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FURANOCOUMARINS WITH AFFINITY TO BRAIN BENZODIAZEPINE RECEPTORS *IN VITRO*

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Key Word Index—*Angelica dahurica*; Umbelliferae; roots; furanocoumarins; phellopterin; benzodiazepine receptor.

Abstract—Eight furanocoumarins were isolated from a methanol extract of dried roots of *Angelica dahurica*. One of these, phellopterin, strongly (IC_{50} = 0.36 μM) inhibits the binding of [^{3}H]diazepam to central nervous system benzodiazepine receptors *in vitro*, while the others, despite their structural similarities with phellopterin, are considerably less active. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Radix Angelicae dahuricae, the dry root of *Angelica dahurica* (Boiss.), is listed in the Chinese Pharmacopoeia and is used as an antipyretic and analgesic for colds, headaches and toothache [1]. It is known to contain a large number of coumarins and furanocoumarins, e.g., coumarin, scopoletin, psoralen, xanthotoxin, bergapten (**1**) and imperatorin (**5**) [2, 3]. During screening of Chinese medicinal plants for activity on central nervous system (CNS) receptors *in vitro*, methanol extracts of radix Angelicae dahuricae were noted to have an inhibitory effect on the binding of [^{3}H]diazepam to the benzodiazepine receptor. In the present paper, we report the isolation and identification of the active principle, as well as the inhibitory activity of this and seven similar furanocoumarins from the same source on the binding of the following CNS receptor ligands: [^{3}H]diazepam to the benzodiazepine receptor, [^{3}H]SCH 23390 (8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol) to the dopamine D_1 receptor, [^{3}H]QNB to the muscarine acetylcholine receptor, and [^{3}H]kainic acid to the kainic acid sensitive glutamate receptor subtype. For comparison, coumarin and six coumarin derivatives were also assayed.

RESULTS AND DISCUSSION

The active principle, phellopterin (**6**), was isolated by bioassay-guided fractionation of a methanol extract of the dry root, as described in the Experimental section. Bergapten (**1**), isoimperatorin (**2**), oxypeucedanin (**3**), 5-(2-hydroxy-3-methoxy-3-methylbutoxy)psoralen (**4**), imperatorin (**5**), byakangelicol (**7**) and 9-(2-hydroxy-3-methoxy-3-methylbutoxy)bergapten (**8**) were also isolated from the same extract. Compounds **4** and **8** are believed to be extraction artefacts, because they are formed when oxypeucedanin (**3**) and byakangelicol (**7**) are left in methanol for an extended period of time. All compounds have been reported previously from various sources [1]. As NMR data for some of the compounds were difficult to find in the literature, the structures of compounds **1–8** were unambiguously determined by means of two-dimensional NMR experiments (COSY, NOESY, HMQC and HMBC). In order to facilitate future studies of furanocoumarins, the NMR data are given in Tables 1 (^{1}H NMR) and 2 (^{13}C NMR).

Phellopterin (**6**) is a potent inhibitor of the binding of [^{3}H]diazepam to the benzodiazepine receptor, with an IC_{50} value of 0.36 ± 0.03 μM (mean ± S.E.M., n = 5). However, it has no effect on the binding of any of the other ligands at concentrations up to 50 μM. On the same receptor, the IC_{50} for imperatorin (**5**) was found to be 8.0 ± 0.8 μM (n = 3) and for byakangelicol (**7**) 12 ± 3 μM (n = 3). None of the other furanocoumarins isolated in this investigation had an effect on the binding of any of ligands at concentrations up to 10 μg ml^{-1} (30–46 μM). In addition, the following commercially available coumarins and furanocoumarins were assayed at concentrations up to 10 μg ml^{-1} and found to be inactive: coumarin, 7-methylcoumarin, 7-ethoxycoumarin, scopoletin, psoralen, xanthotoxin and isopimpinellin.

