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Anti-Inflammatory and Chemopreventive Effects of Triterpene Cinnamates and Acetates from Shea Fat

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Abstract: Four triterpene acetates, α-amyrin acetate (1a), β-amyrin acetate (2a), lupeol acetate (3a), and butyrospermol acetate (4a), and four triterpene cinnamates, α-amyrin cinnamate (1c), β-amyrin cinnamate (2c), lupeol cinnamate (3c), and butyrospermol cinnamate (4c), were isolated from the kernel fat (*n*-hexane extract) of the shea tree (*Vitellaria paradoxa*; Sapotaceae). Upon evaluation of these eight triterpene esters for inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation (1 μg/ear) in mice, all of the compounds tested exhibited marked anti-inflammatory activity, with ID_{50} values in the range of 0.15–0.75 μmol/ear, and among which compound 3c showed the highest activity with ID_{50} of 0.15 μmol/ear. Compound 3c (10 mg/kg) further exhibited anti-inflammatory activity on rat hind paw edema induced by carrageenan, with the percentage of inflammation at 1, 3, and 5 h of 35.4, 41.5, and 45.5%, respectively. The eight triterpene esters were then evaluated for their inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) in Raji cells as a primary screening test for inhibitors of tumor promoters. All the compounds showed moderate inhibitory effects. Furthermore, compound 3c exhibited inhibitory effect on skin tumor promotion in an *in vivo* two-stage carcinogenesis test using 7,12-dimethylbenz [*a*] anthracene (DMBA) as an initiator and TPA as a promoter. The biological activities of triterpene acetate and cinnamate esters, together with the exceptionally high levels of these triterpenes in shea fat, indicate that shea nuts and shea fat (shea butter) constitute a significant source of anti-inflammatory and anti-tumor promoting compounds.

Key words: *Vitellaria paradoxa*, shea fat, triterpene ester, anti-inflammatory activity, chemopreventive effect

1 INTRODUCTION

The shea tree[*Vitellaria paradoxa* C. F. Gaertn.; synonyms *Butyrospermum paradoxum*(C. F. Gaertn.)Hepper, *Butyrospermum parkii*(G. Don)Kotschy; family Sapotaceae]is indigenous to the savanna belt extending across sub-Saharan Africa north of the equator, ranging from Mali in the west to Ethiopia and Uganda in the east (extending from 16° W to 34° E longitude and 1° N to 15° N latitude)[1-4]. The most valued product of shea tree is shea fat(shea butter)extracted from the kernels. Processed shea fat is used primarily as a cocoa butter additive in chocolate manufacture, although it is increasingly popular in skin care products and cosmetic product formulations in part due to the unusually high level of non-glyceride constituents in the fat[5]. The main non-glyceride constituents of shea fat have been reported to be triterpene alcohols such as α-amyrin(**1**), β-amyrin(**2**), lupeol(**3**), and butyrospermol(**4**)[6-9], most of which occur as the acetic acid and cinnamic acid esters[4, 5, 8](Fig. 1). We have recently determined the contents and composition of triterpene alcohol fractions of the non-saponifinable lipids(NSL)along with fatty acid composition of the kernel fats(*n*-hexane extracts)of the shea tree for 36 nut samples from seven sub-Saharan countries[10]. Since naturally occurring triterpene alcohols and their derivatives exhibit a variety of biological activities including anti-inflammatory, antitumor, chemopreventive, and antimycobacterial activities[11-15], we were especially interested to undertake investigation of the trit-

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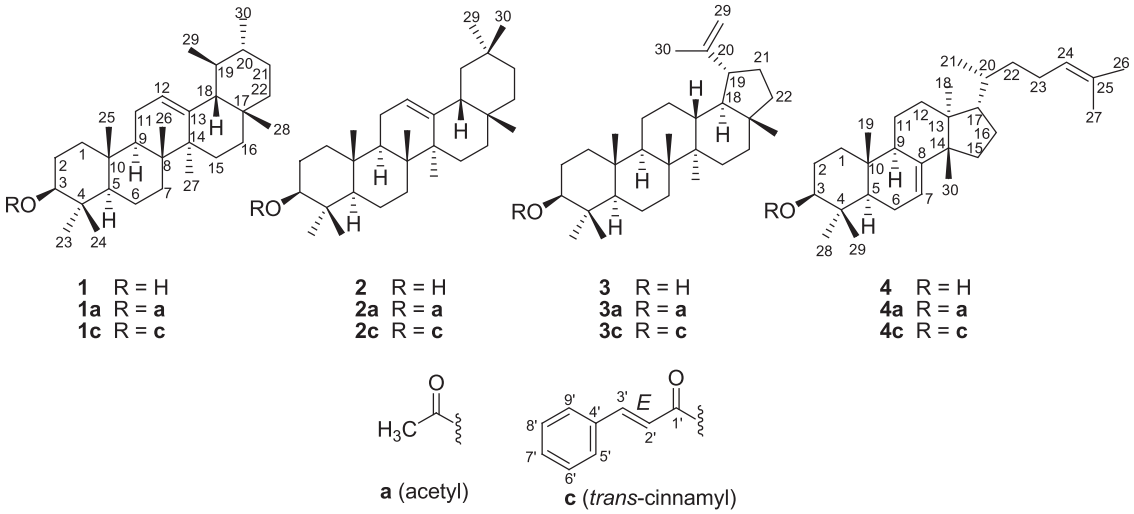


Fig. 1 Structures of Compounds **1-4**, **1a-4a**, and **1c-4c**.

erpene ester constituents of shea fat in order to evaluate their further pharmacological potential. We now report, in this paper, the isolation of four triterpene acetates (**1a-4a**) and four triterpene cinnamates (**1c-4c**) from shea fat and the evaluation of their inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced inflammation in mice, and on the Epstein-Barr virus early antigen (EBV-EA) activation induced by TPA. In addition, we report the inhibitory effects of compound **3c** on the anti-inflammatory activity on rat hind paw edema induced by carrageenan, and on an *in vivo* two-stage mouse skin carcinogenesis.

