Phytochemistry, Vol. 27, No. 12, pp. 3781–3785, 1988.
Printed in Great Britain.

0031–9422/88 $3.00+0.00

SPINOSIDES A AND B. TWO CYTOTOXIC CUCURBITACIN GLYCOSIDES FROM *DESFONTAINIA SPINOSA*

KALAKOTA S. REDDY, ASHOK J. AMONKAR, THOMAS G. MCCLOUD, CHING-JER CHANG, and JOHN M. CASSADY*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

(*Received in revised form* 12 *April* 1988)

Key Word Index—*Desfontainia spinosa*; Desfontainiaceae; spinoside A; spinoside B; 23,24-dihydro-11-deoxycucurbitacin.

Abstract—Fractionation of the active ethanol extract of *Desfontainia spinosa* based on cytotoxicity has led to the discovery of three new derivatives of the parent compound, 11-deoxocucurbitacin I. The structures of 23,24-dihydro-11-deoxocucurbitacin I and the glycosides, spinosides A and B were elucidated on the basis of extensive analysis of their high field ^{1}H NMR, ^{13}C NMR, high resolution mass spectra (fast atom bombardment, field desorption, chemical ionization, electron impact) and chemical interconversions. Spinosides A and B were cytotoxic in the KB cell culture assay.

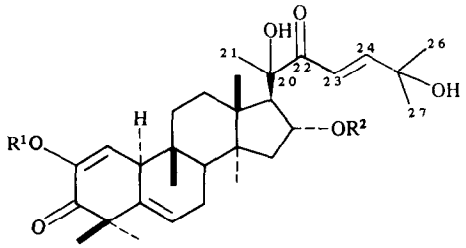
INTRODUCTION

In the course of a continuing search for tumour inhibitors of plant origin, an ethanolic extract of *Desfontainia spinosa* Ruiz and Pav. (Desfontainiaceae) from Costa Rica was found to show significant activity against KB cells in culture and the P388 mouse leukemia [1]. Schultes [2] has reported the use of this plant as a narcotic and hallucinogen by certain Chilian Indian tribes. Prior work by our group led to the isolation and characterization of the novel cytotoxic triterpene, 11-deoxocucurbitacin I (**1**) [3]. Houghton *et al.* [4, 5] recently reported the phytochemical investigation of *Desfontainia spinosa* and the isolation of several iridoids and pentacyclic triterpenes. This paper describes further examination of the cytotoxic fractions obtained from the 95% EtOH extract of *D. spinosa* which has resulted in the isolation and characterization of two new cytotoxic cucurbitacin glycosides, **3** and **4**, and 23,24-dihydro-11-deoxocucurbitacin I (**2**).

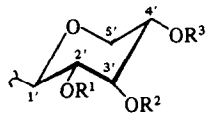
RESULTS AND DISCUSSION

Structures of spinosides A (**3**) *and B* (**4**)

Spinosides A and B were crystallized from *n*-hexane and ether. Both compounds gave positive Liebermann–Burchard and Molisch tests, consistent with the formulation of **3** and **4** as terpenoid glycosides [6]. The high resolution (field desorption, fast atom bombardment) mass spectra of spinosides A and B established the molecular formula $C_{39}H_{56}O_{12}$ (*m/z* 716) and $C_{37}H_{54}O_{11}$ (*m/z* 674) respectively. The M^+ ion of spinoside A at *m/z* 716 was 42 mass units higher than spinoside B (M^+ at *m/z* 674) suggesting that spinoside A contained an additional acetoxyl group, probably in the sugar moiety. Characteristic UV and IR absorptions due to α,β-unsaturated ketone and diosphenol moieties in compounds **3** and **4** indicated that they were closely related to 11-deoxocucurbitacin I (**1**) [3].



