ENANTIOSELECTIVE SYNTHESIS OF POISON-FROG ALKALOID 237D AND DETERMINATION OF ABSOLUTE STEREOCHEMISTRY

Naoki Toyooka,*1 Masashi Kawasaki,2 Hideo Nemoto,*1 John W. Daly,3 Thomas F. Spande,3 and H. Martin Garraffo3

1: Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama 930-0194, Japan; 2: Department of Liberal Arts and Sciences, Faculty of Engineering, Toyama Prefectural University, Kurokawa 5180, Kosugi-Machi, Toyama 939-0398, Japan; 3: Laboratory of Bioorganic Chemistry, National Institutes of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, DHHS, Bethesda, Maryland 20892, USA

Abstract - An enantioselective synthesis of the 5-heptyl-8-methylindolizidine ((−)-7) has been achieved. Alkaloid ((−)-7, (MW 237)) was used to determine the relative and absolute stereochemistry of the natural indolizidine 237D from frog skin as 5S, 8S, 9R using GC-IR and GC-MS. Chiral gas-chromatographic comparisons with catalytically reduced (+)-235B″ and (−)-235B′ indicated (−)-7 had the same absolute stereochemistry as the dihydro-product resulting from (−)-235B′ and is naturally occurring in certain extracts of Panamanian poison frogs (Dendrobates).

The 5,8-disubstituted indolizidines are one of the major subclasses of poison frog alkaloids, and over sixty such alkaloids have been detected to date.1 Recently, a 5,8-disubstituted indolizidine was detected in a mixed collection of small arthropods and one group of these arthropods is presumed to be the source of the 5,8-disubstituted indolizidines found in the skin of poison frogs.2 Alkaloid 237D, detected in extracts of Dendrobates pumilio and D. speciosus,3 had the above indolizidine core, and the relative stereochemistry was expected to be 5,9-Z due to the intense Bohlmann bands observed in their GC-FTIR spectra.1 However, the relative stereochemistry at the 8-position was not known, nor was the absolute stereochemistry.

We now report the synthesis of indolizidine ((−)-7) and its use to establish both the relative and absolute stereochemistry of natural 237D using GC-IR, GC-MS and GC with a chiral column.

The synthesis (see Scheme 1) began with the known 2,3,6-trisubstituted piperidine (1),5 prepared stereoselectively by our original Michael-type conjugate addition reaction to the enaminoester as the key step,5 which was then converted to the α,β-unsaturated ester (2). Hydrogenation of 2 followed by reduction of the ester moiety with Super-Hydride gave the alcohol (3). Treatment of 3 with MOMCl in the presence
of the Hünig base provided the MOM ether (4), which was treated with TBAF to provide the alcohol (5). Swern oxidation of 5 and Wittig olefination of the resulting aldehyde gave rise to the olefin (6) as a mixture of E- and Z-isomers. Hydrogenation of the double bond in 6 followed by indolizidine formation using a 3-step sequence furnished the indolizidine ((−)-7). Synthetic (−)-7 was co-chromatographed on a non-chiral GC-column with natural 237D found in an extract of *Dendrobates speciosus*, and had exactly the same mass and infrared spectrum as the natural product.

Scheme 1: Reagents and conditions: 

- a: Swern ox.; b: (EtO)₂P(O)CH₂CO₂Et, NaH, THF, 0 °C-rt (95%);
- c: 10% Pd-C, H₂, EtOAc, 4 atm;
- d: Super-Hydride, THF, 0 °C (93%);
- e: MOMCl, Hünig base, CH₂Cl₂, 0 °C-rt (85%);
- f: TBAF, THF, 0 °C-rt (95%);
- g: Swern ox.; h: Me(CH₂)₅P⁺Ph₃Br⁻, n-BuLi, THF, 0 °C-rt (66%);
- i: 10% Pd-C, H₂, EtOAc, 1 atm;
- j: n-PrSLi, HMPA, THF, 0 °C-rt;
- k: conc. HCl, MeOH, reflux;
- l: CBr₄, Ph₃P, then Et₃N, CH₂Cl₂, 0 °C-rt (42%)

Lacking racemic 237D, to demonstrate enantiomer separation on a chiral column, we prepared (+)-237D by catalytic reduction of (+)-235B'' ([α]D +11.3°), which previously had been isolated from *D. pumilio*³ and (−)-237D by reduction of (−)-235B' ([α]D −61°) present in an extract of *D. speciosus*³b (see below). Gas chromatography using flame-ionization detection and a chiral column, permethylated β-cyclodextrin (SGE, 30 m x 0.25 mm; 130°-200°C at 0.5°C/ min), resulted in a baseline separation of (+)- and (−)-237D prepared in this way. The retention times were 31.9 and 32.4 min. respectively. The alkaloid 237D present in *D. pumilio* or *D. speciosus* was co-chromatographed with (−)-7 on the chiral column using the above conditions.
The synthetic (-)-7 and reduced 235B’ co-chromatographed on GC-MS with a non-chiral column (Zebron-5 (Phenomenex) 100°C-280°C at 5°C/ min) and had identical GC-EIMS and GC-FTIR spectra proving that they had the same relative stereochemistry. We conclude that the absolute stereochemistry of 237D occurring naturally in D. pumilio or D. speciosus is the same as that of (-)-7 and has the 5S, 8S, 9R absolute stereochemistry as indicated in Scheme 1.

ACKNOWLEDGMENT

This work was supported in part by The Research Foundation for Pharmaceutical Sciences.

REFERENCES AND NOTES


3 a) T. Tokuyama, N. Nishimori, A. Shimada, M. W. Edwards, and J. W. Daly, Tetrahedron, 1987, 43, 643.; b) M. W. Edwards, J. W. Daly, and C. W. Myers, J. Nat. Prod., 1988, 51, 1188. The detection of 237D in the Dendrobates pumilio extract is a new finding. Alkaloid 235B' from D. pumilio and (-)-7 could not be separated with a non-chiral column, indicating the likelihood that the small amount of (-)-237D in the two extracts recently examined was missed earlier, but is detectible with the chiral column and the very slow temperature program used in the present work.


6 The spectral data for synthetic (-)-7 are as follows.

IR (neat) 2967, 2934, 2879, 2787, 2706, 1461, 1378, 1163, 1133 cm⁻¹; ¹H NMR (500 MHz) δ 0.88 (3H, d, J = 6.8 Hz), 0.89 (3H, t, J = 6.8 Hz), 0.97 (1H, q-like, J = 11.5 Hz), 1.27-1.38 (13H, br), 1.51-2.18 (10H, br m), 3.31 (1H, br); ¹³C NMR (75 MHz) δ 14.16 (q), 18.93 (q), 20.36 (t), 22.72 (t), 25.89 (t), 29.03 (t), 29.32 (t), 30.05 (t), 31.15 (t), 31.88 (t), 33.67 (t), 34.53 (t), 36.44 (d), 51.78 (t), 63.60 (d),
71.37 (d); MS: 237 (M\(^+\)), 138 (100); HRMS Calcd for C\(_{16}\)H\(_{33}\)N 237.2455, Found 237.2458; \([\alpha]\)\(_D\) -98.9° (c 1.59, CHCl\(_3\)).