COMPONENTS OF THE HEARTWOOD OF *Populus euphratica* FROM AN ANCIENT TOMB

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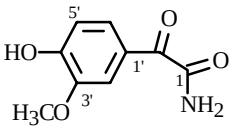
To probe the organic constituents of over 2000-year-preserved Populus euphratica *found in an ancient tomb, a chemical investigation was undertaken, which led to the isolation of a new compound, 2-(4′-hydroxy-3′-methoxyphenyl)-2-oxoacetamide (**1**), together with 12 known compounds (**2–13**) by column chromatography. Their structures were elucidated on the basis of spectroscopic evidence. It is the first time that compounds **2–13** were isolated from this plant.*

Key words: *Populus euphratica*, 2-(4′-hydroxy-3′-methoxyphenyl)-2- oxoacetamide.

Populus euphratica is a salt tolerant tree species, which belongs to the family of *Salicaceae* and is mainly distributed in the desert regions in northwest China [1]. This plant is the only large tree species that can survive and develop into forest in these arid and saline-alkali areas. Glucosides and topolins from this genus have been reported previously [2, 3]. Some wood of *P. euphratica* was found in an ancient tomb belived to be over 2000 years old in the desert of Xinjiang Province, China, raising questions about its phytochemical constituents. The chemical study on long-term preserved plants showed the presence of natural constituents [4–9]. To explore the organic constituents of over 2000-year-preserved *P. euphratica*, we undertook the chemical investigation of this plant. This paper describes the isolation and elucidation of 13 compounds, including one new compound (**1**) and twelve known compounds (**2–13**).



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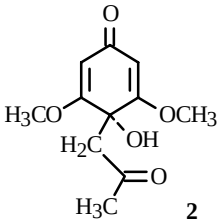

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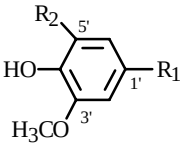

3, 5 - 9

3: $R_1 = COCH_2CH_2OH$, $R_2 = H$; **5:** $R_1 = CHO$, $R_2 = H$
6: $R_1 = COCH_3$, $R_2 = H$; **7:** $R_1 = CH{=}CHCHO$, $R_2 = H$
8: $R_1 = CH{=}CHCHO$, $R_2 = OCH_3$
9: $R_1 = CHO$, $R_2 = OCH_3$

Compound **1** was obtained as a yellow amorphous powder, with a melting point of 105–107°C, and its molecular formula $C_9H_9NO_4$ with an unsaturation degree of n = 6 was determined from the quasi-molecular ion peak at *m/z* 195 in its EI mass spectrum and the ^{13}C NMR (DEPT) spectrum, which was supported by its HR-ESI observed at *m/z* 218.0429 (calculated 218.0429, $[M+Na]^+$). The IR spectrum of compound **1** showed broad absorptions at 3418 and 3159 cm^{-1}, suggesting the presence of an amide group and a hydroxyl group.

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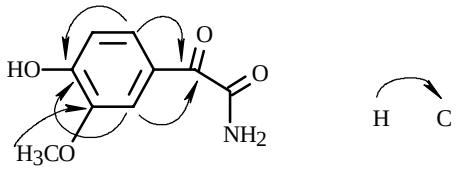
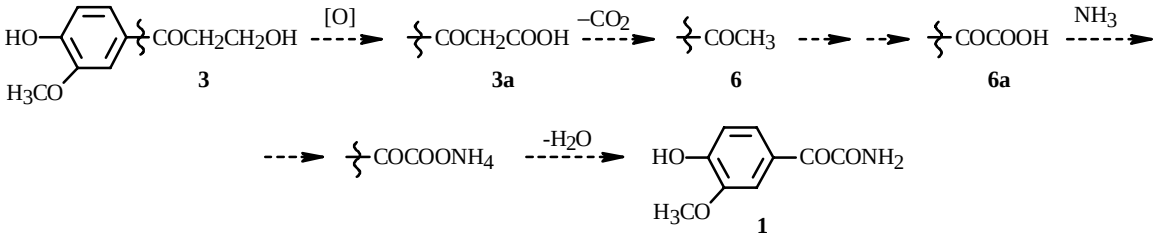


Fig.. 1. Selected HMBC Correlation of **1**.

