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Idesolide: A New Spiro Compound from *Idesia polycarpa*

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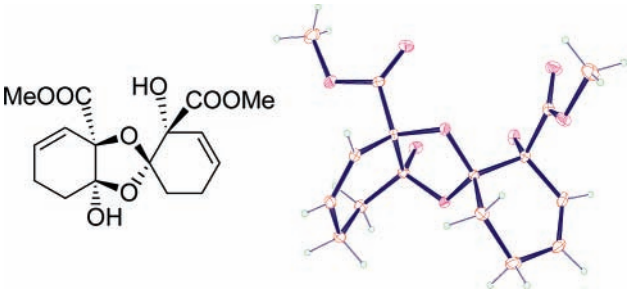
ABSTRACT



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 conturies 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 H_2O and partitioned successively with *n*-hexane, $CHCl_3$, ethyl acetate, and *n*-BuOH. The $CHCl_3$ fraction (32 g) which showed the most potent antiinflammatory activity was subjected to ODS gel column chromatography with a gradient elution of H_2O−MeOH. After several steps of purification using chromatography, a new spiro compound, which has been

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

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

Its HRFABMS exhibited a molecular ion peak at *m*/*z* 341.1246 $[M + H]^+$ (calcd 341.1236), corresponding to $C_{16}H_{20}O_8$ 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 exibiting 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 trioxygenated cyclohexenoic acid methyl ester (Figure 1). Taken into

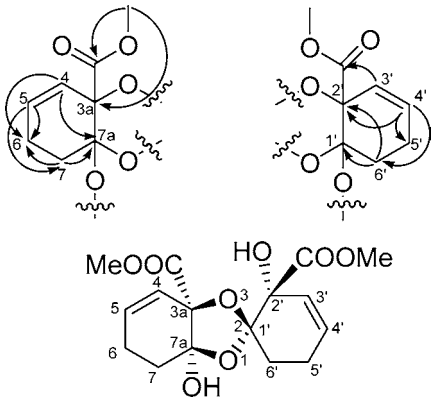

Figure 1. Structures of idesolide and its key HMBC correlations.

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 unstability 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

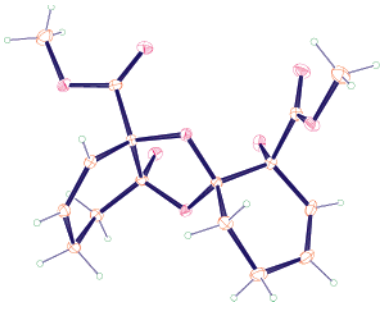

Figure 2. X-ray structure of idesolide showing the relative configuration.

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 previously[9] (Table 2). The amount of nitrite was increased from 1.72 ± 0.17 to 19.07 ± 1.81 μmol after 24 h of exposure to 100 ng/mL of LPS. NO synthesis was decreased to 4.9 ± 0.1 μmol by treatment with 10 μmol of idesolide without affecting cell viabilities. These results show that idesolide suppressed LPS-induced NO production.

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 ± 0.1*

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

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 avaliable free of charge via the Internet at http://pubs.acs.org.

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(7) Single-crystal diffraction data were measured by an Enraf-Nonius CCD single-crystal X-ray diffractometer at room temperature using graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). 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**: $C_{16}H_{18}O_8$ (295 K), M =338.30, orthorhombic, space group $P2_12_12_1$, $a = 7.3062(5)$ Å, $b = 11.4421(8)$ Å, $c = 19.1006(8)$ Å, $V = 1596.78(17)$ Å^3, $Z = 4$, $\rho_{calc} = 1.407$ g/cm^{-3}, absorption coefficient = 0.114 mm^{-1}, total reflections collected 6064, unique 3223 ($R_{int} = 0.0721$), GOF = 1.009, $R_1 = 0.0594$, $R_w = 0.1115$ ($I > 2\sigma(I)$).

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