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N-Benzylated Alkamides Isolated from Maca Planted in China

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Abstract

Lepidium meyenii (maca) is an edible herbaceous biennial plant of the Brassicaceae family that is native to South America and has been marketed for its traditional medicinal effects. *N*-benzylated alkamides, commonly known as "macamides", have been found in maca and are considered as functional component of maca. Macamides could significantly enhance the libido and sexual potency of normal mice, improve anti-fatigue and anti-oxidant status in rats, and function as fatty acid amide hydrolase inhibitors to provide analgesic, anti-inflammatory, and neuroprotective effects. Maca was introduced into China in 2002. Now, China has become an important producer of maca in the world. However, chemical investigations of maca planted in China were rarely reported. Here, seventeen N-benzylated alkamides (macamides) in maca from Wenbi Mountain were identified by liquid chromatography mass spectrometry (LC-MS) and four of them were successfully isolated, namely *N*-3-methoxybenzyl-9Z-octadecenamide (1), *N*-benzyl-9Z-octadecenamide (2), *N*-(3-methoxybenzyl)-hexadecanamide (3), and *N*-benzyl-hexadecanamide (4). The chemical structure of these four compounds were established by spectroscopic and spectrometric methods including ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, 2D NMR, electrospray ionization-high resolution mass spectrometry (ESI-HRMS), ultraviolet–visible spectroscopy (UV-Vis), and fourier transform infrared spectroscopy (FT-IR). These compounds are useful marker compounds for the quality control of *Lepidium meyenii* planted in China.

Keywords

Maca, *Lepidium meyenii*, *N*-benzylated Alkamides, Macamides, Isolation

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1. Introduction

Lepidium meyenii, most commonly known as "maca" or "Peruvian ginseng", is an edible herbaceous biennial plant of the Brassicaceae family that is native to South America [1]. Various preclinical and clinical studies have shown that maca have many heath and medical benefits, such as improvement of sexual performance and fertility, alleviation of menopausal syndrome, alleviation of benign prostatic hyperplasia, enhancement of cognition and memory, anti-osteoporosis, anti-cancer, anti-fatigue, anti-oxidant, and so on [1-4]. *N*-benzylated alkamides, commonly known as "macamides", have been found only from maca [5-7]. In an earlier study, macamides and macaenes were considered as functional component of maca and could significantly enhance the libido and sexual potency of normal mice [8]. Thereafter, Choi et al reported that macamides could improve anti-fatigue and

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anti-oxidant status in rats [9]. Macamides were also reported to function as fatty acid amide hydrolase (FAAH) inhibitors and could take effect on the central nervous system to provide analgesic, anti-inflammatory, and neuroprotective effects [10-12]. Till now, a series of macamides have been successfully isolated from maca [6, 7, 13-15] or determined by LC-MS (Liquid chromatography-mass spectrometry) /MS [5, 16].

In 2002, maca was introduced into China in Lijiang (Yunnan Province) where the geographic environment is similar to its origin Andes mountain (Peru) (Lijiang: 100.25°E, 26.86°N, altitude of above 2400 meters; Andes mountain: 70-80°W, 10°N-40°S, altitude of above 3660 meters). Now, China has become an important producer of maca in the world [17]. However, most chemical investigations of maca were focused on maca from South America and chemical investigations of maca planted in China were rarely reported.

In the present work, we chosed maca from Wenbi Mountain (altitude of above 4350 meters, Lijiang) for chemical investigations. We identified seventeen kinds of macamides in maca from Wenbi Mountain by LC-MS and four of them were successfully isolated and characterized by ^{1}H NMR (nuclear magnetic resonance), ^{13}C NMR, 2D NMR, ESI-HRMS (electrospray ionization-high resolution mass spectrometry), UV-Vis (ultraviolet–visible spectroscopy), and FT-IR (fourier transform infrared spectroscopy). The structural identification based on these characterizations revealed that these four compounds were *N*-3-methoxybenzyl-9Z-octadecenamide (1), *N*-benzyl-9Z-octadecenamide (2), *N*-(3-methoxybenzyl)-hexadecanamide (3), and *N*-benzyl-hexadecanamide (4).

2. Experimental

2.1. Materials

The hypocotyls of *Lepidium meyenii* were purchased from Lijiang Green Enhancer Co., Ltd. A voucher specimen (voucher Huibo-2013-1) has been deposited in the Herbarium of Changzhou University. The materials purchased were positively identified as *Lepidium meyenii*.

2.2. Instruments

NMR spectra were recorded on the Bruker Spectrospin 500 Ultrashield spectrometer and Bruker Spectrospin 300 Ultrashield spectrometer (Bruker, Fallanden, Switzerland). All spectra data were recorded in $CDCl_3$ solution, using the residual solvent signal as internal standard (δ_H 7.26; δ_C 77.28, 77.02, 76.77). Multiplicity determinations (DEPT 45°, 90°, 135°) and 2D NMR (^{1}H-^{1}H COSY and NOESY, ^{1}H-^{13}C HSQC and HMBC) spectra were acquired using standard Bruker pulse programs. ^{1}H-^{15}N HMBC spectra were recorded at 50.7 MHz.

FT-IR spectra were recorded on a Nicolet iS50 FT-IR spectrometer (Thermo Fisher Scientific, MA, America). UV-Vis spectra were recorded on a UV3600-UV-VIS spectrometer (Shimadzu, Japan). Melting points were measured in a X-4B micro melting point instrument (Shanghai Shenguang Instrument Co., Ltd.)

HRMS spectra were obtained by using an Agilent 6540 UHD Q-TOF Accurate-Mass (Agilent Technologies, Inc., California, American) with electrospray ionization (ESI) source operating in positive mode. The capillary voltage was set at 4000V, the temperature of N_2 was maintained at 325°C, the flow rate of drying gas was set at 8 L/min, and the pressure of nebulizer gas was maintained at 40 psi.

LC-MS experiments were performed on Shimadzu Pr-LCMS-2020 (Shimadzu Corporation, Kyoto, Japan) with an ESI source operating in positive mode using an Ultimate LP-C18 column (300 × 4.6 mm, particle size: 5 μm) (Welch Materials, Inc., Shanghai, China). The mobile phase consisted of acetonitrile containing 0.005% (v/v) trifluoroacetic acid (TFA) and water containing 0.005% (v/v) TFA. The flow rate was set at 0.8 mL/min, the injection volume was set at 10 μL, the column temperature was set at 40 °C, and the detection wavelength was set at 210 nm.

The separation of macamides was performed on Shimadzu LC-20AP (Shimadzu Corporation, Kyoto, Japan) using an Ultimate LP-C18 column (250 × 21.2 mm, particle size: 5 μm) (Welch Materials, Inc., Shanghai, China). The mobile phase consisted of acetonitrile containing 0.01% or 0.005% (v/v) TFA and water containing 0.01% or 0.005% (v/v) TFA. The column temperature was set at 25 °C, and the detection wavelength was set at 210 nm. The samples were injected by 1mL for each time.

2.3. Extraction and Isolation

Dried hypocotyls of *Lepidium meyenii* (7.37 kg) were cut with a root slicer and then pulverized with a high-speed comminuter. The powder was successively percolated with petroleum ether (extracting temperature: 40°C, 3 × 18 L, 3 × 24 h) and 50% (v/v) ethanol (extracting temperature: 45°C, 3 × 10 L, 3 × 24 h), and the supernatant was evaporated under reduced pressure to yield 66 g and 903 g of crude extracts, respectively. Then dried ethanol extracts was re-extracted by percolation with petroleum ether (3×1 L) to obtain 25 g petroleum ether soluble fractions.

