Catalytic Hydrogenation of Dehydro Quassinoids and Cytotoxic Antitumor Activity of the Hydrogenation Products

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Two new types of quassinoids (**1a**, **1b**, **2a**, **2b**, **3a**, and **3b**) were obtained by the catalytic hydrogenation of dehydro quassinoids [dehydrobruceantin (**1**), dehydrobruceantinol (**2**), and dehydrobruceantarin (**3**)] which were isolated from *Brucea antidysenterica*. Compounds **1a** and **2b** showed potent selective cytotoxicity against human medulloblastoma TE-671. Significant cytotoxicity was also demonstrated by **3a** for TE-671, for murine leukemia P-388, and **2b** for human melanoma RPMI-7951.

Kupchan et al.[1] isolated eight quassinoids from *Brucea antidysenterica* Mill, including bruceantin (**4**), which showed significant antitumor activity and was in phase II clinical trials as an anticancer drug at the National Cancer Institute in the USA. As a result of our continuing interest in the structure-activity relationship correlation as well as the investigation of further minor bioactive compounds from the same plant,

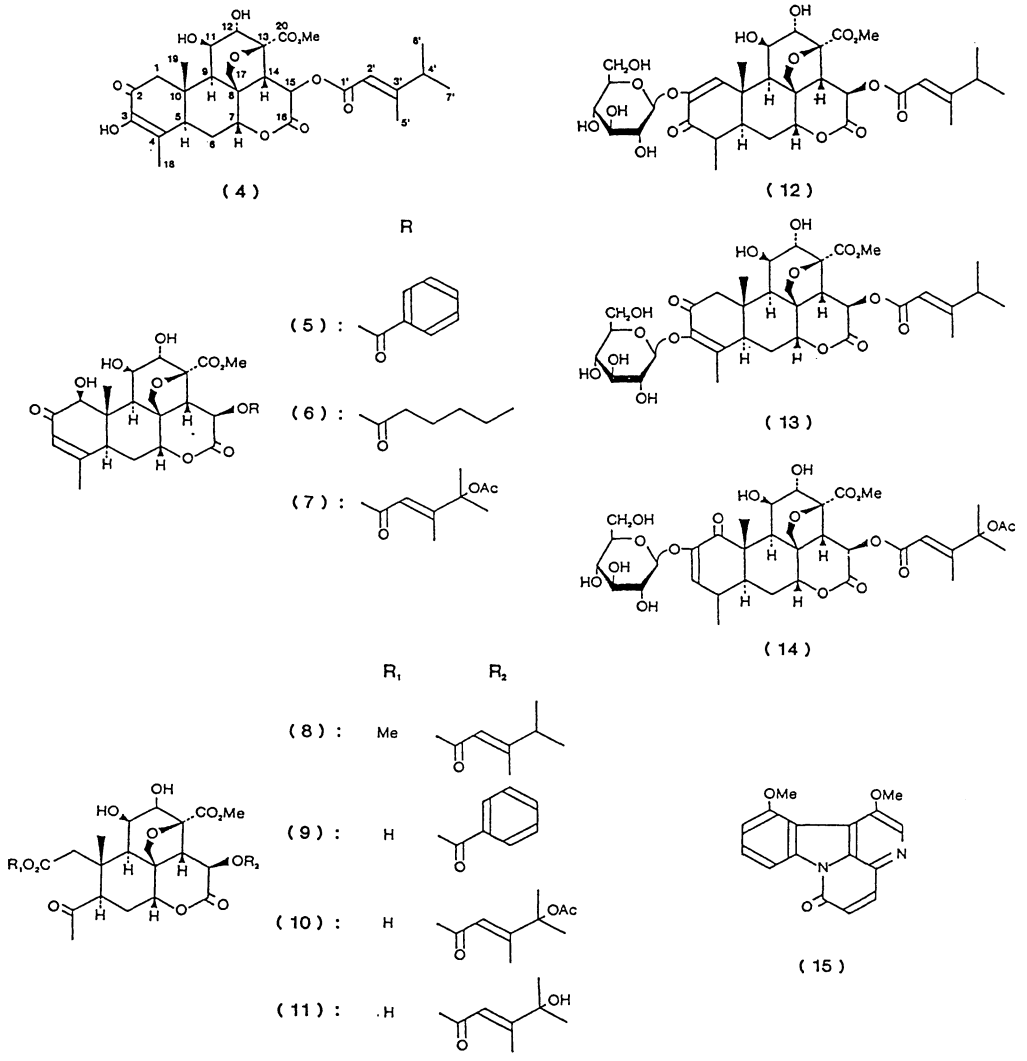

Fig. 1. Structures of Compounds **4**—**15**.

