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5-Fluorouracil Derivatives from the Sponge *Phakellia fusca*

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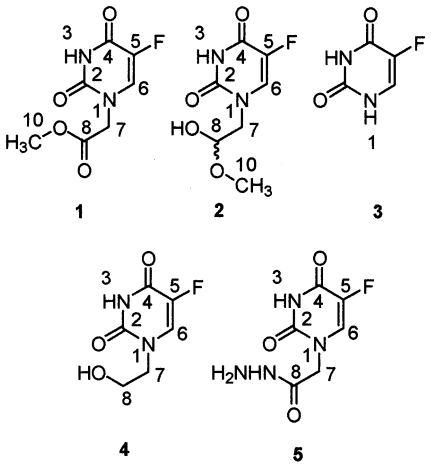
5-Fluorouracil derivatives were isolated from the marine sponge *Phakellia fusca* collected around the Yongxing Island of the Xisha Islands in the South Sea of China. Their structures were determined on the basis of spectral analysis and X-ray diffraction.

It is well known that naturally occurring organohalogen compounds are abundant in plants, fungi, microorganisms, and especially marine invertebrates.[1] Surprisingly, although fluorine is the most abundant halogen in Earth's crust, fluorinated natural products are very rare.[2] Since the first organo-fluorine compound, fluoroacetate, was identified in 1943 from the South African plant *Dichapetalum cymosum*,[3] only 13 fluorine-containing secondary metabolites have been isolated from plants and microorganisms. These include fatty acid homologues,[4] fluorothreonine,[5] and nucleocidin.[6] Recently, in the course of our systematic search for bioactive substances from marine organisms, we investigated the marine sponge *Phakellia fusca* Schmidt, collected in the Yongxing Island of the Xisha Islands in the South China Sea, and isolated five fluorinated natural products. This is the first report of fluorine-containing natural products from a marine source.

Sponges of the genus *Phakellia* have been reported to yield alkaloids,[7] sterols,[8] acetylenic acids,[9] peptides,[10] diterpene isonitriles,[11] polyether macrolides,[12] and polyether acids.[13] We have previously studied the chemistry of the marine sponge *Phakellia fusca* Schmidt and obtained seven *A*-nor-sterols,[14] two known alkaloids, dibromophakellin, monobromophakellin,[15] aldisin,[16] 2-bromoaldisin,[17] and a new cerebroside.[18] In the present study we report the isolation and structures of five 5-fluorouracil alkaloids from the *n*-butanol-soluble fraction of this sponge. Among the five 5-fluorouracil alkaloids, compound **3**[19] and **4**[20] were previously known as synthetic compounds and antitumor agents,[21] whereas compounds **1**, **2**, and **5** are new. In this paper, we report the isolation and structural elucidation of these 5-fluorouracil derivatives.

Sun-dried specimens of the sponge were extracted with 90% aqueous ethanol, and the extract was evaporated under reduced pressure to give a residue that was partitioned between chloroform and water and then between *n*-butanol and water. The *n*-butanol-soluble fractions were condensed and subjected to silica gel column chromatography. Elution with CH_2Cl_2 containing increasing amounts of MeOH yielded 20 fractions. Each fraction was purified further by flash chromatography. Fractions eluted with CH_2Cl_2−MeOH (25:1), CH_2Cl_2−MeOH (20:1), CH_2Cl_2−MeOH (18:1), CH_2Cl_2−MeOH (15:1), and CH_2Cl_2−MeOH (15:1) resulted in compounds **1**−**5**, respectively.

The ^{1}H NMR and ^{13}C NMR spectra of compounds **1**−**5** were all very similar in the low-field region in that they contained two proton resonance signals in the δ_H 7−8 and δ_H 10−11 regions and seven carbon resonance signals in the δ_C 120−160 region, respectively. Therefore, compounds **1**−**5** were suspected of being a series of related derivatives. To determine the structures of compounds **1**−**5**, compound **4** was chosen for initial study because of its larger yield from the sponge.



