PERGAMON

Phytochemistry 50 (1999) 1205–1208

PHYTOCHEMISTRY

1*H*-Indole-3 acetonitrile glycosides from *Capparis spinosa* fruits

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Received in revised form 9 September 1998

Abstract

Two new glucose-containing 1*H*-indole-3-acetonitrile compounds, capparilosides A and B, were isolated from mature fruits of *Capparis spinosa*. On the basis of spectral and chemical evidence, they were shown to be 1*H*-indole-3-acetonitrile 4-*O*-*β*-glucopyranoside and 1*H*-indole-3-acetonitrile 4-*O*-*β*-(6′-*O*-*β*-glucopyranosyl)-glucopyranoside, respectively.

Keywords: Capparis spinosa; Capparidaceae; Fruits; Indole acetonitrile glycosides; Capparilosides A and B

1. Introduction

Capparis spinosa is a wide spread plant in the flora of Turkey (Davis, 1965). It is used under the names of 'kapari and kebere' in folk medicine as a diuretic, constipant, antihypertensive, poultice and tonic. The floral buttons of *C. spinosa* are also used as a flavouring in cooking and for making pickles (Baytop, 1984).

Previous studies have shown the presence of indole and aliphatic glucosinolates, polyprenols, flavonoids and alkaloids in *C. spinosa* (Ahmed, Rızk, Hammouda, & Seif El-Nasr, 1972; Sadykov & Khodzhimatov, 1981; Al-Said, Abdelsattar, Khalifa, & El-Feraly, 1988; Schraudolf, 1989; Türköz, Toker, & Şener, 1995; Benkinouar, Rhouati, & Jay, 1996). There are only a few reports on the fruits of this species. The present paper reports on the isolation and structural elucidation of two indole-3-acetonitrile glycosides.

2. Result and discussion

The methanolic extract of the mature fruits of *C. spinosa* was separated into several fractions, which were subjected to repeated column chromatography on reverse and normal phase silica gel, affording the indole-3-acetonitrile glycosides, capparilosides A (**1**) and B (**2**). Their structures were established from spectral (UV, IR, 1D- and 2D-NMR, ESI and FAB-mass spectrometry) and chemical evidence.

The molecular formula of **1** was established as $C_{16}H_{18}N_2O_6$ on the basis of FAB (*m*/*z* 357 $[M+Na]^+$), positive ion-ESI (*m*/*z* 357 $[M+Na]^+$, 691 $[2M+Na]^+$) and negative ion-ESI (*m*/*z* 333 $[M-H]^-$, 667 $[2M-H]^-$) mass spectrometry (calcd for 334.328), in combination with ^1H and ^{13}C NMR data (Table 1).

The UV spectrum (λ_{max} (MeOH): 267, 278 (sh) and 289 nm) of **1** was characteristic of a 3-substituted-indole chromophore. The IR absorption at 2250 cm^{-1} implied the presence of a nitrile (CN) function in its structure. Additionally, absorptions were observed at 3525, 3495, 3400 and 3359 (OH, NH), 1625, 1590 and 1508 (aromatic), 1170 and 1084 cm^{-1} (C–O–C). The ^1H NMR spectrum showed the following significant aromatic proton signals Table 1: a 1,2,3-trisubstituted benzene (δ 6.69 dd, $J=7.4$ and 1.1 Hz, H-5; δ 7.00 and 6.99 overlapped, H-6 and H-7) and a methine proton (δ 7.20 d, $J=1.3$ Hz, H-2), which was coupled to a one proton signal at δ 11.1 (d, $J=1.3$ Hz). The latter was assigned to NH (H-1) resonance, since it showed no correlation to the carbon resonances in a HSQC experiment. The signals observed as an AB-system at δ 4.17 ($J_{AB}=18.5$ Hz) were indicative of the isolated protons of a methylene group. However, these signals showed correlation with the carbon resonance at the high-field region of the spectrum (δ 15.7) in a HSQC experiment. On the other hand, a quaternary carbon resonance observed at δ 121.0 (C-9) showed long-range correlations to the methylene protons. Thus, these ^1H and ^{13}C NMR spectral data were indicative for the presence of a $-CH_2-CN$ group on the indole moiety.

