# Antitumor Agents. 221.[1] Buceracidins A and B, Two New Flavanones from *Bucida buceras*

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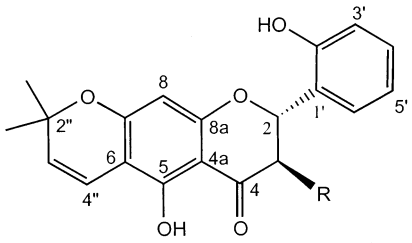
As part of a study on antitumor agents from rainforest plants, two new flavanones, buceracidin A (**1**), 5,2′-dihydroxy-3-methoxy-6,7-(2″,2″-dimethylchromene)flavanone, and buceracidin B (**2**), 5,2′-dihydroxy-6,7-(2″,2″-dimethylchromene)flavanone, were isolated together with four known prenylated flavanones, minimiflorin (**3**), 3-hydroxyminimiflorin (**4**), 3-methoxyminimiflorin (**5**), and mundulinol (**6**), from *Bucida buceras*. The structures of buceracidins A and B were elucidated from detailed 2D NMR analyses. Among the six flavanones, **3**, **5**, and **6** were marginally cytotoxic in a human tumor cell line panel.

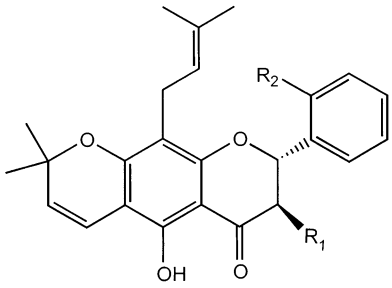
*Bucida buceras* L (Combretaceae) is a timber and shade evergreen tree that is found in tropical regions of northern South America and is commonly called the nonedible black-olive tree.[2] In a continuing collaboration with the National Cancer Institute to discover antitumor agents from rainforest plants, we isolated three new clerodane diterpenes, bucidarasins A−C, as major cytotoxic principles from an extract of *Bucida buceras*.[3] Further investigation of this extract has led to the isolation of two new flavanones (**1** and **2**), together with four known prenylated flavanones (**3**−**6**). The structures of **1** and **2** were determined by detailed NMR analyses including COSY, HMQC, and HMBC techniques. Known flavanones **3**−**6** were isolated previously from the *Lonchocarpus* genus.[4−6] However, cytotoxic activities of these flavanones have not been reported. Herein, we report the isolation, structure elucidation, and biological evaluation of **1**−**6**.

The twigs of *B. buceras* were extracted with a 1:1 mixture of $CH_2Cl_2$−MeOH, which was then concentrated to dryness. This extract showed significant cytotoxic activity against various human tumor cell lines and was fractionated successively with hexane and EtOAc. The hexane-soluble fraction showed cytotoxic activity, and subsequent bioactivity-directed silica gel chromatography yielded several fractions with strong UV absorption, including fractions that did not contain the previously reported cytotoxic bucidarasins. Further purification by repeated silica gel chromatography afforded two new flavanones that were designated as buceracidins A (**1**) and B (**2**), together with known four flavanones, **3**−**6**. By comparison with literature NMR spectral data, **3**−**6** were found to be the known prenylated flavanones minimiflorin, 3-hydroxyminimiflorin, 3-methoxyminimiflorin, and mundulinol, respectively.[4−6]

Compound **1** was obtained as a pale yellow amorphous solid and had a molecular formula of $C_{21}H_{20}O_6$ based on HRFABMS. The IR spectrum of **1** indicated the presence of hydroxy (3400 $cm^{-1}$) and carbonyl (1645 $cm^{-1}$) groups. The $^1H$ NMR spectrum showed signals assignable to two singlet methyl protons at $\delta$ 1.44 and 1.45, a methoxy group at $\delta$ 3.58, two oxymethine protons at $\delta$ 4.17 and 5.47, two olefinic protons with *cis* configuration at $\delta$ 5.52 and 6.62, and two $D_2O$-exchangeable phenolic protons at $\delta$ 6.57 (s) and 11.91 (s). In addition, signals due to 1,2-disubstituted benzene protons at $\delta$ 6.96, 7.03, 7.29, and 7.47 and a singlet benzene proton at $\delta$ 5.99 (s) were observed in the aromatic region. The $^{13}C$ NMR spectrum of **1** contained 21 signals indicating two methyl ($\delta$ 28.4 and 28.5), a methoxy ($\delta$ 61.8), two oxymethine ($\delta$ 77.7 and 81.6), an oxygen-attached tertiary carbon ($\delta$ 78.7), two olefinic methine ($\delta$ 115.0 and 126.6), five aromatic methine ($\delta$ 96.6, 117.7, 121.2, 127.1, and 130.1), seven aromatic tertiary carbons ($\delta$ 101.5, 103.6, 123.7, 153.9, 158.7, 161.4, and 162.6), and a ketone carbonyl carbon ($\delta$ 195.3). The degree of unsaturation, as determined from the molecular formula, together with the partial structures mentioned above suggested that **1** was




