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On the structure of aspongopusin recently isolated from *Aspongopus chinensis*

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ABSTRACT

The 2,5-disubstituted oxazole recently proposed for aspongopusin, a natural product isolated from *Aspongopus chinensis*, was synthesized through an unambiguous route. The synthetic sample showed ^{1}H and ^{13}C NMR entirely different from those in the literature, revealing that the initially assigned structure was incorrect. The spectroscopic data for the given structure are thus made available for the first time.

1. Introduction

Aspongopus chinensis is one of the insects in Pentatomidae family found mainly in southern China [1]. In traditional Chinese medicine, the insect is used to relieve pain and treat nephropathy. It is also known for delicious taste and richness in nutrients [2]. Recently, a novel oxazole, named aspongopusin, was isolated from *A. chinensis* [1], which showed significant activity against several tumor cell lines and encouraged further studies. In efforts to establish the identity of aspongopusin extensive analyses, including ^{1}H and ^{13}C NMR, DEPT, COSY, HMQC, HMBC and HRMS, were performed. And on the basis of the structural data acquired the 2,5-disubstituted (a substitution pattern previously not seen among natural products) oxazole (**1**, Fig. 1) was assigned [1].

For a molecule of this size and complexity, the structure of the compound thus assigned is usually considered unequivocal. However, to our surprise, the data for this planar molecule, without any apparent chiral elements, also included optical rotation ($[\alpha]_D^{24} + 28$ (*c* 0.1, MeOH)). This seems rather peculiar. Was the sample polluted by traces of optically active impurities? Or might there be something really unusual with this particular compound? Or was the structure erroneously assigned? Prompted by these doubts, we synthesized the structure (**1**) proposed for that natural product. Herein we present the details of this endeavor.

2. Experimental

2.1. General

The NMR spectra were recorded on a Bruker Avance NMR spectrometer operating at 400 MHz for ^{1}H with Me_4Si as the internal standard. IR spectra were measured on a Nicolet 380 Infrared Spectrometer. ESI-MS data were acquired on a Shimadzu LCMS-2010EV mass spectrometer. HRMS data were obtained with a Bruker APEXIII 7.0 Tesla FT-MS spectrometer. Melting points were uncorrected (measured on a hot stage melting point apparatus equipped with a microscope). CH_2Cl_2 and toluene were dried with activated 4 Å MS (molecular sieves). All chemicals were reagent grade and used as purchased. Column chromatography was performed on silica gel (300–400 mesh) under slightly positive pressure. PE (for chromatography) stands for petroleum ether (b.p. 60–90 °C).

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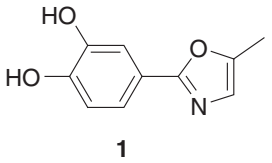

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Fig. 1. The structure proposed in the literature for aspongopusin.

2.2. Condensation of 3 with 4 to afford 5

A mixture of acid **3** (400 mg, 1.68 mmol) and EDCI (420 mg, 2.18 mmol) in dry CH_2Cl_2 (16 mL) was stirred at ambient temperature for 30 min. Aminol **4** (0.13 mL, 1.68 mmol) was then introduced. The mixture was stirred at the same temperature for 4 h, when TLC showed completion of the condensation. Water (5 mL) was added, followed by EtOAc (15 mL). The phases were separated. The aqueous layer was extracted with EtOAc (7 mL×3). The combined organic layers were washed with water and brine before being dried over anhydrous Na_2SO_4. Removal of the solvent by rotary evaporation and column chromatography (1:20 MeOH/CH_2Cl_2) on silica gel gave amide **5** as a colorless oil, which on standing in a freezer became a white solid (396 mg, 1.34 mmol, 81%). Mp 89–91 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.79 (dd, J=2.0, 8.3 Hz, 1H), 7.73 (d, J=2.1 Hz, 1H), 7.35 (d, J=8.3 Hz, 1H), 3.94–4.02 (m, 1H), 3.34 (dd, J=4.8, 13.6 Hz, 1H), 3.33 (dd, J=4.8, 13.6 Hz, 1H) (overlap the solvant residual), 2.32 (s, 3H), 2.31 (s, 3H), 1.22 (d, J=6.2 Hz, 3H). ^{13}C NMR (100, CD_3OD): δ 170.0, 169.8, 168.6, 168.5, 146.4, 143.7, 134.3, 134.2, 126.8, 125.0, 124.2, 67.6, 48.6, 21.3, 20.72, 20.70. FT-IR (film): 3360, 3074, 2972, 2932, 1773, 1647, 1545, 1498, 1371, 1207, 1173, 1012, 901, 587 cm^{-1}. ESI-MS m/z 296.3 ($[M+H]^+$). ESI-HRMS calcd for $C_{14}H_{17}NNaO_6$ ($[M+Na]^+$): 318.0948; found: 318.0935.

