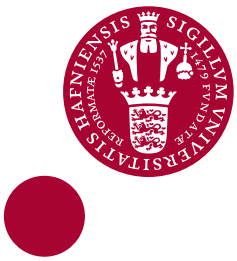


Structural analysis of complex saponins of Balanites aegyptiaca by 800 MHz ¹H NMR spectroscopy

Stærk, Dan; Chapagain, Bishnu P.; lindin, therese; Wiesman, Zeev; Jaroszewski, Jerzy W.

Published in:
Magnetic Resonance in Chemistry

DOI:
10.1002/mrc.1879

Publication date:
2006

MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. 2006; **44**: 923–928
Published online 18 July 2006 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/mrc.1879

MRC

Structural analysis of complex saponins of *Balanites aegyptiaca* by 800 MHz ^{1}H NMR spectroscopy

Dan Stærk,[1]* Bishnu P. Chapagain,[2] Therese Lindin,[1] Zeev Wiesman[2] and Jerzy W. Jaroszewski[1]

[1] Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark
[2] Phyto-Lipid Biotechnology Laboratory, Department of Biotechnology Engineering, The Institutes for Applied Research, Ben-Gurion University of the Negev, P. O. Box 653, Beer Sheva 84105, Israel

Received 15 May 2006; Revised 13 June 2006; Accepted 14 June 2006

The main saponin (1) present in the mesocarp of *Balanites aegyptiaca* fruit is a mixture of 22*R* and 22*S* epimers of 26-(*O*-*β*-D-glucopyranosyl)-3-*β*-[4-*O*-(*β*-D-glucopyranosyl)-2-*O*-(*α*-L-rhamnopyranosyl)-*β*-D-glucopyranosyloxy]-22,26-dihydroxyfurost-5-ene. This structure differs from a previously reported saponin isolated from this source by the site of attachment of the rhamnosyl residue, and presumably represents a structural revision of the latter. The main saponin (2) present in the kernel is a xylopyranosyl derivative of 1. The use of high-field NMR enabled the practically complete assignment of ^{1}H and ^{13}C chemical shifts of these complex saponins, existing as a mixture of C-22 epimers. Moreover, the work represents a new approach to structural elucidation of saponins: direct preparative-scale HPLC-RID of crude extracts followed by high-field NMR investigations supported by ESI-MS^n. Copyright © 2006 John Wiley & Sons, Ltd.

Supplementary electronic material for this paper is available in Wiley InterScience at http://www.interscience.wiley.com/jpages/0749-1581/suppmat/

KEYWORDS: NMR; ^{1}H; ^{13}C; ESI-MS^n; structural analysis; natural products; saponins

INTRODUCTION

Balanites aegyptiaca (L.) Delile (Zygophyllaceae), popularly known as desert date, is a spiny, evergreen tree commonly grown in the arid regions of Africa, the Middle East, India and Burma.[1] Numerous medicinal uses have been reported for this plant.[2] The saponins present in the fruits show molluscicidal activity, including toxicity against snails carrying the Bilharziasis parasites, and it has been suggested that the trees be planted along infested waters so that the fruits could drop into the water spontaneously.[2] Several studies on *B. aegyptiaca* saponins have been reported, describing the content of the spirostan saponins in the root, bark and seeds,[3,4] furostan saponins in the fruit[5–7] and the root,[8] and pregnane derivatives in the fruit.[9]

Saponins are a widely distributed group of secondary metabolites found in plants with numerous uses, traditional as well as modern.[10,11] The complexity and structural diversity of saponins present a considerable challenge to chromatographic separation[12] and to structural elucidation.[10,11] Traditionally, structure elucidation of saponins often required extensive chemical derivatization and degradation in order to identify the aglycone, the number and type of sugar residues present, and their mutual connections. In this article, we describe a procedure for rapid identification of complex saponins from *B. aegyptiaca* fruits based on preparative-scale reversed-phase HPLC, ESI-MS^n, and high-field NMR.

