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The structures and absolute configurations of macrophyllic acids A–E, five new rearranged eudesmane sesquiterpene acids from the bark of *Inula macrophylla*

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Abstract

Macrophyllic acids A–E, five novel sesquiterpene acids with a new rearranged carbon skeleton, have been isolated from the bark of *Inula macrophylla*. Their structures were determined on the basis of spectral evidence (especially by HREIMS and 2D NMR) as well as chemical transformations. The structure of macrophyllic acid A was finally confirmed by an X-ray analysis. The absolute configuration of macrophyllic acid A was determined by appropriate chemical conversion and the means of PGME. The possible biosynthetic pathway for macrophyllic acids A–E is also discussed. © 2000 Elsevier Science Ltd. All rights reserved.

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Aristophyllides A–D, the derivatives of the rearranged *ent*-elemene sesquiterpenes with a new carbon skeleton, have been reported recently.¹ We report herein five novel rearranged eudesmane sesquiterpene acids, macrophyllic acids A–E, which also possess a new kind of carbon skeleton and were isolated from the bark of *Inula macrophylla*. The structures of macrophyllic acids A–E were determined on the basis of spectral evidence, especially by HREIMS and 2D NMR. The structure of macrophyllic acid A (**1**) was finally confirmed by an X-ray analysis. The absolute configuration of macrophyllic acid A was determined by appropriate chemical conversion and the means of PGME. Two pairs of stereoisomers, macrophyllic acids B–E (**2–5**), are the derivatives of macrophyllic acid A (**1**), and their absolute configurations were established by comparison of the spectral data with those of macrophyllic acid A (**1**).

The MeOH extract of the powdered air-dried bark (1.6 kg) of *I. macrophylla* was partitioned between H₂O and CHCl₃. The CHCl₃-soluble fraction was chromatographed over a silica gel column and further

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purified by HPLC and GPC (general permeation chromatography), to give macrophylllic acids A–E (1–5) (950 mg, 25 mg, 14 mg, 10 mg and 11 mg, respectively), (Fig. 1).

