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 (20*S*)-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), (20*R*)-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 (IC_{50} 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 sasanqua oil from *C. sasanqua* THUNB., we have isolated and characterized 27 tetracyclic and pentacyclic triterpenoids[1] 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**; (20*S*)-3β-hydroxy-25,26,27-trisnordammaran-24,20-olide], which is known as a semisynthetic compound[6] but is a new natural product, along with six known trieterpenoids, 3-epicabraleadiol (**2**), ocotillol II (**3**), ocotillol I (**4**), dammarenediol II (**5**), (20*R*)-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 ^{13}C- and ^{1}H-NMR spectra (Table 1) of compound **1a** ($C_{29}H_{46}O_4$) 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.[1] The ^{1}H-NMR signals for the ring system protons of **1a** were very close to those for the corresponding ^{1}H signals of 3-epicabraleadiol 3-acetate (**2a**).[10] Furthermore, analysis of ^{13}C distortionless enhancement by polarization transfer (DEPT)-NMR, ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H-detected multiple-quantum coherence (HMQC), and heteronuclear multiple-quantum coherence (HMBC) spectra of **1a** enabled us to establish its structure as 3β-acetoxy-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-13β–H-21) on the β-face, and (H-28–H-3α–H-5α–H-9α–H-30–H-17α) on the α-face of the molecule, which supported the proposed structure. The structure of **1a** was finally confirmed to be (20*S*)-3β-acetoxy-25,26,27-trisnordammaran-24,20-olide (3-epicabraleahydroxylactone 3-acetate) by direct comparison of the MS and ^{1}H-NMR data with semisynthetic **1a** (20*S*) prepared from **2a** (20*S*, 24*S*) by chromium trioxide oxidation of its 25-hydroxy-20,24-epoxydized C_8-side chain into the trisnor-γ-lactonized C_5-side chain,[11] as described in Experimental.

Compound **6a** ($C_{32}H_{54}O_3$) was identified as taraxastane-3β,20ξ-diol 3-acetate[7,8] by MS and ^{1}H-NMR (Table 1) comparison. Compound **6a** exhibited definite NOE correlations between [H-24–H-25–H-26–H-13β–(H-19β–)H-28–H-30] 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 (20*S*/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 C-3α epimer, cabrareahydroxylactone [(20*S*)-3α-hydroxy-25,26,27-trisnordammaran-24,20-olide],[6] a component of *Cabralea polytricha*[6] and *Cabralea eichleriana* (Meliaceae),[12] to the best of our knowledge this is the first instance of its isolation from

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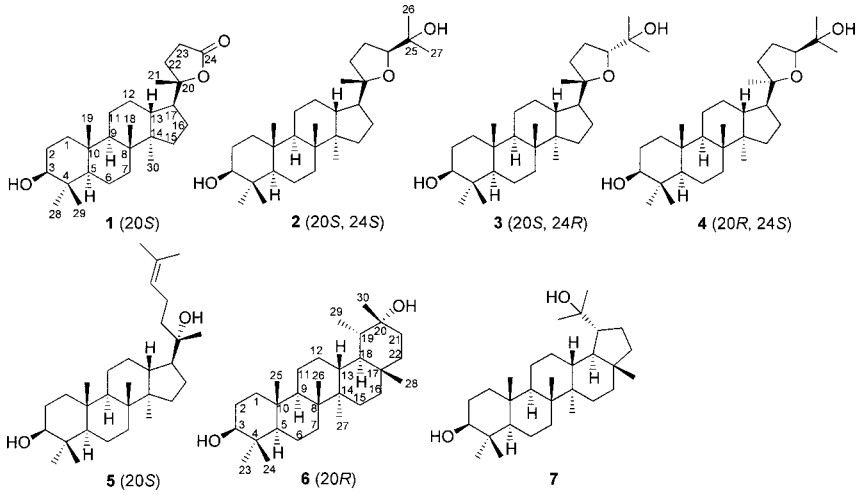

Chart 1. Structures of Compounds **1**—**7** from the Nonsaponifiable Lipid Fraction of Camellia Oil

Table 1. ^{13}C- and ^{1}H-NMR Spectral Data for Triterpene 3-Acetates **1a** and **6a** ($CDCl_3$)

