ORGANIC LETTERS

2000
Vol. 2, No. 4
493−496

# Macrophyllols A and B, Two Unusual Novel Sesquiterpene and Monoterpene Dimers from the Bark of *Inula macrophylla*

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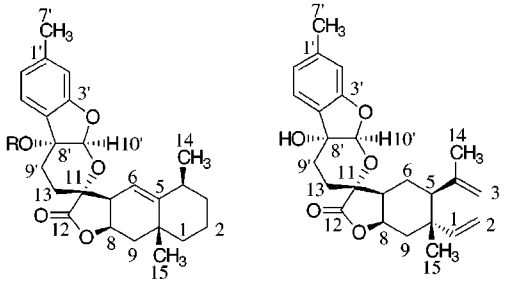
**Received December 14, 1999**

## ABSTRACT




**1** R = H
**1a** R = Ac (acetate of **1**)

**2**

**Two novel sesquiterpene and monoterpene dimers, macrophyllols A (1) and B (2), were isolated from the bark of *Inula macrophylla*. Their structures were determined on the basis of spectral evidence (especially HREIMS and 2D NMR) as well as chemical transformation. The structure of macrophyllol A (1) was confirmed by X-ray analysis. The possible biosynthetic pathways of macrophyllols A (1) and B (2) are discussed.**

The chemical constituents of *Inula macrophylla* (Compositae) have not been investigated to date. In our continuing study on Uzbekistan plants, two unusual novel sesquiterpene and monoterpene dimers, named macrophyllols A (**1**) and B (**2**), were isolated from the bark of *I. macrophylla*. Their structures were determined on the basis of spectral evidence (especially HREIMS and 2D NMR) as well as chemical transformation. The structure of macrophyllol A (**1**) was confirmed by X-ray analysis. Both compounds **1** and **2** contain 25 carbon atoms, and they are in agreement with the isoprene rule of terpenoids; that is to say, macrophyllols A and B apparently are sesterterpenoids, which is a very small group of natural products. However, we prefer to think these two compounds are sesquiterpene and monoterpene dimers, although the two units do not connect to each other by an ether or an ester group as in general natural dimers

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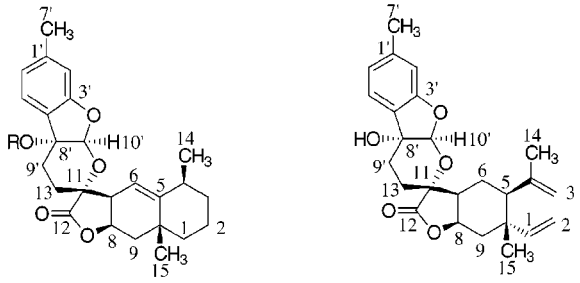
10.1021/ol990401n CCC: $19.00 
**Published on Web 01/28/2000**

but by a C−C bond directly. Macrophyllols A and B were obtained from this plant at the same time as many other related monoterpenes and sesquiterpenes. Furthermore, the characteristic components of the *Inula* genus are sesquiterpenes (especially eudesmanolide and guaianolide)[1−6] and monoterpenes (methoyl derivatives)[7,8] as has been long known. The possible biosynthetic pathways of macrophyllols A (**1**) and B (**2**) are discussed.

The MeOH extract of the powdered air-dried bark (1.6 kg) of *I. macrophylla* was partitioned between $H_2O$ and $CHCl_3$ and then between $H_2O$ and *n*-BuOH. The $CHCl_3$-soluble fraction was chromatographed over a silica gel column and further purified by HPLC and GPC (gel permeation chromatography) to give macrophyllols A (**1**) (21 mg) and B (**2**) (18 mg) (Figure 1).



