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NEW CUCURBITACINS FROM *PHORMIUM TENAX* AND *MARAH OREGANUS**

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Key Word Index—*Phormium tenax*; *Marah oreganus*; Liliaceae; Cucurbitaceae; isocucurbitacin D; 3-epi-isocucurbitacin D; dihydroisocucurbitacin B.

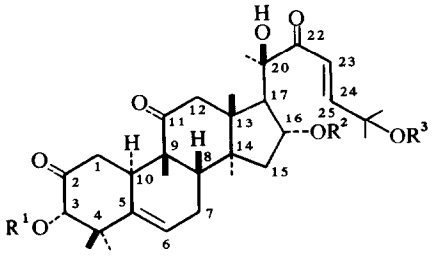
Abstract—Cucurbitacins I and D and two new cucurbitacins, isocucurbitacin D and 3-epi-isocucurbitacin D, were isolated from *Phormium tenax*. A new cucurbitacin, dihydroisocucurbitacin B, was isolated from *Marah oreganus*. The acid sensitivity of the 2β-hydroxy-3-keto system found in cucurbitacin D was demonstrated.

INTRODUCTION

During the course of our continuing search for tumor inhibitors, we have isolated two new cytotoxic cucurbitacins, isocucurbitacin D (**1**) and 3-epi-isocucurbitacin D (**2**), from *Phormium tenax*. Although cucurbitacins have been isolated from several different plant families [1], this appears to be the first report of their presence in the Liliaceae.

RESULTS AND DISCUSSION

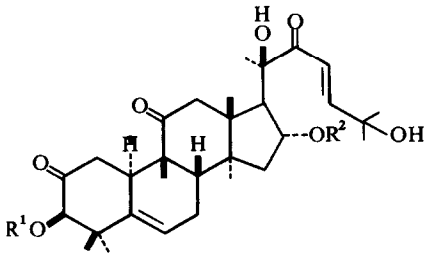
A 95% EtOH extract of the leaves of *P. tenax* demonstrated significant inhibitory activity *in vivo* against P-388 lymphocytic leukemia in the mouse (PS) and *in vitro* against cells derived from human carcinoma of the nasopharynx (KB) [2]. Activity guided fractionation (KB) of the extract showed the activity to be concentrated successively in the $CHCl_3$ phase of a $CHCl_3$–H_2O partition, 10% aq. MeOH phase of a 10% aq. MeOH–Skellysolve B partition, 20% aq. MeOH phase of a 20% aq. MeOH–CCl_4 partition, and finally in the $CHCl_3$ phase of a $CHCl_3$–40% aq. MeOH partition. Extensive CC and TLC of the final $CHCl_3$ phase led to the isolation of four cucurbitacins; two known compounds, cucurbitacin I [3, 4] and cucurbitacin D (**9**) [5] and two new cucurbitacins, isocucurbitacin D (**1**) and 3-epi-isocucurbitacin D (**2**). The two known cucurbitacins were identified by comparison with authentic samples.



1: $R^1 = R^2 = R^3 = H$
3: $R^1 = R^2 = MeCO$, $R^3 = H$
4: $R^1 = R^2 = R^3 = MeCO$
5: $R^1 = R^2 = H$, $R^3 = MeCO$

The structures of the two new cucurbitacins were established primarily by comparison of their NMR spectra to those of known isocucurbitacins (Table 1) and by chemical interconversions to common derivatives. The UV, IR and MS of isocucurbitacin D (**1**) were very similar to those of cucurbitacin D. Acetylation of **1** afforded two main products, a diacetate (**3**) and a triacetate (**4**). The additional acetate in **4** was determined to be at C-25 as indicated by the downfield shift of the C-26 and C-27 methyl group signals in the NMR spectrum of **4** compared to that of **3**. The singlet at δ 3.9 assigned to the C-3 proton in the NMR spectrum of **1** shifted to δ 4.93 in the spectra of both **3** and **4** suggesting a 2-keto-3-hydroxy moiety in ring A [6]. This same shift is found in the isocucurbitacin series (Table 1). As a final proof, acetylation of isocucurbitacin B (**5**) gave a triacetate identical to triacetate **4**.

