Qatar Univ. Sci. J. (1995), 15 (1): 51 - 55

CONSTITUENTS OF PLANTS GROWING IN QATAR: PART XXVIII. CONSTITUENTS OF *CISTANCHE PHELYPAEA*

By

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مكونات نباتات دولة قطر . الجزء ٢٨ . الفحص الكيميائي لنبات السيستانكا (الطرثوث)

تاكيشي دایاما و ك . ياهيكوزاوا و هالة سلطان العيسى و عبد الفتاح محمد رزق

أسفرت دراسة مكونات نبات السيستانكا عن فصل المركبات التالية : ايكيناكوزيد ، تيوبليوزيد ايه ، تيوبليوزيد إي ، اكتيوسيد ، ٢ - اسيتيل اكتيوسيد ، ٦ - ديوكسي كاتالبول ، جلوروسيد ، اجوغول ، سيرينجين ، بيتا سيتوستيرول .

Key Words: Cistanche phelypaea, Orobanchaceae, Echinacoside, Tubuloside A, Tubuloside E,Acteoside, 2'-Acetylacteoside, 6Deoxycatalpol, Gluroside, Ajugol, Syringin, β–'Sitosterol

ABSTRACT

Ten compounds were isolated from the methanolic extract of the aerial parts of *Cistanche phelypaea* (Orobanchaceae) and identified as echinacoside (1), tubuloside A (2), tubuloside E (3), acteoside (4), 2'-acetylacteoside (5), 6-deoxycatalpol (6), gluroside (7), ajugol (8), syringin (9) and β -sitosterol (10).

INTRODUCTION

Cistanche phelypaea (L.) Cout. (Orobanchaceae), a parasitic plant grows on *Arthrocnemun glaucum* and *Seidlitzia rosmarinus,* is a common species in southern Qatar [1]. The whole plant is medicinally used as a remedy for diarrhea, a tonic in speratorrhea impotence and a cataplasm against bruises [2]. Phenylethanoid glycosides have been isolated from *Cistanche tubulosa* [3,4] C. *salsa* [5,6] and *C. deserticola* [7]. Melek, *et. al.* [8] reported the isolation of four phenylethanoid glycosides from *C. phelypaea* growing in Egypt, theses are as follows: tubuloside A, acteoside, 2'-acetylacteoside and pheliposide [8]. The pharmacological studies for the same plant showed that the acute toxicity was minimal. Analgesic, antipyretic and diuretic activities of the extract were significant only at large doses[8].

EXPERIMENTAL

Plant material

Cistanche phelypaea (aerial parts) was collected from north Al-Markhya (3 Km north of Doha), Qatar in March. The plant was identified by Prof. K. H. Batanouny.

Extraction procedure

The plant was air dried, powdered, extracted in a soxhlet with MeOH and freed from solvent under vacuo, yielding (200 g) of crude extract. The extract was then suspended in distilled water and the suspension was extracted with ether, EtOAc and n-BuOH yielding 1.59 g, 0.95 g and 2.0 g extracts respectively.

Isolation of compounds

The EtOAc (0.95 g) extract was chromatographed on silica gel column using $CHCl_3$- MeOH (10:1), and $CHCl_3$-MeOH-H_2O (100:10:1, 80:20:3, and 70:30:5). The separated materials were re-chromatographed and gel filtrated with HW-40. Preparative HPLC (column: Wakosil II HG, 20x250 mm; solvent CH_3CN-H_2O) resulted in the separation of compounds (3),(4) and (5). The same procedure was applied to the n-BuOH extract (2.0 g) yielding two pure compounds (2) and (9). The aqueous layer was subjected to a Diaion HP-20 (Nippon Rensui Co.) column, washed with water, and eluted with 30% MeOH (300 ml) and 100% MeOH (300 ml). The 30% MeOH fraction yielded compound (8), and the 100% MeOH fraction yielded three compounds, (1), (6) and (7).

Apparatus and Techniques

Melting points were determined on a Mitamura-melting point apparatus. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. IR spectra were recorded with a Hitachi 279-30 IR spectrophotometer and ultraviolet (UV) spectra with a Hitachi 200-20 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR were recorded with a JEOL GSX-400 (400 and 100 MHz), respectively. Chemical shifts are given on d (ppm) with a tetramethylsilane (TMS) as an internal standard. HPLC was performed on Hitachi L-6000, L-4200 UV-VIS detector and D-2500 chromato-integrator or Shimadzu LC-6A instrument. Silica gel (Wako-gel, C-300) and polyamide (polyamide C-100) were used for column chromatography. Diaion HP-20 was used for gel filtration. Silica gel 60 F_{254} (Merck) precoated plates were used for TLC and detection was carried out by spraying with 10% H_2SO_4 followed by heating.