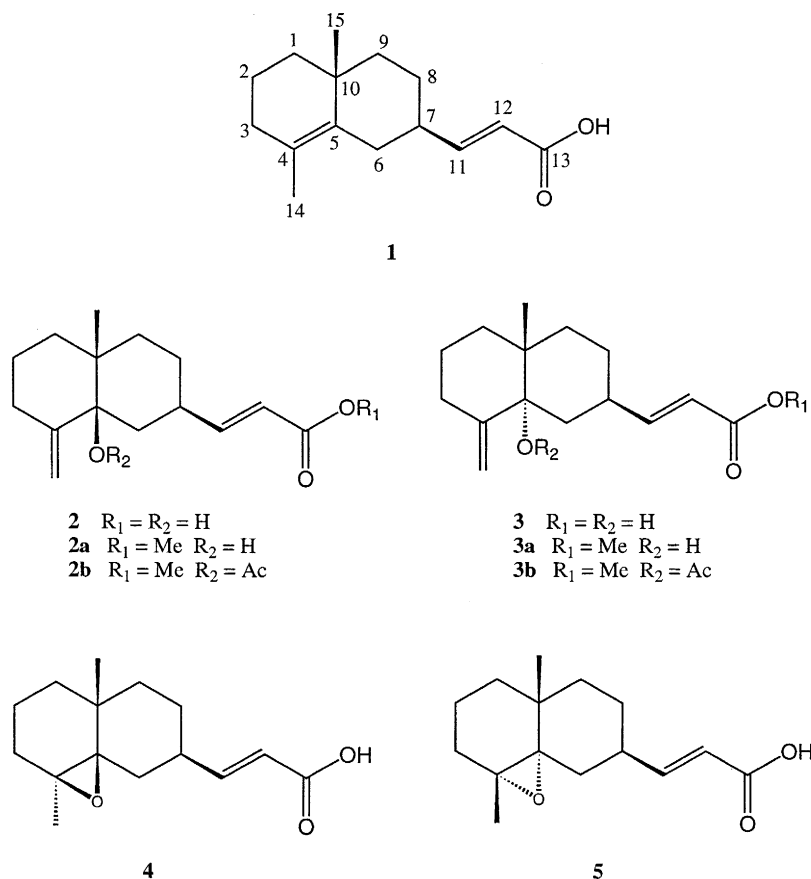


Fig. 1. The structures of compounds 1–5

Macrophylllic acid A (**1**)² was obtained as colorless needles, $[\alpha]_D^{24} -8.0$ ($CHCl_3$, $c=0.85$). Its HREIMS (m/z 234.1608) indicated a molecular formula of $C_{15}H_{22}O_2$ (calcd 234.1620). The 1H NMR spectrum of **1** showed signals of two methyls at δ_H 1.60 (3H, s, H-14) and 1.04 (3H, s, H-15), a *trans* double bond which should be conjugated with a carbonyl group based on their chemical shifts and coupling constants (δ_H 7.08, 1H, dd, $J=15.7, 7.0$ Hz, H-11; δ_H 5.82, 1H, dd, $J=15.7, 0.7$ Hz, H-12), as well as the signals of other methylenes and methine. Its ^{13}C NMR and DEPT spectra (Table 1) displayed signals of four quaternary carbons, three methines, six methylenes and two methyls. Among these signals, the chemical shifts of C-11 (δ_C 156.8, d) and C-12 (δ_C 118.6, d) further supported that this double bond should be conjugated with the carbonyl group (δ_C 172.6, s). There are no other oxygenated carbons except for the carbonyl group in **1**, based on its NMR data, suggesting this carbonyl group was a carboxy group. Compound **1** is an acid, which also can be inferred from experience since it was a long spot on TLC.

In the 1H – 1H COSY spectrum of **1**, the correlations of H-12 to H-11, H-11 to H-7, H-7 to H-6 and H-8, H-8 to H-9, H-1 to H-2, and H-2 to H-3, suggested the structure as shown. This structure was confirmed by the observed correlations of H-11 to C-12, C-13, C-7, C-6 and C-8, H-12 to C-13, C-11 and C-7, H-15 to C-5, C-10, C-1 and C-9, H-14 to C-3, C-4 and C-5, and H-6 to C-7, C-8, C-11, C-4, C-5 and C-10 in the HMBC spectrum. All of the spectral data were assigned on the basis of the correlations of 1H – 1H

Table 1
The ^{13}C NMR and DEPT spectral data of macrophylllic acids A–E (1–5)

No	1	2	3	4	5
1	40.2 t	34.6 t	35.0 t	35.5 t	34.0 t
2	19.0 t	22.5 t	22.2 t	16.7 t	16.0 t
3	33.2 t	32.1 t	31.7 t	31.2 t	28.9 t
4	125.8 s	148.2 s	151.4 s	64.2 s	63.6 s
5	133.2 s	86.5 s	75.3 s	67.9 s	68.9 s
6	30.5 t	29.2 t	35.6 t	31.5 t	30.8 t
7	42.3 d	35.5 d	35.9 d	40.4 d	38.5 d
8	27.6 t	25.9 t	25.9 t	27.1 t	26.6 t
9	41.5 t	33.8 t	33.6 t	35.5 t	36.8 t
10	34.4 s	38.7 s	38.0 s	33.7 s	33.3 s
11	156.8 d	156.2 d	156.9 d	154.8 d	155.9 d
12	118.6 d	118.8 d	118.6 d	119.0 d	118.9 d
13	172.6 s	170.9 s	171.9 s	171.4 s	171.8 s
14	19.4 q	111.7 t	108.2 t	21.4 q	20.7 q
15	24.5 q	21.1 q	20.0 q	23.0 q	20.8 q

100 MHz, CDCl_3 as solvents.

COSY, NOESY, HSQC and HMBC spectra. The structure of macrophylllic acid A was finally verified by an X-ray analysis (Fig. 2).³