2.3. Conversion of 5 into 7 via 6

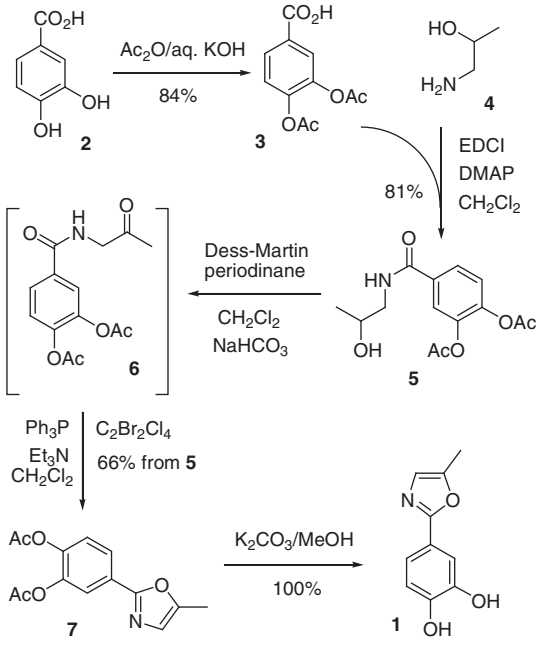
A mixture of alcohol **5** (500 mg, 1.70 mmol), $NaHCO_3$ (2.90 g, 34 mmol), Dess-Martin Periodinane (1.80 g, 4.23 mmol) in dry CH_2Cl_2 (12 mL) was stirred at ambient temperature for 2 h, when TLC showed completion of the oxidation. Aq. sat. Na_2SO_3 (4 mL) was added, followed by EtOAc (10 mL). The phases were separated. The aqueous layer was extracted with EtOAc (10 mL×3). The combined organic layers were washed with water and brine before being dried over anhydrous Na_2SO_4. The mixture was filtered through a pad of silica gel (washing with 1:30 MeOH/CH_2Cl_2). The filtrate was concentrated by rotary evaporation to give the intermediate ketone **6**, which was directly dissolved in CH_2Cl_2 (6.8 mL) and then added to a mixture of PPh_3 (890 mg, 3.4 mmol), $Cl_2BrCCBrCl_2$ (1.11 g, 3.4 mmol) and Et_3N (0.95 mL, 6.8 mmol) in CH_2Cl_2 (34 mL) stirred at ambient temperature. The mixture was stirred at the same temperature for 5 h. Water (10 mL) was added, followed by Et_2O (30 mL). The phases were separated. The aqueous layer was extracted with Et_2O (15 mL×2). The combined organic layers were washed with water and brine before being dried over anhydrous Na_2SO_4. Removal of the solvent by rotary evaporation and column chromatography (1:2 EtOAc/PE) on silica gel afforded **7** as a white solid (305 mg, 1.11 mmol, 66% from **5**). Mp 100–102 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (dd, J=2.1, 8.6 Hz, 1H), 7.83 (d, J=1.7 Hz, 1H), 7.27 (d, J=8.5 Hz, 1H), 6.83 (s, 1H), 2.37 (s, 3H), 2.31 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (100, $CDCl_3$): δ 168.03, 167.99, 159.2, 149.4, 143.4, 142.4, 126.4, 124.1, 124.0, 121.2, 20.6, 20.5, 11.0. FT-IR (film): 2925, 1774, 1604, 1555, 1489, 1370, 1205, 1164, 1111, 1009, 903, 732 cm^{-1}. ESI-MS m/z 276.2 ($[M+H]^+$). ESI-HRMS calcd for $C_{14}H_{13}NNaO_5$ ($[M+Na]^+$): 298.0686; found: 298.0688.

