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# Biomimetic conversion of (−)-fusoxypyridone and (−)-oxysporidinone to (−)-sambutoxin: Further evidence for the structure of the tricyclic pyridone alkaloid, (−)-fusoxypyridone

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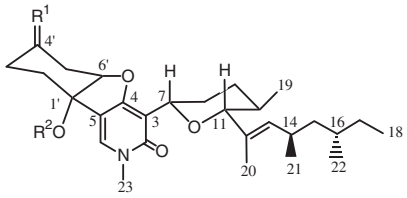
ABSTRACT

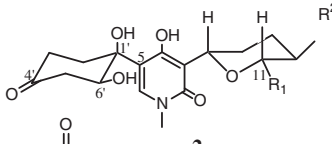
Biomimetic-type reactions of the tricyclic pyridone alkaloid, (−)-fusoxypyridone [(−)-4,6′-anhydrooxysporidinone] (**1**), recently encountered in an endophytic strain of *Fusarium oxysporum*, and (−)-oxysporidinone (**2**) afforded (−)-sambutoxin (**3**) and an analogue of **1**, identified as (−)-1′(6′)-dehydro-4,6′-anhydrooxysporidinone (**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**).



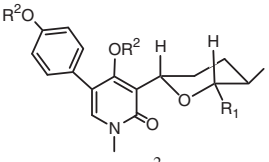
4-Hydroxy-2-pyridones are a small group of fungal-derived alkaloids bearing the central 2-pyridone ring linked to two substituents at C-3 and C-5 positions. The members of this group display a range of biological activities and include (−)-4,6′-anhydrooxysporidinone (**1**)[1] [now named as (−)-fusoxypyridone], (−)-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 A[7] 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 antiviral[6] activities, respectively. We have recently encountered the first tricyclic *N*-methyl-4-hydroxy-2-pyridone alkaloid, (−)-fusoxypyridone (**1**), in an endophytic strain of *Fusarium oxysporum*.[1] Subsequent to our report two analogues 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]



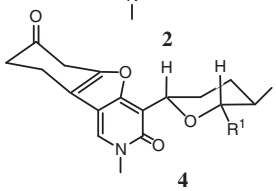
**1** $R^1$ = O; $R^2$ = H
**7** $R^1$ = O; $R^2$ = Me
**8** $R^1$ = OMe,OMe; $R^2$ = Me



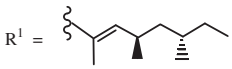
**2**

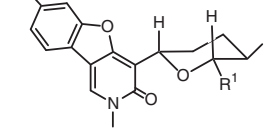


**3** $R^2$ = H
**6** $R^2$ = Me



**4**





**5**

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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 $^1$H 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** ($C_{28}H_{42}O_5N$). $^1$H and $^{13}$C NMR spectroscopic data of **4** were similar to those of **1**[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 $\delta$ 77.2 to 109.8 and from $\delta$ 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 (Fig. 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 $C_{28}H_{37}O_4N$. $^1$H 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′-anhydosambutoxin (**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 ($^1$H and $^{13}$C NMR and HRFABMS).

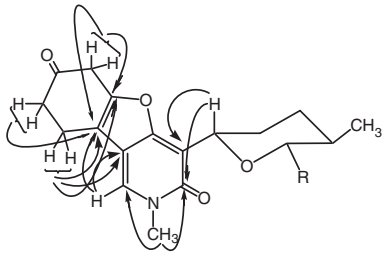
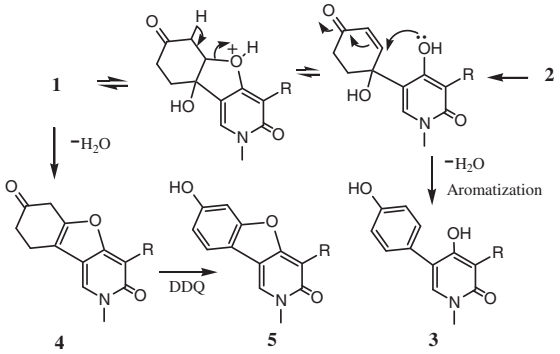


**Figure 1.** Selected HMBC correlations for **4**.




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

## Acknowledgments

This work was supported by a grant from the National Cancer Institute (Grant No. R01CA090265-06A1). We are also thankful to Dr. N. P. D. Nanayakkara (University of Mississippi) for providing samples and copies of spectroscopic data of **2** and **3**.

