Pergamon 0031-9422(95)00582-X

Phytochemistry, Vol. 41, No. 1, pp. 225–227, 1996
Elsevier Science Ltd
Printed in Great Britain
0031-9422/96 $15.00 + 0.00

NEW PENTAPEPTIDES FROM *ASTER TATARICUS*

DONG-LIANG CHENG,* YU SHAO, R. HARTMANN,† E. ROEDER‡ and K. ZHAO§

Department of Chemistry, State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, People's Republic of China; †Institut für Physiologische Chemie, D-53115 Bonn, Germany; ‡Pharmazeutisches Institut, Universitaet Bonn, D-53121, Germany; §Department of Chemistry, New York University, New York, NY 10003, U.S.A.

(*Received in revised form* 5 *June* 1995)

Key Word Index—*Aster tataricus*; Compositae; pentapeptides; asterinin D, E and F.

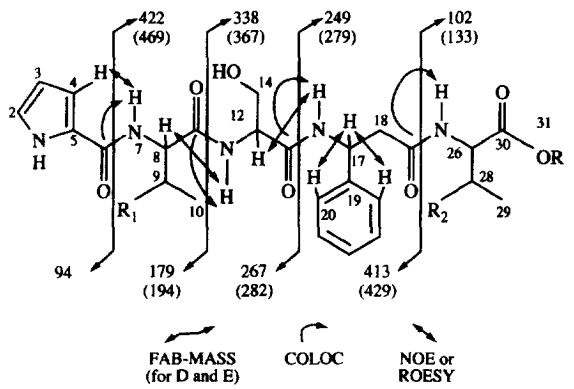
Abstract—Three pentapeptides have been isolated from the roots of *Aster tataricus* and their structures elucidated on the basis of spectroscopic analysis as well as chemical and enzymatic methods.

INTRODUCTION

In the last few years monoterpenoids, triterpenoids and oligopeptides have been isolated from the roots of *Aster tataricus* L.f. by Japanese researchers and ourselves [1–7]. In our previous papers we have reported on the isolation of some terpenoids as well as three new oligopeptides, asterinin A(**1**), B(**2**) and C(**3**), from the ethyl acetate and butanolic extracts of *A. tataricus* roots, respectively [6, 7]. Further investigation of the remaining fraction of the ethyl acetate extract gave the new pentapeptides, asterinin D(**4**), E(**5**) and F(**6**).

RESULTS AND DISCUSSION

Asterinin D (**4**) was assigned the molecular formula $C_{25}H_{33}N_5O_7$ by FAB-MS ($[M + 1]^+$ *m/z* 516) and by counting the carbons and hydrogens in the ^1H and ^{13}C NMR spectra. The IR spectrum showed the presence of hydroxyl (3303 and 3493 cm^{-1}) and amide (1651 and 1553 cm^{-1}) groups. All 25 carbons appeared in the ^{13}C NMR spectrum including the typical signals for five carbonyl carbons at δ 167.80, 170.50, 171.66, 172.52 and 174.86 and one oxygen-linked methylene and two methyl carbons. The ^1H NMR spectrum showed signals of five NH protons at δ 12.92, 9.60, 9.40, 9.05 and 8.79 (Table 1), indicating that compound **4** was a pentapeptide. Amino acid analysis of **4** after hydrolysis with either 6N HCl or α-chymotrypsin in pH 9 buffered solution at 37° revealed the presence of L-Ser(leq), L-βPhe (β-amino, β-phenylpropanoic acid) (leq), L-Abu (α-amino butyric acid) (2eq) and $\Delta^{2,4}$Pro (pyrrole-2-carboxylic acid) (leq). The presence of the pyrrole derivative was confirmed by a positive Ehrlich reaction [8]. The L-configuration of all amino acids was tentatively assigned by Masfey's method [9], as well as by comparison of the ^1H and ^{13}C NMR data with those reported natural oligopeptides [4, 5]. The presence of these amino acid residues was also supported by the ^1H and ^{13}C NMR spectra whose ambiguous assignments were resolved by a combination of ^1H–^1H COSY and ^{13}C–^1H COSY. The amino acid sequence of asterinin D was disclosed by the COLOC spectra which indicated the correlation between the signals of NH protons and those of carbonyl carbons and by a detailed analysis of its differential NOEs and ROESY spectra. The amino acid sequence was also corroborated by a series of fragment peaks at *m/z* 422 and 94, 338 and 179, 267 and 249, and 413 and 102 in the FAB-mass spectrum. Consequently,



	R	R_1	R_2
1	H	OH	H
2	Me	H	OH
3	Me	OH	H
4	H	H	H
5	Me	OH	OH
6	Me	H	H

*Author to whom correspondence should be addressed.