Compound **4** was obtained as colorless needle crystals. Its ^{1}H NMR spectrum showed five proton resonance signals at δ_H 11.73 (1H, d, J = 4.5 Hz), 7.96 (1H, d, J = 6.5 Hz), 4.91 (1H, t, J = 5 Hz), 3.68 (2H, t, J = 5 Hz), and 3.58 (2H, dd, J = 5 Hz). Among them, δ_H 11.73 and 4.91 were considered to be active protons on the basis of the HMQC spectrum. IR absorptions at 3520, 3410, and 1061 cm^{-1} further indicated the existence of NH or OH functional groups. In a DEPT experiment, nine resonance lines in the ^{13}C NMR were assigned to two methylenes (δ_C 58.4, 50.3), two methines (δ_C 131.3, 131.0), and five quaternary carbons (δ_C 157.7, 157.5, 149.7, 140.1, 138.3). Two conjugated amide carbonyl groups and a carbamido carbonyl group were suggested by ^{13}C NMR signals at δ_C 157.7, 157.5, and 149.7, and IR absorptions at 1688, 1666, and 1510 cm^{-1} supported this suggestion. The cross-peak for δ_H 3.58 (2H, dd, J = 5 Hz, H-8) to δ_H 4.91 (1H, t, J = 5 Hz, H-8-OH) and δ_H 3.68 (2H, t, J = 5 Hz, H-7) in the ^{1}H−^{1}H COSY spectrum indicated the existence of the partial structure "−N−CH_2−CH_2−OH". The proton ratio in the ^{1}H NMR spectrum (from low field to high field) was 1:1:1:2:2; however, it could be seen from the HMQC spectrum that the proton signal at δ_H 7.96 (1H, d, J = 6.5 Hz) in the ^{1}H NMR was correlated to two methine signals (δ_C 131.3, 131.0). Therefore, the actual number of protons should be 2:2:2:4:4. According to this deduction, compound **4** should contain three "C=O", two "C=", two "CH=", two "NH", and two partial structures "−N−CH_2−CH_2−OH". The possible molecular formula

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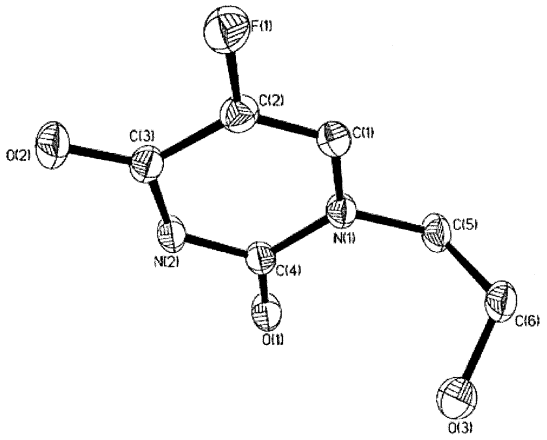

Figure 1. Computer-generated perspective view and X-ray numbering system for compound **4**.

became $C_{11}H_{14}N_4O_5$ with a molecular weight of 282. However, positive FABMS gave a quasi molecular ion peak $[M + 1]^+$ at *m/z* 175. Initially, we thought that the molecular ion peak was not being displayed in the FABMS; however, subsequent alternate MS methods, such as EIMS, ESIMS, and negative FABMS, of compound **4** all gave the same $[M + 1]^+$ *m/z* 175 or $[M]^+$ *m/z* 174. For compound **1**−**3** and **5**, the same conflicts between MS and NMR were observed. Consequently, we concluded that there was a more complex process involving carbon coupling that resulted in these anomalies in these compounds. Because of these uncertainties, an X-ray crystal structure determination was carried out on compound **4**.

