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ANTITUMOR AGENTS, 85.[1] CICUTOXIN, AN ANTILEUKEMIC PRINCIPLE FROM *CICUTA MACULATA*, AND THE CYTOTOXICITY OF THE RELATED DERIVATIVES

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Cicuta maculata L. (Umbelliferae), known as spotted water-hemlock, spotted cowbane, or wild-parsnip, is one of the stock-poisoning plants of North Carolina (1). Cases of *C. maculata* poisoning in humans have been reported (2,3). The roots and leaves of this plant have also been used as an herbal remedy for the treatment of scirrhous mammary cancer and scirrhous tumors, respectively (4). As a result of our continuing search among medicinal plants for novel, naturally occurring, potential antitumor agents,[1] the methanolic extract of the whole plant of *C. maculata* was found to show significant in vitro cytotoxicity in the 9 KB (human nasopharyngeal carcinoma) cell culture assay (5). Bioassay-directed fractionation of the active extract led to the isolation and characterization of cicutoxin (**1**) as the cytotoxic [ED_{50} (KB)=2.0 μg/ml] and antileukemic [T/C=165% (P-388 lymphocytic leukemia in mice) at 1 mg/kg, 3-day dosing][2] principle. Cicutoxin was previously isolated as the poisonous principle from *Cicuta virosa* (the water hemlock) (6-10);[3] however, its potent antileukemic activity is revealed for the first time.

Cicutoxin (**1**) was isolated as a yellowish oil in 0.12% yield. It is very unstable on exposure to light, heat, and air. Structural characterization of **1** was based upon the fact that it has a molecular formula of $C_{17}H_{22}O_2$ as determined by hrms. The ir spectrum showed characteristic bands of acetylene groups at 2225 and 2135 cm^{-1}. The ^{13}C-nmr spectrum revealed the presence of seventeen carbon signals, which include six olefinic (δ 144.3, 139.2, 135.4, 131.6, 129.7, and 109.9) and four acetylenic (δ 85.2, 77.0, 75.1, and 65.9) carbons. This spectrum also showed the presence of four methylene (δ 39.4, 30.9, 18.6, and 16.3), one methyl (δ 13.9), one primary alcoholic (δ 61.4), and one secondary alcoholic (δ 72.3) carbons, which were determined by the method of distortionless enhancement by polarization transfer (DEPT). The formation of a diacetate (**2**, $C_{21}H_{26}O_4$) by acetylation of **1** with Ac_2O in pyridine and of a saturated diol (**3**, $C_{17}H_{36}O_2$) by hydrogenation of **1** with 10% Pd-C with 7 moles of hydrogen absorbed, further confirmed the presence of two alcoholic and seven unsaturated functions in **1**.

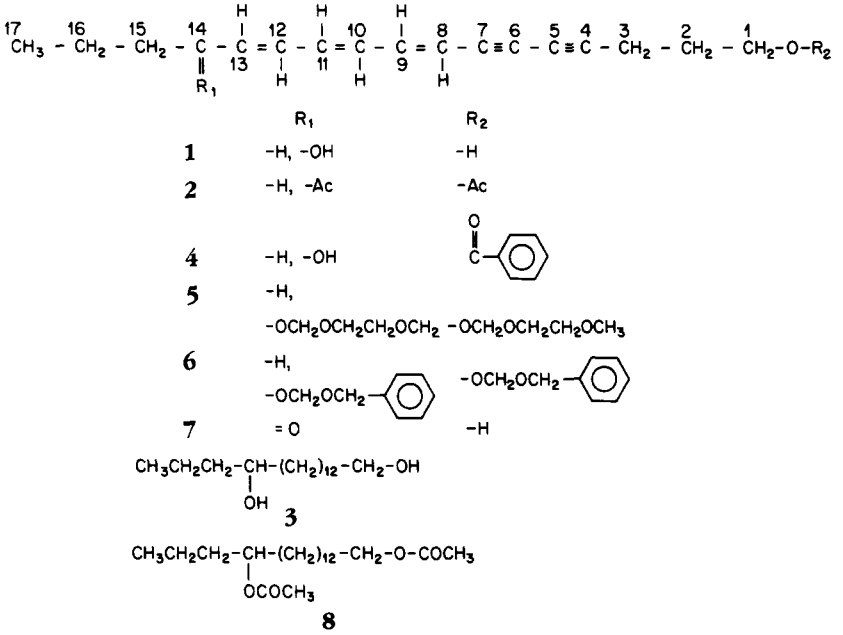
The foregoing evidence coupled with the ^1H nmr (Table 1) and other data which are in accord with the structures of **1** and its related derivatives (**2,4-7**) led to the assignment of **1** for cicutoxin; the configuration at C-14 in **1** remains to be determined.

