Sesquiterpenoids, Triterpenoids, and Flavonoids from the Fruits of *Schinus molle*

Masateru ONO^{1*}, Masakazu YAMASHITA¹, Kaori MORI¹, Chikako MASUOKA¹, Masashi ETO¹, Junei KINJO², Tsuyoshi IKEDA³, Hitoshi YOSHIMITSU⁴, and Toshihiro NOHARA⁴

¹ School of Agriculture, Tokai University, 5435 Minamiaso, Aso, Kumamoto 869-1404, Japan

² Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

³ Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan

⁴ Faculty of Pharmaceutical Sciences, Sojo University, 4-22-2 Ikeda, Kumamoto 860-0082, Japan

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Twelve sesquiterpenoids, one of which was a new compound, six tirucallane-type triterpenoids, and four flavonoids were isolated from the fruits of *Schinus molle* L. (Anacardiaceae). Their chemical structures were determined on the basis of spectroscopic data and X-ray analysis. Among them, three flavonoids exhibited almost identical antioxidative activity as that of α -tocopherol by the ferric thiocyanate method. In addition, one flavonoid showed a stronger radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl than that of α -tocopherol.

Keywords: *Schinus molle*, Anacardiaceae, sesquiterpenoid, triterpenoid, flavonoid, antioxidative activity, radical-scavenging effect

Introduction

Schinus molle L. (Anacardiaceae) is native to South and Central America, but has been acclimated to most of the tropical and subtropical areas of the world. The fruits of this plant are used as a pepper substitute and a source of vinegar and beverages. Furthermore, the fruits, leaves, and sap of the trunk are used as traditional medicines in Peru (Olfasson *et al.*, 1997). With regard to the chemical constituents of the fruits, tirucallane- and euphane-type triterpenoids, an aromadendrane-type sesquiterpenoid, and a biflavonoid have been previously reported (Terhune *et al.*, 1974; Pozzo-Balbi *et al.*, 1978; Olfasson *et al.*, 1997; Yueqin *et al.*, 2003). In the course of our studies on the constituents of Peruvian plants (Ono *et al.*, 2008), we have investigated the constituents of the fruits of *S. molle*. Here we report the isolation and structural elucidation of a new sesquiterpenoid along with 11 known sesquiterpenoids, six known tirucallane-type triterpenoids, and four known flavonoids, as well as the antioxidative activity of the flavonoids.

Materials and Methods

General experimental procedures Optical rotations were

measured with a JASCO DTP-1000 KUY digital polarimeter (JASCO, Tokyo, Japan). Proton (¹H)- and carbon-13 (¹³C)-nuclear magnetic resonance (NMR) spectra were recorded using a JEOL Alpha 500 spectrometer (JEOL, Tokyo, Japan). Mass spectra were obtained on a JEOL JMS-DX-303HF instrument (JEOL). Visible absorptions were measured with a JASCO UV-530 UV/vis spectrometer (JASCO). Column chromatography was carried out over silica gel 60 (Merck, Art. 1.07734 and Art. 1.09385; Merck, Darmstadt, Germany), Diaion HP20 (Mitsubishi Chemical Industries Co., Ltd., Tokyo, Japan), MCI gel CHP20P (Mitsubishi Chemical Industries Co., Ltd.), Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden), Chromatorex ODS (Fuji Silysia Chemical Ltd., Aichi, Japan), and Bio-Beads SX2 (BIO-RAD Laboratories, Hercules, CA, USA). High performance liquid chromatography (HPLC) separation was run on a Micro pump LC-10AS (Shimadzu, Kyoto, Japan) with a RI-Detector RID-10A (Shimadzu). For HPLC column chromatography, a COSMOSIL 5C18 AR-II column (Nacalai Tesque, Inc., Kyoto, Japan, 250 mm × 20 mm i. d.) was used. Linoleic acid was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan) and the other reagents were purchased from Nacalai Tesque, Inc.

Plant material Dried fruits of *Schinus molle* L. were purchased in October (1993) from Fundation Pala la Inves-

*To whom correspondence should be addressed.

Email: mono@agri.u-tokai.ac.jp

tigation Tecnologica del Recurso Agrobiologico Andio, a research institute of Andes Agricultural Bioresources in Peru, and identified by Sokurates Shiota, Executive Director, Fundation Pala la Investigation Tecnologica del Recurso Agrobiologico Andio.

