Arch. Pharm. Res. Vol. 17, No. 4, pp. 284-286, 1994

The NMR Assignments of Torilin from *Torilis japonica*

Sam Sik Kang, Eun Bang Lee, Tae Hee Kim[1], Kyung Ran Kim[1] and Jee Hyung Jung[2]

Natural Products Research Institute, Seoul National University, Seoul 110-460, [1]College of Pharmacy, Sook Myung Women's University, Seoul 140-742 and [2]Korea Ocean Research and Development Institute, Ansan 425-600, Korea

(Received May 30, 1994)

A guaian type sesquiterpene, torilin, was isolated from the hexane extract of the fruits of *Torilis japonica*. The ^{1}H- and ^{13}C-signals of this compound have been fully assigned utilizing ^{1}H-^{1}H COSY, HMQC, and HMBC experiments.

Key words: *Torilis japonica*, Umbelliferae, Sesquiterpene, Torilin, NMR assignment, ^{1}H-^{1}H COSY, HMQC, HMBC

INTRODUCTION

In the course of investigating biologically active natural compounds from the Korean medicinal plants, we have recently isolated a guaian type sesquiterpene, torilin, from the fruits of *Torilis japonica* (Houtt.) DC. (Umbelliferae). Though the structure of this compound has already been elucidated in 1969 (Chimakatsu *et al.*), the NMR asssignment has not been reported so far. The NMR assignment of torillin was made with the aid of 2D correlation NMR techniques including HMQC and HMBC, and we report our results in this paper.

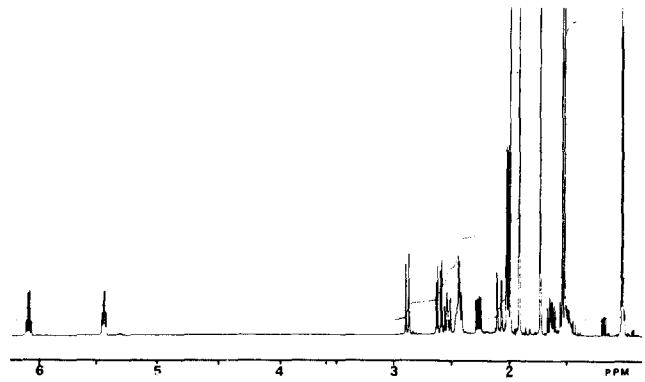


Fig. 1. ^{1}H-NMR spectrum of torilin (**1**) at 500 MHz in $CDCl_3$.

MATERIALS AND METHOD

General Experimental Procedures

Melting point (uncorrected) was obtained with a Mitamura-Riken micromelting point apparatus. UV and IR spectra were taken with a Hitachi U-3210 spectrophotometer and a JASCO FT/IR-5300 spectrometer, respectively. NMR spectra were measured with either a Bruker AM-300 or a Varian UNITY-500 spectrometer, and MS were taken with a Hewlett-Packard 5985B GC/MS system.

Plant Material

The dried fruits of *Torilis japonica* were purchased at herbal drug store in Seoul and were authenticated by Prof. H.J. Chi, Natural Products Research Institute. A voucher specimen is available in Natural Products Research Institute.

Correspondence to: Sam Sik Kang, Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

Isolation of torilin (1)

The dried fruits (6 kg) of *Torilis japonica* were refluxed three times with 70% MeOH for 7 hr. Concentration of the extracts under reduced pressure afforded a residue, which was subsequently partitioned between *n*-hexane and water. The hexane soluble fraction (100 g) was chromatographed on a silica gel column.

Elution with hexane containing increasing amounts of EtOAc yielded five subfractions. Subfraction No. 2 was recrystallized from MeOH to afford **1** as colorless stout needles (25 g).

mp 78-79°, IR ν_{max} (KBr) 1726 (ester), 1696, 1640 (α,β-unsaturated C=O), 1258 (acetate), 1125 cm^{-1}; UV λ_{max}^{MeOH} (log ε) 235 (4.38) nm; EIMS *m/z* (rel. int., %) 376 $[M]^+$ (0.2), 316 $[M\text{-}HOAc]^+$ (7.9), 245 (2.1),

