Chemical constituents of *Tephrosia purpurea*

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ABSTRACT

In continuation of our chemical investigation on some medicinal plants of the genus *Tephrosia*, reinvestigation of the methylenechloride/methanol (1:1) extract of the aerial parts of *Tephrosia purpurea* yielded an aromatic ester 1, a sesquiterpene 2 and prenylated flavonoid 3. The structures of the compounds were established by comprehensive NMR studies, including DEPT, COSY, NOE, HMQC, HMBC, EIMS and CIMS.

Key words: Aromatic ester, prenylated flavonoid, sesquiterpene, *Tephrosia purpurea*

INTRODUCTION

Tephrosia purpurea (Dil.) Pers, belongs to the family Fabaceae, subfamily Faboideae, tribe Millettieae, and it is a highly branched suberect herbaceous perennial, up to 60 m in height with spreading branches; the leaves are imparipinnate, with narrow, oblanceolate leaflets; the flowers are red or purple in extra-axillary racemes, the pods are slightly curved, 3–4.5 cm long, grey, smooth and containing 5–10 seeds per pod.[1,2] The plant grows abundantly in the upper Gangetic plains, and western Himalayas. The herb is commonly grown as a green manure in paddy fields in India and in tobacco and rubber plantation in other countries. It grows ubiquitously in all soils, sandy, rocky and loamy.[3] In India and South Africa, it is used as a fodder before flowering, but in Australia it is reported to cause livestock poisoning. In northern India, dry plants are collected for fuel. All parts of the plant have tonic and laxative properties. The dried plant is deobstruent, diuretic and useful in treating bronchitis, bilious febrile attacks and obstructions of the liver, spleen and kidneys. It is also recommended as a blood purifier, in the treatment of boils and pimples and is considered a cordial treatment. In southern India, a decoction of the fruit is given for intestinal worms and a fruit extract is used to relieve bodily pains and inflammatory problems. The roots are bitter and the decoction is used as a nematicide for treatment against *Toxocora canis* larvae which cause a lung disease in Sri Lanka; it is also used for treating dyspepsia, colic, and chronic diarrhoea and as an antihelminthic.[3-5] Several reports of *T. purpurea* have demonstrated the presence of flavones, flavanones and prenylated flavonoids,[6,7] chalcones,[7-11] and rotenoids.[9,10] In continuation of our chemical investigation on some medicinal plants of the genus *Tephrosia*,[12,13] reinvestigation of the methylenechloride extract of aerial parts of *T. purpurea* resulted in isolation and structural elucidation of three compounds: an aromatic ester 1, a sesquiterpene of the rare rotundane skeleton 2 and a prenylated flavonoid 3, isolated for the first time from this species.

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MATERIALS AND METHODS

General

^{1}H-NMR (500 MHz, $CDCl_3$), ^{13}C-NMR (125 MHz, $CDCl_3$) and the 2D spectra were recorded on a JEOL 500 MHz, Lambda spectrometer, with TMS as an internal standard. EIMS was recorded on a JEOL SX102A mass spectrometer.

Plant material

The aerial parts of *T. purpurea* were collected in the spring of 2001 from Aswan Island, Aswan, South of Egypt. A voucher specimen has been deposited in the Herbarium of the Department of Botany, Faculty of Science, South Valley University, Aswan, Egypt.

Extraction and isolation

Air-dried aerial parts (500 g) were crushed and extracted with CH_2Cl_2–MeOH (1:1) at room temperature. After

solvent removal, the residue (35 g) was subjected to CC on silica gel and eluted with *n*-hexane (2 l) followed by a gradient of *n*-hexane–CH_2Cl_2 up to CH_2Cl_2 and CH_2Cl_2–MeOH up to 15% MeOH (2 l each of the solvent mixture). The *n*-hexane–CH_2Cl_2 fraction (1:3) was carefully chromatographed on a Sephadex LH-20 column eluted with *n*-hexane–CH_2Cl_2–MeOH (7:4:0.25) with increasing polarity to give compound 1 (9 mg) and compound 2 (8 mg). The CH_2Cl_2 fraction (100%) was chromatographed on a Sephadex LH-20 column and eluted with *n*-hexane–CH_2Cl_2–MeOH (7:4:0.5), and it gave compound 3 (11mg).

