3-Epicabraleahydroxylactone and Other Triterpenoids from Camellia Oil and Their Inhibitory Effects on Epstein–Barr Virus Activation

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The structure of a triterpenoid isolated from the nonsaponifiable lipid (NSL) of the seed oil of the camellia (Camellia japonica L.; Theaceae) was established to be (20S)-3β-hydroxy-25,26,27-trisnordammaran-24,20-olide (1; 3-epicabraleahydroxylactone) on the basis of spectroscopic and chemical methods. Six other triterpenoids isolated from the NSL were identified as 3-epicabraleadiol (2), ocotillol II (3), ocotillol I (4), dammarenediol II (5), (20R)-taraxastane-3β,20-diol (6), and lupane-3β,20-diol (7). Upon evaluation of the seven triterpenoids (1—7) with respect to their inhibitory effects on the induction of Epstein–Barr virus early antigen (EBV–EA) by 12-O-tetradecanoylphorbol-13-acetate (TPA) in Raji cells, three compounds (5—7) showed potent inhibitory effects against EBV–EA induction (IC50 values of 277—420 mol ratio/32 pmol TPA).

Key words Camellia japonica; 3-epicabraleahydroxylactone; antitumor promoter; triterpenoids; Epstein–Barr Virus early antigen

In our recent study on the monohydroxy triterpenoid (triterpene monol) constituents of the nonsaponifiable lipid (NSL) fraction of camellia oil from Camellia japonica L. (Theaceae) and related sasanka oil from C. sasanka Thumb., we have isolated and characterized 27 tetracyclic and pentacyclic triterpenoids1–3 and six incompletely cyclized triterpenoids.2–5 Upon evaluation of the antiinflammatory effects on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice, 14 tetracyclic and pentacyclic triterpenoids were shown to have inhibitory effects.1 In this paper, we report the isolation and characterization of a triterpenoid, 3-epicabraleahydroxylactone [1; (20S)-3β-hydroxy-25,26,27-trisnordammaran-24,20-olide], which is known as a semisynthetic compound6 but is a new natural product, along with six known triterpenoids, 3-epicabraleadiol (2), ocotillol II (3), ocotillol I (4), dammarenediol II (5), (20R)-taraxastane-3β,20-diol (6), and lupane-3β,20-diol (7), from the dihydroxy triterpenoid (triterpene diol) fraction of the NSL fraction obtained from camellia oil, as well as their inhibitory effects on Epstein–Barr virus early antigen (EBV–EA) activation induced by TPA, as a primary screening for antitumor promoters.

Seven triterpenoids, 1—7, among which compound 1 was a new naturally occurring compound, were isolated and characterized from the dihydroxy triterpenoid fraction obtained from the NSL fraction of camellia oil in this study. Compound 6 was previously isolated from Canarium strictum (Dipterocarpaceae)7 and from Mangifera indica (Anacardiaceae),8 but the stereochemistry at C-20 remained undetermined. Characterization of a new naturally occurring triterpenoid 1 and the determination of the stereochemistry at C-20 of 6, as described below, were performed on their 3-acetyl derivatives, 1a and 6a, respectively.

The 13C- and 1H-NMR spectra (Table 1) of compound 1a (C39H46O4) showed the presence of a γ-lactone ring,9 a tertiary methyl adjacent to the lactone ring, five other tertiary methyls, and a secondary acetoxyl group oriented equatorially (β) at C-3.10 The 1H-NMR signals for the ring system protons of 1a were very close to those for the corresponding 1H signals of 3-epicabraleadiol 3-acetate (2a).10a,b Further, analysis of 13C distortionless enhancement by polarization transfer (DEPT)-NMR, 1H–1H correlation spectroscopy (COSY), H-detected multiple-quantum coherence (HMQC), and heteronuclear multiple-quantum coherence (HMBD) spectra of 1a enabled us to establish its structure as 3β-ace-toxy-25,26,27-trisnordammaran-24,20-olide. The phase-sensitive nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum of compound 1a showed significant NOE correlations between (H-29–H-19–H-18–H-13α–H-21) on the β-face, and (H-28–H-3α–H-5α–H-9α–H-17α) on the α-face of the molecule, which supported the proposed structure. The structure of 1a was finally confirmed to be (20S)-3β-acetoxy-25,26,27-trisnordammaran-24,20-olide (3-epicabraleahydroxylactone 3-acetate) by direct comparison of the MS and 1H-NMR data with semisynthetic 2a prepared from 2a (20S, 24S) by chromium trioxide oxidation of its 25-hydroxy-20,24-epoxydized C20-side chain into the trisnor-γ-lactonized C20-side chain,11 as described in Experimental.