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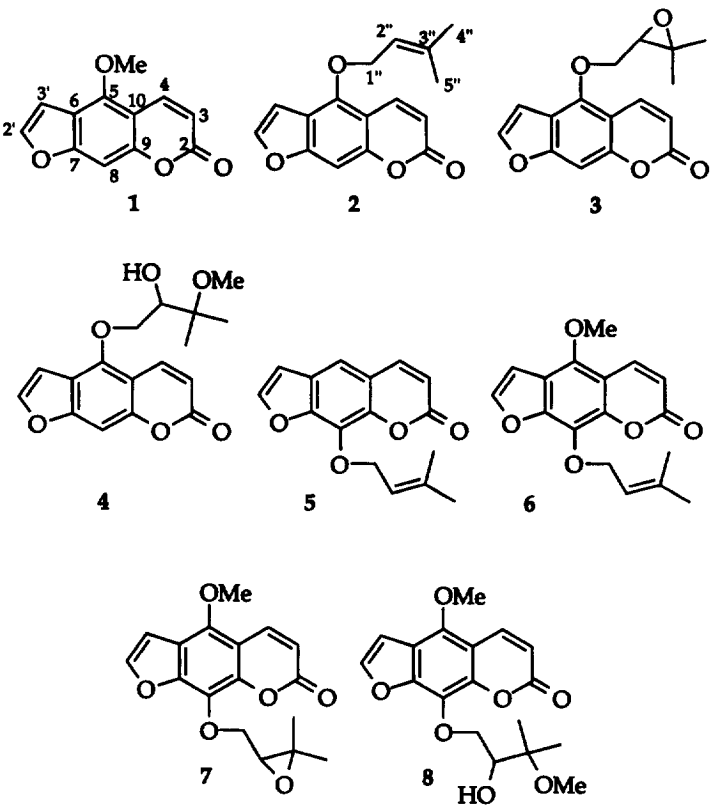

Several of the assayed compounds are structurally very similar and the selectivity of the benzodiazepine receptor for phellopterin (**6**) is quite remarkable. Even if phellopterin (**6**) is less potent than diazepam itself (IC_{50} = 0.018 μM), its potency and specificity make it of interest for further study. The recent discovery of a variety of receptor subunits (α_1–α_5, β_1–β_3, γ_1–γ_3, δ and ρ), which in various compositions may form the GABA–benzodiazepine receptor complex, suggests the existence of a multiplicity of benzodiazepine receptors in the mammalian CNS [4]. It is possible that the furanocoumarin **6** has an even higher affinity towards one specific receptor subtype, an effect that would be masked in our assay system, since the rat cortical membrane preparation contains all receptor subtype combinations. To our knowledge, this is the first report of a coumarin or furanocoumarin derivative that inhibits a CNS receptor, although such derivatives are known to possess a number of other biological activities [5]. Bergapten (**1**) and xanthotoxin

Table 1. ^{1}H (500 MHz) NMR data (δ; multiplicity; J, Hz) for furanocoumarins **1**–**8***

C	1	2	3	4	5	6	7	8
3	6.27; *d*; 9.8	6.26; *d*; 9.8	6.31; *d*; 9.8	6.29; *d*; 9.8	6.34; *d*; 9.6	6.27; *d*; 9.7	6.27; *d*; 9.8	6.27; *d*; 9.8
4	8.15; *d*; 9.8	8.15; *d*; 9.8	8.19; *d*; 9.8	8.23; *d*; 9.8	7.75; *d*; 9.6	8.12; *d*; 9.7	8.12; *d*; 9.8	8.12; *d*; 9.8
5	–	–	–	–	7.34; *s*	–	–	–
8	7.12; *s*	7.13; *s*	7.13; *s*	7.17; *s*	–	–	–	–
2′	7.58; *d*; 2.4	7.57; *d*; 2.3	7.61; *d*; 2.3	7.59; *d*; 2.3	7.67; *d*; 2.3	7.62; *d*; 2.3	7.62; *d*; 2.3	7.62; *d*; 2.3
3′	7.02; *d*; 2.4	6.95; *d*; 2.3	6.94; *d*; 2.3	7.00; *d*; 2.3	6.80; *d*; 2.3	6.98; *d*; 2.3	7.00; *d*; 2.3	6.99; *d*; 2.3
$1''_a$		4.91; *d*; 7.0	4.60; *dd*; 4.3, 10.9	4.57; *dd*; 3.1, 10.0	4.98; *d*; 7.1	4.83; *d*; 7.3	4.43; *d*; 5.6	4.58; *dd*; 2.8, 10.2
$1''_b$	–	See $1''_a$	4.42; *dd*; 6.5, 10.9	4.38; *dd*; 7.8, 10.0	see $1''_a$	see $1''_a$	see $1''_a$	4.21; *dd*; 8.5, 10.2
2″	–	5.53; *t*; 7.0	3.23; *dd*; 4.3, 6.5	3.94; *m*	5.58; *t*; 7.1	5.59; *t*; 7.3	3.30; *t*; 5.6	3.98; *dd*; 2.8, 8.5
4″	–	1.79; *s*	1.41; *s*	1.27; *s*	1.72; *s*	1.73; *s*	1.32; *s*	1.25; *s*
5″	–	1.69; *s*	1.33; *s*	1.23; *s*	1.69; *s*	1.69; *s*	1.24; *s*	1.25; *s*
5-OMe	4.27; *s*	–	–	–	–	4.17; *s*	4.18; *s*	4.18; *s*
3″-OMe			–	3.27; *s*	–	–	–	3.24; *s*

