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Isolation and Identification of Phase 1 Metabolites of Demethoxycurcumin in Rats

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Abstracts: Curcuminoids are a safe natural food coloring additive with anti-inflammatory, antioxidant and anticarcinogenic activities. Although demethoxycurcumin is one of the major bioactive constituents of curcuminoids, knowledge about its metabolic fate is scant. In the present study, four new metabolites: 5-dehydroxy-hexahydro-demethoxycurcumin-A (**M-1**), 5-dehydroxy-hexahydro-demethoxycurcumin-B (**M-2**), 5-dehydroxy-octahydro-demethoxycurcumin-A (**M-3**) and 5-dehydroxy-octahydro-demethoxycurcumin-B (**M-4**) were isolated from feces of male Wistar-derived rats and from urine, three new metabolites: 5-O-methyl-hexahydro-demethoxycurcumin-A (**M-7**), 5-O-methyl-hexahydro-demethoxycurcumin-B (**M-8**), and 5-dehydroxy-dihydro-demethoxycurcumin-B (**M-9**) and two known metabolites: hexahydro-demethoxycurcumin-A (**M-5**), hexahydro-demethoxycurcumin-B (**M-6**) were isolated. Their structures were established by chemical and spectral methods. All of them were reductive metabolites. Possibly of greater importance is that they occurred as pairs of isomers with a methoxyl group substituted on a different benzene ring. This finding in the metabolism of curcuminoids is reported here for the first. In addition, the 5-dehydroxy or 5-O-methylated metabolites are also novel finding. The fact that the metabolites occurred as pairs of the isomers suggests that demethoxycurcumin possibly undergoes tautomerization between 3-keto-5-enol (form A) and 3-keto-5-enol (form B) (Fig. 1) in rats. On the basis of the metabolite profiles, metabolic pathways of demethoxycurcumin in rats are proposed.

Introduction

Curcuminoids are natural yellow pigments and food-coloring agents present in the rhizomes of the Asian tropical plant *Curcuma longa* which has been used as a traditional medicinal herb for thousands of years. The dried rhizome of *C. longa* has been widely used as an aromatic stomachic, carminative, anthelmintic, laxative, and as condiments in foods as well as for liver ailment (Nurfina et al., 1997). Curcuminoids are responsible for its biological actions. Curcuminoids, consist mainly of three diarylheptanoids: curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Govindaajan et al., 1980) (Fig. 2). These are recognized for their beneficial effects such as a choloretic (Ramprasad et al., 1956; Hermann et al., 1991), as anti-oxidants (Unnikrishnan et al., 1995; Sharma, 1976), anti-inflammatory agents (Arora et al., 1971; Ghatak et al. 1972), for treating human immunodeficiency virus infections (Mazumber et al., 1995; Eigner et al., 1999) and as anticarcinogens (Araujo et al., 2001; Duvoix et al. 2005; Kuttan et al., 1985; Conney et al. 1991). In recent years, their ability to protect neuronal cells from β A insult (Park et al., 2002; Kim et al., 2001) has also attracted great attention. Demethoxycurcumin was found to be the more effective in protecting PC12 and HUVEC cells from β A insult than curcumin. Although numerous aspects of the pharmacology of curcuminoids, in particular its activity as chemopreventive agent, have been studied, their metabolism in humans and experimental animals has not been fully characterized. The metabolism of curcumin has been studied mostly in rats in vivo and in vitro (Holder et al., 1978; Ravindranath et al., 1982; Asai et al., 1999; Pan et al., 2000; Ireson et al., 2001; Ireson et al., 2002). More recently, information on the metabolism of curcumin in humans has been obtained from in vitro studies with hepatic and intestinal cells and subcellular fractions (Ireson et al., 2001; Ireson et al., 2002), as well as from clinical studies in cancer patients (Cheng et al., 2001; Sharma et al., 2001; Garcea et al., 2004; Sharma et al., 2004).

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The metabolism of demethoxycurcumin, which is the major active component in curcuminoids such as curcumin, has only been studied on one report. In that investigation, in vitro studies with tissue slices and subcellular fractions from rat liver were reported (Hoehle et al., 2006). No data have yet been published on the metabolism of demethoxycurcumin in vivo. Therefore, studies of the metabolic products of demethoxycurcumin in feces and urine after oral administration in male Wistar rats were undertaken. The isolation and identification of nine phase 1 reductive metabolites of demethoxycurcumin are described here.

Materials and Methods

Materials. Dry rhizomes of *C. Longa* were collected from Gui Zhou province, China. A voucher specimen was identified by Prof. *Qi-Shi Sun*(No.CL200209) and deposited at the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, China.

Demethoxycurcumin. Dry rhizomes of *C. Longa*(2.5kg) were pulverized then extracted 3 times for 0.5hr/each time by ultrasound in an eight-fold volume (wt/vol) of 80% EtOH. The EtOH solutions were combined and condensed to yield 362g. Then the extract was chromatographed on a silica gel column using a CHCl₃-MeOH gradient solvent system to yield 17 fractions (Fr.A-Q). Fr.E (25.5g) was further subjected to column chromatography on a silica gel with CHCl₃: MeOH 50:1 to yield 4 fractions (Fr.F1-Fr.F4). Demethoxycurcumin (6.8g) was obtained as a yellow-orange amorphous powder from Fr.F4 after repeated precipitation in MeOH. It had a purity of >98% according to HPLC analysis. The structure of demethoxycurcumin was identified and confirmed by comparing the MS and ¹H and ¹³C NMR spectral data with those previously reported (Kiuchi et al., 1993).

Demethoxycurcumin: a yellow-orange amorphous powder, ESI-MS: m/z 337 [M-H]. ¹H-NMR

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(300MHz, DMSO-*d*₆) and ¹³C-NMR (75MHz, DMSO-*d*₆) (Table 5).

Chemicals The purity of MeOH for HPLC was 99.9% was from Jiangsu Hanbang Chemical Factory (Jiangsu, China), silica gel for column chromatography (200–300 mesh), and silica gel G₆₀ for thin-layer chromatography (300-400mesh), preparative thin-layer chromatography (300-400mesh) and macroporous resin D101 from Qingdao Marine Chemical Factory (Shandong, China), reverse-phase preparatory TLC from Merck Co. Sephadex LH-20 and ODS from Pharmacia Company.gy Co. Ltd. Other chemicals were analytical grade, provided by Shenyang Chemical reagent factory (Shenyang, China).

Animals Male Wistar-derived rats (200-250g) were provided by the Institute of Jingfeng Medical Animal Center (Beijing, China). Subjects were judged to be in good health and housed in conditions of temperature-(22±2°C), humidity-(55±10%), and light-(8:00-20:00) in a controlled breeding room where they were acclimated for 7 days prior to study. Normal food and water were available ad libitum, but withdrawn 24hr prior to intragastric administration of demethoxycurcumin. Demethoxycurcumin was orally administered as 30% aq. 1,2- propylene glycol solution. Urine and feces were collected for 48 hr. from animals housed in stainless steel metabolism cages equipped with a urine and feces separator.

Preliminary Studies For the sample group (four rats), a solution of demethoxycurcumin (50mg/kg) was administered orally by direct stomach intubation in a volume of 10ml/kg body weight. For the control group (four rats), the solvent 30% aq. 1,2-propylene glycol only was orally administered in rats by the same method. Pooled urine and feces of the sample group and those of the control group were simultaneously treated with parallel procedures. Urine was subjected to macroporous resin D101 chromatography and eluted with H₂O, 50% EtOH and 95% EtOH in turn after filtration. Each elution

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was concentrated to nearly 1.0ml in vacuo and detected by T.L.C in CHCl₃: MeOH (15:1) and CHCl₃: MeOH: H₂O (7:3:0.5), and spraying with 10% H₂SO₄. After heating, two metabolite spots [R_F 0.45 (spot 1) and 0.50 (spot 2)] were observed in 50% EtOH and 95% EtOH fraction of the sample group in CHCl₃: MeOH (15:1) and two metabolite spots were observed at R_F 0.05 and 0.40 in 50% EtOH fraction of the sample group in CHCl₃: MeOH: H₂O (7:3:0.5), but not in those of the control group. Feces were extracted with EtOAc (100ml) then MeOH (100ml) for 2hr. for twice. The combined EtOAc and MeOH extracts were concentrated to nearly 1.0ml under vacuum, then detected by T.L.C in CHCl₃: MeOH (15:1) and CHCl₃: MeOH: H₂O (7:3:0.5), and spraying with 10% H₂SO₄. After heating, the same metabolite spot [R_F 0.57 (spot 3)] was observed both in EtOAc and MeOH extracts of the sample group and one metabolite spot [R_F 0.40 (spot 4)] was observed in MeOH extracts of the sample group in CHCl₃: MeOH (15:1), but not in those of the control group. There was not other metabolite spot observed in CHCl₃: MeOH: H₂O (7:3:0.5).