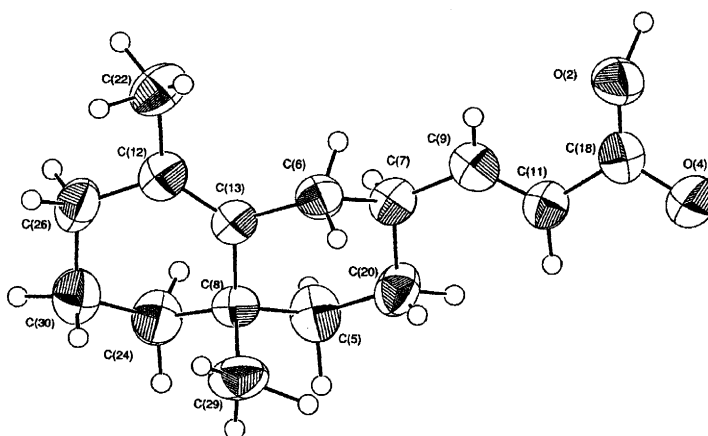


Fig. 2. ORTEP drawing of compound 1

In order to determine the absolute configuration of macrophylllic acid A, **1** was converted to **1a** (Fig. 3, the structure was confirmed by ^1H , ^{13}C NMR, ^1H – ^1H COSY and HSQC spectral data). The (*S*)- and (*R*)-PGME (phenylglycine methyl ester) amides were obtained after **1a** was treated with (*R*)- and (*S*)-PGME,

respectively (Fig. 3). Thus, the absolute configuration of **7R** and **10R** for **1** can be elucidated according to the $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$) (Fig. 3).⁴

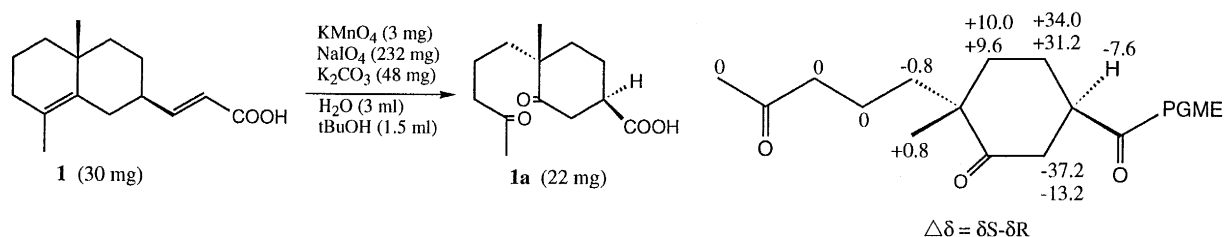


Fig. 3. The structures of **1a** and (S)- and (R)-PGME amides and their $\Delta\delta$ values

The NMR data of macrophylllic acid B (**2**)⁵ were similar to those of compound **1**. Its ¹H NMR spectrum exhibited signals of exomethylene hydrogens at δ_H 5.05 (1H, s, H-14) and 4.74 (1H, s, H-14), and one methyl signal at δ_H 0.91 (3H, s, H-15). The ¹³C NMR and DEPT spectra (Table 1) of **2** indicated an oxygenated quaternary carbon at δ_C 86.5 (C-5). These NMR data suggested the exocyclic double bond was located between C-4 and C-14, and a hydroxyl group attached to C-5 in **2**. The HIEIMS (m/z 250.1587) gave a molecular formula of C₁₅H₂₂O₃, which was identical with the proposed structure. The observed HMBC correlations of H-14 to C-3, C-4 and C-5, H-6 to C-4, C-5, C-7, C-8, C-10 and C-11, and H-15 to C-1, C-9, C-5 and C-10 confirmed its structure.

The HREIMS (m/z 250.1561) of macrophylllic acid C (**3**)⁶ indicated a molecular formula of C₁₅H₂₂O₃, the same as that of **2**. The NMR spectral data of **3** were very similar to those of **2**, and the evident difference between them were the chemical shifts of C-5 (Table 1). The hydroxyl groups should adopt an equatorial orientation and an axial orientation in **2** and **3** based on the chemical shifts of C-5, because the axial orientation hydroxyl group has a stronger shielding effect. For confirming the structures of **2** and **3**, they have been treated with MeOH and DCC in a CH₂Cl₂ solution at rt for 2 h, which gave their methyl esters **2a** and **3a**. The methyl esters **2a** and **3a** were further acetylated using acetic anhydride and pyridine in the presence of a catalytic amount of 4-dimethylamoniopyridine at rt overnight; **2b** and **3b** were obtained.

