Inhibitory Effects of Quassinoid Derivatives on Epstein-Barr Virus Early Antigen Activation

Shakila RAHMAN,[a] Narihiko FUKAMIYA,[a] Nobuhiro OHNO,[a] Harukuni TOKUDA,[b] Hoyoku NISHINO,[b] Kiyoshi TAGAHARA,[c] Kuo-Hsiung LEE,[d] and Masayoshi OKANO*,[a]

Department of Interdisciplinary Studies on Natural Environment, Faculty of Integrated Arts and Sciences, Hiroshima University,[a] 1–7–1 Kagamiyama, Higashi-Hiroshima 739, Japan, Department of Biochemistry, Kyoto Prefectural University of Medicine,[b] Kawaramachi-dori, Kamigyo-ku, Kyoto 602, Japan, Faculty of Pharmaceutical Sciences, Kobe Pharmaceutical University,[c] 1–19–4 Motoyamakita-machi, Higashinada-ku, Kobe 658, Japan, and Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina,[d] Chapel Hill, N.C. 27599–7360, U.S.A. Received September 19, 1996; accepted November 21, 1996

Short-term *in vitro* assays for tumor promoters and antitumor promoters (Epstein-Barr virus activation test) were carried out for semisynthetic quassinoids (3—7), which were obtained by esterification of the C-15 OH group of deacetylated isobrucein-B (2). All the ester derivatives showed higher antitumor promoting activity than that of the potent compound 2. A compound containing a fluorinated aliphatic ester showed the highest potency.

Key words Epstein-Barr virus early antigen activation test; antitumor promoter; fluorinated quassinoid

Recently, 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, as potential antitumor promoting agents. In the previous paper,[6] we reported the inhibitory activities of forty-five natural quassinoids, which we had isolated from plants of the Simaroubaceae family, such as *Brucea javanica*, *Brucea antidysenterica*, and *Picrasma ailanthoides*. The ten most potent quassinoids were obtained from *B. antidysenterica*. These ten compounds are aglycones with C=O and OH groups in ring A, a $-CH_2O-$ 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. This fact suggested that the added suger moiety decreases the activity. The quassinoid glycosides were obtained mainly from *B. javanica*. The ten least active compounds, obtained from *P. ailanthoides*, lacked the $-CH_2O-$ bridge and the ester side chain.

In structure–activity relationship studies of antitumor[7,8] and anti-inflammatory[9] quassinoids, we found that the lipophilicity of the ester side chain at C-15 is very important for the activity. However, incubation of bruceantin (a quassinoid obtained from *B. antidysenterica*) in *Streptomyces griseus* gave three metabolites, an epoxide and two alcohols, which might have been formed by enzymatic oxidation of the C-15 ester side chain.[10] Introducing fluorine into the quassinoid side chain might increase both lipophilicity and bond strength; the modified quassinoid then could be transported easily into the cell, but might not be decomposed by enzymatic oxidation.

In this paper, we report the esterification of the C-15 OH group of deacetylated isobrucein-B (**2**) and the inhibitory effect of these ester derivatives, including fluorinated esters, on EBV-EA activation.

Results and Discussion

The quassinoid derivatives (compounds **3**—**7**) used in the inhibition assay were obtained by acylation of the C-15 OH group of compound **2**. The following acid chlorides were used: isovaleryl chloride, 3,3-dimethylacryloyl chloride, 4,4,4-trifluoro-3-methylbutanoyl choloride, 3-fluorobenzoyl chloride, and 3-trifluoromethylbenzoyl chloride. Compound **2** was obtained by alkaline hydrolysis of isobrucein-B (**1**) in 66% yield. Compound **3** is isobrucein-A, which has been isolated from *Soulamea tomentosa* (Simaroubaceae)[10]; however, we prepared a synthetic sample, since we could not obtain the compound as a natural product. The yields of compounds **3**, **4**, **5**, **6**, and **7** were 26%, 7%, 14%, 4.8%, and 27%, respectively. The yields in the acylation reaction were not very high. This was because acylation also occurred at other hydroxy groups.

