

STUDIES ON INHIBITORS OF SKIN-TUMOR PROMOTION. INHIBITORY EFFECTS OF TRITERPENES FROM *COCHLOSPERMUM* *TINCTORIUM* ON EPSTEIN-BARR VIRUS ACTIVATION

BILO DIALLO, M. VANHAELEN,* R. VANHAELEN-FASTRÉ,

Department of Pharmacognosy, U.L.B. Campus de la Plaine B205-4,
Bd. Triomphe, 1050 Bruxelles, Belgium

TAKAO KONOSHIMA, MUTSUO KOZUKA,

Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan

and HARUKUNI TOKUDA

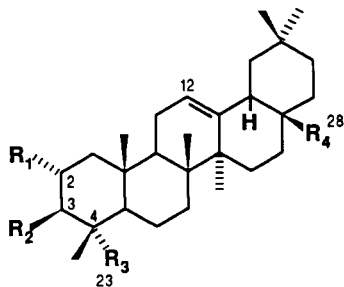
Department of Microbiology, Faculty of Medicine, Kyoto University,
Yoshida, Sakyo-ku, Kyoto 606, Japan

ABSTRACT.—Arjunolic acid, an oleanene-type triterpene isolated from the rhizome of *Cochlospermum tinctorium*, its triacetate derivative, and their methyl esters were tested using the short-term in vitro assay on EBV-EA activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Their inhibitory effects on skin tumor promoters were found to be greater than those of previously studied natural products.

In continuation of our search for biologically active constituents from the rhizomes of *Cochlospermum tinctorium* A. Rich. (syn. *Cochlospermum niloticum* Oliv.) (Cochlospermaceae) (1,2), combined chromatographic methods allowed the purification of three pentacyclic triterpenoids. Among the three, one major constituent was elucidated as arjunolic acid, 2 α ,3 β ,23-trihydroxyolean-12-en-28-oic acid [**1**]; this triterpene was previously isolated from *Terminalia arjuna* (3), *Mitragyna ciliata* (4), *Tristania conferta* (5), and *Psidium guajava* (6); its presence in *Dryobalanops aromatica* was also presumed (7). Identification of **1** was achieved using ¹H-nmr, ¹³C-

nmr, and ms data of its triacetate and methyl ester derivatives. These data have not been published until now and were compared to those reported for methyl asiaticate (methyl 2 α ,3 β ,23-trihydroxyursan-12-en-28-oate) and for methyl 2 α ,3 α ,23-trihydroxyolean-12-en-28-oate (8).

As the inhibitory activity of several related triterpenes on skin tumor promoters was recently reported (9), arjunolic acid and three derivatives, compounds **2–4**, obtained by methylation and/or acetylation were tested using the short-term in vitro assay of Epstein-Barr virus early antigen (EBV-EA) activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The four triterpenes showed stronger inhibitory effects than those of triterpenes studied earlier (9). Triacetate **3** and its methyl ester **4** exhibited complete inhibition on EBV-EA activation at 5 × 10² mol ratio and strong inhibitory effects even at 1 × 10² mol ratio (Table 1). As all values of activation were identical to that of negative control (negative control = 0.1; positive control with TPA 20 ng/ml = 34.5), it was concluded that the four compounds did not exhibit any activation.



- 1** R₁=R₂=R₃=OH, R₄=COOH
- 2** R₁=R₂=R₃=OH, R₄=COOMe
- 3** R₁=R₂=R₃=OAc, R₄=COOH
- 4** R₁=R₂=R₃=OAc, R₄=COOMe