Extraction and isolation The dried and powdered fruits of *S. molle* (720 g) were extracted with methanol (MeOH) (1000 ml $\times$ 6) under reflux for 2 h, and the solvent was removed under reduced pressure to give a syrup (148.7 g). The MeOH extract was chromatographed over a Diaion HP20 column (50% MeOH, MeOH, acetone) to give Fraction 1 (70.72 g), Fraction 2 (47.59 g), and Fraction 3 (19.60 g). Fraction 2 was treated with hexane (755 ml) to yield a hexane-soluble fraction (15.50 g) and a hexane-insoluble fraction (30.42 g). Chromatography of the hexane-insoluble fraction over a silica gel column [Art. 1.07734, CHCl₃-MeOH-H₂O (14:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1, 0:1:0)] furnished Fractions 4 - 13. Fraction 4 (4366 mg) was subjected to silica gel column chromatography [Art. 1.09385, hexane-acetone (20:1, 10:1, 5:1, 3:1, 1:1, 0:1), MeOH] to afford Fractions 14 - 20. Fraction 17 (553 mg) was separated by chromatography over a Chromatorex ODS column (80% MeOH, 90% MeOH, MeOH) to give Fractions 21 - 24. High performance liquid chromatography (COSMOSIL 5C18 AR-II, 95% MeOH) of Fraction 23 gave **4** (131 mg). Fraction 18 (918 mg) was separated by chromatography over a Chromatorex ODS column (90% MeOH, MeOH) to furnish **18** (290 mg) and Fractions 25 - 27. Chromatography of Fraction 26 (262 mg) over a silica gel column [Art. 1.09385, CHCl₃, MeOH] gave **3** (25 mg). Fraction 6 (3502 mg) was separated by chromatography over a Chromatorex ODS column (70% MeOH, MeOH) to give Fractions 28 - 35. Fractions 29 (79 mg), 30 (21 mg), 32 (93 mg), and 34 (78 mg) were each subjected to HPLC under similar conditions (70% MeOH) to Fraction 23 to give **16** (29 mg) and **11** (12 mg) from Fraction 29, **12** (8 mg) from Fraction 30, **1** (6 mg) and **14** (61 mg) from Fraction 32, and **9** (20 mg) and **10** (15 mg) from Fraction 34. Fraction 7 (2480 mg) was separated by chromatography over a Chromatorex ODS column (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) to yield Fractions 36 - 44. Fractions 37 (41 mg), 40 (85 mg), and 41 (87 mg) were each subjected to HPLC under the same conditions as for Fraction 29 to yield **16** (11 mg) from Fraction 37, **1** (41 mg) and **14** (8 mg) from Fraction 40, and **10** (18 mg) and **9** (25 mg) from Fraction 41. Similar HPLC (65% MeOH) of Fraction 39 (79 mg) to Fraction 23 furnished **13** (21 mg). Fraction 8 (4757 mg) was subjected to Sephadex LH-20 column chromatography (MeOH) to give **22** (1442 mg). Fraction 9 (4329 mg) was separated by chromatography over a Chromatorex ODS column (50% MeOH, 60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH,

MeOH) to give Fractions 45 - 51. Fraction 46 (223 mg) and Fraction 50 (115 mg) were each subjected to HPLC [COSMOSIL 5C18 AR-II, Fraction 46 (40% MeOH), Fraction 50 (70% MeOH)] to give **7** (17 mg) from Fraction 46 and **8** (10 mg) from Fraction 50. Fraction 48 (2140 mg) was successively subjected to silica gel [Art. 1.09385, CHCl₃-MeOH-H₂O (20:1:0, 10:1:0, 14:2:0.1, 6:4:1, 0:1:0)] and MCI gel CHP20P (H₂O, MeOH, acetone) column chromatography to yield **21** (29 mg). Fraction 11 (1503 mg) and Fraction 12 (1595 mg) were each subjected to Sephadex LH-20 column chromatography (MeOH) to furnish **19** (35 mg) from Fraction 11 and Fraction 52 and Fraction 53 from Fraction 12. Chromatography of Fraction 53 (240 mg) over a silica gel column [Art. 1.09385, CHCl₃-MeOH-H₂O (14:2:0.1)] furnished **20** (129 mg). The hexane-soluble fraction was separated by chromatography over a silica gel column [Art. 1.09385, hexane-acetone (20:1, 15:1, 10:1, 5:1, 3:1, 1:1)] to give Fractions 54 - 61. Fraction 55 (121 mg) was successively subjected to silica gel [Art. 1.09385, hexane-acetone (30:1, 20:1, 10:1, 5:1, 3:1, 1:1)] and Bio-Beads SX2 [hexane-acetone (1:1)] column chromatography, and similar HPLC (93% MeOH) to Fraction 23 to yield **15** (16 mg). Fraction 57 (1464 mg) was successively subjected to Chromatorex ODS column chromatography (50% MeOH, 60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) and HPLC under the same conditions as for Fraction 29 to give **14** (13 mg) and **10** (39 mg). Fraction 58 (280 mg) was subjected to HPLC under the same conditions as for Fraction 29 to furnish **16** (32 mg), **2** (11 mg), and Fraction 62. Fraction 62 (58 mg) was separated by chromatography over a silica gel column [Art. 1.09385, hexane-acetone (10:1, 5:1)] to give **1** (34 mg). Fraction 59 (501 mg) was successively subjected to Chromatorex ODS column chromatography (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) and HPLC under the same conditions as for Fraction 29 to give **6** (8 mg), **5** (9 mg), **17** (10 mg), and **18** (108 mg).

Acetylation of 1 Compound **1** (5 mg) in Ac₂O-pyridine (1:1, 1 ml) was left to stand at room temperature overnight. After removal of the reagent under a stream of N₂, the residue was partitioned between ether and H₂O. The ether layer was concentrated to give **1a** (5 mg).

