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Two Novel Resveratrol Trimers, Leachianols A and B, from *Sophora leachiana*

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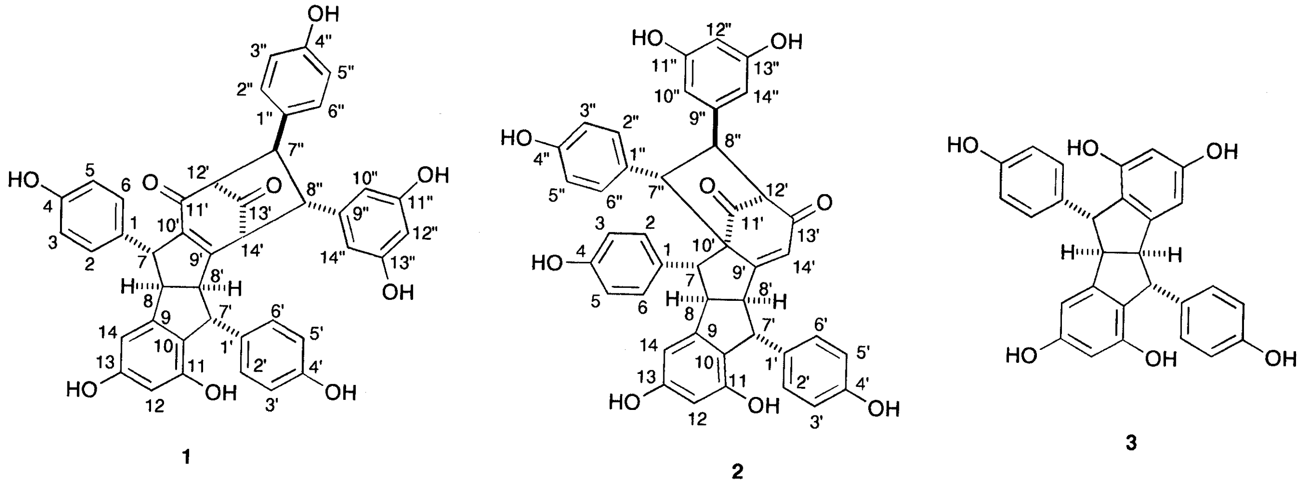
Two novel resveratrol trimers, named leachianols A (1) and B (2), were isolated from the roots of *Sophora leachiana*. Their structures were determined by spectroscopy using correlation spectroscopy involving long range coupling and nuclear Overhauser effect experiments. The resveratrol trimers 1 and 2, which are formed by characteristic oligomerization *via* a pallidol (3), are the first naturally occurring oligostilbenes to be reported.

Keywords *Sophora leachiana*; Leguminosae; stilbene; resveratrol trimer; leachianol A; leachianol B

Our previous studies of the chemical constituents of the roots of *Sophora leachiana* Peckwere (Leguminosae) native to the northwest region of North America have characterized the structures of flavonostilbenes,[1,2)] flavanones,[2-5)] with a lavandullyl (C_{10}) and/or an isoprenyl (C_5) group, *C*-methylated flavonoid glycosides,[6)] and stilbene oligomers,[1)] the occurrence of which suggests that this species is closely related to *S. moorcroftiana*[7,8)] which is found in Nepal and the Xinjiang-Xizang region of China. More precise study of the stilbene oligomers found in the species, in addition to the above-mentioned flavanone derivatives, is required for a fuller understanding of the chemosystematics of the genus *Sophora*. To clarify the chemical relationships between *S. leachiana* and *S. moorcroftiana*, resveratrol oligomers in an ethyl acetate extract of the roots of *S. leachiana* were investigated and this resulted in the isolation of two new trimers, named leachianols A (**1**) and B (**2**), together with a known dimer pallidol (**3**).[9)] In the present paper, the structural elucidation of **1** and **2** using correlation spectroscopy involving long range coupling (COLOC) spectra and nuclear Overhauser effect (NOE) experiments is described.

