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New Macrocyclic Diamide from *Rauvolfia Yunnanensis* Tsiang

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Abstract A new macrocyclic diamide, 22-membered macrocyclic diamide, named cyclodicaprylamide(**2**), and five known compounds, bis(2-ethylhexyl) phthalate(**1**), ethyl 3,4,5-trimethoxybenzoate(**3**), ethyl 3,4,5-trimethoxycinnamate (**4**), (+)-syringaresinol(**5**), loliolide(**6**), were isolated from the roots of *Rauvolfia yunnanensis* Tsiang. Their structures were elucidated based on NMR, 2D NMR, and MS spectrum, respectively. They were obtained from it for the first time.

Keywords *Rauvolfia yunnanensis*; Cyclodicaprylamide; (+)-Syringaresinol; Loliolide

1 Introduction

Rauvolfia yunnanensis Tsiang is commonly used as a folklore herb to treat hypertension, snake bites, and insanity, in Southwest China[1]. It is an important raw material resource for the drugs of reserpine and has been known for a long time. But the wild resources have reduced gradually in recent years. For the sustainable development, we introduced and cultivated it at the arid-hot valley region of Nujiang River[2]. In the process, we further investigated the chemical constituent's variety of it. A new 22-membered macrocyclic diamide-cyclodicaprylamide(**2**) and five known compounds, bis(2-ethylhexyl) phthalate(**1**)[3], ethyl 3,4,5-trimethoxybenzoate(**3**)[4], ethyl 3,4,5-trimethoxycinnamate(**4**)[5], (+)-syringaresinol(**5**)[6], and loliolide(**6**)[7], were isolated from the root and were determined. They were obtained from the herb for the first time. The isolation and elucidation of these compounds are explained in this article.

2 Results and Discussion

Cyclodicaprylamide**(2)** was obtained as a needle crystal, m. p. 183 °C. The molecular formula $C_{20}H_{38}N_2O_2$ was derived using positive HR-FAB-MS (339.3016[M+1]$^+$) in combination with ^{13}C NMR (DEPT) spectroscopy. The IR spectrum of it shows absorptions at 1641 cm^{-1} (C═O) and 3289 cm^{-1}(NH). The ^{13}C NMR(DEPT) spectrum of compound **2** shows nine CH_2 signals at δ 39.0(t), 36.7(t), 29.1(t), 29.0(t), 28.7(t), 28.4(t), 28.2(t), 26.2(t), 25.7(t), and one C═O signal at δ 173.2(s). The ^1H NMR spectrum of it indicates the signal of two NH at δ 5.50(2H, bs, HN), the signal of two N—CH_2— groups at δ 3.30(4H, dd, J=12.06, 5.97 Hz), the signal of two CH_2—C═O groups at δ 2.18(4H, t, J=7.0 Hz), and the signals of fourteen CH_2 groups at δ 1.56—1.76(4H, m), 1.48—1.50(4H, m), 1.25—1.31(20H, m). The results of ^1H NMR, ^{13}C NMR, and HR-FAB-MS spectra indicate the preferable symmetry in the structure and suggested compound **2** as a macrocyclic dilactamide. The HMQC experiment of it confirms the connections of δ_C 39.0(t) with δ_H 3.30, 36.7(t) with 2.18, 25.7(t) with 1.56—1.76(4H, m), and 29.0(t) with 1.48—1.50 (4H, m). In the HMBC experiment(see Fig.1) of it, the carbon signal at δ 173.2(s) is correlated with the proton signals at δ 5.50(w), 3.30, 2.18, 1.56—1.76(4H, m), δ_C 39.0(t) with δ 5.50(w), 1.48—1.50(4H, m), the

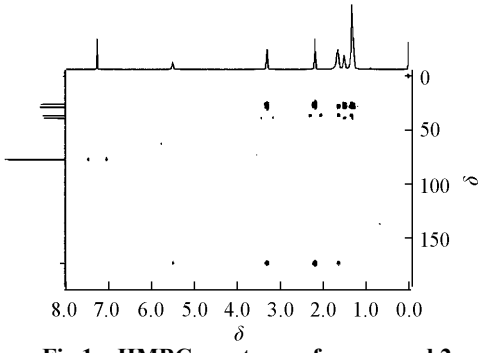

Fig.1 HMBC spectrum of compound 2

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proton signal at δ 3.30 is correlated with the carbon signals at δ 173.2(s), 26.2(t), and 29.0(t), δ 2.18 with δ 173.2(s), 25.7(t)(see Fig.2), respectively.

