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NOTE

# Structure Determination of *O*-Methyllaureolol From *Skimmia Laureolas* sp. *Multinervia* by 2D HMQC and HMBC Techniques

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**The $^1H$ and $^{13}C$ NMR spectra of *O*-methyllaureolol were assigned utilizing the 2D HMQC and HMBC correlation techniques. The hydrogens and carbons of the steroid nucleus and its side-chain were completely assigned using the HMQC and HMBC in concert, with the aid of DEPT spectra which give the types of all carbon atoms. A NOESY spectrum confirmed its stereoconfiguration.** © **1997 by John Wiley & Sons, Ltd**

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## INTRODUCTION

*Skimmia* (Rutaceae), containing about 14 species,[1] is widely distributed in the temperate and subtropical zones of Asia, such as China, Japan, India, Nepal and the Philippines, *Skimmia laureolas* sp. *multinervia*, a subspecies of the genus *Skimmia*, is used in folk medicine.

Our research for biologically active constituents of this plant led to isolation of a new triterpenol,[2,3] together with four known compounds. This paper presents the results of the isolation and structural elucidation of the new compound, *O*-methyllaureolol (Fig. 1), by 1D NMR and 2D HMQC and HMBC techniques.

## EXPERIMENTAL

### Isolation of *O*-methyllaureolol

The dry and powdered material was percolated with light petroleum. After chromatography on silica gel and elution with $CH_2Cl_2$–EtOH, the main fraction was chromatographed on $Al_2O_3$ and purified by crystallization in $CHCl_3$. Colorless crystals of *O*-methyllaureolol with m.p. 222–224 °C were obtained.

### NMR method

All 1D and 2D NMR experiments were run on a Bruker ARX 300 spectrometer. 1D $^{13}C\{^1H\}$ DEPT and $^{13}C\{^1H\}$ inverse gated decoupling data sets were collected using a 5 mm $^1H/^{13}C$ dual probe-head. The $^{13}C$ and $^1H$ 90° pulse widths for observation and decoupling channels were 11.2 and 6.8 μs, respectively. The composite pulse WALTZ-16 used for decoupling in the DEPT experiment was set at 10 s. $^1H$ and $^{13}C$ chemical shifts of the sample in $CDCl_3$ are quoted relative to those of internal TMS. Data were collected for 192 values of $t_1$ and zero-filling was applied in both dimensions. The 2D $^1H$ phase-sensitive DQF-COSY data were recorded over the range 0.5–2.8 ppm with 320 $t_1$ increments and the $t_1$ domain was extended to 1024 points by zero-filling. The 2D $^1H$ phase-sensitive NOESY data were collected with a mixing time $\tau_m$ = 800 ms; 256 increments were collected. Zero-filling was applied to $t_1$ to extend it to 512 points. The 2D $^1H$–$^{13}C$

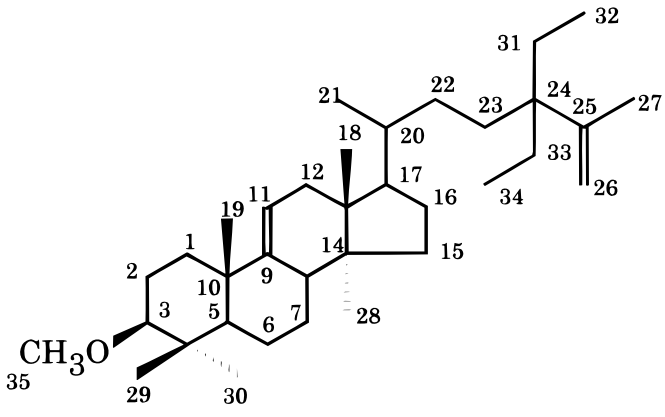


**Figure 1**. Structure of *O*-methyllaureolol.

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HMQC and HMBC spectra were recorded with 384 and 256 values in $t_1$, respectively. The $t_1$ domains were extended to 512 points by zero-filling. $^{13}C$ decoupling during acquisition in the HMQC experiment was achieved with composite pulse GARP decoupling. The delay time for optimizing two- and three-bond correlation in HMBC experiments was 55 ms. Quadrature detection during $t_1$ in all 2D phase-sensitive experiments was achieved by the TTPI method, and 1024 points were collected in $t_2$. Double zero-filling was applied to the $t_2$ dimension before Fourier transformation. A $\pi/2$ shifted sine-bell window function was applied in both dimensions to spectra recorded in DQF-COSY, NOESY and HMQC experiments, and a $\pi/4$ shifted sine-square window function was used for the HMBC spectrum.

