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Spatozoate and varninasterol from the brown alga *Spatoglossum variabile*

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Abstract

Two new compounds, spatozoate and varninasterol were isolated from *Spatoglossum variabile*. Four known compounds, fucosterol, cholesta-5-ene-23-yne-3β-ol, apiole and nothoapiole were also isolated for the first time from the methanolic extracts of this alga. The structure elucidation of the new compounds were carried out with the help of modern spectroscopic techniques.

Keywords: *Spatoglossum variabile*; Marine brown alga; Arabian sea; Spatozoate; Varninasterol

1. Introduction

In recent years several new bioactive compounds have been isolated from marine sources (Atta-ur-Rahman et al., 1997). Despite recent progress in the area of marine chemistry, marine organisms of the Arabian sea are little explored, which stimulated us to investigate the chemical constituents of such marine organisms for bioactive constituents. *Spatoglossum variabile* Figari et De Notar. is a brown alga belonging to the family Dictyotaceae (class Phaeophyceae and order Dictyotales) (Shameel, 1990). The genus *Spatoglossum* was first described by Kutz, while *S. variabile* was first described from Karachi coast by Nizamuddin and Perveen (1986). It grows on mid and littoral rocks along the coastline of Pakistan near Karachi city. We now report the isolation of two new compounds, spatozoate (**1**) and varninasterol (**2**) along with four known compounds, fucosterol (**3**), cholesta-5-ene-23-yne-3β-ol (**4**), apiole (**5**) and nothoapiole (**6**) from this marine alga.

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2. Results and discussion

The HREI MS of compound **1** showed the $[M]^+$ at m/z 312.1360, corresponding to the molecular formula $C_{19}H_{20}O_4$ (calc. 312.1362) indicating ten degrees of unsaturation in the molecule. The mass spectrum of **1** also showed the base peak at m/z 149.0293 which was due to the fragment $[M–C_8H_5O_3]^+$. Significant ions were present at m/z 239 $[M–C_4H_9O]^+$, m/z 207 $[M–C_7H_5O]^+$, 206 $[C_{12}H_{14}O_3]^+$ and 178 $[C_{11}H_{14}O_2]^+$. The UV spectrum exhibited absorptions at 265, 256 and 195 nm which indicated the presence of a conjugated aromatic system (Phillips & Nachod, 1959). The IR spectrum of **1** exhibited strong absorptions at 1083, 1135 (C–O), 1724 (ester carbonyl) and 2912 (C–H) cm^{-1}. Analysis of the ^{1}H-NMR data of **1** indicated the presence of nine aromatic protons, three sets of methyl and eight methylene protons. Signals in the aromatic region indicated the presence of two benzene rings, one disubstituted and one mono-substituted. The aromatic protons which resonated at δ 7.74 (1H, dd, $J_{6,5}=7.5$ Hz, $J_{6,4}=1.5$ Hz), 7.70 (1H, m), 7.53 (1H, td, $J_{5,4}=7.5$ Hz, $J_{5,6}=7.5$ Hz, $J_{5,3}=1.5$ Hz) and 7.51 (1H, td, $J_{4,3}=7.5$ Hz, $J_{4,5}=7.5$ Hz, $J_{4,6}=1.5$ Hz) were assigned to H-6, H-3, H-5 and H-4, respectively.

