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ISOLATION AND IDENTIFICATION OF A NEW PHYTOSTEROL ESTER FROM *TEPHROSIA PURPUREA* (LINN.) *PERS. ROOT*

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ABSTRACT

Tephrosia purpurea (Linn.) Pers. (Fabaceae) is herb or undershrub being used for the treatment of various disorders since time immemorial in the indigenous system of medicine in India. The ethanol extract of the roots were subjected to column chromatograpy so as to yield pale yellow crystals from the petroleum ether : chloroform (1 : 1) fraction a new phytosterol ester identified by means of spectroscopy as Stigmast-5, 22-dien-3β, 21diol-3β, 21-dihexadecanoate.

Key words: Phytosterol ester, *Tephrosia purpurea*.

INTRODUCTION

Tephrosia purpurea (Linn.) Pers (Fabaceae) is herb commonly found on road sides, pathways open waste lands and fields in the desert area[1]. Traditionally the drug is considered to be useful in cough, asthma. It is also used as bitter, astringent, acrid, thermogenic, anthelmic, digestive, laxative, diuretic, uterine tonic and anti-inflammatory. Internally it is used as a blood purifier, in the treatment of bronchitis, boils, pimples, bleeding piles; kidney disorders[2]**.** In Unani system of medicine, it is believed to resolve stone in kidney; it acts as diuretic, stomachic, emmenagogue, and used in the treatment of bronchitis, asthma, liver and spleen disorders. In Ayurveda and Sidha system of medicine, it is considered as Ushna veeryam, Katu rasam, Katu vipakam, and used in the diseases of the teeth, salivation, to treat poisoning due to snake bite[3]**.** The roots of *Tephrosia purpurea* is reported to contain purpurenone, (+)-purpurin, (-)-purpurin, dehydroisodericin, (-)-macckiain, pseudosemiglabrin, (-)-semiglabrin[4]. Purpuritenin, purpureamethide are

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reported from seeds[5] and serratin from stem[6]. Semiglabrin, semiglabrinol, pseudosemiglabrin, pseudosemiglabrinol, methylpseudoglabrinate and, methylpseudomultijuginate have been reported from whole plant[7,8]. Tephrosin, pongaglabol and semiglabrin have been identified in the aerial parts[9]**.** The present work reports a new phytosterol ester in the roots of *Tephrosia purpurea.*

EXPERIMENTAL

UV spectrum was recorded in methanol on Perkin Elmer EZ301 spectrophotometer. IR spectrum was recorded on a Shimadzu FTIR 8201 spectrophotometer. ^{1}H NMR and ^{13}C NMR were recorded on Bruker Avance 400 spectrometer. Mass spectra (FABMS) data was recorded on JEOL SX 102/DA-6000 mass spectrometer.

Plant material

The root of *Tephrosia purpurea* (Linn.) Pers. was collected during the month of the September 2006 from Govt. Polytechnic, Hisar (Haryana). The plant material was taxonomically identified and authenticated at the National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, by Dr. H.B. Singh. The voucher specimen has been deposited in the herbarium section of the Pharmacognosy Division, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, for future reference.

Extraction, isolation and characterization[10-12]

The coarsely powdered root of *Tephrosia purpurea* (2 kg) was subjected to hot extraction process with ethanol (95%) for 72 hrs. The combined extracts were concentrated in rotary vacuum evaporator. A dark brown extract (154 g) obtained was chromatographed over silica gel (100-120 mesh) in a column using various solvents in order of increasing polarity. Elution of the column with petroleum ether: chloroform (1 : 1) gave yellowish crystals of compound, which were recrystallized from pure chloroform, 220 mg (1.19% yield). R_f: 0.57 [petroleum ether : chloroform (1 : 1)], m.p.: 299-304°C.

UV λ_{max} (Methanol): 274-380 nm

IR ν_{max} (KBr): 1707, 1592, 1549, 1465, 1292, 1259, 1058, 970, 932, 801, 773 cm^{-1}.

^{1}H NMR ($CDCl_3$), ^{13}C NMR ($CDCl_3$): Position of carbon is shown in

Tables 1 and 2

EIMS *m/z* (rel. int.): 902 $[M]^+$ ($C_{61}H_{108}O_4$).

