HETEROCYCLES, Vol. 65, No. 5, 2005, pp. 1215 - 1220
Received, 20th January, 2005, Accepted, 24th February, 2005, Published online, 1st March, 2005

TOTAL SYNTHESIS AND ANTIOXIDATIVE ACTIVITY OF MAGNOLAMIDE FROM *MAGNOLIA COCO*

Wen-Fei Chiou,[a] Chien-Chang Shen,[a] Hsi-Jung Yu,[b] Chang-Han Chiang,[b] Ching-Chuan Chen,[b] Wenyi Chang,[a] and Ming-Jaw Don[a]*

[a]National Research Institute of Chinese Medicine, 155-1, Section 2, Li-Nung Street, Taipei 112, Taiwan, Republic of China
E-mail: mjdon@nricm.edu.tw
[b]Department of Chemistry, Chinese Culture University, 55, Hwa-Kang Road, Yang-Ming-Shan, Taipei 111, Taiwan, Republic of China

Abstract – A simple total synthesis of naturally occurring magnolamide, *N*-[4-(2-formyl-5-hydroxymethylpyrrol-1-yl)butyl]-3-(4-hydroxy-3-methoxy-phenyl)-2-propenamide, from pyrrole-2,5-dicarbaldehyde was performed in six steps and its antioxidant activity was compared with probucol and resveratrol. Results showed that magnolamide markedly inhibited $CuSO_4$-induced oxidation of human low-density lipoprotein (IC_{50} = 9.7 μM) with similar activity to probucol and resveratrol.

