Alkaloids and Flavone Acyl Glycosides from *Acanthus arboreus*

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O estudo fitoquímico de *Acanthus arboreus* resultou no isolamento de 3 novos alcalóides: 6-hidróxi-2-benzoxazolinona, 4-hidróxi-acantamina (3,4-di-hidróxi-1,4-benzoaxino-2-onha) e acantaminósideo (3-O-glicopiranosídeo-1,4-benzoxazino-2-onha). Além destes alcalóides foi também isolado o novo flavonóide 7-O-β-D-(6”-trans-p-cumaroil)3”-O-acetilglicopiranosídeo apigenina e os seguintes compostos de estruturas já conhecidas: apigenina, 7-O-β-D-(6”-trans-p-cumaroil)3”-O-glicopiranosídeo apigenina, ácido vanilico, lupeol, estigmasterol e 3-β-glicopiranosídeo sitosterol. As estruturas dos compostos foram determinadas por métodos espectroscópicos e transformações químicas.

**Keywords:** *Acanthus arboreus*, Acanthaceae, alcalóides, flavoné acil glicosídeos, atividade antimicrobiana

**Introduction**

The Acanthaceae is a large family with more than 250 genera and 2700 species. Chemical investigation of genus *Acanthus* resulted in the isolation of flavonoids, alcalóides, triterpenoids and sterols. *A. ilicifolius* is used as anticonvulsant, hypnotic and skeletal muscle relaxant due to the presence of benzoxazolinone; an alcalóide with CNS depressant activity. Benzoxazolinone also exhibited antiprotozoal activity against *Leishmania donovani in vitro*; while its ribose derivatives were active as anticancer and antiviral agents.

**Results and Discussion**

The HRCI-MS of 1 showed an M+1 at m/z 579.151 for the molecular formula C_{30}H_{26}O_{12}. Physical and spectral data of 1 were identical with those reported for apigenin O-β-D-(6”-trans-p-cumaroil) glucoside isolated from *Pogostemon cablin*. However, Singh et al in 1986 reported the same compound as a novel product from *Echinops echinatus* under the name echinacin. Based on COSY and HMCO experiments, complete assignments for the sugar protons and all carbons were achieved. Our assignments were in complete agreement with first publication. However, some of the 13C-NMR assignments in the latter publication must be revised.

The HRCI-MS (M+1 at m/z 621.161 for the molecular formula C_{32}H_{28}O_{13}) and other spectral data of 4 (see experimental) indicated an additional acetyl group compared with 1. Complete assignments of the sugar protons could be achieved by a combination of COSY and HMCO experiments. A major difference was observed in the chemical shift of H-3” (δ 4.97, t) compared to that of 1 (δ 3.30, m), indicating that the acetyl group is located at C-3”. Further evidence for the position of the acetyl group was obtained from the 13C-NMR, where a substantial downfield shift ∆δC-3” = 1.20 ppm and the upfield shift of C-2” and C-4” (∆δC-2” = 1.66 ppm and ∆δC-4” = 1.67 ppm) relative to those of 1 were observed. A literature search revealed that 4 (apigenin 7-O-β-D-(6”-trans-p-cumaroil)3”-O-acetilglucopiranosídeo) is a previously unreported natural compound.

The new alcalóide 2 gave positive reactions with FeCl₃, Lassaigne and ninhydrin reagents indicating its phenolic activity.

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nitrogenous nature. HREI-MS (experimental) showed an M⁺ at m/z 151.027 for the molecular formula C₇H₅O₃N. It also showed characteristic mass fragments at m/z 105, 95, 91 and 77, suggesting the presence of 2-benzoxazolinone.¹² In the ¹³C-NMR spectrum (Table 1) the seven carbons were resolved as three aromatic methines (δ 99.1, 111.0 and 111.6) assigned to C-7, C-5 and C-4 respectively, three quaternary aromatic carbons (δ 129.9, 146.1 and 154.8) attributed to C-9, C-8 and C-6 respectively, and a fourth quaternary carbon at δ 157.7 assigned to the lactone carbonyl. The ¹H-NMR spectrum (Table 1) showed an ABX system at δ 6.68 (1H, d, J 2.3 Hz), 6.85 (1H, d, J 8.5 Hz) and 6.60 (1H, dd, J 2.3, 8.5 Hz) indicating a trisubstituted aromatic system. Compound 2 was identified as the demethyl derivative of 6-methoxy-benzoxazolinone i.e. 6-hydroxy-benzoxazolinone. Even the known 6-methoxy-benzoxazolinone is a rare natural product isolated only once from Corn plants.¹³

