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CONIOIDINES A AND B, NOVEL DNA-INTERACTING PYRROLIDINES FROM *CHAMAESARACHA CONIOIDES*

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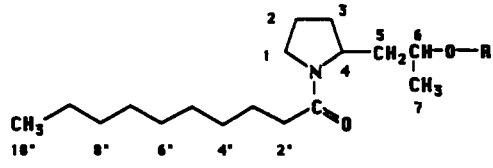
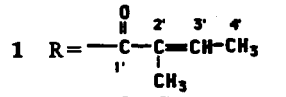
ABSTRACT—Two novel pyrrolidine compounds, conioidines A [**1**] and B [**2**], have been isolated from the Texas plant, *Chamaesaracha conioides* (Solanaceae). Their structures were determined by spectroscopic methods and hydrolysis studies. Both natural products, like doxorubicin, showed DNA-specific KB cell cytotoxicity. Dose-response data indicated a K_d value of 2.8 μM for binding of conioidine A [**1**] to calf thymus DNA.

The ability to bind DNA with resultant change in DNA structure has provided the basis for the biological activity of many antimicrobial and antineoplastic agents (1–6). For that reason, natural products that can interact with DNA by novel mechanisms have attracted much attention and research interest (7–10). In the course of our search for such natural products, we have designed a bioassay for high throughput screening of crude extracts from natural sources. In principle, this bioassay detects in a crude extract any cytotoxic compounds that bind tightly to DNA. Our extracts or compounds of interest show cytotoxicity to the transformed human cell line, KB, only in the absence of the DNA. With the addition of exogenous calf thymus DNA, the cytotoxic compounds are either partially or totally DNA-bound, and the observed KB cell cytotoxicity is therefore significantly reversed or reduced. It is this DNA-reversal bioassay that led us to the selection of the Texas plant, *Chamaesaracha conioides* (Dun.) Britt. (Solanaceae), which upon fractionation gave the active principles conioidines A [**1**] and B [**2**]. We report here their isolation, structure determination, and the biological activity.

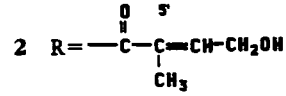
RESULTS AND DISCUSSIONS

The CH_2Cl_2 extract of *C. conioides* exhibited cytotoxicity (IC_{50}=34 μg/ml; 50% reduced with exogenous calf thymus DNA at 25 μg/ml) towards KB cells and was selected for fractionation. Bioassay-guided purification proceeded through Si gel and sequential reversed-phase chromatography and afforded two novel compounds, coniodine A [**1**] and coniodine B [**2**].





1 R=



2 R=

3 R=H

Conioidine A [**1**], isolated as a colorless oil, exhibited an hreims molecular ion at *m/z* 365.2938, corresponding to a molecular formula of $C_{22}H_{39}NO_3$ (calcd 365.2946). With the olefinic and carbonyl signals from the gated spin echo spectrum (GASPE) at δ 129.0, 136.8, 167.5, and 171.8, the formula suggested one site of unsaturation due to a monocyclic ring. The ^{1}H 2D COSY spectrum showed three isolated spin systems, one of which clearly belonged to a pyrrolidine moiety. The cross peaks showed that the nitrogen -CH_2 at δ 3.35 was connected to the nitrogen -CH at δ 4.08 through two sets of methylenes, with the first methylene at δ 1.90 and the second at δ 1.85. The nitrogen methine, in turn, was connected to a methylene with nonequivalent protons at δ 2.03 and 1.62, and then an oxygen-bearing methine at δ 4.90. The oxygen methine further showed a cross-peak to an Me signal at δ 1.31, thus accounting for all proton connectivity within the pyrrolidine moiety.