RESULTS AND DISCUSSION

Compound 1 [Figure 1], showed ion peak $[M+1]^+$ at m/z 441 corresponding to the molecular formula $C_{24}H_{24}O_8$ in its CIMS. The low-resolution EIMS showed the molecular ion peak $[M]^+$ at m/z 440.147066 (calcd. 440.147118) that corresponds with the molecular formula $C_{24}H_{24}O_8$. The structure of compound 1 was determined from careful investigation of the 1D and 2D NMR data. The ^{1}H-NMR spectrum [Table 1] showed a doublet at δ 7.06 (J = 8.5 Hz, H-2), which showed a correlation in ^{1}H–^{1}H COSY with a doublet of doublet at δ 7.12 (J = 8.5, 2 Hz, H-1); also, it showed a doublet of doublet at δ 6.44 (J = 15.8 Hz, H-8) and a doublet at δ 7.70 (J = 15.8 Hz, H-7). Four singlet signals appeared at δ 2.31, 2.32, 3.85, 3.86, respectively, for the two acetyl groups and the two methoxyl groups. Additionally, it revealed the presence of the other olifinic protons as a doublet of doublet of doublet at δ 6.30 for H-11 (J = 16, 13, 13 Hz). The methylene protons H-10 appeared as two broad doublets at δ 4.85 and 4.87 (J = 17.5 Hz). The ^{13}C-NMR data [Table 1] revealed the presence of 24 carbon signals that were resolved by DEPT experiments into 4 methyls, 10 methines, 1 methylene and 9 quaternary carbons. Moreover, all proton and carbon signals were established from the results of ^{1}H–^{1}H COSY, HMBC. The HMBC showed important correlations namely, H-1 with C-5, C-3, C-2, C-1 and C-7 and H-14' with C-18, C-16 and C-12. Also, the spectrum showed correlations of H-12 with

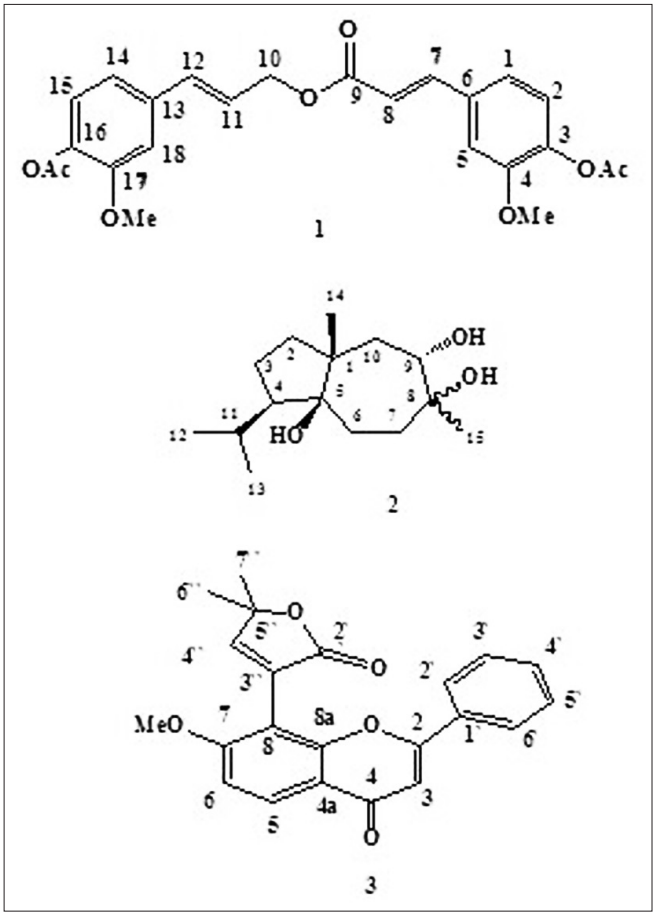

Figure 1 : Structures of compounds 1, 2 and 3

Table 1: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data in $CDCl_3$ for compounds 1 and 2 (δ_H and δ_C in ppm, J in Hz)

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1	7.2 dd (8.5, 2.0)	111.3 d	–	51.9 s
2	7.06 d (8.5)	123.3 d	1.70–150 m	20.5 t
3	–	141.5 s	1.70–150 m	23.1 t
4	–	151.5 s	–	55.3 d
5	7.14 d (2.0)	121.3 d	–	81.2 t
6	–	133.3 s	1.70- 150 m	29.6 d
7	7.7 d (15.8)	144.4 d	1.70- 150 m	40.9 t
8	6.44 d (15.8)	118.1 d	–	75.6 s
9	-	166.5 s	4.12 dd (5.0, 10)	71.4 d
10	4.85 d (17.5) 4.87 d (17.5)	65.0 t	–	47.9 t
11	6.30 d (16.27)	123.6 d	a: 1.26 d (14.0, 10) b: 1.80 d (14.0, 5)	45.6 d
12	6.69 d (16.2)	133.7 d	0.95 d (7.0)	21.1 q
13		135.3 s	1.00 d (7.0)	21.5 q
14	7.01 dd (8.2, 1.8)	110.3 d	1.27 s	22.0 q
15	6.99 d (8.2)	122.9 d	1.24 d (15.8)	22.9 q
16	–	139.7 s	–	–
17	–	151.2 s	–	–
18	6.94 d (1.8)	119.4 d	–	–
OAc	2.31 (3H, s)	168.7 s, 20.6 q	–	–
OAc	2.32 (3H, s)	169.0 s, 20.6 q	–	–
2 OMe	3.85 (3H, s) 3.86 (3H, s)	20.6 q	–	–

