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Phenolic compounds from *Anomianthus dulcis*

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Abstract

p-Coumaroyl-β-phenethylamine (**1**) and 2′,3′-dihydroxy-4′,6′-dimethoxydihydrochalcone (**2**) were isolated by preparative HPLC from a methanolic extract of *Anomianthus dulcis* leaves. This is the first time that these compounds were isolated from a natural source. In addition, the known compounds chrysin (**3**), pinocembrin (**4**), 5,7-dimethoxy-8-hydroxyflavanone (**5**) and 2′,3′-dihydroxy-4′,6′-dimethoxychalcone (**6**) were isolated for the first time from the genus *Anomianthus*. The isolated compounds were identified by ^{1}H and ^{13}C NMR, MS, UV and IR.

Keywords: *Anomianthus dulcis*; Annonaceae; Flavonoids; Chalcone; 2′,3′-Dihydroxy-4′,6′-dimethoxydihydrochalcone; Hydroxycinnamoylamide; *p*-Coumaroyl-β-phenethylamine

1. Introduction

The Annonaceae are a large family of aromatic trees, shrubs or climbers (ca. 120 genera and more than 2000 species), which are widely distributed in tropical and subtropical regions (Heywood, 1978).

The climber *Anomianthus dulcis* (Dun.) Sinclair is a member of the Annonaceae family that grows in many parts of South East Asia. Recently, we reported the isolation of alkaloids from the leaves and stem (Sinz et al., 1998b), as well as the isolation of the Annonaceous acetogenin squamocin from the stem of *Anomianthus dulcis* (Sinz, Matusch, Santisuk, Chaichana, & Reutrakul, 1998a). Our present phytochemical investigations of this species reveal the presence of several phenolic constituents in the leaves of *Anomianthus dulcis*.

2. Results and discussion

A crude methanolic extract from the leaves of *Anomianthus dulcis* was evaporated and dissolved in water. The suspension was acidified to pH 1.5 and successively extracted with *n*-hexane and methylene chloride. Alkalization of the water phase to pH 10.0 and extraction with methylene chloride lead to the isolation of the alkaloids as recently reported (Sinz et al., 1998b). From the fraction extracted with methylene chloride at pH 1.5 compounds **1**–**6** were isolated by preparative HPLC. To avoid the isolation of artifacts, analytical HPLC was performed before and after acid and base treatment. No difference in the chromatograms was observed.

Compound **1** gave a UV spectrum with strong absorption maxima at $\lambda = 300$, 277, 223 and 217 nm. The EI-mass spectrum of **1** exhibited a $[M]^+$ peak at $m/z = 267$ and two characteristic fragment ions at $m/z = 148$ and 120. High resolution mass spectrometry revealed the $[M]^+$ peak at $m/z = 267.1259$ suggesting that compound **1** possesses the molecular formula $C_{17}H_{17}NO_2$. The fragment ion at $m/z = 120.0600$ was assigned to the molecular formula C_8H_8O which constitutes a fragment formed by the cleavage of the C-8′ carbonyl bond, while the fragment ion at $m/z = 148.0768$ was assigned to the molecular formula $C_9H_{10}NO$.

The ^{1}H NMR spectrum of **1** showed two signals at δ 7.02 (d, 2H, $J = 8.7$ Hz) and 6.69 (d, 2H, $J = 8.7$ Hz) that are due to the C-2′ and C-6′ and the C-3′ and C-5′ protons, respectively. The C-3 and C-5 protons appeared at δ 7.40 (2H, dd, $J = 7.4$ Hz, $J = 6.8$ Hz), while the C-2 and C-6 protons exhibited a dd at δ 7.55 (2H, $J = 7.4$ Hz, $J = 1.7$ Hz). A tt at δ 7.38 (1H, $J = 6.8$ Hz, $J = 1.7$ Hz) was assigned to the C-4 proton. The downfield doublet at δ 7.41 ($J = 15.7$ Hz) was assigned to the olefinic proton

