

Thymol Derivatives from *Carpesium divaricatum*

Ok Pyo Zee¹, Dae Keun Kim² and Kang Ro Lee¹

Natural Products Laboratory, College of Pharmacy, Sung Kyun Kwan University, Suwon 440-746, Korea and Department of Natural Products Chemistry, College of Pharmacy, Woosuk University, Samrye 565-800, Korea

(Received June 30, 1998)

Four thymol derivatives, 2,5-dimethoxythymol (**1**), 2-methoxythymol isobutyrate (**2**), 10-isobutyloxy-8,9-epoxythymolisobutyrate (**3**) and 10-(2-methylbutyloxy)-8,9-epoxythymolisobutyrate (**4**) were isolated from the aerial parts of *Carpesium divaricatum*. The structures were elucidated by high field 1D and 2D NMR techniques.

Key words : *Carpesium divaricatum*, 2,5-Dimethoxythymol, 2-Methoxythymol isobutyrate, 10-Isobutyloxy-8,9-epoxythymolisobutyrate, 10-(2-Methylbutyloxy)-8,9-epoxythymolisobutyrate

INTRODUCTION

Carpesium divaricatum S. et Z. (Compositae) has been used as a folk medicine in Korea for its antipyretic, analgesic and anti-inflammatory properties (Yook, 1981). As a perennial herb, this plant grows in mountainous areas of Korea and is widely distributed (Lee 1989). In previous phytochemical investigations of the aerial parts of this plant, we reported the isolation of four new cytotoxic sesquiterpene lactones (Kim *et al.*, 1997), two new and two known sesquiterpene lactones (Kim *et al.*, 1997) from the methanol extract of *Carpesium divaricatum*. Further investigation of the same extract of the plant yielded four thymol derivatives. Although thymol derivatives **1-4** were previously isolated from other plant sources (Schulte *et al.*, 1963; Bohlmann *et al.*, 1969, Bohlmann *et al.*, 1981), no high resolution ¹H-NMR and no ¹³C-NMR data were available. Furthermore, compound **4** was previously isolated as mixture with compound **3**. This paper reports their isolation and structural elucidation.

MATERIALS AND METHODS

General experimental procedures

Melting points were measured on a Gallenkamp melting point apparatus (uncorr.). ¹H- and ¹³C-NMR were recorded on a Bruker AMX-500 spectrometer. UV spectra were obtained on a Shimadzu UV-Visible recording spectrophotometer. IR spectra were measured on a Shimadzu IR-435 Infrared spectrophotometer. Mass

spectra were recorded on a Hewlett-Packard 5985B GC/MS system and high resolution mass spectrum was obtained on a VG70-VSEQ mass spectrometer (VG Analytical, UK).

Plant materials

Carpesium divaricatum was collected in August 1994 from Samyeong Mt., Kangwondo, Korea. A voucher specimen was deposited at the herbarium of College of Pharmacy, Sung Kyun Kwan University

Extraction and isolation

The air-dried plant material (3.5 kg) was finely ground and extracted with MeOH for 2 weeks at room temperature. The resulting MeOH extract (110 g) was suspended in H₂O (1 L) and successively partitioned with CH₂Cl₂ and BuOH (each 2×1 L). The concentrated extract (30 g) of CH₂Cl₂ soluble fraction was applied over silica gel (800 g) eluting sequentially with n-hexane-EtOAc (5:1, 800 ml), n-hexane-EtOAc (3:1, 650 ml), n-hexane-EtOAc (1:1, 600 ml), and EtOAc (500 ml). The eluates were fractionated by TLC to yield fractions designated as MC1-MC5: void volume (450 ml), MC1 (520 ml), MC2 (350 ml), MC3 (410 ml), MC4 (380 ml), and MC5 (420 ml). The second subfraction MC2 (2.5 g) was chromatographed on silica gel (200 g) eluted with n-hexane-EtOAc (15:1) to give five subfractions (a-e). Subfraction b (30 mg) and e (100 mg) were rechromatographed on Lobar[®]-A column (n-hexane-EtOAc, 30:1) to yield **1** (6 mg), **3** (50 mg) and **4** (10 mg), respectively. Subfraction c (40 mg) afforded 10 mg of **2** by Lobar[®]-A column (n-hexane-acetone, 30:1).