Single crystal X-ray analysis of 3 A colorless needle crystal having approximate dimensions of 0.10 $\times$ 0.20 $\times$ 1.00 mm was mounted on a glass fiber. All measurements were made on a Rigaku RAXIS RAPID imaging plate area detector (Rigaku Co., Tokyo, Japan) with graphite monochromated Mo-K α radiation. The data were collected at a temperature of 23 $\pm$ 1°C to a maximum 2 θ value of 55.0°. The structure was solved by a direct method (SHELXS) (Sheldrick, 1990) and hydrogen atoms were placed at the calculation. A full-matrix

least-squares technique was used with anisotropic thermal parameters for non-hydrogen atoms and a riding model was used for hydrogen atoms. All calculations were performed using SHELXL (Sheldrick and Schneider, 1997).

Antioxidative activity by the ferric thiocyanate method

The antioxidative activity of the test sample was measured using the previously described ferric thiocyanate method (Kikuzaki and Nakatani, 1993). A mixture of 2.51% linoleic acid ethanol (EtOH) solution (0.80 ml), 0.05 M phosphate buffer (pH 7.0, 1.60 ml), EtOH (0.60 ml), and H₂O (0.80 ml) was added to 10 mM EtOH solution (0.20 ml) of each sample in a vial with a cap and placed in the dark at 40°C to accelerate the oxidation. At intervals during the incubation, this assay solution (0.05 ml) was diluted with 75% EtOH (4.85 ml), which was followed by adding 30% ammonium thiocyanate (0.05 ml). Precisely 6 min after the addition of 0.02 M ferrous chloride in 3.5% hydrochloric acid (0.05 ml) to the reaction mixture, the absorbance of the developed red color was measured at 500 nm.

Assay of scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) The method of Uchiyama *et al.* (1968) was applied in a slightly modified manner. The EtOH solution (1.00 ml) of each test sample was added to a mixture of 0.1 M acetic acid buffer (pH 5.5, 1.00 ml) and 0.5 mM DPPH EtOH solution (0.50 ml) in a test tube and left standing at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm. The control sample was prepared from the mixture containing all ingredients except a test sample. The scavenging effect on DPPH was expressed as follows: $\Delta O. D. = O. D. \text{ of control} - O. D. \text{ of sample}$. α -Tocopherol was used as a standard sample. Results presented are the means of two experiments.

Results and Discussion

The MeOH extract of the fruits of *S. molle* was successively subjected to Diaion HP20, silica gel, Chromatorex ODS, Sephadex LH-20, MCI gel CHP20P and Bio-Beads SX2 column chromatography, as well as HPLC on ODS, to yield 22 compounds: 12 sesquiterpenoids (**1**, **2**, **7** - **16**), six tirucallane-type triterpenoids (**3** - **6**, **17**, **18**), and four flavonoids (**19** - **22**).

Structure elucidation of **1** - **22** Compounds **7** - **22** were identified as teuclatriol (**7**) (Bruno *et al.*, 1993), 10-epiteuclatriol (**8**) (Bruno *et al.*, 1993), teucladiol (**9**) (Bruno *et al.*, 1993), 4 β ,6 β -dihydroxy-1 α ,5 β (*H*)-guai-9-ene (**10**) (Mahmoud, 1997), 1 β ,6 α -dihydroxy-7-epi-eudesm-3-ene (**11**) (Mahmoud, 1997), 1 β ,6 β -dihydroxy-7-epi-eudesm-3-ene (**12**) (Mahmoud, 1997), oplodiol (**13**) (Takahashi and Takani, 1976), isocalamediol (**14**) (Nawamaki and Kuroyanagi, 1996), α -cadinol methyl ether (**15**) (Dupré *et*

al., 1991), oplopanone (**16**) (Herz and Watanabe, 1983), 3 β -hydroxy-8,24-tirucalladien-21-oic acid (**17**) (Badria *et al.*, 2003), 3 α -hydroxy-8,24*Z*-tirucalladien-26-oic acid (**18**) (Olfasson *et al.*, 1997), engeletin (**19**) (Silva *et al.*, 1997), quercetin 3-*O*-rhamnoside (**20**) (Harbone and Mabry, 1982), agathisflavone (**21**) (Chari *et al.*, 1977), and 2,3,2'',3''-tetrahydroamentoflavone (**22**) (Ahmad *et al.*, 1981), based on comparisons of their physical and spectral data with those in the literature, although ¹H- and ¹³C-NMR spectral data in pyridine-*d*₅ of **7** - **14** and **16** - **18** and ¹³C-NMR data of **15** have not been reported in the literature (Fig. 1, Tables 1 - 5).

