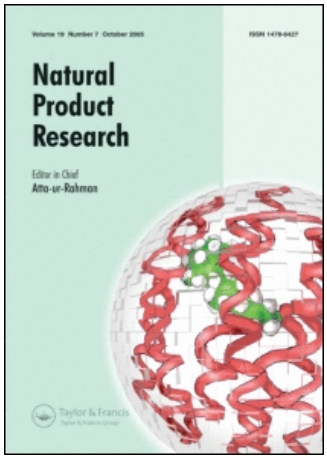

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Antifungal agent and other constituents from *Cynanchum otophyllum*

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Seven constituents were isolated from the ethyl acetate extract of the rhizome of *Cynanchum otophyllum* Schneid (Asclepiadaceae). Their structures were determined as 1-(4-methoxy-3-(6-methoxy-3-acetylphenylperoxy)phenyl)ethanone (**1**), 1-(3-hydroxy-7-acetylnaphthalen-2-yl)ethanone (**2**), 1-(3,4-dihydroxyphenyl)ethanone (**3**), 1-(2,4-dihydroxyphenyl)ethanone (**4**), 1-(3-(3,6-dihydroxy-2-methylbenzoyl)-2,4-dihydroxyphenyl)ethanone (beishouwubenzophenone) (**5**), N,N-dimethylethanamine (**6**), and 2-oxo-2-phenylacetic acid (**7**), respectively, by spectral methods. Among them, **1** and **2** were new compounds; **1** had antifungal activity.

Keywords: Peroxide; Antifungal agent; *Cynanchum otophyllum*; Asclepiadaceae

1. Introduction

Cynanchum otophyllum Schneid, Qingyangshen, is a species of the genus *Cynanchum* L. (Asclepiadaceae), and a traditional Chinese medicine distributed extensively over southwest China. Pharmacodynamic and clinical experiments have established that the chloroform extract and the ethyl acetate extract of the rhizome are particularly effective against epilepsy and chronic hepatitis [1–8]. Since 1984, Qingyangshen Tablets (the total saponins of *C. otophyllum*) have been manufactured by Lijiang Pharmaceutical Co., Yunnan Baiyao Group, Lijiang, Yunnan, China. The constituents from the genus *Cynanchum* L. have been reported [9]. From the rhizome of *C. otophyllum*, Mu *et al.* isolated nine constituents [10–12]. Consequently, Mu and co-workers developed *C. otophyllum* into three novel medicines (Patents of China: ZL 98 1 18938.5, ZL 98 1 18173.2, and ZL 96 1 11270.0). For maintaining the lead in the research of *C. otophyllum*, the authors carried out further investigations, which were very important. From the ethyl acetate extract (the total glycosides) and its acidic hydrolysis part of the rhizome of *C. otophyllum*, the authors isolated four new carbohydrates [13], and three new C_{21} steroidal glycosides [14,15]. Furthermore, this article aimed to study

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the constituents except saponins in *C. otophyllum*, and reports seven chemical constituents from the EtOAc extract of its rhizome. Among them, **1** and **2** were new compounds; **1** had antifungal activity.

2. Results and discussion

Compound **1** was obtained as a white powder. The molecular formula was determined as $C_{18}H_{18}O_6$ by HRFAB-MS. The ^{13}C NMR and DEPT spectra showed one carbonyl, three pairs of double bond, one methoxy group, one methyl (linked with a carbonyl), three methines, two quaternary carbons (linked with oxygens), and one quaternary carbon (linked with a carbonyl). Thus, **1** was a derivative of biphenol; the substituents were methoxy and acetyl. δ 152.2 was the biggest, so the carbon at δ 152.2 was C-1 (table 1). The proton at δ 3.90 correlated with the signal at δ 56.2 in the HMQC, and had a long-range correlation with the carbon at δ 148.2 in the HMBC, so this methoxy group was linked with the carbon, which was C-2. Since the proton at δ 7.51 corresponded to the carbon at δ 111.4 in the HMQC, and had long-range correlations with the carbon resonances at δ 152.2, 148.2, 124.2, 130.7, and 196.2 in the HMBC, so the carbon at δ 111.4 was C-3. The carbon at δ 124.2 corresponded to the proton at δ 7.56 in the HMQC, which had long-range correlations with the carbons at δ 196.2, 152.2, and 111.4 in the HMBC. Consequently, the carbon at δ 124.2 was C-4. Since the proton at δ 6.90, which correlated with the resonance at δ 115.3 in the HMQC, showed a long-range correlation with the carbons at δ 152.2, 148.2, and 130.7 in the HMBC, so the carbon at δ 115.3 was C-6. In this case, the carbons of **1** were found (Table 1). Therefore, **1** was elucidated as 1-(4-methoxy-3-(6-methoxy-3-acetylphenylperoxy)-phenyl)ethanone (O,O-bi(2-methoxy-5-acetylphenol) in Table 1).