Table 1 shows the inhibitory effects of the five semisynthetic quassinoids on TPA-induced EBV-EA activation compared with that of the starting material, com-

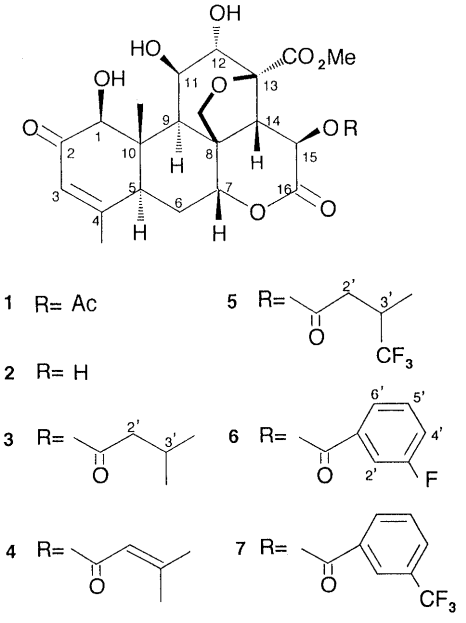

Fig. 1

* To whom correspondence should be addressed.

Table 1. Inhibitory Effects of Quassinoid Derivatives on TPA-Induced EBV-EA Activation

Compound	EBV-EA-positive cells (%)					EC_{50}
	Compound concentration (mol ratio/TPA[a])				Control	
	1000	500	100	10		
2	0(80)[b]±0	0±2	19±3	36±2	40±2	94.7
3	0(80)[b]±0	0±2	11±2	33±2	40±2	63.2
4	0(80)[b]±0	0±1	13±2	36±2	40±2	72.6
5	0(80)[b]±0	0±1	4±1	12±2	40±2	7.1
6	0(80)[b]±0	0±1	6±1	19±3	40±2	9.5
7	0(80)[b]±0	0±2	8±2	24±2	40±2	32.5
Curcumine	0(70)[b]±0	10±2	29±3	40±2	40±2	289
β-Carotene	3(60)[b]±1	12±3	32±2	40±1	40±2	340
Glycyrrhizin	5(60)[b]±0	21±2	32±2	40±1	40±2	531

a) TPA = 32 pmol. *b*) Values in parentheses are viability percentages of Raji cells.

pound **2**. Esterification of the C-15 group of these quassinoids enhances the activity. The inhibitory effect of compound **3** is almost the same as that of compound **4**. This fact indicates that saturation and unsaturation of the aliphatic side chain essentially do not affect the activity. Compound **5**, a quassinoid containing a fluorinated aliphatic side chain, was the most potent compound among these semisynthetic quassinoids, followed by quassinoids containing fluorinated aromatic side chains, compounds **6** and **7**. Thus, fluorination of the side chain enhances the antitumor promoting activity.

Materials and Methods

General Experimental Procedures Melting points were determined on an MRK air-bath melting point apparatus and are uncorrected. IR and UV spectra were recorded on a Jasco IR-810 spectrometer and a Hitachi 320-S spectrometer, respectively. ^{1}H- and ^{13}C-NMR spectra were measured on Varian VXR-500 and JEOL ALPHA-400 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 were detected on TLC by using UV light (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 or MeOH/H_2O/AcOH. Preparative HPLC was carried out on a Tosoh or Gilson liquid chromatograph equipped with a reverse-phase column (Dynamax-60A and/or Lichrosorb RP-18) at 254 nm using the same solvents as used for analytical HPLC.

Compound 2 A methanolic 0.5 M-KOH solution (3.0 ml) was added to a solution of isobrucein-B (**1**, 100 mg, 0.209 mM) in MeOH (10 ml) at 0 °C. The reaction was monitored by analytical HPLC (MeOH/H_2O, 3 : 7). After 1 h, the reaction mixture was neutralized with Dowex 50W-X2 (cation exchange resin) and the resin was filtered off. The solution was evaporated *in vacuo* to give a residue, which was subjected to preparative HPLC (MeOH/H_2O, 3 : 7) to afford compound **2** (60.4 mg, 66%) as a colorless amorphous solid: mp 279—282 °C. $[\alpha]_D^{27}$ +34.2 (c = 0.076, EtOH). UV λ_{max}^{EtOH} nm (ε): 240 (9800). IR (KBr) cm^{-1}: 3400 (OH), 1740 (ester, δ-lactone C=O), 1660 (α,β-unsaturated C=O). ^{1}H-NMR (C_5D_5N) δ: 1.44 (s, 3H, Me-10), 1.72 (s, 3H, Me-4), 2.44 (dd, 1H, J=12, 2 Hz, H-6α), 2.89 (d, 1H, J=5 Hz, H-9), 3.07 (d, 1H, J=12 Hz, H-5), 3.61 (dd, 1H, J=12, 2 Hz, H-14), 3.79 (s, 3H, OMe-20), 3.93 (d, 1H, J=7 Hz, H-17α), 4.53 (s, 1H, H-1), 4.86 (s, 1H, H-7), 5.07 (s, 1H, H-12), 5.13 (d, 1H, J=7 Hz, H-17β), 5.53 (d, 1H, J=4 Hz, H-11), 6.12 (s, 1H, H-3). EI-MS m/z (rel. int. %): 438 (M^+, 100). HR-EI-MS m/z 438.1538 (M^+, 438.1526 Calcd for $C_{21}H_{26}O_{10}$).

