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A Pyridyl Alkaloid and Benzoic Acid Derivatives from the Rhizomes of *Anemarrhena asphodeloides*

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Abstract – A pyridyl alkaloid, 3-pyridylcarbinol (**1**) and benzoic acid derivatives, 4-hydroxy benzoic acid (**2**), 4-hydroxyactophenone (**3**), vanilic acid (**4**), and benzoic acid (**5**) were isolated from the rhizomes of *Anemarrhena asphodeloides* on the basis of spectroscopic and physicochemical analyses including 1D- and 2D- NMR techniques as well as by comparison of their data with the published values. Compounds **1** - **5** were isolated for the first time from this plant source.
Keywords – *Anemarrhena asphodeloides*, Liliaceae, benzoic acid derivatives, 3-pyridylcarbinol

Introduction

The rhizomes of *Anemarrhena asphodeloides* Bunge (Liliaceae) have been used as a traditional medicine for its antidiabetic, antiphlogistic, antipyretic, sedative, diuretic and anodyne properties in Korea, China, and Japan (Duke *et al*., 2002). There have been phytochemical reports on this species including xanthones (Pardo-Andreu *et al*., 2006), norlignans (Iida *et al*., 2000; Park *et al*., 2003), and steroidal saponins (Nakashima *et al*., 1993; Sy *et al*., 2008; Ren *et al*., 2006), associated with various biological activities such as antidiabetic (Nakashima *et al*., 1993), anticancer (Sy *et al*., 2008), antioxidant (Pardo-Andreu *et al*., 2006), antifungal (Iida *et al*., 2000; Park *et al*., 2003), and antidepressant (Ren *et al*., 2006). As a continuing study of the constituents from the rhizomes of *A. asphodeloides,* we additionally isolated a pyridyl alkaloid and benzoic acid derivatives. This paper deals with the isolation and structure elucidation of **1** - **5**.

Experimental

General – Melting points were measured by using an Electrothermal apparatus. UV and IR spectra were recorded on a U-3000 spectrophotometer (Hitachi, Japan) and a FTS 135 FT-IR spectrometer (Bio-Rad, CA), respectively. 1D and 2D NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA) with tetramethylsilane (TMS) as internal standard. Thin layer chromatography (TLC) was performed on precoated silica gel 60 F254 (0.25 mm, Merck). Silica gel (230 - 400 mesh, Merck, Germany) and RP-18 (YMC-pack ODS-A, 12 nm, S150 μm) were used for column chromatography. Preparative HPLC was run on an Acme 9000 HPLC (Young Lin, South Korea) using the YMC-pack ODS-A column and the flow rate was 1 ml/min.

Plant material – The rhizomes of *A. asphodeloides* were purchased from Omni Herb.com Oriental Herb Store in Seoul, South Korea in September 2008, and were identified by Professor Je-hyun Lee (College of Oriental Medicine, Dongguk University). A voucher specimen (no. EA270) was deposited at the Natural Product Chemistry Laboratory, College of Pharmacy, Ewha Womans University.

Extraction and Isolation – The rhizomes of *A. asphodeloides* (20 kg) were extracted with MeOH three times under reflux for 4 h. The MeOH solutions were concentrated in vacuo to yield a dried MeOH-soluble extract (4 kg). This extract was suspended in distilled water and fractionated with *n*-hexane, EtOAc, and *n*-BuOH, successively. The EtOAc extract (75 g) was chromatographed over a silica gel (1875 g) column, eluting with a gradient solvent system of *n*-hexaneEtOAc (100 : 1 to 1 : 1), to afford 25 fractions (E1 - E25). Fraction E8 (10 g) was chromatographed on a silica gel (200 g) column eluting with $CHCl_3$ - MeOH (50 : 1 to 5 : 1) to afford eight subfractions (E8.1 to E8.8). Subfraction E8.3 (0.5 g) was chromatographed on a silica gel (50 g) column eluting with $CHCl_3$ - MeOH (50 : 1 to 10 : 1) to

