

Ternifolide A, a New Diterpenoid Possessing a Rare Macrolide Motif from *Isodon ternifolius*

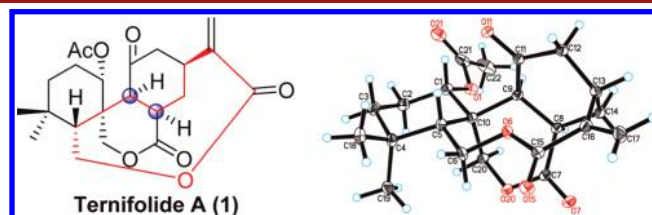
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ABSTRACT



Ternifolide A (1), a new diterpenoid featuring a unique 10-membered lactone ring formed between C-6 and C-15, along with ternifolide B (2), a *nor*-diterpenoid, and ternifolide C (3) were isolated from the leaves of *Isodon ternifolius*. Both H-8 and H-9 being α -orientations in compound 1 were found for the first time. The absolute configurations of 1 and 3 were confirmed by X-ray diffraction study. Compounds 1 and 3 were evaluated for their cytotoxicity.

The genus *Isodon* is famous for producing bioactive diterpenoids with diverse skeletons, especially *ent*-kaurane diterpenoids. To discover novel natural products for cancer treatment, our group has phytochemically investigated more than 66 *Isodon* (Labiateae) plants and isolated and characterized more than 600 new diterpenoids.¹ Some structures are very interesting, such as maoecrystal V,² bisrubescensins A–C,³ maoecrystal Z,⁴ and neolaxiflorins A and B.⁵ Particularly, maoecrystal V has attracted great

attention from synthetic chemists,⁶ due to its unusual skeleton and highly selective inhibitory activity against HeLa cells (IC₅₀ = 0.02 μ g/mL).

Morphologically, *Isodon ternifolius* (D. Don) Kudô is readily distinguished from all other *Isodon* species by having verticillate leaves, while other species typically have opposite leaves. This species has been used as folk medicine for the treatment of enteritis, icterohepatitis, and other types of inflammation,⁷ and it is also the major ingredient

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(1) Sun, H. D.; Huang, S. X.; Han, Q. B. *Nat. Prod. Rep.* **2006**, *23*, 673–698.

(2) Li, S. H.; Wang, J.; Niu, X. M.; Shen, Y. H.; Zhang, H. J.; Sun, H. D.; Li, M. L.; Tian, Q. E.; Lu, Y.; Cao, P.; Zheng, Q. T. *Org. Lett.* **2004**, *6*, 4327–4330.

(3) Huang, S. X.; Xiao, W. L.; Li, L. M.; Li, S. H.; Zhou, Y.; Ding, L. S.; Lou, L. G.; Sun, H. D. *Org. Lett.* **2006**, *8*, 1157–1160.

(4) Han, Q. B.; Cheung, S.; Tai, J.; Qiao, C. F.; Song, J. Z.; Tso, T. F.; Sun, H. D.; Xu, H. X. *Org. Lett.* **2006**, *8*, 4727–4730.

(5) Wang, W. G.; Du, X.; Li, X. N.; Wu, H. Y.; Liu, X.; Shang, S. Z.; Zhan, R.; Liang, C. Q.; Kong, L. M.; Li, Y.; Pu, J. X.; Sun, H. D. *Org. Lett.* **2012**, *14*, 302–305.