The petroleum ether (PE) extracts (66 g) and PE-soluble fractions (25 g) were subjected to column chromatography over silica gel using $CHCl_3$ followed by increasing concentration of ethyl acetate (EA) (0-15%) in $CHCl_3$ as

eluent, to give 55 main fractions. All fractions were analyzed by LC-MS. Fractions 10-11 and 22-24 (22.53 g, PE: EA = 7: 1, R_f = 0.25) contained crude *N*-benzyl macamides fractions, and fractions 12-15 and 26-28 (3.39 g, PE: EA = 7:1, R_f = 0.20) contained crude *N*-methoxybenzyl macamides fractions.

Fractions 10-11 and 22-24 were further subjected to preparative RP-HPLC on Ultimate LP-C18 column and eluted with acetonitrile containing 0.005% TFA and water containing 0.005% TFA by a linear gradient from 80:20 to 100:0 (v/v) (flow rate: 6 mL/min), to separate into sub-fraction I-A ~ sub-fraction I-L. Fractions 12-15 and 26-28 were chromatographed on the same column and eluted with the same solvent (flow rate: 5 mL/min), to afford sub-fraction II-A ~ sub-fraction II-L.

Finally, sub-fraction I-H (319.56 mg) was subjected to preparative RP-HPLC on Ultimate LP-C18 column and eluted with acetonitrile containing 0.005% TFA and water containing 0.005% TFA by a linear gradient from 90:10 to 72:28 (v/v) (flow rate: 12 mL/min), to obtain two purifications. Sub-fraction II-F (87.39 mg) were continually chromatographed on the same column and eluted with acetonitrile containing 0.01% TFA and water containing 0.01% TFA by a linear gradient from 90: 10 to 65: 35 (v/v) (flow rate: 12 mL/min), to obtain another two purifications. These four purifications were concentrated under reduced pressure to yield pure compounds 1 (25.30 mg), 2 (11.43 mg), 3 (10.25 mg), and 4 (76.27 mg).

N-3-methoxybenzyl-9Z-octadecenamide (1)

White powder; m.p. 59-60°C; UV (methanol) λ_{max} (log ε) 212 (3.99) nm, 273 (3.14) nm; IR (film) ν_{max} (cm^{-1}) 3302(N-H), 2921, 2849, 1642(C=O), 1539(N-C), 1466 (N-C=O), 1265(C-O-C), 1156, 1050, 693; ^{1}H NMR ($CDCl_3$, 500MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2 and Table 3; ESI-HRMS, m/z found 402.3366 ($[M+H]^+$), calc. for $C_{26}H_{44}NO_2$ ($[M+H]^+$): 402.3372.

N-benzyl-9Z-octadecenamide (2)

White powder; m.p. 57-58°C; UV (methanol) λ_{max} (log ε) 208 (4.00) nm, 274 (2.85) nm; IR (film) ν_{max} (cm^{-1}) 3300 (N-H), 3054, 2923, 2853, 1639 (C=O), 1552 (N-C), 1430 (N-C=O), 1351, 1252, 1128, 1065, 1002, 812, 731; ^{1}H NMR ($CDCl_3$, 300MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2 and Table 3; ESI-HRMS, m/z found 372.3263 ($[M+H]^+$), calc. for $C_{25}H_{42}NO$ ($[M+H]^+$): 372.3266.

N-(3-methoxybenzyl)-hexadecanamide (3)

White powder; m.p. 71-72°C; UV (methanol) λ_{max} (log ε) 212 (4.01) nm, 274 (3.14) nm; IR (film) ν_{max} (cm^{-1}) 3297 (N-H), 2920, 2850, 1640 (C=O), 1537 (N-C), 1466 (N-C=O), 1263 (C-O-C), 1156, 1050, 783, 693; ^{1}H NMR ($CDCl_3$, 300MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2 and Table 3; ESI-HRMS, m/z found 376.3023 ($[M+H]^+$), calc. for $C_{24}H_{42}NO_2$ ($[M+H]^+$): 376.3216.

N-benzyl-hexadecanamide (4)

White powder; m.p. 94-95°C; UV (methanol) λ_{max} (log ε) 208 (3.41) nm; IR (film) ν_{max} (cm^{-1}) 3306 (N-H), 2917, 2848, 1633 (C=O), 1548 (N-C), 1455 (N-C=O), 729, 697; ^{1}H NMR ($CDCl_3$, 300MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2 and Table 3; ESI-HRMS, m/z found 346.3105 ($[M+H]^+$), calc. for $C_{23}H_{40}NO$ ($[M+H]^+$): 346.3110.

Table 1. Macamides from maca planted in china determined by LC-MS.

Retention time (min)	Molecular weight	Macamides
7.433	383	—
8.381	383	—
12.300	397	*N*-(3-methoxybenzyl)-9Z,12Z,15Z-octadecatrienamide
12.383	367	*N*-benzyl-9Z,12Z,15Z-octadecatrienamide
14.367	373	—
14.444	343	—
15.300	399	*N*-(3-methoxybenzyl)-(9Z,12Z)-octadecadienamide
15.383	369	*N*-benzyl-9Z,12Z-octadecadienamide
18.633	375	*N*-(3-methoxybenzyl)-hexadecanamide
18.901	345	*N*-benzyl-hexadecanamide
19.033	401	*N*-(3-methoxybenzyl)-9Z-octadecenamide
19.252	371	*N*-benzyl-9Z-octadecenamide
21.491	359	*N*-benzyl-heptadecanamide
23.573	373	—
24.100	403	—
24.307	373	—
36.032	455	—

Table 2. ^{1}H NMR spectral data and coupling constants (δ in ppm, J in Hz) of compound 1-4 (300 MHz or 500 MHz, $CDCl_3$)[a].

Proton	1	2	3	4
1’	4.42 *d*[b] (7.6)	4.43 *d* (5.7)	4.42 *d* (5.7)	4.44 *d* (5.7)
2’	—	—	—	—
3’	6.87 *d* (7.6)	7.27 *m*	6.87 *d* (8.1)	7.29 *m*

Proton	1	2	3	4
4’	7.24 *m*	7.33 *m*	7.24 *m*	7.34 *m*
5’	6.85 *d* (7.6)	7.31 *m*	6.84 *d* (8.1)	7.32 *m*
6’	—	7.33 *m*	—	7.34 *m*
7’	6.82 *dd* (4.4, 2.0)	7.27 *m*	6.82 *dd* (4.5, 2.0)	7.29 *m*
8’	3.80 *s*	—	3.80 *s*	—
1	—	—	—	—
2	2.21 *t* (7.6)	2.20 *t* (7.6)	2.21 *t* (7.6)	2.22 *t* (7.6)
3	1.64 *m*	1.64 *m*	1.65 *m*	1.65 *m*
8	2.01 *q* (6.9)	2.01 *q* (6.5)	—	—
9	5.34 *ddt* (5.9, 3.5, 1.7)	5.35 *td* (4.7, 3.9, 1.4)	—	—
10	5.34 *ddt* (5.9, 3.5, 1.7)	5.35 *td* (4.7, 3.9, 1.4)	—	—
11	2.01 *q* (6.9)	2.01 *q* (6.5)	—	—
16	—	—	0.87 *t* (6.9)	0.88 *t* (7.0)
17	—	—	—	—
18	0.88 *t* (6.9)	0.88 *t* (6.7)	—	—
N-H	5.69 *br s*	5.84 *br s*	5.74 *s*	5.73 *br s*
Other protons	1.20-1.37 *m* (H-4—H-7, H-12—H-17, 10×CH_2)	1.21-1.39 *m* (H-4—H-7, H-12—H-17, 10×CH_2)	1.14-1.39 *br s* (H-4—H-15, 12×CH_2)	1.14-1.39 *br s* (H-4—H-15, 12×CH_2)

[a] Spectra for compound 1–2 were recorded at 500 MHz in $CDCl_3$, and spectra for compound 3–4 were recorded at 300 MHz in $CDCl_3$
[b] Multiplicities of protons were determined by DEPT 45°, 90°, 135°, COSY, HSQC and HMBC spectra.