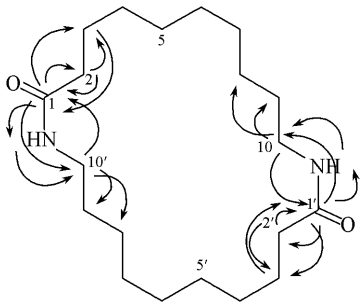

Fig.2 Key HMBC correlations of compound 2

On the basis of IR, MS, 1D NMR, and 2D NMR data, the structure of compound **2** is thus established as 22-membered macrocyclic dilactamide, named cyclodicaprylamide.

The known compounds were identified by comparison of their spectroscopic data with those reported in the literature[3—7], their structures are shown in Fig.3. The loliolide(**6**), which has been isolated from certain terrestrial plants and sea seeds, shows a strong activity of immunosuppression, antirepellence, germination inhibition, and growth inhibition against human nasopharynx carcinoma and murine lymphocytic leukemia[7] but has not been obtained previously from *Rauvolfia*.

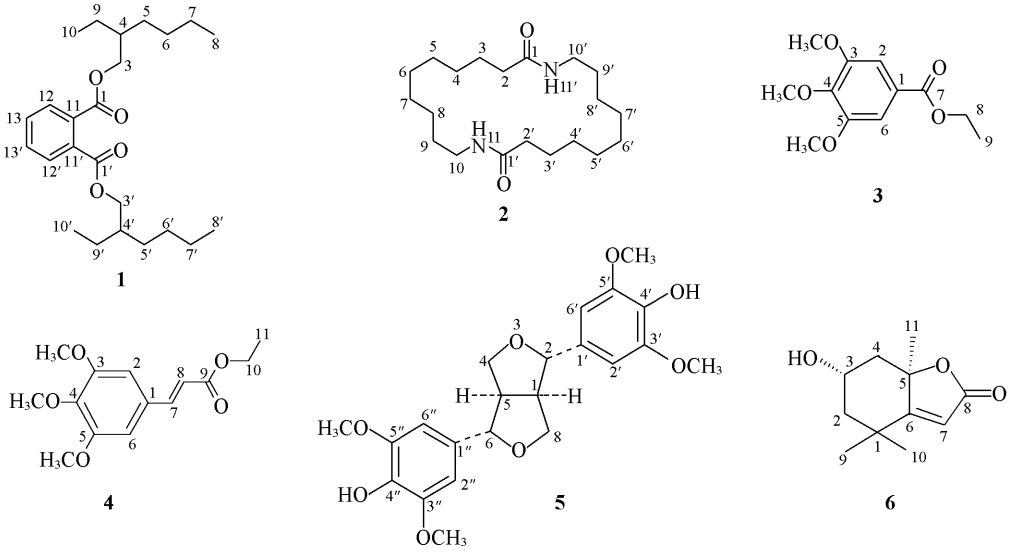

Fig.3 Structures of compounds 1—6

3 Experimental

3.1 General Procedures

Melting points were uncorrected. The IR spectra were recorded on a BioRad FTS-35 spectrometer with KBr pellets. The MS data were obtained on an Autospec-3000 spectrometer in a positive ion model. 1D NMR and 2D NMR spectra were measured on a Bruker AM-400 or a Bruker DRX-500 spectrometer with TMS as the internal standard.

3.2 Plant Material

The roots of *Rauvolfia yunnanensis* Tsiang were collected in Nujiang, Yunnan Province, China, in December 2005, which was cultivated in 2003.

