

PHCOG MAG.: Research Article

Prenylated flavonoids from the roots of *Tephrosia tinctoria* Pers.

P. Lakshmi^a, K. Lakshminarayana^a, S. Ganapaty^{b*} and P. Steve Thomas^b

^aDepartment of Botany and ^bDepartment of Pharmaceutical Sciences

Andhra University, Visakhapatnam- 530 003. A.P. India

*Author for Correspondence: ganapatyseru@yahoo.co.in

ABSTRACT

The genus *Tephrosia* is known for its unusual flavonoids. Chemical examination of the roots of *Tephrosia tinctoria* led to the isolation of β -sitosterol, lupeol, tephtrone, 7-O-methyl glabranin, 2-Hydroxy tephrosin, rotenone and dehydrodeguelin. The compounds were isolated by sequential chromatography and the structures were established by 2D NMR analysis and MS spectral data. All the compounds are reported for the first time from the species *T. tinctoria* and the candidate 2-Hydroxy tephrosin is new to the species and also to the genus *Tephrosia* as well.

KEYWORDS: Prenylated flavonoids, *Tephrosia tinctoria*, 2-Hydroxy tephrosin

INTRODUCTION

The genus *Tephrosia* is a pantropical taxa with about 400 species distributed chiefly in Asia, Africa, Australia and America (1, 2). About twenty-four species of *Tephrosia* were recorded in India (3,4). Most of the *Tephrosia*'s are herbs to undershrub and are grown as weeds. The genus is well known for its richness in prenylated flavonoids and is considered to possess insect repellent, larvicidal, piscicidal, antimicrobial and anticancer properties (5-8). In the present study we report the chemical examination of the roots of *Tephrosia tinctoria*, a perennial undershrub and the compounds obtained therein.

Materials and methods

All chemicals and solvents used were of analytical grade and obtained from Ranbaxy Fine Chemicals and Merck Ltd., Mumbai.

Plant Material

The air-dried roots (2kg) of *T. tinctoria* were collected at Talacona hills of Chittoor district, Andhra Pradesh in March 2004 and were authenticated by Prof. T. Pulliah, Taxonomist, Department of Botany, Sri Krishnadevaraya University, Anantapur, India. A voucher specimen (SG/TTR /06/126) has been deposited at the Herbarium, Department of Botany, Andhra University, Visakhapatnam, India.

The roots were separated, air-dried and powdered in a Wiley mill. The root powder (2kg) was extracted with chloroform and methanol successively and subsequently concentrated under reduced pressure to get their corresponding residues. The chloroform and methanol residues showed a positive Lieberman-Burchard test for sterols and triterpenes, olive green

colour with ferric chloride and an orange colour with Shinoda's test indicating the presence of flavonoids.

Isolation and Characterization of the compounds

The chloroform (14g) and methanol (18g) concentrates showed similar spots on TLC (sys: Chloroform: petroleum ether, 80:20) and hence were combined and column chromatographed over silica gel (Acme, 100-200 mesh) and successively eluted with petroleum ether; petroleum ether-chloroform, chloroform and chloroform-methanol mixtures. The compounds β -sitosterol (1) lupeol (2) tephtrone (3), 7-O-methylglabrin (4), 2-Hydroxy tephrosin (5), rotenone (6), dehydrodeguelin (7) were isolated and identified by chemical tests and spectral means.

Results

β -sitosterol (1) was crystallized from hexane as colourless fine needles, m.p. 136- 138°C and analyzed for the formula $C_{29}H_{50}O$. It showed positive colour reaction for sterols with Lieberman-Burchard test. The 1H NMR spectrum showed the peaks at δ 0.80-1.25 (methyl), δ 3.45 (1H broad C_3 α -H) and δ 5.30 (1H, *m*, C_5 -H). Based on the data, it was identified as β -sitosterol.

Lupeol (2) was crystallized from chloroform-petroleum ether as needles, m.p. 211-213°C, and analyzed for the formula $C_{30}H_{50}O$. It gave pink colour in Lieberman-Burchard reaction and yellow colour with tetranitromethane test. The IR ν_{max}^{KBr} spectrum showed absorption band at 890 cm^{-1} due to the presence of vinyl methylene group and the 1H NMR spectrum ($CDCl_3$, 90MHZ) exhibited the peaks at δ

0.78, 0.80, 0.83, 0.90 and 1.02 (18H, s, 6xCH₃), 1.63 (3H, s, CH₃), 2.25 (1H, d, 19-H), 3.15 (1H, m, 3α-H), 4.5 (2H, d, CH₂). The above data was in good agreement with that of lupeol.

