Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 1773–1778

BIOORGANIC & MEDICINAL CHEMISTRY

Anti-Tumor Promoting Effects of Sesquiterpenes from *Maytenus cuzcoina* (Celastraceae)

Antonio G. González,[a] Benigna M. Tincusi,[a] Isabel L. Bazzocchi,[a,*] Harukuni Tokuda,[b] Hoyoku Nishino,[b] Takao Konoshima,[c] Ignacio A. Jiménez[a] and Angel G. Ravelo[a]

[a]*Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Avda. Astrofísico Francisco Sánchez 2, La Laguna, 38206 Tenerife, Canary Islands, Spain*
[b]*Kyoto Prefectural University of Medicine, Kamigyo-Ku, Kyoto 602-0841, Japan*
[c]*Kyoto Pharmaceutical University, Yamashina-Ku, Kyoto 607-8414, Japan*

Received 27 January 2000; accepted 21 March 2000

Abstract—Ten sesquiterpenoids (**1**–**10**), with a dihydro-β-agarofuran skeleton, were isolated from *Maytenus cuzcoina* (Celastraceae). Their structures were elucidated on the basis of spectral analysis, including homo- and heteronuclear correlations NMR experiments (COSY, ROESY, HMQC and HMBC), and chemical correlations. The compounds have been tested for their inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), as a test for potential cancer chemopreventive agents. Compounds **1**–**3**, **6** and **7** showed strong inhibitory effects on EBV-EA induction (100% inhibition at 1000 mol ratio/TPA). Their structure–activity relationship is discussed.

Introduction

Inhibition of the tumor promotion stage in the multistage of chemical carcinogenesis have been regarded as the most promising method for cancer chemoprevention.[1] In the search for cancer chemopreventive agents, the inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) induction by the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), have been conducting as a primary screening test.[2] Recently, studies on antitumor promoting activities of several natural products,[3–6] using this in vitro assay, which have correlated well with those in some animal models,[7,8] have been reported.

Species of the family Celastraceae have a long history in traditional medicine.[9] As part of an intensive study of the bioactive metabolites from species of this family, we had previously reported quinone-methide[10,11] and dimeric triterpenes,[12] showing antimicrobial and cytotoxic activities. On the other hand, sesquiterpene esters, based on the dihydro-β-agarofuran [5,11-epoxy-5β,10α-eudesman-4(14)-ene] skeleton, are chemotaxonomic indicators of the family,[13] and they have attracted a great deal of interest because of their immunosuppressive,[14] cytotoxic,[15] insect-antifeedant and insecticidal activities.[16] Recently, anti-HIV[17] and reversal multidrug-resistence[18] sesquiterpenes of this type, have been reported. In addition, dihydro-β-agarofuran sesquiterpenes with antitumor-promoting activity have been described.[19–21]

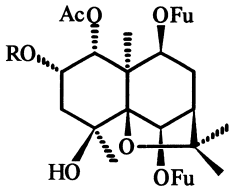
This paper reports the inhibitory effects produced by ten dihydro-β-agarofuran sesquiterpenes (**1**–**10**) isolated from the fruits of *Maytenus cuzcoina* (Celastraceae) on the Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The structure of the new compounds (**1**–**8**) were determined on the basis of spectroscopic data, including ^{1}H–^{13}C heteronuclear correlation (HMQC), long-range correlation with inverse detection (HMBC), and ROESY NMR experiments. The known compounds **9** and **10** were identified as eumaytenol[22] and euonymine[23] by comparison of their spectral with data reported in the literature.

Results and Discussion

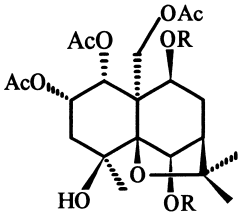
Repeated chromatography of the *n*-hexane:Et_2O (1:1) extracts of the fruits of *M. cuzcoina* on Sephadex LH-20 and Si gel afforded, in addition to the known compounds eumaytenol[22] and euonymine,[23] the new compounds **1**–**8** (Fig. 1).

*Corresponding author. Tel.: +34-922-318576; fax: +34-922-318571; e-mail: ilopez@ull.es

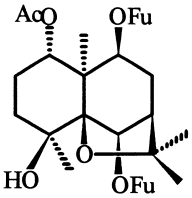
0968-0896/00/$ - see front matter
PII: S0968-0896(00)00107-3



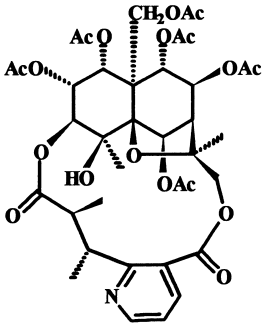
1 R = Fu ; **4** R = Bz
2 R = Ac; **5** R = Pr
3 R = H; **6** R =MeBut



7 R = Fu
8 R = Bz



9 Eumaytenol



10 Euonymine

Ac= Acetate; Bz= Benzoate; Fu= 2-Furoate;
MeBut= 2-Methylbutyrate; Pr=Propionate

Figure 1.

