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(1*S*,5*R*)-(−)-2,4,4-Trimethylbicyclo[3.1.1]hept-2-en-6-one, from the essential oil of the Ethiopian plant *Laggera tomentosa*

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Abstract

Enantiomerically pure (1*S*,5*R*)-(−)-2,4,4-trimethylbicyclo[3.1.1]hept-2-en-6-one has been found along with chrysanthenone, thymoquinol dimethyl ether and two stereoisomers of dehydrocitral in the essential oil of *Laggera tomentosa*, which is endemic to Ethiopia. (1*S*,5*R*)-(−)-2,4,4-trimethylbicyclo[3.1.1]hept-2-en-6-one has been identified for the first time from a natural source. Chrysanthenone which is the major constituent of the oil (58%), occurs as the (+)- and (−)-enantiomers in a ca 92:8 ratio. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Laggera tomentosa; Asteraceae; Essential oil; Monoterpene; (1*S*,5*R*)-(−)-2,4,4-trimethylbicyclo[3.1.1]hept-2-en-6-one

1. Introduction

The genus *Laggera* Sch. Bip. ex Benth. & Hook. (Asteraceae) comprises about 17 species confined to the Old World Tropics. *L. tomentosa* Sch. Bip. ex Oliv. & Hiern is a perennial fragrant bushy herb about 0.5–1.2 m high, and occurs in Ethiopia only (Tadesse, 1999, in press). Locally, it is a well-known medicinal plant. The juice of the crushed plant is ingested as a treatment for stomach-ache, and is also used against migraine. It can also be used as a fumigant and for cleansing milk containers (Tadesse, 1999, in press; Mercier, 1979; Mercier, 1980–1982). A literature survey indicated that there are no published reports on the chemistry of the plant.

As part of our study on the essential oil plants of Ethiopia, we report on the isolation and characterisation of some monoterpenes, namely, (1*S*,5*R*)-(−)-2,4,4-trimethylbicyclo[3.1.1]-hept-2-en-6-one (**1**) which is a new natural product, (1*S*,5*R*)-(+)-chrysanthenone (**2**; 2,7,7-trimethylbicyclo[3.1.1]hept-2-en-6-one), thymoquinol dimethyl ether (**3**; 1,4-dimethoxy-5-methyl-2-(1-methylethyl)benzene), and two stereoisomers of dehydrocitral (**4** and **5**; (2*E*,4*E*)- and (2*Z*,4*E*)-3,7-dimethylocta-2,4,6-trienal) from the essential oil of *L. tomentosa*.

2. Results and discussion

The essential oil of *L. tomentosa* was isolated by steam distillation and further separated by column chromatography on Si-gel into an oxygenated and a hydrocarbon fraction. Additional column chromatography of the oxygenated fraction yielded fractions containing mixtures of (i) compounds **1** and **2**, (ii) compound **2** with a small amount of **1**, and (iii) compounds **3** and filifolone (**6**, $[\alpha]^{22}_{D}$ −636° ($CHCl_3$; *c* 0.30), artefact ?). The fractions were subjected to preparative GC. The enriched fractions were investigated by enantioselective GC, spectroscopic methods, and polarimetry.

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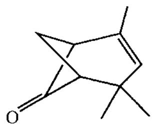
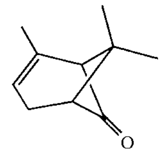
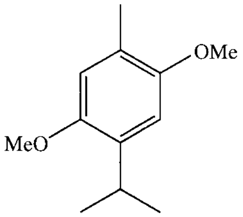


(1*S*, 5*R*)-(-)-**1**



(1*S*, 5*R*)-(+)-**2**



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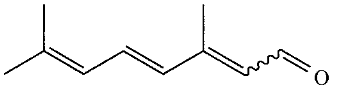
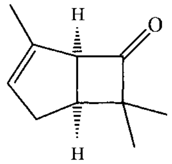


4 and **5**



(*S*, *S*)-(-)-**6**

Compound **1**, $C_{10}H_{14}O$ (*m*/*z* 150, M^+), appeared to be enantiomerically pure as judged by enantioselective GC. Baseline separation was obtained for a partially racemised authentic sample. The specific rotation, $[\alpha]_D^{21}$ −67° ($CHCl_3$; *c* 0.47), was calculated taking into account the presence of racemic chrysanthenone (**2**) and racemic filifolone (**6**). The latter compound (**6**) (in the mixture from preparative GC) was found to be racemic by enantioselective GC. The formation of racemic **6** in the GC column is probably due to thermal rearrangement of chrysanthenone (**2**) as previously demonstrated by Bates, Onore, Paknikar and Steelink (1967) and Erman, Treptow, Bakuzis and Wenkert (1971) (see also below). Authentic (−)-**6** showed no loss of optical activity when subjected to preparative GC whereas (+)-**2** racemised.