*Correspondence to: Dan Stærk, Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark.
E-mail: ds@dfuni.dk

RESULTS AND DISCUSSION

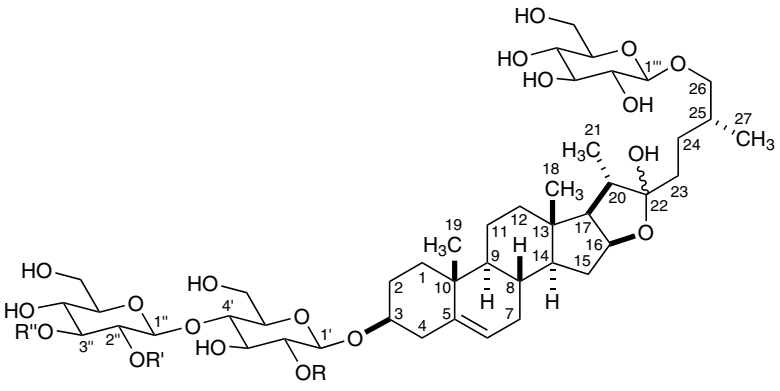
The mesocarps were separated manually and extracted with cold methanol. The extracts were defatted and resolved directly on a preparative HPLC column packed with octadecylsilyl silica, using methanol–water (7 : 3) as eluent and a refractive index detector (RID). This procedure, which contrasts elaborate fractionation schemes used in the previous studies,[3–8] enabled rapid purification of *B. aegyptiaca* saponins using a minimum number of manipulations and chromatographic separation steps. The extract contained a large proportion of sugars, which were eluted with the front band, whereas saponins were differentially retarded on the column, yielding pure compounds in a single purification step.

The main saponin of *B. aegyptiaca* fruit mesocarp, isolated in a yield corresponding to 0.55% of the mesocarp weight, was injected directly into an electrospray ionization mass spectrometer (ESI-MS) operating in the negative-ion mode.[13] Multi-stage experiments (MS^n) showed that the molecular ion ($[M-H]^-$, m/z 1063) was cleaved with a loss of Glc (glucose) and Rha (rhamnose), as well as of (Glc + H_2O), (Glc + Rha), (Glc + Rha + H_2O), (2Glc + Rha + H_2O),

(3Glc + Rha), and (3Glc + Rha + H_2O). The intensity of the peak at m/z 901 ([M − H − Glc]$^-$) was somewhat higher than that at m/z 917 ([M − H − Rha]$^-$), corresponding to preferential expulsion of Glc from the C-26 position of the [M − H]$^-$ ion.[13] The MS^3 spectrum of the [M − H − Glc]$^-$ ion (m/z 901) produced ions corresponding to the loss of Rha (m/z 755), Glc (m/z 739), and (Glc + Rha) (m/z 593). Further fragmentation (MS^4) of the [M − H − Glc − Rha]$^-$ ion (m/z 755) resulted in the loss of H_2O (m/z 737), Glc (m/z 593), and (Glc + H_2O) (m/z 575). In addition, MS of the [M − H − Glc]$^-$, [M − H − Glc − Rha]$^-$, and [M − H − Glc − Rha − Glc]$^-$ ions showed elimination of H_2O, but the resulting ions always had low intensity.