The ir data (1705, 1630 cm^{-1}) and ^{13}C-nmr data (167.5, 171.8 ppm) of **1** indicated one amide and one ester function on the pyrrolidine moiety. The ms fragments at *m/z* 282 and *m/z* 83 suggested the presence of an angelate or tiglate group of C_5H_7O. The ^{1}H resonances at δ 6.80, 1.80, and 1.78 and the ^{13}C signals at δ 167.5, 129.0, 136.8, 14.1, and 12.0 were consistent with the *E* stereochemistry as described for tiglate in the literature (11). There were two possibilities for a tiglate attachment to the pyrrolidine moiety, one with an amide bond and the other through an ester linkage. When coioidine A [**1**] was treated with methanolic KOH, the hydrolysis product **3** had a molecular formula of $C_{17}H_{33}NO_2$ (hreims *m/z* 283.2485, calcd 283.2511), therefore confirming an ester linkage for the tiglate. With the pyrrolidine and tiglate systems defined, a decanoyl function on the nitrogen was an obvious conclusion. Methyl decanoate was isolated and identified when **1** was degraded with 6 N methanolic HCl. All the ion peaks as outlined in Figure 1 supported the proposed conioidine A [**1**] structure. The relative stereochemistry at C-4 and C-6, however, cannot be deduced from the present spectral data.

The eims of conioidine B [**2**], $[M+H]^+$ *m/z* 382, showed that it differed from conioidine A [**1**] by one oxygen atom. In the eims of **2**, the appearance of the ion peak at *m/z* 226 and the disappearance of the ion peak at *m/z* 210 as in conioidine A [**1**] suggested that the oxygen atom resided in the pyrrolidine and tiglate portion of the molecule. A strong ion peak at *m/z* 282 of an *N*-decanoyl pyrrolidine moiety was also

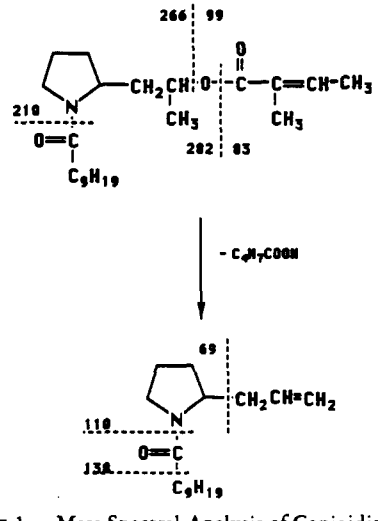


FIGURE 1. Mass Spectral Analysis of Conioidine A [**1**].

observed for **2**. This observation implied, therefore, an oxygenated tiglate group. The ^{1}H-nmr spectrum implied, therefore, an oxygenated tiglate group. The ^{1}H-nmr spectrum of conioidine B [**2**] is like that of **1**, except for the loss of the allylic Me doublet signal at δ 1.78 and the appearance of a new methylene muliplet at δ 4.28. These data, together with the retention of the Me singlet signal at δ 1.82, suggested that the OH resided on a distal rather than a proximal Me in the tiglate.

The two structurally related compounds, conioidine A [**1**] and conioidine B [**2**], gave identical bioassay results. Figure 2 shows the dependence of cytotoxicity on the