we have isolated the new bruceanol-A(**5**),[2] -B(**6**),[2] -C(**7**),[3] methylester of bruceanic acid-A(**8**),[4] bruceanic acid-B(**9**),[4] -C(**10**),[4] -D(**11**),[4] bruceantinoside-A(**12**),[5] -B(**13**),[5] and -C(**14**),[6] as well as 1,11-dimethoxycanthin-6-one(**15**)[7] in addition to the known yadanzioside-G,[6] yadanzioside-N,[6] yadanzioside-M,[7] canthin-6-one,[8] 11-hydroxycanthin-6-one,[8] 1-methoxycanthin-6-one,[9] 11-hydroxy-1-methoxycanthin-6-one,[9] and 1-hydroxy-11-methoxycanthin-6-one,[9] which were obtained from this plant for the first time. The structures of compounds **1**—**3** and **4**—**15** were shown in Fig. 2 and Fig. 1, respectively.

The known dehydrobruceantin (**1**), dehydrobruceantinol (**2**), and dehydrobruceantarin (**3**) were isolated along with the above compounds. They were considered by Kupchan et al.[1] to be dehydrogenation products of bruceantin (**4**), bruceantinol, and bruceantarin, respectively. As part of our structure-activity relationship studies for antitumor agents, we report herein on the formation of two new types of quassinoids (**1a**, **2a**, **3a**, and **1b**, **2b**, **3b**) by catalytic hydrogenation of **1**, **2**, and **3** and the cytotoxicity of these new compounds.

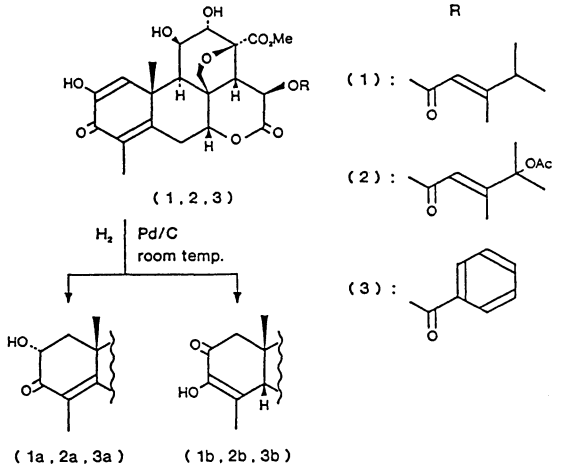

Fig. 2. Catalytic Hydrogenation of Compounds **1**, **2**, and **3**.

Results and Discussion

Compounds **1a** and **1b** were obtained by catalytic hydrogenation of dehydrobruceantin (**1**). The electron impact mass (EIMS) spectrum of **1a** showed fragment ion peaks at m/z 530 ($M^+ - H_2O$) and 111 (side chain, $C_7H_{11}O$). On the other hand, the EIMS spectrum of **1** exhibited fragment ion peaks at m/z 528 ($M^+ - H_2O$) and 111 (side chain, $C_7H_{11}O$). This result suggested that one of the A-ring double bonds of **1** was hydrogenated and also the side chain double bond remained intact.

The ^1H NMR spectrum of **1a** showed that **1a** has new signals of H-1α (δ=2.18, dd, J=13, 13 Hz), H-1β (δ=3.04, dd, J=13, 6 Hz), and H-2 (δ=4.70, dd, J=13, 6 Hz), instead of the signal of H-1 (δ=7.10, s) as seen in **1** of Table 1. This result suggested that only $\Delta^{1,2}$ of **1** was hydrogenated. Furthermore, the Me-4 signal (δ=2.06, s) of **1a** remained, because $\Delta^{4,5}$ of **1** was not hydrogenated.