The unique structure plus the potent antileukemic activity of **1** prompted us to study its structure-cytotoxicity relationships. Thus, in addition to the above-mentioned diacetate **2** and saturated diol **3**, new derivatives of **1** were also prepared according to literature

[1]For part 84, see T. Hayashi, J. Koyama, K.H. Lee, and A.T. McPhail, *Phytochemistry*, in press.

[2]In vitro cytotoxicity and in vivo antileukemic assays were carried out according to standard National Cancer Institute procedures described in the literature (5). The control, 5-fluorouracil, had T/C=135% (200 mg/kg, 1-day dosing).

[3]Cicutoxin is a spasmodic with high toxicity to the central nervous system. It accelerates respiration causing respiratory paralysis and death (13).



methods (10-12). These include a monobenzoate (**4**), a methoxyethoxymethyl ether (**5**), a benzyloxymethyl ether (**6**), and a keto alcohol (**7**). Since all of these compounds (**2-7**) demonstrated ED_{50} (KB)>4.0 μg/ml and were less cytotoxic than **1**, it is concluded that the structural requirement for the potent cytotoxic antileukemic activity of **1** is due to the presence of the conjugated double and triple bonds as well as the two hydroxyl groups.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fischer-Johns melting point apparatus and are uncorrected. Ir spectra were measured on Perkin-Elmer 1320 ir spectrometer. ^{1}H- and ^{13}C- nmr spectra were recorded on a Bruker 250 MHz spectrometer using TMS as an internal standard. Mass spectra were determined on a V.G. Micromass 70-70 instrument at 70 eV using a direct inlet system. Silica gel (Merck, type G 60, 70-230 mesh) was used for column chromatography, and precoated silica gel plates were used for analytical (Merck, 60 F-254, 0.25 mm) and preparative (Analtech, G. 100 m) tlc. Compounds were visualized by uv light or spraying with 15% H_2SO_4 solution followed by heating.

PLANT MATERIAL.—The whole plant of *C. maculata* was collected by Raymond Bowkley in Watavga County, NC in July 1985. A voucher specimen was placed on file at Wilcox Drug Co., Boone, NC.

EXTRACTION OF *C. maculata*.—The ground, air-dried, whole plant of *C. maculata* (1.5 kg) was exhaustively extracted with MeOH. After the solvent was removed in vacuo at <40°, a dark green syrup remained. Extraction and fractionation of the active compounds were monitored by an in vitro KB assay (5). The active syrup was suspended in H_2O-MeOH (10:1) and extracted several times with *n*-hexane. The aqueous layer was concentrated and then extracted with $CHCl_3$ (5 liters). The combined active $CHCl_3$ layers (ED_{50} <8.0 μg/ml) were dried over anhydrous $MgSO_4$ and then evaporated in vacuo to give a residue (35 g).