3.3 Extraction and Isolation

The dried roots powder(2386.0 g) of *Rauvolfia yunnanensis* Tsiang was extracted using 95% EtOH (5 L×3) at room temperature. The EtOH extract was evaporated under reduced pressure to afford a residue (247.5 g). The residue was suspended in H_2O, and the pH was adjusted to 3.0 using HCl. The insoluble material was removed by filtration. The pH of the acidic system was adjusted to 10.0 by the addition of NaOH and was then extracted with EtOAc. The EtOAc extract(28.8 g) was separated by initial silica gel column chromatography with CH_3Cl-MeOH gradient elution(volume ratio 9:1—1:1) to afford three fractions(Fr.1—Fr.3). Fraction 1 (13 g) was further subjected to column chromatography on silica gel with petrol ether-acetone gradient elution(volume ratio 10:1—1:1), and on Al_2O_3 with CH_3Cl-MeOH gradient elution(volume ratio 30:1—20:1) to afford compounds **1**(20 mg), **2**(5 mg), **3**(4 mg), **4**(6 mg), **5**(7 mg), and **6**(20 mg).

Cyclodicaprylactam(**2**), $C_{20}H_{38}N_2O_2$, a needle crystal, m. p. 183 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3289, 3086,

2923, 2852, 1641, 1559, 1467, 1440, 723, 690. EIMS, *m/z*: 338(M^+, 35%), 86(44%), 100(41%), 114(31%), 128(32%), 142(48%), 156(56%), 170(46%), 184 (53%), 198(26%), 84(28%), 98(31%), 112(24%), 126 (27%), 140(44%), 154(45%), 168(27%), 182(25%), 196(26%), 210(28%), 224(25%), 238(26%), 252 (34%), 266(37%). HR-FAB-MS, *m/z*: 339.3016 (calcd. for $C_{20}H_{39}N_2O_2$, 339.301154). ^{1}H NMR($CDCl_3$, 400 MHz), *δ*: 5.50(2H, bs, 2NH), 3.30(4H, dd, *J*=12.0, 6.0 Hz, H-10, 10'), 2.18(4H, t, *J*=7.0 Hz, H-2,2'), 1.56—1.76(4H, m, H-3,3'), 1.48—1.50(4H, m, H-9,9'), 1.25—1.31(20H, m, H-4,4' to H-8,8'). ^{13}C NMR ($CDCl_3$, 100 MHz), *δ*: 173.2(s, C-1,1'), 39.0(t, C-10,10'), 36.7(t, C-2,2'), 29.1(t), 28.7(t), 28.4(t), 28.2(t) (C-4,4' to C-7,7'), 25.7(t, C-3,3') , 26.2(t, C-8,8'), 29.0 (t, C-9,9').

Bis(2-ethylhexyl) phthalate(**1**), $C_{24}H_{38}O_4$, colourless liquid. EIMS, *m/z*: 390(w), 279(46%), 167(75%), 149(100%), 113(16%), 71(27%). ^{1}H NMR ($CDCl_3$, 400 MHz), *δ*: 0.85—0.92(12H, m, H-8, 10,8',10'), 1.23—1.47(16H, m, H-5,6,7,9,5',6',7',9'), 1.66(2H, dt, *J*=12.4, 6.3 Hz, H-4,4'), 4.14—4.28 (4H, m, H-3,3'), 7.48—7.55(2H, m, H-13,13'), 7.74—7.66(2H, m, H-12, 12'). ^{13}C NMR($CDCl_3$, 100 MHz), *δ*: 167.7(s, C-1,1'), 68.0(t, C-3,3'), 38.7(d, C-4,4'), 30.3(t, C-55'), 28.9(t, C-6,6'), 22.9(t, C-7,7'), 14.0(q, C-8,8'), 23.7(t, C-9,9'), 10.9(q, C-10,10'), 132.4(s, C-11,11'), 130.8(d, C-12,12'), 128.7(d, C-13, 13').

Ethyl 3,4,5-trimethoxybenzoate(**3**), $C_{12}H_{16}O_5$, white needle, m. p. 55 °C. ^{1}H NMR($CDCl_3$, 400 Hz), *δ*: 7.23(2H, s, H-2,6), 4.31(2H, q, *J*=7.1 Hz, 8-H), 3.84(6H, s, 3,5-OCH_3), 3.83(3H, s, 4-OCH_3), 1.33(3H, t, *J*=7.1 Hz, H-9). ^{13}C NMR($CDCl_3$, 100 MHz), *δ*: 125.5(s, C-1), 106.3(d, C-2), 152.9(s, C-3), 142.0(s, C-4), 152.9(s, C-5), 106.6(d, C-6), 168.2(s, C-7), 61.1(t, C-8), 14.4(q, C-9), 61.0(q, 4-OMe), 56.2(q, 3,5-OMe).