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0031-9422/99/$ - see front matter
PII: S0031-9422(98)00669-4

Table 1
^{1}H and ^{13}C NMR data for compounds **1**, **1a** and **2** ($^{1}H = 600$ MHz; $^{13}C = 150$ MHz, **1** and **2** in DMSO-d_6, **1a** in $CDCl_3$)[a]

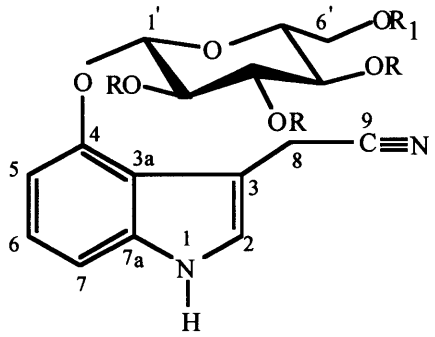
Position		**1**		**1a**	**2**	
		δ_H, J (Hz)	δ_C	δ_H	δ_H, J (Hz)	δ_C
1	NH	11.1 d (1.3)	–	8.19 d (1.2)	11.0 d (1.3)	–
2	CH	7.20 d (1.3)	123.6	7.20 d (1.2)	7.17 d (1.3)	123.4
3	C	–	104.6	–	–	104.2
3a	C	–	117.5	–	–	116.9
4	C	–	152.7	–	–	151.9
5	CH	6.69 dd (7.4/1.1)	104.2	6.62 dd (6.3/2.3)	6.77 dd (6/2.6)	104.1
6	CH	7.00[b]	123.3	7.10[b]	7.01[b]	123.4
7	CH	6.99[b]	106.9	7.10[b]	7.01[b]	106.6
7a	C	–	138.8	–	–	138.4
8	CH_2	4.17 AB system ($J_{AB} = 18.5$)	15.7	4.00 dd (3.5/1.0)	4.12 AB system ($J_{AB} = 18.4$)	15.4
9	C	–	121.0	–	–	120.9
Glucose						
1′	CH	4.89 d (7.8)	102.1	5.42–5.33	4.89 d (7.8)	101.3
2′	CH	3.35[b]	74.4	5.42–5.33	3.40[b]	72.5
3′	CH	3.33[b]	77.5	5.42–5.33	2.98[b]	74.0
4′	CH	3.23 t (9.3)	70.6	5.22 dd (9.9/9.2)	3.02[b]	70.6
5′	CH	3.29[b]	77.9	3.92 m	3.60[b]	76.3
6′	CH_2	3.73 dd (11.8/1.9)	61.6	4.16 dd (12.3/2.4)	4.0 br d (10.6)	68.7
		3.50 dd (11.8/5.8)		4.30 dd (12.3/5.3)	3.63[b]	
Terminal glucose						
1″	CH				4.26 d (7.8)	103.5
2″	CH				3.39[b]	73.9
3″	CH				3.10[b]	76.9
4″	CH				3.22[b]	70.1
5″	CH				3.00[b]	77.1
6″	CH_2				3.64[b]	61.5
					3.40[b]	
Aliphatic						
OAc				2.09 (3H)		
				2.07 (6H)		
				2.04 (3H)		

[a] Assignments are based on DEPT, COSY, HSQC and HMBC.
[b] Signal pattern unclear due to overlapping.

