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The monoterpenes of *Artemisia tridentata* ssp. *vaseyana*, *Artemisia cana* ssp. *viscidula* and *Artemisia tridentata* ssp. *spiciformis*

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Abstract

Monoterpenes from three different members of the Anthemideae family, *Artemisia tridentata* ssp. *vaseyana*, *Artemisia cana* ssp. *viscidula* and *Artemisia tridentata* ssp. *spiciformis* were isolated and their structures determined using spectroscopic techniques. A total of 26 irregular and regular monoterpenes were identified. Among these, 20 had previously been identified in the Anthemideae family. Of the remaining six, four were known, but previously unidentified in this family. 2,2-Dimethyl-6-isopropenyl-2*H*-pyran, 2,3-dimethyl-6-isopropyl-4*H*-pyran and 2-isopropenyl-5-methylhexa-*trans*-3,5-diene-1-ol were isolated from both *A. tridentata* ssp. *vaseyana* and *A. cana* ssp. *viscidula*. The irregular monoterpene 2,2-dimethyl-6-isopropenyl-2*H*-pyran has a carbon skeleton analogous to the biologically important triterpene squalene. Two additional irregular monoterpenes, artemisia triene and *trans*-chrysanthemal were isolated from *A. cana* ssp. *viscidula* and lavandulol was isolated from *A. tridentata* ssp. *spiciformis*. This is the first time a compound possessing a lavandulyl-skeletal type has been found in the Anthemideae family. © 2002 Published by Elsevier Science Ltd.

Keywords: *Artemisia tridentata* ssp. *vaseyana*; *Artemisia cana* ssp. *viscidula*; *Artemisia tridentata* ssp. *spiciformis*; Compositae; Monoterpenes; Irregular monoterpenes; Squalene analogous C-10 skeleton; Lavandulyl skeleton; Chrysanthemol; Fraganol

1. Introduction

Members of the plant family Asteraceae are widespread in the arid and semiarid western USA. The woody sagebrushs of the genus *Artemisia* are Anthemideae tribe members of this family (McArthur et al., 1981). Genus *Artemisia* subgenus *Tridentatae* is composed of 11 species of differing geographic distribution (McArthur et al., 1981; Klobus, 1990), including the following three subspecies of two species: *Artemisia tridentata* ssp. *vaseyana*, *Artemisia cana* ssp. *viscidula* and *Artemisia tridentata* ssp. *spiciformis*. McArthur and Goodrich (1986) have suggested that *A. tridentata* ssp. *spiciformis* is probably a stabilized hybrid between *A. tridentata* ssp. *vaseyana* and *A. cana* ssp. *viscidula*. It was originally identified as *Artemisia tridentata* ssp. *rothrockii* but later reclassified to *A. tridentata* ssp. *spiciformis*.

In the present study, *A. tridentata* ssp. *vaseyana*, *A. cana* ssp. *viscidula* and *A. tridentata* ssp. *spiciformis* growing proximally at a Utah location were collected and the monoterpenoids isolated and identified for taxonomic and statistical study. Identification of isolated monoterpenes was accomplished by comparative spectral analysis (^{1}H NMR, ^{13}C NMR and IR spectra) to known compounds or synthesized standards.

2. Results and discussion

Twenty of the 26 monoterpenoids identified from *Artemisia* were known and 12 of those 26 were found in two or more of the species investigated. Two previously unidentified monoterpenoids, 2,2-dimethyl-6-isopropenyl-2*H*-pyran **1** and 2,3-dimethyl-6-isopropyl-4*H*-pyran **3** were isolated from *A. cana* ssp. *viscidula* and *A. tridentata* ssp. *vaseyana*. Four known, but never naturally isolated monoterpenoids, 2-isopropenyl-5-methylhexa-*trans*-3,5-diene-1-ol **4**, artemisia triene **5**, *trans*-chrysanthemal **6** and lavandulol **7** were isolated from one or more of the species investigated. Further, this is the first time a compound containing a lavandulyl skeletal system has been identified in the Anthemideae family (Fig. 1).