Compound **1** was obtained as a colorless syrup, [α]_D²² + 21.7° (*c* = 5.3, CHCl₃), and it exhibited an [M+Na]⁺ ion peak at *m/z* 261 in the positive fast atom bombardment mass spectrum (FAB-MS) and an [M-H]⁻ ion peak at *m/z* 237 in the negative FAB-MS. The molecular formula of **1** was determined to be C₁₅H₂₆O₂ by high-resolution (HR)-negative FAB-MS (*m/z* 237.1860, Δ + 0.6 mmu). The ¹H-NMR spectrum of **1** showed signals due to two tertiary methyl groups (δ 1.62, 1.25), two secondary methyl groups [δ 0.91 (d, *J* = 6.5 Hz), 0.90 (d, *J* = 6.5 Hz)], and one oxygenated methine proton [δ 4.17 (d, *J* = 4.5 Hz)]. The ¹³C-NMR spectrum of **1** showed 15 carbon signals, which were elucidated as signals due to two oxygenated quaternary carbons (δ 74.3, 73.3), one oxygenated methine carbon (δ 76.3), four methine carbons (δ 69.1, 53.3, 38.7, 33.3), four methylene carbons (δ 48.8, 37.9, 24.5, 20.7), and four methyl carbons (δ 26.6, 22.4, 21.2, 20.4) using the distortionless enhancement by polarization transfer (DEPT) method. These ¹H- and ¹³C-NMR signals (Tables 1 and 2) were assigned with the aid of ¹H-¹H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) spectra, and the planar structure of **1**, which was a guaiane-type sesquiterpenoid, could be determined by these observations (Fig. 2). A key HMBC correlation between H-6 and C-10 indicated that an epoxy bridge was located between C-6 and C-10. Furthermore, ordinary acetylation of **1** with acetic anhydride and pyridine gave a monoacetyl derivative (**1a**), which was a colorless syrup, of **1**. The signal of H-6 in the ¹H-NMR spectrum of **1a** exhibited no acetylation shift, whereas the signal due to C-4 in the ¹³C-NMR spectrum of **1a**, in comparison with that of **1**, indicated a downfield shift. Thus, the epoxy bridge between C-6 and C-10 was established and H-1/H-5 was assumed to have a *cis*-configuration by forming the epoxy bridge. In the difference nuclear Overhauser effect (NOE) and nuclear Overhauser and exchange spectroscopy (NOESY) spectra of **1**, NOEs were observed between the respective protons as illustrated in Figure 3, while no correlations between H-1 and H₃-14, between H-5 and H-11, between H-5 and H₃-12, and between H-5 and

Fig. 1. Structures of 1 - 22.

H₃-13 were detected; this suggested all of the configurations of H-1, H-5, and the methyl group at C-4 to be α and that of the isopropyl group at C-7 to be β , and NOE correlations in the NOESY spectrum of **1a** supported these configurations. Consequently, the structure of **1** was established as 6 β ,10 β -epoxy-4 β -hydroxy-1 α ,5 α ,7 α (H)-guaiane (Fig. 1). Recently, the structure of chrysanthol was reported to be 6 β ,10 β -epoxy-4 α -hydroxy-1 β ,5 α ,7 β (H)-guaiane (Ahmed *et al.*, 2006). However, the ¹H- and ¹³C-NMR data of **1a** were superimposable on those of the monoacetate of chrysanthol, and the sign and the value of the specific rotation of **1** was similar to those of chrysanthol. Therefore, **1** was considered to be identical to chrysanthol.

Compound **2** was obtained as a colorless syrup, $[\alpha]_{\text{D}}^{13}$ +

25.0° ($c = 0.9$, CHCl₃). The positive FAB-MS and negative FAB-MS exhibited an $[\text{M}+\text{Na}]^+$ ion peak at m/z 293 and an $[\text{M}-\text{H}]^-$ ion peak at m/z 269, respectively. The HR-negative FAB-MS (m/z 269.2100, $\Delta - 1.6$ mmu) of **2** indicated the molecular formula of **2** to be C₁₆H₃₀O₃. The ¹H- and ¹³C-NMR signals (Tables 1 and 2) of **2** were quite similar to those of **8**, except for signals due to C-1, C-9, C-10, and C-14, and the appearance of signals due to one methoxyl group, although the assignments at C-1 and C-7 of **8** in the literature (Bruno *et al.*, 1993) should be interchanged. From these data, **2** was considered to be a derivative of **8**, in which a hydroxyl group at C-10 was replaced by a methoxyl group. In the NOESY spectrum of **2**, key NOEs were observed between H-1 and the methoxyl group, between H-5 and H₃-14, and between

Table 1. ¹H-NMR Data for **1**, **1a**, **2** and **7-10** (500 MHz).