	R¹	R²	Other
1	H	H	
2	H	H	23,24-dihydro



		R¹	R²	R³
3	H	Ac	H	Ac
4	H	Ac	H	H
5	Ac	Ac	Ac	Ac

The structural relationship between spinosides A and B was established by acetylation with pyridine–acetic anhydride to give the identical peracetate **5** based on ^{1}H NMR and mass spectra (M^+ ion at *m/z* 823.3855, calcd for $C_{43}H_{60}O_{14}+Na$, 823.3880).

The detailed analysis of high field ^{1}H NMR spectra of **3** and **4** confirmed the presence of the 11-deoxycucurbitacin I moiety. All the functional groups of **1**, including the α,β-

Table 1. ^{1}H NMR (470 MHz) chemical shifts of relevant protons of compounds **1**, **3**, **4** and **5** isolated from *Desfontainia spinosa**

Assignment	**1**	**3**†	**4**†	**5**†
H-1	6.13 *d*	6.13 *d*	6.12 *d*	6.53 *d*
	(2.3)	(2.4)	(2.4)	(2.2)
H-6	5.67 *t*	5.69 *t*	5.68 *t*	5.74 *t*
	(2.1)	(2.5)	(2.4)	(2.4)
H-16	4.37 *t*	4.44 *t*	4.47 *t*	4.45 *t*
	(7.5)	(7.1)	(7.3)	(7.4)
H-23	6.63 *d*	6.58 *d*	6.59 *d*	6.62 *d*
	(15.2)	(15.2)	(15.2)	(15.1)
H-24	7.07 *d*	7.17 *d*	7.11 *d*	7.15 *d*
	(15.2)	(15.2)	(15.2)	(15.2)
tertiary methyls	1.39 *s*	1.41 *s*	1.39 *s*	1.46 *s*
	1.33 *s*	1.36 *s*	1.34 *s*	1.36 *s*
	1.30 *s*	1.35 *s*	1.33 *s*	1.34 *s*
	1.24 *s*	1.32 *s*	1.31 *s*	1.28 *s*
	1.22 *s*	1.22 *s*	1.21 *s*	1.27 *s*
	1.15 *s*	1.12 *s*	1.11 *s*	1.14 *s*
	1.09 *s*	1.09 *s*	1.06 *s*	1.05 *s*
	0.83 *s*	0.85 *s*	0.84 *s*	0.86 *s*
H-1′	—	4.30 *d*	4.20 *d*	4.20 *d*
		(5.8)	(6.4)	(7.6)
H-2′	—	4.69 *dd*	4.62 *dd*	4.88 *dd*
		(5.8, 8.1)	(6.4, 8.4)	(7.6, 10.5)
H-3′	—	3.79 *ddd*	3.84 *m*	4.95 *dd*
		(3.6, 8.1, 9.1)	(8.4, 3.6, 9.5)	(3.5, 10.5)
H-4′	—	5.03 *m*	3.57 *ddd*	5.20 *m*
		(3.6, 4.8, 10.5)	(5.8, 8.4, 10.7)	(2.0, 3.5, 10.6)
H_a-5′		3.90 *dd*	3.83 *dd*	4.00 *dd*
		(4.8, 12.9)	(5.8, 12.8)	(2.0, 13.5)
H_b-5′	—	3.45 *m*	3.42 *dd*	3.46 *dd*
		(10.5, 12.9)	(10.7, 12.8)	(10.6, 13.5)
2′-OAc	—	2.15 *s*	2.15 *s*	2.16 *s*
3′-OAc		—	—	1.97 *s*
4′-OAc	—	2.04 *s*	—	1.96 *s*

*Spectra were taken in $CDCl_3$. Chemical shifts are in δ units relative to $SiMe_4$. Coupling constants (J) in parentheses are given in Hz.

†Protons in the sugar moiety assigned by selective decoupling experiments.

unsaturated ketone in the side chain (H-23, δ6.58, *d*, J = 15.2 Hz; H-24, 7.17, *d*, J = 15.2 Hz), a trisubstituted double bond between C-5 and C-6 (H-6, 5.69, *t*, J = 2.5 Hz) and the carbinyl proton (H-16) at δ4.44, *t*, J = 7.1 Hz, could be readily identified (see Table 1), suggesting that **3** and **4** differ only in their sugar moieties.