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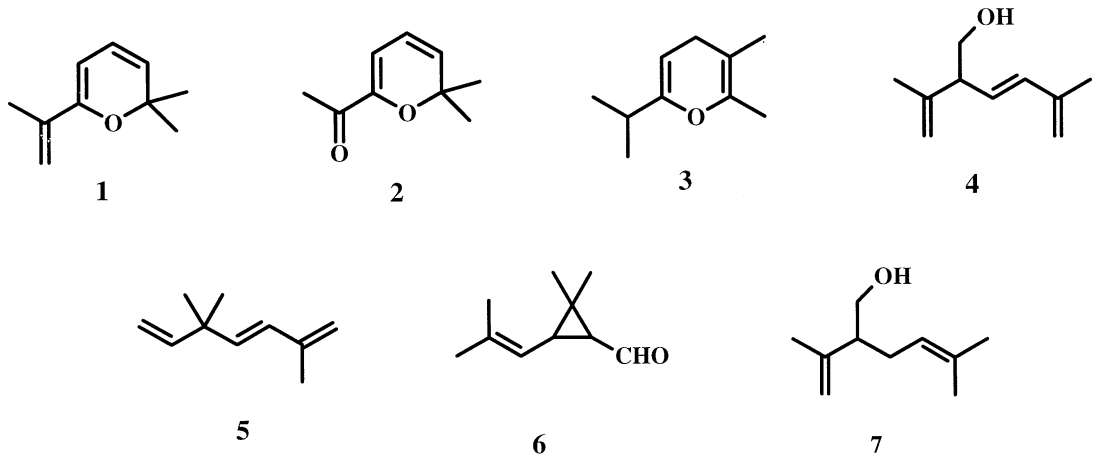

1 2 3 4 5 6 7

Fig. 1. Structures of isolated and synthesized compounds: 2,2-dimethyl-6-isopropenyl-2*H*-pyran **1**, 2,2-dimethyl-6-acetyl-2*H*-pyran **2**, 2,3-dimethyl-6-isopropyl-4*H*-pyran **3**, 2-isopropenyl-5-methylhexa-*trans*-3,5-dien-1-ol **4**, artemisia triene **5**, chrysanthemal **6** and lavandulol **7**.

Comparisons to previous monoterpene isolations from *A. cana* ssp. *viscidula* and *A. tridentata* ssp. *spiciformis* reveal that many compounds were isolated in both studies. However, a few differences exist. In previous work, monoterpenes isolated from *A. cana* ssp. *viscidula* included β-pinene, *p*-cymene, artemisia ketone, yomogi alcohol, lyratyl acetate and *trans*-3-(1-oxo-2-methyl-1-propyl)-2,2-dimethylcyclopropylmethanol but were not found in the latest study. Conversely, rothrockene, artemiseole, isolyratol, lyratol, fragranyl acetate and fragranol were found in this study but not previously (Gaudioso, 1980; Klobus, 1990). From *A. tridentata* ssp. *spiciformis*, results of earlier work indicated the presence of limonene, β-pinene and *p*-cymene but were not found in the latest study. Chrysanthemyl acetate, sabinol and lavandulol were found in this study but not previously (Gaudioso, 1980; Klobus, 1990).

2.1. *Artemisia tridentata ssp. vaseyana*

The neutral pentane extract from the ground leaves and stems of *A. tridentata* ssp. *vaseyana* was vacuum short path distilled to afford a fragrant yellow oil, which was separated into three major fractions by column chromatography. GC analysis of the first chromatographic fraction indicated the presence of four constituents. Two major compounds were isolated and identified by comparison of spectral data to literature values. The first was 1,8 cineole (Dev et al., 1982) and the second was *trans*-3-(1-oxo-2-methyl-2-propenyl)-2,2-dimethylcyclopropylmethanol which is thermally unstable and isolated as its GC artifact 2,4-diisopropenyl-5*H*-furan (Epstein et al., 1991).

A third compound **1** had a molecular weight of 150 (GC–MS), corresponding to the molecular formula $C_{10}H_{14}O$ and confirmed by HRMS. The GC–MS exhibited diagnostic losses of 15 (M–CH_3, m/z 135) and 43 (M–CH_3–C=O, m/z 107). The olefinic region of the ^{1}H NMR spectrum integrated to five protons, three of which show an ABC pattern [two-hydrogen doublets at 5.84 ppm ($J = 10.30$ Hz), 6.16 ppm ($J = 15.20$ Hz) and a one-hydrogen doublet of doublets at 6.33 ppm ($J =$ 10.30 and 15.20 Hz)]. The remaining two olefinic protons appear as a single peak at 4.90 ppm. However, analysis of the ^{13}C NMR spectrum indicated six olefinic carbons (142.6, 136.3, 132.9, 125.8, 125.5 and 115.6 ppm) or three double bonds. Since the molecular formula $C_{10}H_{14}O$ requires four degrees of unsaturation, one was due to a ring. A single alkane carbon attached to oxygen was also indicated by the ^{13}C NMR spectrum (77.2 ppm).

