JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 8987−8992 **8987**

Chemistry and Bioactivity of Royal Jelly from Greece

ELENI MELLIOU AND IOANNA CHINOU*

Division of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis Zografou, GR-15771 Athens, Greece

Twenty-five compounds were identified from the dichloromethane and methanol extracts of royal jelly from Greece. Among them, 16 compounds are reported for the first time as royal jelly constituents, whereas 7 of them are isolated for the first time as natural products. The 7 new compounds were fatty acid derivatives: 10-acetoxydecanoic acid (**1**), *trans*-10-acetoxydec-2-enoic acid (**2**), 11-oxododecanoic acid (**3**), (11*S*)-hydroxydodecanoic acid (**4**), (10*R*,11*R*)-dihydroxydodecanoic acid (**5**), 3,11-dihydroxydodecanoic acid (**6**), and (11*S*),12-dihydroxydodecanoic acid (**7**). The structures of the isolated compounds were determined by spectroscopic methods, mainly by the concerted application of 1D and 2D NMR techniques (HMQC, HMBC) and mass spectrometry. The studied sample and the isolated compounds were tested for their antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi and exhibited interesting activities.

KEYWORDS: Royal jelly; fatty acids; decanoic and dodecanoic acid derivatives; antimicrobial activities

INTRODUCTION

Royal jelly is a secretion of the mandibular and hypopharyngeal glands of worker bees, *Apis mellifera* (Hymenoptera, Apidae), involved in the sexual determination and longetivity of the queen. This secretion is a yellowish, creamy, and acidic material with a slightly pungent odor and taste. Royal jelly is fed temporarily (for up to but not more than 3 days) to the brood of workers and drones, but it is a sole food of the queen bee for both her larval and adult life (*1*). Royal jelly has been demonstrated to possess several pharmacological activities in experimental animals, including antitumor activity (*2*), antimicrobial (*3*), vasodilative, and hypotensive activities, increase in growth rate, disinfectant action, anti-hypercholesterolemic activity, and anti-inflammatory activity (*4*). Therefore, royal jelly has been widely used in commercial medical products, health foods, and cosmetics in many countries for more than 30 years.

The unique and chemically most interesting feature of royal jelly is its fatty acids contents. Unlike fatty acids of most animal and plant materials, which consist mainly of their tri-, di-, and monoglyceride forms along with minor amounts of free fatty acids, royal jelly fatty acids are free short-chain fatty acids containing 8−12 carbons and are usually either hydroxyl or dicarboxylic fatty acids. The major fatty acid in royal jelly is *trans*-10-hydroxydec-2-enoic acid, and it is noteworthy that no other natural product containing *trans*-10-hydroxydec-2-enoic acid has been reported, not even other bee products. Previous studies with deuterated substrates have revealed that the royal jelly fatty acids are de novo biosynthesized in the mandibular glands of the honey bees (*5*, *6*).

Additionally, a substantial part of royal jelly is made of proteins, which form ∼50% of its dry mass. The most studied proteins of royal jelly are the major royal jelly proteins (MRJPs) and royalisin, which have immunoregulatory and antibacterial effects (*7*, *8*).

Despite numerous studies, beginning in 1852, dedicated to the chemical composition of royal jelly, which have resulted in the identification of the principal classes of components, knowledge in this field has remained incomplete. One possible reason for the incomplete chemical characterization of royal jelly could be the fact that it is a very expensive raw material. The aim of this study was the isolation, structure elucidation, and biological testing of the secondary metabolites of Greek royal jelly.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Perkin-Elmer Paragon 500 instrument. NMR spectra were recorded on Bruker DRX 400 and Bruker AC 200 spectrometers [^{1}H (400 and 200 MHz) and ^{13}C (50 MHz)]; chemical shifts are expressed in parts per million downfield from TMS. The ^{1}H−^{1}H and ^{1}H−^{13}C NMR experiments were performed using standard Bruker microprograms. GC-MS analysis was carried out on a Hewlett-Packard 6890-5973 system operating in EI mode, equipped with a 30 m × 0.25 mm i.d., 0.25 μm HP-5 MS capillary column. The temperature program was as follows: FROM 60 °C (5 min) to 280 °C at a rate of 3 °C/min; the injection temperature was 200 °C. The identification of the compounds by GC-MS was based on comparison of their retention indices (RI), obtained using *n*-alkanes (C_9−C_{25}), and on comparison of their EI-mass spectra with the NIST/NBS, Wiley library spectra, and the literature (*9*). Additionally, the identification of compounds by GC-MS was performed by co-injection with commercially available samples. CI-MS spectra were determined on a Finnigan GCQ *Plus* mass spectrometer using

* Author to whom correspondence should be addressed (telephone +30210 7274595; fax +30210 7274115; e-mail ichinou@pharm.uoa.gr).

10.1021/jf051550p CCC: $30.25
Published on Web 10/22/2005

CH_4 as the CI ionization reagent, and ESMS and HRMS were performed on a AEI MS-90 spectrometer.

Medium-pressure liquid chromatography (MPLC) was performed with a Büchi model 688 apparatus on columns containing 20−40 μm of silica gel 60 (Merck). Thin-layer chromatography (TLC) was performed on plates coated with silica gel 60 F_{254}, 0.25 mm (Merck).

Material. The samples of royal jelly were collected in northwestern Greece (Arta region) in May 2002. The samples were kept at −20 °C until analysis.

Extraction and Isolation of Compounds. One kilogram of fresh royal jelly was lyophilized and gave 331.6 g of dry powder that was then extracted for 48 h with CH_2Cl_2 (3 × 2 L) and then with MeOH (3 × 2 L). After evaporation of the solvent from the dichloromethane extract, the residue (32.0 g) was submitted to column chromatography (7.0 cm) containing 900 g of 40−63 μm silica gel 60 (Merck) with cyclohexane/EtOAc (from 100:0 to 70:30 gradient) as eluent to afford 200 fractions of 200 mL each: fractions A1−A10, cyclohexane eluate; fractions A11−A70, cyclohexane/EtOAc (90:10) eluate; fractions A71−A150, cyclohexane/EtOAc (80:20) eluate; fractions A151−A200, cyclohexane/EtOAc (70:30) eluate. Fractions A75−A82 afforded sebacic acid (400 mg) (*10*), fractions A113−A120 afforded 10-hydroxydecanoic acid (850 mg) (*10*), and fractions A132−A200 afforded *trans*-10-hydroxydec-2-enoic acid (12 g) (*11*).