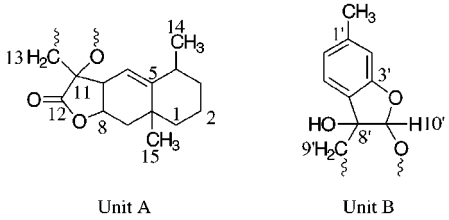
**1** R = H
**1a** R = Ac (acetate of **1**)

**2**

**Figure 1.** Structures of macrophyllol A (**1**) and macrophyllol B (**2**).

Macrophyllol A (**1**) was obtained as colorless needles, $[\alpha]_D^{25}$−49.2° ($CHCl_3$, *c* 0.52); UV ($CHCl_3$) $\lambda_{max}$ 279.8 (log $\epsilon$ 3.27); IR (KBr) $\nu_{max}$ 3465, 2950, 1762, 1209, 1105, 1087 $cm^{-1}$. The $^1H$ NMR spectrum of compound **1** showed the presence of a 1,3,4-trisubstituted aromatic ring at $\delta_H$ 6.71 (1H, br s, H-2′), 7.22 (1H, d, $J$ = 7.6 Hz, H-5′) and 6.85 (1H, br d, $J$ = 7.6 Hz, H-6′), an olefinic proton at $\delta_H$ 4.93 (1H, d, $J$ = 3.4 Hz, H-6), an oxygenated methine at $\delta_H$ 5.10 (1H, m, H-8), an acetal proton at $\delta_H$ 5.76 (1H, s, H-10′), and three methyls at $\delta_H$ 1.06 (3H, d, $J$ = 7.8 Hz, H-14), 1.21 (3H, s, H-15) and 2.36 (3H, s, H-7′), as well as other signals belonging to other methylenes and methines. Its HREIMS (*m*/*z* 410.2093) indicated a molecular formula of $C_{25}H_{30}O_5$ (calcd 410.2093). The $^{13}C$ NMR spectral data are in good agreement with the above analysis. However, it only showed 24 carbon signals [including the signal of C-8 ($\delta_c$ 76.9) which was overlapped with $CDCl_3$ peaks and distinguished by DEPT spectra] when $CDCl_3$ was used as solvent. Just as expected, the $^{13}C$ NMR spectrum of compound **1** showed 25 signals when $CD_3OD$ was used as solvent, indicating that not only the above-mentioned C-8 but also another quaternary carbon (C-8′) was overlapped with $CDCl_3$ peaks when $CDCl_3$ was used as solvent.

The $^1H-^1H$ COSY spectrum of **1** showed correlations from H-8 to H-7 and H-9, H-7 to H-6, H-4 to H-14 and H-3, and H-1 to H-2. Combined with the observed HMBC correlations from H-7 to C-11, C-12, C-13, C-9, C-6, and C-5; H-13 to C-11, C-12, and C-7; H-8 to C-10, C-9, C-7, C-6, and C-11; H-15 to C-1, C-10, and C-9; H-14 to C-4, C-3, and C-5; and H-9 to C-10, C-15, C-7, and C-5, the presence of a partial structure of unit A (Figure 2, sesquiterpene unit) in the structure of **1** was suggested. The relative configuration of unit A was determined on the basis of proton splitting patterns and coupling constants as well as the observed cross-peaks in the NOESY spectrum. The remaining signals of **1** were a methyl (C-7′), a 1,3,4-trisubstituted aromatic ring, an oxygenated quaternary carbon (C-8′), a methylene (C-9′), and a methine which is with a very downfield chemical shift ($\delta_c$ 110.4 in $CDCl_3$, 112.2 in $CD_3OD$). In the HMBC spectrum of **1**, H-7′ correlated to C-1′, C-2′, and C-6′; H-2′ correlated to C-3′, C-4′, and C-6′; H-5′ correlated to C-3′, C-1′, and C-8′; H-10′ correlated to C-3′, C-8′, C-9′; and C-4′, and H-9′ correlated to C-8′, C-10′, and C-4′. On the other hand, $\delta_H$ 2.33 (H-7′) clearly correlated to $\delta_H$ 6.66 (H-2′) and 6.84 (H-6′) in the NOESY spectrum of **1**. These correlations suggested the existence of another partial structure of unit B (Figure 2, monoterpene unit) in the structure of **1**.