Compound 3 A solution of compound **2** (50 mg, 0.114 mM) in dry C_5H_5N (2.0 ml) was added to a solution of isovaleryl chloride (27.5 mg, 0.228 mM) in dry $CHCl_3$ (1.3 ml) at 0 °C under N_2. The reaction mixture was stirred at 0 °C for 1 h and at 25 °C for 3 h. The reaction was monitored by analytical HPLC (MeOH/H_2O/AcOH, 50 : 50 : 1). Then, MeOH (3 ml) was added to the reaction mixture, and the solution was evaporated *in vacuo* to dryness. The residue was extracted with $CHCl_3$, washed with H_2O, and dried ($MgSO_4$). The solution was concentrated to give a crude product, which was purified by preparative HPLC (MeOH/H_2O/AcOH, 50 : 50 : 1) to afford pure compound **3** (isobrucein-A) as a colorless amorphous solid (15.3 mg, 26%); mp 146—148 °C. $[\alpha]_D^{27}$ +30.3 (c=0.119, EtOH). UV λ_{max}^{EtOH} nm (ε): 240 (11000). IR (KBr) cm^{-1}: 3450 (OH), 1740 (ester, δ-lactone C=O), 1660 (α,β-unsaturated C=O). ^{1}H-NMR (C_5D_5N) δ: 0.94 (d, 3H, J=7 Hz, Me-3′), 0.97 (d, 3H, J=7 Hz, Me-3′), 1.37 (s, 3H, Me-10), 1.71 (s, 3H, Me-4), 1.72 (dd, 1H, J=13, 13 Hz, H-6β), 2.20 (m, 1H, H-3′), 2.22 (d, 1H, J=9 Hz, H-6α), 2.33 (t, 1H, J=7 Hz, H-2′), 2.76 (d, 1H, J=4 Hz, H-9), 2.94 (br s, 1H, H-5), 3.77 (s, 3H, OMe-20), 3.82 (d, 1H, J=8 Hz, H-17α), 3.82 (br s, 1H, H-14), 4.17 (s, 1H, H-1), 4.95 (br s, 1H, H-7), 5.00 (br s, 1H, H-12), 5.05 (d, 1H, J=8 Hz, H-17β), 5.39 (d, 1H, J=4 Hz, H-11), 6.08 (s, 1H, H-3), 6.55 (br s, 1H, H-15). EI-MS m/z (rel. int. %): 522 (M^+, 52). These physical and spectral data coincided with those of authentic isobrucein-A.[11]