The anomeric proton of spinoside A (**3**) resonated as a doublet at δ4.30. The spin–spin coupling constants (5.8 Hz, for H-1′ and H-2′, and 162 Hz for C-1′ and H-1′) suggested that spinoside A has an equatorial glycosidic bond [7–9]. A similar conclusion could also be drawn for spinoside B. The anomeric proton was coupled to a proton at δ4.69 (*dd*, J = 5.8, 8.1 Hz) indicating that H-2′ must be on a carbon bearing an acetoxy group. H-2′ further coupled to H-3′ which appeared at δ3.79 *ddd*, J = 9.1, 8.1, 3.6 Hz) and changed to a doublet of doublets on D_2O shake and upon acetylation, suggesting that it is a carbinol methine proton. Since H-3′ was weakly coupled to H-4′ the dihedral angle between H-3′ and H-4′ is close to 90°. The latter proton appeared at δ5.03 (*m*, J = 3.6, 4.8, 10.5 Hz) which was shifted 1.3 ppm to lower field when compared to H-3′ indicating that it is geminal to the acetoxy group. H-4′ also coupled to the 5′-methylene protons at δ3.90 (*dd*, J = 4.8, 12.9 Hz) and δ3.45 (*m*, J = 10.5, 12.9 Hz). Complete hydrolysis [10] of compounds **3** and **4** in refluxing methanolic HCl (8%) and subsequent purification (silica gel flash column [11], $CHCl_3$–MeOH) of the resulting residue yielded an aglycone and a methyl glycoside. The less polar crystalline product was identified as 11-deoxocucurbitacin I (**1**) by high field ^{1}H NMR, high resolution mass spectrum and by direct comparison with an authentic sample [3]. The second product from the methanolysis crystallized from aqueous methanol as colourless needles, mp 165–166° $[\alpha]_D$ +232 (H_2O; c 0.2). Analysis by high field ^{1}H NMR and high resolution mass spectral data [12, 13] estab-

lished the structure of this compound as methyl-β-L-arabinopyranoside (**6**). Complete characterization of **6** was achieved by direct comparison to an authentic sample and by acid hydrolysis (MeOH–HCl) which gave α-L-arabinopyranoside. The furanose form of arabinose was ruled out based on the ^{1}H NMR spectrum in which only H-4′ was affected during D_2O shake as well as by the acetylation. Based on the above results the stereochemistry of the sugar moiety in spinosides A (**3**) and B (**4**) was assigned as 2, 4-di-*O*-acetyl-α-L-arabinopyranose and 2-*O*-acetyl-α-L-arabinopyranose, respectively.

The position of the glycosidic linkage in both compounds **3** and **4** was established as C-16 based on the formation of **1** during acid hydrolysis, the presence of common structural features in biogenetically related compounds [14] and ^{13}C NMR analysis of spinosides A and B, and their peracetate **5**. Chemical shifts were assigned with the aid of proton coupled and decoupled spectra, by application of known chemical shift rules, and comparison to the ^{13}C NMR spectrum of the aglycone (Table 2) [3, 15, 16]. The sp^2 carbon C-1 (δ133.6) in the peracetate **5** showed a strong downfield shift of 15 ppm due to the deshielding effect by the acetoxyl group at C-2. The typical shifts (6–7 ppm) [15] were observed for the C-16 methine carbon in both spinosides A and B compared to their aglycone **1**. The chemical shifts of C-2′,3′,4′ and 5′ in the peracetate **5** were identical with literature values [10, 17].