Crystals suitable for X-ray diffraction were obtained by slow evaporation of a methanol−dichloromethane solution of compound **4**. The crystal structure was readily solved and is shown in Figure 1. The final standard residual *R* value was 0.0432, corresponding to a Sheldrick residual, wR2 = 0.1105. It can be seen from Figure 1 that compound **4** has one six-membered pyrimidine ring, a fluorine atom, two oxygen atoms, and a 2-hydroxylethyl group attached to C (2), C(3), C(4), and N (1), respectively. To corroborate the existence of the fluorine atom, ^{19}F NMR was undertaken on compound **4**, and a strong resonance signal at δ_F −91.3 (s) was detected. The very strong absorption at 1233 cm^{-1} exhibited in its IR spectrum further supported the presence of a C−F bond. It is a consequence of the presence of the fluorine atom that makes C(2), C(3), and C(4) split into two carbon resonance signals, respectively. The bonds of O(1)−C(4) and O(2)−C(3) were assigned as carbonyl groups on the basis of their bond lengths (1.228 and 1.225 Å, respectively). Otherwise, the bond of O(3)−C(6) (1.418 Å) was assigned to a single carbon−oxygen bond, and O(3) was a hydroxyl. The $^1H-^1H$ COSY, HMQC, and HMBC of compound **4** were consistent with this crystal structure. Thus, the conflict observed in the NMR and MS was solved, and compound **4** was determined to be 1-(2-hydroxylethyl)-5-fluoro-2,4-[1*H*,3*H*]pyrimidine dione, also known as the synthetic product 1-(2-hydroxylethyl)-5-fluorouracil.[19]

As mentioned above, compounds **1**, **2**, **3**, and **5** were similar to compound **4** in the low-field portions of their ^{13}C NMR spectra, suggesting that all of them contain a 5-fluorouracil nucleus. Their ^{19}F NMR spectrum confirmed this deduction (see Experimental Section**)**. The distinct IR absorption at ∼1230 cm^{-1} in each further supported these results. The main difference between compounds **1**−**5** was the substituent in the 1-position of the 5-flurouracil nucleus. This deduction was confirmed by 2D NMR of each.

Compared with that of compound **4**, there were no signals in the high-field region (δ_C <120) of the ^{13}C NMR spectrum of compound **3**. Therefore, compound **3** was composed of only a 5-flurouracil nucleus, and there were no substituent groups. The ^1H NMR spectrum of compound **3** showed only three signals [δ_H 11.51 (1H, br, s, H-3), 10.72 (1H, s, H-1), and 7.76 (1H, t, J = 6.0 Hz, H-6)] in the low field, further indicating that compound **3** was the known compound 5-flurouracil.[19] Its molecular formula was confirmed to be $C_4H_3FN_2O_2$ by HRFABMS.

The molecular formula of compound **1** was confirmed to be $C_7H_7FN_2O_4$ by HRFABMS. Compared with that of compound **4** the ^{13}C NMR spectrum of compound **1** showed an ester carbonyl group [168.4 (s)]; this was also shown by an IR absorption at 1736 cm^{-1}. The presence of a methoxy group could be deduced from the chemical shift of H-10 [δ_H3.69 (3H, s)], corresponding to C-10 [δ_C52.4 (q, C-10), and IR absorptions at 1376 cm^{-1} ($-CH_3$) and 1010 cm^{-1} (C−O). The HMBC spectrum of compound **1** showed cross-peaks for H-10 (δ_H 3.69, 3H, s) and H-7 (δ_H 4.47, 2H, s) to C-8 (δ 168.4, s), suggesting the existence of the partial structure "$-CH_2-C(=O)-OCH_3$". Cross-peaks for H-7 (δ_H 4.47, 2H, s) to C-2 (δ 149.6, s) and C-6 (δ 130.3, d) indicated this partial structure was connected to the N-1 position.

The molecular formula of compound **5**, $C_6H_7FN_4O_3$, was confirmed by HRFABMS. Its ^1H NMR and ^{13}C NMR were very similar to that of compound **1**, except that the methoxy group signal of compound **1** was absent, and a new broad peak [δ_H 3.36 (3H, s, H-8-$NHNH_2$)] appeared in its ^1H NMR spectrum. Therefore, the ester bond in compound **1** was replaced by a hydrazine group and resulted in compound **5**. The cross-peak of δ_H 4.02 (2H, s, H-7) to δ_C 149.8 (s, C-2) and δ_C 131.7 (d, C-6, J_{C-F} = 32.4 Hz) in the HMBC spectrum indicated the partial structure "$NH_2NH-C(=O)-CH_2-$" was connected to the N-1 position of the flurouracil nucleus.