Fractions A15−A70 (110 mg) were rechromatographed on MPLC (1.8 × 23 cm) with cyclohexane/EtOAc (95:5 to 93:7) to afford 50 fractions (B1−B50) of 10 mL each: fractions B1−B20, cyclohexane/EtOAc (95:5) eluate; fractions B21−B50, cyclohexane/EtOAc (93:7) eluate. Fractions B30−B45 afforded methyl *p*-hydroxybenzoate (50 mg) (*10*).

Fractions A83−A95 (1.2 g) were rechromatographed on MPLC (3.0 × 23 cm) with cyclohexane/EtOAc (90:10 to 85:15) to afford 75 fractions (C1−C75) of 10 mL each: fractions C1−C23, cyclohexane/EtOAc (90:10) eluate; fractions C24−C75, cyclohexane/EtOAc (85:15) eluate. Fractions C21−C28 afforded 10-acetoxydecanoic acid (**1**) (120 mg), and fractions C37−C54 afforded *trans*-10-acetoxydec-2-enoic acid (**2**) (170 mg).

GC-MS analysis of fractions C18−C20 also revealed the presence of *p*-hydroxyacetophenone, acetovanillone, δ-decalactone, δ-octalactone, octanoic acid, 8-hydroxyoctanoic acid, and methyl 3-hydroxydecanoate.

Fractions C60−C72 (540 mg) were rechromatographed on MPLC (1.8 × 23 cm) with cyclohexane/EtOAc (85:15) to afford 40 fractions (D1−D40) of 10 mL each: fractions D25−D34 afforded 11-oxododecanoic acid (**3**) (185 mg).

Fractions A96−A105 (1.6 g) were rechromatographed on MPLC (3 × 23 cm) with CH_2Cl_2/MeOH (100:0 to 98:2) to afford 60 fractions (E1−E60) of 10 mL each: fractions E1−E10, CH_2Cl_2 eluate; fractions E11−E30, CH_2Cl_2/MeOH (99:1) eluate; fractions E31−E60, CH_2Cl_2/MeOH (98:2) eluate. Fractions E15−E26 afforded 9-hydroxy-2-nonanone (265 mg).

Fractions E31−E60 (940 mg) were rechromatographed on MPLC (3 × 23 cm) with CH_2Cl_2/MeOH 98:2 to afford 75 fractions (F1−F75) of 10 mL each: fractions F20−F33 afforded 3-(4-hydroxy-3-methoxyphenyl)propionic acid (155 mg) (*12*), and fractions F45−F68 afforded (11*S*)-hydroxydodecanoic acid (**4**) (168.5 mg).

After evaporation of the solvent from the methanol extract, the residue (141.65 g) was diluted with distilled water (1 L) and extracted with EtOAc (3 × 1 L). The EtOAc residue (7.8 g) was submitted to column chromatography (5.0 cm) containing silica gel 60 (Merck) (300 g, 40−63 μm) to afford 59 fractions of 200 mL each: G1−G11, cyclohexane/EtOAc (85:15) eluate; fractions G12−G43, cyclohexane/EtOAc (50:50) eluate; fractions G44−G59, EtOAc eluate. Fractions G5−G8 afforded 24-methylenecholesterol (307 mg) (*13*, *14*).

GC-MS analysis of fractions G13−G20 also revealed the presence of sitosterol and isofucosterol.

Fractions G32−G54 afforded 3,10-dihydroxydecanoic acid (810 mg).

Fractions G23−G31 (300.6 mg) were rechromatographed on MPLC (1.8 × 23 cm) with CH_2Cl_2/MeOH (99:1) to afford 152 fractions (H1−H152) of 10 mL each. Fractions H72−H80 afforded (10*R*,11*R*)-dihydroxydodecanoic acid (**5**) (15 mg).

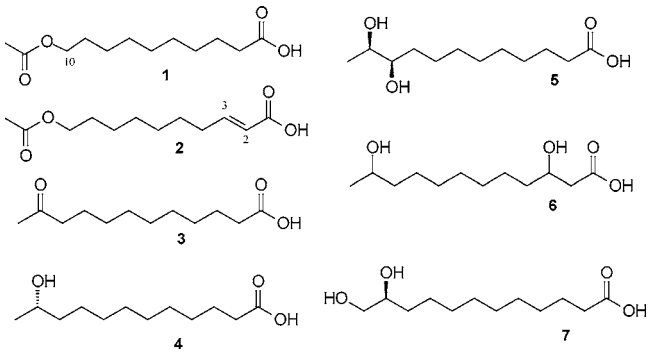

Figure 1. Structures of isolated fatty acids (**1**–**7**).

Fractions H40−H64 (100 mg) were rechromatographed on MPLC (1.8 × 23 cm) with EtOAc/MeOH (99.5:0.5) to afford 42 fractions of 10 mL each (I1−I42). Fractions I20−I29 afforded 3-hydroxydodecanedioic acid (12.6 mg).Fractions H93−H123 (166 mg) were rechromatographed on MPLC (1.8 × 23 cm) with CH_2Cl_2/MeOH (99.5:0.5) to afford 50 fractions of 10 mL each (J1−J50). Fractions J26−J32 afforded 3,11-dihydroxydodecanoic acid (**6**) (5.3 mg). Fractions J39−J50 afforded (11*R*),12-dihydroxydodecanoic acid (**7**) (10 mg).

All of the known compounds were identified by comparison of their NMR and MS data with literature values. All collected fractions were estimated by TLC using eluent systems similar to those used in the relative column chromatographies.

3,10-Dihydroxydecanoic acid: white powder; $[\alpha]^{25}_{D}$ 1.1° (*c* 0.15, MeOH); $[\alpha]^{25}_{436}$ 8.2° (*c* 0.15, MeOH); $[\alpha]^{25}_{365}$ 12.9° (*c* 0.15, MeOH); IR (CaF_2, MeOH) υ_{max} = 1711 cm^{-1}; ^1H NMR (CD_3OD/TMS, 400 MHz, δ, *J* in Hz) 3.92 (1H, m, H-3), 3.49 (2H, t, *J* = 6.0 Hz, H-10), 2.40 (1H, dd, *J* = 15.2, 4.7 Hz, H-2a), 2.32 (1H, dd, *J* = 15.2, 8.1 Hz, H-2b), 1.48 (2H, m, H-9), 1.43 (2H, br s, H-4), 1.30 (8H, br s, H5−8); ^{13}C NMR (CD_3OD, 50 MHz, δ) 175.9 (C-1), 69.3 (C-3), 62.9 (C-10), 43.3 (C-2), 38.0 (C-4), 33.6 (C-9), 30.6 (C-6), 30.5 (C-7), 26.8 (C-5), 26.6 (C-8); EIMS (70 eV) 168 (5%), 155, 138, 109, 98, 89, 84, 71, 55 (100%); ES-MS, *m*/*z* 227 $(M + Na)^+$, 205 $(M + H)^+$; HRESMS, *m*/*z* 227.1256 (calcd for $C_{10}H_{20}NaO_4$: 227.1259).

