New Nitrogenous Compounds from *Anisotes trisulcus*

Mohamed A. El-Shanawany, Hanaa M. Sayed, Sabrin R. M. Ibrahim*, and Marwa A. A. Fayed

Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt.
Fax: +2-088-2332776. E-mail: sabrinshaur@gmail.com

* Author for correspondence and reprint requests

Z. Naturforsch. **69c**, 209 – 218 (2014) / DOI: 10.5560/ZNC.2013-0116
Received July 7, 2013 / January 21, 2014 / published online May 21, 2014

Re-investigation of the methanolic extract of *Anisotes trisulcus* (Forssk.) Nees aerial parts led to the isolation of two new tricyclic quinazoline alkaloids, 8-amino-7,8,9,11-tetrahydro-6*H*-pyrido[2,1-*b*]-quinazoline-2,6-diol (**4**) and 8-amino-3,6-dihydroxy-7,8,9-trihydro-6*H*-pyrido[2,1-*b*]quinazoline-11-one (**5**), and two quaternary ammonium compounds, (dimethylamino)-*N*-(hydroxymethyl)-*N*,*N*-dimethyl methanaminium chloride (**6**) and *N*-[(carboxyamino)methyl]-*N*,*N*-dimethyl ethanaminium chloride (**7**), together with three known compounds, peganine (**1**), vasicinone (**2**), and anisotine (**3**). The structures of these compounds were established on the basis of physical, chemical, and spectral data (UV, IR, MS, 1D and 2D NMR), as well as by comparison with authentic samples. GC-MS analysis of the fatty acid methyl esters and unsaponifiable matter revealed the presence of 46 fatty acids, 53 hydrocarbons, and 18 sterols. The different extracts were evaluated for their antihyperglycaemic activities. The MeOH, *n*-hexane, and EtOAc extracts exhibited a significant hypoglycaemic effect.

Key words: Anisotes trisulcus, Quinazoline Alkaloids, Antihyperglycaemic

Introduction

The genus *Anisotes* (Acanthaceae) comprises 23 species (El-Shanawany *et al.*, 2011). A survey of the traditional and folk uses of *A. trisulcus* showed that the plant has been used as antidiabetic, bronchodilator, hypotensive, appetite suppressant, and local anaesthetic (Al-Rehaily *et al.*, 2011; El-Shanawany *et al.*, 2011). In our previous phytochemical study of *A. trisulcus* we reported on the isolation and identification of one new and two known alkaloids (El-Shanawany *et al.*, 2011). In continuation of our investigation of *A. trisulcus* aerial parts, we report here the isolation and structural elucidation of two new tricyclic quinazoline alkaloids and two quaternary ammonium compounds, together with the isolation of three known compounds (Fig. 1), as well as the gas chromatography/mass spectroscopy (GC-MS) analysis of the fatty acid methyl esters and unsaponifiable matter. In addition, the antihyperglycaemic activities of the different extracts were evaluated.

Materials and Methods

General

Melting points were not corrected and carried out on an Electrothermal 9100 digital melting point apparatus (Electrothermal Engineering, Essex, UK). EI and FAB mass spectra were recorded on a Jeol JMS.600 H mass spectrometer (Peabody, MA, USA). UV spectra were recorded in MeOH on a Shimadzu 1601 UV/VIS spectrophotometer (Kyoto, Japan). IR spectra were measured on a Shimadzu Infrared-400 spectrophotometer. NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on a Varian NMRYH-400 instrument (Oxford, UK) using DMSO-d_6, $CDCl_3$, and C_5D_5N as solvents. Column chromatographic separation was performed on silica gel 60 (0.04 – 0.063 mm; Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was performed on TLC plates precoated with silica gel 60 F254 (0.2 mm; Merck). The following solvent systems were used:

n-hexane/EtOAc (70:30, v/v; S_1), $CHCl_3$/MeOH (95:5; S_2), $CHCl_3$/MeOH (90:10; S_3), $CHCl_3$/MeOH (80:20; S_4), and $CHCl_3$/MeOH (70:30; S_5). Authentic alkaloids were obtained from the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt. Gliclazide, as a reference antidiabetic, was purchased from Sigma-Aldrich (St. Louis, MO, USA). Spots were detected by spraying with Dragendorff's reagent.

Plant material

Aerial parts of *Anisotes trisulcus* (Forssk.) Nees were collected during the flowering season in March 2005 from AL-Baha, Al-Abnaa escarpment, Kingdom of Saudi Arabia. The plant was kindly identified by Professor Abdel-Aziz Ali Fayed, Professor of Plant Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt. A voucher sample (AT-20051) was deposited in the herbarium of the Faculty of Pharmacy, Assiut University.

Extraction and isolation

The air-dried powdered aerial parts of *A. trisulcus* (2.5 kg) were exhaustively extracted by cold percolation with MeOH. The methanolic extract was concentrated under reduced pressure to get a viscous residue (270 g) which was suspended in 500 mL distilled H_2O and subjected to solvent fractionation using *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, respectively. The fractions were separately concentrated to give 60, 40, 20, and 70 g, respectively. The $CHCl_3$ fraction (40 g) was subjected to vacuum-liquid chromatography (VLC) using *n*-hexane/EtOAc gradient elution; five subfractions were obtained, CA-I to CA-V. Subfraction CA-III (8 g) was chromatographed on a silica gel column (250 g, 100 cm × 5 cm) using an *n*-hexane/EtOAc gradient. The fractions eluted with *n*-hexane/EtOAc (90:10, v/v) yielded compounds **1** (50 mg, colourless needles) and **2** (40 mg, colourless needles). Subfraction CA-IV (6.5 g) was subjected to a silica gel column (250 g, 100 cm × 5 cm) using an *n*-hexane/EtOAc gradient. Similar fractions were grouped together, and those eluted with *n*-hexane/EtOAc (80:20) were further purified over a silica gel column to yield compound **3** (20 mg, orange needles). The EtOAc fraction (20 g) was subjected to VLC with $CHCl_3$/MeOH gradient elution. Six subfractions were obtained, EA-I to EA-VI. Subfractions EA-IV (3.5 g) and EA-V (1.5 g) were subjected to a silica gel column eluted with a $CHCl_3$/MeOH gradient to afford compounds **4** (12 mg, brown needles) and **5** (10 mg, brown fine needles), respectively. A part of the *n*-BuOH fraction (8 g) was subjected to a silica gel column (320 g, 100 cm × 5 cm) eluted with a $CHCl_3$/MeOH gradient. Four subfractions were obtained, BA-I to BA-IV. Subfraction BA-I (2.5 g) was chromatographed on an aluminium oxide column (80 g, 100 cm × 5 cm) and eluted with a $CHCl_3$/MeOH gradient. Fractions eluted with $CHCl_3$/MeOH (95:5) afforded compound **6** (5 mg, colourless needles). Subfraction BA-II (2.5 g) was subjected to aluminium oxide column chromatography using $CHCl_3$/MeOH as an eluent. Fractions eluted with $CHCl_3$/MeOH (90:10) yielded compound **7** (20 mg, colourless needles). The other subfractions had been previously investigated by us (El-Shanawany *et al.*, 2011).