Compound 6a (C39H46O4) was identified as taraxastane-3β,20-diol 3-acetate12 by MS and 1H-NMR (Table 1) comparison. Compound 6a exhibited definite NOE correlations between [H-24–H-25–H-26–H-13β–H-21] on the β-face, and (H-23–H-3α–H-5α–H-9α–H-27–H-18α) on the α-face of the molecule in the phase-sensitive NOESY spectrum, which indicated that the methyl group at C-20 is oriented to the β-face of the ring system. Thus we conclude that the compound is (20S/α)-taraxastane-3β,20-diol 3-acetate.

This study demonstrated the presence of a triterpenoid 1 in the NSL fraction of camellia oil. Although compound 1 has previously been semisynthesized from its C3α epimer, cabrareahydroxylactone [(20S)-3α-hydroxy-25,26,27-trisnordammaran-24,20-olide],6 a component of Cabrlea polytricha9 and Cabrlea eichleriana (Meliaceae),13 to the best of our knowledge this is the first instance of its isolation from
a natural source. Lactones are susceptible to hydrolytic ring opening in basic solution, and the isolation of compound 1 in this study may be attributed to its elution from the base-catalyzed saponification of the oil.

The inhibitory effects of compounds 1—7 on EBV-EA activation induced by TPA were examined for the primary screening of antitumor-promoting activities, and the results are shown in Table 2. Compounds 5—7 showed potent inhibitory effects, with IC50 values of 277—420 mol ratio/32 pmol TPA, while preserving high viability of Raji cells. The inhibitory effects of compounds 5—7 were found to be almost equivalent to those of β-carotene (IC50 value 397 mol...
performed by 13C-NMR comparison with data in the literature. 16) TP A was agents.

Crude camellia seed oil was prepared at Oshima Tsubaki Co. (Tokyo, Japan) KOH in MeOH) were performed at room temperature overnight. Pressed alkaline hydrolysis of in 2000. Reference 3-epicabraleadiol [7-]

carbamoyl reagents, purchased from ChemSyn Laboratories (Lenexa, KS, U.S.A.). The cell cul-

ative index detector was used for reverse-phase HPLC. Alkaline hydrolysis of chroma
tography (HPLC) was carried out on a 25 cm

ated by spectral comparison with reference com-
mounts. The following three compounds were identified by spectral compar-

Experimental Crystallizations were performed from methanol (MeOH), and melting


crude camellia seed oil was prepared at Oshima Tsubaki Co. (Tokyo, Japan) from the seeds of C. japonica L. cultivated on Oshima Island (Tokyo, Japan) in 2000. Reference 3-epicabraleadiol [2, (20S,24R)-20,24-epoxydammarane-3β,25-diol] was isolated from chrysanthemum flower. 10) Dammarenediol II (3.2 mg) from fraction B3; 3-epicabraleadiolhydroxylactone 3-acetate (1.0 mg), ocotillol II 3-acetate (4.5 mg), and (20R)-taraxastane-3β,20-diol 3-acetate (6a; 4.5 mg, tR 48.4 min) from fraction B4; and 1a (12.2 mg) from fraction B5. Alkaline hydrolysis of the acetates 1a—7a afforded the corresponding free alcohols 1—7.

Preparation of 3-Epicabraleadiolhydroxylactone 3-Acetate (1a) 3-Epicabraleadiol 3-acetate (2a; 10 mg) was added to a stirred solution of CrO3 (5 mg) in pyridine (2 ml). After stirring for 48 h, water was added and the product was extracted with diethyl ether and washed with water. The solvent was evaporated and the residue was subjected to preparative HPLC to give 1a (2 mg; mp 230—233 °C). The MS and 1H-NMR data of the semi-
synthetic 1a were essentially the same as those of 1a isolated from the NSL fraction of camellia oil. Identification and Characterization Identification of 2 and 5 was performed by spectral (MS, 1H-, 13C-NMR) comparison with reference com-

Table 2. Percentage of Epstein–Barr Virus Early Antigen Induction in the Presence of 1—7 with Respect to a Positive Control (100%)a,b,c