ISOLATION OF CICUTOXIN (**1**).—The foregoing residue (400 mg) was separated by preparative tlc on silica gel [solvent: MeOH-$CHCl_3$ (7:93)] to afford seven zones. These zones were subjected to the above-mentioned cytotoxicity assay. Purification of the most active zone ($ED_{50}\simeq$3.0 μg/ml; Rf=0.4 with fluorescence produced under short uv light) by repeated preparative tlc yielded 20 mg of cicutoxin (**1**) as a slightly yellowish syrup: $[\alpha]_D^{25}=-11.8°$ (*c*=0.55, EtOH) lit. (9) reported $[\alpha]_D^{15}=-14.5°$ (*c*=1.7, EtOH); ir ($CHCl_3$) 3620 (OH), 2225, 2135 (C≡C), 1600, 999 (C$\overset{+}{=}$C-C$\overset{+}{=}$C-C$\overset{+}{=}$C) cm^{-1}; and ^{1}H nmr (see Table 1).

ACETYLATION OF CICUTOXIN.—Cicutoxin (10 mg) was acetylated with Ac_2O (1 ml) and pyridine (1 ml) at room temperature for 18 h. The reaction product was worked up by the usual

TABLE 1. ^{1}H-nmr Spectral Data[a] of **1**, **2**, and **4-7**.

Compounds	Protons										
	$^1CH_2$	$^2CH_2$	$^3CH_2$	$^8CH=$	$^9CH=$	^{10}CH-^{12}CH	$^{13}CH=$	^{14}CH-	$^{15}CH_2$-$^{16}CH_2$	$^{17}CH_2$-	
1	3.76 (t,7.0)	1.81 (qn-like, 7.0)	2.48 t, 7.0)	5.60 (d, 14.0,	6.71 (dd, 14.0, 10.0)	6.40-6.10 (m)	5.81 (dd, 14.0, 7.0)	4.19 (q-like, 7.0)		0.93 (t, 7.0)	
2	4.16 (t, 6.3)	1.88 (qn-like, 6.5)	2.46 (t, 6.3)	5.60 (d, 15.3)	6.70 (dd, 15.3, 8.6)	6.38-6.18 (m)	5.71 (dd, 14.8, 7.1)	5.29 (q-like, 7.1)	1.70-1.25 (m)	0.91 (t, 7.1)	CH_3CO 2.05 2.06
4	4.42 (t, 6.2)	2.03 (qn-like, 6.5	2.55 (t, 6.7)	5.60 (d, 15.4)	6.70 (dd, 15.4, 9.7)	6.30-6.17 (m)	5.82 (dd, 14.5, 6.7)	4.19 (q-like, 6.2)		0.93 (t, 7.1)	C_6H_5CO 8.10-7.40 (5H, m)
5	overlapped with other signals	1.83 (qn-like, 6.5)	2.47 (t, 6.8)	5.61 (d, 15.4)	6.69 (dd, 15.4, 9.4)	6.38-6.14 (m)	5.64 (dd, 14.6, 7.6)	4.11 (q-like, 6.3)		0.91 (t, 7.3)	
6	3.68 (t, 6.2)	1.84 (qn-like, 6.3)	2.48 (t, 6.8)	5.60 (d, 15.4)	6.68 (dd, 15.4, 9.4)	6.35-6.15 (m)	5.65 (dd, 14.6, 7.6)	4.14 (q-like, 6.3)		0.92 (t, 7.3)	$2\times C_6H_5CH_2$ 7.40-7.29 (10H, m)
7	3.76 (t, 6.1)	1.82 (qn-like, 6.3)	2.51 (t, 7.5)	5.80 (d, 14.9)	6.75 (dd, 14.9, 11.0)	6.60, 6.40 (dd, each; 14.4, 11.0; H-10, H-11) 7.15 (dd, 15.5, 11.0; H-12)	6.23 (d, 15.5)		2.54 (t, 7.2; H-15) 1.66 (si-like, 7.3; H-16)	0.94 (t, 7.3)	

[a]Measured in $CDCl_3$. The chemical shifts are given in δ values. Coupling constants (J in Hz) are given in parentheses. The abbreviations s, d, t, qn, q, dd, si, and m refer to singlet, doublet, triplet, quartet, quintet, doublet of doublet, sixtet, and multiplet, respectively.