Table 3. ^{13}C NMR spectral data (δ in ppm) of compound 1-4 (300 MHz or 125 MHz, $CDCl_3$)[a].

Carbon	1	2	3	4
1’	43.57	43.61	43.57	43.64
2’	139.99	138.47	139.97	138.32
3’	120.03	127.81	120.02	127.84
4’	129.75	128.70	129.75	128.73
5’	113.40	127.48	113.38	127.54
6’	159.91	128.70	159.90	128.73
7’	112.98	127.81	112.98	127.84
8’	55.24	—	55.23	—
1	172.96	173.03	173.07	173.13
2	36.86	36.82	36.84	36.84
3	25.79	25.79	25.80	25.79
8	27.23	27.20	—	—
9	129.85	129.85	—	—
10	129.94	129.94	—	—
11	27.80	27.24	—	—
14	—	—	31.94	31.93
15	—	—	22.70	22.70
16	31.79	31.80	14.13	14.14
17	22.69	22.68	—	—
18	14.12	14.11	—	—
Other carbon	28.97-29.83 (C-4—C-7, C-12—C-17, 10×CH_2)	28.94-29.87 (C-4—C-7, C-12—C-17, 10×CH_2)	29.30-29.80 (C-4—C-13, 10×CH_2)	29.16-29.92 (C-4—C-13, 10×CH_2)

[a] Spectra for compound 1–2 were recorded at 125 MHz in $CDCl_3$, and spectra for compound 3–4 were recorded at 300 MHz in $CDCl_3$.

3. Results

The PE extracts and PE-soluble portion of the ethanol extracts of *L. meyenii* hypocotyls were separated by column chromatography and preparative RP-HPLC system, to give seventeen macamides (Table 1). Four of them were purified, namely compound 1-4 (Figure 1). The structures of these four compounds were established by HRMS and NMR experiments, including ^1H, ^{13}C, ^1H-^1H COSY and NOESY, ^1H-^{13}C HMBC and HSQC, DEPT45°, 90°, 135°, and ^1H-^{15}N HMBC experiments.

Compound 1 was isolated as white powder. The HRMS spectra displayed a molecular formula of $C_{26}H_{43}NO_2$, indicating six degrees of unsaturation. The ^1H NMR spectra showed four aromatic protons (δ_H 6.82-7.24, 4H) (Table 2) which were correlated to four carbons at δ_C 129.75, 120.03, 113.40, and 112.98 (Table 3) in ^1H-^{13}C HMBC and HSQC experiments, suggesting the presence of disubstituted benzene ring. The ^1H signals at δ_H 3.80 (3H, *s*) and ^{13}C signals at δ_C 55.24 were corresponded to a methoxyl group. The protons of methoxyl group and a carbon at δ_C 159.91 (C-6’) showed $^3J_{C\text{-}H}$ correlation in DEPT and HSQC spectra, indicating that the methoxyl group was connected to the benzene ring. The ^1H signals at δ_H 5.69 (*br*, *s*, H) and ^{13}C signals at δ_C 172.96 (C-1) suggested that compound 1 had secondary amide group. The ^1H-^{15}N HMBC spectra showed that the nitrogen at δ_N 119.22 and two protons at δ_H 4.42 (H-1’, *d*, *J*=6.7 Hz) had $^2J_{N\text{-}H}$

coupling relationship, indicating that a methylene group was connected to the nitrogen of the amide bond. The combination of HMBC and ^{1}H-^{1}H COSY experiments showed that the secondary amide group had coupling relationships with a carbon at δ_C 139.99 (C-2'), two protons at δ_H 4.42 (H-1'), and two protons at δ_H 2.21 (H-2). These data showed that compound 1 featured an *N*-(3-methoxybenzyl) amide moiety.

The benzene ring and amide group accounted for five unsaturation degrees. Therefore, compound 1 might have a double bond. The ^{1}H NMR spectra showed two cis form olefinic protons at δ_H 5.34 (H-9 and 10, *ddt*, *J*=5.9, 3.5, 1.7 Hz) and two methylene groups at δ_H 2.01 (H-8 and 11, *q*, *J*=6.9 Hz), and they had coupling in the COSY, indicating the presence of cis double bond. In ^{1}H-^{13}C HSQC and HMBC, DEPT 90° and 135° spectra, two methine groups and two methylene groups exhibited strong correlations, further confirming the presence of cis double bond. The presence of cis double bond was also verified by signal of NOE correlations between H-9 and H-8, H-7, as well as H-10 and H-11, H-12 (Figure 2) in ^{1}H-^{1}H NOESY spectra.

The ^{1}H NMR spectra showed a terminal methyl group at δ_H 0.88 (*t*, *J*=6.9 Hz), and the terminal methyl group was coupled with a group of ten protons at δ_H 1.20-1.37 (*m*) in ^{1}H-^{1}H COSY experiment. In HMBC experiment, a methylene group (H-2, δ_H 2.21, *t*, *J*=7.6 Hz) was found to be adjacent to the carbonyl group (C-1, δ_C, 172.96) and linked to another methylene group (H-3, δ_H 1.64, *m*). According to ^{13}C NMR and DEPT spectra, the alkyl moiety had fourteen methylene groups, two methine groups, and a methyl, and compound 1 had a straight alkyl chain with a cis double bond. According to all information and data, an alkyl moiety of $-CH_2CH_2(CH_2)_4CH_2CH=CHCH_2(CH_2)_6CH_3$ was determined.

In the ^{1}H-^{1}H NOESY, a medium resonance between the proton at δ_H 5.69 (N-H) and the proton at δ_H 2.21 (H-2) appeared, indicating that the alkyl moiety was connect to the *N*-(3-methoxybenzyl) amide moiety. The UV spectra exhibited absorption at 212 and 273 nm, which were correlated to the double bond and benzene ring, respectively. The FT-IR spectra showed strong absorption bands at 3302, 1642, 1539, and 1466 cm^{-1}, which were attributed to N-H, amide bond, and benzene ring. From the aforementioned spectroscopic data, compound 1 was unquestionably assigned as *N*-3-methoxybenzyl-9Z-octadecenamide.

Compound 2 was obtained as white powder, and its HRMS spectra displayed a molecular formula of $C_{25}H_{42}NO$, indicating six degrees of unsaturation. The ^{1}H signals at δ_H 7.33 (H-6' and 4', *m*), 7.31 (H-5', *m*), 7.27 (H-7' and 3', *m*) (Table 2) and ^{13}C signals at δ_C 138.47 (C-2'), 128.70 (C-6' and 4'), 127.81 (C-7' and 3') (Table 3) suggested that compound 2 had a monosubstituted benzene ring. The DEPT 90° and HSQC spectra confirmed that the aromatic protons were correlated to the aromatic carbons. The ^{13}C signals at δ_C 173.03 and a broad singlet at δ_H 5.84 (N-H, *br*, *s*) were found to be same as compound 1, suggesting the presence of an amide bond. The ^{1}H-^{13}C HMBC experiment showed that aromatic carbons at δ_C 138.47, 127.81 (C-2', 3', and 7') and two protons at δ_H 4.43 (H-1') had $^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$ correlations, indicating that a methylene group was connected to the benzene ring. The ^{1}H-^{1}H COSY spectra showed a strong correlation between the methylene protons and N-H protons, which demonstrated that compound 2 had an *N*-benzyl amide fragment.

