# Diterpenoids of *Isodon macrophylla*

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Two new *ent*-abietane diterpenoids, macrophynin E (**1**) and macrophynin F (**2**), and a known related *ent*-abietanoid (−)-lambertic acid (**3**), together with four known *ent*-kauranoids, were isolated from the roots and aerial parts of *Isodon macrophylla*, respectively. The structures of the new compounds were elucidated on the basis of spectroscopic-data analysis and chemical correlations.

**Introduction.** – The genus *Isodon*, which includes *ca.* 150 species, is one of the most widespread members of the family Labiatae (Lamiaceae) and has attracted considerable attention as a prolific source of new natural products with diverse structures and biological properties, including antibacterial, anti-inflammatory, and, especially, antitumor activities. For the past 30 years, more than 50 *Isodon* species distributed in mainland China have been phytochemically investigated, and hundreds of new diterpenoids (mainly *ent*-kauranoids) have been isolated and characterized [1][2]. *Isodon macrophylla* (Migo) was mainly distributed in the southern part of Jiangsu Province, China. The chemical investigation of this plant was seldom reported, since six *ent*-kaurane type diterpenoids were isolated from the aerial parts of this plant in 1980s [3–5]. As part of our ongoing research on the biologically active constituents of traditional Chinese herbal medicine [6][7], we have re-investigated the chemical constituents of the plant *I. macrophylla*, collected from Zhenjiang, Jiangsu Province, China. In the course of this study, two new *ent*-abietane type diterpenoids, macrophynin E (**1**) and macrophynin F (**2**), and a known related diterpene, (−)-lambertic acid (**3**) [8], were isolated from the roots of *I. macrophylla*, while four known *ent*-kaurane diterpenoids, rubescensin A (**4**) [9], parvifoline E (**5**) [10], lasiodonin (**6**) [11], and effusanin E (**7**) [12], were obtained from the aerial parts of the title plant. Here, we report the isolation and structural elucidation of the two new diterpenoids.

**Results and Discussion.** – The air-dried, powdered roots of *I. macrophylla* were extracted exhaustively with 95% EtOH. The EtOH extract was partitioned consecutively between $H_2O$ and petroleum ether, $H_2O$ and AcOEt, and $H_2O$ and BuOH. The AcOEt-soluble fraction was subjected to repeated column chromatography (silica gel and *Sephadex LH-20*) to give two new compounds **1** and **2**, and the known diterpene **3**. In a similar manner, the AcOEt-soluble fraction of the EtOH extract of the aerial parts of the title plant yielded four known compounds **4**–**7**.

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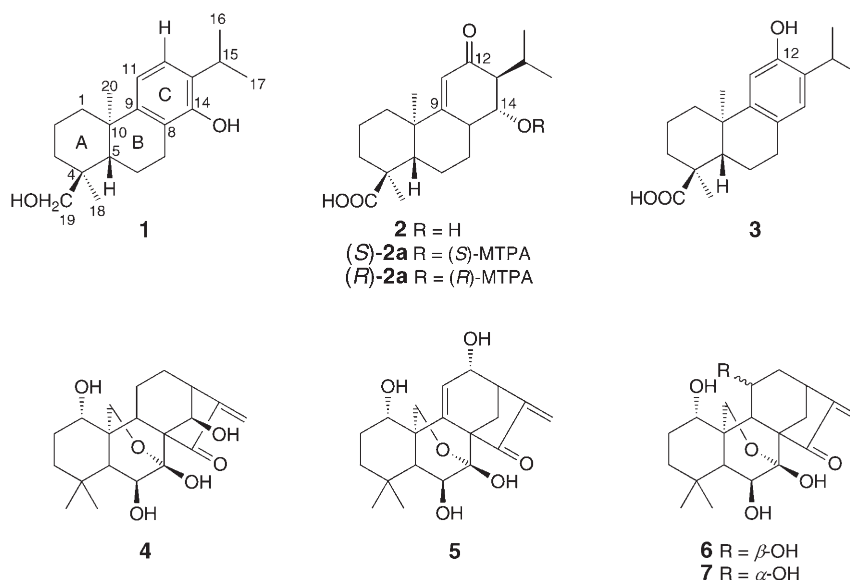


The known compounds were readily identified as (−)-lambertic acid (**3**) [8], rubescensin A (**4**) [9], parvifoline E (**5**) [10], lasiodonin (**6**) [11], and effusanin E (**7**) [12], by analysis of their NMR spectra and by comparison with the data reported in literature.