RESULTS AND DISCUSSION

Echinacoside (1)

It was isolated as an amorphous powder. The IR spectrum suggested the presence of hydroxyl groups at 3424 cm^{-1}, a carboxyl group at 1698 cm^{-1} and aromatic rings at 1634, 1608 and 1528 cm^{-1}. The UV spectrum showed absorption maxima at l218(sh), 246(sh), 292, and 333 nm, which suggested the presence of caffeoyl group. The ^{1}H-NMR (in MeOH-d_4) spectrum gave signals of one methyl group of rhamnose at δ 1.08 (3H, δ, J=6.2 Hz), δ 2.97 (2H, t, J=7.0 Hz, Ar-CH_2), two anomeric protons of glucose at d 4.29 (1H, δ, J=7.7 Hz), and d 4.39 (1H, δ, J=7.8 Hz) and one of rhamnose at δ 5.18 (1H, δ, J=1.7 Hz), δ 6.28 (1H, d, J=15.8 Hz, Ar-CH=CH-CO),d 6.56-7 (6H, m, Ar-H) and δ 7.60 (1H, δ, J=15.8 Hz, Ar-CH=CH-CO). The ^{13}C-NMR spectrum gave δ signals at: 131.5 (C-1), 117.2 (C-2), 146.1 (C-3), 144.7 (C-4), 116.6 (C-5), 121.4 (C-6), 72.1(C-α), 36.3 (C-β), 127.7 (C-1`), 115.3 (C-2`), 146.9 (C-3`), 149.9 (C-4`), 116.4 (C-5`), 123.3 (C-6`), 168.5 (C-α`), 114.7 (C-β),148.3 (C-γ`), 104.2 (C-glu-1), 76.2 (C-glu-2), 81.7 (C-glu-3), 70.5 (C-glu-4), 74.8 (C-glu-5), 69.4 (C-glu-6), 103.1 (C-rhm-1), 72.4 (C-rhm-2), 72.4 (C-rhm-3), 73.8 (C-rhm-4), 70.6 (C-rhm-5), 18.5 (C-rhm-6), 104.7 (C-glu-1`), 75.1 (C-glu-2`), 78.0 (C-glu-3`), 71.5 (C-glu-4`), 77.8 (C-glu-5`), and 62.7 (C-glu-6`). These evidences suggested the structure of phenylethanoid glycoside for compound (1). This compound was identified as echinacoside (1) by direct comparison of TLC, HPLC, IR and 1H-NMR with authentic sample. The obtained data were indentical with that reported in literature [3, 9,10].

Tubuloside A (2)

