Phorbol

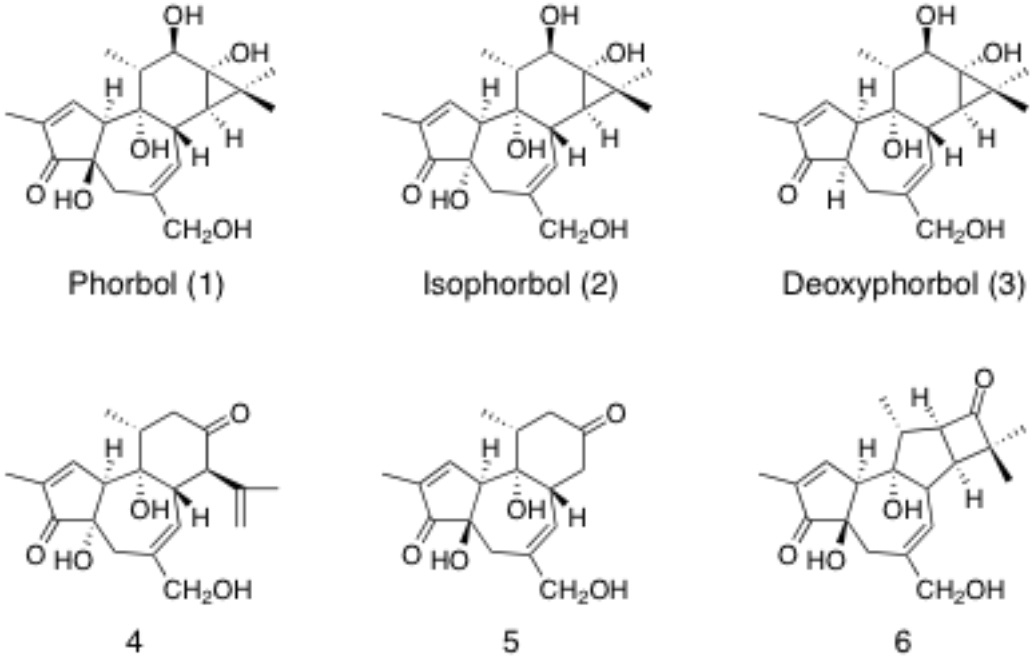

Chart 1. Phorbol and its intestinal bacterial metabolies **2**-**6**

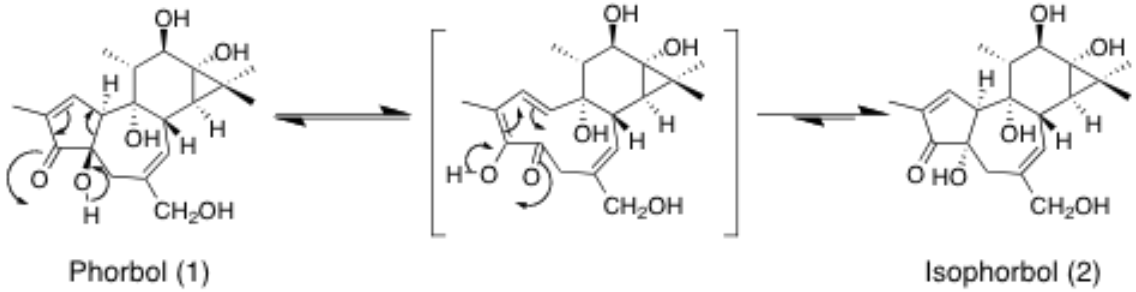

Chart 2. Possible mechanism of conversion of phorbol to isophorbol

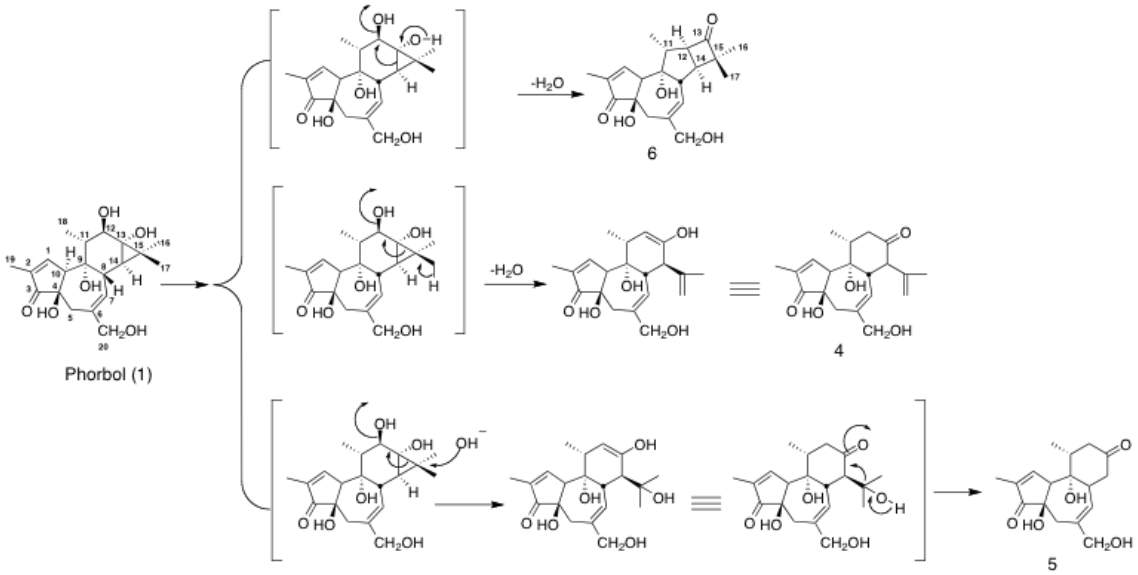

Chart 3.　Possible mechanism of conversion of phorbol to metabolite **4-6**

代謝実験

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単一化合物　phorbol

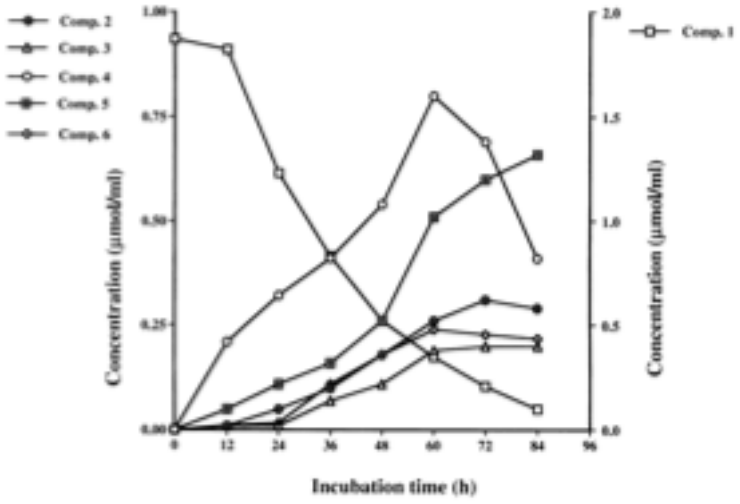

Fig. 1.　Time course of metabolism of phorbol (1) by human intestinal bacteria

Preparation of a human intestinal bacterial (HIB) mixture

A fresh feces (5 g) obtained from a healthy subject was mixed with 50 mM K-phosphate buffer (50 ml, pH 7.3), and the sediments were removed by decantation. The suspension

was made up to 100 ml with the same buffer, and was used as an HIB mixture in this experiment. [Abdel-Hafez *et al., Cmem. Pharm. Bull*., **50**, 160-164 (2002)]

Transformation of 1 by an HIB mixture

A precultured bacterial suspension (300 ml) was added to GAM broth (3000 ml) and the mixture was cultured for 24 h at 37 °C under anaerobic conditions. The culture was centrifuged at 8000 × g for 10 min. The pellets were washed with saline and suspended in GAM broth (1000 ml). Phorbol (**1**, 1 g in 2 ml MeOH) was added to the bacterial suspension, and the mixture was anaerobically incubated at 37 °C for 3 d. The reaction mixture was extracted with ethyl acetate (500 ml × 4). The residue (2.4 g) after evaporation of ethyl acetate *in vacuo* was chromatographed over silica gel eluted with $CHCl_3$-MeOH (9:1) to give fractions A (150 mg) and B (315 mg). Fraction A was separated by MPLC using a LiChroprep RP-18 column with MeOH-H_2O (4 :6) as a mobile phase to yield compounds **2** (9 mg) and **3** (5 mg). Fraction B was also separated by MPLC with RP-18 (MeOH-H_2O, 3 : 7) to afford compounds **4** (28 mg), **5** (16 mg) and **6** (4 mg). [Abdel-Hafez *et al., Cmem. Pharm. Bull*., **50**, 160-164 (2002)]

