Two New Cinnamic Acid Esters from Marine Brown Alga *Spatoglossum variabile*

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Two new natural products, *n*-butyl and isopropyl 3,5-dimethoxy-4-hydroxycinnamate were isolated from *Spatoglossum variabile*. Three known compounds, methyl 3,4,5-trihydroxybenzoate, 2-deoxyinosine and 9-β-(D-ribofuranosyl)adenine were isolated for the first time from the methanolic extracts of this alga. The structure elucidations of the new compounds were carried out with the help of modern spectroscopic techniques.

Key words *Spatoglossum variabile*; marine brown alga; Arabian sea; 3,5-dimethoxy-4-hydroxycinnamic acid ester

In recent years several new bioactive compounds have been isolated from marine organisms.[1] However, despite recent progress in the area of marine chemistry, organisms of the Arabian Sea are little explored, which stimulated us to investigate the chemical constituents of such marine organisms. *Spatoglossum variabile* FIGARI et DE NOTAR. is a brown alga belonging to the family Dictyoaceae.[2] *S. variabile* grows on the mid and littoral rocks along the coastline of Pakistan near Karachi city.[3] As a part of our ongoing phytochemical studies on *S. variabile*, we have recently reported some new natural products from this alga.[4—6] We now report the isolation of two new compounds, *n*-butyl 3,5-dimethoxy-4-hydroxycinnamate (**1**) and isopropyl 3,5-dimethoxy-4-hydroxycinnamate (**2**) along with three known compounds, methyl 3,4,5-trihydroxybenzoate (**3**), 2-deoxyinosine (**4**) and 9-β-(D-ribofuranosyl)adenine (**5**) from this marine alga.

The HREI-MS (high resolution electron impact mass spectrum) of compound **1** showed the M^+ at *m*/*z* 280.1319, corresponding to the molecular formula $C_{15}H_{20}O_5$ (Calcd 280.1311) indicating six degrees of unsaturation in the molecule. Significant ion at *m*/*z* 224.0688 ($C_{11}H_{12}O_5$) was due to the loss of *n*-butyl group (C_4H_9) from the parent molecular ion at *m*/*z* 280.1319. The ions at *m*/*z* 207.0661 ($C_{11}H_{11}O_4$) and 180.0776 ($C_{10}H_{12}O_3$) were observed, corresponding to the losses of hydroxy group (–OH) from the ion at *m*/*z* 224.0688 and –CO group from the ion at *m*/*z* 207.0661, respectively. Furthermore, the ions at *m*/*z* 149.0807 ($C_9H_9O_2$) and 120.0581 (C_8H_8O) corresponded to the subsequent losses of a methoxy group from the ion at *m*/*z* 180.0776 and as well as from the ion at *m*/*z* 149.0807, respectively.

The UV (ultraviolet) spectrum exhibited absorptions at 313, 227 and 212 nm which indicated the presence of an aromatic conjugated system.[7] The IR (infrared) spectrum of **1** showed strong absorptions at 3275 (O–H), 2918 (C–H), 1673 (C=O), 1608, 1526 (aromatic C=C) and 1144 (C–O) cm^{-1}.[8]

Analysis of the ^{1}H-NMR spectrum of **1** indicated the presence of two aromatic protons, two olefinic protons, six methylene protons, three sets of methyl and six sets of methoxy methyl protons. This indicated the cinnamic nature of compound in hand.[8] The aromatic protons resonated as a singlet at δ 6.71 was due to H-2/H-6. A singlet integrating for 6H at δ 3.85 was assigned to OCH_3-1a and OCH_3-1b. The C-10 methylene protons, geminal to the ester oxygen, appeared as a triplet at δ 4.12 ($J_{10,11}$=6.6 Hz), whereas the C-11 and C-12 methylene protons exhibited a multiplet centered at δ 1.64 and 1.37, respectively. The terminal methyl protons appeared as a triplet at δ 0.91 ($J_{13,12}$=7.5 Hz, H_3-13). The spectrum also showed the presence of a pair of downfield doublets at δ 7.52 (H-7) and 6.24 (H-8) with coupling constants of 15.8 Hz each, indicating the presence of a double bond in *trans*-configuration,[9] The COSY-45° spectrum of **1** exhibited coupling between olefinic H-7 (δ 7.52) and H-8 (δ 6.24). The terminal methyl protons resonating at δ 0.91 (H_3-13) displayed the cross-peaks with the H_2-12 which resonated at δ 1.37. Coupling between the three pairs of vicinal methylene protons, resonating at δ 4.12, 1.64 and 1.37, were also observed.