The structural relationship between spinoside A and spinoside B was further established based on the analysis of high resolution FAB mass spectral data of their peracetate **5**. A prominent peak at *m/z* 259 due to 2′,3′,4′-tri-*O*-acetylpentoseoxonium ion and its loss from the M$^+$ (*m/z* at 800) was also noticed in the mass spectrum of acetate **5**. Since compound **4** gave a M$^+$ ion at *m/z* 674 which is 42 mass units fewer than spinoside A, it can be concluded that it contained a monoacetyl arabinose as its sugar moiety and the location of the acetoxy group was established at C-2′ from its high field ^{1}H NMR spectral data. All the proton assignments in the peracetate **5** were made by double resonance experiments and were consistent with peracetylated α-arabinopyranoside [12, 18]. On the basis of the accumulated data, the structures of spinosides A and B were established as **3** and **4** respectively. Although there has been one recent example [19] of a naturally occurring 2′,4′-diacetoxyxylopyranoside moiety in terpenoid glycosides, this is the first report of 11-deoxocucurbitacin glycosides having a 2′,4′-diacetoxyarabinopyranose at C-16.

Table 2. ^{13}C NMR chemical shifts* for compounds **1**, **3**, **4**, and **5**

C	1†	3‡	4‡	5
1	117.4	118.1	118.1	133.6
2	144.6	145.3	145.3	143.1
3	199.4	198.0	198.0	195.4
4	43.3	47.6	47.6	48.9
5	138.7	139.3	139.3	138.1
6	121.2	120.4	120.6	121.6
7	24.1	23.8	23.8	24.0
8	41.5	41.0	41.1	41.5
9	34.8	34.3	34.3	34.8
10	37.5	36.9	36.9	37.4
11	30.3[a]	30.9[a]	30.9[a]	31.0[a]
12	31.0[a]	30.3[a]	30.3[a]	30.3[a]
13	48.7[b]	47.5[b]	47.5[b]	48.8[b]
14	49.0[b]	47.3[b]	47.3[b]	48.1[b]
15	46.0	40.8	40.8	40.3
16	71.9	77.7	77.2	76.1
17	53.2	59.4	60.4	56.2
18	20.2[c]	20.5[c]	20.5[c]	20.5[c]
19	18.0[c]	16.5[c]	161.5[c]	17.2[c]
20	78.6	78.6	78.8	78.1
21	29.5[d]	29.2[d]	29.2[d]	29.2[d]
22	203.2	204.2	204.8	201.1
23	119.3	119.7	119.7	119.4
24	115.3	153.7	153.5	154.4
25	71.0	69.6	69.4	70.4
26	27.6	27.2	27.2	27.0
27	27.6	27.2	27.2	27.5
30	18.2[c]	18.4[c]	18.5[c]	18.0[c]
31	28.9[d]	29.0[d]	29.0[d]	28.5[d]
1′	—	99.1	99.0	98.0
2′	—	70.9	70.6	69.6
3′	—	72.3	72.5	70.1
4′	—	68.5	68.0	68.0
5′	—	63.6	66.0	63.8
2′-OCOMe	—	169.9	169.9	169.9
3′-OCOMe	—	—	—	169.9
4′-OCOMe	—	168.8	—	169.8
2′-OCOMe	—	20.9	20.9	20.8
3′-OCOMe	—	—	—	20.2
4′-OCOMe	—	21.0	—	20.8
2-OCOMe	—	—	—	170.0
2-OCOMe	—	—	—	20.7
$^1J_{CH}$ —	162.0	162.0	160.5	

*Spectra were recorded in $CDCl_3$ on a Chemagnetics A-200 FT spectrometer operating at 50.16 MHz.

†Taken from ref. [2].

‡Spectra measured in d_6-DMSO.

[a–d]Assignments with the same symbol may be interchanged.