*Spectra recorded in $CDCl_3$; solvent signal (7.26 ppm) used as reference.

Table 2. ^{13}C (125 MHz) NMR data (δ; multiplicity) for furanocoumarins **1–8***

C	1	2	3	4	5	6	7	8
2	161.2; *s*	161.3; *s*	161.0; *s*	161.2; *s*	160.5; *s*	160.5; *s*	160.3; *s*	160.3; *s*
3	112.5; *d*	112.5; *d*	113.0; *d*	112.8; *d*	114.6; *d*	112.8; *d*	112.8; *d*	112.9; *d*
4	139.3; *d*	139.6; *d*	139.0; *d*	139.3; *d*	144.3; *d*	139.4; *d*	139.4; *d*	139.4; *d*
5	149.5; *s*	148.9; *s*	148.3; *s*	148.8; *s*	113.1; *d*	144.3; *s*	144.8; *s*	144.6; *s*
6	112.6; *s*	114.1; *s*	114.1; *s*	114.0; *s*	125.8; *s*	114.5; *s*	114.4; *s*	114.5; *s*
7	158.3; *s*	158.1; *s*	157.9; *s*	158.1; *s*	148.5; *s*	150.8; *s*	150.5; *s*	150.3; *s*
8	93.8; *d*	94.1; *d*	94.7; *d*	94.5; *d*	131.6; *s*	126.8; *s*	126.6; *s*	127.1; *s*
9	152.7; *s*	152.6; *s*	152.4; *s*	152.6; *s*	143.7; *s*	144.3; *s*	144.1; *s*	144.0; *s*
10	106.3; *s*	107.4; *s*	107.3; *s*	107.3; *s*	116.4; *s*	107.5; *s*	107.4; *s*	107.5; *s*
2′	144.8; *d*	144.8; *d*	145.2; *d*	145.0; *d*	146.7; *d*	145.1; *d*	145.2; *d*	145.2; *d*
3′	105.0; *d*	105.0; *d*	104.4; *d*	104.9; *d*	106.7; *d*	105.0; *d*	105.2; *d*	105.2; *d*
1″	–	69.7; *t*	72.2; *t*	74.2; *t*	70.1; *t*	70.4; *t*	72.7; *t*	75.8; *t*
2″	–	119.0; *d*	61.0; *d*	76.1; *d*	119.7; *d*	119.8; *d*	61.3; *d*	75.5; *d*
3″	–	139.8; *s*	58.3; *s*	75.9; *s*	139.7; *s*	139.7; *s*	58.1; *s*	76.0; *s*
4″	–	25.8; *q*	24.5; *q*	20.7; *q*	25.8; *q*	25.8; *q*	24.5; *q*	21.4; *q*
5″	–	18.2; *q*	19.0; *q*	20.7; *q*	18.1; *q*	18.0; *q*	18.4; *q*	20.5; *q*
5-OMe	60.0; *q*	–	–	–	–	60.7; *q*	60.6; *q*	60.7; *q*
3″-OMe	–	–	–	49.2; *q*	–	–	–	49.3; *q*

*Spectra recorded in $CDCl_3$; solvent signal (77.0 ppm) used as reference.

have been used together with long-wave UV radiation to treat psoriasis [6]. However, the compounds are also known to give rise to photosensitizing reactions [7]; mutagenic and carcinogenic activities of psoralens have been reported after photoactivation [8, 9].