The ^{13}C-NMR, BB (broad-band decoupled) and DEPT (distortionless enhancement by polarization transfer) spectra of **1** showed resonances for all fifteen carbon atoms comprising three methylene, four methine, three methyl and five quaternary carbon atoms. The downfield signal at δ 167.2 (C-9) indicated the presence of ester carbonyl carbon, while signals at δ 144.8 (C-7) and 116.1 (C-8) indicated that one double bond was present in the molecule. The downfield signals at δ 105.2 (C-2/C-6), 126.0 (C-1), 137.2 (C-4) and 147.3 (C-3/C-5) suggested the presence of benzene ring which comprise of two methine and four quaternary carbons of which three were oxygen-bearing centers.[10]

In the HMQC (heteronuclear multiple quantum coherence) spectrum, the protons which resonated at δ 7.52 (H-7), 6.71 (H-2/H-6), 6.24 (H-8), 4.12 (H_2-10), 3.85 (OCH_3-1a/OCH_3-1b), 1.64 (H_2-11), 1.37 (H_2-12) and 0.91 (H_3-13) were found to be coupled with the carbon atoms at δ 144.8 (C-7), 105.2 (C-2/C-6), 116.1 (C-8), 64.3 (C-10), 56.4 (OCH_3-1a/OCH_3-1b), 30.8 (C-11), 19.2 (C-12) and 13.7 (C-13), respectively. The HMBC (heteronuclear multiple bond connectivity) experiment showed that H_3-13 (δ 0.91) was coupled with C-12 (δ 19.2) and C-11 (δ 30.8). H_2-10 (δ 4.12) further showed the coupling with C-11 (δ 30.8), C-12 (δ 19.2) and C-9 (δ 167.2), suggested that *n*-butyl unit is substituted on an ester carbonyl group. The C-8 olefinic proton (δ 6.24) was found to be coupled with C-1 (δ 126.0), C-7 (δ 144.8) and C-9 (δ 167.2). Similarly, H-7 (δ 7.52) showed the coupling with C-8 (δ 116.1), C-1 (δ 126.0), C-2/C-6 (δ 105.2) and C-9 (δ 167.2), thereby indicating that there was a double bond which inter-connected the ester carbonyl carbon (C-9) and benzene ring (C-1). The downfield H-2/H-6 (δ 6.71) displayed correlations with C-1 (δ 126.0), C-7 (δ 144.8) and C-

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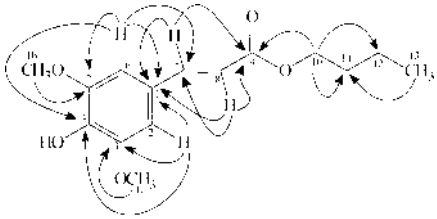

Fig. 1. Important HMBC Interactions in **1**

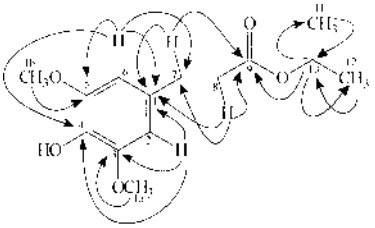

Fig. 2. Important HMBC Interactions in **2**

Table 1. ^{1}H- and ^{13}C-NMR (250 MHz and 63 MHz in $CDCl_3$) Data of Compounds **1** and **2**