Peganine (**1**): Colourless needles. – Yield: 50 mg (MeOH). – R_f 0.63 (S_1). – M.p. 188 – 190 °C. – UV (MeOH): λ_{max} = 280, 295 nm. – IR (KBr): ν_{max} = 3240 (OH), 1685 (C-N), 1625 (C=N), 1610, 1570, 1500 (aromaticity) cm^{-1}. – ^1H NMR (DMSO-d_6, 400 MHz): δ_H = 3.48 (1H, m, H-1A), 3.83 (1H, m, H-1B), 2.46 (1H, m, H-2A), 1.92 (1H, m, H-2B), 4.11 (1H, brs, H-3), 7.23 – 7.51 (4H, m, H-5,6,7,8), 4.89 (2H, dd, J = 15.0, 15.0 Hz, H-9), 6.02 (1H, d, J = 5.0 Hz, 3-OH). – EIMS (rel. int.): m/z = 188 $[M]^+$ (25), 187 $[M - H]^+$ (5), 171 $[M - OH]^+$ (4), 130 $[M - C_3H_6O]^+$ (18), 69 (25), 58 (13).

Vasicinone (**2**): Colourless needles. – Yield: 40 mg (MeOH). – R_f 0.58 (S_1). – M.p. 175 – 177 °C. – UV (MeOH): λ_{max} = 265, 295, 309 nm. – IR (KBr): ν_{max} = 3150 (OH), 1665 (-O=C-N-), 1630 (C=N), 1610, 1485 (aromaticity) cm^{-1}. – ^1H NMR (DMSO-d_6, 400 MHz): δ_H = 3.84 (1H, m, H-1A), 4.04 (1H, m, H-1B), 2.46 (1H, m, H-2A), 1.92 (1H, m, H-2B), 4.86 (1H, m, H-3), 7.38 – 7.51 (3H, m, H-5,6,7), 7.53 (1H, d, J = 6.7 Hz, H-8), 5.99 (1H, d, J = 4.9 Hz, 3-OH). – ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C = 43.0 (C-1), 30.0 (C-2), 71.6 (C-3), 158.0 (C-3a), 142.7 (C-4a), 124.2 (C-5), 109.2 (C-6), 109.2 (C-7), 129.2 (C-8), 122.0 (C-8a), 160.2 (C-9). – EIMS (rel. int.): m/z = 202 $[M]^+$ (5), 185 $[M - OH]^+$ (3), 146 (3), 135 (100), 105 (77), 69 (10), 58 (13).

Anisotine (**3**): Orange needles. – Yield: 20 mg ($CHCl_3$). – R_f 0.76 (S_2). – M.p. 189 – 190 °C. – UV ($CHCl_3$): λ_{max} = 225, 298, 310, 359 nm. – IR (KBr): ν_{max} = 3340 (N-H), 1740 (C=O), 1625 (C=N), 1610,

1584, 1500 (aromaticity) cm^{-1}. – ^1H NMR (DMSO-d_6, 400 MHz): δ_H = 3.30, 3.42 (2H, m, H-1), 2.31, 2.66 (2H, m, H-2), 4.8 (1H, s, H-3), 7.33 – 7.42 (3H, m, H-5,6,7), 8.10 (1H, d, J = 8.8 Hz, H-8), 8.0 (1H, d, J = 4.8 Hz, H-11), 6.84 (1H, d, J = 8.8 Hz, H-14), 7.30 (1H, s, H-15), 3.86 (3H, s, H-17), 2.92 (3H, d, J = 4.8 Hz, H-18), 9.01 (1H, s, NH). – ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C = 45.7 (C-1), 29.1 (C-2), 49.9 (C-3), 140.2 (C-3a), 144.9 (C-4a), 127.3 (C-5), 132.6 (C-6), 128.2 (C-7), 128.5 C-8), 122.3 (C-8a), 159.9 (C-9), 109.1 (C-10), 131.4 (C-11), 152.6 (C-12), 152.3 (C-13), 113.4 (C-14), 134.9 (C-15), 169.2 (C-16), 51.9 (C-17), 29.4 (C-18). – EIMS (rel. int.): m/z = 349 $[M]^+$ (30), 348 $[M-H]^+$ (10), 317 $[M-CH_3OH]^+$ (10), 316 $[M-H-CH_3OH]^+$ (30), 290 $[M-CO_2CH_3]^+$ (25), 186 $[M-C_9H_{10}NO_2]^+$ (5), 166 (20), 98 (32).

8-Amino-7,8,9,11-tetrahydro-6H-pyrido[2,1-b]quinazoline-2,6-diol (**4**): Brown fine needles. – Yield: 12 mg (MeOH). – R_f 0.67 (S_4). – M.p. 98 – 100 °C. – IR (KBr): ν_{max} = 3320 (OH), 1690 (C-N), 1630 (C=N), 1610, 1550, 1500 (aromaticity) cm^{-1}. – ^1H and ^{13}C NMR: see Table I. – EIMS (rel. int.): m/z = 233 $[M]^+$ (3), 217 $[M-OH]^+$ (4), 197 $[M-2H_2O]^+$ (10), 173 (90), 164 (100), 138 (25), 120 (40), 86 (10), 72 (5%).

8-Amino-3,6-dihydroxy-7,8,9-trihydro-6H-pyrido[2,1-b]quinazoline-11-one (**5**): Brown needles. – Yield: 10 mg (MeOH). – R_f 0.56 (S_2). – M.p. 120 – 122 °C. – IR (KBr): ν_{max} = 3350 (OH), 1690 (C-N), 1630 (C=N), 1615 (C=O), 1620, 1560, 1500 (aromaticity) cm^{-1}. – ^1H and ^{13}C NMR: see Table I. – EIMS (rel. int.): m/z = 247 $[M]^+$ (50), 230 $[M-OH]^+$ (5), 213 $[M-2OH]^+$ (8), 173 (92), 164 (100), 138 (25), 120 (42), 86 (10).