## RESULTS AND DISCUSSION

### Characterization of *O*-methyllaureolol

The molecular formula of *O*-methyllaureolol is $C_{35}H_{60}O$, which was determined by elemental analysis and high-resolution mass spectrometry. The IR spectrum indicates that carbonyl and hydroxyl groups are absent but double bonds are present (1640 $cm^{-1}$). The number of rings and double bonds is six. The $^{13}C$ NMR spectrum reveals that there are two double bonds, the chemical shifts of which are 148.7 ppm (quaternary carbon atom), 114.8 ppm (CH), 149.7 ppm (quaternary carbon atom) and 112.0 ppm ($CH_2$). The remaining four degrees of unsaturation constitute the tetracyclic nucleus of the steroid. The DEPT data indicate different forms of the carbon atoms (see Table 1). Integration of the carbon atom at 7.9 ppm, which was measured quantitatively by using the inverse-gated decoupling technique, relative to the carbon atom at 57.5 ppm showed that there are two identical $CH_3$ groups (7.90 ppm) in the molecule.

### Determination of $^1H$–$^{13}C$ chemical shift correlation

All of the $^1H$–$^{13}C$ correlations can be determined from the HMQC spectrum (see Table 1). The chemical shifts of two H atoms on each of C-1, C-2, C-6, C-7, C-12, C-26 and C-33 are not identical. They are labelled as $\alpha$, $\beta$ (in the nucleus) and a, b (on the side-chain).

### Determination of $^1H$–$^1H$ correlation

In order to obtain further assignments of NMR signals, the H–H COSY spectrum was measured. Owing to the serious overlap of peaks in the $^1H$ spectrum, only part of the correlation peaks can be observed (Table 2). From these correlation peaks it can be deduced that C-2 is attached to C-3, C-5 to C-6, C-7 to C-8 and C-20 to C-22.

### Resolution of long-range $^{13}C$-$^1H$ correlation in the steroid ring system

Table 3 gives the long-range correlations of $^{13}C$–$^1H$ from the HMBC spectrum. From the chemical shift of

**Table 1. $^{13}C$ and $^1H$ NMR chemical shifts (300 MHz, $CDCl_3$, ppm relative to internal TMS)**

| Carbon | $\delta_c$ (ppm) | DEPT | $\delta_H$ (ppm) | Carbon | $\delta_c$ (ppm) | DEPT | $\delta_H$ (ppm) |
|---|---|---|---|---|---|---|---|
| 1 | 36.0 | $CH_2$ | 1.83 ($\alpha$)<br>1.40 ($\beta$) | 19 | 22.2 | $CH_3$ | 1.04 |
| 2 | 22.5 | $CH_2$ | 1.45 ($\alpha$)<br>1.92 ($\beta$) | 20 | 36.3 | CH | 1.32 |
| 3 | 88.6 | CH | 2.65 | 21 | 18.7 | $CH_3$ | 0.87 |
| 4 | 39.0 | C | | 22 | 29.6 | $CH_2$ | 1.24 |
| 5 | 53.0 | CH | 0.87 | 23 | 25.9 | $CH_2$ | 1.34 |
| 6 | 21.2 | $CH_2$ | 1.65 ($\alpha$)<br>1.43 ($\beta$) | 24 | 44.4 | C | |
| 7 | 28.1 | $CH_2$ | 1.30 ($\alpha$)<br>1.65 ($\beta$) | 25 | 149.7 | C | |
| 8 | 41.8 | CH | 2.16 | 26 | 112.0 | $CH_2$ | 4.89 (a)<br>4.66 (b) |
| 9 | 148.7 | C | | 27 | 19.5 | $CH_3$ | 1.63 |
| 10 | 39.4 | C | | 28 | 18.5 | $CH_3$ | 0.73 |
| 11 | 114.8 | CH | 5.21 | 29 | 28.2 | $CH_2$ | 0.96 |
| 12 | 37.1 | $CH_2$ | 1.88 ($\alpha$)<br>2.06 ($\beta$) | 30 | 16.4 | $CH_3$ | 0.80 |
| 13 | 44.2 | C | | 31 | 26.0 | $CH_2$ | 1.34 |
| 14 | 47.0 | C | | 32 | 7.9 | $CH_3$ | 0.67 |
| 15 | 33.9 | $CH_2$ | 1.33 | 33 | 28.1 | $CH_2$ | 1.30 (a)<br>1.90 (b) |
| 16 | 30.2 | $CH_2$ | 1.42 | 34 | 7.9 | $CH_3$ | 0.67 |
| 17 | 50.8 | CH | 1.60 | 35 ($OCH_3$) | 57.3 | $CH_3$ | 3.37 |
| 18 | 18.3 | $CH_3$ | 0.65 | | | | |

