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TWO NEW NATURAL AZAFLUORENE ALKALOIDS AND A CYTOTOXIC APORPHINE ALKALOID FROM *POLYALTHIA LONGIFOLIA*

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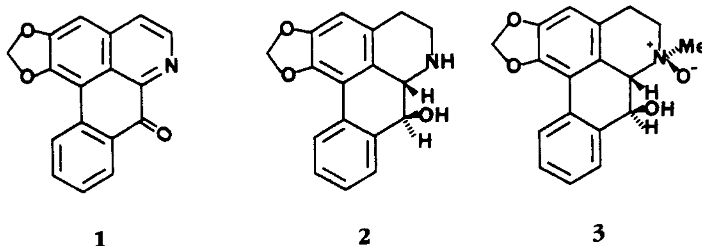
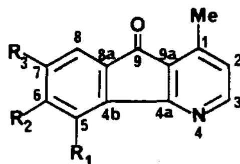
ABSTRACT.—The stem and stem bark of *Polyalthia longifolia* afforded the cytotoxic aporphine alkaloid liriodenine [**1**], as well as two aporphine alkaloids, noroliveroline [**2**] and oliveroline-β-*N*-oxide [**3**] and three azafluorene alkaloids, darienine [**4**], polyfothine [**6**], and isooncodine [**7**], which are not bioactive. Polyfothine [**6**] and isooncodine [**7**] are new natural compounds.

In a previous paper, the leaves of *Polyalthia longifolia* (Sonn.) Thwaites (Annonaceae) were found to contain a novel azafluorene alkaloid, polylongine (5-hydroxy-6-methoxy-1-methyl-4-azafluoren-9-ol) and three new aporphine *N*-oxide alkaloids named (+)-*O*-methylbulbocapnine-β-*N*-oxide, (+)-*O*-methylbulbocapnine-α-*N*-oxide, and (+)-*N*-methylnandigerine-β-*N*-oxide, along with four known alkaloids (1). As part of our continuing search for novel plant antitumor agents, the MeOH extract of stem parts of *P. longifolia* was found to show significant cytotoxicity against the KB nasopharyngeal system and the P-388 murine lymphocytic leukemia system in cell cultures when assessed using standard protocols (2). Bioassay-directed fractionation traced the active fractions to alkaloid components. We report herein the isolation and identification of one cytotoxic aporphine alkaloid, liriodenine [**1**], in addition to two apor-





4 $R_1=R_2=OMe$, $R_3=OH$
5 $R_1=R_2=OMe$, $R_3=OAc$
6 $R_1=H$, $R_2=R_3=OMe$
7 $R_1=H$, $R_2=OMe$, $R_3=OH$
8 $R_1=H$, $R_2=OH$, $R_3=OMe$
9 $R_1=H$, $R_2=OMe$, $R_3=OAc$

phinoid alkaloids, noroliveroline [2] and oliveroline-β-*N*-oxide [3] and three azafluorenoid alkaloids, which are not bioactive. The three azafluorenoid alkaloids are darienine [4], polyfothine [6], and isooncodine [7]. The later two compounds were obtained from natural sources for the first time.

The MeOH extract of stem parts of *P. longifolia* was fractionated by solvent partitioning followed by in vitro KB cytotoxicity tests. Further separation and purification by chromatography furnished the principal active component, liriodenine [**1**]. The identity of **1** was confirmed by direct comparison with an authentic sample and by spectral analyses. Liriodenine [**1**] (3–6) demonstrated potent cytotoxicity against KB, A-549, HCT-8, P-388, and L-1210 cells with ED_{50} values of 1.00, 0.72, 0.70, 0.57, and 2.33 μg/ml, respectively. This compound has recently been shown to be the main cytotoxic principal in *Artabotrys uncinatus* (7) and *Thalictrum sessile* (8). The aporphinoid alkaloids, noroliveroline [**2**] (9) and oliveroline-β-*N*-oxide [**3**] (1), were identified by comparison with authentic samples and spectral analyses, respectively. The known azafluorene alkaloid, darienine [**4**], was readily identified by comparison of its spectral data (uv, ir, ms, ^{1}H nmr, and ^{13}C nmr) with those in the literature (10). Moreover, acetylation of darienine [**4**] with AC_2O and pyridine gave *O*-acetyldarienine [**5**], which was identified by comparison (uv, ms, and ^{1}H nmr) with literature data (10).