Leachianol A (**1**), isolated as a pale yellow solid, showed an $[M-H]^-$ ion at m/z 679 [negative ion fast atom bombardment mass spectrometry (FAB-MS)] corresponding to the molecular formula $C_{42}H_{32}O_9$ expected for a tristilbene. The ^{1}H-nuclear magnetic resonance (NMR) spectrum showed the presence of six sets of *ortho*-coupled aromatic hydrogens assignable to three 4-hydroxyphenyl groups [δ_H 6.71 (2H, d, $J=8$ Hz), 7.03 (2H, d, $J=8$ Hz); 6.69 (2H, d, $J=8$ Hz), 6.98 (2H, d, $J=8$ Hz); 6.54 (2H, d, $J=9$ Hz), 6.29 (2H, d, $J=9$ Hz)], a set of *meta*-coupled aromatic hydrogens on a 1,2,3,5-tetrasubstituted benzene [δ_H 6.49 (1H, d, $J=1$ Hz), 6.77 (1H, br s)], AX_2-type aromatic hydrogens due to a 3,5-dihydroxyphenyl group [δ_H 6.24 (2H, br s) and 6.25 (1H, t, $J=1$ Hz)], a sequence of aliphatic methine hydrogens successively coupled in this order [δ_H 3.52 (1H, dd, $J=7$, 1 Hz), 3.70 (1H, dd, $J=7$, 6 Hz) and 3.20 (1H, d, $J=6$ Hz)], a set of mutually coupled aliphatic methine hydrogens [δ_H 3.78 (1H, br d, $J=5$ Hz) and 4.02 (1H, br d, $J=5$ Hz)], and three aliphatic methine hydrogens [δ_H 3.81 (1H, br s), 4.17 (1H, br s) and 4.77 (1H, br s)] in addition to seven hydroxyl hydrogens [δ_H 8.18, 8.25, 8.33, 8.37 (×2), 8.51 and 8.60]. The absorption bands at 1760 and 1660 cm^{-1} in the infrared (IR) spectrum showed the presence of an isolated and an α,β-unsaturated carbonyl group. In the ^{13}C-NMR spectrum, the signals observed at δ_C 191.7 and 203.6 also supported the presence of these functions.

The ^{13}C–^{1}H shift correlation spectroscopy (COSY) enabled the complete assignment of all protons and carbons to be made and these are listed in Table I. In the



1 **2** **3**

Chart 1

TABLE I. ^{1}H- and ^{13}C-NMR Data for Leachianols A (**1**), B (**2**) and Pallidol (**3**)

Carbon	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		134.0		135.1		137.9
2 (6)	7.03 (d, 8)	128.9	6.68 (d, 8)	129.0	6.98 (d, 8)	129.5
3 (5)	6.71 (d, 8)	115.8	6.85 (d, 8)	115.7	6.71 (d, 8)	115.9
4		157.0		156.5		156.5
7	4.17 (br s)	53.1	3.52 (m)$^{a)}$	53.6	4.57 (br s)	54.2
8	3.78 (br d, 5)	58.7	3.78 (d, 7)	59.4	3.82 (br s)	60.6
9		149.7		147.7		150.4
10		122.0		122.2		123.4
11		155.4		155.1		155.4
12	6.49 (d, 1)	103.1	6.38 (d, 2)	102.8	6.19 (d, 2)	102.7
13		160.1		159.9		159.5
14	6.77 (br s)	103.7	5.95 (br s)	105.3	6.62 (d, 2)	103.5
1′		135.9		136.9		137.9
2′ (6′)	6.98 (d, 8)	129.2	7.01 (d, 8)	128.8	6.98 (d, 8)	129.2
3′ (5′)	6.69 (d, 8)	116.1	6.73 (d, 8)	115.9	6.71 (d, 8)	115.9
4′		156.6		156.6$^{a)}$		156.5
7′	4.77 (br s)	48.9	4.92 (br s)	57.4	4.57 (br s)	54.2
8′	4.02 (br d, 5)	63.6	4.06 (br d, 7)	60.0	3.81 (br s)	60.6
9′		168.1		180.8		150.4
10′		142.5		70.5		123.4
11′		191.7		201.0		155.4
12′	3.52 (dd, 7, 1)	72.7	3.52 (m)$^{a)}$	71.5	6.19 (d, 2)	102.7
13′		203.6		195.5		159.5
14′	3.81 (br s)	58.0	6.69 (br s)	126.3	6.62 (d, 2)	103.5
1″		130.1		133.5		
2″ (6″)	6.29 (d, 9)	130.3	7.01 (d, 8)	128.8		
3″ (5″)	6.54 (d, 9)	116.0	6.73 (d, 8)	115.9		
4″		156.8		157.1$^{b)}$		
7″	3.70 (dd, 7, 6)	51.2	3.02 (br d, 4)	56.5		
8″	3.20 (d, 6)	52.3	3.52 (m)$^{a)}$	51.1		
9″		147.1		141.8		
10″ (14″)	6.24 (br s)	106.2	6.11 (d, 2)	107.8		
11″ (13″)		159.7		159.1		
12″	6.25 (t, 1)	102.2	6.16 (t, 2)	102.2		
OHs	8.18, 8.25, 8.33, 8.37 (×2), 8.51, 8.60		8.19, 8.23 (×3), 8.26, 8.44 (×2)		7.81 (×2), 8.08 (×2), 8.10 (×2)	

