Pergamon

Tetrahedron Letters 42 (2001) 7079–7081

TETRAHEDRON LETTERS

Catharsitoxins from the Chinese remedy qiung laug

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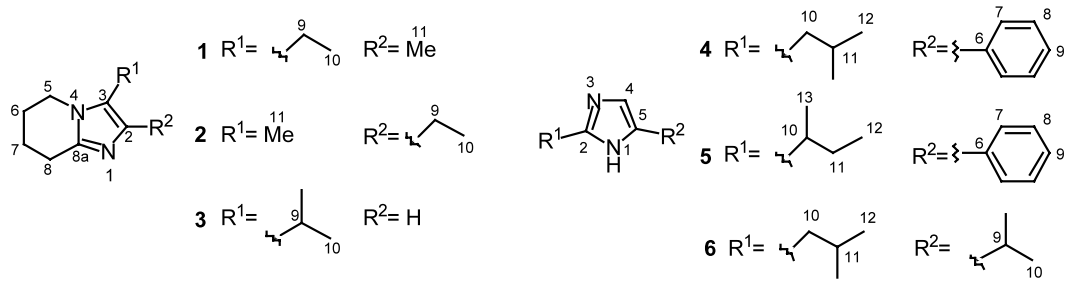
Received 5 July 2001; revised 8 August 2001; accepted 9 August 2001

Abstract—Novel imidazole compounds, catharsitoxins A, B, C, D, E and F, were isolated from the Chinese remedy qiung laug. Their structures were determined by 2D NMR. To confirm their structures and to ensure an adequate supply for further biological studies, catharsitoxins A and D were synthesized. © 2001 Elsevier Science Ltd. All rights reserved.

The Chinese remedy qiung laug is prepared by scalding the dung beetle *Catharsius molossus* L. to death in boiling water and drying with a charcoal fire. While it has been used in China for more than 500 years to treat spasmodic contractions, it carries a risk: it contains poison,[1] and pregnant women must be particularly careful. However, the toxic components of this medicine have not yet been clarified. We have obtained novel imidazole compounds, catharsitoxins A, B, C, D, E and F, by monitoring acute toxicity against mice. We report here the isolation and structures of these compounds.

The Chinese remedy qiung laug (10 kg) was crushed and extracted with 75% ethanol for 2 days. The extract was filtered, concentrated and partitioned between EtOAc and H_2O. Both layers were toxic against mice. The aqueous layer was chromatographed on TSK G-3000S polystyrene gel (50% EtOH), DEAE Sephadex A-25 (0.02 M pH 6.9 phosphate buffer), CM Sephadex C-25 (0.2 M pH 6.9 phosphate buffer), and DEAE Sephadex A-25 (0.02 M pH 6.9 phosphate buffer) again, and reversed-phase HPLC (i. Develosil C8-10, 40% MeOH containing 0.1% trifluoroacetic acid (TFA); ii. Develosil 300C4-HG-5, 20% MeOH containing 0.1% TFA, iii. Develosil ODS-HG-5, 15% MeOH containing 0.1% TFA) guided by acute toxicity against mice to give catharsitoxin A (**1**, 1.5 mg) as a colorless oil. The ethyl acetate layer was extracted with 1% aqueous AcOH, and the aqueous layer was basified (pH 10) with NH_4OH and re-extracted with EtOAc. The resulting alkaloid fraction was purified by chromatography by monitoring acute toxicity against mice to give catharsitoxins B (**2**, 0.5 mg), C (**3**, 0.9 mg), D (**4**, 1.2 mg), E (**5**, 1.1 mg), and F (**6**, 2.3 mg) as colorless oils, respectively.

The molecular formula of **1** was found to be $C_{10}H_{16}N_2$ by HRFABMS (m/z 165.1400, calcd for $C_{10}H_{17}N_2$ $[M+H]^+$ 165.1392). The ^1H NMR spectrum of **1** showed the presence of a methyl group and an ethyl group (Table 1). An analysis of the COSY spectrum of **1** allowed for the construction of four contiguous methylenes (C5–C8). The presence of an imidazole ring was suggested by characteristic carbon NMR data (δ_C 142.5, 130.5, 123.2). The HMBC correlations shown in Table 1 indicated connectivity among the partial structures described above. Although two carbons, C2 and C3, were exchangeable, they were assigned by comparison of their ^{13}C chemical shifts with those of 5,6,7,8-tetrahydroimidazo[1,2-a]pyridine (δ_{C2} 118.3, δ_{C3} 135.6).[2]