*(10R,11R)-Dihydroxydodecanoic acid (**5**):* amorphous colorless solid; $[\alpha]^{25}_{D}$ 6° (*c* 0.2, MeOH); IR (CaF_2, MeOH) υ_{max} = 1710 cm^{-1}; ^1H NMR (CD_3OD/TMS, 400 MHz, δ, *J* in Hz) 3.54 (1H, quint, *J* = 6.4 Hz, H-11), 3.34 (1H, m, H-10), 2.26 (2H, t, *J* = 7.4 Hz, H-2), 1.58 (2H, quint, *J* = 7.4 Hz, H-3), 1.30−1.40 (12H, br s, H4−9), 1.14 (3H, d, *J* = 6.4 Hz, H-12); ^{13}C NMR (CD_3OD, 50 MHz, δ) 178.5 (C-1), 76.3 (C-10), 71.5 (C-11), 33.6 (C-2), 30.4 (C4−7), 30.3 (C-9), 30.2 (C-8), 26.8 (C-3), 18.1 (C-12); EIMS (70 eV) 168 (5%), 155, 138, 109, 98, 89, 84, 71, 55 (100%); ES-MS, *m*/*z* 255 $(M + Na)^+$, 233 $(M + H)^+$; HRESMS, *m*/*z* 255.1569 (calcd for $C_{12}H_{24}NaO_4$: 255.1572).

*3,11-Dihydroxydodecanoic acid (**6**):* amorphous colorless solid; $[\alpha]^{25}_{D}$ 2.2° (*c* 0.2, MeOH); IR (CaF_2, MeOH) υ_{max} =1711 cm^{-1}; ^1H NMR (CD_3OD/TMS, 400 MHz, δ, *J* in Hz) 3.95 (1H, m, H-3), 3.71 (1H, hex, *J* = 6.0 Hz, H-11), 2.42 (1H, dd, *J* = 15.2, 4.7 Hz, H-2a), 2.33 (1H, dd, *J* = 15.2, 8.1 Hz, H-2b), 1.41 (2H, m, H-10), 1.34 (12H, br s, H4−9), 1.15 (3H, d, *J* = 6.0 Hz, H-12); ^{13}C NMR (CD_3OD, 50 MHz, δ) 175.9 (C-1), 69.7 (C-3), 68.6 (C-11), 44.0 (C-2), 40.2 (C-10), 38.1 (C-4), 30.8 (C-7), 30.7 (C-6, C-8), 26.9 (C-5), 26.7 (C-9), 23.5 (C-12); ES-MS, *m*/*z* 255 $(M + Na)^+$, 233 $(M + H)^+$; HRESMS, *m*/*z* 255.1567 (calcd for $C_{12}H_{24}NaO_4$: 255.1572).

*(11S,12)-Dihydroxydodecanoic acid (**7**):* amorphous colorless solid; $[\alpha]^{25}_{D}$ −1.7° (*c* 0.2, MeOH); IR (CaF_2, MeOH) υ_{max} = 1710 cm^{-1}; ^1H NMR (CD_3OD/TMS, 400 MHz, δ, *J* in Hz) 3.53 (1H, m, H-11), 3.45 (2H, dd, *J* = 4.7, 11.3 Hz, H-12b), 3.39 (2H, dd, *J* = 6.4, 11.3 Hz, H-12a), 2.18 (2H, t, *J* = 7.3 Hz, H-2), 1.58 (2H, m, H-3), 1.44 (2H, m, H-10), 1.31 (12H, br s, H4−9); ^{13}C NMR (CD_3OD, 50 MHz, δ) 179.3 (C-1), 71.5 (C-11), 65.3 (C-12), 34.2 (C-2), 33.2 (C-10), 29.7 (C-7,8), 29.5, 29.3, 29.2 (C-4,5,6), 24.7 (C-3), 22.9 (C-9); ES-MS, *m*/*z* 255 $(M + Na)^+$, 233 $(M + H)^+$; HRESMS, *m*/*z* 255.1568 (calcd for $C_{12}H_{24}NaO_4$: 255.1572).

*trans-10-Acetoxydec-2-enoic acid (**2**):* amorphous colorless solid; IR (NaCl, CH_2Cl_2) υ_{max} = 1728, 1697 cm^{-1}; ^1H NMR ($CDCl_3$/TMS, 400 MHz, δ, J in Hz) 7.0 (2H, dt, *J* = 15.5, 7.2 Hz, H-3), 5.80 (1H,

d, J = 15.5 Hz, H-2), 4.03 (2H, t, J = 7.2 Hz, H-10), 2.21 (2H, q, J = 7.2 Hz, H-4), 2.02 (3H, s, CH_3CO), 1.60 (2H, quint, J = 7.2 Hz, H-9), 1.45 (2H, quint, J = 7.2 Hz, H-5), 1.31 (6H, br s, H6−8); ^{13}C NMR ($CDCl_3$, 50 MHz, δ) 171.3 (CH_3CO), 171.0 (C-1), 152.2 (C-3), 120.6 (C-2), 64.6 (C-10), 32.2 (C-4), 29.0 (C-6,9), 28.5 (C-7), 27.7 (C-5), 25.8 (C-8), 21.0 (CH_3CO); CI-MS, m/z 229 $(M + H)^+$; HRESMS, m/z 251.1255 (calcd for $C_{12}H_{20}NaO_4$: 251.1259).

*10-Acetoxydecanoic acid (**1**):* amorphous colorless solid; IR (NaCl, CH_2Cl_2) υ_{max} = 1728, 1713 cm^{-1}; ^{1}H NMR ($CDCl_3$/TMS, 400 MHz, δ, J in Hz) 4.03 (2H, t, J = 7.2 Hz H-10), 2.33 (2H, t, J = 7.4 Hz H-2), 2.03 (3H, s, CH_3CO−), 1.60 (4H, m, H-3,9), 1.25−1.35 (10H, br s, H4−8); ^{13}C NMR ($CDCl_3$, 50 MHz, δ) 179.8 (C-1), 171.2 (CH_3CO), 64.7 (C-10), 33.9 (C-2), 29.7, 29.3, 29.2, 29.0 (C-4,5,6,7), 28.6 (C-9), 25.9 (C-8), 24.7 (C-3), 21.1 (CH_3CO); CI-MS, m/z 231 $(M + H)^+$; HRESMS, m/z 253.1413 (calcd for $C_{12}H_{22}NaO_4$: 253.1416).