Compound 4 Compound **2** (40 mg, 0.091 mM) was reacted with 3,3-dimethylacryloyl chloride (21.6 mg, 0.182 mM) in a similar manner to that described for the synthesis of compound **3**, at 25 °C for 2 h. The reaction mixture was monitored by analytical HPLC (MeOH/H_2O/AcOH, 55 : 45 : 1). HPLC analysis showed starting material remaining, and more 3,3-dimethylacryloyl chloride (43.2 mg, 0.364 mM) in dry $CHCl_3$ (2 ml) was added to the reaction mixture, which was then refluxed for 3 h. After the usual work-up, the crude product (79.5 mg) was subjected to preparative TLC (AcOEt/Et_2O, 1 : 1) to give 7 fractions: fr-1 (5.3 mg), fr-2 (4.0 mg), fr-3 (3.9 mg), fr-4 (11.7 mg), fr-5 (4.6 mg), fr-6 (4.7 mg), and fr-7 (6.1 mg). Fr-4 (11.7 mg) was further subjected to preparative HPLC (MeOH/H_2O, 4 : 6) to afford pure compound **4** as a colorless amorphous solid (3.1 mg, 7%): mp 149—151 °C. $[\alpha]_D^{25}$ +21.7 (c=0.046, EtOH). UV λ_{max}^{EtOH} nm (ε): 223 (26000). IR (KBr) cm^{-1}: 3460 (OH), 1750 (ester, δ-lactone C=O), 1675 (α,β-unsaturated C=O). ^{1}H-NMR (C_5D_5N) δ: 1.45 (s, 3H, Me-10), 1.66 (s, 3H, Me-3′), 1.73 (s, 3H, Me-4), 1.77 (dd, 1H, J=13, 13 Hz, H-6β), 2.16 (s, 1H, H-3′), 2.26 (d, 1H, J=15 Hz, H-6α), 2.88 (d, 1H, J=5 Hz, H-9), 3.05 (br s, 1H, H-5), 3.73 (s, 3H, OMe-20), 3.90 (d, 1H, J=6 Hz, H-17α), 4.01 (br s, 1H, H-14), 4.30 (s, 1H, H-1), 5.12 (br s, 1H, H-12), 5.14 (d, 1H, J=6 Hz, H-17β), 5.52 (d, 1H, J=5 Hz, H-11), 6.13 (s, 1H, H-3), 6.71 (br s, 1H, H-15). EI-MS m/z (rel. int. %): 520 (M^+, 0.7). These physical and spectral data coincided with those of a derivative of yadanzioside-E.[12]

Compound 5 Compound **2** (51.2 mg, 0.118 mM) was reacted as above with 4,4,4-trifluoro-3-butanoyl chloride (41.4 mg, 0.237 mM). After 1 h at 1 °C and 2 h at 25 °C, an additional portion of 4,4,4-trifluro-3-butanoyl chloride (41.4 mg, 0.237 mM) was added, and the solution was refluxed for 3 h. The reaction was monitored by analytical HPLC (MeOH/H_2O/AcOH, 50 : 50 : 1). The usual work-up gave a crude product, which was purified by preparative HPLC (MeOH/H_2O, 1 : 1) to afford pure compound **5** as a colorless amorphous solid (9.4 mg, 14%): mp 148—150 °C. $[\alpha]_D^{25}$ +8.0 (c=0.05, EtOH). UV λ_{max}^{EtOH} nm (ε): 238 (10600). IR (KBr) cm^{-1}: 3450 (OH), 1740 (ester, δ-lactone C=O), 1660 (α,β-unsaturated C=O). ^{1}H-NMR (C_5D_5N) δ: 1.26 (d, 3H, J=6 Hz, Me-3′), 1.44 (s, 3H, Me-10), 1.74 (s, 3H, Me-4), 1.78 (br s, 1H, H-6β), 2.28 (d, 1H, J=13 Hz, H-6α), 2.52 (m, 1H, H-3′), 2.89 (br s, 1H, H-9),

2.94 (br s, 2H, H-2'), 2.99 (br s, 1H, H-5), 3.79 (s, 3H, OMe-20), 3.93 (d, 1H, $J=6$ Hz, H-17α), 3.97 (br s, 1H, H-14), 4.28 (s, 1H, H-1), 5.11 (br s, 1H, H-12), 5.07 (br s, 1H, H-7), 5.47 (br s, 1H, H-11), 6.14 (s, 1H, H-3), 6.74 (s, 1H, H-15). EI-MS *m/z* (rel. int. %): 576 (M^+, 31). HR-EI-MS *m/z* 576.1817 (M^+, 576.1817 Calcd for $C_{26}H_{31}F_3O_{11}$).