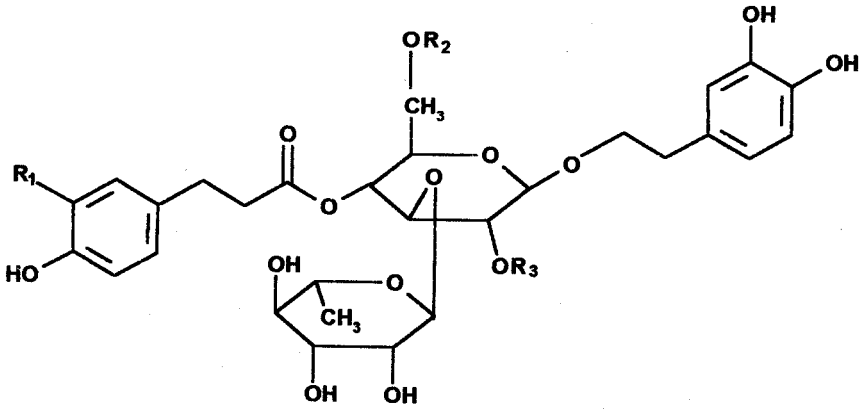
The compound was isolated as an amorphous powder. The IR spectrum suggested the presence of hydroxyl groups at 3424 cm^{-1}, a carboxyl group at 1702 cm^{-1} and aromatic rings at 1634, 1608 and 1522 cm^{-1}. The UV spectrum showed absorption maxima at λ 222, 246(sh), 293 and 337 nm. The ^{1}H-NMR (in MeOH-d_4) spectrum gave signals of methyl group of rhamnose at δ 1.07 (3H, δ, J=5.9 Hz), one acetoxy group at δ 1.98 (3H, s) δ 2.69 (2H, Ar-CH_2-), and two anomeric protons of glucose at δ 4.30 and 4.50 each is (1H, δ, J=7.9 Hz), δ 6.28 (1H, δ, J=15.8 Hz, Ar-CH=CH-CO),d 6.50-7.40 (6H, m, Ar-H) and δ 7.69 (1H, δ, J=15.9 Hz, Ar-CH=CH-CO). The ^{13}C-NMR spectrum gave δ signals at: 131.8 (C-1), 117.2 (C-2), 146.0 (C-3), 144.6 (C-4), 116.3 (C-5), 121.3 (C-6), 72.6 (C-α), 36.3 (C-β), 127.6 (C-1`), 115.3 (C-2`), 146.9 (C-3`), 149.9 (C-4`), 116.6 (C-5`), 123.3 (C-6`), 168.3 (C-α`), 114.6 (C-β`), 148.4 (C-γ`), 101.7 (C-glu-1), 75.1 (C-glu-2), 80.5 (C-glu-3), 70.7 (C-glu-4), 74.8 (C-glu-5), 69.3 (C-glu-6), 103.3 (C-rhm-1), 72.4 (C-rhm-2), 71.4 (C-rhm-3), 73.6 (C-rhm-4), 70.8 (C-rhm-5), 18.5 (C-rhm-6), 104.7 (C-glu-1`), 75.1 (C-glu-2`), 77.9 (C-glu-3`), 71.9 (C-glu-4`), 77.8 (C-glu-5`), 62.6 (C-glu-6`), 20.9 and 171.5 (OAc). The structure was confirmed by comparison with literature data[3].

Tubuloside E (3)

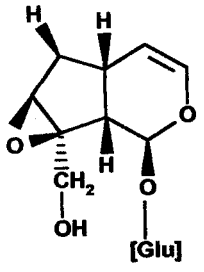
It was isolated as an amorphous powder, and identified directly by comparison with authentic sample. The 13C-NMR spectrum gav δ signals at: 131.7 (C-1), 117.2 (C-2), 146.0 (C-3), 144.5 (C-4), 116.3 (C-5), 121.3 (C-6), 72.6 (C-α), 36.3 (C-β), 127.1 (C-1`), 131.3 (C-2`), 116.9 (C-3`), 161.5 (C-4`), 116.9 (C-5`), 131.3 (C-6`), 168.0 (C-α') 114.7 (β'), 147.7 (C- γ`), 101.7 (C-glu-1), 75.1 (C-glu-2), 80.5 (C-glu-3), 70.7 (C-glu-4), 76.1 (C-glu-5), 62.2 (C-glu-6), 103.3 (C-rhm-1), 71.9 (C-rhm-2), 71.8 (C-rhm-3), 73.6 (C-rhm-4), 70.7 (C-rhm-5), 18.4 (C-rhm-6), 20.9 and 171.5 (OAc). The identification was confirmed by comparison with literature [4].

Acteoside (4)

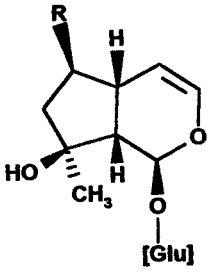
It was isolated as an amorphous powder, and identified directly by comparison with authentic sample. The ^{13}C-NMR spectrum gave δ signals at: 131.6 (C-1), 117.2 (C-2), 144.7 (C-4), 146.1 (C-3), 116.3 (C-5), 121.3 (C-6), 72.4



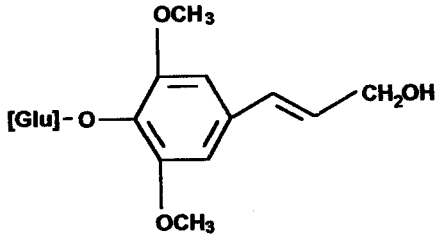
(1)	R_1 = OH	R_2 = Glu	R_3 = H
(2)	R_1 = OH	R_2 = Glu	R_3 = CH_3CO
(3)	R_1 = H	R_2 = H	R_3 = CH_3CO
(4)	R_1 = OH	R_2 = H	R_3 = H
(5)	R_1 = OH	R_2 = H	R_3 = CH_3CO