2 EXPERIMENTAL

2.1 General experimental procedures

Crystallizations were performed in MeOH, and melting points were determined on a Yanagimoto melting point apparatus and are uncorrected (values shown in section **2.3**). NMR spectra were recorded with a JEOL ECX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. High-resolution (HR)-AP-CIMS were recorded with a positive-ion mode on an Agilent 1100 LC/MSD TOF system. Preparative-TLC plates (20 × 20 cm) coated with a 0.5 mm layer of Silica gel G60 (Merck & Co., Inc.) were developed with *n*-hexane-EtOAc (85:15). Reversed-phase preparative HPLC was carried out on ODS columns (25 cm × 10 mm i.d.), on a Pegasil ODS-II 5 μm column (Senshu Scientific Co., Ltd., Tokyo) with MeOH-AcOH (100:0.1; flow rate: 2.5 mL/min) (HPLC system I) or on a TSK ODS-120T column (Toso Co., Ltd., Tokyo) with acetonitrile-acetone (80:20; flow rate: 2.5 mL/min) as the eluting solvent (HPLC system II).

2.2 Materials and chemicals

The shea nut sample selected for detailed analysis and experimentation in this study was collected by one of the authors (E.T.M.) during the 2006 shea season (May through July) from a healthy mature tree at a site (longitude E 7° 27′ 9″, latitude N 9° 40′ 53″, elevation 365 m) in central Nigeria[10]. α-Amyrin acetate (**1a**), β-amyrin acetate (**2a**), lupeol acetate (**3a**), and butyrospermol acetate (**4a**) were used as the reference compounds[6]. Chemicals and reagents were purchased as follows: TPA from ChemSyn Laboratories (Lenexa, KS, U.S.A.), indomethacin, prednisolone acetate, carrageenan (λ type IV), and (*all-trans*)-retinoic acid from Sigma Chemical Co. (St. Louis, MO), Tween® 80 from Strichand United Dispensary (Thailand), and the EBV cell culture reagents and *n*-butanoic acid from Nacalai Tesque, Inc. (Kyoto, Japan).

2.3 Isolation and identification of triterpene esters

Whole nuts were oven-dried (17.7 g) at 60℃ over 72 h and decorticated. Kernels were crushed in a mortar and finely ground in a coffee mill. The pulverized sample (9.91 g) was extracted with *n*-hexane under reflux for 3 h three times. The *n*-hexane extract (4.56 g) was fractionated on preparative-TLC giving five fractions, A-E: fractions-A (616 mg; R_f value 0.81), B (3212 mg; 0.67), C (86 mg; 0.37), D (60 mg; 0.23), and E (97 mg; 0.16), of which fractions-A and B were estimated to consist of triterpene esters and triacylglycerols, respectively. Upon additional preparative-TLC, fraction-A afforded a refined fraction (75 mg), which was separated further using preparative reversed-phase HPLC (HPLC system I), yielding seven additional fractions: fractions-A1 [2.9 mg; retention time (t_R) 28.2 min; lupeol acetate (**3a**)], A2 [4.0 mg; t_R 30.8 min; butyrospermol acetate (**4a**)], A3 [2.0 mg; t_R 35.1 min; β-amyrin acetate (**2a**)], A4 (6.8 mg; t_R 38.1 min), A5 (13.8 mg; t_R 47.1 min), A6 [1.9 mg; t_R 52.9 min; β-amyrin cinnamate (**2c**)], and A7 (11.1 mg; t_R 58.4 min). Fractions-A4, A5, and A7 were subjected to further reversed-phase HPLC (HPLC system II) which

yielded α-amyrin acetate (**1a**) (3.0 mg; t_R 53.9 min), butyrospermol cinnamate (**4c**) (2.8 mg; t_R 27.9 min) and lupeol cinnamate (**3c**) (7.5 mg; t_R 31.9 min), and α-amyrin cinnamate (**1c**) (7.6 mg; t_R 58.1 min), respectively (Fig. 1).

Identification of four triterpene acetates, **1a**-**4a**, was performed by comparison of their ^{1}H NMR and MS data with reference compounds. On the other hand, identification of triterpene cinnamates was undertaken by comparison of ^{13}C and ^{1}H NMR data with those of corresponding or relevant compounds[16,17]. Identification of these cinnamates was supported by the analysis of DEPT, ^{1}H-^{1}H COSY, HMQC, and HMBC spectra, and fully assigned ^{13}C and ^{1}H NMR data for **1c**-**4c** are shown below.