2,5-Dimethoxythymol (1): colorless oil; EI-MS m/z

Correspondence to: Kang Ro Lee, Natural Products Laboratory, College of Pharmacy, Sung Kyun Kwan University, Chunchung-dong 300, Jangan-ku, Suwon, 440-746, Korea

Table I. ^1H - (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of compounds **1** and **2** (in CDCl_3)

position	1		2	
	δ ^1H	δ ^{13}C	δ ^1H	δ ^{13}C
1		124.2		125.0
2		151.8		155.6
3	6.73 (s)	109.2		107.7
4		135.1	6.74(s)	137.9
5		150.5		140.9
6	6.69 (s)	114.2		124.0
7	2.21 (s)	16.0	6.72(s)	15.8
8	3.30 (qq, 7.0)	26.8	2.17(s)	27.4
9	1.21 (d, 7.0)	22.9	2.97(qq, 7.0)	23.0
10	1.21 (d, 7.0)	22.9	1.20(d, 7.0)	23.0
2-OCH ₃	3.81 (s)	56.3	1.20(d, 7.0)	55.6
5-OCH ₃	3.79 (s)	56.2	3.83(s)	
1'				176.1
2'			2.82(qq)	34.2
3'			1.34(d, 7.0)	19.0
4'			1.34(d, 7.0)	19.0

(%): 194 (M^+ , 57), 179 (100), 164 (16), 149 (15); ^1H - and ^{13}C -NMR data: Table I.

2-Methoxythymolisobutyrate (2): colorless plate crystal, m.p. 57~59°C; UV: $\lambda_{\text{max}}^{\text{MeOH}}=219, 284$; IR: $\nu_{\text{max}}^{\text{Nujol}}=1759$ (C=O), 1377; EI-MS: m/z (%)=250 (M^+ , 6), 180 (100), 165 (51), 149 (11), 91 (21), 77 (18); HR-EI-MS: found m/z 250.1566, $\text{C}_{15}\text{H}_{22}\text{O}_3$, calcd.: 250.1569; ^1H - and ^{13}C -NMR data: Table I.

10-Isobutyloxy-8,9-epoxythymolisobutyrate (3): colorless oil; UV: $\lambda_{\text{max}}^{\text{MeOH}}=210, 263$; IR: $\nu_{\text{max}}^{\text{Nujol}}=1758$ (C=O), 1740 (C=O), 1468 (C-O); ^1H - and ^{13}C -NMR data are presented in Table II.

10-(2-Methylbutyloxy)-8,9-epoxythymolisobutyrate (4): colorless oil; UV: $\lambda_{\text{max}}^{\text{MeOH}}=219$; IR: $\nu_{\text{max}}^{\text{Nujol}}=1750$ (C=O), 1738 (C=O), 1460 (C-O); ^1H - and ^{13}C -NMR data are presented in Table II.

RESULTS AND DISCUSSION

The menthol derivative **1** was obtained as a colorless oil. From the ^1H -NMR (18H), ^{13}C -NMR (12C) and EI-MS (M^+ , 194) spectra, the molecular formula of **1** was deduced to be $\text{C}_{12}\text{H}_{18}\text{O}_2$. The formula showed four unsaturated-bond equivalents. The ^1H -NMR spectrum showed two olefinic protons at δ 6.73 (1H, s) and 6.69 (1H, s). The ^{13}C -NMR spectrum showed six olefinic carbons at δ 151.8, 150.5, 135.1, 124.2, 114.2 and 109.2, thus accounting for three double-bond equivalents. Because no other double bond was indicated by the spectral data, one remaining unsaturation was accounted for a ring, suggestive of an aromatic compound. Compound **1** showed a singlet aromatic methyl at δ 2.21, two methoxyl at δ 3.81 and 3.79, and two olefinic protons at δ 6.73 and 6.69 in the ^1H -NMR spectrum. The signals for the ^1H -NMR spectrum were

Table II. ^1H - (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of compounds **3** and **4** (in CDCl_3)