The molecular formula of compound **2** was confirmed to be $C_7H_9N_2O_4F$ by HRFABMS. The chemical shifts of H_3-10 [δ_H 3.25 (3H, s,)], corresponding to C-10 (δ_C 53.8, q), and IR absorption at 1374 cm^{-1} ($-CH_3$), 1077 and 1042 cm^{-1} (C−O) indicated the presence of the methoxy group. The proton resonance signal at δ 6.53 (1H, d, J = 7.5 Hz, H-8-OH) was attributed to a hydroxyl proton on the basis of HMQC data and IR absorptions at 3371 and 1042 cm^{-1}. A coupled spin system [δ_H 3.68 (1H, dd, J = 5.5, 13.5 Hz, H-7α), 3.56 (1H, dd, J = 5.5, 13.5 Hz, H-7β)] in the ^1H NMR spectra, suggested C-7 (δ_C 51.7, t) was adjacent to a chiral center (C-8). On the basis of its chemical shift, this carbon (C-8, δ_C 94.0, d; δ_H 4.64, 1H, dd, J = 5.5, 5 Hz) should be connected with two heteroatoms, one a hydroxy and the other a methoxy group. From a combination of HMBC, HMQC, and $^1H-^1H$ COSY data, the partial structure "$-CH_2-CH(OH)-OCH_3$" could be established, and by chemical shift reasoning, it should be connected to the N-1 position of the uracil ring. Therefore, compound **2** was assigned the structure 1-(2-hydroxyl-2-methyloxylethyl)-5-fluorouracil. The absolute conformation of the chiral center in compound **2** was not determined.

Experimental Section

General Experimental Procedures. Uncorrected melting points were determined on an X-20 micro-melting point apparatus. IR spectra were recorded on a Nicolet 5DX FT-IR and Bruker FT-IR spectrophotometer. UV spectra were recorded on a Cary Bio UV−visible spectrophotometer. The NMR experiments were performed with an INOVA-500 and a Bruker-300 instrument in the indicated solvent. Coupling constants (*J*) are given in Hz. FABMS and ESIMS were obtained using an Autospec-Ultima-ETOF spectrometer, and EIMS was recorded on a VG ZAB-2F 230 instrument (70 V).

The X-ray crystallographic experiments were conducted with a Bruker Smart 1000 diffractometer. Solvents used for chromatographic procedures were redistilled. Silica gel Uniplates for TLC and silica gel Si 60 (230−400 mesh) were employed for chromatographic separation.

Animal Material. The South China Sea sponge *Phakellia fusca* Schmidt belongs to the class Demospongea, the order Axinellida, and the family Phakellia, and was collected around Yongxiong Island of the Xisha Islands in the South China Sea in April 1988 and identified by Professor Li Jin-he, Institute of Oceanology, Chinese Academy of Sciences.

Extraction and Isolation. Sun-dried specimens of this sponge were extracted with 90% aqueous ethanol, and the extract was evaporated under reduced pressure to give a residue that was partitioned between chloroform and water, then between *n*-butanol and water. The *n*-butanol-soluble fractions were condensed and were subjected to silica gel column chromatography. Elution with CH_2Cl_2 containing increasing amounts of MeOH yielded 20 fractions. Each fraction was purified by flash chromatography. The fractions were eluted with CH_2Cl_2−MeOH (25:1), CH_2Cl_2−MeOH (20:1), CH_2Cl_2−MeOH (18:1), CH_2Cl_2−MeOH (15:1), and CH_2Cl_2−MeOH (15:1) to give compound **1**−**5**, respectively.

Compound 1: colorless needle crystals (30 mg); mp 178−179 °C (MeOH); IR (film on KBr) ν_{max} 3454 (−NH), 3181, 3137, 3036, 2834, 1736 (C═O), 1699, 1633, 1470, 1376, 1240, 1159 cm^{-1}; UV λ_{max} (MeOH) 268 (ϵ 12813), 219 (ϵ 5725) nm; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.96 (1H, br, d, J = 4 Hz, H-3), 8.06 (1H, d, J = 2.0 Hz, H-6), 4.47 (2H, s, H-7), 3.69 (3H, s, H-10); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.4 (s, C-8), 157.4 (s, C-4, J_{C-F} = 24.8 Hz), 149.6 (s, C-2), 139.4 (s, C-5, J_{C-F} = 228.8 Hz), 130.3 (d, C-6, J_{C-F} = 34.4 Hz), 52.4 (q, C-10), 48.5 (t, C-7); ^{19}F NMR (282 MHz, DMSO-d_6) δ −89.9 (t, J = 5.9 Hz); HRFABMS m/z 203.1398 $[M + 1]^+$ (calcd for $C_7H_7FN_2O_4$, 203.1400).

