**Note**

1'-Acetoxychavicol Acetate as a Potent Inhibitor of Tumor Promoter-induced Epstein-Barr Virus Activation from *Languas galanga*, a Traditional Thai Condiment

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Chemical carcinogenesis is known to be induced by the initiation-promotion process. Therefore, inhibiting this promotion process could be one of the most promising ways to prevent cancer. We have previously studied the inhibitory properties toward tumor promotion (anti-tumor promoting activity) of common foods, especially of edible plants, and marine algae, and their active components by using an in vitro assay to detect the inhibition of tumor promoter-induced Epstein-Barr virus (EBV) activation.

Some of these in vitro active components have been shown to inhibit skin tumor formation by 12-O-tetradecanoylphorbol-13-acetate in 7,12-dimethylbenz[a]anthracene-initiated mice. In our continuing search for inhibitors (anti-tumor promoters) against tumor promotion, we found potent inhibition of EBV activation in a rhizome extract of *Languas galanga* (Zingiberaceae), a plant used as a ginger substitute and as a stomachic medicine in Thailand. This report describes the isolation and identification of the inhibitor, and the primary structure–activity relationship involved in the inhibition.

The inhibition of EBV activation was tested by the standard method previously reported. After partitioning a methanol extract of the fresh rhizome with *n*-hexane–methanol–water (5:9:1), the active lower layer was purified by preparative TLC and subsequent preparative HPLC through ODS (YMC AM232) to give an inhibitor (1a) as a colorless oil.

This inhibitor (1a), (x) -15.0 - 54.7° (c 1.08, EtOH), showed the following spectral data: EIMS m/z: 234 (M⁺, C₁₃H₁₄O₄); UV λ max (EtOH)nm (ε): 262 (300); IR ν max (CDCl₃) cm⁻¹: 1740, 1600, 1500; H-NMR (90 MHz, CDCl₃) δ ppm: 2.08 (3H, s), 2.28 (3H, s), 5.24 (1H, d, J = 11 Hz), 5.29 (1H, d, J = 17 Hz), 6.01 (1H, ddd, J = 17, 11, and 5 Hz), 6.28 (1H, d, J = 5 Hz), 7.07 (2H, d, J = 8 Hz), and 7.38 (2H, d, J = 8 Hz). These data are in good agreement with those of (-)-1'-acetoxychavicol acetate (ACA; Fig. 1), that has been isolated from this plant by Mitsui et al. as an anti-ulcer principle, and also by Noro et al. as a xanthine oxidase inhibitor.

Figure 2 shows the inhibition of 1a against EBV activation, which was detected by the ratio of the early antigen (EA)-inducing cells by 50 nm of the tumor promoter, telecadin B-4. ACA (1a) completely inhibited EA induction at a concentration of a 100-fold molar equivalent to telecadin B-4, the concentration for 50% inhibition (IC₅₀) being approximately 1.3 μM. This activity is 10-fold higher than that of the inhibitors, oleanolic acid, mokko lactone and gingersal, which have previously been isolated from a green perilla, an edible burdock and a ginger, respectively. No significant activity difference was observed between natural (-)-ACA and synthetic (+)-ACA.

Some compounds related to ACA (Fig. 1) were prepared to evaluate the important structural units for activity. When tested at a concentration of a 100-fold molar equivalent to telecadin B-4, none of the derivatives showed significant activity. Thus, the data primarily present the following conclusions for the structure–activity relationship; both the phenolic and benzylic acyloxy groups (acyclos of the present study) are essential for the activity, and the terminal methylene would also be significant.

ACA is an inhibitor of xanthine oxidase, which generates superoxide anions known to be associated with tumor promotion. Hence, ACA may exhibit anti-tumor promoting activity by inhibiting the generation of anions during the promoting step. Further structure–activity relationships for the inhibition of not only EBV activation but also of xanthine oxidase are being studied. The in vivo anti-tumor promoting activity is also under investigation.

**Experimental**

Inhibition test for EBV activation. The test was done as reported previously. Raji cells (5 × 10⁸/ml) were incubated with telecadin B-4 (50 nm), sodium n-butyrate (3 mm), and a specific amount of the test compound dissolved in 5 μl of DMSO in RPMI 1640 medium at 37°C under a flow of 5% CO₂ for 48 h. Early antigen (EA)-positive cells produced by viral activation were determined by an indirect immunofluorescent method.

