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CUCURBITACINS FROM *ACANTHOSICYOS HORRIDUS*

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Key Word Index—*Acanthosicyos horridus*; Cucurbitaceae; cucurbitacins; tetrahydro-*epi-iso*-cucurbitacin I; *epi-iso*-cucurbitacin R; dihydro-*epi-iso*-cucurbitacin D; tetrahydro-*iso*-elatericin B.

Abstract—Roots of *Acanthosicyos horridus* have yielded, in addition to known triterpenes, cucurbitacins and sterols, dihydro-*epi-iso*-cucurbitacin D (*epi-iso*-cucurbitacin R, tetrahydro-*epi-iso*-cucurbitacin I or tetrahydro-*iso*-elatericin B), identified on the basis of spectroscopic and chemical evidence, and thus shown for the first time to be a naturally occurring material.

INTRODUCTION

The cucurbitacins are a group of highly oxygenated tetracyclic triterpenes having a unique 19(10 → 9β)abeo-10α-lanostane skeleton. This group of compounds possesses a wide range of biological activities [1–5]. In continuing our work on Cucurbitaceae triterpenes [6], we have recently been able to examine roots of an unusual member of the family, *Acanthosicyos horridus* Welw. ex Hook. f., known as !nara or naras.

!Nara occurs in Namibia, South West Africa, particularly in the region of Walvis Bay. Leaves are largely absent and the tendrils are reduced to sharp spines. The fruit is a small gourd containing numerous seeds, and both have been used as food by the desert dwellers in the region for over 8000 years [7]. They are still used locally and traded to some extent, particularly the seeds, which are collected for use in the confectionery industry in South Africa [8]. The unripe fruits, roots and twigs are intensely bitter and the latter two have been used medicinally [9].

Previous chemical work on the plant has shown it to yield principally cucurbitacins B and D, as well as smaller amounts of cucurbitacins G and H, based mainly on paper chromatographic evidence [10]. The seeds are rich in protein [8].

RESULTS AND DISCUSSION

In the present work a methanolic extract of the roots of *Acanthosicyos horridus* was subjected to hydrolysis with elaterase [11] to give a foamy mixture of aglycones, fractionation of which by chromatography on silica gel provided the known materials: cucurbitacin D (isolated after acetylation as the diacetate **1**) [12], dihydrocucurbitacin D (**2**) [12], 3-*epi-iso*-cucurbitacin D (isolated after acetylation as the diacetate **3**) [13], cucurbitacin B (**4**) [14], dihydrocucurbitacin B (isolated after acetylation as the triacetate **5** [14]) and dihydrocucurbitacin D (isolated after acetylation as the diacetate **6** [15]). In addition, a substance, mp 179–181°, was obtained in 0.07% yield. This is assigned structure **7** on the basis of the chemical and spectral evidence below.

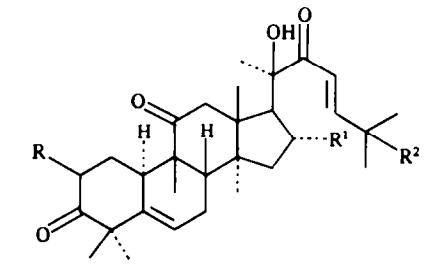
Mass spectroscopy showed the $[M]^+$ peak of this material to be at *m/z* 518 with peaks at *m/z* 500 and 482 (shown by accurate mass measurement to correspond with $C_{30}H_{44}O_6$ and $C_{30}H_{42}O_5$, respectively). The latter arise due to ready dehydration of the compound, showing that at least two hydroxyl groups are present.