The ^{1}H NMR spectrum of compound **1** showed one methoxyl group at δ 3.89 (3H, s) and three aromatic protons at δ 6.88 (1H, d, J = 8.3 Hz), 7.65 (1H, d, J = 1.7 Hz), and 7.69 (1H, dd, J = 1.7, 8.3 Hz), indicating the typical tri-substituted aromatic group. The ^{13}C NMR and DEPT spectrum of compound **1** revealed the presence of nine carbon atoms, including one methoxyl, three tertiary carbons, and five quarternary carbons. The signals of two quarternary carbons at δ 169.9 (s, C-1) and 189.6 (s, C-2) indicated the presence of two carbonyl groups, which were supported by the IR absorptions at 1692 and 1644 cm^{-1}. The carbonyl group at δ 189.6 was linked to the C-1′ in compound **1** based on its HMBC spectrum (Fig. 1), exhibiting the correlations of H-6′ (δ 7.69) and H-2′ (δ 7.65) with C-2 (δ 189.6). The correlations in HMBC of OCH_3 (δ 3.89) with C-3′ (δ 149.2) showed that the methoxyl unit was adjacent to C-3′. The additional partial structure $CONH_2$ attached to C-2 was confirmed by the intense peaks at *m/z* 151 in the EI mass spectrum corresponding to the loss of $CONH_2$ from the molecular ion peak at *m/z* 195. The assignment of compound **1** was further supported by its ^{1}H–^{1}H COSY and HMQC spectrum. Following acetylation of compound **1** with acetic anhydride in pyridine, the acetate was subjected to EI-MS analysis and showed a peak at *m/z* 237 ([195-H+$COCH_3$]$^{+}$), which showed that compound **1** contained one hydroxyl group. Based on the above spectral and chemical evidence, compound **1** was identified as 2-(4′-hydroxy-3′-methoxyphenyl)-2-oxoacetamide.

The known compounds 4-acetonyl-3,5-dimethoxy-*p*-quinol (**2**) [10], *β*-hydroxypropiovanillone (3) [11], hexacosanoic acid (**4**) [12], vanillin (**5**) [13], acetovanillone (**6**) [14], coniferyl aldehyde (**7**) [15], sinapic aldehyde (**8**) [16], syringyl aldehyde (**9**) [16], 3-hydroxy-2-methoxybenzoic acid (**10**) [17], 4-hydroxybenzoic acid (**11**) [18], hexatriacontan-1-ol (**12**) [19], and 3*β*-sitosterol (**13**) [20] were identified by spectral data and comparison with those reported in previous work. The 2000-year-preserved heartwood of *P. euphratica* and its chemical constituents may have experienced various physical, biological, and chemical changes during burial in the ancient tomb. The probable origin of compound **1** isolated from this plant may derive from **3** and / or **6** by the reasonable path shown in Scheme 1.



Scheme 1. Hypothetical synthetic pathway to compound **1**.

EXPERIMENTAL

General Comments. Melting points were measured on an XRC-1 micro-melting apparatus and are uncorrected. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were recorded on a UV 210A spectrometer. MS spectra were carried out on a VG Auto Spec-3000 spectrometer. The 1D and 2D NMR spectra were run on Bruker AM-400 MHz and DRX-500 MHz spectrometers using TMS as an internal. Silica gel (200–300 mesh, Marine Chemical Factory, Qingdao, China) was used for column chromatography.