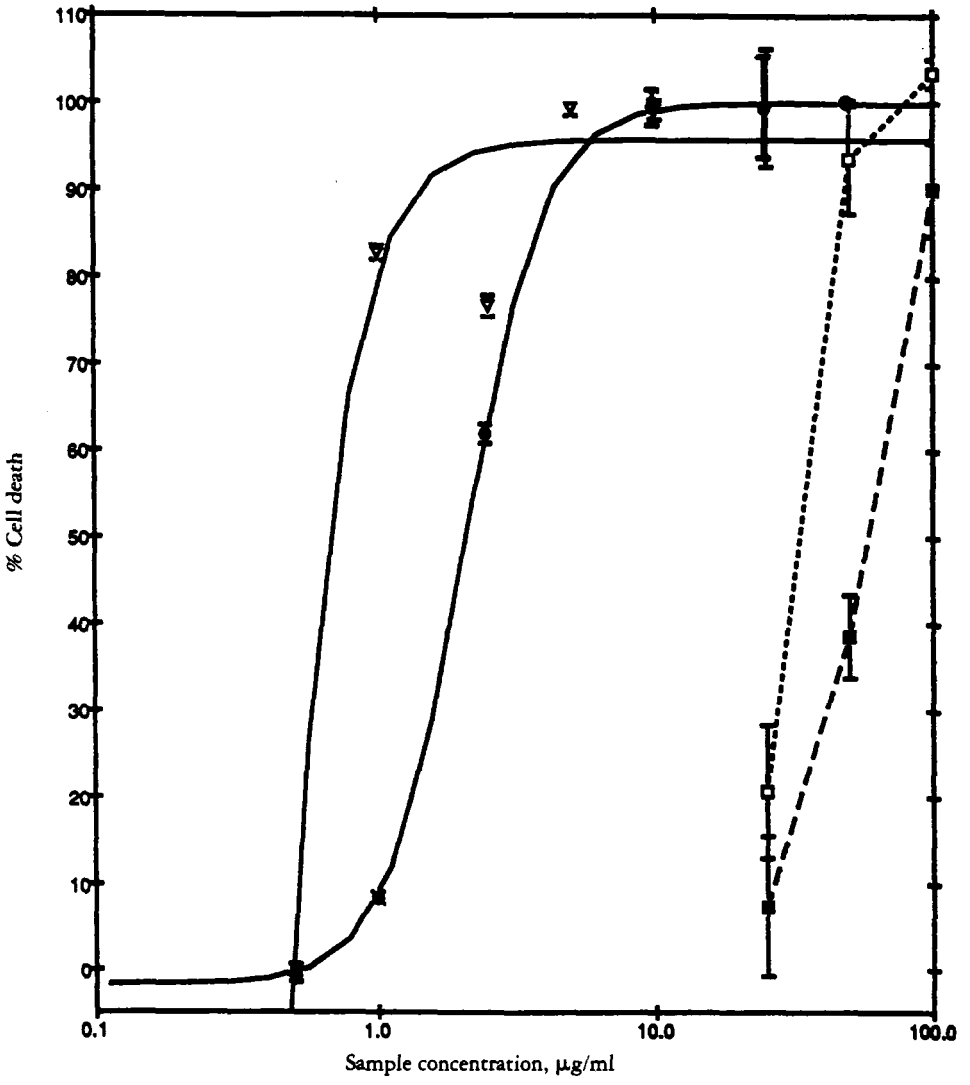


FIGURE 2. Effect of calf thymus DNA on the cytotoxicity of conioidine A [**1**] and crude extract. Conioidine A was tested at seven concentration levels, in the absence of DNA (-△-) and the presence of DNA (-●-). The dose-response curves were fitted by non-linear least squares regression (n=2). The Kd value was determined from a dissociation curve where the response difference (with and without DNA at each concentration) divided by the concentration was plotted against concentration. The crude extract was tested at three concentration levels, in the absence of DNA (-□-) and in the presence of DNA (-■-) to give the dose response curves (n=3).

concentration of **1** as well as the potency over the crude extract. After 48 h exposure, the IC_{50} of **1** in the absence of exogenous DNA was 0.67 μg/ml. In the presence of 25 μg/ml of DNA, it showed a more than threefold increase to 2.1 μg/ml. The reduction in cytotoxicity was in response to DNA binding which ultimately affected the ability of compound **1** to enter the cells and exert its cytopathic effect. From our dose response data, the K_d value was calculated to be 2.8 μM for conioidine A [**1**]. In a control study, the known DNA-interacting compound, doxorubicin, was examined for its KB cell cytotoxicity in the presence and absence of DNA. Our DNA-binding hypothesis was validated when doxourbinic showed a similar DNA reversal effect. The dose-response data gave a binding constant in agreement with the literature value; the data of this finding had been reported (12).