The comparison of the ^{1}H NMR, ^{13}C NMR, DEPT 90° and 135° spectra between compound 1 and 2 suggested that compound 2 had same straight alkyl chain as that of compound 1. In ^{1}H-^{13}C HSQC spectra, two protons at δ_H 5.35 (H-9 and 10, *td*, *J*=4.7, 3.9, 1.4 Hz) were correlated to two carbons at δ_C 129.85-129.94 (C-9 and 10), suggesting the presence of a cis double bond. The ^{2}J correlation between two methine groups and two methylene groups (H-8 and 11, δ_H 2.01, J=5.36 Hz) in ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC experiments further confirmed the presence of cis double bond. This cis double bond configuration was also identified by NOE interaction between H-9 and H-8, H-7, as well as H-10 and H-11, H-12 (Figure 2) from ^{1}H-^{1}H NOESY experiment. According to COSY and HMBC spectra, the linear alkyl chain was connected to the *N*-benzyl amide fragment as compound 1. From the aforementioned spectroscopic data, compound 2 was unambiguously established as *N*-benzyl-9Z-octadecenamide.

Compound 3 was purified as white powder. The HRMS spectra indicated a molecular formula of $C_{24}H_{42}NO$, suggesting that compound 3 exhibited five degrees of unsaturation. The ^{1}H signals at δ_H 7.24 (*m*), 6.87 (*d*, *J*=8.1 Hz), 6.84 (*d*, *J*=8.1 Hz), 6.82 (*dd*, *J*=4.5, 2.0 Hz), 5.74 (s), 4.42 (*d*, *J*=5.7 Hz), 3.80 (*s*) (Table 2) and ^{13}C signals at δ_C 159.90-112.98 (C×6), 173.07, 55.23, 43.57 (Table 3) demonstrated that compound 3 had same *N*-3-methoxybenzyl amide fragment as that of compound 1. In addition, the carbonyl group was also conjugated to the linear alkyl chain in the same way. The ^{1}H NMR spectra displayed a methyl group at δ_H 0.87 (*t*, *J*=6.9 Hz), a methylene group at δ_H 2.21 (*t*, *J*=7.6 Hz), and a methylene group at δ_H 1.65 (*m*). Then, a broad resonance region that contains 24 protons appeared in 1.14-1.39 ppm, suggesting the presence of twelve methylene groups. Based on the above inference, ^{13}C NMR spectra, and literature [7], compound 3 was identified as *N*-(3-methoxybenzyl)-hexadecanamide.

Compound 4 was obtained as white powder, and it possessed a molecular formula of $C_{23}H_{40}NO$ as determined by HRMS spectra, indicating five degrees of unsaturation. The ^{1}H signals of alkyl fragment at δ_H 0.88 (*t*, *J*=7.0 Hz), 1.14-1.39 (CH_2×12, *br*, *s*), 1.62 (*m*), and 2.22 (*t*, *J*=7.6 Hz) (Table 2) were same as compound 3, suggesting that compound 4 had same straight alkyl chain moiety of $-CH_2(CH_2)_{13}CH_3$ as compound 3. The

^{1}H signals at δ_H 7.29 (CH×2, *m*), 7.32 (CH, *m*), 7.34 (CH×2, *m*), and 4.44 (*d*, *J*=5.7 Hz) were same as compound 2, indicating that compound 4 had a benzyl group. The ^{1}H NMR and ^{13}C NMR (Table 3) spectra of 4 also showed the signals of amide proton (N-H) at δ_H 5.73 (*br*, *s*) and carbonyl carbon at δ_C 173.13 as compound 1-3. Base on the above data and literature [6, 7], the structure of compound 4 was established as *N*-benzyl-hexadecanamide.

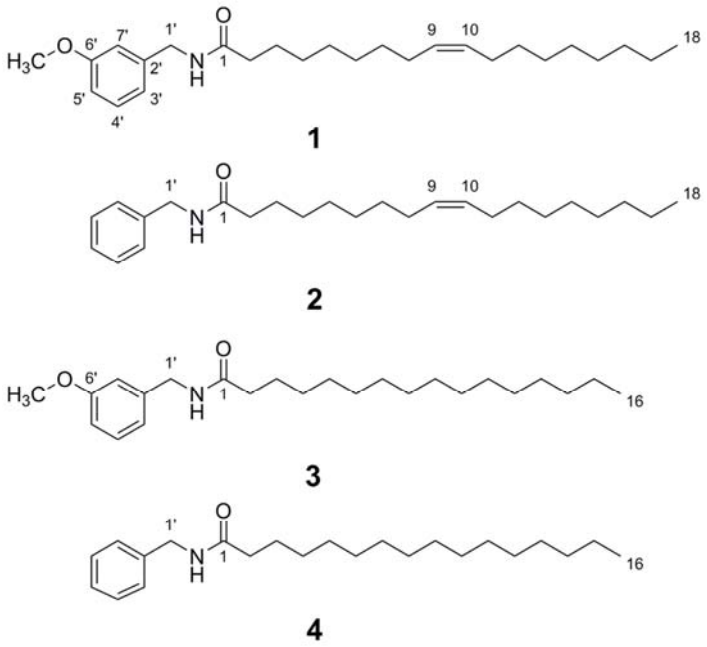

Figure 1. Molecular structures of compound 1-4 from maca planted in China.

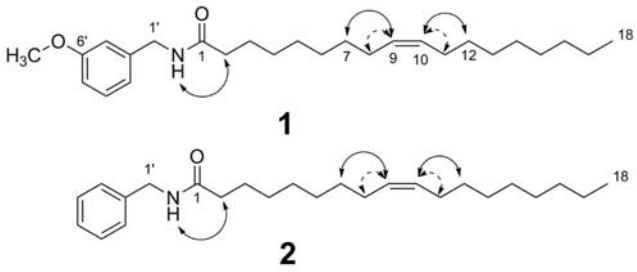

Figure 2. 2D NMR ^{1}H-^{1}H NOESY correlations for compound 1 and 2 Supporting Information for *N*-Benzylated Alkamides Isolated from Maca Planted in China

4. Discussion

Compound 3 and 4 have been isolated from maca in the previous studies [6, 7]. Compound 1 and 2 were identified by HPLC-UV-MS/MS [5], and we appear to be the first to isolate them from maca. In the separation by column chromatography over silica gel, two main fractions of macamides were obtained in different rate of mobile phase and their spots in TLC were very close, indicating two categories of macamides in maca. This was further confirmed by LC-MS that seventeen macamides in this study could be classified into two categories, one had *N*-benzylamide fragment (m/z: 91), and another had *N*-(3-methoxy)-benzylamide fragment (m/z: 121).

5. Conclusion

In conclusion, seventeen N-benzylated alkamides in maca from Wenbi Mountain were identified by LC-MS and four of them were successfully isolated. The chemical structures of these four compounds were established by spectroscopic and spectrometric methods including ^{1}H NMR, ^{13}C NMR, 2D NMR, ESI-HRMS, UV-Vis, FT-IR, and ^{1}H-^{15}N HMBC experiments. The results show that these four compounds were *N*-3-methoxybenzyl-9Z-octadecenamide (1), *N*-benzyl-9Z-octadecenamide (2), *N*-(3-methoxybenzyl)-hexadecanamide (3), and *N*-benzyl-hexadecanamide (4). These compounds may serve as the marker compounds for the quality control of *Lepidium meyenii* planted in China.

Appendix

The 2D NMR spectra of compound 1 and 2 are available in the following part.