(Dimethylamino)-N-(hydroxymethyl)-N,N-dimethyl methanaminium chloride (**6**): Colourless needles. – Yield: 5 mg (MeOH). – R_f 0.51 (S_4). – M.p. 290 – 291 °C. – NMR spectral data (DMSO-d_6, 400 and 100 MHz): see Table II. – HRESITOF-MS (rel. int.): m/z = 169.0998 $[M+H]^+$ (calcd. for $C_6H_{18}N_2OCl$, 169.1029) (78), 171.0893 $[M+2H]^+$ (25), 126.0724 (17), 104.1045 (100), 102.0758 (49).

N-[(Carboxyamino)methyl]-N,N-dimethyl ethanaminium chloride (**7**): Colourless needles. – Yield: 20 mg (MeOH). – R_f 0.78 (S_5). – M.p. 295 – 296 °C. – IR (KBr): ν_{max} = 3475 – 3290 (OH or NH stretching), 1615 (C=O), 1545 (NH bending) cm^{-1}. – NMR spectral data (DMSO-d_6 + TFA, 400 and 100 MHz): see Table II. – HRESITOF-MS (rel. int.): m/z = 169.1016 $[M+H]^+$ (calcd. for $C_5H_{14}N_2O_2Cl$, 169.0666) (70), 171.0919 $[M+2H]^+$ (23), 126.0745 (33), 118.0776 (95), 102.0772 (57).

GC-MS analysis of the unsaponifiable matter and fatty acid composition

Five g of the *n*-hexane fraction were refluxed with 0.5 M ethanolic KOH for 3 h on a boiling water bath. Thereafter the alcohol was distilled off, and the aqueous liquid was diluted with distilled water and then

Table I. NMR data of compounds **4** and **5** [DMSO-d_6, 400 (^1H NMR) and 100 MHz (^{13}C NMR)].

No.	**4**		**5**	
	δ_H [mult., J (Hz)]	δ_C (mult.)	δ_H [mult., J (Hz)]	δ_C (mult.)
1	6.58 d (2.3)	113.2 (CH)	7.55 d (8.7)	109.4 (CH)
2	–	156.1 (C)	7.25 dd (8.7, 2.7)	122.2 (C)
3	6.70 dd (8.6, 2.3)	115.6 (CH)	–	156.6 (CH)
4	7.02 d (8.6)	118.5 (CH)	7.42 d (2.7)	129.3 (CH)
4a	–	122.7 (C)	–	124.3 (C)
5a	–	161.6 (C)	–	158.2 (C)
6	5.01 q (7.9)	70.6 (CH)	4.93 d (6.2)	71.8 (CH)
7	1.94 m	29.4 (CH_2)	1.98 m	30.4 (CH_2)
8	4.05 m	48.5 (CH)	4.10 m	49.3 (CH)
9	3.49 m, 3.62 m	50.1 (CH_2)	3.89 m	43.6 (CH_2)
11	4.75 d (15.8) 4.63 d (15.8)	45.9 (CH_2)	–	160.3 (C)
11a	–	118.3 (C)	–	143.0 (C)
8-NH_2	11.99 brs	–	11.99 brs	–
6-OH	6.41 d (7.5)	–	5.95 d (5.6)	–
2-OH	9.77 s	–	–	–
3-OH	–	–	10.0 s	–

Table II. NMR data of compounds **6** and **7** [DMSO-d_6, 400 (^{1}H NMR) and 100 MHz (^{13}C NMR)].

No.	**6**		No.	**7**		
	δ_H (mult.)	δ_C (mult.)		δ_H (mult.)	δ_C (mult.)	HMBC
1	3.81 m	67.4 (CH_2)	1	–	166.6 (C)	–
2	–	–	2-NH	8.07 s	–	1, 3
3	3.38 m	67.4 (CH_2)	3	4.24 brs	62.9 (CH_2)	1
4-N(CH_3)$_2$	3.10 s	53.6 (CH_3)	N(CH_3)$_3$	3.17 brs	53.1 (CH_3)	3
2-N(CH_3)$_2$	3.32 s	55.6 (CH_3)	–	–	–	–
1-OH	5.42 brs	–	–	–	–	–

extracted with diethyl ether till exhaustion to give unsaponifiable matter. The aqueous solution (soap) that remained after removal of the unsaponifiable matter was treated in the same way as previously mentioned to afford the fatty acid methyl esters (FAMEs) (Ali *et al.*, 2013). GC-MS analysis of the FAMEs and unsaponifiable matter was performed using an Agilent GC-MS spectrometer (Agilent Technology, Waldbronn, Germany). The software controller/integrator was Turbo Mass, version 4.5.0.007 (PerkinElmer, Rodgau, Germany). A GC-MS capillary column [3% methyl phenyl silicon type of stationary phase (OV-17) on 80/100, Carbowax HP (CWHP), 30 m × 0.53 mm ID×3.0 μm df; Perkin Elmer] was used. The carrier gas was helium (purity 99.9999%) at a flow rate of 2 mL/min. The column temperature program was as follows: 160 °C for 2 min, then increase at 15 °C/min till 300 °C, and isothermal for 15 min. The injector temperature was 250 °C, the injection volume was 1.0 μL, and the split ratio was 40:1. The detector temperature was 320 °C using a dual flame ionization detector. MS scan was from m/z 50 to m/z 650.

Antihyperglycaemic activity

Adult male albino mice weighing 20 – 25 g were used. The animals were housed under standardized environmental conditions in the pre-clinical Animal House, Pharmacology Department, Faculty of Medicine, Assuit University, Assiut, Egypt. The animals were fed with standard diet and had free access to water. They were kept at 24 – 28 °C temperature, 60 – 70% relative humidity, and a 12 h:12 h light/dark cycle for one week to acclimatize to the environmental conditions. The work was conducted in accordance with the internationally accepted principles for laboratory animals', use and care as found in the European Community Guidelines and approval of the Institutional Ethical Committee was obtained (Mohammed *et al.*, 2011).