The ^{13}C NMR spectra of the mesocarp saponin showed four quaternary carbon resonances corresponding to C-5, C-10, C-13, and C-22 of a furostan. ^1H NMR spectra at 800 MHz provided excellent dispersion of the resonances, and showed a characteristic signal of the axially positioned H-3 (δ 3.59, triplet of triplets, $J_{3,2ax} = J_{3,4ax} = 11.6$ Hz, $J_{3,2eq} = J_{3,4eq} = 4.6$ Hz), an olefinic signal of H-6 at δ 5.38, and signals of five methyl groups. The resonances for the methyl groups were observed as two singlets (H-18 and H-19) and two doublets (H-21 and H-27) corresponding to the aglycone, and a doublet attributable to the methyl group of rhamnose. The presence of 13 methylene groups and 29 methine groups was inferred from a DEPT135 spectrum. Resonances of three anomeric doublets (δ 4.23, 4.39, and 4.51, $J = 7.8$–7.9 Hz) were attributable to glucose residues, whereas a doublet at δ 5.24 ($J = 1.5$ Hz) was compatible with the presence of a rhamnose residue. Homonuclear (COSY, NOESY) and heteronuclear (HSQC, HMBC) correlations obtained at 800 MHz allowed assignment of the entire set of ^1H and ^{13}C resonances, identifying the main saponin as **1** (Fig. 1, Table 1). In particular, the resonance of C-3 (δ 79.4) showed long-range correlation to H-1′ (δ 4.51) and the latter a COSY correlation to H-2′ (δ 3.42), providing chemical shift of C-2′ (δ 78.7) from the HSQC spectrum. The resonance of C-2′ showed a HMBC correlation to H-1‴′ (δ 5.24), establishing the attachment point of rhamnose. The signal of C-4′ (δ 81.0) showed HMBC correlation to H-1″ (δ 4.39), which established the attachment point of the second glucose moiety. The last glucose residue was attached to C-26 (δ 76.1), as shown by HMBC correlation between C-26 and H-1‴ (δ 4.23). Numerous NOESY correlations confirmed the structure, e.g. NOEs between H-1′ and H-4eq, H-1″ and H-4′, and between H-1‴′ and H-2′. That the saponin was a mixture of 22*R* and 22*S* epimeric hemiacetals (ratio about 1 : 1) was evident from the fact that H-16 appeared as two quartets (δ 4.37 and 4.56, $J = 7.6$ Hz), whereas H-20 gave two pentets (δ 2.10 and 2.18, $J = 6.7$ Hz), and the signals of carbon atoms around C-22 were split into two closely spaced resonances (Table 1). 2D NMR connectivities (800 MHz COSY, NOESY, and HMBC) for **1** and **2** are given in the Supplementary Tables S1 and S2. The saponin was a pure 25*R* epimer, as shown by the resonance of H-27 and the chemical shift difference between the diastereotopic H-26 hydrogens, only a single pair of which was present in the spectrum.[14,15] The order of sugar residues established by NMR is in agreement with the fragmentation pattern observed in the ESI-MS^n experiments.

The major saponin (**2**) isolated from *B. aegyptiaca* fruit kernels contained an additional pentose residue (m/z 1195, [M − H]$^-$), identified as xylopyranosyl attached to *O*-3″ on the basis of 800 MHz 1D and 2D NMR spectra. Thus, the resonance of H-1‴″ of the xylopyranose moiety (δ 4.50, $J = 7.6$ Hz) correlated with that of C-3″ (δ 87.7), and an NOE



1 R = α-L-rhamnopyranosyl, R' = R'' = H
2 R = α-L-rhamnopyranosyl, R' = H, R'' = β-D-xylopyranosyl
3 R = R'' = H, R' = α-L-rhamnopyranosyl

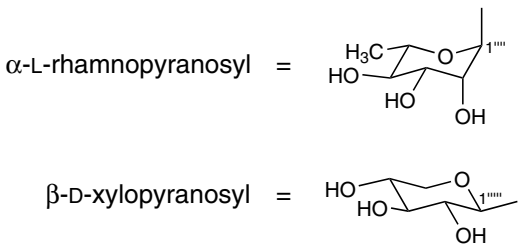


Figure 1. Structure of compounds **1**–**3**.

DOI: 10.1002/mrc

Table 1. 800 MHz ^{1}H and 100 MHz ^{13}C NMR spectroscopic data for *Balanites aegyptiaca* fruit saponins **1** and **2** (CD_3OD)[a]

