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FLAVONE GLYCOSIDES OF *CARALLUMA TUBERCULATA* N. E. BROWN.

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ABSTRACT

Three flavone glycosides have been isolated from the fresh vegetative part of the medicinal herb *Caralluma tuberculata.* The chemical and spectroscopic properties are consistent with structures of Luteolin-4'-O-β-D-glucopyranosyl(2→1)-α-L-rhamnopyranoside [1], Kaempferol-7-O-β-D-glycopyranosyl (2→1)-α-L- rhamnopyranoside [2], Kaempferol-3-O-β-D-glycopynnaryl (6→1)-α-L-rahmnopyranoside (3).

Introduction

Caralluma tuberculata N.E. Brown is one of the important member of the Asclepiadaceae family. This plant is eaten raw or cooked as vegetable (S. I. Ali, 1983) and are reputed in the folkloric medicine for the cure of diabetes, rheumatism and disease of blood (Burkill, 1909; Hoope, 1975). Due to its edible properties the chemical investigation was carried out with the objective to isolate those agents which are medicinally active. In this study an attempt has been made to isolate and identify the flavonoid components from *Caralluma tuberculata.* As such the flavonoid constituents are among the most frequently encountered natural products, and are of repute for their antihaemorrhagic, antisclerotic, antiphlogistic and antioedematic activity. The examples are rutin and hesperidin and related flavonoids which have the ability to decrease capillary fragility by reducing capillary permeability induced by histamine or by tissue injuries and retard destruction of epinephrine on the body tissues. Rutin and hesperidin both exhibit antihaemorrhagic and antisclerotic activity whereas rutin further shows antiphlogistic and antioedema activity (Usmanghani, 1989). In the present communication three flavonoids isolated from *Caralluma tuberculata* are described.

Material and Methods

Melting points were determined on Gallenkamp melting point Electrothermal apparatus model 5-A-6797, optical rotation were measured on Jasco DIP-140 digital Polarimeter. UV and IR spectra were recorded on Shimadzu UV-240 spectrophotometer and Jasco A-302 spectrophotometer respectively, FAB-MS (-ve ion mode) were recorded on Varian MAT-312 mass spectrometer. ^{1}H and ^{13}C-NMR were scanned on Bruker Am-300 and Bruker Am-400 (300, 400 and 75.43, 100.6 MHz) NMR spectrometer. The

purification of compounds were carried out by repeated column chromatography, followed by preparative thin layer chromatography on polyamide plates.

The fresh whole plant of *Caralluma tuberculata* was purchased (25 kg) from the vegetable market of Karachi, and this plant material was identified by Prof. Dr. S. I. Ali, Vice Chancellor, University of Karachi, Karachi. The Chopped pieces of plant material extracted with EtOH, after percolation at room temperature for one month. The EtOH extract was evaporated under reduced pressure which furnished a dark green thick semi-solid residue. For fractionation H2O and EtOAC were added in equal quantity and lipophilic components were separated. After ascertaining through TLC the EtOAC-phase was not processed further, whereas aqueous phase was again treated with n-BuOH and concentrated under reduced pressure and lyophilized to give crude mixture (73 g) which showed different chemical compounds.

A portion (V) of the crude extract (487 mg) was chromatographed over silica gel column using n-hexane--EtOAC (95:5) as eluent. Two fractions Va (201 mg) and Vb (150 mg) were collected separately. Fraction Va: (201 mg) was subjected to polyamide preparative thin layer chromatography and developed in n-Bu OH-HO AC-H_2O (12:3:5) and a pure compound [1] was obtained in 40 mg quantity. Fraction Vb: was also isolated through preparative polyamide thin layer chromatography using n-Bu OH-EtOH-H 0 (7:2:5) as solvent system. As the result of which two flavonoids compounds [2] and [3] were collected. HPTLC plates were finally used for the purification of compounds.

Results and Discussion

Compound [1] was isolated as yellow amorphous powder having m.p. 293-5°C and optical rotation + 196 (MeOH), ultraviolet absorption maxima was recorded at 248, 269 and 337 urn which indicated the presence of chromophoric groups in the molecule. The Infra red spectrum in KBr also provided the evidence for the degree of unsaturation at 1640 (C=C), 1515 and 1440 cm (aromatic ring). The other important absorption were present at 3400 (br, OH) and 1710 (C=0) cm. The FAB-MS showed a molecular ion peak at m/z 595 (M+ H) which corresponded to the molecular formula $C_{27}H_{30}O_{15}$. Other peaks were observed at m/z 551, 458, 432, 278.