The main features of its ^{1}H NMR spectrum were characteristic of the cucurbitacins but peaks for vinyl hydrogens at C-23 and C-24 were absent, implying that the side chain had no double bond. This was confirmed by the mass spectral fragmentation pattern which gave a peak due to loss of 115 mu, showing that the side chain was likely to be Me–C(OH)–CO–$(CH_2)_2$C(OH)Me_2 [16, 17]. An important feature of the ^{1}H NMR spectrum was the singlet at δ3.90 assigned to the methine hydrogen in an α-ketol. The signal was shifted downfield to δ4.93 on acetylation. The signal was a sharp singlet indicative that the compound was a member of the *iso*-cucurbitacin series since 2-hydroxy-3-oxo cucurbitacins show this signal (for H-2 in such cases) as a doubet of doublets [6]. Thus, the corresponding signal can be seen in the ^{1}H NMR spectrum of dihydrocucurbitacin D diacetate (**6**), also isolated in this work, as a doublet of doublets centred at δ5.48 for H-2 (cf. δ5.53 [15]).

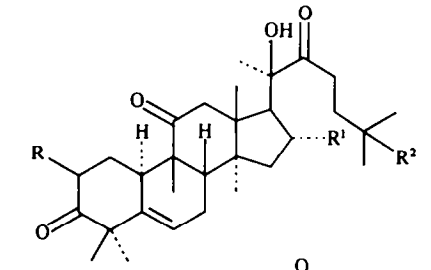
The structure of the rest of the molecule was established primarily by comparison of its ^{1}H NMR spectrum and that of the acetate derivative (**8**) with those of known *iso*-cucurbitacins [13, 15, 18]. Table 1 shows the ^{1}H NMR spectral data of the cucurbitacins (**1–3** and **5–8**) obtained in the present study. Spectra were assigned by comparison with published data [12, 13, 17, 18] as were the ^{13}C NMR data of **2**, **5** and **6–8** (Table 2) [12].

Finally, the fact that H-3 in **8** resonated at higher field (δ4.93) than in the case of its epimer (**9**, δ5.11 [15]) is evidence for an axial hydrogen (i.e. an equatorial acetoxy group) [19]. Thus the structure is **7**, identical with material previously synthesized [15, 18] and epimeric with tetrahydro-*iso*-cucurbitacin I (**10**) isolated from roots of *Bryonia dioica* [15]. The stereochemistry in ring A is confirmed by the difference in the CD curves provided by the epimeric ketols [15]. That of the newly isolated **7** shows a strong Cotton effect, confirming the α-equatorial configuration at C-3 [15].

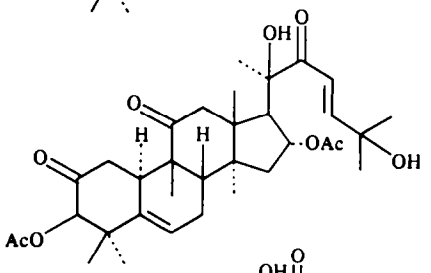
There is some confusion in the report of the Russian



1 R = R¹ = O_2CMe, R² = OH
4 R = R¹ = OH, R² = O_2CMe



2 R = R¹ = R² = OH
5 R = R¹ = R² = O_2CMe
6 R = R¹ = O_2CMe, R² = OH



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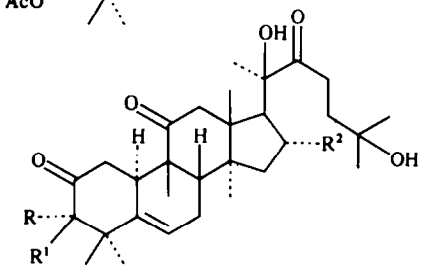