	1		**2**	
Position	^{13}C	^{1}H	^{13}C	^{1}H
1	38.6	ax. 1.08 (m), eq. 1.88 (dt), $J_{1eq,1ax} = 13.4$, $J_{1eq,2eq} \approx J_{1eq,2ax} \approx 3.5$	38.6	ax. 1.08 (m), eq. 1.88 (dt), $J_{1eq,1ax} = 13.4$, $J_{1eq,2eq} \approx J_{1eq,2ax} \approx 3.5$
2	30.8	ax. 1.61, eq. 1.92	30.8	ax. 1.61, eq. 1.93
3	79.4	3.59 (tt), $J_{3,2ax} = J_{3,4ax} = 11.6$, $J_{3,2eq} = J_{3,4eq} = 4.6$	79.4	3.59 (tt), $J_{3,2ax} = J_{3,4ax} = 11.6$, $J_{3,2eq} = J_{3,4eq} = 4.6$
4	39.6	ax. 2.29 (ddd), eq. 2.44 (ddd), $J_{4eq,4ax} = 13.4$, $J_{4ax,3} = 12.0$, $J_{4eq,3} = 4.4$, $J_{4eq,2eq} \approx J_{4ax,6} \approx 2.4$	39.6	ax. 2.29 (ddd), eq. 2.44 (ddd), $J_{4eq,4ax} = 13.4$, $J_{4ax,3} = 12.0$, $J_{4eq,3} = 4.4$, $J_{4eq,2eq} \approx J_{4ax,6} \approx 2.4$
5	142.0	–	141.9	–
6	122.7	5.38 (m)	122.7	5.37 (m)
7	33.2	ax. 1.55, eq. 2.00	33.2	ax. 1.56, eq. 2.00
8	32.8	1.67	32.8	1.65
9	51.8	0.97	51.8	0.97
10	38.1	–	38.1	–
11	22.0	ax. 1.52, eq. 1.57	22.0	ax. 1.51, eq. 1.56
12	40.9	ax. 1.20, eq. 1.78	40.9	ax. 1.20, eq. 1.78
13	41.9	–	41.9	–
14	57.8	1.14	57.8	1.14
15	33.2	1.27, 1.97	33.0	1.27, 1.97
16	82.3,[b] 82.5[c]	4.37 (q),[b] 4.56[c] (q), $J_{16,15A} \approx J_{16,15B} \approx J_{16,17} \approx 7.6$	82.3,[b] 82.5[c]	4.37 (q),[b] 4.57[c] (q), $J_{16,15A} \approx J_{16,15B} \approx J_{16,17} \approx 7.6$
17	64.1,[b] 65.2[c]	1.73,[b] 1.77[c]	64.1,[b] 65.1[c]	1.73,[b] 1.78[c]
18	16.8	0.83 (s)	16.9	0.83 (s)
19	19.9	1.05 (s)	19.9	1.05 (s)
20	40.9,[b] 41.0[c]	2.10 (p),[b] 2.18[c] (p), $J_{20,21} = J_{20,17} = 6.7$	41.0,[b] 41.1[c]	2.10 (p),[b] 2.18[c] (p), $J_{20,21} = J_{20,17} = 6.7$
21	16.3	1.00 (d), $J_{21,20} = 7.0$	16.0,[b] 16.2[c]	1.00 (d), $J_{21,20} = 7.0$
22	112.0,[b] 114.0[c]	–	112.0,[b] 114.0[c]	–
23	31.5	1.68, 1.76	31.4	1.64, 1.83
24	28.7,[b] 29.0[c]	1.15 and 1.58,[b] 1.27 and 1.62[c]	29.0[b] 28.7[c]	1.15 and 1.59,[b] 1.28 and 1.62[c]
25	35.1	1.74	35.0	1.75
26	76.1	3.36,[b] 3.74[c] (dd), $J_{26A,26B} = 9.5$, $J_{26,25} = 6.3$	76.3	3.38,[b] 3.74[c] (dd), $J_{26A,26B} = 9.6$, $J_{26,25} = 6.4$
27	17.5	0.95 (d), $J_{27,25} = 6.7$	17.3,[b] 17.4[c]	0.95 (d), $J_{27,25} = 6.7$
1′	100.5	4.51 (d), $J_{1',2'} = 7.9$	100.5	4.51 (d), $J_{1',2'} = 7.9$
2′	78.7	3.42 (dd), $J_{2',3'} = 9.0$, $J_{2',1'} = 7.9$	78.6	3.42 (dd), $J_{2',3'} = 8.9$, $J_{2',1'} = 7.9$
3′	77.9	3.65	77.8	3.65
4′	81.0	3.54 (t), $J_{4',3'} = J_{4',5'} = 9.0$	81.1	3.56
5′	76.3	3.39	76.3	3.39
6′	62.0	3.83 and 3.87	62.0	3.83 and 3.88
1″	104.7	4.39 (d), $J_{1'',2''} = 7.9$	104.3	4.46 (d), $J_{1'',2''} = 7.8$
2″	75.1	3.20	74.4	3.41
3″	78.0	3.36	87.7	3.52
4″	71.5	3.28	69.7	3.37
5″	78.0	3.24	78.2	3.66
6″	62.5	3.66 and 3.88	62.4	3.65 and 3.89
1‴	104.7	4.23 (d), $J_{1''',2'''} = 7.8$	104.6	4.24 (d), $J_{1''',2'''} = 7.8$
2‴	75.2	3.18	75.2	3.18 (dd), $J_{2''',3'''} = 9.3$, $J_{2''',1'''} = 7.8$
3‴	78.2	3.34	77.8	3.35
4‴	71.8	3.34	71.7	3.33
5‴	78.2	3.25	77.9	3.25
6‴	62.9	3.66 and 3.86	62.9	3.67 and 3.87
1⁗	102.1	5.24 (d), $J_{1'''',2''''} = 1.5$	102.1	5.24 (d), $J_{1'''',2''''} = 1.5$
2⁗	72.3	3.89	72.2	3.90
3⁗	72.4	3.66	72.4	3.66

