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Biomimetic conversion of (–)-fusoxyppyridone and (–)-oxysporidinone to (–)-sambutoxin: further evidence for the structure of the tricyclic pyridone alkaloid, (–)-fusoxyppyridone

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Abstract

Biomimetic-type reactions of the tricyclic pyridone alkaloid, (–)-fusoxyppyridone [(–)-4,6'-anhydrooxysporidinone] (**1**), recently encountered in an endophytic strain of *Fusarium oxysporum*, and (–)-oxysporidinone (**2**) afforded (–)-sambutoxin (**3**) and an analogue of **1**, identified as (–)-1'(6')-dehydro-4,6'-anhydrooxysporidinone (**4**), thus confirming the structure previously proposed for **1** and suggesting that **1** – **3** bear the same relative stereochemistry. Oxidation of **4** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) yielded a hitherto unknown sambutoxin analogue, (–)-4,2'-anhydrosambutoxin (**5**).

Keywords

Biomimetic reaction; 4-hydroxy-2-pyridone alkaloids; (–)-fusoxyppyridone; (–)-oxysporidinone; (–)-4,2'-anhydrosambutoxin

4-Hydroxy-2-pyridones are a small group of fungal-derived alkaloids bearing the central 2-pyridone ring linked to two substituents at C-3 and C-5 positions. The members of this group display a range of biological activities and include (–)-4,6'-anhydrooxysporidinone (**1**)¹ [now named as (–)-fusoxyppyridone], (–)-oxysporidinone (**2**),^{1–3} (–)-6-deoxy-oxysporidinone,¹ oxysporidinone dimethylketal,² 6-*epi*-oxysporidinone,² (–)-sambutoxin (**3**),² *N*-demethyl-sambutoxin,² apiosporamide,⁴ ilicicolin,⁵ funiculosin,⁶ liporins A⁷ and B,⁸ fischerin (YM 215343),⁹ tenellin,¹⁰ militarinone,¹¹ fusarinones A–C,¹² asparidones A and B,¹³ and fusapyridons A (**7**) and B (**8**).¹⁴ Among these, the *N*-methylated members, (–)-oxysporidinone (**2**), (–)-sambutoxin (**3**), fusapyridon A (**7**), and funiculosin are of particular interest as they exhibit antifungal,² mycotoxic,¹⁵ antibacterial,¹⁴ and antiviral⁶ activities, respectively. We have recently encountered the first tricyclic *N*-methyl-4-hydroxy-2-pyridone alkaloid, (–)-fusoxyppyridone (**1**), in an endophytic strain of *Fusarium oxysporum*.¹ Subsequent to our report two analogs of **1**, fusapyridons A (**7**) and B (**8**) have been encountered in another endophytic fungal strain *Fusarium* sp. YG-45.¹⁴ The structure of **1** was elucidated by the application of extensive 2D NMR techniques.¹

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We have now investigated biomimetic-type reactions of both **1** and (–)-oxysporidinone (**2**) which resulted in the formation of (–)-sambutoxin (**3**) and the tricyclic analogue, (–)-1'(6')-dehydro-4,6'-anhydro-oxysporidinone (**4**), providing further evidence for the previously proposed structure and stereochemistry of (–)-fusoxypyridone (**1**) and stereochemical disposition of the methyl groups of the trimethylheptenyl substituent at C-11 of (–)-oxysporidinone (**2**). (–)-Fusoxypyridone (**1**) on treatment with *p*-TsOH (*p*-toluenesulfonic acid) in toluene at 25 °C yielded two products,¹⁶ one of which was identified as (–)-sambutoxin (**3**) by direct comparison (mp, $[\alpha]_D$, and 1H NMR) with an authentic sample.² The HRFABMS of the more polar product **4** exhibited its $[M+H]^+$ at m/z 454.2948, consistent with the molecular formula $C_{28}H_{40}O_4N$, suggesting that it is a dehydration product of **1** ($C_{28}H_{42}O_5N$). 1H and ^{13}C NMR spectroscopic data of **4** were similar to those of **1** except for the chemical shifts of protons and carbons in the disubstituted cyclohexanone ring. In the ^{13}C NMR spectrum of **4**, when compared with (–)-fusoxypyridone (**1**), the signals due to C-1' and C-6' were shifted from δ 77.2 to 109.8 and from δ 92.0 to 150.5, respectively, indicating that a molecule of H_2O has been lost from these positions in **1** to form a double bond. Compound **4** was thus identified as (–)-1'(6')-dehydro-4,6'-anhydro-oxysporidinone which was further confirmed by detailed analysis of its HMBC data (Figure 1).