7 R = R² = OH, R¹ = H
8 R = R² = O_2CMe, R¹ = H
9 R = H, R¹ = R² = O_2CMe
10 R = H, R¹ = R² = OH

workers [17] who state that they have isolated '*iso*-23,24-dihydrocucurbitacin D' from *Bryonia alba* roots but in fact, in their paper, they illustrate the structure of 23,24-dihydrocucurbitacin D (tetrahydrocucurbitacin I). This confusion is further compounded by the fact that the abstract of their paper in *Chemical Abstracts* [20] reports the isolation of the *iso* compound and actually accompanies it with the structure of an *iso*-cucurbitacin with a 3α-hydroxyl group (**7**). However, from the NMR data reported [17] it appears to us that the material really isolated by Panosyan *et al.* [17] is tetrahydrocucurbitacin I (dihydrocucurbitacin D, **2**) (cf. [15]) as indeed may be seen from their previous preliminary report [21] which shows the structure, correctly, as **2**. There are some inconsistencies in their data however, particularly for the CD curve [17] which, surprisingly, does indeed correspond with that expected [15] for an *iso* compound. Thus, the present work is the first report, as far as we are aware, of the isolation of 23,24-dihydro-*epi-iso*-cucurbitacin D from nature. The *iso* compound epimeric at C-3 has been isolated from roots of *Bryonia dioica* [15], but only from extracts which had been stored for several months. This has led to the proposal [25] which has been mentioned by several authors [e.g. 6, 13] that *iso*-cucurbitacins may be artefacts arising from the corresponding materials during the extraction and purification. Dihydro-*epi-iso*-cucurbitacin D was isolated in the present work in relatively high yield which may indicate that it is indeed a truly natural material.

The fact that Panosyan *et al.* [17] used the prefix '*iso*' to describe the material they isolated from *Bryonia alba* has also resulted in a confusion in the literature about the occurrence and distribution of the cucurbitacins. For example, Bauer and Wagner [22] report the isolation by Panosyan *et al.* of both tetrahydrocucurbitacin I (dihydrocucurbitacin D) and tetrahydro-*iso*-cucurbitacin I (dihydro-*iso*-cucurbitacin D) [17, 21] when the evidence shows that the material is of the 'normal' series (tetrahydrocucurbitacin I, **2**) in both cases.

EXPERIMENTAL

Mps were taken with an electrothermal melting point apparatus and are uncorr. ^{1}H NMR spectra were recorded at 250 MHz and 400 MHz in $CDCl_3$ using TMS as internal standard. ^{13}C NMR spectra were recorded at 74.88 MHz on a Bruker WH400 NMR spectrometer in $CDCl_3$ using TMS as internal standard. MS were recorded on an AE1 MS902 high

Table 1. Selected ^{1}H NMR spectral data of compounds 1–3 and 5–8 (δ, $CDCl_3$)

Hydrogen	1 (cf. [12])	2 (cf. [12])	3 (cf. [13])	5	6	7	8
2	5.49 *dd* (13, 6)	4.43 *dd* (12, 6)	—	5.48 *dd* (13, 6)	5.48 *dd* (13, 6)	—	—
3	—	—	5.10	—	—	3.90 (cf. [18])	4.93 (cf. [15, 18])
6	5.78 *m*	5.79 *m*	5.89 *m*	5.89 *m*	5.78 *m*	5.94 *m*	5.94 *m*
12	3.26 *br d* (15)	3.26 *br d* (15)	3.17 *br d* (15)	3.26 *br d* (15)	3.26 *br d* (15)	3.12 *br d* (14.5)	3.12 *br d* (15)
16	5.17 *br t* (8)	4.32 *br t* (7)	5.18 *br t* (8)	5.14 *br t* (8)	5.14 *br t* (7)	4.29 *br t* (7.4)	5.13 *br t* (7.6)
23	6.68 *d* (15)	—	6.65 *d* (15)	—	—	—	—
24	7.15 *d* (15)	—	7.13 *d* (15)	—	—	—	—
methyls	1.04	0.98	1.02	1.01	1.02	0.80	0.96
	1.11	1.08	1.06	1.02	1.10	0.96	0.99
	1.26	1.23	1.10	1.11	1.25	1.18	1.18
	1.30	1.26	1.17	1.29	1.26	1.20	1.21
	1.31	1.28	1.32	1.31	1.29	1.24	1.25
	1.41	1.35	1.41	1.46	1.30	1.27	1.43
	1.43	1.38	1.42	1.47	1.31	1.33	1.43
	1.45	1.44	1.43	1.50	1.46	1.41	1.43
2-OAc	2.15	—	—	2.15	2.14	—	—
3-OAc	—	—	2.19	—	—	—	2.18
16-OAc	1.83	—	1.83	1.95	1.94	—	1.92
25-OAc	—	—	—	1.99	—	—	—

