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Azodyrecins A–C, Azoxides from a Soil-derived *Streptomyces* sp.

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ABSTRACT

Azoxy compounds belong to a small group of natural products sharing a common functional group with the general structure $RN=N^+(O^-)R$. Three new azoxides, azodyrecins A–C (**1–3**), were isolated from a soil-derived *Streptomyces* sp. strain P8-A2. The *cis*-alkenyl unit in **1–3** was found to readily isomerize to the *trans*-congeners (**4–6**). The structures of the new compounds were determined by detailed spectroscopic (1D/2D NMR) and HRMS data analysis. Azodyrecins belong to a new class of natural azoxy compounds and are proposed to derive from L-alanine and alkylamines. The absolute configurations of **1–6** were defined by comparison of ECD spectra. While no antimicrobial effect were observed for **1** against *Staphylococcus aureus*, *Vibrio anguillarum*, or *Candida albicans*, azodyrecin A (**2**) exhibited cytotoxicity against the human leukemia cell line HL-60 with an IC_{50} value of 2.2 μ M.

Natural products containing a nitrogen-nitrogen (N-N) bond, especially those with an azoxy moiety, are not commonly found in Nature.¹ Due to their DNA damaging/chelating effects, these azoxy compounds are considered to be hazardous and carcinogenic.² To date, naturally occurring azoxy compounds have been reported from bacteria, fungi, and plants, with examples shown in Figure 1.^{1,3} Some plants, such as, cycads are capable of producing azoxyglycoside cycasin and macrozamin which may play a role as part of the plants' natural defense against herbivores.⁴

Actinobacteria, a notable source of antibiotics,⁵ live in complex biological communities. Interestingly, soil Actinobacteria appear to be producers of the growing number of natural azoxides exemplified by elaiomycins,⁶ maniwamycins,^{7,8} and others.⁹⁻¹³ These secondary metabolites exhibit a wide array of biological activity being cytotoxic, or having nematicidal, antifungal, or antibacterial effects.⁶⁻¹⁰ Despite the wide spectrum of bioactivity, limited information is available for the impact of structure variations on the biological effects of naturally occurring azoxy compounds. Moreover, microbial secondary metabolites such as azoxides could play a role as a natural defense against other microbes or animals. Thus, it would be of interest to investigate new azoxy compounds and gain insights into the structural diversity as well as biological activity of this class of compound.

As part of an on-going interest in secondary metabolites from soil microorganisms, several Actinobacteria were isolated from a soil sample collected in the Jægersborg Deer Park, Denmark. From the *Streptomyces* strain P8-A2, HPLC-HRMS analysis revealed the presence of several previously unknown azoxides. Here we report the isolation and structure elucidation of three new azoxy compounds (**1-3**) from *Streptomyces* sp. strain P8-A2, along with evaluation of their antibacterial activity as well as toxicity against brine shrimps and the human cancer cell line HL-

60. The discovery of these unusual compounds enrich the structural diversity of natural azoxy compounds.

RESULTS AND DISCUSSION

In the non-polar region, HPLC-HRMS analysis of the crude extract of *Streptomyces* sp. strain P8-A2 we found several mass peaks of N-N containing compounds indicating the presence of azoxy compounds. To elucidate the lipid-like components, the fermentation was scaled up (5 L solid cultivation), and the EtOAc extract was subjected to silica gel column chromatography, followed by reversed-phase HPLC to obtain unusual azoxy compounds **1–3** (Figure 2).

Compound **1** was isolated as a colorless oil with a molecular formula of C₁₈H₃₄N₂O₃, as suggested by (+)-HRESIMS. The characteristic absorption bands at 1467 and 1747 cm⁻¹ in the IR spectrum of **1** suggested the presence of an azoxy moiety and a carbonyl group, respectively.^{7,10,14} The ¹H NMR data (Table 1) of **1** exhibited signals from three methyl groups all appearing as doublets [δ_{H} 0.85 (6H) and 1.54], a methoxy group (δ_{H} 3.72), and four methine hydrogen atoms corresponding to two sp³ methine groups (δ_{H} 1.50 and 4.55) and two olefinic hydrogen atoms (δ_{H} 5.78 and 6.82). From the 1D ¹³C NMR (Table 1) and 2D edited HSQC spectra, 18 carbon resonances could be identified including three methyl groups, nine methylene groups, four methine groups, and one quaternary carbon atom. The ¹³C signal at δ_{C} 171.4 suggested the presence of an ester group in **1**. The fragment of H-2/H₃-3 was readily established in the DQF-COSY spectrum. These data, together with HMBC correlations from H-2, H₃-3 and H₃-4 to the ester carbonyl C-1 constructed the partial structure of a methyl propionate moiety (fragment A, Figure 3). Similarly, further analysis of DQF-COSY data and HMBC correlations from the olefinic H-2' to C-4', from H₂-4' to C-6', from H-12' to C-10', and from both H₃-13' and H₃-14' to C-12' established a fragment

