

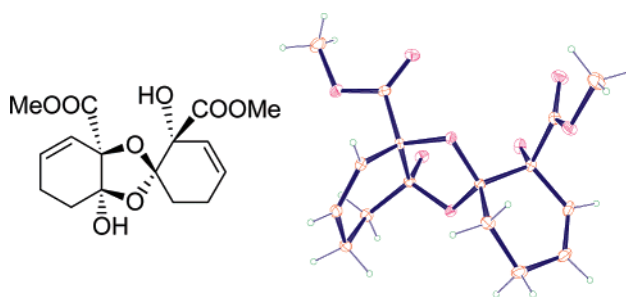
Idesolide: A New Spiro Compound from *Idesia polycarpa*

Seung Hyun Kim,[†] Sang Hyun Sung,[‡] Soo Young Choi,[§] Young Keun Chung,[§]
Jinwoong Kim,[†] and Young Choong Kim^{*†}

College of Pharmacy, Seoul National University, San 56-1, Shillim-Dong, Kwanak-Gu, Seoul 151-742, Korea, Institute for Life Science, Elcom Science Co. Ltd., 235 Guro-Dong, Guro-Gu, Seoul 152-848, Korea, and Department of Chemistry, Seoul National University, San 56-1, Shillim-Dong, Kwanak-Gu, Seoul 151-742, Korea
youngkim@snu.ac.kr

Received May 12, 2005

ABSTRACT



Idesolide, a new spiro compound possessing tetrahydrobenzodioxole structure, was isolated from the fruits of *Idesia polycarpa* Maxim. Its structure was established by NMR and MS spectroscopic analysis with single-crystal X-ray experiments. Idesolide significantly reduced nitric oxide production induced by lipopolysaccharide in BV2 microglial cells.

Idesia polycarpa Maxim. is a deciduous tree of the Flacourtiaceae family.¹ This tree is native to some Asian countries such as Korea, China, Japan, and Taiwan. In Korea, the seeds of this tree have been used as an insecticide, and the leaves have been known to have hemostatic activity.² Several phenolic glycosides including idesin, idescarpin, and salirepin and phenazine derivatives have been reported to be isolated from this plant.^{3,4}

In our search for antiinflammatory substances from natural products, we found that the total methanol extract of the fruits

of *I. polycarpa* effectively inhibited nitric oxide (NO) production induced by lipopolysaccharide (LPS) in BV2 microglial cells. The fruits of *I. polycarpa*⁵ were collected in Beagwoon Mountain, Gwangyang city, Jeollanam-do, Korea, in September 2003 and authenticated by Dr. Jong Hee Park, Professor at Pusan National University.

The fresh fruits (9 kg) were ground and extracted with MeOH (3 × 10 L) at room temperature. The MeOH extract was concentrated in vacuo to give a crude extract (1.2 kg). The methanolic extract was then suspended in H₂O and partitioned successively with *n*-hexane, CHCl₃, ethyl acetate, and *n*-BuOH. The CHCl₃ fraction (32 g) which showed the most potent antiinflammatory activity was subjected to ODS gel column chromatography with a gradient elution of H₂O–MeOH. After several steps of purification using chromatography, a new spiro compound, which has been

* To whom correspondence should be addressed. Phone: (+82) 2-8807842. Fax: (+82) 2-8882933.

[†] College of Pharmacy, Seoul National University.

[‡] Institute for Life Science, Elcom Science Co. Ltd.

[§] Department of Chemistry, Seoul National University.

(1) Chou, C.; Lin, L.; Tsai, W.; Hsu, S.; Ho, Li. *J. Nat. Prod.* **1997**, *60*, 375–377.

(2) Kim, T. W. *The Woody Plants of Korea*; Kyohak Press: Seoul, 1996; p 126.

(3) Zhou, Y.; Zhou, Z.; Cao, P.; Tan, X.; Ding, L. *Tianran Chanwu Yanjiu Yu Kaiifa* **2003**, *15*, 13–14, 17.

(4) Moritake, M.; Ueda, K.; Mori, I. *Tetrahedron Lett.* **1987**, *28*, 1425–1426.

(5) A voucher specimen (CS76) has been deposited at the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University, Koyang, Korea.

named idesolide, was yielded as colorless crystals: mp 141.0–143.0 °C; $[\alpha]_D^{25} = -230.0$ (c 1.0 CHCl_3).