Tephrrinone (3) was obtained as white crystalline compound from chloroform, m.p. 127- 129°C and analyzed for the formula C₂₁H₂₂O₄. It gave a positive result for Shinoda test indicating flavonoid nature. The IR spectrum showed a phenolic hydroxyl at 3375 cm⁻¹ and a chelated carbonyl at 1630 cm⁻¹. Strong olive green colour with alcoholic ferric chloride confirmed phenolic nature. Further the UV absorption maxima (MeOH, λ_{nm}) 285 (4.26) and 345 (3.44) and a bathochromic shift of 40nm in the presence of AlCl₃-HCl indicated the characteristic feature of 5-hydroxy flavonone. The ¹H NMR showed a double doublet at 5.4δ (1H, J_{2,3}=11 Hz and J_{2,3} _{aq} = 6Hz) and a multiplet in the region 2.81-3.1δ (2H) that are assigned to C₂ and C₃ protons respectively of a flavonone. A set of peaks appearing at 1.63 (6H, broad singlet, (CH₃)₂) 3.24 δ (2H, d, J=6Hz, -H₂-) and 5.16δ (1H, t, J =6Hz, -CH-) indicated the presence of C-3-methylbut-2-enyl group. Further the spectra showed the presence of a methoxyl group (3.85 δ-3H, s), a phenyl group (7.45 δ-5H, broad singlet), an up field proton (6.12 δ-1H, s, C₆H) and a hydroxyl group (11.0 δ-1H, s, 5-OH). The above chemical and spectral data was in agreement with that of tephrrinone (9) and hence identified as tephrrinone.

7-O-methylglabranin[4] was obtained as colourless woolly needles. It responded to ferric chloride indicating the phenolic nature of the compound. The UV and IR spectra indicated the presence of an unconjugated aromatic system with hydroxyl group. The MS displayed a molecular ion and a base peak at M/z 337 (M⁺). The ¹H NMR spectrum revealed the peaks at δ 5.29, dd, 2.34M 4.54 t, 6.12, s, 7.39 (M) and 3.26 a singlet, 5.1 (t), 1.62, s, 3.46 (CH₃)₂ and δ 12.2 (OH). The IR showed absorptions at 3430, 1660, 1590, 1560 and 790. Based on the data, it was identified as 7-O- methylglabranin(10)

2-Hydroxy tephrosin (5), the compound crystallised as pale green needles in chloroform, m.p. 204-206°C. The molecular formula was found to be C₂₃H₂₃O₈. It gave negative tests with ferric chloride and also with Shinoda's reagents. The ¹H NMR (CDCl₃, TMS, 300 MHz) δ 1.39(3H, s, H-2''a), 1.44(3H, s, H-2''b), 3.81(3H, s, H-5'a), 3.95(3H, s, H-4'a), 4.46(1H, dd, (J=12.0), H-2a), 4.63(1H, dd, (J=12.0), H-2b), 5.53(1H, d, (J=10.1), H-3''), 6.44(1H, d, (J=8.7), H-6), 6.47(1H, s, H-3'), 6.55(1H, s, H-6'), 6.57(1H, d, (J=10), H-4''), 7.90(1H,

d, (J=8.7), H-5) and the ¹³C NMR spectral data (CDCl₃, 100MHz) 28.0(C-2''a), 28.0(C-2''b), 54.8(C-4'a), 55.3(C-5'a), 63.9(C-2a), 67.4(C-3), 75.2(C-2), 76.9(C-2''), 101.1(C-3'), 108.6(C-1'), 109.1(C-8), 109.6(C-6'), 110.1(C-10), 110.8(C-6), 114.4(C-4''), 128.5(C-5), 126.7(C-3''), 144.0(C-5'), 148.4 (C-2'), 150.2(C-4'), 156.2(C-9), 158.7(C-7), 190.0 (C-4). Based on the spectral properties, the compound was identified as 2-Hydroxy tephrosin. This is the first time report of this compound from *T. tinctoria* in particular and the genus in general (11).

Rotenone (6) was obtained as pale brown needles, m.p. 164- 166°C and analyzed for the formula C₂₃H₂₂O₆. It gave positive Durham's test and negative test with ferric chloride, lead acetate and Shinoda's reagents characteristic of rotenoids. The ¹H NMR spectrum showed ABCD pattern of spin-spin splitting characteristic of a rotenoid arising from 6,6a and 12a protons. The chemical shift of the proton at C-1 appeared at δ 6.78 indicating cis fusion of rings B and C. It also revealed two singlets for methoxys at C-2 and C-3. The para protons of the ring A appeared as singlets at δ 6.78 and 6.45. Two ortho-coupled doublets appearing at δ 6.50 and 7.84 were assigned to the protons on C-10 and C-11 respectively of a rotenoid skeleton. The above data corresponded well with that of rotenone (12).

