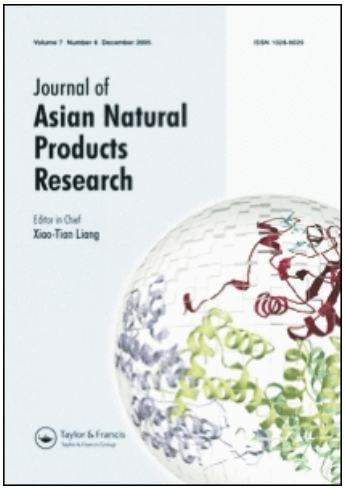

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[a] Department of Biotechnology, National Formosa University, Yunlin, Taiwan [b] Department of Chemistry, National Cheng Kung University, Tainan, Taiwan [c] Department of Cosmetic Science, Chung Hwa University of Medical Technology, Tainan, Taiwan [d] Department of Chemistry, Vinh University, Vinh City, Nghean Province, Vietnam [e] Department of Chemistry, College of Natural Sciences, Hanoi National University, Hanoi, Vietnam [f] National Research Institute of Chinese Medicine, Taipei, Taiwan [g] Department of Applied Chemistry, Providence University, Taichung, Taiwan

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[a]*Department of Biotechnology, National Formosa University, Yunlin 632, Taiwan;* [b]*Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan;* [c]*Department of Cosmetic Science, Chung Hwa University of Medical Technology, Tainan 717, Taiwan;* [d]*Department of Chemistry, Vinh University, Vinh City, Nghean Province, Vietnam;* [e]*Department of Chemistry, College of Natural Sciences, Hanoi National University, Hanoi 10000, Vietnam;* [f]*National Research Institute of Chinese Medicine, Taipei 112, Taiwan;* [g]*Department of Applied Chemistry, Providence University, Taichung 433, Taiwan*

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The investigation on the chemical constituents of the whole plant of *Abutilon indicum* has resulted in the isolation of two new compounds, abutilin A (**1**) and (*R*)-*N*-(1′-methoxycarbonyl-2′-phenylethyl)-4-hydroxybenzamide (**2**), as well as 28 known compounds. The structures of the two new compounds were established on the basis of the spectroscopic analysis, and the known compounds were identified by comparison of their spectroscopic and physical data with those reported in the literature.

Keywords: *Abutilon indicum*; Malvaceae; biphenyl ether; amide

1. Introduction

Abutilon indicum (Malvaceae) is traditionally utilized in many regions of India, Malaya, Philippine Islands, and Indochina for its medicinal uses as febrifuge, anthelmintic, antiemetic, anti-inflammatory, and in urinary and uterine discharges, piles, and lumbago [1–4]. Hypoglycemic, antibacterial, antifungal, antimalarial, and hepatoprotective activities were reported for this genus [5–8]. Alkaloids, flavonoids, steroids, and terpenoids have been isolated and characterized from this genus in the literature survey [4,9–11]. As a part of the integrated program in exploring new active compounds held in Taiwan, *A. indicum* was selected as a target due to the cytotoxicity of its chloroform fraction toward MCF-7 (human breast cancer) (human non-small cell lung cancer), NCI-H460, and SF-268 (human central nervous system cancer) tumor cell lines, with inhibition percentages of 91, 95, and 95%, respectively, at 150 μg/ml. In our investigation on the phytochemical diversity of this plant, the structures of a new biphenyl ether abutilin A (**1**) and a new amide (*R*)-*N*-(1′-methoxycarbonyl-2′-phenylethyl)-4-hydroxybenzamide (**2**) were determined by the spectroscopic methods including 1D- and 2D-NMR and MS techniques. Moreover, 28 known compounds, including 3 alkaloids, 2 amides, 3 coumarins, 12 benzenoids, 2 ionones, 4 nucleosides, and 2 steroids, were identified by comparison of their spectral and physical data with those reported in the literature. The present study reports the structural elucidation of two new compounds **1** and **2**.

2. Results and discussion

The air-dried and powdered whole plants of *A. indicum* were extracted with hot methanol and concentrated to give a dark brown syrup.