The antihyperglycaemic activity of the test extracts was screened in albino mice, which received different doses of glucose [1 – 5 g/kg body weight (BW)] orally (Al-Awadi *et al.*, 1985). Overnight fasting mice (18 h) received solutions of the different extracts (400 mg/kg BW) with glucose load (2.25 g/kg BW) orally using a stomach tube at 0 min. The animals were divided into five groups of six animals each. Group I received glucose load only (2.25 g/kg BW). Group II received glucose load and the *n*-hexane extract at 400 mg/kg BW. Group III received glucose load and the $CHCl_3$ extract at 400 mg/kg BW. Group IV received glucose load and the EtOAc extract at 400 mg/kg BW. Group V received glucose load and the total MeOH extract at 400 mg/kg BW. Group VI received glucose load and gliclazide at a dose of 2 mg/kg BW. Blood samples were withdrawn from the cavernous sinus at 0, 30, 60, 90, 120, and 180 min, and the blood glucose level, in mg/dL, was determined.

Statistical analysis

Data were analysed using Student's *t*-test, and the values were expressed as mean ± S.E. ($n = 6$ animals).

Results and Discussion

Compound **4** was obtained as brown fine needles. It gave positive results for alkaloids with Mayer's and Dragendorff's reagents (Harborne, 1984). Its mass and ^{13}C NMR spectral data suggested the presence of a tricyclic quinazoline system with the molecular formula $C_{12}H_{15}N_3O_2$, which is 29 mass units ($CHNH_2$) more than that of 7-hydroxyvasicine (El-Shanawany *et al.*, 2011). The ^{1}H and ^{13}C NMR spectra combined with the mass spectral data confirmed the presence of the tricyclic quinazoline system (Joshi *et al.*, 1994; Arndt *et al.*, 1967). The ^{1}H NMR spectrum (Table I) exhibited a pattern similar to that of 7-hydroxyvasicine with an additional amino-substituted methine group at δ_H 4.05 ppm (m, H-8)/δ_C 48.5 ppm

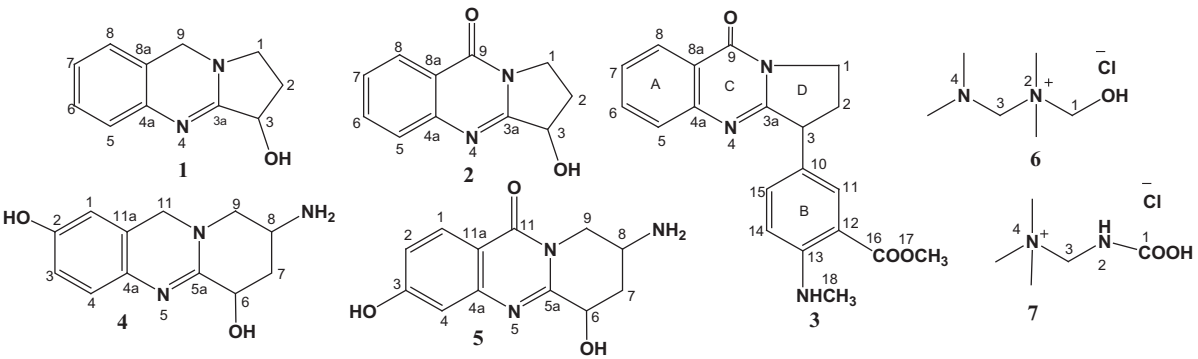

Fig. 1. Chemical structures of the isolated compounds.

(C-8), which was confirmed by a downfield signal at δ_H 11.99 ppm (2H, brs, 8-NH_2). Three aromatic protons at δ_H 6.58 (1H, d, $J = 2.3$ Hz), 6.70 (1H, dd, $J = 8.6$, 2.3 Hz), and 7.02 ppm (1H, d, $J = 8.6$ Hz) were attributed to a trisubstituted benzene ring. Moreover, the ^{1}H NMR spectrum exhibited two hydroxy functionalities at δ_H 6.41 (6-OH) and 9.77 ppm (2-OH), which were confirmed by the appearance of an absorption band in the IR spectrum at 3320 cm^{-1} (El-Shanawany *et al.*, 2012) and fragment ion peaks at m/z 217 ($[M-OH]^+$) and 197 ($[M-2H_2O]^+$). The attachment of the hydroxy and amino functions to C-2, C-6, and C-8 was confirmed by comparing the ^{1}H and ^{13}C chemical shifts of **4** with those of 7-hydroxyvasicine (El-Shanawany *et al.*, 2011). The DEPT and ^{13}C NMR experiments displayed twelve signals; three for methylene groups, five for methine groups, and four for quaternary carbon atoms. On reviewing the literature, the spectral data of **4** were found to be identical with those of 7-hydroxyvasicine (El-Shanawany *et al.*, 2011) except for the presence of an amino-substituted methane group. Thus, the structure was determined as 8-amino-7,8,9,11-tetrahydro-6*H*-pyrido[2,1-*b*]quinazoline-2,6-diol (Fig. 1) and found to be a new natural product.

Compound **5** was isolated as brown needles. It gave positive Mayer's and Dragendorff's tests for alkaloids (Harborne, 1984). Its mass and ^{13}C NMR spectra (Table I) suggested the presence of a tricyclic quinazoline system (Joshi *et al.*, 1994; Arndt *et al.*, 1967). The EI mass, together with ^{1}H and ^{13}C NMR spectra suggested the molecular formula $C_{12}H_{13}N_3O_3$, which is 14 mass units more than the mass of **4**. The DEPT and ^{13}C NMR spectra displayed twelve signals; two for methylene groups, five for methine groups, and five quaternary carbon atoms including one amide carbonyl carbon atom at δ_C 160.3 ppm (C-11). The ^{1}H and ^{13}C NMR spectral data of **5** were similar to those of **4**, with the exception that the signals for the methylene group at δ_H 4.75 (d, $J = 15.8$ Hz, H-11A) and 4.63 ppm (d, $J = 15.8$ Hz, H-11B)/δ_C 45.9 ppm (C-11) were missing and that a new signal appeared for a carbonyl moiety at δ_C 160.3 ppm (C-11). The carbonyl moiety was confirmed by an IR absorption band at 1615 cm^{-1}. Also, two hydroxy functions at δ_H 10.0 (3-OH) and 5.95 ppm (6-OH) were observed. They were confirmed by the appearance of an absorption band in the IR spectrum at 3350 cm^{-1} (El-Shanawany *et al.*, 2012) and fragment ion peaks at m/z 230 ($[M-OH]^+$) and 213 ($[M-2OH]^+$). The ^{1}H NMR spectrum also exhibited three coupled aromatic protons at δ_H 7.25 (1H, dd, $J = 8.7$, 2.7 Hz), 7.42 (1H, d, $J = 2.7$ Hz), and 7.55 ppm (1H, d, $J = 8.7$ Hz) suggesting the presence of an 1,2,4-trisubstituted benzene. On the basis of the above mentioned data, **5** was assigned as 8-amino-3,6-dihydroxy-7,8,9-trihydro-6*H*-pyrido[2,1-*b*]quinazoline-11-one (Fig. 1). To our knowledge, this represents the first report on its identification from a natural source.