Carbon No	**1**		**2**	
	^{1}H-NMR, δ (ppm), J=Hz	^{13}C-NMR, δ (ppm)	^{1}H-NMR, δ (ppm), J=Hz	^{13}C-NMR, δ (ppm)
1	—	126.0	—	125.9
2	6.71 (s)	105.2	6.69 (s)	105.1
3	—	147.3	—	147.3
4	—	137.2	—	137.2
5	—	147.3	—	147.3
6	6.71 (s)	105.2	6.69 (s)	105.1
7	7.52 (d, $J_{7,8}$=15.8)	144.8	7.50 (d, $J_{7,8}$=15.8)	144.6
8	6.24 (d, $J_{7,8}$=15.8)	116.1	6.22 (d, $J_{7,8}$=15.8)	116.4
9	—	167.2	—	167.7
10	4.12 (t, $J_{10,11}$=6.6)	64.3	5.07 (m)	67.6
11	1.64 (m)	30.8	1.21 (d, $J_{11,10}$=6.3)	21.9
12	1.37 (m)	19.2	1.21 (d, $J_{12,10}$=6.3)	21.9
13	0.91 (t, $J_{13,12}$=7.5)	13.7	—	—
1a	3.85 (s)	56.4	3.82 (s)	56.3
1b	3.85 (s)	56.4	3.82 (s)	56.3

3/C-5 (δ 147.3), which indicated that hydroxyl group, two methoxy group and one-olefinic carbon (C-7) are substituted on a benzene ring. The methoxy protons at δ 3.85 showed HMBC interactions with C-3/C-5 (δ 147.3), further indicated the substitution of the methoxy groups on benzene ring.

On the basis of these spectroscopic studies, it was concluded that the compound **1** is a *n*-butyl 3,5-dimethoxy-4-hydroxycinnamate.

Compound **2** was assigned the molecular formula $C_{14}H_{18}O_5$ by HREI-MS, showing the molecular ion peak at *m/z* 266.1157 ($C_{14}H_{18}O_5$, Calcd 266.1154). It was differs from compound **1** only by the presence of a isopropyl unit at C-10 instead of a *n*-butyl unit at C-10. The structure was further supported by mass, IR, ^{1}H- and ^{13}C-NMR experiments. The fragmentation pattern in the EI-MS as well as the IR spectrum ($CHCl_3$) of **2** was identical to **1**. The ^{1}H-NMR spectrum of **2** displayed a methyl signals resonating at δ 1.21 (6H, d, $J_{11/12,10}$=6.3 Hz) which were assigned to H_3-11/H_3-12, whereas the C-10 methine proton, geminal to the ester oxygen which resonated as a multiplet at δ 5.07. Its ^{13}C-NMR spectrum showed two signals at δ 67.6 and 21.9, which were assigned to isopropyl unit C-10 and C-11/C-12, respectively. The HMQC and HMBC spectrums of **2** also revealed the same interactions as in **1**. On the basis of these data compound **2** was identified as isopropyl 3,5-dimethoxy-4-hydroxycinnamate.

Compounds **3**—**5** isolated by us for the first time from the *Spatoglossum variabile* had previously been reported from other natural sources.[11—13] The structures of compounds **3**,[11] **4**,[6] and **5**[13] were determined by comparison of their spectral data with the literature values.

Experimental

The melting point was determined with a Büchi SMP-20 apparatus and is uncorrected. The UV spectra were recorded on a Lambda 5 UV/VIS spectrometer (Perkin-Elmer). The IR spectra were recorded on a Bruker FT-IR IFS 48 spectrophotometer. The EI (electron inpact), FD (field dispersion), FAB (fast atom bombardment) and HREI-MS were recorded on Varian MAT 711 and on a MAT 112S mass spectrophotometer. The ^{1}H-NMR spectra were recorded on Bruker AC 250, AM 400 and AMX 500 spectrometers using UNIX data system at 250, 400 and 500 MHz respectively, while ^{13}C-NMR spectra were recorded at 63, 100 and 125 MHz on the same instruments.

