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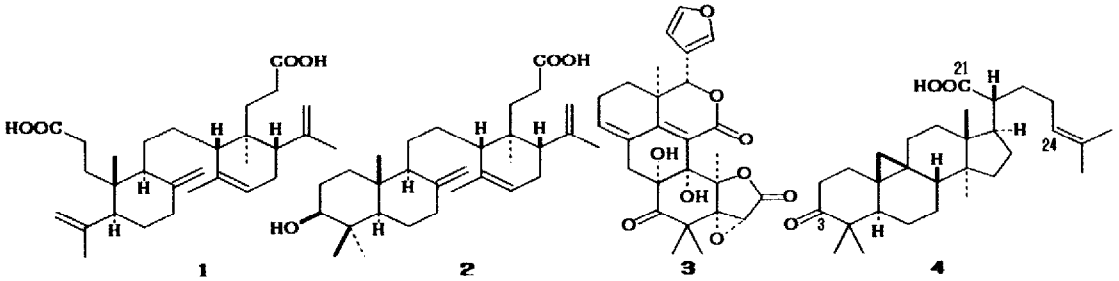
ISOLATION OF A NEW CYCLOARTANOID TRITERPENE FROM LEAVES OF *LANSIUM DOMESTICUM* NOVEL SKIN-TUMOR PROMOTION INHIBITORS

Mugio NISHIZAWA,* Makoto EMURA, Hidetoshi YAMADA, Motoo SHIRO,† CHAIRUL,†† Yuji HAYASHI,†† and Harukuni TOKUDA†††

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-Cho, Tokushima 770, Japan; †Shionogi Research Laboratories, Shionogi & Co. Ltd., Fukushimaku, Osaka 553, Japan; ††Faculty of Science, Osaka City University, Sumiyoshiku, Osaka 558, Japan, †††Department of Microbiology, Faculty of Medicine, Kyoto University, Sakyoku, Kyoto 606, Japan

Summary: A new cycloartanoid triterpene, 3-oxo-24-cycloarten-21-oic acid, has been isolated from leaves of *Lansium domesticum*, and the structure has established on the basis of spectral and an X-ray diffraction studies. Some of the natural product derivatives show significant inhibitory activity of skin-tumor promotion on the basis of Epstein Barr virus activation.

We have been studying constituents of *Lansium domesticum* (Meliaceae, local name duku in Indonesia), and isolated a variety of structurally novel triterpenoids. Although the peel contains a large quantities of seco-onoceranoids such as lansic acid (**1**, major component)[1,2] and lansiolic acid (**2**, minor),[3-6] seed dose not contain this type triterpenoid. Tetranortriterpenoid named dukunolide A (**3**) and analogs have been isolated and characterized as the duku seed metabolites.[7-9] We wish to describe herein the structure and biological activity of the duku leaf metabolites. The major component of duku leaf was lansiolic acid (**2**). The major constituent of duku peel, lansic acid (**1**), was not detected in leaf. The minor triterpene of the leaf was characterized to be a new cycloartanoid type carboxylic acid **4**, named 3-oxo-24-cycloarten- 21-oic acid, by means of spectral and an X-ray diffraction studies. Some chemical derivatives of **4**



1 **2** **3** **4**

showed significant inhibitory activity of skin-tumor promotion on the basis of Epstein-Barr virus associated early antigen (EBV-EA) examination.[10]

A new cycloartanoid **4** was isolated in 0.043% yield from dried duku leaves along with lansiolic acid (**2**) in 1.14% yield by a column chromatography on silica gel and recrystallizations. The compound **4**, obtained as colorless crystals with mp 185-186 ℃, $[\alpha]_D^{12}$ +18.7° (*c* 1.16, $CHCl_3$), gave molecular formula $C_{30}H_{46}O_3$ on the basis of elemental analysis and high resolution mass spectroscopy. A characteristic absorption of carboxylic acid was observed in the IR spectrum (ν_{max} 3200, 1710 cm^{-1}). Thirty signals were observed in the ^{13}C NMR of **4** in $CDCl_3$, and six of them were quartets, eleven were triplets, five were doublets, and eight were singlets. A pair of tri-substituted double bond carbon signals (δ 123.6 d, 132.9 s), and two carbonyl carbon signals (δ 182.6; carboxylic acid, 216.5; ketone) were observed in sp^2 region.[11] In the ^1H NMR spectrum, a characteristic AB doublet signals were detected at δ 0.50 and 0.79 (J = 4.3 Hz), which were assigned to be a geminal proton of 1,1,2,2-tetrasubstituted cyclopropane ring. Four of the methyl signals were observed at high field region (δ 0.90, 1.06, 1.08, 1.08) and two methyls were observed in the vinyl methyl region (δ 1.57, 1.67) as singlets. A vinyl proton was detected as triplet (J = 7.3 Hz) at δ 5.81. From these spectral evidence one can imagine a cycloartanoid triterpene with a ketone and a carboxylic acid moiety.