of a saturated alkyl chain (C-1' along the chain to C-12') with a terminal isopropyl group (fragment B, Figure 3). The ^1H - ^1H coupling constant (9.0 Hz) between H-1' and H-2' indicated a *cis*-configured double bond. The two structural moieties were connected by an azoxy linkage, which was implied by the molecular formula and the IR spectrum of **1**. Furthermore, a weak 4J -HMBC cross peak was observed from H-2 to C-1', which further confirmed the location of the azoxy group between C-2 and the olefinic carbon C-1'. The oxygen atom of the azoxy moiety was determined to be on the side of the olefin group by the ^1H chemical shifts adjacent to the azoxy group,¹⁵ and by comparison of spectroscopic data with related azoxy containing natural products reported in the literature (e.g., elaiomycins, Supporting Information Tables S46 and S47),⁶ maniwamycins,^{7,8} and jietacins.¹⁰ The characteristic UV absorption maximum at 232 nm, which was also observed in similar alkenyl substituted azoxy compounds (maniwamycins and elaiomycins),^{6,8} determined the *Z*-configuration of the azoxy group.¹⁵ In addition, the NOESY spectrum revealed no correlations between H-1' and H-2 or H₃-3, which would be likely observed in an *E*-configuration.¹⁴ It is worth noting that the azoxy moiety in all previously reported azoxides isolated from Actinobacteria has *Z*-configuration.¹ The ECD spectrum of **1** (Figure 4) exhibited a negative Cotton effect at 257 nm ($\Delta\epsilon = -3.38$) and a positive Cotton effect at 227 nm ($\Delta\epsilon = +1.48$), which was similar to those reported for oxidized elaiomycin containing a single stereocenter as in **1**.¹⁶ Notably, oxidized elaiomycin showed a negative Cotton effect at 260 nm and a positive Cotton effect at 230 nm.¹⁶ Despite the existence of a carbonyl group as a another chromophore, the observed Cotton effects derived from a π - π^* excitation from the azoxy moiety, as reported in the extensive ECD studies of LL-BH87 α and elaiomycin.¹⁶ Therefore, the configuration of **1** was defined as *2S*. On the basis of these data, the structure of **1** was established as a new type of azoxide, named azodyrecin A.

Compound **2** was obtained as a colorless oil and was assigned the molecular formula $C_{19}H_{36}N_2O_3$ following analysis of the (+)-HRESIMS data. The 1H and ^{13}C NMR spectra of **2** were similar to those of **1**. Comparison of the NMR and MS data between **1** and **2** revealed that the latter natural product had an additional $-CH_2-$ moiety in the alkyl group. HMBC correlations from H_3-14' to C-13' and C-12' positioned a methyl group at C-13' in **2**. Similarly, HMBC correlations from H_3-15' to C-11', C-12', and C-13' confirmed the location of a branching methyl group at C-12'. The same *2S* configuration previously defined for **1** was also assigned for **2** following ECD spectra comparison (Figure 4). However, the rotatable nature of the alkyl chain rendered the stereochemical determination at C-12' unfeasible using spectroscopic methods. Thus, the structure of **2** was elucidated and named azodyrecin B (**2**).

Azodyrecin C (**3**) was isolated as the third new azoxide, the molecular formula of which was determined as $C_{20}H_{38}N_2O_3$ by (+)-HRESIMS analysis. The NMR and MS data suggested that **3** was structurally similar to **1**, except for the presence of two additional methylene groups in the alkyl substituent in **3**. HMBC correlations from the two overlapped doublet methyl groups at δ_H 0.86 to C-14' revealed the presence of an isopropyl terminus in **3**, as also observed in **1**. The absolute configuration of **3** was judged to be identical to **1** following chiroptical data comparison (Figure 4). Hence, the structure of **3** was established.

Since most natural azoxyalkenes contain a *trans*-alkenyl moiety,¹ and it is generally known that compounds bearing a conjugated *trans*-alkenyl unit are more stable than the *cis*-isomers, we investigated whether the alkene of **1–3** was prone to isomerize. During the spectroscopic data measurement of compounds **1–3**, the alkenyl unit in **1–3** was found to easily isomerize to the *trans*-configuration as seen in compounds **4–6** (after 30 days of storage in $CDCl_3$, middle panel in Figure 5). Compounds **4** and **5** were purified from the mixture by RP-HPLC, and their structures were

elucidated by NMR and MS data analysis (Tables 1 and 2). Compound **6** could not be isolated due to the minute amount. However, the isomerization of compound **3** to **6** was clearly observed in the ^1H NMR spectrum of the mixture of **3** and **6** (Figure S33, Supporting Information). The presence of a *trans*-alkene in compounds **4–6** was obvious from the ^1H - ^1H coupling constant (13.3 Hz) between H-1' and H-2'. This observation provided an additional structural understanding of the natural azoxyalkenes.