Its HRFABMS exhibited a molecular ion peak at m/z 341.1246 $[\text{M} + \text{H}]^+$ (calcd 341.1236), corresponding to $\text{C}_{16}\text{H}_{20}\text{O}_8$ with 7 degrees of unsaturation. IR absorptions at 3364, 2954, and 1735 cm^{-1} implied the existence of hydroxyl groups, methylenes and carbonyl groups. The ^1H NMR spectrum exhibited the presence of two methoxyl groups and four olefinic protons. The ^{13}C NMR spectrum revealed 16 carbon signals due to two ester carbonyl groups, four methines, two dioxygenated quaternary carbons, two monooxygenated quaternary carbons, two methoxyl carbons and four methylenes, which could be possibly divided into two subunits composed of eight carbons according to their chemical shifts. Each of the eight carbons in one subunit has its own counterpart with a similar chemical shift in the other subunit.

The ^1H and ^{13}C NMR spectra of each subunit showed a close resemblance with those of 1-hydroxy-6-oxocyclohex-2-enoic acid methyl ester isolated from *Homalium ceylanicum*⁶ except for the presence of a dioxygenated carbon (δ_{C} 110.81 or 102.03) instead of the ketone (Table 1). The

Table 1. ^1H and ^{13}C NMR Data and Two-Dimensional NMR Correlations of Idesolide in CDCl_3^a

position	δ_{H} (mult, J, Hz)	δ_{C} (mult)	HMBC (H–C)	H–H COSY
2		110.81		
3a		86.29		
4	5.55 (dm, 9.2)	126.06	6, 3a C=O, 7a	5, 6
5	5.94	130.49	6, 7, 3a	4, 6, 7
6	2.18 (m)	22.30	4, 5, 7, 7a	4, 5, 7
7	2.11 (m)	29.61	5, 6, 7a	5, 6
7a		102.03		
2'		77.32		
3'	5.43 (dt, 10.0, 1.8)	126.40	1', 2' C=O, 5'	4', 5'
4'	5.94 (overlap)	132.31	2', 5', 6'	3', 5', 6'
5'	2.28 (m)	24.25	1', 3', 4', 6'	3', 4', 6'
6'	1.80 (m)	30.92	1', 2', 4', 5'	4', 5'
	2.42 (m)			
3a C=O		168.90		
2' C=O		173.04		
3a-OMe	3.73 (3H, s)	52.58	3a C=O, 3a	
2'-OMe	3.81 (3H, s)	54.09	2' C=O, 2'	

^a The NMR measurements were carried out in a Bruker AMX-400 spectrometer. Solvent signals were used as internal signals.

^1H – ^1H COSY spectrum displayed the correlations between H-3', identified at δ_{H} 5.43 (dt, 10.0, 1.8 Hz) and H-4', H-5', and H-6', respectively. The HMBC spectrum enabled the establishment of the cyclohexene ring by exhibiting the correlations of H-4' with C-2', C-5', and C-6' and H-3' with C-1' and C-5'. The attachment of the ester carbonyl carbon which showed a cross-peak with the singlet methoxyl signal at δ_{H} 3.81 (3H, 2'-OMe) was determined by the correlation with H-3' in the HMBC spectrum. Furthermore, the ^1H – ^1H COSY and HMBC spectra of the other subunit also showed

similar correlations. From these data, it could be deduced that eight carbons of each subunit constitute a trioxxygenated cyclohexenoic acid methyl ester (Figure 1). Taken into

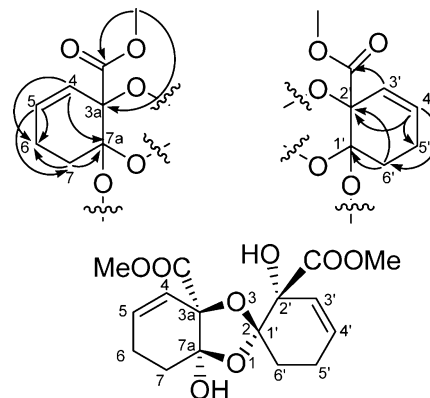


Figure 1. Structures of idesolide and its key HMBC correlations.

account all these spectral data, it seemed reasonable that two oxygen atoms would connect C-3a or C-7a in one subunit to C-1' or C-2' in the other, respectively.

Two possibilities were considered including the one confirmed by X-ray crystallography. The remaining one suggested that the two subunits would be linked by the oxygen atom between C-7a and C-1' making an ethereal bridge with an epoxide constituted by C-3a, C-7a and one oxygen atom. However, it was rejected because of the expected instability due to a free hydroxyl group in C-1'.