Isolation of Metabolites A solution of demethoxycurcumin (6mg/ml) was orally administered at 50mg/kg body weight to eighty rats and then repeated at one week interval. The total administration of demethoxycurcumin was 3g. The urine (approximately 10,000 ml in total) and feces (approximately 385g) were treated by the same methods used in the preliminary tests. The results in T.L.C were essentially identical with those in the preliminary tests. The EtOAc (31.5g) and MeOH (21.0g) extracts of the feces were chromatographed, respectively, on silica gel columns using a CHCl₃-MeOH gradient solvent system to yield 8 (FE1-8) and 14 fractions (FM1-14). FE1 and FM1 (containing spot 3) were combined and subjected to Sephadex LH-20 column chromatography (CHCl₃: MeOH 1:1) followed by C₁₈-ODS reverse-phase open column chromatography (60% MeOH in water) then purified by PTLC (CHCl₃: MeOH 15:1) to yield spot 3 (the mixture of **M-1** and **M-2**, 40.3mg). FM 3 (containing spot 4)

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was applied to Sephadex LH-20 column chromatography (CHCl₃ : MeOH 1:1) followed by C₁₈-ODS reverse-phase open column chromatography (60% MeOH in water) then purified using Sephadex LH-20 column chromatography eluting with MeOH to afford spot 4 (the mixture of **M-3** and **M-4**, 3.1mg). The 50% EtOH fraction (26g) of the urine was dissolved in MeOH then filtered. The filtrate (containing spot 1 and 2) was subjected to Sephadex LH-20 column chromatography, eluted with MeOH followed by C₁₈-ODS reverse-phase open column chromatography (60% MeOH in water) to yield 7 fractions (U01-07). Further purification of U05 (containing spot 1 and 2) was performed using C₁₈-ODS reverse-phase preparative HPLC (55% MeOH in water, 277nm) to afford 5 fractions (U051-U055). U055 was further subjected to C₈-ODS reverse-phase preparative HPLC (50% MeOH in water, 284nm) to yield **M-9** (1.8mg). U051 (containing spot 1 and 2) was applied to PTLC (CHCl₃ : MeOH 15:1) to yield spot 1 (the mixture of **M-7** and **M-8**, 17.3mg) and spot 2 (the mixture of **M-5** and **M-6**, 67.6mg).

Spectroscopic Methods NMR spectra were measured on Bruker ARX-300 or AV-600 spectrometers, using TMS as an internal standard. Electrospray ion trap mass spectrometry was performed on a Agilent 1100 Series LC/MSD Trap instrument whose mass range is 50 to 5000 (the mass was calibrated). The instrument was operated in the both positive and negative ion modes, using nitrogen for nebulizing and dry gas. The ionization was performed applying the following parameters: dry gas temperature, 300°C; dry gas rate: 5L/min; spray voltage, 4000V, atomization, 15psi. Sample solutions were directly introduced into the ESI source at a flow rate of 3uL/min by a syringe pump.

HPLC Instruments Preparative HPLC was performed using a C-8 column (C-8, 250×20 mm, Inertsil Pak) and a C-18 column(C-18, 250×20 mm, Inertsil Pak) in a Waters 600 liquid chromatograph apparatus equipped with a Waters 490 UV detector (Waters, Milford, MA). Analytical HPLC was

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performed using a C-18 column (C-18, 25×20 mm, Inertsil Pak) in a Waters 600 liquid chromatograph apparatus equipped with a Waters 996 UV detector.

Results

Metabolites M-1 and M-2 were obtained together as a viscous oil. The positive ESI-MS showed two quasi-molecular ion peaks at m/z 329 ($[M+H]^+$) and 351 ($[M+Na]^+$), and the negative ESI-MS gave a quasi-molecular ion peaks at m/z 327 ($[M-H]$). The ^{13}C and 1H -NMR spectra revealed two extremely similar groups of signals, which suggested that there were a pair of isomers with the same molecular formula of $C_{20}H_{24}O_4$. For the two groups of signals, the stronger one corresponded to **M-2** and the weaker one corresponded to **M-1**. The ^{13}C and 1H -NMR and HMQC spectra displayed two sets of 1,3,4-trisubstituted benzene ring signals [δ 6.62 (1H, brd, $J=8.0$ Hz), 6.64 (1H, d, $J=1.5$ Hz), 6.81(1H, d, $J=8.0$ Hz) and δ 6.62 (1H, brd, $J=8.0$ Hz), 6.66 (1H, d, $J=1.5$ Hz), 6.81 (1H, d, $J=8.0$ Hz)], two sets of 1,4-bis-substituted benzene ring signals [δ 6.74 (2H, d, $J=8.4$ Hz), 6.99 (2H, d, $J=8.4$ Hz) and δ 6.74 (2H, d, $J=8.4$ Hz), 6.97(2H, d, $J=8.4$ Hz)], two methoxy groups [δ 3.84 (3H, s, OCH_3) and δ 3.82 (3H, s, OCH_3)], two overlapped carbonyl carbonyl signals (δ 211.2) and twelve methylene groups, which suggested that **M-1** and **M-2** were the reduced metabolites of olefinic C-C double bonds of demethoxycurcumin. In the HMBC spectrum of **M-2** (Fig. 3), correlations from H-2', H-6' (δ 6.99) to C-4' (δ 154.1), C-3' and C-5' (δ 115.3); H-3', H-5' (δ 6.74) to C-1' (δ 132.7) and C-4' (δ 154.1) indicated the presence of 4'-hydroxyphenyl (group A). Correlations from H-2'' (δ 6.64) to C-3'' (δ 146.3), C-4'' (δ 143.5) , C-5'' (δ 114.1) and C-6'' (δ 120.8); H-5'' (δ 6.81) to C-1'' (δ 134.2), C-2'' (δ 111.0), C-3'' (δ 146.3), C-4'' (δ 143.5), H-6'' (δ 6.62) to C-2'' (δ 111.0), OCH_3 (δ 3.84) to C-3'' (δ 146.3) suggested the existence of 3''-methoxy-4''-hydroxyphenyl (group B). Correlations from H-1 (δ 2.80) to C-2 (δ 44.5)

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and C-3 (δ 211.2); H-2 (δ 2.67) to C-3 (δ 211.2); H-4 (δ 2.39) to C-3 (δ 211.2) and C-5 (δ 23.3); H-5 (δ 1.57) to C-3 (δ 211.2), C-4 (δ 42.9) and C-7 (δ 35.3); H-6 (δ 1.51) to C-4 (δ 42.9), C-5 (δ 23.3) and C-7 (δ 35.3); H-7 (δ 2.50) to C-5 (δ 23.3) and C-6 (δ 31.1) confirmed the presence of the moiety of 3-heptanone (group C). In addition, correlations from H-2', H-6' (δ 6.99) to C-1 (δ 28.9) justified the connectivity between the group A and group C at C1'/C1, and correlations from H-2'' (δ 6.64) and H-6'' (δ 6.62) to C-7 (δ 35.3) revealed the junction between the group B and group C at C1''/C7 (Fig. 7). Thus, **M-2** was elucidated as 1-(4'-hydroxyphenyl)-7-(3''-methoxy-4''-hydroxyphenyl)-3-heptanone (namely 5-dehydroxy-hexahydro-demethoxycurcumin-B). By the same methods, the assignment of 3'-methoxy-4'-hydroxyphenyl moiety (group A) in **M-1** was confirmed by HMBC correlations of H-2' (δ 6.66) with C-3' (δ 146.4), C-4' (δ 143.8), C-5' (δ 114.3) and C-6' (δ 120.7); H-5' (δ 6.81) with C-1' (δ 132.9), C-2' (δ 111.1), C-3' (δ 146.4), C-4' (δ 143.8), H-6' (δ 6.62) with C-2' (δ 111.1) and protons of methoxy (δ 3.82) with C-3' (δ 146.4). The assignment of 4'-hydroxyphenyl moiety (group B) in **M-1** was supported by the HMBC correlations of H-2'', H-6'' (δ 6.97) with C-4'' (δ 153.8), C-3'' and C-5'' (δ 115.1) and H-3'', H-5'' (δ 6.74) with C-1'' (δ 134.0) and C-4'' (δ 153.8). The presence of 3-heptanone moiety (group C) in **M-1** was suggested by the HMBC correlations of H-1 (δ 2.80) with C-2 (δ 44.6) and C-3 (δ 211.2); H-2 (δ 2.67) with C-3 (δ 211.2); H-4 (δ 2.39) with C-3 (δ 211.2) and C-5 (δ 23.2); H-5 (δ 1.57) with C-3 (δ 211.2), C-4 (δ 42.9) and C-7 (δ 34.7); H-6 (δ 1.51) with C-4 (δ 42.9), C-5 (δ 23.2) and C-7 (δ 34.7) and H-7 (δ 2.50) with C-5 (δ 23.2) and C-6 (δ 31.1). Furthermore, the connectivity between the group A and group C at C1'/C1 was justified by the HMBC correlations of H-2' (δ 6.66) with C-1 (δ 29.5), and the junction between the group B and group C at C1''/C7 demonstrated by the correlations of H-2'', H-6'' (δ 6.97) with C-7 (δ 34.7). Thus, **M-1** was identified as 1-(3'-methoxy-4'-hydroxyphenyl)-7-(4''-hydroxyphenyl)-3-heptanone (namely 5-dehydroxy-

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hexahydro-demethoxycurcumin-A).