Compound 2

White amorphous material. IR (KBr) ν_{max} cm^{-1}: 3450 (OH), 2900 (C-H), 1698 (5-membered ring α-unsaturated C=O) and 1640 (C=C). EI-MS *m/z* (rel. int.): 364 [M^+] (25), 346 [M^+-H_2O] (50), 328 [M^+-$2H_2O$] (75), 310 [M^+-$3H_2O$] (100). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 0.56 (1H, d, *J*=5.6 Hz, H-14), 1.14 (3H, s, H-17), 1.21 (3H, d, *J*=6.4 Hz, H-18), 1.24 (3H, s, H-16), 1.57 (1H, m, H-11), 1.71 (3H, m, H-19), 1.78 (1H, m, H-8), 2.14 (1H, d, *J*= 14.2 Hz, H_a-5), 3.2 (1H, t, *J*= 2.44 Hz, H-10), 3.41 (1H, d, *J*= 14.0 Hz, H_b-5), 3.78 (2H, ABq, *J*= 14.0 Hz, H-20), 4.03 (1H, d, *J*=10.2 Hz, H-12), 5.2 (1H, brs, H-7) and 7.28 (1H, m, H-1). ^{13}C-NMR see Table 1. [Abdel-Hafez *et al., Cmem. Pharm. Bull*., **50**, 160-164 (2002)]

Compound 3

White amorphous material. IR (KBr) ν_{max} cm^{-1}: 3350 (OH), 2900 (C-H), 1695 (5-membered ring α-unsaturated C=O) and 1630 (C=C). EI-MS *m/z* (rel. int.): 364 [M^+] (65), 330 [M^+-H_2O] (100). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 0.52 (1H, d, *J*=5.1 Hz, H-14), 1.12 (3H, s, H-17), 1.22 (3H, d, *J*=6.1 Hz, H-18), 1.24 (3H, s, H-16), 1.56 (1H,

m, H-11), 1.71 (3H, m, H-19), 1.84 (1H, brt, H-8), 2.23 (1H, dd, *J*=18.2, 4.6 Hz, H_a-5), 2.69 (1H, m, H-4), 3.17 (1H, dd, *J*=15.1, 2.4 Hz, H_b-5), 3.5 (1H, m, H-10), 3.83 (2H, ABq, *J*= 14.6 Hz, H-20), 4.02 (1H, d, *J*=10.5 Hz, H-12), 5.13 (1H, brs, H-7) and 7.28 (1H, brs, H-1). ^{13}C-NMR see Table 1.

Compound 4

White amorphous material. $[\alpha]_D$ + 182° (*c* = 0.12, MeOH), IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 2900 (C-H), 1710 (6-membered ring C=O), 1695 (5-membered ring α-unsaturated C=O) and 1640 (C=C). EI-MS *m/z* (rel. int.): 346 [M^+] (42), 328 [M^+-H_2O] (100), 300 [M^+-H_2O-CO] (39). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.02 (3H, d, *J*=7.0 Hz, H-18), 1.64 (3H, s, H-17), 1.81 (3H, m, H-19), 2.31 (2H, dd, *J*=20.0, 5.5 Hz, H-12), 2.5 (2H, m, H-5), 2.68 (1H, m, H-11), 3.13 (1H, t, *J*= 2.5 H-10), 3.39 (1H, d, *J*=13.0 Hz, H-14), 3.71 (1H, m, H-8), 4.0 (2H, q, *J*=13.5 Hz, H-20), 4.82 (1H, brs, H_a-16), 5.03 (1H, t, *J*=1.5 Hz, H_b-16), 5.48 (1H, dd, *J*=5.5, 1.0 Hz, H-7) and 7.56 (1H, m, H-1). ^{13}C-NMR see Table 1. [Abdel-Hafez *et al., Cmem. Pharm. Bull*., **50**, 160-164 (2002)]