The NOE spectrum of **1a** (Fig. 3) revealed that the H-2 (δ=4.70, dd) and Me-10 (δ=2.01, s) signals were enhanced with each other, indicating that the stereochemistry of H-2 is of β-configuration. Therefore, *cis*

Table 1. ^1H NMR Spectral Data for Compounds **1**, **2**, **3**, **1a**, **2a**, **3a**, **1b**, **2b**, **3b**, and **4** in C_5D_5N

Proton	1	2	3
H-1	7.10(1H, s)	7.10(1H, s)	7.10(1H, s)
H-2	—	—	—
H-5	—	—	—
H-6α	3.35(1H, dd, J=14, 3)	3.36(1H, dd, J=14, 3)	3.39(1H, dd, J=13, 3)
H-6β	2.82(1H, d, J=14)	2.80(1H, d, J=14)	2.84(1H, d, J=13)
H-7	5.30(1H, d, J=3)	5.31(1H, d, J=3)	5.36(1H, d, J=3)
H-9	2.60(1H, d, J=4)	2.60(1H, d, J=3)	2.72(1H, d, J=4)
H-11	5.07(1H, d, J=4)	a)	5.11(1H, d, J=4)
H-12	a)	a)	a)
H-14	5.16(1H, d, J=5)	a)	a)
H-15	a)	a)	a)
H-17α	4.07(1H, d, J=7)	4.07(1H, d, J=7)	4.13(1H, d, J=7)
H-17β	5.33(1H, d, J=7)	5.32(1H, d, J=7)	5.36(1H, d, J=7)
H-2′	5.80(1H, s)	6.03(1H, s)	—
H-3′	—	—	8.14(2H, dd, J=7.5, 1.5)
H-4′	2.12(1H, m)	—	7.32(2H, dd, J=7.5, 7.5)
H-5′	—	—	7.47(1H, tt, J=7.5, 1.5)
Me-4	2.23(3H, s)	2.23(3H, s)	2.22(3H, s)
Me-10	1.94(3H, s)	1.92(3H, s)	1.95(3H, s)
Me-3′	2.14(3H, s)	2.24(3H, s)	—
Me-4′	0.82(6H, d, J=6.5)	1.33(3H, s)	—
		1.41(3H, s)	
OMe-20	3.76(3H, s)	3.90(3H, s)	3.47(3H, s)
OAc-4′	—	1.94(3H, s)	—

Table 1. (Continued)

Proton	**1a**	**2a**	**3a**
H-1α	2.18(1H, dd, J=13, 13)	2.14(1H, dd, J=13, 13)	2.11(1H, dd, J=13, 13)
H-1β	3.04(1H, dd, J=13, 6)	3.04(1H, dd, 13, 6)	3.04(1H, dd, J=13, 6)
H-2	4.70(1H, dd, J=13, 6)	4.70(1H, dd, J=13, 6)	4.69(1H, dd, J=13, 6)
H-5	—	—	—
H-6α	3.21(1H, dd, J=15, 3)	3.21 (1H, dd, J=15, 3)	3.24(1H, dd, J=15, 3)
H-6β	2.68(1H, d, J=15)	2.64(1H, d, J=15)	2.68(1H, d, J=15)
H-7	5.21(1H, d, J=3)	5.21(1H, d, J=3)	5.26(1H, d, J=3)
H-9	2.65(1H, d, J=4)	2.57(1H, d, J=4)	a)
H-11	4.91(1H, d, J=4)	4.91(1H, d, J=4)	a)
H-12	a)	a)	a)
H-14	a)	a)	2.65(1H, d, J=4)
H-15	a)	a)	4.93(1H, d, J=4)
H-17α	4.03(1H, d, J=7)	4.03(1H, d, J=7)	4.09(1H, d, J=7)
H-17β	5.26(1H, d, J=7)	5.25(1H, d, J=7)	5.23(1H, d, J=7)
H-2′	5.83(1H, s)	6.06(1H, s)	—
H-3′	—	—	8.16(2H, dd, J=7.5, 1.5)
H-4′	2.13(1H, m)	—	7.34(2H, dd, J=7.5, 7.5)
H-5′	—	—	7.47(1H, tt, J=7.5, 1.5)
Me-4	2.06(3H, s)	2.06(3H, s)	2.05(3H, s)
Me-10	2.01(3H, s)	2.01(3H, s)	2.01(3H, s)
Me-3′	2.15(3H, s)	2.25(3H, s)	—
Me-4′	0.83(6H, d, J=6.5)	1.35(3H, s)	—
		1.42(3H, s)	
OMe-20	3.77(3H, s)	3.90(3H, s)	3.45(3H, s)
OAc-4′	—	1.93(3H, s)	—

Table 1. (Continued)

