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CUCURBITACINS: DIFFERENTIAL CYTOTOXICITY, DEREPLICATION AND FIRST ISOLATION FROM *GONYSTYLUS KEITHII*

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ABSTRACT.—A characteristic pattern of differential cytotoxicity of extracts of *Iberis amara* seeds, predominantly toward renal tumor, brain tumor, and melanoma cell lines in the NCI human disease-oriented tumor screening panel, was traced to cucurbitacins E [**1**] and I [**2**]. This same differential cytotoxicity profile was detected in extracts of *Begonia plebeja* and *Gonystylus keithii*. Computer-assisted recognition of these profiles was followed by a rapid chemical fractionation, thus permitting the efficient dereplication of those extracts containing cucurbitacins B [**3**] and D [**4**], respectively. This is the first report of cucurbitacins from the genus *Gonystylus*.

Our early experiences with natural products research applications of the NCI's human disease-oriented, 60 cell line, in vitro antitumor screening protocol (1,2) have drawn us to several known classes of cytotoxins (3,4). These initial observations, and the realization that previously known "cytotoxic" agents might well exhibit unusual patterns of differential cytotoxicity in the new screen, provided us with both a challenge and an opportunity. The opportunity would lie in the possible discovery that such known, previously considered compounds might possess heretofore unknown activities against important types of tumors now represented in the NCI screen. The challenge would be to make such pattern associations quickly and efficiently, and then to dereplicate (5) all additional leads belonging to that structural class without undue consumption of time or resources. The cucurbitacin studies described herein exemplify this situation. Here we document a differential cytotoxicity profile that is characteristic of the cucurbitacins, the development of efficient separation and dereplication strategies for this class, and the first isolation of cucurbitacins from the genus *Gonystylus* (Thymelaeaceae).

The seed extracts of *Iberis amara* L. were selected for bioassay-guided fractionation from the first group (<2000) of plant and marine extracts screened in the NCI in vitro human tumor cell line panel. These extracts produced a differential cytotoxicity profile in which the renal tumor, brain tumor, and melanoma subpanels exhibited a greater average sensitivity compared to the other tumor type subpanels tested. Our preliminary evaluation protocol (5), comprising a small-scale solvent-solvent partitioning scheme, concentrated the cytotoxicity in the CCl_4 and $CHCl_3$ fractions. For scale-up fractionation, the CCl_4 partition step was omitted for convenience, and the cytotoxic $CHCl_3$-soluble extracts were further separated by gel-permeation chromatography through Sephadex LH-20. ^{1}H-Nmr analyses of the bioactive fractions and a review of the chemical literature strongly suggested that the cytotoxicity was associated with cucurbitacins. Normal-phase elution on a cyano-bonded hplc column provided excellent resolution of cucurbitacins E and I [**1** and **2**]. These compounds were identified by comparison of their spectral properties with those reported in the literature (6–8) and

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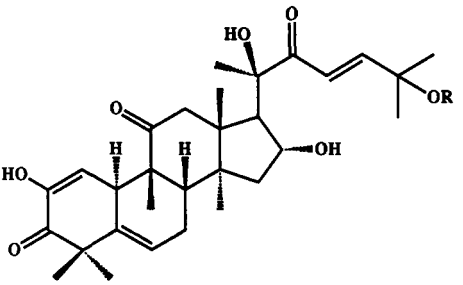

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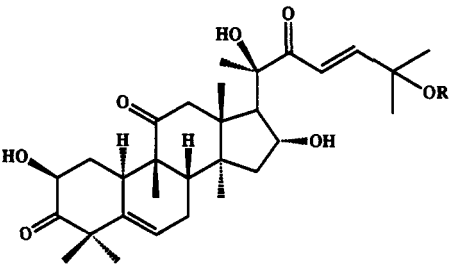
2 R=H



3 R=Ac
4 R=H

with authentic standards. Comparative screening of **1** and **2** revealed that they were virtually identical in both differential and mean panel average cytotoxicity; the renal tumor, brain tumor, and melanoma cell line subpanels, relative to the other subpanels of the NCI screen (1,2), were most sensitive to the compounds.

