A Cytotoxic Acetophenone with a Novel Skeleton, Isolated from *Cynanchum taiwanianum*

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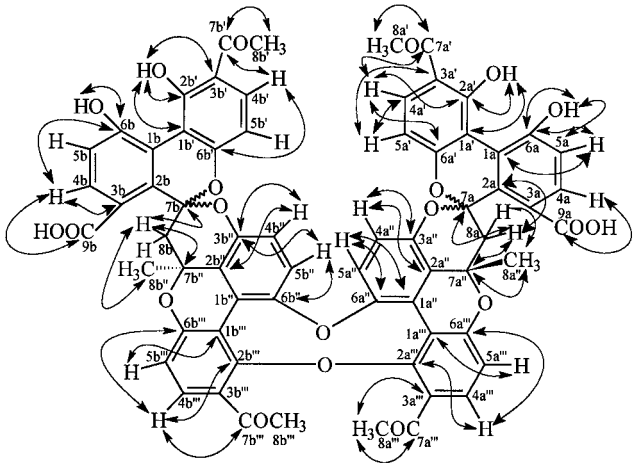
A novel acetophenone, cynantetrone (**1**), was isolated from the rhizome of *Cynanchum taiwanianum* and its structure determined by spectroscopic methods. Compound **1** and cynandione B (**3**) showed significant *in vitro* cytotoxicity against T-24 cell lines, and **3** also against PLC/PRF/5 cell lines.

1. Introduction. – In previous papers [1–4] we have reported the isolation and biological activity of acetophenones, *i.e.*, of cynandiones A–D, cynanchone A, and 2,5-dihydroxyacetophenone, from *Cynanchum taiwanianum* (Asclepiadaceae). In continuation of the investigation on bioactive constituents from this plant, a novel acetophenone, cynantetrone (**1**), was isolated from the rhizome of *C. taiwanianum*, and its structure was elucidated. The cytotoxic activity of **1**, cynandiones A (**2**) and B (**3**), and cynanchone A (**4**) against some cell types are reported.

2. Results and Discussion. – Compound **1**, an orange powder, possesses the molecular formula $C_{66}H_{44}O_{20}$ as determined by DCI mass spectra (negative mode; $[M-H]^-$ at *m/z* 1155) and by H- and C-atom counting in NMR spectra. IR Absorptions were indicative of OH (3260 and 3430 cm^{-1}), carboxylic-acid (1720 and 3560 cm^{-1}), conjugated C=O (1660 cm^{-1}), and aromatic-ring moieties (1580 cm^{-1}). The ^{1}H- and ^{13}C-NMR data (*Table 1*), including NOESY, COSY, HMQC, and HMBC, suggested that **1** is a planar tetraacetophenone derivative with eight aromatic rings and two ether linkages (*Fig. 1*).

The ^{1}H-NMR spectrum of **1** showed eight pairs of *ortho*-coupled aromatic protons at δ 6.45 and 6.85 (J = 8.5 Hz), 6.76 and 7.79 (J = 8.5 Hz), 6.73 and 6.96 (J = 8.5 Hz), 6.73 and 7.79 (J = 8.5 Hz), 6.70 and 6.93 (J = 8.5 Hz), 6.49 and 7.78 (J = 8.5 Hz), 6.99 and 6.71 (J = 8.5 Hz), and 6.19 and 7.70 (J = 8.5 Hz), four acetyl signals at 2.60 and 2.69 (each 6 H, *s*), two Me *s* at 1.66 and 1.78, two CH_2 *d* at δ 2.74 and 3.97 (J_{gem} = 15.2 Hz), and 2.87 and 4.13 (J_{gem} = 15.2 Hz), four phenolic-proton signals at δ 8.60, 8.83, 15.5, and 15.6, and two carboxylic-acid signals at δ 13.6 and 13.7.

