Conversion of Quassinoids for Enhancement of Inhibitory Effect against Epstein–Barr Virus Early Antigen Activation. Introduction of Lipophilic Side Chain and Esterification of Diosphenol

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Introduction of a senecioyl group into shinjulactones B and C, and esterification of the diosphenol moiety in brusatol and brucein A enhanced inhibitory effect against Epstein–Barr virus early antigen activation.

Key words quassinoid conversion; lipophilic side chain; diosphenol esterification; inhibitory effect against Epstein–Barr virus early antigen activation

Several natural products including flavonoids,[1] steroids,[2] triterpenoids,[3] triterpenoid saponins,[4] and quinones[5] have been investigated for their inhibitory effects on 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced Epstein–Barr virus early antigen (EBV-EA) activation and, thus, potential antitumor promoting agents.

Recently, we reported the inhibitory activities of 59 natural quassinoids[6,7] and 3 derivatives[8] isolated from plants of the Simaroubaceous family, such as *Brucea* (*B.*) *javanica*, *B. antidysenterica*, *Picrasma ailanthoides*, and *Ailanthus* (*A.*) *altissima*, and 5 semisynthetic variations.[9] Every quassinoid tested showed inhibitory activity. The most potent ones were aglycones with carbonyl and hydroxyl groups in ring A, a methylenoxy bridge between C-8 and C-13, and an ester side chain at C-15. In contrast, several analogous glycosides, which have these same moieties, showed only moderate activity. The least active quassinoids lacked the methylenoxy bridge and the ester side chain. From the above results, we were interested in the effect of the lipophilic side chain on the inhibitory activity against EBV–EA activation, and so we investigated structure improvement for the less active quassinoids.

In the previous paper,[10] we reported the isolation of bruceanic acids A, B, C, and D. All these compounds may be obtained by oxidative degradation of diosphenol in bruceantin, bruceantarin, bruceantinol, and desacetylbruceantinol, respectively, of which isolation was reported by Kupchan *et al.*[11] Therefore, we were interested in the effect of protected diosphenol for its inhibitory activity against EBV–EA activation.

In this paper, we report the introduction of a lipophilic senecioyl group into shinjulactones B (**1**) and C (**2**), introduction of an ethylsuccinyl group into the diosphenol moiety of brusatol (**5**) and brucein A (**6**) for protection, and their inhibitory activities against EBV–EA activation.

Results and Discussion

Shinjulactone B (**1**) and shinjulactone C (**2**) were isolated from *A. altissima* as colorless amorphous solids. Compounds **3** and **4** were obtained as colorless solids by esterification of **1** and **2** using senecioyl chloride. The molecular formulae of **3** ($C_{24}H_{28}O_8$) and **4** ($C_{25}H_{28}O_8$) were confirmed by high resolution MS spectra. The position of esterification in **3** and **4** was confirmed as C-20 from big differences in the two ^{1}H-NMR signals between 20-CH_2 of **1** (δ 3.92 and 4.08) and that of **3** (δ 4.36 and 4.65), and also between 20-CH_2 of **2** (δ 4.03 and 4.07) and that of **4** (δ 4.56 and 4.61), respectively.

Bruceoside D (**5**) and bruceoside E (**6**) were obtained, respectively, as colorless amorphous solids from *B. javanica*. Compounds **5** and **6** were converted, respectively, into compounds **7** and **8** by acid hydrolysis using *p*-toluenesulfonic acid in MeOH. Compounds **7** and **8** were confirmed, respectively, as brusatol and brucein A by ^{1}H- and ^{13}C-NMR spectral comparison with those of the authentic compounds.