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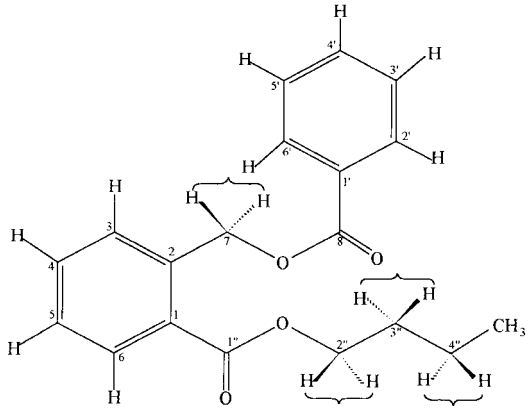
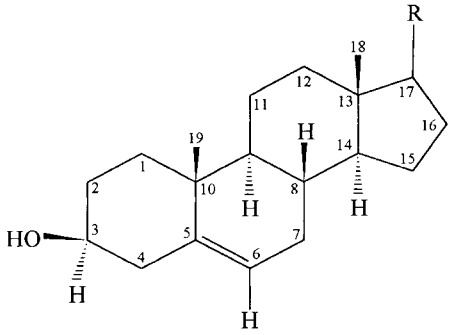
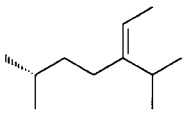
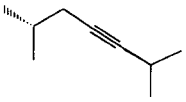
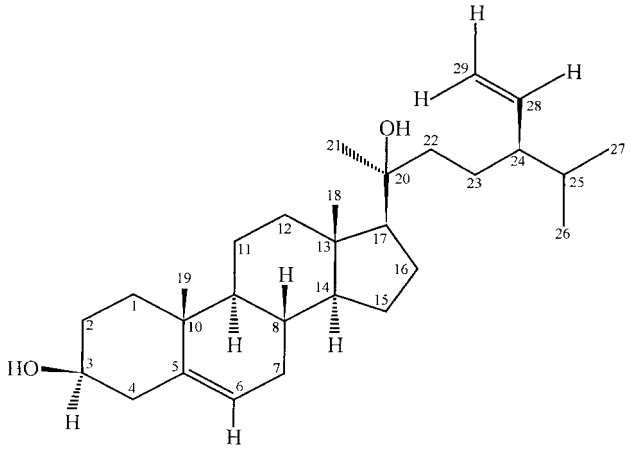


Spatozoate (**1**)

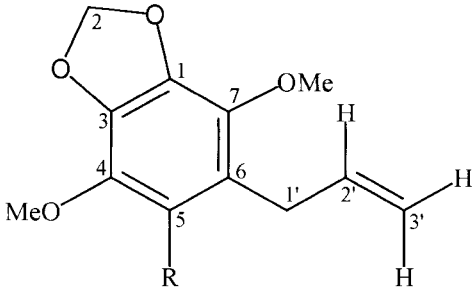


R =  Fucosterol (**3**)

R =  Cholesta-5-ene-23-yne-3β-ol (**4**)



Varninasterol (**2**)



R = H, Apiole (**5**)

R = OMe, Nothoapiole (**6**)

Another set of aromatic signals at δ 7.47 (2H, dd, $J_{2',3'}=8.3$ Hz, $J_{2',4'}=1.8$ Hz), 7.30 (2H, td, $J_{3',2'}=8.3$ Hz, $J_{3',4'}=8.3$ Hz, $J_{3',5'}=1.8$ Hz) and 7.12 (1H, dd, $J_{4',3'}=8.5$ Hz, $J_{4',2'}=1.8$ Hz) were due to H-2′, H-3′ and H-4′, respectively. The C-7 methylene protons, geminal to the ester oxygen and aromatic ring, resonated as a singlet at δ 5.32. Protons H-5″ and H-2″ resonated as triplets at δ 0.90 (3H, t, $J_{5'',4''}=7.0$ Hz) and 4.23 (2H, t, $J_{2'',3''}=7.0$ Hz), respectively. Multiplets centered at δ 1.42/1.75 were assigned to protons H_2-4″/H_2-3″.

The COSY-45° spectrum of **1** exhibited coupling between the aromatic H-3 (δ 7.70), H-4 (δ 7.51), H-5 (δ 7.53) and H-6 (δ 7.74). Coupling between the three pairs of vicinal methylene protons, resonating at δ 4.23, 1.75 and 1.42, were also observed.