Proton	**1b**	**2b**	**3b**	**4**
H-1α	3.39(1H, d, J=16)	3.40(1H, d, J=16)	3.39(1H, d, J=16)	3.31(1H, d, J=16)
H-1β	2.55(1H, d, J=16)	2.54(1H, d, J=16)	2.55(1H, d, J=16)	2.57(1H, d, J=16)
H-2	—	—	—	—
H-5	3.12(1H, brd)	3.10(1H, brd)	3.20(1H, brd)	3.10(1H, brd)
H-6α	2.55(1H, dd, J=16, 3)	2.55(1H, dd, J=16, 3)	2.57(1H, dd, J=16, 3)	2.31(1H, dd, J=14, 3)
H-6β	2.18(1H, dd, J=16, 3)	2.19(1H, dd, J=16,3)	2.22(1H, d, J=16)	1.77(1H, ddd, J=14, 14, 3)
H-7	a)	5.03(1H, d, J=3)	5.06(1H, d, J=3)	5.13(1H, d, J=3)
H-9	a)	2.57(1H, d, J=4)	2.58(1H, d, J=5)	2.63(1H, d, J=4)
H-11	4.90(1H, d, J=4)	4.90(1H, d, J=4)	4.90(1H, d, J=5)	4.82(1H, d, J=4)
H-12	a)	a)	a)	a)
H-14	a)	a)	a)	a)
H-15	a)	6.67(1H, brd)	6.58(1H, d)	6.55(1H, brd)
H-17α	3.92(1H, d, J=7)	3.93(1H, d, J=7)	3.96(1H, d, J=7)	3.95(1H, d, J=7.5)
H-17β	5.11(1H, d, J=7)	5.11(1H, d, J=7)	5.13(1H, d, J=7)	5.11(1H, d, J=7.5)
H-2′	5.75(1H, s)	5.99(1H, s)	—	5.87(1H, s)
H-3′	—	—	8.13(2H, dd, J=7.5, 1.5)	—
H-4′	2.08(1H, m)	—	7.29(2H, dd, J=7.5, 7.5)	2.14(1H, m)
H-5′	—	—	7.44(1H, tt, 7.5, 1.5)	—
Me-4	2.35(3H, s)	2.35(3H, s)	2.34(3H, s)	1.96(3H, s)
Me-10	1.86(3H, s)	1.86(3H, s)	1.87(3H, s)	1.65(3H, s)
Me-3′	2.13(3H, s)	2.22(3H, s)	—	2.17(3H, s)
Me-4′	0.78(6H, d, J=6.5)	1.26(3H, s)	—	0.85(6H, d, J=7)
		1.37(3H, s)		
OMe-20	3.70(3H, s)	3.86(3H, s)	3.40(3H, s)	3.79(3H, s)
OAc-4′	—	1.90(3H, s)	—	—

Values are in ppm. The coupling constants (J values) in parenthesis are in Hz. a) Not assigned.

addition of hydrogen might occur at the β-side of $\Delta^{1,2}$ in **1**.

The EIMS of the other hydrogenation product (**1b**) showed a molecular ion peak at m/z 548 and a side chain signal at m/z 111. On the other hand, the EIMS of the starting material (**1**) exhibited a molecular ion peak at m/z 546 and a side chain peak at m/z 111. This result suggested that one of the A-ring double bonds of **1**, instead of the side-chain double bond, was hydrogenated, as found in the case of **1a**.

The UV spectrum of **1b** showed characteristic absorptions at 220 (ε 20200) and 280 (ε 8640) nm, which were observed for compounds having diosphenol, such as bruceantin (**4**) [220 (ε 15500) and 280 (ε 7760) nm].[1)]

Table 2. Cytotoxicity of Compounds **1a**, **1b**, **2a**, **2b**, **3a**, and **3b**

Compound	$ED_{50}/\mu g\,ml^{-1}$ [a]					
	KB	A-549	HCT-8	RPMI-7951	TE-671	P-388
1a	5.50	6.22	>10	5.06	0.68	3.84
1b	>10	>10	>10	>10	5.56	>10
2a	5.50	>10	8.67	5.36	4.23	5.36
2b	5.50	>10	8.13	1.80	<0.1	4.83
3a	5.20	>10	>10	4.36	3.63	>10
3b	>10	>10	>10	>10	>10	>10

a) $ED_{50} \leqq 4$ μg/ml for minimum significant activity.

