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CYTOTOXIC QUASSINOIDS FROM *CEDRONIA GRANATENSIS*

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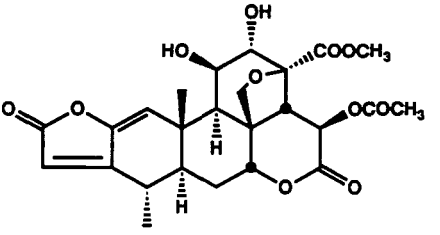
ABSTRACT.—The NCI in vitro primary disease-oriented antitumor screen has been used to select and guide the fractionation of the organic and aqueous extracts of *Cedronia granatensis*. Two quassinoids, sergiolide [**1**] and isobrucein B [**2**], to which the screening panel cell lines exhibited up to a 1000–fold range of differential sensitivity, were isolated. At concentrations of 10^{-5}–10^{-8} M, the compounds typically produced LC_{50}-level responses against a majority of the melanoma lines and several of the colon, lung, and other solid tumor lines. These and related quassinoids may, therefore, be of interest for in vivo evaluation in appropriate xenograft tumor models.

The National Cancer Institute has developed a new in vitro human disease oriented antitumor drug screening program based upon the use of a diverse panel of sixty human tumor cell lines representing seven cancer types—lung, colon, melanoma, renal, ovarian, central nervous system, and leukemia (1). Some current investigational approaches to data analysis and interpretation from this screen have been provided elsewhere, as have other technical details of the screening procedures (2–4). One goal of this new primary screen is to identify for in vivo testing compounds to which selected individual panel cell lines, or subpanels thereof, show widely differential in vitro growth inhibition or cytotoxicity.

We are exploring the use of the NCI in vitro screen for selection, prioritization, and bioassay-guided fractionation of natural products extracts. Presently, we are placing our highest priority upon extracts to which the panel cell lines exhibit at least a 1–3 $\log_{10}$ range of sensitivity at the LC_{50} response level [for definitions of the various response parameters currently used, see Boyd *et al.* (2) and Monks *et al.* (4)]. Additional priority is given to extracts which appear to produce distinctive subpanel-specific responses [for a discussion of statistical approaches to analysis of subpanel specificity, see Boyd *et al.* (2)]. Examination of the crude organic and aqueous extracts of various parts of the previously unstudied Simaroubaceous tree, *Cedronia granatensis* Cautrec, collected in Colombia, revealed such desired activity. Bioassay-guided fractionation of both the crude organic and aqueous extracts derived from leaf and twig parts provided the quassinoids sergeolide [**1**] and isobrucein B [**2**] as the active components. To our knowledge, this is the first report of secondary metabolites from this monotypic genus.



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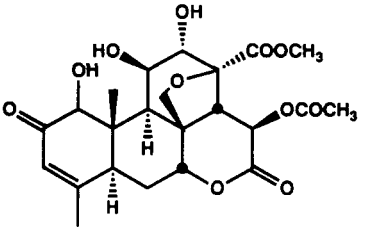

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RESULTS AND DISCUSSION

Isolation of the active constituents from the organic extracts followed solvent-solvent partitioning, gel permeation through Sephadex LH-20, cc on diol-bonded phase, and normal phase hplc. The aqueous extract also provided **1** and **2** after partitioning between *n*-BuOH and H_2O and vlc of the *n*-BuOH-soluble fraction on C_{18}-bonded phase Si gel; final purification required the diol and hplc steps used for the organic extracts. The structures of **1** and **2** were confirmed by spectroscopic comparison to the original literature data. Sergeolide (5) and isobrucein B (6) were previously reported as cytotoxic constituents of the root and stem extracts of *Picrolemma pseudocoffea* Ducke (7).

The crude organic and aqueous extracts exhibited very similar patterns of activity in the primary screen. The extracts were potent, with several melanoma, colon, and non-small-cell lung lines showing responses at the LC_{50} level with extract concentrations in the range of 9–50 μg/ml (Figure 1). [The cell lines used to compile data for Figures 1 and 2 differ somewhat, due to the time interval between screening of the crude extracts and **1** and **2** and refinements in the screening protocols that occurred during the interim.] This pattern persisted, and the differential response was magnified through the separation steps.

