Nakahalene and Cytotoxic principles of Formosan Rhamnus Species

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NAKAHALENE AND CYTOTOXIC PRINCIPLES OF FORMOSAN *RHAMNUS* SPECIES[1]

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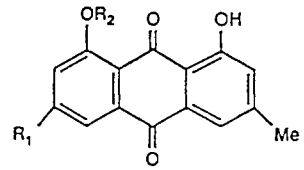
ABSTRACT.—A new naphthalene compound, named nakahalene, has been isolated from the root bark of *Rhamnus nakaharai* (Rhamnaceae) together with chrysophanol, physcion, emodin, rhamnocitrin, and kaempferol. Nakahalene has been characterized as 2-acetyl-3-methyl-6-methoxylnaphthalene-1,8-diol [**4**]. The cytotoxic effects of the isolated anthraquinones of *Rhamnus formosana* and *Rhamnus nakaharai* were investigated.

In a previous paper (1), we reported the isolation of a new flavonol triglycoside, rhamnustrioside, and kaempferol from the fresh fruits of *Rhamnus nakaharai* Hayata (Rhamnaceae). In continuation of this work, the known compounds chrysophanol [**1**], physcion [**2**], emodin [**3**], rhamnocitrin, and kaempferol, and a new naphthalene compound, nakahalene [**4**], were isolated from the fresh root bark of this same plant. In this paper, we report the isolation and characterization of this new naphthalene compound **4**. Because some of the anthraquinones exhibited cytotoxic effects against HL-60 cells (2), compounds **1**–**3**, frangulin B [**6**], and physcion 8-*O*-rhamnosyl-(1→2)-glucoside [**7**] were

TABLE 1. Cytotoxicity[a] of Anthraquinones Against Various Tumor Cell Lines.

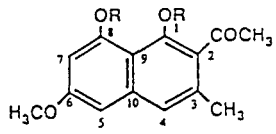


1 $R_1=R_2=H$
2 $R_1=OMe$, $R_2=H$
3 $R_1=OH$, $R_2=H$
6 $R_1=ORha$, $R_2=H$
7 $R_1=OMe$, $R_2=GlcORha$

Compounds	ED_{50} (μg/ml) (N-8)	
	PLC/PRF/5	KB
1	3.23	5.68
2	1.44	1.55
3	5.91	3.46
6	0.80	2.49
7	2.50	3.58
Cisplatin	5.29	0.16

[a]For significant activity of the pure compound, an $ED_{50} \leqq 4.0$ μg/ml is required (10).

[1]Part VI in the series "The Constituents of Formosan *Rhamnus* Species." For part V see C.N. Lin, M.I. Chung, K.H. Gan, and C.M. Lu, *Phytochemistry*, **30**, 3101 (1991).



4 R=H
5 R=Ac

screened for cytotoxic effects against human hepatoma PLC/PRF/5 and KB cell lines. Compounds **6** and **7** were isolated from *Rhamnus formosana* Matsum (3).

RESULTS AND DISCUSSION

Compound **4** showed blue fluorescence under uv. Its uv and ir spectra suggested the presence of a hydroxynaphthalene skeleton (4). Its ^{1}H-nmr spectrum indicated the presence of an aromatic Me signal at δ 2.61, an acetyl signal at δ 2.72, an MeO signal at δ 3.87, a pair of 1H doublet signals (meta coupling) at δ 6.47 (d, J = 2.4 Hz, H-7), and 6.48 (d, J = 2.4 Hz, H-5), a 1H singlet signal at δ 6.83 (H-4) and two chelated phenolic hydroxyl signals at δ 10.42 (1H, s, exchangeable with D_2O, 8-OH) and 17.75 (1H, s, exchangeable with D_2O, 1-OH) (4,5). The ^{1}H nmr spectrum of **4** diacetate [**5**] showed the presence of two singlets for acetyl signals at δ 2.31 and 2.36, an aromatic Me signal at δ 2.38, an acetyl signal at δ 2.50, an MeO signal at δ 3.89, a pair of 1H doublet signals (meta coupling) at δ 6.80 (d, J = 2.4 Hz, H-7) and 6.98 (d, J = 2.4 Hz, H-5) and a 1H singlet signal at δ 7.47. The downfield shift of H-4, H-5, and H-7 of **4** by acetylation supported the characterization of **4**.