2.3.1 α-Amyrin cinnamate (**1c**)

Compound **1c** gave fine needles upon crystallization, mp 230-233 ℃ (MeOH); ^{13}C and ^{1}H NMR: C-1 [δ_C 38.5; δ_H 1.13, 1.67], C-2 [23.6; 1.70 (2H)], C-3 [81.0; 4.65 (dd, J = 6.0, 10.1 Hz)], C-4 [37.9], C-5 [55.2; 0.89 (br d, J = 6.0 Hz)], C-6 [18.2; 1.38, 1.53], C-7 [32.9; 1.33, 1.55], C-8 [40.0], C-9 [47.6; 1.57], C-10 [36.8], C-11 [23.4; 1.92 (2H)], C-12 [124.3; 5.13 (t, J = 3.8 Hz)], C-13 [139.6], C-14 [42.0], C-15 [26.7; 0.98, 1.82], C-16 [28.2; 1.44; 2.00], C-17 [33.7], C-18 [59.0; 1.32], C-19 [39.6; 0.91], C-20 [39.7; 1.31], C-21 [31.2; 1.25, 1.39], C-22 [41.5; 1.27, 1.42], C-23 [28.1; 0.92 (s)], C-24 [16.9; 0.95 (s)], C-25 [15.7; 1.01 (s)], C-26 [16.9; 1.03 (s)], C-27 [23.2; 1.08 (s)], C-28 [28.8; 0.80 (s)], C-29 [17.5; 0.80 (d, J = 5.9 Hz)], C-30 [21.4; 0.92 (s)], C-1′ [166.8], C-2′ [144.2; 7.67 (d, J = 16.0 Hz)], C-3′ [118.8; 6.44 (d, J = 16.0 Hz)], C-4′ [134.5], C-5′ , 9′ [128.0; 7.53], C-6′ ,8′ [128.8; 7.38], C-7′ [130.1; 7.38]; HR-APCIMS *m/z* 409.3811 (calculated for $C_{30}H_{49}$ [MH-$C_9H_8O_2$ (cinnamic acid)]$^+$, 409.3834).

2.3.2 β-Amyrin cinnamate (**2c**)

Compound **2c** gave fine needles upon crystallization, mp 156-160℃ (MeOH); ^{13}C and ^{1}H NMR: C-1 [δ_C 38.2; δ_H 1.08, 1.65], C-2 [23.6; 1.69 (2H)], C-3 [80.1; 4.63 (t, J = 8.0 Hz)], C-4 [37.9], C-5 [55.2; 0.87], C-6 [18.2; 1.40, 1.55], C-7 [32.6; 1.32, 1.54], C-8 [39.8], C-9 [47.5; 1.57], C-10 [38.8], C-11 [23.5; 1.88 (2H)], C-12 [121.6; 5.17 (t, J = 3.7 Hz)], C-13 [145.2], C-14 [41.7], C-15 [26.1; 0.94, 1.73], C-16 [26.9; 1.43; 1.97], C-17 [32.5], C-18 [47.2; 1.93], C-19 [46.7; 1.01, 1.65], C-20 [31.1], C-21 [34.7; 1.08, 1.34], C-22 [37.1; 1.20, 1.41], C-23 [28.1; 0.90 (s)], C-24 [16.9; 0.93 (s)], C-25 [15.7; 0.97 (s)], C-26 [16.8; 0.96 (s)], C-27 [26.0; 1.13 (s)], C-28 [28.4; 0.82 (s)], C-29 [33.3; 0.86 (s)], C-30 [23.7; 0.85 (s)], C-1′ [166.3], C-2′ [144.3; 7.65 (d, J = 16.0 Hz)], C-3′ [118.8; 6.43 (d, J = 16.0 Hz)], C-4′ [134.5], C-5′ , 9′ [128.0; 7.52], C-6′ , 8′ [128.8; 7.37], C-7′ [130.1; 7.37]; HR-APCIMS *m/z* 409.3811 (calculated for $C_{30}H_{49}$ [MH-$C_9H_8O_2$ (cinnamic acid)]$^+$, 409.3834).

2.3.3 Lupeol cinnamate (**3c**)

Compound **3c** gave fine needles upon crystallization, mp 238-241 ℃ (MeOH); ^{13}C and ^{1}H NMR: C-1 [δ_C 38.4; δ_H 1.02, 1.68], C-2 [23.8; 1.69 (2H)], C-3 [81.3; 4.61 (dd, J = 6.4, 11.8 Hz)], C-4 [38.0], C-5 [55.4; 0.84], C-6 [18.2; 1.39, 1.53], C-7 [34.2; 1.41 (2H)], C-8 [40.8], C-9 [50.3; 1.32], C-10 [37.1], C-11 [20.9; 1.21, 1.41], C-12 [25.1; 1.07, 1.66], C-13 [38.0; 1.64], C-14 [42.8], C-15 [27.4; 1.09, 1.67], C-16 [35.6; 1.33, 1.47], C-17 [43.0], C-18 [48.3; 1.35], C-19 [48.0; 2.38 (dt, J = 5.8, 11.0 Hz)], C-20 [151.0], C-21 [29.8; 1.34, 1.91], C-22 [40.0; 1.19, 1.38], C-23 [28.0; 0.89 (s)], C-24 [16.7; 0.92 (s)], C-25 [16.2; 0.88 (s)], C-26 [16.0; 1.04 (s)], C-27 [14.5; 0.94 (s)], C-28 [18.0; 0.79 (s)], C-29 [109.3; 4.58 (dd, J = 1.4, 2.3 Hz), 4.69 (d, J = 2.3 Hz)], C-30 [19.3; 1.69 (s)], C-1′ [166.8], C-2′ [144.2; 7.66 (d, J = 16.0 Hz)], C-3′ [118.8; 6.44 (d, J = 16.0 Hz)], C-4′ [134.5], C-5′ , 9′ [128.0; 7.52], C-6′ , 8′ [128.8; 7.37], C-7′ [130.1; 7.37]; HR-APCIMS *m/z* *m/z* 409.3811 (calculated for $C_{30}H_{49}$ [MH-$C_9H_8O_2$ (cinnamic acid)]$^+$, 409.3834).