Chemical reactions and solubility in NaOH, followed by recovery after acidification, suggested that 3 is a nitrogenous compound with a lactone function. This was confirmed by the IR absorption bands for carbonyl (1670 cm⁻¹), hydroxy and/or NH groups (3325 cm⁻¹). The HRCI-MS showed an M⁺+1 at m/z 182, and an M⁺ at m/z 181.144 for the molecular formula C₈H₇O₄N. The ¹³C-NMR spectrum (Table 1) showed five methine signals and three quaternary carbons. In the ¹H-NMR spectrum the coupling pattern of the 4 aromatic protons suggested the presence of an O-disubstituted benzene ring. In the ¹H-NMR spectrum the singlet at δ 5.68, diagnostic for a proton flanked by two electronegative atoms, was assigned for H-3. This was supported by a ¹³C-NMR signal which appeared at δ 93.7 and was attributed to the oxymethine carbon. The remaining three quaternary carbon absorptions at δ 129.7, 142.5 and 160.1 were assigned to C-10, C-9 and the carbonyl group respectively. The violet colour with FeCl₃,¹⁴ as well as the M⁺ of the diacetate derivative 3a at m/z 265 indicated the presence of a hydroxylamine group. The exact positions of the carbonyl and hydroxyl groups were established from alkaline hydrolysis and by the inability of 3 to give O-aminophenol after fusion with KOH. This clearly distinct the new alkaloid 3 (3,4-dihydroxy-1,4-benzoxazine-2-one) from blepharigenin (2-hydroxy-1,4-benzoxazine-3-one) a compound with very close ¹H-NMR data.¹⁵

The HRCI-MS of the third new alkaloid 5 showed M⁺ at m/z 327.095 for the molecular formula C₁₄H₁₇O₈N. The

<p>| Table 1. ¹H- and ¹³C-NMR spectral data of compounds 2, 3 and 5 (coupling constants in Hz)¹⁶ |</p>
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¹ Assignments are based on HMQC experiments; ² Spectra were measured in CD₃OD; ³ Spectra were measured in CD₃OD/DMSO mixture
14 carbon signals were clear in the $^{13}$C-NMR spectrum (Table 1), of which 8 signals were accounted for the aglycone part, while the remaining 6 carbon signals were assigned for the sugar moiety. The 1,4-benzoxazine-2-one skeleton was assigned to 5 rather than the 1,4-benzoxazin-3-one as indicated from its reaction with KOH solution and fusion test. The spectral data of 5 aglycone (Table 1 and Experimental) showed a close similarity to 3. However, the CI-MS and the negative reaction with FeCl$_3$ suggested the absence of a hydroxyl group attached to nitrogen atom. Consequently, the only possible site for glycosylation is the C-3 hydroxyl. The identity of the sugar moiety was established as glucose by spectral evidences and by TLC comparison after acid hydrolysis. On the basis of the chemical shift and coupling constant of the anomeric proton ($J_{1',2'}$ 7.8 Hz), the glucosidic linkage should have the $\beta$-orientation. The identity of 5 was therefore established as 1,4-benzoxazine-2-one-3-O-glucoside.

The known compounds were identified by direct comparison with reference materials (Aldrich).

Compounds 1 and 3-5 were subjected to antimicrobial testing using 10 microorganisms. Only compounds 1 and 4 were active against Bacillus subtilis with an MIC 64 and 128 $\mu$g/mL respectively.