*(11S)-Hydroxydodecanoic acid (**4**):* amorphous colorless solid; $[\alpha]^{25}_D$ 1.54° (*c* 0.2, $CHCl_3$); IR (CaF_2, MeOH) υ_{max} =1710 cm^{-1}; ^{1}H NMR ($CDCl_3$/TMS, 400 MHz, δ, J in Hz) 3.77 (1H, hex, J = 6.3 Hz, H-11), 2.32 (2H, t, J = 7.4 Hz, H-2), 1.61 (2H, quint, J = 7.4 Hz, H-3), 1.16 (3H, d, J = 6.3 Hz, H-12), 1.25−1.40 (14H, br s, H4−10); ^{13}C NMR ($CDCl_3$, 50 MHz, δ) 178.8 (C-1), 68.0 (C-11), 38.8 (C-10), 33.7 (C-2), 29.7, 29.6, 29.5, 29.3, 29.2 (C-8,7,5,6,4), 25.7 (C-9), 24.2 (C-3), 23.3 (C-12); CI-MS, m/z 217 $(M + H)^+$; HRESMS, m/z 239.1619 (calcd for $C_{12}H_{24}NaO_3$: 239.1623).

*11-Oxododecanoic acid (**3**):* amorphous colorless solid; IR (NaCl, $CHCl_3$) υ_{max} = 1711 cm^{-1}; ^{1}H NMR ($CDCl_3$/TMS, 400 MHz, δ, J in Hz) 2.40 (2H, t, J = 7.5 Hz, H-10), 2.33 (2H, t, J = 6.9 Hz, H-2), 2.12 (3H, s, H-12), 1.64 (4H, m, H-3,9), 1.25−1.54 (10H, br s, H-4−8); ^{13}C NMR ($CDCl_3$, 50 MHz, δ) 209.5 (C-11), 178.8 (C-1), 43.7 (C-10), 33.2 (C-2), 29.9 (C-12), 29.5, 29.4, 29.3, 29.2, 29.0 (C-4,5,6,7,8), 24.7 (C-3), 23.9 (C-9); CI-MS, m/z 215 $(M + H)^+$; HRESMS, m/z 237.1464 (calcd for $C_{12}H_{22}NaO_3$: 237.1467).

3-Hydroxydodecanedioic acid: amorphous colorless solid; $[\alpha]^{25}_D$ 1.2° (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD/TMS, 400 MHz, δ, J in Hz) 3.96 (1H, m, H-3), 2.42 (1H, dd, J = 4.8, 14.8 Hz, H-2a), 2.34 (1H, dd, J = 8.2, 14.8 Hz, H-2b), 2.27 (2H, t, J = 7.2 Hz, H-11), 1.59 (2H, m, H-10), 1.46 (2H, br s, H-4), 1.33 (10H, br s, H5−9); ^{13}C NMR (CD_3OD, 50 MHz, δ) 177.0 (C-12), 174.0 (C-1), 69.2 (C-3), 43.0 (C-2), 37.8 (C-4), 34.8 (C-11), 30.4, 30.3, 30.1, 29.9 (C-5,6,7,8,9), 25.9 (C-10); HRESMS, m/z 269.1362 (calcd for $C_{12}H_{22}NaO_5$: 269.1364).

9-Hydroxy-2-nonanone: amorphous colorless solid; ^{1}H NMR (CD_3OD/TMS, 400 MHz, δ, J in Hz) 3.62 (2H, t, J = 6.7 Hz, H-9), 2.40 (2H, t, J = 7.4 Hz, H-3), 2.11 (3H, s, H-1), 1.54−1.30 (10H, br s, H4−8); ^{13}C NMR (CD_3OD, 50 MHz, δ) 208.5 (C-2), 62.9 (C-9), 43.7 (C-3), 32.6 (C-8), 29.8 (C-1), 29.6 (C-5), 29.1 (C-6), 25.5 (C-7), 23.6 (C-4); EI-MS (70 eV), m/z 157, 125, 111, 97 (8%), 82 (32%), 71 (30%), 58 (95%), 43 (100%); HRESMS, m/z 181.1200 (calcd for $C_9H_{18}NaO_2$: 181.1204).

Antimicrobial Assay. In vitro antibacterial studies were carried out according to the disk diffusion method (*15*) by measuring the zones of inhibition against two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228); four Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883), and *Pseudomonas aeruginosa* (ATCC 227853); and three human pathogen fungi, *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 13801), and *Candida glabrata* (ATCC 28838). In addition, the tests were performed against the oral pathogens Gram-positive bacteria *Streptococcus mutans* and *Streptococcus viridans*, obtained from the culture collection of the Laboratory of Microbiology of the Anticancer Hospital of Athens "St. Savvas". Standard antibiotics netilmicin, itraconazole, and 5-flucytocine (Sanofi, Diagnostics Pasteur, at concentrations of 30, 15, and 10 μg/mL) were used to control the sensitivity of the tested bacteria and fungi, respectively, whereas standard sanguinarine (Sigma Chemical Co.) was used especially for the oral pathogens. The tested compounds were dissolved in MeOH. For each experiment a control disk with pure solvent was used as blind control. All of the paper disks had a diameter of 6 mm and were deposited on the surface of the seeded trypticase soy agar Petri dishes. The plates were inoculated with the tested organisms to give a final cell concentration of 10^7 cells/mL and were incubated for 48 h at 37 °C. The fungi were grown on Sabouraud's agar at 25 °C for 48 h. The experiments were repeated three times, and the results (diameters in millimeters) were expressed as average values.

The MIC values of the most active compounds, in the previous experiment, were determined using the dilution method (*15*) in 96-hole plates. Stock solutions of the tested extracts and pure compounds were prepared at 10 and 1 mg/mL, respectively. Serial dilutions of the stock solutions in broth medium (100 μL of Müller−Hinton broth or on Sabouraud broth) were prepared in a microtiter plate (96 wells). Then 1 μL of the microbial suspension (the inoculum, in sterile distilled water) was added to each well. For each strain, the growth conditions and the sterility of the medium were checked, and the plates were incubated as referred above. MICs were determined as the lowest concentrations preventing visible growth. The standard antibiotic netilmicin (at concentrations of 4−88 μg/mL) was used to control the sensitivity of the tested bacteria, whereas 5-flucytocine and itraconazole (at concentrations of 0.5−25 μg/mL) were used as controls against the tested fungi.

RESULTS AND DISCUSSION

One kilogram of Greek royal jelly was lyophilized and extracted successively with dichloromethane and methanol. The extracts were submitted to several chromatographic separations, and the isolated compounds were identified by means of spectroscopic and spectrometric data.