Macrophynin E (**1**) was obtained as amorphous powder and had the molecular formula $C_{20}H_{30}O_2$, deduced from its HR-EI-MS exhibiting the molecular ion at *m*/*z* 302.2251 ($M^+$) and indicating six degrees of unsaturation. The IR spectrum revealed an aromatic ring ($\tilde{\nu}_{max}$ 1581.2, 1466.4 cm$^{-1}$) and OH groups ($\tilde{\nu}_{max}$ 3500.3, 3340.6 cm$^{-1}$), and the facile transformation into a diacetate confirmed the presence of two OH functions. The $^1$H-NMR spectrum of the compound showed two aromatic 1-H *doublet*s at $\delta$(H) 6.52 and 6.98 with *J* values of 8.5 and 8.3 Hz, respectively, indicating two vicinal aromatic H-atoms. The presence of an i-Pr group on the benzene ring was obvious from the $^1$H-NMR data ($\delta$(H) 1.34 (*d*, *J* = 7 Me), 1.33 (*d*, *J* = 7 Me), 3.12–3.14 (*m*, CH)). In addition, the $^1$H-NMR spectrum also displayed signals of two Me groups at $\delta$(H) 1.06 (*s*, 3 H) and 1.18 (*s*, 3 H), and of a OH-bearing $CH_2$ moiety due to the presence of a pair of *AB*-type peaks at $\delta$(H) 3.58 and 3.82 (*d*, *J* = 11.1, each 1 H). In the absence of any other sp- and sp$^2$-C-atoms, the gross structure of **1** must be tricyclic. Interpretation of the $^1$H,$^1$H-COSY, HMQC, and HMBC data readily suggested that **1** was an abietane diterpenoid. Observation of a series of diagnostic HMBC correlations from $CH_2$(19) ($\delta$(H) 3.58 (*d*, *J* = 11.1), 3.82 (*d*, *J* = 11.1)) to C(4), and from H−C(12) ($\delta$(H) 6.98 (*d*, *J* = 8.3)) to C(13), C(14), and C(15) permitted the assignment of OH groups at C(18) and C(14), respectively. The NMR data mentioned above are strongly reminiscent of the co-occurring tricyclic diterpene, (−)-lambertic acid (**3**) [8]. A comparison of overall $^1$H- and $^{13}$C-NMR data (*Table*) revealed that **1** differs from **3** only by both the reduction of the C(19)OOH group and the migration of the phenolic OH group from C(12) in **3** to

Table. *$^1$H- and $^{13}$C-NMR Data for Compounds* **1** *and* **2**, *and $^{13}$C-NMR Data for* **3**. Recorded in $CDCl_3$ on a *Bruker DRX-400 NMR* spectrometer; δ in ppm, *J* in Hz. Assignments were accomplished by HMQC and HMBC experiments.