<table>
<thead>
<tr>
<th>Concentration (mol ratio/32 pmol TPA)</th>
<th>Ic50 (mol ratio/32 pmol TPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>1 3-Epicabraleahydroxylactone</td>
<td>18.4±0.9 (60)</td>
</tr>
<tr>
<td>2 3-Epicabraleadiol</td>
<td>16.3±0.7 (60)</td>
</tr>
<tr>
<td>3 Octillol II</td>
<td>17.7±0.5 (60)</td>
</tr>
<tr>
<td>4 Octillol I</td>
<td>16.0±0.7 (60)</td>
</tr>
<tr>
<td>5 Dammarenediol II</td>
<td>0.2±0.3 (70)</td>
</tr>
<tr>
<td>6 (20R)-Taraxastane-3β,20-diol</td>
<td>1.1±0.3 (70)</td>
</tr>
<tr>
<td>7 Lupane-3β,20-diol-β-Carotene</td>
<td>0.2±0.2 (70)</td>
</tr>
<tr>
<td></td>
<td>8.6±0.4 (70)</td>
</tr>
</tbody>
</table>

a) Values represent relative percentages to the positive control value (n=3, and ± S.D.). TP A (32 pmol, 20 ng)=100%. Values in parentheses are viability percentages of Raji cell.
b) Ic50 represents the mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol TPA. c) Reference compound.

eratio/32 pmol TPA), which has been intensively studied in cancer prevention using animal models. 13) It appears that epoxidation at C-20—C-24 of the side chain leads to a decrease in the activity as observed for the dammarane triterpenoids (1—5). The inhibitory effects against EBV-EA activation have been demonstrated to be closely parallel to those against tumor promotion in vitro, and the triterpenoids 5—7 from camellia oil were therefore suggested to be valuable anticancer promoters (potential cancer chemopreventive agents).

Extraction and Isolation Alkaline hydrolysis of the camellia oil (4.4 kg) followed by disopropyl ether extraction yielded a neutral NSL fraction (14.2 g). The NSL was chromatographed on a silica gel (500 g) column with stepwise gradient of n-hexane–EtOAc (1 : 0; 95 : 5; 9 : 1; 4 : 1; 1 : 0; 1 : 0) as eluant. n-Hexane–EtOAc (9 : 1) eluted a fraction (7.6 g; fraction A) and n-hexane–EtOAc (1 : 1) eluted a fraction (1.8 g; fraction B). Upon acetyla-
tion, fractions A and B gave the corresponding acetate fractions A (8.0 g) and B (1.5 g). Fraction A acetate contained acetylated triterpene monols of which the isolation and characterization have been reported recently. 1—5) Upon column chromatography on silica gel [silica gel, 70 g; eluant, n-hexane–EtOAc (4 : 1)], the fraction B acetate yielded six fractions with the ascending order of polarity: fractions B1 (292 mg), B2 (201 mg), B3 (112 mg), B4 (126 mg), B5 (107 mg), and B6 (212 mg). Fractions B1—B6 were subjected to preparative HPLC which yielded: dammarenediol II 3-acetate (5a; 67.0 mg, tR 28.9 min) from fraction B1; 3-epicabraleadiol 3-acetate (2a; 11.0 mg, tR 31.4 min), octillol 1 3-acetate (4a; 2.0 mg, tR 33.4 min), 5a (5.0 mg), and lupane-3β,20-diol (7a; 5.4 mg, tR 37.8 min) from fraction B2; 2a (1.0 mg), octillol 2 3-acetate (3a; 3.3 mg, tR 29.2 min), and 7a (3.2 mg) from fraction B3; 3-epicabraleadiolhydroxylactone 3-acetate (1a; 3.7 mg, tR 12.5 min), 3a (0.5 mg), and (20R)-taraxastane-3β,20-diol 3-acetate (6a; 4.5 mg, tR 48.4 min) from fraction B4; and 1a (12.2 mg) from fraction B5. Alkaline hydrolysis of the acetates 1a—7a afforded the corresponding free alcohols 1—7.
moved. The activated cells were stained with high-titer EBV-EA-positive sera from nasopharyngeal carcinoma patients, and the conventional indirect immunofluorescence technique was employed for detection. In each assay, at least 500 cells were counted and the experiments were repeated three times. The mean extent of EA induction was determined and compared with that on positive control experiments in which the cells were treated with n-butyric acid plus TPA where the extent of EA induction was ordinarily more than around 40%. The viability of treated Raji cells was assayed by the Trypan blue staining method.22)

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