

Structural Elucidation of a New Sesquiterpene Alcohol by Comparative NMR Studies

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Abstract: The principal constituent of the essential oil from the leaves of the *Cordia verbenacea* plants collected in Minas Gerais, Brazil had a GC retention time similar to that of α -bisabolol, a sesquiterpene alcohol with important biological properties. NMR analyses (^1H , ^{13}C , DEPT-135, COSY, HMQC and HMBC) comparing the purified compound with α -bisabolol compound revealed that the new alcohol is an isomer structural of α -bisabolol, due to change in relation to the position of the double bond and the methyl group present in the cyclic chain of these compounds.

Keywords: Sesquiterpene alcohol; *Cordia verbenacea*; α -bisabolol; NMR. © 2015 ACG Publications. All rights reserved.

1. Introduction

Cordia verbenacea, a medicinal plant native to Brazil, belongs to the Boraginaceae family. It is popularly known as erva-baleeira or salicilin and is found along the coast in the southeast region. Its aerial parts possess a strong and persistent odor and have been utilized in the form of alcohol extracts, decoctions and infusions in popular medicine for anti-ulcer, antimicrobial, anti-inflammatory, antirheumatic, and analgesic effects, among others [1]. Tannins, flavonoids, triterpenes and essential oils are among the compounds encountered in the aerial parts of the plant. The essential oil is composed principally of mono- and sesquiterpenes, α -pinene (29.69%) and *E*-caryophyllene (25.27%) being the principal constituents, followed by aloaromadendrene (9.99%), α -humulene (4.64%) and β -gurjunene (4.11%) [2].

The essential oil is presently incorporated as the active principal in the Acheflan® formulation, a national phytomedicine. Anti-inflammatory effects of the extracts of the leaves of *C.*

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verbenacea [3,4] have been demonstrated in many studies. However, recent studies [1] revealed that the essential oil was responsible for this activity because it presented a dose-dependent anti-inflammatory action that reduces the carragenin-induced edema in rats and mice when it was tested in doses of 300 and 600 mg kg⁻¹ per animal. The authors affirmed that the activity was related to the presence of the sesquiterpenes α -humulene and *E*-caryophyllene, major compounds in the essential oil.

Among the compounds from essential oils with herbal applications is α -(-)-bisabolol. It was first found in the essential oil of chamomile flowers (*Matricaria chamomilla*). It has been widely used in various cosmetic formulations, especially dermatological, such as aftershave lotions, hand lotions, body deodorants, lipsticks, and sun screens, among others. Several studies with α -(-)-bisabolol and oils that contain high concentrations of it have shown anti-inflammatory, anti-irritant, antibacterial and antiallergic properties and even anticancer activity [5,6]. The α -(-)-bisabolol can be found in the essential oils of many plants, especially the essential oils from *Matricaria chamomilla*, *Salvia runcinata* [7], *Vanillosmopsis arborea* [8] and *Eremanthus erythropappus*, a species known in Brazil as Candeia. The essential oil extracted from its trunks can contain up to 85% α -bisabolol [9]. Anti-infective, antimutagenic, anti-inflammatory and anticholinesterase activities and the ability to improve the transdermal permeation of drugs are assigned to the chamomile essential oil because of the high levels of α -(-)-bisabolol present in the oil [6]. These activities demonstrate the great potential of α -bisabolol as a compound that can be used in various consumer products, and they can stimulate even greater commercial interest.

α -Bisabolol is a monocyclic sesquiterpene alcohol, whose systematic name is α ,4-dimethyl- α -(4-methyl-3-pentenyl)-3-cyclohexene-1-methanol. In its structure is present two stereocenters, being found four stereoisomers of natural origin, (-)- α -bisabolol, (-)-epi- α -bisabolol, (+)- α -bisabolol and (+)-epi- α -bisabolol [10]. It there is some structural isomers of the bisabolol with changes in the positions of the hydroxyl and double bond present in the aliphatic part of its structure, as β -bisabolol which was isolated from the essential oil of *Santalum* species [11,12], iso- α -bisabolol found in essential oil of *Vanillosmopsis erythropappa* and iso- β -bisabolol, trace constituent of East Indian and Western Australian sandalwood oil [13]. However, any study found natural α -bisabolol isomers with differences in relation the position of the double bond and the methyl group present in the cyclic chain of these compounds.