The ^{13}C-NMR spectra of **1** showed resonances for all nineteen carbon atoms in the molecule comprising one methyl, four methylene, nine methine and five quaternary carbon atoms. In the HMQC spectrum, the protons which resonated at δ 5.32 (H-7), 4.23 (H-2″), 1.75 (H-3″), 1.42 (H-4″) and 0.90 (H-5″) were found to be coupled with the carbon atoms at δ 67.5, 65.6, 30.5, 19.2 and 13.7, respectively. Long-range ^{1}H/^{13}C correlations observed in the HMBC experiment showed that H-2″ (δ 4.23) was coupled with C-1″ (δ 167.4) and C-4″ (δ 19.2). H-6 (δ 7.74) further showed the coupling with C-1″ (δ 167.4) and C-2 (δ 135.0), thereby indicating that one ester carbonyl carbon is substituted on a benzene ring. Similarly, H-7 (δ 5.32) was found to be coupled with C-8 (δ 167.6), C-2 (δ 135.0), C-1 (δ 132.6) and C-3 (δ 130.8). The coupling of H-3 (δ 7.70) with C-7 (δ 67.5) suggested that one methylene is substituted on a benzene ring. On the basis of these spectroscopic studies, it was concluded that the compound **1** is an aromatic ester containing a side chain bearing a hydroxymethylene functionality.

The HREI MS of compound **2** showed the $[M]^+$ at m/z 428.3636, in agreement with the molecular formula $C_{29}H_{48}O_2$ (calc. 428.3654), indicating six degrees of unsaturation. The mass spectrum showed the base peak at m/z 99.0835 in agreement with the fragment $C_6H_{11}O$. The mass fragmentation of **2** was similar to that recognized for the sterol skeleton (Wyllie &

Djerassi, 1968). Significant ions at m/z 273 $[M-C_{10}H_{19}O]^+$ (side chain) and 271 [M– (side chain + 2H)]$^+$ were observed, corresponding to the loss of side chain together with two hydrogen atoms from the steriod nucleus (Wyllie & Djerassi, 1968). The ion at m/z 255 formed by loss of H_2O from $[M-C_{10}H_{19}O-H_2O]^+$ indicated the presence of another hydroxy group in the sterol ring. Furthermore, the ions at m/z 344 $[M-C_6H_{12}]^+$ and 330 $[M-C_7H_{14}]^+$ of **2** were explained by typical McLafferty rearrangements. The IR spectrum of **2** showed bands for the hydroxyl group (3608 and 3411 cm^{-1}) and double bond (1643 cm^{-1}) with a terminal methylene (884 cm^{-1}) (Charles, 1975).

The ^{1}H-NMR spectrum of **2** showed signals for five methyls, four olefinic protons, one-hydroxy-bearing methine and several methylene protons. This indicated the steroidal nature of the compound in hand. A one-proton triplet resonated at δ 5.32 (J = 5.0 Hz) which was assigned to H-6 (Gupta, Ali, Alam, Niwa & Sakai, 1994). The olefinic protons resonated at δ 5.12 (1H, ddd, J_1 = 10.5 Hz, J_2 = 3.0 Hz, J_3 = 1.5 Hz), 5.17 (1H, ddd, J_1 = 16.0 Hz, J_2 = 3.0 Hz, J_3 = 1.5 Hz) and 5.79 (1H, ddd, J_1 = 17.0 Hz, J_2 = 10.5 Hz, J_3 = 6.5 Hz) were assigned to the H-29/H-29′ and H-28, respectively (Shaikh, Shameel & Ahmad, 1995). A multiplet centered at δ 3.50 with $W_{1/2}$ 15.0 Hz was assigned to proton H-3α (Gupta et al., 1994). Two doublets, integrating for three protons each, resonated at δ 0.85 ($J_{26,25}$ = 7.0 Hz, Me-26) and 0.88 ($J_{27,25}$ = 7.0 Hz, Me-27). The Me-18, Me-19 and Me-21 resonated as singlets at δ 0.67, 0.99 and 1.26, respectively. The chemical shift (1.26) and the multiplicity for C-21 Me suggests the presence of a tertiary hydroxy group at C-20 (Cimino, Rosa, Stefano, Scognamiglio & Sodano, 1981; Fukuzawa et al., 1981).

The proton centered at δ 5.32 (H-6) displayed coupling in the COSY 45° spectrum with H-7 resonated at δ 1.23 and 2.01. The terminal methylene protons resonating at δ 5.12 and 5.17 displayed cross-peaks with H-28 which resonated at δ 5.79. H-3α (δ 3.50) was coupled with H-2 (δ 1.81) and H-4 (δ 2.30).