The structures of azodyrecins closely resemble elaiomyacin and valanimycin, suggesting a similar biosynthetic route. Previously, extensive biosynthesis investigation of both elaiomyacin and valanimycin were performed by feeding experiments or/and gene cluster analysis.^{17–26} While L-serine was used as a precursor in both elaiomyacin and valanimycin, L-alanine and alkylamine were hypothesized to be the precursors of azodyrecins A–C (**1–3**). The proposed biosynthetic pathway is shown in Figure 6. In brief, a potential N-hydroxylase could catalyze the formation of a fatty acid-derived amine into a hydroxylamine. Furthermore, L-alanine could be activated by an alanyl-tRNA synthetase, transferring the alanyl residue from alanyl-tRNA to the hydroxy group of the hydroxylamine to produce an ester intermediate, which was proposed to form azodyrecins following a similar arrangement in valanimycin.²³ However, formation of the key azoxy moiety in azodyrecins remain elusive despite the recent progress in the biosynthetic characterization of a group of aromatic azoxy compounds, azoxymycins, where the the azoxy bond formation was confirmed to be an enzymatic and non-enzymatic coupled cascade reaction.^{3,12}

Potent antimicrobial effects for structurally related azoxy natural products (for example, valanimycin, MIC = 10 $\mu\text{g}/\text{mL}$ against *S. aureus*) were previously reported.⁹ Neither azodyrecin A (**1**) nor *l'*-trans-azodyrecin A (**4**) had antibacterial activity against *Staphylococcus aureus* and *Vibrio anguillarum*. Meanwhile, compound **1** was also inactive against *Candida albicans*. This

may be due to the chain length of the azoxides. Several studies have reported that azoxides with a shorter alkyl chain, such as azoxybacilin, and valanimycin tend to exhibit higher activity against microbes.^{27,28,29} Meanwhile, natural azoxides with longer aliphatic chains such as elaiomycin derivatives as well as maniwamycin⁸ exhibited weak to no antimicrobial activity.^{6,30} Thus, the main role of azodyrecins may not be as antimicrobials against other microorganisms.

In view of the potential mutation effects of the azoxides and their toxicity to the other higher organisms, such as nematodes and insects,^{10,28,31,32} cytotoxicity of azodyrecin A (**1**) against brine shrimp *Artemia salina* was evaluated. At a concentration of 10 $\mu\text{g/mL}$, it caused a 100% brine shrimp mortality, stronger than the positive control elaiophylin (10 $\mu\text{g/mL}$, 78%). Further attempts to test compound **1** for *in vitro* cytotoxicity against cell lines were unsuccessful due to the rapid decomposition of **1** during handling and biological testing. Therefore, we tested the *in vitro* cytotoxic effect of the most abundant compound, azodyrecin B (**2**), against the human leukemia cell line HL-60 using the AlamarBlue assay. Compound **2** did exhibit cytotoxicity against the HL-60 cell line, with an IC_{50} value of 2.2 μM (Supporting Information Figure S48).

EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotations were acquired using a Perkin-Elmer 241 polarimeter. ECD spectra were obtained on a JASCO J-1500 CD Spectrometer and processed using the software SDAR.³³ IR data were acquired on Bruker Alpha FTIR spectrometer using OPUS version 7.2. NMR spectra were acquired on 800 MHz Bruker Avance III spectrometer equipped with a TCI CryoProbe using standard pulse sequences. The ^1H and ^{13}C NMR chemical shifts were referenced to the residual solvent peaks at δ_{H} 7.26 and δ_{C} 77.16 ppm for CDCl_3 , and δ_{H} 2.50 and δ_{C} 39.5 ppm for $\text{DMSO-}d_6$. NMR data were processed using MestReNova 11.0.

UHPLC-HRMS was performed on an Agilent Infinity 1290 UHPLC system equipped with a diode array detector. UV-Vis spectra were recorded from 190 to 640 nm. Biotage Isolera One Flash Chromatography system and Biotage SNAP KP-Sil (50 μm , 50 g) Si gel cartridge were used for flash chromatography. All solvents and chemicals used for HRMS and chromatography were VWR Chemicals LC-MS grade, while for metabolites extraction, the solvents were of HPLC grade (VWR Chemicals).

Strain Isolation and Taxonomic Classification. The bacterial strain was isolated from soil collected in September 2018 in the UNESCO World Heritage Site, Jægersborg Deer Park (Dyrehaven in Danish), Denmark. The soil samples (1.0 g) were diluted in sterile water to prepare a 10^{-3} g/mL suspension. The suspension (300 μL) was inoculated onto humic acid-vitamin (HV) agar and incubated at 28 °C for 14 days. HV agar was prepared as previously described.³⁴ Purification of the bacterial strains was performed by re-plating different colonies on coconut mannitol (CM) agar plates containing 20 g L⁻¹ coconut powder (Green Organic), 20 g L⁻¹ mannitol (Sigma Aldrich), and 20 g L⁻¹ agar (Difco); until an axenic culture was obtained. The identification of *Streptomyces* sp. strain P8-A2 was performed using 16S rRNA gene sequence analysis and comparison with NCBI data, which showed 100% similarity to *Streptomyces mirabilis* strain 3662. The nucleotide sequence was deposited in GenBank (www.ncbi.nlm.nih.gov) under the accession number MN826478.

Bacterial cultivation. Spores of strain P8-A2 were inoculated into 500 mL conical flask containing 150 mL of liquid CM medium and incubated at 28 °C for 2 days at 180 rpm to afford a seed culture. A volume of 100 μL of the seed culture was inoculated on CM agar plates and incubated in the dark at 28 °C for 7 days. A total of 200 plates of CM agar (5 L in total) were prepared.