H	1 ^{a)}	1a ^{b)}	2 ^{b)}	7 ^{a)}	8 ^{a)}	9 ^{a)}	10 ^{a)}
1	2.38,ddd(5.5,11.5,14.0)	2.37,ddd(5.5,11.5,14.0)	1.98,ddd(3.0,10.0,10.5)	ca. 2.33	ca. 1.75	2.38,ddd(7.5,7.5,11.5)	2.37,m
2a	ca. 1.61	ca. 1.50	ca. 1.78	ca. 2.27	ca. 2.15	ca. 1.89	ca. 1.80
2b	ca. 1.49	ca. 1.47	1.52,dd(10.0,10.5)	ca. 1.82	1.65,ddd(7.5,10.5,10.5,12.0)	1.72,m	ca. 1.76
3a	2.51,dd(10.0,13.5)	2.45,dd(9.0,14.5)	ca. 1.67	ca. 2.01	2.25,ddd(10.5,10.5,10.5)	2.10,ddd(7.5,9.5,11.5)	ca. 2.01
3b	2.17,ddd(8.5,8.5,13.5)	2.11,ddd(9.0,9.0,14.5)	ca. 1.67	ca. 1.80	ca. 1.77	1.79,m	ca. 1.73
5	2.79,dd(4.5,14.0)	2.29,dd(4.5,14.0)	ca. 1.83	ca. 2.23	3.07,dd(9.0,10.5)	2.27,dd(9.0,11.5)	2.47,dd(9.5,12.0)
6	4.17,d(4.5)	4.09,d(4.5)	4.13,dd(4.5,8.5)	4.44,dd(5.0,7.5)	4.47,dd(4.0,9.0)	4.34,dd(3.5,8.5)	4.37,dd(4.5,9.5)
7	ca. 1.49	ca. 1.52	ca. 1.04	1.33,ddd(5.0,7.5,7.5)	1.14,ddd(4.0,8.0,8.0)	1.41,ddd(1.5,3.5,8.5,10.0)	1.46,ddd(4.5,9.5,9.5)
8a	1.85,ddd(7.5,7.5,14.5)	ca. 1.82	ca. 1.42	ca. 1.77	2.31,ddd(1.5,8.0,13.5,13.5)	ca. 1.91	2.59,m
8b	ca. 1.55	1.60,m	ca. 1.42	1.51,dd(5.0,12.0)	1.42,m	1.63,ddd(6.5,6.5,14.0)	1.91,dd(9.0,17.0)
9a	1.75,ddd(7.5,7.5,12.0)	1.73,ddd(7.5,7.5,12.0)	ca. 1.80	ca. 2.23	ca. 2.16	2.63,ddd(6.0,6.5,14.0)	5.58,br d(8.5)
9b	1.40,ddd(4.0,7.5,12.0)	1.40,ddd(3.5,7.5,12.0)	ca. 1.42		1.50,ddd(2.5,13.5,13.5)	2.18,ddd(7.5,7.5,14.0)	
11	1.67,dqq(6.5,6.5,6.5)	ca. 1.80	ca. 1.68	ca. 2.05	2.05,dqq(8.0,6.5,6.5)	2.01,m	ca. 1.98
12	0.91,d(6.5)	0.98,d(6.5)	1.03,d(6.5)	1.21,d(6.5)	1.26,d(6.5)	1.20,d(6.5)	1.21,d(6.5)
13	0.90,d(6.5)	0.97,d(6.5)	0.99,d(6.5)	1.10,d(6.5)	1.11,d(6.5)	1.06,d(6.5)	1.06,d(6.5)
14a	1.25,s	1.19,s	1.19,s	1.43,s	1.40,s	4.88,s	1.70,s
14b						4.82,s	
15	1.62,s	1.56,s	1.28,s	1.48,s	1.52,s	1.51,s	1.52,s
OCH ₃			3.14,s				
COCH ₃		1.99,s					

δ in ppm from tetramethylsilane (TMS) [coupling constants (*J*) in Hz are given in parentheses]. a) in pyridine-*d*₅, b) in chloroform-*d*. The abbreviations used are as follows: s, singlet; d, doublet; dd, double-doublet; br d, broad doublet; ddd, double-double-doublet; dddd, double-double-double-doublet; dq, double-double-doublet; dq, double-double-double-doublet; m, multiplet.

Table 2. ^{13}C -NMR Data for **1**, **1a**, **2** and **7-16** (125 MHz).

C	1 ^{a)}	1a ^{b)}	2 ^{b)}	7 ^{a)}	8 ^{a)}	9 ^{a)}	10 ^{a)}	11 ^{a)}	12 ^{a)}	13 ^{a)}	14 ^{a)}	15 ^{b)}	16 ^{a)}
1	53.3	52.5	42.1	46.5	45.9	43.2	42.8	76.9	76.4	79.7	55.4	46.6	57.9
2	24.5	23.4	22.8	24.1	24.3	27.5	27.2	33.8	33.7	28.2	23.0	22.6	23.5
3	48.8	46.5	41.2	41.9	41.7	41.4	40.8	121.8	121.9	40.6	35.6	30.9	29.0
4	73.3	82.4	81.2	80.9	81.0	80.4	80.6	136.8	135.6	70.1	147.0	135.0	210.8
5	69.1	66.6	54.9	56.2	54.0	60.6	58.3	50.8	51.9	47.3	47.4	122.5	56.0
6	76.3	78.0	71.5	71.5	71.6	72.2	72.3	69.5	67.9	24.0	75.5	39.6	46.8
7	38.7	37.9	51.9	52.5	52.5	49.2	51.0	46.5	50.2	142.6	52.6	46.7	49.9
8	20.7	20.0	20.3	21.5	20.9	24.1	25.1	20.8	21.4	117.0	20.2	21.6	26.2
9	37.9	37.3	40.7	49.0	48.1	36.8	126.7	34.7	37.1	41.7	44.2	35.5	43.0
10	74.3	74.0	79.5	74.4	72.4	153.9	139.8	37.6	38.2	38.7	71.6	76.2	71.6
11	33.3	32.4	29.6	30.6	30.5	29.9	29.3	27.7	29.4	35.4	25.6	26.0	29.8
12	20.4	20.1	21.5	21.9	21.9	22.0	21.8	23.1	21.3	22.0	24.5	21.5	22.1
13	21.2	21.1	21.2	21.4	21.5	21.7	21.6	21.8	21.2	21.4	19.0	15.2	16.0
14	22.4	22.0	19.2	22.5	30.6	107.5	23.3	15.7	12.8	30.3	23.9	17.5	20.6
15	26.6	21.7	23.1	23.6	23.8	24.3	24.5	23.3	20.8	12.6	100.8	23.8	29.3
OCH ₃			48.4									48.1	