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0040-4039/01/$ - see front matter
PII: S0040-4039(01)01468-X

Table 1. NMR data for catharsitoxin A (**1**) in CD_3OD[a]

Position	^1H[b]	^{13}C[c]	HMBC ($^1H \rightarrow ^{13}C$)
2		123.2	
3		130.5	
5	4.02 (dd, $J=5.9$, 5.9 Hz, 2H)	43.2	C-8a
6	2.09 (m, 2H)	21.3	
7	1.98 (m, 2H)	18.6	
8	2.94 (dd, $J=6.3$, 6.3 Hz, 2H)	22.7	C-8a, 7
8a		142.5	
9	2.67 (q, $J=7.6$ Hz, 2H)	15.3	C-2, 3, 10
10	1.18 (t, $J=7.6$ Hz, 3H)	13.0	C-3, 9
11	2.24 (s, 3H)	8.7	C-2, 3

[a] One drop of TFA was added to the solvent.
[b] Recorded at 800 MHz.
[c] Recorded at 200 MHz.

Thus, the structure of catharsitoxin A was determined to be as shown in formula **1**.

The structure of catharsitoxin D (**4**) was determined as follows. The molecular formula of **4** was found to be $C_{13}H_{16}N_2$ by HRFABMS (m/z 201.1396, calcd for $C_{13}H_{17}N_2$ $[M+H]^+$ 201.1392). The presence of an isobutyl group and a phenyl group was indicated by the ^1H NMR and COSY spectra (Table 2). Characteristic carbon NMR data (δ_C 148.0, 133.5, 114.2) and HMBC correlations from an aromatic singlet proton (δ 7.79) to C2 and C5 suggested the presence of a 2,5-disubstituted imidazole ring. Furthermore, the HMBC correlations, H10/C-2 and H7/C5, allowed for connection of the above partial structures, which established that the structure of catharsitoxin D was as shown in formula **4**. The structures of catharsitoxins B (**2**), C (**3**), E (**5**), and F (**6**) were determined by 2D NMR in the same manner as described above for **1** and **4**.[3]

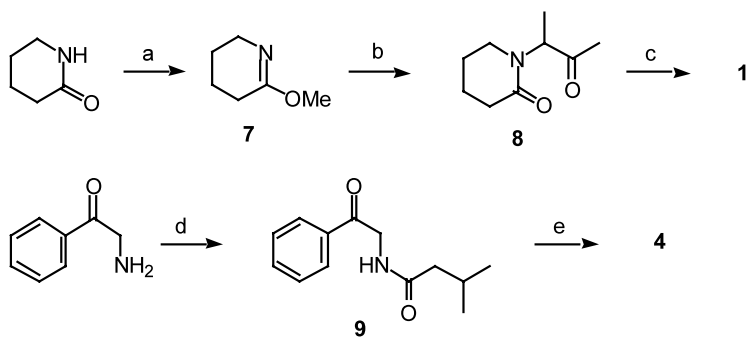
To confirm the structures of catharsitoxins and to ensure an adequate supply for further biological studies, catharsitoxins A (**1**) and D (**4**) were synthesized (Scheme 1). The synthesis of **1** was started from δ-valerolactam. δ-Valerolactam was converted into iminoether **7** with Meerwein reagent,[4] which was alkylated with 3-chloro-2-pentanone (10% in two steps).[5] Cyclization[6] of amide **8** gave catharsitoxin A (**1**) in 56% yield. Catharsitoxin D (**4**) was synthesized from 2-aminoacetophenone. Acylation of 2-aminoacetophenone gave amide **9** (73%), which was cyclized to give catharsitoxin D (**4**) in 85% yield. Synthetic catharsitoxins A (**1**) and D (**4**) were identical to natural **1** and **4** in all respects including spectroscopic data (IR, ^1H and ^{13}C NMR, MS) and acute toxicity against mice ($LD_{99}=$ 100 mg/kg for **1**, 50 mg for **4**).

In conclusion, we isolated catharsitoxins A, B, C, D, E and F, novel imidazole compounds from the Chinese remedy qiung laug, which has been used in China for more than 500 years to treat spasmodic contractions. Structurally related alkaloids containing an imidazole ring[7] such as tolazoline and pilocarpine[8] have been used for clinical drugs. Further biological studies on catharsitoxins are currently in progress.