Compounds **7** and **8** were converted into compounds **9** and **10** by esterification using monoethylsuccinyl chloride. The molecular formulae of **9** ($C_{32}H_{40}O_{14}$) and **10** ($C_{32}H_{42}O_{14}$) were confirmed by high resolution MS spectra. The positions of esterification in **9** and **10** were confirmed, respectively, as C-3 according the big differences of the two ^{13}C-NMR sig-

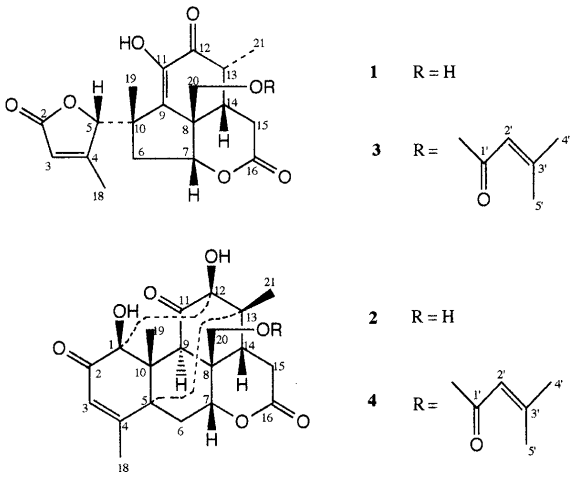

Fig. 1. Introduction of Senecioyl Group into **1** and **3** to Afford **2** and **4**

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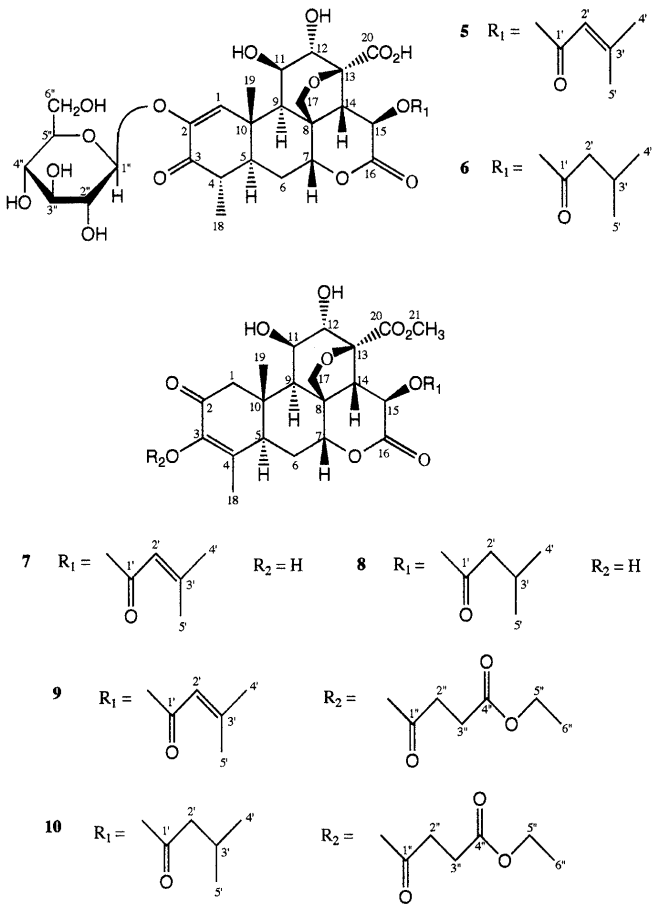

Fig. 2. Acid Hydrolysis of **5** and **6** to Afford **7** and **8**, and Esterification of **7** and **8** to Afford **9** and **10**

Table 1. Inhibitory Effects of Quassinoids (**1**—**6**, **9** and **10**) against TPA-Induced EBV–EA Activation

Compound	%EBV–EA Positive Cells					IC_{50}
	Compound concentration (mol ratio/32 pmol TPA)					
	1000	500	100	10	0[a]	
Shinjulactone B (**1**)	3.1 (70)[b]	18.4	30.5	36.1	40	445
Shinjulactone C (**2**)	0 (80)[b]	4.4	21.8	28.4	40	145
Senecioate of **1** (**3**)	0.9 (70)[b]	16.7	29.4	35.4	40	395
Senecioate of **2** (**4**)	0 (80)[b]	3.9	20.5	28.2	40	115
Bruceoside D (**5**)	0 (80)[b]	2.5	9.4	14.3	40	7.8
Bruceoside E (**6**)	0 (80)[b]	1.6	8.2	12.8	40	7.3
Ethylsuccinate of **7** (**9**)	0 (80)[b]	0	1.0	4.3	40	5.6
Ethylsuccinate of **8** (**10**)	0 (80)[b]	0	0.9	4.1	40	5.5

a) Control. *b*) Values in the parentheses are viability percentage of Raji cells.

nals between C-4 of **7** (δ 128.3) and that of **9** (δ 142.6), and also between C-4 of **8** (δ 128.2) and that of **10** (δ 142.6).

