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SHORT COMMUNICATION

A NEW FLAVONOID GLYCOSIDE FROM THE SEEDS OF *CICER ARIETINUM* LINN.

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Cicer arietinum Linn. (family Fabaceae), commonly known as Bengal gram or Chickpea, is an erect, spreading, much-branched, annual herb covered all over with glandular hair. It is a native to south western Asia and extensively cultivated as a pulp crop throughout northern India (1). It seeds are beaked, semi-round, exalbuminous and an important source of dietary proteins, B group vitamins and certain minerals. The seeds are used in diverse food preparations, as aphrodisiac, anthelmintic, stimulant, tonic and to treat biliousness, bronchitis, diabetes, hyperlipidemia, ear inflammation, diseases of the liver and spleen, leprosy and other skin diseases, headache, sore throat and cough (2, 3). Boiled gram is beneficial in pulmonary, uterine and anal diseases. Seed powder is utilized as a face pack and to remove dandruff (3). The seeds contained carbohydrates (55–62%), proteins (25–29%) including globulin, essential amino acids, fatty oil (5%), isoflavones – biochenin A, daidzein, partensein, pyrimidine nucleotides, saponins, vitamins A–E, ciceritol, cerberoside and β-sitosterol (3–10). The flavonoids daidzein, formononetin, pratensein, liquiritigenin, isoliquiritigenin, its 4'-glucoside, 4',7-dihydroxyflavonol and garbanzol were not present in the ungerminated seeds but synthesized during germination (3). The present paper describes isolation and characterization of a flavonoid glycoside from the seeds of *C. arietinum*.

EXPERIMENTAL

General procedure

Melting point was determined on Perfit melting point apparatus and is uncorrected. Spectra were obtained with following apparatuses: FTIR: Jasco FT/IR-5000; UV: Lambda Bio 20 Spectrophotometer, MeOH; ^{1}H-NMR (400 MHz): Advance DRY 400, Bruker Spectrospin, $CDCl_3$; ^{13}C-NMR (75 MHz): Advance DRY 100, Bruker Spectrospin, $CDCl_3$ with TMS as an internal standard; MS: FAB ionization, JEOL-JMS-DX 303. Column chromatograpy: Silica gel (Qualigens), 60–120 mesh; TLC: Silica gel G (Qualigens). Spots were visualized by exposure to iodine vapors, UV radiation and by spraying reagents.

Plant material

The seeds of *Cicer arietinum,* grown in Punjab, were purchased from the local market of Delhi and identified by Dr M.P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen is deposited in the herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Extraction

The dried seeds (2 kg) were coarsely powdered and exhaustively extracted with methanol. The combined extracts were concentrated on a steam-bath and dried under reduced pressure to get 210 g of dark brown mass. The viscous dark brown mass was dissolved in a little quantity of methanol and adsorbed on silica gel (60–120 mesh) for preparation of slurry. It was dried in air and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol successively in order of increasing polarity.

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Isolation and characterization of 1

