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Flavonoids from Flowers of *Helichrysum buddleioides*

A. G. RAMACHANDRAN NAIR*, R. JAYAPRAKASAM

and

R. SIVAKUMAR

Department of Chemistry, Pondicherry University, Jipmer Campus, Pondicherry-605 006

and

K. P. MADHUSUDANAN

Medicinal Chemistry Division, Central Drug Research Institute, Lucknow-226 001

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HELICHRYSUM buddleioides DC. (Asteraceae), a small shrub, leaves thick, lanceolate, limb short, obtuse and flowers deep yellow in colour, is found in Babubudan Hills of Mysore, Nilgiris and Anamalais[1,2]. The flowers of this plant are popularly called 'Everlasting flowers'. A number of *Helichrysum* species have been known to contain one or more of different types of flavonoids[3-7]. Some species have been recorded[8,9] to possess antimicrobial and anticancer activity.

As a part of the continuing study of flavonoids of South Indian plants and in the absence of any record of work on this species, the flowers of *H. buddleioides* have been examined and the isolation and characterisation of three flavonoids, one flavone and two aurones, are reported here. The compounds identified are 5,7,3′,4′-tetrahydroxyflavone (luteolin), 6,3′,4′-trihydroxy-4-*O*-β-D-glucopyranosylaurone (cernuoside) and 6,3′,4′,5′-tetrahydroxy-4-*O*-β-D-glucopyranosylaurone (bractein).

Experimental

Fresh flowers (400 g) of *H. buddleioides* collected from the Nilgiris in Tamil Nadu, were refluxed with 90% ethanol and concentrated under reduced pressure. The aqueous alcoholic concentrate was processed for flavonoids adopting standard procedures[10-12]. Ether and ethyl acetate fractions showing identical spots were mixed and kept in an ice-chest. The resulting yellow solid was separated into three homogenous fractions by preparative microcrystalline cellulose tlc (R_f of compound (A) 0.65, (B) 0.42, (C) 0.31 ; 50% HOAc). The three homogenous compounds were then crystallised from methanol and subjected to various tests including uv fluorescence and pc in different solvent system. Final structural determination was based on uv, ir, ^{1}H nmr and mass spectral analysis.

Compound-A : It was crystallised as pale yellow needles from MeOH, m.p. 328–30° ; it gave yellow colour with NH_3, olive green with Fe^{3+} ane red with Mg–HCl ; purple under uv and yellow with uv/NH_3 ; λ_{max} (MeOH) 242sh, 253, 267, 291sh and 349 ; (NaOAc) 269, 326sh and 384 ; (NaOAc+H_3BO_3) 259, 301sh, 370 and 432sh ; (NaOMe) 266sh, 329sh and 401 ; ($AlCl_3$) : 274, 300sh, 328 and 426 ; ($AlCl_3$–HCl) 266sh, 275, 294sh, 355 and 385 nm ; ν_{max} (KBr) 3 400br, 2 910, 1 725, 1 650, 1 610, 1 500, 1 450, 1 360, 1 250, 1 200, 1 190, 950, 900, 850, 810 and 750 cm^{-1}. It was identified as 5,7,3′,4′-tetrahydroxy flavone (luteolin) by direct comparison with an authentic sample[13].

Compound B : It revealed λ_{max} (MeOH) 262, 338 and 408 ; (NaOAc) 290, 338, 388sh and 462 ; ($AlCl_3$) 274, 321 and 436 ; ($AlCl_3$–HCl) 261, 339 and 408 cm^{-1} ; ^{1}H nmr (400 MHz, $CDCl_3/CD_3OD$, TMS as internal standard) ; δ 7.22 (1H, d, *J* 2 Hz, H-2′), 6.98 (1H, dd, *J* 8 and 2 Hz, H-6′), 6.60 (1H, d, *J* 8 Hz, H-5′), 6 42 (1H, s, =CH), 6.09 (1H, s, H-7), 6 02 (1H, s, H-5), 4.68 (1H, d, *J* 8 Hz, H-1″ anomeric sugar proton) and 3.68–3.19 (six peaks integrating for six protons of glucose) ; *m/z* (EIMS, 70 eV) 286 (aglycone, M^+, 100%), 268, 258, 153, 152, 137, 134, 125 and 124 ; FDMS 470 $(M+Na)^+$, 449 $(M+H)^+$ and 287 (aglycone+H)$^+$; pc (Whatman 1, 28°, ascending, $R_f \times 100$) 10(H_2O), 14(15% HOAc), 54 (50% HOAc), 45(BAW), 54 (phenol) and 46 (t-BAW) ; acid hydrolysis (2*N* HCl, 100°, 2 h) gave aureusidin and D-glucose. It was identified as 6,3′,4′-trihydroxy-4-*O*-β-D-glucopyranosyl aurone (cernuoside), earlier isolated from *Oxalis cernua*[14].