Among the isolated compounds the most abundant ones were 10-hydroxy-2-decenoic acid, 10-hydroxydecanoic acid, and sebacic acid, in accordance with literature data (*1*), and additionally 3,10-dihydroxydecanoic acid.

The dichloromethane extract after several column chromatographic separations afforded fatty and phenolic compounds: methyl *p*-hydroxybenzoate (methyl paraben), 3-(4-hydroxy-3-methoxyphenyl)propionic acid, 9-hydroxy-2-nonanone, (11*S*)-hydroxydodecanoic acid (**4**), sebacic acid, *trans*-10-hydroxy-2-decenoic acid, 10-acetoxydecanoic acid (**1**), 11-oxododecanoic acid (**3**), 10-acetoxy-2-decenoic acid (**2**), *p*-hydroxyacetophenone, acetovanillone, δ-decalactone, δ-octalactone, octanoic acid, 8-hydroxyoctanoic acid, and methyl 3-hydroxydecanoate. The last seven constituents were identified by GC-MS. It is noteworthy that methyl paraben, which is widely used as a synthetic food preservative, has been found as a natural constituent of royal jelly. It is also interesting that the same compound has been found from queen bees' extract (*16*).

The methanol extract afforded steroids and fatty acid compounds such as 24-methylenecholesterol, sitosterol, isofucosterol, 3-hydroxydodecanedioic acid, (10*R*,11*R*)-dihydroxydodecanoic acid (**5**), 3,11-dihydroxydodecanoic acid (**6**), (11*R*,12)-dihydroxydodecanoic acid (**7**), and 3,10-dihydroxydecanoic acid. The compounds sitosterol and isofucosterol were identified by GC-MS.

From those constituents, (10*R*,11*R*)-dihydroxydodecanoic acid (**5**), 3,11-dihydroxydodecanoic acid (**6**), (11*S*,12)-dihydroxydodecanoic acid (**7**), 10-acetoxydecanoic acid (**1**), 10-acetoxydec-2-enoic acid (**2**), 11-oxododecanoic acid (**3**), and (11*S*)-hydroxydodecanoic acid (**4**) are isolated and described herein for the first time as natural products. Compounds **4** and **6** had been tentatively identified in royal jelly (*1*) and **3** in queen bees' excretions (*17*) as methyl esters without any supporting data. Additionally, the constituents methyl *p*-hydroxybenzoate, 3-(4-hydroxy-3-methoxyphenyl) propionic acid, 9-hydroxy-2-nonanone, *p*-hydroxyacetophenone, acetovanillone, δ-decalactone, δ-octalactone, methyl 3-hydroxyldecanoate, and 3-hydroxydodecanedioic acid are reported for the first time as royal jelly constituents.

Although 3,10-dihydroxydecanoic acid has been isolated earlier (*18*), its ^{1}H NMR and ^{13}C NMR spectra have never been

described. Additionally, ^{1}H and ^{13}C NMR data have never been described for 9-hydroxy-2-nonanone (*19*) and 3-hydroxydecanodioic acid (*20*), which have been identified as natural products by GC-MS.

The molecular formula of **5** was determined by HRMS as $C_{12}H_{24}O_4$. The ES-MS spectrum showed signals at 255 $(M + Na)^+$ and 233 $(M + H)^+$. The IR spectrum of **5** indicated the presence of a carbonyl group at 1710 cm^{-1}. Its ^{1}H NMR spectrum showed a doublet at 1.14 ppm ($J = 6.4$ Hz) integrating for three protons corresponding to a terminal methyl group. Additionally, several aliphatic signals were observed: a broad singlet at 1.30−1.40 ppm (H-4−8), a quintet at 1.58 ppm ($J =$ 7.4 Hz) (H-3), and a triplet at 2.26 ppm ($J = 7.4$ Hz) (H-2) integrating for 10, 2, and 2 protons, respectively. The COSY spectrum showed that the quintet at 1.58 ppm (H-3) was cross-coupled with the peaks at 1.30−1.40 and 2.26 ppm, indicating the presence of an aliphatic long chain. The triplet at 2.26 ppm (H-2) in the HMBC spectrum was correlated with two aliphatic carbons at 26.8 ppm (C-3) and 30.4 ppm (C-4) and with a carbonyl group at 178.5 ppm (C-1), revealing the presence of a carboxylic fatty acid, which was also supported by the appearance of an IR signal at 1710 cm^{-1}. Furthermore. the ^{1}H NMR spectrum of **5** showed a multiplet at 3.34 ppm (H-10) and a quintet at 3.54 ppm ($J = 6.4$ Hz) (H-11). The COSY spectrum showed that the last two protons were cross-coupled. From the HMQC and DEPT spectra it was observed that these two protons corresponded to two hydroxy-bearing methine carbons at 76.3 ppm (C-10) and at 71.5 ppm (C-11). Additionally, the proton at 3.54 ppm (H-11) in the COSY spectrum was cross-coupled with the terminal methyl group protons. From all of these data it is suggested that **5** is a linear aliphatic fatty acid chain of 12 carbons with a carboxylic group on one end and a terminal methyl group on the other, having the two hydroxy-bearing methine carbons at the ω-1 and ω-2 positions. The four possible isomers of **5** have been described by Achmad et al. (*21*) as synthetic products. The NMR data of **5** and the optical rotation $[\alpha]^{25}_D$ 6° were identical with those of the described (10*R*,11*R*)-dihydroxydodecanoic acid (**5**).

The molecular formula of **6** was determined by HRMS as $C_{12}H_{24}O_4$. The ES-MS spectrum showed 255 $(M + Na)^+$ and 233 $(M + H)^+$. The IR spectrum of **6** indicated the presence of a carbonyl group (1711 cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra closely resembled those of **5**, making it obvious that **6** was also a linear C-12 aliphatic fatty acid with a terminal methyl group and with two carbons bearing hydroxyl groups. In the case of **6** the two H-2 protons in the ^{1}H NMR spectrum were observed as a doublet doublet at 2.42 ppm ($J = 15.2$, 4.7 Hz) (H-2a) and 2.33 ppm ($J = 15.2$, 8.1 Hz) (H-2b), which were correlated in the COSY spectrum with a multiplet at 3.95 ppm (H-3). From the HMQC and DEPT spectra it was observed that this multiplet corresponded to an oxygenated methine at 69.7 ppm (C-3). The placement of one of the hydroxyl groups at position 3 was confirmed by the mass spectrum of the synthesized methyl ester of **6**. That compound gave an unusual intense peak at *m*/*z* 103, characteristic of methyl esters of 3-hydroxy acids (*18*). The position of the second hydroxyl group was found from the COSY spectrum. In the COSY spectrum, it has been observed that the peak at 3.71 ppm (H-11) that corresponded to an oxygenated methine at 68.6 ppm (C-11) was cross-coupled with the terminal methyl. From all of these data, it is suggested that **6** is a linear C-12 aliphatic fatty acid with a carboxy group on one end and a terminal methyl group on the other, having the two hydroxy-bearing methine carbons at positions 3 and 11. The optical rotation of **6** was $[\alpha]^{25}_D$ 2.2°. Due to the small amount of the isolated compound the absolute stereochemistry could not be determined.