| | **1** | | **2** | | **3** |
|---|---|---|---|---|---|
| | δ(H) | δ(C) | δ(H) | δ(C) | δ(C) |
| $H_α$–C(1) | 2.01 (*dd*, *J* = 13.0, 8.0) | 19.5 (*t*) | 1.84 (*dd*, *J* = 12.3, 7.1) | 40.2 (*t*) | 39.2 (*t*) |
| $H_β$–C(1) | 1.67–1.69 (*m*) | | 1.26–1.28 (*m*) | | |
| $H_α$–C(2) | 1.62–1.64 (*m*) | 19.2 (*t*) | 1.53–1.54 (*m*) | 19.9 (*t*) | 21.5 (*t*) |
| $H_β$–C(2) | 1.71–1.73 (*m*) | | 1.90–1.93 (*m*) | | |
| $H_α$–C(3) | 1.86 (*d*, *J* = 13.4) | 35.1 (*t*) | 2.15–2.17 (*m*) | 37.7 (*t*) | 32.8 (*t*) |
| $H_β$–C(3) | 1.00 (*dd*, *J* = 13.0, 4.3) | | 0.98–0.99 (*m*) | | |
| C(4) | – | 38.5 (*s*) | – | 44.3 (*s*) | 45.2 (*s*) |
| H–C(5) | 1.42 (*d*, *J* = 13.4) | 50.4 (*d*) | 1.58 (*d*, *J* = 12.1) | 46.5 (*d*) | 54.8 (*d*) |
| $H_α$–C(6) | 1.38 (*d*, *J* = 13.4) | 39.7 (*t*) | 2.38 (*d*, *J* = 12.1) | 38.3 (*t*) | 41.3 (*t*) |
| $H_β$–C(6) | 2.26–2.28 (*m*) | | 2.00–2.02 (*m*) | | |
| $H_α$–C(7) | 2.74–2.76 (*m*) | 29.2 (*t*) | 1.31–1.33 (*m*) | 22.6 (*t*) | 23.0 (*t*) |
| $H_β$–C(7) | 2.94 (*dd*, *J* = 14.1, 6.3) | | 2.33–2.35 (*m*) | | |
| C(8) or H–C(8) | – | 142.7 (*s*) | 2.47–2.50 (*m*) | 42.2 (*d*) | 133.9 (*s*) |
| C(9) or H–C(9) | – | 133.6 (*s*) | – | 174.2 (*s*) | 147.7 (*s*) |
| C(10) | – | 37.6 (*s*) | – | 35.6 (*s*) | 39.8 (*s*) |
| H–C(11) | 6.52 (*d*, *J* = 8.5) | 114.4 (*d*) | 5.93 (*d*, *J* = 1.2) | 124.5 (*d*) | 112.9 (*d*) |
| H–C(12) or C(12) | 6.98 (*d*, *J* = 8.3) | 123.1 (*d*) | – | 199.9 (*s*) | 153.7 (*s*) |
| C(13) | – | 131.0 (*s*) | 2.22 (*dd*, *J* = 11.3, 2.0) | 58.1 (*d*) | 127.5 (*s*) |
| C(14) or $H_β$–C(14) | – | 152.1 (*s*) | 3.58 (*dd*, *J* = 11.9, 8.3) | 74.3 (*d*) | 127.6 (*d*) |
| H–C(15) | 3.12–3.14 (*m*) | 27.2 (*d*) | 2.48–2.51 (*m*) | 24.9 (*d*) | 29.6 (*d*) |
| Me(16) | 1.34 (*d*, *J* = 7.0) | 20.7 (*q*) | 1.04 (*d*, *J* = 6.7) | 19.4 (*q*) | 23.5 (*q*) |
| Me(17) | 1.33 (*d*, *J* = 7.0) | 20.7 (*q*) | 1.08 (*d*, *J* = 7.1) | 19.7 (*q*) | 23.4 (*q*) |
| Me(18) | 1.06 (*s*) | 26.7 (*q*) | 1.23 (*s*) | 28.3 (*q*) | 28.0 (*q*) |
| $CH_2$(19) | 3.82, 3.58 (2*d*, *J* = 11.1) | 65.3 (*t*) | – | 183.2 (*s*) | 182.0 (*s*) |
| Me(20) | 1.18 (*s*) | 26.0 (*q*) | 1.07 (*s*) | 21.9 (*q*) | 24.0 (*q*) |

C(14) in **1**, in agreement with the molecular-weight difference of 14 mass units observed between **1** and **3**.

The relative configuration of **1** was determined through analysis of the correlations observed in ROSEY spectrum. The intense NOE cross-peaks from both $CH_2$(19) to H–C(5) placed them on the same face of the molecule (β), while no ROESY correlation between H–C(5) and Me(20) suggested that Me(20) is oriented opposite to H–C(5). Although the absolute configuration of **1** remained unassigned, the close biogenetic relationship of **1** and **3**–**7** [2] might indicate that the absolute configuration of **1** is the same as that of **3**. Considering the fact that all the *ent*-kauranoids isolated from the genus *Isodon* possess an *ent*-configuration, macrophynin E (**1**) was presumed to be an *ent*-abietanoid. Accordingly, the structure of **1** is proposed as (5β,10α)-abieta-8,11,13-triene-14,19-diol.

