

Structure Elucidation of Pterosupin from *Pterocarpus marsupium*, the First Naturally Occurring C-Glycosyl- β -hydroxy-dihydrochalcone

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The chemical examination of *Pterocarpus marsupium* root afforded pterosupin, a new C-glycosyl- β -hydroxydihydrochalcone along with pseudobaptigenin, liquiritigenin, isoliquiritigenin, garbanzol, 5-deoxy-kaempferol and *p*-hydroxybenzaldehyde.

The genus *Pterocarpus* has received considerable attention in recent years because of its reputation for producing new variants of polyphenolic compounds [1–7]. *Pterocarpus marsupium* Roxb. (Leguminosae) finds use in the Ayurvedic and Unani systems for the treatment of diabetes [8, 9]. Earlier work on the plant has shown the presence of pterostilbene, liquiritigenin and isoliquiritigenin in heartwood and sapwood, 1-epicatechin and pterostilbene in kino bark and two phytosterols, pterocarpol A and B, have also been reported [10]. From the rootwood of *Pterocarpus marsupium* the terpenic constituents β -eudesmol, erythrodil-3-monoacetate and a new sesquiterpene alcohol selin-4(15)-en-1 β , 11-diol, and also pterostilbene were isolated [11] as petroleum ether extractives. We now report detailed account of the isolation and structure elucidation of pterosupin and also the isolation of isoliquiritigenin, liquiritigenin, pseudobaptigenin, *p*-hydroxybenzaldehyde, garbanzol and 5-deoxykaempferol from acetone extract of *P. marsupium* root. The occurrence of dihydroflavonol (garbanzol) and flavonol (5-deoxykaempferol) in *pterocarpus* genus is noticed for the first time.

Results and Discussion

The ethyl acetate soluble part of acetone extract by preparative PC yielded the compound pterosupin

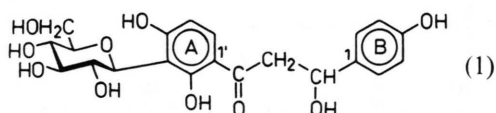
with the molecular formula, $C_{21}H_{24}O_{10}$, as white powder, m.p. 167–9°, $[\alpha]_D^{25} = +113.1^\circ$ (MeOH, $c = 0.76$). Its chromatographic behaviour (R_f 0.60 BAW 4:1:5 and 0.76 15% HOAc) suggested the compound to be a glycoside and the resistance of the compound to acid hydrolysis pointed to C-glycosyl linkage and the sugar was identified as glucose by ferric chloride oxidation [12] and Viscontini-degradation [13]. A violet Fe-III-reaction and UV absorption at λ_{max} 218, 283 and 322 nm and reagent shifts noticed, were typical for a flavanone with free OH in 5 and 7 or a dihydrochalcone with free OH in 2' and 4' positions.

The compound formed an octaacetate (m.p. 94–5°) indicating that it is a monoglycoside and the other 4 hydroxyls are present in the diarylpropanoid skeleton. There are 3 phenolic OH groups detected by 1H -NMR, one of them at δ 13.15 being a chelated hydroxyl. The signals at δ 7.85 and 6.40 ppm (d, $J = 8.5$ Hz) showed the presence of only two *ortho* related protons in the A-ring. The A_2B_2 doublets at δ 7.10 and 6.70 ppm ($J = 9$ Hz) were significant for a *p*-hydroxy substitution in B-ring. So the remaining OH must be alcoholic and present on central propanoid moiety. The ABMX pattern discerned with signals at δ 2.70 (dd, $J = 15$ & 8 Hz), 3.00 (dd, $J = 15$ & 8 Hz), 5.07 (m) and 5.42 (d, $J = 7$ Hz, exchangeable) did not agree with a flavanone structure.