Compound **6** was isolated as colourless needles. It gave a brick red colour with Dragendorff's reagent which suggested it to be a quaternary ammonium compound (Harborne, 1984). Its HRESITOF-mass spectrum exhibited pseudo-molecular ion peaks at m/z 169.0998 ($[M+H]^+$) and 171.0893 ($[M+2H]^+$) in a ratio of 3:1 indicating that it is a chloride salt (Silverstien and Wabster, 1998). The mass, ^{1}H, and ^{13}C NMR spectra (Table II) suggested the molecular formula to be $C_6H_{17}N_2OCl$. The ^{1}H NMR spectrum showed the presence of two singlets at δ_H 3.32 [2-N(C$\underline{H}_3$)$_2$] and 3.10 ppm [4-N(C$\underline{H}_3$)$_2$] assigned to four N-C$\underline{H}_3$ groups. They were confirmed by the ^{13}C

NMR signals at δ_C 55.6 and 53.6 ppm, respectively (Wu *et al.*, 1994). In addition, two multiplets each for two protons at δ_H 3.81 and 3.38 ppm assigned for two nitrogen-bound methylene groups. They were observed at δ_C 67.4 ppm in the ^{13}C NMR spectrum. A broad singlet at δ_H 5.42 ppm indicated the presence of a terminal hydroxy group. Based on the previous spectroscopic data and by comparison with that reported in the literature for quaternary methyl ammonium compounds (Wyn and Storey, 1981; Robertson

Table III. GC-MS analysis of fatty acid methyl esters (FAMEs).

Peak no.	R_t [min]	Relative content (%)[a]	Molecular formula	No. of unsaturations	$[M^+]$ m/z	Compound
1	15.702	0.1	$C_{10}H_{20}O_2$	1	172	Nonanoic acid methyl ester
2	17.19	0.1	$C_{11}H_{22}O_2$	1	186	Decanoic acid methyl ester
3	20.775	0.3	$C_{13}H_{26}O_2$	1	214	Dodecanoic acid methyl ester
4	21.289	1.0	$C_{11}H_{20}O_4$	2	216	Nonanedioic acid dimethyl ester
5	23.2	0.1	$C_{12}H_{22}O_4$	2	230	Decanedioic acid dimethyl ester
6	23.423	0.1	$C_{16}H_{32}O_2$	1	256	3,7,11-Trimethyl-dodecanoic acid
7	24.413	3.1	$C_{15}H_{30}O_2$	1	242	Tetradecanoic acid methyl ester
8	24.752	0.2	$C_{13}H_{24}O_4$	2	244	Undecanedioic acid dimethyl ester
9	25.07	0.3	$C_{17}H_{34}O_2$	1	270	4,8,12-Trimethyl-tridecanoic acid methyl ester
10	25.774	0.5	$C_{16}H_{32}O_2$	1	256	Pentadecanoic acid methyl ester
11	26.272	0.4	$C_{18}H_{36}O_2$	1	284	5,9,13-Trimethyl-tetradecanoic acid methyl ester
12	26.934	0.1	$C_{17}H_{32}O_2$	2	268	11-Hexadecanoic acid methyl ester
13	27.024	15.0	$C_{17}H_{34}O_2$	1	270	Hexadecanoic acid methyl ester
14	27.58	0.4	$C_{17}H_{34}O_2$	2	268	2-Hexadecenoic acid methyl ester
15	27.654	0.8	$C_{20}H_{40}O_2$	1	312	2,6,10,14-Tetramethyl-pentadecanoic acid methyl ester
16	27.844	0.2	$C_{12}H_{20}O_3$	3	212	Methyl-11-oxo-9-undecenoate
17	27.977	0.4	$C_5H_9O_2Br$	4	179	Methyl-2-bromo-isobutyrate
18	28.125	1.7	$C_{23}H_{43}O_3$	3	381	Heptadecanoic acid methyl ester
19	28.374	0.21	$C_{17}H_{34}O_3$	1	286	2-Hydroxy-hexadecanoic acid methyl ester
20	28.792	0.4	$C_{13}H_{24}O_3$	2	228	Methyl-4-oxododecanoate
21	29.089	5.4	$C_{19}H_{36}O_2$	2	296	9-Octadecenoic acid methyl ester
22	29.470	5.9	$C_{19}H_{38}O_2$	1	298	Octadecanoic acid methyl ester
23	29.719	1.4	$C_{19}H_{34}O_2$	3	294	7,10-Octadecadienoic acid methyl ester
24	29.719	1.9	$C_{25}H_{38}O_2$	7	370	9,12-Octadecadienoic acid methyl ester
25	29.798	0.3	$C_{19}H_{36}O_3$	2	312	3-Octyl-oxiraneoctanoic acid methyl ester
26	30.503	3.3	$C_{19}H_{34}O_2$	4	294	9,11-Octadecadienoic acid methyl ester
27	30.879	0.4	$C_{20}H_{38}O_2$	2	310	2-Octyl-cyclopropaneoctanoic acid methyl ester
28	31.147	0.3	$C_{20}H_{40}O_2$	1	312	15-Ethyl-heptadecanoic acid methyl ester
29	31.668	0.9	$C_{18}H_{34}O_4$	2	314	Hexadecanedioic acid dimethyl ester
30	31.768	1.4	$C_{19}H_{32}O_2$	4	292	6,9,12-Octadecatrienoic acid methyl ester
31	33.558	0.8	$C_{17}H_{32}O_2$	2	268	9-Hexadecenoic acid methyl ester
32	34.739	0.4	$C_{20}H_{40}O_2$	1	312	2-Methyl-octadecanoic acid methyl ester
33	35.02	0.2	$C_9H_{16}O_4$	2	188	3-Propyl-glutaric acid monomethyl ester
34	35.274	0.2	$C_{22}H_{44}O_2$	1	340	Heneicosanoic acid methyl ester
35	35.644	0.2	$C_{20}H_{38}O_4$	2	342	Octadecanedioic acid dimethyl ester
36	35.904	0.1	$C_{19}H_{38}O_3$	1	314	9-Hydroxy-octadecanoic acid methyl ester
37	36.243	0.6	$C_{21}H_{38}O_2$	3	322	11,13-Eicosadienoic acid methyl ester
38	36.566	0.3	$C_{20}H_{40}O_2$	2	312	3-Pentyl-oxiraneundecanoic acid methyl ester
39	37.122	0.3	$C_{13}H_{26}O_2$	1	214	2,4,6-Trimethyl-nonanoic acid methyl ester
40	37.424	1.1	$C_{21}H_{42}O_2$	1	326	Eicosanoic acid methyl ester
41	37.424	1.1	$C_{24}H_{48}O_2$	1	368	Tricosanoic acid methyl ester
42	37.964	0.8	$C_{15}H_{30}O_2$	1	242	2-Methyl-tetradecanoic acid methyl ester
43	38.287	3.1	$C_{25}H_{50}O_2$	1	382	Tetracosanoic acid methyl ester
44	39.229	0.5	$C_{26}H_{52}O_2$	1	396	Pentacosanoic acid methyl ester
45	40.331	2.0	$C_{27}H_{54}O_2$	1	410	Hexacosanoic acid methyl ester
46	43.211	1.7	$C_{29}H_{58}O_2$	1	438	Octacosanoic acid methyl ester