Although the gross structure of (–)-oxysporidinone (**2**), including the connectivity and the relative stereochemistry of the trisubstituted tetrahydropyran, has been determined by extensive application of 2D NMR techniques,^{2,3} stereochemistry of the 1,3-dimethyl array at C-14 and C-16 and its absolute configuration have remained undefined. The relative configurations at C-14 and C-16 of some naturally occurring 4-hydroxy-2-pyridone alkaloids have been deduced by comparison with experimental and calculated ^{13}C NMR shifts for *anti*- and *syn*-isomers of model compounds having comparable partial structures.¹⁵ However, this method is known to suffer from a major disadvantage that it is only predictive and not conclusive.¹⁷ Considering the structural relationship between (–)-oxysporidinone (**2**) and (–)-fusoxypyridone (**1**), it was of interest to see if a similar biomimetic-type transformation of **2** would lead to (–)-sambutoxin (**3**). As expected, the treatment of (–)-oxysporidinone (**2**) with *p*-TsOH yielded **3** and (–)-1'(6')-dehydro-4,6'-anhydrooxysporidinone (**4**) (Scheme 1).¹⁶

Formation of (–)-sambutoxin (**3**) from both (–)-fusoxypyridone (**1**) and (–)-oxysporidinone (**2**) suggests that the polyketide-derived trimethylheptenyl substituent at C-11 of the tetrahydropyran ring of **1** and **2** displays the *anti*-1,3-dimethyl relationship as in (–)-sambutoxin (**3**). Since the relative and absolute stereochemistry of the optical antipode of **3** namely, (+)-sambutoxin, has been confirmed by an enantiocontrolled total synthesis,¹⁸ the

relative stereochemistry of the C-11 trimethylheptenyl substituent of compounds **1** – **4** should be as shown.

Oxidation of **4** with DDQ gave **5** as the only product. HRMS data of this compound suggested the molecular formula $C_{28}H_{37}O_4N$. 1H NMR spectroscopic data of **5**, when compared with that of **3**, indicated the absence of the six aliphatic protons assigned to the disubstituted cyclohexenone ring; instead it showed the presence of three aromatic protons suggesting that compound **4** on treatment with DDQ had undergone dehydrogenation followed by aromatization to give (–)-4,2'-anhydrosambutoxin (**5**) (Scheme 1). Methylation of (–)-sambutoxin (**3**) with Me_2SO_4/K_2CO_3 gave its new analogue (–)-dimethylsambutoxin (**6**) as the only product, structure of which was confirmed by analysis of its spectral data (1H and ^{13}C NMR and HRFABMS).

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16. *Experimental*: *p*-TsOH and DDQ were purchased from Aldrich Chemical Co. (Milwaukee, WI) and all solvents from VWR Scientific (San Francisco, CA). All reactions were carried out in an atmosphere of N_2 . Melting points were determined on an electrothermal mp apparatus and were uncorrected. Optical rotations were measured with JASCO Dip-370 polarimeter. UV spectra were recorded with a Shimadzu UV-1601 spectrophotometer. 1H NMR and ^{13}C NMR spectra were obtained on Bruker DRX-500. Low resolution and high resolution MS were recorded on a Shimadzu LCMS QP8000α and an IonSpec FT mass spectrometers, respectively. Reaction of (–)-fusoxypyridone (**1**) with *p*-TsOH: To a stirred solution of (–)-fusoxypyridone (**1**, 3.0 mg) in toluene (0.5 mL) was added *p*-TsOH (1 crystal). After 30 min, toluene was removed under reduced pressure and the residue was separated on preparative TLC (silica gel) using hexane/acetone (1:

2.5) as eluant to give (–)-sambutoxin (**3**) (1.1 mg, 38%) and **4** (1.6 mg, 56%). (–)-1'-(6')-Dehydro-4,6'-anhydrooxysporidinone **4**: mp 175–177 °C; $[\alpha]_D -65.8$ (c 0.3, CH₃OH); UV (EtOH) $\lambda_{\max}$ (log ϵ) 336 (3.53), 264 (4.79), 230 (4.79) nm; ¹H NMR (CDCl₃) δ 7.34 (1H, s, H-6), 5.12 (1H, d, J = 9.1 Hz, H-13), 4.98 (1H, d, J = 8.7 Hz, H-7), 3.62 (3H, s, N-CH₃), 3.52 (2H, brs, H₂-5'), 3.46 (1H, d, J = 9.9 Hz, H-11), 2.77 (2H, t, J = 6.2 Hz, H₂-2'), 2.70 (2H, t, J = 6.2 Hz, H₂-3'), 2.43 (1H, m, H-14), 2.01 (1H, dq, J = 13.8, 3.6 Hz, H-8a), 1.90 (1H, m, H-9a), 1.77 (1H, dq, J = 13.2, 2.5 Hz, H-8b), 1.70 (1H, m, H-10), 1.65 (3H, d, J = 1.0 Hz, H₃-20), 1.38 (1H, m, H-9b), 1.35 (1H, m, H-17a), 1.32 (1H, m, H-16), 1.17 (1H, m, H-15a), 1.03 (2H, m, H-15b, H-17b), 0.86 (3H, d, J = 6.4 Hz, H₃-21), 0.83 (3H, m, H₃-18), 0.82 (3H, d, J = 6.4 Hz, H₃-22), 0.73 (3H, d, J = 6.6 Hz, H₃-19); ¹³C NMR (CDCl₃) δ 204.8 (C, C-4'), 161.9 (C, C-2), 161.2 (C, C-4), 150.4 (C, C-6'), 136.2 (CH, C-13), 132.1 (C, C-12), 126.2 (CH, C-6), 114.6 (C, C-5), 112.1 (C, C-3), 109.8 (C, C-1'), 91.4 (CH, C-11), 72.8 (CH, C-7), 44.9 (CH₂, C-15), 38.8 (CH₃, C-23), 38.7 (CH₂, C-5'), 38.4 (CH₂, C-3'), 32.9 (CH₂, C-9), 31.9 (CH, C-16), 31.8 (CH, C-10), 30.2 (CH₂, C-8), 29.5 (CH₂, C-14), 29.2 (CH₂, C-17), 20.6 (CH₃, C-21), 19.5 (CH₃, C-22), 17.9 (CH₃, C-19), 17.8 (CH₂, C-2'), 11.3 (CH₃, C-18), 11.2 (CH₃, C-20); HRFABMS m/z 454.2948 [M+H]⁺ (calcd for C₂₈H₄₀O₄N, 454.2957). Reaction of (–)-oxysporidinone with *p*-TsOH: To a solution of (–)-oxysporidinone (**2**) (5.0 mg) in toluene (0.5 mL) was added *p*-TsOH (1 crystal) and stirred for 30 min (TLC control). Solvent was then removed under reduced pressure and the residue was separated on preparative TLC (silica gel) using hexane/acetone (1:2.5) as eluant to give (–)-sambutoxin (**3**) (1.8 mg, 39%) and (–)-1'-(6')-dehydro-4,6'-anhydro-oxysporidinone (**4**) (2.7 mg, 58%). Oxidation of **4** to (–)-4,2'-anhydrosambutoxin **5**: To a stirred solution of **4** (0.5 mg) in 1,4-dioxane (0.2 mL) was added DDQ (3.0 mg). After 3 h at 25 °C (TLC control), the reaction mixture was diluted with EtOAc (20 mL), washed with water (3×15 mL), dried (Na₂SO₄), and evaporated. The residue thus obtained was purified by prep. TLC using hexane/acetone (1:2.5) as eluant to give **5** (0.3 mg, 60%) as a white amorphous powder; $[\alpha]_D -78.2$ (c 0.2, CH₃OH); UV (EtOH) $\lambda_{\max}$ (log ϵ) 338 (3.71), 310 (3.89), 258 (4.32), 211 (4.29) nm; ¹H NMR (CDCl₃) δ 7.68 (1H, s, H-6), 7.37 (1H, d, J = 8.3 Hz, H-6'), 