In the ^{13}C-NMR spectrum of **1**, the chemical shift values of C(1a) to C(8a‴) and C(1b) to C(8b‴) were similar to those of cynandiones B (**3**; 7*R*,7″*S*) and D (7*R*,7″*R*), except for C(1a) to C(6a) and C(1b) to C(6b) [3][1]). Comparison of chemical shift values of C(1a) to C(6a) and C(1b) to C(6b) with data reported in [5] and of the phenolic protons with corresponding data of cynandione D [2][3], as well as the presence of ^{1}H,^{1}H-NOESY



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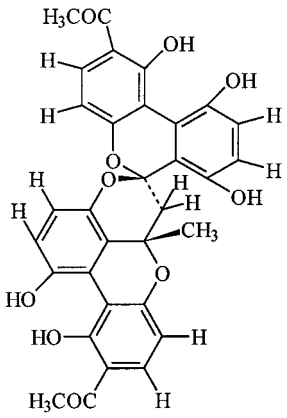

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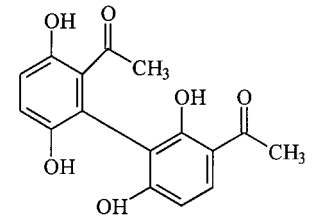

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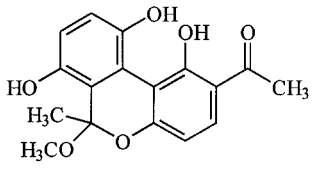

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Fig. 1. *Structures of* **1**–**4** *and* $^{1}H,^{13}C$ *long-range correlations observed in the HMBC spectrum of* **1**

correlations between OH–C(6a) and OH–C(2a′), and OH–C(6b) and OH–C(2b′), suggested that the two COO*H* signals at Δ 13.6 and 13.7 and the four OH signals at δ 8.60, 8.83, 15.5, and 15.6 were assigned to COOH groups at C(3b) and C(3a) and OH groups at C(6b), C(6a), C(2b′), and C(2a′), respectively. These data were consistent with the planar structure of cynantetrone and with two ether linkages between C(6a″) and C(6b″) and C(2a‴) and C(2b‴), or, alternatively, with two ether linkages between C(6a″) and C(2b‴) and C(6b″) and C(2a‴), both kind of linkages being also compatible with the 2D-NMR (*Fig. 1*) and ^{13}C-NMR spectra (*Table 1*).

The base peak at *m/z* 568 in the MS of **1** was attributed to the fragments $[1155 - a - b]^-$ or $[851 - 283]^-$ (*Fig. 2*). These and characteristic peaks at *m/z* 1137 ([1155 –

Table 1. *^{1}H- and ^{13}C-NMR Spectra ($CDCl_3$) of* **1**[a])[1]

	δ(C)	δ(H)		δ(C)	δ(H)		δ(C)	δ(H)		δ(C)	δ(H)
C(1a)	124.8		C(1a″)	112.9		C(1b)	124.8		C(1b″)	115.7	
C(2a)	131.1		C(2a″)	119.7		C(2b)	131.2		C(2b″)	119.9	
C(3a)	108.8		C(3a″)	158.8		C(3b)	109.2		C(3b″)	158.8[b])	
H−C(4a)	119.9	6.85 (*d*, *J* = 8.5)	H−C(4a″)	118.5	6.73 (*d*, *J* = 8.5)	H−C(4b)	120.8	6.93 (*d*, *J* = 8.5)	H−C(4b″)	111.7[b])	6.71 (*d*, *J* = 8.5)
H−C(5a)	120.0	6.45 (*d*, *J* = 8.5)	H−C(5a″)	120.8	6.96 (*d*, *J* = 8.5)	H−C(5b)	118.5	6.70 (*d*, *J* = 8.5)	H−C(5b″)	120.8	6.99 (*d*, *J* = 8.5)
OH−C(6a)	150.0	8.83 (*s*)	C(6a″)	141.2		OH−C(6b)	149.4	8.60 (*s*)	C(6b″)	139.0	
C(7a)	97.5		C(7a″)	74.0		C(7b)	98.1		C(7b″)	74.1	
2 H−C(8a)	44.5	2.87 (*d*, *J* = 15.2) 4.13 (*d*, *J* = 15.2)	Me(8a″)	24.5	1.66 (*s*)	2 H−C(8b)	39.2	2.74 (*d*, *J* = 15.2) 3.97 (*d*, *J* = 15.2)	Me(8b″)	26.3	1.78 (*s*)
C(9a) OOH	182.9	13.6 (*s*)				C(9b)OOH	183.7	13.7 (*s*)			
C(1a′)	112.7		C(1a‴)	112.7		C(1b′)	112.9		C(1b‴)	115.6	
OH−C(2a′)	158.2	15.6 (*s*)	C(2a‴)	158.6[b])		OH−C(2b′)	158.4	15.5 (*s*)	C(2b‴)	158.6[b])	
C(3a′)	114.7		C(3a‴)	114.8		C(3b′)	115.7		C(3b‴)	115.7	
H−C(4a′)	135.4[b])	7.79 (*d*, *J* = 8.5)	H−C(4a‴)	132.0	7.79 (*d*, *J* = 8.5)	H−C(4b′)	135.4	7.78 (*d*, *J* = 8.5)	H−C(4b‴)	132.1	7.70 (*d*, *J* = 8.5)
H−C(5a′)	111.6	6.76 (*d*, *J* = 8.5)	H−C(5a‴)	118.5[b])	6.73 (*d*, *J* = 8.5)	H−C(5b′)	108.8	6.49 (*d*, *J* = 8.5)	H−C(5b‴)	109.2	6.19 (*d*, *J* = 8.5)
C(6a′)	162.2[b])		C(6a‴)	158.9		C(6b′)	162.2		C(6b‴)	162.0[b])	
C(7a′)	204.5		C(7a‴)	203.3		C(7b′)	204.5		C(7b‴)	203.3	
Me(8a′)	26.2	2.69 (*s*)	Me(8a‴)	26.5	2.69 (*s*)	Me(8b′)	26.5	2.60 (*s*)	Me(8b‴)	26.5	2.60 (*s*)