The assignments of ^{13}C-nmr spectra of **4** were obtained by ^{1}H-decoupling spectra and DEPT pulse sequence and assigned by comparison with data reported in the literature (6,7). The presence of peaks at *m/z* 127 and 128 in the eims of **4** also clearly indicated that **4** possessed the naphthalene skeleton (6). Based on the above evidence, compound **4** was characterized as 2-acetyl-3-methyl-6-methoxynaphthalene-1,8-diol.

The cytotoxic effects of **1–3**, **6**, and **7** against human hepatoma PLC/PRF/5 and KB cells were studied (8,9), and the results are listed in Table 1. Compound **6** exhibited the most significant cytotoxic effects against these cell lines. From Table 1, it is clearly indicated that *O*-glycosylation or methylation of the C_6-OH of **3** enhances the cytotoxic effect against human hepatoma PLC/PRF/5 cells in vitro.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mp's are uncorrected. Ft nmr spectra were recorded on a VXR-300/51 Superconducting High Resolution FT NMR system; ir spectra on a Hitachi model 260-30; and ms on a JMS-HX 110 Mass Spectrometer.

EXTRACTION AND SEPARATION.—The fresh root bark (0.7 kg) of *R. nakaharai* was collected at Ali, Wu-Tai Shian, Ping-Tung Hsien, Taiwan, during July 1990, chipped, and extracted with hot MeOH. The MeOH extract was chromatographed on Si gel. Elution with cyclohexane-C_6H_6 (1:4) yielded **1** and **2**. Elution with C_6H_6-EtOAc (5:1) yielded **3**. Elution with C_6H_6-cyclohexane-EtOAc (10:4:1) yielded **4**, kaempferol, and rhamnocitrin. Compounds **1–3**, kaempferol, and rhamnocitrin were identified by uv, ir, nmr, ms, and comparison of the mmp's and spectral data with those of the authentic samples.

2-ACETYL-3-METHYL-6-METHOXYNAPHTHALENE (NAKAHALENE) [**4**].—Yellow needles (EtOAc): mp 207–208°; ir ν max KBr cm^{-1} 3350, 1640, 1580; uv λ max (MeOH) nm (log ε) 275 (3.95), 316 (3.17), 332 (sh) (3.02), 400 (3.36); ^{1}H nmr ($CDCl_3$) see text; ^{13}C nmr ($CDCl_3$) δ 163.6 (C-1), 108.3 (C-2), 139.9 (C-3), 121.0 (C-4), 100.8 (C-5), 160.2 (C-6), 99.2 (C-7), 169.7 (C-8), 112.3 (C-9), 134.2 (C-10), 25.3 (Me), 31.7 (Ac), 55.4 (OMe), 203.5 (Ac); eims *m/z* (rel. int.) $[M]^+$ 246 (62), 231 (100), 185 (12), 128 (10), 127 (9), 115 (12). *Anal.* calcd for $C_{14}H_{14}O_4$ 246.0891, found 246.0907.

COMPOUND **4** DIACETATE [**5**].—Colorless needles (MeOH): mp 190–191°; ir ν max KBr cm^{-1} 1760, 1690, 1635; ^{1}H nmr ($CDCl_3$) see text; ^{13}C nmr ($CDCl_3$) δ 142.9 (C-1), 115.9 (C-2), 138.5 (C-3), 127.8 (C-4), 114.7 (C-5), 159.7 (C-6), 105.6 (C-7), 147.8 (C-8), 133.4 (C-9), 133.8 (C-10), 19.8 (Me), 21.4, 21.6 (OAc), 32.5 (Ac), 55.8 (OMe), 170.5, 170.7 (OAc),

205.4 (Ac); eims *m/z* (rel. int.) $[M]^+$ 330 (15), $[M-42]^+$ 288 (18), $[288-42]^+$ 246 (100), 231 (47), 115 (1). *Anal.* calcd for $C_{18}H_{18}O_6$ 330.1102, found 330.1114.

BIOLOGICAL ASSAYS.—PLC/PRF/5 cells were established from human hepatoma and are known to produce HBs Ag continuously in culture fluids (5). The cells were grown as continuous cultures in a growth medium consisting of Dulbecco's modified Eagle medium (DMEM, GIBCO, Grand Island, NY), 10% fetal bovine serum (FBS, GIBCO), 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. The KB cells were maintained on DMEM (GIBCO) containing with 10% FBS, L-glutamine and antibiotics. For microassays, the growth medium was supplemented further with 10 mM Hepes buffer, pH 7.3. The microassay for anticellular effect was performed as previously described (6,7).

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