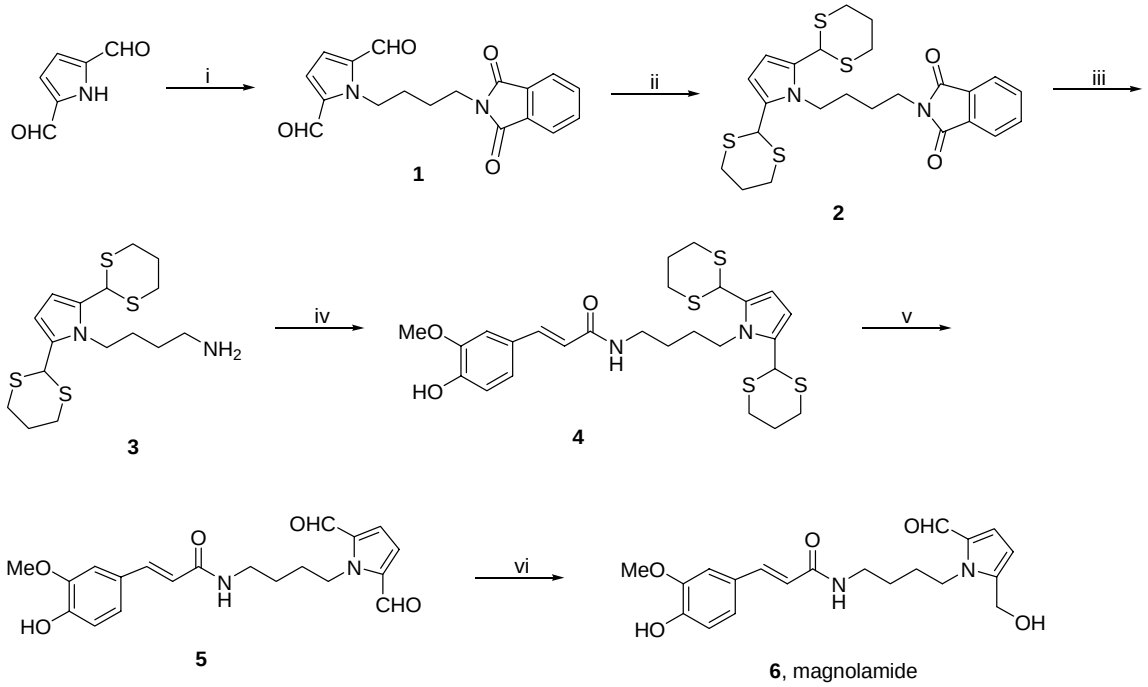
INTRODUCTION

Magnolamide, a phenolic amide, was isolated and characterized from the methanolic extract of the leaves of *Magnolia coco* (Magnoliaceae).[1] The stems and leaves of *M. coco* are used as a Chinese herbal medicine against liver disease. Magnolamide was the first naturally occurring amide that has a [(2-formyl-5-hydroxymethyl)pyrrolyl]butylamine moiety as an amine unit, and its biological activity has not been reported yet. According to the literature reports, hydroxylated and/or methoxylated cinnamic acids conjugated with different kinds of amine (putrescine,[2] spermidine,[2] spermine,[2] tyramine,[2-5] serotonine[6,7]) were found from various plants. A number of amides are known to have biological activities such as inhibitory effects against platelet aggregation,[5] antioxidative activity,[6,8] and inhibition of *in vitro* prostaglandin biosynthesizing enzyme (PG synthetase).[9] In order to obtain more material for further study of the biological activities, we were prompted to investigate the synthesis and antioxidative activity of magnolamide. Herein, we wish to report a facile preparation of magnolamide, the structure of

which was established by spectroscopic analysis and by comparison with published data of the natural product. The inhibitory effect of magnolamide on $CuSO_4$-induced human low-density lipoprotein (LDL) oxidation was also discussed.

RESULTS AND DISCUSSION

The synthetic strategy shown in Scheme 1 was employed for the preparation of magnolamide. The starting pyrrole-2,5-dicarbaldehyde was prepared from pyrrole-2-carbaldehyde by a known literature procedure.[10] *N*-Alkylation of pyrrole-2,5-dicarbaldehyde with *N*-(4-bromobutyl)phthalimide in the presence of K_2CO_3 in acetonitrile gave pyrrole derivative (**1**). Two formyl groups on pyrrole ring of **1** were protected with 1,3-propanedithiol in the presence of anilinium chloride in MeOH to yield 1,3-dithiane derivative (**2**), which was then hydrolyzed with hydrazine hydrate to afford the amine derivative (**3**) in good yield. Condensation of **3** with 4-hydroxy-3-methoxycinnamic acid in the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) and triethylamine in DMF gave amide (**4**).[8] Treatment of **4** with HgO and $HgCl_2$ to remove the dithiane protection regenerated the formyl group on pyrrole ring to give **5**, which was reduced to magnolamide (**6**) using $NaBH_4$ in 92% yield.



Scheme1. Reagents and conditions: (i) *N*-(4-bromobutyl)phthalimide, K_2CO_3, MeCN, reflux; (ii) 1,3-propanedithiol, anilinium chloride, MeOH; (iii) hydrazine hydrate, THF, reflux; (iv) 4-hydroxy-3-methoxycinnamic acid, Et_3N, BOP, CH_2Cl_2; (v) $HgCl_2$, HgO, $MeCN/H_2O$; (vi) $NaBH_4$, MeOH.

The antioxidative activities of magnolamide (**6**) and its analogue (**5**) were evaluated in the inhibitory effects of $CuSO_4$-induced LDL lipid oxidation, and results are shown in Table 1. Both compounds

inhibited conjugated diene formation in a concentration-dependent manner with IC_{50} values of 9.7 and 16.9 μM, respectively. Resveratrol and probucol were used as two reference antioxidants. Results showed that the antioxidative activity of magnolamide was similar to that of probucol and resveratrol.

Table 1. The IC_{50} value of **5** and **6** in the inhibition of Cu^{2+}-induced LDL lipid oxidation.

	5	**6**	resveratrol	probucol
IC_{50} (μM)	16.9±2.3	9.7±2.8	13.1±2.6	8.7±1.4

In conclusion, we have presented here a simple total synthesis of magnolamide in six steps from pyrrole-2,5-dicarbaldehyde with an overall yield of 34**%**. Spectroscopic data of the synthesized product were identical with those of the natural product (**6**).[1] Magnolamide showed significant inhibitory effect in Cu^{2+}/O_2-induced LDL lipid peroxidation. Synthesis of other analogues of magnolamide is in progress with an aim to further improve the antioxadative activity.