Buceracidin A (**1**) R=OMe
Buceracidin B (**2**) R=H




| | |
|---|---|
| Minimiflorin (**3**) | $R_1$=H, $R_2$=OH |
| 3-Hydroxyminimiflorin (**4**) | $R_1$=OH, $R_2$=OH |
| 3-Methoxyminimiflorin (**5**) | $R_1$=OMe, $R_2$=OH |
| Mundulinol (**6**) | $R_1$=OH, $R_2$=H |

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a flavanone containing a dimethylchromene moiety. The presence of the latter moiety was confirmed by HMBC correlations (Me proton/C-2″, C-3″; H-4″/C-2″, C-7; H-3″/C-2″, C-6). The dimethylchromene ring was positioned at C-6 and C-7 of the flavanone skeleton on the basis of HMBC correlations from phenolic protons at C-5 to C-4a, C-5, and C-6, and from H-8 to C-4a, C-6, and C-8a. This assignment was also supported by chemical shifts of H-8 ($\delta$ 5.99; upshifted by $\beta$-effects of two oxygens) and a phenolic proton ($\delta$ 11.91; downshifted by hydrogen bonding with the C-4 ketone). This hydrogen bonding also implied that the ketone was present at C-4a. The 2′-phenolic and methoxy groups were located at C-2 and C-3, respectively, by HMBC correlations from H-2 to C-1′, C-2′, and C-6′, and from methoxy protons to C-3, respectively. The connectivity from C-2 to C-4 was established from H−H COSY (H-2/H-3) and HMBC correlations (H-2/C-3, C-4). The EIMS fragmentation of **1** also gave structural information. A fragment ion at *m*/*z* 353 ($[M - 15]^+$, 39%) occurred from loss of a methyl group in **1**. A retro-Diels−Alder-type cleavage of the molecular ion (*m*/*z* 368, 33%) and of the fragment ion (*m*/*z* 353, 39%) would explain the fragment ions at *m*/*z* 219 (16%) and *m*/*z* 203 (100%), respectively. This type of fragmentation also supported the presence of a dimethylchromene moiety in **1**. The relative stereochemistry of C-2 and C-3 in **1** was elucidated by the observed coupling constants between H-2 and H-3. The coupling constant (10.7 Hz) between H-2 and H-3 suggested a *trans* configuration. Thus, **1** was determined to be 5,2′-dihydroxy-3-methoxy-6,7-(2″,2″-dimethylchromene)flavanone. This assignment was further confirmed by comparison of NMR data of **1** with those published for the related eriotrionol.[7]

Compound **2** was isolated as pale yellow amorphous solid and had a molecular formula of $C_{20}H_{18}O_5$ on the basis of HRFABMS. The IR absorptions of **2** indicated the presence of hydroxy (3400 $cm^{-1}$) and carbonyl (1644 $cm^{-1}$) groups. The $^1H$ NMR and $^{13}C$ NMR spectra of **2** differed from those of **1** only by the absence of the methoxy group, the presence of one ($\delta_C$ 76.6) rather than two oxymethine groups, and the appearance of a methylene group ($\delta_H$ 2.96 and 3.07 and $\delta_C$ 41.8). These NMR data, together with the molecular formula, suggested that **2** is the de-methoxylated analogue of **1**. The HMBC spectrum of **2** contained almost identical correlations with those found in **1**. The connectivities from C-2′ to C-4 (H-2/C-1′, C-2′, C-6′; H-3/C-1′, C-4) were established by HMBC correlations. The chemical shifts of H-8 ($\delta$ 5.99, s) and the phenolic proton ($\delta$ 12.23 s) at C-5 also supported linkage of the dimethylchromene ring at C-6 and C-7, as found in **1**. EIMS fragmentation showed the same retro Diels−Alder-type ions present in **1**. Thus, **2** was determined to be 5,2′-dihydroxy-6,7-(2″,2″-dimethylchromene)flavanone.

Compounds **1**−**6** were assayed for cytotoxicity using a reported procedure.[8] Compounds **3**, **5**, and **6** showed marginal cytotoxic activity against human tumor cell replication. The average $IC_{50}$ values of **3**, **5**, and **6** in nine tumor cell lines tested were 8.3 (±1.3, SD), 7.6 (±0.7), and 8.6 (±1.8) $\mu$g/mL, respectively (Table 1). Interestingly, **5** was more selective toward the U87-MG gliobastoma line compared to **3** and **6**. In addition, **3**, **5**, and **6** did not appear to be substrates for the Pgp drug-efflux pump based on similar activities against KB and KB-VIN lines. Compounds **1**, **2**, and **4** were inactive, with average $IC_{50}$ values of 14.8 ± 1.2, 11.5 + 2.0, and 14.0 ± 1.2 $\mu$g/mL. These data indicate that the isoprenyl group is crucial for activity (cf. **1** vs **5** and **2** vs **3**). In addition, appropriate hydrophobicity is also important for activity as, unlike **3**, **5**, and **6**, the more polar compound **4** has hydroxy groups at both C-2′ and C-3 and is inactive.