2.3.4 Butyrospermol cinnamate (**4c**)

Compound **4c** gave fine needles upon crystallization, mp 105-109 ℃ (MeOH); ^{13}C and ^{1}H NMR: C-1 [δ_C 36.9; δ_H 1.25, 1.69], C-2 [24.4; 1.67 (2H)], C-3 [81.3; 4.67 (dd, J = 4.2, 10.5 Hz)], C-4 [38.2], C-5 [50.9; 1.44], C-6 [23.8; 1.95, 2.16], C-7 [117.6; 5.26 (dd, J = 3.0, 6.6 Hz)], C-8 [146.1], C-9 [48.8; 2.22], C-10 [34.9], C-11 [18.7; 1.49 (2H)], C-12 [33.8; 1.65, 1.80], C-13 [43.6], C-14 [51.8], C-15 [34.0; 1.44 (2H)], C-16 [28.5; 1.27, 1.93], C-17 [53.3; 1.47], C-18 [22.2; 0.79 (s)], C-19 [13.3; 0.77 (s)], C-20 [35.9; 1.41], C-21 [18.8; 0.83 (d, J = 6.4 Hz)], C-22 [35.2; 0.95, 1.06], C-23 [25.4; 1.91, 2.08], C-24 [125.2; 5.10 (tt, J = 1.1, 6.9 Hz)], C-25 [131.1], C-26 [25.9; 1.66 (s)], C-27 [17.8; 1.58 (s)], C-28 [27.7; 0.88 (s)], C-29 [16.1; 1.00 (s)], C-30 [27.4; 0.93 (s)], C-1′ [166.9], C-2′ [144.4; 7.65 (d, J = 16.0 Hz)], C-3′ [118.9; 6.43 (d, J = 16.0 Hz)], C-4′ [134.6], C-5′ , 9′ [128.1; 7.51], C-6′ , 8′ [128.9; 7.36], C-7′ [130.1; 7.36]; HR-APCIMS *m/z* *m/z* 409.3839 (calculated for $C_{30}H_{49}$ [MH-$C_9H_8O_2$ (cinnamic acid)]$^+$, 409.3834).

2.4 Assay of TPA-induced inflammation ear edema in mice

Six-weeks-old specific pathogen-free female ICR mice were obtained from Japan SLC (Shizuoka, Japan). The animals were housed, five per polycarbonate cage, in an air-conditioned specific pathogen-free room at 24 ± 2℃. Food and water were available *ad libitum*.

TPA (1 μg, 1.7 nmol) dissolved in acetone (20 μL) was applied to the right ear only of ICR mice by means of a micropipette. A volume of 10 μL was delivered to both the inner and outer surfaces of the ear. The triterpene alcohol ester samples were dissolved in $CHCl_3$-MeOH (1:1) and were applied topically (20 μL) about 30 min before TPA treatment. Control treatments consisted of the carrier only ($CHCl_3$-MeOH), applied in the same manner. For ear thickness determinations, a pocket thickness gauge with a range of 0-9 mm, graduated at 0.01 mm intervals and modified so that the contact surface area was increased to reduce the tension, was applied to the tip of the ear. The ear thickness

was measured before treatment (a) and 6 h after TPA treatment (b = TPA alone; b' = TPA plus sample). The following values were then calculated:

Edema A as induced by TPA alone (b-a)

Edema B as induced by TPA plus sample (b' -a)

Inhibitory ratio (%) = [(Edema A-Edema B)/Edema A] $\times$100

Each value was the mean of individual determinations from five mice. The 50% inhibitory dose (ID_{50}) values and their 95% confidence intervals (CI 95%)[18] were obtained by nonlinear regression using the GraphPad program 5.0 (Intuitive Software for Science, San Diego, CA, U.S.A.).