A methyl group attached to unsaturated carbon (CH_3C=C–) was indicated by a three-hydrogen singlet at 1.87 ppm and was supported by the ^{13}C NMR spectrum (18.9 ppm). Two methyl groups attached to a quaternary carbon were indicated by the ^{1}H NMR spectrum (two overlapping three-hydrogen singlets at 1.78 ppm) and were confirmed by the ^{13}C NMR spectrum (two overlapping methyl singlets at 26.4 ppm). The unusually high chemical shifts for the two methyl groups can be attributed to the deshielding effect of oxygen. All of these data were consistent with 2,2-dimethyl-6-isopropenyl-2*H*-pyran **1** which was synthesized in two steps from 3-methyl-2-butenal for spectroscopic comparison. Condensation of 3-methyl-2-butenal with 2,3-butanedione in pyridine gave **2** identified by ^{1}H NMR, ^{13}C NMR and IR spectra as 2,2-dimethyl-6-acetyl-2*H*-pyran (De Groot and Jansen, 1975). The acetyl group of **2** was converted to an isopropenyl group using a Wittig reaction (Fitjer and Quabeck, 1985). The ^{1}H NMR spectrum of the synthetic product was identical to **1** isolated from *A. tridentata* ssp. *vaseyana*.

It is important to note that compound **1** has the isoprene units connected in a head-to-head fashion analogous to the biologically important triterpene, squalene. The isolation of **1** strongly supports the unified approach to irregular monoterpene biosynthesis proposed by Epstein and

Poulter (1973). This approach postulates that chrysanthemyl diphosphate is the precursor to a number of different skeletal systems. Compounds representing most of these skeletal systems have previously been isolated and identified, but this is the first representative of the head-to-head skeletal system (Epstein and Poulter, 1973).

The fourth component of fraction one, **3**, has a molecular weight of 152 (CI-MS), corresponding to the molecular formula $C_{10}H_{16}O$. Although the olefinic region of the ^{1}H NMR spectrum integrates to one proton, the olefinic region of the ^{13}C NMR spectrum has four distinct resonances (95.4, 116.2, 135.3 and 158.1 ppm) requiring two double bonds. Thus, three of the olefinic carbons are quaternary. Since the ^{13}C NMR spectrum showed no peak in the carbonyl region, the third unsaturation was due to a ring. Also carbonyl and hydroxyl group bands were absent in the IR spectrum. The enol ether functionality is suggested by the C-O stretching frequency at 1217 cm^{-1} in the IR spectrum.

Two methyl groups attached to unsaturated carbons (CH_3C=C) are indicated by two, three hydrogen singlets at 1.60 and 1.72 ppm in the ^{1}H NMR spectrum. Further, two overlapping methyl doublets at 1.06 ppm coupled to one hydrogen at 2.56 ppm (septet) can be attributed to an isopropyl group [$(CH_3)_2CH$]. The remaining two hydrogens appeared as a doublet at 3.12 ppm. From these data, the structure of **3** was most likely 2,3-dimethyl-6-isopropyl 4*H*-pyran.

For comparative analysis, this compound was synthesized from isopropyl methyl ketone and ethyl orthoformate in CH_2Cl_2 at 0 °C in the presence of $AlCl_3$, resulting in the expected 2,3-dimethyl-6-isopropyl-4*H*-pyran. Isolation by preparative GC gave the pure synthetic compound which exhibited identical spectral properties to that of the naturally isolated compound. Although this method has not previously been reported in the literature, condensation of ethyl orthoformate and aliphatic-aromatic and heterocyclic ketones in the presence of Lewis acid catalysis has been reported to give 2,6-disubstituted pyrylium salts (Mezheritskii and Dorofeenko, 1967).

GC purification followed by spectroscopic studies indicated that thujone (Dev et al., 1982) was the only constituent of the second chromatographic fraction. The third chromatographic fraction contained six compounds, five of which were known. They were identified as sabinol, chrysanthemol, chrysanthemyl acetate, fraganyl acetate and fraganol by comparison of their ^{1}H NMR, ^{13}C NMR and IR spectra with literature data or synthetic standards (Tumlinson et al., 1969; Bohlmann et al., 1973; Dev, 1982; Ellis and Golding, 1985; Curley and Ticoras, 1986; Klobus, 1990).

The sixth compound, **4**, has a molecular weight of 152 (EI–MS) corresponding to the molecular formula $C_{10}H_{16}O$. The IR spectrum of this compound lacks absorptions due to a carbonyl or an ether, but has a broad absorption around 3300 cm^{-1} consistent with a hydroxyl group. The presence of a hydroxyl function is also supported by the ^{1}H NMR spectrum (one hydrogen broad singlet at 1.48 ppm). The off-resonance decoupled ^{13}C NMR spectrum contains six carbons in the olefinic region at 144.6 ppm (doublet), 141.5 ppm (singlet), 135.2 ppm (singlet), 128.3 ppm (doublet), 116.1 ppm (triplet) and 112.3 ppm (triplet) indicating that this compound has three double bonds. The IR spectrum has absorptions due to a terminal alkene (–C=CH_2) at 1430 and 880 cm^{-1}. The presence of two terminal olefin functions is supported by the ^{1}H NMR spectrum (four-hydrogen, four overlapping doublets at 4.82 ppm). The third olefinic function, which proved to be conjugated with a terminal olefin, is indicated by the ^{1}H NMR spectrum (one-hydrogen doublet of doublets at 5.49 ppm and one-hydrogen doublet at 6.20 ppm). This conjugation is also supported by the presence of two carbon–carbon double bond absorptions at 1605 and 1630 cm^{-1} in the IR spectrum.