Compound **2** was obtained as a yellow crystal. $C_{14}H_{12}O_3$ from HRFAB-MS. The ^{13}C NMR and DEPT spectra showed two carbonyls, five pairs of double bond, two methyls, five methines, and five quaternary carbons. Consequently, **2** was a derivative of naphthalene; the substituents were two acetyls and one hydroxy. The proton at δ 7.08 corresponded to the signal at δ 125.6 in the HMQC, and had long-range correlations with δ 156.4 and 116.3 in the HMBC (Table 2). Thus, the carbon at δ 125.6 was C-1; the carbons at δ 156.4 and 116.3 were C-3 and C-8, respectively. The carbon at δ 131.5 correlated with the signal at δ 7.88 in the HMQC, which had long-range correlations with the resonances at δ 162.5, 115.9, and 196.3 in the HMBC, so the carbon at δ 131.5 was C-4, as well as the carbons at δ 162.5 and 115.9 were C-2 and C-5, respectively. The proton at δ 6.91 corresponded to the resonance at δ 115.9 in the HMQC, and had long-range correlations with the signals at δ 162.5 and 130.4 in the HMBC. Consequently, the carbon at δ 115.9 was C-5; the carbon at δ 130.4 was C-9. Since the proton at δ 6.79, which correlated with δ 119.3 in the HMQC, displayed long-range correlations with the resonances at δ 156.4, 120.2, and 150.0 in the HMBC, so the carbon at δ 119.3 was C-7. The proton at δ 7.28 corresponded to δ 116.3 in the HMQC, and showed long-range correlations with δ 125.6, 156.4, 150.0, and 205.5 in the HMBC. Thus, the carbon at δ 116.3 was C-8; the carbon at δ 150.0 was C-6. In this case, the carbons of **2** were found (Table 2). Therefore, **2** was elucidated as 1-(3-hydroxy-7-acetylnaphthalene-2-yl)ethanone (3,6-diacetyl-2-naphthol in Table 2).

Compound **3** was obtained as a white powder. The ^{13}C NMR and DEPT spectra showed one carbonyl, three pairs of double bond, one methyl, three methines, and

Table 1. NMR data for compound **1** in CD_3COCD_3.

Carbon	^{13}C	$^{1}H^{a}$	^{1}H–^{1}H COSY	HMBC	Carbon	^{13}C	$^{1}H^{a}$	^{1}H–^{1}H COSY	HMBC
1	152.2s	–	–	–	1′	152.2s	–	–	–
2	148.2s	–	–	–	2′	148.2s	–	–	–
3	111.4d	7.51m	–	C-1; C-2; C-4; C-5; C-10	3′	111.4d	7.51m	–	C-1′; C-2′; C-4′; C-5′; C-10′
4	124.2d	7.56m	H-6	C-1; C-3; C-10	4′	124.2d	7.56m	H-6′	C-1′; C-3′; C-10′
5	130.7s	–	–	–	5′	130.7s	–	–	–
6	115.3d	6.90d(8.0)	H-4	C-1; C-2; C-5	6′	115.3d	6.90d(8.0)	H-4′	C-1′; C-2′; C-5′
9	56.2q	3.90s; 3H	–	C-2	9′	56.2q	3.90s; 3H	–	C-2′
10	196.2s	–	–	–	10′	196.2s	–	–	–
11	26.3q	2.49d(0.4); 3H	–	C-4; C-5; C-10	11′	26.3q	2.49d(0.4); 3H	–	C-4′; C-5′; C-10′

[a]Coupling constants are in hertz.

