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Note

Chemical examination of the whole plant of *Tephrosia strigosa*

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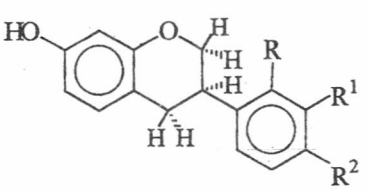
Petroleum ether extract of the whole plant of *Tephrosia strigosa* contains β-amyrin, *n*-triacontanol, β-sitosterol, 3*R*(–)mucronulatol and oleanolic acid.

The genus *Tephrosia* elaborates several types of flavonoid compounds[1]. Chemical investigation of *Tephrosia strigosa* (Fam : Leguminosae) does not seem to have been reported so far. Therefore the chemical examination of *T. strigosa* has been taken up in the present investigation and the results are presented in this note.

The air-dried plants of *T. strigosa* were coarsely powdered and extracted successively with petroleum ether and methanol. The petroleum ether extract after the usual work-up, followed by column chromatography led to the isolation of five compounds designated as TSP-1, -2, -3, -4 and -5. TSP-1 (0.01%), crystallized from MeOH as colourless solid, m.p. 196°C, $C_{30}H_{50}O$, M^+ 426. It gave positive Liebermann-Burchard test indicating it to be a triterpene. It formed a monoacetate, m.p. 175°C, $C_{32}H_{52}O_2$, M^+ 468. From the analytical and spectral data and also by direct comparison, it was identified as β-amyrin. TSP-2 (0.02%), crystallized from C_6H_6 : MeOH mixture as a colourless pluffy solid, m.p. 82°C, $C_{30}H_{62}O$, M^+ 438. From analytical and spectral data, it was identified as *n*-triacontanol. TSP-3 crystallised from methanol as colourless needles (0.01%), m.p. 138°C, $[\alpha]_D^{25}$ –36° ($CHCl_3$), $C_{29}H_{50}O$, M^+ 414, and gave positive Libermann-Burchard test. It formed monoacetate, m.p. 134°C. TSP-3 and its acetate were found to be identical in all respects with an authentic sample of β-sitosterol and its acetate respectively. TSP-4 (0.01%) crystallised from chloroform as colourless crystals, m.p. 146°C, $[\alpha]_D^{25}$ –12.203° ($CHCl_3$) : C, 0.59), $C_{17}H_{18}O_5$, M^+ 302. It gave blue colour in Gibbs test[2] indicating the presence of *para* unsubstituted phenol moiety. IR spectrum of TSP-4 showed absorptions at 3379 (hydroxyl group), 1622 cm^{-1} (aromatic C=C str). The UV spectrum showed absorption maxima at 226 (4.21) and 283 (3.90) which are characteristic of isoflavans[3]. The overall signal pattern of TSP-4 in its ^1H NMR spectrum (200 MHz, $CDCl_3$) was found to be similar to those of isoflavans[4-6]. The ^1H NMR spectrum exhibited signals at δ 2.80-3.1 (2H, m, H-4), 3.53 (1H, m, H-3_{ax}), 3.80 (3H, s, –OCH_3), 3.85 (3H, s, –OCH_3), 4.0 (1H, t, $J_{2ax,3ax}$=10 Hz, $J_{2ax,2eq}$=10 Hz, H-2_{ax}), 4.3 (1H, m, H-2_{eq}), 4.92 (1H, broad singlet, –OH), 5.9 (1H, s, –OH), 6.30-6.42 (2H, m, H-6 & 8), 6.59 (1H, d, *J*=9 Hz, H-5′), 6.9 (1H, d, *J*=9 Hz, H-6′), 7.0 (1H, d, *J*=9 Hz, H-5). The mass spectrum of TSP-4 showed M^+ at m/z 302 (35%), and base peak at m/z 180 (100%) and another ion at m/z 122 (5%). The ion at m/z 122 (RDA fragmentation) is characteristic of ring-A of isoflavan[7] with only one hydroxyl group at C_7 while the ion at m/z 180 arises from trioxygenated ring-B with one hydroxyl and two methoxyl groups. The mass and ^1H NMR spectral data of TSP-4 permitted three isomeric structures (**1, 2, 3**) for it, differing in the orientation of one hydroxyl group and two methoxyl groups in ring-B. These three compounds are already known in litrature and are called as mucronulatol[10] **1**, isomucronulatol[11] **2** and laxiflorin[8] **3**. The identity of TSP-4 to laxiflorin was ruled out as laxiflorin does not give Gibb's test by not containing a *para* unsubstituted phenol moiety.



1. $R = R^2 = OCH_3$; $R^1 = OH$
2. $R^1 = R^2 = OCH_3$; $R = OH$
3. $R = R^1 = OCH_3$; $R^2 = OH$

The C.D. spectrum of TSP-4 showed positive cotton curve in the region 270-300 nm indicating *R*-configuration at C-3 position[9,11,12]. The sign and magnitude are identical to that of 3*R*(–)mucronulatol but different from that of 3*R*(–)ismucronulatol. Thus from the above spectral data TSP-4 was characterised as 3*R*(–)mucronulatol [3*R*(–) 3′,7-dihydroxy-2′,4′-dimethoxyisoflavan]. 3*R*(–)Mucronulatol was first isolated from *Dalbergia cearensis*[10,13], subsequently from *Oxytropis glabra*[14] and *Pterocarpus soyauxil*[14] only. This is the first report of the isolation and characterization of 3*R*(–)mucronulatol from *Tephrosia* species. TSP-5 crystallised from methanol as colourless needles (0.04%), m.p. 284°C, $[\alpha]_D^{25}$ + 61.8, $C_{30}H_{48}O_3$, M^+ 456. It gave positive Liebermann-Burchard test for triterpenes. It formed a monoacetate, m.p. 266°C, $C_{32}H_{50}O_4$, M^+ 498 and a monomethyl ester, m.p. 201°C, $C_{31}H_{50}O_3$, M^+ 470. The ^{1}H NMR spectrum of TSP-5 revealed seven quarternary methyl groups, a β-hydroxyl group and one olefinic proton. All this indicated it to be a pentacyclic olean type of triterpenoid. From the analytical and spectral data it was identified as oleanolic acid which was confirmed by directed comparison with an authentic sample. Methanol extract contained mainly water soluble simple sugars, tanins etc. Hence it was not investigated further.

All the compounds were tested for their antifeedant activity by the non-choice test method[15] using 6 h pre-starved fourth instar larva of *Spodoptera litura*. Among the compounds isolated from *T. strigosa* only 3*R*(–)mucronulatol exhibited the highest antifeedant activity (100%).

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