6.86 (1H, d, J = 2.2 Hz, H-3'), 6.68 (1H, dd, J = 8.3, 2.2 Hz, H-5'), 5.14 (1H, d, J = 9.9 Hz, H-13), 5.02 (1H, dd, J = 11.7, 2.3 Hz, H-7), 3.63 (3H, s, N-CH₃), 3.49 (1H, d, J = 9.8 Hz, H-11), 2.45 (1H, m, H-14), 2.16 (1H, m, H-8a), 1.93 (1H, dq, J = 13.2, 3.4 Hz, H-9a), 1.76 (1H, m, H-10), 1.73 (3H, s, H₃-20), 1.68 (1H, m, H-8b), 1.40 (1H, m, H-9b), 1.35 (1H, m, H-17a), 1.32 (1H, m, H-16a), 1.17 (1H, m, H-15a), 1.04 (1H, m, H-15b), 1.03 (1H, m, H-17b), 0.86 (3H, d, J = 6.7 Hz, H₃-21), 0.84 (3H, m, H₃-18), 0.82 (3H, d, J = 6.3 Hz, H₃-22), 0.76 (3H, d, J = 6.6 Hz, H₃-19); HRFABMS m/z 452.2795 [M+H]⁺ (calcd for C₂₈H₃₈O₄N, 452.2801). Methylation of (–)-sambutoxin **3**: To a solution of (–)-sambutoxin (**3**) (2.0 mg) in acetone (0.6 mL) were added K₂CO₃ (20 mg) and Me₂SO₄ (20 μ L). After 3 h under reflux (TLC control), the reaction mixture was filtered, the filtrate evaporated under reduced pressure, and the resulting residue was purified on prep TLC using hexane/acetone (1:2) as eluant to give **6** (2.1 mg, 98%) as a white amorphous powder; $[\alpha]_D -92.4$ (c 0.8, CH₃OH); UV $\lambda_{\max}$ (log ϵ) 315 (3.61), 260 (4.14), 215 (4.11) nm; ¹H NMR (CDCl₃) δ 7.29 (2H, d, J = 8.6 Hz, H-2' and H-6'), 7.12 (1H, s, H-6), 6.90 (2H, d, J = 8.6 Hz, H-3' and H-5'), 5.11 (1H, d, J = 9.0 Hz, H-13), 4.96 (1H, d, J = 11.6 Hz, H-7), 3.82 (3H, s, OCH₃), 3.49 (3H, s, OCH₃), 3.41 (1H, d, J = 9.8 Hz, H-11), 3.38 (3H, s, N-CH₃), 2.39 (1H, m, H-14a), 2.37 (1H, m, H-8a), 1.89 (1H, dd, J = 12.9, 3.0 Hz, H-9a), 1.67 (1H, m, H-10), 1.62 (3H, s, H₃-20), 1.56 (1H, m, H-8b), 1.38 (1H, m, H-9b), 1.30 (1H, m, H-16), 1.30 (1H, m, H-17a), 1.16 (1H, m, H-15a), 1.02 (2H, m, H-15b, H-17b), 0.84 (3H, d, J = 6.5 Hz, H₃-21), 0.81 (3H, d, J = 6.6 Hz, H₃-22), 0.80 (3H, m, H₃-18), 0.70 (3H, d, J = 6.5 Hz, H₃-19); ¹³C NMR (CDCl₃) δ 165.7 (C, C-4), 162.8 (C, C-2), 158.9 (C, C-4'), 136.8 (CH, C-13), 136.3 (CH, C-6), 132.1 (C, C-12), 129.7 (CH, C-2' and C-6'), 127.0 (C, C-1'), 122.4 (C, C-5), 117.5 (C, C-3), 114.0 (CH, C-3' and C-5'), 92.2 (CH, C-11), 73.7 (CH, C-7), 44.9 (CH₂, C-15), 37.6 (CH₃, C-23), 33.5 (CH₂, C-9), 32.0 (CH, C-10), 31.8 (CH, C-16), 29.5 (CH₂, C-8), 29.3 (CH₂, C-17), 29.1 (CH, C-14), 20.6 (CH₃, C-21), 19.5 (CH₃, C-22), 18.0 (CH₃, C-19), 11.6 (CH₃, C-20), 11.2 (CH₃, C-18); HRFABMS m/z 482.3247 [M+H]⁺ (calcd for C₃₀H₄₄O₄N, 482.3270).

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Figure 1.
Selected HMBC correlations for **4**

Scheme 1.

Proposed pathway for biomimetic-type reactions of **1** and **2** giving **3** – **5**.