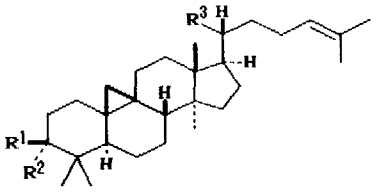
The correct structure was finally derived by a single crystal X-ray diffraction study. Crystal data are M = 454.7, monoclinic, space group *C2*, a = 23.355(7), b = 7.142(2), c = 16.568(4) Å, β = 103.37°, V = 2689(1) $Å^3$, D_c = 1.123 g cm^{-3}, Z = 4, and crystal size 0.45 × 0.15 × 0.1 mm. Intensity data were collected on a Rigaku AFC-5R diffractometer with graphite-monochromated Cu $K\alpha$ radiation (λ = 1.54178 Å). A total 2138 unique reflections in the range of $2\theta \leq 120°$ were measured; 1936 were employed for structure determination by direct method (MULTAN84).[12] The structure was refined by block-diagonal least-squares technique to R = 0.059 and Rw = 0.080 for 1918 reflections.[13]

Investigation on the considerable overlap between Epstein-Barr virus associated early antigen (EBV-EA) inducing compounds and tumor promoters of mouse skin carcinogenesis have been reported.[14] We have tested natural 3-oxo-24-cycloarten-21-oic acid (**4**) along with seventeen kinds of derivatives **5-21**, prepared from natural **4** by simple chemical transformations, using the short-term *in vitro* assay of EBV-EA activation in Raji cells induced by 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA).[15] The inhibitory effect on activation and the viabilities of Raji cell are summarized in Table I. Samples showing grater than 60% viability were employed for evaluation of the inhibitory activity. In this experiments, dose response relationship were observed in the range of 0.32 to 32 nM. For example at 3.2 nM concentration, chemical derivatives **5, 6, 8, 13, 14, 17, 18, 19,** and **20** showed higher activities than natural product **4**. Very active compounds **5, 14, 17,** and **18**, have ketone moiety at C-3. Although the stereoisomers **15** and **16** were separated by using HPLC (Develosil ODS-5 column, CH_3CN-$CHCl_3$ 10:1 as eluant) with *Rt* 12.1 and 13.4 min, respectively, separations of isomeric epoxy carboxylic acids **13** or epoxy esters **14** were very difficult, and 1:1 mixtures were employed for the biological examinations. The stereochemistry at C-24 of **15** and **16** have not determined yet.

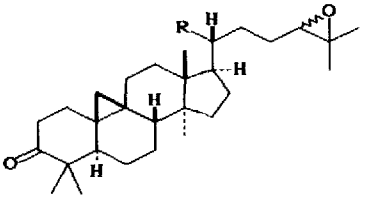
Analogous *in vitro* assay of the other duku metabolites, **1–3**, along with *in vivo* experiment of the anti-skin tumor promoting activity of compounds **5**, **14**, and **17**, are currently undertaken by using mouse skin.

Table I. Inhibitory effects % to control and (% viability) of cycloartanoid triterpenes on EBV. Positive control 100% on the basis of the activation by TPA (32 pM).

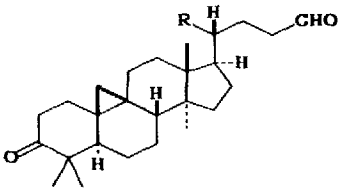
Sample	Concentration			
	32 nM	16 nM	3.2 nM	0.32 nM
4	0 (30)	30.5 (60)	86.9 (>60)	100 (>60)
5	0 (60)	0 (>60)	74.3 (>60)	95.7 (>60)
6	6.7 (60)	43.7 (>60)	77.1 (>60)	92.1 (>60)
7	28.0 (70)	63.6 (>70)	90.6 (>70)	100 (>70)
8	0 (50)	20.5 (60)	80.4 (>60)	100 (>60)
9	38.6 (40)	67.9 (60)	100 (>60)	100 (>60)
10	46.5 (60)	71.5 (>60)	91.4 (>60)	100 (>60)
11	84.0 (70)	100 (>70)	100 (>70)	100 (>70)
12	0 (60)	20.5 (>60)	90.3 (>60)	100 (>60)
13	0 (20)	0 (40)	78 (80)	100 (>80)
14	0 (50)	0 (60)	32.7 (>60)	71.4 (>60)
15	42.5 (70)	79.5 (>70)	100 (>70)	100 (>70)
16	0 (50)	38.5 (60)	92.4 (>60)	100 (>60)
17	0 (20)	0 (60)	37.5 (>60)	92.7 (>60)
18	0 (10)	0 (60)	57.5 (>60)	100 (>60)
19	20.2 (60)	70.1 (>60)	100 (>60)	100 (>60)
20	0 (0)	35.5 (60)	72.9 (>60)	90.1 (>60)
21	0 (10)	68.5 (70)	83.0 (>70)	100 (>70)