Table 2. NMR data for catharsitoxin D (**4**) in CD_3OD[a]

Position	^1H[b]	^{13}C[c]	HMBC ($^1H \rightarrow ^{13}C$)
2		148.0	
4	7.79 (s, 1H)	114.2	C-2, 5
5		133.5	
6		126.8	
7	7.70 (dd, $J=1.4$, 7.6 Hz, 2H)	125.2	C-5, 6, 8
8	7.53 (dd, $J=7.6$, 7.6 Hz, 2H)	129.1	C-7, 9
9	7.47 (t, $J=7.6$ Hz, 1H)	129.3	C-8
10	2.89 (d, $J=7.3$ Hz, 2H)	34.1	C-2, 11, 12
11	2.19 (m, 1H)	28.4	C-10, 12
12	1.04 (d, $J=6.5$ Hz, 6H)	21.1	C-10, 11

[a] One drop of TFA was added to the solvent.
[b] Recorded at 800 MHz.
[c] Recorded at 200 MHz.



Scheme 1. (a) Me_3OBF_4, i-Pr_2NEt, CH_2Cl_2, rt, 2 h; (b) 2-chloropentanone, KBr, DMF, 110°C, 24 h, 10% in two steps; (c) $AcONH_4$, AcOH, reflux, 8 h, 56%; (d) isovaleric acid, Et_3N, EDCI·HCl, CH_2Cl_2, rt, 2 h, 73%; (e) $AcONH_4$, AcOH, reflux, 5 h, 85%.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (No. 11558079) and Scientific Research on Priority Areas (A) (No. 12045235) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan. We are indebted to Wako Pure Chemical Industries Ltd., and Banyu Pharmaceutical Co., Ltd. for their financial support. We thank Professors R. Noyori and M. Kitamura (COE, Nagoya University) for performing the NMR experiments.

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3. Compound **2**: ^{1}H NMR (800 MHz, CD_3OD containing one drop of TFA) δ 3.92 (dd, $J=5.9$, 5.9 Hz, 2H), 2.96 (dd, $J=6.3$, 6.3 Hz, 2H), 2.66 (q, $J=7.6$ Hz, 2H), 2.24 (s, 3H), 2.10 (m, 2H), 2.00 (m, 2H), 1.23 (t, $J=7.6$ Hz, 3H); HRFABMS m/z 165.1415, calcd for $C_{10}H_{17}N_2$ $(M+H)^+$ 165.1392.
   Compound **3**: ^{1}H NMR (800 MHz, CD_3OD containing one drop of TFA) δ 7.12 (s, 1H), 4.09 (dd, $J=5.9$, 5.9 Hz, 2H), 2.97 (dd, $J=6.3$, 6.3 Hz, 2H), 2.97 (m, 1H), 2.09 (m, 2H), 2.01 (m, 2H), 1.31 (d, $J=7.2$ Hz, 6H); HRFABMS m/z 165.1395, calcd for $C_{10}H_{17}N_2$ $(M+H)^+$ 165.1392.
   Compound **5**: ^{1}H NMR (800 MHz, CD_3OD containing one drop of TFA) δ 7.79 (s, 1H), 7.70 (dd, $J=8.0$, 1.4 Hz, 2H), 7.53 (dd, $J=7.6$, 7.6 Hz, 2H), 7.47 (t, $J=7.6$ Hz, 1H), 3.19 (m, 1H), 1.86 (m, 2H), 1.47 (d, $J=9.6$ Hz, 3H), 0.96 (t, $J=7.2$ Hz, 3H); HRFABMS m/z 201.1412, calcd for $C_{13}H_{17}N_2$ $(M+H)^+$ 201.1392. This compound was found to be racemic by chiral HPLC analysis.
   Compound **6**: ^{1}H NMR (800 MHz, CD_3OD containing one drop of TFA) δ 7.79 (s, 1H), 3.02 (m, 1H), 2.79 (d, $J=6.3$ Hz, 1H), 2.10 (m, 1H), 1.32 (d, $J=7.4$ Hz, 6H), 0.98 (d, $J=6.5$ Hz, 6H); HRFABMS m/z 167.1553, calcd for $C_{10}H_{18}N_2$ $(M+H)^+$ 167.1548.
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