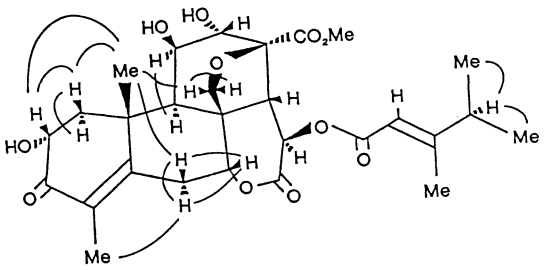

Fig. 3. NOE of Compound **1a**.

This suggested that **1b** is not a stereoisomer at C-2 of **1a**. Indeed, the ^1H NMR spectrum of **1b** (Table 1) nearly coincided with that of **4** except for the lower chemical shift by δ=0.39, 0.24, and 0.41 each from those Me-4, H-6α, and H-6β signals of **4**. This result indicated that stereochemistry of H-5 of **1b** is different from that of **4** (H-5α). The foregoing lower chemical shift of the signals might be influenced by the magnetic anisotropy of the D-ring (δ-lactone), because the A/B ring junction is of *cis* configuration. For these reasons, **1b** should be a stereoisomer at C-5 of **4**.

Structures of compounds **2a**, **2b**, **3a**, and **3b** (Fig. 2) were also elucidated by MS, ^1H NMR, and NOE spectra. Thus, two types (**a**-type and **b**-type) of quassinoids were obtained again by the hydrogenation of dehydro quassinoids (**2** and **3**).

Generally, unsaturated bonds are hydrogenated by *cis*-addition of hydrogen in catalytic hydrogenation. Therefore, an **a**-type compound might be obtained by the *cis*-addition of hydrogen from the β-direction of the A-ring to the double bond between C-1 and C-2 in the dehydro quassinoid. On the other hand, a **b**-type compound might be obtained through two steps: At first *cis*-addition of hydrogen from the β-direction of the A-ring to the double bond between C-4 and C-5 in the dehydro quassinoid (forming an intermediate compound), then the keto–enol exchange at the A-ring from the intermediate compound (which formed the diosphenol structure). These formation mechanisms are shown in Fig. 4.

The in vitro cytotoxicity assay of these compounds (**1a**, **1b**, **2a**, **2b**, **3a**, and **3b**) against KB (epidermoid carcinoma of nasopharynx), TE-671 (human medulloblastoma), A-549 (human lung carcinoma). HCT-8

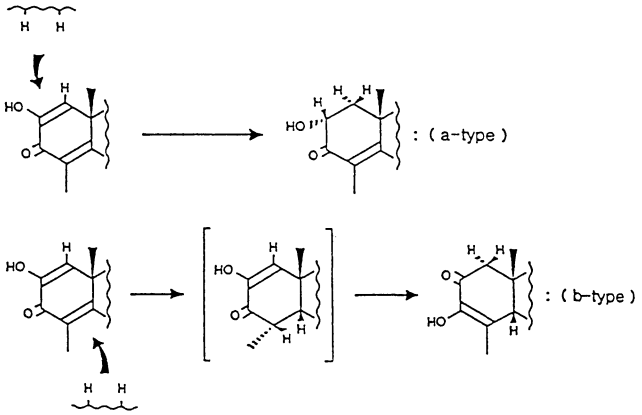

Fig. 4. Mechanism for Formation of **a**- and **b**-Type Compounds.

(human colon carcinoma), RPMI-7951 (human melanoma), and P-388 (murine leukemia) tumor cells was carried out. As shown in Table 2, compounds **1a** and **2b** demonstrated selective cytotoxicity against TE-671 with ED_{50} of 0.68 and <0.1 $\mu g\,ml^{-1}$, respectively. Significant cytotoxicity was also demonstrated by **3a** (for TE-671), **1a** (for P-388), and **2b** (for RPMI-7951).

Experimental

General Experimental Procedure. All melting points were determined on an MRK air-bath type melting point apparatus and were uncorrected. Specific rotations were obtained on a JASCO DIP-370 digital polarimeter. IR and UV spectra were recorded on a JASCO IR-810 spectrometer and a Hitachi 320-S spectrometer, respectively. ^1H NMR spectra were determined on a VARIAN VXR-500 or a JASCO GSX-500 in C_5D_5N, using TMS as an internal standard. MS spectra were recorded on a Hitachi M80 instrument. Analytical HPLC was performed on a TOSOH liquid chromatograph equipped with a UV detector at 254 nm and a reversed phase column (TSK-gel $80T_M$), using a mixed solvent of MeOH–H_2O. Preparative HPLC was carried out on a Gilson and/or Waters Associates liquid chromatograph equipped with a reversed phase column (Dynamax-60A and/or Lichrosorb RP-18) at 254 nm. The solvents used for analytical HPLC were also used for preparative HPLC.