[a] Determination based on the percentage of each component relative to the total peak area of the oil contents.

Table IV. GC-MS analysis of unsaponifiable matter.

Peak no.	R_t [min]	Relative content (%)[a]	Molecular formula	No. of unsaturations	$[M^+]$ m/z	Compound
1	12.519	0.4	$C_{10}H_{22}$	0	142	3-Ethyl-2,5-dimethylhexane
2	13.054	0.1	C_7H_{16}	0	100	3-Methylhexane
3	13.245	0.6	C_7H_{14}	1	98	3-Methyl-1-hexene
4	13.441	2.0	C_7H_{14}	1	98	Ethylcyclopentane
5	13.504	1.3	C_7H_{14}	1	98	2-Methyl-3-hexene
6	13.573	1.5	$C_{10}H_{20}$	1	140	1-Methyl-2-(3-methylpentyl)-cyclopropane
7	13.79	0.6	C_7H_{14}	1	98	2,4-Dimethyl-2-pentene
8	13.838	0.6	C_7H_{14}	1	98	4,4-Dimethyl-2-pentene
9	13.891	0.9	C_7H_{14}	1	98	2,3-Dimethyl-1-pentene
10	14.013	1.3	C_7H_{14}	1	98	5-Methyl-1-hexene
11	14.15	2.5	C_7H_{14}	1	98	3-Ethyl-1-pentene
12	14.246	1.4	C_7H_{14}	1	98	1,2-Dimethylcyclopentane
13	14.304	0.4	C_7H_{14}	1	98	1,1-Dimethylcyclopentane
14	14.426	0.5	C_9H_{18}	1	126	1,2,3-Trimethylcyclohexane
15	14.473	0.8	$C_{11}H_{24}$	0	156	2,2,6-Trimethyloctane
16	14.521	0.1	$C_{12}H_{24}$	1	168	6-Methyl-4-undecene
17	14.722	0.6	$C_{13}H_{26}$	1	182	4,5-Dimethyl-2-undecene
18	14.865	0.2	$C_{12}H_{24}$	1	168	8-Methyl-1-undecene
19	14.908	0.3	C_8H_{16}	1	112	1-Butyl-2-methylcyclopropane
20	15.077	0.8	C_8H_{16}	1	112	3,3-Dimethyl-1-hexene
21	15.141	1.0	C_8H_{16}	1	112	1-Ethyl-3-methylcyclopentane
22	15.225	1.2	C_7H_{14}	1	98	4,4-Dimethyl-1-pentene
23	15.273	1.2	C_8H_{16}	1	112	4-Methyleneheptane
24	15.315	0.5	C_8H_{16}	1	112	Cyclooctane
25	15.395	0.8	C_8H_{16}	1	112	4-Octene
26	15.49	0.2	$C_{18}H_{36}$	1	252	5-Octadecene
27	15.538	0.7	C_9H_{18}	1	126	1,1,2-Trimethylcyclohexane
28	15.575	0.7	$C_{10}H_{20}$	1	140	5-Methyl-4-nonene
29	15.623	0.6	C_8H_{16}	1	112	Trimethylcyclopentane
30	15.776	0.5	C_8H_{16}	1	112	1-Ethyl-1-methylcyclopentane
31	15.813	0.9	C_8H_{16}	1	112	1,4-Dimethylcyclohexane
32	15.855	0.1	C_8H_{16}	1	112	Propylcyclopentane
33	15.882	0.1	C_8H_{16}	1	112	Methylcycloheptane
34	16.004	0.1	$C_{17}H_{34}$	1	238	1-Heptadecene
35	16.353	0.1	C_9H_{18}	1	126	3,3,5-Trimethyl-1-hexene
36	16.523	0.2	C_9H_{18}	1	126	2,4,4-Trimethyl-1-hexene
37	17.812	0.4	$C_{28}H_{48}O_4$	5	448	Ergost-25-ene-3,5,6,12-tetrol
38	18.315	0.6	$C_{29}H_{46}O$	7	410	4,22-Stigmastadiene-3-one
39	18.538	0.5	$C_{28}H_{42}O_2$	8	410	Epoxy-methylcholesta-4,6-diene-3-one
40	18.728	0.2	$C_{29}H_{50}O$	5	414	4,4-Dimethyl-5-α-cholestan-3-one
41	18.76	0.3	$C_{27}H_{46}O$	5	386	16,22-Epoxycholestane
42	21.0	0.5	$C_{28}H_{46}O$	6	398	5-Ethenyl-5-β-A-norcholestan-3-one
43	22.432	0.1	$C_{10}H_{18}$	2	138	2,6-Dimethyl-2,6-octadiene
44	23.004	0.1	$C_{11}H_7NO$	12	169	1-Isocyanatonaphthalene
45	23.047	0.1	$C_{12}H_{24}$	0	168	Cyclododecane
46	24.241	0.6	$C_{27}H_{46}O$	5	386	Cholest-5-en-3-ol
47	24.41	0.3	$C_{27}H_{48}O$	4	388	Cholestan-3-ol
48	24.535	0.1	C_7H_{28}	1	196	7-Tetradecene
49	24.815	0.8	$C_{13}H_{26}$	8	182	Phenanthrene
50	25.938	0.1	$C_{11}H_{18}O_3$	3	240	Heptadecane
51	26.001	1.6	C_8H_{18}	0	114	3,3-Dimethylhexane
52	26.075	0.1	$C_{10}H_{20}$	1	140	Ethylpropylcyclopentane
53	26.221	3.4	$C_{28}H_{48}O$	5	400	Ergost-5-en-3-ol
54	26.478	0.1	$C_{14}H_{28}$	0	196	Cyclotetradecane
55	26.841	3.5	$C_{16}H_{32}$	1	224	5-Cyclohexyldecane

Table IV. Continued.