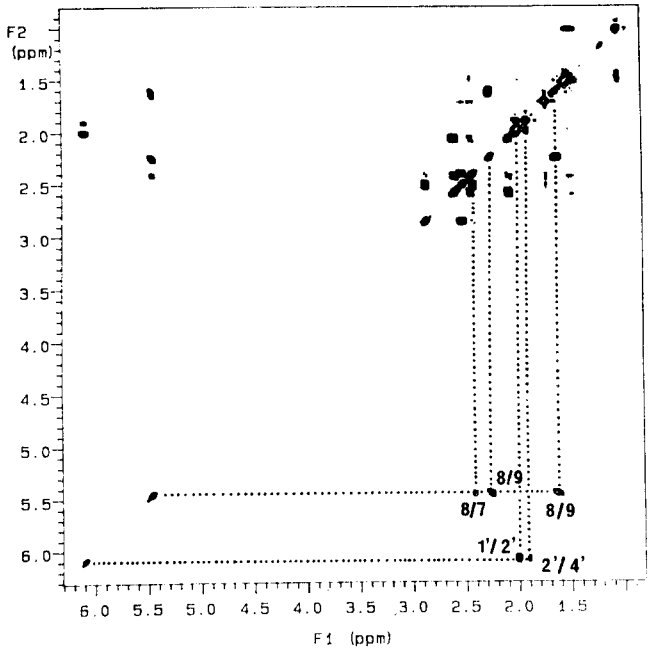


Fig. 2. Contour plot of ^{1}H-^{1}H COSY-45 spectrum for torilin (**1**) in $CDCl_3$.

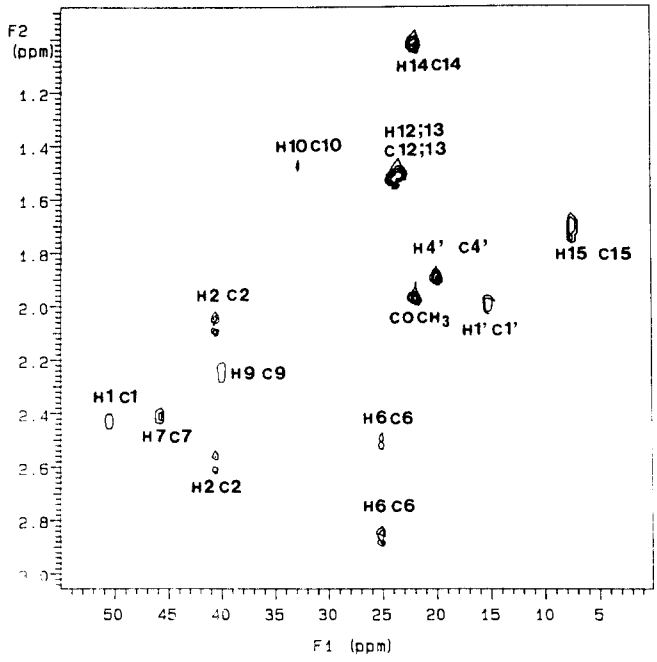


Fig. 4. HMQC spectrum of torilin (**1**).

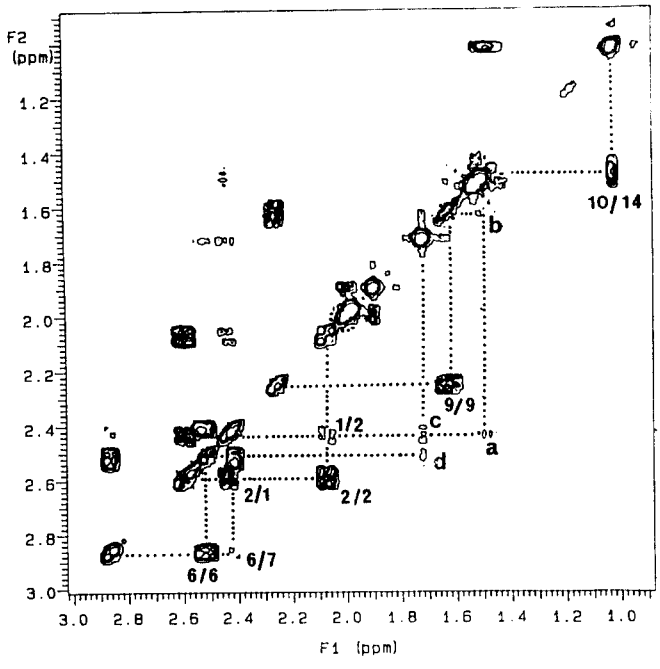
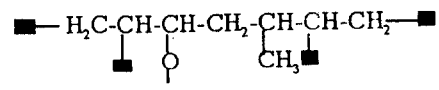


Fig. 3. Contour plot of partial ^{1}H-^{1}H COSY-45 spectrum for torilin (**1**) in $CDCl_3$.

234 [M-(HOAc+$CH_3CH{=}CCH_3C{=}O$)]$^+$ (6.9), 216 [234-H_2O]$^+$ (23.0), 188 [234-(H_2O+CO)]$^+$ (16.4), 173 [234-(H_2O+CO+CH_3)]$^+$ (13.1), 158 (7.0), 83 [$CH_3CH{=}CCH_3C{=}O$]$^+$ (100), 55 (58.3).