Compound **1** showed the molecular formula $C_{32}H_{34}O_{13}$ by HR-EI/MS. The IR spectrum showed absorption bands for hydroxyl groups (3547 cm^{-1}) and ester groups (1721 cm^{-1}). The presence of a band a 235 nm in its UV spectrum, a fragment at *m/z* 112 (C_4H_3OCOOH) in the EI/MS, and signals for nine aromatic protons between δ 6.68 and 8.19 and twelve aromatic carbons in the ^1H and ^{13}C NMR spectra, respectively, suggested the presence of three furoylate groups in the molecule; while a singlet at δ 1.75 (3H, s) in the ^1H NMR spectrum and fragments at 60 *m/z* (CH_3COOH) and 42 *m/z* (CH_2=CO) in the EIMS suggested the existence of an acetate group. When **1** was treated with acetic anhydride in pyridine, the compound was unaltered, data which together with the presence of a singlet at δ 3.06 in the ^1H NMR spectrum, interchangeable with D_2O, confirmed the presence of a *tertiary* hydroxyl group. All these data indicated that compound **1** was a pentasubstituted dihydro-β-agarofuran sesquiterpene. In its ^1H NMR spectrum (Table 1) also were observed an ABX_2 system with signals at δ 5.71 (1H, m), δ 5.49 (1H, d, J = 3.5 Hz), δ 2.12 and 2.17 (2H, m), assignable to protons H-2, H-1 and H-3, respectively, and signals at δ 5.68 (1H, s) and δ 4.95 (1H, d, J = 6.8 Hz), assigned to protons H-6 and H-9, respectively. A *tertiary* methyl at δ 1.57 binding to a quaternary carbon at δ 69.9 in the ^{13}C NMR spectrum (Table 2) and three angular methyls, were also observed. The relative stereochemistry of **1** was established on the basis of the coupling constants and confirmed by a ROESY experiment (Fig. 2), showing NOE effects between H-1 and H-2 and between H-15 and H-6, H-9 and H-14. The regiosubstitution was established by an HMBC experiment, showing three-bond correlation between the carboxyl signal of the acetate group at δ 169.7 and the ^1H signal at δ 5.49 assigned to H-1. All these data established the structure of **1** as 1α-acetoxy-2α,6β,9β-trifuroyloxy-4β-hydroxy-dihydro-β-agarofuran.

The structure of compounds **2**–**4** were elucidated by spectral methods, ^1H and ^{13}C NMR studies (Tables 1 and 2), 2D ^1H–^1H and ^1H–^{13}C correlations, ROESY experiment

Table 2. ^{13}C NMR (100 MHz) data (δ, $CDCl_3$) of **1–8**[a]

Position	1	2	3	4	5	6	7	8
C-1	70.0 d	70.0 d	72.4 d	70.1 d	70.0 d	69.9 d	68.2 d	68.8 d
C-2	68.8 d	68.6 d	67.8 d	69.4 d	68.3 d	68.2 d	68.0 d	68.0 d
C-3	42.3 t	42.3 t	44.2 t	42.5 t	42.3 t	41.5 t	42.2 t	42.2 t
C-4	69.9 s	69.9 s	70.1 s	70.0 s	69.9 s	69.9 s	69.7 s	69.8 s
C-5	91.6 c	91.2 s	91.5 s	91.3 s	91.2 s	91.2 s	90.9 s	91.0 s
C-6	79.2 d	79.3 d	79.3 d	79.3 d	79.2 d	79.3 d	77.9 d	78.6 d
C-7	48.9 d	48.9 d	48.8 d	48.9 d	48.8 d	48.9 d	48.9 d	49.0 d
C-8	31.1 t	31.2 t	31.1 t	31.2 t	31.1 t	31.3 t	34.5 t	34.6 t
C-9	72.0 d	72.1 d	72.7 d	72.0 d	72.0 d	72.0 d	70.2 d	70.4 d
C-10	51.0 s	51.1 s	51.1 s	51.0 s	51.0 s	51.1 s	54.6 s	54.9 s
C-11	84.7 s	84.6 s	84.3 s	84.8 s	84.6 s	84.7 s	84.5 s	84.6 s
C-12	25.6 c	25.7 c	25.6 c	25.7 c	25.6 c	25.7 c	25.5 c	25.7 c
C-13	29.5 c	29.7 c	29.6 c	29.7 c	29.6 c	29.7 c	29.3 c	29.4 c
C-14	25.5 c	25.1 c	25.4 c	25.4 c	25.0 c	25.1 c	24.7 c	24.7 c
C-15	21.5 c	21.4 c	21.8 c	21.8 c	21.4 c	21.7 c	65.3 t	65.4 t

[a]Data are based on DEPT and ^1H–^{13}C (HMQC and HMBC) experiments.

