Journal of Natural Products
Vol. 53, No. 6, pp. 1575-1577, Nov-Dec 1990

CYTOTOXIC PYRIDONE ALKALOIDS FROM THE LEAVES OF *PIPER ABORESCENS*

CHANG-YIH DUH,* YANG-CHANG WU,

School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan 80731, Republic of China

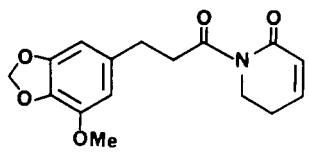
and SHANG-KWEI WANG

Department of Microbiology, Kaohsiung Medical College, Kaohsiung, Taiwan 80731, Republic of China

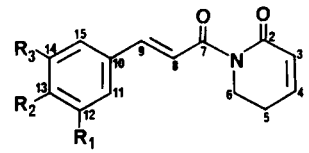
ABSTRACT.—Bioactivity-guided fractionation of a $CHCl_3$ extract of the leaves of *Piper aborescens* afforded a new cytotoxic pyridone alkaloid, *N*-(3-methoxy-4,5-methylenedioxydihydrocinnamoyl)-Δ^3-pyridin-2-one [**1**], as well as three known cytotoxic pyridone alkaloids, *N*-(3-methoxy-4,5-methylenedioxycinnamoyl)-Δ^3-pyridin-2-one [**2**], piplartine [**3**], and piplartine dimer A [**4**].

Piper aborescens Roxb. (Piperaceae) is a creeping shrub native to Lan-yu Island of Taiwan (1). In a previous paper (2), the stems of this plant were reported to contain two new cytotoxic pyridone alkaloids, *N*-(3-methoxy-4,5-methylenedioxycinnamoyl)-Δ^3-pyridin-2-one [**2**] and *N*-(3,4-dimethoxycinnamoyl)-Δ^3-pyridin-2-one [**5**]. As a result of a continuing search for novel plant antitumor agents, the $CHCl_3$ extract of the leaves of *P. aborescens* was found to show significant (ED_{50}<20 μg/ml) cytotoxicity against the KB nasopharyngeal carcinoma and P-388 lymphocytic leukemia systems in cell cultures when assessed using standard protocols (3). Bioactivity-guided chromatographic fractionation in the KB cell culture system led to the isolation and characterization of a new cytotoxic pyridone alkaloid, *N*-(3-methoxy-4,5-methylenedioxydihydrocinnamoyl)-Δ^3-pyridin-2-one [**1**], as well as three known cytotoxic pyridone alkaloids, piplartine [**3**], piplartine dimer A [**4**], and **2**.

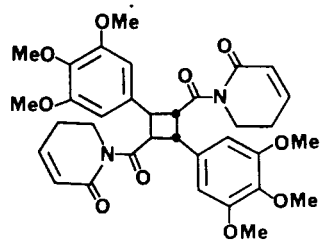
Compound **1** was obtained as white needles, mp 80–81°, and exhibited a molecular formula of $C_{16}H_{17}NO_5$ as determined by hrms. Its ir spectrum showed a carbonyl absorption at 1670 cm^{-1}. The uv spectrum, with absorption maxima at 242 and 285 nm, indicated that it possessed an α,β-unsaturated amide functionality. In the ^{1}H-nmr spectrum, two doublets of triplets at δ 6.88 (J = 9.7, 4.2 Hz) and δ 5.98 (J = 9.7, 1.8 Hz), a triplet at δ 3.95 (J = 6.5 Hz) and a multiplet at δ 2.37 suggested the presence of a Δ^3-2-pyridone ring (2). This was confirmed by the ^{13}C-nmr spectrum (Table 2) (2). The proton signals at δ 3.86 (s), 5.90 (s), 6.40 (d, J = 1.4 Hz), and 6.42 (d, J = 1.4 Hz) indicated that **1** possessed a 1-substituted 3-methoxy-4,5-methylene-



1



2 R_1=OMe; R_2,R_3=OCH_2O
3 R_1=R_2=R_3=OMe
5 R_1=R_2=OMe, R_3=H



4

TABLE 1. Cytotoxicity[a] of Compounds **1–4**.

