Chemical Studies on Zizyphus rugosa Lam.

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Summary: From the bark of Zizyphus rugosa, 3-O-rhamnosides of kaempferol, quercetin and myricetin together with β-sitosterol and β-sitosterol glucoside have been isolated.

Introduction

Zizyphus rugosa (Family Rhamnaceae) is an armed shrub distributed throughout India and Ceylon. Various medicinal properties have been attributed to this plant in the Indian system of medicine [1]. Survey of literature revealed that betulic, oleanolic, alphitolic, 2 α-hydroxy ursolic acids, zizyphoside [2] and a cyclopeptide alkaloid, amphibine-D [3] have been isolated from this plant species. The present investigation deals with the isolation and characterisation of 3-O-rhamnosides of kaempferol, quercetin and myricetin, together with β-sitosterol and β-sitoterol glucoside from its bark.

Experimental

Air dried powdered bark (5 Kg) of Z.rugosa, collected from Coimbatore district, South India, were extracted in a Soxhlet extractor with benzene and methanol respectively. The methanol extract was concentrated to brown semi-solid mass. It was stirred mechanically with aqueous citric acid (5%) and extracted successively with ether and ethyl acetate. The ethyl acetate fraction was chromatographed over silica gel column eluting with chloroform-methanol-water (65:35:10) organic phase, and collecting the fractions of 50 ml each.

Results and Discussion

Fractions 5-10, 15-19, and 25-31 furnished kaempferol-3-O-rhamnoside, quercetin-3-O-rhamnoside and myricetin-3-O-rhamnoside respectively. Chromatographic resolution of the acid insoluble fraction of methanol extractive over silica gel column furnished β-sitosterol and β-sitosterol glucoside from benzene-chloroform (4:2) and chloroform-methanol (9:1) eluants respectively.

Kaempferol-3-O-rhamnoside

It crystallised from methanol as yellow granules (75 mg), m.p. 178-80°. UV λ_{max}(methanol, nm) 264 (ε 6720), 313 sh (ε 11520), 343 (ε 13440); λ_{max} (methanol+aluminium trichloride, nm) 274, 304, 345, 400; λ_{max} (methanol + aluminium trichloride + hydrochloric acid, nm) 274, 302, 342, 396; λ_{max} (methanol + sodium methoxide, nm) 272, 325, 388; λ_{max} (methanol + sodium acetate, nm) 273, 308 sh, 350; λ_{max} (methanol + sodium acetate + boric acid, nm) 265, 313 sh, 344.

IR (KBr, cm^{-1}): ν_{max} 3200-3600, 1660, 1610. ^{1}H-NMR (90 MHz,

DMSO-d_6,): δ 6.10 (1H, d, J=2Hz, C-6-H), 6.28 (1H, d, J=2Hz, C-8-H), 6.93 (2H, d, J=9Hz, C-5'-H and C-6'-H), 7.75 (2H, d, J=9Hz, C-2'-H and C-3'-H), 12.66 (1H, br, C-5-OH) and signals for one proton of one molecule of rhamnose [5.32 (C-1-H), 4.88 (OH), 4.00 (C-2-H), 3.46 (C-3-H, 3.13 (C-4-H and C-5-H), 0.81 (C-5-CH_3)].

Hydrolysis with sulphuric acid (2N) furnished rhamnose and kaempferol, M^+ at m/z 286. This was identified as kaempferol-3-O-rhamnoside [4] from analysis of spectral data and by direct comparison of the aglycone and sugar residues.

Quercetin-3-O-rhamnoside

It crystallised from methanol as yellow granules (500 mg), m.p. 165-68°, UV: λ_{max} (methanol, nm) 255 (ε 2004), 265 sh (ε 1813), 301 sh (ε 859), 350(ε 1718); λ_{max} (methanol + aluminium trichloride, nm) 275, 304, sh, 330 sh, 432; λ_{max} (methanol + sodium methoxide, nm) 270, 325 sh, 393; λ_{max} (methanol + sodium acetate, nm) 270, 322 sh, 363; λ_{max} (methanol + sodium acetate + boric acid, nm) 260, 300 sh, 365.

IR: (KBr, cm^{-1}): ν_{max} 3200-3500, 1640, 1610. ^{1}H-NMR (90 Mhz, DMSO-d_6) δ 6.02 (1H, d, J=2Hz, C-6-H), 6.38 (1H, d, J=2Hz, C-8-H), 6.86 (1H, d, J=9Hz, C-5'-H), 7.22(1H, d, J=2H, C-2'-H), 7.27 (1H, d, J=9Hz, C-6'-H), 12.66 (1H, br, C-5-OH) and signals for proton of one molecule of rhamnose [5.27 (C-1-H), 5.00 (OH), 3.97 (C-2-H), 3.51 (C-3-H), 3.17 (C-4-H and C-5-H), 0.80 (C-5-CH_3)]. Hydrolysis with sulphuric acid (2N) furnished rhamnose and quercetin, M^+ 302. This was identified as quercetin-3-O-rhamnoside [5] by direct comparison with the authentic sample available in our B.H.U. Labs.

Myricetin-3-O-rhamnoside

It crystallised from methanol as yellow solid (350 mg), m.p. 212-15°. UV: λ_{max} (methanol, nm) 258 (ε 17468), 305 sh (ε 6734), 353 (ε 14739); λ_{max} (methanol + aluminium trichloride, nm) 272, 313 360 sh, 433; λ_{max} (methanol + aluminium trichloride + hydrochloride, nm) 272, 310, 365 sh, 405; λ_{max} (methanol + sodium methoxide, nm) 265, 330 sh; λ_{max} (methanol + sodium acetate, nm) 265, 323 sh, 357; λ_{max} (methanol + sodium acetate + boric acid, nm) 257, 304 sh, 372.

IR (KBr, cm^{-1}): ν_{max} 3000-3600,1665, 1610. ^{1}H-NMR (90 MHz, DMSO-d_6, δ 6.21 (1H, d, J=2Hz, C-6-H), 6.38 (1H,d, J=2Hz, C-8-H), 6.92 (2H, br s, C-2'-H and C-6'-H), 12.75 (1H, br s, C-5-OH) and signals for protons of molecule of rhamnose [5.23 (C-1-H), 4.02 (C-2-H), 3.59 (C-3-H), 3.36 (C-4-H and C-5-H) and 0.86 (C-5-CH_3)]. Hydrolysis with sulphuric acid (2N) furnished rhamnose and myricetin, M^+ 318. This was identified as myricetin-3-O-rhamnoside [6] by direct comparison with the authentic sample available in our labs.

β-Sitosterol

It crystallised from ethanol as colourless needles (46 mg) m.p. 134-35°; acetate: m.p. 126°. It was confirmed by direct comparison with the authentic sample.

β-Sitosterol glucoside

It crystallised from methanol as colourless granules (86 mg). m.p.

295-96°,; Hydrolysis with hydrochloric acid (2N) furnished glucose and β-sitosterol. It was identified by direct comparison with authentic sample available in our labs.

References

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