RESULTS AND DISCUSSION

Chromatographic separation of *n*-hexane soluble fraction with hexane-EtOAc gave torilin (**1**) as colorless stout needles. The ^{1}H- (Fig. 1) and ^{13}C-NMR spectra of **1** were assigned through 2D NMR experiments. The ^{13}C- and DEPT NMR spectra showed seven methyl carbons, three methylene carbons, five methine carbons, and four quaternary carbons together with one ketonic and two ester carbonyls. The ^{1}H-^{1}H COSY-45 and HMQC (Summers *et al.*, 1986) spectra (Figs. 2, 3 and 4) along with the above data led to the following partial structure,



the α-methyl-α, β-unsaturated carbonyl moiety, the angeloyl and the acetoxyl groups. The presence of the α-methyl-α, β-unsaturated carbonyl moiety in **1**

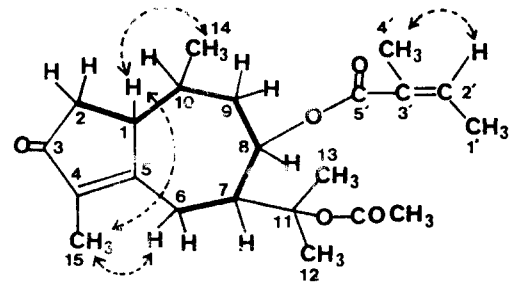


Fig. 5. Structure of Torilin (**1**).
— Connectivities deduced by ^{1}H-^{1}H COSY-45 in $CDCl_3$
--- Long-range ^{1}H-^{1}H correlations observed in ^{1}H-^{1}H COSY-45 in $CDCl_3$

Table I. Chemical shifts and correlations of torilin (**1**) in $CDCl_3$

Position	^1H shift	DEPT	^{13}C shift	HMBC
C-1	2.43 (m)	CH	51.0	C10
C-2	2.60 (dd, 6.4, 18.5)	CH_2	41.2	C5
	2.06 (dd, 3.4, 18.5)			
C-3	–	C	207.9	
C-4	–	C	135.2	
C-5	–	C	173.6	
C-6	2.53 (br. dd, 10.4, 13.8)	CH_2	25.7	C1; C4; C5; C7; C11
	2.87 (d, 13.8)			
C-7	2.42 (dd, 3.7, 10.4)	CH	46.4	C11
C-8	5.46 (br. ddd, 3.7, 7.7, 8.4)	CH	70.7	C6; C7; C5′
C-9	1.63 (ddd, 8.4, 10.4, 14.6)	CH_2	40.6	C1; C7; C8; C14
	2.26 (ddd, 1.3, 7.7, 14.6)			
C-10	1.50 (m)	CH	33.5	
C-11	–	C	84.4	
C-12, 13	1.52 (s), 1.54 (s)	CH_3	23.9; 24.5	C7; C11
C-14	1.03 (d, 6.7)	CH_3	22.6	C1; C9; C10
C-15	1.73 (d, 2.0)	CH_3	8.0	
C-1′	2.01 (dq, 1.3, 7.2)	CH_3	15.7	
C-2′	6.09 (qq, 1.3, 7.2)	CH	138.1	C1′; C4′
C-3′	–	C	127.8	
C-4′	1.91 (q, 1.3)	CH_3	20.5	C2′; C3′; C5′
C-5′	–	C	166.8	
$COCH_3$	1.99 (s)	CH_3	22.5	$\underline{C}OCH_3$
		C	170.2	

could be inferred from the presence of a strong absorption at 235 nm in UV spectrum (Scott, 1964). Weak cross peaks belonging to correlations between the protons at C-10 and C-1 (peak a) and also between protons at C-10 and C-9 (peak b) along with two homoallylic cross peaks belonging to correlations between protons at C-1 and methyl protons (C-15) of α-methyl-α, β-unsaturated carbonyl moiety (peak c), and also between one proton at C-6 and the same C-15 methyl protons (peak d) clearly showed that the structure of **1** could be determined to be a derivative of 8,11-dihydroxy-4-guaien-3-one. After assigning the carbon chemical shifts for protonated carbons with the aid of an HMQC experiment, from an HMBC (Bax and Summers, 1986) experiment we obtained long-range hydrogen to carbon connectivies to determine the carbon skeleton of **1**. Long-range coupling from the H-8 to an angeloyl carbonyl carbon was observed. Further long-range couplings from the H-6 methylene protons to carbons C-5, C-4 and C-1 established the α-methyl-α, β-unsaturated carbonyl moiety. All observed HMBC connectivities are given in Table I. Although sesquiterpenes are one of the major groups in natural products (Connolly and Hill, 1991), a limited number of NMR spectral data for the guaiane type sesquiterpenes have so far been published(Atta-ur-Rahman and Ahmad, 1992).

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