The ^{1}H-NMR spectrum (CD_3 OD, 300 MHz, ppm) of this compound exhibited the signals which were characteristic of a flavonoid compound. On comparing the data (Mabry *et al,* 1970; Harborne *et al*., 1982; Nakano *et al.*, 1983). It was found to be identical to Luteolin-4'-glycoside. The major characteristics signals were present at 6.20 d (d, J = 2.2 Hz, H-6), 6.44 (d, J = 2.2 Hz, H-8), 6.60 (s, H-3), 5.18 (d, J= 7.5 Hz, H-1), 5.26 (d, J = 1.8Hz,H-2), 4.56 (s, C-1), 7.26 (d, J= 8.8 Hz, H-6) and 7.45 (d, J = 2.2, 8.8 Hz, H-2, 5) ppm. The ^{13}C-NMR spectrum showed the presence of 27 signals, out of which 9 were alloted to the major component of flavan chromophore of luteolin, 6 to aromatic ring and the rest of 12 signals to the two sugars i.e. a glucose and a rhamnose

unit. The ^{13}C-NMR data showed the aromatic ring was attached to the main flavan skeleton at C-2 (116.22 ppm) while the sugar in a disaccharide manner was attached to an aromatic ring through C-4 (149.16 ppm). The sugars were proved as glucose and rhamnose through ^{13}C-NMR assignments. It was also noted that rhamnose was a terminal sugar. The details of ^{13}C-NMR data are given in Table 1.

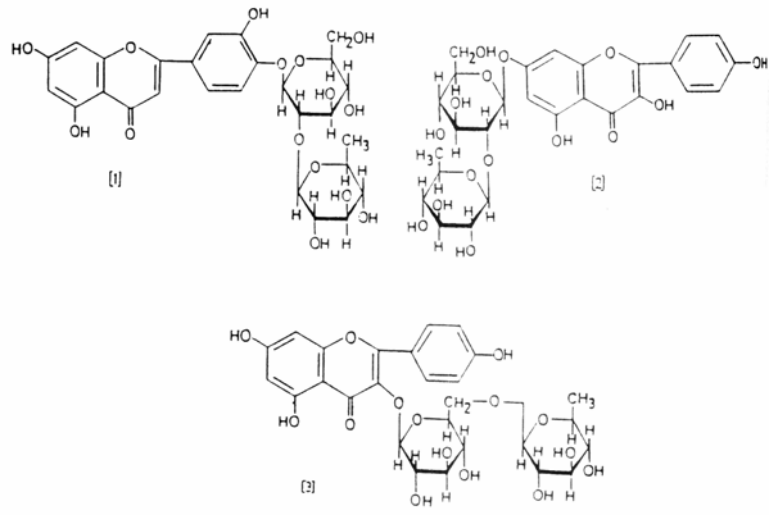
Table: 1

^{13}C-NMR chemical shift of compound [1] in (CD3 OD, 75.5 MHz),[2] and [3] in (C5D5N, 100 MHz) ppm.

Carbon No.	[1]	[2]	[3]
C-2	166.22	158.22	158.42
C-3	103.16	135.88	132.00
C-4	183.89	179.13	79.13
C-5	159.52	162.12	161.77
C-6	100.34	99.93	99.93
C-7	165.62	162.12	64.38
C-8	95.16	94.82	94.82
C-9	139.52	158.22	157.06
C-10	105.19	105.14	103.77
C-1'	27.41	122.76	121.51
C-2'	118.21	131.52	131.52
C-3'	149.70	116.41	116.41
C-4'	149.18	158.22	158.42
C-5'	115.25	116.41	116.41
C-6'	19.81	131.52	131.52
C-1''	101.39	101.66	101.61
C-2''	80.99	79.16	74.15
C-3'''	78.25	78.36	76.81
C-4''	70.63	71.63	70.46
C-5''	78.60	77.05	76.01
C-6''	62.40	62.86	69.76
C-1'''	100.34	100.07	100.32
C-2'''	71.40	71.77	70.90
C-3'''	72.30	71.34	70.18
C-4'''	71.40	72.09	72.04
C-5'''	70.63	69.74	69.74
C-6'''	17.83	18.51	18.20

Therefore compound [1] was identified as Luteolin-4'-O-β-D-glucopyranosyl (2→1)-α-rhamnopyranoside on the basis of spectroscopic data reported for Luteolin-4'-O-β-D-glycopyranosyl (2→1)-a-L-rhamnopyranoside (Mabry *et al,* 1970; Harborne *et al,* 1982; Nakano *et al*., 1983), and on the basis of direct comparison with an authentic sample by TLC monitoring.