Unit A Unit B

**Figure 2.** Partial structures of macrophyllol A (**1**).

The connected positions of the above determined two units were established according to the following key correlations: in the $^1H-^1H$ COSY spectrum, H-9′ correlated to H-13; in the HMBC spectrum, both H-10′ and H-9′ correlated to C-11, and H-13 correlated to C-8′ and C-9′. Thus, C-10′ and C-11 were connected by an oxygen ether group, C-10′ was an acetal carbon, and C-9′ and C-13 were connected by a C−C bond directly. To verify the determined structure, compound **1** was acetylated using acetic anhydride and pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine at room temperature overnight to give the acetate (**1a**) (EIMS: $[M]^+$ *m*/*z* 452). The $^1H$ NMR signals of H-10′, H-9′, and H-5′ of **1a** showed a significant downfield shift compare to those of **1** (Table 1), suggesting that a hydroxyl group was attached to C-8′. A colorless crystal of macrophyllol A was obtained for X-ray crystallographic analysis (Figure 3),[9] which finally confirmed the determined structure.

Macrophyllol B (**2**) was obtained as a white amorphous powder. $[\alpha]_D^{25}$ −131.5° ($CHCl_3$, *c* 0.50); UV ($CHCl_3$) $\lambda_{max}$ 280.0 (log $\epsilon$ 3.31), 239.5 (log $\epsilon$ 2.11); IR (KBr) $\nu_{max}$ 3460, 2968, 1780, 1225, 1160, 1126, 1079 $cm^{-1}$. Its HREIMS (*m*/*z* 410.2119) indicated a molecular formula of $C_{25}H_{30}O_5$ (calcd 410.2093), the same as that of **1**. The NMR data of **2** (Table 2) showed that it is also a sesquiterpene and monoterpene

**Table 1.** NMR Spectral Data[a] of Compounds **1** and **1a**

| | **1** ($CDCl_3$) | | **1** ($CD_3OD$) | | **1a** ($CDCl_3$) |
|---|---|---|---|---|---|
| no. | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1a | 1.59, br d (13.2) | 42.3 t | 1.60, br d (13.2) | 43.3 t | 1.60, br d (13.2) |
| 1b | 1.11, dd (13.2, 3.4) | | 1.13, dd (13.2, 3.4) | | 1.10, dd (13.2, 3.4) |
| 2a | 1.82, m | 16.9 t | 1.82−1.86, m | 17.8 t | 1.82, m |
| 2b | 1.43, m | | 1.41, m | | 1.43, m |
| 3 | 1.54, m | 32.9 t | 1.55, m | 33.9 t | 1.52, m |
| 4 | 2.37, m | 38.6 d | 2.40, m | 39.8 d | 2.36, m |
| 5 | | 152.3 s | | 153.1 s | |
| 6 | 4.93, d (3.4) | 113.2 d | 4.94, d (3.3) | 114.7 d | 4.92, d (3.3) |
| 7 | 3.01, dd (5.4, 3.4) | 46.7 d | 3.08, dd (5.4, 3.3) | 48.0 d | 3.02, dd (5.2, 3.3) |
| 8 | 5.10, m | 76.9 d | 5.09, m | 78.4 d | 5.11, m |
| 9a | 2.13, dd (14.6, 3.4) | 42.6 t | 2.08−2.13, m | 43.6 t | 2.13, dd (14.8, 3.4) |
| 9b | 1.47, m | | 1.53, m | | 1.48, m |
| 10 | | 33.0 s | | 34.0 s | |
| 11 | | 79.5 s | | 80.8 s | |
| 12 | | 174.7 s | | 176.6 s | |
| 13a | 1.92, ddd (13.2, 4.8, 3.9) | 22.8 t | 1.82−1.86, m | 23.3 t | 1.91, ddd (13.2, 4.8, 3.8) |
| 13b | 1.51, m | | 1.50, m | | 1.52, m |
| 14 | 1.06, d (7.8) | 22.8 q | 1.08, d (7.8) | 23.1 q | 1.06, d (7.8) |
| 15 | 1.21, s | 28.5 q | 1.19, s | 29.0 q | 1.21, s |
| 1′ | | 141.6 s | | 142.1 s | |
| 2′ | 6.71, br s | 111.4 d | 6.66, br s | 111.5 d | 6.71, br s |
| 3′ | | 158.5 s | | 159.6 s | |
| 4′ | | 126.2 s | | 128.0 s | |
| 5′ | 7.22, d (7.6) | 123.3 d | 7.20, d (7.6) | 124.6 d | 7.44, d (7.6) |
| 6′ | 6.85, br d (7.6) | 123.0 d | 6.84, br d (7.6) | 123.7 d | 6.82, br d (7.6) |
| 7′ | 2.36, s | 21.8 q | 2.33, s | 21.7 q | 2.35, s |
| 8′ | overlapped with $CDCl_3$ peaks | | | 77.9 s | |
| 9′a | 2.66, ddd (13.2, 13.2, 3.9) | 27.9 t | 2.44, ddd (13.2, 13.2, 3.7) | 28.8 t | 2.73−2.77, m |
| 9′b | 2.22, ddd (13.2, 4.4, 3.9) | | 2.08−2.13, m | | 2.73−2.77, m |
| 10′ | 5.76, s | 110.4 d | 5.68, s | 112.2 d | 5.98, s |
| OAc | | | | | 2.00, s |