(6) (a) Gong, J.; Lin, G.; Li, C. C.; Yang, Z. *Org. Lett.* **2009**, *11*, 4770–4773. (b) Krawczuk, P. J.; Schone, N.; Baran, P. S. *Org. Lett.* **2009**, *11*, 4774–4776. (c) Peng, F.; Yu, M. L.; Danishefsky, S. J. *Tetrahedron Lett.* **2009**, *50*, 6586–6587. (d) Baitinger, I.; Mayer, P.; Trauner, D. *Org. Lett.* **2010**, *12*, 5656–5659. (e) Gong, J. X.; Lin, G. A.; Sun, W. B.; Li, C. C.; Yang, Z. *J. Am. Chem. Soc.* **2010**, *132*, 16745–16746. (f) Lazarski, K. E.; Hu, D. X.; Stern, C. L.; Thomson, R. J. *Org. Lett.* **2010**, *12*, 3010–3013. (g) Nicolaou, K. C.; Dong, L.; Deng, L. J.; Talbot, A. C.; Chen, D. Y. K. *Chem. Commun.* **2010**, *46*, 70–72. (h) Singh, V.; Bhalerao, P.; Mobin, S. M. *Tetrahedron Lett.* **2010**, *51*, 3337–3339. (i) Dong, L.; Deng, L. J.; Lim, Y. H.; Leung, G. Y. C.; Chen, D. Y. K. *Chem.—Eur. J.* **2011**, *17*, 5778–5781. (j) Gu, Z. H.; Zakarian, A. *Org. Lett.* **2011**, *13*, 1080–1082. (k) Peng, F.; Danishefsky, S. J. *Tetrahedron Lett.* **2011**, *52*, 2104–2106.

(7) Wu, Z. Y.; Li, X. W. *Flora Republicae Popularis Sinicae*; Science Press: Beijing, 1977; Vol. 66, pp 436–439.

of a Chinese patent medicine “Fufang Sanyexiangchacai Pian”, which is used to treat acute and chronic hepatitis and hepatitis B. Previous chemical investigations of *I. ternifolius* have led to the isolation of a series of *ent*-kaurane diterpenoids.⁸ In our search for biologically active secondary metabolites from this plant, three new diterpenoids, ternifolides A–C (**1–3**), were discovered.⁹ Compound **1** bore an unprecedented 10-membered lactone ring formed between C-6 and C-15, and both H-8 and H-9 being α -orientations in compound **1** were opposite to those of 8,15-*seco-ent*-kaurane diterpenoids: laxiflorin F (**4**),¹⁰ rubescensin T,¹¹ and rubescensin U.¹² It is the first time that we discovered a diterpenoid having such configurations in the reported *ent*-kaurane diterpenoids (Figure 1); thus we classified compound **1** to a new diterpene type, ternifonane. In addition, ring A of compound **3** existed in two types of conformation (type I and type II, Figure 2) in solvent, as in the case of trichorabdals A, B, D, and H,¹³ and the NMR data could not be recorded clearly at normal temperature (+22 °C). However, the NMR spectra of **3** showed that the data of the type-II conformation could be recorded at about +60 °C, while those of both conformations could be observed at about –30 °C (1:1.56) (Scheme 1).

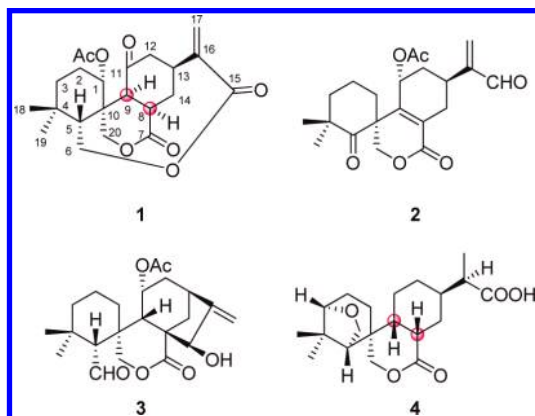


Figure 1. Structure of compounds **1–4**.

Compound **1** was obtained as colorless laminate crystals (MeOH). Its molecular formula was determined as $C_{22}H_{28}O_7$ by HR-ESI-MS ($[M + Na]^+$, 427.1731, calcd

(8) (a) Sun, H. D.; Lin, Z. W.; Minami, Y.; Takeda, Y.; Fujita, T. *Yakugaku Zasshi* **1982**, *102*, 887–890. (b) Takeda, Y.; Takeda, K.; Fujita, T.; Sun, H. D.; Minami, Y. *Chem. Pharm. Bull.* **1990**, *38*, 439–442. (c) Takeda, Y.; Takeda, K. I.; Fujita, T.; Sun, H. D.; Minami, Y. *Phytochemistry* **1994**, *35*, 1513–1516. (d) Li, G. Y.; Wang, Y. L.; Song, W. Z.; Ji, Q. Y. *Zhongyao Tongbao* **1983**, *8*, 28–29. (e) Wang, Z. M.; Feng, H.; Liang, X. T.; Yuan, S. T.; Xu, M. J. *Acta Pharmaceutica Sinica* **1996**, *31*, 764–769. (f) Na, Z.; Xiang, W.; Zhao, Q. S.; Mei, S. X.; Li, C. M.; Lin, Z. W.; Sun, H. D. *Acta Bot. Sin.* **2002**, *24*, 267–272.