The molecular formula of **7** was determined by HRMS as $C_{12}H_{24}O_4$. The ES-MS spectrum showed a structure revealing peaks at *m*/*z* 255 $(M + Na)^+$ and 233 $(M + H)^+$. The IR spectrum of **7** indicated the presence of a carbonyl group at 1710 cm^{-1}. The ^{1}H NMR spectrum was to a large extent similar to that of **5**, making it obvious that **7** was also a dihydroxylated linear C-12 aliphatic fatty acid. One significant difference was the absence of the signal of the terminal methyl protons. Additionally, two double doublets at 3.39 ppm ($J = 6.4$, 11.3 Hz) (H-12a) and 3.45 ppm ($J = 4.7$, 11.3 Hz) (H-12b) and a multiplet at 3.53 ppm (H-11) were observed. All of these peaks were cross-coupled in the COSY spectrum. The HMQC and DEPT spectra revealed that the two peaks at 3.39 and 3.45 ppm corresponded to a hydroxymethylene group, whereas the peak at 3.53 ppm corresponded to a hydroxy-bearing methine. The absence of a terminal methyl group led to the placement of the hydroxymethylene group at the terminal position. From all of these data and especially from the COSY spectrum, the second hydroxyl group should be placed at the ω-1 position. Consequently, the structure of **7** was determined to be 11,12-dihydroxydodecanoic acid. The optical rotation of **7** was $[\alpha]^{25}_D$ −1.7°. The absolute configuration of C-11 is proposed as *S*, on the basis of the optical rotation of several closely related structures such as (7*S*,8)-dihydroxyoctanoate (*22*).

The empirical formula of **2** was established by accurate mass measurement as $C_{12}H_{20}O_4$. The CI-MS spectrum showed a peak at 233 $(M + H)^+$. The IR spectrum showed a characteristic peak of a conjugated carboxy group at 1697 cm^{-1} and one additional acetoxy carbonyl at 1728 cm^{-1}. The NMR profile of **2** was very similar to that of *trans*-10-hydroxydec-2-enoic acid. Indeed, the ^{1}H NMR spectrum displayed the characteristic signals of the trans double bond, namely, a doublet triplet at 7.0 ppm ($J = 15.5$, 7.2 Hz, H-3), a doublet at 5.80 ppm ($J =$ 15.5 Hz, H-2), and a quartet at 2.21 ppm (H-4) as well as a series of peaks corresponding to a long linear chain at 1.31−1.60 ppm. The most important differences in the ^{1}H NMR spectrum were the presence of one singlet at 2.02 ppm corresponding to three protons and the downfield shift of the triplet, which corresponded at H-10. In the case of **2**, that triplet was observed at 4.03 ppm, whereas in the case of *trans*-10-hydroxydec-2-enoic acid it was at 3.55 ppm. The singlet at 2.02 ppm, indicative of an acetoxy group, in combination with the change in the chemical shift of H-10, showed that the hydroxyl group at position 10 was acetylated. Indeed, the HMBC spectrum showed that the carbonyl of the acetoxy moiety was correlated with H-10. Consequently, compound **2** is *trans*-10-acetoxydec-2-enoic acid, which has been reported only as a synthetic derivative (*23*). It should be noted that previous studies (*1*) on royal jelly that included saponification and subsequent methylation of fatty acids could not lead to the identification of this type of compound.

The molecular formula of **1** was determined by HRMS as $C_{12}H_{22}O_4$. The CI-MS spectrum showed a peak at *m*/*z* 231 $(M + H)^+$. The IR spectrum of **1** indicated the presence of a carbonyl group at 1713 cm^{-1} and an acetoxy carbonyl at 1728 cm^{-1}. The ^{1}H NMR spectrum was almost similar to that of **2**, but in the ^{1}H NMR spectrum of **1**, the two peaks corresponding to the double bond were absent. It was obvious that the product **1** had the same structure as that of **2** but that it was saturated. From all of these data it is suggested that **1** is 10-acetoxydecanoic acid. The NMR and MS data of **1** were identical with those reported for synthetic 10-acetoxydecanoic acid (*24*).

Table 1. Antimicrobial Activities (Zones of Inhibition/MIC mg/mL, $n = 3$) of the Studied Royal Jelly and Its Main Components

tested compound	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*S. mutans*	*S. viridans*	*C. albicans*	*C. tropicalis*	*C. glabrata*
MeOH extract	16/0.67	16/0.74	14/0.94	12/1.20	12/0.98	10/0.95	14/0.72	15/0.58	10/1.25	11/0.95	11/0.98
CH_2Cl_2 extract	14/0.70	14/0.72	12/0.98	10/1.33	10/1.26	10/0.98	12/0.80	12/0.70	11/1.34	13/0.97	14/0.80
methyl paraben	16/0.45	13/0.78	13/0.75	8/1.50	12/0.90	10/1.10	12/0.98	13/0.80	11/1.10	13/0.90	14/0.85
3,10-dihydroxydecanoic acid	14/0.55	16/0.40	13/0.65	13/0.77	9/1.45	9/1.40	11/0.78	10/0.84	9/1.25	10/0.90	10/1.17
trans-10-hydroxydec-2-enoic acid	12/0.99	12/0.95	14/0.67	12/0.90	9/1.64	8/1.50	12/0.78	12/0.74	9/1.10	11/0.95	10/1.25
sebacic acid	11/0.95	11/0.88	12/0.90	10/1.10	9/1.35	9/1.45	10/1.14	10/0.95	16/0.20	18/0.18	18/0.15
3-hydroxydodecanedioic acid	16/0.35	18/0.25	14/0.64	14/0.57	15/0.47	12/1.10	17/0.35	18/0.18	14/0.7	15/0.49	15/0.35
9-hydroxy-2-nonanone	16/0.35	15/0.40	10/1.18	9/1.50	10/1.32	11/1.18	14/0.67	14/0.78	NA[a]	NA	NA
24-methylenecholesterol	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3-(4-hydroxy-3-methoxyphenyl)-propionic acid	15/0.67	11/0.99	11/1.12	8/1.63	10/1.25	10/1.15	10/1.37	11/0.90	9/1.45	11/1.12	10/1.17
1	16/0.35	13/0.57	14/0.60	11/0.88	10/1.35	9/1.37	11/0.95	15/0.54	10/1.18	10/1.20	11/1.00
2	18/0.17	16/0.35	10/1.10	12/0.89	9/1.37	9/1.45	15/0.36	16/0.28	NA	NA	NA
3	14/0.45	12/0.83	14/0.70	14/0.65	13/0.80	12/0.83	15/0.45	16/0.37	12/0.67	14/0.45	15/0.26
4	17/0.27	16/0.36	13/0.68	14/0.55	14/0.50	13/0.98	18/0.29	17/0.25	13/0.85	14/0.55	15/0.39
5	14/0.60	14/0.57	13/0.70	12/1.10	10/1.25	10/1.17	12/0.80	12/0.78	9/1.35	10/1.27	9/1.87
6	14/0.64	15/0.47	13/0.49	12/0.98	11/0.80	9/1.55	10/1.20	12/0.80	9/1.40	12/0.55	12/0.67
7	14/0.64	13/0.59	12/0.75	13/0.98	9/1.45	10/1.10	13/0.85	14/0.70	9/1.47	9/1.35	10/1.55
sanguinarine	NT[a]	NT	NT	NT	NT	NT	28/0.015	28/0.015	NT	NT	NT
netilmicin	21/0.004	25/0.004	20/0.088	23/0.008	22/0.008	24/0.010	24/NT	25/NT	NT	NT	NT
itraconazole	NT	NT	NT	NT	NT	NT	NT	NT	20	22	23
5-flucytocine	NT	NT	NT	NT	NT	NT	NT	NT	0.01	0.001	0.0001