Peak no.	R_t [min]	Relative content (%)[a]	Molecular formula	No. of unsaturations	$[M^+]$ m/z	Compound
56	26.843	0.1	$C_{15}H_{32}$	0	212	2,6,11-Trimethyldodecane
57	27.458	0.3	$C_{14}H_{18}$	6	186	1,2,3,4,5,6,7,8-Octahydrophenanthrene
58	28.307	10.8	$C_{29}H_{50}O$	5	414	Ethylcholest-5-en-3-β-ol
59	28.44	0.9	$C_{29}H_{52}O$	4	416	23-Ethylcholestanol
60	28.877	0.1	$C_{18}H_{36}$	1	252	1-Octadecene
61	29.827	0.9	$C_{29}H_{50}O$	6	412	4,4-Dimethylcholest-7-en-3-one
62	30.219	0.8	$C_{31}H_{52}O$	6	440	3-Methoxy-3-β-olean-12-ene
63	31.021	10.1	$C_{29}H_{50}O$	5	414	β-Sitosterol
64	31.601	0.6	$C_{31}H_{52}O$	6	440	24-Methylene-3-β-9,19-cyclolanostan-3-ol
65	31.853	7.2	$C_{29}H_{48}O$	6	412	Stigmasterol
66	32.438	0.6	$C_{29}H_{46}O$	7	410	Stigmasta-4,6,22-trien-3-α-ol
67	34.566	0.4	$C_{29}H_{48}O$	6	412	3-Acetylcholestene
68	34.797	0.5	$C_{14}H_{28}$	0	196	1,2,4,5-Tetraethylcyclohexane
69	35.639	0.1	$C_{31}H_{60}$	2	432	1-(1-Decylundecyl)decahydro-naphthalene
70	37.932	0.2	$C_{12}H_{22}$	2	166	3,4,5,6-Tetramethyl-2,5-octadiene
71	39.547	0.3	$C_{15}H_{28}$	2	208	Decahydro-1,6-dimethyl-4-(1-methylethyl)naphthalene

[a] Determination based on the percentage of each component relative to the total peak area of the oil contents.

et al., 1990), **6** was identified as (dimethylamino)-*N*-(hydroxymethyl)-*N*,*N*-dimethyl methanaminium chloride (Fig. 1) and considered a new compound. According to the available literature, quaternary ammonium compounds were traced in *Acanthus montanus* and *Crossandra nilitoca* (subfamily: Acanthoideae) (Hegnauer and Kooiman, 1978). To the best of our knowledge, this represents the first report on the identification of such a compound from a natural source. The presence of such compounds in plants has been related to salt stress conditions (Wyn and Storey, 1981), and some mangrove plants have been found to accumulate large amounts of quaternary methyl ammonium compounds (Hegnauer and Kooiman, 1978).

Compound **7** was obtained as colourless needles. It gave a brick red colour with Dragendorff's reagent suggesting that it is a quaternary ammonium salt (Harborne, 1984). The HRESITOF-mass spectrum displayed pseudo-molecular ion peaks at m/z 169.1016 ($[M+H]^+$) and 171.0919 ($[M+2H]^+$) in a ratio of 3:1 indicating that it is a chloride salt (Silverstien and Wabster, 1998). Its molecular formula was determined to be $C_5H_{13}N_2O_2Cl$, representing one degree of unsaturation attributed to a carbonyl group, which was confirmed by an IR absorption band at 1615 cm^{-1}. Also, the IR spectrum exhibited absorption bands at 3475 – 3290 (OH or NH stretching) and 1545 (NH bending) cm^{-1}. The ^1H NMR spectrum (Table II) showed two singlets at δ_H 4.24 and 3.17 ppm, which were assigned to N-CH_2 and trimethyl ammonium groups, respectively. The singlet at δ_H 8.07 ppm was attributed to an NH group and confirmed by an IR absorption band at 3475 cm^{-1}. The ^{13}C and DEPT NMR spectra together with the HMQC spectrum showed the presence of three signals at δ_C 53.1 (3 × N-CH_3), 62.9 (C-3), and 166.6 ppm (C-1). The chemical shifts of N-CH_2 and trimethyl ammonium groups were consistent with those in the literature (Robertson *et al*., 1990; Jensen *et al.*, 1988). The assignment of **7** was secured from HMBC correlations of H-3 to C-1, N-CH_3 to C-1 and C-3, and N-CH_3 to C-3 (Fig. 1). By comparison with the literature data together with those obtained from 1D and 2D NMR spectra, the structure of **7** was unambiguously elucidated as *N*-[(carboxyamino)methyl]-*N*,*N*-dimethyl ethanaminium chloride (Fig. 1).

The other isolated compounds were identified as peganine (**1**) (Joshi *et al*., 1994; Al-Azizi, 1997; Al-Rehaily *et al*., 2002), vasicinone (**2**) (Joshi *et al*., 1994; Al-Azizi, 1997; Al-Rehaily *et al*., 2002), and anisotine (**3**) (Arndt *et al*., 1967; Al-Azizi, 1997) (Fig. 1) by comparison of their physical and spectral data with those in the literature.

GC-MS analysis of the FAMEs (fatty acid methyl esters) of the *n*-hexane extract revealed the presence of 46 fatty acids (Table III). The major fatty acids identified were hexadecanoic acid methyl ester (15.0%), octadecanoic acid methyl ester (5.9%), (*Z*)-9-octadecenoic acid methyl ester (5.4%), 9,11-octadecadienoic acid methyl ester (3.3%), tetradecanoic acid methyl ester (3.1%), and tetracosanoic acid methyl ester (3.1%). While, GC-MS analysis of

Table V. Effect of *A. trisulcus* extracts on blood glucose levels (mg/dL).