In studies of the essential oil from the leaves of native *Cordia verbenacea* plants collected in rural Itumirim, Minas Gerais, Brazil, the chromatographic profile was very different from the essential oil extracted from plants of the same species grown for therapeutic purposes. A major constituent in the essential oil from wild plants had the same retention time as that of the α -bisabolol standard, which suggested that a structural similarity existed between these compounds. Therefore, this study sought to isolate this constituent from the essential oil of native *Cordia verbenacea* plants and determine the chemical structure through comparative studies of unidimensional NMR spectra and bidimensional contour maps of this compound with those of α -bisabolol.

2. Materials and Methods

2.1. General

The α -bisabolol (standard) was used as a reference compound, being submitted to the same spectroscopic analysis as the purified sample. The Nuclear Magnetic Resonance spectra were obtained on a Bruker Avance DRX 400 MHz spectrometer; deuterated chloroform was used as the solvent for all the samples, and tetramethylsilane as the internal reference standard ($\delta=0$ ppm) for ¹H and ¹³C. Scalar coupling constants (*J*) are expressed in hertz (Hz), and NMR experiments were recorded at 300 K. To aid in data interpretation, the DEPT 135 technique, where CH₃ = positive sign, CH = positive sign, CH₂ = minus sign, C not bound to hydrogen = zero intensity signal. The infrared spectrum were recorded on a Perkin Elmer spectrophotometer and low-resolution mass spectrum was recorded on a Shimadzu QP5050A spectrometer using the electron impact ionization mode.

2.2. Plant material

The leaves of *Cordia verbenacea* (Verbenaceae) were collected in October 2007 in the morning in the municipality of Itumirim, Minas Gerais, Brazil. The species was identified by Prof. Dr. Mariana Esteves Mansanares, and its voucher specimen is recorded in the ESAL Herbarium, located in the Department of Biology, UFLA under registration number 13035.

2.3. Extraction of essential oil

The essential oil was extracted by hydrodistillation using a modified Clevenger apparatus. Fresh leaves were chopped, placed in a one-liter flask and covered with water. The extraction process was conducted by boiling the mixture for a period of 2 hours [14]. The hydrolate was collected and centrifuged in a centrifuge with a horizontal crosspiece at 950g for 5 minutes. The essential oil was removed with the aid of a Pasteur pipette and dried over anhydrous sodium sulfate. After purification, it was packed in an amber flask and stored at low temperature (3 °C).

2.4. Purification of the major constituent of the essential oil from *Cordia verbenacea*

Fractionation was performed on a 1 cm by 25 cm column. Silica gel (230 mesh) was used as the stationary phase and methylene chloride as the mobile phase. The essential oil (70 mg) was applied, and thirty 5-mL fractions were collected at a flow rate of approximately 1 drop per second. The fractions were subjected to spray drying to evaporate the mobile phase and were monitored by gas chromatography using a gas chromatograph (Shimadzu G-17A) equipped with a FID detector. Based on the chromatographic profile, the fraction numbers 19-29 were pooled to furnish 23.0 mg of the major compound with 96.0% purity.

3. Results and Discussion

3.1. Structure elucidation

The novel compound, a tertiary sesquiterpene alcohol, 6-methyl-2-(3-methycyclohex-3-enyl)hept-5-en-2-ol is an isomer of α -bisabolol, a compound already described in the literature [15,16]. The new compound was isolated as a clear oil and had a retention time (32.53 min), similar to the standard substance α -bisabolol when it was subjected to analysis by GC-FID. The infrared spectrum was obtained in KBr pallets and presented absorptions at 3413, 1453, 1453 and 1377 cm^{-1} , characteristic of hydroxyl groups, olefinic groups, methylene groups and methyl groups, respectively. The main peaks in the low resolution mass spectrum obtained for the new sesquiterpene alcohol isolated from the essential oil of *C. verbenacea* were: m/z (%), 43 (100); 41.05 (92.82); 67.05 (30.15); 69.05 (76.14); 79.05 (20.13); 93.00 (46.68); 109.10 (43.56); 119.15 (41.90); 204 (11.73). The presence of the molecular ion peak at m/z 222 was not observed. However, a peak at m/z 204, corresponding to loss of one molecule of H_2O from the molecular ion was present, given that molecular ions are usually not observed for tertiary alcohols because of their low stabilities. A similar result was observed in the structural elucidation of a new bisabolene derivative [17].

Because of this similarity, the proposed structure for this sesquiterpene alcohol was based on a comparison of the NMR (^1H , ^{13}C and DEPT 135) spectra and two dimensional NMR experiments (COSY, HMQC, HMBC) with those of α -bisabolol, furnishing the following chemical structure (Figure 1).