Compound 5

White amorphous material. $[\alpha]_D$ + 109° (*c* = 0.15, dioxan), IR (KBr) ν_{max} cm^{-1}: 3450 (OH), 2900 (C-H), 1709 (6-membered ring C=O), 1695 (5-membered ring α-unsaturated C=O) and 1630 (C=C). EI-MS *m/z* (rel. int.): 306 [M^+] (35), 289 [M^+-OH] (18), 276 [M^+-CH_3] (68). ^{1}H-NMR (CD_3OD, 500 MHz) δ: 0.92 (3H, d, *J*=7.0 Hz, H-18), 1.76 (3H, m, H-19), 2.06 (1H, ddd, *J*=18.6, 8.55, 5.8 Hz, H_a-12), 2.18 (1H, ddd, *J*=18.6, 7.95, 5.2 Hz, H_b-14), 2.41 (1H, m, H_a-5), 2.50 (1H, m, H_b-12), 2.52 (1H, m, H_b-5), 2.59 (1H, m, H-11), 2.84 (1H, t, *J*= 15.0 Hz, H_b-14) 3.16 (1H, m, H-10), 3.6 (1H, m, H-8), 3.9 (2H, s, H-20), 5.34 (1H, m, H-7) and 7.61 (1H, m, H-1). ^{13}C-NMR see Table 1. [Abdel-Hafez *et al., Cmem. Pharm. Bull*., **50**, 160-164 (2002)]

Compound 6

White amorphous material. $[\alpha]_D$ - 13.2° (*c* = 0.18, MeOH), IR (KBr) ν_{max} cm^{-1}: 3450 (OH), 2900 (C-H), 1765 (4-membered ring C=O), 1705 (5-membered ring α-unsaturated C=O) and 1635 (C=C). EI-MS *m/z* (rel. int.): 346 [M^+] (24), 300 [M^+-H_2O-CO] (55), 285 [M^+-CH_3] (14). ^{1}H-NMR (CD_3OD, 500 MHz) δ: 0.96 (3H, d,

J=7.4 Hz, H-18), 1.22 (3H, s, H-16), 1.23 (3H, s, H-17), 1.76 (3H, m, H-19), 1.95 (1H, m, H_a-5), 2.45 (1H, m, H-14), 2.7 (1H, m, H_b-5), 2.87 (1H, m, H-11), 3.12 (1H, t, *J*= 3.0 Hz, H-10), 3.86 (1H, m, H-12), .3.93 (1H, m, H-8), 4.59 (2H, s, H-20), 5.45 (1H, d, *J*= 6.0 Hz, H-7) and 7.57 (1H, brs, H-1). ^{13}C-NMR see Table 1. [Abdel-Hafez *et al., Cmem. Pharm. Bull*., **50**, 160-164 (2002)]

Time Course of the metabolism of phorbol (1) by a human intestinal bacterial mixture from human faces

To a suspension of the precultured bacterial mixture (3 ml) in GAM broth (10 ml), phorbol (10 mg) was added, and the mixture was anaerobically incubated at 37°C. A portion (1 ml) of it was taken each day for 4 d and extracted with ethyl acetate (1 ml). An aliquot (10 ml) of the ethyl acetate extract was applied to a TLC plate (Merk Kieselgel F_{245}, 0.25 mm thickness) and the plate was developed with $CHCl_3$-MeOH (9:1) and analyzed with a TLC scanner at 260 nm. The amounts of phorbol and its metabolites were determined by using calibration lines prepared with authentic samples. [Abdel-Hafez *et al., Cmem. Pharm. Bull*., **50**, 160-164 (2002)]

Screening of Bacterial Strains for Their Abilities to Metabolize Phorbol (1)

Each precultured bacterial suspension (0.2 ml) was added to GAM broth (10 ml) and cultured for 24 h at 37°C under anaerobic conditions. A portion (3 ml) of each culture was diluted to 10 ml with the same medium and incubated for further 24 h. Phorbol (**1**, 10 mg) was added to each culture and the mixture was incubated for 3 d at 37°C under anaerobic conditions. The incubation mixture was extracted with EtOAc (10 ml x 3). The extract was evaporated *in vacuo* to afford a residue. The residue was dissolved in MeOH (1 ml) and analyzed quantitatively by TLC-densitometry. [Abdel-Hafez *et al., Cmem. Pharm. Bull*., **50**, 160-164 (2002)]

参考文献

1) Abdel-Hafez A. A., Nakamura N., and Hattori M.: Biotransformation of phorbol by human intestinal bacteria. *Cmem. Pharm. Bull*., **50**, 160-164 (2002).