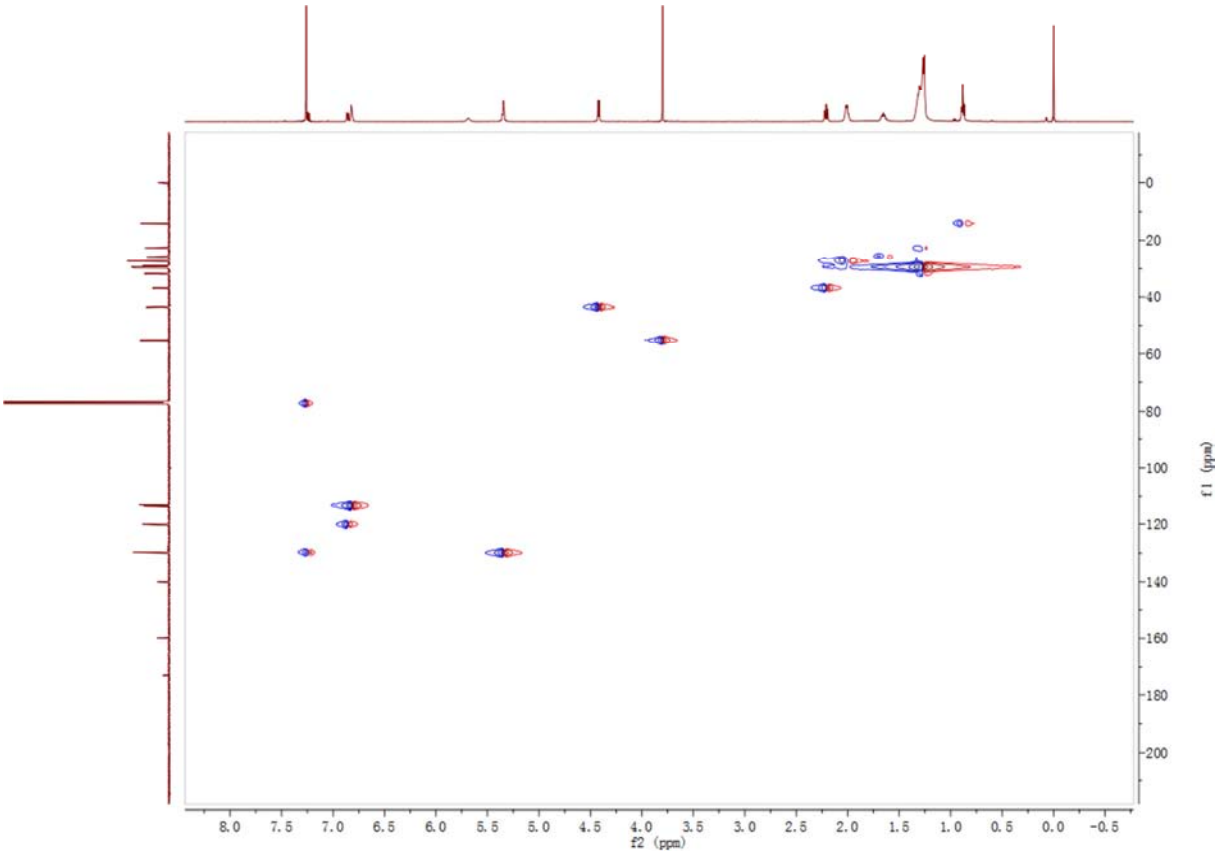

Figure 3. The ^{1}H-^{13}C HSQC spectra of the compound 1.

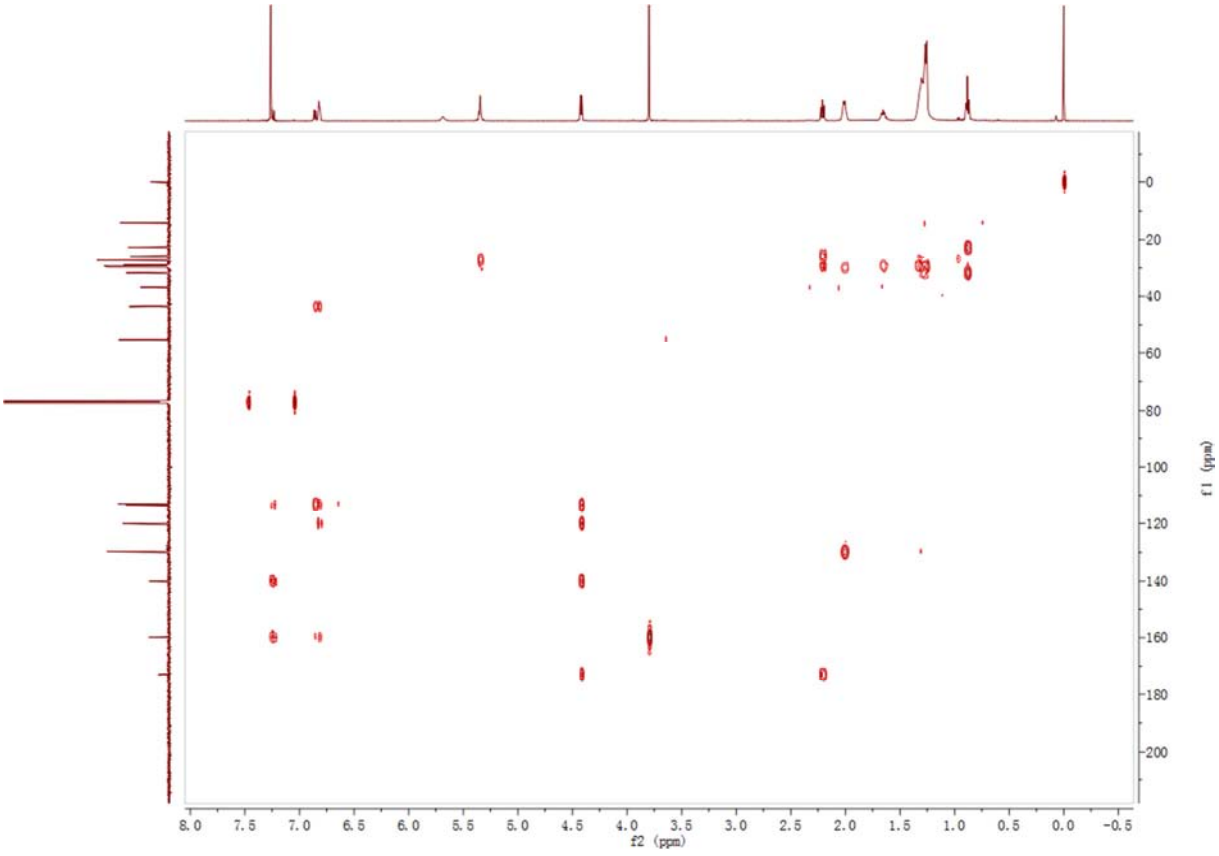

Figure 4. The ^{1}H-^{13}C HMBC spectra of the compound 1.