Of the two compounds, sergeolide [**1**] was the more potent, with concentrations of 10^{-7}–10^{-8}M eliciting strong differential responses at the LC_{50} level from nearly all the melanoma cell lines and from several of the colon and lung cell lines (Figure 2A). Isobrucein B [**2**] gave a similar pattern but required higher concentrations (see Figure 2B), as did samples of isobrucein A and desacetyl sergeolide, obtained from the NCI repository (data not shown). Quassinoids (8), the bitter principles originally isolated from other members of the Simaroubaceae and derived biogenetically via the degradation of tetracyclic triterpenes (9), have been reported previously to display a diverse array of biological activities, including antitumor (10), antiviral (11), antimalarial (12), amoebicidal (13), and antifeedant properties (14). However, to our knowledge, none of the quassinoids has been subjected to detailed in vivo evaluation in xenograft tumor models of melanoma, colon, lung, or other human solid tumors, which may be of particular interest based upon the current in vitro screening data.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Gel permeation chromatography was carried out using Sephadex LH-20 (Pharmacia). Diol and C_{18} bonded phases (YMC) were used for cc. Hplc was performed on a Rainin system employing a Rainin Dynamax silica column (1 × 25 cm), monitored at 254 nm, at a flow rate of 4 ml/min. Nmr spectra were recorded on a Varian VXR 500S spectrometer at 500 MHz and 125 MHz for ^1H and ^{13}C spectra, respectively, using TMS, $\delta = 0$ ppm, as the internal standard. Ir spectra were recorded on a Perkin-Elmer model 1600 FT-IR.

PLANT MATERIAL AND EXTRACTION.—The leaves and twigs of *C. granatensis* were collected in the Department de Antioquia, Colombia. A voucher specimen, Q65R0005, has been deposited in the Botany Department of the Museum of Natural History, Smithsonian Institution, Washington, DC. The plant material (453 g) was ground and extracted, first with CH_2Cl_2-MeOH (1:1), followed by MeOH (combined organic extract, 67 g), then H_2O (aqueous extract, 32 g).

ISOLATION AND PURIFICATION.—The crude organic extract (15.4 g) was subjected to a solvent-solvent partition, yielding hexane (3.7 g), CCl_4 (1.2 g), $CHCl_3$ (1.8 g), and aqueous MeOH (8.1 g) fractions, with the bulk of the activity found in the $CHCl_3$ solubles. A portion of the $CHCl_3$-soluble fraction (0.5 g) was chromatographed on Sephadex LH-20 (2.5 × 100 cm) with MeOH-MeCN (3:1), affording 0.19 g of an active fraction. This whole fraction was further subjected to cc (gravity, 2.5 × 15 cm) on diol bonded phase, using hexane- EtOAc (1:5), followed by final purification via semi-preparative hplc on silica [EtOAc-hexane (3:1)] to yield sergeolide [**1**] (6.9 mg, 0.024% of dried plant) as a white solid, as well as isobrucein B [**2**] (29.3 mg, 0.10%) as a white solid. The crude aqueous extract (5 g) was dissolved in 250 ml of H_2O and extracted with *n*-BuOH (3 × 250 ml); the bulk of the activity was found in the *n*-BuOH phase (2.3 g). Gradient elution vacuum chromatography on C18-silica [H_2O, H_2O-MeOH (2:1), H_2O-

