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Pentapeptides from the roots of *Aster tataricus*

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Asterinin D and E, pentapeptides, have been isolated from the roots of *Aster tataricus* L.f. (Compositae, syn. Asteraceae, subtribe Asteroideae) and their structures have been elucidated on the basis of spectroscopic analysis, as well as chemical and enzymatic methods.

Pentapeptide aus den Wurzeln von *Aster tataricus*

Die Pentapeptide D und E wurden aus den Wurzeln von *Aster tataricus* (Compositae, syn. Asteraceae, Asteroideae) isoliert und deren Struktur auf der Basis von spektroskopischen und enzymatischen Methoden aufgeklärt.

1. Introduction

A popular strategy for the development of efficient drugs is to modify the structural feature of biologically active natural products such as in taxol [1], calicheamicin [2], and qinghaosu [3]. Indigenous plants used in traditional remedies can be convenient natural sources to isolate active compounds. We investigated one of those plants known as *Aster tataricus* L.f. Its roots; named Ziwan, has expectorative and remediable cough actions [4] and the use of Ziwan in Chinese medicines was mentioned as early as 1596 in Ben Cao Gang Mu [5]. Identification of active components from the roots of *Aster tataricus* L.f. was attempted by a Japanese researcher and resulted in the discovery of monoterpenes and triterpenoids [6–10]. We have systematically examined the chemical compositions of Ziwan and found eleven new compounds, including nine oligopeptides from butanolic extract [11].
Two terpenoids and three oligopeptides with their structures have been previously presented [11, 12]. In this paper, we describe the identification of Asterinin D and E. A comparative study of the structural features of isolated peptides will be the topic of another study.

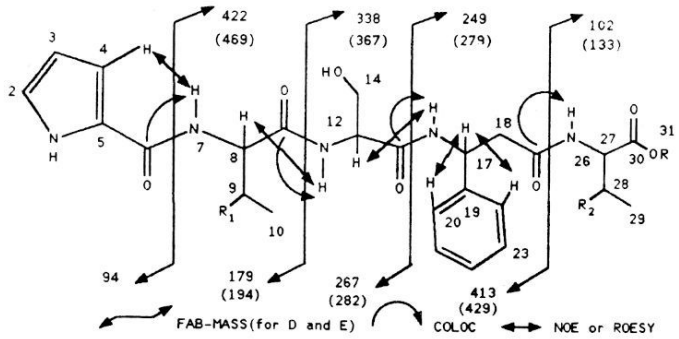
2. Investigations, results and discussion

The butanolic extract of air-dried Ziwan was chromatographed on a silicagel column using chloroform/methanol as the eluent. Repeated silicagel CC and preparative TLC yielded Asterinin D and Asterinin E.
Asteridin D was determined as $C_{25}H_{33}N_5O_7$ by FAB-MS ($[M + 1]^+$ at m/z 516) and by counting carbons and hydrogens from the data of its ^1H, ^{13}C NMR spectra. The IR spectrum showed the presence of hydroxyls, amides and an ester carboxyl group, indicating that Asterinin D is a oligopeptide. The total assignment of ^1H, ^{13}C NMR chemical shift were given on the basis of ^1H, ^1H COSY and ^1H, ^{13}C COSY (Table). Amino acid analyses after acidic and enzymatic hydrolyses revealed one equivalent of L-serine, one equivalent β-phenylalanine, one equivalent 2-pyrrolocarboxylic acid and two equivalents α-aminobutyric acid after comparing with authentic samples. The presence of the pyrrole derivative was confirmed by positive Ehrlich's reaction [13] and the L-configuration of all amino acids were tentatively assigned by Masfey's method [14] as well as by the ^1H, ^{13}C NMR studies of the reported natural oligopeptides [10]. The amino acid sequence of Asterinin D was disclosed by COLOC, differential NOE's and ROESY and its FAB-MS fragmentation pattern (Fig.) [16]. Consequently, the structure of Asterinin D was $\Delta^{2,4}$Pro-L-Abu-L-Ser-L-βPhe-L-Abu.
Asterinin E was determined as $C_{26}H_{35}N_5O_9$ by FAB-mass spectrum ($[M + 1]^+$ at m/z 562) and elemental analysis.