Table 1. ^1H NMR (400 MHz) data (δ, $CDCl_3$, *J* are given in Hz in parentheses) of **1–8**

Position	1	2	3	4	5	6	7	8
H-1	5.49 d (3.5)	5.39 d (3.5)	5.33 d (3.3)	5.53 d (3.6)	5.42 d (3.6)	5.43 d (3.6)	5.53 d (3.4)	5.63 d (3.4)
H-2	5.71 m	5.49 m	4.35 m	5.80 m	5.55 m	5.55 m	5.50 m	5.54 m
H-6	5.68 s	5.63 s	5.63 s	5.71 s	5.65 s	5.65 s	6.18 s	6.29 s
H-7	2.36 t (3.1)	2.32 t (2.9)	2.33 t (3.1)	2.36 t (3.0)	2.34 t (3.2)	2.34 t (3.2)	2.34 t (3.3)	2.38 t (3.1)
H-8	2.17 m, 2.57 m	2.15 m, 2.51 m	2.05 m, 2.52 m	2.22 m, 2.58 m	2.18 m, 2.55 m	2.18 m, 2.52 m	2.33 m, 2.61 m	2.24 m, 2.69 m
H-9	4.95 d (6.8)	4.91 d (6.7)	4.94 d (6.8)	4.95 d (6.8)	4.93 d (6.8)	4.93 d (6.8)	5.36 d (7.2)	5.50 d (7.2)
H-15	1.59 s	1.52 s	1.58 s	1.64 s	1.54 s	1.54 s	4.40,4.96 d_{AB} (12.9)	4.46, 4.99 d_{AB} (13.0)

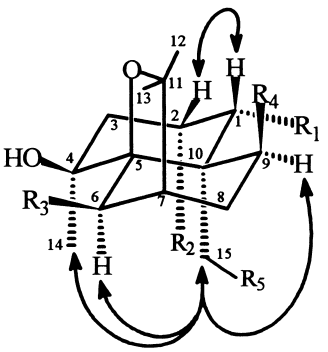


Figure 2. ROESY experiment of compounds **1**–**8**.

(Fig. 2), and chemical correlations (Scheme 1). Thus, acetylation and benzoylation of **3** gave two derivatives (Scheme 1), the spectral data of which were identical to those of **2** and **4**, respectively.

Compound **5** showed the molecular formula $C_{30}H_{36}O_{12}$ by HREIMS. The EIMS contained fragmentation ions attributable to losses of *m*/*z* 18, 60, 74 and 112, suggesting the presence in the molecule of hydroxyl, acetate, propionate and furoylate groups. This was confirmed by the ^{1}H NMR spectrum, showing an acetate methyl at δ 1.74 as singlet, a methyl at δ 1.13 as triplet and a methylene at δ 2.31 as quartet assigned to a propionate group, and six aromatic protons between δ 6.74 and 8.18, data that were in accordance with the ^{13}C NMR spectrum. When **5** was acetylated under standard conditions, it afforded the product unaltered, confirming the existence of a tertiary alcohol. The relative stereochemistry of **5** was established by a ROESY experiment, while an HMBC experiment, showing long-range correlations between the carboxyl signals of the acetate group at δ 169.6 and the propionate group at δ 173.1 with the ^{1}H signals at δ 5.42 (H-1) and δ 5.55 (H-2), respectively, confirmed the regiosubstitution in the molecule.

A detailed study of the spectroscopic data of compound **6**, which had the molecular formula $C_{32}H_{40}O_{12}$ (HR-EI/MS), showed it to be related to **5**. The most notable differences being the existence of one 2-methylbutyrate with signals at δ 0.88 t (3H, $J = 7.4$ Hz), 1.12 d (3H, $J = 7.0$ Hz), 1.61 m (2H) and 2.34 m (1H) in the ^{1}H NMR spectrum, instead of the signals corresponding to the propionate group in **5**.

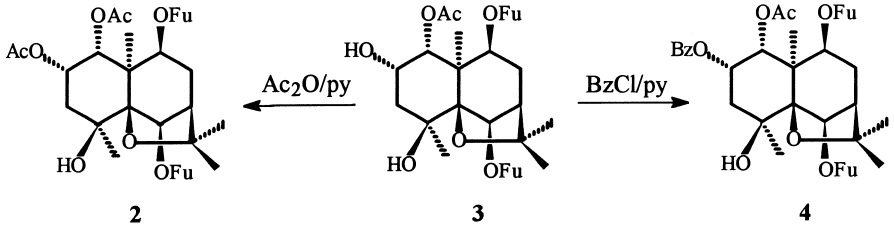
Compound **7**, with the molecular formula $C_{31}H_{36}O_{14}$ (HREIMS), in a study of its IR, UV, ^{1}H and ^{13}C NMR data (Tables 1 and 2), and 2D experiments was shown to be a dihydro-β-agarofuran sesquiterpene with three acetate, two furoylate and one *tertiary* hydroxyl group, located at 1α, 2α, 4β, 6β, 9β and 15. An HMBC experiment established the regiosubstitution partners and the relative stereochemistry was resolved by analysis of a ROESY experiment (Fig. 2).

The spectroscopic data of compound **8** and comparison with these of **7** allowed to determine its structure. Compounds **1**–**6** and **7**–**8** have the basic polyhydroxylated skeletons of 2α,4β-dihydroxy-celorbicol[24] and 3-deoxy-maytol,[25] respectively.

Antitumor-promoting activity

Compounds **1**–**10** were tested for their inhibitory effects on Epstein-Barr virus early antigen (EBV-EA), induced by the tumor promoter, TPA, in Raji cells. Their inhibitory effects on the activation of the early antigen and the viability of Raji cells are shown in Table 3.