[a]) All assignments were confirmed by HMQC, HMBC, and NOESY data. Coupling constants *J* in Hz. [b]) Assignments may be reversed.
[1]) Arbitrary numbering; for the systematic name, see *Exper. Part.*

$H_2O]^-$), 851 ([1155 – a – H_2O – CO]$^-$), 552 [568 – H_2O + 2 H]$^-$), 284 ([b – COOH]$^-$), 283 ([b – H_2O – CO]$^-$), and 260 ([a + 2 H]$^-$) [6], as well as the presence of a NOESY correlation between OH–C(2a′) and OH–C(2b′), established finally that the novel cynantetrone corresponds to structure **1**.

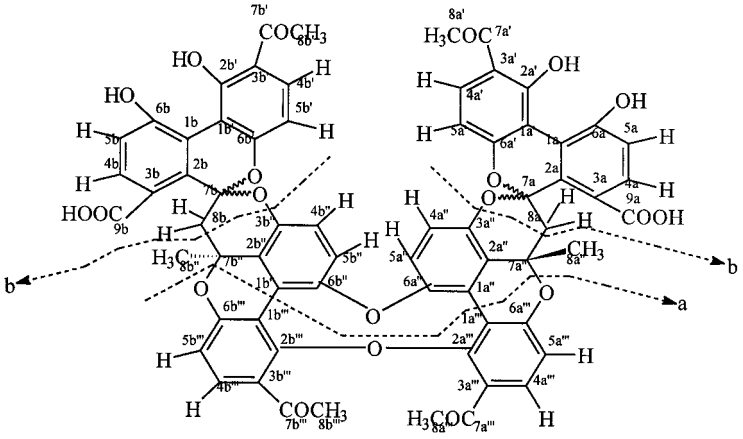

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Fig. 2. *DCI-MS Fragmentation patterns of* **1**

The NOESY spectrum also indicated correlations between Me(8a″) and one geminal proton at C(8a) (δ 4.13), and between Me (8b″) and one geminal proton at C(8b) (δ 2.74), suggesting β- and α-configurations for Me(8a″) and Me(8b″), respectively.

The cytotoxic activity of **1**–**4** against PLC/PRF/5, KB, and T-24 cells was studied *in vitro* [7][8]. Compounds **1** and **3** exhibited significant cytotoxic effect against T-24 cell lines with ED_{50} values of *ca*. 3.5 and 2.5 μg/ml, respectively, and **3** also against PLC/PRF/5 cell lines (ED_{50} = 2.7 μg/ml) (*Table 2*). Thus, the presence of two moieties of a biacetophenone such as **2** or **4** in a dimer such as **3** enhanced the *in vivo* cytotoxic activity against PLC/PRF/5, KB, and T-24 cells, but the presence of four such moieties in a tetramer such as **1** did not enhance the cytotoxic activity. This clearly indicates that

Table 2. *Cytotoxicity of* **1**–**4** *against Different Cell Lines*[a])

	ED_{50} [μg/ml]		
	PLC/PRF/5	KB	T-24
1	6.6	NS	3.5
2	10.3	9.0	11.0
3	2.7	5.5	2.5
4	17.7	NS	NS
Cisplatin	5.29	0.16	–[c])
Mitomycin C	–[c])	–[c])	0.042

[a]) For significant activity of the pure compounds, an $ED_{50} < 4.0$ μg/ml is required. [b]) NS, no significant activity of the pure compounds. [c]) Not determined.

these acetophenone derivatives need a reasonable molecular size, such as given in **3**, for cytotoxic activity against tumor cells.