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yield compound **1** (3 mg, 0.00008% w/w). Fraction E14 (5.0 g) was chromatographed on a silica gel (150 g) column eluting with $CHCl_3$ - MeOH (50 : 1 to 3 : 1) to afford nine subfractions (E14.1 to E14.9). Subfraction E14.5 (0.2 g) was subjected to a silica gel (50 g) column eluting with $CHCl_3$ - MeOH (50 : 1 to 3 : 1) to give **2** (20 mg, 0.00041% w/w). Fraction E11 (3.0 g) was chromatographed on a silica gel (90 g) column eluting with $CHCl_3$ - MeOH (50 : 1 to 5 : 1) to afford five subfractions (E11.1 to E11.5). Subfraction E11.4 (0.25 g) was subjected to semi-preparative HPLC (MeO - HH_2O, 75 : 25) to yield **4** [10 mg (0.00021% w/w), t_R 125 min). Fraction E6 (15 g) was chromatographed on a silica gel (250 g) column, eluted with $CHCl_3$ - MeOH (50 : 1 to 10 : 1), to afford six subfractions (E6.1 to E6.6). Subfraction E6.2 (0.2 g) was subjected to a silica gel (40 g) column eluting with $CHCl_3$ - MeOH (50 : 1 to 10 : 1) to give **3** (3 mg, 0.00008% w/w) and **5** (10 mg, 0.00021% w/w).

3-Pyridylcarbinol (1) – White needle. IR ν_{max} (KBr) 3300, 1610 cm^{-1}; UV λ_{max} (log ε) (MeOH) 280 (3.54), 210 (3.44) nm; ^{1}H-NMR (400 MHz, CD_3OD) δ: 4.66 (2H, s, H-7), 7.42 (1H, dd, J = 7.6, 8.0 Hz, H-5), 7.84 (1H, d, J = 8.0 Hz, H-4), 8.44 (1H, d, J = 7.6 Hz, H-6), 8.53 (1H, s, H-2); ^{13}C-NMR (100 MHz, CD_3OD) δ: 148.9 (C-2), 148.8 (C-6), 139.4 (C-3), 137.1 (C-4), 125.3 (C-5), 62.6 (C-7); ESIMS *m/z* 109 $[M]^+$.

4-Hydroxybenzoic acid (2) – White powder. IR ν_{max} (KBr) 2900, 1675, 1460, 1300, 690 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; LREIMS *m/z* (rel. int., %) 138 $[M]^+$ (75), 121 (100), 110 (5), 93 (24), 77 (5), 65 (26), 63 (10), 53 (7), 51 (5).

4-Hydroxyacetophenone (3) – White powder. IR ν_{max} (KBr) 3400, 1750, 1657, 1603, 1513, 1460, 1277, 1169, 839, 760 cm^{-1}; UV λ_{max} (log ε) (MeOH) 275 (3.54), 219 (3.44) nm; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; LREIMS *m/z* (rel. int., %) 136 $[M]^+$ (25), 121 (85), 93 (33), 85 (57), 71 (89), 57 (100), 55 (30).

Vanilic acid (4) – Yellow powder, UV (MeOH) λ_{max} (log ε) 288 (3.98) nm; IR ν_{max} (KBr) 3400, 1670, 1620, 1510, 930 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; EI-MS *m/z* 168 $[M]^+$.

Benzoic acid (5) – Amorphous powder. mp 121 - 122 °C; UV λ_{max} (log ε) (MeOH) 256 (3.5), 209 (3.1) nm; IR ν_{max} (KBr) 3202 (OH), 2363, 1675, 1246 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESIMS *m/z* 121 $[M - H]^-$.