Structure of 23,24-*dihydro*-11-*deoxycucurbitacin* I (**2**)

This compound co-eluted with compound **1** during silica gel centrifugal chromatography. Final purification was effected by preparative TLC and recrystallization. Compound **2** resembles compound **1** in some respects but it differs significantly based on the ^{1}H NMR spectrum which lacked the signals due to α-and β-protons of the α,β-unsaturated ketone. Instead, compound **2** showed two sets of triplets at δ2.58 and 2.95 consistent with the 23,24-dihydro derivative. As expected the molecular ion in the chemical ionization mass spectrum of **2** was observed at *m/z* 503 which is 2 higher than for compound **1**. The mass spectrum further showed a prominent peak at *m/z* 115 due to the ion fragment $^+O{\equiv}CCH_2CH_2C(Me)_2OH$, and the corresponding fragment was observed at *m/z* 113 in all previously isolated cucurbitacins-[20] having an α,β-unsaturated ketone in the side chain. The rest of the mass spectral data, as well as ^{1}H NMR spectral data, was identical with its congener 11-deoxocucurbitacin I. Therefore, compound **2** is 23,24-dihydro-11-deoxocucurbitacin I.

Biological assays

The spinosides, 11-deoxocucurbitacin I and its dihydro derivative were isolated by following the cellular toxicity of fractionated materials against Eagle KB strain of human carcinoma of nasopharynx [21]. The ED_{50} of compounds **1**–**4** as shown in the scheme indicated that glycosidation of the C-16 position in the cucurbitacin skeleton reduced the cytotoxicity to some extent and the reduction of the side chain double bond led to complete loss of activity.

EXPERIMENTAL

All mps: uncorr. CC was performed on C-18 phase bonded Hiflosil silica gel. IR spectra were obtained in KBr. UV spectra were recorded in the solvents indicated. ^{1}H NMR spectra were obtained in the solvent indicated on either a Varian XL-200 or the Nicolet 470 MHz spectrometer at the Purdue University Biological Magnetic Resonance Laboratory (NIH Grant RR1077). Chemical shifts are given as ppm with reference to TMS and J values are reported in Hz. ^{13}C NMR spectra were recorded at 50.16 MHz in d_6-DMSO. Chemical shifts are in ppm downfield from TMS. For sugars, TLC was performed on Merck silica gel 60 F254 pre-coated plates (0.2 mm) developed with MeOH–$CHCl_3$ (1:5) and 2-propanol–EtOAc–H_2O (7:2:1). TLC spots were visualized with 5% methanolic sulphuric acid, and vanillin–sulphuric acid or aniline–hydrogen phthalate. Fast atom bombardment mass spectra were taken in lithium sulpholane.

Air-dried, finely ground plant material (10 kg) was percolated with 95% EtOH, yielding 1.6 kg extract. This extract which gave an ED_{50} = 8.8 μg/ml was first partitioned between $CHCl_3$ and H_2O to give an active (ED_{50} = 5.5 μg/ml) $CHCl_3$ fraction (758 g). This fraction was next partitioned between 10% aq. MeOH and petrol to give 530 g aq. MeOH solubles, ED_{50} = 4 μg/ml. Partition fractions were also tested in the P388 mouse leukemia assays. The 90% MeOH-soluble fraction was found to be active (132% T/C at 50 μg/kg in P388), and was selected for further fractionation. This fraction (530 g) was triturated with 4 l MeOH–H_2O–MeCN (2:2:1), the residue separated and the soluble portion filtered through a column of C-18 phase bonded Hiflosil silica. The material (218 g) which passed through the column in the MeOH–H_2O–MeCN (2:2:1) solvent system was cytotoxic (ED_{50} = 0.3 μg/ml, 9KB). A portion of this material (104 g) was chromatographed on 2.5 kg MN 81532 silica gel. This gravity column was developed with a gradient of increasing MeOH in $CHCl_3$ and the 90 l of eluate was collected in 60 ml fractions and pooled (fractions A–R) based on TLC. These 18 pooled fractions were tested in P388 *in vivo* and *in vitro* revealing a zone of cytotoxicity from fractions F to N. Fractions G (12 g, ED_{50} = 0.4 μg/ml), H (2.8 g, ED_{50} = 1 μg/ml) and L (2.16 g, ED_{50} = 6 μg/ml) yielded yellow ppts, respectively, upon concentration. Further purification of fraction G by chromatography over silica gel in EtOAc–CH_2Cl_2 followed by chromatotron yielded 300 mg of 11-deoxocucurbitacin I (**1**) [1] and 25 mg of 23,24-dihydro-11-deoxocucurbitacin I (**2**). The ppt. obtained from fraction H was repeatedly triturated with Me_2CO to remove the coloured material and to leave the crude spinoside A. Final purification of spinoside A (**3**) (0.22 g) was effected by silica gel centrifugal chromatography on a chromatotron rotor in hexane –CH_2Cl_2–EtOAc followed by recrystallization from hexane –Et_2O. In a similar manner fraction L yielded spinoside B (**4**) (0.2 g). Compound **1** had an ED_{50} = 0.002 μg/ml, **2** was inactive, **3** had an ED_{50} = 0.1 μg/ml and **4** showed ED_{50} = 0.3 μg/ml.

