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INHIBITION OF KAINIC ACID BINDING TO GLUTAMATE RECEPTORS BY EXTRACTS OF *GASTRODIA*

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Key Word Index—*Gastrodia elata*; Orchidaceae; kainic acid; glutamate receptor; glutathione derivative.

Abstract—*S*-(4-hydroxybenzyl)glutathione was isolated as the major principle responsible for the inhibition of the *in vitro* binding of kainic acid to brain glutamate receptors by water extracts of the plant *Gastrodia elata*. The affinity (IC_{50} value) of the compound is slightly lower compared to glutamate and glutathione.

INTRODUCTION

Gastrodia elata Bl. is used as an anticonvulsant, an analgesic and a sedative against vertigo, general paralysis, epilepsy and tetanus, and is listed in the Chinese Pharmacopoeia [1]. During a screening of the inhibitory activity of extracts of Chinese medicinal plants in a CNS receptor radioligand binding assay *in vitro*, water extracts of *G. elata* inhibited the binding of [^{3}H]kainic acid. Kainic acid is a potent neurotoxin in the CNS and its neurotoxic activity is presumed to be mediated by binding and subsequent activation of the kainic acid sensitive glutamate receptor subtype present on neurons [2, 3]. The active constituent was isolated by a bioassay-guided fractionation with reversed phase HPLC. It is soluble in both water and methanol and the NMR data suggested it to be a small peptide. Amino acid analysis indicated that it consists of equimolar amounts of glutamic acid, glycine and a possible substituted cysteine. The identity of the three amino acids was confirmed by NMR spectroscopy, which also indicated that the group attached to cysteine is a *para*-substituted benzyl group. The ^{1}H and ^{13}C NMR data together with long-range heteronuclear correlations observed in methanol-d_4 are summarized in Table 1 and Fig. 1, and these data indicate that the tripeptide is glutathione. The chemical shift for H-2 varied considerably between different experiments even in the same solvent, indicating that both the α carboxylic acid and the amine groups are free and that the glutamate is bound at the γ position. In DMSO-d_6, which was not used for the correlation experiments because of signal overlapping, signals for two amide and one phenol protons were visible at δ8.26, 8.63, and 9.31. The comparison with data published for similar compounds [4, 5] suggests that the cysteine substituent is a 4-hydroxybenzyl group linked at the thioether. This could be confirmed by the long-range correlations observed between the β protons in cysteine and the benzylic carbon, which protons in turn correlate to the ring carbons. This is the first report of the isolation of *S*-(4-hydroxybenzyl)-glutathione as a natural product. It has previously been

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data for *S*-(4-hydroxybenzyl)glutathione (in CD_3OD, the chemical shifts are given in ppm relative to the solvent signals, 3.31 and 49.0 ppm, and the coupling constants, *J* in Hz)

C	^{1}H	^{13}C
1	—	174.0 *s*
2	3.65 *m*	55.3 *d*
3	2.16 *m*	27.8 *t*
4	2.53 *m*	32.9 *t*
5	—	175.3 *s*
6	4.57 *dd* (8.8, 5.4)	54.2 *d*
7	2.68 *dd* (14, 8.8)	33.9 *t*
	2.92 *dd* (14, 5.3)	
8	3.69 *s*	36.6 *t*
9	—	130.1 *s*
10 and 14	7.14 *d* (8.7)	131.2 *d*
11 and 13	6.71 *d* (8.7)	116.3 *d*
12	—	157.6 *s*
13	6.71 *d* (8.7)	116.3 *d*
15	—	173.5 *s*
16	3.91 *s*	42.2 *t*
17		173.2 *s*

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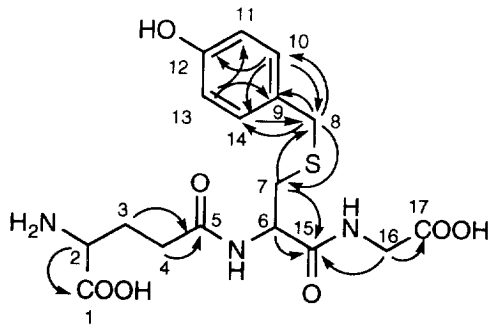

Fig. 1. Long-range heteronuclear correlations (indicated with arrows) observed in a HMBC spectrum of *S*-(4-hydroxybenzyl)glutathione (see Experimental).

prepared [6] during a study of the inhibition of glutathione transferase, although it was never characterized by spectroscopic methods. There were no detectable levels of glutathione itself in this investigation. A number of derivatives of 4-hydroxybenzyl alcohol have previously been isolated from methanol extracts of *G. elata* [7].