Extraction and Isolation. The cultured agar was extracted with EtOAc (2 x 2.5 L) under ultrasonication for 30 min. The EtOAc phase was filtered and removed under reduced pressure to obtain 2.4 gram of extract. The extract was chromatographed over a Si-gel flash cartridge eluted with a stepwise *n*-heptane/EtOAc solvent system (100% *n*-heptane to 100% EtOAc) at a flow rate of 50 mL/min to give 22 fractions (132 mL each). Similar fractions were pooled following (+)-HRESIMS analysis to afford 12 fractions (A1–A12). Fraction A4 (775.2 mg) was further fractionated using a semi-preparative phenyl-hexyl HPLC column (250 x 10 mm, Luna-Phenomemex 5 μ m 100 Å). A linear gradient from 67% CH₃CN-H₂O (50 ppm TFA) to 80% CH₃CN-H₂O (50 ppm TFA) at a flowrate of 4 mL/min was run over 60 min to yield compounds **1** (9.5 mg, *t*_R 30–31 min), **2** (22.3 mg, *t*_R 36–37min), and **3** (2.3 min, *t*_R 42–43 min). The mixture (9.5 mg) of compounds **1** and **4** was separated using a semi-preparative phenyl-hexyl HPLC column (250 x 10 mm, Gemini-Phenomemex 5 μ m 100 Å) with a linear gradient from 75% CH₃CN-H₂O (50 ppm TFA) to 83 % CH₃CN-H₂O (50 ppm TFA) over 20 min to afford compound **4** (0.8 mg). The mixture (22.3 mg) of **2** and **5** was further separated using a semi-preparative phenyl-hexyl HPLC column (250 x 10 mm, Gemini-Phenomemex 5 μ m 100 Å). A gradient from 78% CH₃CN-H₂O (50 ppm TFA) to 85 % CH₃CN-H₂O (50 ppm TFA) over 20 min was used to afford compound **5** (2.5 mg).

Antimicrobial activity test. The antimicrobial activity of pure **1** and **4** were assessed against the Gram-negative fish pathogen *V. anguillarum* strain 90-11-287³⁵ and the Gram-positive human pathogen *S. aureus* strain 8325³⁶ in a well diffusion agar assay. The pathogens were seeded in Instant Ocean (IO) agar plates containing 3% Instant Ocean (Aquarium Systems Inc.), 3.33 g L⁻¹ Casamino acids, 4 g L⁻¹ glucose, and 10 g L⁻¹ agar.³⁷ A volume of 30 μ L of compounds **1** and **4** solutions in MeOH (100 μ g/100 μ L) were added to wells (diameter = 4 mm; distance between

the wells = 32–38 mm) punched into the agar plate alongside medium and MeOH control. Sterile filtered supernatant from 3 day Marine Broth (Difco 2216) cultures of a *Phaeobacter inhibens* DSM17395 producing tropodithietic acid was used as positive control. Following 24 h incubation at 25 °C, the diameters of the resulting inhibition zones were measured.

Compound **1** was also evaluated for its antifungal effect against *Candida albicans* IBT 654.³⁸ The test plates for *C. albicans* were prepared by pouring 14 ml of media (40 g glucose, 10 g Bacto-Peptone, 1 L Tap water, pH 4.8, 36g agar) as a base layer; after solidifying, this was inoculated with the fungus. Paper disks with a diameter of 6 mm were impregnated with 80 µg of pure compound **1** (dissolved in CH₂Cl₂); the disks were dried under sterile conditions and placed on the surface of test plates. The test plate was incubated at 28 °C for 16–24 h. After incubation, the diameter of inhibition zones was measured.

Brine shrimp toxicity assay. Toxicity assay was carried out using brine shrimp mortality test according to the Meyer method with slight modifications.³⁹ The eggs of *Artemia salina* were hatched in a beaker filled with artificial seawater. Seawater (4.95 mL) containing 30–40 nauplii was added to each glass test tube. A volume of 50 µL solution of azodyrecin A (**1**) in dimethylsulfoxide (DMSO) was added to the test tube with a final concentration of 10 µg/mL. Elaiophyllin was used as a positive control. DMSO (50 µL) was used as a negative control. After incubation for 24 h, the mortality of brine shrimps was calculated.

Cytotoxicity assay. Cytotoxicity against the human cell line HL-60 was evaluated at 48 hours for azodyrecin B (**2**), using AlamarBlue (Thermo Scientific, Kansas, USA). The assay was performed in 96 well plates (Costar 3595, Corning, New York, USA), with an assay volume of 200 µL. HL-60 cells were cultured at 37 °C in a 5% CO₂ incubator and seeded in the plate with a cell density of 2 x 10⁵ cells/mL. The cells were treated with 200 nL azodyrecin B (**2**) (dissolved in

DMSO) using an Echo 550 liquid handler (Labcyte, California, USA) to dispense. After 48 hours of incubation in a 5% CO₂ incubator at 37 °C, 20 μL of AlamarBlue were added to each well and the cells were further incubated under the same conditions for 5 hours. Fluorescence (excitation: 544 nm, emission: 590 nm) was detected by an Infinite M200 plate reader (Tecan, Switzerland). The dose-response of the compound was tested in 10 concentrations, with a starting concentration of 10 μM and 1:2 dilutions in DMSO. Control wells were treated with DMSO only and used as the maximal cellular growth reference, while wells containing only media were used to perform the background signal correction. The software Prism 5.03 was used for data analysis (GraphPad Software, USA).