a) Overlapped signals. *b*) May be interchanged.

^{1}H–^{1}H long range COSY spectrum, the mutually coupled methine hydrogens at δ_H 3.78 (H-8) and 4.02 (H-8′) correlated with the methine hydrogens at δ_H 4.17 (H-7) and 4.77 (H-7′), respectively, and were coupled with the *ortho*-coupled aromatic hydrogens at δ_H 7.03 [H-2(6)] and 6.98 [H-2′(6′)] in the two 4-hydroxyphenyl groups. The broad *meta*-coupled aromatic hydrogen at δ_H 6.77 (H-14) in a 1,2,3,5-tetrasubstituted benzene ring was coupled with the aliphatic methine hydrogen at δ_H 3.78 (H-8) in the ^{1}H–^{1}H long range COSY spectrum, and with the quaternary carbon at δ_C 122.0 (C-10) which was correlated with the benzyl methine hydrogen at δ_H 4.77 (H-7′) in the COLOC spectrum (Fig. 1). Furthermore, the methine hydrogen signals at δ_H 4.17 (H-7) and 4.02 (H-8′) were correlated with the carbon signals at δ_C 142.5 (C-10′) and 168.1 (C-9′) attributable to the α- and β-positions in the α,β-unsaturated carbonyl group.

In the ^{1}H–^{1}H long range COSY spectrum, the methine hydrogens at δ_H 3.70 (H-7″) and 3.20 (H-8″) exhibited respective long range correlations between *ortho*-coupled aromatic hydrogens at δ_H 6.29 [H-2″(6″)] in a 4-hydroxyphenyl group and *meta*-coupled aromatic hydrogens at δ_H 6.24 [H-10″(14″)] in a 3,5-dihydroxyphenyl group. The COLOC spectrum gave two-bonded correlations between the signals at δ_C 72.7 (C-12′)–δ_H 3.70 (H-7″), δ_C 58.0 (C-14′)–δ_H 3.20 (H-8″), and also exhibited three-bonded correlations between the signals at δ_C 191.7 (C-11′)–δ_H 3.70 (H-7″); δ_C 168.1 (C-9′)–δ_H 3.20 (H-8″); δ_C 203.6 (C-13′)–δ_H 3.20 (H-8″); δ_C 142.5 (C-10′)–δ_H 3.81 (H-14′), indicating a planar structure for leachianol A as illustrated in **1**.

The relative stereochemistry of **1** was clarified by means of difference NOE experiments (Fig. 2). NOE interactions were observed between H-8/H-8′, H-8/H-2(6) and H-8′/H-2′(6′), which showed that two 4-hydroxyphenyl groups at C-7 and C-7′ were *cis*-oriented to H-8 and H-8′. In addition, NOEs appeared between H-7″/H-10″(14″) and H-8″/H-2″(6″) suggesting that the aryls at C-7″ and C-8″ were *trans*-oriented. The NOE enhancement between H-8′/H-14′, H-7′/H-8″ and H-7″/H-10″(14″) demonstrated the relative stereostructure of **1** to be *rel*-(7*R*, 8*R*, 7′*R*, 8′*R*, 12′*S*, 14′*S*, 7″*S*, 8″*S*). An unusual chemical shift of *ortho*-coupled hydrogens at δ_H 6.29 [H-2″(6″)] on the 4-hydroxyphenyl group attached to C-7″ was explained by the shielding effect of the carbonyl group at C-11′, which also supported the above configuration.