Compounds **1**–**3**, **6** and **7** have strong anti-tumor promoting activity, even at 10 mol ratio/TPA (100% inhibitory activity at 1000 mol ratio/TPA, and more than 70% and 25% even at 500 mol ratio/TPA and 100 mol ratio/TPA, respectively), and preserved high viability of Raji cells (more than 70% at 10 to 1000 mol ratio/TPA).



Scheme 1.

Table 3. Percentage of Epstein-Barr virus early antigen induction in the presence of compounds 1–10 with respect to a positive control[c]

Concentration (mol ratio/TPA)[a]	1	2	3	4	5	6	7	8	9	10
1000	0[b] (70)	0 (70)	0 (70)	11.7 (60)	6.3 (70)	0 (70)	0 (70)	12.6 (60)	10.5 (70)	26.4 (70)
500	30.2	27.4	28.5	42.9	34.9	32.7	25.9	48.0	40.2	59.6
100	76.9	74.8	76.7	87.1	77.9	78.0	72.0	86.9	84.6	86.7
10	94.6	93.0	95.8	100	100	96.2	91.0	100	100	100

[a]Values in parentheses represent viability percentages of Raji cells; unless otherwise stated, the viability percentages of Raji cells were more than 80%.
[b]Mol ratio/TPA (32 pmol = 20 ng/mL), 1000 mol ratio = 32 nmol, 500 mol ratio = 16 nmol, 100 mol ratio = 3.2 nmol, and 10 mol ratio = 0.32 nmol.
[c]Values represent percentages of EBV-EA induction to the positive control values (100%) (n = 3).

Compounds **7** and **8** based on the same 3-deoxy-maytol skeleton showed, however, a very different inhibitory activity. Thus, while **7** was the most active of the compounds assayed (9.0% inhibition of induction at 10 mol ratio/TPA), compound **8** showed an insignificant inhibitory activity (87.4% at 1000 mol ratio/TPA). Compound **9** with a skeleton based on 4β-hydroxy-celorbicol,[22] showed an inhibitory activity comparable to **8**.

Compounds **2**, **1**, **3** and **6**, with a basic skeleton of 2α,4β-dihydro-celorbicol, showed increasing inhibitory activity in this order. These results clearly indicated that the acetoxy and furoyloxy groups increase the inhibition of Epstein-Barr virus activation for this type of compounds. Furthermore, the substitution of the acetate group in compound **2** (7.0% inhibition of induction at 10 mol ratio/ATP) for a furoyloxy in **1**, an hydroxy in **3** or a methylbutyroyloxy group in **6**, decrease progressively the activity (5.4%, 4.2% and 3.8% inhibition of induction at 10 mol ratio/TPA, respectively). On the other hand, when a benzoyloxy or a propionyloxy group are present on C-2, as in **4** and **5**, respectively, compounds show less activity. It suggests that the substituent at C-2 plays an important role in the inhibitory activity. Such strict structural requirements remind us of the specific target(s) of sesquiterpenes being involved in the action mechanisms of EBV activation,[19] and thus deserve further mechanistic studies.

It is interesting to note that compound **10**, with a macrolide bridge showed the lowest inhibitory activity (73.6% at 1000 mol ratio/TPA). It seems that the macrolide bridge leads to a decrease in inhibitory activities, as was pointed out by Tokuda et al.,[19] and suggests that the size of the molecule could strongly affect the inhibitory activity.

The inhibitory activities of these compounds are greater than those of glycyrrhizin[26] and retinoic acid,[27] which are known as typical antitumor promoters, and also stronger than other dihydro-β-agarofuran sesquiterpenes previously reported.[19–21] From these results, it was concluded that these sesquiterpenes might be valuable chemopreventive agents, and the fruits of *Maytenus cuzcoina* would be effective resources of preventive agents.

Experimental

General

IR spectra were recorded in $CDCl_3$ on a Bruker IFS 55 spectrophotometer, and UV spectra were collected in absolute EtOH on a JASCO V-560. ^1H and ^{13}C NMR spectra were recorded on a Bruker at 400 MHz and 100 MHz, respectively. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter, and $[\alpha]_D$ values are given in 10^{-1} °cm^2/g. CD spectra on a JASCO J-600 spectropolarimeter. EI/MS and HR-EI/MS were recorded on a Micromass Autospec spectrometer. TLC 1500/LS 25 Schleicher and Schuell foils were used for thin layer chromatography, while silica gel (0.2–0.63 mm) and Sephadex LH-20 were used for column chromatography.

Plant material

M. cuzcoina (local name "paltay-paltay") was collected at Huayllabamba-Urquillos, Province of Urubamba, Cusco (Peru), in December 1993. A voucher ("cuz" 02765 A.T. 1004 MO) is deposited in the herbarium of Vargas, Department of Botany, in the National University of San Antonio Abad de Cusco.

Bioassays

Chemicals. The cell culture reagent and *n*-butyric acid were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) was obtained from Sigma Chemical Co. (St. Louis, MO).