In Table 1, senecioyl esters at C-15 (**3** and **4**) of compounds **1** and **2** showed higher inhibitory activity against EBV–EA activation than those of the starting materials. This fact indicates that the introduction of a lipophilic side chain is very important for enhancement of the inhibitory activity of quassinoids.

Ethylsuccinyl esters at C-3 (**9** and **10**) of compounds **7** and **8** showed the highest inhibitory activity against EBV–EA activation, as shown in Table 1. This fact indicates that the esterification of diosphenol is very important for enhancement of the inhibitory activity of quassinoids.

Materials and Methods

General Experimental Procedures Melting points were determined on an MRK air-bath type melting point apparatus and are uncorrected. Specific rotations were obtained on a JASCO DIP-370 digital polarimeter (length= 0.5 dm). IR spectra were recorded on a JASCO IR-810 spectrophotometer and UV spectra were obtained on a Hitachi 320-S or Shimadzu UV 3101 PC spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were determined on JEOL JNM-A400 or Varian VXR-500 instruments in C_5D_5N using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were recorded on a Hitachi M-80 instrument. Precoated silica gel plates (Merck, $60F_{254}$) of 0.25 mm thickness were used for analytical TLC, and plates of 1 mm thickness were used for preparative TLC. Components on TLC were detected using a UV lamp (254 and 365 nm). Analytical HPLC was performed on a Tosoh liquid chromatograph equipped with a UV detector set at 254 nm and a reverse-phase column (TSK-gel ODS-80Ts) using a mixed solvent of MeOH–H_2O. Preparative HPLC was carried out on a Tosoh or Gilson liquid chromatograph equipped with a reverse-phase column (Lichrosorb RP-18) at 254 nm using the same solvent as used for analytical HPLC.

Compound 1 This compound (**1**, shinjulactone B) was isolated as a colorless amorphous solid from *A. altissima*.[12,13] ^{1}H-NMR (C_5D_5N) δ: 1.05 (3H, d, J=7 Hz, 13-Me), 1.60 (3H, s, 10-Me), 1.95 (3H, d, J=1 Hz, 4-Me), 3.92 (1H, d, J=12 Hz, 20-H$_\alpha$), 4.08 (1H, d, J=12 Hz, 20-H$_\beta$).

Compound 2 This compound (**2**, shinjulactone C) was isolated as a colorless amorphous solid from *A. altissima*.[12,14] ^{1}H-NMR (C_5D_5N) δ: 1.05 (3H, s, 10-Me), 1.28 (3H, s, 13-Me), 1.98 (3H, s, 4-Me), 4.03 (1H, d, J=12 Hz, 20-H$_\alpha$), 4.08 (1H, d, J=12 Hz, 20-H$_\beta$).