Atom no.	**1a**		**6a**	
	δ_C	δ_H [a]	δ_C	δ_H [a]
1	38.8	1.04 (α), 1.66 (β)	38.4	0.99 (α), 1.64 (β)
2	23.8	1.68 (α), 1.56 (β)	23.8	1.62 (2H)
3	80.9	4.47 (dd, 5.7, 10.8)	81.1	4.47 (dd, 5.7, 10.8)
4	38.0		37.8	
5	56.1	0.82	55.3	0.78 (br d, 9.6)
6	18.2	1.54 (α), 1.44 (β)	18.3	1.50 (α), 1.35 (β)
7	35.5	1.55 (α), 1.26 (β)	34.4	1.34 (α), 1.39 (β)
8	40.5		41.3	
9	50.6	1.33	49.4	1.25
10	37.1		36.9	
11	21.5	1.50 (α), 1.20 (β)	21.4	1.44 (α), 1.26 (β)
12	26.9	1.23 (α), 1.75 (β)	28.5	1.15 (α), 1.99 (β)
13	43.2	1.57	38.9	1.82 (ddd, 4.1, 10.1, 13.3)
14	50.2		43.1	
15	31.2	1.12 (α), 1.51 (β)	26.6	0.97 (α), 1.73 (β)
16	25.1	1.82 (2H)	38.4	1.32 (α) 1.17 (β)
17	49.4	1.98 (ddd, 5.7, 10.3, 16.5)	35.7	
18	15.5	0.95 (s)	48.0	1.02
19	16.3	0.86 (s)	42.0	1.51
20	90.2		75.3	
21	25.4	1.35 (s)	37.8	1.47 (α), 1.70 (β)
22	31.3	1.92 (α, ddd, 4.6, 10.3, 12.8) 2.11 (β, ddd, 9.2, 10.3, 12.8)	40.3	1.28 (α), 1.28 (β)
23	29.2	2.54 (α, ddd, 4.6, 10.3, 18.3) 2.64 (β, ddd, 9.2, 10.3, 18.3)	28.0	0.85 (s)
24	176.8		16.6	0.84 (s)
25			16.2	0.86 (s)
26			16.1	1.03 (s)
27			14.7	0.93 (s)
28	28.0	0.85 (s)	18.5	0.89 (s)
29	16.6	0.84 (s)	17.4	1.05 (d, 6.2)
30	16.4	0.88 (s)	21.5	1.07 (s)
COMe	21.3	2.04 (s)	21.4	2.03 (s)
COMe	171.1		171.1	

a) Figures in parentheses denote *J* values (Hertz).

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 IC_{50} 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 (IC_{50} value 397 mol

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

Compound		Concentration (mol ratio/32 pmol TPA)				IC_{50}[b)]
		1000	500	100	10	(mol ratio/32 pmol TPA)
1	3-Epicabraleahydroxylactone	18.4±0.9 (60)	59.0±1.7	87.3±2.1	100±0.3	587
2	3-Epicabraleadiol	16.3±0.7 (60)	56.1±1.5	83.5±2.0	100±0.2	553
3	Ocotillol II	17.7±0.5 (60)	58.0±1.7	85.7±2.1	100±0.2	571
4	Ocotillol I	16.0±0.7 (60)	53.0±1.2	82.1±1.7	100±0.4	525
5	Dammarenediol II	0±0.3 (70)	27.5±1.2	71.3±1.8	90.2±0.5	300
6	(20*R*)-Taraxastane-3β,20-diol	1.1±0.3 (70)	42.3±1.5	72.3±1.9	100±0.3	420
7	Lupane-3β,20-diol	0±0.2 (70)	22.2±1.3	68.4±1.7	91.7±0.5	277
	β-Carotene[c)]	8.6±0.4 (70)	34.2±1.4	82.1±2.0	100±0.3	397

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

ratio/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 vivo*,[14] and the triterpenoids **5**—**7** from camellia oil were therefore suggested to be valuable antitumor promoters (potential cancer chemopreventive agents).