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**Table 2. Long-range correlation (HMBC) for *O*-methyllaureolol[a]**

| | | | |
|---|---|---|---|
| For A ring | H(C-3)–C-1 | H(C-3)–C-29 | H(C-3)–C-35 |
| | H(C-3)–C-30 | H(C-29)–C-5 | H(C-30)–C-5 |
| | H(C-19)–C-1 | H(C-19)–C-5 | |
| For B ring | H(C-19)–C-9 | H(C-19)–C-10 | |
| For C ring | H(C-11)–C-13 | H(C-11)–C-8 | H(C-18)–C-12 |
| | H(C-18)–C-14 | | |
| For D ring | H(C-28)–C-13 | H(C-28)–C-15 | H(C-18)–C-29 |
| | H(C-18)–C-17 | H(C-16)–C-13 | H(C-16)–C-13 |
| For the side-chain | H(C-17)–C-18 | H(C-27)–C-26 | H(C-27)–C-24 |
| | $H_{a,b}$(C-26)–C-27 | $H_{a,b}$(C-26)–C-24 | H(C-21)–C-17 |
| | H(C-20)–C-23 | H(C-20)–C-16 | H(C-23)–C-25 |
| | H(C-32 or C-34)–C-24 | | |

[a] H(C-*m*)–C-*n* means that the hydrogen atom attached to the *m*th carbon atom is correlated with the *n*th carbon atom in the HMBC spectrum.

C-3 (88.6 ppm) it can be deduced that this carbon atom is attached to the methoxy group, verified by the correlation between C-3 and the hydrogen atoms attached to C-35. The position of C-3 in the A ring can be determined by the correlation of C-3 with the hydrogen atom attached to the tertiary carbon atom C-5 and the hydrogen atoms attached to primary carbon atoms C-29 and C-30. C-4 must be a quaternary carbon atom. Long-range correlation between hydrogen atoms attached to C-19 and C-5 indicates that C-19 is a $\beta$-oriented angular methyl group in position 19. According to the long-range correlation between hydrogen atoms attached to C-19, C-1 and C-9, the positions of C-1 in the A ring and C-9 in the C ring were determined because C-9 is in the double bond which cannot be in A ring. The other carbon atom forming the double bond with C-9 in the C ring must be in the position 11 of the C ring with $\delta_C = 114.8$ ppm. Otherwise the chemical value of C-8 would be $>4$. C-11 is a methine carbon whose hydrogen atom ($\delta_H = 5.20$ ppm) has a long-range correlation with C-8. This verifies the validity of the position mentioned above.

The primary carbon atom C-18 has a chemical shift of 14.3 ppm, which is characteristic of an angular methyl group at position 18 of a steroid. In the HMBC spectrum the correlation between hydrogen atoms attached to this angular $CH_3$ and methylene C-12 (37.1 ppm), methine C-17 (50.8 ppm) and C-14 (47.0 ppm) suggests the position of C-12 in the C ring and of C-14 and C-17 in the D ring, as shown in Fig. 1. The correlation between hydrogen atoms attached to C-11 and C-13 indicates that the chemical shift at 44.2 ppm belongs to C-13. In general, there is a methyl group attached to C-14 atom in steroids. In our experiment, a methyl group (C-28) with chemical shifts $\delta_C = 18.9$ ppm and $\delta_H = 0.73$ ppm showed a single peak in the 1D $^1$H NMR spectrum. This suggests that the methyl group is attached to C-14, confirmed by the correlation between the hydrogen of this methyl and C-13 and methylene (C-15). Position 15 in the D ring was determined as C-15. From another correlation between C-13 and the hydrogen atoms attached to the methylene ($\delta_C$ 30.2 ppm), the chemical shift of position 16 was determined. The chemical shift of the quaternary carbon atom at position 4 can be determined based on the fact that C-25 is in the double bond with $\delta_C = 149.7$ ppm, which cannot be at position 4, and C-24 at 44.4 ppm has a long-range correlation with hydrogen attached to C-26 in the double bond. The assignments of $\delta_C$ and $\delta_H$ of the steroid nucleus are completed.