In vitro EBV-EA induction effect. The EBV genome-carrying lymphoblastoid cells, Raji cells, derived from Barkitt's lymphoma, were cultivated in RPMI-1640 medium. The Raji cells were incubated for 48 h at 37 °C in a medium containing *n*-butyric acid (4 mmol), TPA (32 pmol), and various amounts of test compounds. Smears were made from the cells suspension, and the EBV-EA-inducing cells were stained by means of an indirect immunofluorescence technique. The details of the in vitro assay on EBV-EA induction have been reported previously.[7,8]

Extraction and isolation

The fruits (400 g) of *M. cuzcoina* were extracted with *n*-hexane:Et_2O (1:1) in a Soxhlet apparatus. Removal of the solvent under vacuum gave 70 g of residue, which was chromatographed on a Si-gel column, using mixtures of *n*-hexane-EtOAc of increasing polarity as solvent. In this way, after several chromatographies on Sephadex LH-20 and Si gel, the known compounds eumaytenol[22] and euonymine,[23] and the new compounds **1** (26 mg), **2** (620 mg), **3** (393 mg), **4** (58 mg), **5** (61 mg), **6** (123 mg), **7** (54 mg), and **8** (68 mg), were obtained.

1α-Acetoxy-2α,6β,9β-trtifuroyloxy-4β-hydroxy-dihydro-β-agarofuran (1). Amorphous solid; $[\alpha]_D^{25}$ +34.10 (*c* 1.0, $CHCl_3$); UV λ_{max} nm: 235, 221; IR ν_{max} cm^{-1}: 3547, 2928, 1721, 1576, 1508, 1309, 1232, 1161, 768; ^1H NMR δ: 1.54 (3H, s), 1.56 (3H, s), 1.57 (3H, s), 1.75 (3H, s), 2.12 (1H, m), 2.17 (1H, m), 3.06 (1H, br s), 6.68 (1H, d, J = 2.0 Hz), 6.75 (1H, d, J = 2.0 Hz), 6.84 (1H, d, J = 2.0 Hz), 7.44 (3H, m), 7.95 (1H, s), 8.04 (1H, s), 8.18 (1H, s), for other signals, see Table 1; ^{13}C NMR δ: 20.4 (q); 109.5 (d); 109.7 (d); 109.8 (d); 118.6 (s); 119.2 (2 × s); 143.7 (d); 143.8 (2×d); 147.5 (d); 148.5 (d); 149.0 (d); 161.8 (s); 161.9 (s); 162.2 (s); 169.7 (s), for other signals, see Table 2; EI/MS *m*/*z*% : 626 (M^+, 1); 611 (1); 514 (1); 499 (3); 472 (1) 402 (11); 290 (4); 233 (3); 192 (11); 95 (100); 57 (3); HR-EI/MS: *m*/*z* 626.20728 (calcd for $C_{32}H_{34}O_{13}$, 626.19994).

1α,2α-Diacetoxy-6β,9β-difuroyloxy-4β-hydroxy-dihydro-β-agarofuran (2). Amorphous solid; $[\alpha]_D^{25}$ +20.10 (*c* 2.4, $CHCl_3$); UV λ_{max} nm :235, 221; IR ν_{max} cm^{-1}: 3550, 3029, 2957, 1747, 1712, 1577, 1366, 1309, 1243, 1160, 761; ^1H NMR δ: 1.49 (3H, s), 1.50 (3H, s), 1.51 (3H; s), 1.72 (3H, s), 1.98 (1H, m), 2.15 (1H, m), 2.02 (3H, s),

2.30 (1H, m), 3.00 (1H, br s), 6.72 (1H, d, $J=1.8$ Hz), 6.82 (1H, d, $J=1.8$ Hz), 7.41 (1H, d, $J=1.8$ Hz), 7.43 (1H, d, $J=1.8$ Hz), 8.00 (1H, s), 8.16 (1H, s), for other signals, see Table 1; ^{13}C NMR δ: 20.4 (q); 21.1 (q); 109.7 (d); 109.8 (d); 118.7 (s); 119.3 (s); 143.8 (d); 144.0 (d); 148.6 (d); 149.1 (d); 161.9 (s); 162.2 (s); 169.7 (2 × s), for other signals, see Table 2; EI/MS *m/z*%: 574 (M^+, 1) 559 (1), 514 (1), 470 (2), 447 (5), 402 (13), 290 (5), 233 (5), 192 (14), 105 (17), 95 (100), 57 (2); HR-EI/MS: *m/z* 574.20554 (calcd for $C_{29}H_{34}O_{12}$ 574.20503).

1α-Acetoxy-6β,9β-difuroyloxy-2α,4β-dihydroxy-dihydro-β-agarofuran (3). Amorphous solid; $[\alpha]_D^{25}$ +11.20 (*c* 0.5, $CHCl_3$); UV λ_{max} nm: 236, 222; IR ν_{max} cm^{-1}: 3520, 3136, 2927, 2855, 1721, 1576, 1508, 1310, 1160, 1077, 874, 762; ^{1}H NMR δ: 1.50 (3H, s), 1.53 (3H, s), 1.56 (3H, s), 1.84 (3H, s), 2.05 (1H, m), 2.18 (1H, m), 2.98 (1H, s), 6.74 (1H, d, $J=1.8$ Hz), 6.83 (1H, d, $J=1.8$ Hz), 7.42 (1H, d, $J=1.8$ Hz), 7.43 (1H, d, $J=1.8$ Hz), 8.02 (1H, s), 8.18 (1H, s), for other signals, see Table 1; ^{13}C NMR δ: 20.7 (q), 109.6 (d), 109.7 (d), 118.7 (s), 119.2 (s), 143.7 (d), 143.9 (d), 148.5 (d), 149.1 (d), 161.9 (s), 162.2 (s), 169.9 (s), for other signals, see Table 2; EI/MS *m/z* (%): 518 (M^+-14, 1), 472 (5), 453 (5), 446 (6), 416 (32), 361 (29), 312 (45), 287 (66), 252 (99), 125 (97), 95 (100), 57 (10); HR-EI/MS: *m/z* 518.17702 (calcd for $C_{26}H_{30}O_{11}$ 518.17881).