Results and Discussion

Repeated chromatography of the EtOAc-soluble fraction of the MeOH extract from the rhizomes of *A. asphodeloides* on silica gel, YMC-pack RP-C_{18} columns led to the isolation of five compounds (**1** - **5**). The isolated compounds were identified as 3-pyridylcarbinol (**1**) (Cho *et al*., 2004), 4-hydroxy benzoic acid (**2**) (Li and Wu, 2002), 4-hydroxyacetophenone (**3**) (Yamauchi *et al*., 1972), vanilic acid (**4**) (Abramovitch *et al*., 1968), and benzoic acid (**5**) (Li and Wu, 2002). Compounds **1** - **5** were isolated for the first time from this plant source. In

Table 2. ^{13}C-NMR Spectroscopic Data for **2** - **5**

Position	2	3	4	5
1	123.0	130.5	123.2	129.6
2	133.1	131.2	115.9	130.4
3	116.1	115.5	148.8	128.7
4	163.4	160.5	152.8	134.1
5	116.1	115.5	113.9	128.7
6	133.1	131.2	125.4	130.4
7	170.3	197.4	170.1	172.8
OCH_3-6			56.5	
CH_3-7		26.5		

Spectrum recorded at ^{13}C-NMR (100 MHz) in CD_3OD.

Table 1. ^{1}H-NMR Spectroscopic Data for **2** - **5**

Position	2	3	4	5
	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1				
2	7.88 (d, 8.8)	7.91 (d, 8.8)	7.55 (m)	8.13 (dd, 1.6, 8.8)
3	6.82 (d, 8.8)	6.90 (d, 8.8)		7.47 (t, 7.6)
4				7.61 (tt, 1.6, 7.6)
5	6.82 (d, 8.8)	6.90 (d, 8.8)	6.82 (d, 8.4)	7.47 (t, 7.6)
6	7.88 (d, 8.8)	7.91 (d, 8.8)	7.55 (m)	8.13 (dd, 1.6, 8.8)
OCH_3-6			3.81 (s)	
CH_3-7		2.56 (s)		

Spectrum recorded at ^{1}H-NMR (400 MHz) in CD_3OD.

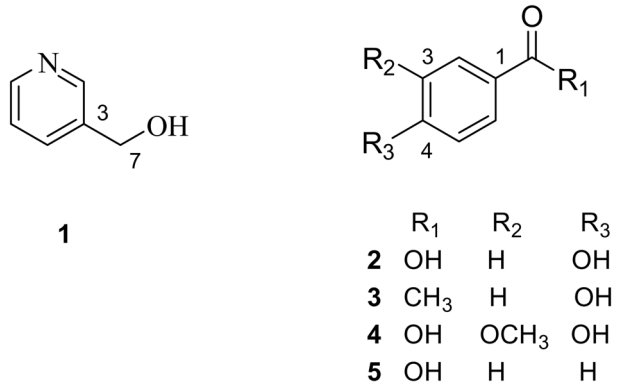

Fig. 1. Chemical structures of compounds **1** - **5** from rhizomes of *A. asphodeloides*.

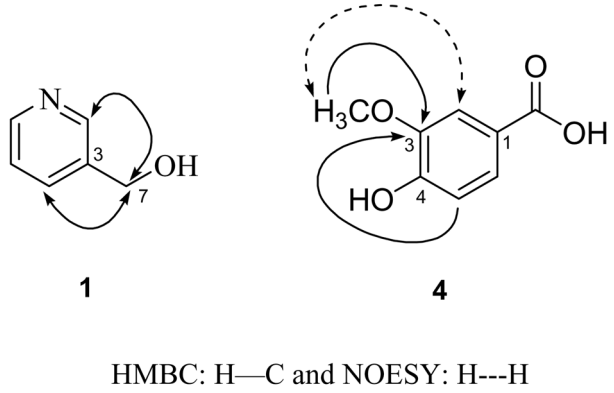

Fig. 2. Important HMBC and NOESY correlations of **1** and **4**.

particular, pyridyl alkaloid, **1** was rarely reported in the nature.