Table: ^1H and ^{13}C NMR chemical shifts of Asterinin D und E

	Asterinin D*		Asterinin E	
	δH (ppm, J = Hz)	δC (ppm)	δH (ppm, J = Hz)	δC (ppm)
1	12.92 br s		12.95 br s	
2	7.12 br s	111.14	7.10 br s	111.09
3	6.23 br s	109.78	6.30 br s	109.07
4	7.24 br s	122.40	7.38 br s	121.85
5		127.48		128.02
6		162.50		161.58
7	8.80 d (8.0)		8.83 d (8.8)	
8	4.99 dt (12.0, 8.0)	55.44	5.52 t (8.5)	59.38
9	2.20 ddq (12.0, 8.0, 4.0) 2.06 ddq (12.0, 8.0, 4.0)	26.33	4.63 dq (8.5, 6.2)	68.58
10	0.96 t (7.0)	10.88	1.55 d (6.2)	20.55
11		173.37		171.62
12	9.40 d (8.0)		9.42 d (8.8)	
13	4.66 m	55.49	5.28 t (7.5)	56.54
14	4.40 m 4.47 m	63.11	4.20 dd (10.5, 5.5) 4.45 m	61.62
15		170.85		172.12
16	9.61 d (8.0)		9.25 d (8.8)	
17	5.60 dt (8.0, 6.0)	51.59	6.14 ddd (8.8, 7.6, 4.0)	50.71
18	3.16 m	43.10	3.14 dd (12.4, 7.6, 4.8)	42.78
19		143.25		142.37
20	7.45 d (7.5)	127.27	7.68 d (7.5)	126.57
21	7.10–7.24 m	128.04	7.10–7.34 m	128.02
22	7.10–7.24 m	127.39	7.10–7.34 m	126.61
23	7.10–7.24 m	128.04	7.10–7.34 m	128.02
24	7.45 d (7.5)	127.27	7.68 d (7.5)	126.57
25		170.94		170.26
26	9.06 d (8.0)		9.12 d (8.8)	
27	4.79 dt (12.0, 8.0)	54.63	5.06 dd (8.8, 7.0)	59.18
28	1.85 ddq (12.0, 7.0, 4.0) 2.13 m	25.81	4.45 m	67.60
29	0.95t (7.0)	10.53	1.43 d (6.2)	20.06
30		175.32		170.09
31			3.67 s	51.28

* ^1H NMR spectra data of Asterinin D at 250 MHz



Asterinin D: $R = R_1 = R_2 = H$; E: $R_1 = R_2 = OH$, $R = Me$

Fig.: Fragmentation pattern of Asterinin D and E

The IR spectrum showed the presence of hydroxyls, amides and an ester carboxyl group. The total assignment of ^1H and ^{13}C NMR chemical shifts were given on the basis of ^1H, ^1H COSY and ^1H, ^{13}C COSY (Table), indicating Asterinin E as a pentapeptide. The amino acid analysis of Asterinin E revealed the presence of one equivalent L-serine, two equivalents L-allothreonine, one equivalent L-β-phenylalanine, and one equivalent $\Delta^{2,4}$ pyrrolocarboxylic acid, absenting the presence of L-α-aminobutyric acid. The sequence of amino acid residues, $\Delta^{2,4}$ Pro-L-allo-Thr-L-Ser-L-βPhe-L-allo-ThrOMe, was deducted by the COLOC AND ROESY spectra.

A series of fragment peaks at m/z: 468, 94, 367, 195, 282, 131 and 429 in the FAB-mass 469, 194, 367, 282, 279, 429, 133 spectrum (Fig.) supported also the sequence of amino acid residues mentioned above.

3. Experimental

3.1. Procedures

Extraction and fractionation procedures were described in the literature [12]. Mp's are uncorrected. The results of the elemental analyses (C, H, N) were in an acceptable range. The acidic hydrolysis of Asterinin D and E was carried out at 110 °C, 6 N HCl for 2 h under which the pyrrolocarboxylic acid was unstable, α-Chymotrypsin was used for enzymatic hydrolysis in pH 9 buffer solutions [12].

3.2. Equipment

NMR spectra: Bruker spectrometer AMX 500 with standard software by Bruker. Solvent: C_5D_5N; internal standard TMS. ^1H NMR: using at 500 MHz, ^{13}C NMR: using at 125 MHz. FAB-MS: Fast atom bombardment MS were obtained on ZAB I Hf of Vacuum Generators. IR: Pye-Unicam PU 9800 with KBr. Optical rotation.: Polarimeter 241 Perkin Elmer (589 nm at 20 °C).

3.3. Asterinin D

Amorphous powder, mp. 261–265 °C;
$[\alpha]_D$-19.8 °C (70% EtOH, c 0.20). IR (ν^{KBr}, cm^{-1}): 3494 (OH), 3304 (OH), 1722 (CO_2), 1651, 1553 (HNCO). UV (λ^{MeOH} nm): 210 (log ε 4.12) and 267 (log ε 4.25). FAB-MS: m/z: 538 $[M + Na]^+$, 516 $[M + H]^+$, 422, 413, 338, 266, 249, 179, 103, 94. ^1H- and ^{13}C-NMR (Table 1).

3.4. Asterinin E

Amorphous powder, mp. 277–281 °C; $[\alpha]_D$ + 43.1° (70% EtOH, c 0.31). IR (ν^{KBr}, cm^{-1}): 3521 (OH), 3402 (OH), 3290, 1729 (CO_2), 1651 (HNCO), 1581, 1553 (HNCO), 1433, UV (λ^{MeOH}, nm) 220 (log ε, 4.14) and 267 (log ε 4.34). FAB-MS: m/z: 584 $[M + Na]^+$, 562 $[M + H]^+$, 544 $([M + H]^+ - H_2O$, 495 $([M + H]^+ - 2H_2O)$, 469, 429, 384, 391, 382, 367, 282, 279, 195, 194, 133, 131.
^1H and ^{13}C NMR: see Table.
$C_{26}H_{35}N_5O_9$

Acknowledgements: We are greatly indebted to professor Yin-Suo Zhou (Faculty of Pharmacy, Lanzhou Medical College, PR China) for his help in identification of the plant material, and to Dr. J. Peter-Katalinic of Institute of Physiological Chemistry, University Bonn, FRG for FAB-MS and useful discussions about the elucidation of two structures. This work was supported by the National Science Foundation of China.

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Received August 1, 1995
Accepted October 2, 1995

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