(*continued overleaf*)

DOI: 10.1002/mrc

Table 1. (*Continued*)

Position	1		2	
	^{13}C	^{1}H	^{13}C	^{1}H[b,c]
4‴″	74.0	3.39	74.0	3.39
5‴″	69.8	4.12 (m)	69.7	4.13
6‴″	18.0	1.23 (d), $J_{6''''',5''''} = 6.3$	18.0	1.24 (d), $J_{6''''',5''''} = 6.3$
1‴″	–	–	106.0	4.50 (d), $J_{1''''',2''''} = 7.6$
2‴″	–	–	75.3	3.27
3‴″	–	–	78.2	3.34
4‴″	–	–	71.0	3.51
5‴″	–	–	67.2	3.24 and 3.91

[a] Assignments supported by 800 MHz 2D NMR data; chemical shift values without stated multiplicity, due to overlap in the ^{1}H NMR spectrum, were obtained from 2D correlations; multiplicities given as apparent splittings: d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; coupling constants are given as line separations in Hz; ax. and eq. designate axial and equatorial hydrogen atom, respectively.

[b,c] Different values for C-22 epimers.

between H-1‴″ and H-3″ (δ 3.52) was observed. Apart from these differences, the spectra of **1** and **2** were very similar, with the largest differences observed for the glucose residue bearing the xylopyranose ring (Table 1). All hydrogen atoms of the five sugar moieties could be uniquely assigned from 2D correlations in spite of considerable signal overlap (Fig. 2). Compound **2** was the major saponin of *B. aegyptiaca* fruit kernels corresponding to 0.27%, but significant amounts of **1** (0.22%) were also present in the kernels. In addition to **1** and **2**, all extracts contained small amounts of their derivatives in which the hydroxy group attached to C-22 was exchanged for a methoxy group, presumably during extraction of the plant material with methanol.

Previous investigations of *B. aegyptiaca* fruit resulted in a report of saponin **3**, named balanitoside and differing from **1** by the position of attachment of the rhamnose residue

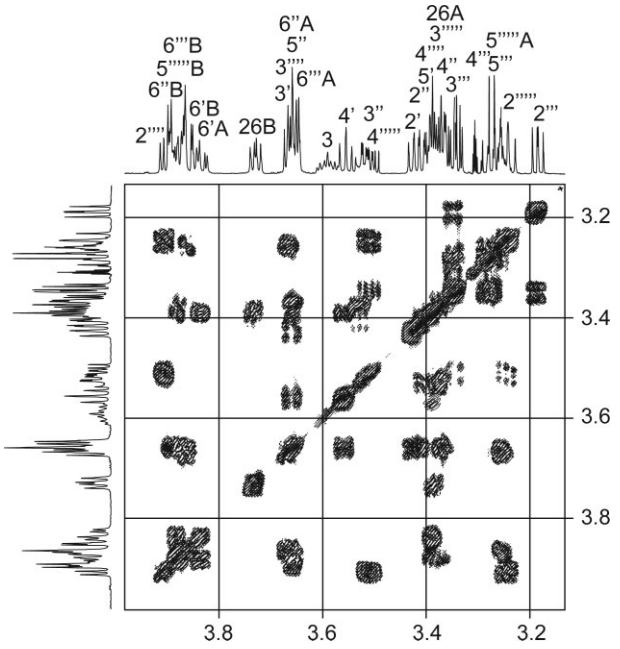


Figure 2. Fragment of 800 MHz ^{1}H NMR spectrum of **2** showing signals of the carbohydrate residues (CD_3OD).