Pyrrolidine alkaloids in plants have been extensively studied and reviewed (13,14). A variety of bioactive pyrrolidine compounds have also been isolated from animal, fungal, and bacterial sources (15–22). However, the DNA binding property has not been reported for this class of compounds until the present study. Unlike most other DNA interacting agents reported in the literature, conioidines A and B are not aromatic structures. It is not certain whether these pyrrolidine natural products bind to DNA in the same manner as doxorubicin. Precisely how and where these compounds exert their binding ability will require further mechanistic studies.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were obtained using a Bruker WM360 spectrometer operating at 360.13 MHz and 90.56 MHz, respectively. For ^{13}C spectra, the GASPE technique and INEPT experiments were employed to distinguish multiplicity. All chemical shifts are reported with respect to TMS (δ 0). The proton correlation data were acquired using a standard Bruker COSY-45 sequence, which collected 256 spectra consisting of 1K data points over a spectral width of 3400 Hz. Each spectrum resulted from signal-averaging 64 scans. The data were zero-filled to 512 within the F1 domain and were apodized using a sinebell multiplier in each domain prior to double Fourier transformation. The lreims were obtained on a Finnigan MAT 4610 mass analyser with the samples applied to a direct exposure probe heated at 10 mA/sec, with the source operated at a temperature of 150° and ionization energy of 70 eV. The lreims were recorded and processed by a SuperIncos 2100D data system. The hreims data were obtained by peak matching on a Varian MAT CH-5DF mass spectrometer using a solids probe and operated at a resolution of 10,000 (m/Δm, 10% valley). Analysis by gc-ms was performed on a Finnigan 3300 equipped with an INCOS data system and chemical ionization (ci) detector. Uv spectra were obtained from a Beckman DU-7 spectrometer. Specific optical rotations were recorded with a Jasco DIP 360 digital polarimeter. Ir spectra were taken from KBr pellets on a Nicolet 20DXB Fourier transform infrared spectrometer equipped with an rt DTGS detector.

PLANT MATERIAL.—*C. conioides* was collected in Texas in April 1981; a voucher specimen SPJUT 6611 is preserved in the National Herbarium, Washington, DC. The extracts for screening were prepared from dry pulverized roots, stems, leaves, and flowers of *C. conioides* (3.5 lb) through sequential solvent processing with hexane, CH_2Cl_2, MeCOEt, and MeOH. Only the CH_2Cl_2 extract (3.9 g) showed cytotoxicity that could be reversed by exogenous DNA.

ISOLATION AND PURIFICATION.—All solvents used were hplc or reagent grade. The crude CH_2Cl_2 extract (1.4 g) was chromatographed on Si gel 60 (E.M. Science, 70–230 mesh), eluting with hexane-CH_2Cl_2-MeCN (8:2:1). The active fractions were further chromatographed on reversed-phase hplc (Whatman Partisil 10, ODS, M-9) eluting with 80% aqueous MeOH to give fractions of conioidine B [**2**] and with 85% aqueous MeOH to give fractions of conioidine A [**1**]. The final purification of conioidine A [**1**] (13.2 mg, 0.94% yield) was made by rechromatographing on reversed-phase (Beckman Ultrasphere ODS, 10×250 mm) with 80% aqueous MeCN; conioidine B [**2**] (3.5 mg, 0.25% yield) was by 70% MeCN.

DNA-BINDING ASSAYS.—KB cells were routinely grown in antibiotic-free minimal essential medium (GIBCO, Laboratories, Grand Island, NY) containing 20 mM HEPES and 10% fetal bovine serum (Flow Laboratories, McLean, VA). For cytotoxicity assays, cells were added to 96-well microtiter plates at 5×10^4 cells/well and allowed to attach overnight. Test substances (crude extract, isolation fractions, or pure compounds) were dissolved at 10 mg/ml in DMSO and mixed with equal volume of H_2O or 10 mg/ml calf thymus DNA (Sigma Chemical Co., St. Louis, MO) dissolved in H_2O. This mixture was incubated at room

temperature for 18–20 h before adding to the cells. DNA premixed with DMSO only was included as a control, and cell cultures were tested with a final DMSO concentration of 1%. Cultures were incubated in whole medium at 37° for 48 h, the medium was removed, and the remaining cells were stained with 0.5% crystal violet in 70% MeOH. The amount of stain (proportional to the number of live cells remaining) was quantified by absorbance using a Dynatech MR 600 microplate reader and expressed as a percentage of controls that were not exposed to the drug.