Compound 3 Senecioyl chloride (33 mg, 0.28 mM) dissolved in $CHCl_3$ (0.3 ml) was added dropwise into compound **1** (20 mg, 0.055 mM), dissolved in C_5H_5N (1.2 ml) at 0 °C, and stirred at room temperature (24 °C) for 6 d. Then, MeOH (2 ml) was added to the reaction mixture for quenching. The reaction product was isolated first by preparative TLC (EtOAc–Et_2O, 50 : 50, v/v) and then by preparative HPLC (MeOH–H_2O, 60 : 40, v/v) to afford compound **3** (2.9 mg, 0.0065 mM, yield 11.8%) as a colorless amorphous solid, mp 186—188 °C. UV λ_{max}^{MeOH} nm (ε): 277 (8200). IR (KBr) cm^{-1}: 3400 (OH), 1760 (γ-lactone C=O), 1710 (C=O), 1680 (α,β-unsaturated C=O). ^{1}H-NMR (C_5D_5N) δ: 1.12 (3H, d, J=6.8 Hz, 13-Me), 1.60 (3H, s, 10-Me), 1.94 (3H, s, 4-Me), 4.36 (1H, d, J=12 Hz, 20-H$_\alpha$), 4.65 (1H, d, J=12 Hz, 20-H$_\beta$), 1.78 (3H, s, 3′-Me), 2.19 (3H, s, 3′-Me), 5.81 (1H, s, 2′-H). ^{13}C-NMR (C_5D_5N) δ: 12.5 (C-21, Me), 16.2 (C-18, Me), 24.0 (C-19, Me), 63.8 (C-20, CH_2), 20.2 (C-4′, Me), 27.1 (C-5′, Me), 115.6 (C-2′, CH), 158.9 (C-3′, C), 165.9 (C-1′, C=O). HREIMS *m/z*: 444.1793 (M^+) ($C_{24}H_{28}O_8$, error 1.1).

Compound 4 Senecioyl chloride (64 mg, 0.54 mM) dissolved in $CHCl_3$ (0.3 ml) was added dropwise into compound **2** (10 mg, 0.0267 mM) dissolved in C_5H_5N (2.0 ml) at 0 °C and stirred at 45—50 °C for 24 h. Then, MeOH (2 ml) was added into the reaction mixture for quenching. The reaction product was isolated by preparative HPLC (MeOH–H_2O, 50 : 50, v/v) to afford compound **4** (3.9 mg, 0.0085 mM, yield 31.9%) as a colorless amorphous solid, mp 296—298 °C (dec.). UV λ_{max}^{MeOH} nm (ε): 221 (15280). IR (KBr) cm^{-1}: 3400 (OH), 1760 (γ-lactone C=O), 1710 (C=O), 1660 (α,β-unsaturated C=O). ^{1}H-NMR ($CDCl_3$) δ: 0.99 (3H, s, 10-Me), 1.02 (3H, s, 13-Me), 2.08 (3H, s, 4-Me), 4.07 (1H, d, J=12 Hz, 20-H$_\alpha$), 4.13 (1H, d, J=12 Hz, 20-H$_\beta$), 1.91 (3H, s, 3′-Me), 2.17 (3H, s, 3′-Me), 5.65 (1H, s, 2′-H). ^{13}C-NMR ($CDCl_3$) δ: 12.1 (C-19, Me), 13.9 (C-21, Me), 20.4 (C-18, Me), 61.6 (C-20, CH_2), 23.2 (3′-Me), 27.5 (3′-Me), 114.7 (C-2′, CH), 158.9 (C-3′, C), 165.4 (C-1′, C=O). HREIMS *m/z*: 456.1780 (M^+) ($C_{25}H_{28}O_8$, error 0.3). ^{1}H-NMR (C_5D_5N) δ:1.14 (3H, s, 10-Me), 1.31 (3H, s, 13-Me), 2.01 (3H, s, 4-Me), 4.56 (1H, d, J=12 Hz, 20-H$_\alpha$), 4.61 (1H, d, J=12 Hz, 20-H$_\beta$), 1.69 (3H, s, 3′-Me), 2.13 (3H, s, 3′-Me), 5.74 (1H, s, 2′-H).

Compound 5 This compound (**5**, bruceoside D) was isolated as a colorless amorphous solid from *B. javanica*.[15] ^{1}H- and ^{13}C-NMR (C_5D_5N) spectra coincided with those of the authentic bruceoside D.

Compound 6 This compound (**6**, bruceoside E) was isolated as a colorless amorphous solid from *B. javanica*.[15] ^{1}H- and ^{13}C-NMR (C_5D_5N) spectra coincided with those of the authentic bruceoside E.