C-10, C-14, C-18 and C-12; H-10 with C-15, C-11, C-12 and C-10; OMe with C-14, C-18 and C-17; H-15 with C-14, C-18, C-12 and C-17; H-5 with C-1, C-5, C-3 and C-7, H-2 with C-6, and C-4 and C-3. While comparing the spectral data of compound 1 with those of the compounds isolated before,[14] compound 1 was identified as 2-propenoic acid, 3-(4-(acetyloxy) -3-methoxypheny)-3(4-actyloxy)-3-methoxyphenyl)-2-propenyl ester.

Compound 2 [Figure 1], was assigned to be a sesquiterpene of the rare rotundane skeleton, 4-isopropyl-1,8-dimethyl-decahydro-azulene-5,8,9-triol.[15] Its EI mass spectrum showed the molecular ion peak at $m/z = 256$, corresponding to the molecular formula $C_{15}H_{28}O_3$, some important fragments were observed at m/z 238, 220 and 195 due to the loss of water, isopropyl radical and another water molecule, respectively. The ^{1}H-NMR spectrum [Table 1] showed two doublet signals at δ 0.95 and 1.00 that revealed the presence of the isopropyl moiety. Additionally, the proton singlet at δ 1.24 was assigned for H-15 and that at δ 1.27 for H-14. A doublet at δ 4.12 suggested the presence of a carbon bearing oxygen. The ^{13}C-NMR spectrum [Table 1] revealed the presence of 15 nonequivalent carbon atoms, which resolved by DEPT experiments. It was determined that compound 2 possess four methyls, five methylenes and three methines. On the basis of these results, the structure of compound 2 was assigned to the sesquiterpene of rotundane skeleton 4-isopropyl-1,8-dimethyl-decahydro-azulene-5,8,9-triol, previously isolated from *Ferula sinaica*.[15]

Compound 3 [Figure 1], was established based on analysis of ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HMBC and EIMS data. The EIMS spectrum showed a molecular ion peak $[M]^+$ at m/z 362 corresponding to the molecular formula $C_{22}H_{18}O_5$. Examination of the ^{1}H-NMR spectroscopic data [Table 2] of compound 3 indicated the presence of a flavone structure. Two multiplets at δ 7.43 and 7.74 established the presence of B-ring flavone protons at H-2', H-4' and H-6', as well as at H-3' and H-5'. The signals at δ 1.65 (6H, s) and at δ 3.94 (3H, s), correspond to a gem-dimethyl group and a methoxy group, respectively. Also, it showed a singlet signal at δ 7.52 (1H, H-4") and a doublet signal at δ 8.26 (1 H, d, J = 9 Hz, H-5), showed a correlation in ^{1}H–^{1}H COSY with a doublet signal at δ 7.08 (1H, d, J = 9 Hz, H-6). The ^{13}C-NMR and DEPT spectrum [Table 2] showed 22 carbon signals with two carbonyl carbon signals at δ 177.72 and 170.62: three methyls, nine methines and eight quaternary carbon atoms. HMBC analysis showed correlations between gem-dimethyl and C-5" with H-4", H-5 with C-4, C-7 and C-8a; H-6 with C-8 and C-4a; H-3 with C-2, C-4b and C4a; and OMe with C-7. Comparing the spectral data of compound 3 with those of the compounds isolated before,[16] identified compound 3 as apollinine.

Table 2: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data in $CDCl_3$ for compound 3 (δ_H and δ_C in ppm, J in Hz)

Position	3	
	δ_H	δ_C
2	–	161.69 s
3	6.74 s	107.35 d
4	–	177.72 s
4a	–	118.04 s
5	8.26 d (9)	128.13 d
6	7.08 d (9)	109.36 d
7	–	163.18 s
8	–	109.7 s
8a	–	154.87 s
1′	–	131.92 s
2′, 6′	7.43 m	126.20 d
3′, 5′	7.74 m	128.99 d
4′	7.43 m	131.51 d
gem-Me_2	1.65 (6H, s)	25.83 q
OMe	3.94 (3H, s)	56.60 q
2″	–	170.62 s
3″	–	124.24 s
4″	7.52 s	159.89 d
5″	–	84.92 s

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