Note

Inhibitory Effects of Cembratriene-4,6-diol Derivatives on the Induction of Epstein-Barr Virus Early Antigen by 12-*O*-Tetradecanoylphorbol-13-acetate

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We have reported that a short-term *in vitro* assay system to test inhibitory effects on the induction of the early antigen (EA) of Epstein-Barr virus (EBV) in lymphoblastoid Raji cells which carried the EBV-genome is a very useful means of screening inhibitors of tumor promotion.^{1~3)} Using this assay system, we have isolated two diastereoisomers of 2,7,11-cembratriene-4,6-diol (α - and β -CBT) which showed potent inhibitory effects on the induction of EBV-EA by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a potent tumor promoter, from ciga-

rette smoke condensate.²⁾ α - and β -CBT also inhibited the induction of ornithine decarboxylase activity by TPA in mouse epidermis²⁾; this short-term *in vivo* assay system is used for detecting tumor promoters⁴⁾ and anti-tumor promoters.⁵⁾ Furthermore, these CBT inhibited the promoting effect of TPA on skin tumor formation initiated with 7,12-dimethylbenz[*a*]anthracene in mice.²⁾ These results prompted us to examine the relationship between the anti-tumor promoting activity and the structure of CBT and its derivatives. In this study, we tested the inhibitory effects of CBT derivatives on the induction of EBV-EA by TPA.

The structures of the CBT derivatives tested in this study are shown in Fig. 1. Compounds **6**,⁶⁾ **8**,⁷⁾ and **9**⁷⁾ were gifts from Dr. K. Koseki of this institute, which were obtained from the neutral fraction of an acetone extract of tobacco flowers and buds.⁸⁾ The other CBT derivatives were synthesized from α - or β -CBT. Compound **1a** was prepared by treatment of α -CBT (**1**) with an excess of acetic anhydride in pyridine (at room temperature for 3 hr) followed by work-up and HPLC. MS, IR, and ¹H-NMR data of the product were identical to those of the 6-acetate.⁹⁾ Compounds **1b**~**1e** were prepared in the same manner using propionic anhydride, butyric anhydride, varellic anhydride, or benzoic anhydride instead of acetic anhydride. The structures were established by ¹H-NMR (CDCl₃); **1b**: δ 0.78~0.85 (6H, dd), 1.13 (3H, t), 1.40 (3H, s), 1.52 (3H, s), 1.73 (3H, s), 2.30 (2H, q), 5.54 (1H, dt); **1c**: δ 0.78~0.85 (6H, dd), 0.93 (3H, t), 1.38 (3H, s), 1.52 (3H, s), 1.73 (3H, s), 5.55 (1H, dt); **1d**: δ 0.79~0.84 (6H, dd), 0.90 (3H, t), 1.38 (3H, s), 1.52 (3H, s), 1.72 (3H, s), 5.54 (1H, dt); **1e**: δ 0.80~0.86 (6H, dd), 1.43 (3H, s), 1.53 (3H, s), 1.80 (3H, s), 5.79 (1H, dt), 7.40~8.03 (5H, m).

FIG. 1. Structures of CBT and Its Derivatives.

Compounds **3**, **4**, and **10** were prepared by the methods of Roberts *et al.*¹⁰⁾ Compound **3** can also be obtained using pyridine chlorochromate (PCC) as an oxidizing agent. Compound **5** was obtained by treatment of α -CBT (1 mmol) with methanesulfonyl chloride (5 mmol) in dichloromethane and triethylamine (at room temperature for 7 hr), followed by the usual work-up and HPLC. Structures were found by MS and ¹H-NMR;* MS: 288 (M⁺), 227 (12), 133 (31), 107 (100), 81 (89), 43 (94); ¹H-NMR (CDCl₃): δ 0.81~0.86 (6H, dd), 1.31 (3H, s), 1.52 (3H, s), 4.93 (1H, s), 4.97 (1H, s), 5.05 (1H, t), 5.50 (2H, m), 5.64 (1H m), 6.14 (1H, d, *J* = 16 Hz). Compound **7** was prepared by the method of Behr *et al.*¹¹⁾ The spectral data was identical in all respects to **11**, 12-*cis*-epoxide.¹¹⁾

All other chemicals used for the EBV-EA assay were of the purest grade available from commercial sources. The lymphoblastoid Raji cells were supplied by the late Dr. Y. Ito, Kyoto University, Kyoto, and serum from a patient with nasopharyngeal carcinoma (NPC) by Dr. S. Sawaki, Yokohama City University, Yokohama.