Polyfothine [**6**] was obtained from $CHCl_3$ as a pale yellowish amorphous powder. Its molecular formula was established as $C_{15}H_{13}NO_3$ by hreims (found 255.0893, calcd 255.0895) and ^{13}C nmr. The presence of a carbonyl group in the polyfothine molecule was indicated by an ir band at 1705 cm^{-1} and a signal appearing at δ 191.0 (s) in the ^{13}C-nmr spectrum. Its uv spectrum exhibited several absorption maxima in the same regions as darienine [**4**] and 2,6-dimethoxy-7-hydroxyonychine (11); these underwent no bathochromic shifts on adding base, suggesting that polyfothine [**6**] should be a nonphenolic derivative of onychine. The ^{1}H-nmr spectrum of **6** (Table 1) revealed the presence of a methyl group bonded to an aromatic ring, two methoxyl groups, an AB pair of hydrogen atoms assignable to the α and β positions of a pyridine ring, and two uncoupled aromatic ring hydrogen atoms. This indicated that both methoxy groups were located on the aromatic ring at positions C-6 and C-7, allowing the protons to be para-distributed (H-5 and H-8). Furthermore, the ^{13}C-nmr spectrum of polyfothine (Table 2) was also consistent with the proposed structure in accordance

TABLE 1. ^{1}H-nmr Chemical Shifts of Compounds **4**–**7** and **9** (at 200 MHz, in $CDCl_3$, TMS as internal standard).[a]

Proton	Compound				
	4	5	6	7	9
H-2	6.85 d (5.2)[a]	6.95 d (5.3)	6.89 d (5.4)	6.87 d (5.2)	7.11 d (5.9)
H-3	8.41 d (5.2)	8.49 d (5.3)	8.31 d (5.4)	8.32 d (5.2)	8.35 d (5.9)
H-5	—	—	7.36 s	7.36 s	7.34 s
H-8	7.11 s	7.23 s	7.23 s	7.22 s	8.04 s
1-Me	2.58 s	2.62 s	2.61 s	2.59 s	2.76 s
5-OMe	4.08 s	4.08 s	—	—	—
6-OMe	4.08 s	4.01 s	3.98 s[b]	3.98 s	3.89 s
7-OH	—	—	—	6.34 s (br.)	—
7-OMe	—	—	4.05 s[b]	—	—
7-OAc	—	2.36 s	—	—	2.30 s

[a]Coupling constants in Hz are in parentheses; chemical shifts are in δ values.
[b]Assignments may be interchanged.

TABLE 2. ^{13}C-nmr Chemical Shifts of Compounds **6** and **7** (at 50 MHz, in $CDCl_3$, TMS as internal standard).

Carbon	6	7
1-Me	17.2	17.2
C-1	146.9	147.0
C-2	125.0	125.6
C-3	152.7	152.7
C-4a	165.0	165.2
C-4b	137.0	137.0
C-5	106.0	106.5
C-6	156.0	156.2
C-7	151.4	152.4
C-8	107.8	107.9
C-8a	126.5	126.8
C-9	191.0	191.8
C-9a	128.2	129.0
OMe	56.6	56.7
OMe	56.8	

with those of Cassels *et al.* (12). The above data led us to propose that the structure of polyfothine should be represented as **6** (6,7-dimethoxy-1-methyl-4-azafluoren-9-one).

The molecular formula of isooncodine [**7**], deduced from the hreims of its *O*-acetyl derivative, is $C_{14}H_{11}NO_3$. The presence of a carbonyl group in the isooncodine molecule was also indicated by an ir band at 1708 cm^{-1} and a signal which appeared at δ 191.8 (s) in the ^{13}C-nmr spectrum (Table 2). The ^{1}H-nmr spectrum of isooncodine (Table 1) also showed the presence of a methyl group bonded to an aromatic ring, one methoxyl group, one hydroxyl group, an AB pair of hydrogen atoms assignable to the α and β positions of a pyridine ring, and two uncoupled aromatic ring hydrogen atoms. This indicated that both oxygenated substituents (hydroxy and methoxy groups) were also located on the aromatic ring at positions allowing the protons to be para-distributed, i.e., structure **7** or **8**. Its uv spectrum also exhibited several absorption maxima in the same regions as polyfothine [**6**]; these underwent bathochromic shifts and revealed a remarkable color change and absorption around 480 nm in basic solution. Obviously, this involved the disassociation of the phenolic proton and the formation of an enolate with an extended conjugated system. Positioning of the hydroxy at C-7 allowed for a greater delocalization than if it were placed at C-6 (11). Proof of the phenolic character of isooncodine was obtained by preparation of the *O*-methyl and *O*-acetyl derivatives, **6** and **9**, respectively. The *O*-methyl derivative of isooncodine was identified by comparison (ir, tlc, and ^{1}H nmr) with an authentic sample, polyfothine [**6**]. Moreover, comparison of the ^{1}H-nmr spectra (Table 1) of isooncodine [**7**] and its *O*-acetyl derivative **9** indicated a downfield acetylation shift of the H-8 proton resonance, while the methoxyl group appeared to be shielded by the ester function, strongly suggesting that the phenolic group is located at C-7 (10). Therefore, it is proposed that the structure of isooncodine should be represented as **7** (6-methoxy-7-hydroxy-1-methyl-4-azafluoren-9-one).

Following the structural elucidation of polyfothine [**6**] and isooncodine [**7**] by spectral means, it was determined that these two compounds have recently been prepared synthetically by Bou-Abdallah *et al.* (13). Comparison of the spectral data (uv, ir, ms, and ^{1}H nmr) of natural polyfothine [**6**] and isooncodine [**7**] with those of the synthetic compounds (13) proved they are identical.