Metabolites M-3 and M-4 were obtained together as a viscous oil. The positive ESI-MS showed a quasi-molecular ion peak at m/z 353 ($[M+Na]^+$) and the negative ESI-MS gave a quasi-molecular ion peaks at m/z 329.0 ($[M-H]^-$). The ^{13}C and 1H -NMR spectra also revealed two extremely similar groups of signals which suggested that there were a pair of isomers with the same molecular formula of $C_{20}H_{26}O_4$. For the two groups of signals, the stronger one corresponded to **M-4** and the weaker one corresponded to **M-3**. The ^{13}C and 1H -NMR data of **M-4** were similar to those of **M-2** except for the upfield shifts of C-3 by 139.9ppm and the appearance of the H-3 [δ 3.62(1H, m)], which showed that **M-4** was the 3-hydroxy reductive product of **M-2**. In addition, the ^{13}C and 1H -NMR data of **M-4** were nearly identical with those previously reported (Li et al., 2004). Thus, **M-4** was established as 1-(4'-hydroxyphenyl)-7-(3''-methoxy-4''-hydroxyphenyl)-3-heptitol (namely 5-dehydroxy-octahydro-demethoxycurcumin-B). The chemical shifts of **M-3** were similar to those of **M-1** except for those around C-3. In the ^{13}C -NMR spectrum, the signal of C-3 was shifted to higher field (δ 71.4) compared with that of **M-1** (δ 211.2). In the 1H -NMR spectrum, the signal of H-3 [δ 3.62(1H, m)] occurred. These results suggested that **M-3** was the 3-hydroxy reductive product of **M-1**. Therefore, **M-3** was established as 1-(3'-methoxy-4'-hydroxyphenyl)-7-(4''-hydroxyphenyl)-3-heptitol (namely 5-dehydroxy-octahydro-demethoxycurcumin-A).

Metabolites M-5 and M-6 were obtained together as a viscous oil. The positive ESI-MS showed a quasi-molecular ion peak at m/z 367 ($[M+Na]^+$) and the negative ESI-MS gave a quasi-molecular ion peak at m/z 343 ($[M-H]^-$). Similarly, the ^{13}C and 1H -NMR spectra also revealed two extremely similar groups of signals which suggested that there were a pair of isomers with the same molecular formula of $C_{20}H_{24}O_5$. For the two groups of signals, the stronger one corresponded to **M-6** and the weaker one

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corresponded to **M-5**. In the ^1H and ^{13}C -NMR spectra, the signal pattern of **M-5** and **M-6** was nearly identical with that of **M-1** and **M-2**, except that the signals of C-5 and H-5 of **M-5** were shifted to lower fields [$\delta_{\text{C}}66.9$ and $\delta_{\text{H}}4.03(1\text{H},\text{m})$] compared with those of **M-1** [$\delta_{\text{C}}23.2$ and $\delta_{\text{H}}1.57(2\text{H},\text{m})$] and the signals of C-5 and H-5 of **M-6** were shifted to lower fields [$\delta_{\text{C}}67.0$ and $\delta_{\text{H}}4.03(1\text{H},\text{m})$] compared with those of **M-2** [$\delta_{\text{C}}23.3$ and $\delta_{\text{H}}1.57(2\text{H},\text{m})$], respectively. These results suggested that **M-1** and **M-2** were the 5-dehydroxylated products of **M-5** and **M-6**. In addition, the MS, ^1H and ^{13}C -NMR data of **M-5** were nearly identical with those previously reported (Kikuzaki et al., 1991) and those of **M-6** were same as those previously reported (Shin et al., 2002). Thus, **M-5** and **M-6** were determined to be hexahydro-demethoxycurcumin-A and hexahydro-demethoxycurcumin-B, respectively.

Metabolites M-7 and **M-8** were obtained together as a viscous oil. The positive ESI-MS showed a quasi-molecular ion peak at m/z 381 ($[\text{M}+\text{Na}]^+$) and the negative ESI-MS gave a quasi-molecular ion peaks at m/z 357 ($[\text{M}-\text{H}]^-$). Similarly, the ^{13}C and ^1H -NMR spectra also revealed two extremely similar groups of signals which suggested that there were a pair of isomers with the same molecular formula of $\text{C}_{21}\text{H}_{26}\text{O}_5$. For the two groups of signals, the stronger one corresponded to **M-8** and the weaker one corresponded to **M-7**. The chemical shifts of **M-7** were nearly the same as those of **M-5** except for the following findings: the proton signal of H-5 ($\delta_{\text{H}}3.71$) was shifted upfield by 0.32ppm, the carbon signal of C-5 ($\delta_{\text{C}}76.7$) was shifted downfield by 9.7ppm, and the signals of methoxy [$\delta_{\text{C}}56.9$ and $\delta_{\text{H}}3.31(3\text{H}, \text{s})$] were present which indicated that **M-7** was the 5-O-methyl ether of **M-5**. Therefore, **M-7** was elucidated as 5-O-methyl-hexahydro-demethoxycurcumin-A. By the same method, **M-8** was determined to be the 5-O-methyl ether of **M-6**, and the MS, ^1H and ^{13}C -NMR data of **M-8** were nearly the same as those previously reported (Li et al., 2003). Therefore, **M-8** was established as 5-O-methyl-hexahydro-demethoxycurcumin-B.

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Metabolite M-9 was obtained as a yellow amorphous powder. The positive ESI-MS showed two quasi-molecular ion peaks at m/z 325 ($[M+H]^+$) and 347 ($[M+Na]^+$) and the negative ESI-MS gave a quasi-molecular ion peaks at m/z 323 ($[M-H]^-$), corresponding to the molecular formula $C_{20}H_{20}O_4$, which was further supported by the 1H -NMR and ^{13}C -NMR spectral data. The ^{13}C and 1H -NMR and HMQC spectra displayed a set of 1,3,4-trisubstituted benzene ring signals [δ 6.76 (1H, d, $J=8.1$ Hz), 6.96 (1H, brd, $J=8.1$ Hz), 7.16(1H, brs)], a set of 1,4-bis-substituted benzene ring signals [δ 6.64 (2H, d, $J=8.1$ Hz), 7.00 (2H, d, $J=8.1$ Hz)], one pair of *trans* conjugated olefinic protons [δ 6.22 (1H, d, $J=15.5$ Hz), 7.35 (1H, dd, $J=10.4, 15.4$ Hz) and δ 6.93 (1H, dd, $J=10.2, 15.4$ Hz), 6.96 (1H, d, $J=16.0$ Hz)], a methoxy group [δ 3.80 (3H, s, OCH_3)], a carbonyl signal (δ 199.2) and two methylene groups. The HMBC correlations of H-1 (δ 2.71), H-2 (δ 2.84), H-4 (δ 6.22) and H-5 (δ 7.35) with C-3 (δ 199.2) suggested that H-1 and H-5 are three bonds away from the C-3 carbonyl group while H-2 and H-4 are adjacent to the carbonyl group. The HMBC spectrum revealed H-2', 6' (δ 7.00) to be correlated with C-1 (δ 29.0), and H-2'' (δ 7.16) correlated with C-7 (δ 141.9). Thus, **M-9** was identified as 5-dehydroxy-dihydro-demethoxycurcumin-B. It is a 5-dehydroxy product with reduction of the double bond between C-1 and C-2 of demethoxycurcumin. The chemical shifts of **M-9** were nearly identical with those previously reported (Li et al., 2004).

Discussion

This is the first study on the metabolism of demethoxycurcumin in vivo. Nine phase 1 metabolites were obtained and identified by ESI-MS spectra and NMR spectroscopy including 1H -NMR, ^{13}C -NMR, and two-dimensional NMR (HMQC, HMBC). Compared with earlier reports on the metabolism of demethoxycurcumin in vitro (Hoehle et al., 2006), an identical result was obtained in that reduction of

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the aliphatic moiety is the only pathway in phase 1 metabolism and no oxidative metabolites were discovered. However, there were two new discoveries in the present study: First the existence of the dehydroxy or methylated metabolites was demonstrated and secondly the existence of the isomers with a methoxy group substituted on a different benzene ring. In this study, the major metabolites of demethoxycurcumin in urine were hexahydro-demethoxycurcumins (**M-5** and **M-6**) and the 5-O-methyl-hexahydro-demethoxycurcumins (**M-7** and **M-8**) together with traces of 5-dehydroxy-dihydro-demethoxycurcumins (**M-9**). While the major metabolites in feces were 5-dehydroxy-hexahydro-demethoxycurcumin (**M-1** and **M-2**) and 5-dehydroxy-octahydro-demethoxycurcumin (**M-3** and **M-4**). Combined with previous reports on the metabolism of curcuminoids (Hoehle et al., 2006; Holder et al., 1978; Wahlstrom et al., 1978; Ireson et al., 2001), it might be presumed that some demethoxycurcumin should initially undergo reduction to form dihydro, tetrahydro, hexahydro (**M-5** and **M-6**) and octahydro-demethoxycurcumin in a stepwise fashion (Ireson et al., 2002) followed by dehydroxylation (Bokkenheuser et al., 1981; Feighner et al., 1980; Kasahara et al., 1995) to form 5-dehydroxy-dihydro (**M-9**), 5-dehydroxy-hexahydro (**M-1** and **M-2**) and 5-dehydroxy-octahydro-demethoxycurcumins (**M-3** and **M-4**); on the other hand, some demethoxycurcumin might be initially methylated (Yang et al., 2005) followed by reduction to form 5-O-methyl-hexahydro-demethoxycurcumins (**M-7** and **M-8**). On the basis of the metabolite profiles, the metabolic pathways of demethoxycurcumin in rats are proposed (Fig. 4).