EXPERIMENTAL

Melting points were determined with a Yanaco micromelting point apparatus and are uncorrected. IR spectra were obtained on a Nicolet Avatar 320 FTIR spectrophotometer. NMR spectra were recorded on a Varian Unity Inova-500 and Gemini-200 spectrometers. Chemical shifts are reported in parts per million (δ) units relative to internal tetramethylsilane. The EIMS spectra were measured from a Finnigan GCQ GC/MS spectrometer at 30 eV. HREIMS was recorded on a Finnigan MAT 95S mass spectrometer. Elemental analyses were performed on Perkin-Elmer CHN-2400 analyzer. Column chromatography was performed with E. Merck 230-400 mesh silica gel.

1-[4-(1,3-Dioxoisoindolin-2-yl)butyl]-1*H*-pyrrole-2,5-dicarbaldehyde (1). To a solution of pyrrole-2,5-dicabaldehyde (1.58 g, 12.8 mmol) in acetonitrile (60 mL) were added *N*-(4-bromobutyl)phthalimide (4.0 g, 14.2 mmol) and K_2CO_3 (3.0 g, 21.7 mmol), and the mixture was refluxed for overnight. After cooling, the solution was filtered and evaporated. The residue was taken in chloroform (100 mL) and washed with water (100 mL). The aqueous layer was extracted once again with $CHCl_3$ (100 mL). The combined organic extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, and evaporated. The residue was recrystallized from $CHCl_3$/hexane to give **1** (3.32 g, 80 %) as a white solid: mp 195-197 °C; IR (KBr) 2938, 1767, 1717, 1670, 1397, 1199, 1051, 724 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.66-1.72 (m, 2H), 1.76-1.82 (m, 2H), 3.67 (t, *J* = 7.0 Hz, 2H), 4.78 (t, *J* = 7.0 Hz, 2H), 6.93 (s, 2H), 7.67-7.70 (m, 2H), 7.79-7.82 (m, 2H), 9.78 (s, 2H); EIMS *m/z* (%) 324 (57) [M^+], 295 (68), 148 (96), 136 (100), 120 (56); *Anal.* Calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.43; H, 4.94; N, 8.58.

2-{4-[2,5-Di(1,3-dithian-2-yl)-1*H*-pyrrol-1-yl]butyl}isoindoline-1,3-dione (2). To a solution of **1** (3.30

g, 10.2 mmol) in MeOH (100 mL) were added 1,3-propanedithiol (2.32 g, 21.4 mmol) and anilinium chloride (70 mg), and the mixture was stirred at rt for 3 h. The solution was then filtered and evaporated. The residue was taken in chloroform (100 mL) and washed with water (100 mL). The organic layer was dried over Na_2SO_4, and evaporated. The residue was recrystallized from $CHCl_3$/hexane to give **2** (4.83 g, 94 %) as a white solid: mp 183-185 °C; IR (KBr) 1772, 1717, 1396, 1363, 1036, 721 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.78-1.91 (m, 6H), 2.08-2.13 (m, 2H), 2.77-2.81 (m, 4H), 3.01-3.06 (m, 4H), 3.76 (t, *J* = 6.5 Hz, 2H), 4.06 (t, *J* = 7.0 Hz, 2H), 5.15 (s, 2H), 6.24 (s, 2H), 7.68-7.71 (m, 2H), 7.80-7.83 (m, 2H); EIMS *m/z* (%) 504 (100) [M^+], 430 (30), 398 (42), 385 (26), 324 (16), 119 (18); *Anal.* Calcd for $C_{24}H_{28}N_2O_2S_4$: C, 57.11; H, 5.59; N, 5.55; S, 25.41. Found: C, 56.85; H, 5.71; N, 5.47; S, 25.54.

4-[2,5-Di(1,3-dithian-2-yl)-1*H*-pyrrol-1-yl]butyl-1-amine (3). To a solution of **2** (2.50 g, 4.96 mmol) in THF (100 mL) was added hydrazine hydrate (0.6 g, 12.0 mmol), and the mixture was refluxed for overnight. After the mixture was cooled, the pH of the mixture was adjusted to 4.0 with 1N HCl, and the precipitate was then filtered. The filtrate was evaporated to small amount under reduced pressure and adjusted pH to 9.0 with 2N NaOH solution. The resulting solution was extracted with chloroform three times. The combined extracts were washed with water, dried over Na_2SO_4, and concentrated. The residue was recrystallized from $CHCl_3$/hexane to give **3** (1.60 g, 86 %) as a white solid, which was used in the next step without further purification: mp 121-122 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.49-1.60 (m, 2H), 1.80-2.17(m, 6H), 2.73-3.09 (m, 10H), 4.03 (t, *J* = 8.0 Hz, 2H), 5.16 (s, 2H), 6.26 (s, 2H); EIMS *m/z* (%) 374 (25) [M^+], 255 (100), 119 (72).