Irradiation at $\delta = 5.07$ produced two doublets at $\delta = 2.76$ and 3.00 ppm while decoupling at $\delta = 2.90$

resulted in a singlet at 5.07 ppm. These shift values are in a good agreement with the ^1H -NMR-data of gliricidol [14] (2',4',3,5, β -pentahydroxy-4-methoxy-dihydrochalcone) which showed δ = 5.21, 2.77, and 3.01 ppm for the corresponding signals but not with the data reported for nubigeniol [15] (2',4',6',4, α -pentahydroxydihydrochalcone) which showed δ = 4.40, 3.10 and 3.45 ppm.

The ^{13}C -NMR interpretation clarified the presence of C-glucopyranosyl sugar and 2',4'-dihydroxy substitution of A-ring. Since the $\alpha\text{-CH}_2$ signal at δ 40.1 and $\beta\text{-CHO}$ at 73.3 ppm (triplet and doublet in off resonance spectrum) show a similar absorbance to the shifts assigned to flavanone C-3 and C-2 respectively [15], a dihydrochalcone with a hydroxy group in α -position to the carbonyl can be excluded. Thus pterosupin is 3'- β -D-glucopyranosyl-2',4', β -tetrahydroxy-dihydrochalcone or 3'-glucosyl- β -hydroxydavidigenin (1), and the first naturally occurring C-glycoside with a β -hydroxydihydrochalcone structure, in a group with the known dihydrochalcone-C-glycosides nothofagin [16] and aspalathin [17].



Interestingly the flavonoids obtained in this work are 5-deoxy type and this observation has biogenetic significance.

Experimental

Mps were determined on Gallenkemp hot stage melting block and are uncorrected. IR and NMR spectra were recorded in KBr and DMSO- d_6 or CDCl_3 respectively. ^{13}C -NMR was recorded at 20.15 MHz and MS at 70 eV by direct probe insertion.

Plant material: The main root of the plant was collected at the Mamandur forests, Andhra Pradesh, India.

Extraction and fractionation: The chips of the root (3.3 kg) were extracted with acetone and the concentrate was fractionated with light petroleum (60–80°), benzene, ether and ethyl acetate. The light petroleum soluble part on work up afforded the terpenic compounds β -eudesmol, selin-4-(15)-en-1 β ,11-diol and erythrodil-3-monoacetate, and also ptero-

stilbene [11]. The benzene soluble part was once again fractionated into 5% Na_2CO_3 -, 1% NaOH-soluble and neutral fractions. The Na_2CO_3 soluble fraction when taken up in methanol yielded compound A, mp 276°, which was identified as pseudobaptigenin. The mother liquor on preparative TLC (silica gel) in benzene:dioxan:acetic acid (90:25:4) and further work up on column chromatography over silica gel yielded compounds B (benzene), C and D (benzene:ethyl acetate) and they were identified as *p*-hydroxybenzaldehyde, liquiritigenin and isoliquiritigenin respectively. The NaOH soluble part did not yield any crystalline material. The neutral fraction on further work up yielded some more amounts of the terpenic compounds. The ether soluble fraction on preparative TLC gave compounds E and F which were identified as garbanzol and 5-deoxykaempferol respectively. The identity of all the compounds was done by mp, UV, IR, NMR and MS studies and confirmation by mmp and co-chromatography with authentic samples. The ethyl acetate soluble fraction on repeated preparative PC in BAW (4:1:5) and 15% HOAc resulted in compound G (200 mg), which is named pterosupin.