Plant Materials. The heartwoods of *Populus euphratica* Oliver were collected from an ancient tomb in the desert of Xinjiang province in September 2004 and identified by Dr. Hong-En Jiang. A voucher specimen (No. M 8-3) was deposited in the Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The heartwood of *P. euphratica* (1.5 Kg) was extracted with hot acetone (6 L × 3) and filtered. The acetone extraction was evaporated in vacuum. The residue (62 g) was subjected to column chromatography on silica gel (200–300 mesh) and eluted with gradient petroleum–acetone (from 4:1 to 1:4) to yield 10 fractions. Fraction 8 (1.6 g) was purified by repeated column chromatography on Sephadex LH-20 and silica gel with petroleum–acetone–$CHCl_3$ (2:1:2) to afford compounds **1** (6 mg), **2** (13 mg), and **3** (18 mg). Fraction 6 (0.5 g) was chromatographed on Sephadex LH-20 and silica gel with petroleum–EtOAc (3:1) to yield compounds **4** (36 mg), **5** (45 mg), and **6** (8 mg). Fraction 7 (2.2 g) was separated over Sephadex LH-20 and silica gel with petroleum–EtOAc (3:2) to give compounds **7** (5 mg), **8** (12 mg), **9** (7 mg), **10** (52 mg), and **11** (85 mg). Fraction 3 (1.8 g) was subjected to column chromatography on silica gel with petroleum–$CHCl_3$ (3:1) to afford compound **12** (25 mg), and fraction 4 (0.72 g) was chromatographed on Sephadex LH-20 to yield compound **13** (35 mg).

Acetylation of Compounds 1 and 12. Each sample (1 mg) was dissolved in Ac_2O–pyridine (1:0.5) in a sealed micro-tube. After reacting at 60–70°C for 2 h, the acetate of compound **1** was subjected to EI-MS analysis and showed *m/z* 237 ($[M]^+$ + Ac); the acetate of compound **12** was subjected to ESI-MS analysis and showed *m/z* 564 ($[M]^+$ + Ac).

2-(4′-Hydroxy-3′-methoxyphenyl)-2-oxoacetamide (1). Yellow amorphous powder, mp 105~107°; IR bands (KBr, ν, cm^{-1}): 3418, 3159, 2959, 2927, 1692, 1644, 1589, 1518, 1471, 1458, 1285, 1136, 1015, 872, 625; UV (MeOH, λ_{max}, nm): 203, 231, 289, 315. ^{1}H NMR (400 MHz, δ, CD_3OD, J/Hz): 3.89 (3H, s, OC<u>H</u>$_3$), 6.88 (1H, d, J = 8.3, H-5′), 7.65 (1H, d, J = 1.7, H-2′), 7.69 (1H, dd, J = 1.8, 8.3, H-6′); ^{13}C NMR (100 MHz, δ, CD_3OD): 169.9 (s, C-1), 189.6 (s, C-2), 126.3 (s, C-1′), 113.3 (d, C-2′), 149.2 (s, C-3′), 154.8 (s, C-4′), 116.1 (d, C-5′), 127.5 (d, C-6′), 56.4 (q, O<u>C</u>H$_3$); HR-EIMS calcd for $C_9H_9NO_4$ 218.0429 ($[M+Na]^+$), found 218.0429; EIMS *m/z* (%): 195 ($[M]^+$, 23), 151 (100), 136 (4), 123 (33), 108 (12), 80 (4), 77 (5).

4-Acetonyl-3,5-dimethoxy-*p*-quinol (2). Colorless needles, ^{1}H NMR (500 MHz, δ, $CDCl_3$): 2.18 (3H, s, H-3′), 3.02 (2H, s, H-1′), 3.75 (6H, s, OC<u>H</u>$_3$), 5.43 (2H, s, H-2,6); ^{13}C NMR (125 MHz, δ, $CDCl_3$): 31.6 (q, C-3′), 47.5 (t, C-1′), 56.4 (q, O<u>C</u>H$_3$), 71.1 (s, C-4),100.3 (d, C-2,6), 170.5 (s,C-3,5), 186.9 (s, C-1), 207.0 (s, C-2′); EIMS *m/z* (%): 226 ($[M]^+$, 16), 183 (7), 169 (100), 154 (22), 141 (7), 126 (6), 69 (7).