Azodyrecin A (1): colorless oil; $[\alpha]_D^{20}$ -66.7 (c 0.39, CH₃OH), UV (CH₃CN/H₂O) λ_{\max} (%) 232 (100%) nm; ECD λ_{ext} ($\Delta\epsilon$) (CH₃OH) 227 (+1.48), 257 (-3.38) nm; IR (ATR) ν_{\max} 2952, 2925, 2854, 1747, 1557, 1468, 1434, 1378, 1320, 1198, 1171 cm⁻¹; ¹H NMR see Table 1; ¹³C NMR see Table 1; (+)-HRESIMS m/z 349.2471 [M + Na]⁺ (calcd for C₁₈H₃₄N₂O₃Na, 349.2462).

Azodyrecin B (2): colorless oil; $[\alpha]_D^{20}$ -64.6 (c 0.46, CH₃OH), UV (CH₃CN/H₂O) λ_{\max} (%) 235 (100%) nm; ECD λ_{ext} ($\Delta\epsilon$) (CH₃OH) 227 (+1.69), 257 (-3.63) nm; IR (ATR) ν_{\max} 2925, 2854, 1747, 1556, 1434 cm⁻¹; ¹H NMR see Table 1; ¹³C NMR see Table 1; (+)-HRESIMS m/z 363.2621 [M + Na]⁺ (calcd for C₁₉H₃₆N₂O₃Na, 363.2618).

Azodyrecin C (3): colorless oil; $[\alpha]_D^{20}$ -52.2 (c 0.23, CH₃OH), UV (CH₃CN/H₂O) λ_{\max} (%) 235 (100%) nm; ECD λ_{ext} ($\Delta\epsilon$) (CH₃OH) 226 (+1.89), 257 (-4.20) nm; IR (ATR) ν_{\max} 2925, 2854, 1748, 1557, 1468, 1436, 1320, 1198, 1172, 1132 cm⁻¹; ¹H NMR see Table 1; ¹³C NMR see Table 1; (+)-HRESIMS m/z 377.2783 [M+Na]⁺ (calcd for C₂₀H₃₈N₂O₃Na 377.2775).

l'-trans-Azodyrecin A (4): colorless oil; $[\alpha]_D^{20}$ -6.3 (c 0.08, CH₃OH), UV (CH₃CN/H₂O) λ_{\max} (%) 236 (100%) nm; ECD λ_{ext} ($\Delta\epsilon$) (CH₃OH) 229 (+0.80), 257 (-0.82) nm; IR (ATR) ν_{\max}

2926, 2854, 1747, 1557, 1468, 1433, 1321, 1171, 1128 cm⁻¹; ¹H NMR see Table 1; ¹³C NMR see Table 1; (+)-HRESIMS *m/z* 349.2467 [M+Na]⁺ (calcd for C₁₈H₃₄N₂O₃Na, 349.2462).

l'-trans-Azodyrecin B (**5**): colorless oil; [α]_D²⁰+5.0 (c 0.26, CH₃OH), UV (CH₃CN/H₂O) λ_{max} (%) 237 (100%) nm; ECD λ_{ext} (Δε) (CH₃OH) 256 (−0.03) nm; IR (ATR) ν_{max} 2925, 2854, 1748, 1558, 1434, 1322 cm⁻¹; ¹H NMR see Table 1; ¹³C NMR see Table 1; (+)-HRESIMS *m/z* 341.2805 [M+H]⁺ (calcd for C₁₉H₃₇N₂O₃, 341.2799).

ASSOCIATED CONTENT

Supporting Information

NMR spectra of compounds **1–5** and ECD curves for compounds **4–6**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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Table 1. ^1H (800 MHz) and ^{13}C (200 MHz) NMR data for **1–5** (**1–3** in CDCl_3 ; **4–5** in $\text{DMSO}-d_6$)