Plant Material *S. variabile* Figari et De Notar. (35 kg) was collected from the Buleji coastline of Karachi in December 1996. A voucher specimen has been deposited in the Department of Botany, University of Karachi.

Extraction and Isolation The alga was washed with water, air-dried for five days and then soaked in methanol (50 l) for one week. The methanolic extract of the alga was filtered and then evaporated under vacuum. The residue (512 g) was triturated with distilled water and successively fractionated with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH. The concentrated 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. The fraction obtained on elution with *n*-hexane–$CHCl_3$ (7 : 3) was subjected to preparative TLC using *n*-hexane–$CHCl_3$–EtOAc–NH_4OH (6 : 2 : 2 : 3 drops) as the solvent system to afford the new compounds **1** (18.3 mg) and **2** (38.5 mg). The fraction which eluted with *n*-hexane–$CHCl_3$ (5 : 5) gave one major spot which was further purified by chromatography over silica gel using *n*-hexane–$CHCl_3$–EtOAc (5 : 3 : 2) as eluent to provide **3** (86.7 mg). The fraction eluted with $CHCl_3$–MeOH (7 : 3) gave two major spots, which was further purified by preparative TLC in the solvent system $CHCl_3$–MeOH–NH_4OH (6.5 : 3.5 : 3 drops) to give compounds **4** (26.4 mg) and **5** (31.5 mg).

n-Butyl 3,5-Dimethoxy-4-hydroxycinnamate (**1**): Yellow oil (18.3 mg, 3.5×10^{-3}%). *Rf*=0.61. IR ($CHCl_3$) cm^{-1}: 3275 (O–H), 2918 (C–H), 1673 (C=O), 1608, 1526 (aromatic C=C), 1144 (C–O), 945, 735. UV λ_{max}

($CHCl_3$) nm (log ε): 313 (5.07). ^{1}H- and ^{13}C-NMR (Table 1). EI-MS: *m/z*: 280, 224, 207, 180, 175, 167, 149, 135, 120, 119, 104, 91, 77, 65, 57, 41. FD-MS *m/z*: 280. HR-EI-MS *m/z*: 280.1319 ($C_{15}H_{20}O_5$, Calcd 280.1311), 224.0688 ($C_{11}H_{12}O_5$), 207.0661 ($C_{11}H_{11}O_4$), 180.0776 ($C_{10}H_{12}O_3$), 149.0807 ($C_9H_9O_2$), 120.0581 (C_8H_8O), 104.0632 (C_8H_8).

Isopropyl 3,5-Dimethoxy-4-hydroxycinnamate (**2**): Colorless oil (38.5 mg, 7.5×10^{-3} %). *Rf*=0.50. IR ($CHCl_3$) cm^{-1}: 3404 (O–H), 2921 (C–H), 1685 (C=O), 1611, 1521 (aromatic C=C), 1134 (C–O), 892, 741. UV λ_{max} ($CHCl_3$) nm (log ε): 282 (4.6). ^{1}H- and ^{13}C-NMR (Table 1). EI-MS *m/z*: 266, 224, 207, 205, 180, 175, 163, 149, 135, 120, 119, 104, 91, 76, 65, 55, 43. FD-MS *m/z*: 266. HR-EI-MS *m/z*: 266.1157 ($C_{14}H_{18}O_5$, Calcd 266.1154), 224.0691 ($C_{11}H_{12}O_5$), 207.0663 ($C_{11}H_{11}O_4$), 180.0778 ($C_{10}H_{12}O_3$), 149.0809 ($C_9H_9O_2$), 120.0579 (C_8H_8O), 104.0631 (C_8H_8).