Macrophynin F (**2**), was obtained as colorless needles (M.p. 190–193°, $[\alpha]^{D}_{20}$ = +44.8 (*c* = 0.39, $CHCl_3$)). The molecular formula of **2** was determined to be $C_{20}H_{30}O_4$ from the HR-EI-MS data exhibiting the molecular ion at *m/z* 334.2146 ($M^+$) and indicating six degrees of unsaturation. Its NMR data are similar to those of **1**

and **3**, suggesting **2** to be an *ent*-abietanoid. A careful analysis of the 2D-NMR spectra and comparison with those of (−)-lambertic acid (**3**) revealed that both **2** and **3** shared the same partial structure of rings A and B, and differed from each other at the ring C. Immediately identifiable from the NMR data for **2** (*Table*) were resonances consistent with one $\alpha$,$\beta$-unsaturated enone moiety ($\delta$(C) 199.9 (*s*, C(12)), 124.5 (*d*, C(11)), 174.2 (*s*, C(9))), an i-Pr group ($\delta$(H) 1.04 (*d*, $J$ = 6.7, Me(16)), 1.08 (*d*, $J$ = 7.1, Me(17))), as well as a OH group ($\delta$(C) 74.3 (*d*, C(14))).

A series of diagnostic HMBC correlations as depicted in *Fig. 1* led to locate the C=C bond at C(9), the oxo group at C(12), the i-Pr group at C(13), and the OH group at C(14). Moreover, because of the ROESY correlations from H−C(14) to H−C(15) and from H−C(13) to H−C(8) (*Fig. 1*), the OH group at C(14) was determined to be in an $\alpha$-orientation. The large coupling constants between H−C(14) and H−C(8) ($J$ = 8.3 Hz), and H−C(14) and H−C(13) ($J$ = 11.9 Hz) indicated that all these H-atoms were axial. Accordingly, the structure of **2** was assigned to be (5$\beta$,8$\xi$,10$\alpha$,13$\beta$,14$\alpha$)-14-hydroxy-12-oxoabiet-9(11)-en-19-oic acid.

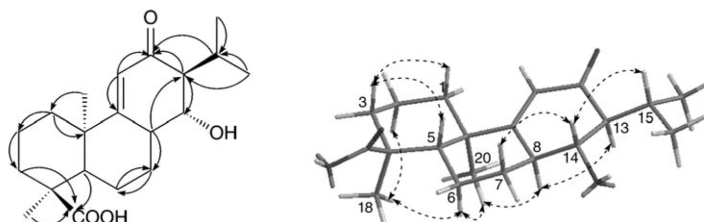


Fig. 1. *Selected HMBC* (H → C) *and ROESY* (←---→) *correlations of compound* **2**

As described above, the *ent*-nature of diterpenoids **1** and **2** was deduced mainly based on biogenetic considerations. To confirm this, we tried to determine the absolute configuration at C(14) by using an advanced *Mosher*'s method [13][14]. Unfortunately, the Δ$\delta$ ($\delta$((*S*)-MTPA ester) − $\delta$((*R*)-MTPA ester)) values for the vicinal H-atoms of the stereogenic center C(14) were irregularly distributed (*Fig. 2*), suggesting that *Mosher*'s methodology is not applicable to compound **2**.

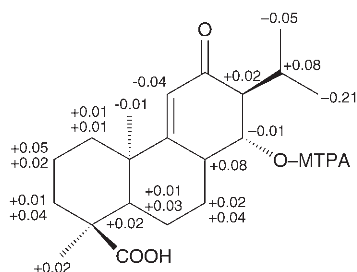


Fig. 2. *Δδ Values* ($\delta_{(S)} - \delta_{(R)}$) [ppm] *for the H-atoms near C(14) of* (S)*- and* (R)*-MTPA esters of* **2**

Although the *ent*-kaurane type diterpenoids are predominant metabolites of the genus *Isodon*, the discovery of *ent*-abietane diterpenoids were reported in recent years. It may be worth to point out that the *ent*-abietane-type diterpenes were found only in

the roots of *I. macrophylla*, while the aerial part of the plant contained only the *ent*-kauranoids. This is the first report of *ent*-abietanoids from *I. macrophylla*.