*Corresponding author. Email: tswu@mail.ncku.edu.tw

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The methanolic extract was suspended in H_2O and partitioned with $CHCl_3$. The successive purification of $CHCl_3$- and H_2O-soluble extracts by a combination of conventional chromatographic techniques afforded two new compounds (**1** and **2**). In addition, 28 known compounds including a mixture of β-sitosterol (**3**) and stigmasterol (**4**), vanillin (**5**), methylcoumarate (**6**), 4-hydroxyacetophenone (**7**), *p*-hydroxybenzaldehyde (**8**), aurantiamide acetate (**9**) [12], methyl indole-3-carboxylate (**10**) [13], 3,7-dihydroxychromen-2-one (**11**) [14], methylparaben (**12**), scoparone (**13**) [15], scopoletin (**14**) [16], syringaldehyde (**15**), 1-methoxycarbonyl-β-carboline (**16**) [17], 4-hydroxy-3-methoxy-*trans*-cinnamic acid methyl ester (**17**), *trans*-*p*-coumaric acid (**18**), thymine (**19**), adenine (**20**), methyl 4-hydroxyphenylacetate (**21**), riboflavin (**22**), 1-lycoperodine (**23**) [18], 3-hydroxy-β-damascone (**24**) [19], adenosine (**25**), *p*-hydroxybenzoic acid (**26**), 3-hydroxy-β-ionol (**27**) [20], *N*-feruloyl tyrosine (**28**) [21], vanillic acid (**29**), and benzoic acid (**30**) were also isolated from the study plant, and the structures were established on the basis of spectroscopic analysis. Among them, aurantiamide acetate (**9**) was reported to inhibit calf DNA polymerase [22] and may be the source of cytotoxicity of this plant material. The structures of the new compounds **1** and **2** were elucidated on the basis of the spectroscopic evidence as follows.

Abutilin A (**1**), obtained as a colorless syrup, was shown to have a molecular formula of $C_{15}H_{12}O_4$ from the pseudomolecular ion peak $[M + H]^+$ at *m/z* 257.0811 in the HR-FAB-MS analysis. The UV spectrum of **1** in MeOH showed absorption maxima at 225 and 275 nm and suggested that **1** is a benzenoid derivative [23]. The broad and strong IR absorption bands at 3401 and 1600 cm^{-1} indicated the presence of hydroxyl and conjugated carbonyl functions, respectively. In addition, absorptions at 773 cm^{-1} revealed the presence of *para*-substituted phenyl ring system. The ^1H NMR spectrum of **1** revealed two sets of mutually coupled doublets at δ 6.72 (2H, d, $J = 8.7$ Hz) and 7.63 (2H, d, $J = 8.7$ Hz), and δ 6.67 (2H, d, $J = 8.4$ Hz) and 7.07 (2H, d, $J = 8.4$ Hz), respectively, characteristic of the AA′BB′ system of proton signals attributed to two *para*-substituted benzene rings. In addition, one methylene singlet at δ 3.35 (2H) and one downfield singlet at δ 9.52 (1H) were also appeared in the ^1H NMR spectrum. The two moieties of the *para*-substituted phenyl rings connected to an ether linkage were suggested by the molecular formula and also the two oxygenated quaternary aromatic carbons at δ 156.5 and 170.3. For the A-ring in the molecule of **1**, one $-CH_2CO_2H$ fragment was attached at C-4 position through the analyses for the 2D-NMR experiments. One set of aromatic protons at δ 7.07 (H-3 and H-5) exhibited HMBC ^3J correlations with carbons at δ 156.5 (C-1) and 45.5 (C-7). One singlet at δ 3.35 (2H, H-7) correlated with the carbon at δ 45.5 (C-7) in the HMQC spectrum for the methylene group displayed ^2J correlations with carbonyl carbon for the carboxylic acid at δ 180.2 (C-8) and aromatic quaternary carbon at δ 131.1 (C-4) in the HMBC analysis. There are also HMBC cross-peaks between another set of aromatic protons at δ 6.67 (H-2 and H-6) and the quaternary carbon mentioned above. Moreover, in the HMBC analysis, aromatic protons at δ 7.63 (H-3′ and H-5′) exhibited ^3J correlations with carbons at δ 170.3 (C-1′) and 192.3 (C-7′); another upfield set of aromatic protons at δ 6.72 (H-2′ and H-6′) and an aldehydic proton at δ 9.52 displayed ^3J and ^2J correlations with one quaternary carbon at δ 130.4 (C-4′). These HMBC cross-peaks confirmed the substitution pattern of B-ring for the biphenyl ether as 4-formyl-substituted phenoxyl moiety. Accordingly, **1** was confirmed to be a new biphenyl ether as shown in Figure 1 and assigned the trivial name abutilin A.