Table 2. NMR data for compound **2** in CD_3COCD_3.

Carbon	^{13}C	$^{1}H^{a}$	^{1}H–^{1}H COSY	HMBC	Carbon	^{13}C	$^{1}H^{a}$	^{1}H–^{1}H COSY	HMBC
1	125.6d	7.08dd(2.8, 6.0)	H-5; H-7; H-8	C-3; C-8	8	116.3d	7.28d(2.8)	H-1	C-1;C-3; C-6; C-11
2	162.5s	–	–	–	9	130.4s	–	–	–
3	156.4s	–	–	–	10	120.2s	–	–	–
4	131.5d	7.88dd(2.0, 4.8)	H-5	C-2; C-5; C-13	11	205.5s	–	–	–
5	115.9d	6.91m	H-1; H-4; H-7	C-2; C-9	12	26.3q	2.48s; 3H	H-14	C-9; C-13
6	150.0s	–	–	–	13	196.3s	–	–	–
7	119.3d	6.79(9.2)	H-1; H-5	C-3; C-6; C-10	14	26.9q	2.59s; 3H	H-12	C-3; C-8; C-10; C-11

[a]Coupling constants are in hertz.

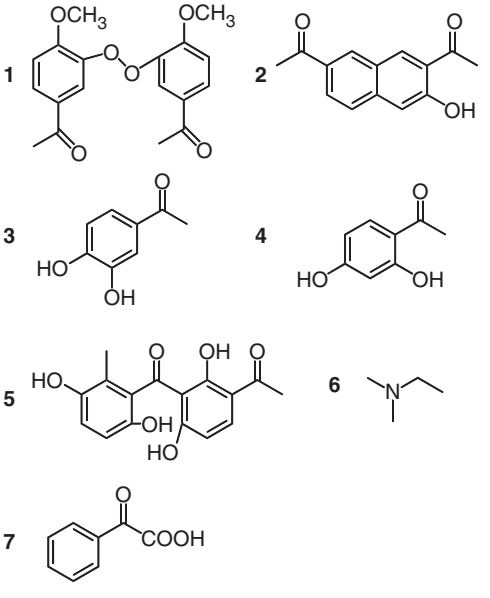
three quaternary carbons, so **3** was a benzene of three substituents. δ_C data were considered, and the substituents were two hydroxies and one acetyl. Therefore, **3** was elucidated as 1-(3,4-dihydroxyphenyl)ethanone. Literature search confirmed that **3** was a known structure.

Compound **4** was obtained as a white powder. The ^{13}C NMR and DEPT spectra showed one carbonyl, three pairs of double bond, one methyl, three methines, and three quaternary carbons, so **4** was a benzene of three substituents. δ_C data were considered, and the substituents were two hydroxies and one acetyl. Therefore, **4** was elucidated as 1-(2,4-dihydroxyphenyl)ethanone. Compound **4** was also a known structure.

Compound **5** was obtained as a yellow crystal. The ^{13}C NMR and DEPT spectra showed two carbonyls, six pairs of double bond, two methyls, four methines, and eight quaternary carbons; thus, **5** was a benzophenone of six substituents, which were four hydroxies, one methyl, and one acetyl. The NMR data were compared with those in a literature [16], **5** was elucidated as a known structure: 1-(3-(3,6-dihydroxy-2-methylbenzoyl)-2,4-dihydroxyphenyl)ethanone (beishouwubenzophenone).

Compound **6** was obtained as a white powder. The ^{13}C NMR, DEPT spectra, and the integral high in the ^{1}H NMR showed three methyls (including two same) and one methylene. The coupling splittings were considered, and they were two same methyls and one ethyl, so **6** was elucidated as N,N-dimethylethanamine. Literature search confirmed that it was isolated from natural products for the first time.