Spinoside A (**3**). Crystallized from hexane–Et_2O as colourless needles, mp 194–196°, $[\alpha]_D^{25}$ –77° (MeOH; c 0.6), UV λ_{max}^{MeOH} nm (log ε): 270 (4.15), 232 (4.33), 200 (4.24); IR ν_{max}^{KBr} cm^{-1}: 3480, 1740, 1680, 1660, 1620, 1380, 1220, 1080, 1040. FDMS m/z: 716.377 (calcd for $C_{39}H_{56}O_{12}$; 716.374), 698 $[M-H_2O]^+$, 563, 396, 217, 164, 113. ^{1}H NMR: see Table 1.

Peracetylation of spinoside A (**3**). A soln of spinoside A (**3**, 5.0 mg) in dry pyridine (0.5 ml) and Ac_2O (0.5 ml) was stirred overnight at room temp under N_2. The soln was evapd *in vacuo* and the residual pyridine and Ac_2O were co-distilled with toluene (3 × 1.5 ml). The resulting residue (6.0 mg) was recrystallized from MeOH to give the tetraacetate **5** (3.8 mg, 70%) as colourless needles, mp 150–152°. UV λ_{max}^{MeOH} nm (log ε): 233 (4.57), 204 (4.34); IR ν_{max}^{KBr} cm^{-1}: 3380, 1704, 1680, 1665, 1620, 1380, 1220, 1080, 1040. FABMS m/z: 807 $[M+Li+H]^+$, 783 $[M-H_2O+H]^+$, 723 $[M-H_2O-HOAc+H]^+$, 671, 651, 620, 547, 525 $[M-H_2O-$sugar$]$, 259 [acetylated pentose], 199, 183, 157, 139, 113, 97; HRMS (FAB) m/z: 823.3880 (calcd. for $C_{43}H_{60}O_{14}+Na$, 823.3855). ^{1}H NMR: see Table 1.

23,24-Dihydro-11-deoxocucurbitacin I (**2**). This compound was co-eluted with compound **1** during silica gel centrifugal chromatography and was purified further by prep. RP-2 TLC. Crystallization from Et_2O and hexane afforded **2** as colourless plates, mp 140–142° UV λ_{max}^{MeOH} nm (log ε): 268 (4.08), 233 (3.9), 203 (4.35); IR ν_{max}^{KBr} cm^{-1}: 3420, 2990, 1700, 1680, 1450, 1380, 1210, 1080, 1040; ^{1}H NMR (470 MHz) ($CDCl_3$): δ6.12 (*d*, 1H, J = 2.4 Hz, H-1), 5.67 (*t*, 1H, J = 2.3 Hz; H-6), 4.31 (*t*, 1H, J = 7.5 Hz, H-16), 2.95 (*t*, 1H, J = 7.5 Hz, Ha-23), 2.58 (*t*, 1H, J = 7.5 Hz, Ha-24), 2.54 (*t*, 1H, J = 7.5 Hz, Hb-24), 2.40 (*d*, 1H, J = 7.5 Hz, H-17), 1.41 (*s*, 3H), 1.32 (*s*, 3H), 1.23 (*s*, 3H), 1.22 (*s*, 3H), 1.21 (*s*, 3H), 1.19 (*s*, 3H), 1.09 (*s*, 3H), 0.84 (*s*, 3H); FDMS m/z: 503 $[M+H]^+$, 485 $[M-H_2O+H]^+$, 387 $[M-115]^+$, 369, 343 $[M-$side chain$]^+$, 321, 273, 234, 183, 164, 141, 129, 115 $[^+O{\equiv}CCH_2CH_2C(CH_3)_2OH]$, 98.

