Pergamon

Tetrahedron Letters 41 (2000) 2395–2400

TETRAHEDRON LETTERS

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

Bao-Ning Su,[a] Yoshihisa Takaishi,[a,*] Tetsuya Yabuuchi,[a] Takenori Kusumi,[a] Motoo Tori [b] and Shigeru Takaoka [b]

[a] *Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima 770-8505, Japan*
[b] *Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan*

Received 16 December 1999; revised 14 January 2000; accepted 21 January 2000

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.

Keywords: Inula macrophylla; compositae; macrophyllic acids A–E; sesquiterpene acid; new carbon skeleton; NMR data; chemical transformation; X-ray analysis; absolute configuration; biosynthetic pathway.

Aristophyllides A–D, the derivatives of the rearranged *ent*-elemane 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 H_2O and $CHCl_3$. The $CHCl_3$-soluble fraction was chromatographed over a silica gel column and further

* Corresponding author. Tel: 0081-88-6337275; fax: 0081-88-6339501; e-mail: takaishi@ph.tokushima-u.ac.jp (Y. Takaishi)

0040-4039/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(00)00172-6

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

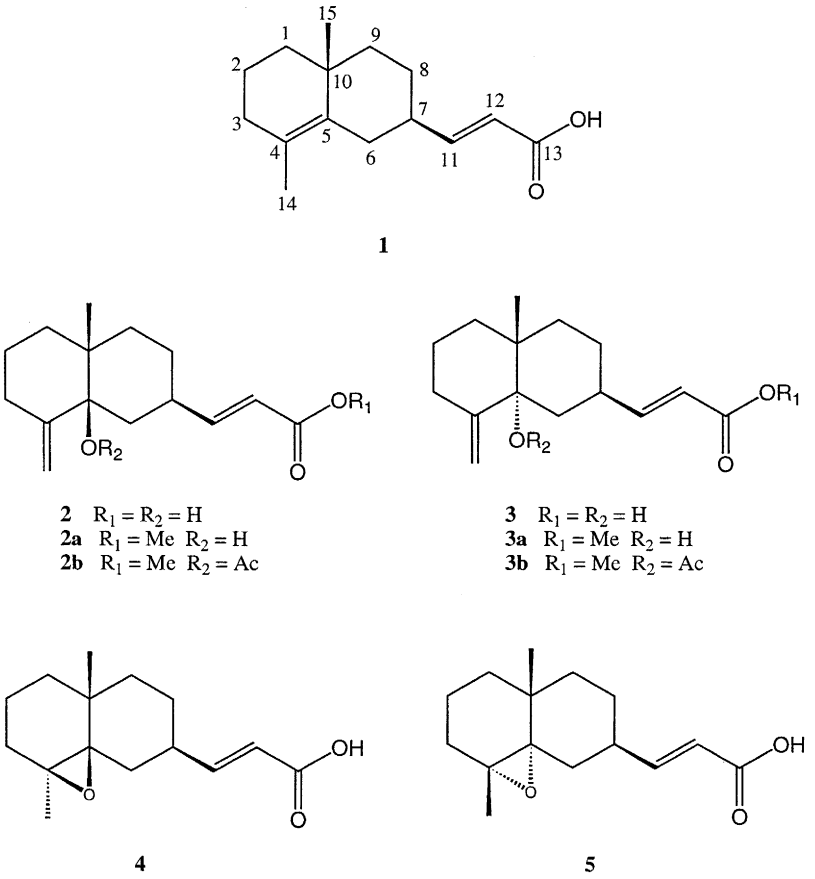

Fig. 1. The structures of compounds **1**–**5**

Macrophyllic 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 macrophyllic 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 macrophyllic acid A was finally verified by an X-ray analysis (Fig. 2).[3]