pos.	1		2		3		4		5	
	δ_{C} , type	δ_{H} , (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)
1	171.4, C	–	171.4, C	–	171.4, C	–	170.5, C	–	170.5, C	–
2	58.8, CH	4.55, q (7.2)	58.8, CH	4.55, q (7.2)	58.8, CH	4.56, q (7.2)	58.4, CH	4.47, q (7.2)	58.4, CH	4.47, q (7.2)
3	15.8, CH_3	1.54, d (7.2)	15.8, CH_3	1.54, d (7.2)	15.8, CH_3	1.55, d (7.2)	15.6, CH_3	1.43, d (7.2)	15.6, CH_3	1.43, d (7.2)
4	52.3, CH_3	3.72, s	52.3, CH_3	3.73, s	52.4, CH_3	3.73, s	51.9, CH_3	3.61, s	51.9, CH_3	3.61, s
1'	135.1, CH	6.82, dt (9.0, 1.8)	135.2, CH	6.82, dt (9.1, 1.7)	135.2, CH	6.72, dt (9.0, 1.8)	137.0, CH	7.29, dt (13.3, 1.5)	137.0, CH	7.23, dt (13.3, 1.5)
2'	135.6, CH	5.78, dt (9.0, 7.5)	135.6, CH	5.78, dt (9.1, 7.5)	135.7, CH	5.78, dt (9.0, 7.5)	135.7, CH	6.91, dt (13.3, 7.5)	135.7, CH	6.91, dt (13.3, 7.5)
3'	27.3, CH_2	2.64, m	27.3, CH_2	2.64, m	27.3, CH_2	2.64, m	27.64*, CH_2	2.21, m	27.6, CH_2	2.21, m
4'	29.1, CH_2	1.45, m	29.1, CH_2	1.45, m	29.1, CH_2	1.45, m	27.60*, CH_2	1.43, m	27.7, CH_2	1.43, m
5'	29.4, CH_2	1.31, m	29.4, CH_2	1.32, m	29.4, CH_2	1.32, m	28.5, CH_2	1.21–1.29, m	28.7, CH_2	1.18–1.32, m
6'	27.3, CH_2	1.22–1.29, m	27.2, CH_2	1.20–1.30, m	27.3, CH_2	1.22–1.29, m	28.7, CH_2	1.21–1.29, m	28.6, CH_2	1.18–1.32, m
7'	29.5, CH_2	1.22–1.29, m	29.5*, CH_2	1.20–1.30, m	29.5, CH_2	1.22–1.29, m	28.9#, CH_2	1.21–1.29, m	26.5*, CH_2	1.18–1.32, m
8'	29.8, CH_2	1.22–1.29, m	29.7*, CH_2	1.20–1.30, m	29.7*, CH_2	1.22–1.29, m	29.0#, CH_2	1.21–1.29, m	28.9*, CH_2	1.18–1.32, m
9'	30.0, CH_2	1.22–1.29, m	29.8, CH_2	1.20–1.30, m	29.78*, CH_2	1.22–1.29, m	29.3, CH_2	1.21–1.29, m	29.0, CH_2	1.18–1.32, m
10'	27.5, CH_2	1.22–1.29, m	30.1, CH_2	1.20–1.30, m	29.81*, CH_2	1.22–1.29, m	26.8, CH_2	1.24, m	29.4, CH_2	1.18–1.32, m
11'	39.2, CH_2	1.13, m	36.8, CH_2	1.07, m	30.1, CH_2	1.22–1.29, m	38.5, CH_2	1.13, m	36.0, CH_2	1.06, m
12'	28.1, CH_2	1.50, m	34.5, CH	1.28, m	27.6, CH_2	1.22–1.29, m	27.4, CH_2	1.49, m	33.7, CH	1.28, m
13'	22.8, CH_3	0.85, d (6.7)	29.6, CH_2	1.11, m	39.2, CH_2	1.15, m	22.5, CH_3	0.84, d (6.6)	28.9, CH_2	1.10, m
				1.25, m						1.24, m

14'	22.8, CH ₃	0.85, d (6.7)	11.5, CH ₃	0.85, t (7.2)	28.1, CH	1.51, m	22.5, CH ₃	0.84, d (6.6)	11.2, CH ₃	0.82, t (7.2)
15'	–	–	19.5, CH ₃	0.83, d (6.4)	22.8, CH ₃	0.86, d (6.6)	–	–	19.1, CH ₃	0.81, d (6.5)
16'	–	–	–	–	22.8, CH ₃	0.86, d (6.6)	–	–	–	–

*,#Interchangeable signals

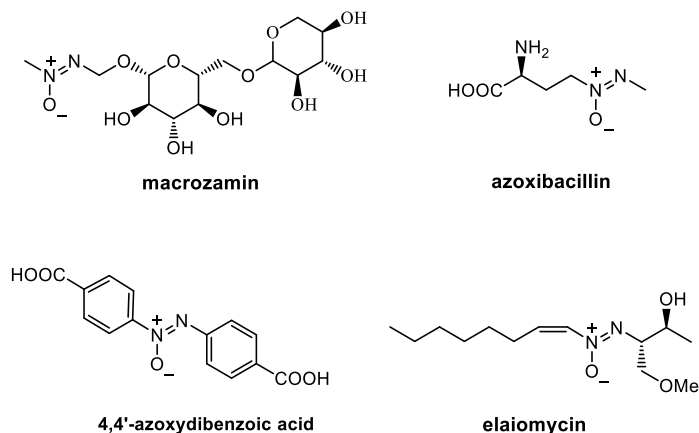


Figure 1. Structures of selected naturally occurring azoxides. Macrozamin from cycad plant; azoxibacillin produced by *Bacillus cereus*; 4,4'-azoxydibenzoic acid from entomopathogenic zygomycete *Entomophthora virulenta*; and elaiomycin produced by *Streptomyces hepaticus*.