2.5 Assay of carrageenan-induced rat hind paw edema

Stock 0.5% (w/v) lupeol cinnamate (**3c**) suspension was prepared by dispersing **3c** in 1% Tween® 80 in distilled water. Prednisolone acetate at 2.0 mg/kg was used as a positive control. Six-to-seven-weeks-old male Sprague rats (200-250 g body weight) obtained from National Laboratory Animal Centre, Mahidol University (Bangkok, Thailand) were divided into 5 groups (n = 3-7). In group 1, rats were fed with 0.5 mL of 1% Tween® 80 in distilled water as the negative control. In groups 2-5, rats were fed with test sample (**3c**) suspensions at 5, 10, 20, and 30 mg/kg bw. After 1 h oral administration of the above samples to the animals, acute carrageenan paw edema was induced by subcutaneous injection of 0.05 mL of 1% (w/v) carrageenan solution in the right hind paw. The paw volume was measured using a plethysmometer (Ugo Basile-7150, U.S.A.) before injection (a) and 1, 3, and 5 h after carrageenan injection (b = carrageenan alone; b' = carrageenan plus sample). The following values were then calculated:

% Edema A as induced by carrageenan alone (b-a)/a

% Edema B as induced by carrageenan plus sample (b' -a)/a

Inhibitory ratio (%) =

[(% Edema A - % Edema B)/% Edema A] $\times$ 100

2.6 *In vitro* EBV-EA activation experiment

The EBV genome-carrying lymphoblastoid Raji cells, derived from Burkitt's lymphoma, were cultured in 10% fetal bovine serum RPMI-1640 medium. The Raji cells were incubated for 48 h at 37℃ in a medium containing 4 mmol n-butanoic acid, 32 pmol TPA (20 ng/mL in DMSO), and various amounts of each test compound. Smears were made from the cell suspension, and the EBV-EA-inducing cells were stained by means of an indirect immunofluorescence technique. Details of this *in vitro* assay on EBV-EA induction have been reported previously[19].

2.7 *In vivo* two-stage carcinogenesis assay on mouse-skin papillomas

Each group was composed of 15 mice, housed five per cage, and given water *ad libitum*. The back of each mouse was shaved with surgical clippers, and the mice were topically treated with DMBA (100 μg, 390 nmol) in acetone (0.1 mL) for the initiation treatment. One week after initiation, papillomas formation was promoted twice a week by application of TPA (1 μg, 1.7 nmol) in acetone (0.1 mL) on the skin. The control group received the TPA treatment alone, and the test group received a topical application of test sample (85 nmol) in acetone (0.1 mL) 1 h before each TPA treatment. The incidence and numbers of papillomas were observed and detected weekly over 20 weeks; only typical papillomas larger than ca. 1 mm in diameter were counted.

3 RESULTS AND DISCUSSION

Four triterpene acetates (**1a**-**4a**) and four triterpene cinnamates (**1c**-**4c**) were isolated from the shea fat (n-hexane extract of shea kernel). These were evaluated with respect to their anti-inflammatory activity against TPA-induced inflammation in mice, and the inhibitory effects were compared with those of a commercially available anti-inflammatory drug, indomethacin, as shown in **Table 1**. All of the triterpene esters tested markedly inhibited the TPA-induced inflammation [ID_{50} (50% inhibitory dose) 0.15-0.75 μmol/ear], which is more inhibitory than indomethacin (ID_{50} 0.91 μmol/ear; CI 95% 0.76-1.09 μmol/ear). The cinnamates (**1c**-**4c**) exhibited stronger activity (ID_{50} 0.15-0.35 μmol/ear) than those of the corresponding acetates (**1a**-**4a**) (0.54-0.75 μmol/ear). Among the cinnamates, lupeol cinnamate (**3c**) exhibited the strongest inhibitory activity (ID_{50} 0.15 μmol/ear; CI 95% 0.12-0.18 μmol/ear).

Our results suggest that topical application of triterpene esters exert a strong and rapid onset inhibition of TPA-induced inflammation. These effects seem to be associated with the suppression of skin prostaglandin E_2 (PGE_2) levels by mechanisms involving the suppression of cyclooxygenase (COX)-2 expression, via inhibition of upstream protein kinases, namely, extracellular signal-regulated protein kinase (ERK), p38 mitogen-activated protein kinase (MAPK), and protein kinase C (PKC) α, and blocking nuclear factor-κB (NF-κB) activation[20].

Compound **3c** was then evaluated for its anti-inflammatory activity at 5.0, 10.0, 20.0, and 30.0 mg/kg oral administration dose levels by rat hind paw edema induced by the injection of 0.05 mL of 1% (w/v) carrageenan. The results are shown in **Table 2**. Compound **3c** inhibited the carrageenan-induced inflammation especially at 10 mg/kg with the highest activity at 3 and 5 h, with about 41.5 and 45.5% inhibition, respectively[21]. These values were lower than prednisolone acetate (2.0 mg/kg) which gave 74.3 and 80.6% inhibition, respectively, at the same time intervals. At the higher doses, **3c** exhibited lower activity which may be due to its low solubility in water thereby affecting the gastric absorption and bioavailability of **3c**. Compound **3c**

may precipitate in the gastric epithelial region and obstruct its absorption in solution.

Paw edema caused by subcutaneous injection of carrageenan is due to vasodilation and increased vascular permeability. This event is caused by the release of various inflammatory mediators such as PGs and thromboxane (TX)[22]. Compound **3c** markedly inhibited paw edema formation induced by carrageenan 1 h after **3c** administration. It is suggested that **3c** probably exerted anti-inflammatory activity through the inhibition of those inflammatory mediators of the acute inflammation. Inhibition of the synthesis or release of inflammatory mediators may be the main mechanisms of action of **3c**.

Consistent with our observations, a cinnamate fraction containing α-amyrin cinnamate (**1c**) and lupeol cinnamate (**3c**) obtained from a *n*-hexane extract of *Himatanthus sucuuba* (Apocynaceae) stem bark has been reported to possess anti-inflammatory activity in the carrageenan-induced rat paw edema[23].