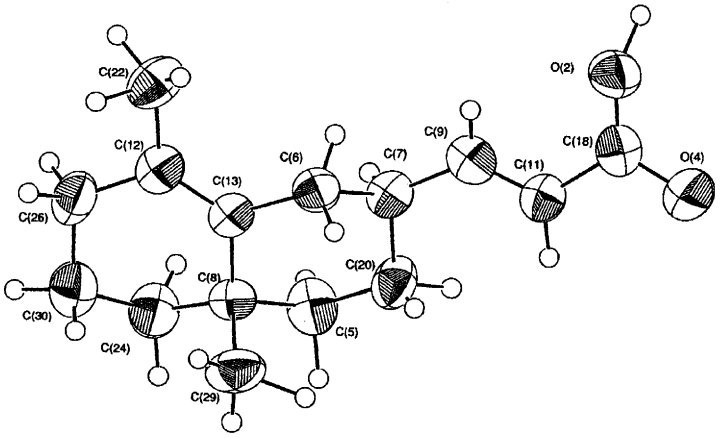

Fig. 2. ORTEP drawing of compound **1**

In order to determine the absolute configuration of macrophyllic 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 **7*R*** and **10*R*** for **1** can be elucidated according to the $\Delta\delta$ values ($\Delta\delta=\delta S-\delta R$) (Fig. 3).[4]

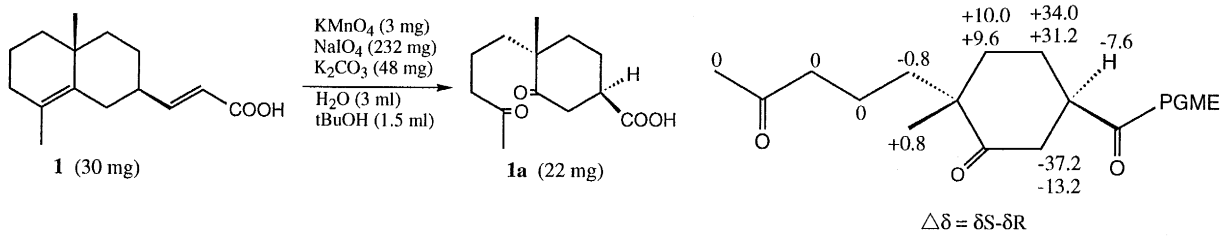

Fig. 3. The structures of **1a** and (*S*)- and (*R*)-PGME amides and their $\Delta\delta$ values

The NMR data of macrophyllic acid B (**2**)[5] were similar to those of compound **1**. Its ^{1}H 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 ^{13}C 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 $C_{15}H_{22}O_3$, 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 macrophyllic acid C (**3**)[6] indicated a molecular formula of $C_{15}H_{22}O_3$, 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 CH_2Cl_2 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 $C_{15}H_{22}O_3$ 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, macrophyllic acid D (**4**) is an isomer of macrophyllic 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, macrophyllic 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 macrophyllic acid A (**1**) from the related 11(13)-eudesmen-12-oic acid to be as shown in Fig. 4.

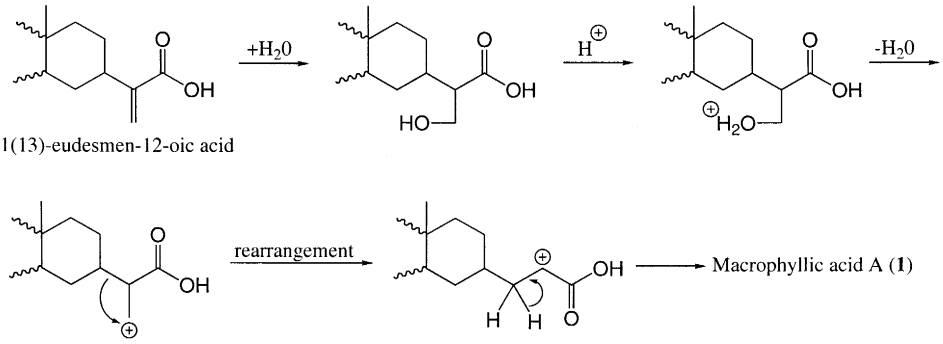

Fig. 4. The possible biosynthetic pathway of macrophyllic acid A (**1**)

It is worth to point out that macrophyllic 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. Macrophyllic acids B–E (**2**–**5**) were more possible derived from macrophyllic 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.