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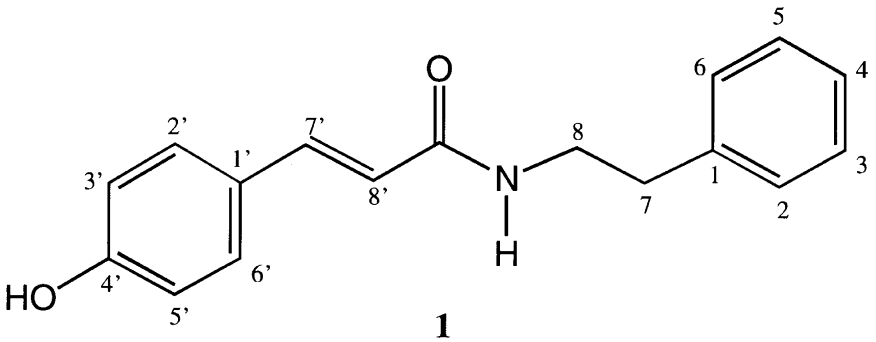

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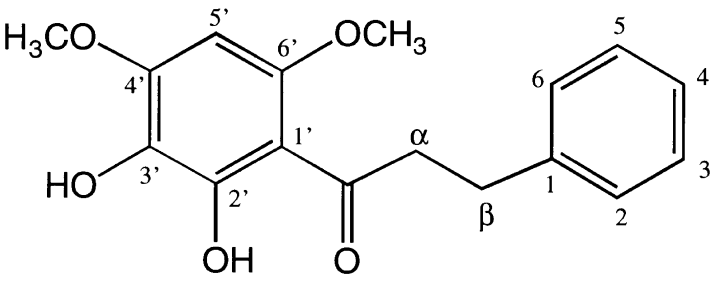

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at C-7′ which showed *trans*-coupling with the olefinic proton at C-8′ that appeared as doublet at δ 6.62 ($J=15.7$ Hz). The C-7 protons showed a triplet at δ 2.67 ($J=7.1$ Hz). At room temperature, the presence of two amide rotamers in **1** was confirmed by the C-8 protons which exhibited two triplets ($J=7.1$ Hz) at δ 3.36 for the *Z* rotamer and at δ 3.34 for the *E* rotamer, respectively. Integration of the signals revealed an approximate 1:1 distribution of the two rotamers.

The ^{13}C NMR spectrum of **1** showed 13 carbon resonances (Table 1). The assignments of the carbon atoms were made by means of the DEPT pulse sequence, HMQC and gated decoupling experiments. Paprazine, a similar alkaloid from *Fumaria indica* (Atta-ur-Rahman, Bhatti, Akhtar, & Choudhary, 1992) differs from **1** only by the presence of an additional hydroxyl group at C-4.

Chrysin (**3**) (Shen, Chang, & Ho, 1993), pinocembrin (**4**) (Wagner & Chari, 1976) and 5,7-dimethoxy-8-hydroxyflavanone (**5**) (Bhaskar & Seshadri, 1974; Vieira, De Alvarenga, Gottlieb, & Gottlieb, 1980) were unambiguously identified by comparison with reported data.

Compound **6**, obtained as orange crystals, revealed a strong UV absorption maximum at $\lambda=342$ nm which is characteristic of a chalcone structure. The spectral data were in close agreement with those of 2′,3′-dihydroxy-4′,6′-dimethoxychalcone (Bhaskar & Seshadri, 1974; Vieira et al., 1980; Ichino, Tanaka, & Ito, 1988).