The inhibitory effects on EBV-EA activation induced by TPA were examined as a preliminary evaluation of the potential antitumor-promoting activities[12,13] for compounds **1a**-**4a** and **1c**-**4c**. The results are shown in Table 1, together with comparable data for retinoic acid, one of the reti-

Table 1 Inhibitory Effects of Esterified Triterpene Alcohols from Shea Nut Extracts and Reference Compounds on TPA-induced Inflammation in Mice and on the Induction of Epstein-Barr Virus Early Antigen

Compound	Inhibition of inflamation		Percentage of EBV-EA induction[c] (% viability)				IC_{50}[d]
	ID_{50}[a]	95% CI[b]	Concentration (mol ratio/TPA)				(mol ratio/32 pmol TPA)
	(μmol/ear)		1000	500	100	10	
α-Amyrin acetate (**1a**)	0.61	0.54–0.68	3.3(70)	43.3	78.4	100	401
β-Amyrin acetate (**2a**)	0.75	0.67–0.85	3.9(70)	44.6	79.6	100	405
Lupeol acetate (**3a**)	0.54	0.47–0.62	2.1(70)	42.6	75.3	98.4	383
Butyrospermol acetate (**4a**)	0.71	0.60–0.84	2.0(70)	41.3	74.1	97.2	380
α-Amyrin cinnamate (**1c**)	0.35	0.31–0.40	2.2(70)	45.7	77.2	96.3	470
β-Amyrin cinnamate (**2c**)	0.27	0.23–0.33	3.1(70)	47.1	78.2	97.0	452
Lupeol cinnamate (**3c**)	0.15	0.12–0.18	0(70)	43.0	74.3	92.1	379
Butyrospermol cinnamate (**4c**)	0.21	0.16–0.26	0(70)	42.6	73.1	91.7	373
Indomethacin[e]	0.91	0.76–1.09					
Retinoic acid[e]			15.3(60)	49.3	76.3	100	482

a) 50% Inhibitory dose.
b) 95% confidence intervals.
c) Values represent percentage relative to the positive control value. TPA (32 pmol, 20 ng) =100%. Values in parentheses are viability percentages of Raji cells.
d) IC_{50} represents the molar ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol of TPA.
e) Reference compound.

Table 2 Anti-inflammatory Activity of Hind Paw Edema in Male Rats by Lupeol Cinnamate (**3c**) and Prednisolone Acetate, a Reference Compound

Sample	Group no.	Dose (mg/kg)	*n*	Mean % edema inhibition[a]		
				1 h	3 h	5 h
Negative control[b]	1			0.0	0.0	0.0
Lupeol cinnamate (**3c**)	2	5.0	3	21.8 ± 16.21	26.5 ± 16.40	20.8 ± 6.03
	3	10.0	7	35.4 ± 10.52	41.5 ± 15.13	45.5 ± 13.72
	4	20.0	5	39.5 ± 37.62	29.8 ± 11.90	37.0 ± 15.01
	5	30.0	3	35.6 ± 14.08	33.0 ± 12.48	31.8 ± 15.35
Prednisolone acetate[c]	6	2.0	3	44.1 ± 45.76	74.3 ± 13.52	80.6 ± 11.46

a) Mean ± SEM.
b) 1% v/v Tween® 80 in distilled water.
c) Reference compound.

noids that has been studied as a cancer chemoprevention strategy for various organ site cancers[24]. All of the compounds tested showed inhibitory effects with IC_{50} values (concentration of 50% inhibition with respect to positive control) of 373-470 mol ratio/32 pmol TPA, while preserving the high viability of Raji cells. These values are equivalent to or more potent than retinoic acid (IC_{50} 482). Among the eight compounds tested, both acetates and cinnamates of butyrospermol (**3**) and lupeol (**4**) showed potent inhibitory effects (IC_{50} 373-383). Since the inhibitory effects against EBV-EA induction have been demonstrated to correlate with inhibition of tumor promotion *in vivo*[19], these compounds may have potential as antitumor agents.

Subsequently, we determined the inhibitory effects of compound **3c** in a two-stage carcinogenesis test on mouse skin using 7,12-dimethylbenz[*a*]anthracene (DMBA) as an initiator and TPA as a promoter. The incidence (%) of papilloma-bearing mice and the average numbers of papillomas per mouse are presented in Figs. 2A and 2B, respectively. The incidence of papillomas in group I (untreated) was 100% in mice after 11 weeks of promotion. Further, more than four and eight papillomas were formed per mouse at 11 and 20 weeks of promotion, respectively. In contrast, the formation of papillomas in mouse skin was delayed and the mean number of papillomas per mouse was reduced by treatment with **3c**. Thus, in group II (treated with **3c**), the percentage ratios of papilloma-bearing mice were only 33% at 11 weeks, and 93% at 20 weeks, and the mean papillomas per mouse were 1.9 at 11 weeks, and 4.1 at 20 weeks.

Several studies reported that skin applications of TPA result in several histological and biochemical alterations including inflammatory responses such as development of edema, hyperplasia and induction of COX-2 expression[25], and generation of reactive oxygen species, which play a critical role in oxidation of many macromolecules and help in initiation as well as promotion of tumorigenesis[26]. TPA induces the enzyme ornithine decarboxylase (ODC) activity, which is a biomarker of skin tumorigenesis[27]. Administration of TPA results in stimulation of Ras signaling pathway along with activation of a number of kinases subsequently activates the cell proliferation pathway and alters the expression of anti-apoptotic Bcl-2 and pro-apoptotic Bax protein[28].