Elution of the column with chloroform-methanol (93:7) mixture afforded pale yellow crystals of **1**, recrystallized with methanol, 850 mg (0.043 % yield) R_f value: 0.77 (petroleum ether); m.p.: 67–68°C; UV λ_{max} (MeOH): 213, 262, 317 nm (log ε 3.2, 6.3, 1.9); UV λ_{max} (MeOH+NaOMe): 213, 263, 362 nm; UV λ_{max} (MeOH+$AlCl_3$): 213, 263, 354 nm; UV λ_{max} (MeOH+$AlCl_3$+HCl): 213, 273, 369 nm; UV λ_{max} (MeOH+NaOAc): 207, 262, 321 nm; UV λ_{max} (MeOH+NaOAc+H_3BO_3): 217, 221, 262, 337 nm; IR ν_{max} (KBr): 3410, 3372, 3315, 2925, 2857, 1734, 1680, 1618, 1580, 1494, 1452, 1368, 1301, 1253, 1176, 1076, 821, 782 cm^{-1}; ^{1}H-NMR (DMSO-d_6, δ, ppm): 8.46 (1H, d, *J* = 2.5 Hz, H-2'), 7.53 (1H, d, *J* = 8.1 Hz, H-5'), 7.02 (1H, dd, *J* = 2.5, 8.1 Hz, H-6'), 6.73 (1H, d, *J* = 1.5 Hz, H-8), 6.49 (1H, d, *J* = 1.5 Hz, H-6), 6.46 (1H, s, H-3), 5.07 (1H, d, *J* = 6.0 Hz, H-1''), 4.66 (1H, m, H-5''), 4.50 (1H, d, *J* = 6.0 Hz, H-2''), 3.69 (1H, m, H-4'''), 3.28 (1H, m, H-3''), 3.16 (1H, d, *J* = 9.5 Hz, H_2-6''a), 3.09 (1H, d, *J* = 9.5 Hz, H_2-6''b), 2.50 (2H, brs, H_2-2'''), 1.51 (2H, brs, CH_2), 1.22 (12H, brs, 6×CH_2), 0.84 (3H, t, *J* = 6.2 Hz, Me-10'''); ^{13}C-NMR (DMSO-d_6, δ, ppm): 180.57 (C-4), 171.06 (C-1'''), 163.19 (C-2), 161.76 (C-7), 159.38 (C-5), 157.38 (C-9), 155.04 (C-4'), 130.34 (C-3'), 127.88 (C-6'), 122.84 (C-5'), 122.40 (C-1'), 113.89 (C-2'), 106.24 (C-3), 106.24 (C-10), 99.97 (C-1''), 99.78 (C-6), 94.72 (C-8), 77.31 (C-5''), 76.50 (C-2''), 73.20 (C-4''), 69.72 (C-3''), 60.75 (C-6''), 55.32 (C-2'''), 29.13 (C-3'''), 28.57 (C-4'''), 28.48 (C-5'''), 26.73 (C-6'''), 25.33 (C-7'''), 24.51 (C-8'''), 22.25 (C-9'''), 14.13 (C-10'''); Positive ion FAB MS m/z (rel. int.): 602 $[M]^+$ ($C_{31}H_{38}O_{12}$) (1.2), 447 (12.3), 317 (9.6), 285 (100), 178 (18.3), 155 (25.2), 152 (23.3), 150 (47.5), 136 (56.2), 134 (19.6), 108 (41.9).

Hydrolysis of 1

Compound **1** (20 mg) was dissolved in MeOH (5 mL), 2M HCl (2 mL) was added and heated till half volume was left. The solution was first extracted with petroleum ether to separate capric acid (Co-TLC comparable, petroleum ether : chloroform, 1:1, v/v, sprayed with ceric sulfate) and then with EtOAc (3×10 mL). The organic layer was washed with H_2O (2×10 mL), dried over anhydrous Na_2SO_4 and evaporated to give luteolin, m.p. 326–328°C (decomp.) (TLC comparable, MeOH:*n*-BuOH:HCOOH, 7:2:1, v/v/v).The spot was detected under UV light. The aqueous phase was concentrated and analyzed by paper chromatography along with standard samples of monosacchrides. *n*-Butanol:ethanol:water (4:1:2.2, v/v/v) was used as the developing solvent system. The paper was sprayed with aniline hydrogen phthalate. The sugar was identified as D-glucose.

