxygen electrode method (4).

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