Compound **7** was obtained as a white powder. The ^{13}C NMR and DEPT spectra displayed two carbonyls, three pairs of double bond, five methines, and one quaternary carbon. Consequently, **7** was a benzene of one carbonyl, which was linked with another carbonyl, i.e. carboxyl. Compound **7** was elucidated as 2-oxo-2-phenylacetic acid.



3. Experimental

3.1. *General*

Melting points were determined on a WC-1 micromelting point apparatus (Instrument Plant of Sichuan University, Sichuan, China) and were uncorrected. Optical rotations were measured on a Horiba Sepa-300 digital polarimeter. The UV spectra were measured on a Shimadzu double-beam 210A spectrometer. The IR spectra were measured on a Perkin-Elmer 577 spectrophotometer. Bruker Am-400 and DRX-500 instruments were used to record ^{1}H NMR and 2D NMR (400 MHz), and ^{13}C NMR. C_5D_5N, CD_3COCD_3, or CD_3OD was the solvent and the internal standard at room temperature. EIMS and FABMS were performed on a VG AutoSpec-3000 spectrometer; EIMS were under 70 eV. Column chromatography (CC) was carried out on silica gel. Silica gel (200–300 mesh) for column chromatography and silica gel plate (GF-254) for thin-layer chromatography were the products of Qingdao Haiyang Chemical Group Co., Qingdao, China.

3.2. *Plant materials*

The rhizome of *C. otophyllum* was bought from a drug market in Kunming. It was identified by Dr Yue-Mao Shen and a voucher specimen (KUN No. 0776933) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

3.3. *Extraction and isolation*

The dried powder of the rhizome of *C. otophyllum* (40 kg) was extracted from water at boiling temperature (the above completed at the processing factory of the Institute). The extract was evaporated, and was extracted with EtOH. The extract of EtOH was evaporated, and was extracted with *n*-BuOH. The extract of *n*-BuOH was evaporated, and was extracted with EtOAc to result in the extract of EtOAc (140 g).

We mainly isolated red points on silica gel plate colorated by 5% H_2SO_4–EtOH solution. The extract (140 g) was separated into ten fractions, A1–A10, through column chromatography over silica gel (1.1 kg) by elution with $CHCl_3$–CH_3OH (95 : 5, 94 : 6, 9 : 1). Fraction A4 (77 g, 95 : 5 needed) was separated into eleven fractions, B1–B11, through CC (488 g) eluted with petroleum ether–$(CH_3)_2CO$ (7 : 3, 6 : 4, 1 : 1). Fraction B5 (3.4 g, 7 : 3 needed) was subjected to CC (161 g) eluted with $CHCl_3$–CH_3OH (100 : 0, 100 : 1, 100 : 2), and then to CC (35.5 g) eluted with petroleum ether–$(CH_3)_2CO$ (8 : 2, 7 : 3, 0 : 10), and to CC (24 g) eluted with $CHCl_3$–CH_3OH (100 : 4.5, 100 : 9), to afford **6** (10 mg). Fraction B4 (7.4 g, 7 : 3) was separated into ten fractions, C1–C10, through CC (286 g) eluted with $CHCl_3$–CH_3OH (100 : 0, 100 : 1, 100 : 2). Fraction C3 (448 mg, 100 : 0) was subjected to CC (29.4 g) eluted with petroleum ether–$(CH_3)_2CO$ (8 : 2), to yield **1** (9 mg). Fraction C5 (635 mg, 100 : 0) was subjected to CC (33.5 g) eluted with petroleum ether–$(CH_3)_2CO$ (8 : 2, 7 : 3), to afford **3** (90 mg). Fraction C9 (339 mg, 100 : 2) was subjected to CC (30.5 g) eluted with petroleum ether–$(CH_3)_2CO$ (7 : 3), to yield **5** (50 mg). The crystal I (0.9 g) in the extract of EtOAc produced **7** (100 mg) through CC (75 g) eluted with $CHCl_3$–CH_3OH (100 : 1, 100 : 3, 100 : 4.5, 100 : 9).