Compound-C : It revealed λ_{max} (MeOH) 250, 267, 340sh, 389 and 406 ; (NaOAc) 285, 338, 386 and 455 ; ($AlCl_3$) 255, 287, 338sh and 446 ; ($AlCl_3$–HCl) 254, 273, 338sh, 389 and 406 ; ^{1}H nmr (400 MHz, $CDCl_3/CD_3OD$, TMS as internal standard) δ 6.71 (2H, s, H-2′ and H-6′), 6.31 (1H, s, =CH), 6.04 (1H, d, *J* 2 Hz, H-7), 5.98 (1H, d, *J* 2 Hz, H-5), 4.66 (1H, d, *J* 8 Hz, H-1″ anomeric sugar proton) and 3.65–3.17 (six peaks integrating for six protons of glucose) ; *m/z* (EIMS, 70 eV) 302 (aglycone, M^+, 100%), 284, 274, 153, 152, 150, 134, 126, 125 and

124 ; FDMS : 487 $(M+Na)^+$, 465 $(M+H)^+$ and 303 (aglycone+H)$^+$; pc (Whatman 1, 28°, ascending, $R_f \times 100$) 7(H_2O), 9(15% HOAc), 41 (50% HOAc), 33 (phenol) and 26 (t-BAW) ; acid hydrolysis yielded bracteatin and D-glucose. It was identified as 6,3',4',5'-tetrahydroxy-4-*O*-β-D-glucopyranosylaurone (bractein), earlier reported from *Helichrysum bracteatum*[15].

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Chemical Investigation of the Roots of *Flueggea microcarpa* Blume

SUJATA KUNDU, M. SAHAI and A. B. RAY*

Department of Medicinal Chemistry, I.M.S., Banaras Hindu University, Varanasi-221 005

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FLUEGGEA microcarpa Blume (Euphorbiaceae) is an indigenous medicinal plant distributed throughout India[1,2] and several publications on the chemical constituents of different parts of this plant have appeared[3-6], the last one coming from Murthy and Jairaj[7]. However, contrary to the claim of the last two authors[7], the occurrence of bergenin in this plant was recorded earlier and we reported in our previous communication[8] the isolation of gallic acid, ellagic acid, quercetin, β-sitosterol glucoside and N_b-methyl-tetrahydro-β-carboline in addition to bergenin, the major constituent of the leaves. The present paper reports the characterisation of a relatively rare plant phenolic, norbergenin (**1**) and an alkaloid, securinine[8] isolated from the roots of *F. microcarpa*.

The air-dried and powdered roots of *F. microcarpa* were successively extracted with petroleum ether (b.p. 60 - 80°) and acetone. The concentrated acetone extract on keeping, yielded a solid which was crystallised from methanol to afford colourless prisms, $C_{13}H_{14}O_9$ (HRMS : M^+, 314.0637), m.p. 281° ; it gave positive phenolic reaction. It showed in its ^1H nmr spectrum (methanol-d_4), 1H singlet at δ 7.07 for a lone aromatic hydrogen and a series of multiplets spread over the region δ 3.2 - 4.9 for seven hydrogens. While the signal for a single aromatic hydrogen suggested the presence of a pentasubstituted phenyl nucleus, the appearance of signals for only eight out of fourteen hydrogens present in the molecule indicated the probable presence of six exchangeable hydrogens as hydroxyl groups. This assumption was proved to be correct by acetylation of the compound which yielded a hexaacetate (**1a**), m.p. 212°. The ^1H nmr spectral data of this hexaacetate compared well with those of bergenin pentaacetate with the only difference that the former lacked the aromatic methoxyl signal of the latter and instead showed an extra acetoxymethyl signal. Based on these observations, the isolated compound was considered to be des-*O*-methylbergenin (norbergenin) which was confirmed from the observation that the trimethyl ether of the isolated compound was identical in all respects with the dimethyl ether of bergenin.

Though preparation of norbergenin[9] from bergenin was reported in 1936, its natural occurrence was first reported[10] in 1981 and this is the third report of its isolation from a plant. It is interesting to note that while the leaves of *F. microcarpa* are rich in bergenin, its roots yielded only norbergenin.

The acetone extract, left after removal of norbergenin, responded to Dragendorff's reagent for alkaloids and the alkaloid fraction separated by standard procedure, on chromatographic resolution over silica gel yielded a pale yellow solid which crystallised from methanol as cubes, $C_{18}H_{15}NO_3$ (M^+, 217) m.p. 145° ; $[\alpha]_D$ - 1283.33 ($CHCl_3$). Colour of the alkaloid, its melting point, high negative rotation and above all the perfect matching of its ^1H nmr signals with the reported values[8] established its identity as securinine. Isolation of securinine from the roots of *F. microcarpa* is of interest in view of the fact that beside flueggine, an uncharacterised alkaloid, the only other alkaloid that was isolated from the leaves of this plant was an indole alkaloid. The occurrence of securinine