Compound 7 A mixture of compound **5** (64 mg, 0.0957 mM) and *p*-toluenesulfonic acid (18 mg) was dissolved in MeOH (6 ml) and stirred under reflux condition for 20 h. Then, the reaction mixture was subjected to preparative HPLC (MeOH–H_2O, 40 : 60, v/v) to afford compound **7** (brusatol, 24.3 mg, 0.0467 mM, yield 48.8%) as a colorless amorphous solid,

mp 249—251 °C. UV λ_{max}^{MeOH} nm (ε): 227 (7350). ^{1}H-NMR (C_5D_5N) δ: 1.67 (3H, s, 10-Me), 1.95 (3H, d, J=1.2 Hz, 4-Me), 3.76 (3H, s, OMe), 1.63 (3H, s, 3′-Me), 2.15 (3H, s, 3′-Me), 5.85 (1H, s, 2′-H). ^{13}C-NMR (C_5D_5N) δ: 13.4 (C-18, Me), 15.8 (C-19, Me), 42.5 (C-5, CH), 52.4 (OMe), 128.3 (C-4, C), 146.0 (C-3, C), 193.0 (C-2, C=O), 20.2 (3′-Me), 27.0 (3′-Me), 116.0 (C-2′, CH), 158.4 (C-3′, C), 165.4 (C-1′, C=O). The ^{1}H- and ^{13}C-NMR spectra of compound **7** coinsided with those of brusatol.[8]

Compound 8 A mixture of compound **6** (80 mg, 0.119 mM) and *p*-toluenesulfonic acid (23 mg) was dissolved in MeOH (6 ml) and stirred under the reflux condition for 20 h. Then, the reaction mixture was subjected to preparative HPLC (MeOH–H_2O, 45 : 55, v/v) to afford compound **8** (brucein A, 23.4 mg, 0.0448 mM, yield 37.6%) as a colorless amorphous solid, mp 144—146 °C. UV λ_{max}^{MeOH} nm (ε): 278 (5120). ^{1}H-NMR (C_5D_5N) δ: 1.62 (3H, s, 10-Me), 1.94 (3H, d, J=1.6 Hz, 4-Me), 3.83 (3H, s, OMe), 0.95 (3H, d, J=6.8 Hz, 3′-Me), 0.97 (3H, d, J=6.4 Hz, 3′-Me). ^{13}C-NMR (C_5D_5N) δ: 13.3 (C-18, Me), 15.7 (C-19, Me), 128.2 (C-4, C), 146.0 (C-3, C), 193.0 (C-2, C=O), 22.4 (3′-Me), 22.5 (3′-Me), 25.9 (C-3′, CH), 43.3 (C-2′, CH_2), 171.7 (C-1′, C=O). The ^{13}C-NMR spectrum of compound **8** coincided with that of brucein A.[16]

Compound 9 Monoethylsuccinyl chloride (7.69 mg, 0.0467 mM) dissolved in $CHCl_3$ (0.1 ml) was added dropwise into compound **7** (brusatol, 24.3 mg, 0.0467 mM), dissolved in C_5H_5N (1.0 ml), and stirred at 24 °C for 17 h. Then, the reaction mixture was subjected to preparative HPLC (MeOH–H_2O, 50 : 50, v/v) to afford compound **9** (9.7 mg, 0.0149 mM, yield 32%) as a colorless amorphous solid, mp 200—202 °C. UV λ_{max}^{MeOH} nm (ε): 222 (11090). IR (KBr) cm^{-1}: 3420 (OH), 1740 (ester C=O), 1710 (C=O), 1675 (α,β-unsaturated C=O), 1640 (C=C). ^{1}H-NMR (C_5D_5N) δ: 1.77 (3H, s, 10-Me), 1.80 (3H, s, 4-Me), 3.75 (3H, s, OMe), 1.66 (3H, s, 3′-Me), 2.14 (3H, s, 3′-Me), 5.85 (1H, s, 2′-H), 1.12 (3H, t, J=6.8 Hz, 6″-Me), 2.80 (2H, t, J=6.4 Hz, 2″-CH_2), 3.05 (2H, t, J=6.4 Hz, 3″-CH_2), 4.12 (2H, q, J=6.8 Hz, 5″-CH_2). ^{13}C-NMR (C_5D_5N) δ: 14.2 (C-18, Me), 15.8 (C-19, Me), 52.3 (OMe), 142.6 (C-4, C), 146.6 (C-3, C), 189.9 (C-2, C=O), 20.2 (3′-Me), 27.0 (3′-Me), 115.9 (C-2′, CH), 158.5 (C-3′, C), 168.2 (C-1′, C=O), 14.3 (C-6″, Me), 29.0 (C-2″, CH_2), 29.1 (C-3″, CH_2), 60.7 (C-5″, CH_2), 170.8 (C-4″, C=O), 172.2 (C-1″, C=O). HREIMS *m/z*: 648.2417 (M^+) ($C_{32}H_{40}O_{14}$, error 0.1).