References

1. Wu, T.-S.; Chan, Y.-Y.; Leu, Y.-L.; Wu, P.-L.; Li, C.-Y.; Mori, Y. *J. Nat. Prod*. **1999**, *62*, 348–351.
2. ^{1}H NMR data (400 MHz, $CHCl_3$, δ, ppm): 7.08 (1H, dd, *J*=15.7, 7.0 Hz, H-11), 5.82 (1H, dd, *J*=15.7, 0.7 Hz, H-12), 2.58 (1H, dd, *J*=13.7, 1.4 Hz, H-6a), 2.12 (1H, m, H-7), 1.83–2.04 (2H, m, H-3), 1.77 (1H, dd, *J*=13.7, 12.6 Hz, H-6b), 1.63 (2H, m, H-8), 1.60 (3H, s, H-14), 1.58 (1H, m, H-9a), 1.57 (2H, m, H-2), 1.55 (1H, m, H-1a), 1.30 (1H, m, H-1b), 1.27 (1H, m, H-9b), 1.04 (3H, s, H-15).
3. 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 Å^3, space group *P*1(*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 *Rw* values were 0.075 and 0.099, respectively, for 1866 observed reflections.
4. Nagai, Y.; Kusumi, T. *Tetrahedron Lett*. **1995**, *36*, 1853–1856.
5. $[\alpha]_D^{24}$ +125.40 ($CHCl_3$, *c*=0.86); ^{1}H NMR data (400 MHz, $CHCl_3$, δ, ppm): 7.07 (1H, dd, *J*=15.8, 6.6 Hz, H-11), 5.85 (1H, dd, J=15.8, 1.2 Hz, H-12), 5.05 (1H, s, H-14), 4.74 (1H, s, H-14), 2.73 (1H, m, H-7), 2.49 (1H, m, H-3a), 2.18 (1H, br d, *J*=13.2 Hz, H-3b), 2.15 (1H, dd, *J*=12.6, 2.4 Hz, H-6a), 1.81 (1H, m, H-1a), 1.78 (1H, m, H-9a), 1.61–1.68 (3H, m, H-2 and H-8a), 1.49 (1H, dddd, *J*=13.2, 12.7, 12.7, 3.8 Hz, H-8b), 1.46 (1H, dd, *J*=13.2, 12.6 Hz, H-6b), 1.18 (1H, ddd, *J*=13.2, 4.0, 2.4 Hz, H-9b), 1.03 (1H, br d, *J*=14.0 Hz, H-1b), 0.91 (3H, s, H-15).
6. $[\alpha]_D^{24}$ +49.3 ($CHCl_3$, *c*=0.92); ^{1}H NMR data (400 MHz, $CHCl_3$, 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).
7. $[\alpha]_D^{24}$ −18.1 ($CHCl_3$, *c*=0.74); ^{1}H NMR data (400 MHz, $CHCl_3$, 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 ($CHCl_3$, *c*=0.95); ^{1}H NMR data (400 MHz, $CHCl_3$, 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).
9. Oksuz, S.; Topcu, G. *Phytochemistry* **1992**, *31*, 195–197.
10. Ulubelen, A.; Oksuz, S.; Goren, N. *Phytochemistry* **1987**, *26*, 1223–1224.
11. Guilhon, G. M. S. P.; Muller, A. H. *Phytochemistry* **1998**, 47, 227–229.
12. Bohlmann, F.; Jakupovic, J.; Ahmed, M.; Schuster, A. *Phytochemistry* **1983**, *22*, 1623–1636.
13. Bohlmann, F.; Jakupovic, J.; Schuster, A. *Phytochemistry* **1983**, *22*, 1637–1644.
14. Zdero, C.; Bohlmann, F.; King, R. M; Robinson, H. *Phytochemistry* **1987**, *26*, 187–190.