**Experimental**

**General procedure**

The CI-MS of 3a was measured on a Finnigan SSQ7000 mass spectrometer. NMR spectra were recorded on a JEOL 500 NMR instrument at 500 MHz for $^1$H and 125 MHz for $^{13}$C. Other experimental conditions were as previously described.

**Plant material**

The whole plants of Acanthus arboresus Forssk. growing wild in Wadi Dhar, Sana’a, Yemen was collected during the flowering stage in August 1998 and was identified by Prof. Nabil El-Hadidy, Department of Plant Taxonomy, Faculty of Science, Cairo University. A voucher sample (YA1) is preserved in the Department of Pharmacognosy, Faculty of Pharmacy, University of Alexandria, Egypt.

**Extraction and isolation**

The air-dried powdered whole plant of Acanthus arboresus Forssk (3.5 kg) were extracted by 95% ethanol at room temperature. The concentrated ethanolic extract was partitioned between CHCl$_3$ (1L) and water (1L). The CHCl$_3$ fraction (80 g) was again partitioned between 90% MeOH (1L) and petroleum ether (1L). The aqueous fraction was extracted with EtOAc (3x500 mL), then with n-butanol (3x500 mL).

A sample (10 g) of the 90% methanolic extract (26 g) was chromatographed on silica gel column (200 g, 4 cm) eluted with petroleum ether/CH$_2$Cl$_2$ (1:1) with increasing content of CH$_2$Cl$_2$, then methanol. Fractions of 250 ml each were collected, screened by TLC and similar fractions were combined. Fractions 6-15 (1.9 g, petroleum ether/CH$_2$Cl$_2$, 40:60) afforded lupeol (800 mg) upon crystallization from petroleum ether. Crystallization of fractions 16-20 (0.8 g, petroleum ether/CH$_2$Cl$_2$, 25:75) from methanol gave stigmasterol (100 mg). Fractions 36-42 (0.9 g, CH$_2$Cl$_2$/MeOH 92.5:7.5) were rechromatographed on silica gel column (9:1)(double development) and the zone with an $R_f$ value of 0.54 was scraped off, eluted with a mixture of chloroform and methanol (1:1) to afford 1 (160 mg). Fractions 43-47 (1.1 g, CH$_2$Cl$_2$/MeOH 90:10) gave sitosterol glucoside (230 mg) on crystallization from methanol.

The EtOAc extract (12 g) was fractionated on silica gel column (400 g, 3 cm) eluted with CH$_2$Cl$_2$ and CH$_2$Cl$_2$/MeOH mixtures with gradual increase of methanol content. Ninety fractions (150 mL each) were collected. Repeated crystallization of fraction 12 (0.85 g, CH$_2$Cl$_2$/MeOH 98:2) from methanol gave vanillic acid (200 mg). Crystallization of fractions 25-29 (0.78 g, CH$_2$Cl$_2$/MeOH 97:3) from methanol afforded apigenin (10 mg); while PTLC of the supernatant on silica gel plates developed with CHCl$_3$/MeOH (9:1)(double development) and the zone with an $R_f$ value of 0.54 was scraped off, eluted with a mixture of chloroform and methanol (1:1) to afford 3 (1L). Fractions 36-42 (1.1 g, CH$_2$Cl$_2$/MeOH 96:4) were obtained by crystallization from methanol to give 3 (850 mg) ($R_f$ = 0.34 EtOAc/MeOH/H$_2$O 30:5:2). Additional crystalization of fraction 49-55 (1.6 g, CH$_2$Cl$_2$/MeOH 94:6) from methanol gave 4 (620 mg) ($R_f$ = 0.30 EtOAc/MeOH/H$_2$O 30:5:2). Additional quantity of 1 (210 mg) was obtained by crystallization of fractions 78-81(1.4 g, CH$_2$Cl$_2$/MeOH 90:10) from MeOH.

A sample (8 g) of the n-butanol extract (30 g) was chromatographed of a silica gel column (160 g, 2 cm) with a gradual increase of methanol content. Twenty five fractions (100 mL each) were collected. Fractions 13-16 (0.7 g, CH$_2$Cl$_2$/MeOH 80:20) afforded 5 (120 mg) ($R_f$ = 0.47 EtOAc/MeOH/H$_2$O 30:5:4) on crystallization from MeOH.