Compound 2: yellow armophorous powder (27 mg); mp 160−162 °C; IR (film on KBr) ν_{max} 3371, 2992, 2829, 1700, 1657, 1517, 1475, 1374, 1294, 1234, 1135, 1077, 1042, 944, 814, 756, 720, 609, 548, 471, 431 cm^{-1}; UV λ_{max} (MeOH) 271 (ϵ 8895), 219 (ϵ 4479) nm; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.79 (1H, br, d, J = 5 Hz, H-3), 7.93 (1H, d, J = 7 Hz, H-6), 6.53 (1H, d, J = 7.5 Hz, H-8-OH), 4.64 (1H, dd, J = 5.5, 5 Hz, H-8), 3.68 (1H, dd, J = 13.5, 5.5 Hz, H-7β), 3.56 (1H, dd, J = 13.5, 5.5 Hz, H-7β), 3.25 (3H, s, H-10); ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.4 (s, C-4, J_{C-F} = 13.6 Hz), 149.7 (s, C-2), 139.1 (s, C-5, J_{C-F} = 227.4 Hz), 131.2 (d, C-6, J_{C-F} = 32.1 Hz), 94.0 (d, C-8), 53.8 (q, C-10), 51.7 (t, C-7); ^{19}F NMR (282 MHz, DMSO-d_6) δ −91.0 (t, J = 5.9 Hz); FABMS m/z 205.1560 $[M + 1]^+$ (calcd for $C_7H_9N_2O_4F$, 205.1558).

Compound 3: yellow solid (20 mg); mp 210 °C (decomp); IR (film on KBr) ν_{max} 3108, 2992, 2815, 1717, 1652, 1497, 1426, 1345, 1276, 1239, 1217, 1170, 989, 958, 869, 807, 745, 640, 548, 467, 436 cm^{-1}; UV λ_{max} (MeOH) 265 (ϵ 11344), 220 (ϵ 4920) nm; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.51 (1H, br, s, H-3), 10.72 (1H, s, H-1), 7.76 (1H, t, J = 6.0 Hz, H-6); ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.0 (s, C-4, J_{C-F} = 24.9 Hz), 150.1 (s, C-2), 139.9 (s, C-5, J_{C-F} = 225.0 Hz), 126.3 (d, C-6, J_{C-F} = 32.4 Hz); ^{19}F NMR (282 MHz, DMSO-d_6) δ −91.7 (d, J = 5.4 Hz); FABMS m/z 131.0773 $[M + 1]^+$ (calcd for $C_4H_3N_2O_2F$, 131.0773).

Compound 4: colorless needle crystals (170 mg); mp 153−154 °C; IR (film on KBr) ν_{max} 3520, 3410, 2994, 2282, 1688, 1666, 1510, 1233, 1133, 1061, 882, 743, 663 cm^{-1}; UV λ_{max} (MeOH) 219 (ϵ 4823), 272 (ϵ 9159) nm; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.73 (1H, br, d, J = 4.5 Hz, H-3), 7.96 (1H, d, J = 6.5 Hz, H-6), 4.91 (1H, t, J = 5 Hz, H-8-OH), 3.68 (2H, t, J = 5 Hz, H-7), 3.58 (2H, dd, J = 5 Hz, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.6 (s, C-4, J_{C-F} = 26.1 Hz), 149.7 (s, C-2), 139.2 (d, C-5, J_{C-F} = 225.4 Hz), 131.1 (d, C-6, J_{C-F} = 32.3 Hz), 58.4 (t, C-8), 50.3 (t, C-7); ^{19}F NMR (282 MHz, DMSO-d_6) δ −91.3 (s); MS (EI, 70 eV) 174 (M^+, 25.8), 156 ($M^+ - H_2O$, 8), 144 (22.7), 143 (23.5), 131 ($M^+ - CH_2CH_2OH$, 48.0), 130 (40.7), 101 (11.6), 100 (100), 87 (11.2), 72 (12.21), 45 (19.78), 43 (5.96); FABMS m/z 175.1295 $[M + 1]^+$ (calcd for $C_6H_7FN_2O_3$, 175.1299).