Pterosupin (1): White amorphous powder, m.p. 167–9°, $[\alpha]_D^{25} = +113.1^\circ$ (MeOH, $c = 0.76$). PC: R_f 0.60 (BAW, 4:1:5), 0.76 (15% HOAc). With alc. FeCl_3 it gave violet colour but did not answer colour reactions with Zn/HCl , Mg/HCl and Na-Hg/HCl . With dil. alkali it gave yellow colouration. UV: λ_{max} (MeOH): 218, 241 sh, 283, 322 nm; + AlCl_3 : 225, 245 sh, 312, 360 nm; + AlCl_3/HCl : 225, 245 sh, 310, 362 nm; + NaOAc : 264, 285 sh, 341 nm; + $\text{NaOAc}/\text{H}_3\text{BO}_3$: 278, 330 nm; + NaOMe : 220, 241 sh, 283, 323 nm. IR: $\tilde{\nu}_{\text{max}}$ (KBr) 3380 (OH), 1620 (C=O), 1518, 1500, 1445 (Ar), 1255 cm^{-1} (C–O). ^1H -NMR: (80 MHz, DMSO- d_6 , TMS int. standard) δ 13.15 (s, 1 H, 2'-OH), 10.27 (br, 1 H, 4'-OH), 9.1 (s, 1 H, 4-OH), 7.85 (d, $J = 8.5$ Hz, 1 H, H-6'), 7.10 (d, $J = 9$ Hz, 2 H, H-2 & 6), 6.70 (d, $J = 9$ Hz, 2 H, H-3 & 5), 6.40 (d, $J = 8.5$ Hz, 1 H, H-5'), 5.40 (d, $J = 7$ Hz, 1 H, β -OH), 5.07 (m, 1 H, β -CH), 3.00 (dd, $J = 15$ & 5 Hz, 1 H, α -CH), 2.76 (dd, $J = 15$ & 8 Hz, 1 H, α -CH). MS: m/z (% rel. int.) 436 M^+ (0.002), 418 (2), 400 (14.1), 312 (52.6), 294 (33.3), 281 (59.0), 263 (35.9), 245 (46.2), 233 (62.8), 219 (14.1), 217 (15.38), 215 (12.8), 191 (23.1), 189 (19.2), 179 (26.9), 163 (29.5), 162 (25.6), 161 (28.2), 136 (23.1), 135 (33.3), 108 (51.3), 107 (100), 106 (30.8), 91 (25.6), 77 (61.5). ^{13}C -NMR: (20.15 MHz, DMSO- d_6 , TMS int. standard) δ

204.7 (C = O), 164.1 (C-4'), 163.8 (C-2'), 159.3 (C-4), 132.1 (C-6'), 130.4 (C-2 & 6), 128.1 (C-1), 115.2 (C-3 & 5), 112.5 (C-1'), 108.5 (C-3'), 108.1 (C-5'), 81.4 (C-5''), 79.0 (C-3''), 73.8 (C-1''), 73.3 (C- β), 71.2 (C-2''), 70.6 (C-4''), 61.4 (C-6''), 40.1 (C- α).

Pterosupin octaacetate: (Py/Ac₂O); m.p. 94-5°
¹H-NMR: (CDCl₃, TMS int. standard) δ 7.80 (d, J = 8.5 Hz, 1H, H-6'), 7.25 (d, J = 8.5, 2H, H-2 & 6), 7.02 (d, J = 8.5, 2H, H-3 & 5), 6.72 (d, J = 8.5 Hz, 1H, H-5'), 5.27 (m, 1H, β -CH), 4.76 (br. d, J = 10 Hz, 1H, H-1''), 3.12 (m, 2H, α -CH₂), 2.44, 2.40, 2.30, (9H, 2', 4,4'-OAc), 2.23, 2.1, 1.80, (15 H, 2'', 3'', 4'', 6'', β -OAc).

FeCl₃ oxidation: A mixture of pterosupin (50 mg) and ferric chloride (250 mg) in water (1.5 ml) was heated at 125° in an oil bath for 6 h. After usual

work up and chromatographic examination the sugar was identified as glucose.

Viscontini degradation: To 2 mg of pterosupin in 0.1 ml of aqueous DMSO, 2 mg of sodium metaperiodate was added and kept at room temperature for 4 h. Then 2 mg of sodium borohydride in 0.1 ml of water was added and left over night. After usual work up the product was identified as glycerol by chromatography.

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