The spectra of 3-epi-isocucurbitacin D (**2**) were similar to those of both cucurbitacin D (**9**) and, particularly, isocucurbitacin D (**1**). The major difference between the NMR spectra of **1** and **2** was the signal assigned to the



2: $R^1 = R^2 = H$
6: $R^1 = R^2 = MeCO$

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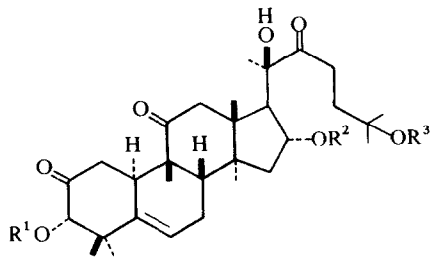
Table 1. NMR data for cucurbitacins **1**–**8**. Chemical shifts are given δ values, coupling constants (Hz) are quoted in parentheses

	1	3	4	2	6	5	7	8
4,4-Me	1.18	1.18	1.18	1.08	1.1	1.18	1.14	1.18
	1.25	1.21	1.22	1.26	1.16	1.26	1.24	1.22
9,13-Me	0.83	0.96	0.96	0.85	1.05	0.81	0.78	0.96
	0.97	1.01	1.01	0.97	1.01	0.96	0.93	1.0
14-Me	1.34	1.25	1.25	1.31	1.31	1.32	1.28	1.25
21-Me	1.34	1.41	1.4	1.39	1.4	1.41	1.35	1.42
25,25-Me	1.34	1.41	1.58	1.37	1.4	1.53	1.39	1.45
	1.34	1.41	1.59	1.37	1.4	1.55	1.39	1.48
3-H	3.9	4.93	4.93	4.14	5.09	3.89	3 8	4.93
6-H	5.95 *m*	5.92 *m*	5.91 *m*	5.89	5.88 *m*	5.91 *m*	5.83 *m*	5.92 *m*
16-H	4.33 *m*	5.16 *m*	5.17 *m*	4.38 *m*	5.18 *m*	4.35 *m*	4.2 *m*	5.13 *m*
23,24-H	6.58 *d*, 7.13 *d* (15.2)	6.64 *d*, 7.13 *d* (15.1)	6.38 *d*, 7.16 *d* (15.6)	6.63 *d*, 7.12 *d* (15.2)	6.64 *d*, 7.13 *d* (15.3)	6.43 *d*, 7.06 *d* (15.6)	obsc	obsc
OCOMe		1.81	1.85		1.83	2.0	1.9	1.93
		2.18	2.02		2.18			1.97
			2.18					2.18

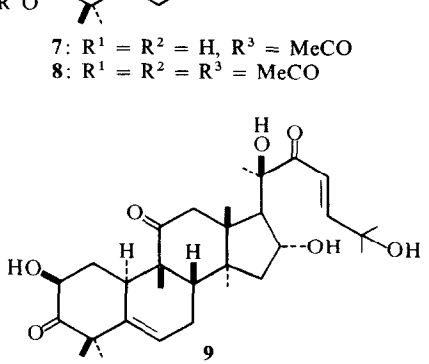
C-3 proton. In the NMR spectrum of **2**, this signal was a singlet at δ 4.14 which shifted to δ 5.09 in the NMR spectrum of diacetate **6**. This was indicative of the 2-keto-3-hydroxy moiety in ring A and of **2** being epimeric to **1** at C-3.

Attempts were made to interconvert isocucurbitacin D (**1**), 3-epi-isocucurbitacin D (**2**), and cucurbitacin D employing both basic and acidic conditions. Cucurbitacin D was heated in a methanolic solution of KOH in an oxygen-free atmosphere, conditions known to isomerize 2β-hydroxy-3-keto systems [6]. Although these conditions apparently did accomplish the isomerization of ring A to give both the 2-keto-3β-hydroxy and the 2-keto-3α-hydroxy moieties, the C-23,24 double bond also reacted. Only unidentifiable products were isolated.