The off resonance decoupled ^{13}C NMR spectrum shows another triplet at 63.7 ppm and a doublet at 52.9 ppm. The corresponding peaks in the ^{1}H NMR spectrum are at 3.57 ppm (multiplet) and 2.92 ppm (multiplet). The 63.7 ppm peak can be attributed to –CH_2OH and the 52.9 ppm peak should represent an sp^3 –CH group. Two methyl groups attached to unsaturated carbons (CH_3C=C) were indicated by two three-hydrogen singlets, one at 1.73 ppm and the other at 1.83 ppm in the ^{1}H NMR spectrum. This is confirmed by the presence of two quartets (18.6 and 20.8 ppm) in the off resonance decoupled ^{13}C NMR spectrum.

Thus, the structure of **4** was assigned as 2-isopropenyl-5-methylhexa-*trans*-3,5-diene-1-ol. A literature survey revealed that **4** had been synthesized from chrysanthemic acid but never naturally isolated (Crombie et al., 1972). Proton NMR spectral data of the synthetic material and naturally isolated material are consistent. In order to established the stereochemistry, compound **4** was synthesized using the four-step literature method but starting with the ethyl instead of the methyl ester (Crombie et al., 1972). The optical rotation for the alcohol derived from *trans*-(1*R*,3*R*)-chrysanthemic acid is $[\alpha]_D = -6.74°$. The alcohol obtained from *A. cana* ssp. *viscidula* has a rotation of $[\alpha]_D = -6°$, indicating the alcohol is $\geqslant 90\%$ *R* enantiomer.

2.2. *Artemisia cana ssp. viscidula*

The neutral pentane extract from the ground leaves and stems of the Manti LaSal National Forest species was vacuum short path distilled to afford a fragrant yellow oil. Four major chromatographic fractions were collected. GC analysis of the hydrocarbon fraction ($R_f = 0.8$) indicated the presence of four constituents and three were identified as santolina triene, α-pinene, and rothrockene by comparison of ^{1}H NMR, ^{13}C NMR and IR spectra

with literature data (Dev et al., 1982; Epstein et al., 1984).

The remaining compound [EI–MS = 136] corresponded to the molecular formula $C_{10}H_{16}$ and proved to be a colorless oil with no appreciable optical rotation. Lack of absorptions for a carbonyl, ether or alcohol group, and the absence of a carbonyl peak in the ^{13}C NMR spectrum confirmed this compound to be a hydrocarbon. The ^{13}C NMR spectrum indicated six olefinic carbons (147.7, 142.1, 138.7, 129.2, 115.0 and 110.6 ppm) implying three double bonds. The IR spectrum indicated the presence of absorptions due to a terminal alkene (–C=CH_2) at 1430 and 880 cm^{-1} and the absorption related to the vinyl double bond system (CH_2=CH) at 910 and 1000 cm^{-1}. The presence of a vinyl olefin function is also supported by the ^1H NMR spectrum (one-hydrogen doublet of doublets at 4.90 ppm, one-hydrogen doublet of doublets at 4.91 ppm and one-hydrogen doublet of doublets at 5.78 ppm). The terminal olefin (CH_2=C–) is also indicated by the ^1H NMR spectrum (two-hydrogen broad singlet at 4.89 ppm). The third olefinic function, which proved to be conjugated with the terminal olefin, was indicated by the ^1H NMR spectrum (one-hydrogen doublet at 5.59 ppm and one-hydrogen doublet at 6.04 ppm). Conjugation is consistent with the presence of two carbon double bond absorptions at 1605 and 1630 cm^{-1} in the IR spectrum. Two methyl groups attached to a quaternary carbon were indicated by the ^1H NMR spectrum (two overlapping three-hydrogen singlets at 1.12 ppm). This is also supported by ^{13}C NMR spectroscopy (two methyl singlets at 27.0 ppm). A methyl group attached to unsaturated carbon (CH_3C=C) was indicated by the ^1H NMR spectrum (three-hydrogen singlet at 1.82 ppm) and confirmed by the ^{13}C NMR spectrum (singlet at 18.7 ppm).