The crystal II (5.9 g) in the extract of EtOAc was subjected to CC (75 g) eluted with petroleum ether–EtOAc (8 : 2), to CC (75 g) eluted with $CHCl_3$–CH_3OH (100 : 0, 100 : 1), and to CC (50 g) eluted with petroleum ether–EtOAc (75 : 25, 6 : 4), to afford **2** (91 mg) and **4** (50 mg).

Compound **1** was obtained as a white powder: m.p. 108.5–112.5°C, $-H_2O$; decomposition at high temperature; $[\alpha]_D^{20.0}$ +20.4° (*c* 0.21, $(CH_3)_2CO$); UV (EtOH) λ_{max} (log ε) 204.5 (4.45), 229.5 (4.49), 276 (4.35), 303.5 (4.27) nm; IR (KBr) ν_{max} 3310, 1661, 1576, 1515, 1462, 1421, 1357, 1291, 1219, 1192, 1162, 1116, 1077, 1027, 964, 903, 851, 794, 739, 681, 637, 600, 566 cm^{-1}; ^1H, ^{13}C, and 2D NMR data, see table 1; EIMS *m*/*z* 330 $[M]^+$ (5.5), 315 (4.5), 279 (5), 256 (5), 182 (32.5), 167 (62), 151 (100), 123 (25), 55 (28); HREIMS *m*/*z* 330.1118 (Calcd for $C_{18}H_{18}O_6$, 330.1103).

Compound **2** was obtained as a yellow crystal (acetone): m.p. 98–101°C; 133°C, $-H_2O$; >152°C, decomposition; $[\alpha]_D^{20.0}$ +32.1° (*c* 0.21, $(CH_3)_2CO$); UV (EtOH) λ_{max} (log ε) 226.5 (4.36), 257 (4.02), 365 (3.78) nm; IR (KBr) ν_{max} 3309, 1663, 1609, 1577, 1504, 1358, 1218, 1165, 1022, 962, 921, 888, 849, 817, 788, 741, 643, 590, 567, 447 cm^{-1}; ^1H, ^{13}C, and 2D NMR data, see table 2; FAB–MS *m*/*z* 228 $[M]^-$ (94), 183 (4.5), 135 (100), 81 (1); HRFABMS *m*/*z* 227.0694 (Calcd for $C_{14}H_{11}O_3$, 227.0708).

Compound **3** was obtained as a white powder: UV (EtOH) λ_{max} (log ε) 226.5 (4.11), 256 (3.84), 364 (3.60) nm; IR (KBr) ν_{max} 3260, 1617, 1579, 1497, 1371, 1302, 1214, 968, 921, 887, 834, 788, 740, 643, 481, 446 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ 11.7 (1H, s), 8.25 (1H, s), 7.28 (1H, d, $J = 3.2$ Hz), 7.08 (1H, dd, $J = 5.6$, 3.2 Hz), 6.79 (1H, d, $J = 8.8$ Hz), 3.05 (3H, s), 2.60 (3H, d, $J = 24.8$ Hz); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 205.5 (C, C-7), 156.6 (C, C-1), 150.2 (C, C-2), 125.7 (CH, C-3), 120.4 (C, C-4), 119.4 (CH, C-5), 116.4 (CH, C-6), 26.9 (CH_3, C-8); EIMS *m*/*z* 152 $[M]^+$ (93), 137 (100), 109 (55), 81 (71.5), 69 (52.5), 63 (35.5), 53 (96).

Compound **4** was obtained as a white powder: UV (EtOH) λ_{max} (log ε) 212 (3.90), 230.5 (3.95), 276.5 (4.18), 315 (3.91) nm; IR (KBr) ν_{max} 3298, 1632, 1176, 1141, 1065, 1024, 984, 953, 880, 866, 840, 610, 513, 454, 439 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 13.31 (1H, s), 7.71 (2H, d, $J = 8.0$ Hz), 6.73 (2H, m), 2.51 (6H, s); ^{13}C NMR (C_5D_5N, 100 MHz) δ 203.1 (C, C-7), 167.0 (C, C-3), 166.3 (C, C-1), 134.1 (CH, C-5), 114.1 (C, C-4), 109.4 (CH, C-2), 104.0 (CH, C-6), 26.4 (CH_3, C-8); EIMS *m*/*z* 152 $[M]^+$ (66), 137 (100), 123 (11.5), 108 (22.5), 81 (25), 69 (9).