[a] NT, not tested; NA, not active.

Accurate mass measurement of compound **4** showed that its molecular formula was $C_{12}H_{24}O_3$. The IR spectrum of **4** indicated the presence of a carbonyl group at 1710 cm^{-1}. The NMR profile of **4** was very similar to that of (10*R*,11*R*)-dihydroxydodecanoic acid (**5**). Indeed, the ^1H NMR spectrum displayed the characteristic signal of the terminal methyl group, namely, a doublet at 1.16 ppm (H-12), a quintet at 1.61 ppm (H-3), a hexaplet at 3.77 ppm (H-11), and a broad singlet at 1.25−1.40 ppm (H-4−10) corresponding to a long linear methylene chain. The only important difference in its ^1H NMR spectrum was that in the case of **4**, the multiplet at 3.34 ppm was absent. All of these evidences revealed that product **4** is a linear fatty C-12 aliphatic fatty acid with a carboxy group on one end and a terminal methyl group on the other, having only one hydroxy-bearing methine carbon at the ω-1 position. The precise position of the hydroxyl group was confirmed from the COSY spectrum. In the COSY spectrum it was observed that the proton at 3.77 ppm (H-11) was cross-coupled with the terminal methyl. Consequently, the product **4** is 11-hydroxydodecanoic acid. The absolute stereochemistry of **4** was found as *S* on the basis of the comparison of optical rotation with that of (9*R*)-hydroxydecanoic acid (*25*).

The empirical formula of **3** was established by accurate mass measurement as $C_{12}H_{22}O_3$. The IR spectrum of **3** indicated the presence of two overlapped carbonyl groups at 1711 cm^{-1}. Its ^1H NMR spectrum showed a singlet at 2.12 ppm integrating for three protons. From the HMQC and DEPT spectra it was observed that this singlet corresponded to a methyl group at 29.3 ppm (C-12). The HMBC spectrum showed that this methyl group was correlated with a carbonyl group at 209.5 ppm (C-11), revealing the presence of a CH_3CO group. Furthermore, the ^1H NMR spectrum of **3** showed a pair of triplets each integrating for two protons, at 2.40 ppm ($J = 7.5$ Hz) (H-10) and at 2.33 ppm ($J = 6.9$ Hz) (H-2). These peaks corresponded to methylene groups next to carbonyl groups. Indeed, the HMBC spectrum showed that the triplet at 2.33 ppm was correlated with a carbonyl group at 178.8 ppm (C-1) and that the triplet at 2.40 ppm was correlated with a carbonyl group at 209.5 ppm (C-11). Additionally, the ^1H NMR spectrum of **3** showed a quintet at 1.64 ppm (H-3,9) and a broad singlet at 1.25−1.54 ppm (H-4−8) corresponding to a long linear chain. From these data, it was obvious that compound **3** was also a linear C-12 aliphatic fatty acid, with a carbonyl group at the ω-1 position. Consequently, the product **3** is 11-oxododecanoic acid.

The aforementioned isolation procedure was repeated two more times for royal jelly samples collected from the same region in years 2003 and 2004, and all of the above-described constituents were identified.

Extracts of royal jelly as well as its isolated compounds were studied for their antimicrobial activity against six Gram-negative and Gram-positive bacterial strains, two oral pathogens (*S. mutans* and *S. viridans*), and three human-pathogen fungi (*C. albicans*, *C. tropicalis*, and *C. glabrata*). The results of these tests (**Table 1**) showed interesting and promising antimicrobial activity.

Some of the isolated compounds such as 3-hydroxydodecanedioic acid, **3**, and **4** exhibited very strong antimicrobial activity against all assayed microorganisms. The most active constituents against *S. aureus*, *S. epidermidis*, and the oral pathogens *S. mutans* and *S. viridans* were 3-hydroxydodecanedioic acid **2** and **4**, with MIC values ranging from 0.17 to 0.36 mg/mL. Methyl paraben and 9-hydroxy-2-nonanone especially showed specific activity against *S. aureus* and *S. epidermidis*. The Gram-negative bacteria appeared to be the most resistant against all pure compounds and extracts assayed, with 3-hydroxydodecanedioic acid presented as the most active one (MIC = 0.47 mg/mL against *K. pneumoniae*). The higher activity against all fungi was exhibited by sebacic acid, with MIC values ranging from 0.15 to 0.20 mg/mL, followed by compound **3** (MIC values of 0.26−0.67 mg/mL), whereas compound **2** (*trans*-10-acetoxydec-2-enoic acid) and 9-hydroxy-2-nonanone were shown to be completely inactive against all fungi. It is noteworthy that both MeOH and the CH_2Cl_2 extracts also showed a distinguished antimicrobial spectrum of activity (MICs ranging from 0.67 to 1.3 mg/mL), whereas only the above-mentioned pure compounds exhibited much stronger activities. Especially compounds **3** and **4** showed the highest as well as widest spectrum of antimicrobial activities compared with all assayed extracts and pure compounds.