(*O*-2″ rather than *O*-2′).[6] The structure of **3** was based on acetylation-induced shifts without rigorous assignment of all carbon resonances.[6] Unfortunately, ^{13}C NMR data reported for balanitoside do not allow any certain conclusions about its identity with **1**, and the optical rotation of balanitoside was not reported.[6] However, since **1** is the major saponin of *B. aegyptiaca* fruit, we believe that it represents a structural revision of balanitoside. The saponin **1** was previously isolated several times from other plants: *Dioscorea deltoides* Wall. (the saponin was named deltoside),[16] *Ophiopogon planiscapus* Nakai (glycoside F),[17] *Rhapis* species and *Sabal causiarum* Becc. (as a 22-methoxy derivative designated methyl protodeltonin),[18,19] *Lilium* species,[20–22] *Trigonella foenum-graecum* L. (along with its 25*S* epimer named *trigoneoside IVa*),[23] and *Allium nutans* L.[24] Compound **2** was previously reported as a mixture of 25*S* and 25*R* epimers in *B. aegyptiaca* root,[8] but the pure 25*R* epimer was not isolated prior to this work.

The present work, in addition to elucidation of structures of *B. aegyptiaca* saponins, describes a useful, compact protocol for rapid isolation of saponins based on HPLC-RID, and demonstrates the power of high-field NMR spectroscopy, supported by ESI-MS^n, for unambiguous *de novo* structure determination of complex saponins. Structure elucidation of complex saponins usually requires chemical manipulation, typically acid hydrolysis for separate analysis of aglycones and sugars or acetylation in order to increase dispersion of ^{1}H NMR resonances.[25–35] Unambiguous structure elucidation, including complete relative stereochemistry and sugar connectivities as shown in the present work, can be performed only seldom with instrumental methods alone.[36–38]

EXPERIMENTAL

General experimental procedures

NMR spectra were recorded at 25 °C using CD_3OD as solvent and TMS as internal reference. Proton-decoupled ^{13}C NMR and DEPT135 spectra were acquired on a Bruker Avance 400 spectrometer (carbon frequency 100.62 MHz) equipped with a 5-mm, normal-configuration, dual ^{1}H–^{13}C

DOI: 10.1002/mrc

probe. A total of 10k transients with 105 260 data points were collected using a spectral width of 26 316 Hz for the proton-decoupled ^{13}C NMR spectrum and 8k transients with 71 426 data points were collected using a spectral width of 23 809 Hz for the DEPT135 spectrum. All 1D ^{1}H NMR and homo- and heteronuclear 2D NMR experiments were performed on a Varian Unity Inova 800 spectrometer (proton frequency 799.798 MHz) equipped with a 5-mm, inverse-detection, triple-resonance probe, using standard Varian pulse programs. For 1D ^{1}H NMR spectra of **1** and **2**, 256 and 128 transients, respectively, were recorded with a spectral width of 10 000 Hz using 37 840 data points. Phase-sensitive $^{1}H-^{1}H$ COSY and NOESY experiments were performed with a spectral width of 4685 Hz using 2k data points for f_2 and 512 increments for f_1, and a relaxation delay of 1 s. A total of 32 and 16 transients for **1** and **2**, respectively, were collected. NOESY spectra were obtained with mixing times of 700 ms. Gradient-selected, sensitivity-enhanced $^{1}H-^{13}C$ HSQC and gradient-selected HMBC experiments were performed with a spectral width of 4685 Hz using 2k data points for f_2, and a spectral width of 30 036 Hz using 512 increments for f_1. The HSQC experiment was optimized for $^{1}J_{C,H} = 145$ Hz, and the HMBC experiment for $^{n}J_{C,H} = 5$ Hz. Relaxation delays of 1 s were used and a total of 96 transients were recorded for each f_1 increment. Data processing was carried out on an SGI workstation using Bruker XWINNMR version 3.1 software.