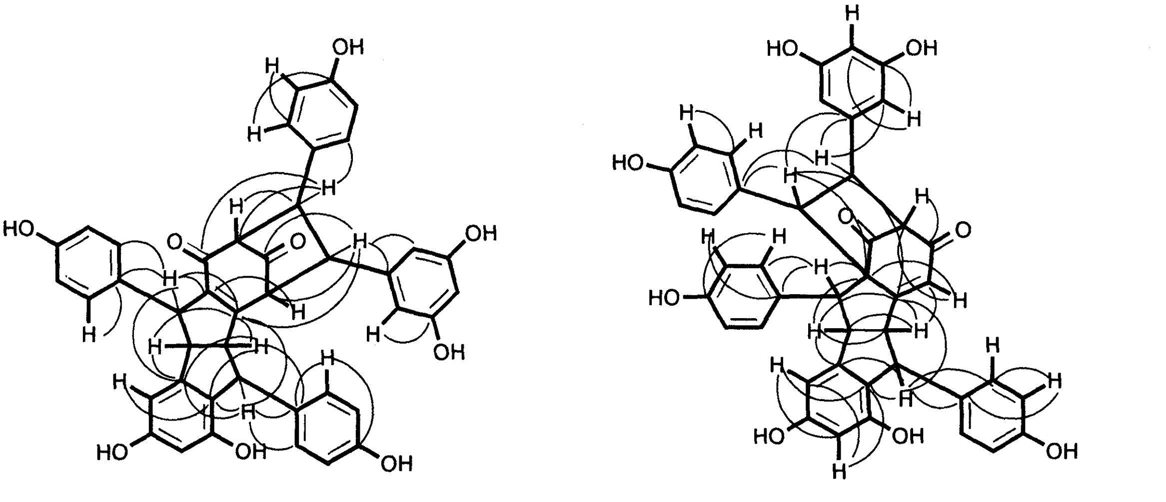


Fig. 1. ^{13}C–^{1}H Long Range Correlations in the COLOC Spectrum of Compounds **1** and **2**

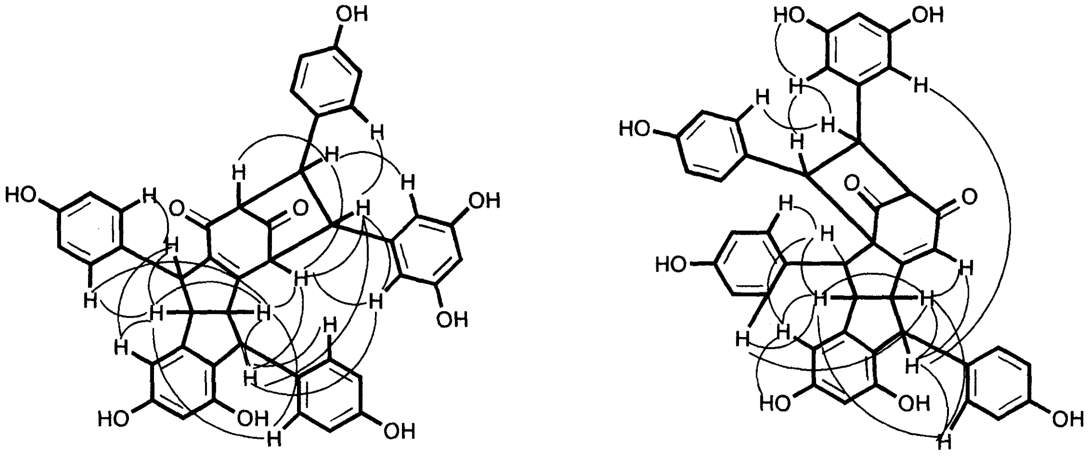


Fig. 2. NOE Interactions in the Difference NOE Experiments Involving **1** and **2**