The HREIMS of **4** (m/z 250.1556) and **5** (m/z 250.1580) gave the same molecular formula of C₁₅H₂₂O₃ and their NMR data were also very similar to each other. The NMR data of compounds **4**⁷ and **5**⁸ indicated that two methyls were attached to the quaternary carbons. The chemical shifts of C-4 (**4**: δ_C 64.2, s; **5**: δ_C 63.6, s) and C-5 (**4**: δ_C 67.9, s; **5**: δ_C 68.9, s) suggested the existence of epoxy groups in **4** and **5**, which were also identical with their molecular formulas. The HMBC correlations of H-14 to C-3, C-4 and C-5, and H-15 to C-1, C-5, C-9 and C-10 were observed for both **4** and **5**; these correlations verified that their epoxy groups were between C-4 and C-5. In the NOESY spectrum of **5**, H-14 correlated to H-15, suggested the two methyls were in *cis* relationship in **5**. The signal of H-14 of **4** (δ_H 1.33) showed a downfield shift relative to that of **5** (δ_H 1.26), and the NOESY correlation between H-14 and H-15 was not observed for **4**. Thus, macrophylllic acid D (**4**) is an isomer of macrophylllic acid E (**5**).

All of the H-7 of **1–5** should adopt the axial orientations by analysis of the splitting patterns and coupling constants of H-6 (H-6eq: **1**, dd, $J=13.7, 1.4$ Hz; **2**, dd, $J=12.6, 2.4$ Hz; **3**, dd, $J=13.2, 2.8$ Hz; **4**, dd, $J=13.8, 3.2$ Hz; **5**, dd, $J=14.0, 3.7$ Hz; H-6ax: **1**, dd, $J=13.7, 12.6$ Hz; **2**, dd, $J=13.2, 12.6$ Hz; **3**, dd, $J=13.2, 12.7$ Hz; **4**, dd, $J=13.8, 12.5$ Hz; **5**, dd, $J=14.0, 12.8$ Hz). The absolute configurations of C-7 of **2–5** should be the same as that of **1**, since all of these compounds were isolated from the same plant and all of the H-7 of **1–5** adopt the axial orientations. Thus, the absolute configurations of **2–5** are as shown (Fig. 1).

11(13)-Eudesmen-12-oic acid is a common natural product, and a number of these types of sesquiterpenes have been isolated from various plant materials.^{9–14} However, macrophylllic acids A–E (**1**–**5**) possess a new rearranged carbon skeleton, and this is the first report of this kind of sesquiterpene acids. We speculate the possible biosynthetic pathway of macrophylllic acid A (**1**) from the related 11(13)-eudesmen-12-oic acid to be as shown in Fig. 4.

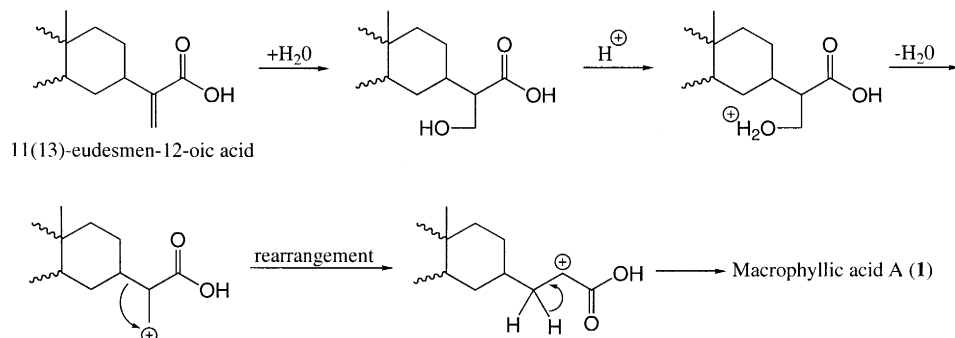


Fig. 4. The possible biosynthetic pathway of macrophylllic acid A (**1**)

It is worth to point out that macrophylllic acid A (**1**) is one of the main components (950 mg **1** has been isolated from 1.6 kg material) of the bark of *Inula macrophylla*. This suggests the biological transformation from general 11(13)-eudesmen-12-oic acids to the presently reported new rearranged carbon skeleton sesquiterpene acids (**1**–**5**) is a main and easy biological reaction in the studied plant. Macrophylllic acids B–E (**2**–**5**) were more possible derived from macrophylllic acid A (**1**) than from the related 11(13)-eudesmen-12-oic acids, due to **1** is one of the main components of this plant.