RESULTS AND DISCUSSION

Compound (**1**), named, luteolin glucocapriate, was obtained as light yellow crystals from chloroform-methanol (93:7) eluants. It responded positively to the tests for flavonoids. The UV spectrum of **1** displayed absorption maxima at 262 and 317 nm indicating flavone type nature of the molecule (11). A batochromic shift of band I from 317 nm to 362 on addition of sodium methoxide suggested free 4'-hydroxyl group in the flavone (12). Shifting of band I to 354 nm on addition of $AlCl_3$ indicated free 5-hydroxyl group and B-ring *o*-dihydroxyl groups. The absence of any shift on addition of NaOAc in band II at 262 nm supported glycosidic linkage at C-7. The shifting of band I with addition of NaOAc/H_3BO_3 reagents from 317 nm to 337 nm supported B-ring *o*-dihydroxyl groups (13). Its IR spectrum displayed characteristic absorption bands for hydroxyl groups (3410, 3372 and 3315 cm^{-1}), ester group (1734 cm^{-1}), carbonyl group (1680 cm^{-1}) and long aliphatic chain (782 cm^{-1}). The mass spectrum of **1** exhibited a molecular ion peak at m/z 602 consistent with a molecular formula of flavonoidal glycoside $C_{31}H_{38}O_{12}$. The generation of prominent ion fragments at m/z 155 $[CO(CH_2)_8CH_3]^+$, 317 $[C_6H_{10}O_5CO\ (CH_2)_8CH_3]^+$ and 447 $[M-CO(CH_2)_8CH_3]^+$ indicated the location of glycosidic linkage esterified with capric acid. The existence of the ion peaks at m/z 134, 152 $[C_{3,4}$-C_2O fission$]^+$, 150, 136 $[C_{3,4}$-C_9,O fission$]^+$ and 178, 108 $[C_{4,10}$-C_9,O fission$]^+$ of the flavone residue suggested the presence of two hydroxyl groups in each ring A and B. The ^{1}H-NMR spectrum of **1** exhibited a one-proton *meta*-coupled doublet at δ 8.46 ppm, and a one-proton *ortho*-coupled doublet at δ 7.53 ppm (*J* = 8.1 Hz) and a one-proton *ortho*-, *meta*-coupled double doublet at δ 7.02 ppm (*J* = 2.5, 8.1 Hz) ascribed to aromatic protons H-2', H-5' and H-6', respectively, supporting ABX-type coupling system of ring B. The presence of two *meta*-related aromatic protons at δ 6.73 and 6.49 ppm with coupling interaction of 1.5 Hz each suggested that ring A had two oxygenated functions. A one-proton sharp signal at δ 6.46 ppm was accounted to H-3. A one-proton doublet at δ 5.07 ppm (*J* = 6.0 Hz) was attributed to anomeric H-1'' proton. Three one-proton multiplets at δ 4.66, 4.50 and 3.28 ppm and a one-proton doublet at δ 3.69 ppm (*J* = 6.0 Hz) were associated with the carbinol protons of the sugar moiety. The

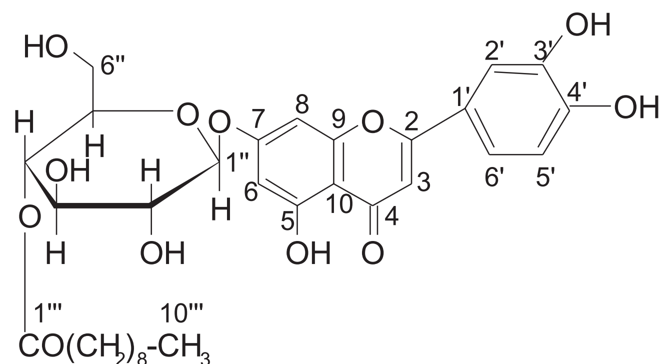

Figure 1.

appearance of H-4'' in the deshielded region at δ 4.50 ppm suggested location of the ester group at this carbon. The oxygenated H_2-6'' methylene protons resonated as one-proton doublets each at δ 3.16 (J = 9.5 Hz) ppm and δ 3.09 ppm (J = 9.5 Hz). A three-proton triplet at 0.84 ppm with coupling interaction of 6.2 Hz was accounted to terminal C-10 primary methyl protons. A two-proton broad signal at δ 2.50 ppm was accommodated to methylene H_2-2''' protons adjacent to the ester group. The remaining methylene protons appeared at δ 1.51 (2H) and 1.22 (12H) ppm. The ^{13}C-NMR spectrum of **1** displayed important signals for carbonyl carbon at δ 180.57 ppm (C-4) supporting the flavone type carbon framework of the molecule and aromatic carbon between δ 163.19–94.72 ppm. The anomeric carbons appeared at δ 99.97 ppm (C-1'') and sugar carbons resonated between δ 77.31–69.72 ppm. The signals at δ 171.06 and 14.13 ppm were ascribed to the ester carbon C-1''' and methyl carbon C-10''', respectively. The methylene carbons resonated between δ 55.32–22.55 ppm. The HSQC experiment correlated all protons to their respective carbons. The HMBC spectrum exhibited correlation between the proton signal at δ 6.46 ppm and the three quaternary carbon resonances at δ 122.40 (C-1'), 163.19 (C-2) and 180.57 (C-4) ppm, which allowed us to assign this proton at position 3 of the flavone core (14, 15). Acid hydrolysis of **1** gave luteolin, capric acid and sugar – glucose. On the basis of spectral data analyses and chemical reactions, the structure of **1** has been elucidated as 5,7,3',4'-tetrahydroxyflavonyl 7-(4'''-n-decanoyl)-β-D-glucopyranoside (Figure 1). It is a new flavone glucoside ester isolated from a natural source for the first time.

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