Compound **2**, obtained as colorless crystals, showed UV maxima at $\lambda=350$, 291 and 242 nm. Treatment with $AlCl_3$ exhibited a bathochromic shift that was stable in acid solution. The ^{13}C NMR spectrum of **2** Table 1 appeared similar to that of **6**. The proton NMR spectrum of **2** showed a characteristic downfield signal for the C-2′-OH group at δ 13.8 which is caused by a hydrogen bridge formation with the carbonyl oxygen. In addition, the ^{1}H NMR spectrum of **2** showed signals corresponding to the protons of the unsubstituted B ring and a singlet appeared at δ 6.06 for the C-5′-proton. However, instead of signals corresponding to the *trans*-double bond present in **6**, two triplets at δ 3.34 and δ 3.00 ($J=8.0$ Hz) were observed suggesting the presence of a saturated bond between C-α and C-β. This assignment was confirmed by a DEPT NMR spectrum. Therefore, the structure of compound **2** was determined to be 2′,3′-dihydroxy-4′,6′-dimethoxydihydrochalcone. The structure was verified by the IR, UV and MS data which correspond with data

Table 1
^{13}C NMR spectral data of compounds **1** and **2**, 100 MHz

Carbon	**1** (DMSO-d_6)	**2** ($CDCl_3$)
1	135.5	135.5
2	128.0	128.5
3	129.4	129.0
4	129.9	130.3
5	129.4	129.0
6	128.0	128.5
7	34.9	
8	41.2	
C-α		45.9
C-β		30.6
1′	130.1	106.4
2′	130.0	152.7
3′	115.6	128.5
4′	156.2	155.8
5′	115.6	87.7
6′	130.0	152.1
7′	138.9	
8′	122.9	
C=O	165.3	193.3
OCH_3		56.3, 56.2

reported by Bhaskar and Seshadri who obtained **2** from **6** by catalytic hydrogenation (Bhaskar & Seshadri, 1974).

3. Experimental

3.1. General

M.p.'s: uncorrected; NMR spectra: Jeol GX 400 Delta spectrometer using TMS as internal standard; UV spectra: UV-VIS photometer 2101 PC (Shimadzu); IR spectra: Nicolet 510 P FT-IR spectrometer; EI-mass spectra: 70 eV; prep. HPLC: Waters prep LC 4000 system with 990 Diode Array Detector.

3.2. Plant material

Leaves were collected in July 1995 at Mae Rim-Samoeng road (Thailand). The identity of the plant material was confirmed by Dr. P. Kessler, Rijksherbarium Leiden (L), where a voucher specimen has been deposited. Voucher specimen: Coll. A. Nuntasan s.n., Thailand, Mae Rim-Samoeng Road, Chiangmai, 7 July 1995 (L 997.104 782).

3.3. Extraction and isolation

The plant material was dried (4 kg d.w.), crushed and percolated at 30°C with MeOH. After evaporation of MeOH, the residue (687 g) was dissolved in H_2O, acidified with 1% HCl (pH 1.5) and filtered. The solution was extracted with *n*-hexane (3 × 500 ml) and CH_2Cl_2 (3 × 500 ml). The CH_2Cl_2 phase, evaporated to dryness, afforded 9.85 g of a gummy material. This was fractionated by prep. HPLC (250 × 25 mm, RPselect B, 15 μm (Merck), 32 ml min^{-1}, MeOH–H_2O (6:4)) into 8 frs (fr. 1–8). Fr. 2 consisted of 5,7-dimethoxy-8-hydroxyflavanone (**5**)(1.169 g), fr. 3 was separated further by prep. HPLC (250 × 25 mm, RPselect B, 15 μm (Merck), 32 ml min^{-1}, MeOH–H_2O (1:1)) to yield 78 mg of compound **1**, fr. 5 consisted of 297 mg pinocembrin (**4**), fr. 6 was separated by prep. HPLC (250 × 25 mm, DIOL, 7 μm (Merck), 40 ml min^{-1} *tert*·butylmethylether–hexane (4:6), to give 149 mg of 2′,3′-dihydroxy-4′,6′-dimethoxychalcone (**6**) and 140 mg of 2′,3′-dihydroxy-4′,6′-dimethoxydihydrochalcone (**2**). Fr. 7 consisted of 205 mg chrysin (**3**).