Strong IR absorption at 1774 cm^{-1} indicated the presence of a cyclobutanone moiety. The ^1H NMR data established the presence of a vinylic methyl group at δ 1.72 (*d, J* = 1.3 Hz), geminal methyl groups at δ 1.05 (*s*) and 1.09 (*s*), an olefinic proton at δ 5.01 (*br s*), and two bridgehead protons (*t*) at δ 2.83 and 2.93. The ^{13}C NMR spectra revealed 10 carbon atoms which were identified as three methyl-, one methylene-, three methine-, and three quaternary carbon atoms. The spectral data obtained for compound **1** are consistent with those reported for synthetic 2,4,4-trimethylbicyclo[3.1.1]hept-2-en-6-one (Torrance & Steelink, 1974; Erman, 1967). An authentic sample of (+)-2,4,4-trimethylbicyclo[3.1.1]hept-2-en-6-one (**1**) was obtained by irradiation of (−)-chrysanthenone as previously reported (Torrance & Steelink, 1974; Erman, 1967). Its NMR spectra were consistent with those of compound **1** isolated from the *L. tomentosa* oil.

2,4,4-Trimethylbicyclo[3.1.1]hept-2-en-6-one (**1**) was chromatographically (GC) detected in *n*-pentane and diethyl ether extracts of the plant excluding the possibility that **1** had been formed during the steam distillation process. Furthermore, **1** was present (^1H- and ^{13}C-NMR) in the crude oil obtained by steam distillation ruling out the possibility of its formation under GC conditions.

The major constituent (**2**) of the oil (58 %) which was isolated from the oxygenated fraction by liquid column chromatography on Si-gel, was optically active ($[\alpha]_D^{20}$ +97.6° ($CHCl_3$; *c* 4.0)), and its MS, IR and NMR spectra were found to be consistent with those reported for chrysanthenone (**2**) (Erman, 1967; Coxon, Hydes & Steel, 1984; Uchio, 1978).

Photochemically induced allylic rearrangement of (−)-verbenone in cyclohexane using a Pyrex filter (Erman, 1967) yielded an enantiomeric mixture of (−)-chrysanthenone as well as other products including 2,4,4-trimethylbicyclo[3.1.1]hept-2-en-6-one. The identity of the enantiomers in the oil of *L. tomentosa* was established by co-injection of racemic synthetic and natural chrysanthenone on an enantioselective GC column. The (+)- and (−)-enantiomers were found to be present in the oil in a 92: 8 ratio (baseline separation of the enantiomers).

The oxygenated fraction containing compounds **3** and **6**, was subjected to preparative GC yielding samples of **3** (99% pure by GC) and **6** (94% pure by GC). They were identified as thymoquinol dimethyl ether and filifolone, respectively, by comparison of their NMR spectra with those reported for **3** and **6** (Uchio, 1978; Lasswell & Hufford, 1977; Beereboom, 1965; Marotta, Pagani, Righi & Rosini, 1994; Adams, Bhatnagar, Cookson & Tuddenham, 1975). Filifolone (**6**) was not detected in the in *n*-pentane and diethyl ether extracts of the plant and, thus, may possibly be an artefact formed/liberated during the steam distillation. Studies on its origin are in progress.

The ^1H NMR spectra of compounds **4** and **5** showed resonance signals at δ 10.07 (1H, *d, J* = 8.2 Hz) and at 10.16 (1H, *d, J* = 8.1 Hz) characteristic of aldehydic protons adjacent to vinylic protons. The compounds were subsequently identified as an approximate 1:1 ratio of (2*E*)- and (2*Z*)-dehydrocitral (**4** and **5**), by comparison with ^1H NMR spectra reported for these compounds (Duhamel, Duhamel & Lecouve, 1987; Sheves & Nakanishi, 1983; Bohlmann & Kapteyn, 1973).