To induce isomerization without affecting the side chain, mild acidic conditions were employed. A solution of cucurbitacin D was allowed to stand on a column of Si gel for 138 hr. Elution of the column then afforded unreacted cucurbitacin D, isocucurbitacin D and 3-epi-isocucurbitacin D. The demonstrated base and particularly the acid sensitivity of the 2β-hydroxy-3-keto moiety found in cucurbitacin D and other cucurbitacins, suggests that the isocucurbitacins may be artifacts of the isolation procedures used.



7: $R^1 = R^2 = H$, $R^3 = MeCO$
8: $R^1 = R^2 = R^3 = MeCO$



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In the process of purifying a sample of isocucurbitacin B (**5**) from fractions obtained from *Marah oreganus* (Cucurbitaceae) [7] for chemical interrelation to isocucurbitacin D (**1**), another new cucurbitacin, dihydroisocucurbitacin B (**7**), was isolated. The molecular formula ($C_{32}H_{48}O_8$), IR, UV, NMR and MS all indicated a cucurbitacin monoacetate with a hydrogenated side chain and with the acetate located at C-25. Acetylation afforded a triacetate (**8**), and the C-3 proton singlet at δ 3.8 in the NMR spectrum of **7** shifted to δ 4.93 in the spectrum of **8** indicating an isocucurbitacin. Finally, hydrogenation of isocucurbitacin B (**5**) gave a dihydro product identical to **7**, thus confirming that the compound was dihydroisocucurbitacin B (**7**).

EXPERIMENTAL

General procedure. Mps were uncorr. NMR spectra were recorded at 100 MHz in $CDCl_3$ using TMS as int. stand. TLC and PLC were on Si gel 60 plates (0.25 mm). Leaves of *Phormium tenax* Forst. were collected in California in 1971. We thank Dr Robert E. Perdue, Jr., USDA. Beltsville, for supplying the dried plant material in accordance with the program developed by the National Cancer Institute. Biological testing was conducted under the auspices of the National Cancer Institute [2]. Isocucurbitacin D and 3-epi-isocucurbitacin D showed cytotoxicity against KB cell culture (ED_{50} = 0.024 and 0.24 μg/ml, respectively), and 3-epi-isocucurbitacin D showed tumor inhibitory activity against P-388 lymphocytic leukemia in the mouse (PS), T/C 134 at 1 mg/kg.

Extraction and isolation. Dried and ground leaves (2 kg) of *P. tenax* were extracted with 95% EtOH (Soxhlet) for 22 hr. The extract was evapd *in vacuo* and the residue partitioned as above. The residue from the final $CHCl_3$ partition phase (11 g) was chromatographed on a column of SilicAR CC-7 eluting successively with 50, 60, 70, 80 and 90% EtOAc–CH_2Cl_2. The 60% EtOAc–CH_2Cl_2 eluate, after PLC eluting with MeOH–$CHCl_3$ (1:9), yielded cucurbitacin I, identical (PMR, IR, UV, MS, mmp, TLC) with an authentic sample. CC of the 70 and 80% EtOAc–CH_2Cl_2 eluates over Si gel 40 eluting with MeOH–$CHCl_3$ (1:32 increased to 1:24) yielded, after PLC of the appropriate column fraction eluting with EtOAc, EtOAc–C_6H_6 (3:1) or

MeOH–$CHCl_3$ (1:1), isocucurbitacin D (**1**), 3-epi-isocucurbitacin D (**2**) and cucurbitacin D (**9**) (identical with an authentic sample by PMR, IR, MS, UV, mmp and TLC), in that order.

Isocucurbitacin D (**1**). Mp 188–191° (Et_2O). $[\alpha]_D$ +37° (*c* 0.8, $CHCl_3$). ν_{max}^{KBr} cm^{-1}: 3456, 1720, 1689, 1629, 1369, 1276. λ_{max}^{EtOH} nm: 230 (ε 9050). MS (probe, C.I.-CH_4) high resolution *m/e* 517.3160 (M^+ +1) calc. for $C_{30}H_{45}O_7$ *m/e* 517.3165, 499, 481, 463, 357.