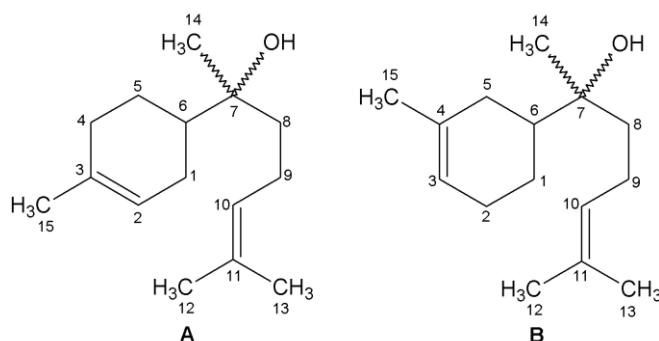


Figure 1. Struture of α -bisabolol (A) e new sesquiterpene alcohol (B).

Although the structure of α -bisabolol is already known, its elucidation is discussed in this article because there are no reports or discussion of the results of two-dimensional NMR spectroscopic analysis in the literature for this compound [16]. Carle et al. [18] elucidated the structure of α -bisabolol through chemical reactions and unidimensional ¹H and ¹³C NMR spectroscopic data. Hence, the results of the NMR analysis of the isolated sesquiterpene alcohol and the standard α -bisabolol (Fluka - 95.0%) will be compared.

The ¹H spectrum of α -bisabolol presents four singlets corresponding to methyl groups (H-12 at δ 1.63, H-13 at δ 1.57, H-14 at δ 1.05 and H-15 at δ 1.60, Table 1). The chemical shift values higher than the characteristic values for methyl hydrogens are due to the presence of unsaturation near the hydrogens H-12, H-13 and H-15 and the proximity of a hydroxyl group to H-14. There are three signals assigned to methine hydrogens, these being assigned to two hydrogens attached directly to sp^2 carbons, thus being more highly deshielded (H-2 at δ 5.32, multiplet; H-10 at δ 5.08, triple triplet, J = 6.8), and a signal (multiplet, at δ 1.55-1.48) corresponding to H-6. Other signals are observed for ten methylene hydrogens, four of them being diastereotopic because different absorptions are observed for a single carbon (H-1, m, 1.85-1.73 Ha and 2.00 to 1.95, Hb; H-5, m, 1.29-1.16, Ha and 1.90-1.84, Hb, Table 1). Three signals observed at δ 1.94-1.93, δ 1.47-1.42, and δ 2.04-1.97 correspond to H-4, H-8 and H-9 (Table 1).

Four signals corresponding to methyl carbons are observed in the combined analysis of the ¹³C and DEPT135 spectra with their respective displacements: C-12 (δ 25.66), C-13 (δ 17.62), C-14 (δ 23.16) and C-15 (δ 23.34). Five signals corresponding to methylene carbons can also be seen: C-1 (δ 26.92), C-4 (δ 31.03), C-5 (δ 23.03), C-8 (δ 40.13), C-9 (δ 22.07) (Table 1). There are three signals corresponding to methine carbons: C-2 (δ 120.58), C-6 (δ 43.00) and C-10 (δ 124.64) (Table 1), and signals relating to three quaternary carbons: C-3 (δ 134.02), C-7 (δ 74.24), C-11 (δ 131.54) (Table 1). The presence of double bonds between C-2 and C-3 and between C-10 and C-11 justifies the high chemical shift values observed in the ¹³C spectrum for these carbons. The signal at δ 74.24 ppm corresponding to carbon C-7 refers to a carbon linked to a tertiary hydroxyl group; the high electronegativity of the oxygen results in deshielding of this carbon. The results of the ¹H and ¹³C spectra are in agreement with those reported in many studies that elucidate the chemical structure of α -bisabolol [15-19].

Based on the spectroscopic analysis of the new sesquiterpene alcohol, the acyclic portion of the structure was concluded to be very similar to the same portion of the α -bisabolol structure.

The similarity of the chemical shift values of the hydrogen signals presented by H-8, H-9, H-10, H-12, H-13 and H-14 and the aliphatic carbons (C-6 to C-14) can be observed in Table 1. However, the differences in the signals corresponding to the cyclic moiety of the sesquiterpene alcohol justify the proposed structure (Figure 1). These differences are related to changes in the chemical shift values of hydrogens and carbons, together with observed changes in HMBC, HSQC and COSY correlations.