Compound **1** was obtained as white needle and it showed a molecular ion peak at *m/z* 109 $[M]^+$ in the ESIMS. The IR spectrum showed the presence of hydroxyl group at 3300 cm^{-1} and C = N stretch at 1610 cm^{-1}. The ^1H- and ^{13}C-NMR signals for four aromatic methines at δ_H 8.53/ δ_C 148.9 (C-2), δ_H 8.44/ δ_C 148.8 (C-6), δ_H 7.84/ δ_C 137.1 (C-4), and δ_H 7.42/ δ_C 125.3 (C-5), and an oxygenated methylene proton at δ 4.66 (2H, s)/ δ_C 62.6 (C-7), were indicative of the presence of a *ortho*- or *meta*-substituted pyridine derivative. This observation was further supported by the ^1H-^1H COSY experiment, connectivity from δ_H 8.44 (H-6) to δ_H 7.84 (H-4). The HMBC experiment showed a hydroxymethyl group substituted at C-3 in the pyridine molecule, a methylene functionality resonated at δ_H 4.66/ δ_C 62.6, which was correlated with C-2, C-3, and C-4, and two aromatic protons at δ_H 8.53 (H-2) and δ_H 7.84 (H-4) with C-7 (Fig. 2). Therefore, compound **1** was identified as 3-pyridyl-carbinol, which had previously been synthesised from (pyridine-3-yl)carboxylic acid (Cho *et al*., 2004).

Compound **2** was obtained as white powder and it showed a molecular ion peak at *m/z* 138 $[M]^+$ in the LREIMS. The IR spectrum showed the presence of hydroxyl group at 3000 cm^{-1} and carboxylic function at 1675 cm^{-1}. The ^1H- and ^{13}C-NMR spectra of **2** showed the presence of a *para*-substituted benzene group at δ_H 7.88 (2H, d, J = 8.8 Hz)/ δ_C 133.1 (C-2, 6) and δ_H 6.82 (2H, d, J = 8.8 Hz)/ δ_C 116.1 (C-3, 5), an oxygenated aromatic carbon at δ_C 163.4 (C-4), a quarternary carbon at δ_C 123.0 (C-1), and a carbonyl carbon at δ_C 170.3, were characteristics for a *para*-hydroxyl group substituted in benzoic acid. Thus, compound **2** was identified as 4-hydroxybenzoic acid, which had previously been isolated from *Crocus sativus* L. (Li and Wu, 2002).

Compound **3** was obtained as white powder and it showed a molecular ion peak at *m/z* 136 $[M]^+$ in the LREIMS. The IR spectrum showed the presence of hydroxyl group at 3400 cm^{-1} and carboxylic function at 1657 cm^{-1}. The ^1H- and ^{13}C-NMR spectra of **3** showed the characteristic methyl group at δ_H 2.56 (3H, s)/ δ_C 26.5 ($CO\underline{CH_3}$) substituted in benzocarbonyl moiety. In addition, **3** showed the similar patterns compare to the compound **2**. Thus, the structure of compound **3** was confirmed as 4-hydroxyacetophenone, which had previously been isolated from *Nerium odorum* (Yamauchi *et al*., 1972).

Compound **4** was obtained as yellow powder and it showed a molecular ion peak at *m/z* 288 $[M]^+$ in the EIMS. The IR spectrum showed the presence of hydroxyl group at 3400 cm^{-1} and carbonyl group at 1670 cm^{-1}. The ^1H- and ^{13}C-NMR signals for three aromatic protons at δ_H 6.82 (1H, d, J = 8.4 Hz) and δ_H 7.55 (2H, m), an aromatic methoxyl group at δ_H 3.81 (3H, s)/ δ_C 56.5 (OCH_3), and two oxygenated aromatic carbons at δ_C 148.8 (C-3) and 152.8 (C-4), and an ester carbonyl carbon at δ_C 170.1, were indicative of the presence of the vanilic acid or isovanilic acid (Lai *et al*., 1985). The position of methoxyl group in the aromatic ring was clarified by the NOESY correlation between H-2 and OCH_3 (Fig. 2). Thus, the methoxyl group was substituted at C-3 in the molecule. On the basis of the above evidence, compound **4** was identified as vanilic acid, which had previously been isolated from *Opuntia fragilis* (Abramovitch *et al*., 1968).

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