Compound **5** was obtained as a yellow crystal (acetone): UV (EtOH) λ_{max} (log ε) 213.5 (4.48), 230.5 (4.36), 279 (4.21), 318 (4.20) nm; IR (KBr) ν_{max} 3535, 3344, 1696, 1500, 1435, 1206, 1180, 1151, 1085, 1055, 978, 938, 889, 810, 716, 659, 611, 569, 485, 437 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ 7.84 (1H, d, $J = 8.8$ Hz, H-4), 7.07 (1H, d, $J = 9.2$ Hz, H-4′), 6.84 (1H, d, $J = 8.8$ Hz, H-3′), 6.59 (1H, d, $J = 8.8$ Hz, H-5), 2.57 (3H, s, H-10), 2.08 (3H, s, H-9); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 206.0 (C, C-7), 204.0 (C, C-8), 163.7 (C, C-2), 163.0 (C, C-6), 154.6 (C, C-5′), 148.8 (C, C-2′), 133.9 (CH, C-4), 124.8 (C, C-6′), 123.4 (CH, C-4′), 120.1 (C, C-1′), 118.8 (CH, C-3′), 114.4 (C, C-3), 113.0 (C, C-1), 108.8 (CH, C-5), 30.5 (CH_3, C-9), 26.3 (CH_3, C-10); EIMS *m*/*z* 302 $[M]^+$ (27), 284 (63.5), 269 (26), 266 (23), 245 (21), 149 (16), 77 (18), 64 (100), 55 (30).

Compound **6** was obtained as a white powder: UV (EtOH) λ_{max} (log ε) 205 (3.08) nm; IR (KBr) ν_{max} 3400, 2435, 1573, 1418, 1383, 1118, 1052, 1024, 836, 661, 623 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 3.03 (2H, dd, $J = 7.6$, 7.2 Hz, H-1), 1.90 (6H, s, H-3, H-4), 1.29 (3H, t, $J = 7.4$ Hz, H-2); ^{13}C NMR (CD_3OD, 100 MHz) δ 43.5 (CH_2, C-1),

24.2 (CH_3, C-3, C-4), 11.6 (CH_3, C-2); EIMS m/z 73 $[M]^+$ (1.5), 69 (6.5), 60 (33), 58 (13.5), 55 (19.5).

Compound **7** was obtained as a white powder: UV (EtOH) λ_{max} ($\log \varepsilon$) 202 (3.97), 225.5 (3.75), 249.5 (3.58), 293.5 (3.40) nm; IR (KBr) ν_{max} 3172, 2708, 2603, 1604, 1515, 1473, 1367, 1199, 1095, 1004, 948, 857, 832, 761, 701, 684, 623, 585 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 8.42 (1H, d, $J = 8.8$ Hz), 7.25 (5H, m); ^{13}C NMR (C_5D_5N, 100 MHz) δ 169.4 (C, C-1), 163.5 (C, C-2), 152.0 (C, C-3), 133.0 (CH, C-5, C-7), 117.5 (CH, C-4, C-8), 116.4 (CH, C-6); EIMS m/z 149 $[M - 1]^+$ (3.5), 138 (38.5), 121 (31.5), 110 (100), 93 (11.5), 81 (16), 55 (25), 53 (39.5).

3.4. *Antifungal bioassay*

The drug-sensitive test of compound **1** was agar plate diffusion method with Sabouraud's agar. *Candida albicans* came from clinical samples of respiratory tract infection, and was identified by Mrs Gen-Chun Wang, Pharmaceutical Department, Kunming General Hospital, Chengdu Military Command, Kunming, China. The inhibition zone had a diameter of 12 mm, which was similar to that of Fluconazole Injection.

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