All these data made possible the assignment of structure **5**, artemisia triene, for this compound. A *trans* relationship between the olefinic hydrogens is suggested on the basis of an IR band at 960 cm^{-1} and the coupling constant between the two olefinic hydrogens H_a and H_b (both are 16.0 Hz doublets). This compound has been synthesized but not isolated from natural sources. Although *cis*- and *trans*-chrysanthemol and their methyl ethers gave *trans*-artemisia triene when heated with *p*-$CH_3C_6H_4SO_3H$ in C_6H_6 (Crombie et al., 1972), only ^1H NMR spectral data were reported. For comparison purposes chrysanthemol was heated with *p*-$CH_3C_6H_4SO_2Cl$ in CH_2Cl_2 to yield *trans*-artemisia triene (Marshall et al., 1986). The ^1H NMR and ^{13}C NMR spectra of the synthetic product were identical when compared to those from the material isolated from *A. cana* ssp. *viscidula*.

The second of four chromatographic fractions from *A. cana* ssp. *viscidula* gave five components after isolation by preparative GC. Three of the compounds were identified as artemiseole, 1,8-cineole, and *trans*-3-(1-pylmethanol which is thermally unstable and isolated as its GC artifact 2,4-diisopropenyl-5*H*-furan by comparison of ^1H NMR, ^{13}C NMR and IR spectra with literature data (Dev et al., 1982; Klobus, 1990; Epstein et al., 1991). The other two compounds were identified as 2,2-dimethyl-6-isopropenyl-2*H*-pyran **1** and 2,3-dimethyl-6-isopropyl-4*H*-pyran **3** by comparison with synthetic samples as described previously.

Preparative GC analysis of the third chromatographic fraction from *A. cana* ssp. *viscidula* gave two components. One compound was identified as lyratal by comparison of ^1H NMR, ^{13}C NMR and IR spectral data with literature data (Epstein et al., 1991). The second compound proved to be a previously unidentified compound. The ^1H NMR spectrum showed two singlets at 1.15 and 1.30 ppm corresponding to two methyl groups attached to a saturated carbon. Two more methyl singlets at 1.67 and 1.69 ppm indicated methyl groups attached to double bonds. The olefinic region of the ^1H NMR spectrum showed one olefinic hydrogen as a doublet of septets. In the IR spectrum, a –CH stretch occurs at 3020 cm^{-1} and double bond CH out-of-plane bending at 750 cm^{-1} consistent with the presence of a C–C double bond. The ^{13}C NMR spectrum contained two olefinic absorptions at 120.3 and 136.2 ppm confirming the presence of one double bond. A one proton doublet at 9.37 ppm in the ^1H NMR spectrum indicated the presence of an aldehyde group, which was confirmed by a strong IR absorption at 1695 cm^{-1} and a ^{13}C signal at 201.0 ppm. The ^1H NMR spectrum showed two more protons (both doublet of doublets at 1.57 and 2.28 ppm).

The presence of 16 hydrogens, 10 carbons and one oxygen is indicated by the spectral data and is consistent with a formula of $C_{10}H_{16}O$. The three degrees of unsaturation must be due to one double bond, one aldehyde group and one ring. By summation of the above spectral data and comparison with literature data, the likely structure of this molecule was *trans*-chrysanthemal **6**. This compound has been synthesized but not naturally isolated. However, no NMR spectroscopic data was cited in the literature procedure (Crombie and Crossley, 1963) and naturally isolated material was obtained in insufficient quantities to obtain a rotation. Thus, chrysanthemal was synthesized according to the literature procedure and the spectroscopic data obtained were identical with those of compound **6** isolated from *A. cana* ssp. *viscidula*.

Eight compounds were isolated by preparative GC from the fourth chromatographic fraction of *A. cana* ssp. *viscidula*. They were identified as camphor, isolyratol, lyratol, chrysanthemol, chrysanthemyl acetate, fraganyl acetate, fraganol and 2-isopropenyl-5-methylhexa-*trans*-3,5-dien-1-ol **4** by comparisons of IR, ^{13}C and ^1H NMR spectra with literature data or synthetic standards (Tumlinson et al., 1969; Dev et al., 1982; Ellis and Golding, 1990; Curley and Ticoras, 1986; Klobus, 1990).

2.3. Artemisia tridentata ssp. spiciformis

Volatile oils obtained from the neutral pentane extract of *A. tridentata* ssp. *spiciformis* were flash chromatographed into five separate fractions to give mainly known compounds. All fractions were identified by comparison of ^{1}H NMR, ^{13}C NMR and IR spectra with literature data. The first fraction containing hydrocarbons was analyzed by preparative GC and contained santolina triene, α-pinene, camphene and rothrockene. Fraction two contained artemiseole, 1,8-cineole and oxidosantolina triene, fraction three contained lyratal, thujone and camphor and fraction four contained sabinyl acetate and chrysanthemyl acetate. The final alcohol fraction contained α-santolina alcohol, sabinol, chrysanthemol, isolyratol, lyratol and an unidentified compound (Gaudioso, 1980; Dev et al., 1982; Epstein et al., 1984, 1991; Klobus, 1990).