Coupling constants in Hz are quoted in parentheses. Signals are singlets unless otherwise indicated.

resolution mass spectrometer having a direct inlet system and operating at 18 and 70 eV. IR spectra were obtained in KBr discs using a Unicam SP200 spectrometer. Analytical TLC was on silica gel 60 GF plates and PLC was on silica gel 60 PF_{254} (0.5 mm). CD measurements were obtained on a JASCO J.40 CS instrument using MeOH as solvent.

Roots of *Acanthosicyos horridus* were collected in 1981 near Walvis Bay, South West Africa. The dried powdered roots (3 kg) were exhaustively extracted successively with petrol (bp 60–80°) and then with MeOH. Part of the MeOH extract was hydrolysed with elaterase enzyme for 24 hr at 30°, diluted with a large vol. of H_2O and extracted into $CHCl_3$ [11]. The resultant foamy residue (about 32.5 g) was adsorbed onto a column of silica gel 60 (1 kg). The material was chromatographed using the following solvents: petrol (bp 40–60°); petrol–$CHCl_3$ (1:1); $CHCl_3$; $CHCl_3$–EtOAc (1:1); EtOAc; EtOAc–MeOH (1:1) and MeOH; 425 fractions of either 100 ml or 250 ml were collected. Similar fractions were grouped together according to TLC examination. In this way, dihydrocucurbitacin D (**2**) [12] and cucurbitacin B (**4**) [14] were isolated. Acetate derivatives of the following compounds were isolated after acetylation: cucurbitacin D (**1**) [12], 3-*epi*-*iso*-cucurbitacin D (**3**) [13], dihydrocucurbitacin B (**5**) [14] and dihydrocucurbitacin D (**6**) [15]. Known compounds were identical in every respect with values reported in the literature. ^{1}H NMR and ^{13}C NMR data are presented in Tables 1 and 2. Fractions 284–297 (2.82 g, collected by elution with $CHCl_3$–EtOAc (1:9), were adsorbed onto another column of silica gel 60 and eluted using the following solvents: $CHCl_3$; $CHCl_3$–EtOAc (1:1); EtOAc; and MeOH. Elution with increasing concns of EtOAc in $CHCl_3$ allowed isolation (after prep. TLC, cyclohexane–EtOAc, 3:2) of dihydro-*epi*-*iso*-cucurbitacin D (**7**) as white needles from $CHCl_3$–EtOAc (1:1); mp 179–181° (lit. 173–176° [18] and 168–172° [15]); $[\alpha]_D$ +91° (*c* 0.35; $CHCl_3$) (lit. +59° [18] and +55° [15]); IR ν_{max}^{KBr} cm^{-1}: 3440, 1725, 1705, 1689, 1465, 1440, 1375, 1265, 1215, 1100, 1030, 925; ^{1}H NMR ($CDCl_3$): see Table 1; ^{13}C NMR: see Table 2; MS *m/z* (rel. int.): 500 (0.6), 482 (11), 464 (2), 449 (3), 403 (4), 369 (3), 360 (4), 325 (4), 316 (2), 203 (2), 189 (4), 188 (2), 187 (4), 166 (11), 115 (2), 113 (100); Accurate mass measurements: Found: 518.3228, Calc. for $C_{30}H_{46}O_7$: 518.3244; Found: 500.3124, Calc. for $C_{30}H_{44}O_6$: 500.3138; Found: 482.3019, Calc. for $C_{30}H_{42}O_5$: 482.3032; Found: 464.2925, Calc. for $C_{30}H_{40}O_4$: 464.2926; Found: 369.2055, Calc. for $C_{23}H_{29}O_4$: 369.2066; Found: 166.1001, Calc. for $C_{10}H_{14}O_2$: 166.0994; Found: C, 67.64; H, 8.99%. Calc. for $C_{30}H_{46}O_7 \cdot H_2O$: C, 67.14; H, 9.01%. CD: (MeOH; *c* 1.0 mg/ml): $\Delta\varepsilon_{297}$ +4.0, $\Delta\varepsilon_{258}$ −0.5 (cf. $\Delta\varepsilon_{302}$ +3.367, $\Delta\varepsilon_{266}$ −0.6993 [17]).

