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METABOLITES PRODUCED BY AN ENDOPHYTE *Alternaria alternata* ISOLATED FROM *Maytenus hookeri*

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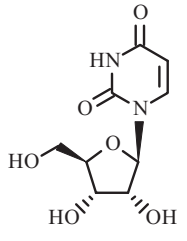
Endophytes are bacteria or fungi that live in the intercellular spaces of the tissues of host plants without causing discernible manifestation of disease [1]. Recently, endophytes have been recognized as important sources of a variety of structurally novel and biologically active secondary metabolites, including terpenoids, steroids, alkaloids, and isocoumarin derivatives.

In the course of our search for new biologically active secondary metabolites from endophytic microorganisms residing in *Maytenus hookeri* (Celastraceae) [2, 3], we studied chemical substances from an endophytic fungus *Alternaria alternata* of *Maytenus hookeri* and isolated and characterized nine compounds, alternariol (**1**), alternariol monomethyl ether (**2**), 5′-epialtenuene (**3**), altenuene (**4**), uridine (**5**), adenosine (**6**), ACTG toxin-E (**7**), ergosta-4,6,8,22-tetraen-3-one (**8**), and ergosta-7,24(28)-dien-3-ol (**9**) from the ethyl acetate–methanol–acetic acid extract of the solid-state fermentations of this fungus. Among them, compounds **5** and **6** were isolated for the first time from this genus *Alternaria*, both **8** and **9** first being obtained from the species. In this paper, we describe the isolation and structure elucidation of these compounds **1–9**.

With the use of column chromatography over silica gel, and Sephadex LH-20 and reversed-phase RP-18, as well as preparative TLC on silica gel, further separation of methanol - and petroleum ether–soluble parts of the ethyl acetate–methanol–acetic acid extract of the cultures of *A. alternata* afforded nine compounds **1–9**.

Compound **1** was isolated as a white powder. ESI-MS (negative mode) showed a molecular ion at *m/z* 257 $[M-H]^-$. From the ^{13}C NMR (DEPT) spectra, the molecular formula was deduced as $C_{14}H_{10}O_5$, with 10 degrees of unsaturation. The ^{13}C NMR and DEPT indicated 14 skeletal C atoms, including one methyl, four methines, and nine quarternary C atoms. In the ^1H NMR spectrum, the downfield signals appeared at δ 6.88 (1H, d, J = 1.69 Hz, H-4), 6.94 (1H, d, J = 2.36 Hz, H-5′), 6.99 (1H, d, J = 2.48 Hz, H-3′), and 7.44 (1H, d, J = 1.71 Hz, H-6), which suggested it was a biphenyl-type compounds. The above spectral data were in agreement with values reported in the literature [4] for 3,4′,5-trihydroxy-6′-methyldibenzo-α-pyrone.

Compound **2**, white powder, $C_{15}H_{12}O_5$ was identified as alternariol monomethyl ether by comparison of physicochemical data and spectral data (EI-MS, ^1H NMR and ^{13}C NMR) with those reported in the literature [4, 5].



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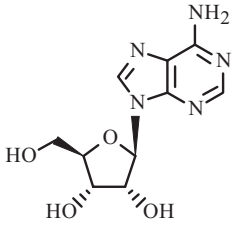

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Compound **3** was isolated as a white needle. Its EI-MS spectra gave a molecular ion at *m/z* 292 $[M]^+$, corresponding to the molecular formula $C_{15}H_{16}O_6$ based on ^{13}C NMR (DEPT), with 8 degrees of unsaturation. From the spectra of ^{13}C NMR and DEPT, the compound contained 15 skeletal C atoms: two methyls, one methylene, and five methines, and seven quarternary C atoms. The ^1H NMR and ^{13}C NMR spectra displayed the presence of one benzene ring at δ 133.7, 101.3, 164.8, 101.5, 167.2, 103.1, a δ-lactone ring at δ 169.6, and an exocyclic double bond at δ 6.30 (1H, d, J = 2.92 Hz; 140.4 and 131.5). The above data were very similar to those reported in the literature [5, 6] for (2*R*,3*S*,4a*S*)-2,3,4,4a-tetrahydro-2,3,7-trihydroxy-4a,9-dimethylbenzo[c]chromen-6-one.

Compound **4**, isolated as white crystals, had the same molecular formula of $C_{15}H_{16}O_6$ as compound **3** on the basis of a molecular ion at *m/z* 292 $[M]^+$ in EI-MS and ^{13}C NMR (DEPT). Careful comparison of the ^1H NMR and ^{13}C NMR spectra of **4** with those of compound **3** revealed that **4** is an isomer of **3**. The coupling constant value between H-6′ and H-5′ is 2.44, indicating the pseudoequatorial orientation of the hydroxyl group at C-5′ [6]. The above data were very similar to those reported in the literature [6, 7] for (2*R*,3*S*,4a*S*)-2,3,4,4a-tetrahydro-2,3,7-trihydroxy-4a,9-dimethylbenzo[c]chromen-6-one.

Compound **5**, white crystal (methanol), $C_9H_{12}N_2O_6$. Its spectral data (ESI-MS, ^1H NMR, ^{13}C NMR) and physicochemical data were identical to those recorded for uridine in the literature [8, 9].