Leachianol B (**2**), obtained as a pale yellow solid, also showed an $[M-H]^-$ ion at *m/z* 679 in the negative ion FAB-MS which is consistent with the molecular formula $C_{42}H_{32}O_9$ suggesting a resveratrol trimer. The ^{1}H-NMR spectrum of **2**, which was generally similar to that of **1**, exhibited the presence of six sets of *ortho*-coupled aromatic hydrogens assignable to three 4-hydroxyphenyl groups [δ_H 6.68 (2H, d, $J=8$ Hz), 6.85 (2H, $J=8$ Hz); 6.73 (4H, $J=8$ Hz), 7.01 (4H, d, $J=8$ Hz)], a set of *meta*-coupled aromatic hydrogens adjacent to a 1,2,3,5-tetrasubstituted benzene [δ_H 6.38 (1H, d, $J=2$ Hz), 5.95 (1H, br s)], AX_2-type *meta*-coupled aromatic hydrogens attributable to a 3,5-dihydroxyphenyl group [δ_H 6.11 (2H, d, $J=2$ Hz), 6.16 (1H, t, $J=2$ Hz)], a set of mutually coupled aliphatic methine hydrogens [δ_H 3.78 (1H, d, $J=7$ Hz), 4.06 (1H, br d, $J=7$ Hz)], five aliphatic methine hydrogens [δ_H 3.52 (3H, m, overlapped), 4.92 (1H, br s), 3.02 (1H, br d, $J=4$ Hz)] and seven hydroxyl groups [δ_H 8.19, 8.23 ($\times 3$), 8.26, 8.44 ($\times 2$)] as well as an olefinic methine hydrogen [δ_H 6.69 (1H, br s)] which was not observed in **1**. The IR (ν_{max} 1650 and 1760 cm^{-1}) and ^{13}C-NMR (δ_c 195.5 and 210.0) spectra showed the existence an α,β-unsaturated ketone and an isolated ketone. The ^{13}C-NMR spectral data of **2** were closely compatible with those of **1**, except for the carbon signals due to a 2-cyclohexen-1,5-dione ring, which indicated that **2** is an isomer of **1** involving the resveratrol unit was substituted with the pallidol skeleton in a different way from **1**. Detailed analysis of the ^{1}H–^{1}H long range COSY and COLOC spectra revealed long range correlations between the signals at δ_H 4.06 (H-8′)–δ_H 6.69 (H-14′), δ_C 70.5 (C-10′)–δ_H 6.69 (H-14′), δ_C 70.5 (C-10′)–δ_H 3.02 (H-7″) and δ_C 180.8 (C-9′)–δ_H 3.02 (H-7″), demonstrating the planar structure for leachianol B to be **2**.

The relative stereochemistry was established in a similar way to **1**. The NOE enhancements between H-8/H-8′, H-8/H-2(6) and H-8′/H-2′(6′) indicated a *cis*-orientation of the two 4-hydroxyphenyl groups at C-7 and C-7′ to H-8 and H-8′. NOEs between H-8″/H-2″(6″) and H-7″/H-10″(14″) in the difference NOE spectrum suggested a *trans*-orientation of the two aryl groups attached to C-7″ and C-8″. Irradiation of the H-7′ at δ_H 4.92 enhanced the H-10″(14″) *meta*-coupled aromatic hydrogen signal at δ_H 6.11. This enhancement would be possible if the aryl group at C-8″ was in a *cis*-configuration to H-7′. Thus, the relative stereochemistry of **2** was shown to be *rel*-(7*R*, 8*R*, 7′*R*, 8′*R*, 12′*S*, 7″*S*, 8″*R*). The relative structure was further confirmed by the unusual chemical shifts of δ_H 5.95 (H-14) caused by the shielding effect of the spatial proximity of the 4-hydroxyphenyl group at C-7″.

Up to now, two main biogenetic pathways for the formation of oligostilbenes have been proposed,[10] one is for oligomers such as α-viniferin[11] and hopeaphenol[12] which are formed by condensation of a resveratrol(s) with an ε-viniferin as a precursor, and another is for copalliferol A[13] and stemonoporol[13] which are formed by direct oligomerization of resveratrol molecules. The resveratrol oligomers such as α-viniferin and miyabenol C[14] from the roots of *S. moorcroftiana*[8] have been reported to be formed through the former pathway. However, **1** and **2** are the first naturally occurring examples of resveratrol oligomerization *via* pallidol (**3**) as a precursor. Thus, oligomerization plays an important role in clarifying the crucial difference between the highly polar phenolic components in *S. leachiana* and those in *S. moorcroftiana*.