δ in ppm from TMS. a) in pyridine-*d*₅. b) in chloroform-*d*.

Table 3. ^1H -NMR Data for **11-14** and **16** (in pyridine-*d*₅, 500 MHz).

H	11	12	13	14	16
1	3.87,dd(6.5,10.0)	3.84,dd(6.5,10.0)	3.63,dd(3.5,12.5)	1.72,dd(2.5,12.5)	1.74,ddd(5.5,12.5,12.5)
2a	ca. 2.51	2.53,m	2.51,dddd(3.5,12.5,12.5,12.5)	2.38,m	ca. 1.56
2b	2.35,m	ca. 2.44	1.89,dddd(3.5,3.5,3.5,12.5)	1.81,m	ca. 1.56
3a	5.46,br s	5.53,br s	1.99,ddd(3.5,3.5,12.5)	2.44,br d(12.5)	1.89,m
3b			1.64,ddd(3.5,12.5,12.5)	2.08,ddd(4.5,12.5,12.5)	ca. 1.56
4					2.63,ddd(5.0,9.0,11.5)
5a	ca. 2.52	2.01,s	1.41,dd(5.0,12.5)	2.75,d(13.0)	
5b				2.00,d(13.0)	
6a	4.30,dd(4.0,10.0)	4.56,s	ca. 2.64		ca. 1.98
6b			2.20,dd(5.0,11.0)		
7	ca. 1.76	ca. 0.99		1.23,br d(9.0)	ca. 1.13
8a	ca. 1.76	1.88,dddd(3.5,13.0,13.0,13.0)	5.49,d(5.5)	ca. 1.84	ca. 2.05
8b	ca. 1.76	1.76,dddd(3.5,3.5,3.5,13.0)		ca. 1.58	ca. 1.52
9a	ca. 1.94	2.44,ddd(3.5,3.5,13.0)	2.60,dd(5.5,15.5)	ca. 2.13	ca. 1.96
9b	ca. 1.94	1.34,ddd(3.5,13.0,13.0)	2.15,br d(15.5)	ca. 1.84	1.66,m
11	2.17,m	1.97,m	ca. 2.25	2.27,m	1.66,m
12	1.25,d(6.5)	1.09,d(6.5)	1.06,d(6.5)	1.10,d(6.5)	0.89,d(7.0)
13	1.03,d(6.5)	1.02,d(6.5)	1.06,d(6.5)	0.98,d(6.5)	0.81,d(7.0)
14	1.24,s	1.56,s	1.58,s	1.59,s	1.33,s
15a	2.23,s	2.06,s	1.37,s	4.86,s	2.16,s
15b				4.74,s	

δ in ppm from TMS [coupling constants (*J*) in Hz are given in parentheses]. The abbreviations used are as follows: s, singlet; br s, broad singlet; d, doublet; dd, double-doublet; br d, broad doublet; ddd, double-double-doublet; dddd, double-double-double-doublet; m, multiplet.

Table 4. ^{13}C -NMR Data for **3** - **6**, **17** and **18** (125 MHz).