Uridine (5). White crystal (methanol), $C_9H_{12}N_2O_6$. ESI-MS (positive mode) *m/z*: 289 $[M + COOH]^-$; ^1H NMR (500 MHz, MeOD-d_4, δ, ppm, J/Hz): 5.68 (1H, d, J = 8.12, H-5), 8.00 (1H, d, J = 8.09, H-6), 5.89 (1H, d, J = 4.63, H-1′), 4.18 (2H, m, H-2′, H-3′), 4.00 (1H, m, H-4′), 3.73 (1H, dd, J_1 = 3.02, J_2 = 12.24, H-5′a), 3.84 (1H, dd, J_1 = 2.62, J_2 = 12.21, H-5′b); ^{13}C NMR (125 MHz, MeOD-d_4, δ): 152.5 (s, C-2), 166.2 (s, C-4),102.6 (d, C-5), 142.7 (d, C-6), 90.7 (d, C-1′), 71.3 (d, C-2′), 75.2d, C-3'), 86.4 (d, C-4′), 62.3 (t, C-5′).

Compound **6**, white powder, $C_{10}H_{13}N_5O_4$. The physicochemical data and spectral data (ESI-MS, ^1H NMR, ^{13}C NMR) were identical with those reported for an authentic specimen of adenosine [10].

Adenosine (6). White powder, $C_{10}H_{13}N_5O_4$. ESI-MS (positive mode) *m/z*: 268 $[M + H]^+$; ^1H NMR (500 MHz, C_5D_5N, δ, ppm, J/Hz): 8.61 (1H, s, H-2), 8.34 (1H, s, H-8), 6.71 (1H, d, J = 5.8, H-1′), 5.51 (1H, t, J = 5.28, H-2′), 5.06 (1H, t, J = 3.81, H-3′), 4.76 (1H, d, J = 2.5, H-4′), 4.32 (1H, dd, J_1 = 2.12, J_2 = 12.35, H-5′a), 4.14 (1H, d, J = 12.44, H-5′b); ^{13}C NMR (125 MHz, C_5D_5N, δ): 152.6 (d, C-2), 149.2 (s, C-4), 119.5 (s, C-5), 156.3 (s, C-6), 140.2 (d, C-8), 88.2 (d, C-1′), 73.7(d, C-2′), 70.9(d, C-3′), 86.1 (d, C-4′), 61.9 (t, C-5′).

Compound **7** was isolated as an oil. Its ESI-MS (positive mode) indicated a molecular ion peak at *m/z* 347 $[M – H]^+$, corresponding to the molecular formula $C_{21}H_{30}O_4$, with 7 degrees of unsaturation. Analysis of the ^{13}C NMR and DEPT spectra established 21 carbons in the molecule, consisting of three methyls, seven methylenes, five methines, and six quaternary carbons. The 7 degrees of unsaturation of the molecule indicated that the compound has three rings in addition to an *α,β*-unsaturated ketone unit at δ 108.0 (s, C-13), 171.8 (s, C-14), and 196.6 (s, C-18) and two double bonds at δ 136.4 (s, C-2), 124.8 (d, C-3), 151.0 (s, C-7), and 119.6 (d, C-8). It showed a positive color reaction against H_2SO_4–EtOH reagent, suggesting that this compound was characteristic of terpenoids [11]. It has the same skeleton as the analogs of ATCG. The above data were in accordance with those reported in the literature [11] for 3,3a,6,7,9,9a-hexahydro-5-hydroxy-1[(*E*)-7-hydroxy-6-methylhept-5-en-2-yl]-3a-methylcyclopenta[b]chromen-8(5H)-one.

Compound **8** was isolated as a yellowish crystal. EI-MS showed a molecular ion peak at *m/z* 392, corresponding to the molecular formula $C_{28}H_{40}O$. The ^1H NMR spectrum clearly showed two tertiary methyl signals at δ 0.99 and 0.97 and four secondary methyl signals at 0.83, 0.85, 0.93, and 1.06, which suggested an ergostane skeleton [12, 13]. This was supported by the fact that the chemical shift of the signals for the secondary methyl groups together with resonances for a *trans*-disubstituted double bond at δ 5.29 (H-22 and H-23) were consistent with those of ergosterol. The above data were similar to those described in the literature [12]. Thus, this compound was determined as ergosta-4,6,8,22-tetraen-3-one.

Compound **9**, white needles (methanol), $C_{28}H_{46}O$. Its structure was elucidated as ergosta-7,24(28)-dien-3-ol by spectral data (ESI-MS, ^1H NMR, ^{13}C NMR) and comparison with literature values [14, 15].

Ergosta-7,24(28)-dien-3-ol (9). White needles (methanol), $C_{28}H_{46}O$. EI-MS (70 eV) *m/z* (%): 398 $[M]^+$ (5), 383 (16), 314 (23), 271 (100); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 5.15 (1H, bs, H-7), 4.71 (1H, s, H-24′a), 4.66 (1H, s, H-24′b), 3.63 (1H, m, H-3), 0.54 (3H, s, H-18), 0.79 (3H, s, H-19); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 37.2 (t, C-1), 31.5 (t, C-2), 71.3 (d, C-3), 38.1 (t, C-4), 40.3 (d, C-5), 29.7 (t, C-6), 117.5 (d, C-7), 139.6 (s, C-8), 49.5 (d, C-9), 34.3 (s, C-10), 21.6 (t, C-11), 39.6 (t, C-12), 43.5 (s, C-13), 55.1 (d, C-14), 23.0 (t, C-15), 27.9 (t, C-16), 56.1 (d, C-17), 11.9 (q, C-18), 13.0 (q, C-19), 36.2 (d, C-20), 18.9 (q, C-21), 31.1 (t, C-22), 34.3 (t, C-23), 156.9 (s, C-24), 106.0 (t, C-24′), 33.9 (d, C-25), 21.9 (q, C-26), 22.0 (q, C-27).

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