Spinoside B (**4**). Crystallized from hexane–Et_2O as an amorphous powder mp 229–231°, $[\alpha]_D^{25}$ –76° (MeOH; c 0.6), UV λ_{max}^{MeOH} nm (log ε): 270 (4.18), 234 (4.66); IR ν_{max}^{KBr} cm^{-1}: 3460, 1740, 1680, 1640, 1620, 1370, 1080, 1050. FDMS m/z: 674 $[M]^+$, 657, 603, 561, 519 $[M-$side chain$]^+$; CIMS (NH_3) m/z: 692 $[M+NH_3+H]^+$ 675 $[M+H]^+$, 500 (aglycone), 175 (sugar moiety). HRMS (FAB) m/z: 697.3564 (calcd. for $C_{37}H_{54}O_{11}+Na$, 697.3563). ^{1}H NMR: see Table 1. The tetracetate **5** of spinoside B (**4**) was prepared by the procedures similar to the preparation of spinoside A peracetate and silica gel preparative layer chromatography (5% MeOH in $CHCl_3$) to give compound **5** (8 mg, 72%) which was recrystallized from MeOH as colourless needles mp 150–152°. This compound was identical with the acetate obtained from spinoside A by co-TLC (R_f 0.6; $CHCl_3$–MeOH, 19:1) and spectral data (UV, IR, NMR and MS).

Methanolysis of spinoside A. A sample of spinoside A (45 mg, 0.06 mmol) was heated to reflux with 8% methanolic HCl (10 ml) for 4 hr. The reaction mixture was cooled and the HCl was removed under a stream of N_2. The resulting residue was loaded on a silica gel flash column (1.0 × 15 cm) and eluted with $CHCl_3$–MeOH (41:9) to give two main products. The first one (8.0 mg) was identified as 11-deoxocucurbitacin I by ^{1}H NMR, MS and finally by direct comparison with an authentic sample (co-TLC, mmp). The second product **6** (10 mg) was crystallized from aq. MeOH as colourless needles, mp 165–166° (Lit [22] mp 165–166°), R_f = 0.45 ($CHCl_3$–MeOH, 80:20); ^{1}H NMR (D_2O): δ4.65 (*d*, 1H, J = 2.9 Hz, H-1′), 3.81 (*m*, 1H, H-4′), 3.70 (*dd*, 1H, J = 1.0, 12.8 Hz, H-5′a), 3.65 (*t*, 2H, J = 2.5 Hz, H-2′ and H-3′), 3.47 (*dd*, 1H, J = 2.5, 12.8 Hz, H-5′b), 3.23 (*s*, 3H, –OMe); HRMS (CI, *i*-butane) m/z 165.076 $[M+H]^+$ calcd. for $C_6H_{12}O_5$ + H, 165.076, 133 $[M-OMe]^+$, 115 $[M-MeOH-H_2O]^+$, 73 which was identified as methyl-β-L-arabinopyranoside by direct

comparison with an authentic sample co-TLC, silica gel, $CHCl_3$–MeOH, 4:1) and its conversion to α-L-arabinopyranoside by treating with MeOH–HCl at room temp.