Compound [2] was obtained from fraction Vb as yellow amorphous powder. It melted at 161-162°C. FAB-MS (negative ion mode) spectrum exhibited a molecular ion peak at m/z 593 [M-H] which corresponded to the molecular formula $C_{27}H_{30}O_{15}$.



In the UV spectrum absorption of molecules occurred at 208, 248, 269, 337 nm due to the chromophoric groups. The degree of unsaturation further supported by IR which displayed absorption at 3400 (b r, 011), 1710 (C=0), 1640 (C=C), 1520, 1440 (aromatic ring) cm_{-1}. The ^{1}H-NMR spectrum (400 MHz, C_5D_5N) of the compound [2] showed the chemical shifts for three different units. The chemical shifts for one unit were similar to flavan of kaempferol, another to an aromatic ring and the last unit was matched with sugar moieties i.e. glucose and rhamnose in a dissachride fashion. The important signals for kaempferol unit were found at 6.20 (d, J = 3.5 Hz, H-6), 6.24 (d, J = 3.5 Hz, H-8) ppm. The signals for aromatic ring were present at 8.02 (d, J = 8.9 H, H-2, 6), and 7.25 (d, J = 8.9 Hz, H-3, 5) ppm. The anomeric protons signal for glucose and rhamnose were observed at 5.22 (d, J = 7.6 H, 14-1) and 5.05 (d, J = 1.3 Hz, H-1"") ppm.

The ^{13}C-NMR of this compound [2] can be correlated with signals ^{1}H-NMR and on these basis the presence of flavan moiety of kaempferol, aromatic ring, glucose and rhamnose units were deduced. The detail of chemical shifts i1 given in Table 1, and here the discussion is being put forth only on the point of attachment. The aromatic ring was linked to kaempferol unit at C-2 (158.22 ppm) and sugar (glucose) was found on kaempferol unit at C-7 (162.40 ppm). It was also noticed that the rhamnose was a terminal sugar and was linked with a glucose unit through C-2 (79.16 ppm). Further evidences were obtained from reported values (Mabry *et al.,* 1970; Harborne *et al*, 1982;

Nakano *et al*., 1983) and comparison on TLC plate with authentic sample. Therefore, the isolated compound [2] was identified as kaempferol-7-O-β-D-glucopyranosyl-(2→1)-α-L-rhamnopyranoside.

Compound [3] was obtained as yellow needle-shape crystals, having melting point 154-8°C, optical rotation $[\alpha]D_{25}$ 100° (c = 100, MeOH) and UV absorption maxima at 261 and 359 nm (log ε =4.32, 4.27). The IR (KBr) spectrum revealed absorption at 3400 (OH), 1705 (C = O), 1635 (C = C), 1520 and 1440 (aromatic ring) cm^{1}. The FAB-MS (negative ion mode) exhibited a molecular ion peak at m/z 593 $(M-H)^{-}$, which corresponded to the molecular formula $C_{27}H_{30}O_{15}$. The ^{1}H-NMR (400 MHz, C_5D_5N) spectrum showed signals at 6.20 (d, J = 1.98 Hz, H-6), 6.24 (d, J = 1.98 Hz, H-8) ppm. These signals were found similar in all respect to kaempferol unit as given in the literature (Mabry *et al,* 1970; Harborne *et. al,* 1982; Nakano *et al* 1983). The signals present at 8.02 (d, J = 9.0 Hz, H-2, 8) and 7.25 (d, J = 9.0 Hz, 11-3,5) ppm represents an aromatic ring substituted at C-4. The other signals were displayed at 5.16 (d, J = 7.2 Hz, H-1) and 5.04 (d, J = 1.2 Hz, H-1) ppm and were denoted as the protons of anomeric carbon, while the former signal belong to glucose unit and the later signal to rhamnose unit. The methyl signal of Macanese was found at 1.40 (d, J = 6.5 Hz, H-6) ppm.

The details of ^{13}C-NMR chemical shifts are given in Table 1. Some of the specific signals of the isolated compound were observed at 158.42 (C-2), 132.00 (C-3), 158.42 (C-4), 101.61 (C-1), 69.76 (C-6), 100.32 (C-1), 18.20 (C-6) ppm. The comparison with authentic sample was also carried out and found unvarying with literature values (Mabry *et al,* 1970; Harborne *et al,* 1982; Nakano *et al,* 1983).

This compound [3] was therefore proved as a derivative of kaempferol, and was identified as kaempferol-3-O-β-D-glucopyranosyl(6→1)-α-L-rhamnopyranoside.

Till to date the presence of such flavone glycosides have not been reported from *Caralluma tuberculata,* and therefore this is the first report of its occurrence in this plant.

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