**Table 3. H–H COSY data for *O*-methyllaureolol**

| | |
|---|---|
| $H_{\alpha,\beta}$(C-2)–H(C-3) | H(C-5–H(C-6) |
| $H_{\alpha,\beta}$(C-7)–H(C-8) | H(C-20)–H(C-22) |

## HMBC spectrum of the side-chain

The correlation between H of C-27 and C-25 in the double bond can be seen in the HMBC spectrum. This, coupled with the correlation between $H_{a,b}$ (C-26) and C-27, suggests that there is a 1-methylvinyl group in the side-chain. By the correlation between the quaternary C-24 and hydrogen atoms attached to C-26 and C-27 it can be deduced that C-24 is attached to C-25.

In the 1D $^1$H NMR spectrum, the signals of $CH_3$ ($\delta_C = 18.7$ ppm, $\delta_H = 0.87$ ppm) split into a double peak, which fits the characteristic of methyl group at position 21 connected with a methine at position 20 in the steroid. The correlation between hydrogen atoms attached to C-21 and C-17 also confirms the above assignment. C-20 can be easily determined because C-20 is the only methine group in the unassigned signals. The correlation between H (C-20) and C-16 confirms this assignment. The hydrogen atom attached to C-20 is correlated with methylene C-23 and the hydrogen atom attached to C-23 is correlated with quaternary C-25, which suggests the position of C-23 which is attached to C-24. From the correlation of hydrogen atoms attached to C-21 with C-22, the position of C-22 is determined. Hence the main part of the side-chain has been resolved, except for two groups attached to C-24 which include two methyl (C-32, C-34) and two methylene (C-31, C-33) groups. The correlation of hydrogen atoms attached to C-32 or C-34, whose chemical shift $\delta_H =$ 0.67 ppm, with C-24 in the HMBC spectrum suggests that there must be a methyl group at the $\beta$-site of C-24, so this side-chain must be an ethyl group. The same holds true for the other side-group because the two groups attached to C-24 are identical.

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**Table 4. NOESY correlation for *O*-methyllaureolol**

| | | |
|---|---|---|
| H(C-3)–H(C-5) | H(C-8)–H(C-19) | H(C-8)–H(C-18) |
| H(C-17)–H(C-28) | H(C-11)–$H_{\alpha,\beta}$(C-1) | |

## Determination of configuration from the NOE spectrum

NOESY spectra can give the stereo character of chemical compounds. It is known that the angular methyl group at site 19 in a steroid is on the $\beta$-face. The fact that there is no correlation between the hydrogen atoms attached to C-19 and the hydrogen atom attached to C-5 suggests that this hydrogen atom is on the $\alpha$-face (Table 4). From the correlation between the hydrogen atom attached to C-3 and that attached to C-5, it can be derived that the methoxy group is $\beta$. The correlation between the hydrogen atom attached C-8 and those attached to C-19 suggests the former is $\beta$, and the correlation between the hydrogen atom attached to C-8 and those attached to C-18 suggests that the angular methyl C-18 is $\beta$. Because the hydrogen atom at site 17 in steroids is $\alpha$, the correlation between the hydrogen atom attached to position 17 and those attached to C-28 suggests the angular methyl group of C-28 is $\alpha$. The correlation between the hydrogen atom attached to C-11 and those attached to C-1 confirms the position of a double bond at position 9(11). It is interesting that the assignments for the structure of *O*-methyllaureolol are similar to those of the analogous compound 24$\beta$-ethyl-5$\alpha$-lanosta-9(11), 25-dien-3$\beta$-ol acetate.[4]

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