Compound	ED_{50} (μg/ml) ($N = 8$)			
	Cell line			
	A-549	HT-29	KB	P-388
1	5.76	3.80	4.99	2.21
2	2.57	2.15	2.62	0.43
3	0.60	0.45	1.80	0.90
4	2.21	2.49	3.90	3.06

[a]For significant activity of pure compounds, an ED_{50} value of ≤ 4.0 μg/ml is required (3).

dioxybenzene moiety. The nOe difference spectrum of **1** revealed that on irradiation of aromatic methoxyl protons at δ 3.86 ppm a significant degree of nOe enhancement was observed for H-11 (at δ 6.40 ppm) with a negligible degree of nOe enhancement being observed for H-15 (at δ 6.42 ppm). The two symmetrical 2H triplets at δ 2.88 and δ 3.20 (J = 7.6 Hz), the additional carbonyl signal at δ 176.3 in the ^{13}C-nmr spectrum, and fragment ions at m/z 207 and 179 in the eims pointed to the presence of a $-CH_2CH_2-C=O$ group linking the aromatic and pyridone moiety. The assignment of the ^{1}H-nmr and ^{13}C-nmr chemical shifts of **1** was achieved by the application of COSY, DEPT, and HETCOR experiments (4). The structure of **1** is in full accord with all of the spectral data.

The identities of **2** and **3** were confirmed by direct comparison with authentic samples isolated from the stems of *P. aborescens* by tlc and spectral data (2). Compound **4** was identified by spectral comparison (ir, uv, ^{1}H nmr, and ms) with the literature data (5).

Compounds **2**, **3**, and **4** showed significant cytotoxicity against the growth of A-549, HT-29, KB, and P-388 cells (Table 1). Compound **1** exhibited significant cytotoxicity against P-388 and HT-29 cells and showed marginal cytotoxicity in KB and A-549 cells.

TABLE 2. ^{13}C-nmr Spectral Data (ppm) of Compound **1**[a]

Carbon	Chemical Shift
C-2	166.2 (s)[b]
C-3	126.5 (d)
C-4	146.0 (d)
C-5	25.0 (t)
C-6	41.5 (t)
C-7	176.3 (s)
C-8	41.5 (t)
C-9	31.5 (t)
C-10	134.2 (s)
C-11	108.2 (d)
C-12	149.4 (s)
C-13	136.4 (s)
C-14	144.1 (s)
C-15	103.2 (d)
-OMe	57.0 (q)
$-OCH_2O-$	101.8 (t)

[a]At 50 MHz, in $CDCl_3$, TMS as internal standard. Assignments of carbons bearing hydrogen atoms were confirmed by the ^{13}C-^{1}H HETCOR experiment.

[b]SFORD and DEPT multiplicity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Yanagimoto micro-melting point apparatus and were uncorrected. The uv spectra were obtained on a Hitachi 200-20 spectrophotometer and ir spectra measured on a Hitachi 260-30 spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were recorded with a Varian Gemini NMR spectrometer at 200 MHz and 50 MHz, respectively, in $CDCl_3$ using TMS as the internal standard. Eims spectra were obtained with a Jeol JMS-HX110 mass spectrometer at 70 eV. Silica gel 60 (Merck, 230–400 mesh) was used for cc, pre-coated Si gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical tlc, and pre-coated Si gel plates (Merck, Kieselgel 60 F-254, 0.50 mm) were used for preparative tlc.

PLANT MATERIAL.—Leaves of *P. aborescens* were collected in Lan-yu Island of Taiwan in February 1989. Voucher specimens are kept in the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

EXTRACTION AND SEPARATION.—The air-dried, milled leaves (1.3 kg) of *P. aborescens* were extracted repeatedly with MeOH. After removal of solvent in vacuo, the MeOH-soluble residue was partitioned between H_2O and $CHCl_3$. The dried $CHCl_3$ extract (90 g) was found to exhibit

activity against the KB cell system and the P-388 lymphocytic leukemia system in cell cultures (3) with ED_{50} of 5.4 and 1.4 μg/ml, respectively. Cc of the $CHCl_3$ extract was undertaken using $CHCl_3$ and $CHCl_3$/MeOH mixtures of increasing polarity. A total of 55 fractions (0.2 liters each) was collected. Elution by $CHCl_3$-MeOH (98:4) afforded fractions containing **1** and **2** from which these two amides were separated by preparative tlc with *n*-hexane–EtOAc (1:1). Elution of the chromatographic column by $CHCl_3$-MeOH (92:8) led to the isolation of a mixture of **3** and **4**, which were further purified by cc over Si gel with *n*-hexane–EtOAc (1:1).