2.4. Removal of acetyl groups in 7 to afford 1

A mixture of **7** (61 mg, 0.22 mmol) and K_2CO_3 (15 mg, 0.11 mmol) in MeOH (de-aired by bubbling argon into the MeOH before use, 5 mL) stirred at ambient temperature under argon for 25 min, when TLC showed completion of the reaction. Aq. HCl (1 N, 10 drops from a pipette) was added. The mixture was concentrated on a rotary evaporator. The residue was chromatographed (1:1 EtOAc/PE) on silica gel to give oxazole **1** as a white powder (42 mg, 0.22 mmol, 100% from **7**). Mp 192–194 °C. $[\alpha]_D^{27}$ −0.3 (c 0.23, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.39 (d, J=2.0 Hz, 1H), 7.34 (dd, J=2.2, 8.3 Hz, 1H), 6.87 (d, J=8.1 Hz, 1H), 6.82 (dd, J=1.1, 2.3 Hz, 1H), 2.38 (d, J=1.1 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 163.7, 150.9, 150.1, 147.6, 124.8, 121.1, 120.4 117.4, 115.0, 11.5. FT-IR (KBr): 3499, 3459, 3124, 2927, 2869, 2704, 2586, 1618, 1610, 1564, 1510, 1454, 1327, 1289, 1216, 1171, 1115, 869, 737 cm^{-1}. UV (MeOH) λ_{max} (log ε): 213.0 (4.26), 285.0 (4.18), 303.0 (4.13) nm. ESI-MS m/z 192.1 ($[M+H]^+$). ESI-HRMS calcd for $C_{10}H_{10}NO_3$ ($[M+H]^+$): 192.0655; found: 192.0655.

3. Results and discussion

The synthesis is outlined in Scheme 1. The commercially available acid **2** was acetylated with acetic anhydride in aqueous KOH to afford diacetate **3** as described in a literature



Scheme 1. Synthesis of oxazole 1.

[3]. The resulting phenol-protected acid **3** was then connected to commercially available aminol **4**, using EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) [4] to give the desired amide **5**.

The alcohol **5** could be readily transformed into corresponding dihydrooxazole, but further elaboration into end product **1** was not successful. Therefore, an oxidation under the Dess-Martin [5] conditions was performed to yield the intermediate ketone **6**. Conversion of similar α-amido-ketones into oxazoles using $Ph_3P/Et_3N/CH_2Cl_2$ in the presence of e.g. I_2 [6], or C_2Cl_6 [7] are known. In this work we tried $C_2Br_2Cl_4$ (1,2-dibromo-tetrachloro-ethane) [8] to obtained equally good results. Thus, treatment of the crude intermediate ketone **6** afforded the expected **7** in 66% yield over two steps. Finally, the acetyl protecting groups were removed with K_2CO_3 in MeOH [9] to give oxazole **1**.

The ^1H and ^{13}C NMR for the synthetic **1** were measured in the same solvent (CD_3OD) as reported in the literature. Surprisingly, neither ^1H nor ^{13}C NMR was compatible with that reported in the literature (cf. Table S-1 and Fig. S-1). UV–vis was also apparently different from what was measured on the natural product. These distinct data discrepancies show beyond all doubts that aspongopusin and oxazole **1** are two different compounds. The genuine structure for that natural aspongopusin is therefore to be established. Judging from the information available, the natural product is most likely to be a derivative of compound **1** with some (^1H and ^{13}C) NMR-silent chiral subunit attached to one or both phenolic hydroxyl groups.

4. Conclusion

A novel 2,5-disubstituted oxazole was synthesized in an effort to confirm the identity of an antitumor natural product recently isolated from insect *A. chinensis*. The ^1H and ^{13}C NMR as well as UV–vis data for the synthetic **1** were measured under the same conditions as reported for the natural product. The results unambiguously show that oxazole **1** and the natural product are not the same compound. The identity of the latter is therefore still to be established. All the spectroscopic data previously reported for structure **1** should no longer be related to this compound from now on; the new data set acquired in this work should be used instead.

Acknowledgment

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Appendix A. Supplementary data

^1H and ^{13}C NMR as well as IR spectra for compounds **5**, **7** and **1** are available as Supporting Information. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2012.12.012.

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