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded in acetone-d_6 solutions on JNM-EX-400 (JEOL) spectrometers. Chemical shifts are shown as δ-values with tetramethylsilane as the internal reference. Peaks multiplicities are quoted in Hz. The COLOC spectra were measured both at $J=5$ and 10 Hz, although only the data measured at 10 Hz are given in the figure. Negative ion FAB-MS was measured on a JMS-DX-300 spectrometer equipped with a JMA 3500 data analysis system (JEOL). UV spectra were recorded on a UV-2200 spectrophotometer (Shimadzu), IR spectra (KBr) on an IR-A-102 (JASCO) instrument, optical rotations on a DIP-370 (JASCO) instrument ($[\alpha]_D$-values are given in units of $10^{-1}\,\mathrm{deg \cdot cm^2 \cdot g^{-1}}$), and CD spectra on a J-20 (JASCO) instrument. Silica gel 60 (70—230 mesh ASTM, Merck) and Sephadex LH-20 (Pharmacia) were used for column chromatography; Silica gel 60H (Merck) was used for vacuum liquid chromatography.

Isolation and Purification of Leachianols A (1) and B (2) The dried and ground roots (580 g) of *Sophora leachiana* PECKWERE, collected at Medford in Oregon, U.S.A. in June 1992, were extracted successively with acetone and methanol. The acetone solution was concentrated under reduced pressure and the residue was poured into water. The suspended solution was partitioned successively with benzene, ethyl acetate and *n*-butanol. After concentration, the ethyl acetate residue (45 g) was separately subjected to silica gel column chromatography eluting with benzene–acetone, and monitoring the eluate using three known flavanone derivatives, sophoraflavanone (SF) G,[15] H[7] and I.[7] Finally, the residue was divided into five fractions, and subfractions 1—3 mainly contained SFG, SFI and SFH, respectively; whereas 4—5 were rich in oligostilbenes following monitoring by thin-layer chromatography. Fraction 4 was further subjected to vacuum liquid chromatography on Silica gel 60H (Merck) and eluted with chloroform–methanol (50 : 1), and finally purified using Sephadex LH-20 (Pharmacia) with acetone–water (4 : 1) as mobile phase to give **1** (184 mg), **2** (554 mg) and **3** (175 mg).

Leachianol A (1) A pale yellow solid. FAB-MS *m*/*z*: 679 $[\mathrm{M-H}]^-$. $[\alpha]_D^{23}$ −159.8° ($c=0.11$, MeOH). CD ($c=7.32\times10^{-5}$, MeOH) Δ_ε^{24}: −11.2 (235), −11.2 (275), −9.2 (295), +1.4 (335). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 214, 224, 278. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3300, 1760, 1660, 1600. ^{1}H- and ^{13}C-NMR spectral data are shown in Table I.

Leachianol B (2) A pale yellow solid. FAB-MS *m*/*z*: 679 $[\mathrm{M-H}]^-$. $[\alpha]_D^{23}$ +147.4° ($c=0.25$, MeOH). CD ($c=7.23\times10^{-5}$, MeOH) Δ_ε^{22}: −15.9 (231), +2.9 (243), −7.8 (265), +3.6 (285), +7.5 (320). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 211, 224, 279. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3300, 1760, 1650, 1610. ^{1}H- and ^{13}C-NMR spectral data are listed in Table I.

Pallidol (3) A brown solid. FAB-MS *m*/*z*: 453 $[\mathrm{M-H}]^-$. $[\alpha]_D^{23}$ −36.3° ($c=0.13$, MeOH). CD ($c=2.67\times10^{-4}$, MeOH) Δ_ε^{23}: −5.5 (240), +3.9 (283), +1.7 (318). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 226, 285, 321sh. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3300, 1600. ^{1}H- and ^{13}C-NMR spectral data are shown in Table I.

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