Based on present knowledge concerning the metabolism of curcuminoids, curcuminoids as well as their reduced metabolites appear to be easily conjugated *in vivo* and *in vitro*. The reported conjugates include monoglucuronides, monosulfates, and mixed sulfate/glucuronides (Hoehle et al., 2006; Holder et al., 1978; Ravindranath et al., 1982; Asai et al., 2000). In this study, seven new phase 1 metabolites

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were discovered, which provide new types of precursors for research on phase 2 metabolites of curcuminoids. In addition, because of the low bioavailability of curcumin, some previous research suggested that the pharmacological activities of curcumin was in part mediated by its metabolites (Ireson et al., 2001; Ireson et al., 2002). This has been confirmed by recent experiments with the activities of the phase I metabolites (Murugan et al., 2006; Leyon et al., 2004; Pari et al., 2004; Limtrakul et al., 2006; Lee et al., 2005). Further studies of the nine phase I reductive metabolites should clarify whether they remain the activity or not.

From the comparison of the ^{13}C -NMR spectra of the four pairs of isomers, it was always found that there were two very similar groups of signals and one stronger than the other. These findings suggest that, in rats, demethoxycurcumin possibly undergoes tautomerization between 3-keto-5-enol (form A) and 3-keto-5-enol (form B) (Fig. 1) and that the forms of these two isomers were not equal, one form (form B) was the major one and that the other form (form A) was the minor one. In the HMQC spectrum of **M-9**, the proton signals δ_{H} 7.40 (d, 8.6Hz) associated with δ_{C} 129.2 (Fig. 8) and δ_{H} 3.76 with δ_{C} 55.7 were observed in addition to the proton and carbon signals of **M-9**, which could be assigned to the isomer of **M-9**. The reason that the other correlative signals of the isomer were not entirely displayed in the ^{13}C and ^1H -NMR spectra of **M-9** might be due to the scant amount of the compound available.

M-5 and **M-6** presumably would begin to transfer partly to **M-7** and **M-8** when placed in MeOH for more than two weeks. However, the spot 1 (the mixture of **M-7** and **M-8**), which was observed in TLC in the preliminary study and at that time, the urine sample had not yet been dealt with MeOH. The data demonstrated that **M-7** and **M-8** were the actual metabolites of demethoxycurcumin in rats. However, a portion of **M-7** and **M-8** probably transferred from **M-5** and **M-6** because a large quantity of

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MeOH was utilized during the course of the subsequent separation.

Structural elucidation of metabolites is an important task in drug metabolism studies. In recent years, comparisons of ESI-MSⁿ data and retention times in HPLC with synthesized standards are usually used to identify the structures of metabolites. However, the structures of some metabolites deduced only from LC/MSⁿ data might not be correct, especially in the case of the existence of isomerism of the metabolites. In this study, four groups of isomers (**M-1** and **M-2**; **M-3** and **M-4**; **M-5** and **M-6**; **M-7** and **M-8**) were obtained that have the same chromatographic behaviors and identical data in LC/MSⁿ. So the findings could not be validated just by LC/MSⁿ data (Hoehle et al., 2006). In these cases, preparation of metabolites and further identification based on NMR data must be done. Of course, the direct isolation of the metabolites from urine, bile, or feces of humans or animals has difficulties, but it is the most reliable method for the identification of metabolites.

In summary, we have determined the definitive structures of nine phase I reductive metabolites of demethoxycurcumin by mass spectra and NMR spectroscopy. In the urine, the major reductive metabolites are the hexahydro-demethoxycurcumin and the methyl ether products of hexahydro-demethoxycurcumin. In the feces, the dehydroxy products of hexahydro and octahydro-demethoxycurcumin are predominant. These results are important for the understanding of demethoxycurcumin metabolism in rats and should provide information and reference for the further metabolic investigation of demethoxycurcumin in humans. Screening of the bioactivities of the novel metabolite is presently under study.

References

- Araujo CAC, Leon LL (2001) Biological Activities of *Curcuma longa* L. *Mem Inst Oswaldo Cruz* **96**:723-728.
- Arora RB, Kapoor V, Basu N, Jain AP(1971) Anti-inflammatory studies on *Curcuma longa* (Turmeric). *Indian J Med Res* **59**:1289-1295.
- Asai A, Miyazawa T (2000) Occurrence of orally administered curcuminoid as glucuronide and glucuronide/sulfate conjugates in rat plasma. *Life Sci* **67**:2785-2793.
- Bokkenheuser VD, Winter J, Hylemon PB, Ayengar NKN, and Mosbach EH.(1981) Dehydroxylation of 16 α -hydroxyprogesterone by fecal flora of man and rat. *J.Lipid Res* **22**: 95-102.
- Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, Ko JY, Lin JT, Lin BR, Ming-Shiang W, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, Lai MK, Pu YS, Pan MH, Wang YJ, Tsai CC, Hsieh CY (2001) Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res* **21**:2895-2900.
- Conney AH, Lysz T, Ferraro T, Abidi TF, Manchand PS, Laskin JD, Huang MT (1991) Inhibitory effect of curcumin and some related dietary compounds on tumor promotion and arachidonic acid metabolism in mouse skin. *Adv Enzyme Regul* **31**:385-396.
- Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, Dicato M, Diederich (2005) Chemopreventive and therapeutic effects of curcumin. *Cancer Lett* **223**:181-190.
- Eigner D, Scholz, D (1999) *Ferula asa-foetida* and *Curcuma longa* in traditional medical treatment and diet in Nepal. *J Ethnopharmacol* **67**:1-6.
- Feighner SD, Hylemon PB (1980) Characterization of a corticosteroid 21-dehydroxylase from the intestinal anaerobic bacterium, *Eubacterium lentum*. *J. Lipid Res* **21**:585-593.

DMD #15008

- Garcea G, Jones DJ, Singh R, Dennison AR, Farmer PB, Sharma RA, Steward WP, Gescher AJ, Berry DP (2004) Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. *Br J Cancer* **90**:1011-1015.
- Ghatak G, Basu N (1972) Sodium curcumin as an effective anti-inflammatory agent. *Indian J Exp Biol* **10**:235-236.
- Govindarajan VS (1980) Turmeric-chemistry, technology, and quality. *Crit Rev Food Sci Nutr* **12**:199-301.
- Gupta KK, Bharné SS, Rathinasamy K, Naik NR, Panda D (2006) Dietary antioxidant curcumin inhibits microtubule assembly through tubulin binding. *FEBS J* **273**:5320-5332.
- Hermann PT, Ammon and Martin AW (1991) Pharmacology of *Curcuma longa*. *Planta Med.* **57**:1-7.
- Hoehle SI, Pfeiffer E, Solyom AM, Metaler M (2006) Metabolism of Curcuminoids in Tissue Slices and Subcellular Fractions from Rat Liver. *J Agric Food Chem* **54**:756-764.
- Holder GM, Plummer JL, Ryan AJ (1978) The metabolism and excretion of curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) in the rat. *Xenobiotica* **8**:761-768.
- Huang MT, Ma W, Lu YP, Chang RL, Fisher C, Manchand PS, Newmark HL, Conney AH (1995) Effects of curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin on 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion. *Carcinogenesis* **16**:2493-2497.
- Ireson C, Orr S, Jones DJ, Verschoye R, Lim CK, Luo JL, Howells L, Plummer S, Jukes R, Williams M, Steward WP, Gescher A (2001) Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat in vivo, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2 production. *Cancer Res* **61**:1058-1064.
- Ireson CR, Jones DJ, Orr S, Coughtrie MW, Boocock DJ, Williams ML, Farmer PB, Steward WP,