Conioidine A [**1**].—A colorless oil: ir ν max cm^{-1} (KBr) 2950–2860, 1705, 1630, 1275, 1160, 780; uv λ max (MeOH) 210 nmr (ϵ=7400); $[\alpha]^{25}D$+48.2 (MeOH, *c*=0.2); hreims 365.2938 ($C_{22}H_{39}NO_3$, calcd 365.2946); eims *m/z* (rel. int.) $[M]^+$ 365 (0.3), 282 (1), 265 (8), 210 (7), 138 (28), 110 (30), 100 (1), 83 (22), 70 (100); ^{13}C nmr (Me_2CO-d_6) 46.7 (t, C-1), 29.4 (t, C-2), 31.8 (t, C-3), 55.1 (d, C-4), 34.9 (t, C-5), 69.5 (d, C-6), 27.4 (q, C-7), 167.5 (s, C-1′), 129.0 (s, C-2′), 136.8 (d, C-3′), 12.0 (q, C-4′), 14.1 (q, C-5′), 171.8 (s, C-1″), 39.2 (t, C-2″), 22.6, 24.1, 24.8, 29.1, 29.2, 29.4, 29.5 (t, C-3″ to C-9″), 14.3 (q, C-10″); ^1H nmr see Table 1.

TABLE 1. ^1H-nmr Data for Conioidines A [**1**] and B [**2**].[a]

Proton	Compound	
	1	**2**
H-1	3.35 (2H, m)	3.41 (2H, m)
H-2	1.90 (2H, m)	1.90 (2H, m)
H-3	1.85 (2H, m)	1.85 (2H, m)
H-4	4.08 (1H, m)	4.10 (1H, m)
H_2-5	1.62, 2.03 (2H, m)	1.75, 2.28 (2H, m)
H-6	4.90 (1H, m)	4.96 (1H, m)
H-7	1.31 (3H, d, *J*=7.2)	1.34 (2H, d, *J*=7.5)
H-3′	6.80 (1H, dq, *J*=7.5, 1.1)	6.79 (1H, dt, *J*=7.0, 1.0)
H-4′	1.78 (3H, d, *J*=7.5)	4.28 (2H, d, *J*=7.0)
H-5′	1.80 (3H, d, *J*=1.1)	1.82 (3H, d, *J*=1.0)
H-2″	2.15 (2H, t, *J*=6.0)	2.20 (2H, t, *J*=6.0)
H-3″–H-9″	1.20–1.61 (14H, m)	1.22–1.50 (14H, m)
H-10′	0.86 (3H, t, *J*=6.5)	0.87 (3H, t, *J*=6.5)

[a]Spectra in $CDCl_3$. Table entries are in ppm from δ0 (multiplicity, *J* in Hz). Assignments are based on 2D COSY and decoupling data.

Conioidine B [**2**].—A colorless oil: ir ν max cm^{-1} (KBr) 3650–3500, 2960–2860, 1710, 1625, 1270, 1160, 780; uv λ max (MeOH) 211 nm (ϵ=7200); $[\alpha]^{25}D$+20.9 (MeOH, *c*=0.1); eims *m/z* (rel. int.) $[MH]^+$ 382 (0.7), 282 (1.6), 265 (5.4), 226 (0.8), 138 (32), 110 (35), 70 (100); ^1H nmr see Table 1.

Hydrolysis product **3**.—Conioidine A (0.4 mg) was heated in 600 μl of 0.2 M methanolic KOH in a sealed tube at 70° for 6 h . The mixture was evaporated to dryness, and the residue was chromatographed on reversed-phase hplc [Whatman ODS-3, 4.6×250 mm, MeOH-H_2O (8:2)] to give **3**: hreims 283.2485 ($C_{17}H_{33}NO_2$, calcd 283.2511); eims *m/z* (rel. int.) $[MH]^+$ 284 (0.4), 283 (0.9), 266 (0.4), 224 (2), 70 (100).

Acid degradation of [**1**].—Conioidine A (0.8 mg) was heated in 200 μl of 4 N methanolic HCl in a sealed tube at reflux for 2 h. After cooling to room temperature the mixture was evaporated to dryness. The residue was analyzed to gc-ms and found to contain methyl decanoate.

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