Acetylation of 3. Ac_2O (4 drops) was added to compound **3** (5.0 mg) dissolved in pyridine (2 drops), and the mixture left at room temperature for 16 h. EtOH (3×2 mL) was added and carried almost to dryness in a rotavapor, and this process was repeated with $CHCl_3$ (3×2.0 mL), and purified by preparative TLC with a mixture of *n*-hexane: EtOAc (1:1), to give product **2** (5.0 mg).

Benzoylation of 3. Compound **3** (5.0 mg) was dissolved in dry pyridine (0.5 mL) and benzoyl chloride (6 drops), and some crystals of 4-(dimethylamino)-pyridine were added under argon atmosphere. The mixture was heated at 60 °C for 15 h, poured over H_2O extracted with EtOAc, and purified on preparative TLC with a mixture of *n*-hexane:EtOAc (1:1) to give **4** (4.5 mg).

1α-Acetoxy-2α-benzoyloxy-6β,9β-difuroyloxy-4β-dihydro-β-agarofuran (4). Pale yellow amorphous solid; $[\alpha]_D^{25}$ +40.90 (*c* 0.8, $CHCl_3$); UV λ_{max} nm: 231, 217; IR ν_{max} cm^{-1}: 3544, 2956, 2931, 1747, 1721, 1507, 1395, 1160, 761, 712; ^{1}H NMR δ: 1.53 (3H, s), 1.56 (3H, s), 1.61 (3H, s), 1.73 (3H, s), 2.22 (2H, m), 3.06 (1H, s), 6.74 (1H, d, $J=2.0$ Hz), 6.83 (1H, d, $J=1.8$ Hz), 7.43 (3H, m), 7.56 (2H, m), 7.94 (1H, d, $J=2.0$ Hz), 7.96 (2H, m), 8.10 (1H, s), 8.18 (1H, s), for other signals, see Table 1; ^{13}C NMR δ: 20.5 (q), 109.7 (2×d), 118.7 (s), 119.2 (s), 128.6 (2×d), 129.5 (2×d), 129.9 (s), 132.2 (d), 143.8 (d), 144.0 (d), 148.6 (d), 149.1 (d), 161.9 (s), 162.3 (s), 165.7 (s), 169.8 (s), for other signals, see Table 2; EI/MS *m/z* (%): 621 (M^+-15, 1), 509 (3), 402 (14), 290 (4), 247 (2), 230 (3), 215 (3), 192 (16), 105 (68), 95 (100), 57 (1), HR-EI/MS: *m/z* 621.19492 (calcd for $C_{33}H_{33}O_{12}$ 621.19720).

1α-Acetoxy-6β,9β-difuroyloxy-2α-propyonyloxy-4β-hydroxy-dihydro-β-agarofuran (5). Pale yellow amorphous solid; $[\alpha]_D^{25}$ +17.40 (*c* 1.8, $CHCl_3$); UV λ_{max} nm: 235, 221; IR ν_{max} cm^{-1}: 3550, 2970, 2931, 1721, 1577, 1508, 1309, 1232, 1160, 874, 762; ^{1}H NMR δ: 1.13 (3H, t, $J=7.6$ Hz), 1.51 (3H, s), 1.52 (3H, s), 1.53 (3H, s), 1.74 (3H, s), 1.99 (1H, m), 2.18 (1H, m), 2.31 (2H, m), 6.74 (1H, d, $J=1.6$ Hz), 6.84 (1H, d, $J=1.8$ Hz), 7.43 (1H, d, $J=1.6$ Hz), 7.45 (1H, d, $J=1.8$ Hz), 8.03 (1H, s), 8.18 (1H, s), for other signals, see Table 1; ^{13}C NMR δ: 9.02 (q), 20.4 (q), 27.8 (t), 109.6 (d), 109.7 (d), 118.6 (s), 119.2 (s), 143.7 (d), 143.9 (d), 148.5 (d), 149.0 (d), 161.8 (s), 162.2 (s), 169.6 (s), 173.1 (s), for other signals, see Table 2; EI/MS *m/z* (%): 588 (M^+, 1), 587 (3), 514 (2), 475 (5), 460 (0.4), 454 (0.4), 402 (27), 290 (5), 233 (6), 192 (21), 105 (4), 95 (100), 57 (8); HR-EI/MS: *m/z* 587.21143 (calcd for $C_{30}H_{35}O_{12}$ 587.21285).