MS^n experiments were performed with a Bruker Daltonik Esquire 3000 Plus single ion-trap mass spectrometer equipped with an electrospray interface (ESI) and operating in the negative-ion mode. MS conditions were optimized in order to achieve maximum sensitivity. Full-scan spectra from m/z 25 to 1500 were obtained with scan time 1 s. The ion-trap condition was acquisition in automatic gain control mode with an accumulation time 159 μs. For the MS^n analysis, ions were isolated with an isolation width of 3 m/z units and fragmented using an activation amplitude of 0.95–1.12. Preparative HPLC separations were performed on a 25 × 2.12 cm i.d. Phenomenex Luna C18 (2) 5 μm column eluted isocratically with 5 ml/min of MeOH–H_2O (7 : 3), using a Shimadzu RID-6A refractive index detector. Optical rotations were measured on a Perkin Elmer model 241 polarimeter.

Plant material

Ripe fruits of *B aegyptiaca* were collected from an orchard in Samar, southern Israel, and authenticated by Prof. Uzi Plitman; a voucher specimen (no. 76 816) was deposited in Herbarium HUJ (Department of Evolution, Systematics and Ecology, Hebrew University of Jerusalem, Israel).

Extraction and isolation

The epicarps (outer covers) of the fruits were removed, after which the mesocarps were collected (total of 414 g), and extracted with 2 × 750 ml of MeOH. The combined extracts were concentrated, and the residue (312 g) was dissolved in H_2O (400 ml) and defatted by shaking with 3 × 300 ml of light petroleum (b.p. 60–80 °C). A portion (1.14 g) of the residue (250 g), dissolved in MeOH–H_2O (7 : 3), was repeatedly injected into the preparative HPLC column (200 mg of extract per injection). Yield 8.4 mg of **1** ($t_R = 35$ min), corresponding to 0.55% of the mesocarp; $[\alpha]_D^{25} - 68.6°$ (*c* 0.55, MeOH), lit.[16] −51.2° (*c* 0.72, pyridine). Using a similar procedure, 55 g of kernels separated from shells (endocarps) yielded 8.5 g of extract, defatted by shaking with 3 × 150 ml of light petroleum; fractionation of 600 mg sample of the extract (total amount 6.3 g) by preparative HPLC as above gave 12 mg (0.22%) of **1** and 18 mg (0.27%) of **2** ($t_R = 44$ min); $[\alpha]_D^{25} - 72.5°$ (*c* 0.45, MeOH). The compounds were freeze-dried from D_2O for NMR analysis.

Supplementary material

Supplementary electronic material for this paper is available in Wiley InterScience at: http://www.interscience.wiley.com/jpages/0749-1581/suppmat/

Acknowledgments

The 800 MHz NMR spectra were obtained using the Varian Unity Inova spectrometer of the Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules, Copenhagen. The MS analyses were performed at the Interdisciplinary Equipment Unit, Ben-Gurion University of the Negev. The authors like to thank Mr. Igal Gilboa for valuable assistance with the MS analysis. We are grateful to the personnel of the Kibbutz Samar, Israel, for maintaining *B. aegyptiaca* plants. This study was supported by the Dibner Fund.