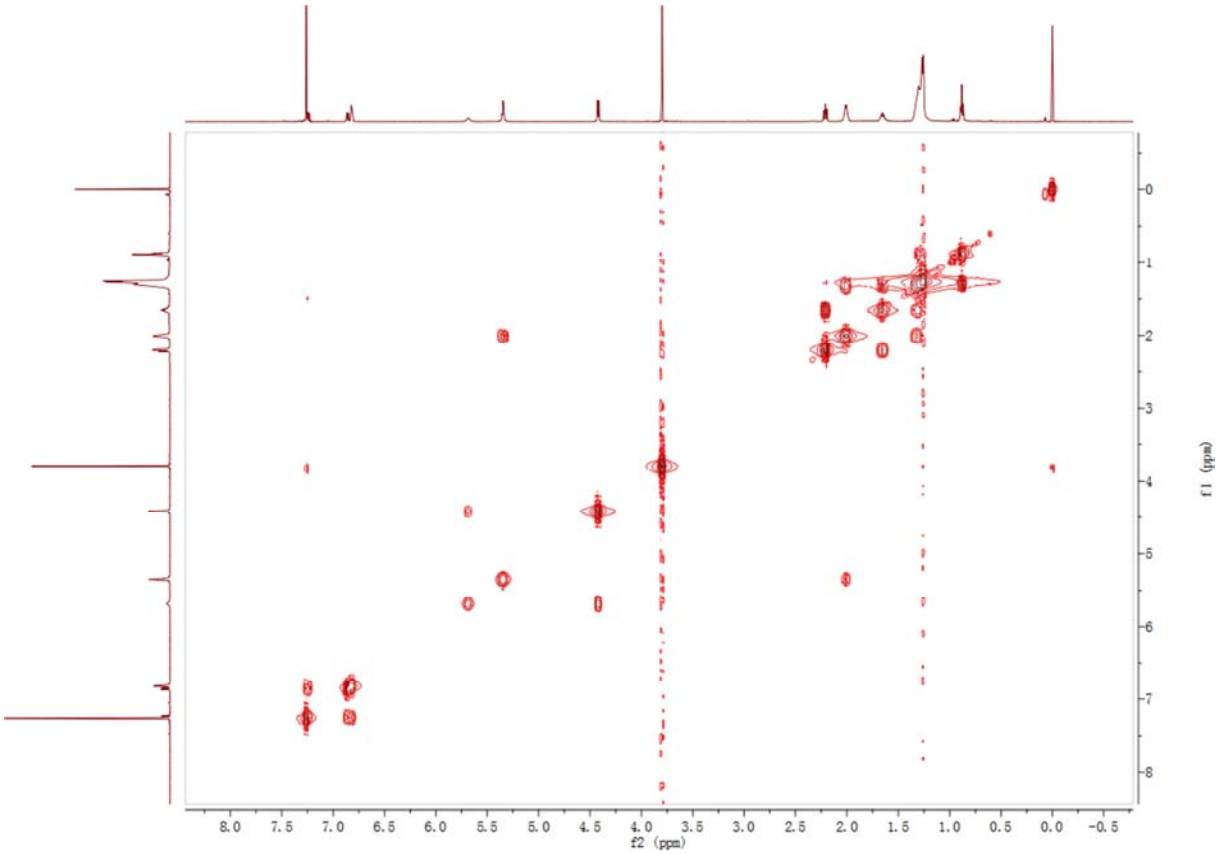

Figure 5. The ^{1}H-^{1}H COSY spectra of the compound 1.

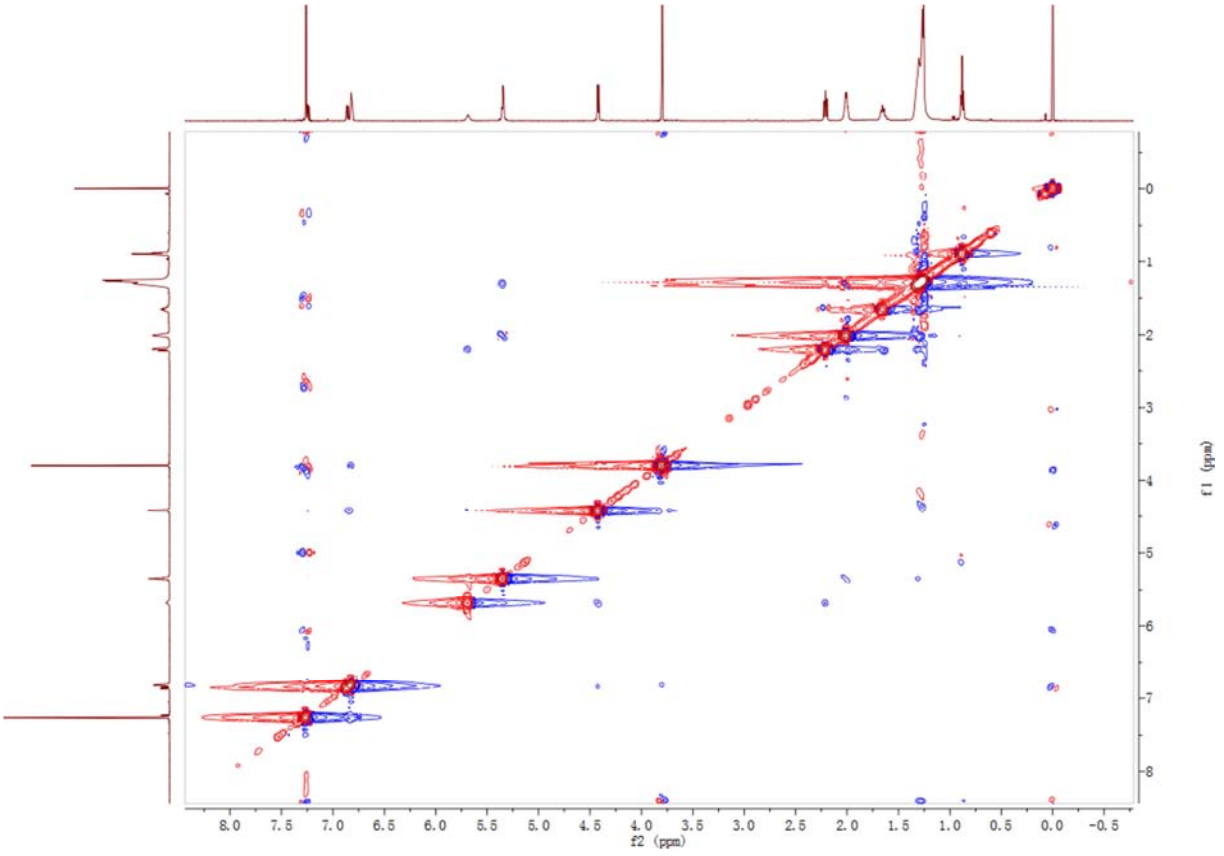

Figure 6. The ^{1}H-^{1}H NOESY spectra of the compound 1.

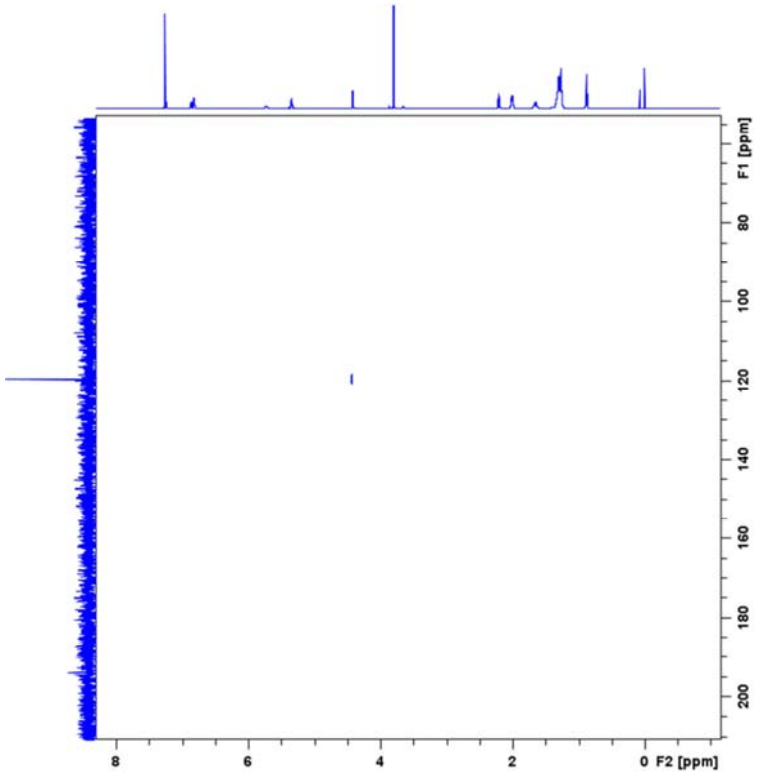

Figure 7. The ^{1}H-^{15}N HMBC spectra of the compound 1.