Furthermore, the proton and carbon resonances at δ 4.89 (d, $J = 7.8$ Hz) and δ 102.1, together with the other resonances in the same spin-system, indicated the presence of a glucose moiety. The positions of these two substituents were determined by a HMBC experiment. Long-range correlations were observed from methylene protons (δ 4.17 (H_2-8)) to carbons at δ 123.6 (C-2), 104.6 (C-3), 117.5 (C-3a) and from the anomeric proton of glucose (δ 4.89, H-1′) to an oxygenated aromatic carbon at δ 152.7 (C-4). The doublet of the methine proton at δ 7.20 (d, $J = 1.3$ Hz, H-2) was also long-range coupled to carbons at δ 104.6 (C-3), 15.7 (C-9), 117.5 (C-3a) and 138.8 (C-7a). Furthermore, correlations were observed from the proton resonance assigned as H-1 (δ 11.1, d, $J = 1.3$ Hz) to the sp^2 quaternary carbons at δ 104.6 (C-3), 117.5 (C-3a) and 138.8 (C-7a) and to the carbon at δ 123.6 (C-2). All other significant long-range correlations are shown on Fig. 1. These HMBC data indicated that the acetonitrile and glucose units should be attached at C-3 and C-4, respectively. This observation was also supported by a NOESY experiment; NOE correlations were observed from H-1 to H-2 and H-7, and from H-1′ (anomeric proton of glucose) to H-5 (δ 6.69 dd, $J = 7.4$ and 1.1 Hz).

Acetylation of **1** yielded a tetra-*O*-acetyl derivative (**1a**). The ^{1}H NMR spectrum of **1a** exhibited only four aliphatic acetoxyl resonances arising from the glucose moiety. All the other resonances supported the proposed structure for **1** Table 1. Thus, the structure of **1** was determined to be 1*H*-indole-3-acetonitrile 4-*O*-β-glucopyranoside.

Compound **2** was obtained as an amorphous colourless powder. The ESI-mass spectrum exhibited a $[M + Na]^+$ peak at m/z 519 corresponding to a molecular formula of



1: $R = R_1 = H$
1a: $R = R_1 = COCH_3$
2: $R = H, R_1 = glucose$

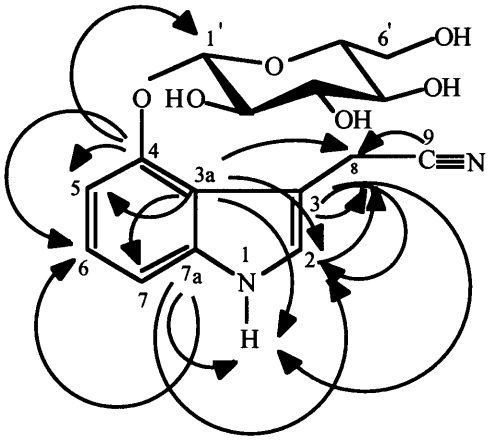


Fig. 1. HMBC od Cappariloside A.

$C_{22}H_{28}N_2O_{11}$. The UV (λ_{max} (MeOH): 272, 279 (sh) and 289 nm) and IR (ν_{max} (KBr): 3390, 2855, 2255, 1625, 1540, 1510 and 1120 cm^{-1}) spectra were similar to those of **1**. The ^1H and ^{13}C NMR spectra of **2** Table 1 displayed many similarities with those of **1**, especially for the resonances assigned to the indole-3-acetonitrile moiety and the glucose unit. However, the set of additional protons, apart from the β-anomeric proton at δ 4.26 (d, $J=7.8$ Hz) and the corresponding carbon signals, were in agreement with the presence of another hexose unit, which was identified as β-glucose by COSY and HSQC experiments. In the ^{13}C NMR spectrum of **2**, the presence of a typical carbon signal at δ 68.7 (CH_2) suggested that the additional glucose unit was attached to C-6′ of the other glucose unit, since the down-field shift (7.1 ppm) in comparison to that of **1** is due to the α-effect of glycosidation. Furthermore, in the ^1H NMR spectrum of **2**, the chemical shift values for the anomeric protons of two glucose units at δ 4.89 (H-1′) and 4.26 (H-1″) suggested the sites of glycosidation of the sugar units should be on the aromatic and aliphatic hydroxyl groups, respectively. Thus, these data supported the presence of a biosidic unit, 6-*O*-β-glucopyranosyl-glucose, on the indole moiety. This observation was further confirmed by an HMBC experiment, which showed long-range correlations between C-6′ (δ 68.7) and H-1″ (δ 4.26, d, $J=7.8$ Hz, anomeric proton of terminal glucose) and C-4 (δ 151.9) of the indole moiety and H-1′ (δ 4.89, d, $J=7.8$ Hz, anomeric proton of the inner glucose). Consequently, compound **2** was established as 1*H*-indole-3-acetonitrile 4-*O*-β-(6′-*O*-β-glucopyranosyl)-glucopyranoside.