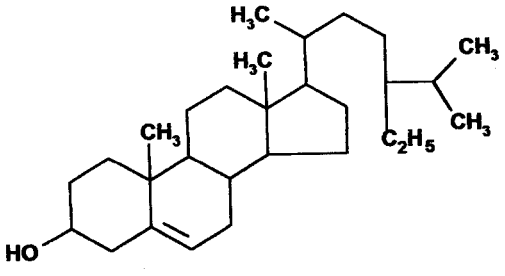
(6) Deoxycatalpol



(7) Gluroside
(8) Ajugol



(9) Syringin



(10) β-Sitosterol

(C-α) 36.6 (C-b) 127.7 (C-1`), 115.3 (C-2`), 146.9 (C-3`), 149.8 (C-4`), 116.5 (C-5`), 123.3 (C-6`), 168.4 (C-α`), 114.8 (C-β`), 148.0 (C-γ`), 104.3 (C-glu-1), 76.1 (C-glu-2), 80.7 (C-glu-3), 70.5 (C-glu-4), 76.3 (C-glu-5), 62.4 (C-glu-6), 103.1 (C-rhm-1), 72.3 (C-rhm-2), 72.1 (C-rhm-3), 73.9 (C-rhm-4), 70.7 (C-rhm-5), and 18.5 (C-rhm-6). The results were in agreement with literature [3].

2'-Acetylacteoside (5)

It was isolated as an amorphous powder, and was identified by comparison with authentic sample. The ^{13}C-NMR spectrum gav δ signals at: 131.6 (C-1), 117.2 (C-2), 146.1 (C-3), 144.7 (C-4), 116.4 (C-5), 121.3 (C-6), 72.4 (C-α), 36.6 (C-b), 127.7 (C-1`), 115.3 (C-2`), 146.9 (C-3`), 149.8 (C-4`), 116.6 (C-5`), 123.3 (C-6`), 168.4 (C-α`),114.8 (C-β`),148.0 (C-γ`), 104.3 (C-glu-1), 76.1 (C-glu-2), 81.7 (C-glu-3), 70.5 (C-glu-4), 76.3 (C-glu-5), 62.4 (C-glu-6), 103.1 (C-rhm-1), 72.3 (C-rhm-2), 72.1 (C-rhm-3), 73.9 (C-rhm-4), 70.7 (C-rhm-5), and 18.5 (C-rhm-6).

6-Deoxycatalpol (6)

It was isolated as an amorphous powder. The IR spec-

trum suggested the presence of hydroxyl groups at 3418 cm^{-1}. The ^{1}H-NMR (in D_2O) spectrum gave signals at d 1.58 (1H, J=13.5, 9.1 Hz, H-6), δ 2.35 (1H, dd, J=13.5, 7.3 Hz, H-6), d 2.47 (1H, m, H-5), δ 2.53 (1H, dd, J=9.0, 7.5 Hz, H-9),d 3.78 (1H, δ, J=13.2 Hz, H-10), δ 4.35 (1H, δ, J=13.2 Hz, H-10), δ 4.90 (1H, δ, J=7.9 Hz, H-1-glu), δ 5.11 (1H, δ, J=9.3 Hz, H-1) and δ 6.35 (1H, dd, J=5.9, 1.7 Hz, H-3). These data suggested the presence of an iridoid. The ^{13}C-NMR spectrum gave δ signals at: 97.3 (C-1), 142.2 (C-3), 108.7 (C-4), 33.6 (C-5); 36.9 (C-6), 63.5 (C-7), 71.8 (C-8), 45.2 (C-9), 63.5 (C-10), 101.5 (C-glu-1), 75.7 (C-glu-2), 79.1 (C-glu-3), 72.4 (C-glu-4), 78.5 (C-glu-5), 64.5 (C-glu-6). This compound was identified by direct comparison of TLC, HPLC, IR, ^{1}H-NMR and ^{13}C-NMR with authentic sample and the reported literature[4,11,12]

Gluroside (7)