The inhibitory activity of *S*-(4-hydroxybenzyl)glutathione on the binding of kainic acid to the glutamate receptor is slightly lower compared with that of glutamate and glutathione. The concentrations of the substances causing 50% inhibition of the specific [^{3}H]kainic acid binding (IC_{50} value, see ref. [8]) were 2×10^{-6}, 2×10^{-7} and 8×10^{-7} M for *S*-(4-hydroxybenzyl)glutathione, glutamate and glutathione, respectively.

EXPERIMENTAL

Dried specimens of *Gastrodia elata* Bl. were purchased in a Chinese pharmacy in Beijing and identified by JA and AL. A voucher specimen is deposited in the herbarium of the Chinese Academy of Traditional Chinese Medicine in Beijing. The plant material was extracted with H_2O at room temp. by ultrasonics (100 ml H_2O per 5 g plant material), and the extract was fractionated by HPLC on reversed phase columns eluted with mixts of MeCN and H_2O and with UV (215 nm) detection. The active fr. was further purified by chromatography and pure *S*-(4-hydroxybenzyl)-glutathione was obtained as the sole active constituent. The NMR experiments were carried out with a Bruker ARX500 spectrometer with an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC and HMBC experiments were performed with gradient enhancements using sine shaped gradient pulses, and for the 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH} = 145$ Hz and $^2J_{CH} = 10$ Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 930901). The amino acid analysis was carried out by the Amino Acid Analysis Central at BMC, University of Uppsala (Sweden).

Preparation of the brain tissue. The cerebral cortex of male Wistar rats was homogenized for 10 sec in a 50 mM (pH 7.1) Tris–HCl buffer (10 ml per cortex). The suspension was centrifuged at 30 000 *g* for 10 min, and the pellet was washed twice with buffer. The final pellet was frozen and stored at −20°.

The binding assay. The binding assay was carried out as described previously [8]. Briefly, to 0.5 ml of the membrane prepn (50 ml g^{-1} original tissue), 25 μl of test soln and 25 μl of [^{3}H]kainic acid (final concn 2 nM) was added. The mixt. is incubated for 1 hr at 0–4°. Non-specific binding was determined by adding L-glutamate (final concn 0.6 mM) to control samples. After the incubation, 5 ml of ice-cold buffer was added to the samples and the mixt. was poured directly into Whatman GF/C glass fibre filters and washed twice. The amount of radioactivity on the filters was determined by conventional liquid scintillation counting. The specific binding is calcd as the total binding minus the non-specific binding.

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REFERENCES

1. Tang, W. and Eisenbrand, G. (1992) in *Chinese Drugs of Plant Origin*, p. 545. Springer, Berlin.
2. Young, A. B. and Fagg, G. E. (1990) *TIPS* **11**, 126.
3. Meldrum, B. and Garthwaite, J. (1990) *Trends Pharmacol. Sci.* **11**, 379.
4. Huckerby, T. N., Tudor, A. J. and Dawber, J. G. (1985) *J. Chem. Soc., Perkin Trans. II* 759.
5. York, M. J., Beilharz, G. R. and Kuchel, P. W. (1987) *Int. J. Peptide Protein Res.* **29**, 638.
6. Shiotsuki, T., Koiso, A. and Eto, M. (1990) *Pesticide Biochem. Phys.* **37**, 121.
7. Taguchi, H., Yosioka, I., Yamasaki, K. and Kim, I. H. (1981) *Chem. Pharm. Bull.* **29**, 55.
8. Honoré, T., Drejer, J. and Nielsen, M. (1986) *Neurosci. Letters* **65**, 47.