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SHORT COMMUNICATION

FLAVONOIDS FROM *CENTAUREA SENEGALENSIS* DC (COMPOSITAE)

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ABSTRACT. Chemical investigation of the methanolic extract of *Centaurea senegalensis* DC afforded the known flavonoids 6-hydroxykaempferol, 6-methoxykaempferol, eupalitin and jaceosidin, and two new flanonoids, 7-hydroxy-3,5,6,8-4'-pentamethoxyflavone (centaflavone A) and 7,4'-dihydroxy-3,5,6,8-tetramethoxyflanone (centaflavone B). The structures of centaflavone A and B were elucidated by spectroscopic methods and chemical evidence.

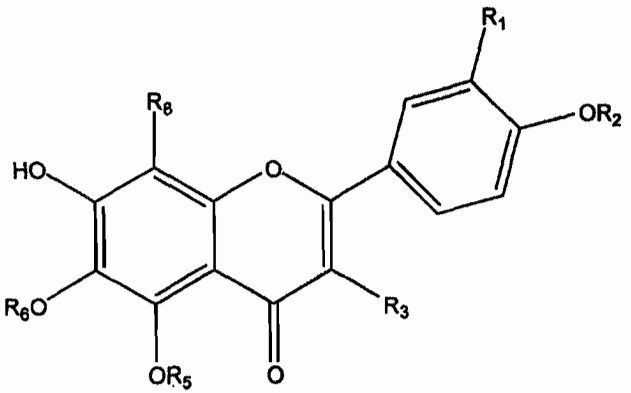
INTRODUCTION

Centaurea senegalensis is an annual branhced scabrid herb, erect 1.2 ft high or prostrate: stem-leaves semi-amplexical upto 2 inch long, the basal leaves of early growth larger; florets purple or mave in spiny heads about 1/2 inch broad terminating leafy branches [1]. It is valued in the indegenous system of medicine in Nigeria where it is used as a crude drug for the treatment of stomach ache, kidneys, micturition diuresis, dropsy, gout and pains [2]. This is the first report on this plant and describes the isolation and characterisation of six flavonoids including two new, named centaflavone A and centaflavone B.

RESULTS AND DISCUSSION

The methanolic extract of *centaurea senegalensis* was chromatographed over silica gel (CC, prep. TLC) to afford six flavonoids. The identities of known compounds - eupalitin (**3**), jaceosidin (**4**), 6-methoxykaempferol (**5**) and 6-hydroxykaempferol (**6**) were established by direct comparison (TLC, UV, ^{1}H NMR, MS) with authentic samples and the reported values in the literature [3-6]. The positive colour reactions [7] and λ_{max} at 338, 266 nm and at 355, 267 nm suggested (**1**) and (**2**) to be flavonoid compounds. The absence of any bathochromic shifts with $AlCl_3$, $AlCl_3$-HCl, NaOMe or NaOAc-H_3BO_3, ruled out the possibility of hydroxyl groups at C-5, C-3, C-4 positions or an O-dihydroxyl system in the molecule [8]. Compound (**1**) dissolved in 10% sodium carbonate, showed a minor bathochromic shift or +4 nm (band II) with NaOMe and a shoulder peak at 328 nm (band I) with NaOMe, which was indicative of a free hydroxyl group at C-7 in the presence of a methoxyl function at C-6 and C-8 positions [9, 10]. This was further supported by the chemical shifts at δ 6.48 (broad signal for 1H) and δ 141.60 in ^{1}H NMR and ^{13}C NMR, respectively [11, 12].

