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GYMNOSPERMAE

CUPRESSACEAE

HYPOLAETIN 7-GLUCOSIDE FROM *JUNIPERUS MACROPODA*

S. A. SIDDIQUI and A. B. SEN

Dept. of Chemistry, University of Lucknow, Lucknow, India

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Abstract—The structure of the flavone glucoside isolated from berries of *Juniperus macropoda* has been established as 7-*O*-β-D-glucoside of 5,7,8,3′,4′-pentahydroxyflavone (hypolaetin 7-glucoside).

OUR INITIAL investigations[1] on the berries of *J. macropoda* (Cupressaceae) showed that in addition to the known constituents, nonacosanol, β-sitosterol glucoside and sugiol, a new flavone-*O*-glucoside (I) was present. Acidic hydrolysis of I gave an aglycone, II, $C_{15}H_{10}O_7$, m.p. 291° identical with hypolaetin recently isolated from *Hypolaena fastigiata*.[2] The observations recorded here provide additional evidence for the structure of hypolaetin and establish the structure of I as 7-*O*-β-glucoside of II.

The u.v. spectral data of I and its aglycone II established[1] that I contained free hydroxyl groups at C-5 and C-4′, two hydroxyl groups in *ortho* position, and the glucose moiety at C-7. One of the two hydroxyl groups of the *o*-dihydroxy system should be either at 6- or 3′-position, the 3′-position being favoured by the typical splitting of 258, 280 nm of the lower wavelength band. The fifth hydroxyl group may occupy one of the positions 6 or 8 in the A-ring or the positions 2′, 5′ or 6′ in the B-ring.

The mass spectral fragmentation of II showed a parent peak at *m/e* 302 (M^+, $C_{15}H_{10}O_7$). Loss of carbonyl from C-ring gave an ion at *m/e* 274 ($C_{14}H_{10}O_6{}^+$, 6·4%) and the cleavage of the C-ring following a *retro*-Diels-Alder rupture gave two complementary ions at *m/e* 168 ($C_7H_4O_5{}^+$, 69·8%) and *m/e* 134 ($C_8H_6O_2{}^+$, 10·8%) indicating the presence of three hydroxyls in the A-ring and two hydroxyls in the B-ring.[3] Stepwise loss of two CO functions (28 mass units) from *m/e* 168 ion gave the fragments at *m/e* 140 ($C_6H_4O_4{}^+$, 22·5%) and *m/e* 112 ($C_5H_4O_3{}^+$, 20·5%). Thus mass spectral data showed that the fifth hydroxyl must be at either position 6 or 8. Position 8 was finally ascertained for this hydroxyl group because alkaline reductive degradation[4] of II gave pyrogallol (co-TLC) indicating the presence of 5,7,8-hydroxylation pattern, and two other products corresponding to the degradation products obtained from dihydroquercetin, indicating the presence of 3′,4′-hydroxylation system. Therefore, II was confirmed as 5,7,8,3′,4′-pentahydroxyflavone.

The NMR spectrum of trimethylsilyl ether[5] of I further corroborated the above findings. It showed, *inter alia*, signals at δ 6·36 (singlet, H-6), 6·45 (singlet, H-3), 6·86 (doublet, J = 8 c/s, H-5′), 7·38 (singlet, H-2′), 7·45 (doublet obscured by 2′-proton signal, H-6′), 5·0

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(broad singlet, H-1 of glucose), 3·20–3·98 (multiplet, 6 protons of glucose) and 12·3 (singlet, OH-5, present due to partial decomposition of the C-5 ether linkage during record of the spectrum). The absence of a signal for H-7 supports the attachment of the sugar to the hydroxyl group at this position. The characteristic broad signal near 5·0 ppm of the H-1 sugar proton indicated the β-configuration of the glucose moiety which was supported by the facile hydrolysis of I by β-glucosidase.

The chemical and spectroscopic evidence thus indicated I to be 7-*O*-β-D-glucoside of 5,7,8,3′,4′-pentahydroxyflavone.

EXPERIMENTAL

M.ps determined in open capillaries are uncorrected; NMR spectrum was recorded in the form of trimethylsilyl ether and u.v. spectra were recorded in 95% ethanol.

Hypolaetin Glucoside

The coarsely powdered berries (2·75 kg) were extracted with 80% methanol (5·5 l.) and after removal of most of the solvent the residue (600 ml) was extracted successively with petroleum, ether and butanol. The butanol extract after chromatography over silica gel (methanol–benzene, 8:2) and several crystallizations of the product afforded golden yellow needles (205 mg) of I, m.p. 243–45° (Found: C, 50·67; H, 5·03. $C_{21}H_{20}O_{12}\cdot 2H_2O$ required: C, 50·40; H, 4·80%).

Acidic Hydrolysis of I

I (80 mg) was refluxed for 3 hr with 7% alcoholic H_2SO_4 and the aglucone extracted with ether. After repeated crystallizations from methanol the residue furnished bright yellow crystals of II, m.p. 291°, (Found C, 59·54; H, 3·35. $C_{15}H_{10}O_7$ required: C, 59·60; H, 3·31%). II had identical m.p. (lit. value 296°), R_fs and u.v. spectra to those reported for hypolaetin.[2]

Enzymatic Hydrolysis of I

I (15 mg, 10 ml, 50% ethanol) was treated (pH 6, 30–35°, 24 hr) with a freshly prepared enzyme solution (1 ml, 1%). During this period the glycoside was fully hydrolysed as observed by paper chromatography.

Octacetate of I

I (85 mg) was acetylated (Py/Ac_2O, 120°) and the crude product on repeated crystallizations (MeOH/$CHCl_3$) yielded colourless plates of the octacetate, m.p. 247–48°, $[\alpha]_D^{20} \pm 0°$ ($CHCl_3$) (Found: C, 53·60; H, 4·97. $C_{37}H_{36}O_{20}\cdot 2H_2O$ required: C, 53·11; H, 4·78%).

Pentacetate of II

II (70 mg) on acetylation (*vide supra*, 18 hr, room temp.) furnished colourless crystals of the pentacetate, m.p. 217–20°, (Found: C, 58·80; H, 4·04. $C_{25}H_{20}O_{12}$ required: C, 58·79; H, 3·90%).

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