Table 1. ^{1}H and ^{13}C NMR chemical shift of asterinin D and E (TMS as int. st. in C_5D_5N at 500 MHz for H and 125 MHz for C)

	Asterinin D*		Asterinin E	
H/C	^{1}H (J = Hz)	^{13}C	^{1}H (J = Hz)	^{13}C
1	12.92 *br s*		12.95 *br s*	
2	7.21 *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*	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)	26.33	4.63 *dq* (8.5, 6.2)	68.58
	2.06 *ddq* (12.0, 8.0, 4.0)			
10	0.96 *t* (7.0)	10.88	1.55 *d* (6.2)	20.65
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*	63.11	4.20 *dd* (10.5, 5.5)	61.62
	4.47 *m*		4.45 *m*	
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 *ddd* (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*	128.02	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)	25.81	4.45 *m*	67.60
	2.13 *m*			
29	0.95 *t* (7.0)	10.53	1.43 *d* (6.2)	20.06
30		175.32		170.09
31			3.67 *s*	51.28

*^{1}H NMR spectra data of asterinin D measured at 250 MHz.

the structure of Asterinin D is $\Delta^{2,4}$Pro-L-Abu-L-Ser-L-βPhe-L-Abu.

Asterinin E (**5**) was found to have the molecular formula $C_{26}H_{35}N_5O_7$ (FAB–MS: $[M + 1]^+$ *m/z* 562; elemental analysis found: C, 55.53; H, 6.30, N, 12.36%). The IR spectrum showed the presence of hydroxyl (3300 and 3490 cm^{-1}) and amide (1651 and 1553 cm^{-1}) groups and an ester carbonyl group (1729). The complete assignment of the ^{1}H and ^{13}C NMR chemical shifts was achieved on the basis of ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY (Table 1), and indicated that compound **5** was also a pentapeptide. Amino acid analysis of **5**, after acidic and enzymatic hydrolysis, revealed the presence of L-Ser(1 eq), L-allo-Thr(2 eq), L-βPhe(1 eq) and $\Delta^{2,4}$Pro (1 eq), but no L-Abu. The sequence of amino acid residues, $\Delta^{2,4}$Pro-L-allo-Thr-L-Ser-L-Pro-L-allo-Thr-OMe, was deduced by means of COLOC, NOEs and ROESY. These established the correlation between the signals of the NH protons and those of the carbonyl carbons, and the NOEs of long range protons. A series of fragment peaks at *m/z* 468, 94, 367, 195, 279, 282, 131 and 429 in the FAB–MS supported the sequence of amino acid residues given above.

Asterinin F (**6**) was shown to have the molecular formula $C_{26}H_{35}N_5O_7$ (FAB–MS: $[M + 1]^+$ *m/z* 530; elemental analysis found: C, 59.01; H, 6.69; N, 13.13%). The amino acid composition of compound **6** was shown to be identical with that of **4** by means of acid and enzymatic hydrolysis. The IR and NMR spectral data were very close to those of **4**. However, a three-proton singlet at δ 3.60 due to a methoxyl group, indicated that **6** was the methyl ester of **4**. This proposal was further confirmed by the M_r of **6** (14 amu higher than that of **4**). Furthermore, methylation of **4** with diazomethane afforded **6**. Therefore, asterinin F was determined to be ^{2,4}Pro-L-Abu-L-Ser-L-βPhe-L-Abu-OMe.

EXPERIMENTAL

Mps: uncorr; ^1H and ^{13}C NMR: 500 MHz and 125 MHz, respectively, in pyridine-d_6 using TMS as int. st.; ^1H NMR: 250 MHz for asterinin D; FAB–MS: VG.ZAB-HS mass spectrometer.

Isolation of asterinin D (**4**) *E* (**5**) *and F* (**6**). The extraction and fractionation of the plant material are described in Ref. [6]. The fraction eluted with $CHCl_3$–MeOH (5:1) was further purified by CC with C_6H_6–MeCOEt–MeOH (8:2:1 to 6:2:1) and prep. TLC to give compounds **5** (300 mg) and **6** (150 mg), respectively. The fraction eluted with $CHCl_3$–MeOH (2:1) was further purified by CC with $CHCl_3$–MeOH–H_2O (18:5:1) and Sephadex LH-20 to give 200 mg compound **4**.

Asterinin D (**4**). Amorphous powder, mp 261–265° (dec.) (from 70% EtOH), $[\alpha]_D$ −19.8° (70%, EtOH *c* 0.20); IR ν^{KBr} cm^{-1}: 3494, 3304, 1722, 1651, 1553; UV λ^{MeOH} nm 210 (log ε, 4.10) and 269 (log ε, 4.35); FAB–MS *m/z*: 538 $[M + Na]^+$, 516 $[M + Na]^+$, 422, 413, 338, 266, 249, 179, 103, 94; ^1H and ^{13}C NMR: Table 1.