From the results of the *in vivo* anti-inflammatory tests, *in vitro* EBV-EA induction test, and *in vivo* two-stage carcinogenesis, it appears that the triterpene cinnamates and triterpene acetates isolated from shea fat, especially lupeol cinnamate (**3c**), could be valuable as anti-inflammatory agents and chemopreventive agents in chemical carcinogenesis. Although these compounds can be found in other plants, shea kernels are a particularly attractive source due to exceptionally high levels of triterpene alcohols (up to 6.2% as the NSL constituents of fat)[10] and the availability of shea nuts and shea kernel fat (shea butter) as export commodities. The results of this study will be of value for further utilization of shea fat in product applications in the cosmetic and pharmaceutical fields in the future.

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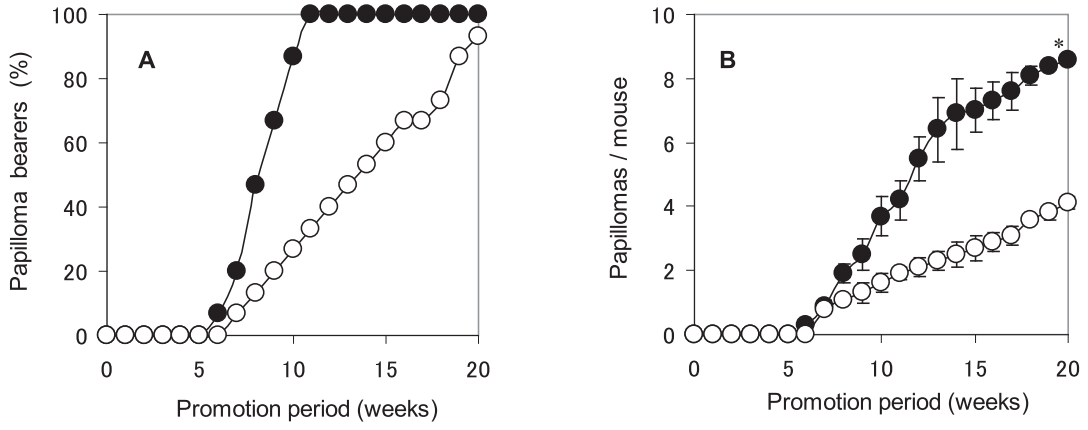


Fig. 2 Inhibition of TPA-induced Tumor Promotion by Multiple Applications of Lupeol Cinnamate (**3c**; ○). Mice (n = 15 for each of groups I and II) were initiated with DMBA (390 nmol) and promoted with TPA (1.7 nmol) given twice weekly starting 1 week after initiation. (A) Percentage of mice with papillomas. (B) Average number of papillomas per mouse. ●, control TPA alone (group I); ○, TPA + 85 nmol of **3c** (group II). After 20 weeks of promotion, a significant difference in the number of papillomas per mouse between the groups treated with compound **3c** and the control group was evident ($p < 0.05$, using the Student's *t*-test).