Ethyl 3,4,5-trimethoxycinnamate(**4**), $C_{14}H_{18}O_5$, white needle, m. p. 57 °C. EIMS, *m/z*: 266(M^+, 100%), 251(60%), 221(25%), 223(19%), 177(21%), 163 (14%), 135(11%). ^{1}H NMR($CDCl_3$, 400 MHz), *δ*: 7.58(1H, d, *J*=16.0 Hz, H-7), 7.02(1H, s, H-2,6), 6.50(1H, d, *J*=16.0 Hz, H-8), 4.18(2H, q, *J* =7.1 Hz, H-10), 3.88(6H, s, 3,5-OMe), 3.75(3H, s, 4-OMe), 1.27(3H, t, *J*=7.1 Hz, H-11). ^{13}C NMR($CDCl_3$, 100 MHz), *δ*: 130.9(s, C-1), 106.7(d, C-2), 154.7(s, C-3), 154.7(s, C-4), 154.7(s, C-5), 106.7(d, C-6), 145.4(d, C-7), 118.3(d, C-8), 167.2(s, C-9), 60.7(t, C-10), 60.6 (q, OMe-4), 56.6(q, 3,5-OMe), 14.6(q, C-11).

(+)-Syringaresinol(**5**), $C_{22}H_{26}O_8$, white needle. m. p. 172 °C. EIMS, *m/z*: M^+=418(70%), 181(100%), 167(85%), 193(25%), 210(15%), 154(26%), 123 (14%). ^{1}H NMR(Acetone-d_6, 400 MHz), *δ*: 7.14(2H, s, 4',4"-OH), 6.66(4H, s, H-2',6',2",6"), 4.66(2H, d, *J*=6.2 Hz, H-2,6), 4.12—4.29(2H, m, H-4*α*,8*α*), 3.81—3.82(2H, m, H-4*β*,8*β*), 3.80(12H,s, 3',5',3", 5"-OMe), 2.99—3.16(2H, m, H-1,5). ^{13}C NMR (Acetone-d_6, 100 MHz), *δ*: 55.3(C-1,5), 86.8(C-2,6), 72.3(C-4,8), 133.15(C-1',1"), 104.4(C-2', 6',2",6"), 148.6(C-3',5',3",5"), 136.12(C-4',4") .

Loliolide(**6**), $C_{11}H_{16}O_3$, white needle, m. p. 153 °C. EIMS, *m/z*: 197($[M+1]^+$, 15%), 196(M^+, 18%), 178(100%), 163(65%), 153(32%), 150(20%), 140(56%), 135(57%), 111(80%), 107(35%), 95(33%), 85(23%), 67(24%), 57(18%). ^{1}H NMR(Acetone-d_6, 400 MHz), *δ*: 5.67(1H, s, H-7), 4.28(1H, q, *J*=3.4 Hz, H-5), 4.16(1H, d, *J*=3.2 Hz, 3-OH), 2.38(1H, ddd, *J*=14.1, 3.4, 1.8 Hz, H-3), 2.00(2H, ddd, *J*=14.5, 3.4, 1.8 Hz, H-4), 1.72(3H, s, H-11), 1.72(1H, dd, *J*=14.1, 3.4 Hz, H-2), 1.51(1H, dd, *J*=14.5, 3.4 Hz, H-2), 1.46 (3H, s, H-9), 1.25(3H, s, H-10). ^{13}C NMR(Acetone-d_6, 100 MHz), *δ*: 183.4(s, C-6), 171.6(s, C-8), 113.2(d, C-7), 87.0(s, C-5), 66.7(d, C-3), 47.8(t, C-2),46.4(t, C-4), 36.6(s, C-1), 31.0(q, C-9), 27.4(q, C-11), 26.8(q, C-10).

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