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- X-Ray crystallographic analysis data of (**1**): A colorless triclinic crystal was obtained from *n*-hexane:EtOAc (4:1). Crystal size=0.35×0.20×0.15 mm, cell parameters: a =7.621000 (0) Å, b =8.402000 (0) Å, c =12.184000 (0) Å, V =687.200012 Å³, space group $P1(Z=2)$. Data collection was performed on a DIP Image plate, and the structure was solved by direct method (maXus SIR92) and the final R and R_w values were 0.075 and 0.099, respectively, for 1866 observed reflections.
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- $[\alpha]_D^{24}$ +49.3 (CHCl₃, c =0.92); ¹H NMR data (400 MHz, CHCl₃, δ , ppm): 7.09 (1H, dd, J =15.7, 6.7 Hz, H-11), 5.85 (1H, dd, J =15.7, 1.3 Hz, H-12), 4.84 (1H, s, H-14), 4.69 (1H, s, H-14), 2.83 (1H, m, H-7), 2.59 (1H, ddd, J =15.8, 13.5, 6.7 Hz, H-3a), 2.14 (1H, dd, J =13.2, 2.0 Hz, H-3b), 1.87 (1H, m, H-1a), 1.85 (1H, m, H-9a), 1.73 (1H, dd, J =13.2, 12.7 Hz, H-6a), 1.67 (1H, dd, J =13.2, 2.8 Hz, H-6b), 1.63 (2H, m, H-2 and H-8a), 1.51 (1H, dddd, J =13.2, 12.7, 12.7, 3.6 Hz, H-8b), 1.23 (1H, ddd, J =13.2, 4.1, 2.3 Hz, H-9b), 1.09 (1H, br d, J =15.3 Hz, H-1b), 0.88 (3H, s, H-15).
- $[\alpha]_D^{24}$ –18.1 (CHCl₃, c =0.74); ¹H NMR data (400 MHz, CHCl₃, δ , ppm): 7.04 (1H, dd, J =15.7, 6.8 Hz, H-11), 5.81 (1H, dd, J =15.7, 1.2 Hz, H-12), 2.29 (1H, m, H-7), 1.86 (1H, m, H-9a), 1.83 (1H, dd, J =13.8, 12.5 Hz, H-6a), 1.80 (2H, m, H-3),

- 1.76 (1H, m, H-8a), 1.56 (1H, dd, $J=13.8, 3.2$ Hz, H-6b), 1.53 (1H, m, H-8b), 1.47 (3H, m, H-1a, H-9b and H-2a), 1.41 (1H, m, H-2b), 1.33 (3H, s, H-14), 1.06 (3H, s, H-15), 1.05 (1H, m, part overlapped with H-15, H-1b).
8. $[\alpha]_D^{24} +21.3$ (CHCl₃, $c=0.95$); ¹H NMR data (400 MHz, CHCl₃, δ , ppm): 7.04 (1H, dd, $J=15.7, 6.7$ Hz, H-11), 5.81 (1H, dd, $J=15.7, 1.2$ Hz, H-12), 2.54 (1H, m, H-7), 1.91 (1H, m, H-3a), 1.79 (1H, m, H-3b), 1.77 (1H, dd, $J=14.0, 12.8$ Hz, H-6a), 1.72 (1H, m, H-8a), 1.53–1.60 (4H, m, H-8b, H-9a, H-2a and H-1a), 1.48 (1H, dd, $J=14.0, 3.7$ Hz, H-6b), 1.39 (2H, m, H-9b and H-2b), 1.26 (3H, s, H-14), 1.07 (3H, s, H-15), 0.95 (1H, m, H-1b).
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