(*E*)-*N*-{4-[2,5-Di(1,3-dithian-2-yl)-1*H*-pyrrol-1-yl]butyl}-3-(4-hydroxy-3-methoxyphenyl)-2-propenamide (4). To a stirred solution of 4-hydroxy-3-methoxycinnamic acid (388 mg, 2.0 mmol) in DMF (8 mL) and Et_3N (0.28 mL) cooled to 0 °C were added **3** (860 mg, 2.3 mmol) and a solution of BOP (884 mg, 2.0 mmol) in CH_2Cl_2 (8 mL). The mixture was stirred at 0 °C for 30 min and then at rt for 2 h. The solution was poured into a mixture of H_2O (100 mL) and $CHCl_3$ (100 mL). The layer was separated, and the aqueous phase was extracted with $CHCl_3$. The combined organic extracts were washed successive with 1 N HCl, water, 1N $NaHCO_3$, water, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel chromatography eluting with 5% acetone in $CHCl_3$ to give **4** as a white solid (890 mg, 81%): mp 192-194 °C (recrystallized from $CHCl_3$); IR (KBr) 3302, 2935, 1688, 1658, 1513, 1272, 1183, 816 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.61-2.15 (m, 8H), 2.77-3.09 (m, 8H), 3.42 (q, *J* = 6.2 Hz, 2H), 3.89 (s, 3H), 4.07 (t, *J* = 7.6 Hz, 2H), 5.15 (s, 2H), 5.84 (br t, 1H, NH), 6.20 (d, *J* = 15.6 Hz, 1H), 6.28 (s, 2H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.95 (d, *J* = 1.6 Hz, 1H), 7.07 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.50 (d, *J* = 15.6 Hz, 1H); EIMS *m/z* (%) 550 (40) [M^+], 431 (100), 251 (97), 177 (63); *Anal.* Calcd for $C_{26}H_{34}N_2O_3S_4$: C, 56.69; H, 6.22; N, 5.09; S 23.29. Found: C, 56.46; H, 6.16; N, 5.09; S, 23.12.

(*E*)-*N*-[4-(2,5-Diformyl-1*H*-pyrrol-1-yl)butyl]-3-(4-hydroxy-3-methoxyphenyl)-2-propenamide (5).

To a solution of **4** (750 mg, 1.36 mmol) in acetonitrile/H_2O (85:15, 50 mL) were added HgO (1.20 g, 5.44 mmol) and $HgCl_2$ (1.50 g, 5.44 mmol), and the mixture was stirred at rt for 2 h. The solution was then filtered and evaporated. The residue was purified by silica gel chromatography eluting with 3% methanol in CH_2Cl_2 to give **5** as a white solid (360 mg, 71%): mp 141-143 °C (recrystallized from methanol); IR (KBr) 3317, 2954, 2930, 1650, 1619, 1548, 1513, 1269, 1218, 1125, 971, 790 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.61-1.65 (m, 2H), 1.75-1.80 (m, 2H), 3.43 (t, *J* = 6.5 Hz, 2H), 3.87 (s, 3H), 4.73 (t, *J* = 7.5 Hz, 2H), 6.01 (s, 1H, OH), 6.24 (br t, 1H, NH), 6.27 (d, *J* = 15.5 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 6.95 (s, 2H), 6.95 (d, *J* =1.5 Hz, 1H), 7.03 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.50 (d, *J* = 15.5 Hz, 1H), 9.79 (s, 2H); EIMS *m/z* (%) 370 (100) [M^+], 177 (61), 165 (60), 149 (99), 135 (73), 121 (40); *Anal.* Calcd for $C_{20}H_{22}N_2O_5$•1/2 CH_3OH: C, 63.73; H, 6.22; N, 7.25. Found: C, 63.69; H, 6.54; N, 6.95.