Chromatography of the $CHCl_3$ Fraction. The crude $CHCl_3$ fraction (372 g), which is part of the $CHCl_3$ extract of the ground wood of *B. antidysenterica* (4228 lbs) reported previously,[5] was subjected to column chromatography on

silica gel (2 kg. 10×60 cm). It was eluted first with $CHCl_3$ and then with increasing amounts of MeOH in $CHCl_3$ to yield 28 fractions.

Isolation of Dehydrobruceantin (1). Fraction 20 (31.2 g) was further subjected to column chromatography on silica gel (500 g, 4×90 cm and eluted with EtOAc–Et_2O, 1 : 1, v/v) to yield 10 fractions. The 4th fraction (1.52 g) was further subjected to repeated preparative TLC and preparative HPLC to afford dehydrobruceantin (**1**, 27.0 mg) as colorless solid.

Compound 1: Colorless amorphous solid; mp 175—177 °C. $[\alpha]_D^{23}$ + 70.3° (c=0.065, EtOH). UV (EtOH) 210 (ε 16200) and 252 (ε 9160) nm. IR (KBr) 3500 (OH), 1735 (δ-lactone and ester C=O), 1720 (α,β-unsaturated C=O), 1635 (C=C) cm^{-1}. For ^1H NMR see Table 1. EIMS m/z (rel intensity) 546 ($[M]^+$, 0.3%), 528 ($[M-H_2O]^+$, 0.3%), 111 ($[C_7H_{11}O]^+$, 46.7%).

Isolation of Dehydrobruceantinol (2) and Dehydrobruceantarin (3). Part (24.4 g) of fraction 21 (128 g) was subjected to column chromatography on silica gel (500 g, 4×90 cm and eluted with EtOAc–Et_2O, 1 : 2, v/v) to yield 15 fractions. The 10th fraction (2.46 g) was further subjected to repeated HPLC to afford dehydrobruceantinol (**2**, 49.2 mg) as amorphous solid. The 8th and the 9th fractions (2.17 g, total) were further subjected to repeated HPLC to afford dehydrobruceantarin (**3**, 29.5 mg) as amorphous solid.

Compound 2: Colorless amorphous solid; mp 146—148 °C. $[\alpha]_D^{22}$ +53.6° (c=0.10, EtOH). UV (EtOH) 210 (ε 18400) and 252 (ε 11000) nm. IR (KBr) 3450 (OH), 1740 (δ-lactone and ester C=O), 1720 (α,β-unsaturated C=O), 1635 (C=C) cm^{-1}. For ^1H NMR see Table 1. EIMS m/z (rel intensity) 586 ($[M-H_2O]^+$, 0.1%), 526 ($[M-AcOH-H_2O]^+$, 3.0%), 127 ($[C_7H_{11}O_2]^+$, 29%), 109 ($[C_7H_{11}O_2-H_2O]^+$, 100%).

Compound 3: Colorless amorphous solid; mp 175—177 °C. $[\alpha]_D^{23}$ +97.1° (c=0.089, EtOH). UV (EtOH) 229 (ε 16800) and 253 (ε 11900) nm. IR (KBr) 3450 (OH), 1740 (δ-lactone and ester C=O), 1720 (α,β-unsaturated C=O), 1635 (C=C), 710 (monosubstituted phenyl) cm^{-1}. For ^1H NMR see Table 1. EIMS m/z (rel intensity) 540 ($[M]^+$, 0.2%), 522 ($[M-H_2O]^+$, 7.8%), 105 ($[C_7H_5O]^+$, 100%).

Catalytic Hydrogenation of Dehydrobruceantin (1). A solution of **1** (22.8 mg, 0.042 mmol) and Pd/C (5%) in MeOH (20 ml) was stirred under H_2 gas at room temperature for 5 h. The catalyst was removed by filtration and the residual solution was subjected to analytical HPLC (TSK-gel 80T_M, MeOH : H_2O=1 : 1) which showed to contain two reduction products. The products were isolated by preparative HPLC, as both colorless amorphous solids, **1a** (2.2 mg, 9.6%) and **1b** (8.8 mg, 38.6%).