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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. $^1$H NMR and $^{13}$C NMR spectra were obtained on Bruker DRX-500. Low resolution and high resolution MS were recorded on a Shimadzu LC–MS 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, $CH_3OH$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 336 (3.53), 264 (4.79), 230 (4.79) nm; $^1H$ NMR ($CDCl_3$) $\delta$ 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-$CH_3$), 3.52 (2H, br s, $H_2$-5′) 3.46 (1H, d, *J* = 9.9 Hz, H-11), 2.77 (2H, t, *J* = 6.2 Hz, $H_2$-2′), 2.70 (2H, t, *J* = 6.2 Hz, $H_2$-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, $H_3$-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, $H_3$-21), 0.83 (3H, m, $H_3$-18), 0.82 (3H, d, *J* = 6.4 Hz, $H_3$-22), 0.73 (3H, d, *J* = 6.6 Hz, $H_3$-19); $^{13}C$ NMR ($CDCl_3$) $\delta$ 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 ($CH_2$, C-15), 38.8 ($CH_3$, C-23), 38.7 ($CH_2$, C-5′), 38.4 ($CH_2$, C-3′), 32.9 ($CH_2$, C-9), 31.9 (CH, C-16), 31.8 (CH, C-10), 30.2 ($CH_2$, C-8), 29.5 ($CH_2$, C-14), 29.2 ($CH_2$, C-17), 20.6 ($CH_3$, C-21), 19.5 ($CH_3$, C-22), 17.9 ($CH_3$, C-19), 17.8 ($CH_2$, C-2′), 11.3 ($CH_3$, C-18), 11.2 ($CH_3$, C-20); HRFABMS *m*/*z* 454.2948 $[M+H]^+$ (calcd for $C_{28}H_{40}O_4N$, 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′-anhydosambutoxin **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 (3x15 mL), dried ($Na_2SO_4$), 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, $CH_3OH$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 338 (3.71), 310 (3.89), 258 (4.32), 211 (4.29) nm; $^1H$ NMR ($CDCl_3$) $\delta$ 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–$CH_3$), 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, $H_3$-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, $H_3$-21), 0.84 (3H, m, $H_3$-18), 0.82 (3H, d, *J* = 6.3 Hz, $H_3$-22), 0.76 (3H, d, *J* = 6.6 Hz, $H_3$-19); HRFABMS *m*/*z* 452.2795 $[M+H]^+$ (calcd for $C_{28}H_{38}O_4N$, 452.2801).

Methylation of (−)-sambutoxin **3**: To a solution of (−)-sambutoxin (**3**) (2.0 mg) in acetone (0.6 mL) were added $K_2CO_3$ (20 mg) and $Me_2SO_4$ (20 μL). 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, $CH_3OH$); UV $\lambda_{max}$ (log $\varepsilon$) 315 (3.61), 260 (4.14), 215 (4.11) nm; $^1H$ NMR ($CDCl_3$) $\delta$ 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, $OCH_3$), 3.49 (3H, s, $OCH_3$), 3.41 (1H, d, *J* = 9.8 Hz, H-11), 3.38 (3H, s, N–$CH_3$), 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, $H_3$-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, $H_3$-21), 0.81 (3H, d, *J* = 6.6 Hz, $H_3$-22), 0.80 (3H, m, $H_3$-18), 0.70 (3H, d, *J* = 6.5 Hz, $H_3$-19); $^{13}C$ NMR ($CDCl_3$) $\delta$ 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 ($CH_2$, C-15), 37.6 ($CH_3$, C-23), 33.5 ($CH_2$, C-9), 32.0 (CH, C-10), 31.8 (CH, C-16), 29.5 ($CH_2$, C-8), 29.3 ($CH_2$, C-17), 29.1 (CH, C-14), 20.6 ($CH_3$, C-21), 19.5 ($CH_3$, C-22), 18.0 ($CH_3$, C-19), 11.6 ($CH_3$, C-20), 11.2 ($CH_3$, C-18); HRFABMS *m*/*z* 482.3247 $[M+H]^+$ (calcd for $C_{30}H_{44}O_4N$, 482.3270).

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