method, and further purified by preparative tlc [silica gel, MeOH-$CHCl_3$ (1:99)] to furnish a diacetate (**2**, 7 mg) as a colorless oil: ir ($CHCl_3$) 2225, 2130 (C≡C), 1730 ($OCOCH_3$), 1600, 999 ($C\overset{\pm}{=}C\text{-}C\overset{\pm}{=}C\text{-}C\overset{\pm}{=}C$) cm^{-1}; ^1H nmr (see Table 1); eims *m/z* 342.1839 (M^+, $C_{21}H_{26}O_4$ requires *m/z* 342.1824), 282 (M^+-HOAc), 239 (M^+-HOAC-$CH_3C{\equiv}O$), 227 (M^+-$CH_3CH_2CH_2$-CHOAc), 193 (M^+-C≡C-C≡C-CH_2CH_2-CH_2OAc), 167 (193-C_2H_2), 165 (193-C_2H_4), 152 (167-CH_3), 139 (167-C_2H_4), 141 (167-C_2H_2).

HYDROGENATION OF CICUTOXIN.—A solution of cicutoxin (**1**, 80 mg) in MeOH (30 ml) was hydrogenated with 10% Pd-C (30 mg) at room temperature for 20 min. The mixture was filtrated and evaporated in vacuo. The residue was purified by preparative tlc [silica gel; MeOH-$CHCl_3$ (5:95)] and recrystallized from petroleum ether to yield a 1,14-heptadecadiol (**3**, 45 mg) as colorless flakes: mp 67-69°; ir ($CHCl_3$) 3620 (OH) cm^{-1}; ^1H nmr ($CDCl_3$) 0.93 (3H, t, *J*=6.9 Hz, $^{17}CH_3$), 3.62 (1H, m, ^{14}CH), 3.64 (2H, t, *J*=6.4 Hz, $^1CH_2$); eims *m/z* 272 (M^+), 236, (M^+-$2H_2O$), 229 (M^+-C_3H_7), 211 (M^+-C_3H_7-H_2O), 193 (M^+-C_3H_7-$2H_2O$), 73 ($CH_3CH_2CH_2CHOH$), 55 (73-H_2O, base peak).

Compound **3** was further acetylated in the same manner as described above for the preparation of **2** to afford a diacetate (**8**) as a colorless oil: ir ($CHCl_3$) 1725 ($OCOCH_3$), 1250 (C-O-C) cm^{-1}; ^1H-nmr ($CDCl_3$) δ 4.87 (1H, gn, *J*=6.2 Hz, ^{14}CH-OAc), 4.05 (2H, t, *J*=6.7 Hz, $^1CH_2OAc$), 2.04, 2.05 (3H each, s, $COCH_3{\times}2$), 0.90 (3H, t, *J*=7.2 Hz, $^{17}CH_3$).

BENZOYLATION OF CICUTOXIN.—To a mixture of **1** (30 mg) in pyridine (5 ml) was added benzoic acid anhydride (100 mg). After the mixture was stirred for 30 h at room temperature, it was poured into ice-water and extracted with Et_2O. The Et_2O layer was washed with 1% HCl, 3% aqueous $NaHCO_3$, and H_2O, dried over anhydrous $MgSO_4$, and evaporated in vacuo at a temperature lower than 30° to yield a yellowish oil (25 mg). Purification of this oil by preparative tlc [silica gel; MeOH-$CHCl_3$ (1:99)] afforded a monobenzoate (**4**, 12 mg) as a colorless oil: ir ($CHCl_3$) 3600 (OH), 2225, 2140 (C≡C), 1720 (R-COOR), 1275, 1120 (C-O-C), 1650, 999 ($C\overset{\pm}{=}C\text{-}C\overset{\pm}{=}C\text{-}C\overset{\pm}{=}C$) cm^{-1}; ^1H nmr (see Table 1); eims *m/z* 344.1778 (M^+-H_2O, $C_{24}H_{24}O_2$ requires *m/z* 344.1770), 315 (M^+-H_2O-C_2H_5), 239 (M^+-H_2O-C_6H_5 C O), 210 (315-C_6H_5 $C{\equiv}O$), 193 (315-C_6H_5COOH), 178 (193-CH_3), 165 (193-C_2H_4), 152 (178-C_2H_2), 122 ($C_6H_5\text{-}\overset{+}{C}OOH$), 105 ($C_6H_5\ C{\equiv}\overset{+}{O}$), 77 ($C_6H_5^+$).