3. Experimental

3.1. General

IR was recorded on Magna IR Spec 550; 1D and 2D NMR were measured on DPX 300 MHz and DRX 500 MHz Bruker instruments.

3.2. Plant material

L. tomentosa was collected near Chancho, about 40 km Northwest of Addis Ababa, Ethiopia. A voucher specimen has been authenticated and deposited at the National Herbarium, Biology Department, Addis Ababa University.

3.3. Isolation and analysis

The leaves and inflorescence of *L. tomentosa* were subjected to steam distillation for 3 h using a Clevenger-type apparatus yielding a pale yellow oil (0.5% v/w). The essential oil (1.5 g) was subjected to low temperature (5°C) column chromatography in a glass column packed with 40 g Si-gel (63–200 mesh), using *n*-pentane and increasing amounts of diethyl ether in pentane as eluents. Further fractionation was accomplished using preparative GC on a Hewlett Packard GC instrument equipped with a glass column (15% OV-17, 2.26 m × 4 mm). Injector and detector temperatures were 250 and 260°, respectively. The column temperature was 150°. Nitrogen was used as carrier gas at a flow rate of 100 ml min^{-1}.

The oil samples were analysed by GC and GC-MS. A Supelcowax 10 (30 m × 0.25 mm, 0.2 μm film thickness) fused silica capillary column with helium as carrier gas at a flow rate of 1 ml min^{-1}, and with a split ratio of 82, was used for GC analysis. The column temperature was programmed from 70° (5 min), 70–180° (5° min^{-1}), 180–240° (10° min^{-1}) and 240° (10 min). An FID detector was used at a temperature of 260° and the injector temperature was 210°.

Enantioselective GC analyses were performed on β-DEX-120 and γ-DEX-120 (30 m × 0.25 mm, 0.25 μm film thickness) fused silica capillary columns with the temperature programmed as follows: 100° (10 min), 1.5° min^{-1} to 220° (15 min), and on a CP Chirasil-DEX CB (25 m × 0.25 mm, 0.25 μm film thickness) fused silica capillary column programmed as follows: 100° (10 min), 1.5° min^{-1} to 180° (15 min). Carrier gas flow rate was 1 ml min^{-1}. Injector and detector temperatures were 210 and 260°, respectively. GC/MS analyses were carried out using MassLab VI.1 system equipped with FI 8000 GC. A Supelcowax 10 (30 m × 0.25 mm, 0.2 μm film thickness) fused silica capillary column was used with a temperature program of 60° (5 min) 60–240° at 5° min^{-1}, with injector temperature at 210°.

The essential oil of *L. tomentosa* obtained by direct extraction with *n*-pentane and diethyl ether was compared (GC) with the oil isolated by steam distillation.

3.3.1. (1S,5R)-(−)-2,4,4-Trimethylbicyclo[3.1.1]hept-2-en-6-one (*1*)

$[\alpha]_D^{21}$ −67° (40% **1**, 8% (±)-**6**, 51% (±)-**2**; $CHCl_3$; *c* 0.47); IR ($CHCl_3$): ν_{max} 1774 cm^{-1}; GC-MS 70 eV, *m*/*z*: 150 (M^+) (2), 122 (18), 108 (13), 107 (100), 105 (22), 93 (12), 91 (69), 80 (18), 79 (26), 77 (13), 41 (11), 39 (16). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.07 (3H, *s*, Me-4), 1.09 (3H, *s*, Me-4), 1.68 (1H, *m*, H-7), 1.72 (3H, *d*, $J = 1.3$ Hz, Me-2), 1.92 (1H, *m*, H-7), 2.84 (1H, *t*, $J = 6$ Hz, H-1), 2.94 (1H, *t*, $J = 6$ Hz, H-5), 5.02 (1H, *br s*, H-3); ^{13}C NMR ($CDCl_3$, 500 MHz): δ 203.2 (C-6), 139.2 (C-2), 128.4 (C-3), 68.2 (C-1), 60.3 (C-5), 44.8 (C-4), 27.9 (4-Me), 27.4 (4-Me), 24.9 (C-7), 22.1 (2-Me). Detectable amounts of (+)-**1** was not observed (enantioselective GC). Baseline separation was obtained for an enantiomeric mixture of **1** prepared photochemically from an enantiomeric mixture of chrysanthenone (**2**).

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