Methyl 3,4,5-Trihydroxybenzoate (**3**): Crystalline compound (86.7 mg, 1.7×10^{-2} %). mp 199 °C. *Rf*=0.59. IR (KBr) cm^{-1}: 3404 (O–H), 1708 (C=O), 1632, 1484 (aromatic C=C), 1132 (C–O), 845. UV λ_{max} (MeOH) nm (log ε): 280 (5.13). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.16 (3H, s, OH), 7.07 (2H, s, H-2/H-6), 3.72 (3H, s, OCH_3). EI-MS *m/z*: 184, 153, 125, 107, 79, 77, 59, 51, 31. FD-MS *m/z*: 184. HR-EI-MS *m/z*: 184.0379 ($C_8H_8O_5$, Calcd 184.0371).

2-Deoxyinosine (**4**): Colorless compound (26.4 mg, 5.1×10^{-3} %). *Rf*=0.41. IR (KBr) cm^{-1}: 3564 (O–H), 3348 (N–H), 1625 (C=C), 1548 (C=N), 1058 (C–O), 942, 746. UV λ_{max} (MeOH) nm (log ε): 308 (5.11). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 11.06 (1H, br s, NH), 8.32 (1H, s, H-2), 8.08 (1H, s, H-8), 6.32 (1H, dd, $J_{1',2'a}$=7.5 Hz, $J_{1',2'b}$=6.5 Hz, H-1′), 4.40 (1H, br s, C3′-OH), 3.85 (1H, m, H-4′), 3.62 (2H, dd, $J_{5'a,5'b}$=10.8 Hz, $J_{5'a/5'b,4'}$=2.8 Hz, H-5′a/H-5′b), 3.50 (1H, m, H-3′), 2.71 (2H, m, H_2-2′). EI-MS *m/z*: 252, 235, 163, 135, 126, 107, 107, 77, 62, 61, 55. FAB-MS *m/z*: 253 (+ve mode). HR-EI-MS *m/z*: 252.0863 ($C_{10}H_{12}N_4O_4$, Calcd 252.0858).

9-β-(D-Ribofuranosyl)adenine (**5**): Colorless amorphous (31.5 mg, 6.1×10^{-3} %). *Rf*=0.36. IR (KBr) cm^{-1}: 3425 (O–H), 3354 (N–H), 1608 (C=C), 1532 (C=N), 1064 (C–O), 905, 743. UV λ_{max} (MeOH) nm (log ε): 259 (4.10). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 8.30 (1H, s, H-2), 8.10 (1H, s, H-8), 5.95 (1H, d, $J_{1',2'}$=6.5 Hz, H-1′), 4.59 (1H, dd, $J_{2',1'}$=8.4 Hz, $J_{2',3'}$=5.8 Hz, H-2′), 4.36 (1H, br s, C3′-OH), 4.31 (1H, br s, C2′-OH), 4.08 (1H, br d, $J_{3',2'}$=5.8 Hz, H-3′), 3.92 (1H, dd, $J_{4',3'}$=6.5 Hz, $J_{4',5'}$=3.1 Hz, H-4′), 3.62 (1H, dd, $J_{5'a,5'b}$=11.4 Hz, $J_{5'a/5'b,4'}$=3.1 Hz, H-5′a/H-5′b), 3.15 (2H, br s, NH_2). EI-MS *m/z*: 267, 237, 165, 136, 135, 108, 77, 63, 61, 57. FAB-MS *m/z*: 268 (+ve mode). HR-EI-MS *m/z*: 267.0961 ($C_{10}H_{13}N_5O_4$, Calcd 267.0967).

Acknowledgements Financial assistance from the Office of Naval Research, U.S.A. (ONR Grant # N00014-86-G-0229 to A. R. and Grant # NP-14 to M. I. C.) is gratefully acknowledged. The authors are grateful to Prof. Mustafa Shameel, Department of Chemistry, University of Karachi, for identification of the alga.

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