METHOXYETHOXYMETHYL ETHER OF CICUTOXIN.—To a solution of **1** (100 mg) in anhydrous CH_2Cl_2 (15 ml), diisopropylethyl amine (0.5 ml) and methoxyethoxymethyl chloride (0.5 ml) were added. The reaction mixture was sealed and allowed to stand at room temperature for 15 h. The mixture was poured into ice-water and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over anhydrous $MgSO_4$, and evaporated in vacuo at a temperature below 30° to give a yellowish oil. This oil was further purified by preparative tlc [silica gel; MeOH-$CHCl_3$ (1:99)] to yield **5** (42 mg) as a colorless oil: ir ($CHCl_3$) 2230, 2140 (C≡C), 1600, 1000 ($C\overset{\pm}{=}C\text{-}C\overset{\pm}{=}C\text{-}C\overset{\pm}{=}C$) cm^{-1}; ^1H nmr (see Table 1); eims *m/z* 434.2665 (M^+, $C_{25}H_{38}O_6$ requires *m/z* 434.2669), 345 (M^+-$CH_2OCH_2CH_2OCH_3$), 329 (M^+-OCH_2OCH_2-CH_2OCH_3), 328 (M^+-$HOCH_2OCH_2CH_2$-OCH_3), 269 (328-$CH_2CH_2OCH_3$), 253 (328-$OCH_2CH_2OCH_3$), 239 (328-$CH_2OCH_2CH_2$-OCH_3), 89 ($CH_2OCH_2CH_2OCH_3$), 59 ($CH_2CH_2OCH_3$).

BENZYLOXYMETHYL ETHER OF CICUTOXIN.—To a solution of **1** (100 mg) in anhydrous CH_2Cl_2 (50 ml), diisopropylethyl amine (0.5 ml) and benzyloxymethyl chloride (0.5 ml) were added. After the reaction mixture was stirred at room temperature for 20 h, it was worked up in an analogous manner as described above for the preparation of **5** to yield a benzyloxymethyl ether (**6**, 30 mg, oil) after purification of the product by silica gel column chromatography in MeOH-$CHCl_3$ (5:95): ir ($CHCl_3$) 2220, 2140, 1600, 1500, 1450, 1380, 1160, 1110, 1040, 999 cm^{-1}; ^1H nmr (see Table 1); eims *m/z* 498 (M^+).

OXIDATION OF CICUTOXIN.—To a solution of **1** (30 mg) in $CHCl_3$ (20 ml), activated MnO_2 (200 mg) was added. After the mixture was stirred at room temperature for 5 h, it was filtered, and evaporated in vacuo at a temperature below 20° to furnish a yellowish syrup (15 mg). This syrup was crystallized from $CHCl_3$/*n*-hexane to give 14-oxocicutoxin (**7**, 7 mg) as yellow crystals: mp 69-71°; ir ($CHCl_3$) 3630, 2220, 1650, 1600, 999 cm^{-1}; ^1H nmr (see Table 1); eims *m/z* 256 (M^+), 238 (M^+-H_2O), 213 (M^+-C_3H_7), 185 (M^+-C_3H_7 C=O), 71 ($C_3H_7C{=}O$), 43 (C_3H_7).

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