The advent of a rapid, computerized method of matching or comparing patterns of differential cytotoxicity has provided us with a convenient tool to facilitate the dereplication of such leads. By combining data from COMPARE analyses [e.g., see Paull *et al.* (9)] with chemical literature surveys on the taxon or taxa in question, we could rapidly identify screening profiles of extracts whose cytotoxicity was likely due to cucurbitacins. For example, the root extracts of *Begonia plebeja* Liebm. exhibited a cytotoxicity profile with a high correlation coefficient (e.g., consistently higher than 0.8) to the cucurbitacins and *Iberis* extracts. The literature survey indicated that cucurbitacin B [**3**] was known from this genus (10). We quickly confirmed that our extracts contained the same compound by application of the separation protocol used for *I. amara* (7,11).

We then found that twig extracts of *Gonystylus keithii* Airy Shaw produced essentially the same screening profile, although this genus was not known to contain cucurbitacins. Using the same separation scheme described above, we isolated and identified cucurbitacin D [**4**] from these extracts (7,8).

Computerized analyses of the characteristic screening profiles revealed high correlations not only among **1–4**, but also with other cucurbitacins. Figure 1 provides the TGI- and LC_{50}-centered mean graph profiles (9,12) of pure cucurbitacin E [**1**], which are characteristic of the cucurbitacins in general, as well as of cucurbitacin-containing extracts.

The combination of the differential cytotoxicity profile analyses, literature surveys, and our preliminary evaluation protocol provides an effective, rapid means of implicating the presence of a known class of bioactive compounds. This dereplication could be further confirmed by hplc with diode-array detection or hplc-ms, if one desired or required more specific identification of the bioactive components(s) of an extract.

The relatively potent in vitro cytotoxicity of the cucurbitacins, particularly to human tumor lines of the NCI's brain tumor, renal tumor, and melanoma subpanels, encourages the further evaluation of cucurbitacins in appropriate in vivo xenograft models. The Biological Evaluation Committee (BEC) of the NCI's Developmental Therapeutics Program has selected cucurbitacin E [**1**] for such consideration.

EXPERIMENTAL

PLANT MATERIAL.—Seeds of *Iberis amara* were obtained from Jelitto in Hamburg, Germany, by G.M.C. Roots of *Begonia plebeja* were collected in the Cayo District, Belize, by M.J. Balick of the New York Botanical Garden in November, 1987. Twigs of *Gonystylus keithii* were collected in the

