Furan Oligomers and β-Carbolines from Terrestrial Streptomycetes

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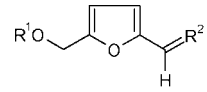
2,5-Bis(hydroxymethyl)furan monoacetate (**3**) and 2,5-bis(hydroxymethyl)furan diacetate (**4**) were obtained as new natural products from an ethyl acetate extract of the terrestrial *Streptomyces* sp. isolate GW11/1695. Another *Streptomyces* isolate, GW21/1313, delivered a dimer (**6**) and a trimer (**7**) of (hydroxymethyl)furfural. The latter strain also produced 4-hydroxy-2-(5-(hydroxymethyl)furan-2-ylmethylene)-5-methylfuran-3-one (**5**), perlolyrin (**8**), and two new β-carboline derivatives, **9** and **10**. 2,5-Bis(hydroxymethyl)furan diacetate (**4**) exhibited weak cytotoxic activity against brine shrimp larvae.

Furan derivatives are very widespread in nature. The unsubstituted furan is one of the products obtained by thermolysis of wood, and 5-(hydroxymethyl)furfural (**1**) is produced on heating hexoses with acid. The corresponding alcohol, 2,5-bis(hydroxymethyl)furan (**2**) was reported from a fungus[1] and was recently also obtained from a streptomycete.[2] Further natural furan derivatives were found to display various biological activities: potamogetonyde, potamogetonol, and potamogetonin from *Potamogeton malaianus* were reported to exhibit antiviral, insecticidal, and cytotoxic activity.[3] 2-Formyl-5-pentylfuran and 2-formyl-5-(4-pentenyl)furan from the fungus *Irpex lacteus*[4,5] demonstrated nematocidal activity. Furan fatty acid esters were isolated from the marine sponge *Dictyonella incisa* and showed inflammatory activity.[6] Other bioactive furans include wyeronic acid[7] and its derivatives,[8] omphalone,[9] and (1*R*,2*R*)-1-(5′-methylfur-3′-yl)propane-1,2,3-triol.[10]

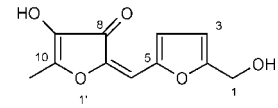
While furfural derivatives are certainly sugar-derived, C-1-substituted β-carbolines are amino acid metabolites that can be formed easily under very mild conditions from tryptamine or tryptophan and the respective aldehydes by a Pictet−Spengler reaction. These compounds are less widespread in nature than furans and appear to occur mostly in plants and marine animals.[11] Several simple furans and two new β-carbolines have now been isolated from two *Streptomyces* spp.

An ethyl acetate extract of the terrestrial *Streptomyces* sp. isolate GW11/1695 delivered two compounds, which displayed a gray to black color reaction on TLC with anisaldehyde/sulfuric acid. This behavior, the UV/vis spectra, and the NMR data of the isolated compounds were very similar to those of a commercial sample of **2**, the main differences in the ^{13}C NMR spectrum being one and two, respectively, additional acetyl signals. The molecular weight of the first compound was found to be *m*/*z* 170, which corresponds to a monoacetate of **2**. The structure **3** was indeed confirmed by partial acetylation of **2**. The main product **4** obtained in this reaction was found to be identical with the second furan derivative obtained from *Streptomyces* sp. GW11/1695. Both compounds were isolated from bacteria for the first time now; however, **4** had been synthesized previously.[12]

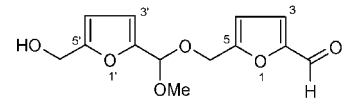
A second terrestrial *Streptomyces* sp. isolate, GW21/1313, yielded in M_2 medium a mixture of actinomycins C_2 and D as main components. During variation of the culture conditions, the strain produced, however, in a calcium-rich medium instead of actinomycins a completely different metabolite pattern. Separation of the extract from a shaker culture delivered adenine, tyrosol,[13] and a yellow compound, which showed a dark yellow, then brown, and finally yellowish-green color reaction with anisaldehyde/sulfuric acid on TLC.