The structure of the unknown compound was established as follows. The presence of 10 carbons, 18 hydrogens and one oxygen is indicated by the spectral data and is consistent with a formula of $C_{10}H_{18}O$. The IR spectrum of this compound lacks absorptions due to a carbonyl or an ether, but it has a broad absorption around 3389 cm^{-1} indicating a hydroxyl group.

The olefinic region of the ^{1}H NMR spectrum showed three protons at 4.80, 4.90 and 5.00 ppm coupled with four olefinic peaks in the ^{13}C NMR spectrum requires two double bonds. The IR spectrum has absorptions due to a terminal alkene (–C=CH_2) at 1438 and 888 cm^{-1}. The presence of the terminal olefin function is also supported by the ^{1}H NMR spectrum (one-hydrogen broad singlet at 4.80 ppm and one-hydrogen broad singlet at 4.90 ppm). The ^{13}C NMR spectrum also showed a peak at 63.9 ppm indicating a CH_2OH group which is confirmed by a two hydrogen peak at 3.40 ppm in the ^{1}H NMR spectrum. The ^{1}H NMR spectrum showed a three hydrogen singlet at 1.60 ppm and two, three-hydrogen singlets at 1.70 ppm indicating that these three methyl groups are attached to unsaturated carbons. By comparing these data with literature data, lavandulol **7** was selected as the best possible structure. Co-injection studies with synthetic lavandulol definitively proved the structure (Epstein et al., 1991). Lavandulol **7** isolated from *A. tridentata* ssp. *spiciformis* has a rotation of −2.72° ($CHCl_3$, ca. 0.50) while lavandulol isolated from *Lavandula vera* D. C. has a rotation of −10.20° (petroleum ether, ca. 2.5; Schinz and Seidel, 1942). This suggests that the lavandulol isolated from *A. tridentata* ssp. *spiciformis* is a partial racemate.

The precursor of lavandulyl is not known. Epstein and Poulter (1973) suggested that chrysanthemyl diphosphate might be an intermediate in the biosynthesis of lavandulyl monoterpenes. The isolation of two naturally occurring lavandulyl compounds from the same *Anthemideae* tribe species would lend support for this proposal, but the only other lavandulyl carbon skeletal type isolated from an *Anthemideae* tribe member was a GC artifact (Epstein et al., 1991). Another possible explanation for lavandulol biosynthesis is the direct condensation of two dimethylallyl diphosphate (DMAPP) molecules to form lavandulol diphosphate. This mechanism avoids invoking chrysanthemyl diphosphate as a intermediate (Fig. 2). At this point in time, the antecedent of lavandulol will remain a mystery.

3. Experimental

3.1. General

TLC analyses were performed on precoated sheets (0.20 mm thick) of silica gel on aluminum backing with detection by staining with 5% phosphomolybdic acid in EtOH or with a vanillin/H_2SO_4 reagent followed by heating or with I_2 stain or with anisaldehyde stain followed by heating. Preparative GC was performed on a Varian Aereograph A-90-P instrument under the following experimental conditions: injector temp. of 250 °C, detector temp. of 250 °C and He carrier gas (60 psi regular pressure, 37 ml/min flow rate). The column used was a 30×3/8″ Carbowax 20M (5%) on silanized 60–80 mesh Chromasorb W. Column chromatography was performed with 60–200 mesh silica gel. All solvents were distilled prior to use, and spectral grade solvents were used for all spectroscopic measurements. Mass spectra

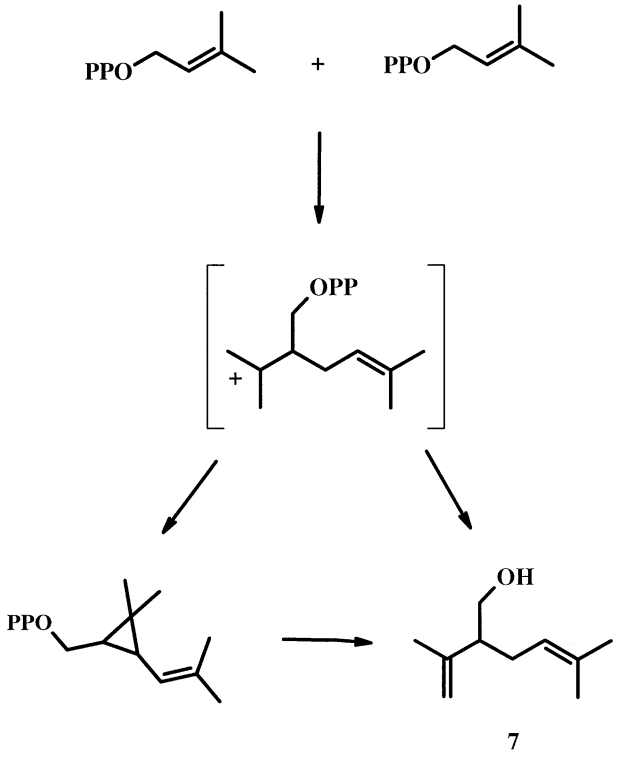

Fig. 2. Possible explanations of lavandulol biosynthesis.

