

Tephrospinosinol, a flavonol glucoside from *Tephrosia spinosa*

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ABSTRACT

A flavonol glycoside tephrospinosinol 1 has been isolated from the MeOH extract of the *Tephrosia spinosa* by various chromatographic methods. The structure of tephrospinosinol¹ was established by physical and spectroscopic analysis. Application of 2D NMR techniques was useful for complete characterization of the new tephrospinosinol 1 and it is identified as 5,4'-dihydroxy,6,7-dimethoxy,flavone 3-o- β -glucopyranoside. Tephrospinosinol 1 shows moderate antibacterial and low antifungal activity.

Keywords: Tephrospinosinol, 5,4'-dihydroxy,6,7-dimethoxy,flavone3-O- β -glucopyranoside, *Tephrosia spinosa*, antimicrobial activity.

INTRODUCTION

Tephrosia (Papilionaceae) is a large genus of perennial woody shrubs, which were well distributed in tropical and sub – tropical regions of the world¹. Between 300 and 400 species are known², of which occur in India, 30 are native of South America, 70 are South Africa and 50 in equatorial America of which 30 are found in Kenya^{3,4,5}. Some of the species have been used in herbal remedies, insecticides and rat, fish and human poisons by the various indigenous people of Kenya^{1,6}. Various rotenoids and other flavonoids have been isolated from the genus *Tephrosia*⁷. Some possess insecticidal activity⁸. *Tephrosia spinosa* resulted in the isolation of spinochalcone A and B⁹, diisoprenylated chalcone¹⁰, flavonoids and chalcone derivatives¹¹. In the course of chemical studies of *Tephrosia spinosa* from the Warangal, Andhra Pradesh, India region as potential sources of a new bio active secondary metabolites, We

investigated the MeOH extract of *Tephrosia spinosa*. Chemical studies of this extract led to the isolation of a new flavonolglucoside named tephrospinosinol¹. It was previously reported^{12,13}. This report describes the isolation, structure elucidation and antimicrobial activity of compound¹.

EXPERIMENTAL

Tephrosia spinosa was collected and identified from Waddepalli tank of Warangal district, Andhra Pradesh, India in November 2004 by Prof.V.S.Raju, Department of Botany, Kakatiya University, Warangal. Voucher specimen (reference Rao -61) have been deposited in the Chemistry Department herbarium. The dried sample of *Tephrosia spinosa* (1.3 kg) was extracted with MeOH (5LX2) for 48h. The resulting crude MeOH extract (50 g) was subjected to c_{18} functionalized silica gel flash column chromatography with a stepwise gradient of 30-100% MeOH in EtOAc and

the fraction that was eluted with 70% MeOH was further subjected to purification by repeated recrystallization from MeOH to yield tephrospinosinol¹ [130 mg].

RESULTS AND DISCUSSION

Tephrospinosinol(1)¹² was isolated as pale yellow colored powder and analyzed for the molecular formula C₂₃H₂₄O₁₂ by EIMS(probe) 70 eV data m/z 493.13[M⁺]; and this formula was fully supported by ¹H NMR (Bruker; DMSO at 400.13MHz) and ¹³C NMR (DMSO at 75MHz) data (Table 1). The ¹H NMR spectrum revealed the presence of two methoxy groups, 5 aromatic methines, five SP³ methylenes (two oxygenated) units. Flavonoid skeletal protons appeared between

δ 6.84-8.02 while one glycoside proton signals are in the range δ 3.37-5.42. Peaks at δ 6.86 and 6.84 are corresponds to OH of C-4' and C-5'. In addition to the signals corresponding to the above carbons, analysis of ¹³C NMR spectrum revealed the presence of one carbonyl (δ 177.7) in the molecule of flavonol signal at δ 101.5 indicates the presence of sugar unit in the molecule (glycoside linkage). Signals corresponding to δ 131.6 (C-1'), δ 130.9(C-2',C-6'), δ 115.01(C-3',C-5'), δ 160.3(C-4') indicate the presence of the aromatic ring in the flavonol molecule. Several carbon signals corresponding to methine and methylene carbons of the sugar portion appeared in the region δ 60-80. The assignments of these sets of protons was supported by the NOESY spectrum, where by H-8 showed spatial contours with H-1''' and H-2', H-2' with H-3' and H-

Table 1: The ¹H, ¹³C NMR, NOESY and HMBC spectral data for tephrospinosinol (1)