(*R*)-*N*-(1′-Methoxycarbonyl-2′-phenylethyl)-4-hydroxybenzamide (**2**) was obtained as colorless optically active powder, with mp 130–132°C and $[\alpha]_D^{25}$ − 13.1. The pseudomolecular ion peak at *m/z* 300.1234 $[M + H]^+$ in its HR-FAB-MS corresponded with the molecular formula $C_{17}H_{17}NO_4$. The UV absorption

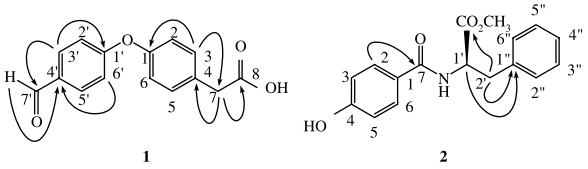

Figure 1. Structures and significant HMBC correlations of compounds **1** and **2**.

maxima of **2** at 228 and 324 nm implied the presence of conjugated double bond system and aromatic skeleton [23]. The IR spectrum displayed absorbance's for hydroxyl group (3324 cm^{-1}), carbonyl group in ester (1741 cm^{-1}), and carbonyl group in conjugated amide (1646 cm^{-1}), respectively. In the ^1H NMR spectrum of **2**, one AA′BB′ system of proton signals at δ 7.72 (2H, d, $J = 7.7$ Hz) and 7.13 (2H, d, $J = 7.7$ Hz) suggested that there is one *para*-substituted phenyl ring. The remaining signals in the aromatic region of the ^1H NMR spectrum appeared as a typical mono-substituted benzene ring at δ 7.30 (2H, dd, $J = 7.6, 2.0$ Hz), 7.43 (2H, dd, $J = 7.6, 7.2$ Hz), and 7.51 (1H, dd, $J = 7.2, 2.0$ Hz). The remaining six aliphatic protons including deshielded methylene protons at δ 3.30 (1H, dd, $J = 14.0, 6.1$ Hz) and 3.23 (1H, dd, $J = 14.0, 5.2$ Hz), a deshielded methine proton at δ 5.10 (1H, dd, $J = 6.1, 5.2$ Hz), and a methoxyl group at δ 3.77 (3H, s) together with the carbon signals in the ^{13}C NMR spectrum at δ 37.9, 52.4, 53.5, and 172.0 could be assigned to the fragment for $-CH_2CH(NH)CO_2CH_3$. This fragment was connected with the mono-substituted benzene ring due to the HMBC correlation between protons at δ 3.30 (H-2′) and 5.10 (H-1′) with the carbon at δ 135.8 (C-1″). Moreover, the HMBC analysis also displayed the ^3J correlation between protons at δ 7.72 (H-2 and H-6) and amide carbonyl at δ 166.8 (C-7). The full assignment of the ^1H and ^{13}C NMR signals were substantiated by extensive 2D-NMR experiments including the COSY, NOESY, HMQC, and HMBC spectra. These spectral elucidations defined the structure of compound **2** as *N*-(1′-methoxycarbonyl-2′-phenylethyl)-4-hydroxybenzamide (Figure 1). In the previous report [24], *N*-(1′-methoxy-carbonyl-2′-phenylethyl)-4-hydroxybenzamide was synthesized via (1′*S*)-*N*-1′-(1″-methoxy-carbonyl-2″-phenylethyl)-1-hydroxy-4-oxo-2-cyclohexene-1-carbonamide, so that the product was defined as (*S*) configuration. However, compared with the optical rotation value of **2** with another natural product *N*-(4-methoxybenzoyl)-D-phenylalanine [25], both compounds exhibited the negative value of optical rotation power. These spectral data determined that the stereo configuration at 1′-position of **2** as *R* and thus also confirmed that **2** is a new amide that is reported from the natural source for the first time.