were recorded on a Finnigan MAT 95 GC–MS at 70 eV. *Spectral data*: NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C. IR spectra were obtained from a Perkin Elmer 298 (grating) spectrometer or Mattson Galaxy series FTIR 3000 and UV spectra were obtained from a Hewlett-Packard 8452A Diode Array UV-visible spectrometer using a 1-cm quartz cell. Optical rotations were obtained on a Perkin-Elmer-241 MC polarimeter (sodium D line) using a 1 dm microcell at ambient temperature. *Collections*: *A. tridentata* ssp. *vaseyana*, *A. cana* ssp. *viscidula* and *A. tridentata* ssp. *spiciformis* were collected from the Manti LaSal National Forest on July 18, 1990 at 8200 foot elevation and were identified by one of the authors (W.E.). Voucher specimens of the organisms used in this study are located in the University of Utah Herbarium.

For each plant sample, 500 g of air-dried ground leaves and flower heads were extracted with pentane in a large soxlet extractor for five days. The extracts were concentrated in vacuo, and vacuum short path distilled (0.1 mm Hg) to yield yellowish oils. The oils isolated from *A. tridentata* ssp. *vaseyana*, *A. cana* ssp. *viscidula* and *A. tridentata* ssp. *spiciformis* corresponded to 0.38% of dry wt. of plant (1.93 g), 0.39% of dry wt. of plant (1.98 g) and 1.14% of dry wt. of plant (5.72 g), respectively. Each oil was separated by flash chromatography on silica gel using 19:1 hexane:EtOAc followed by 4:1 hexane:EtOAc except for the oil isolated from *A. tridentata* ssp. *spiciformis* which was flash chromatographed with 9:1 hexane:EtOAc as the second solvent system. Chromatographic fractions were separated by GC and each compound was identified by comparison of ^{1}H NMR, ^{13}C NMR and IR spectral data with literature data or with synthetic standards.

3.2. Naturally isolated 2,2-dimethyl-6-isopropenyl-2H-pyran **1**

2,2-Dimethyl-6-isopropenyl-2*H*-pyran **1** was isolated as a colorless oil via preparative GC on the Carbowax 20M column: IR (in $CDCl_3$) 734, 758, 910, 1057, 1111, 1217, 1338, 1468, 1616, 1653, 2876, 2933, 2973, 3020 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78 (6H, *s*, C-2α), 1.87 (3H, *s*, C-6β), 4.90 (2H, *bd*, C-6β'), 5.84 (1H, *d*, $J=10.3$ Hz, C-3), 6.16 (1H, *d*, $J=15.2$ Hz, C-5), 6.33 (1H, *dd*, $J=15.2$ and 10.3 Hz, C-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.9 (C-6β), 26.4 (C-2α), 26.4 (C-2α), 77.2 (C-2), 115.6 (C-6β'), 125.5 (C-3), 125.8 (C-5), 132.9 (C-4), 136.3 (C-6α), 142.6 (C-6); MS (GC) 135, 107, 91, 79; HRMS calcd for $C_{10}H_{14}O$ *m*/*z* 150.1045, found *m*/*z* 150.10392.

3.3. Naturally isolated 2,3-dimethyl-6-isopropyl-4H-pyran **3**

2,3-Dimethyl-6-isopropyl-4*H*-pyran **3** was isolated as a colorless oil via preparative GC on the Carbowax 20M column: IR ($CDCl_3$): 1217, 1458, 2933, 2972, 3020 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06 (6H, *d*, C-6β), 1.60 (3H, *s*, C-2α), 1.72 (3H, *s*, C-3α), 2.56 (1H, *h*, C-6α), 3.12 (2H, *d*, C-4), 5.25 (1H, *thd*, C-5); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.1 (C-6β), 18.4 (C-6β), 25.8 (C-2α), 29.1 (C-3α), 40.3 (C-6α), 40.3 (C-4), 95.4 (C-5), 116.2 (C-3), 135.3 (C-2), 158.1 (C-6).