Experimental

Crystallizations were performed from methanol (MeOH), and melting points are uncorrected. Optical rotations were measured on a JASCO DIP-370 polarimeter in $CHCl_3$ at 25 °C. IR spectra were recorded on a JASCO IR-300 spectrometer in KBr disks. NMR spectra were recorded with a JEOL LA-500 spectrometer at 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C-NMR), unless otherwise stated, in $CDCl_3$ with tetramethylsilane (TMS; ^{1}H-NMR) and $CDCl_3$ at δ 77.0 (^{13}C-NMR) as internal standards. EI-MS and HR-MS were recorded on JEOL JMS-GC mate spectrometer (70 eV) using a direct inlet system. Silica gel (Silica gel 60, 220—400 mesh, Merck) was used for open-column chromatography. Reverse-phase preparative high-performance liquid chromatography (HPLC) was carried out on a 25 cm×10 mm i.d. Pegasil ODS II (Senshu Scientific Co., Ltd., Tokyo, Japan) C_{18} silica column at 25 °C with MeOH–H_2O (95 : 5, v/v) as mobile phase at 3 ml/min. A refractive index detector was used for reverse-phase HPLC. Alkaline hydrolysis of the oil was performed with 5% (w/v) KOH in MeOH under reflux for 3 h. Acetylation (acetic anhydride–pyridine) and hydrolysis of acetates (5% KOH in MeOH) were performed at room temperature overnight. Pressed 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**; (20*S*,24*S*)-20,24-epoxydammarane-3β,25-diol] was isolated from chrysanthemum flower.[10] Dammarenediol II [**5**; (20*R*)-dammar-24-ene-3β,20-diol] was isolated from dammar resin, which was courteously donated by Ogawa & Co., Ltd. (Tokyo, Japan), by the procedure described in the literature,[15] followed by preparative HPLC as the acetyl derivative **5a** [retention time (t_R) 28.9 min]. Identification of **5a** was performed by ^{13}C-NMR comparison with data in the literature.[16] TPA was purchased from ChemSyn Laboratories (Lenexa, KS, U.S.A.). The cell culture reagents, *n*-butyric acid, and other reagents were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Extraction and Isolation Alkaline hydrolysis of the camellia oil (4.4 kg) followed by diisopropyl 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 : 1, 0 : 1; v/v) 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 acetylation, 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, t_R 28.9 min) from fraction B1; 3-epicabraleadiol 3-acetate (**2a**; 11.0 mg, t_R 31.4 min), ocotillol I 3-acetate (**4a**; 2.0 mg, t_R 33.4 min), **5a** (5.0 mg), and lupane-3β,20-diol (**7a**; 5.4 mg, t_R 37.8 min) from fraction B2; **2a** (1.0 mg), ocotillol II 3-acetate (**3a**; 3.3 mg, t_R 29.2 min), and **7a** (3.2 mg) from fraction B3; 3-epicabraleadiolhydroxylactone 3-acetate (**1a**; 3.7 mg, t_R 12.5 min), **3a** (0.5 mg), and (20*R*)-taraxastane-3β,20-diol 3-acetate (**6a**; 4.5 mg, t_R 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 CrO_3 (5 mg) in pyridine (2 ml).[11] 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 ^{1}H-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, ^{1}H-, ^{13}C-NMR) comparison with reference compounds. The following three compounds were identified by spectral comparison with the literature: **3** (MS, ^{1}H-, ^{13}C-NMR),[3,17,18] **4** (MS and ^{1}H-NMR),[19] and **7** (MS, ^{1}H-, ^{13}C-NMR).[20] The stereochemistry at C-20 of compound **6**, identified by MS and ^{1}H-NMR comparison with those of taraxastane-3β,20-diol[7,8,21] and by two-dimensional NMR experiments, was determined based on a NOESY experiment. The spectral data along with some physical characteristics are given below for compounds **1** and **6**, along with those for their 3-acetyl derivatives.

3-Epicabraleahydroxylactone (**1**) and Its 3-Acetate (**1a**) **1**: Fine needles, mp 155—157 °C (lit.[6] 216—219 °C). $[\alpha]_D^{25}$ +6.7° (c=0.21, $CHCl_3$). IR ν_{max} cm^{-1}: 3442 (OH), 1766 (γ-lactone). ^{1}H-NMR: δ: 0.78 (3H, s, H-29), 0.86 (3H, s, H-19), 0.89 (3H, s, H-18), 0.96 (3H, s, H-30), 0.98 (3H, s, H-28), 1.36 (3H, s, H-21), 3.20 (1H, dd, J=5.1, 10.1 Hz, H-3α). EI-MS m/z (%): 416 (M^+, 7), 398 (49), 383 (16), 355 (41), 315 (21), 299 (18), 247 (10), 229 (12), 220 (12), 207 (43), 189 (88), 95 (100). HR-MS: m/z 416.3275 [Calcd for $C_{27}H_{44}O_3$ (M^+): 416.3290]. **1a**: Fine needles, mp 229—231 °C (lit.[6] 244—246 °C). $[\alpha]_D^{25}$ −15.7° (c=0.14, $CHCl_3$). IR ν_{max} cm^{-1}: 1765 (γ-lactone), 1730 (OAc). ^{13}C- and ^{1}H-NMR spectra, see Table 1. EI-MS m/z (%): 458 (M^+, 3), 398 (46), 383 (12), 355 (15), 299 (13), 249 (7), 217 (7), 203 (19), 189 (100), 175 (22). HR-MS: m/z 458.3384 [Calcd for $C_{29}H_{46}O_4$ (M^+): 458.3396].

(20*R*)-Taraxastane-3β,20-diol (**6**) and Its 3-Acetate (**6a**) **6**: Fine needles, mp 259—262 °C (epi-ψ-taraxastanediol, lit.[7] 261—263 °C; ψ-taraxastanediol, lit.[8] 270—273 °C). $[\alpha]_D^{25}$ +4.0° (c=0.20, $CHCl_3$). **6a**: Fine needles, mp 254—257 °C. $[\alpha]_D^{25}$ +2.4° (c=0.25, $CHCl_3$).