C	3 ^{a)}	3 ^{b)}	4 ^{b)}	5 ^{a)}	6 ^{a)}	17 ^{a)}	18 ^{a)}
1	35.7	35.4	35.6	35.9	29.5	35.9	30.5
2	34.6	34.3	34.6	28.9	26.5	28.9	27.7
3	216.4	217.7	218.1	78.1	74.1	78.1	75.0
4	47.2	47.1	47.3	39.6	38.0	39.6	37.7
5	51.5	51.4	51.6	51.5	42.8	51.5	45.1
6	20.4	20.0	20.3	19.4	36.2	19.4	19.2
7	27.3	27.3	27.5	28.1	197.8	27.5	27.0
8	134.7	134.3	134.8	134.8	138.9	134.9	133.4
9	133.2	132.5	132.8	133.7	166.2	133.6	135.2
10	37.3	37.0	37.2	37.7	39.6	37.7	38.2
11	21.6	21.2	21.4	21.8	23.6	21.8	21.8
12	29.2	28.5	29.9	30.2	30.3	30.3	30.1
13	44.5	43.8	44.2	44.5	44.9	44.6	44.4
14	50.0	49.6	50.1	50.3	48.2	50.3	50.3
15	29.8	29.2	30.8	31.2	32.3	31.5	31.2
16	26.8	26.7	28.1	28.3	29.0	28.1	28.3
17	47.5	46.8	50.2	50.5	49.2	46.9	50.4
18	16.2	15.7	15.6	15.8	15.7	16.3	15.7
19	19.7	19.5	19.8	20.5	18.6	20.5	20.4
20	48.9	47.5	36.5	36.8	36.7	49.9	36.8
21	178.6	182.4	18.6	18.9	19.0	178.3	18.9
22	33.3	32.3	35.9	36.5	36.5	32.4	36.5
23	26.7	25.8	26.9	27.1	27.0	26.9	27.0
24	124.9	123.5	147.1	142.5	142.4	125.0	142.6
25	131.7	131.9	125.8	128.7	128.8	131.8	128.6
26	25.8	25.5	172.5	170.7	170.8	25.8	170.6
27	17.7	17.5	20.5	21.5	21.5	17.8	21.5
28	27.7	26.5	26.8	28.7	28.1	28.7	29.0
29	21.2	21.0	21.1	16.5	22.0	16.5	22.7
30	24.4	24.1	24.2	24.6	24.7	24.6	24.7

δ in ppm from TMS. a) in pyridine- d_5 . b) in chloroform- d .

H-6 and H₃-15 (Fig. 3). Consequently, **2** was concluded to be 4 β ,6 β -dihydroxy-10 α -methoxy-1 α ,5 β ,7 α (H)-guaiane (Fig. 1).

Compound **3** was obtained as colorless needles (MeOH), mp 222 - 223°C, $[\alpha]_{\text{D}}^{14} + 35.4^\circ$ ($c = 2.7$, CHCl_3), and indicated an $[\text{M}]^+$ ion peak at m/z 454 in the electron impact (EI)-MS. The ^1H -NMR spectrum (in pyridine- d_5) of **3** showed signals due to one olefinic proton [δ 5.34 (dd, $J = 6.0$, 6.0 Hz)] and seven tertiary methyl groups (δ 1.68, 1.63, 1.15, 1.09, 1.05, 0.97, 0.94). The ^{13}C -NMR spectrum of **3** gave 30 carbon signals, including two carbonyl carbons (δ 216.4, 178.6) and four olefinic carbons (δ 134.7, 133.2, 131.7, 124.9). From these data, **3** was assumed to be a tirucallane- or euphane-type triterpenoid, which was previously isolated from this plant. Finally, the structure of **3** was elucidated by X-ray crystallography (Fig. 4, Table 6). Consequently, the structure of **3** was confirmed as 3-oxo-8,24-tirucalladien-21-oic acid, which was previously isolated from *Boswellia carterii* Birdwood (Badria *et al.*, 2003) (Fig. 1). However, the assignment of ^{13}C -NMR data (in chloroform- d) in the literature (Badria *et al.*, 2003) should be corrected as shown in Table 4, and ^1H - and ^{13}C -NMR data in pyridine- d_5 have not been reported.

Compound **4** was obtained as a white powder, $[\alpha]_{\text{D}}^{14} + 13.2^\circ$ ($c = 1.4$, CHCl_3), and showed the same $[\text{M}]^+$ ion peak as that of **3** at m/z 454 in the EI-MS. The ^1H - and ^{13}C -NMR signals were similar to those of **3**, except for signals due to C-17 and the side chain moiety, which were quite similar to those of **18** (Olfasson, 1997) (Tables 4 and 5). Thus, **3** was identified as isomasticadienoic acid (Paraschos, 2007) (Fig. 1). Recently, the ^{13}C -NMR data of **3** have been reported (Paraschos, 2007), although the assignments in the literature

Table 5. ^1H -NMR Data for **3** - **5**, **17** and **18** (500 MHz).

H	3 ^{a)}	4 ^{a)}	5 ^{b)}	17 ^{b)}	18 ^{b)}
3			3.47,dd(5.5,10.5)	3.48,dd(5.5,11.0)	3.63,br s
18	0.82,s	0.76,s	0.85,s	0.97,s	0.97,s ^{c)}
19	1.03,s	1.05,s	1.04,s	1.04,s	1.05,s
21		0.93,d(6.0)	1.05,d(6.0)		1.01,d(6.0)
24	5.09,dd(5.0,5.0)	6.09,dd(7.5,7.5)	6.06,dd(7.5,7.5)	5.37,dd(7.5,7.5)	6.03,dd(7.0,7.0)
26	1.58,s			1.65,s	
27	1.67,s	1.92,s	2.14,s	1.67,s	2.12,s
28	1.09,s	1.10,s	1.07,s	1.07,s	1.21,s
29	1.05,s	1.06,s	1.25,s	1.25,s	0.93,s ^{c)}
30	0.91,s	0.89,s	0.94,s	0.96,s	0.94,s ^{c)}

δ in ppm from TMS [coupling constants (J) in Hz are given in parentheses]. a) in chloroform- d . b) in pyridine- d_5 .

c) Assignments may be interchanged. The abbreviations used are as follows: s, singlet; br s, broad singlet; d, doublet; dd, double-doublet.