DMD #15008

- Gescher AJ (2002) Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidemiol Biomarkers Prev* **11**:105-111.
- Kasahara H, Miyazawa M, Kameoka H (1995) Biotransformation of an Acyclic Neolignan in rats. *Phytochemistry*, **38**(2): 343-346.
- Kikuzaki H, Kobayashi M, Nakatani N (1991) Diarylheptanoids from Rhizomes of *Zingiber officinale*. *Phytochemistry* **30**:3647-3651.
- Kim DS, Park SY, Kim JY (2001) Curcuminoids from *Curcuma longa* L. (Zingiberaceae) that protect PC12 rat pheochromocytoma and normal human umbilical vein endothelial cells from β A (1-42) insult. *Neurosci lett* **303**:57-61.
- Kim JM, Araki S, Kim DJ, Park CB, Takasuka N, Baba-Toriyama H, Ota T, Nir Z, Khachik F, Shimidzu N, Tanaka Y, Osawa T, Uraji T, Murakoshi M, Nishino H, Tsuda H (1998) Chemopreventive effects of carotenoids and curcumins on mouse colon carcinogenesis after 1,2-dimethylhydrazine initiation. *Carcinogenesis* **19**:81-85.
- Kiuchi F, Goto Y, Suqimoto N, Akao N, Kondo K, Tsuda Y (1993) Nematocidal Activity of Turmeric: Synergistic Action of Curcuminoids. *Chem Pharm Bull* **41**:1640-1643.
- Kuttan R, Bhanumathy P, Nirmala K, George MC (1985) Potential anticancer activity of turmeric (*Curcuma longa*). *Cancer lett* **29**:197-202.
- Lee SL, Huang WJ, Lin WW, Lee SS, Chen CH (2005) Preparation and anti-inflammatory activities of diarylheptanoid and diarylheptylamine analogs. *Bioorg Med Chem* **13**:6175-6181.
- Leyon PY, Kuttan G (2003) Studies on the role of some synthetic curcuminoid derivatives in the inhibition of tumour specific angiogenesis. *J Exp Clin Cancer Res* **22**:77-83.
- Li G, Seo CS, Lee SH, Jahng Y, Chang HW, Lee CS, Woo MH, Son JK (2004) Diarylheptanoids from

DMD #15008

- the Roots of *Juglans mandshurica*. *Bull Korean Chem Soc* **25**:397-399.
- Li G, Xu ML, Choi HG, Lee SH, Jahng YG, Lee CS, Moon DC, Woo MH, Son JK (2003) Four New Diarylheptanoids from the Roots of *Juglans mandshurica*. *Chem Pharm Bull* **51**:262-264.
- Limtrakul P, Chearwae W, Shukla S, Phisalpong C, Ambudkar SV (2007) Modulation of function of three ABC drug transporters, P-glycoprotein (ABCB1), mitoxantrone resistance protein (ABCG2) and multidrug resistance protein 1 (ABCC1) by tetrahydrocurcumin, a major metabolite of curcumin. *Mol Cell Biochem* **296**:85-95.
- Mazumber A, Raghavan K, Weinstein, J, Kohn KW, Pommer Y (1995) Inhibition of human immunodeficiency virus type-I integrase by curcumin. *Biochem Pharmacol* **49**:1165-1170.
- Murugan P, Pari L (2006) Antioxidant effect of tetrahydrocurcumin in streptozotocin-nicotinamide induced diabetic rats. *Life Sci* **79**:1720-1728.
- Nurfina AN, Reksোধadiprodjo MS, Timmerman H, Jenie UA, Sugiyanto D, Van der Goot H (1997) Synthesis of some symmetrical curcumin derivatives and their anti-inflammatory activity. *Eur J Med Chem* **32**: 321-328.
- Pan MH, Huang TM, Lin JK (1999) Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metab Dispos* **27**:486-494.
- Pari L, Murugan P (2004) Protective role of tetrahydrocurcumin against erythromycin estolate-induced hepatotoxicity. *Pharmacol Res* **49**:481-486.
- Park SY, Kim DS (2002) Discovery of Natural Products from *Curcuma longa* that Protect Cells from Beta-Amyloid Insult: A Drug Discovery Effort against Alzheimer's Disease. *J Nat Prod* **65**:1227-1231.
- Ramprasad CE, Sirsi M (1956) Studies on Indian medicinal plants *Curcuma longa* Linn-Effects of

DMD #15008

- curcumin and essential oil of *C. longa* on bile secretion. *J Sci Res Inst* **15C**:262-265.
- Ravindranath V, Chandrasekhara N (1982) Metabolism of curcumin-studies with [³H]curcumin. *Toxicology* **22**: 337-344.
- Sharma RA, McLelland HR, Hill KA, Ireson CR, Euden SA, Manson MM, Pirmohamed M, Marnett LJ, Gescher AJ, Steward WP (2001) Pharmacodynamic and pharmacokinetic study of oral curcuma extract in patients with colorectal cancer. *Clin Cancer Res* **7**:1894-1900.
- Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, Marczylo TH, Morgan B, Hemingway D, Plummer SM, Pirmohamed M, Gescher AJ, Steward WP (2004) Phase I clinical trial oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res* **10**: 6847-6854.
- Sharma OP (1976) Antioxidant activity of curcumin and related compounds. *Biochem Pharmacol* **25**:1811-1812.
- Shin D, Kinoshita K, Koyama K, Takahashi K (2002) Antiemetic Principles of *Alpinia officinarum*. *J Nat Prod* **65**:1315-1318.
- Unnikrishnan MK, Rao MN (1995) Inhibition of nitrite induced oxidation of hemoglobin by curcuminoids. *Pharmazie* **50**:490-492.
- Yang B, Meng ZY, Dong JX, Yan LP, Zou LB, Tang ZM, Dou GF (2005) Metabolic profile of 1,5-dicaffeoylquinic acid in rats, an in vivo and in vitro study. *Drug Metab. Dispos.* **33**:930-936.

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Footnotes

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Figure Legends

Fig. 1. Chemical structure of demethoxycurcumin

Fig. 2. Structure of curcumin, demethoxycurcumin and bisdemethoxycurcumin

Fig. 3. Significant HMBC (H→C) correlations of **M-1** and **M-2**

Fig. 4. Structures of demethoxycurcumin metabolites in rat urine and feces and possible metabolic pathways for their production

Fig. 5. ESI-MS spectra of [M-H]⁻ ion of **M-1** and **M-2** (A), **M-3** and **M-4** (B), **M-5** and **M-6** (C), **M-7** and **M-8** (D), **M-9** (E) and demethoxycurcumin (F)

Fig. 6. ¹³C-NMR spectra of **M-1** and **M-2** (A), **M-3** and **M-4** (B), **M-5** and **M-6** (C), **M-7** and **M-8** (D)

Fig. 7. Partial HMBC spectrum of **M-1** and **M-2**

Fig. 8. Partial HMQC spectrum of **M-9**

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Table 1. Assignments of carbon and proton signals of **M-1** and **M-3**

NO.	Carbon Signals		Proton Signals	
	M-1	M-3	M-1	M-3
1	29.5	31.7	2.80(2H, m)	2.60(1H, m); 2.70(1H, m)
2	44.6	39.4	2.67(2H, m)	1.72(2H, m)
3	211.2	71.4		3.62(1H, m)
4	42.9	37.4	2.39(2H, t, 6.8Hz)	1.49(2H, m)
5	23.2	25.2	1.57(2H, m)	1.35(1H, m); 1.47(1H, m)
6	31.1	31.8	1.51(2H, m)	1.60(2H, m)
7	34.7	34.9	2.50(2H, m)	2.54(2H, t, 7.6Hz)
1'	132.9	134.0		
2'	111.1	111.0	6.66(1H, d, 1.5Hz)	6.69(1H, br.s)
3'	146.4	146.4		
4'	143.8	143.7		
5'	114.3	114.3	6.81(1H, d, 8.0Hz)	6.83(1H, d, 7.8Hz)
6'	120.7	120.9	6.62(1H, brd, 8.0Hz)	6.67(1H, br.d, 7.8Hz)
1''	134.0	134.7		
2'', 6''	129.3(2C)	129.4(2C)	6.97(2H, d, 8.4Hz)	7.02(2H, d, 8.3Hz)
3'', 5''	115.1(2C)	115.1(2C)	6.74(2H, d, 8.4Hz)	6.74(2H, d, 8.3Hz)
4''	153.8	153.6		
-OCH ₃	55.8	55.9	3.82(3H, S)	3.87(3H, s)

Notes: a) all spectra were recorded on an AV-600 spectrometer, in CDCl₃

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- b) The carbon and proton signals were assigned unambiguously on ^1H NMR, ^{13}C NMR, HMQC
and HMBC
- c) m, multiplet.