(9) For detailed experimental procedures, physical–chemical properties, and 1H NMR data for compounds **1–3** (Table 1S), see the Supporting Information.

(10) Niu, X. M.; Li, S. H.; Zhao, Q. S.; Lin, Z. W.; Sun, H. D.; Lu, Y.; Wang, C.; Zheng, Q. T. *Tetrahedron Lett.* **2002**, *43*, 661–664.

(11) Han, Q. B.; Li, R. T.; Zhang, J. X.; Sun, H. D. *Helv. Chim. Acta* **2004**, *87*, 1119–1124.

(12) Han, Q. B.; Zhang, J. X.; Zhao, A. H.; Sun, H. D.; Lu, Y.; Wu, Y. S.; Zheng, Q. T. *Tetrahedron* **2004**, *60*, 2373–2377.

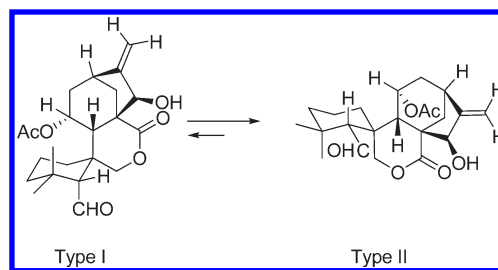


Figure 2. Two conformations of compound **3**.

427.1732), corresponding to 9 degrees of unsaturation. The IR spectrum showed the absorption bands at 1739 and 1683 cm^{-1} indicating the existence of carbonyl groups for lactone and conjugated lactone, respectively. The ^{13}C NMR and DEPT spectrum data (Table 1) of **1** displayed six quaternary carbons (including an isolated ketone, an α,β -unsaturated lactone, a lactone residue, and a terminal double bond), seven methylenes (including two oxymethylenes and a terminal double bond), five methines (including an oxymethine), two methyl groups, and one acetoxy group. On the basis of these data and chemotaxonomic considerations, compound **1** was initially presumed to be a tetracyclic diterpenoid containing two lactone rings.

The observed correlations in the HMBC spectrum of **1**: from the geminal methyls Me-18 (δ_H , 0.74) and Me-19 (δ_H , 0.92) to C-3, C-4, and C-5; from H-1 (δ_H , 5.92) to C-3, C-5, C-20, and 1- O $COCH_3$; from H-6a (δ_H , 4.52) and H-6b (δ_H , 4.44) to C-4, C-5, and C-10, and from H-20a (δ_H , 4.94) and H-20b (δ_H , 4.52) to C-1, C-5, and C-10, together with the $^1H-^1H$ COSY correlations of H-1/ H_2-2/H_2-3 , and of H-5/ H_2-6 gave partial structure **1a** (Figure 3).

The HMBC correlations from H-17a (δ_H , 6.36) and H-17b (δ_H , 5.56) to C-13, C-15, and C-16; from H-9 (δ_H , 3.58) to C-8 and C-12; from H-8 (δ_H , 3.44) to C-11 (δ_C , 207.1) and C-13; and from H-13 (δ_H , 3.05) to C-8, C-11, C-16, and C-17, along with the $^1H-^1H$ COSY correlations of H-9/ $H_8/H_2-14/H_2-12$, established partial structure **1b** (Figure 3).