The cytotoxic activities of new compounds **1** and **2** against the growth of tumor cell lines A549 (human lung adenocarcinoma) and HL-60 (human leukemia) were evaluated. Unfortunately, the results indicated that both the tested compounds were inactive against the above cancer cells (50% effective dose of clonal inhibition ($ED_{50}$) > 10 mg/ml).

## Experimental Part

*General*. Column chromatography (CC): Commercial silica gel (*Qing Dao Hai Yang Chemical Group Co.*; 200–300 mesh) and *Sephadex LH-20* (*Amersham Biosciences*). TLC: Precoated silica-gel plates (*Yan Tai Zi Fu Chemical Group Co.*; *G60*, *F-254*). M.p.: *X-5* apparatus, uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *756 CRT* spectrophotometer; $\lambda_{max}$ (log $\varepsilon$) in nm. IR Spectra: *Nicolet Magna FT-IR 750* spectrophotometer; $\tilde{\nu}_{max}$ in $cm^{-1}$. $^1H$- and $^{13}C$-NMR spectra: *Varian Mercury 400* ($^1H$: 400 MHz and $^{13}C$: 100 MHz) spectrometer; chemical shifts $\delta$ in ppm, with residual $CHCl_3$ ($\delta$(H) 7.26, $\delta$(C) 77.0) or $CD_3OD$ ($\delta$(H) 3.30, $\delta$(C) 49.5) as internal standards, coupling constant $J$ in Hz. ESI- and HR-ESI-MS: *Q-TOF Micro* LC-MS-MS spectrometer, in $m/z$.

*Plant Material. I. macrophylla* (MIGO) were collected in Zhenjiang, Jiangsu Province, China, in September, 2005, and identified by Assoc. Prof. *J.-G. Shen* of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (P06-38) is available for inspection at the Herbarium of Shanghai Institute of Materia Medica, CAS.

*Extraction and Isolation*. The air-dried, powdered roots (2.1 kg) of *I. macrophylla* were exhaustively extracted with 95% EtOH at r.t. The extract was then concentrated under reduced pressure to give a dark residue, which was partitioned consecutively between $H_2O$ and petroleum ether, $H_2O$ and AcOEt, and $H_2O$ and BuOH. The AcOEt-soluble fraction was chromatographed on a silica-gel column using eluents of increasing polarity, from petroleum ester to acetone to MeOH. The fractions eluted with petroleum ether/acetone 8:2 afforded compound **1** (21.3 mg).

The fractions eluted with petroleum ether/acetone 7:3 were further purified by a *Sephadex-LH-20* CC with $CHCl_3$/MeOH 1:1 to afford **2** (6.7 mg) and **3** (12.7 mg). Similar to the isolation process of the roots of *I. macrophylla*, the AcOEt-soluble fraction of the EtOH extract of the aerial parts of the title plant was subjected to silica-gel CC with eluents of increasing polarity, from petroleum ether to acetone to MeOH. Compounds **4** (11.3 mg), **5** (8.3 mg), **6** (12.5 mg), and **7** (19.6 mg) were obtained from the fractions eluted with petroleum ether/acetone 8:2.

*Macrophynin E* (*=(5β,10α)-Abieta-8,11,13-triene-14,19-diol=(1*S**,4a*R**,10a*S**)-1,2,3,4,4a,9,10,10a-Octahydro-8-hydroxy-1,4a-dimethyl-7-(1-methylethyl)phenanthrene-1-methanol*; **1**). Amorphous powder. $[\alpha]_D^{20}=+28.4$ ($c=0.32$, $CHCl_3$). UV (MeOH): 280 (3.48). IR (KBr): 3500.3, 3340.6, 1581.2, 1466.4. $^1H$- and $^{13}C$-NMR: see *Table*. EI-MS: 302 ($M^+$). HR-EI-MS: 302.2251 ($M^+$, $C_{20}H_{30}O_2^+$; calc. 302.2246).