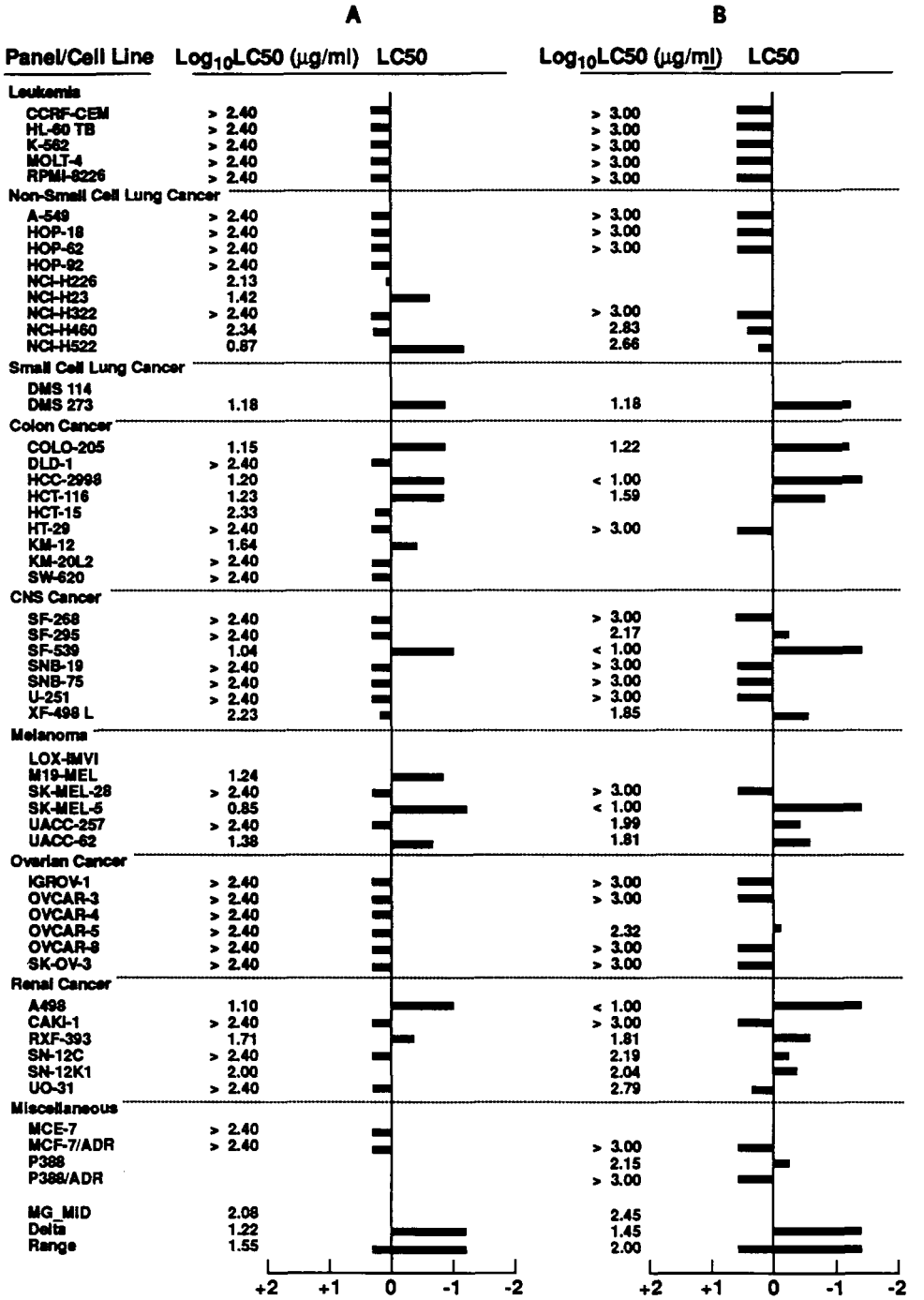

FIGURE 1. LC_{50} mean graphs from the human disease-oriented cancer cell line screening panel for (A) crude organic extract, *Cedronia granatensis*; (B) crude aqueous extract, *C. granatensis*.

MeOH (1:1), H_2O-MeOH (1:2), and MeOH] placed the bulk of the activity in the third fraction (874 mg). Cc of this material on diol-bonded phase using increasingly polar mixtures of EtOAc/MeOH, followed by hplc on silica [EtOAc-hexane (3:1)] furnished sergeolide [**1**] 9.2 mg (0.16%), and isobrucein B [**2**] 14.1 mg (0.26%).