Table 1. ^{1}H NMR ($CDCl_3$), ^{13}C NMR ($CDCl_3$) of teprosteryl dipalmitate

Position of carbon	^{1}H NMR		^{13}C NMR
	Alpha	Beta	
1	1.90 ddd (*J*= 5.6,10.1,6.4)	1.92 ddd (*J*=5.6, 10.1, 6.4)	37.070
2	1.60 m	1.58 m	27.880
3	4.53 brm ($w_{1/2}$ = 18.5 Hz)	-	73.730
4	1.88 m	1.86 m	38.230
5	-	-	139.76
6	5.30 d (*J*= 1.6)	-	122.64
7	1.94 dd (*J*= 5.6,10.1)	1.59 m	31.980
8	-	1.49 dddd (*J*=10.4, 2.4, 2.0, 3.2)	31.600
9	1.54 ddd (*J* = 10.8, 4.8, 4.4)	-	50.090
10	-	-	36.660
11	1.10 ddd (*J*= 3.6, 9.2, 2.8)	1.16 ddd (*J*=6.4, 5.2, 2.4)	22.650
12	1.47	1.54 m	36.220
13	-	-	42.370
14	1.43	-	56.750
15	1.54	1.50 m	24.360

Cont…

Position of carbon	^{1}H NMR		^{13}C NMR
	Alpha	Beta	
16	1.48	1.42 m	38.320
17	1.43	-	56.100
18	0.60 brs	-	12.320
19	0.95 brs	-	19.380
20	-	2.73 m	39.790
21	4.07 d (*J*= 7.2)	4.04 d (*J*=7.2)	60.190
22	5.29 dd (*J*= 4.0, 2.4)	-	130.25
23	5.26 dd (*J*= 2.4, 5.2)	-	128.09
24	2.17 m	-	45.890
25	1.47 m	-	34.460
26	0.80 d (*J*=6.4)	-	22.760
27	0.79 d (6.8)	-	19.880
28	1.21 m	1.19 m	25.490
29	0.78 dd (*J*=4.2, 2.1)	-	12.690

Table 2. ^{1}H NMR ($CDCl_3$), ^{13}C NMR ($CDCl_3$) of teprosteryl dipalmitate

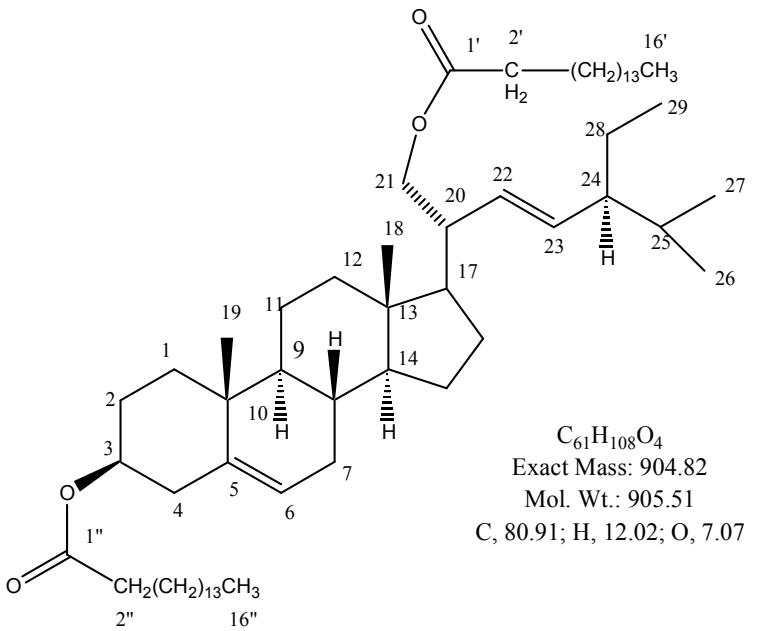
Position of carbon	^{1}H NMR		^{13}C NMR
	Alpha	Beta	
1’	-	-	173.96
2’	2.24 d (*J*=6.4)	2.22 d (*J*=6.4)	34.770
3’	1.29 brs	1.29 brs	29.770

Cont…

Position of carbon	^{1}H NMR		^{13}C NMR
	Alpha	Beta	
4’	1.28 brs	1.28 brs	29.770
5’	1.22 brs	1.22 brs	29.770
6’	1.21 brs	1.21 brs	29.670
7’	1.19 brs	1.19 brs	29.530
8’	1.18 brs	1.18 brs	29.400
9’	1.18 brs	1.18 brs	29.340
10’	1.18 brs	1.18 brs	29.220
11’	1.18 brs	1.18 brs	28.990
12’	1.18 brs	1.18 brs	27.270
13’	1.16 brs	1.16 brs	25.060
14’	1.16 brs	1.16 brs	21.090
15’	1.14 brs	1.14 brs	17.100
16’	0.84 t (*J*= 6.4)	-	14.320
1”	-	-	173.32
2”	2.21 d (*J*= 7.6)	2.19 d (*J*= 7.6)	34.010
3”	1.29 brs	1.29 brs	29.770
4”	1.28 brs	1.28 brs	29.770
5”	1.22 brs	1.22 brs	29.770
6”	1.21 brs	1.21 brs	29.670
7”	1.19 brs	1.19 brs	29.530
8”	1.18 brs	1.18 brs	29.400
9”	1.18 brs	1.18 brs	29.340