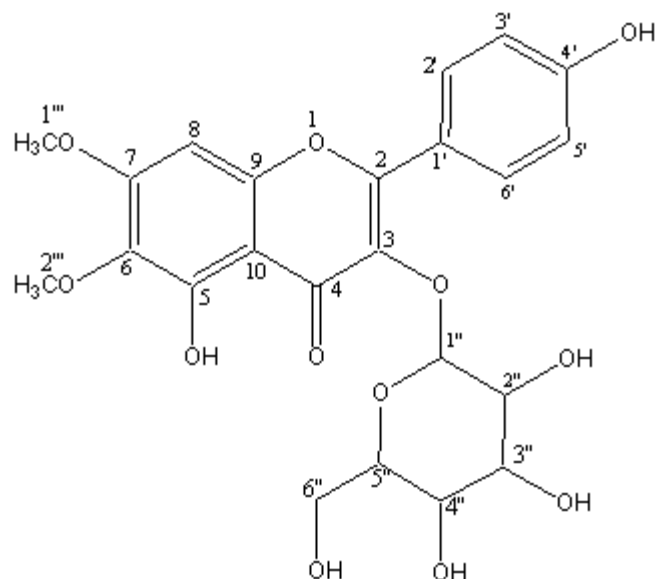
C n°	δ _c	δ _H in ppm	NOESY with H at	HMBCC with H at
2	156.71	-	-	-
3	133.2	-	-	-
4(CO)	177.7	-	-	-
5	151.7	-	-	-
6	131.6	-	-	-
7	160.3	-	-	-
8	91.6	6.85 (1H,s)	1''',2'''	-
1'	131.6	-	-	-
2'	130.9	8.01(1H,d)	2',3',6'	3'
3'	115.1	6.89(1H,dd)	3',2',5'	2'
4'	160.03	-	-	-
5'	115.01	6.81 (1H, d)	8	-
6'	130.9	8.01 (1H, d)	5',6'	2',5',6'
OH-5	-	6.89(1H, s)	-	-
OH-4'	-	8.01 (1H, s)	3', 5'	-
1''	101.5	5.4 (1H, d)	-	2''
2''	71.1	3.55 (1H, m)	1'',2''	2''
3''	73.06	3.38(1H, m)	2'',3''	2'', 4''
4''	67.8	3.65 (1H, m)	3'',4''	3'',5''
5''	75.7	3.37(1H, m)	4'',5'',6''	4'',6''
6''	62.1	4.19(2H,m)	5',6'	2',5',6'
1'''	60.15	3.42(3H,s)	8,2'	-
2'''	60.03	3.32(3H,s)	-	-

Table 3: Antifungal activity of tephrospinosinol (1)

Compound	Dose in mg	Organisms and zone of inhibition in mm	
		<i>Aspergillus parasiticus</i>	<i>Candida albicans</i>
Tephrospinosinol	100	-	-
	50	-	-
	33.3	9.0	15.0
	16.7	6.5	9.0
Clotrimazole	10	18.0	27.0

Table 2: Antibacterial activity of tephrospinosinol (1)

Compound	Dose in mg	Organisms and zone of inhibition in mm			
		Gram positive		Gram negative	
		<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>
Tephrospinosinol	100	-	-	-	-
	50	-	-	-	-
	33.3	18.0	16.0	15.0	14.0
	16.7	10.0	14.0	13.5	12.5
Streptomycin	20	20.0	18.0	18.0	20.0
Ampicillin	20	20.0	22.0	18.0	17.0



Tephrospinosinol (1)

6', H-6' with H-5' and H-2', these assignments were supported by 3-bond correlation in the HMBC spectrum (Table 1).

Tephrospinosinol¹ was evaluated for antimicrobial activity^{13,14} (anti bacterial and antifungal) against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two fungi (*Aspergillus parasiticus* and *Candida albicans*). Tephrospinosinol¹ exhibited more activity against gram positive bacteria than gram negative

bacteria (Table 2). It shows lower antifungal activity (Table 3). However it doesn't show antimicrobial activity at higher concentrations.

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- Tephrospinosinol(1): Pale yellow colored powder (bp 173-176 °C); UV(CH₃OH) λ_{max} at 227 nm, 290 and 323; FTIR in KBr at 1658 cm⁻¹ (CO), 1549, 1482, 1361 cm⁻¹ (aromatic/benzene), 1272 cm⁻¹ (aromatic C-O-C), 1135 cm⁻¹ (aliphatic C-O-C) and 3445 cm⁻¹ (OH); ¹H, ¹³C and 2D NMR data, Table 1; EIMS m/z 493.13 (M⁺) (calc. for C₂₃H₂₄O₁₂, 493.156).
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