The experimental procedure to test inhibitory effects on the induction of the EBV-EA in Raji cells was essentially the same as described previously.³⁾ Raji cells (2×10^6 cells/ml) were cultured in PRMI-1640 medium containing 4% fetal bovine serum, 4 mM *n*-butyrate and various amounts of the test compound with 25 ng/ml of TPA. After 48 hr, the EA was stained with EA (+) Viral capsid antigen(+) serum from a patient with NPC using an indirect immunofluorescence technique. In each assay, at least 500 cells were counted and the ratio of EBV-EA-positive cells was recorded. The concentrations of the compounds necessary to produce 50% inhibition of the EBV-EA induction of TPA (IC₅₀) were estimated from the response curves, and these concentrations are presented as results. Surviving cells were counted by the trypan-blue staining method.

The results are summarized in Table I. The concentration of **1** (α -CBT) required to produce 50% inhibition of the EBV-EA induction by TPA (IC₅₀) was estimated to be 25.2 μ M. Esterification of the hydroxy group at C-6 of **1** to acetoxy (**1a**), propionyloxy (**1b**), butyryloxy (**1c**), valeryloxy (**1d**), and benzoyloxy groups (**1e**) resulted in an increase in IC₅₀ compared with that of **1**. Though IC₅₀ of the four aliphatic esters (**1a**~**d**) were about the same, the cytotoxicity increased with the increase in the number of carbons in the acyl group. By oxidation of the hydroxy group at C-6 of **1** to a carbonyl group (**3**), IC₅₀ increased slightly compared with that of **1**. This effect was also observed for the oxidation product (**4**) of β -CBT (**2**); IC₅₀ of **2** and β -hydroxyketone (**4**) was 21.9 and 26.3 μ M, respectively. Compound **5**, which was formed by the dehydration at C-6 of **1**, accompanied by double bond migration, inhibited EA induction less potently than **1**. β -4,8-Diol (**6**) failed to produce 50% inhibition at concentrations of up to 20 μ g/ml (65 μ M). These results suggest

TABLE I. INHIBITORY EFFECTS OF CBT AND ITS DERIVATIVES ON EBV-EA INDUCTION BY TPA

Compound	IC ₅₀ ^a (μ M)	Survival ^c (% of control)
1 (α -CBT)	25.2	98
1a	36.5	100
1b	35.9	82
1c	38.0	82
1d	37.7	80
1e	31.7	92
2 (β -CBT)	21.9	98
3	28.6	100
4	26.3	88
5	42.4	94
6	65 ^b (40%)	96
7	47 ^b (2%)	95
8	48.2	48
9	54.1	78
10	48 ^b (2%)	104

Each value is the mean of duplicate experiments.

^a The concentrations of the compounds producing 50% inhibition of EBV-EA induction by TPA.

^b For compounds which failed to produce 50% inhibition, the maximum concentration used for the compound is indicated, and the inhibition rate at this concentration is indicated in parentheses.

^c Survival at the IC₅₀ of each compound. For **6**, **7** and **10**, survival at the concentration indicated in the table.

that the hydroxy group at C-6 of CBT (**1** and **2**) makes a considerable contribution to the inhibition of EBV-EA induction by TPA.

Compound **7**, 11, 12-epoxide of **1**, showed no inhibitory effect at concentrations of up to 15 μ g/ml (47 μ M). Compound **8**, 11-hydroperoxide containing an exocyclic double bond was cytotoxic and the inhibition of EA-induction was less potent than that of **1**. 12-Hydroperoxide of **1** (**9**) also decreased the inhibitory potency. These results suggest that the addition of a functional group at C-11 or C-12 to CBT decreases the inhibitory potency.

Compound **10**, the saturated diol of **1**, had no inhibitory effect. This result indicates that the double bond in the fourteen-membered ring is essential for the inhibition of EBV-EA induction by TPA.

Among CBT and its derivatives tested in this study, CBT showed the highest potency for inhibition of EBV-EA induction by TPA. Based on the results of this study, it would be desirable to make other modifications of CBT in seeking a more potent inhibitor of EBV-EA induction by TPA.

* Details of the structure determination will be reported separately elsewhere.

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