position	3		4	
	δ ^1H	δ ^{13}C	δ ^1H	δ ^{13}C
1		139.8		139.9
2	6.86(s)	122.9	6.87(s)	122.9
3		148.6		148.6
4		126.0		126.0
5	7.35(d, 7.8)	128.9	7.35(d, 8.0)	128.9
6	7.04(d, 7.8)	126.6	7.04(d, 8.0)	126.7
7	2.34(s)	21.0	2.34(s)	21.1
8		56.8		56.9
9	2.84(d, 5.3)	50.6	2.78(d, 5.3)	50.7
10	3.03(d, 5.3)	64.8	3.02(d, 5.3)	64.8
	4.59(d, 12.2)		4.57(d, 12.3)	
	4.19(d, 12.2)		4.19(d, 12.3)	
1'		176.3		176.4
2'	2.84(qq, 7.0)	34.1	2.85(qq, 7.0)	34.2
3'	1.32(d, 7.0)	18.8	1.33(d, 7.0)	18.9
4'	1.32(d, 7.0)	18.8	1.33(d, 7.0)	18.8
1''		175.2		175.3
2''	2.52(qq, 7.0)	33.8	2.52(m)	40.8
3''	1.11(d, 7.0)	18.9	1.57(m)	26.6
			1.43(m)	
4''	1.09(d, 7.0)	18.8	0.81(t, 7.4)	16.4
5''			1.11(d, 7.4)	11.4

very similar to 2-hydroxy-acetylthymol (Mohamed *et al.*, 1986) except for the presence of two methoxyl groups instead of acetyl group. The position of two methoxyl groups was confirmed by HMBC experiment; ^1H - ^{13}C long-range correlation between δ 3.81 proton signal and δ 151.8 carbon signal, and the correlation between δ 3.79 proton signal and δ 150.5 carbon signal were observed. Consequently, **1** was characterized as 2,5-dimethoxythymol. This compound was previously isolated from *Arnica montana* (Schulte *et al.*, 1963).

The thymol derivative **2** was obtained as colorless

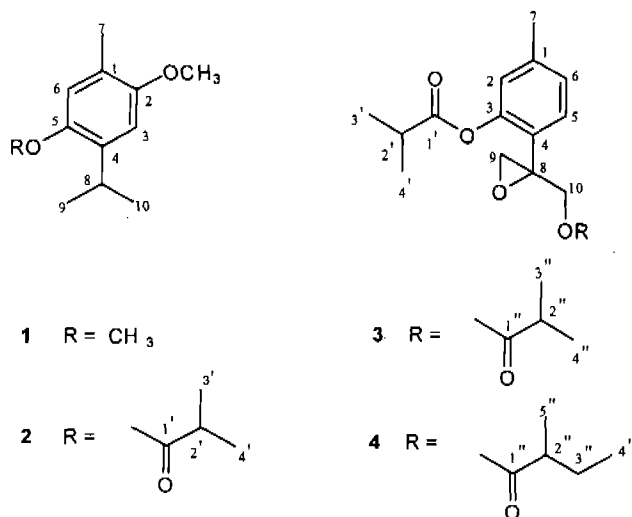
**Fig. 1.** Structures of compounds **1-4**.

plate crystals. From the $^1\text{H-NMR}$ (22H), $^{13}\text{C-NMR}$ (15C) and EI-MS (M^+ , 250) spectra, the molecular formula of **2** was deduced to be $\text{C}_{15}\text{H}_{22}\text{O}_3$. This was reassured by high resolution mass spectrometry. The formula showed five unsaturated-bond equivalents in the molecule. The $^{13}\text{C-NMR}$ spectrum showed one carbonyl group (δ 176.1) and six olefinic carbons (δ 155.6, 140.9, 137.9, 125.0, 124.0, 107.7), thus accounting for the four double-bond equivalents. A remaining unsaturation was accounted for one ring suggestive of an aromatic compound. According to the $^1\text{H-NMR}$ and COSY spectra of compound **2**, the signals at δ 1.34 (6H, d, $J=7.0$ Hz) and δ 2.82 (1H, qq) were attributable to the isopropyl group attached to the carbonyl carbon. The signals at δ 1.20 (6H, d, $J=7.0$ Hz) and δ 2.97 (1H, qq, $J=7.0$ Hz) were assigned to the isopropyl group attached to aromatic ring. The olefinic protons at δ 6.74 (1H, s) and δ 6.72 (1H, s) were not correlated each other. The signals of compound **2** for the $^1\text{H-NMR}$ spectrum were very similar to compound **1** except for the presence of isobutanoyl group (δ 1.34, d and δ 2.82, qq) instead of one methoxyl group (δ 3.79, s) of compound **1** (Mohamed *et al.*, 1986). These observations led to the structure **2**. The positions of the methoxyl and isobutanoyl groups were confirmed by HMBC and $^1\text{H-NMR}$ data; $^1\text{H-}^{13}\text{C}$ long-range correlation between δ 3.83 proton signal and δ 155.6 carbon signal in the HMBC spectrum and singlet peaks at δ 6.72 (H-6) and 6.74 (H-3) in the $^1\text{H-NMR}$ spectrum. Therefore, compound **2** was elucidated as 2-methoxy-thymolisobutyrate. The compound **2** was also reported by Bohlmann *et al.*, (1981).