Compound 5: brown solid (8 mg); mp >300 °C; IR (film on KBr) ν_{max} 3437, 3123, 2996, 2800, 1701, 1680, 1607, 1506, 1478, 1438, 1383, 1319, 1241, 1234, 1141, 986, 891, 802, 763, 674, 583, 551 cm^{-1}; UV λ_{max} (MeOH) 274 (ϵ 10909), 219 (ϵ 5763) nm; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.65 (1H, br, H-3), 7.90 (1H, d, J = 6.0 Hz, H-6), 4.02 (2H, s, H-7), 3.36 (3H, s, H-8-$NHNH_2$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.5 (s, C-8), 157.6 (s, C-4, J_{C-F} = 24.8 Hz), 149.8 (s, C-2), 138.9 (s, C-5, J_{C-F} = 225 Hz), 131.7 (d, C-6, J_{C-F} = 32.4 Hz), 50.7 (t, C-7); ^{19}F NMR (282 MHz, DMSO-d_6) δ −91.6 (s); FABMS m/z 203.1425 $[M + 1]^+$ (calcd for $C_6H_7FN_4O_3$, 203.1433).

X-ray Crystal Structure Determination of Compound 4. A colorless needle crystal of compound **4** with approximate dimensions of 0.30 × 0.25 × 0.20 mm was selected for data collection on a Bruker SMART 1000 diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). A total of 1295 reflections were collected in the range 2.40° < θ < 25.02° by using the ω scan technique at 293(2) K. A total of 1105 observable reflections with $I > 2\sigma(I)$ were used in the succeeding refinements (R_{int} = 0.0187). *Lp* corrections were applied to the data.