It was isolated as an amorphous powder, and was identified by comparison of TLC, IR, ^{1}H-NMR and ^{13}C-NMR with authentic sample. The IR spectrum suggested the presence of hydroxyl groups at 3416 cm^{-1}. The ^{1}H-NMR (in D_2O) spectrum gave signals at δ 1.36 (3H, s, CH_3), d 2.48-2.88 (1H, m, H-9), d 4.87-4.92 (1H, m, H-4), δ 5.50 (1H, d, J=1.8 Hz, H-1) and δ 6.23 (1H, dd, J=6.4, 2.0 Hz, H-3). The ^{13}C-NMR spectrum gave δ signals at: 96.3 (C-1), 140.7 (C-3), 111.4 (C-4), 32.6 (C-5), 31.6 (C-6), 43.0 (C-7), 82.6 (C-8), 54.0 (C-9), 26.5 (C-10), 100.9 (C-glu-1), 75.6 (C-glu-2), 79. 1(C-glu-3), 72.5 (C-glu-4), 78.5 (C-glu-5), 63.6 (C-glu-6). The obtained results are in agreement with literature [13].

Ajugol (8)

It was isolated as an amorphous powder, and was identified by comparison of TLC, IR, 1H-NMR and ^{13}C-NMR with authentic sample. The IR spectrum suggested the presence of hydroxyl groups at 3400 cm^{-1}. The ^{1}H-NMR (in MeOH-d_4) spectrum gaves signals at δ 1.31 (3H, s, CH_3),δ 1.79 (1H, dd, J=13.4 , 4.6 Hz, H-7), δ 2.04 (1H, dd, J=13.4, 5.7 Hz, H-7), d 2.54 (1H, dd, J=9.5, 2.0 Hz, H-9), δ 2.71-2.73 (1H, m, H-5), d 4.63 (1H, d, J=8.1 Hz, H-1-glu), δ 5.46 (1H, d, J=2.4 Hz, H-1) and δ 6.15 (1H, dd, J=6.2, 2.0 Hz, H-3). The ^{13}C-NMR spectrum gave δ signals at: 93.8 (C-1), 140.5 (C-3), 106.0 (C-4), 41.3 (C-5), 78.3 (C-6), 50.1 (C-7), 79.5 (C-8), 51.9 (C-9), 25.3 (C-10), 19.5 (C-glu-1), 74.9 (C-glu-2), 77.8 (C-glu-3), 71.8 (C-glu-4), 78.1 (C-glu-5), 62.9 (C-glu-6). The ^{13}C-NMR, data are in agreement with that reported in literature[14].

Syringin(9)

It was isolated as a white powder, m. p. 186-7°C. The IR spectrum suggested the presence of a hydroxyl group at 3444 cm^{-1}, and an aromatic ring at 1636, 1592 and 1512 cm^{-1}. The ^{1}H-NMR spectrum (in DMSO-d_6) gave signals at δ 3.76 (6H, s, OCH3), δ 6.3-6.4 (2H, m, -CH=CH-) and δ 6.7 (2H, s, Ar-H) representing two aromatic protons. The ^{13}C-NMR spectrum gave δ signals at: 132.5 (C-1), 104.3 (C-2), 152.6 (C-3), 133.7 (C-4), 152.6 (C-5), 104.3 (C-6), 60.8 (C-α), 130.1 (C-β), 128.3 (C-γ), 102.5 (C-glu-1), 74.1 (C-glu-2), 76.4 (C-glu-3), 69.8 (C-glu-4), 77.1 (C-glu-5), 61.4 (C-glu-6) and 56.2 (OCH_3). All data are in agreement with that reported in literature[15].

b- Sitosterol (10)

Colorless powder, $C_{33}H_{25}O$. The IR spectrum suggested the presence of a hydroxyl group at 3432 cm^{-1} and methyl groups at 1468 and 1386 cm^{-1}. The compound was identified by direct comparison with authentic sample.

Previous investigation of this species [8] showed the presence of acteoside, 2'- acetylacteoside, tubuloside A and pheliposide, their identification as phenylethanoid glycosides was mentioned without any data. References were given only for acteoside, 2'- acetylacteoside and tubuloside A. In our work the first three phenylethanoid glycosides were isolated in addition to two other phenylethanoid glycosides: echinacoside and tubuloside E, three iridoids: 6-deoxycatalpol, gluroside and ajugol, together with syringin and (-sitosterol. The identification of these compounds was based on the melting points, IR spectra, ^{1}H-NMR ^{13}C-NMR and UV spectral data. The phenylethanoid glycoside (pheliposide) was not detected in our extract, but another compound has been isolated and its identification is underway.

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