EXPERIMENTAL

Materials. Radix Angelicae dahuricae was purchased at a local market in Beijing and identified by J. A. A voucher specimen is deposited at the China Academy of Traditional Chinese Medicine. Coumarin, 7-methylcoumarin, 7-ethoxycoumarin, scopoletin, psoralen, xanthotoxin and isopimpinellin were purchased from Extrasynthese (France). [^{3}H]Diazepam (83.5 C_i mmol^{-1}), [^{3}H]kainic acid (58 C_i mmol^{-1}), [^{3}H]QNB (44.9 C_i mmol^{-1}) and [^{3}H]SCH 23390 (70.7 C_i mmol^{-1}) were obtained from New England Nuclear (U.S.A.).

Isolation and identification. Pulverized roots were extracted repeatedly with MeOH at room temp. and compound **6** was isolated after bioassay-guided fractionation of the extract by reverse-phase HPLC. The columns were eluted with different mixtures of MeCN and H_2O. NMR were recorded at 500 MHz. MS (EI and CI) were recorded.

Binding assay. Brain tissue from male Wistar rats (*ca* 200 g) was prepd for the binding study as described previously [10, 11]. In brief, the cerebral cortex was homogenized in Tris-citrate buffer (50 mM, pH 7.1) with an Ultra-Turrax. The homogenate was centrifuged at 30 000*g* for 10 min and the pellet obtained washed ×2 by homogenization and centrifugation. The final pellet was resuspended in Tris-citrate buffer at 2 mg orginal tissue ml^{-1}. Portions (1 ml) of this membrane prepn were used for binding assays, which were carried out as described previously [10, 11]. The concn of [^{3}H]diazepam was 0.6 nM in the assay. Nonspecific binding was defined in the presence of midazolam (2×10^{-6} M).

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REFERENCES

1. Tang, W. and Eisenbrand, G., *Chinese Drugs of Plant Origin, Chemistry, Pharmacology, and Use in Traditional and Modern Medicine*. Springer, Berlin, 1992, p. 113.
2. Saiki, Y., Morinaga, K., Okegawa, O., Sakai, S., Amaya, Y., Ueno, A. and Fukushima, S., *Yakugaku Zasshi*, 1971, **53**, 1313.
3. Zang, H., Yuan, C., Chen, G., Ding, Y., Chen, S. and Deng, Y., *Chinese Pharmacology Bulletin*, 1980, **2**, 1817.
4. Olsen, R. W. and Tobin, A. J., *FASEB Journal*, 1990, **4**, 1469.
5. Egan, D., O'Kennedy, R., Moran, E., Cox, D., Prosser, E. and Thornes, R. D., *Drug Metabolism Reviews*, 1990, **5**, 503.
6. Parrish, J. A., Fitzpatrick, T. B. and Tannenbaum, L., *New England Journal of Medicine*, 1974, **518**, 1207.
7. Dall'acqua, F., Vedaldi, D., Caffieri, S., Guiotto, A., Bordin, F. and Rodighiero, G., *National Cancer Institute Monograph*, 1984, **1818**, 55.
8. Babudri, N., Pani, B., Venturini, S. and Monti-

Bragadin, C., *Journal of Environmental Pathology, Toxicology and Oncology*, 1986, **19**, 123.
9. Mullen, M. P., Pathak, M. A., West, J. D., Harris, T. J. and Dall'acqua, F., *National Cancer Institute Monograph*, 1984, **1818**, 205.
10. Nielsen, M., Honoré, T. and Braestrup, C., *Biochemistry and Pharmacology*, 1985, **65**, 3633.
11. Nielsen, M., Frokjaer, S. and Braestrup, C., *Biochemistry and Pharmacology*, 1988, **619**, 3285.