3.4. p-*Coumaroyl-β-phenethylamine* (*1*)

Colorless crystals (78 mg), m.p.: 146–148°C, UV λ^{MeOH}_{max} nm (log ε): 300 sh (4.12), 277 (4.51), 223 (4.47), 217 (4.49). IR (KBr, cm^{-1}): 3278, 1668, 1626, 1535. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.12 (1H, s, OH), 7.55 (2H, dd, J = 7.4, 1.7, H-2, H-6), 7.41 (1H, d, J = 15.7, H-7′), 7.40 (2H, dd, J = 7.4, 6.8, H-3, H-5), 7.38 (1H, tt, J = 6.8, 1.7, H-4), 7.02 (2H, d, J = 8.7, H-2′, H-6′), 6.69 (2H, d, J = 8.7, H-3′, H-5′), 6.62 (1H, d, J = 15.7, H-8′), 5.74 (1H, s, NH), 3.36 (*Z* rotamer, t, J = 7.1, H-8), 3.34 (*E* rotamer, t, J = 7.1, H-8), 2.67 (1H, t, J = 7.1, H-7). ^{13}C NMR (100 MHz, DMSO-d_6): Table 1. EI-MS (70 eV): m/z (rel. int.) 267 $[M]^+$ (4), 148 (58), 132 (10), 131 (97), 121 (10), 120 (100), 107 (18), 103 (47), 77 (32). HR-MS: found: 267.1259; calculated for $C_{17}H_{17}NO_2$: 267.1259. Fragment C_8H_8O: found: 120.0600; calculated: 120.0575. Fragment $C_9H_{10}NO$: found: 148.0768; calculated: 148.0762.

3.5. 2′,3′-*Dihydroxy-4′,6′-dimethoxydihydrochalcone* (*2*)

Colorless crystals (140 mg), m.p.: 134–136°C, UV λ^{MeOH}_{max} nm (log ε): 350 (3.59), 291 (4.27), 242 (4.07); + $AlCl_3$: 414, 318, 252; + $AlCl_3$/HCl: 401, 367, 316, 246; + NaOMe: 372, 298 sh, 262; + NaOAc: 355, 298, 247; + NaOAc/H_3BO_3: 351, 313 sh, 293, 245. IR (KBr, cm^{-1}): 1635 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.84 (1H, brs, OH-2′), 7.30–7.22 (5H, m, H-2, H-3, H-4, H-5, H-6), 6.06 (1H, s, H-5′), 4.04 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 3.34 (2H, t, J = 8.0, H-α), 3.00 (2H, t, J = 8.0, H-β). ^{13}C NMR (100 MHz, $CDCl_3$): Table 1. EI-MS (70 eV): m/z (rel. int.) 303 $[M+H]^+$ (6), 302 $[M]^+$ (32), 198 (10), 197 (100), 182 (17), 170 (27), 167 (4), 153 (4), 139 (4), 104 (5), 91 (24), 77 (6), 69 (10). HR-MS: found: 302.1142, calculated for $C_{17}H_{18}O_5$: 302.1154.

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References

Atta-ur-Rahman, Bhatti, M. K., Akhtar, F., & Choudhary, M. I. (1992). *Phytochemistry*, *31*, 2869.

Bhaskar, A., & Seshadri, T. R. (1974). *Indian J. Chem.*, *12*, 557.

Heywood, V. H. (1978). *Flowering plants of the world*. Oxford: University Press.

Ichino, K., Tanaka, H., & Ito, K. (1988). *J. Nat. Prod.*, *51*, 90.

Shen, C. C., Chang, Y. S., & Ho, L. K. (1993). *Phytochemistry*, *34*, 843.

Sinz, A., Matusch, R., Santisuk, T., Chaichana, S., & Reutrakul, V. (1998). *Biochem. Syst. Ecol.*, *26*, 361.

Sinz, A., Matusch, R., Witte, L., Santisuk, T., Chaichana, S., & Reutrakul, V. (1998). *Biochem. Syst. Ecol.*, *26*, 139.

Vieira, P. C., De Alvarenga, M. A., Gottlieb, O. R., & Gottlieb, H. E. (1980). *Planta Med.*, *39*, 153.

Wagner, H., & Chari, V. M. (1976). *Tetrahedron Lett.*, *21*, 1799.