Dehydrodeguelin(7) a yellow crystalline compound obtained from chloroform-methanol. The ¹H NMR spectral data (CDCl₃, TMS, 300 MHz) showed signals at δ 8.46(1H, s, H-1), 8.06(1H, d (J=8.07) H-11), 6.86(1H, d (J=8.07) H-10), 6.77(1H, d (J=10.0) H-4'), 6.56(1H, s, H-4), 5.74(1H, d (J=10.0) H-3'), 5.02(2H, s, H₂- 6), 3.96(3H, s, 3-OCH₃), 3.87 (3H, s, 2- OCH₃), 1.52(6H, s, 2'-(CH₃)₂) and the ¹³C NMR spectral data (CDCl₃, 100MHz) exhibited peaks at δ 174.1 (C-12), 157.2(C-9), 156.0 (C-6a), 151.1(C-7), 149.2(C-3), 146.3(C-4a), 144.1 (C-2), 130.5(C-11), 126.5(C-3'), 118.5(C-11a), 115.3 (C-4'), 114.7(C-10), 111.8(C-12a), 110.5(C-8), 110.4 (C-1), 109.2(C-1a), 100.6(C-4), 77.7(C-6'), 64.8(C-6), 56.3 (3-OCH₃), 55.9(2-CH₃), 28.1 (2'-(CH₃)₂). The chemical properties and the spectroscopic data tallied well with that of dehydrodeguelin.

ACKNOWLEDGEMENT

One of the authors (P.L) is thankful to University Grants Commission (U.G.C) New Delhi, for a fellowship (SRF).

REFERENCES

1. R. Geesink. Tephrosieae. In: Polhill M. and P.H. Ravan (Eds.), *Advances in Legume Systematics*, Part 1 (Royal Botanic Gardens, Kew, U.K., 1981) pp. 245-260.

2. J.B. Gillett. Tephrosieae. In: Milne- E. Readhead and R.M. Polhill. (Eds.) *Flora of Tropical East Africa*, Leguminosae, Part 3, Sub family Papilionoideae(1), (Crown Agents, London, U.K., 1971)
3. J.S. Gamble, C.E.C. Fischer. *Flora of Presidency of Madras*, (3Vols), (Repr. Ed.1957). (Botanical Survey of India, Howrah. 1981)
4. C.J. Saldanha, B.G. Singh. (1984). Leguminosae, In: C.J. Saldanha (Ed.) *Flora of Karnataka* Vol:I,) Oxford and IBH,1984) pp. 495-499.
5. A.B. Gokhale, M.N. Saraf. *Tephrosia purpurea*, A review of contemporary literature and medicinal properties. *Indian Drugs*. **37**: 12 (2000).
6. M.D. Bentley, A. Hassanali, W. Lwande, P.E.W. Njoroge, M. Yatagai. Insect antifeedants from *Tephrosia elata* Deflers. *Insect. Sci. Appl.* **8(1)**: 85-88 (1987).
7. Chen Yuh-Lin. New piscicidal flavonoids from *Tephrosia obovata* merr. *Asian. J. Pharm.* **3(4)**:18 (1978).
8. P.S. Sarin jagat, S. Singh, H. Garg, N.M. Khanna, M.M. Dhar. A flavonol glycoside with anticancer activity from *Tephrosia candida*. *Phytochemistry*, **15(1)**:232-234 (1976).
9. G Srimannarayana, P Pulla Rao. Tephrinone, a new flavanone from *Tephrosia villosa*. *Curr. Sci. India*. **50(7)**:319-320 (1981).
10. F. Gomez-Garibay, S. Jose Calderon, Q. Leovigildo, S. Morales, R. Tirso. Prenyl flavanols from *Tephrosia quercetorum*. *Phytochemistry*. **27**:2971-2973 (1988).
11. V.U. Ahmad, Z. Ali, S.R. Hussaini, F. Iqbal, M. Zahid, M. Abbas, N. Saba. Flavonoids of *Tephrosia purpurea*. *Fitoterapia*. **70(4)**: 443-445 (1999).
12. K. Roy Mita, S. Pradip Bhattacharya, A. Chowdhuri, N. Adityachaudhury. Dehydrodihydroteneone and flemichapparin B in *Tephrosia candida*. *Phytochemistry*. **26(8)**:2423-2424 (1987).