Compound 6 Compound **2** (50 mg, 0.114 mM) was reacted with 3-fluorobenzoyl chloride (23 mg, 0.14 mM) in the usual manner. After 24 h at 25 °C, more 3-fluorobenzoyl chloride (23 mg, 0.14 mM) in dry $CHCl_3$ (0.3 ml) was added. This addition of the reagent was repeated four times in 24 h, and the reaction mixture was stirred for an additional 24 h at the same temperature. The reaction was monitored by analytical TLC ($CHCl_3$/EtOAc, 1 : 1). After the usual work-up, the crude product was subjected to preparative TLC ($CHCl_3$/EtOAc, 1 : 1) to give fr-1 (27.7 mg), which contained the desired product, and fr-2 (95 mg) which contained mainly the reactant. The fr-1 was subjected to preparative HPLC (MeOH/H_2O, 6 : 4) to afford pure compound **6** as a colorless amorphous solid (3.1 mg, 4.8%): mp 105—108 °C. $[\alpha]_D^{25}$ −25.0 ($c=0.016$, EtOH). UV λ_{max}^{EtOH} nm (ε): 235 (10700). IR (KBr) cm^{-1}: 3450 (OH), 1745 (ester, δ-lactone C=O), 1660 (α,β-unsaturated C=O). ^{1}H-NMR (C_5D_5N) δ: 1.44 (s, 3H, Me-10), 1.76 (s, 3H, Me-4), 1.82 (ddd, 1H, $J=14.8$, 13.2, 3.0 Hz, H-6β), 2.32 (ddd, 1H, $J=14.8$, 2.8, 2.8 Hz, H-6α), 2.95 (d, 1H, $J=4.8$ Hz, H-9), 3.05 (d, 1H, $J=13.2$ Hz, H-5), 3.59 (s, 3H, OMe-20), 3.98 (d, 1H, $J=7.6$ Hz, H-17α), 4.11 ($J=13.2$ Hz, H-14), 4.28 (s, 1H, H-1), 5.12 (s, 1H, H-7), 5.13 (br s, 1H, H-12), 6.16 (s, 1H, H-3), 7.39 (m, 2H, H-4', H-5'), 8.12 (d, 1H, $J=9.6$ Hz, H-2'), 8.18 (d, 1H, $J=7.6$ Hz, H-6'). EI-MS *m/z* (rel. int. %): 560 (M^+, 18). HR-EI-MS *m/z* 560.1678 (M^+, 560.1682 Calcd for $C_{28}H_{29}FO_{11}$).

Compound 7 Compound **2** (20 mg, 0.046 mM) in dry C_5H_5N (0.6 ml) and 3-trifluoromethylbenzoyl chloride (40 mg, 0.18 mM) were reacted in an identical manner to that described for the synthesis of compound **6**. Five additional portions of 3-fluoromethylbenzoyl chloride (40 mg, 0.18 mmol) in dry $CHCl_3$ (0.3 ml) were added. The reaction was monitored by analytical TLC ($CHCl_3$/EtOAc, 1 : 1). After the usual work-up, the crude product was subjected to preparative TLC ($CHCl_3$/EtOAc, 1 : 1) to give fr-1 (36.4 mg), which contained the desired product, and fr-2 (119 mg), which contained mainly the reactant. The fr-1 was subjected to preparative HPLC (MeOH/H_2O, 6 : 4) to afford pure compound **7** as a colorless amorphous solid (7.7 mg, 27%): mp 130—133 °C. $[\alpha]_D^{21}$ −37.5 ($c=0.048$, EtOH). UV λ_{max}^{EtOH} nm (ε): 237 (10700). IR (KBr) cm^{-1}: 3450 (OH), 1745 (ester, β-lactone C=O), 1660 (α,β-unsaturated C=O). ^{1}H-NMR (C_5D_5N) δ: 1.46 (s, 3H, Me-10), 1.78 (s, 3H, Me-4), 1.83 (ddd, 1H, $J=14.4$, 13.2, 3.6 Hz, H-6β), 2.98 (d, 1H, $J=4.4$ Hz, H-9), 3.09 (d, 1H, $J=13.2$ Hz, H-5), 3.63 (s, 3H, OMe-20), 4.00 (d, 1H, $J=7.2$ Hz, H-17α), 4.13 (d, 1H, $J=11.6$ Hz, H-14), 4.29 (s, 1H, H-1), 5.12 (br s, 1H, H-7), 5.16 (br s, 1H, H-12), 5.18 (d, 1H, $J=7.2$ Hz, H-17β), 5.54 (br s, 1H, H-11), 6.17 (s, 1H, H-3), 6.69 (br s, 1H, H-15), 7.50 (t, 1H, $J=8.0$ Hz, H-5'), 7.81 (d, 1H, $J=8$ Hz, H-4'), 8.35 (d, 1H, $J=8$ Hz, H-6'). EI-MS *m/z* (rel. int. %): 610 (M^+, 15). HR-EI-MS *m/z* 610.1644 (M^+, 610.1604 Calcd for $C_{29}H_{29}F_3O_{11}$).