3.4. Preparation of 2,3-dimethyl-6-isopropyl-4H-pyran **3**

To a stirred, cooled (0 °C) solution of 1.55 g (0.012 mol) of $AlCl_3$ in 30 ml of CH_2Cl_2, 1.72 g (0.012 mol) of ethyl orthoformate followed by 1g (0.012 mol) of methyl isopropyl ketone was added. The cooled solution was stirred for 30 min and a 10% HCl solution and ice were added. The organic layer was separated, the aqueous layer was extracted twice with CH_2Cl_2, the combined organic layers were dried over $MgSO_4$ and the solvent was removed in vacuo. The product was purified by flash chromatography (silica gel; 1.5×15 cm column; Et_2O/CH_2Cl_2/hexanes 2:1:5) to give 0.27 g (30%) of **3** as a light yellow oil: R_f 0.83 (Et_2O/CH_2Cl_2/hexanes 2:1:5); spectral comparisons were identical to the naturally occurring compound.

3.5. Naturally isolated 2-isopropenyl-5-methylhexa-trans-3,5-dien-1-ol **4**

2-Isopropenyl-5-methylhexa-*trans*-3,5-dien-1-ol **4** was isolated as a colorless oil via preparative GC on the Carbowax 20M column: IR ($CDCl_3$) 880, 950, 1020, 1360, 1430, 1605, 1630, 2940, 2960, 3060, 3300 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (1H, *bs*, C-1α O*H*), 1.73 (3H, *s*, C-5α), 1.83 (3H, *s*, C-2α CH_3), 2.92 (1H, *ddd*, $J=7.0$, 15.0, and 0.52 Hz, C-2), 3.57 (2H, *m*, C-1), 4.82 (4H, *m*, C-2α CH_2 and C-6), 5.49 (1H, *dd*, $J=15.62$ and 7.82 Hz, C-3), 6.20 (1H, *d*, $J=15.62$, C-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.6 (*q*, C-5α), 20.8 (*q*, C-2α CH_3), 52.9 (*d*, C-2), 63.7 (*t*, C-1), 112.3 (*t*, C-6), 116.1 (*t*, C-2α CH_2), 128.3 (*d*, C-3), 135.2 (*s*, C-5), 141.5 (*s*, C-2α), 144.6 (*d*, C-4); MS (EI) 152 (17), 134 (3), 122 (32), 121 (75), 107 (41), 105 (28), 93 (100), 91 (57), 79 (44), 77 (45).

3.6. Naturally isolated artemisia triene **5**

Artemisia triene **5** was isolated as a colorless oil via preparative GC on the Carbowax 20M column: IR (neat) 880, 910, 960, 1000, 1430, 1605, 1630, 2960, 3080 cm^{-1}, ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (6H, *s*, C-5α), 1.82 (3H, *s*, C-2α), 4.89 (2H, *bs*, C-1), 4.90 (1H, *dd*, $J=1.3$ and 17.4 Hz, C-7), 4.91 (1H, *dd*, $J=1.3$ and 10.5 Hz, C-7), 5.59 (1H, *d*, $J=16.1$ Hz, C-4), 5.78 (1H, *dd*, $J=10.6$ and 17.4 Hz, C-6), 6.04 (1H, *d*, $J=16.0$ Hz, C-3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.7 (C-2α), 27.0 (C-5α), 27.0 (C-5α), 39.1 (C-5), 110.6 (C-1), 115.0 (C-4), 129.2 (C-7), 138.7 (C-6), 142.1 (C-2), 147.7 (C-3); MS

(EI) 136.1 (21), 121.0 (51.8), 107.1 (24.6), 105.1 (20.2), 93.1 (100), 91 (36.8), 79 (37.3), 77 (32.9), 41 (42), 39 (36.3).

3.7. Naturally isolated chrysanthemal **6**

Chrysanthemal **6** was isolated as a colorless oil via preparative GC on the Carbowax 20M column: IR (neat) 750, 1110, 1379, 1448, 1695, 2733, 2928, 3020 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.15 (3H, *s*, C-2α), 1.30 (3H, *s*, C-2α), 1.57 (1H, *dd*, $J = 5.2$ and 5.13, C-3), 1.67 (3H, *s*, C-3β CH_3), 1.69 (3H, *s*, C-3β CH_3), 2.28 (1H, *dd*, $J = 4.98$ and 7.77, C-1), 4.87 (1H, *dh*, $J = 7.76$, C-3α), 9.37 (1H, *d*, $J = 5.42$, C-1α); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.4 (C-3β CH_3), 21.6 (C-3β CH_3), 22.2 (C-2α), 25.6 (C-2α), 31.5 (C-2), 34.6 (C-3), 45.1 (C-1), 120.3 (C-3β), 136.2 (C-3α), 201.0 (C-1α).

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