***In Vitro* EBV-EA Activation Experiment** The inhibition of EBV-EA activation was assayed using Raji cells (EBV genome-carrying human lymphoblastoid cells, nonproducer type), cultivated in 10% fetal bovine serum (FBS) RPMI-1640 medium (Sigma, St. Louis, MO, U.S.A.). The indicator cells (Raji cells; 1×10^6 cells/ml) were incubated in 1 ml of the medium containing 4 mM *n*-butyric acid as an inducer, 32 pM of TPA [20 ng/ml in dimethyl sulfoxide (DMSO)], and a known amount (32, 16, 3.2, 0.32 nmol) of the test compound at 37 °C in a CO_2 incubator. After 48 h, the cell suspensions were centrifuged at 1000 rpm for 10 min, and the supernatant was re-

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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References

1) Akihisa T., Yasukawa K., Kimura Y., Takase S., Yamanouchi S., Tamura T., *Chem. Pharm. Bull.*, **45**, 2016—2023 (1997).
2) Akihisa T., Yasukawa K., Kimura Y., Yamanouchi S., Tamura T., *Phytochemistry*, **48**, 301—305 (1998).
3) Akihisa T., Kimura Y., Koike K., Shibata T., Yoshida Z., Nikaido T., Tamura T., *J. Nat. Prod.*, **61**, 409—412 (1998).
4) Akihisa T., Arai K., Kimura Y., Koike K., Kokke W. C. M. C., Shibata T., Nikaido T., *J. Nat. Prod.*, **62**, 265—268 (1999).
5) Akihisa T., Koike K., Kimura Y., Sashida N., Matsumoto T., Ukiya M., Nikaido T., *Lipids*, **34**, 1151—1157 (1999).
6) Cascon S. C., Brown K. S., Jr., *Tetrahedron*, **28**, 315—323 (1972).
7) Hinge V. K., Paknikar S. K., Jr., Das K. G., Bose A. K., *Tetrahedron*, **22**, 2861—2868 (1966).
8) Anjaneyulu V., Prasad H., Ravi K., Connolly J. D., *Phytochemistry*, **24**, 2359—2367 (1985).
9) Nagaya H., Tobita Y., Nagai T., Itokawa H., Takeya K., Halim A. F., Abdel-Halim O. B., *Phytochemistry*, **44**, 1115—1119 (1999).
10) Ukiya M., Akihisa T., Yasukawa K., Kasahara Y., Kimura Y., Koike K., Nikaido T., Takido M., *J. Agric. Food Chem.*, **49**, 3187—3197 (2001).
11) Warnhoff E. W., Halls C. M. M., *Can. J. Chem.*, **43**, 3311—3321 (1965).
12) Rao M. M., Heshulam H., Zelnik R., Lavie D., *Tetrahedron*, **31**, 333—339 (1975).
13) Murakami A., Ohigashi H., Koshimizu K., *Biosci. Biotech. Biochem.*, **60**, 1—8 (1999).
14) Akihisa T., Yasukawa K., "Studies in Natural Products Chemistry, Vol. 25, Bioactive Natural Products (Part F)," ed. by Atta-ur-Rahman, Elsevier Science B.V., Amsterdam, 2001, pp. 43—87.
15) Mills J. S., *J. Chem. Soc.*, **1956**, 2196—2203 (1956).
16) Asakawa J., Kasai R., Yamasaki K., Tanaka O., *Tetrahedron*, **33**, 1935—1939 (1977).
17) Gonzalez A. G., Cortes M., Suarez E., *Tetrahedron Lett.*, **1974**, 2791—2792 (1974).
18) Ohmoto T., Nikaido T., Ikuse M., *Chem. Pharm. Bull.*, **26**, 1437—1442 (1978).
19) Fuchino H., Konishi S., Satoh T., Yagi A., Saitsu K., Tatsumi T., Tanaka N., *Chem. Pharm. Bull.*, **44**, 1033—1038 (1996).
20) Ulubelen A., Topcu G., Lotter H., Wagner H., Eris C., *Phytochemistry*, **36**, 413—415 (1994).
21) Anjaneyulu V., Satyanarayana P., Viswanadham K. N., Jyothi V. G., Rao K. N., Radhika P., *Phytochemistry*, **50**, 1229—1236 (1999).
22) Takaishi Y., Ujita K., Tokuda H., Nishino H., Iwashima A., Fujita T., *Cancer Lett.*, **65**, 19—26 (1992).