The key HMBC correlations from H_2-6 to C-15 indicated that a 10-membered lactone ring formed between C-6 and C-15, and correlations from H_2-20 to C-7 suggested a δ -lactone ring formed between C-7 and C-20. The HMBC correlations from H-1, H-5, and H_2-20 to C-9; from H-9 to C-1; and from H-8 to C-10 permitted subunits **1a** and **1b** to be joined to the gross structure of **1**. In the ROESY spectrum, the presence of the correlations of H-1 with H-3 β , H-5 β , and H-9; of H-9 with H-8 and H-14 α ; of H-8 with H-20b; and of H-13 α with H_2-12 , H_2-14 , and H-17b determined H-1 as being in a β -orientation, while

(13) (a) Fujita, E.; Fuji, K.; Sai, M.; Node, M.; Watson, W. H.; Zabel, V. *J. Chem. Soc. Chem. Commun.* **1981**, 899–900. (b) Node, M.; Sai, M.; Fuji, K.; Fujita, E.; Takeda, S.; Unemi, N. *Chem. Pharm. Bull.* **1983**, *31*, 1433–1436. (c) Node, M.; Sai, M.; Fuji, K.; Fujita, E.; Shingu, T.; Watson, W. H.; Grossie, D. *Chem. Lett.* **1982**, 2023–2026. (d) Kashyap, R. P.; Watson, W. H.; Grossie, D. A.; Node, M.; Sai, M.; Fujita, E.; Fuji, K. *Acta Crystallogr. Sect. C* **1984**, *40*, 515–517.

Scheme 1. ^1H NMR of Compound **3** (500 MHz Pyridine- d_5)

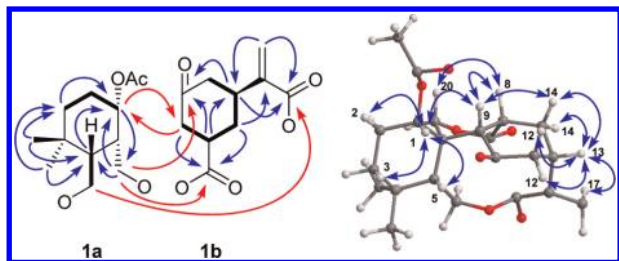
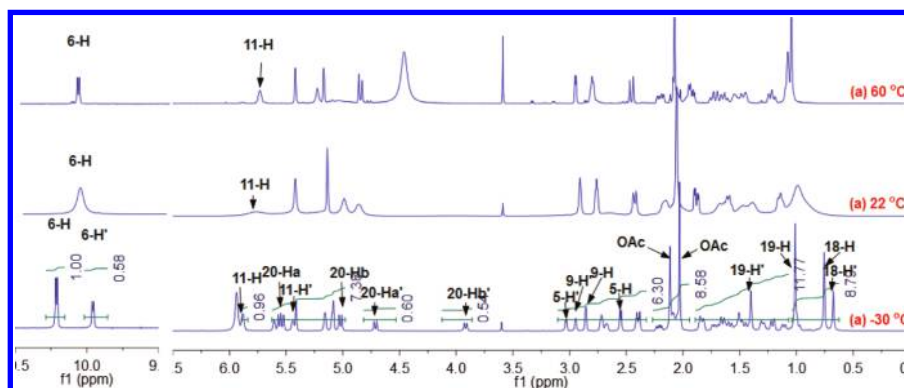


Figure 3. Selected 2D NMR correlations of compound **1** (\rightarrow , HMBC; bold — , ^1H - ^1H COSY; \leftrightarrow , ROESY).

H-8 and H-9 as being in α -orientations, respectively (Figure 3). The structure of **1** was finally confirmed by a single-crystal X-ray diffraction using anomalous scattering of Cu K α radiation (CCDC 880635),¹⁴ which indicated the absolute stereochemistry of **1** to be C-1 (*S*), C-5 (*R*), C-8 (*R*), C-9 (*S*), C-10 (*R*), C-13 (*S*) (Figure 4). The configurations of C-8 and C-9 in **1** were opposite to those of laxiflorin F (**4**).¹⁰ Therefore, the structure of **1** was determined as shown and given the trivial name ternifolide A.