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 were 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 in $CDCl_3$ using TMS as internal standard. Eims spectra were obtained with a JEOL JMS-HX110 mass spectrometer at 70 eV. Si 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.—Stems of *P. longifolia* were collected from the Taipei Botanical Garden of the Taiwan Forestry Research Institute in January 1988. Voucher specimens are kept in the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.

EXTRACTION AND SEPARATION.—The ground air-dried stem parts (2.5 kg) of *P. longifolia* were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated and partitioned to yield *n*-hexane, $CHCl_3$, and aqueous extracts as guided by bioassays in KB and P-388 cells. The final, active $CHCl_3$ extract (37 g) was chromatographed on a Si gel (1500 g, 40 × 6 cm) column using $CHCl_3$/MeOH mixtures of increasing polarity to yield 50 fractions of 100 ml, each of which was monitored by tlc and the KB cell bioassay. The fractions (457 mg) eluting with $CHCl_3$ were further purified by neutral Al_2O_3 cc ($CHCl_3$), and preparative tlc [$CHCl_3$-MeOH (20:1)] provided liriodenine [**1**]. Fractions (986 mg) eluting with $CHCl_3$-MeOH (50:1) were further separated and purified by Si gel cc, and preparative tlc [$CHCl_3$-MeOH, (15:1)] gave darienine [**4**], polyfothine [**6**], and isooncodine [**7**], respectively. Fractions (269 mg) eluting with $CHCl_3$-MeOH (20:1) were further purified by Si gel cc, and preparative tlc [$CHCl_3$-MeOH (10:1)] yielded noroliveroline [**2**] and oliveroline-β-*N*-oxide [**3**].

LIRIODENINE [**1**].—The alkaloid (57 mg), mp 280–281° and $[\alpha]D$ ±0° (c = 0.1, $CHCl_3$), was identified by comparison with an authentic sample available in our laboratory (mp, ir, tlc, and ^{1}H nmr).

NOROLIVEROLINE [**2**].—The alkaloid (9 mg), mp 139–141° and $[\alpha]D$ −56° (c = 0.05, $CHCl_3$), was characterized by spectral (uv, ir, ^{1}H nmr, and ms) analyses and comparison with literature data (9).

OLIVEROLINE-β-*N*-OXIDE [**3**].—The alkaloid (7 mg), $[\alpha]D$ −63° (c = 0.1, $CHCl_3$), was identified by comparison with an authentic sample available in our laboratory (uv, ir, tlc, ^{1}H nmr, and ms).

DARIENINE [**4**].—The alkaloid (14 mg), a yellow-reddish amorphous powder, and its acetate derivative **5**, mp 140–143°, were characterized by spectral [uv, ir, ms, ^{13}C- and ^{1}H nmr (Table 1)] analyses and comparison with literature data (10).

POLYFOTHINE [**6**].—Pale yellowish amorphous powder (7.8 mg): uv λ max (MeOH) (log ε) 220 (3.43), 268 (3.79), 293 (3.38), 314 (3.32), 328 nm (3.30); ir ν max (Nujol) 1705, 1600, 1570 cm^{-1}; ^{1}H nmr ($CDCl_3$) see Table 1; ^{13}C nmr ($CDCl_3$) see Table 2; eims *m/z* $[M]^+$ 255 (100%), 241 (1.25), 240 (6.25), 212 (0.83); hreims *m/z* $[M]^+$ 255.0893 (calcd for $C_{15}H_{13}NO_3$, 255.0895).

ISOONCODINE [**7**].—Yellowish amorphous powder (3.7 mg): uv λ max (MeOH) (log ε) 218 (3.45), 264 (3.78), 293 (3.39), 314 (3.30), 328 (3.28), 340 sh nm (3.12); λ max (MeOH + NaOH) log ε) 225 (3.46), 284 (3.58), 318 (3.56), 343 (3.28), 354 nm (3.23); ir ν max (Nujol) 3400, 2930, 1708, 1600, 1575 cm^{-1}; ^{1}H nmr ($CDCl_3$) see Table 1; ^{13}C nmr ($CDCl_3$) see Table 2.

O-METHYLISOONCODINE [**6**].—From isooncodine [**7**] with CH_2N_2, it was identified by comparison (tlc, ir, and ^{1}H nmr) with polyfothine [**6**].

O-ACETYLISOONCODINE [**9**].—From isooncodine [**7**] with Ac_2O/pyridine: ir ν max (Nujol) 1760, 1705, 1596, 1560 cm^{-1}; ^{1}H nmr ($CDCl_3$) see Table 1; eims *m/z* $[M]^+$ 283 (13.3%), $[M-Ac]^+$ 241 (100), $[M-Ac-Me]^+$ 226 (20.0); hreims *m/z* $[M]^+$ 283.0847 (calcd for $C_{16}H_{13}NO_4$, 283.0845).

CYTOTOXICITY TESTING.—The cytotoxicity assays were carried out according to procedures described in the literature (2, 14).

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