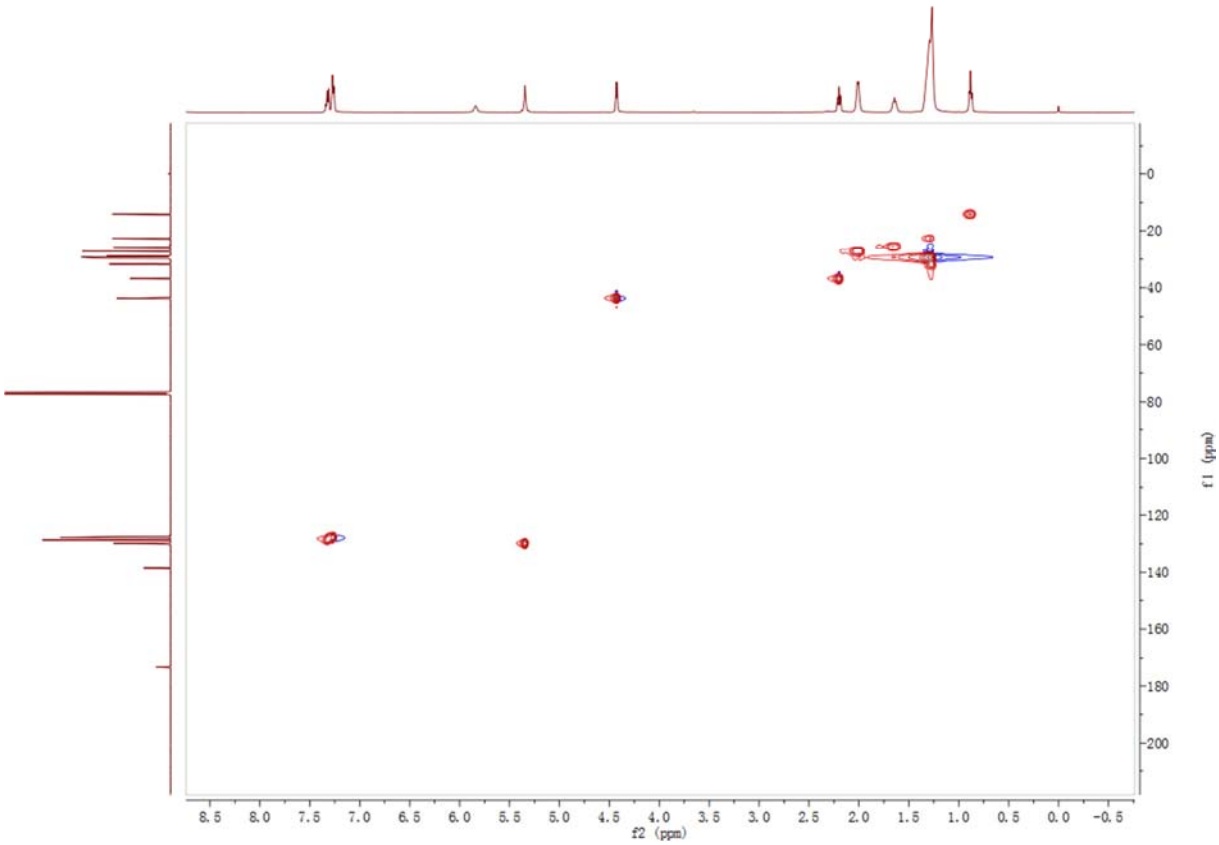

Figure 8. The ^{1}H-^{13}C HSQC spectra of the compound 2.

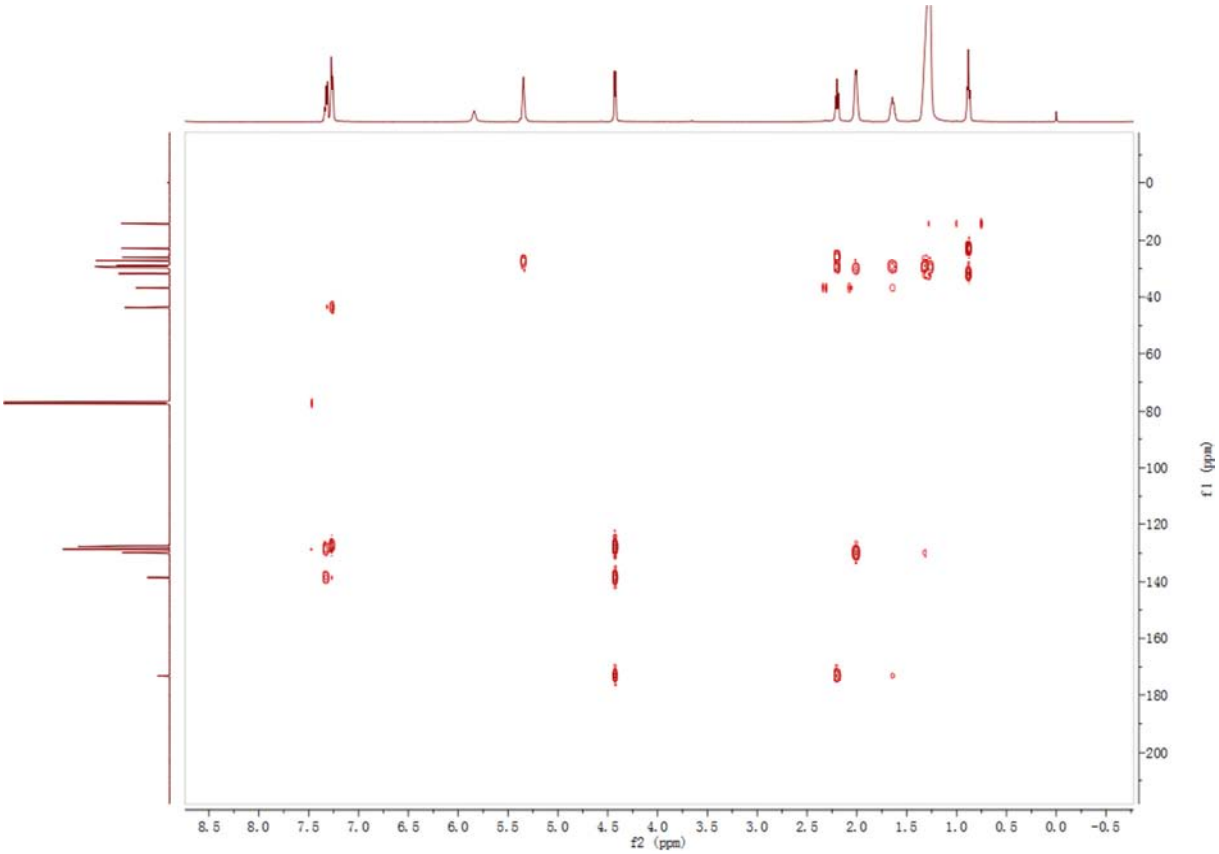

Figure 9. The ^{1}H-^{13}C HMBC spectra of the compound 2.