The first changes are observed in the ¹H spectrum, where signals corresponding to the H-1 and H-5 hydrogens have chemical shifts that are different from those observed for α -bisabolol (Table 1). While H-1 suffers a slight protective effect, H-5 absorbs at higher fields. The position of the methine hydrogen that absorb at δ 5.35 ppm is also different. In the structure of α -bisabolol, this value corresponds to H-2, but it corresponds to H-3 in the structure of the sesquiterpene alcohol. Such evidence corroborates the change of position of the double bond in the six-carbon ring, which occurs between C-3 and C-4 in the sesquiterpene alcohol. These values are related to the inversion of the

alpha and beta positions of the hydrogens on the double bond [20]. In addition, the absorption of the H-2 methylene hydrogens (δ 1.92-1.84 ppm) in the sesquiterpene alcohol is shifted to a value similar to that of H-4 (δ 1.94-1.93 ppm) in α -bisabolol.

According to the ^{13}C spectra for the sesquiterpene alcohol and α -bisabolol, the only differences in the two structures are in the cyclic portion. This evidence suggests that the positions of the alpha and beta carbons relative to the double bond in the ring are changed. The C-2 (δ 25.81) and C-5 (δ 30.80) carbons in the structure of the sesquiterpene alcohol occupy the position alpha to the double bond, while these positions are occupied by C-1 (δ 26.92) and C-4 (δ 31.03) in α -bisabolol. The C-1 (δ 23.73) in the new compound is in the beta position opposite the unsaturation in the ring, while this same position refers to C-5 (δ 23.30) in α -bisabolol. Thus, the signals near 120 ppm and 134 ppm that refer to the positions of sp^2 carbons assume different positions in the sesquiterpene alcohol and correspond to carbons C-3 and C-4.

Table 1. ^1H NMR (400 MHz), ^{13}C NMR (100 MHz) and DEPT 135 NMR spectral data for α -bisabolol and new sesquiterpene alcohol in CDCl_3 .

Carbons	^{13}C (ppm)	α -Bisabolol C (DEPT)	^1H (ppm) (J/Hz)*	^{13}C (ppm)	New sesquiterpene alcohol C (DEPT)	^1H (ppm) (J/Hz)*
1	26.92	CH_2	1.85-1.73 (m, 1H, Ha) 2.00-1.95 (m, 1H, Hb)	23.73	CH_2	1.30-1.20 (m, 1H, Ha) 1.81-1.75 (m, 1H, Hb)
2	120.58	CH	5.32 (m, 1H)	25.81	CH_2	1.92-1.84 (m, 1H, Ha) 2.07-2.00 (m, 1H, Hb)
3	134.02	C	-	120.52	CH	5.37 (m, 1H)
4	31.03	CH_2	1.94-1.93 (m, 2H)	133.50	C	-
5	23.30	CH_2	1.29-1.16 (m, 1H, Ha) 1.90-1.84 (m, 1H, Hb)	30.80	CH_2	1.96-1.95 (m, 2H)
6	43.00	CH	1.55-1.48 (m, 1H)	43.07	CH	1.58-1.53 (m, 1H)
7	74.24	C	-	74.03	C	-
8	40.13	CH_2	1.47-1.42 (m, 2H)	39.09	CH_2	1.51-1.47 (m, 2H)
9	22.07	CH_2	2.04-1.97 (m, 2H)	22.01	CH_2	2.07-2.00 (m, 2H)
10	124.64	CH	5.08 (t, $J = 6.8$, 1H)	124.34	CH	5.11 (t, $J = 7.2$, 1H)
11	131.54	C	-	131.37	C	-
12	25.66	CH_3	1.63 (s, 3H)	25.40	CH_3	1.66 (s, 3H)
13	17.62	CH_3	1.57 (s, 3H)	17.67	CH_3	1.60 (s, 3H)
14	23.16	CH_3	1.05 (s, 3H)	23.65	CH_3	1.11 (s, 3H)
15	23.34	CH_3	1.60 (s, 3H)	23.02	CH_3	1.65 (s, 3H)

In the HSQC contour map, the signals for the hydrogens correlate with the respective carbons (Table 1). The HMBC 2J and 3J correlations confirm the positions of the double bonds between C-2 and C-3 and between C-10 and C-11. The principal 2J correlations observed are H-1 (δ 2.00-1.95, Hb) with C-2 (δ 120.58), H-4 (δ 1.94 – 1.93) with C-3 (δ 134.02), H-9 (δ 2.04 – 1.97) with C-10 (δ 124.64), H-12 (δ 1.63) and H-13 (δ 1.57) with C-11 (δ 131.54), and H-15 (δ 1.60) with C-3 (δ 134.02) (Figure 2). The principal 3J correlations observed are H-1 (δ 26.92) with C-3 (δ 134.02), H-4 (δ 31.03) with C-2 (δ 120.58), H-8 (δ 40.13) with C-10 (δ 124.64), and H-12 (δ 25.66) and H-13 (δ 17.62) with C-10 (δ 124.64) (Figure 2). The 3J correlations of H-2 (δ 5.32) with C-6 (δ 43.00), H-10 (δ 5.08) with C-8 (δ 40.13) and H-5 (δ 23.30) with C-7 (δ 74.24) confirm the position of the double bonds (Figure 1). Other important correlations are those observed between the methyl hydrogens (H-14, δ 1.05) and the carbons C-6 (δ 43.00, 3J), C-8 (δ 40.13, 3J) and C-7 (δ 74.24, 2J), thereby confirming the position of C-7 bound to the hydroxyl group (Figure 2).