REFERENCES

1. Hall JB, Walker DH. *Balanites Aegyptiaca: A Monograph*. University of Wales: Banger, 1991.
2. Iwu MM. *Handbook of African Medicinal Plants*. CRC Press: Boca Raton, 1993.
3. Liu HW, Nakanishi K. *Tetrahedron* 1982; **38**: 513.
4. Pettit GR, Doubek DL, Herald DL, Numata A, Takahashi C, Fujiki R, Miyamoto T. *J. Nat. Prod.* 1991; **54**: 1491.
5. Kamel MS, Ohtani K, Kurokawa T, Assaf MH, El-Shanawany MA, Ali AA, Kasai R, Ishibashi S, Tanaka O. *Chem. Pharm. Bull.* 1991; **39**: 1229.
6. Hosny M, Khalifa T, Çalis I, Wright AD, Sticher O. *Phytochemistry* 1992; **31**: 3565.
7. Kamel MS, Koskinen A. *Phytochemistry* 1995; **40**: 1773.
8. Farid H, Haslinger E, Kunert O, Wegner C, Hamburger M. *Helv. Chim. Acta* 2002; **85**: 1019.
9. Kamel MS. *Phytochemistry* 1998; **48**: 755.
10. Hostettmann K, Marston A. *Saponins*. Cambridge University Press: Cambridge, 1995.
11. Oleszek W, Marston A (eds). *Saponins in Food Feedstuffs and Medicinal Plants*. Kluver Academic Publishers: Dordrecht, 2000.
12. Oleszek WA. *J. Chromatogr., A* 2002; **967**: 147.
13. Liu Y, Liang F, Cui L, Xia M, Zhao L, Yang Y, Shi J, Abliz Z. *Rapid Commun. Mass Spectrom.* 2004; **18**: 235.
14. Agrawal PK. *Magn. Reson. Chem.* 2004; **42**: 990.
15. Agrawal PK. *Steroids* 2005; **70**: 715.
16. Sviridov AF, Paseshnichenko VA, Kadentsev VI, Chizhov OS. *Bull. Acad. Sci. USSR, Div. Chem. Sci.* 1975; **24**: 80.
17. Watanabe Y, Sanada S, Ida Y, Shoji J. *Chem. Pharm. Bull.* 1983; **31**: 3486.
18. Harai Y, Sanada S, Ida Y, Shoji J. *Chem. Pharm. Bull.* 1984; **32**: 4003.
19. Idaka K, Hirai Y, Shoji J. *Chem. Pharm. Bull.* 1986; **34**: 1783.
20. Ori K, Mimaki Y, Mito K, Sashida Y, Nikaido T, Ohmoto T, Masuko A. *Phytochemistry* 1992; **31**: 2767.
21. Mimaki Y, Satou T, Kuroda M, Sashida Y, Hatakeyama Y. *Chem. Pharm. Bull.* 1998; **46**: 1829.
22. Mimaki Y, Nakamura O, Sashida Y, Satomi Y, Nishino A, Nishino H. *Phytochemistry* 1994; **37**: 227.
23. Yoshikawa M, Murakami T, Komatsu H, Yamahara J, Matsuda H. *Heterocycles* 1998; **47**: 397.

24. Akhov LS, Musienko MM, Piacente S, Pizza C, Oleszek W. *J. Agric. Food Chem.* 1999; **47**: 3193.
25. Ye W, Liu X, Zhang Q, Che CT, Zhao S. *J. Nat. Prod.* 2001; **64**: 232.
26. Plock A, Beyer G, Hiller K, Grundemann E, Krause E, Nimtz M, Wray V. *Phytochemistry* 2001; **57**: 489.
27. D'Acquarica I, Di Giovani MC, Gasparrini F, Misiti D, D'Arrigo C, Fagnano N, Guarnieri D, Iacono G, Bifulco G, Riccio R. *Tetrahedron* 2002; **58**: 10 127.
28. Ouyang MA, Li DP. *J. Asian Nat. Prod. Res.* 2002; **4**: 25.
29. Teng RW, Xie HY, Liu XK, Wang DZ, Yang CR. *J. Asian Nat. Prod. Res.* 2003; **5**: 75.
30. Sang S, Mao S, Lao A, Chen Z, Ho CT. *Food Chem.* 2003; **83**: 499.
31. Mehta BK, Mehta D, Itoriya A. *Carbohydr. Res.* 2004; **339**: 2871.
32. Kang WY, Du ZZ, Hao XJ. *J. Asian Nat. Prod. Res.* 2004; **6**: 1.
33. Liu X, Ye W, Mo Z, Yu B, Wu H, Zhao S, Che C, Hsiao WLW. *Planta Med.* 2005; **71**: 880.
34. Bialy Z, Jurzysta M, Mella M, Tava A. *J. Agric. Food Chem.* 2006; **54**: 2520.
35. Ahn MJ, Kim CY, Yoon KD, Ryu MY, Cheong JH, Chin YW, Kim J. *J. Nat. Prod.* 2006; **69**: 360.
36. Teng RW, Xie HY, Wanf DZ, Yang CR. *Magn. Reson. Chem.* 2002; **40**: 603.
37. Santos RP, Viana FA, Lemos TLG, Silveira ER, Braz-Filho R, Pessoa ODL. *Magn. Reson. Chem.* 2003; **41**: 735.
38. Magid AA, Voutquenne L, Moretti C, Long C, Lavaud C. *J. Nat. Prod.* 2006; **69**: 196.

DOI: 10.1002/mrc