Compound **2** was isolated as a white powder. The molecular formula $\text{C}_{21}\text{H}_{26}\text{O}_6$ was determined by HR-ESI-MS ($[\text{M} + \text{Na}]^+$ m/z 397.1623, calcd 397.1627). The ^{13}C NMR and DEPT spectral data of **2** (Table 1) showed seven quaternary carbons (including one carbonyl carbon and two olefinic carbons), three methines (including one aldehyde carbon and one oxymethine), seven methylenes (including one olefinic carbon and one oxymethylene), one acetyl carbon, and three methyls. The above data unveiled compound **2** as a *nor*-diterpenoid sharing structural features with the known 6,7:8,15-*seco-ent*-kaurane diterpene skeleton.¹⁰ In the HMBC spectrum, correlations from H-1, H-3, H₃-18, and H₃-19 to the quaternary carbon (δ_{C} , 213.9) confirmed the carbonyl carbon assigned to C-5. Correlations from H-11 (δ_{H} , 5.76) to C-8, C-13, and

OCOCH_3 determined C-11 is connected with the acetoxy group. Correlations from H-11 and H₂-14 to the quaternary carbon (δ_{C} 130.6) and from H-11, H-12, H₂-14, and H-20 to the quaternary carbon (δ_{C} 149.1) suggested a double bond formed between C-8 and C-9. The ROESY correlations of H-11 with H-12 β and H-1 β suggested that the H-11 was in a β -orientation. Accordingly, the structure of **2** was established as shown and has been accorded the trivial name ternifolide B.

Compound **3**, obtained as colorless columnar crystals, has the molecular formula $\text{C}_{22}\text{H}_{30}\text{O}_6$ on the basis of its HR-ESI-MS data at m/z 413.1941 $[\text{M} + \text{Na}]^+$ (calcd 413.1940). The

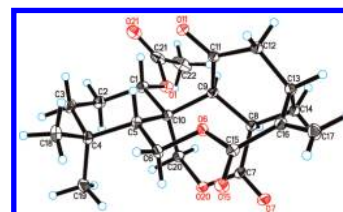


Figure 4. X-ray crystallographic structure of compound **1**.

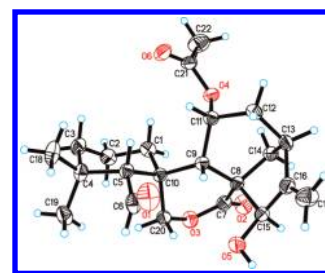


Figure 5. X-ray crystallographic structure of compound **3**.

(14) (a) Flack, H. D. *Acta Crystallogr., Sect. A* **1983**, *39*, 876–881. (b) Flack, H.; Bernardinelli, G. *Acta Crystallogr., Sect. A* **1999**, *55*, 908–915.

^{13}C NMR and DEPT data (Table 1) of **3** were quite similar to those of trichrabdal A,^{13b} and the main differences were the

Scheme 2. Proposed Biogenetic Pathway of Compounds 1–3

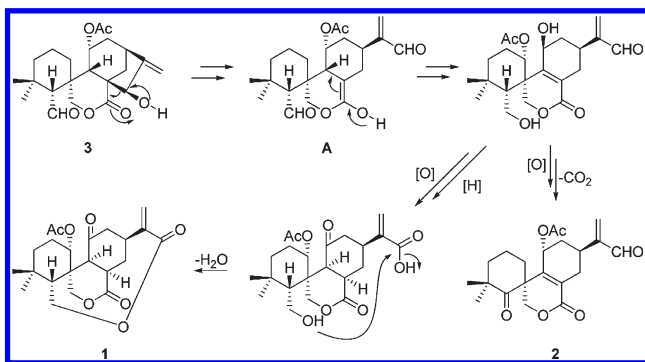


Table 1. ^{13}C Spectroscopic Data (δ in ppm) of Compounds 1–3 (Pyridine- d_5 , δ in ppm)^a