**Table 1.** Cytotoxicity Data for **1**−**6** against Parental and Drug-Resistant Human Tumor Cell Lines

| | compound/$IC_{50}$ ($\mu$g/mL)[a] | | | | | |
|---|---|---|---|---|---|---|
| cell line[b] | **1** | **2** | **3** | **4** | **5** | **6** |
| KB | >10 (31)[c] | 9.5 | 7.3 | 16.5 | 6.5 | 8.5 |
| KB-VIN | 15.0 | 9.5 | 7.7 | 13.3 | 7.0 | 7.5 |
| A549 | 14.1 | 9.9 | 8.0 | 13.5 | 7.6 | 7.5 |
| 1A9 | 14.9 | 11.5 | 7.6 | 15.0 | 7.8 | 8.2 |
| HCT-8 | 15.5 | 12.8 | 7.2 | 12.1 | 7.2 | 7.0 |
| MCF-7 | 13.6 | 9.3 | 7.5 | 12.8 | 7.0 | 7.5 |
| PC-3 | 16.3 | 13.0 | 9.8 | 15.4 | 8.4 | 9.3 |
| U87-MG | 16.1 | 14.2 | 11.0 | 14.8 | 8.6 | 13.0 |
| SK-MEL-2 | 15.6 | 14.0 | 9.0 | 14.8 | 7.8 | 8.5 |

[a] $IC_{50}$ = concentration that causes a 50% reduction in absorbance at 562 nm relative to untreated cells using SRB assay. [b] KB, nasopharyngeal; KB-VIN, vincristine resistant; A549 lung; 1A9, ovarian; HCT-8, ileococal; MCF-7, breast; PC-3, prostate; U87-MG, gliobastoma; SK-MEL-2, melanoma. [c] The percent inhibition observed at 10 $\mu$g/mL is the value in parentheses.

## Experimental Section

**General Experimental Procedures.** A DIP-1000 digital polarimeter (cell length 10 mm, unless otherwise indicated) was used for optical rotations. $^1H$ and $^{13}C$ NMR spectra were taken on Varian Gemini 2000 300 MHz and JEOL Λ500 500 MHz NMR spectrometers with TMS as internal reference. The EIMS spectra were measured with a VG-70E double-focus high-resolution GC mass spectrometer. The FABMS spectra were measured with a JEOL Mastation.

**Plant Material.** The twigs of *Bucida buceras* were collected in Belize on May 1989 by Dr. Rosita Arvigo and the New York Botanical Garden under contract to the National Cancer Institute for plant collection.

**Isolation of Flavanones from *Bucida buceras*.** Air-dried branches (345 g) were extracted with $CH_2Cl_2$−MeOH (1:1) and concentrated in vacuo to give a crude extract (15.17 g; sample number Q65S0650). This extract was adsorbed on Celite and packed into a column. The column was eluted successively with *n*-hexane and EtOAc. The hexane layer was evaporated in vacuo, and the concentrate (7.0 g) was fractionated using silica gel CC (*n*-hexane−EtOAc stepwise gradient) to give 10 fractions on the basis of TLC behavior. Mundulinol (**6**; 115 mg) was isolated as a yellowish oil from the second fraction using silica gel CC with hexane−EtOAc (9:1) as eluent. The fourth fraction was subjected to silica gel CC with a gradient solvent system (hexane−EtOAc) to give seven fractions (fractions 4-1 to 4-7). Minimiflorin (**3**; 37 mg) was obtained as a yellowish oil from fraction 4-1. Fraction 4-2 was rechromatographed on silica gel to afford 3-methoxyminimiflorin as yellow crystals (**5**; 43 mg) and buceracidin B as a pale yellow amorphous solid (**2**; 20 mg). Buceracidin A (**1**; 280 mg) was obtained from the fifth fraction as a pale yellow amorphous solid by silica gel CC eluting with hexane−acetone (9:1). The sixth fraction was purified with silica gel CC with $CHCl_3$−acetone (95:5) as eluent to obtain three fractions. Fraction 6-2 was re-subjected to silica gel CC to give 3-hydroxyminimiflorin as a yellowish oil (**4**; 230 mg).