*Macrophynin F* (*=(5β,8ξ,10α,13β,14α)-14-Hydroxy-12-oxoabiet-9(11)-en-19-oic Acid=(1*S**,4a*R**,7*S**,8*R**,10a*S**)-1,2,3,4,4a,6,7,8,8a,9,10,10a-Dodecahydro-8-hydroxy-1,4a-dimethyl-7-(1-methylethyl)-6-oxophenanthrene-1-carboxylic Acid*; **2**). Colorless needles. M.p. 190–193°. $[\alpha]_D^{20}=+44.8$ ($c=0.39$, $CHCl_3$). UV (MeOH): 243 (3.96). IR (KBr): 3411.5, 1695.1, 1646.9, 1465.7, 1367.3, 1228.5, 1172.5, 1056.8, 756.0. $^1H$- and $^{13}C$-NMR: see *Table*. EI-MS: 334 ($M^+$). HR-EI-MS: 334.2146 ($M^+$, $C_{20}H_{30}O_4^+$; calc. 334.2144).

*Preparation of (*S*)- and (*R*)-MTPA Esters*. The derivative ($S$)-**2a** was obtained by treating **2** (4.0 mg) with ($S$)-MTPA-Cl in dry pyridine for *ca.* 16 h under stirring at r.t. The mixture was purified by CC (silica gel) to afford pure ($S$)-**2a** (2.8 mg). In a similar manner, ($R$)-**2a** (2.6 mg) was prepared from ($R$)-MTPA-Cl.

*Data of (*S*)-**2a***: $^1H$-NMR ($CDCl_3$, 400 MHz): 0.85 ($d$, $J=7.1$, Me(17)); 1.03–1.05 ($m$, $H_\beta$–C(3)); 1.05 ($d$, $J=6.9$, Me(16)); 1.10 ($s$, Me(20)); 1.23–1.25 ($m$, $H_\beta$–C(1)); 1.27 ($s$, Me(18)); 1.56–1.57 ($m$, $H_\alpha$–C(2)); 1.56–1.74 ($m$, $H_\alpha$–C(7)); 1.60 (overlapped, H–C(5)); 1.86–1.87 ($m$, $H_\alpha$–C(1)); 1.96–1.98

(*m*, $H_β$–C(2)); 2.13–2.15 (*m*, $H_α$–C(3)); 2.39 (*dd*, *J* = 3.6, 10.4, H–C(13)); 2.43–2.44 (*m*, $H_α$–C(6)); 2.52–2.54 (*m*, $H_β$–C(6)); 2.54–2.56 (*m*, $H_β$–C(7)); 2.60–2.61 (*m*, H–C(15)); 2.74–2.76 (*m*, H–C(8)); 5.25 (*dd*, *J* = 7.0, 10.8, H–C(14)); 5.92 (*s*, H–C(11)).

*Data of (*R*)-**2a***: $^1$H-NMR ($CDCl_3$, 400 MHz): 1.00–1.01 (*m*, $H_β$–C(3)); 1.06 (*d*, *J* = 7.1, Me(17)); 1.10 (*d*, *J* = 7.0, Me(16)); 1.11 (*s*, Me(20)); 1.22–1.24 (*m*, $H_β$–C(1)); 1.25 (*s*, Me(18)); 1.51–1.53 (*m*, $H_α$–C(2)); 1.58 (overlapped, H–C(5)); 1.72–1.74 (*m*, $H_α$–C(7)); 1.86–1.88 (*m*, $H_α$–C(1)); 1.95–1.97 (*m*, $H_β$–C(2)); 2.12–2.14 (*m*, $H_α$–C(3)); 2.37 (*dd*, *J* = 3.7, 10.4, H–C(13)); 2.41–2.43 (*m*, $H_α$–C(6)); 2.49–2.50 (*m*, $H_β$–C(6)); 2.51–2.52 (*m*, $H_β$–C(7)); 2.52–2.53 (*m*, H–C(15)); 2.66–2.68 (*m*, H–C(8)); 5.26 (*dd*, *J* = 7.1, 10.5, H–C(14)); 5.96 (*s*, H–C(11)).

The research work was financially supported by the *National '863' Project* (No. 2006AA09Z412), National Science Foundation of China (Nos. 30730108 and 20721003), *CAS Key Project* (grant KSCX2-YW-R-18), and *STCSM Projects* (No. 054307062 and 06DZ22028), and partially funded by the grants from *Syngenta-SIMM-PhD Studentship Project.*

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*Received June 12, 2007*