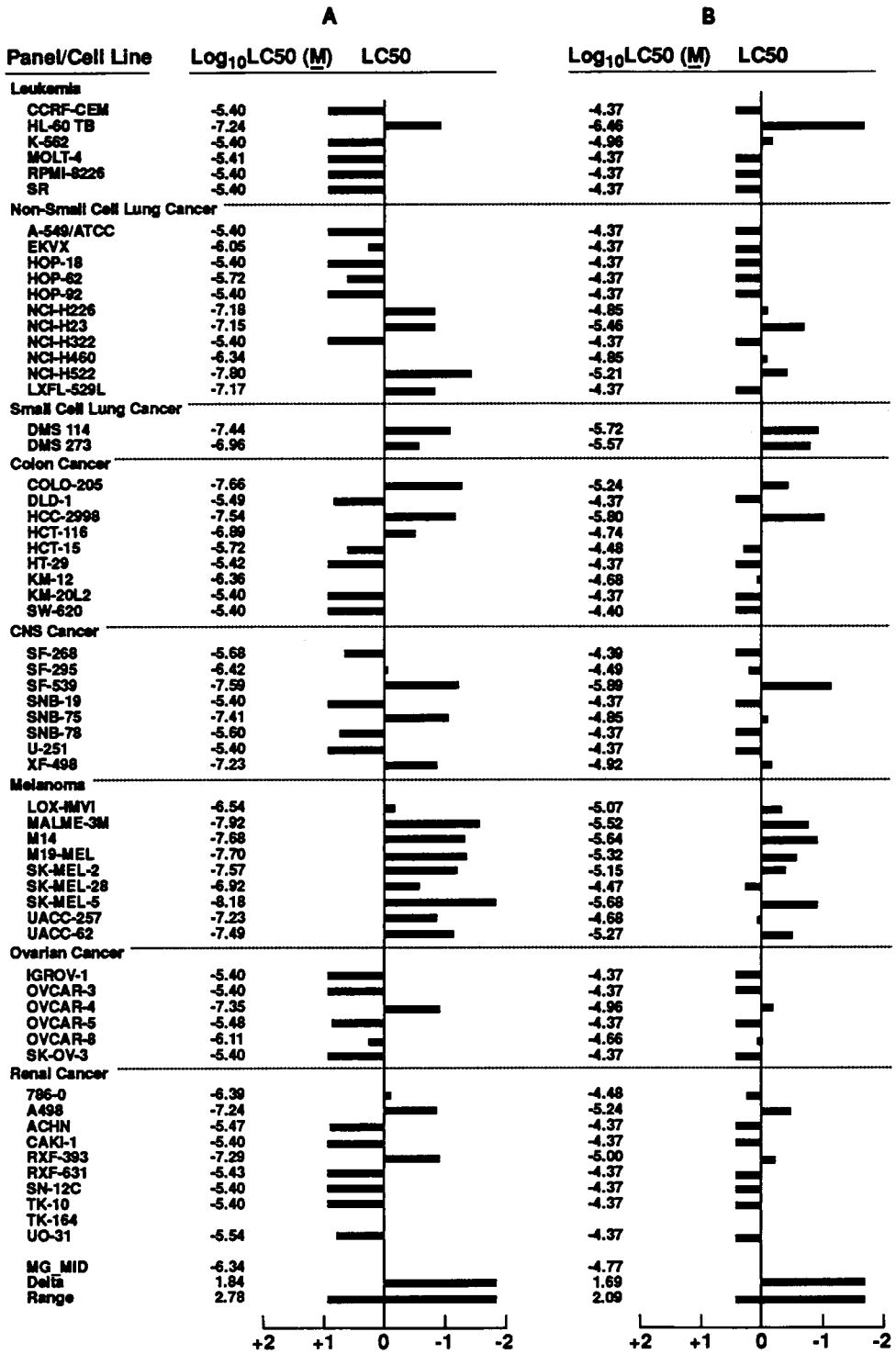

FIGURE 2. LC_{50} mean graphs from the human disease-oriented cancer cell line screening panel for (A) sergeolide [**1**]; (B) isobrucein B [**2**].

ACKNOWLEDGMENTS

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