Crystal data: $C_6H_7FN_2O_3$, triclinic with space group $P\bar{1}$, with a = 5.213(2) Å, b = 8.376(3) Å, c = 8.943(3) Å, α = 104.690(6)°, β = 96.583(7)°, γ = 105.118(6)°, V = 1800.1(2) Å^3, ρ_c = 1.617 Mg/m^3 for Z = 2 and fw = 174.14, $F(000)$ = 180. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement process with the SHELXL-97 software package. The final standard residual R value for the fluoro-model was 0.0432 for observed data (1105 reflections) and 0.0692 for all data (1295 reflections). The corresponding Sheldrick R values were wR2 of 0.1105 and 0.1223, respectively. A final difference Fourier map showed significant residual electron density, the largest difference peak and hole being 0.174 and −0.207 e Å^{-3}, respectively. Final bond distances and angels were within acceptable limits. The final structure of compound **4** is shown in Figure 1.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Gribble, G. W. *J. Nat. Prod.* **1992**, *55*, 1353−1395. (b) Gribble, G. W. *Pure Appl. Chem.* **1996**, *68*, 1699−1712. (c) Gribble, G. W. *Acc. Chem. Res.* **1998**, *31*, 141−152.
(2) (a) O'Hagan, D.; Harper, D. B. *J. Fluorine Chem.* **1999**, *100*, 127−133. (b) Harper, D. B.; O'Hagan, D. *Nat. Prod. Rep.* **1994**, *11*, 123−133.
(3) (a) Dembitsky, V. M.; Srebnik, M. *Progr. Lipid Res.* **2002**, *41*, 315−367. (b) Marais, J. S. C. *Onderstepoovt J. Vet Animo. Ind.* **1943**, *18*, 203−209. (c) Marais, J. S. C. *Onderstepoovt J. Vet Animo. Ind.* **1944**, *20*, 67−71.
(4) (a) Peter, R. A.; Hall, R. J.; Ward, P. F. V.; Shappard, N. *Biochem. J.* **1960**, *77*, 17−22. (b) Ward, P. F. V.; Hall, R. J.; Peter, R. A. *Nature* **1964**, *201*, 611−612.
(5) Sanda, M.; Miyceno, T.; Iwadare, S.; Williamson, J. M.; Arison, B. H.; Smith, J. L.; Douglas, A. W.; Leisch, J. W.; Inamine, E. *J. Antibiotics* **1986**, *39*, 259−265.
(6) (a) Morton, G. O.; Lancaster, J. E.; Van Lear, G. E.; Fulmor, W.; Meyer, W. E. *J. Am. Chem. Soc.* **1969**, *91*, 1535−1357. (b) Thomas, S. O.; Singleton, V. L.; Lowery, J. A.; Sharpe, R. W.; Preuss, L. M.; Porter, J. N.; Mowat, J. H.; Bohonos, N. *Antibiot. Ann.* **1956**, *1956−1957*, 716−721.
(7) (a) Higa, T.; Tanaka, J.; Kitamura, A.; Koyama, T.; Takahashi, M.; Uchia, T. *Pure Appl. Chem.* **1994**, *66*, 2227−2230. (b) Pettit, G. R.; McNulty, J.; Herald, D. L.; Doubek, D. L.; Chapuis, J.-C.; Schmidt, J. M.; Tackett, L. P.; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 180−183.
(8) Malik, S.; Djerassi, C. *Steroids* **1989**, *53*, 271−284.
(9) (a) Burke, S. D.; Junge, K. W.; Phillips, J. R.; Perri, R. E. *Tetrahedron Lett.* **1994**, 703−706. (b) Barrow, R. A.; Capon, R. J. *Aust. J. Chem.* **1994**, *47*, 1901−1918.
(10) (a) Pettit, G. R.; Tan, R.; Herald, D. L.; Cerny, R. L.; Williams, M. D. *J. Org. Chem.* **1994**, *59*, 1593−1559. (b) Pettit, G. R.; Tan, R.; Ichihara, Y.; Williams, M. D. *J. Nat. Prod.* **1995**, *58*, 961−965.
(11) Wolf, D.; Schmidtz, F. J. *J. Nat. Prod.* **1998**, *61*, 1524−1527.
(12) Pettit, G. R.; Ichihaara, Y.; Wurzel, G.; Williams, M. D.; Schmidt, J. M.; Chapuis, J.-C. *J. Chem. Soc., Chem. Commun.* **1995**, 383.
(13) Sakai, R.; Rinehart, K. L. *J. Nat. Prod.* **1995**, *58*, 773−777.
(14) Zeng, L. M.; Fu, X.; Su, J. Y.; Zhou, H. *Chem. J. Chin. Univ.* **1991**, *12*, 924−926.

(15) Sharma, G. M.; Magdoff-Fairchild, B. *J. Org. Chem.* **1977**, *42*, 4118−4124.
(16) Schmidtz, F. J.; Gunasekera, S. P.; Lakshmi, V.; Tillekeratne, L. M. V. *J. Nat. Prod.* **1985**, *48*, 47.
(17) Xu, X. H.; Chen, X.; Liao, R. A.; Xie, Q. L. *Chin. J. Struct.* **2001**, *20*, 173−176.
(18) Xu, X. H.; Yao, G. M.; Lu, J. H.; Li, Y. M.; Lin, Ch. J. *Chem. J. Chin. Univ.* **2001**, *22* (Suppl.), 116−119.
(19) (a) Robert, D.; Edward, P.; Charle, H. *J. Am. Chem. Soc.* **1957**, *79*, 4559−4560. (b) Charles, H.; Robert, D. U.S. Patent 2,808,005, 1957.
(20) (a) Ozaki, S.; Watanabe, Y.; Hoshiko, T.; Mizuno, H.; IshiRawa, K.; Mori, H. *Chem. Pharm. Bull.* **1984**, *32*, 733−738. (b) Mitsui Toatsu Chemicals Inc., Japan. Patent Open 49-36685, 1974.
(21) Ignoffo, R. *J. Am. Health-Syst Pharm.* **1999**, *56*, 2417−2428.

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