Acetylation of (**1**). Acetylation of isocucurbitacin D (10 mg) with C_6H_5N–Ac_2O at room temp. for 48 hr gave the diacetate **3** (6 mg), mp 140–151°. ν_{max}^{KBr} cm^{-1}: 1730, 1720, 1690. MS *m/e* 582 (M^+ −18), 540, 522, 507, 487, 479, 444, 427, 411 (100%), 385 367. Also obtained was the triacetate **4** (3 mg, mp 123–125°, Et_2O–hexane). ν_{max}^{KBr} cm^{-1}: 1732, 1718, 1689. MS *m/e* 582 (M^+ −60), 522, 507, 487, 447, 444, 427, 411 (100%), 385, 384, 367. Triacetate **4** was found to be identical with the triacetate formed by acetylation of isocucurbitacin B (**5**).

3-Epi-isocucurbitacin D (**2**). Mp 179–181° (Me_2CO–Et_2O). $[\alpha]_D$ −76° (*c* 0.6, $CHCl_3$). ν_{max}^{KBr} cm^{-1}; 3440, 1717, 1690, 1629, 1370, 1280. λ_{max}^{EtOH} nm: 230 (ε12800). MS (probe, C.I.-CH_4) high resolution *m/e* 517.3160 (M^+ +1) calc. for $C_{30}H_{45}O_7$ *m/e* 517.3165.

Acetylation of (**2**). Acetylation of 3-epi-isocucurbitacin D **2**, 16 mg) with C_6H_5N–Ac_2O at room temp. for 20 hr gave a diacetate (**6**, 7 mg), mp 137.5–139° (C_6H_6). ν_{max}^{KBr} cm^{-1}: 1730, 1717, 1690. MS *m/e* 582 (M^+ −18), 551, 522, 487, 479, 444, 427, 411.

Isomerization of cucurbitacin D (**9**). A soln of cucurbitacin D (**9**, 23 mg) in EtOAc–$CHCl_3$ (1:20) was allowed to stand on a column of Si gel 40 for 138 hr. The column was then eluted with EtOAc, the eluate evapd *in vacuo*, and the residue subjected to PLC eluting with EtOAc–C_6H_6 (3:1) to yield isocucurbitacin D (**1**, 1 mg), 3-epi-isocucurbitacin D (**2**, 1 mg) and cucurbitacin D (**9**, 9 mg).

Dihydroisocucurbitacin B (**7**). PLC of a column fraction from a previous fractionation of an extract of *M. oreganus* [7] eluting with EtOAc–C_6H_6 (1:3) × 2 yielded isocucurbitacin B (**5**) and dihydroisocucurbitacin B (**7**), mp 230–232° (dec., Et_2O–CH_2Cl_2). $[\alpha]_D$ +54.5° (*c* 1.3, $CHCl_3$). ν_{max}^{KBr} cm^{-1}: 3480, 1724, 1698, 1366, 1257. MS (probe, C.I.-CH_4) high resolution *m/e* 501.3205 (M^+ -HOAc +1) calc. for $C_{30}H_{45}O_6$ *m/e* 501.3216, 483, 465.

Hydrogenation of isocucurbitacin B (**5**). Hydrogenation of isocucurbitacin B (**5**) at room temp. and atm. press. over 10% Pd/C gave **7**.

Acetylation of (**7**). Acetylation of dihydroisocucurbitacin B (**7**, 27 mg) with Ac_2O–C_6H_5N at room temp. for 18 hr gave a triacetate (**8**, 18 mg), mp 117–119° (Et_2O–hexane). ν_{max}^{KBr} cm^{-1}: 1730 (*br*), 1698. MS *m/e* 584 (M^+ −60), 566, 524, 509, 506, 491, 487, 481, 444, 427.

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