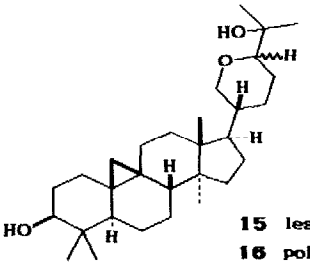
5 $R^1, R^2 = O$ $R^3 = COOCH_3$
6 $R^1 = OH$ $R^2 = H$ $R^3 = COOCH_3$
7 $R^1 = H$ $R^2 = OH$ $R^3 = COOCH_3$
8 $R^1 = OH$ $R^2 = H$ $R^3 = COOH$
9 $R^1 = OH$ $R^2 = H$ $R^3 = CH_2OH$
10 $R^1 = H$ $R^2 = OH$ $R^3 = CH_2OH$
11 $R^1 = OAc$ $R^2 = H$ $R^3 = CH_2OAc$
12 $R^1 = OAc$ $R^2 = H$ $R^3 = COOH$
19 $R^1, R^2 = O$ $R^3 = CHO$
20 $R^1 = OH$ $R^2 = H$ $R^3 = CHO$
21 $R^1, R^2 = O$ $R^3 = CH_2OH$



13 R = COOH (1 : 1 mixture)
14 R = $COOCH_3$ (1 : 1 mixture)



17 R = $COOCH_3$
18 R = CHO



15 less polar isomer
16 polar isomer

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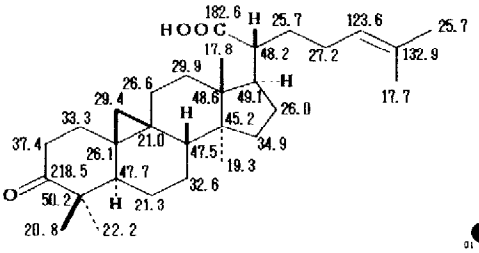
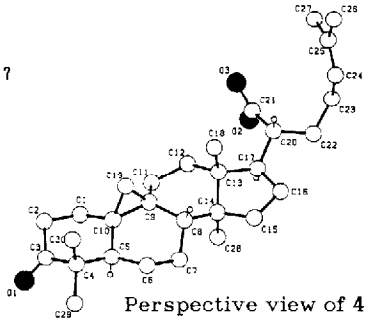
11. Assignment of ^{13}C NMR spectrum of **4** based on C-H COSY, H-H COSY, and C-H long range spectra are as follows. The assignment of C-9 (δ 21.0) and C-10 (δ 26.1) may have to be interchanged.





Perspective view of **4**

12. P. Main, G. Germain, and M. M. Woolfson, MULTRAN84, A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data. University of York, England, and Louvain, Belgium.
13. Atomic coordinates, bond lengths and angles, and thermal parameters have deposited at the Cambridge Crystallographic Data Centre. The absolute configuration of **4** is supposed to be the normal cycloartanoid structure.
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15. The inhibition of EBV-EA activation was assayed using the EBV genome-carrying nonproducer Raji cells, which were employed as a "marker" for the test system. The cells were cultivated in RPMI 1640 medium containing 8% fetal calf serum supplemented with penicillin and streptomysin. The marker cells (1×10^6 mL^{-1}) were incubated at 37 °C for 48 h in 1 mL of medium containing *n*-butyric acid (4 mM), TPA (32 pM) and a DMSO solution of test compound with each concentration. After 48 h, smears were made from the cell suspension. The active cells were detected by indirect immunofluorescent method using EA-positive sera from NPC patients. The EA induction was compared to that of positive control experiments (100%) with *n*-butyric acid and TPA.

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