[a] 400 MHz for $^1H$ NMR, 100 MHz for $^{13}C$ NMR and DEPT. Figures in parentheses are coupling constants in hertz.

dimer, and the monoterpene units of **1** and **2** are nearly identical with each other. Macrophyllols A (**1**) and B (**2**) appeared to differ in the sesquiterpene units. Compound **2** bore an elemanolide type of sesquiterpene unit, whose structure was determined on the basis of $^1H$ and $^{13}C$ NMR data and the observed correlations of 2D NMR. In the $^1H-^1H$ COSY spectrum of **2**, H-9′ correlated to H-13, and in its HMBC spectrum, both H-10′ and H-9′ correlated to C-11 and H-13 correlated to C-8′ and C-9′. These correlations verified that the two units of **2** were connected to each other in the same way as those of **1**. The relative configuration of

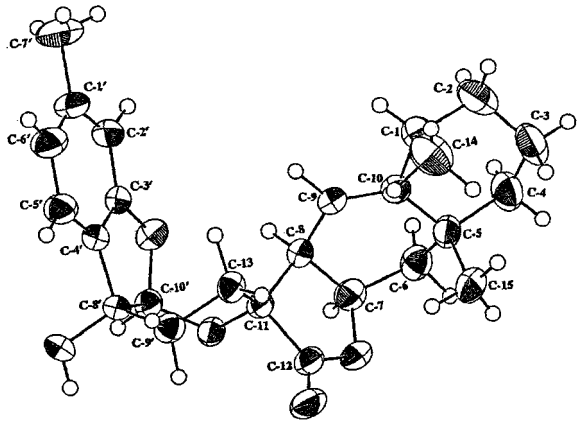



**Figure 3.** X-ray structure of macrophyllol A (**1**).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(9) X-ray crystallographic analysis data of macrophyllol A (**1**): orthorhombic crystal was obtained from a solvent system of *n*-hexane−EtOAc (4:1). Crystal size = 0.35 × 0.20 × 0.15 mm. cell parameters: $a$ = 6.448 000 (0) Å, $b$ = 11.490 000 (0) Å, $c$ = 28.976 999 (0) Å, $V$ = 2146.800 049 Å$^3$, space group $P2_12_12_1$ ($Z$ = 4). Data collection was performed on a DIP Image plate, the structure was solved by direct methods (maXus SIR92), and the final $R$ and $R_w$ values were 0.044 and 0.193 for 2017 observed reflections.