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References and Notes

1. Masters, E.T.; Yidana, J.A.; Lovett, P.N. Reinforcing sound management through trade: Shea tree products in Africa. *Unasylva No. 219*, **55**, 46-52 (2004).
2. Maranz, S.; Wiesman, Z.; Bisgaard, J.; Bianchi, G. Germplasm resources of *Vitellaria paradoxa* based on variations in fat composition across the species distribution range. *Agrofor. Sys.* **60**, 61-69 (2004).
3. Maranz, S.; Kpikpi, W.; Wiesman, Z.; de Sait Sauveur, A.; Chapagain, B. Nutritional values and indigenous preferences for shea fruits (*Vitellaria paradoxa* Gaertn. F.) in African agroforestry parklands. *Econ. Bot.* **58**, 588-600 (2004).
4. di Vincenzo, D.; Maranz, S.; Serraiocco, A.; Vito, R.; Wiesman, Z.; Bianchi, G. Regional variation in shea butter lipid and triterpene composition in four African countries. *J. Agric. Food Chem.* **53**, 7473-7479 (2005).
5. Alander, J. Shea butter - a multifunctional ingredient for food and cosmetics. *Lipid Technol.* **16**, 202-205 (2004).
6. Itoh, T.; Tamura, T.; Matsumoto, T. Sterols, methylsterols, and triterpene alcohols in three Theaceae and some other vegetable oils. *Lipids* **9**, 173-184 (1974).
7. Itoh, T.; Tamura, T.; Matsumoto, T. 24-Methylenedammarenol: a new triterpene alcohol from shea butter. *Lipids* **10**, 808-813 (1975).
8. Peers, K.E. The non-glyceride saponifiables of shea butter. *J. Sci. Fd. Agric.* **28**, 1000-1009 (1977).
9. Itoh, T.; Uetsuki, T.; Tamura, T.; Matsumoto, T. Characterization of tritierpene alcohols of seed oils from some species of Theaceae, Phytolaccaceae and Sapotaceae. *Lipids* **15**, 407-411 (1980).
10. Akihisa, T.; Kojima, N.; Katoh, N.; Ichimura, Y.; Suzuki, H.; Fukatsu, M.; Maranz, S.; Masters, E. T. Triterpene alcohol and fatty acid composition of shea nuts from seven African countries. *J. Oleo Sci.* **59** (7), (2010).
11. Akihisa, T.; Yasukawa, K.; Kimura, Y.; Takase, S.; Yamanouchi, S.; Tamura, T. Triterpene alcohols from camellia and sasanqua oils and their anti-inflammatory effects. *Chem. Pharm. Bull.* **45**, 2016-2023 (1997).
12. Fernández, M.A.; de las Heras, B.; García, M.D.; Sáenz, M. T.; Villar, A. New insights into the mechanism of action of the anti-inflammatory triterpene lupeol. *J. Pharm. Pharmacol.* **53**, 1533-1539 (2001).
13. Akihisa, T.; Yasukawa, K. Anti-inflammatory and anti-allergic properties of triterpenoids from plants. in *Biomaterials from Aquatic and Terrestrial Organisms* (Fingerman, M.; Nagabhushanam R. ed.). Science Publ. Enfield. pp. 63-114 (2006).
14. Akihisa, T. Anti-inflammatory, antitumor, and chemopreventive effects of triterpenes from plants and fungi, *Oleoscience*, **7**, 445-453 (2007).
15. Akihisa, T.; Yasukawa, K.; Oinuma, H.; Kasahara, Y.; Yamanouchi, S.; Takido, M.; Kumaki, K.; Tamura, T. Triterpene alcohols from the flowers of Compositae and their anti-inflammatory effects. *Phytochem.* **43**, 1255-1260 (1996).
16. Goad, L.J.; Akihisa, T. *Analysis of Sterols*. Blackie Academic & Professional. London. pp. 357-422 (1997).
17. Wood, C.A.; Lee, K.; Vaisberg, A.J.; Kingston, D.G..I.; Neto, C.C.; Hammond, G.B. A bioactive spirolactone iridoid and triterpenoids from *Himatanthus sucuuba*. *Chem. Pharm. Bull.* **49**, 1477-1478 (2001).
18. Yasukawa, K.; Sun, Y.; Kitanaka, S.; Tomizawa, N.; Miura, M.; Motohashi, S. Inhibitory effect of rhizomes of *Alpinia officinarum* on TPA-induced inflammation and tumor promotion in two-stage carcinogenesis in mouse skin. *Nat. Med.* **62**, 374-78 (2008).
19. Takaishi, Y.; Ujita, K.; Tokuda, H.; Nishino, H.; Iwashima, A.; Fujita, T. Inhibitory effects of dihydroagarofuran sesquiterpenes on Epstein-Barr virus activation. *Cancer Lett.* **65**, 19-26 (1992).
20. Medeiros, R.; Otuki, M.F.; Avellar, M.C.W.; Calixto, J.B. Mechanisms underlying the inhibitory actions of the pentacyclic triterpene α-amyrin in the mouse skin inflammation induced by phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate. *Eur. J. Pharmacol.* **559**, 227-235 (2007).
21. The standard deviation values obtained for **3c** were very large. This was because of the lack of sufficient amount of **3c** for this anti-inflammation experiment. Thus, the standard error of the mean (SEM) is appropriate for this experiment. The results shown in **Table 2** represent mean ± SEM.
22. Guay, J.; Bateman, K.; Gordon, R.; Mancini, J.; Riendeau, D. Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E_2 (PGE_2) response in the central nervous system associated with the induction of microsomal PGE_2 synthase-1. *J. Biol. Chem.* **279**, 24866-24872 (2004).
23. de Miranda, A.L.P.; Silva, J.R.A.; Rezende, C.M.; Neves, J. S.; Parrini, S.C.; Pinheiro, M.L.B.; Cordeiro, M.C.; Tamborini, E.; Pinto, A.C. Anti-inflammatory and analgesic activities of the lates containing triterpenes from *Himatanthus sucuuba*. *Planta Med.* **66**, 284-286 (2000).
24. Niles, R.M. Biomarker and animal models for assessment of retinoid efficacy in cancer chemoprevention. *Acta Pharmacol. Sin.* **28**, 1383-1391 (2007).
25. Afaq, F.; Saleem, M.; Krueger, C.G.; Reed, J.D.; Mukhtar, H. Anthocyanin and hydrolysable tannin-rich pomegranate fruit extract modulates MAPK and NF-

κB pathways and inhibits skin tumorigenesis in CD-1 mice. *Intl. J. Cancer* **113**, 423-433(2005).
26. Bicker, D.R.; Athar, M. Oxidative stress in the pathogenesis of skin disease. *J. Invest. Dermatol*. **126**, 2565-2575(2006).
27. O'Brien, T.G.; Megosh, L.C.; Gilliard, G.; Soler, A.P. Ornithine decarboxylase overexpression is a sufficient condition for tumor promotion in mouse skin. *Cancer Res*. **57**, 2630-2637(1997).
28. Kalra, N.; Bhui, K.; Roy, P.; Srivastava, S.; George, J.; Prasad, S.; Shukla, Y. Regulation of p53, nuclear factor κB and cyclooxygenase-2 expression by bromelain through targeting mitogen-activated protein kinase pathway in mouse skin. *Toxicol. Appl. Pharmacol*. **226**, 30-37(2008).