Compound 10 Monoethylsuccinyl chloride (6.08 mg, 0.0369 mM) dissolved in $CHCl_3$ (0.1 ml) was added dropwise into compound **8** (brucein A, 19.3 mg, 0.0369 mM) dissolved in C_5H_5N (1.0 ml) and stirred at 24 °C for 24 h. Then, the same amount of monosuccinyl chloride was added into the reaction mixture and the reaction was continued in the same way. This procedure was repeated twice. Finally, the reaction mixture was subjected to preparative HPLC (MeOH–H_2O, 50 : 50, v/v) to afford compound **10** (7.2 mg, 0.0111 mM, yield 30%) as a colorless amorphous solid, mp 204—206 °C. UV λ_{max}^{MeOH} nm (ε): 246 (11650). IR (KBr) cm^{-1}: 3400 (OH), 1740 (ester C=O), 1710 (C=O), 1660 (α,β-unsaturated C=O), 1635 (C=C). ^{1}H-NMR (C_5D_5N) δ: 1.75 (3H, s, 10-Me), 1.80 (3H, s, 4-Me), 3.82 (3H, s, OMe), 0.95 (3H, d, J=6.4 Hz, 3′-Me), 0.98 (3H, d, J=7.2 Hz, 3′-Me), 2.22 (1H, m, 3′-H), 2.35 (2H, m, 2′-CH_2), 1.12 (3H, t, J=7.2 Hz, 6″-Me), 2.81 (2H, t, J=6.8 Hz, 2″-CH_2), 3.06 (2H, t, J=6.8 Hz, 3″-CH_2), 4.12 (2H, q, J=7.2 Hz, 5″-CH_2). ^{13}C-NMR (C_5D_5N) δ: 14.2 (C-18, Me), 15.8 (C-19, Me), 52.3 (OMe), 142.6 (C-4, C), 146.5 (C-3, C), 189.9 (C-2, C=O), 22.4 (C-4′, Me), 22.5 (C-5′, Me), 25.9 (C-3′, CH), 43.3 (C-2′, CH_2), 171.7 (C-1′, C=O), 14.3 (C-6″, Me), 29.0 (C-2″, CH_2), 29.0 (C-3″, CH_2), 60.7 (C-5″, CH_2), 170.8 (C-4″, C=O), 172.2 (C-1″, C=O). HREIMS *m/z*: 650.2587 (M^+) ($C_{32}H_{42}O_{14}$, error 1.5).

EBV–EA Activation The inhibition of EBV–EA activation was assayed using a literature method described in literature.[17] The cells were incubated at 37°C for 48 h in a medium containing butyric acid (4 nM), TPA (32 pM), and various amounts of test compound. Smears were made from the cell suspension and the EBV–EA inducing cells were stained by means of an indirect immunofluorescence technique.[18]

In each assay, at least 500 cells were counted, and the number of stained cells (positive cells) was recorded. Triplicate assays were performed for each data point. The EBV–EA inhibitory activity of the test compound was compared with that of the control experiment with butyric acid plus TPA. In the control experiments, the EBV–EA activities were ordinarily around 40%, and these values were taken as a positive control. The viability of the cells was assayed by the trypan-blue staining method. For the determination of cytotoxicity, the cell viability was required to be more than 60%.[19]

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