The ^{13}C-NMR spectrum of **2** showed resonances for all twenty nine carbon atoms in the molecule. The DEPT spectra revealed the presence of five methyl, eleven methylene, nine methine and four quaternary carbon atoms. The two downfield methine carbon signals at δ 71.8 (C-3) and 77.6 (C-20) indicated the presence of two hydroxyl functionalities, while signals at δ 121.7 (C-6), 140.7 (C-5), 112.9 (C-29) and 142.6 (C-28) indicated that two double bonds were present in the molecule. In the HMQC spectrum, the protons observed at δ 3.50 (H-3), 5.32 (H-6), 5.79 (H-28), 5.12 (H-29) and 5.17 (H-29′) were found to be coupled with the carbon atoms resonating at δ 71.8, 121.7, 142.6 and 112.9. In the HMBC spectrum, H-3 (δ 3.50) was coupled with C-2 (δ 31.9), C-4 (δ 42.4) and C-5 (δ 140.7). H-6 (δ 5.32) showed coupling with C-8 (δ 31.4), C-4 (δ 42.4) and C-10 (δ 36.1), which indicated that there was a double bond between C-5 and C-6. The coupling of protons at δ 5.12 (H-29)/5.17 (H-29′) with C-24 (δ 55.9) and of δ 5.79 with C-25 (δ 35.9) and C-23 (δ 29.1), suggested that the vinyl group should be placed at C-24 in the side chain. The H_3-21 (δ 1.26) protons were found to be coupled with C-22 (δ 40.0), C-17 (δ 56.8) and C-20 (δ 77.6), further indicating the presence of a hydroxyl group at C-20 in the side chain (Cimino et al., 1981; Fukuzawa et al., 1981). On the basis of these findings, structure **2** (stigmasta-5,28β-diene-3β,20-diol) was deduced for the compound.

Compounds **3**–**6** isolated by us for the first time from the *Spatoglossum variabile* had previously been reported from other natural sources (Heilbron, Phipers & Wright, 1934; Itoh & Djerassi, 1983; Saiki, Okamoto, Ueno, Uchida & Fukushima, 1970; Sethi, Rao, Morton, Chowdhury & Kapadia, 1976). The structures of compounds **3** (Ahmad et al., 1992), **4** (Steiner et al., 1977), **5** and **6** (Formacek & Kubeczka, 1982) were detemined by comparison of their spectral data with the literature values.

3. Experimental

3.1. General

The IR spectra were recorded on a JASCO A-302 spectrophotometer. The UV spectra were measured on a Hitachi U-3200 spectrophotometer. The melting point was measured on a BUCHI-535 apparatus. Optical rotation was measured on JASCO-DIP-360 digital polarimeter in the MeOH. The EI MS and HREI MS were recorded on JMS HX 110 with the data system DA 5000 and on MAT 112S mass spectrometer. The ^{1}H-NMR spectra were recorded on Bruker AM 300, AM 400 and AMX 500 spectrometers using UNIX data system at 300, 400 and 500 MHz respectively, while ^{13}C-NMR spectra were recorded at 100 MHz and 125 MHz on the same instruments, respectively.

3.2. Plant material

S. variabile (35 kg dry weight) was collected from sub-littoral rocks during mid December 1994 of Buleji Coast near the Karachi City.

3.3. Extraction and isolation

The alga *S. variabile* (25 kg) was washed with water, air dried for 5 days and then soaked in methanol for

one week. The methanolic extract of the alga was filtered and then evaporated under vacuum. The residue (512 g) was dissolved in distilled water and successively fractionated using *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH. The EtOAc extract (49.2 g) was loaded onto a silica gel (804 g) column and then subjected to gradient elution with mixtures of *n*-hexane: $CHCl_3$ and $CHCl_3$: MeOH using increasing polarity. The fraction obtained on elution with the $CHCl_3$ was subjected to preparative TLC using $CHCl_3$ as a solvent system to yield the new compounds, **1** and **2**.