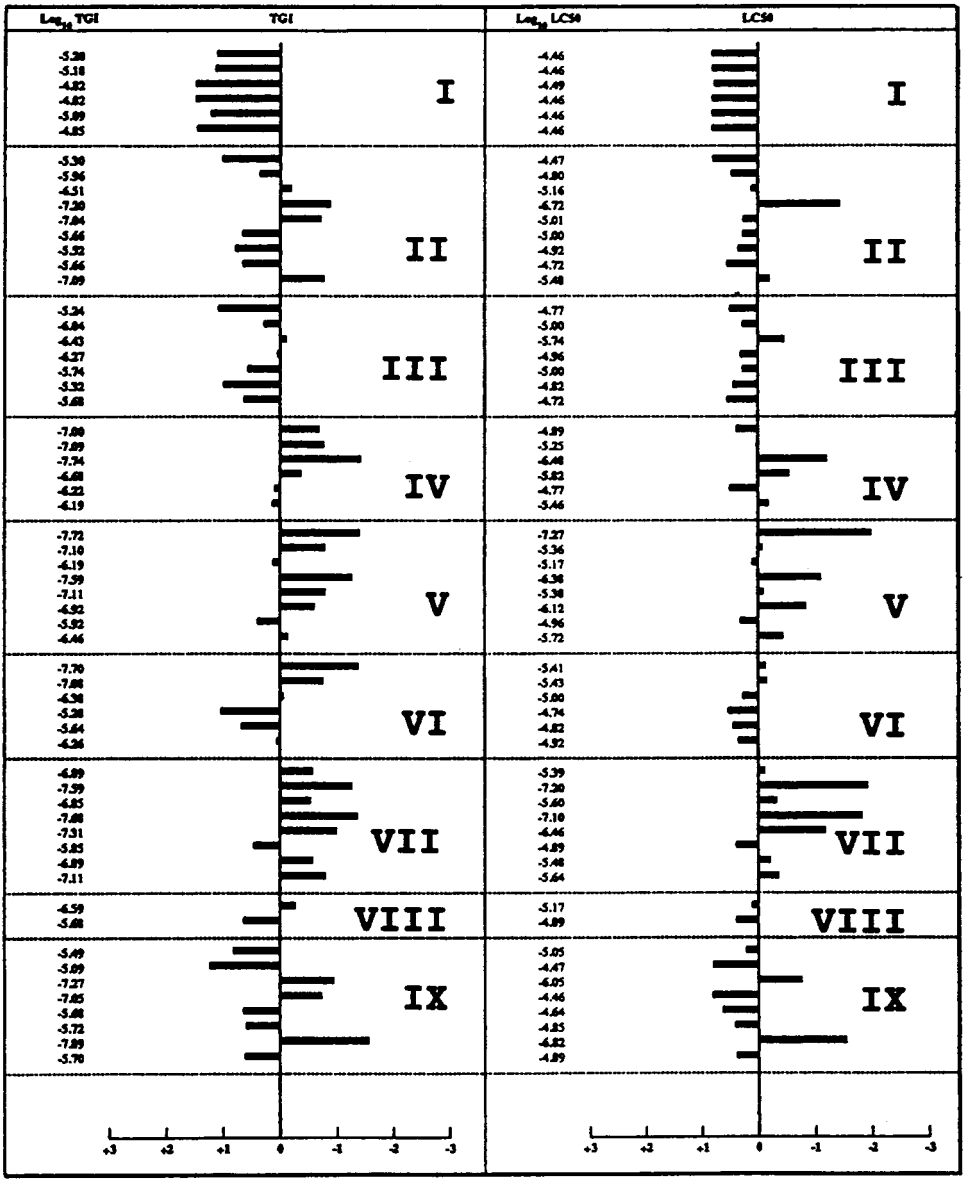


FIGURE 1. Averaged TGI- and LC_{50}-centered mean graph derived from quadruplicate testing of pure cucurbitacin E [1] in the NCI screen. The individual $\log_{10}$ TGI and $\log_{10}$ LC_{50} values for each cell line are provided in the vertical columns to the left of each mean graph. The subpanel and individual cell line identifiers are presented top-to-bottom as follows: I (leukemia) CCRF-CEM, HL-60 (TB), K-562, MOLT-4, RPMI-8226, SR: II (non small cell lung) A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522; III (colon) COLO205, HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620; IV (brain) SF-268, SF-295, SF-539, SNB-19, SNB-75, U251; V (melanoma) LOX IMVI, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62; VI (ovary) IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3; VII (renal) 786-0, A498, ACHN, CAKI-1, RXF-393, SN12C, TK-10, UO-31; VIII (prostate) PC-3, DU-145; IX (breast) MCF7, MCF7/ADR-RES, MDA-MB-231/ATCC, HS578T, MDA-MB-435, MDA-N, BT-549, T-47D.

TABLE 1. Isolation Data for Cucurbitacins.

Organism	mass (g)			R_t[a]	Cucurbitacin[b]: yield
	Crude extract	$CHCl_3$ fraction	LH-20 fraction		
I. amara	5.63	0.66	0.42	24.3	I[**2**]: 148 mg
				29.1	E[**1**]: 34 mg
B. plebeja	8.36	1.01	0.35	21.3	B[**3**]: 95 mg
G. keithii	6.15	1.23	0.44	25.9	D[**4**]: 114 mg

[a]Retention time in minutes on hplc system (see Experimental).
[b]Compounds were identified by comparison with spectral data in the literature.

Gunong Mulu National Park, Sarawak, by J.S. Burley of the Arnold Arboretum in September 1987. All plant materials were air-dried and ground to a fine powder prior to successive extractions with CH_2Cl_2-MeOH (1:1) and MeOH. The combined organic extracts were evaporated *in vacuo*. Yields: *I. amara* 14.49 g (from 45 g seeds); *B. plebeja* 7.75 g (from 232 g roots); *G. keithii* 56.72 g (from 494 g twigs).

Separation and identification of cucurbitacins.—An abbreviated solvent-solvent partitioning scheme [hexane vs. MeOH-H_2O (9:1), then $CHCl_3$ vs. MeOH-H_2O (6:4)] concentrated the cytotoxicity in the $CHCl_3$-soluble fraction. Gel permeation of the $CHCl_3$ solubles through Sephadex LH-20 with CH_2Cl_2-MeOH (1:1) concentrated the observed differential cytotoxicity in one fraction. The active constituents were readily purified by hplc on a cyano-bonded phase (Rainin-Dynamax-Cyano, 4.1×25 cm); elution with hexane-*i*-PrOH (7:3) at 49 ml/min provided the purified cucurbitacins with R_ts ranging from 21 to 30 min. For details in each case, see Table 1.

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