N-(3-METHOXY-4,5-METHYLENEDIOXYDIHYDROCINNAMOYL)-Δ^3-PYRIDIN-2-ONE [**1**].—White needles (24 mg); mp 80–81°; uv (MeOH) λ max (log ε) 220 (3.51), 242 sh (3.22), 285 nm (2.53); ir (Nujol) ν max 1670 (CO), 1615, 1490, 1420, 1360, 1315, 1305, 1280, 1250, 1200, 1160, 1120 cm^{-1}; ^1H nmr ($CDCl_3$) δ 2.37 (m, 2H, H-5), 2.88 (t, J = 7.6 Hz, 2H, H-9), 3.20 (t, J = 7.6 Hz, 2H, H-8), 3.86 (s, 3H, MeO-12), 3.95 (t, J = 6.5 Hz, 2H, H-6), 5.90 (s, 2H, $-OCH_2O-$), 5.98 (dt, J = 9.7, 1.8 Hz, 1H, H-3), 6.40 (d, J = 1.4 Hz, 1H, H-11), 6.42 (d, J = 1.4 Hz, 1H, H-15), 6.88 (dt, J = 9.7, 4.2 Hz, 1H, H-4); ^{13}C nmr ($CDCl_3$) see Table 2; eims *m/z* $[M]^+$ 303 (52%), 207 (11), 206 (95), 179 (10), 178 (100), 165 (40), 149 (15), 135 (21), 107 (14), 98 (20), 77 (18); hreims found 303.1111, calcd 303.1107 for $C_{16}H_{17}NO_5$.

CYTOTOXICITY TESTING.—KB and P-388 cells were kindly provided by Prof. J.M. Pezzuto, University of Illinois at Chicago; A-549 (lung carcinoma) and HT-29 (colon carcinoma) were purchased from ATCC.

The P-388 cells were cultured in Fisher's medium supplemented with 10% heat-inactivated (56° for 30 min) fetal calf serum (FCS). The KB cells were maintained in Basal Medium Eagle (BME) containing 10% heat-inactivated FCS. The A-549 cell line was cultured in Eagle Minimum Essential Medium (EMEM) containing Earle's salts and supplemented with 0.1 mM of nonessential amino acids and 10% heat-inactivated FCS. The HT-29 cell line was maintained in Rosewell Park Memorial Institute (RPMI) 1640 Medium containing 10% heat-inactivated FCS. All the cell lines were maintained in an incubator at 37° in humidified air containing 5% CO_2.

The cytotoxic activities of tested compounds against P-388, KB, A-549, and HT-29 were assayed with modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method described by Alley *et al.* (6). For P-388 cells, 200 μl of culture was established at 1500 cells/well in 96-well tissue culture plates (Falcon). Tested compounds were dispensed subsequently to the established culture plate at eight concentrations each with three repeats. After 3 days of incubation, P-388 cells were enumerated with MTT.

To measure the cytotoxic activities of purified compounds against KB, A-549, and HT-29, each cell line was initiated at 1,000 cells/well in 96-well microtiter plates. Eight concentrations encompassing a 128-fold range were performed on each cell line. KB, A-549, and HT-29 cells were enumerated using MTT after the exposure to tested compounds for 3, 6, and 6 days, respectively. Fifty μl of 1 mg/ml MTT was added to each well, and plates were incubated at 37° for a further 5 h. Formazan crystals were redissolved in DMSO (Merck) for 10 min with shaking, and the plate was read immediately on an enzyme-linked immunosorbent assay reader (Titertek Multiskan, Flow) at a wavelength of 540 nm. The ED_{50} was defined as 50% reduction of absorbance in the MTT assay. Results are given in Table 1.

ACKNOWLEDGMENTS

We thank Professor J.M. Pezzuto, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, for the provision of KB and P-388 cell lines. This work was supported by a grant from the National Science Council of the Republic of China (NSC79-0208-M037-01) awarded to C.-Y. Duh.

LITERATURE CITED

1. F.-L. Wang, in: "Flora of Taiwan." Ed. by H.-L. Li, T.-S. Liu, T.-C. Huang, T. Koyama, and C.E. Devol, Epoch Publishing, Taipei, Taiwan, Republic of China, 1976, Vol. 2, p. 560.
2. C.-Y. Duh, Y.-C. Wu, and S.-K. Wang, *Phytochemistry*, **29**, 2689 (1990).
3. R.I. Geran, N.H. Greenberg, M.M. MacDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3**, 1 (1972).
4. A. Bax and G.A. Morris, *J. Magn. Reson.*, **42**, 501 (1981).
5. R.B. Filho, M.P. De Souza, and M.L.O. Mattos, *Phytochemistry*, **20**, 345 (1981).
6. M.C. Alley, D.A. Scudiero, A. Monks, M.L. Hursey, M.J. Czerwinski, D.L. Fine, B.J. Abbott, J.G. Mayo, R.H. Shoemaker, and M.R. Boyd, *Cancer Res.*, **48**, 589 (1988).

Received 28 February 1990