The EI-MS of (**1**) exhibited a molecular ion peak at m/z 388 [M^+] (40%) for $C_{20}H_{20}O_8$ in accord with one hydroxyl and five methoxyl groups. The fragment peaks at m/z 387 [M-1]$^+$



Centaflavone A (**1**): $R_1 = H, R_2 = R_5 = R_6 = CH_3, R_3 = R_8 = OCH_3$
Centaflavone B (**2**): $R_1 = R_2 = H, R_5 = R_6 = CH_3, R_3 = R_8 = OCH_3$
Eupalitin (**3**): $R_1 = OCH_3, R_2 = R_6 = CH_3, R_3 = R_5 = R_8 = H$
Jaceosidin (**4**): $R_1 = OCH_3, R_6 = CH_3, R_2 = R_3 = R_5 = R_8 = H$
6-Methoxykaempferol (**5**): $R_1 = R_2 = R_5 = R_8 = H, R_3 = OH, R_6 = CH_3$
6-Hydroxykaempferol (**6**): $R_1 = R_2 = R_5 = R_6 = R_8 = H, R_3 = OH$

(35%), m/z 373 $[M\text{-}Me]^+$ (100%), m/z 370 $[M\text{-}18]^+$ (20%) and m/z 345 $[M\text{-}COMe]^+$ (30%) indicated the possibility of methoxyl groups at C-3, C-5, C-6, and C-8 positions which was supported by the fragment peak at m/z 211 $[A_1\text{-}Me]^+$ (13%) [13, 14]. The absence of proton signals in the ^1H NMR spectrum for H-3, H-5, H-6 and H-8 and in ^{13}C NMR spectra, the downfield shift of the same carbons as compared to those induced by hydroxyl groups, suggested the presence of methoxyl groups at these carbon atoms [15].

The observation of an A_2B_2 pattern of signals in the ^1H NMR spectrum of (**1**) indicated a C-4' substituted ring B and the presence of fifth methoxyl group at C-4' which was supported by the fragment peak at m/z 135$[B_2]^+$ and by the shift of C-4' carbon atom in ^{13}C NMR spectra to downfield was compared to that induced by a hydroxyl group [15]. The compound (**1**) is thus characterised as 7-hydroxy-3,5,6,8,4'-pentamethoxyflavone (centaflavone A).

Compound (**2**) was obtained as yellowish crystals. The spectral data indicated that (**2**) was very similar to (**1**) except for the presence of a hydroxyl group in (**2**) in C-4' position in place of the methoxyl group in (**1**). This was supported by a fragment peak at m/z 121 $[B^+_2]$ (22%) in the EI-MS of (**2**) and a bathochromic shift of 63 nm (band I) with NaOMe relative to band I.

The ^{13}C NMR spectrum of (**2**) revealed a signal due to C-4' carbon atom, shifted upfield by *ca.* 4.30 ppm while ortho carbons (C-3,5) and meta carbons (C-2,6) had shifted downfield by *ca.* 2 ppm and *ca.* 0.50 ppm, respectively, in comparison with those of (**1**). The result further confirmed the presence of a hydroxyl group at C-4' position in (**2**) and hence compound (**2**) is 7,4'-dihydroxy-3,5,6,8-tetramethoxyflanvone (centaflavone B).

EXPERIMENTAL

Mps were uncorrected. UV spectra were run in MeOH by using standard procedures [8]. ^1H NMR spectra were recorded in $CDCl_3$ at 60 MHz. ^{13}C NMR spectra were recorded in

DMSO-d_6 at 50 MHz. Chemical shifts are given in ppm relative to internal tetramethylsilane (TMS) in ^1H and ^{13}C NMR spectra. Column and thin layer chromatography were performed using silica gel (BDH).

Plant material. The whole plants of *Centaurea senegalensis* were procured in November 1994 from Dikwa town, Dikwa Local Government Borno State and was identified by Professor B.V. Gopal, Department of Biological Sciences, Unimaid, Maiduguri, Nigeria.