**Table 2.** NMR Spectral Data[a] of Compound **2**

| | **2** ($CDCl_3$) | | **2** ($CD_3OD$) | |
|---|---|---|---|---|
| no. | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 5.71, dd (17.5, 10.9) | 148.6 d | 5.78, dd (17.1, 11.2) | 150.4 d |
| 2a | 4.97, br d (10.9) | 111.5 t | 4.95, br d (11.2) | 111.7 t |
| 2b | 4.93, br d (17.5) | | 4.91, br d (17.1) | |
| 3a | 4.83, br s | 113.1 t | 4.81, br s | 113.4 t |
| 3b | 4.54, br s | | 4.56, br s | |
| 4 | | 146.3 s | | 148.0 s |
| 5 | 1.91, dd (11.4, 4.0) | 49.7 d | 1.97−2.06, m | 50.7 d |
| 6 | 1.27−1.30, m | 23.5 t | 1.27, m | 24.8 t |
| 7 | 2.41, m | 46.7 d | 2.50, m | 48.0 d |
| 8 | 5.03, m | 77.0 d | 5.00, m | 78.7 d |
| 9a | 2.08, m | 39.7 t | 1.97−2.06, m | 40.9 t |
| 9b | 1.69, m | | 1.80, dd (13.8, 4.0) | |
| 10 | | 38.5 s | | 39.6 s |
| 11 | | 79.9 s | | 81.4 s |
| 12 | | 174.9 s | | 177.0 s |
| 13a | 2.05, m | 21.4 t | 1.97−2.06, m | 22.0 t |
| 13b | 1.39, ddd (13.8, 13.2, 3.4) | | 1.34, ddd (13.7, 13.5, 3.3) | |
| 14 | 1.66, s | 24.7 q | 1.67, s | 25.2 q |
| 15 | 1.02, s | 18.1 q | 0.99, s | 18.5 q |
| 1′ | | 141.7 s | | 142.3 s |
| 2′ | 6.71, br s | 111.3 d | 6.68, br s | 111.7 d |
| 3′ | | 158.4 s | | 159.7 s |
| 4′ | | 126.2 s | | 128.1 s |
| 5′ | 7.20, d (7.7) | 123.3 d | 7.20, d (7.6) | 124.8 d |
| 6′ | 6.84, br d (7.7) | 123.1 d | 6.84, br d (7.6) | 123.9 d |
| 7′ | 2.36, s | 21.8 q | 2.33, s | 21.8 q |
| 8′ | overlapped with $CDCl_3$ peaks | | | 78.1 s |
| 9′a | 2.58, ddd (13.2, 13.2, 3.5) | 28.0 t | 2.41, ddd (13.2, 13.2, 3.7) | 29.2 t |
| 9′b | 2.18, ddd (13.2, 4.3, 3.4) | | 2.10, ddd (13.2, 4.4, 3.3) | |
| 10′ | 5.75, s | 110.5 d | 5.68, s | 112.3 d |

[a] 400 MHz for $^1H$ NMR, 100 MHz for $^{13}C$ NMR and DEPT. Figures in parentheses are coupling constants in hertz.

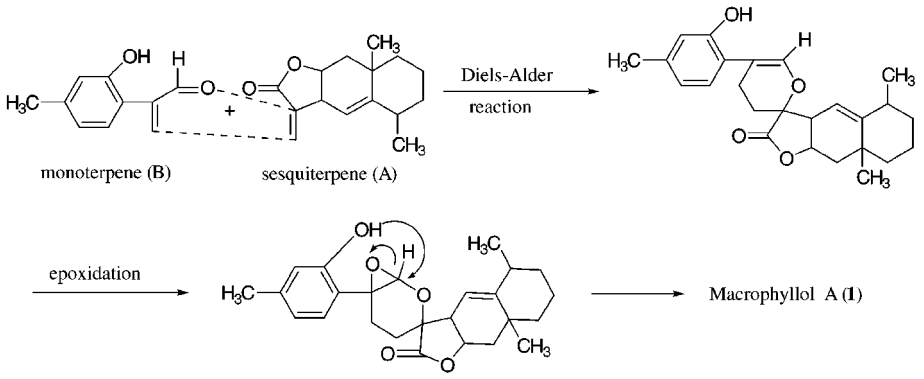


**Figure 4.** Possible biosynthetic pathway of macrophyllol A (**1**).

**2** was determined by comparison of its NMR data with those of **1** and the observed cross-peaks in its NOESY spectrum.

We speculate the possible biosynthetic pathway of macrophyllol A (**1**) from the related sesquiterpene (A) and monoterpene (B) to be as shown in Figure 4. Macrophyllol B (**2**) should be derived in the same way as macrophyllol A (**1**).

**Supporting Information Available:** $^1H$ and $^{13}C$ NMR and DEPT spectra (in $CDCl_3$ and $CD_3OD$) as well as $^1H-^1H$ COSY, NOSEY, HSQC, HMBC, and HREIMS for macrophyllols A (**1**) and B (**2**), the X-ray data for macrophyllol A (**1**), and the $^1H$ NMR spectrum (in $CDCl_3$) for the acetate (**1a**) of macrophyllol A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

OL990401N