Since C-1', C-2', C-3a, and C-7a were fully substituted carbons, the NMR spectra including two-dimensional NMR spectra did not provide sufficient information to confirm our hypothesis. Thus, a single-crystal X-ray diffraction study of idesolide was carried out, from which its uncertain connectivity between the subunits was established.⁷ The drawing

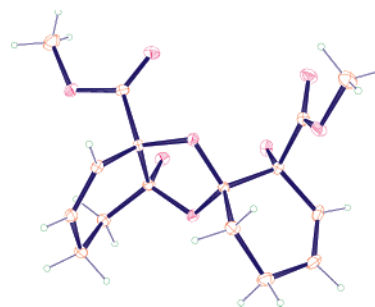


Figure 2. X-ray structure of idesolide showing the relative configuration.

depicted in Figure 2 supports the deduction concerning the structure made by both NMR and mass spectrometry.

The relative stereochemistry of idesolide was also determined by X-ray analysis. There are four chiral centers in

(6) Ekabo, O. A.; Farnsworth, N. R. *J. Nat. Prod.* **1993**, *56*, 699–707.

idesolide, 2, 2', 3a, and 7a. According to the IUPAC sequence rule,⁸ the chiral center with the lowest locant, C-2, has the (*S*)-chirality. The relative stereochemistry of the other three carbons was assigned as 2'*R**, 3a*R**, 7a*S**. Thus, idesolide was identified as dimethyl (2*S**, 2'*R**, 3a*R**, 7a*S**)-2', 7a-dihydroxy-6,7,3a,7a-tetrahydrospiro[1,3-benzodioxole-2,1'-cyclohex-3-ene]-2',3a-dicarboxylate.

To evaluate the antiinflammatory effect of idesolide, the inhibitory activity of this compound against LPS-induced NO production in BV2 microglial cells was tested using the Griess assay as reported previously⁹ (Table 2). The amount of nitrite was increased from 1.72 ± 0.17 to 19.07 ± 1.81

(7) Single-crystal diffraction data were measured by an Enraf-Nonius CCD single-crystal X-ray diffractometer at room temperature using graphite-monochromated Mo K α radiation ($\lambda = 0.71073 \text{ \AA}$). Preliminary orientation matrixes and unit cell parameters were obtained from the peaks of the first 10 frames and then refined using the whole data set. Frames were integrated and corrected for Lorentz and polarization effects using DENZO. The structure was solved by direct methods using SHELXS-97 and refined by full-matrix least-squares with SHELXL-97. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms on the oxygen atoms could not be found in the Fourier map or refined. The rest hydrogen atoms were treated as idealized contributions. Crystal data for **1**: C₁₆H₁₈O₈ (295 K), $M = 338.30$, orthorhombic, space group $P2_12_12_1$, $a = 7.3062(5) \text{ \AA}$, $b = 11.4421(8) \text{ \AA}$, $c = 19.1006(8) \text{ \AA}$, $V = 1596.78(17) \text{ \AA}^3$, $Z = 4$, $\rho_{\text{calc}} = 1.407 \text{ g/cm}^{-3}$, absorption coefficient = 0.114 mm^{-1} , total reflections collected 6064, unique 3223 ($R_{\text{int}} = 0.0721$), GOF = 1.009, $R_1 = 0.0594$, $R_w = 0.1115$ ($I > 2\sigma(I)$).

(8) IUPAC. *Nomenclature of Organic Chemistry*; Pergamon: New York, 1979; Sections A–H. Recommendation for section A, Spiro hydrocarbons.

Table 2. Effect of Idesolide on LPS-Induced NO Production in BV2 Microglial Cells

	concentration (μM)	nitrite ^a (μM)
control		1.7 ± 0.2
LPS		19.1 ± 1.8
idesolide	0.1	17.6 ± 0.7
	1.0	16.4 ± 1.5
	10.0	$4.9 \pm 0.1^*$

^a Each value represented the mean \pm S.D. of independent trials ($n = 4$). * $P < 0.001$ vs LPS-treated cells (one-way ANOVA).

μmol after 24 h of exposure to 100 ng/mL of LPS. NO synthesis was decreased to $4.9 \pm 0.1 \mu\text{mol}$ by treatment with 10 μmol of idesolide without affecting cell viabilities. These results show that idesolide suppressed LPS-induced NO production.

Supporting Information Available: Experimental details on the isolation of idesolide; NMR, MS and IR spectra of idesolide; and X-ray data of idesolide (CIF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL051105F

(9) Woo, M. S.; Jung, S. H.; Hyun, J. W.; Kim, H. S. *Neurosci. Lett.* **2004**, *356*, 187–190.