***β*-Hydroxypropiovanillone (3)**. Yellow needles, ^{1}H NMR (400 MHz, δ, $CDCl_3$, J/Hz): 3.17 (2H, t, J = 5.2, H-2), 4.01 (2H, t, J = 5.2, H-3), 6.93 (1H, d, J = 8.0, H-5′), 7.51 (1H, d, J = 1.9, H-2′), 7.53 (1H, dd, J = 1.9, 8.0, H-6′), 3.93 (3H, s, OC<u>H</u>$_3$); ^{13}C NMR (100 MHz, δ, $CDCl_3$): 197.2 (s, C-1), 39.7 (t, C-2), 58.3 (t, C-3), 130.5 (s, C-1′), 109.6 (d, C-2′), 146.7 (s, C-3′), 150.8 (s, C-4′), 114.0 (d, C-5′), 123.6 (d, C-6′), 56.0 (q, O<u>C</u>H$_3$); EIMS *m/z* (%): 196 ($[M]^+$, 34), 178 (5), 151 (100), 123 (28), 108 (9).

Hexacosanoic Acid (4). White amorphous powder, ^{1}H NMR (400 MHz, δ, C_5D_6N): 0.83 (3H, t, H-26), 1.23~1.37 (m, H-4 ~ H-25), 1.77 (2H, m, H-3), 2.50 (2H, t, H-2). EIMS *m/z* (%): 396 ($[M]^+$, 3), 368 (12), 354 (8), 340 (20), 185 (23), 171 (14), 129 (75), 73 (90), 57 (100).

Vanillin (5). Yellow needles, ^{1}H NMR (500 MHz, δ, $CDCl_3$, J/Hz): 3.95 (3H, s, OC<u>H</u>$_3$), 7.03 (1H, dd, J = 1.2, 8.4, H-6), 7.79 (1H, d, J = 8.4, H-5), 7.43 (1H, d, J = 1.2, H-2), 9.82 (1H, s, C<u>H</u>O); EIMS *m/z* (%): 152 ($[M]^+$, 93), 151 (100), 149 (40), 123 (20), 109 (17), 93 (14), 81 (28), 69 (41).

Acetovanillone (6). Yellow needles, ^{1}H NMR (400 MHz, δ, $CDCl_3$, J/Hz): 2.93 (3H, s, H-2), 7.32 (1H, d, J = 8.6, H-5′), 7.90 (1H, d, J = 2.1, H-2′), 7.91 (1H, dd, J = 2.1, 8.6, H-6′), 4.31 (3H, s, OC<u>H</u>$_3$); ^{13}C NMR (100 MHz, δ, $CDCl_3$): 196.8 (s, C-1), 26.1 (q, C-2), 130.2 (s, C-1′), 109.8 (d, C-2′), 146.6 (s, C-3′), 150.4 (s, C-4′), 113.8 (d, C-5′), 124.0 (d, C-6′), 56.1 (q, O<u>C</u>H$_3$); EIMS *m/z* (%): 166 ($[M]^+$, 49), 151 (100), 123 (26), 85 (18).

Coniferyl Aldehyde (7). Yellow needles, ^{1}H NMR (500 MHz, δ, CD_3OD, J/Hz): 3.91 (3H, s, OC<u>H</u>$_3$), 7.24 (1H, d, J = 1.8, H-2′), 7.16 (1H, dd, J = 8.2, 1.8, H-6′), 6.84 (1H, d, J = 8.2, H-5′), 9.56 (1H, d, J = 7.9, H-1), 7.57 (1H, d, J = 15.7, H-3), 6.64 (1H, dd, J = 15.7, 7.9, H-2); ^{13}C NMR (125 MHz, δ, CD_3OD): 56.5 (q, O<u>C</u>H$_3$), 192.9 (d, C-1), 156.2 (d, C-3), 151.7 (s, C-4′), 149.5 (s, C-3′), 127.9 (d, C-2), 127.8 (s, C-1′), 116.7 (d, C-5′), 125.1 (d, C-6′), 112.3 (d, C-2′); EIMS *m/z* (%): 178 ($[M]^+$, 100), 177 (29), 163 (11), 161 (20), 135 (25), 107 (18).