Methanolysis of spinoside B. A solution of 5 mg of spinoside B in 10 ml of 8% methanolic HCl was heated to reflux for 4 hr. After usual work-up the products were purified by flash CC. These products (**1** and **6**) were identical with those obtained from spinoside A (co-TLC and mp and spectral data).

Acknowledgements—This research was supported by grant CA33326 awarded by the National Cancer Institute, HHS. High resolution (470 MHz) proton spectra were recorded at the Purdue University Biochemical Magnetic Resonance Laboratory (NIH RR 1077). Dr John L. Occolowitz (Eli Lilly Laboratories, Indianapolis, Indiana) provided FDMS. *In vitro* testing was performed by Dr Linda Jacobsen in the Cell Culture Laboratory, Purdue Cancer Center. The animal testing data are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, NCI, Bethesda, Maryland.

REFERENCES

1. Geran, R. I., Greenberg, N. H., MacDonald, N. N., Schumacher, A. A. and Abbott, B. J. (1972), *Cancer Chemother. Rep.*, **3**, 1.
2. Schultes, R. E. (1977), *Bot. Mus. Leafl. Harvard Univ.* **25**, 99.
3. Amonkar, A. J., McCloud, T. G., Chang, C., Saenz-Renauld, J. A. and Cassady, J. M. (1985) *Phytochemistry* **24**, 1803.
4. Houghton, P. J. and Lian, L. M. (1986) *Phytochemistry* **25**, 1939.
5. Houghton, P. J. and Lian, L. M. (1985) *Phytochemistry* **24**, 1841.
6. Schneider, L. F. (1964) *Qualitative Organic Microanalysis, Cognition and Recognition of Carbon Compounds*, p. 172. Academic Press, New York.
7. Massiot, G., Lavand, C., Guillaume, D., LeMen-Oliver, L. and Binst, B. V. (1986) *J. Chem. Soc., Chem. Commun.* 1485.
8. Crombie, L., Crombie, W. M. L. and Whiting, D. A. (1986) *J. Chem. Soc. Perkin Trans. I* 1917.
9. Bock, K. and Pedersen, C. (1975) *Acta Chim. Scand. B* **29**, 258.
10. Liu, H. W. and Nakanishi, K. (1981) *J. Am. Chem. Soc.* **103**, 7005.
11. Still, W. C., Khan, M. and Mitra, A. (1978) *J. Org. Chem.* **43**, 2923.
12. Lahloub, M. F., Gross, G.-A., Sticher, O., Winkler, T. and Schulten, H.-R. (1986) *Planta Med.* 352.
13. DeJongh, D. C. and Biemann, K. (1963) *J. Am. Chem. Soc.* **85**, 2289.
14. Kupchan, S. M., Sigel, C. W., Gultman, L. J., Restivo, R. J. and Bryan, R. F. (1972) *J. Am. Chem. Soc.* **94**, 1353.
15. Velde, V. V. and Lavie, D. (1983) *Tetrahedron* **39**, 317.
16. Tori, K., Seo, S., Yoshimura, Y., Nakamura, M., Tomita, Y. and Ishii, H. (1976) *Tetrahedron Letters* **46**, 4167.
17. Liptak, A., Szurmai, Z., Nanasi, P. and Neszmelyi, A. (1982) *Tetrahedron* **23**, 3489.
18. Lang, H., Li, S., McCabe, T. and Clardy, J. (1984) *Planta Med.* 501.
19. Gan, L.-X., Han, X.-B. and Chen, Y.-Q. (1986) *Phytochemistry* **25**, 2389.
20. Audier, H. and Das, B. (1966) *Tetrahedron Letters* 2205.
21. Oyami, V. I. and Eagle, H. (1956) *Pro. Soc. Exp. Biol. Med.* **91**, 305.
22. Cook, A. H., Bunbuny, H. M. and Hey, D. H. (1965) *Dictionary of Organic Compounds* **1**, 270. Oxford University Press, New York.