Isolation of ACA (1a). The fresh rhizomes (10 g) of *L. galanga* collected

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*Fig. 1. Structures of ACA and Related Compounds.

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Fig. 2. Inhibitory Activity of ACA against EBV Activation by Teledocin B-4 at 50 nM.

Values are the mean ± SD of three experiments, each conducted in triplicate.

In Thailand were extracted with MeOH. The extract (710 mg) was partitioned with 200 ml of n-hexane-MeOH-water (5:9:1). A part (41 mg) of the active lower layer (590 mg) was separated by preparative TLC (silica gel), developing with toluene-acetone (3:1), to give an active fraction (25 mg), which was then purified by preparative HPLC (YMC AM322) with CH₃CN-water (35:65) to afford ACA (1a; 11 mg).

Syntheses of (±)-ACA and related compounds. (±)-1′-Hydroxychavicol (1; 500 mg) was synthesized from p-hydroxybenzaldehyde (1.5 g) and vinylmagnesium bromide (3.3 g) by the method reported by Mitsu et al.⁹ 1: EIMS m/z: 150 (M⁺); UV λmax (EtOH) nm (ε): 272 (1500); IR νmax (CH₃Cl) cm⁻¹: 3550, 1620, 1520; ¹H-NMR (90 MHz, CDCl₃ + d₅-acetone) δ ppm: 4.8-5.2 (1H + 1H, m), 5.15 (1H, d, J = 17 Hz), 5.6-6.1 (1H, m), 6.65 (2H, d, J = 8 Hz), 7.04 (2H, d, J = 8 Hz). (±)-2,3′-Dihydro-ACA (H₂-ACA, 2a; 4.2 mg) was obtained by catalytic hydrogenation of (±)-ACA (24 mg) over Pt. 2a: EIMS m/z: 236 (M⁺); UV λmax (EtOH) nm (ε): 261 (300); IR νmax (CH₃Cl) cm⁻¹: 1750, 1600, 1500; ¹H-NMR (400 MHz, CDCl₃) δ ppm: 0.88 (3H, t, J = 7.3 Hz), 1.84 (2H, m), 2.07 (3H, s), 2.29 (3H, s), 5.66 (1H, t, J = 6.9 Hz), 7.06 (2H, d, J = 8.7 Hz), 7.33 (2H, d, J = 8.7 Hz). Chavicol (3; 1.9 g) was obtained from estragol (6.0 g) with methyl iodide (7.2 g) and magnesium (1.3 g) as previously reported.¹³ 3: EIMS m/z: 134 (M⁺); UV λmax (EtOH) nm (ε): 278 (2600); IR νmax (CH₃Cl) cm⁻¹: 3550, 1640, 1510; ¹H-NMR (90 MHz, CDCl₃) δ ppm: 3.35 (2H, br. d, J = 5 Hz), 4.9-5.3 (1H + 1H, m), 6.3-5.8 (1H, m), 6.82 (2H, d, J = 8 Hz), 7.11 (2H, d, J = 8 Hz). The synthesis of 1-phenyl-2-propene-1-ol (5; 1.5 g) from benzaldehyde (1.3 g) and vinylmagnesium bromide (3.3 g) was done by the same procedure as that for 1 from p-hydroxybenzaldehyde. 5: EIMS m/z: 134 (M⁺); UV λmax (EtOH) nm (ε): multiple maxima from 240-270 centered at 258 (200); IR νmax (CH₃Cl) cm⁻¹: 3550, 1600, 1450; ¹H-NMR (90 MHz, CDCl₃) δ ppm: 5.0-5.2 (1H + 1H, m), 5.20 (1H, br. d, J = 17 Hz), 5.95 (1H, ddd, J = 17, 10, 6 Hz), 7.20 (5H, br. s).

p-Propylphenol acetate (4a) was obtained from commercially available p-propylphenol (4) by the usual treatment with pyridine-acetic anhydride. Other acetylated and methylated derivatives of 1, 3, and 5 were obtained by the usual reactions with pyridine-acetic anhydride (for 1a, 1b, 3a, and 5a) and diazomethane (for 1d and 3b) or methyl iodide–Ag₂O (for 1e and 5b), and their structures were confirmed by EIMS, UV, IR, and ¹H-NMR.

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