Cont…

Position of carbon	^{1}H NMR		^{13}C NMR
	Alpha	Beta	
10”	1.18 brs	1.18 brs	29.220
11”	1.18 brs	1.18 brs	28.990
12”	1.18 brs	1.18 brs	26.120
13”	1.16 brs	1.16 brs	25.690
14”	1.16 brs	1.16 brs	23.120
15”	1.14 brs	1.14	18.840
16”	0.8 t (*J*= 7.2)	-	14.180



$C_{61}H_{108}O_4$
Exact Mass: 904.82
Mol. Wt.: 905.51
C, 80.91; H, 12.02; O, 7.07

Scheme

RESULTS AND DISCUSSION

The isolated phytosterol ester designated as teprosteryl dipalmitate, was obtained as yellowish crystals from petroleum ether : chloroform (1 : 1) eluent. It responded positively to Liebermann Burchard test indicating steroidal nature of the molecule. Its IR

spectrum exhibited characteristic absorption bands for ester group at 1737 cm^{-1}, unsaturation at 1640 cm^{-1}, and long aliphatic chain at 805 cm^{-1} and 723 cm^{-1}. Its UV absorption maxima at 250-330 nm indicated flavone nature of the molecule. The mass spectrum of teprosteryl dipalmitate showed a molecular ion peak at 904 *m/z* consistent to the molecular formula of a diester derivative of sterol, $C_{61}H_{108}O_4$. It indicated eight double bond equivalents. Four of them were adjusted in the tetracyclic carbon framework of the steroidal moiety and two each in the vinylic linkages and ester groups. A two-proton doublet at 5.30 (J=1.6Hz) and two one-proton double doublet at δ 5.29 (J=4.0, 2.4 Hz) and 5.26 (J=2.4, 5.2 Hz) were assigned to vinylic H-6, H-22 and H-23 respectively at δ 4.53 with half-width of 18.5 Hz was attributed to α-oriented H-3 carbinol proton. Two one-proton doublets at δ 4.07 (J=7.2 Hz) and 4.04 (J=7.2 Hz) were ascribed to oxygenated methylene H_2-71 protons. Four one-proton doublet at δ 2.24 and 2.22 with coupling interactions of 6.4 Hz each and at 2.21 and 2.19 with coupling constants of 7.6 Hz each were associated with methylene H_2-2' and H_2-2", respectively, both adjacent to the ester groups. Two three-proton broad signals at δ 0.60 and 0.95 were accounted to tertiary C-18 and C-19 methyl protons. Two three-proton double doublets at δ 0.78 (J=4.2, 2.1 Hz) were associated with the C-26, C-27 secondary and C-29 primary methyl protons. The absence of any there-proton doublet near δ 0.93 supported oxygenated methylene nature of C-21. Two three-proton triplets at δ 0.84 (J=6.4 Hz) and 0.82 (J=7.2 Hz) were due to C-16" primary methyl protons. The remaining methylene and methane protons resonated between δ 1.98-1.14. The presence of all methyl signals in the range 0.95-0.60 indicated that all these functionalities were attached to the saturated carbons. The ^{13}C NMR spectrum of teprosteryl dipalmitate exhibited signals for vinylic carbons at δ 139.76 (C-5), 122.64 (C-6), 130.25 (C-22) and 128.09 (C-23), ester carbons at δ 173.96 (C-1') and 173.32 (C-1"), carbinol carbon at δ 73.73 (C-3), oxygenated methylene carbon at δ 12.32 (C-18), 19.38 (C-19), 22.76 (C-26), 19.88 (C-27), 12.19 (C-29), 14.32 (C-16') and 14.18 (C-16"). The ^{1}H and ^{13}C NMR spectral values were compared with the related steroidal molecules like β-sitosterol, stigmasterol and lawsaritol[12]**.** Alkaline hydrolysis of teprosteryl dipalmitate yielded palmitic acid.

On the basis of spectral data analysis and chemical reactions, the structure of teprosteryl dipalmitate has been formulated as Stigmast-5, 22-dien-3β, 21diol-3β, 21-dihexadecanoate. This is a new phytosterol ester isolated from plant source and being reported for the first time.

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