In the COSY experiment, correlations of hydrogen H-10 with hydrogens H-12 and H-13 of the methyl groups and with hydrogens H-9 and H-8 can be seen. These correlations collaborate to define the position of the double bond between carbons C-10 and C-11. The correlations observed between hydrogen H-2 and the diastereotopic hydrogens H-1 and H-5, plus the correlation with H-15, confirm the position of the double bond between C-2 and C-3 of the ring in α -bisabolol (Figure 2).

The analysis of the HSQC, HMBC and COSY contour maps for the new sesquiterpene alcohol confirmed the presence of small structural differences, principally a shift in the position of the double bond to C-3 and C-4. The signal observed at δ 26.93 (C-1) for α -bisabolol correlates with hydrogen signals at δ 1.85-1.73 and δ 2.00-1.95, and the ^{13}C signal observed at δ 23.31 (C-5) correlates with the hydrogen signals at δ 1.29-1.16 and δ 1.90- 1.84. However, the ^{13}C signal observed at δ 25.81 (C-2)

for the new compound correlates with the hydrogen signals at δ 1.92-1.84 and δ 2.07- 2.00, and the ^{13}C signal observed at δ 23.73 (C-1) correlates with the hydrogen signals at δ 1.30-1.20 and δ 1.81-1.75.

Based on these correlations, H-1 in the proposed structure for the new sesquiterpene alcohol was shown to have a chemical shift similar to that presented by H-5 in the structure of α -bisabolol. This fact is related to the exchange of alpha and beta positions relative to unsaturation in the cyclic structures, whereas the aliphatic chain is not altered.

The 3J correlations between H-6 (δ 1.58) and C-4 (δ 133.50, attached to the methyl group) and C-2 (δ 25.81) observed in the HMBC contour map show the difference in position of the double bond. In addition, the 3J correlations of H-5 (δ 1.96) with C-3 (δ 120.52) and C-1 (δ 23.73) corroborate the proposed structure. In the contour map for α -bisabolol, there is no correlation between the H-6 (δ 1.42) and the C-3 (δ 134.02) attached to the methyl group. However, a 3J correlation is observed between H-2 (δ 5.32) and C-6 (δ 43.00) that is not observed for the new compound. Such differences confirm the difference in the structures of the two isomers.

Correlations observed in the COSY contour map of the sesquiterpene alcohol differ from those of α -bisabolol at H-1 (δ 1.30- 1.20, Ha and 1.81-1.75, Hb), H-5 (δ 1.96), and H-2 (δ 1.84-1.92, Ha and 2.00 to 2.07, Hb). These correlations indicate that the position of the double bond in the cyclic sesquiterpene alcohol occurs between C-3 and C-4, thereby confirming the observations in the ^1H and ^{13}C spectra and HMBC contour map mentioned above (Figure 2).

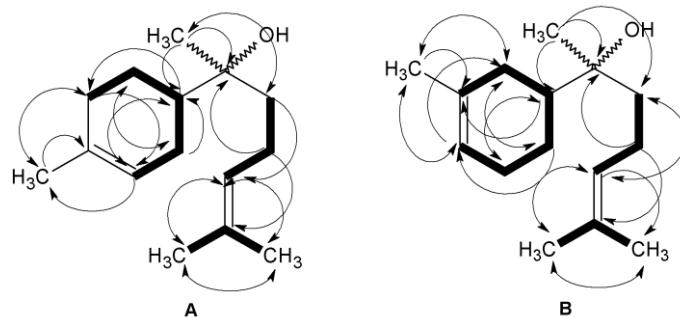


Figure 2. Principal HMBC (→) and COSY correlations (↔) for α -bisabolol (A) and new sesquiterpene alcohol (B)

Thus it was isolated from the essential oil of *C. verbenacea* and characterized for the first time, one new sesquiterpene alcohol from natural origin, which differs structurally of α -bisabolol in relation positions of unsaturation and of methyl group present in the cyclic part of the chain. This feature day not yet been observed between the isomers of α -bisabolol.

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