The compound **3** was obtained as colorless oil. The $^{13}\text{C-NMR}$ spectrum showed two carbonyl groups (δ 175.2, 176.3), six olefinic carbons (δ 139.8, 122.9, 148.6, 126.0, 128.9, 126.6), three carbinol carbons (δ 56.8, 50.6, 64.8) and seven aliphatic carbons. According to the $^1\text{H-NMR}$ and COSY spectra of compound **3**, the signals at δ 1.32 (6H, d, $J=7.0$ Hz) and δ 2.84 (1H, qq) and the signals at δ 1.09 (3H, d, $J=7.0$ Hz), δ 1.11 (3H, d, $J=7.0$ Hz) and δ 2.52 (1H, qq) were attributable to two isopropyl groups. The signals at δ 2.84 (1H, d, $J=5.3$ Hz) and δ 3.03 (1H, d, $J=5.3$ Hz) in the $^1\text{H-NMR}$ spectrum and the signals at δ 50.6, 56.8 in the DEPT spectrum indicated an oxirane ring. Two olefinic protons at δ 7.35 (1H, d, $J=7.8$ Hz) and 7.04 (1H, d, $J=7.8$ Hz) in the $^1\text{H-NMR}$ spectrum showed AB system. This can be assigned to the aromatic ortho positioned protons. These observations and comparison of their UV, IR, NMR spectral data and physical properties with those reported in the

literature (Bohlmann *et al.*, 1969) yielded the structure of compound **3** to be 10-isobutyloxy-8, 9-epoxythymolisobutyrate (**3**).

The spectral data (UV, IR, NMR) and physical properties of compound **4** were very similar to those of compound **3**, apart from the additional methylene signals at δ 1.43 (1H) and 1.57 (1H) in compound **4**. The above evidence and comparison of its spectral data with those reported in the literature (Bohlmann *et al.*, 1969) led to the structure **4**, 10-(2-methylbutyloxy)-8,9-epoxythymol isobutyrate (**4**).

Though thymol derivatives **1-4** were previously isolated from other plants sources (Schulte *et al.*, 1963; Bohlmann *et al.*, 1969; Bohlmann *et al.*, 1981), the authors reported only the $^1\text{H-NMR}$ data. We, therefore, reanalysed the spectra of these compounds and assigned by means of 2D-NMR ($^1\text{H-}^1\text{H}$ COSY, $^{13}\text{C-}^1\text{H}$ COSY and HMBC) data (Table I, II).

ACKNOWLEDGEMENT

This work was supported by the research grant from Korea Science & Engineering Foundation (KOSEF: 93-0400-07).

REFERENCES CITED

- Bohlmann, F., Niedballa, U. and Schulz, J., Ueber einige Thymol derivate aus *Gaillardia- und Helenium-Arten*. *Chem. Ber.* 102, 864-871 (1969).
- Bohlmann, F., Zdero, C., Jakupovic, J., Robinson, H. and King, R. M., Eriolanolides, eudesmanolides and a rearranged sesquiterpene from *Eriophyllum species*. *Phytochemistry*, 20, 2239-2244 (1981).
- Kim, D. K., Lee, K. R. and Zee, O. P., Sesquiterpene lactones from *Carpesium divaricatum*. *Phytochemistry* 46, 1245-1247 (1997).
- Kim, D. K., Baek, N. I., Choi, S. H., Lee, K. R. and Zee, O. P., Four new cytotoxic germacranolides from *Carpesium divaricatum*. *J. Nat. Prod.* 60, 1199-1202 (1997).
- Lee, T. B., *Illustrated Flora of Korea*. Hayngmoonsa, Seoul, p.731, 1989.
- Mohamed, M., Abdel-Aziz, D. and Saud, M., A new thymol derivative from *Pulicaria undulata*. *Chem. Pharm. Bull.*, 34, 378-379 (1986).
- Schulte, K. E., Reisch, J. and Ruecker, G., Einige neue Inhaltstoffe der Wurzel von *Arnica montana*. *Arch. Pharm.* 296, 273-281 (1963).
- Yook, C. S., *Medicinal Plants of Korea*. Jin Myeong Publishing Co., Seoul, p. 392, 1981.