(*E*)-*N*-[4-(2-Formyl-5-hydroxymethyl-1*H*-pyrrol-1-yl)butyl]-3-(4-hydroxy-3-methoxyphenyl)-2-propenamide (Magnolamide, 6). To a solution of **5** (290 mg, 0.78 mmol) in MeOH (20 mL) was added $NaBH_4$ (14.4 mg, 0.39 mmol), and the mixture was stirred at rt for 15 min. The solution was then evaporated, and the residue was purified by silica gel chromatography eluting with 5% acetone in $CHCl_3$ to give **6** (267 mg, 92%, hygroscopic) as a colorless oil: IR (neat) 3419, 1650, 1515, 1272, 773 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 1.56-1.62 (m, 2H), 1.76-1.82 (m, 2H), 3.31 (t, *J* = 7.0 Hz, 2H), 3.86 (s, 3H), 4.38 (t, *J* = 7.5 Hz, 2H), 4.62 (s, 2H), 6.24 (d, *J* = 4.0 Hz, 1H), 6.39 (d, *J* =15.5 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 4.0 Hz, 1H), 7.00 (d, *J* = 8.5 Hz, 1H), 7.09 (s, 1H), 7.42 (d, *J* = 15.5 Hz, 1H), 9.40 (s, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 27.7, 29.8, 40.0, 46.3, 56.3, 56.5, 111.5, 116.7, 118.3, 123.4, 126.4, 127.7, 133.5, 142.2, 144.5, 149.6, 150.8, 169.3, 180.9; EIMS *m/z* (%) 372 (95) [M^+], 343 (99), 195 (19), 177 (100), 145 (30); HR-EIMS *m/z* 372.1694 (calcd for $C_{20}H_{24}N_2O_3$, 372.1680).

Assay of LDL lipid peroxidation

LDL (d = 1.019-1.063) was isolated from freshly prepared human plasma and stored in the dark at 4 °C under nitrogen for up to 2 weeks. LDL concentration was determined by using Biorad protein assay kit (USA) and the purity was assessed by agarose gel electrophoresis (Paragen, Beckman LIPO kit).

Aliquots of LDL (50 μg/mL) was incubated with copper sulphate (10 μM final concentration) at 37 °C for 6 h, by monitoring every 15 min the absorbance at 234 nm of conjugated dienes.[11] Oxidations were performed in the presence or absence of tested drugs concentrations ranging from 1 to 25 μM. The IC_{50} was calculated as the 50 % inhibition of peak absorbance at 234 nm.

ACKNOWLEDGEMENTS

This research was supported by grants from the National Science Council of Republic of China (NSC 91-2113-M-077-003).

REFERENCES

1. H.-J. Yu, C.-C. Chen, and B.-J. Shieh, *J. Nat. Prod.*, 1998, **61**, 1017.
2. J. Martin-Tanguy, F. Cabanne, E. Perdrizet, and C. Martin, *Phytochemistry*, 1978, **17**, 1927.
3. H. Tanaka, T. Nakamura, K. Ichino, and K. Ito, *Phytochemistry*, 1989, **28**, 2516.
4. C.-Y. Chen, F.-R. Chang, H.-F. Yen, and Y.-C. Wu, *Phytochemistry*, 1998, **49**, 1443.
5. T. Okuyama, S. Shibata, M. Hoson, T. Kawada, H. Osada, and T. Noguchi, *Planta Medica*, 1986, **52**, 171.
6. H. L. Zhang, A. Nagatsu, T. Watanabe, J. Sakakibara, and H. Okuyama, *Chem. Pharm. Bull.*, 1997, **45**, 1910.
7. H. Sato, H. Kawagishi, T. Nishimura, S. Yoneyama, Y. Yoshimoto, S. Sakamura, A. Furusaki, S. Katsuragi, and T. Matsumoto, *Agric. Biol. Chem.*, 1985, **49**, 2969.
8. P. Rajan, I. Vedernikova, P. Cos, D. V. Berghe, K. Augustyns, and A. Haemers, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 215.
9. C.-F. Tseng, S. Iwakami, A. Mikajiri, M. Shibuya, F. Hanaoka, Y. Ebizuka, K. Padmawinata, and U. Sankawa, *Chem. Pharm. Bull.*, 1992, **40**, 396.
10. C. E. Loader and H. J. Anderson, *Synthesis*, 1978, 295.
11. H. Puhl, G. Wäg, and H. Esterbauer, *Methods Enzymol.*, 1994, **233**, 425.