1α-Acetoxy-6α,9β-difuroyloxy-2α-(2)-methylbutyroyloxy-4β-hydroxy-dihydro-β-agarofuran (6). Pale yellow amorphous solid; $[\alpha]_D^{25}$ +18.30 (*c* 0.8, $CHCl_3$); UV λ_{max} nm 238, 222; IR ν_{max} cm^{-1}: 3548, 3136, 2966, 2931, 1720, 1577, 1366, 1308, 1160, 874, 762; ^{1}H NMR δ: 0.88 (3H, t, $J=7.4$ Hz), 1.12 (3H, d, $J=7.0$ Hz), 1.50 (3H, s), 1.51 (3H, s), 1.53 (3H, s), 1.61 (2H, m), 1.73 (3H, s), 1.97 (1H, m), 2.18 (1H, m), 2.34 (1H, m), 3.00 (1H, s), 6.74 (1H, d, $J=1.7$ Hz), 6.83 (1H, d, $J=1.7$ Hz), 7.42 (1H, d, $J=1.7$ Hz), 7.44 (1H, d, $J=1.7$ Hz), 8.02 (1H, s), 8.17 (1H, s), for other signals, see Table 1; ^{13}C NMR δ: 11.5 (q), 16.7 (q), 20.4 (q), 26.5 (t), 42.6 (d), 109.7 (d), 109.8 (d), 118.7 (s), 119.3 (s), 143.8 (d), 144.0 (d), 148.6 (d), 149.1 (d), 161.9 (s), 162.3 (s), 169.6 (s), 175.6 (s), for other signals, see Table 2; EI/MS *m/z* (%): 601 (M^+-15, 1), 514 (1), 489 (5), 402 (14), 290 (7), 233 (6), 215 (4), 192 (17), 95 (100), 85 (13), 57 (38); HR-EI/MS: *m/z* 601.22971 (calcd for $C_{31}H_{37}O_{12}$ 601.22850).

1α,2α,15-Triacetoxy-6β,9β-difuroyloxy-4β-hydroxy-dihydro-β-agarofuran (7). Amorphous solid; $[\alpha]_D^{25}$: +23.90 (*c* 0.8, $CHCl_3$); UV λ_{max} nm: 234, 220; IR ν_{max} cm^{-1}: 3549, 2930, 1749, 1722, 1576, 1367, 1309, 1158, 762; ^{1}H NMR δ: 1.49 (3H, s), 1.54 (3H, s), 1.57 (3H, s), 1.72 (3H, s), 2.01 (1H, m), 2.11 (3H, s), 2.17 (1H, m), 2.30 (3H, s), 2.93 (1H, br s), 6.74 (1H, d, $J=1.8$ Hz), 6.81 (1H, d, $J=1.7$ Hz), 7.43 (1H, d, $J=1.8$ Hz), 7.44 (1H, d, $J=1.7$ Hz), 8.02 (1H, s), 8.14 (1H; s), for other signals, see Table 1; ^{13}C NMR δ: 20.3 (q), 21.0 (q), 21.2 (q), 109.7 (2×d), 128.5 (s), 129.0 (s), 143.8 (d), 143.9 (d), 148.7 (d), 148.9 (d), 161.4 (s), 162.3 (s), 169.3 (s), 169.6 (s), 171.4 (s), for other signals, see Table 2; EI/MS *m/z* (%): 617 (M^+-15, 1), 572 (1), 505 (2), 460 (11), 275 (9), 192 (12), 105 (38), 95 (100), 57 (7); HR-EI/MS: *m/z* 617.18915 (calcd for $C_{30}H_{33}O_{14}$ 617.18703).

1α,2α,15-Triacetoxy-6β,9β-dibenzoyloxy-4β-hydroxy-dihydro-β-agarofuran (8). Amorphous solid; $[\alpha]_D^{25}$ +40.20 (*c* 0.7, $CHCl_3$); UV λ_{max} nm: 274, 232, 202; IR ν_{max} cm^{-1}: 3552, 2928, 1750, 1716, 1366, 1275, 714; ^{1}H NMR δ: 1.53 (3H; s), 1.57 (3H, s), 1.59 (3H, s), 1.71 (3H, s), 2.04 (1H, m), 2.19 (1H, m), 2.10 (3H, s), 3.09 (1H, br s), 7.46 (4H, m), 7.58 (2H, m), 8.05 (2H, m), 8.19 (2H, m), for other signals, see Table 1; ^{13}C NMR δ: 20.2 (q), 21.0 (q), 21.2 (q), 128.3 (2×d), 128.6 (2×d), 128.9 (s), 129.7 (s), 130.0 (4×d), 133.3 (d), 133.5 (d), 165.1 (s), 165.9 (s), 169.1 (s), 169.5 (s), 170.5 (s), for other signals, see Table 2; EIMS *m/z* (%): 637 (M^+-15, 2), 592 (1), 515 (4), 470 (15), 275

(7), 202 (5), 122 (3), 105 (100), 57 (5); HREIMS: m/z 637.22868 (calcd for $C_{34}H_{37}O_{12}$ 637.22850).

Acknowledgements

This work has been supported by DGES (Projects PB96-1039 and PB96-1033) and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture and the Ministry of Health and Welfare of Japan.