EBV-EA Activation Experiment EBV-genome-carrying lymphoblastoid cells (Raji cells derived from Burkitt's lymphoma) were cultured in RPMI 1640 medium (Nissui) as described in the literature.[13] Spontaneous activation of EBV-EA in our subline of Raji cells was less than 0.1%.

The inhibition of EBV-EA activation was assayed using the literature method.[13] TPA (32 pmol) and a test compound were added to the medium containing the cells and *n*-butyric acid (4 mM), and the mixture was incubated at 37 °C for 48 h. Smears were made from the cell suspension, and the cells were stained by means of an indirect immunofluorescence technique,[14] using nasopharyngeal carcinoma patient's serum and anti-human IgG conjugate FITC (goat). 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 evaluated by comparison with that of the control, containing *n*-butyric acid plus TPA. In the control experiments, the EBV-EA activities were usually around 40%. The viability of cells was assayed by the trypan-blue staining method. For the determination of cytotoxicity, the cell viability was required to be more than 60%.[15]

Acknowledgements The authors thank Drs. M. Sugiura, K. Saiki, and T. Sai, Kobe Pharmaceutical University for their measurements of ^{1}H-NMR and MS (EI, HR-EI) spectra. This study was supported in part by Grants-in Aid from the Ministry of Education, Science and Culture, and from the Ministry of Health and Welfare for the Second-term Comprehensive Ten-year Strategy for Cancer Control, Japan.

References

1) Konoshima T., Takasaki M., Kozuka M., Inada A., Nakanishi T., Tokuda H., Matsumoto T., *Shoyakugaku Zasshi*, **43**, 135—141 (1989).
2) Inada A., Nakanishi T., Konoshima T., Kozuka M., Nishino H., Iwashiama A., *Shoyakugaku Zasshi*, **44**, 215—218 (1990).
3) Diallo H., Vanhaelen M., Vanhaelen-Fastre R., Konoshima T., Kozuka M., Tokuda H., *J. Nat. Prod.*, **53**, 879—881 (1989).
4) Tokuda H., Konoshima T., Kozuka M., Kimura T., *Cancer Lett.*, **40**, 309—317 (1988).
5) Konoshima T., Kozuka M., Koyama J., Okatani T., Tagahara K., Tokuda H., *J. Nat. Prod.*, **52**, 987—995 (1989).
6) Okano M., Fukamiya N., Tagahara K., Tokuda H., Ishikawa A., Nishino H., Lee K. H., *Cancer Lett.*, **94**, 139—146 (1995).
7) Lee K. H., Okano M., Hall I. H., Brent D. A., Soltmann B., *J. Pharm. Sci.*, **71**, 338—345 (1982).
8) Okano M., Fukamiya N., Lee K. H., "Studies in Natural Products Chemistry, Vol. 7, Structure and Chemistry (Part A)," ed. by Atta-ur-Rahman, Elsevier, Amsterdam, 1990, pp. 369—404.
9) Hall I. H., Lee K. H., Imakura Y., Okano M., Johnson S., *J. Pharm. Sci.*, **72**, 1282—1284 (1983).
10) Chien M. M., Rosazza J. P., *J. Chem. Soc., Perkin Trans., 1*, **1981**, 1352—1356.
11) Polonsky J., Varon Z. B., Seveenet T., *Experientia*, **15**, 1113—1248 (1975).
12) Sasaki T., Yoshimura S., Ishibashi M., Tsuyuki T., Takahashi T., Honda T., Nakanishi T., *Bull. Chem. Soc. Jpn.*, **58**, 2680—2686 (1985).
13) Ito Y., Yanase S., Fujita J., Harayama T., Takashima M., Imanaka H., *Cancer Lett.*, **13**, 29—37 (1981).
14) Henle G., Henle W., *J. Bacteriol.*, **91**, 1248—1256 (1966).
15) Ohigashi H., Takamura H., Koshimizu K., Tokuda H., Ito Y., *Cancer Lett.*, **30**, 143—151 (1986).