**Buceracidin A (1), 5,2′-dihydroxy-3-methoxy-6,7-(2″,2″-dimethylchromene)flavanone:** pale yellow amorphous solid; mp >200 °C; $[\alpha]_D$ +29.7° (*c* 0.59, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 313 (4.05), 300 (4.02), 277 (4.51) nm; IR (neat) 3400, 2982, 1645, 1631, 1574, 1489, 1457 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.44 (s, 3H, 2″-Me), 1.45 (s, 3H, 2″-Me), 3.58 (s, MeO), 4.17 (d, *J* = 10.7, H-3), 5.47 (d, *J* = 10.7, H-2), 5.52 (d, *J* = 10.2, H-3″), 5.99 (s, H-8), 6.57 (s, 2′-OH), 6.62 (d *J* = 10.2 Hz, H-4″), 6.96 (d, *J* = 8.3 Hz, H-3′), 7.03 (dd, *J* = 8.3, 8.2 Hz, H-5′), 7.29 (ddd, *J* = 8.3, 8.2, 1.7 Hz, H-4′), 7.47 (dd, *J*= 8.3, 1.7 Hz, H-6′), 11.91 (s, 5-OH); $^{13}C$ NMR (500 MHz, $CDCl_3$) $\delta$ 28.4, 28.5 (t, 2″-Me), 61.8 (t, OMe), 77.7 (d, C-2), 78.7 (s, C-2″), 81.6 (d, C-3), 96.6 (s, C-8a), 101.5 (s, C-4a), 103.6 (s, C-6), 115.0 (s, C-4″),

117.7 (s, C-3′), 121.2 (s, C-5′), 123.7 (s, C-1′), 126.6 (s, C-3″), 127.1 (s, C-6′), 130.1 (s, C-4′), 153.9 (s, C-2′), 158.5 (s, C-5), 161.4 (s, C-9), 162.6 (s, C-7), 195.3 (C-4); HRFABMS *m*/*z* 391.1152 $[M + Na]^+$ (calcd for $C_{21}H_{20}O_6Na$ (Δ 0.5 mmu)).

**Buceracidin B (2), 5,2′-dihydroxy-3-hydroxy-6,7-(2″,2″-dimethylchromene)flavanone:** pale yellow amorphous solid; mp 142−143 °C; $[\alpha]_D$ −25.1° (*c* 0.65, $CHCl_3$); UV (MeOH) λmax (log ε) 310 (4.01), 298 (4.07), 276 (4.58) nm; IR (neat) 3400, 2981, 1644, 1632, 1572, 1492, 1446 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 3H, 2″-Me), 1.45 (s, 3H, 2″-Me), 2.96 (dd, *J* = 19.2, 3.2, H-3), 3.07 (dd, *J* = 19.2, 12.0, H-3), 5.70 (dd, *J* = 12.0, 3.2, H-2), 5.52 (d, *J* = 10.6, H-3″), 5.99 (s, H-9), 6.61 (s, 2′-OH), 6.62 (d *J* = 10.6 Hz, H-4″), 6.87 (d, *J* = 8.2 Hz, H-3′), 6.96 (dd, *J* = 8.2, 8.1 Hz, H-5′), 7.23 (ddd, *J* = 8.2, 8.1, 1.9 Hz, H-4′), 7.36 (dd, *J* = 8.1, 1.9 Hz, H-6′), 12.23 (s, 5-OH); $^{13}C$ NMR (500 MHz, $CDCl_3$) δ 28.4, 28.5 (t, 2″Me), 41.8 (d, C-3), 76.6 (d, C-2), 78.5 (s, C-2″), 96.4 (s, C-8a), 102.9 (s, C-4a), 103.4 (s, C-6), 115.2 (s, C-4″), 116.5 (s, C-3′), 120.9 (s, C-5′), 124.4 (s, C-1′), 126.5 (s, C-3″), 126.8 (s, C-6′), 129.9 (s, C-4′), 153.5 (s, C-2′), 158.6 (s, C-5), 162.0 (s, C-9), 162.2 (s, C-7), 196.4 (C-4); HRFABMS *m*/*z* 391.1152 $[M + Na]^+$ (calcd for $C_{21}H_{20}O_6Na$ (Δ 0.5 mmu)).

The known compounds (**3**−**6**) were identified by comparison of physical and spectral data with those reported in the literature.[4−6]

**Cytotoxicity Assay.** All stock cultures were grown in T-25 flasks (5 mL of RPMI-1640 medium supplemented with 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, and 100 μg/mL kanamycin). Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 1500−7500 cells per well with test compounds from DMSO-diluted stock. After 3 days in culture, cells attached to the plastic substratum were fixed with cold 50% trichloroacetic acid and then stained with 0.4% sulforhodamine B (SRB). The absorbency at 562 nm was measured using a microplate reader after solubilizing the bound dye. The $IC_{50}$ is the concentration of test compound that reduced cell growth by 50% over a 3-day assay period.

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## References and Notes

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