Diacetyldihydro-epi-iso-cucurbitacin D (**8**). Dihydro-*epi*-*iso*-cucurbitacin D (**7**) (8 mg) was acetylated with Ac_2O in pyridine in the usual way to provide **8** (4.8 mg), mp 123–125°; IR ν_{max}^{KBr} cm^{-1}: 3449, 1740, 1730, 1700, 1470, 1380, 1245; 1180, 1130, ^{1}H NMR ($CDCl_3$): see Table 1; MS *m/z* (rel. int.): 584 (0.1), 566 (2), 551 (0.2), 527 (5), 524 (3), 509 (10), 487 (30), 453 (2), 451 (1), 443 (2), 427 (5), 425 (3), 385 (14), 367 (40), 325 (33), 268 (8), 142 (67), 115 (1), 113 (100); Found: 584.3338, Calc. for $C_{34}H_{48}O_8$: 584.3349; Found: 487.2694, Calc. for $C_{28}H_{39}O_7$: 487.2694; Found: 208.1102, Calc. for $C_{12}H_{16}O_3$: 208.1099.

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Table 2. ^{13}C NMR signals of cucurbitacins **2**, **5**, **6**–**8** (δ, $CDCl_3$)

Carbon	2 (cf. [12])	5	6	7	8
1	35.99	31.97	31.97	38.78	39.68
2	71.65	73.23	73.26	210.53	203.44
3	211.93	205.37	205.41	80.19	81.36
4	50.22	49.94	49.96	46.64	44.50
5	140.59	139.82	139.81	138.17	138.00
6	120.32	120.39	120.36	121.77	121.71
7	23.90	23.74	23.70	23.77	23.69
8	42.41	42.11	42.11	42.67	42.32
9	48.43	47.89	47.89	48.59	47.73
10	33.87	34.31	34.30	36.25	36.09
11	212.87	212.20	213.30	211.86	210.82
12	48.73	48.60	48.56	48.31	48.28
13	48.43	48.42	48.36	48.10	48.45
14	50.80	51.20	51.20	50.65	49.93
15	45.45	43.25	43.24	45.33	43.24
16	71.08	74.01	74.07	70.86	74.07
17	57.83	54.05	54.03	57.69	53.94
18	19.76	19.64	19.61	19.80	19.56
19	18.91	18.82	18.90	18.79	18.85
20	79.21	78.62	78.69	79.15	78.64
21	24.52	24.24	23.74	24.48	23.70
22	215.26	211.50	211.60	215.38	213.36
23	30.91	30.42	30.70	30.88	30.60
24	36.96	35.13	37.12	36.87	37.13
25	70.30	80.90	69.89	70.22	69.86
26	28.90*	26.02*	29.47*	28.71*	24.99
27	29.77*	25.81*	29.58*	29.83*	29.47
28	21.26	21.32	21.30	20.91	22.41
29	29.34	28.76	28.68	24.05	24.24
30	20.02	19.96	19.98	20.03	19.98
$\underline{Me}CO_2$	—	20.58	20.57	—	20.56
		20.85	20.92		20.91
		22.29			
$\underline{Me}CO_2$	—	169.90	169.89	—	169.89
		169.90	169.89		170.03
		169.90			

*Signals which in any vertical column may be interchanged.

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