Fig. 2. ^1H - ^{13}C long-range correlations observed for **1** (in pyridine- d_5) and **2** (in chloroform- d) in the HMBC spectra (500 MHz).

Fig. 3. Selected NOE correlations observed for **1** (in pyridine- d_5) and **2** (in chloroform- d) (500 MHz).

should be corrected as shown in Table 4.

Compound **5** was obtained as a white powder, $[\alpha]_{\text{D}}^{24} - 8.1^\circ$ ($c = 1.0$, CHCl_3), and was determined to have the molecular formula $\text{C}_{30}\text{H}_{48}\text{O}_3$ by HR-EI-MS (m/z 456.3596, $\Delta - 1.7$ mmu). The ^1H - and ^{13}C -NMR signals were similar to those of **4**, except for the appearance of a signal due to one oxygenated methine group [δ_{H} 3.47 (1H, dd, $J = 5.5$, 10.5 Hz), δ_{C} 78.1], the loss of a signal due to one ketone carbonyl carbon, and the resonances of signals due to an A-ring moiety (Tables 4 and 5). From these data, **5** was identified as 24Z-isomasticadienolic acid. Although the ^1H -NMR spectral data of methyl ester of **5** was previously reported (Pozzo-Balbi *et al.*, 1978), ^1H - and ^{13}C -NMR data of **5** have not been reported in the literature.

Compound **6** was obtained as a white powder. In the EI-MS, **6** gave an $[\text{M}]^+$ ion peak at m/z 456. The ^1H - and ^{13}C -NMR signals were impossible on those of **18**, with an additional signal due to one ketone carbonyl carbon and the loss of a signal due to one methylene group; the ^{13}C -NMR data due to a side chain moiety was almost the same as those of **18** (Tables 4 and 5). The location of a ketone carbonyl carbon was confirmed by techniques similar to those of **1**. Consequently, **6** was elucidated as 7,11-dioxo-3 α -hydroxy-8,24Z-

tirucalladien-26-oic acid (Fig. 1), which has been recently reported as a constituent of *Juliania adstringens*, and its ^1H -NMR data were quite similar to those of **6**, but the ^{13}C -NMR data were slightly different from those of **6** (Makino *et al.*, 2004).

Antioxidative effect of 19 - 22 The antioxidative activity of **19 - 22** was evaluated using linoleic acid as the substrate in the ferric thiocyanate method. Compounds **19**, **20**, and **21** exhibited almost the same activity as that of α -tocopherol (Fig. 5). Furthermore, the scavenging effect of **19 - 22** on the stable free radical DPPH was examined. Compound **20** exhibited more potent activity than α -tocopherol at a concentration of 0.02 mM (Fig. 6).

In conclusion, 12 sesquiterpenoids, one of which was a new compound (**2**), six tirucallane-type triterpenoids and four flavonoids were isolated from the fruits of *S. molle*. Tirucallane- and euphane-type triterpenoids, a sesquiterpenoid, and a biflavonoid have been previously isolated from the fruits of *S. molle* (Terhune *et al.*, 1974; Pozzo-Balbi *et al.*, 1978; Olfasson *et al.*, 1997; Yueqin *et al.*, 2003), but this is the first report on the isolation of **1**, **3**, **5 - 17**, and **19 - 22** from the fruits. Yokozawa *et al.* (1998) reported that the 3,4-dihydroxyl group in the B-ring of the flavonoid skeleton is important for

Fig. 4. ORTEP drawing of **3**. The ellipsoid probability level of **3** is 50%.

Table 6. Crystal Data and Intensity Measurement of **3**.

formula	C ₃₀ H ₄₆ O ₃
formula weight	454.67
crystal system	orthorhombic
lattice parameters	
<i>a</i> (Å)	7.0807 (9)
<i>b</i> (Å)	9.9782 (14)
<i>c</i> (Å)	39.079 (5)
<i>V</i> (Å ³)	2761.1 (6)
space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁ (#19)
<i>Z</i>	4
<i>D_c</i> (g/cm ³)	1.09
unique data used (<i>I</i> > 2.0 σ <i>I</i>)	6289
<i>R</i>	0.089
<i>R_w</i>	0.185

the DPPH radical-scavenging effect. The results in this study supported this structure-activity relationship, because **20** showed a higher effect than those of **19**, **21**, and **22**.

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Fig. 5. Antioxidative activity of **19** - **22**, α-tocopherol and BHA after the fifth day of lipid peroxidation. The final concentration of the sample was 0.5 mM. The value of control represents 100% lipid peroxidation.

Fig. 6. DPPH radical-scavenging effect of **19** - **22** and α-tocopherol. The final concentration of each sample tested was 0.02 mM. ΔO. D. = O. D. of control at 517 nm (1.115) – O. D. of sample.

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