Eventhough indoleacetonitriles are known as thermal degradation products of indole glucosinolates (Slominski & Campbell, 1988), compounds **1** and **2**, 1*H*-indole-3-acetonitrile glycosides were isolated from nature for the first time, for which capparilosides A and B are proposed as trivial names, respectively.

3. Experimental

3.1. General

UV were determined in MeOH and IR in KBr disks. NMR spectra were recorded in DMSO-d_6 at 600 MHz for ^1H and 150 MHz for ^{13}C. Chemical shifts are given in δ relative to TMS as int. ref. Complete proton and carbon assignments were based on 1D (^1H, ^{13}C and DEPT) and 2D (^1H–^1H COSY, ^1H–^{13}C HSQC and ^1H–^{13}C HMBC) NMR experiments. TLC was carried out on pre-coated silica gel 60F-254 aluminium sheets (Merck). For CC, silica gel 60 (0.063–0.200 mm, Merck) was used. Compounds were detected by UV fluorescence and/or after spraying with vanillin–H_2SO_4 followed by heating at 100°C for 5–10 min.

3.2. Plant material

Mature fruits of *C. spinosa* L. were collected from Mut-Içel, Turkey in September, 1993. A voucher specimen has been deposited in the Herbarium of Pharmaceutical Botany, Faculty of Pharmacy, Hacettepe University (HUEF 94-008).

3.3. Extraction and isolation

Plant material was stored frozen at −20°C. Freeze-dried (1.2 kg) and sliced plant material was homogenized in MeOH (2 × 2.5 l) and kept overnight at room temp. The combined MeOH extracts were concd to dryness in vacuo. The H_2O-sol. part of the MeOH extract was chromatographed over LiChroprep RP-18 (VLC) using a H_2O–MeOH gradient. The frs eluted with 50% MeOH

were purified repeatedly by CC on silica gel using $CHCl_3$–MeOH–H_2O mix. (85:15:1–80:20:2–70:30:3) to yield compounds **1** (80 mg) and **2** (28 mg).

3.4. Capparilosiside A (*1*)

Amorphous $[\alpha]^{20}_D$ −58.8° (*c* 0.4, MeOH). FAB-MS *m/z* 357 $[M+Na]^+$, positive ion-ESI-MS *m/z* 357 $[M+Na]^+$, 691 $[2M+Na]^+$, negative ion-ESI-MS *m/z* 333 $[M-H]^-$, 667 $[2M-H]^-$ (calcd for $C_{16}H_{18}N_2O_6$: 334.32). UV λ_{max} (MeOH) nm: 267, 278, 289. IR ν_{max} (KBr) cm^{-1}: 3525, 3495, 3400, 3359 (OH, NH), 2250 (CN), 1625, 1590, 1508 (arom.), 1170, 1084 (C–O–C). ^1H and ^{13}C NMR (DMSO-d_6): Table 1.

3.5. Acetylation of *1*

Treatment of **1** (8 mg) with Ac_2O (1 ml) and pyridine (1 ml) at room temp. overnight, followed by the usual work-up, yielded **1a**. ^1H NMR ($CDCl_3$): Table 1.

3.6. Capparilosiside B (*2*)

Amorphous $[\alpha]^{20}_D$ −23.7° (*c* 0.3, MeOH). ESI-MS *m/z* 519 $[M+Na]^+$. UV λ_{max} (MeOH) nm: 272, 279, 289. IR ν_{max} (KBr) cm^{-1}: 3390 (OH, NH), 2855, 2255 (CN), 1625, 1540, 1510 (arom.), 1120. ^1H and ^{13}C NMR (DMSO-d_6): Table 1.

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