This work was supported by a grant from the *National Science Council of R. O. C.* (NSC 88-2314-B037-112).

Experimental Part

General. M.p. uncorrected. UV Spectra: *Jasco-UV-VIS* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi 260-30* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) Spectra: *Varian-Unity-400* spectrometer; δ in ppm rel. to $SiMe_4$ (= 0 ppm), *J* in Hz. MS: *JMS-HX-100* mass spectrometer; *m/z* (rel. %).

Plant Material. Fresh rhizomes (5 kg) of *C. taiwanianum* were collected at Kaohsiung Hsieng, Taiwan, in July 1993. A voucher specimen is deposited in the laboratory of medicinal chemistry.

Extraction and Isolation. The fresh rhizomes (5 kg) of *C. taiwanianum* were chipped and extracted with acetone at r.t. several times. The extract was subjected to column chromatography (silica gel, cyclohexane/$CHCl_3$/MeOH 1:9:1): 15 mg of *cynantetrone* (= *2,2″,7′,9′-tetraacetyl-3′,3′a,12′a,13′-tetrahydro-1,1″,10,10″-tetrahydroxy-3′a,12′a-dimethyldispiro[6*H*-dibenzo[*b,d*]pyran-6,2′-[2*H*,14*H*]-[1,4,8,12,15,18]hexaoxadibenzo[*j:k′,j′k′]-cyclodeca[1,2,3,4-*def:*6,7,8,9-*d′e′f′]anthracene-14′,6″-[6*H*]dibenzo[*b,d*]pyran]-7,7″-dicarboxylic acid*; **1**). Orange powder ($CHCl_3$). M.p. > 300°. $[\alpha]_D^{25} = -39$ (*c* = 0.18, $CHCl_3$). UV (MeOH): 276 (4.68). IR (KBr): 3560, 3430, 3260, 1720, 1660, 1580. ^{1}H- and ^{13}C-NMR: *Table 1*. DCI-MS (neg.; see *Fig. 2*): 1155 (0.2, $[M-1]^-$), 1137 (9, $[1155-H_2O]^-$), 851 (3, $[1155-a-H_2O-CO]^-$), 568 (100, $[851-283]^-$ or $[1155-a-b]^-$), 552 (25, $[568-H_2O+2H]^-$), 284 (15, $[b-COOH]^-$), 283 (7, $[b-H_2O-CO]^-$), 260 (3, $[a+2H]^-$).

Tumor Cell Growth Inhibition Assays. A microassay for cytotoxicity was performed using a MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-1*H*-tetrazolium bromide) assay [7][8]. Briefly, $1-3\cdot10^3$ cells/100 µl were seeded in 96-well microplates (*Nunck*, Roskilde, Denmark) and preincubated for 6 h to allow cell attachment. This medium was then aspirated, and fresh medium (100 µl) containing various concentrations of the test drug was added to the cultures. The cells were incubated with each drug for 6 days. Cell survival was evaluated by adding 10 µl of tetrazolium salt soln. (1 mg of MTT/ml in PBS (phosphate buffered saline soln.)). After 4 h incubation at 37°, DMSO (100 µl) was added to dissolve the precipitate of reduced MTT. Microplates were then shaken for 15 min, and the absorbance was determined at 550 nm with a multiwell scanning spectrophotometer (*Dynex MR 5000*, Chantilly, VA).

PLC/PRF/5 Cells were established from a human hepatoma and known to produce HBs Ag continuously in culture fluids [9]. Human hepatoma PLC/PRF/5 cells, epidermoid carcinoma KB cells, and human bladder carcinoma T-24 cells were maintained in *Dulbecco*'s modified *Eagle* medium (DMEM, *Gibco BRL*, Grand Island, NY, USA) [7][8], containing 10% fetal bovine serum (FBS, *Gibco BRL*), 2 mM L-glutamine, penicillin (100 units/ml) and streptomycin (100 µg/ml). For the microassay, the growth medium was supplemented with 10 mM HEPES (= 1-(2-hydroxyethyl)piperazine-4-ethanesulfonic acid) buffer (pH 7.3) and incubated at 37° in a CO_2 incubator.

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Received June 7, 1999