3.4. Spatozoate (*1*)

Oily compound (12 mg, 2.3×10^{-3}%); $R_f = 0.41$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1083, 1135 (C–O), 1724 (C=O), 2912 (C–H); UV $\lambda_{max}^{CHCl_3}$ 195 nm ($\log \varepsilon = 4.47$); EI MS: *m/z* 311.8, 239.0, 205.8, 149.9, 105.0; HREI MS: *m/z* 312.1360 ($C_{19}H_{20}O_4$, calc. 312.62), 239.0681 ($C_{15}H_{11}O_3$), 206.0963 ($C_{12}H_{14}O_3$), 178.0978 ($C_{11}H_{14}O_2$), 149.0293 ($C_8H_5O_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.74 (1H, dd, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.5$ Hz, H-6), 7.70 (1H, m, H-3), 7.53 (1H, td, $J_{5,4} = 7.5$ Hz, $J_{5,6} = 7.5$ Hz, $J_{5,3} = 1.5$ Hz, H-5), 7.51 (1H, td, $J_{4,3} = 7.5$ Hz, $J_{4,5} = 7.5$ Hz, $J_{4,6} = 1.5$ Hz, H-4), 7.47 (2H, dd, $J_{2',3'} = 8.3$ Hz, $J_{2',4'} = 1.8$ Hz, H-2′), 7.30 (2H, td, $J_{3',2'} = 8.3$ Hz, $J_{3',4'} = 8.3$ Hz, $J_{3',5'} = 1.8$ Hz, H-3′), 7.12 (1H, dd, $J_{4',3'} = 8.5$ Hz, $J_{4',2'} = 1.8$ Hz, H-4′), 5.32 (2H, s, H-7), 4.23 (2H, t, $J_{2'',3''} = 7.0$ Hz, H-2″), 1.42 and 1.75 (4H, m, H-4″/3″), 0.90 (3H, t, $J_{5'',4''} = 7.4$ Hz, H-5″); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 132.6 (C-1), 135.0 (C-2), 130.8 (C-3), 128.3 (C-4), 129.1 (C-5), 131.1 (C-6), 67.5 (C-7), 167.6 (C-8), 131.9 (C-1′), 128.6 (C-2′), 128.4 (C-3′), 128.0 (C-4′), 128.4 (C-5′), 128.6 (C-6′), 167.4 (C-1″), 65.6 (C-2″), 30.5 (C-3″), 19.2 (C-4″), 13.7 (C-5″).

3.5. Varninasterol (*2*)

Colorless compound (15 mg, 3.0×10^{-3}%); m.p. 225°C; $[\alpha]_D^{25}$ −39° (1×10^{-3} g/ml $CHCl_3$); $R_f = 0.61$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 884, 1053 (C–O), 1643, 2813 (C–H), 3411, 3608 (O–H); UV $\lambda_{max}^{CHCl_3}$ 198 nm ($\log \varepsilon = 3.89$); EI MS: *m/z* 428, 410, 385, 367, 273, 255, 213, 147, 123, 119, 99; HREI MS: *m/z* 428.3636 ($C_{29}H_{48}O_2$, calc. 428.3654), 410.3535 ($M^+ - H_2O$), 367.2979 ($M^+ - C_3H_7 - H_2O$), 273.2178 ($M^+ - C_{10}H_{19}O$), 255.2107 ($M^+ - C_{10}H_{19}O - H_2O$), 213.1635 ($M^+ - C_{13}H_{52}O - H_2O$), 147.1154 ($M^+ - C_{18}H_{33}O - H_2O$), 119.0835 ($M^+ - C_{20}H_{35}O - H_2O$), 99.0825 ($M^+ - C_{23}H_{37}O$); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.79 (1H, ddd, $J_1 = 17.0$ Hz, $J_2 = 10.5$ Hz, $J_3 = 6.5$ Hz, H-28), 5.32 (1H, t, $J = 5.0$ Hz, H-6), 5.17 (1H, ddd, $J_1 = 16.0$ Hz, $J_2 = 3.0$ Hz, $J_3 = 1.5$ Hz, H-29′), 5.12 (1H, ddd, $J_1 = 10.5$ Hz, $J_2 = 3.0$ Hz, $J_3 = 1.5$ Hz, H-29), 3.50 (1H, ddd, $J_1 = 15.0$ Hz, $J_2 = 10.5$ Hz, $J_3 = 4.5$ Hz, H-3), 1.26 (3H, s, H-21), 0.99 (3H, s, H-19), 0.88 (3H, d, $J_{27,25} = 7.0$ Hz, H-27), 0.85 (3H, d, $J_{26,25} = 7.0$ Hz, H-26), 0.67 (3H, s, H-18); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 35.0 (C-1), 31.9 (C-2), 71.8 (C-3), 42.4 (C-4), 140.7 (C-5), 121.7 (C-6), 33.9 (C-7), 31.4 (C-8), 50.1 (C-9), 36.1 (C-10), 21.1 (C-11), 37.3 (C-12), 42.4 (C-13), 50.8 (C-14), 24.3 (C-15), 28.1 (C-16), 56.8 (C-17), 11.8 (C-18), 18.8 (C-19), 77.6 (C-20), 24.5 (C-21), 40.0 (C-22), 29.1 (C-23), 55.9 (C-24), 31.9 (C-25), 16.5 (C-26), 17.5 (C-27), 142.6 (C-28), 112.9 (C-29).