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Table 2. Assignments of carbon and proton signals of **M-2** and **M-4**

NO.	Carbon Signals		Proton Signals	
	M-2	M-4	M-2	M-4
1	28.9	31.1	2.80(2H, m)	2.60(1H, m); 2.70(1H, m)
2	44.5	39.3	2.67(2H, m)	1.72(2H, m)
3	211.2	71.3		3.62(1H, m)
4	42.9	37.4	2.39(2H, t, 6.8Hz)	1.49(1H, m)
5	23.3	25.2	1.57(2H, m)	1.35(1H, m); 1.47(1H, m)
6	31.1	31.8	1.51(2H, m)	1.60(2H, m)
7	35.3	35.6	2.50(2H, m)	2.54(2H, t, 7.6Hz)
1'	132.7	134.2		
2', 6'	129.3(2C)	129.5(2C)	6.99(2H, d, 8.4Hz)	7.05(2H, d, 8.3Hz)
3', 5'	115.3(2C)	115.2(2C)	6.74(2H, d, 8.4Hz)	6.75(2H, d, 8.3Hz)
4'	154.1	153.7		
1''	134.2	134.6		
2''	111.0	110.9	6.64(1H, d, 1.5Hz)	6.66(1H, br.s)
3''	146.3	146.3		
4''	143.5	143.5		
5''	114.1	114.1	6.81(1H, d, 8.0Hz)	6.82(1H, d, 7.7Hz)
6''	120.8	120.9	6.62(1H, br.d, 8.0Hz)	6.66(1H, br.d, 7.7Hz)
-OCH ₃	55.8	55.9	3.84(3H, s)	3.87(3H, s)

Notes: a) all spectra were recorded on an AV-600 spectrometer, in CDCl₃

b) The carbon and proton signals were assigned unambiguously on ¹H NMR, ¹³C NMR, HMQC

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and HMBC

c) m, multiplet.

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Table 3. Assignments of carbon and proton signals of **M-5** and **M-7**

NO.	Carbon Signals		Proton Signals	
	M-5	M-7	M-5	M-7
1	29.2	29.2	2.82(2H, m)	2.81(2H, m)
2	45.3	45.7	2.70(2H, m)	2.72(2H, m)
3	211.6	209.4		
4	49.3	47.3	2.53(2H, m)	2.69(1H, m); 2.43(1H, m)
5	66.9	76.7	4.03(1H, m)	3.71(1H, m)
6	38.2	35.8	1.62(1H, m); 1.76(1H, m)	1.75(2H, m)
7	30.7	30.4	2.59(1H, m); 2.71(1H, m)	2.59(2H, m)
1'	133.1	133.5		
2'	111.1	111.1	6.69(1H, br.s)	6.67(1H, br.s)
3'	146.4	146.4		
4'	143.9	144.0		
5'	114.4	114.3	6.81(1H, d, 7.9Hz)	6.81(1H, d, 7.9Hz)
6'	120.6	120.7	6.64(1H, br.d, 7.9Hz)	6.64(1H, br.d, 7.9Hz)
1''	132.5	132.8		
2'', 6''	129.4(2C)	129.3(2C)	7.01(2H, d, 8.1Hz)	6.99(2H, d, 8.6Hz)
3'', 5''	115.3(2C)	115.2(2C)	6.74(2H, d, 8.1Hz)	6.74(2H, d, 8.6Hz)
4''	154.1	154.0		
3'-OCH ₃	55.9	55.8	3.85(3H, s)	3.83(3H, s)
5-OCH ₃		56.9		3.31(3H, s)

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Notes: a) all spectra were recorded on an AV-600 or ARX-300 spectrometer, in CDCl₃.

b) The carbon and proton signals were assigned unambiguously on ¹H NMR, ¹³C NMR and
HMQC.

c) m, multiplet.

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Table 4. Assignments of carbon and proton signals of **M-6** and **M-8**

NO.	Carbon Signals		Proton Signals	
	M-6	M-8	M-6	M-8
1	28.7	28.6	2.80(2H, m)	2.80(2H, m)
2	45.3	45.6	2.70(2H, m)	2.72(2H, m)
3	211.6	209.4		
4	49.2	47.3	2.53(2H, m)	2.71(1H, m); 2.46(1H, m)
5	66.9	76.7	4.03(1H, m)	3.71(1H, m)
6	38.2	36.0	1.62(1H, m); 1.76(1H, m)	1.75(2H, m)
7	31.4	31.0	2.59(1H, m); 2.71(1H, m)	2.60(2H, m)
1'	132.2	132.6		
2', 6'	129.3(2C)	129.3(2C)	6.99(2H, d, 8.1Hz)	7.00(2H, d, 8.6Hz)
3', 5'	115.4(2C)	115.3(2C)	6.74(2H, d, 8.1Hz)	6.73(2H, d, 8.6Hz)
4'	154.4	154.2		
1''	133.6	133.7		
2''	111.0	111.0	6.69(1H, br.s)	6.67(1H, br.s)
3''	146.4	146.4		
4''	143.6	143.6		
5''	114.3	114.3	6.81(1H, d, 7.9Hz)	6.82(1H, d, 8.0Hz)
6''	120.9	120.8	6.64(1H, br.d, 7.9Hz)	6.64(1H, br.d, 8.0Hz)
3''-OCH ₃	55.8	55.8	3.85(3H, s)	3.85(3H, s)
5-OCH ₃		57.0		3.31(3H, s)

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Notes: a) all spectra were recorded on an AV-600 or ARX-300 spectrometer, in CDCl₃.

b) The carbon and proton signals were assigned unambiguously on ¹H NMR, ¹³C NMR and
HMQC.

c) m, multiplet.

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Table 5. Assignments of carbon and proton signals of **M-9** and demethoxycurcumin

NO.	Carbon Signals		Proton Signals	
	M-9	demethoxycurcumin	M-9	demethoxycurcumin
1	29.0	140.8	2.71(2H, t, 7.5Hz)	7.55(1H, d, $J=16.2$ Hz)
2	41.6	120.9	2.84(2H, t, 7.5Hz)	6.77(1H, d, $J=16.2$ Hz)
3	199.2	183.4		
4	127.7	101.0	6.22(1H, d, 15.5Hz)	6.05(1H, s)
5	143.6	183.3	7.35(1H, dd, 10.4, 15.4Hz)	
6	124.2	121.1	6.93(1H, dd, 10.2, 15.4Hz)	6.70(1H, d, $J=15.8$ Hz)
7	141.9	140.5	6.96(1H, d, 16.0Hz)	7.54(1H, d, $J=15.8$ Hz)
1'	131.4	125.9		
2', 6'	129.2	130.5	7.00(2H, d, 8.1Hz)	7.57(2H, d, $J=8.0$ Hz)
3', 5'	115.1	116.0	6.64(2H, d, 8.1Hz)	6.82(2H, d, $J=8.0$ Hz)
4'	155.5	159.9		
1''	128.1	126.4		
2''	110.3	111.3	7.16(1H, brs)	7.33(1H, brs)
3''	148.4	149.5		
4''	148.0	148.1		
5''	115.7	115.8	6.76(1H, d, 8.1Hz)	6.82(1H, d, $J=8.0$ Hz)
6''	121.9	123.3	6.96(1H, brd, 8.1Hz)	7.15(1H, brd, $J=8.0$ Hz)
-OCH ₃	55.7	55.7	3.80(3H, s)	3.84(3H, s)

Notes: all spectra were recorded on an AV-600 or ARX-300 spectrometer, in DMSO-*d*₆.

Fig. 1

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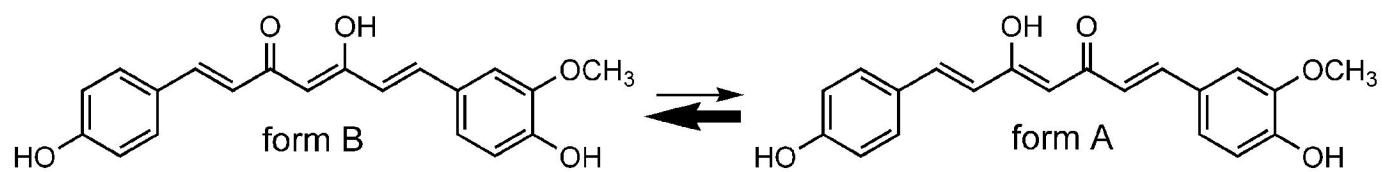
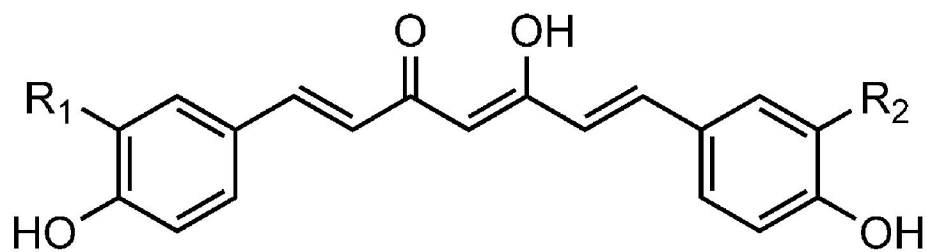


Fig. 2

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Curcumin: $R_1=R_2=OCH_3$
Demethoxycurcumin: $R_1=H$; $R_2=OCH_3$
Bisdemethoxycurcumin: $R_1=R_2=H$