In conclusion, our studied samples as well as most of the isolated chemical compounds have shown antimicrobial activity, confirming its traditional reputation as an antimicrobial agent.

ACKNOWLEDGMENT

We thank Dr. E. Chinou from the Laboratory of Microbiology of the Anticancer Hospital of Athens "St. Savvas" for her help concerning the oral pathogen bacteria. We acknowledge the "Attiki" Bee-Culturing Co. for the offer of royal jelly samples.

LITERATURE CITED

(1) Lercker, G.; Capella, P.; Conte, L. S.; Ruini, F.; Giordani, G. Components of royal jelly: I. Identification of the organic acids. *Lipids* **1981**, *12*, 912−919.

(2) Townsend, G. F.; Morgan, J. F.; Hazlett, B. Activity of 10-hydroxydecenoic acid from royal jelly against experimental leukaemia and ascitic tumours. *Nature* **1959**, *183*, 1270−1271.

(3) Blum, M. S.; Novak, A. F.; Taber, S. 10-Hydroxy-2Δ-decenoic acid, an antibiotic found in royal jelly. *Science* **1959**, *130*, 452−453.

(4) Nagai, T.; Inoue, R. Preparation and the functional properties of water extract and alkaline extract of royal jelly. *Food Chem*. **2004**, *84*, 181−186.

(5) Plettner, E.; Slessor, K. N.; Winston, M. L.; Oliver, J. E. Caste-selective pheromone biosynthesis in honeybees. *Science* **1996**, *271*, 1851−1853.

(6) Plettner, E.; Slessor, K. N.; Winston, M. L. Biosynthesis of mandibular acids in honeybees (*Apis mellifera)*: de novo synthesis, route of fatty acid hydroxylation and caste selective β-oxidation. *Insect Biochem. Mol. Biol.* **1998**, *28*, 31−42.

(7) Okamoto, I.; Taniguchi, Y.; Kunikata, T.; Kohno, K.; Iwaki, K.; Ikeda, M.; Kurimoto, M. Major royal jelly protein 3 modulates immune responses in vitro and in vivo. *Life Sci*. **2003**, *73*, 2029−2045.

(8) Fujiwara, S.; Imai, J.; Fujiwara, M.; Yaeshima, T.; Kawashima, T.; Kobayashi, K. A potent antibacterial protein in royal jelly. *J. Biol. Chem.* **1990**, *265*, 11333−11337.

(9) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectometry*; Allured Publishing: Carol Stream, IL, 2001.

(10) The Aldrich library of FT-NMR spectra; 1997.

(11) Villieras, J.; Rambaud, M.; Graff, M. La reaction de wittig-horner en milieu heterogene VI. Selectivite de la reaction sur des composes bifonctionnels. *Tetrahedron Lett.* **1985**, *26*, 53−56.

(12) Doyle, M.; Hu, W.; Valenzuela, M. V. Total synthesis of (*S*)-(+)-imperanene. Effective use of regio- and enantioselective intramolecular carbon−hydrogen insertion reactions catalyzed by chiral dirhodium(II) carboxamidates. *J. Org. Chem.* **2002**, *67*, 2954−2959.

(13) Kalinowska, M.; Nes, W. R.; CrumLey, F. G.; Nes, W. D. Stereochemical differences in the anatomical distribution of C-24 alkylated sterols in *Kalanchoe daigremoutiana*. *Phytochemistry* **1990**, *11*, 3427−3434.

(14) Kazuo, I.; Shuichi, S.; Yasuzi, Y. Novel 19-oxygenated sterols from the Okinawan soft coral *Litophyton viridis*. *Chem. Pharm. Bull*. **1989**, *9*, 2553−2554.

(15) Melliou, E.; Chinou, I. Chemical analysis and antimicrobial activity of Greek propolis. *Planta Med*. **2004**, *70*, 515−519.

(16) Barbier, M.; Lederer, E.; Reichstein, T.; Schindler, O. Auftrennung der sauren anteile von extrakten aus bienenköniginnen (*Apis mellifera* L.); Isolierung des als königinnen-substanz bezeichneten pheromones *Helv. Chim. Acta* **1960**, *207*, 1682−1689.

(17) Engels, W.; Rosenkranz, P.; Adler, A.; Taghizadeh, T.; Lubke, G.; Francke, W. Mandibular gland volatiles and their ontogenetic patterns in queen honey bees, *Apis mellifera carnica*. *J. Insect Physiol*. **1997**, *43*, 307−313.

(18) Weaver, N.; Johnston, N. C.; Benzamin, R.; Law, J. H. Novel fatty acids from the royal jelly of honeybees (*Apis mellifera*, L.). *Lipids* **1968**, *6*, 535−538.

(19) Katsiotis, S. T.; Langezaal, C. R.; Sceffer, J. J. C.; Verpoorte, R.; Comparative study of the essential oils from hops of various *Humulus lupulus* L. cultivars. *Flavour Fragrance J.* **1989**, *4*, 187−191.

(20) Wilkins, A. L.; Lu, Y.; Tan, S. T. Extractives from New Zealand honeys. 5. Aliphatic dicarboxylic acids in New Zealand rewarewa (*Knightea excelsa*) honey. *J. Agric. Food Chem*. **1995**, *43*, 3021−3025.

(21) Achmad, S.; Høyer, T.; Kjær, A.; Makmur, L.; Norrestam, R. Molecular and crystal structure of hyptolide, a naturally occurring α,β-unsaturated δ-lactone. *Acta Chem. Scand*. **1987**, *41*, 599−609.

(22) Lees, W. J.; Whitesides, G. M. Diastereoselectivity (enantioselectivity) of aldol condensations catalyzed by rabbit muscle aldolase at c-2 of RCHOHCHO if R has an appropriately placed negatively charged group. *J. Org. Chem*. **1993**, *58*, 1887−1894.

(23) Chiron, R. New synthesis of royal jelly acid. *J. Chem. Ecol.* **1982**, *8*, 709−713.

(24) Blay, G.; Gardona, L.; Garcia, B.; Garcia, C. L.; Pedro, J. R. Reductive cleavage of 2,2,2-trichloroethyl esters with sodium telluride. *Synth. Commun.* **1998**, *28*, 1405−1414.

(25) Hamada, T.; Daikai, K.; Irie, R.; Katsuki, T. Insect pheromone synthesis using Mn-salen catalysed asymmetric epoxidation as a key step. *Tetrahedron Asymm.* **1995**, *6*, 2441−2451.

Received for review June 29, 2005. Revised manuscript received September 10, 2005. Accepted September 12, 2005. This study was supported by the General Secretariat of Research and Technology of Greece (PENED project) as well as by "Korres" Natural Products, S.A.

JF051550P