Asterinin E (**5**). Amorphous powder, mp. 277–281° (dec.) (from 70% EtOH). $[\alpha]_D$ +43.1° (70%, EtOH *c* 0.31); IR ν^{KBr} cm^{-1}: 3521, 3402, 3290, 1729, 1651, 1581, 1553, 1433; UV λ^{MeOH} nm 212 (log ε 4.54) and 267 (log ε, 4.34); FAB–MS *m/z*: 584, $[M + Na]^+$, 562 $[M + 1]^+$, 544 ($[M + H]^+ - 18$), 495 ($[M + H]^+ - 2H_2O$), 469, 429, 384, ,391, 382, 367, 282, 279, 195, 194, 133, 131. Anal. calc. for $C_{26}H_{35}N_5O_9$ (%): C, 55.61; H, 6.24; N, 12.48. C, 55.53; H, 6.30; N, 12.36. Found: C, 55.53; H, 6.30; N, 12.36. ^1H and ^{13}C NMR: Table 1.

Asterinin F (**6**). Amorphous powder, mp. 236–241° (dec.) (from 70% EtOH). $[\alpha]_D$ −30.9° (70% EtOH, *c* 0.16); IR ν^{KBr} cm^{-1} 3402, 3310, 1750, 1651, 1553, 1454; UV λ^{MeOH} nm 210 (log ε, 4.49) and 267 (log ε, 4.43); FAB–MS *m/z*: 552 $[M + Na]^+$, 530 $[M + 1]^+$, 437, 413, 352, 263, 265, 266, 235, 179, 131; ^1H and ^{13}C NMR: Table 2.

Acidic and enzymatic analyses of compounds **4–6**, *and methylation of compound* **4**. The procedures used were as described in ref. [7]. Each sample was analysed for amino acids by means of an amino acid analyser.

Acknowledgements—The authors are greatly indebted to Prof. Zhou Yin-Suo (Faculty of Pharmacy, Lanzhou Medical College, People's Republic of China) for his help in identification of the plant material, and to Dr Frau Peter-Katalinic of Institute of Physiological Chemistry (Germany) for FAB–MS and useful discussions about the structural elucidation of these compounds. This work was supported by the National Natural Science Foundation of China and the W.M. Keck Foundation of the U.S.A.

Table 2. ^1H and ^{13}C NMR spectral data of asterinin F (in pyridine-d_6)*

C/H	^1H	^{13}C
1	12.72 *br s*	
2	7.53 *br s*	110.53
3	6.10 *br s*	109.05
4	7.58 *br s*	121.74
5		126.52
6		161.73
7	9.83 *d* (8.0)	
8	5.15 *m*	54.65
9	1.91 *ddq* (12.0, 8.0, 4.0)	25.73
	2.13 *ddq* (12.0, 8.0, 4.0)	
10	0.97 *t* (7.2)	10.12
11		172.53
12	8.70 *d* (8.0)	
13	5.12 *m*	55.99
14	4.16 *dd* (7.0, 3.5)	62.36
	4.22 *dd* (7.0, 3.5)	
15		170.20
16	9.51 *d* (8.0)	
17	5.95 *ddd* (8.0, 7.2, 4.4)	50.71
18	3.00 *ddd* (12.2, 7.2, 4.4)	42.13
19		142.28
20	7.35 *d* (7.5)	126.59
21	7.10 *m*	128.11
22	7.10 *m*	126.45
23	7.10 *m*	128.11
24	7.35 *d* (7.5)	126.59
25		170.37
26	9.18 *d* (8.0)	
27	4.65 *dt* (8.0, 7.5)	53.61
28	1.68 *ddq* (12.0, 8.0, 4.0)	24.68
29	0.81 *t* (7.2)	9.72
30		170.72
31	3.45 *s*	51.25

*Coupling constants (*J* in Hz) in parentheses.

REFERENCES

1. Nagao, T., Okabe, H. and Yamaochi, T. (1988) *Chem. Pharm. Bull.* **36**, 571.
2. Nagao, T., Hashiyama, S., Okabe, H. and Yamaochi, T. (1989) *Chem. Pharm. Bull.* **37**, 1977.
3. Nagao, T., Okabe, H. and Yamaochi, T. (1990) *Chem. Pharm. Bull.* **38**, 783.
4. Kosemura, S. (1993) *Tetrahedron Lett.* **34**, 1291.
5. Morita, H., Nagashima, S., Takeya, K. and Itokawa, H. (1993) *Chem. Pharm. Bull.* **41**, 993.
6. Cheng, D.-L. and Shao, Y. (1994) *Phytochemistry* **35**, 173.
7. Cheng, D.-L. and Shao, Y. (1994) *Phytochemistry* **36**, 945.
8. Mattocks, A. R. (1967) *J. Chromatogr.* **27**, 505.
9. Ruterbories, K. J. and Nurok, D. (1987) *Anal. Chem.* **59**, 2735.