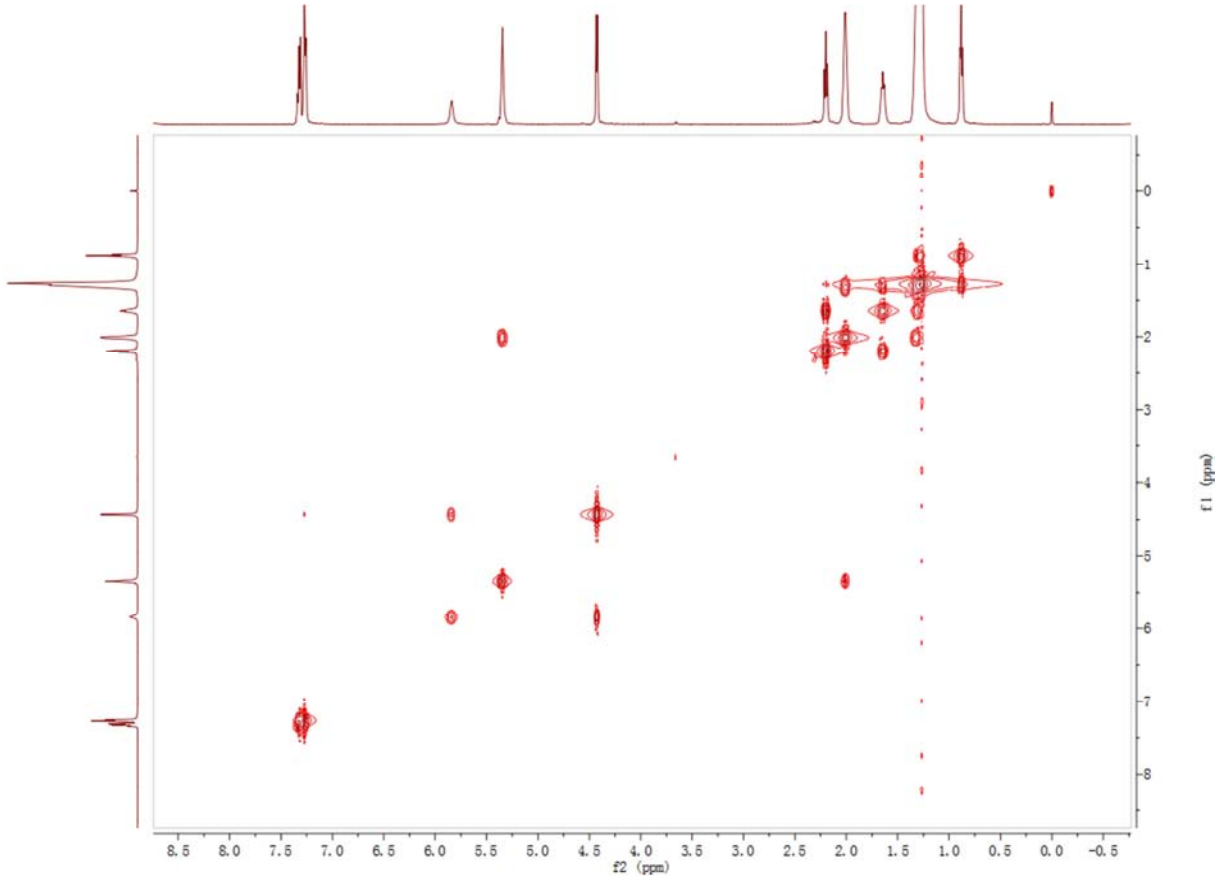

Figure 10. The ^{1}H-^{1}H COSY spectra of the compound 2.

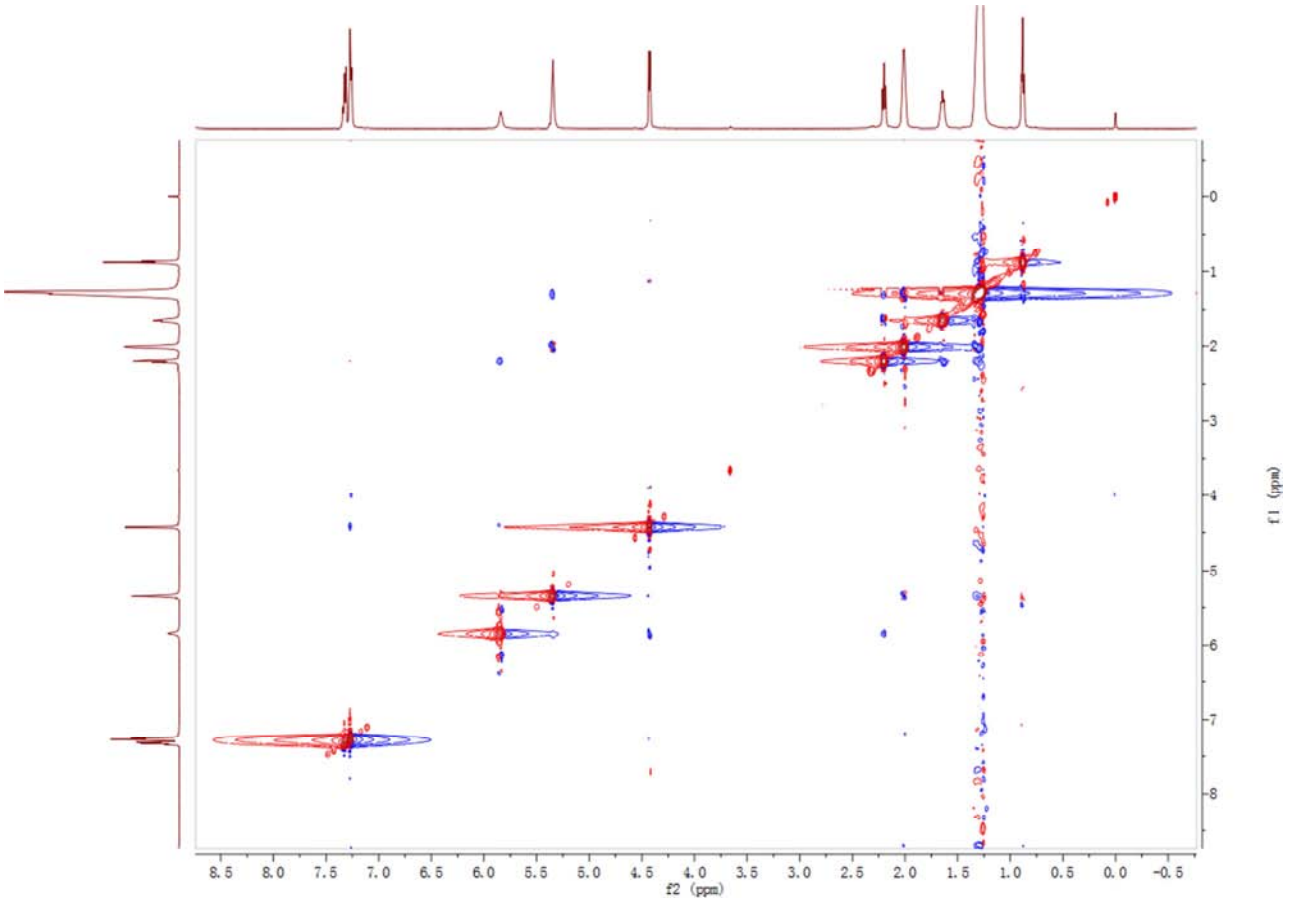

Figure 11. The ^{1}H-^{1}H NOESY spectra of the compound 2.

Author Contributions

Jing Gan and Chuan He contributed equally to this work.

Conflict of Interest

The authors declare no conflict of interest.

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