Apigenin 7-O-β-D-(6”-trans-p-coumaryl) glucoside (1). White crystals, mp 267 °C (lit. 260-264 °C).
Cl-MS m/z (rel. Int.): 579 (6, [M+1]⁺), 489 (2), 433 (4), 417 (16), 416 (8), 350 (2), 311 (6), 309 (18), 299 (30), 271 (100), 270 (25) 192 (8), 165 (78), 147 (40), 121 (18), 99 (5). HRCl-MS m/z 579.151 (M⁺+1), calcd for C₁₈H₁₇O₁₃N, 579.150. H-NMR of sugar protons (ppm, DMSO-d₆, J 3.30 (1H, m, H-3”), 3.32 (1H, m, H-4”), 3.36 (1H, m, H-2”), 3.83 (1H, dd, J 7.8, 9.4 Hz, H-5”), 4.17 (1H, dd, J 4.8, 10.3 Hz, H-6”), 4.45 (1H, d, J 10.3 Hz, H-6”a), 5.16 (1H, d, J 8.0 Hz, H-1”). 13C-NMR of sugar carbons (ppm, DMSO-d₆, J (C-6”), 4.43 (1H, H-6”a), 4.97 (1H, t, J 9.6 Hz, H-3”), 5.30 (1H, d, J 7.8 Hz, H-1”), 6.35 (1H, d, J 16 Hz, H-c), 6.50 (1H, d, J 1.9 Hz, H-6), 6.66 (2H, d, J 8.5 Hz, H-3”**, 5”), 6.84 (1H, d, J 1.9 Hz, H-8), 6.85 (1H, s, H-3), 6.92 (2H, d, J 8.9 Hz, H-3”, 5”), 7.39 (2H, d, J 8.5 Hz, H-2”**, 6”), 7.49 (1H, d, J 16 Hz, H-β), 7.94 (2H, d, J 8.9 Hz, H-2’, 6’). 13C-NMR (ppm, DMSO-d₆): δ 21.1 (COCH₃), 63.3 (C-6”), 68.3 (C-4”), 71.3 (C-2”), 73.9 (C-5”), 77.4 (C-3”), 95.1 (C-8), 99.8 (C-1”), 103.4 (C-3”), 105.9 (C-10), 114.0 (C-α), 115.9 (C-3”**, 5”), 116.2 (C-3”, 5”), 121.4 (C-1”), 128.5 (C-1”), 130.3 (C-2”), 145.4 (C-β), 157.3 (C-9”), 160.1 (C-4”), 161.4 (C-4”), 161.7 (C-5), 162.8 (C-7), 164.7 (C-2), 166.8 (C=O), 170.2 (COCH₃), 182.3 (C-4”). Acanthaminoside (1,4-benzoxazin-2-one-3-O-glucoside) (5). Colourless needles, mp 213-214 °C. IR (KBr): ν max/cm⁻¹: 3475 (OH), 3125, 2960, 1700 (CO), 1600, 1375. UV λ max/nm (MeOH): 283, 275, 250. CI-MS m/z (rel. Int.): 328 (14, [M+1]⁺), 327 (14, M⁺), 166 (100), 165 (15.5), 164 (16, aglycone), 163 (13, glucosy1), 148 (100); 145 (30), 136 (18). HRCl-MS m/z 327.095 (M⁺), calcd for C₁₄H₁₇O₈N, 327.095. 1H and 13C-NMR (Table 1).

Antimicrobial testing

The MIC were determined for compounds 1 and 3-5 using a procedure described elsewhere. Twelve microorganisms: Bacillus subtilis, Micrococcus luteus, Sarcina lutea, Staphylococcus aureus, Bordetella bronchiseptica, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Serratia marcescens and Shigella sonnie were used in the study. The antibiotics ampicillin, ciprofloxacin, erythromycin and gentamicin were used as controls.

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References


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