Compound 1a: Colorless amorphous solid; mp 190—192 °C. $[\alpha]_D^{23}$ +92.2° (c=0.13, EtOH). UV (EtOH) 220 (ε 19400) nm. IR (KBr) 3450 (OH), 1740 (δ-lactone and ester C=O), 1670 (α,β-unsaturated C=O), 1640 (C=C), cm^{-1}. For ^1H NMR see Table 1. EIMS m/z (rel intensity) 530 ($[M-H_2O]^+$, 0.4%), 420 ($[M-H_2O-C_7H_{10}O+1]^+$, 1.0%), 111 ($[C_7H_{11}O]^+$, 100%).

Compound 1b: Colorless amorphous solid; mp 143—145 °C. $[\alpha]_D^{22}$ −7.23° (c=0.083, EtOH). UV (EtOH) 220 (ε 20200) and 280 (ε 8640) nm. IR (KBr) 3450 (OH), 1740 (δ-lactone and ester C=O), 1720 (α,β-unsaturated C=O), 1640 (C=O) cm^{-1}. For ^1H NMR see Table 1. EIMS m/z (rel intensity) 548 ($[M]^+$, 1.2%), 111 ($[C_7H_{11}O]^+$, 100%). HREIMS Found: m/z 548.2264 ($[M]^+$). Calcd for $C_{28}H_{36}O_{11}$: 548.2255.

Catalytic Hydrogenation of Dehydrobruceantinol (2). A solution of **2** (46.7 mg, 0.077 mmol) and Pd/C (5%) in MeOH (20 ml) was stirred under H_2 gas at room temperature for 5 h. The catalyst was removed by filtration and the residual solution was subjected to analytical HPLC (TSK-gel 80T_M, MeOH : H_2O=1 : 1), which showed to contain two reduction products. The products were isolated by a preparative HPLC, as colorless amorphous solids, **2a** (5.4 mg, 11.6%) and **2b** (20.5 mg, 43.9%), respectively.

Compound 2a: Colorless amorphous solid; mp 151—153 °C. $[\alpha]_D^{22}$ +102.7° (c=0.029, EtOH). UV (EtOH) 220 (ε 22800) nm. IR (KBr) 3450 (OH), 1740 and 1735 (δ-lactone and ester C=O), 1670 (α,β-unsaturated C=O), 1640 (C=C) cm^{-1}. For ^1H NMR see Table 1. EIMS m/z (rel intensity) 528 ($[M-H_2O-C_2H_4O_2]^+$, 3.6%), 127 ($[C_7H_{11}O_2]^+$, 45%), 109 ($[C_7H_{11}O_2-H_2O]^+$, 100%). FDMS m/z (rel intensity) 607 ($[M+1]^+$, 2.8%), 588 ($[M-H_2O]^+$, 6.5%), 547 ($[M-C_2H_4O_2+1]^+$, 15%), 529 ($[M-H_2O-C_2H_4O+1]^+$, 26%). HREIMS Found: m/z 528.2005. Calcd for $C_{28}H_{32}O_{10}$: 528.1994.

Compound 2b: Colorless amorphous solid; mp 135—137 °C. $[\alpha]_D^{23}$ −15.09° (c=0.15, EtOH). UV (EtOH) 217 (ε 15100) and 277 (ε 6470) nm. IR (KBr) 3450 (OH), 1740 and 1735 (δ-lactone and ester C=O), 1670 (α,β-unsaturated C=O), 1640 (C=C) cm^{-1}. For ^1H NMR see Table 1. EIMS m/z (rel intensity) 546 ($[M-C_2H_4O_2]^+$, 2.0%), 127 ($[C_7H_{11}O_2]^+$, 20%), 109 ($[C_7H_{11}O_2-H_2O]^+$, 100%). FDMS m/z (rel intensity) 606 ($[M]^+$, 18%), 605 ($[M-1]^+$, 22%), 546 ($[M-C_2H_4O_2]^+$, 54%). HREIMS Found: m/z 546.2097 ($[M]^+$). Calcd for $C_{28}H_{34}O_{11}$: 546.2098.