3. Experimental

3.1 General experimental procedures

Melting points were determined using Yanagimoto MP-S3 micro melting point apparatus without correction. UV spectra were obtained on a Hitachi UV-3210 spectrophotometer, and IR spectra were recorded on a Shimadzu FT-IR DR-8011 spectrophotometer. Optical rotations were measured using a Jasco DIP-370 digital polarimeter. ^1H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on the Bruker Avance-300 NMR spectrometer, using TMS as the internal standard. Standard pulse sequences and parameters were used for the NMR experiments and all chemical shifts were reported in parts per million (ppm, δ). All the FAB mass and high-resolution mass spectra were obtained on a JEOL JMS-700 spectrometer. TLC was conducted on precoated Kieselgel 60 F 254 plates (Merck, Whitehouse Station, NJ, USA) and the spots were detected either by examining the plates under a UV lamp or by treating the plates with a 10% methanolic

solution of phosphomolybdic acid followed by heating at 110°C.

3.2 Plant material

The whole plant of *A. indicum* (Malvaceae) was collected from Vietnam and the plant material identified and authenticated by Association Professor Dr Vu Xuan Phuong, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology. A voucher specimen (TSWVIE 2004001) has been deposited in the herbarium of the Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hanoi, Vietnam.

3.3 Extraction and isolation

Whole plant material of *A. indicum* (5 kg) was shade dried, ground, extracted with methanol under reflux, and filtered to give the residue. The combined filtrate was concentrated under reduced pressure to obtain a dark brown crude extract (430 g) that was suspended in water. The suspension was then treated with chloroform, after removal of the solvent, to give $CHCl_3$- and H_2O-soluble residues. The $CHCl_3$-soluble residue (180 g) was chromatographed over a silica gel column, which was then developed by gradient elution with *n*-hexane and increasing concentrations of ethyl acetate to afford 12 fractions. Fraction 3 (10 g) gave a mixture of **3** and **4** (20 mg) when subjected to silica gel column chromatography eluting with benzene–acetone (19:1). Chromatography of fraction 7 (15 g) on silica gel column by eluting with a mixture of *n*-hexane–acetone (19:1) and step gradient with acetone led to the isolation of pure compounds **2** (5 mg), **5** (2 mg), **6** (3 mg), **7** (2 mg), **8** (2 mg), **9** (50 mg), **10** (3 mg), **11** (1 mg), and **12** (1 mg). Separation of fraction 9 (5 g) by silica gel column chromatography with *n*-hexane–acetone (9:1) afforded compound **13** (5 mg). Fraction 11 (10 g) was subjected to a series of silica gel column chromatographic separation using $CHCl_3$/-MeOH. Final purifications of the resulting fractions was achieved through preparative TLC on silica gel ($CHCl_3$–MeOH, 19:1) to obtain pure compounds **14** (3 mg), **15** (5 mg), and **16** (2 mg).