Group	0 min	30 min	60 min	90 min	120 min	180 min
I	85.00 ± 3.00	215.00 ± 10.00	224.50 ± 4.50	194.50 ± 6.50	163.00 ± 1.00	120.00 ± 5.00
II[a]	98.11 ± 6.99	121.89 ± 5.91	135.99 ± 2.36***	98.61 ± 5.86***	95.98 ± 4.48***	78.99 ± 6.76**
III[a]	84.59 ± 1.98	200.47 ± 9.33	218.21 ± 7.34	194.19 ± 1.29	161.17 ± 2.49	115.95 ± 5.66
IV[a]	106.12 ± 5.77	203.38 ± 6.56***	91.15 ± 5.67***	105.00 ± 6.93***	70.15 ± 5.67***	65.02 ± 1.53***
V[a]	89.23 ± 5.19	125.38 ± 8.45	141.28 ± 4.32	129.29 ± 4.31	85.65 ± 9.46	75.18 ± 7.56
VI	92.45 ± 6.10	121.11 ± 5.72	133.97 ± 3.86	112.78 ± 3.62	76.43 ± 5.59	71.65 ± 6.24

Data are expressed as mean ± S.E., $n = 6$; $^{**}P > 0.05$, $^{***}P > 0.001$ (using Student's t-test).
[a] 400 mg/kg BW.
Group I received glucose load only. Group II received glucose load and the *n*-hexane extract. Group III received glucose load and the $CHCl_3$ extract. Group IV received glucose load and the EtOAc extract. Group V received glucose load and the total MeOH extract. Group VI received glucose load and gliclazide (2 mg/kg BW).

the unsaponifiable matter showed the presence of 53 hydrocarbons representing 34.0 % and 18 sterols representing 38.9 % (Table IV). The compounds were identified by comparison of their retention time and molecular weight with those of reference standards. This study is the first report on the composition of the lipids of the studied plant.

To determine the antihyperglycaemic activity, the MeOH extract and its *n*-hexane, $CHCl_3$, and EtOAC extracts were given to mice concurrently with the glucose load at a dose of 400 mg/kg BW. A significant hypoglycaemic effect was observed after 1 h and this continued for 2 h, while the $CHCl_3$ extract was least active (Table V). These results support the use of *A. trisulcus* (Forssk.) Nees as an antidiabetic in folk medicine. These are likely due to the extracts' high content of sterols and their corresponding glycosides, flavonoids, and other phenolic compounds.

Conclusion

Two new tricyclic quinazoline alkaloids and two quaternary ammonium compounds, together with three known compounds, were isolated from aerial parts of *A. trisulcus* (Forssk.) Nees and their structures elucidated. GC-MS analysis of the fatty acid methyl esters and unsaponifiable matter is reported for the first time. The MeOH, *n*-hexane, and EtOAc extracts exhibited significant hypoglycaemic effects.

Acknowledgement

We would like to express our deep thanks to Prof. Samir A. Ross (University of Mississippi, School of Pharmacy, National Center for Natural Products Research) for conducting HRESITOF-MS spectral measurements.

Al-Awadi F. M., Khattar M. A., and Gumaa K. A. (1985), On the mechanism of the hypoglycaemic effect of a plant extract. Diabetologia **28**, 432 – 434.

Al-Azizi M. M. (1997), Quinazoline alkaloids from *Anisotes trisulcus* L. Egypt. J. Biotechnol. **2**, 1 – 6.

Al-Rehaily A. J., El-Sayed K. A., Al-Said M. S., and Ahmed B. (2002), Trisulcusine: A novel spiro quinazoline alkaloid from *Anisotes trisulcus*. Indian J. Chem. **41B**, 2385 – 2389.

Al-Rehaily A. J., Al-Said M. S., and El-Tahir K. E. H. (2011), Peganine isolated from *Anisotes trisulcus* as a smoking deterrent and anorexigenic agent. Afr. J. Pharm. Pharmacol. **5**, 1342 – 1348.

Ali A. A., Sayed H. M., Ibrahim S. R. M., and Zaher A. M. (2013), Chemical constituents, antimicrobial, analgesic, antipyretic, and anti-inflammatory activities of *Euphorbia peplus* L. Phytopharmacology **4**, 69 – 80.

Arndt R. R., Eggers S. H., and Jordaan A. (1967), The alkaloids of *Anisotes sessiliflorus* C. B. Cl. (Acanthaceae) – five new 4-quinazolone alkaloids. Tetrahedron **23**, 3521 – 3532.

El-Shanawany M. A., Sayed H. M., Ibrahim S. R. M., and Fayed M. A. A. (2011), 5-Hydroxy vasentine, a new pyrroloquinazoline alkaloid from *Anisotes trisulcus* (Forssk.) Nees. J. Nat. Prod. Plant Res. **1**, 80 – 85.

El-Shanawany M. A., Sayed H. M., Ibrahim S. R. M., Fayed M. A. A., Radwan M. M., and Ross S. A. (2012), A new isoflavone from *Blepharis ciliaris* of an Egyptian origin. Med. Chem. Res. **19**, 2346 – 2350.

Harborne J. B. (1984), Phytochemical Methods. Chapman and Hall Ltd., London, UK.

Hegnauer R. and Kooiman P. (1978), Die systematische Bedeutung von Iridoiden Inhaltsstoffen im Rahmen von Wettstein's Tubiflorae. Planta Med. **33**, 1 – 33.

Jensen H. F. W., Jensen S. R., and Nielsen B. J. (1988), Chemotaxonomy of the Acanthaceae iridoids and quaternary amines. Phytochemistry **27**, 2581 – 2589.

Joshi B. S., Bai Y., Puar M. S., Dubose K. K., and Pelletier S. W. (1994), ^{1}H and ^{13}C NMR assignments for some pyrrolo{2,1-*b*}quinazoline alkaloids of *Adhatoda vasica*. J. Nat. Prod. **57**, 953 – 962.

Mohammed A. F., Hussein M. A., Abdel-Moty S. G., and Abdel-Alim A. M. (2011), Synthesis and biological evaluation of some benzimidazo-1,2,4-triazole derivatives as antimicrobial and anti-inflammatory agents. Bull. Pharm. Sci. Assiut University **34**, 77 – 92.

Robertson D. E., Noll D., Roberts M. F., Menaia J. A., and Boone D. R. (1990), Detection of the osmoregulator betaine in methanogens. Appl. Environ. Microbiol. **56**, 563 – 565.

Silverstien R. M. and Wabster F. X. (1998), Spectrometric Identification of Organic Compounds, 6th ed. John Wiley & Sons, Inc., New York, USA.

Wu T. S., Ou L. F., and Ten C. M. (1994), Aristolochic acids, aristolactam alkaloids and amides from *Aristolochia kankauensis*. Phytochemistry **36**, 1063 – 1068.

Wyn J. R. G. and Storey R. (1981), The Physiology and Biochemistry of Drought Resistance in Plants. Academic Press, New York, USA.