Fig. 3

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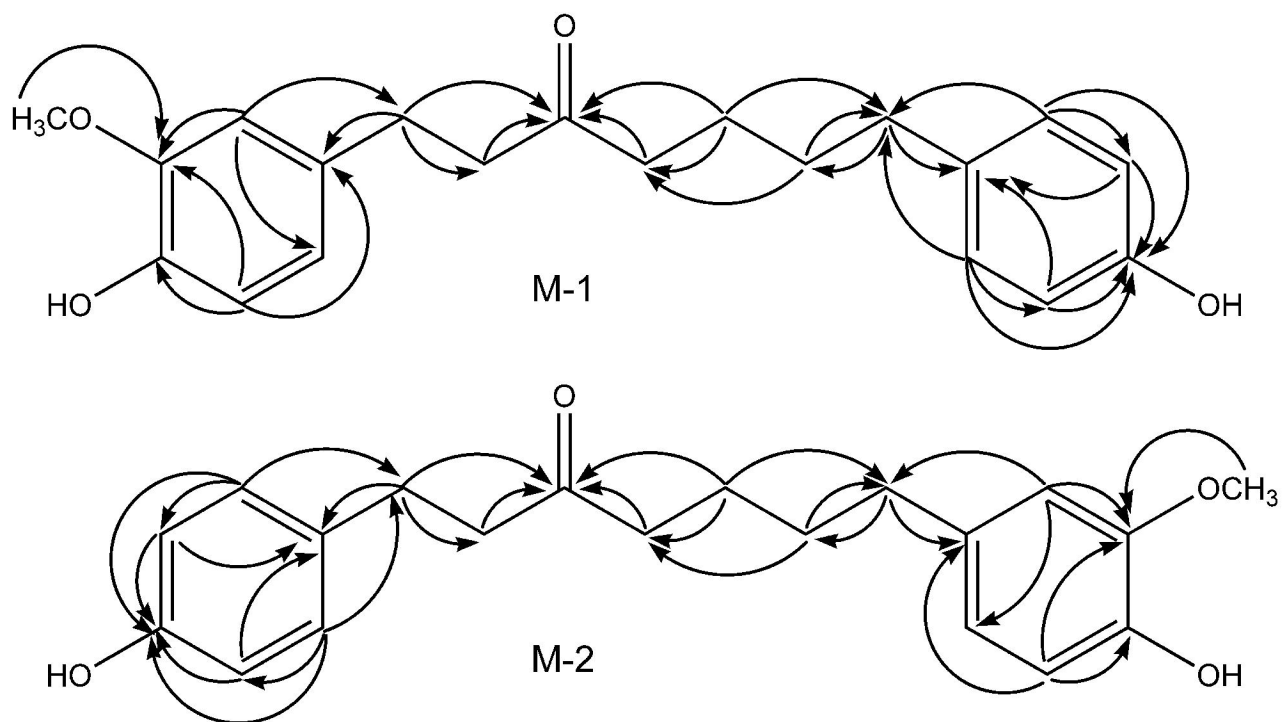
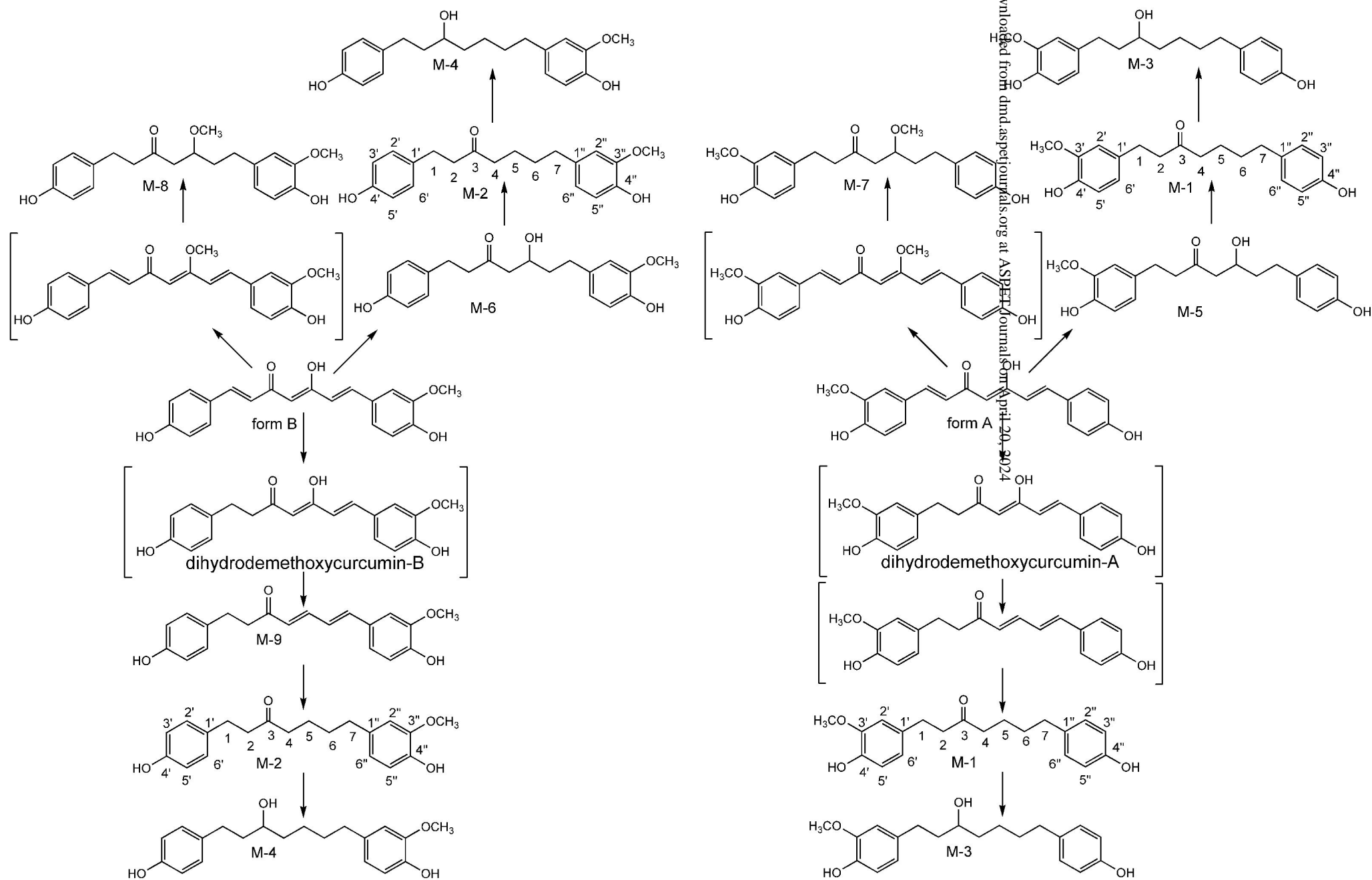