Sinapic Aldehyde (8). Yellow needles, ^{1}H NMR (400 MHz, δ, CD_3OD, J/Hz): 3.89 (6H, s, OC<u>H</u>$_3$), 6.68 (1H, dd, J = 7.8, 15.7, H-2), 7.57 (1H, d, J = 15.7, H-3), 6.99 (2H, s, H-2′, 6′), 9.58 (1H, d, J = 7.8, H-1); ^{13}C NMR (100 MHz, δ, CD_3OD): 56.9 (q, O<u>C</u>H$_3$), 107.6 (d, C-2′, 6′), 126.5 (s, C-1′), 127.1 (d, C-2), 149.6 (s, C-4′), 156.4 (d, C-3), 156.5 (s, C-3′, 5′), 196.1 (d, C-1); EIMS m/z (%): 208 ($[M]^+$, 100), 180 (25), 177 (25), 165 (48), 137 (33), 122 (11), 91 (14), 77 (17).

Syringyl Aldehyde (9). Yellow needles, ^{13}C NMR (125 MHz, δ, $CDCl_3$): 56.5 (q, O<u>C</u>H$_3$), 106.7 (d, C-2′, 6′), 128.4 (s, C-1′), 140.9 (s, C-4′), 147.4 (s, C-3′, 5′), 190.7 (d, <u>C</u>HO); EIMS *m/z* (%): 182 ($[M]^+$, 100), 181 (62), 167 (12), 139 (10), 111 (12), 96 (10), 79 (7).

3-Hydroxy-2-methoxybenzoic Acid (10). White needles, ^{1}H NMR (400 MHz, δ, CD_3OD, J/Hz): 3.89 (3H, s, $OC\underline{H}_3$), 6.81 (1H, dd, J = 8.6, 8.3, H-5), 6.84 (1H, d, J = 8.6, H-4), 7.55 (1H, d, J = 8.3, H-6), 7.87 (1H, s, O<u>H</u>); ^{13}C NMR (100 MHz, δ, CD_3OD): 56.4 (q, $O\underline{C}H_3$), 113.8 (d, C-4), 115.8 (d, C-5), 125.2 (d, C-6), 123.0 (s, C-1), 148.6 (s, C-3), 152.6 (s, C-2), 169.9 (s, <u>C</u>OOH); EIMS *m/z* (%): 168 ($[M]^+$, 100), 167 (8), 153 (87), 151 (25), 125 (30), 123 (10), 97 (25), 79 (7).

4-Hydroxybenzoic Acid (11). Colorless needles, ^{1}H NMR (400 MHz, δ, CD_3OD, J/Hz): 6.82 (2H, d, J = 9.5, H-3, 5), 7.88 (2H, d, J = 9.5, H-2,6), 9.90 (1H, s, O<u>H</u>); ^{13}C NMR (100 MHz, δ, CD_3OD): 116.0 (d, C-3,5), 122.6 (s, C-1), 133.0 (d, C-2,6), 163.3 (s, C-4), 170.1 (s, <u>C</u>OOH); FABMS *m/z* (%): 139 ($[M+1]^+$, 65), 121 (20), 102 (2).

Hexatriacontan-1-ol (12). White amorphous powder, ^{13}C NMR (500 MHz, δ, C_5D_6N): 62.4 (t, C-1), 14.3 (q, C-36), 23.0~36.9 (C-2~C-35). On acetylation with acetic anhydride in pyridine, compound **12** gave a monoacetate with ESI-MS *m/z* 564 $[M+COCH_3]^+$.

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