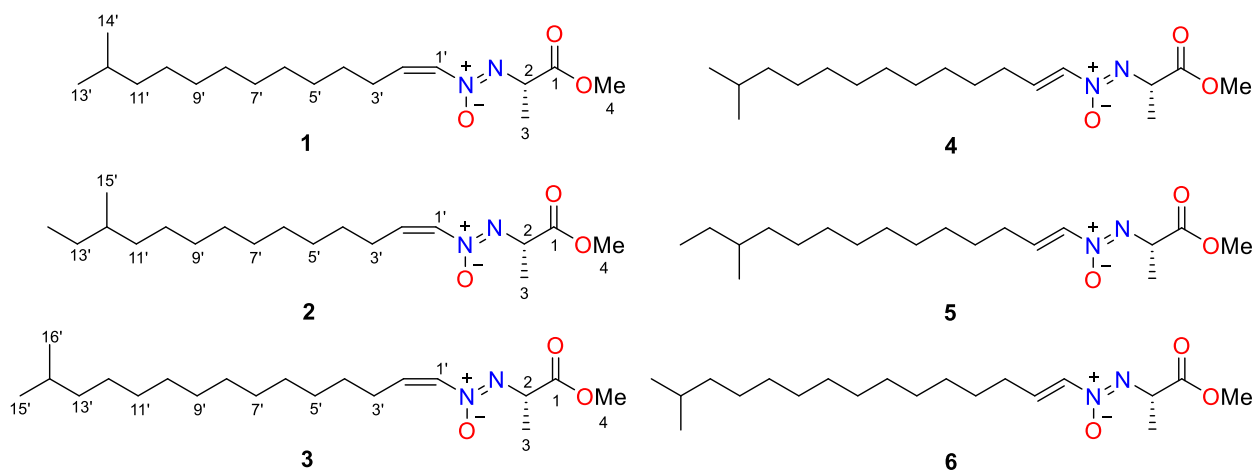


Figure 2. Structures of compounds 1–6.

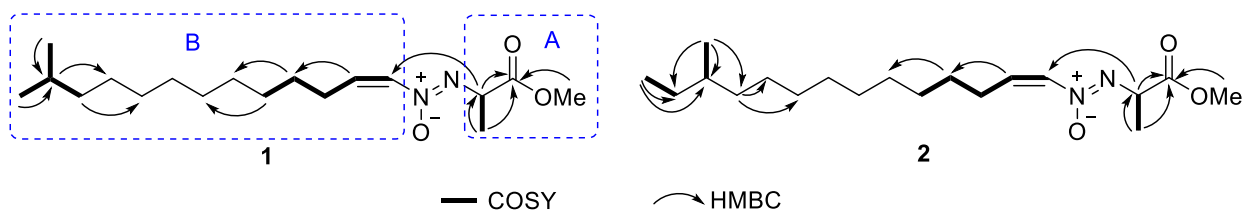


Figure 3. Selected COSY and HMBC correlations for compounds 1 and 2.

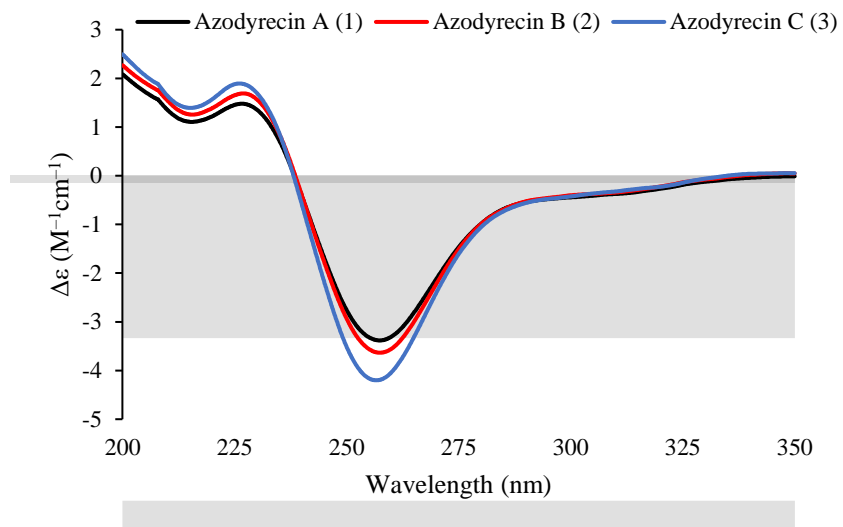


Figure 4. ECD curves of **1–3**.