Fig. 4



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Fig. 5

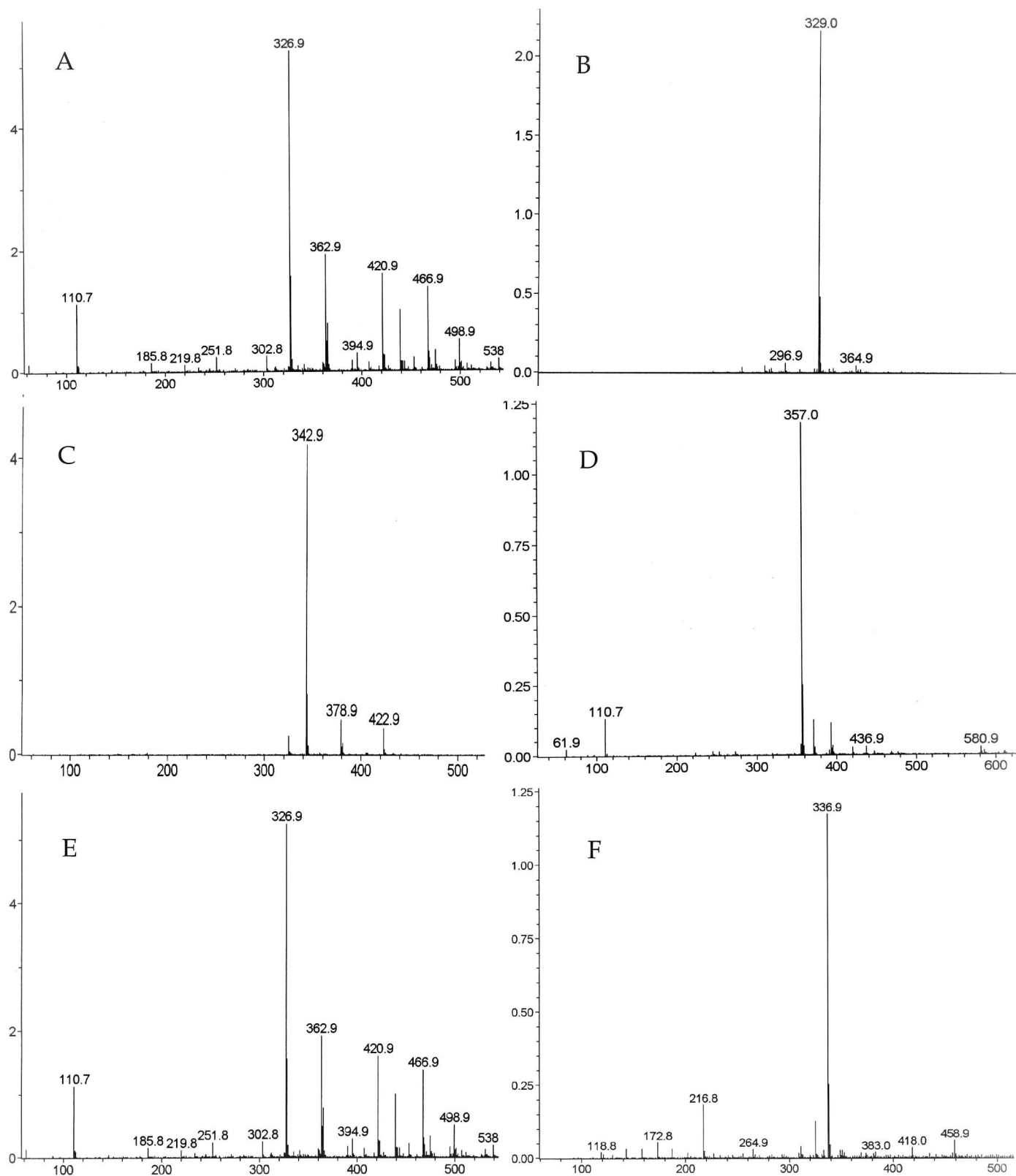


Fig. 6

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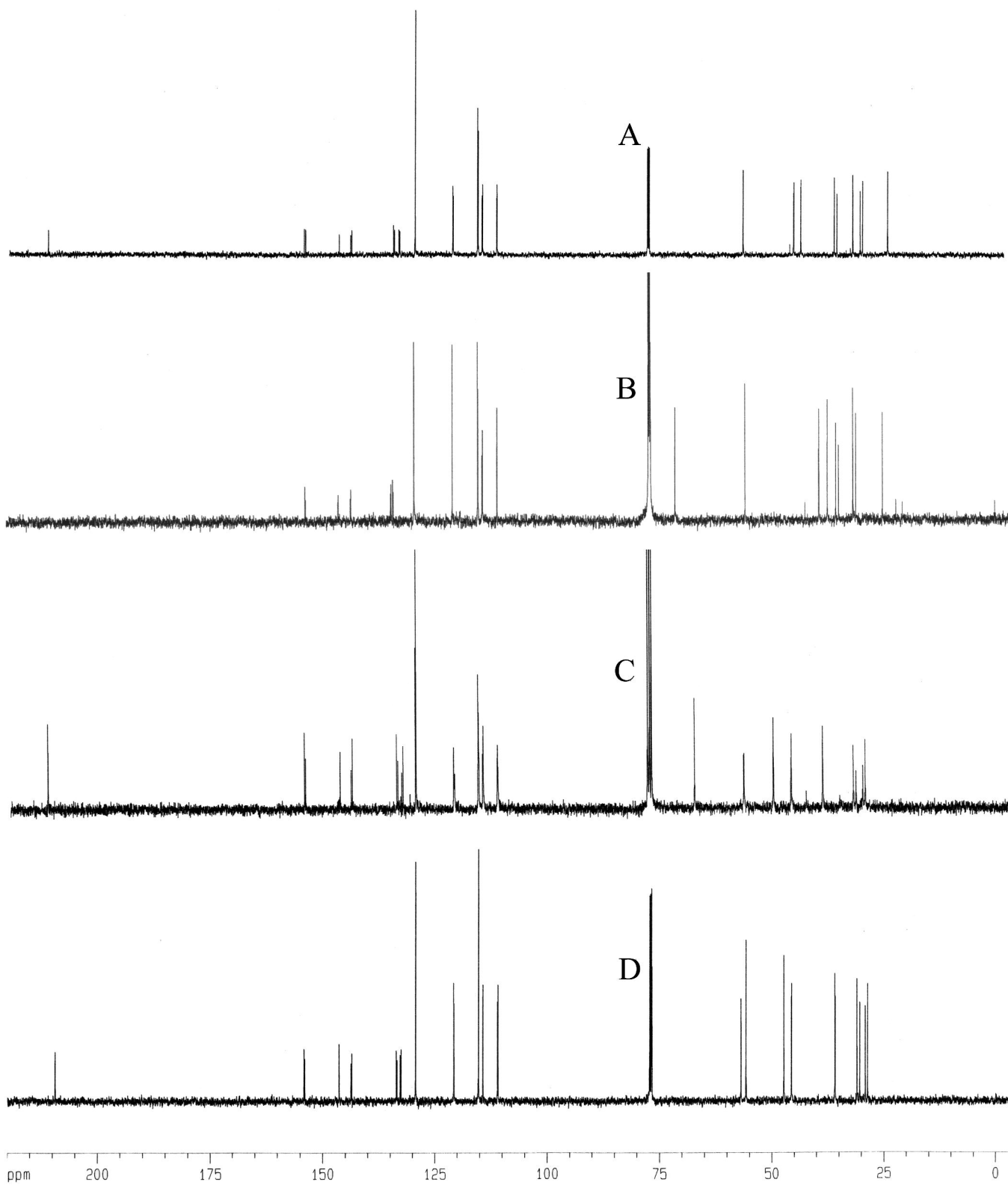


Fig. 7

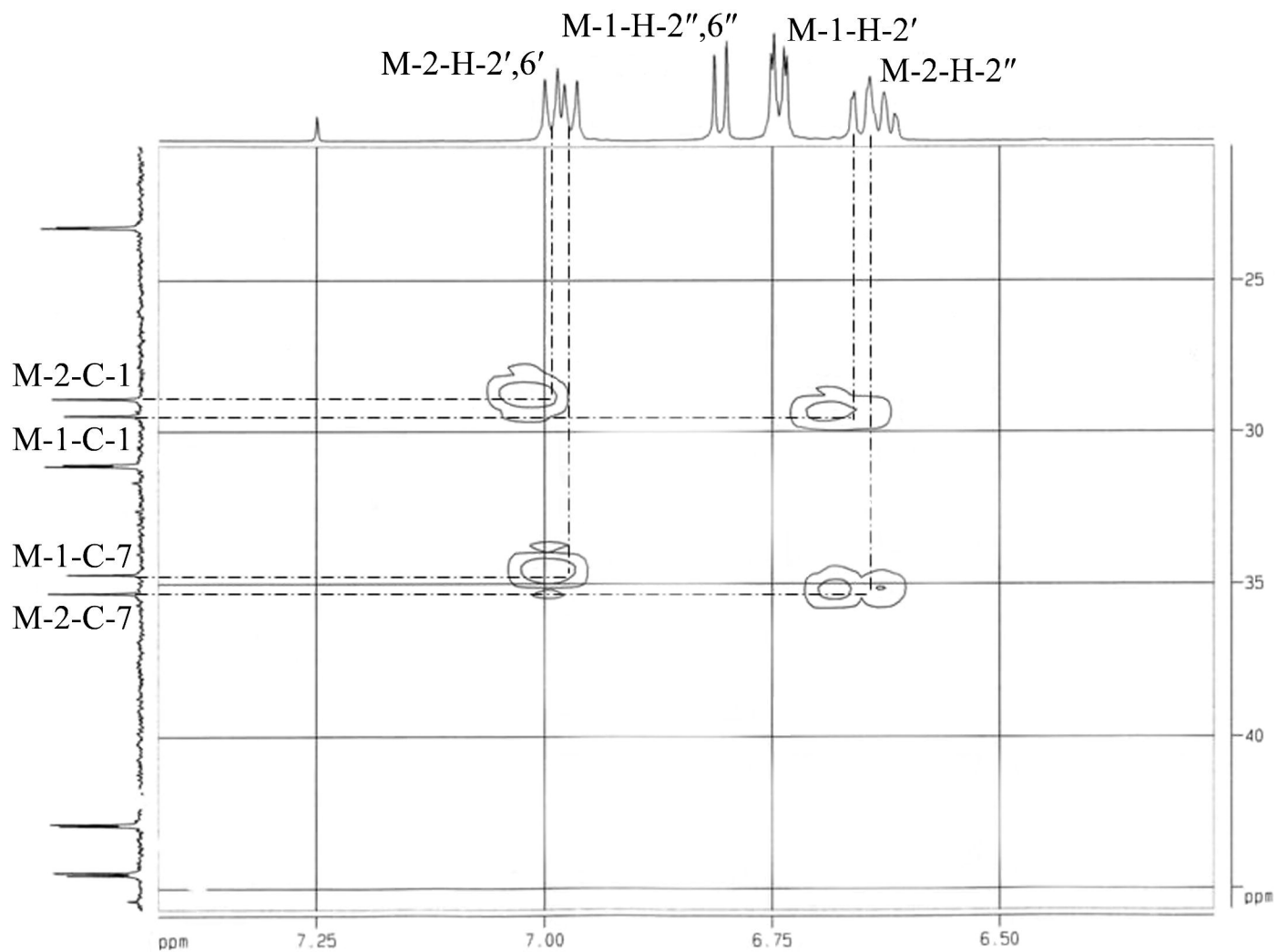


Fig. 8

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The possible signal of the isomer of M-9