Extraction and isolation. The air dried whole plant powdered material (800 g) was extracted in MeOH in a Soxhlet apparatus for 10 h. The solvent was removed *in vacuo* and residue was partitioned between EtOAc and H_2O. The EtOAc extract upon concentration yielded 40 g of a dark oily residue which was subjected to column chromatography over silica gel (180 g, BDH). Elution was carried out using petroleum ether (40-60°) and $CHCl_3$-MeOH mixtures of increasing polarities. A total of 55 fractions each 150 mL, were collected and separated into six groups: fractions 1-5 (petroleum ether), 6-15 ($CHCl_3$), 16-25 (10% MeOH in $CHCl_3$), 26-35 (20% MeOH), 36-45 (40% MeOH) and 46-55 (50% MeOH). The residue obtained from groups 2-6, responded positive to flavonoid colour reactions [3]. This flavonoid mixture was purified by preparative thin layer chromatography (prep. TLC) over silica gel, using the solvent system: toluene-ethylformate-formic acid, 5:4:1 (TEF). This afforded compounds **1** (centraflavone A, 120 mg), **2** (centaflavone B, 150 mg), **3** (eupalitin, 60 mg), **4** (jaceosidin, 50 mg), **5** (6-methoxykaempferol, 50 mg) and **6** (6-hydroxy-kaempferol, 40 mg).

7-Hydroxy-3,5,6,8,4'-pentamethoxyflavone or centaflavone A (1). Yellowish amorphous solid, m.p. 193-195 °C, UV λ_{max}(MeOH) nm: 338, 266; (MeOH + $AlCl_3$) nm: 340, 267; $AlCl_3$-HCl nm: 339, 266; (MeOH + NaOMe) nm: 340, 328 (sh), 268; (MeOH + NaOAc) nm: 340, 270; (MeOH + NaOAc-H_3BO_3) nm: 336, 266. ^1H NMR (60 MHz, $CDCl_3$) δ 3.84, 3.90, 3.96, 4.02 (3 x 3 H and 1 x 6 H, s, i.e. 5 x OMe), 6.48 (1 H, br, s, OH-7), 6.96 (2 H, d, J = 9.0 Hz, H-3',5',), 7.98 (2H, d, J = 9.0 Hz H-2',6'); ^{13}C NMR (50 MHz, DMSO-d_6) δ 155.50 (C-2), 137.40 (C-3), 178.60 (C-4), 148.10 (C-5), 136.20 (C-6), 141.60 (C-7), 136.70 (C-8), 139.10 (C-9),105.00 (C-10), 122.50 (C-1'), 130.40 (C-2',6'), 114.20 (C-3',5'), 161.50 (C-4'), 60.30 (O$\underline{C}H_3$-8), 60.50 (O$\underline{C}H_3$-6), 59.70 (O$\underline{C}H_3$-3), 55.60 (O$\underline{C}H_3$-4') and 61.20 (O$\underline{C}H_3$-5); EI-MS, m/z (rel. int.) 388 [M^+] $C_{20}H_{20}O_8$ (40), m/z 387 [M-H]$^+$(35), m/z 373 [M-Me]$^+$(100), m/z 370 [M-18]$^+$(20), m/z 345 [M-COMe]$^+$(30), m/z 211 [A_1-Me]$^+$(13), m/z 183 [A_1-COMe]$^+$(9) and m/z 135 [B_2]$^+$(23).

7,4'-Dihydroxy-3,5,6,8-tetramethoxyflavone or Centaflavone B (2). Crystallized from Me_2CO-C_6H_{12}, gave yellowish crystals (80 mg), m.p. 208-210 °C; UV λ_{max} (MeOH) nm: 355, 267; (MeOH + NaOMe) nm: 418, 270; (MeOH + NaOAc) nm: 358, 270: ^1H NMR (60 MHz, $CDC1_3$) δ 3.86, 3.90, 3.94, 4.00 (4 x 3 H, s, 4 x OMe), 6.88 (2 H, d, J = 9 Hz, H-3',5'), 7.82 (2 H, d, J = 9 Hz, H-2',6'); ^{13}C NMR (50 MHz, DMSO-d_6); δ 155.80 (C-2), 137.50 (C-3), 178.70 (C-4), 148.20 (C-5), 136.30 (C-6), 141.60 (C-7), 136.90 (C-8), 139.20 (C-9), 105.20 (C-10), 122.80 (C-1'), 130.90 (C-2',6'), 115.80 (C-3',5') ,157.20 (C-4'), 60.40 (O$\underline{C}H_3$-8), 60.60 (O$\underline{C}H_3$-6), 59.80 (O$\underline{C}H_3$-3) and 61.20 (O$\underline{C}H_3$-5).

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