no.	1	2	3 (type-II)	3' (type-I)
1	77.5 d	31.0 t	31.1 t	34.3 t
2	24.6 t	17.7 t	18.4 t	19.2 t
3	39.4 t	38.8 t	41.3 t	36.2 t
4	45.1 s	45.4 s	34.3 s	33.8 s
5	43.6 d	213.9 s	64.0 d	56.0 d
6	64.4 t	—	206.4 d	205.1 d
7	174.6 s	164.0 s	175.8 s	175.1 d
8	38.2 d	130.6 s	51.7 s	50.0 s
9	47.2 d	149.1 s	42.5 d	38.8 s
10	32.7 s	53.6 s	41.3 s	38.5 s
11	207.1 s	67.0 d	67.3 d	69.8 d
12	44.6 t	32.5 t	41.4 t	41.6 t
13	38.0 d	27.3 d	37.0 d	36.2 d
14	28.0 t	30.0 t	31.1 t	29.4 t
15	164.3 s	194.3 d	81.8 d	81.3 d
16	144.1 s	152.6 s	157.7 s	158.1 s
17	127.6 t	133.9 t	109.5 t	109.3 t
18	33.5 q	27.6 q	33.8 q	31.4 q
19	21.0 q	26.8 q	23.3 q	29.5 q
20	68.2 t	71.8 t	69.3 t	72.7 t
OAc	170.3 s	169.9 s	175.8 s	175.1 s
	21.0 q	17.7 q	21.7 q	21.7 q

^aData of 1–3 were recorded at 100 MHz, and the assignments were based on DEPT, 2D NMR experiments.

appearance of an oxymethine (δ_{C} 81.8) and the absence of a carbonyl carbon (δ_{C} 201.6) in 3, which were further confirmed by the HMBC correlations from H-11 to C-8, C-12, C-13, and 11- $\text{O}(\text{CO})\text{CH}_3$ and from H-15 to C-9 and C-17, coupled with ^1H – ^1H COSY correlations of H-9/H-11/H₂-12/H-13/H₂-14. The ROESY correlations of H-11 with H-1 β ,

(15) Fujita, E.; Fujita, T.; Shibuya, M. *Tetrahedron* **1969**, *25*, 2517–2530.

(16) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.

Table 2. Cytotoxic Activity of Compounds 1 and 3 (IC_{50} in μM)^a

compd	HL-60	SMMC-7721	A-549	MCF-7	SW-480
1	>40	>40	>40	>40	>40
3	3.38	4.27	3.16	3.46	3.60
<i>cis</i> -platin	1.96	16.23	17.50	17.77	12.83

^a*cis*-Platin was used as positive control.

H-5 β , H-9 β , and H-12 β and of H-15 with H-13 α suggested that H-11 and OH-15 were in β -orientations, respectively. The structure of 3 was finally determined by a single-crystal X-ray diffraction using anomalous scattering of Cu K α radiation (CCDC 880924), which indicated the absolute configurations of 3 to be 5*R*, 8*S*, 9*S*, 10*S*, 11*R*, 13*S*, 15*R* (Figure 5). Thus, the structure of 3 (ternifolide C) was fully determined as shown.

In the reported 8,15-*seco-ent*-kaurane diterpenoids, both H-8 and H-9 were in β -orientations.^{10–12} However, the configurations of H-8 and H-9 in compound 1 were opposite to those of the *ent*-kaurane diterpenoids. Compound 1 may be derived from the *ent*-kaurane diterpenoids. A plausible biogenetic pathway of 1–3 was postulated to explain their origins (Scheme 2). This pathway involved retro-aldol,¹⁵ oxidation, reduction, dehydration, and decarboxylic reaction to form compounds 1 and 2, and the retro-aldol reaction of the formation of intermediate A was the key step to convert the configurations of H-8 and H-9 in compound 1.

Compounds 1 and 3 were evaluated for their cytotoxicity against several human tumor cell lines, including HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines by the MTT method reported (Table 2).¹⁶ Compound 3 showed significant cytotoxicity against four of the above-mentioned tumor cell lines with $\text{IC}_{50} < 4.3 \mu\text{M}$, and compound 1 showed no cytotoxicity against all the assayed cell lines.

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Supporting Information Available. Detailed experimental procedures, method of cytotoxicity test, physicochemical properties, 1D and 2D NMR, MS, UV, IR spectra of compounds 1–3, and X-ray crystal data of 1 and 3. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.