References and Notes

1. Murakami, A.; Ohigashi, H.; Koshimizu, K. *Biosci. Biotech. Biochem.* **1996**, *60*, 1.
2. Ohigashi, H.; Takamura, H.; Koshimizu, K.; Tokuda, H.; Ito, Y. *Cancer Lett.* **1986**, *30*, 143.
3. Konishi, T.; Takasaki, M.; Kiyosawa, S.; Tokuda, H.; Konoshima, T. *Biol. Pharm. Bull.* **1998**, *21*, 993.
4. Konishi, T.; Takasaki, M.; Tokuda, H.; Nishino, H.; Duc, N. M.; Kasai, R.; Yamasaki, K. *Biol. Pharm. Bull.* **1998**, *21*, 834.
5. Konishi, T.; Takasaki, M.; Tokuda, H.; Masuda, K.; Ara, Y.; Shiojima, K.; Ageta, H. *Biol. Pharm. Bull.* **1996**, *19*, 962.
6. Konishi, T.; Kokumai, M.; Kozuka, M.; Tokuda, H.; Nishimo, H.; Iwashima, A. *J. Nat. Prod.* **1997**, *20*, 865.
7. Takaishi, M.; Konoshima, T.; Yasuda, I.; Hamano, T.; Tokuda, H. *Biol. Pharm. Bull.* **1997**, *20*, 776.
8. Konoshima, T. In *Saponins Used in Traditional and Modern Medicine*; Waller, G. R.; Yamasaki, K. Eds.; Plenum: New York, 1996; pp 73–100.
9. Muñoz, O.; Peñaloza, A.; González, A. G.; Ravelo, A. G.; Crespo, A.; Bazzocchi, I. L.; Alvarenga, N. L. In *Celastraceae, Bioactive Metabolites*; Series Studies in Natural Products, Elsevier Science: Amsterdam, 1996, Vol. 18, pp 739–785.
10. Moujir, L. M.; Navarro-Gutiérrez, A. M.; González, A. G.; Ravelo, A. G.; Luis, J. G. *Antimicrob. Agents Chemother.* **1991**, *35*, 211.
11. González, A. G.; Alvarenga, N. L.; Ravelo, A. G.; Bazzocchi, I. L.; Ferro, E. A.; Navarro, A. G.; Moujir, L. M. *Bioorg. Med. Chem.* **1996**, *4*, 815.
12. González, A. G.; Alvarenga, N. L.; Estévez-Braun, A.; Ravelo, A. G.; Bazzocchi, I. L.; Moujir, L. M. *Tetrahedron* **1996**, *52*, 9597.
13. Brüning, R.; Wagner, H. *Phytochemistry* **1978**, *17*, 1821.
14. Zheng, Y. L.; Xu, Y.; Lin, J. F. *Acta Pharm. Sin* **1989**, *24*, 568.
15. Kuo, Y.; King, M.; Chen, G.; Chen, H.; Chen, C.; Chen, L.; Lee, K. *J. Nat. Prod.* **1994**, *57*, 263.
16. González, A. G.; Jiménez, I. A.; Ravelo, A. G.; Coll, J.; González, J. A.; Lloria, J. *Biochem. Systematic. Ecol.* **1997**, *25*, 513.
17. Duan, H.; Takaishi, Y.; Bando, M.; Kido, M.; Imakura, Y.; Lee, K. *Tetrahedron Lett.* **1999**, *40*, 2969.
18. Pérez-Victoria, J. M.; Tincusi, B. M.; Jiménez, I. A.; Bazzocchi, I .L.; Gupta, M. P.; Castanys, S.; Gamarro, F.; Ravelo, A. G. *J. Med. Chem.* **1999**, *42*, 4388.
19. Takaishi, Y.; Ujita, K.; Tokuda, H.; Nishino, H.; Iwashima, A.; Fujita, T. *Cancer Lett.* **1992**, *65*, 19.
20. Takaishi, Y.; Ohshima, S.; Nakano, K.; Tomimatsu, T. *J. Nat. Prod.* **1993**, *56*, 815.
21. Ujita, K.; Takaishi, Y.; Tokuda, H.; Nishino, H.; Iwashima, A.; Fujita, T. *Cancer Lett.* **1993**, *68*, 129.
22. Becerra, J.; Gaete, L.; González, F.; Silva, M.; Bohlman, F.; Jakupovio, J. Nuevos eudesmanos aislados de semillas de *Maytenus boaria* Mol. Libro de Resúmenes XVIII Congreso Latinoamericano de Química 1988, Santiago de Chile: Chile.
23. Wada, H.; Shizuri, Y.; Suguria, K.; Yamada, K.; Hirata, Y. *Tetrahedron Lett.* **1971**, *33*, 3131.
24. Katsuhiro, U.; Naoto, M.; Iwao, M. *Phytochemistry* **1993**, *33*, 1993.
25. Kupchan, S. M.; Smith, R. M. *J. Org. Chem.* **1977**, *42*, 115.
26. Percentages of EBV-EA induction in the presence of glycyrrhetic acid were 15.6, 54.3, 100, and 100% at 1×10^3, 5×10^2, 1×10^2, and 1×10 mol ratio/TPA, respectively, and the viability percentage of Raji cells was more than 80% at each concentration.
27. Ohigashi, H.; Takamura, H.; Koshimizu, K.; Tokuda, H.; Ito, Y. *Cancer Let.* **1986**, *30*, 143.