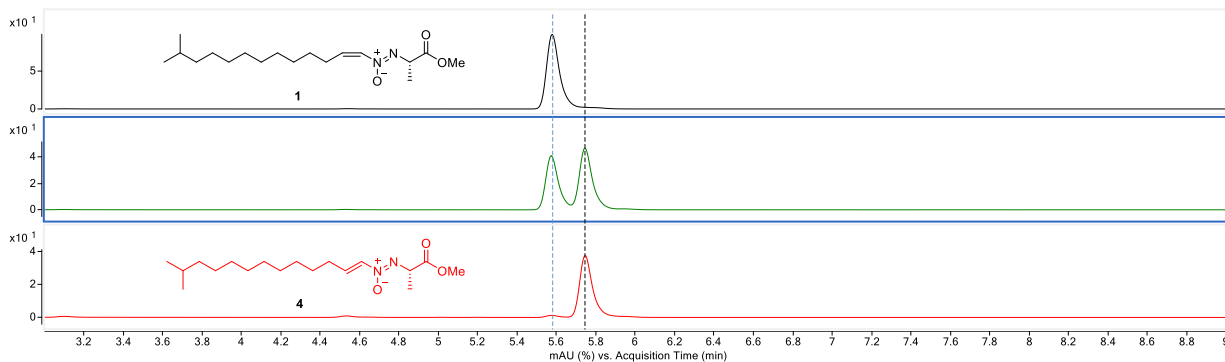


Figure 5. HPLC-UV (280 nm) chromatograms showing the conversion of compound **1** (top panel) to compound **4** (bottom panel). Middle panel shows the mixture of compounds **1** and **4**.

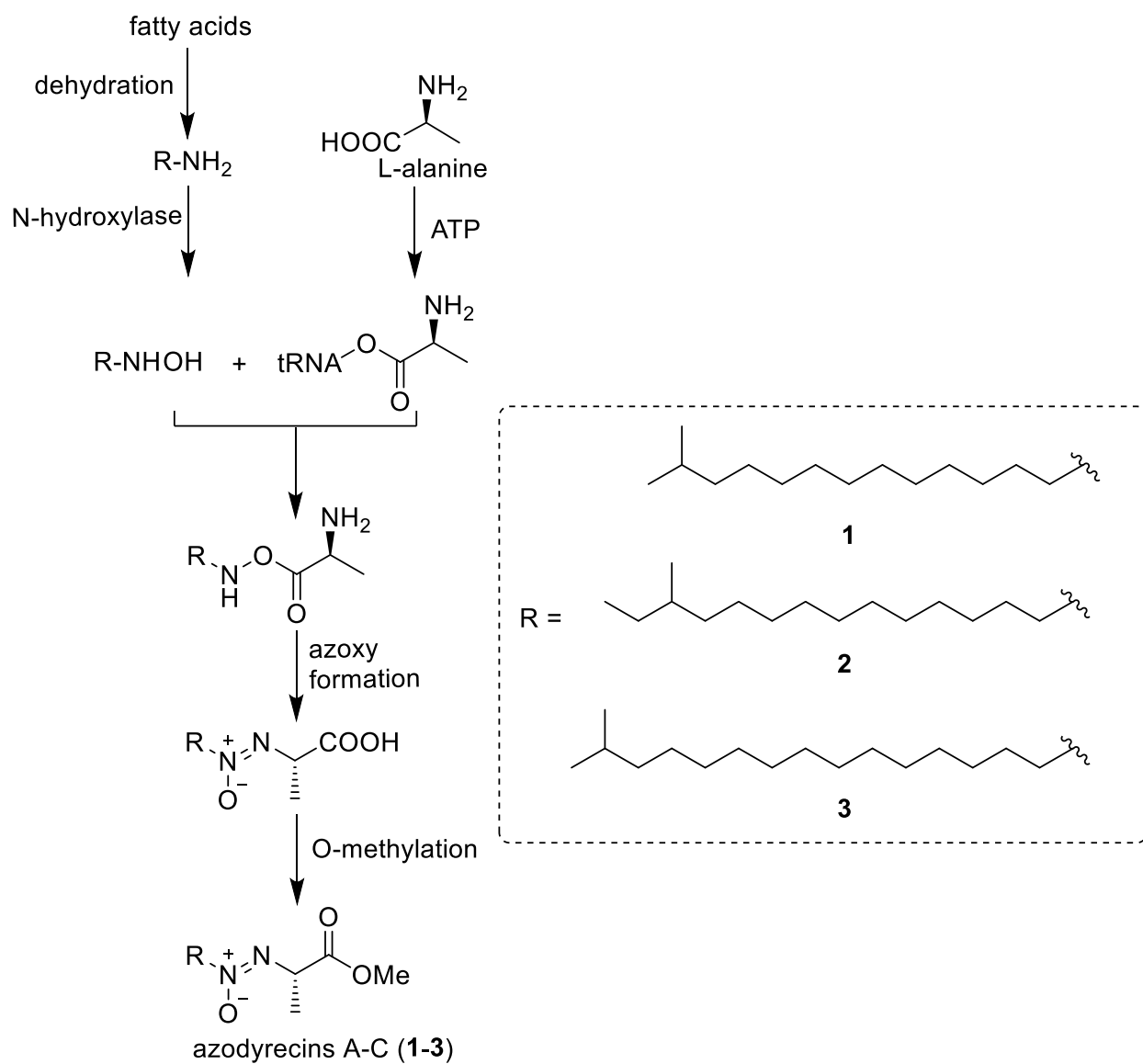


Figure 6. Plausible biosynthetic pathway for azodyrecins A–C (1–3).

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