The H_2O-soluble residue (250 g) was subjected to Diaion HP-20 column chromatography eluting with increasing concentrations of MeOH in H_2O to give six fractions. Chromatography of fraction 3 (10 g) over Sephadex LH-20 eluting with a mixture of H_2O–MeOH and successive purification by preparative TLC on silica gel with a mixture of $CHCl_3$–MeOH (9:1) saturated with water gave compounds **17** (2 mg), **18** (2 mg), **19** (10 mg), and **20** (2 mg). Purification of fraction 4 (20 g) on Sephadex LH-20 column chromatography eluting with a mixture of H_2O–MeOH followed by a series of column chromatography separations over silica gel led to the isolation of **1** (1 mg), **21** (1 mg), **22** (3 mg), **23** (2 mg), **24** (5 mg), **25** (3 mg), **26** (2 mg), **27** (5 mg), and **28** (5 mg). Separation of fraction 6 (10 g) by repeated column chromatography over silica gel using $CHCl_3$–MeOH gradients followed by purification with preparative TLC on silica gel with $CHCl_3$–MeOH–H_2O (19:1:0.05) yielded compounds **29** (3 mg) and **30** (3 mg).

3.3.1 Abutilon A (*1*)

Colorless syrup. UV λ_{max} (MeOH) nm (log ε): 275 (2.02), 225 (2.36, sh). IR (KBr) ν_{max} cm^{-1}: 3401 (OH), 2924, 2830, 1600 (C = O), 1359, 773. ^1H NMR (CD_3OD, 300 MHz) δ (ppm): 3.35 (2H, s, CH_2-7), 6.67 (2H, d, $J = 8.4$ Hz, H-2 and H-6), 6.72 (2H, d, $J = 8.7$ Hz, H-2′ and H-6′), 7.07 (2H, d, $J = 8.4$ Hz, H-3 and H-5), 7.63 (2H, d, $J = 8.7$ Hz, H-3′ and H-5′), 9.52 (1H, s, CHO-7′). ^{13}C NMR (CD_3OD, 75 MHz) δ (ppm): 45.5 (C-7), 115.9 (C-2 and C-6), 118.8 (C-2′ and C-6′), 130.4 (C-4′), 131.1 (C-3 and C-5), 131.1 (C-4), 133.9 (C-3′ and C-5′), 156.5 (C-1), 170.3 (C-1′), 180.2 (C-8), 192.3 (C-7′). FAB-MS *m/z*: 257 ($[M + H]^+$, 5). HR-FAB-MS *m/z*: 257.0811 $[M + H]^+$ (calcd for $C_{15}H_{13}O_4$, 257.0814).

*3.3.2 (*R*)-N-(1′-Methoxycarbonyl-2′-phenylethyl)-4-hydroxybenzamide (**2**)*

Colorless powder, mp 130–132°C. $[\alpha]_D^{25}$ − 13.1 ($c = 0.7$, MeOH). UV λ_{max} (MeOH) nm (log ε): 324 (2.31), 228 (3.14, sh). IR (KBr) ν_{max} cm^{-1}: 3324, 2923, 1741, 1646, 1529, 1216, 702. ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.23 (1H, dd, $J = 14.0$, 5.2 Hz, H-2′), 3.30 (1H, dd, $J = 14.0$, 6.1 Hz, H-2′), 3.77 (3H, s, OCH_3), 5.10 (1H, dd, $J = 6.1$, 5.2 Hz, H-1′), 6.56 (1H, br s, D_2O exchangeable, NH), 7.13 (2H, d, $J = 7.7$ Hz, H-3 and H-5), 7.30 (2H, dd, $J = 7.6$, 2.0 Hz, H-2″ and H-6″), 7.43 (2H, dd, $J = 7.6$, 7.2 Hz, H-3″ and H-5″), and 7.51 (1H, dd, $J = 7.2$, 2.0 Hz, H-4″), 7.72 (2H, d, $J = 7.7$ Hz, H-2 and H-6). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 37.9 (C-2′), 52.4 (C-1′), 53.5 (OCH_3), 127.0 (C-2 and C-6), 127.2 (C-1), 128.6 (C-2″ & C-6″, and C-3″ and C-5″), 129.3 (C-3 and C-5), 131.8 (C-4″), 133.9 (C-4), 135.8 (C-1″), 166.8 (C-7), 172.0 (CO_2CH_3). FAB-MS *m/z*: 300 ($[M + H]^+$, 2). HR-FAB-MS *m/z*: 300.1234 $[M + H]^+$ (calcd for $C_{17}H_{18}NO_4$, 300.1236).

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