Catalytic Hydrogenation of Dehydrobruceantarin (3). A solution of **3** (27.3 mg. 0.051 mmol) and Pd/C (5%) in MeOH (20 ml) was stirred under H_2 gas at room temperature for 1.5 h. The catalyst was removed by filtration and the residual solution was subjected to analytical HPLC (TSK-gel 80T_M, MeOH : H_2O=4 : 6) which showed to contain two reduction products. The products were isolated by preparative HPLC as colorless amorphous solids, **3a** (2.7 mg, 9.9%) and **3b** (16.2 mg, 59.3%), respectively.

Compound 3a: Colorless amorphous solid; mp 180—182 °C. $[\alpha]_D^{23}$ +127.0° (c=0.052, EtOH). UV (EtOH) 229 (ε 11000) nm. IR (KBr) 3450 (OH), 1740 (δ-lactone and ester C=O), 1720 (α,β-unsaturated C=O), 1640 (C=C), 710 (monosubstituted phenyl) cm^{-1}. For ^1H NMR see Table 1. EIMS (rel intensity) 524 ($[M-H_2O]^+$, 6.0%), 105 ($[C_7H_5O]^+$, 100%).

Compound 3b: Colorless amorphous solid; mp 156—158 °C. $[\alpha]_D^{23}$ −34.16° (c=0.064, EtOH). UV (EtOH) 228 (ε 12900) and 277 (ε 7560) nm. IR (KBr) 3450 (OH), 1735 (δ-lactone and ester C=O), 1720 (α,β-unsaturated C=O), 1640 (C=C), 710 (monosubstituted phenyl) cm^{-1}. For ^1H NMR see Table 1. EIMS m/z (rel intensity) 542 ($[M]^+$, 0.8%), 524 ($[M-H_2O]^+$, 1.1%), 105 ($[C_7H_5O]^+$, 100%).

Biological Activity of 1a, 1b, 2a, 2b, 3a, and 3b. The in vitro cytotoxicity assay was carried out according to the procedures described in Geran et al.[10] and Ferguson et al.[11] The assay against KB (epidermoid carcinoma of nasopharynx), TE-671 (human medulloblastoma), A-549 (human lung carcinoma), HCT-8 (human colon carcinoma), RPMI-7951 (human melanoma), and P-388 (murine leukemia) tumor cells was based on a method reported by Lee et al.[12] The cytotoxicity of compounds **1a**, **1b**, **2a**, **2b**, **3a**, and **3b** is shown in Table 2.

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References

1) S. M. Kupchan, R. W. Britton, J. A. Lacadie, M. F. Ziegler, and C. W. Sigel, *J. Org. Chem.*, **40**, 648 (1975).

2) M. Okano, N. Fukamiya. T. Aratani, M. Juichi, and K. H. Lee, *J. Nat. Prod.*, **48**, 972 (1985).

3) N. Fukamiya. M. Okano, K. Tagahara, T. Aratani, and K. H. Lee, *J. Nat. Prod.*, **51**, 349 (1988).

4) T. Toyota, N. Fukamiya, M. Okano, K. Tagahara, and K. H. Lee, *J. Nat. Prod.*, **53**, 1526 (1990).

5) M. Okano. K. H. Lee, I. H. Hall, and F. E. Boettner, *J. Nat. Prod.*, **44**, 470 (1981).

6) N. Fukamiya, M. Okano, K. Tagahara, T. Aratani, Y. Muramoto, and K. H. Lee, *J. Nat. Prod.*, **50**, 1075 (1987).

7) M. Okano, N. Fukamiya, T. Toyota, K. Tagahara, and K. H. Lee, *J. Nat. Prod.*, **52**, 398 (1989).

8) N. Fukamiya, M. Okano, T. Aratani, K. Negoro, A. T. McPhail, M. Juichi, and K. H. Lee, *J. Nat. Prod.*, **49**, 428 (1986).

9) N. Fukamiya, M. Okano, T. Aratani, K. Negoro, Y. M. Lin, and K. H. Lee, *Planta Med.*, **53**, 140 (1987).

10) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3(2)**, 1 (1972).

11) P. J. Ferguson, M. H. Fisher, J. Stephenson, D. H. Li, B. S. Zhaou, and Y. C. Cheng, *Cancer Res.*, **48**, 5956 (1988).

12) K. H. Lee, Y. M. Lin, T. S. Wu, D. C. Zhang, T. Yamagishi, T. Hayashi, I. H. Hall, J. J. Chang, R. Y. Wu, and T. H. Yang, *Planta Med.*, **54**, 308 (1988).