3.6. Fucosterol (*3*)

Colorless compound (21.5 mg, 4.2×10^{-3}); m.p. 124°C; $[\alpha]_D^{25}$ −38° (2×10^{-3} g/ml, $CHCl_3$); $R_f = 0.51$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3407, 2856; EI MS: *m/z* 412, 379, 314, 229, 213, 145, 55; HREI MS: *m/z* 412.3692 ($C_{29}H_{48}O$, calc. 412.3690); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.30 (1H, m, H-6), 5.17 (1H, q, $J_1 = 13.5$ Hz, $J_2 = 6.7$ Hz, H-28), 3.39 (1H, m, H-3), 1.54 (3H, d, $J_{29,28} = 7.5$ Hz, H-29), 1.02 (3H, d, $J_{21,20} = 6.4$ Hz, H-21), 1.01 (3H, s, H-19), 0.96 (6H, d, $J_{26,27/25} = 6.5$ Hz, H-26/H-27), 0.72 (3H, s, H-18).

3.7. Cholesta-5-ene-23-yne-3β-ol (*4*)

Colorless compound (10.5 mg, 2.0×10^{-3}); m.p.120°C; $[\alpha]_D^{25}$ −37° (2×10^{-3} g/ml, $CHCl_3$); $R_f = 0.31$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3408, 2853, 2143 (C=C); EI MS: *m/z* 382, 367, 339, 314, 271, 213, 127, 55; HREI MS: *m/z* 382.3230 ($C_{27}H_{42}O$, calc. 382.3236); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.30 (1H, m, H-6), 3.40 (1H, m, H-3), 1.14 (3H, d, $J_{26,27/25} = 7.0$ Hz, H-26/H-27), 1.04 (3H, d, $J_{21,20} = 6.5$ Hz, H-21), 1.01 (3H, s, H-19), 0.72 (3H, s, H-18).

3.8. Apiole (*5*)

Oily compound (11.3 mg, 2.2×10^{-3}); $R_f = 0.31$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1612, 1052 (C=C); EI MS: *m/z* 222, 207, 191, 177, 149, 121; HREI MS: *m/z* 222.0895 ($C_{12}H_{14}O_4$, calc. 222.0880); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 6.30 (1H, s, H-5), 5.90 (1H, m, H-2′), 5.81 (2H, s, H-2), 5.01 (2H, m, H-3′), 4.05 (s, OCH_3), 3.70 (s, OCH_3), 3.22 (2H, dt, $J_1 = 6.5$ Hz, $J_2 = 1.5$ Hz, $J_3 = 1.5$ Hz, H-1′).

3.9. Nothoapiole (*6*)

Oily compound (13.2 mg, 2.5×10^{-3}); $R_f = 0.21$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1052, 1453, 1612 (C=C); EI MS: *m/z* 252, 237, 207, 179, 151; HREI MS: *m/z* 252.0989 ($C_{13}H_{16}O_5$, calc. 252.0983); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.90 (1H, m, H-2′), 5.80 (2H, s, H-2), 4.92 (2H, m, H-3′), 4.05 (s, OCH_3), 3.81 (s, OCH_3), 3.70 (s, OCH_3), 3.21 (2H, dt, $J_1 = 6.5$ Hz, $J_2 = 2.0$ Hz, $J_3 = 2.0$ Hz, H-1′).

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