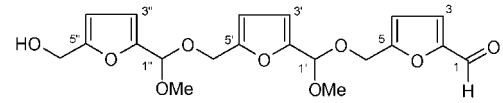
1: R^1 = H; R^2 = O
2: R^1 = H; R^2 = H,OH
3: R^1 = H; R^2 = H,OAc
4: R^1 = Ac; R^2 = H,OAc



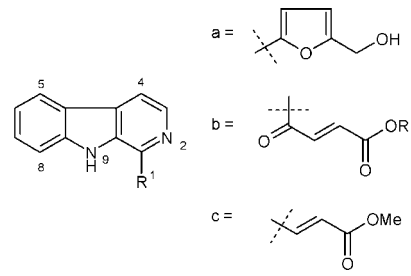
5



6



7



8: R^1 = a
9: R^1 = b; R^2 = Me
10: R^1 = b; R^2 = H
11: R^1 = c

The molecular weight of this compound was determined as *m*/*z* 222; the ^{1}H NMR spectrum depicts two doublets each with a coupling constant of 3.4 Hz at δ 7.03 and 6.52, which could be assigned to a disubstituted furan or pyrrole. Additionally, it displayed three singlets of an olefinic methine, an oxygen-bearing methylene, and an sp^2-connected methyl group. The ^{13}C NMR spectrum showed signals of six quaternary and three methine carbon atoms in the sp^2 region, of which the signal at δ 182.8 could be assigned to a carboxylic acid derivative or a conjugated ketone. In the aliphatic region, only two signals of a methylene and a methyl group were observed. The structure was finally derived from the 2D NMR spectra as 4-hydroxy-2-(5-(hydroxymethyl)furan-2-yl-methylene)-5-methylfuran-3-one (**5**), a new natural product that had,

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however, been obtained earlier by Maillard reaction of amino acids and sugars.[14]

In the fermentor, the strain produced additionally tryptophol,[15] *p*-hydroxybenzoic acid, uracil, 5-hydroxymethylfurfural (**1**),[16] two new furans (**6** and **7**), perlolyrin (**8**),[17] and two new β-carbolines (**9** and **10**).

The oily compounds **6** and **7** exhibited strong UV-absorbing bands similar to those of **1**, which turned black with anisaldehyde/sulfuric acid and decomposed partially during chromatography on silica gel with formation of **1**. The high-resolution EI mass spectrum of **6** delivered the molecular formula $C_{13}H_{14}O_6$. The ^{1}H NMR spectrum indicated an aldehyde singlet as in **1**; however, instead of one furan system, two rings and two oxygen-bound methylene groups and a further methoxy singlet at δ 3.35 were present. The molecular weight and the ^{1}H and ^{13}C NMR data indicated it to be a dimer of 5-(hydroxymethyl)furfural, where the **1** units were connected via an acetal bond. The structure **6** was finally confirmed by the H−H COSY, HMQC, and HMBC spectra (see Supporting Information).

The molecular peak in the EIHRMS of **7** gave the formula $C_{20}H_{22}O_9$. The ^{1}H and ^{13}C NMR spectra were similar to those of **1** and **6**, except that three furan units with two additional methoxy signals and two acetal carbon atoms were indicated. The 2D spectra confirmed finally the trimer **7**. Compounds **6** and **7** have not been described previously in the literature and represent the first natural examples of acetal-connected hydroxyaldehydes. Both compounds are levorotatory; however, the stereochemistry of these compounds is still unknown.

It seems plausible that **6** and **7** are formed from **1** during the separation on silica gel in the presence of MeOH, and **5** may be the product of an aldol condensation with **1**. Indeed **6** and **7** were found by HPLC/MS in an aged sample of **1**.[18] When freshly distilled **1** was treated under conditions used for chromatography of the crude bacterial extract, **6** and **7** were not formed; however, both compounds were already detectable in the crude extract by TLC. As also the optical activity indicates, an abiotic origin of these acetals is less likely.

Compound **8** was obtained on repeated chromatography of the extracts as a yellow solid with a strong blue fluorescence on TLC at 366 nm and a blue coloration with Ehrlich's reagent. By the molecular weight (*m*/*z* 264) and the characteristic ^{1}H NMR spectrum with signals of an acidic proton at δ 11.18, an oxygen-connected methylene group, sp^2 protons of a 1,2-disubstituted benzene, a pair of *ortho*-coupled protons of an electron-deficient aromatic system, and an electron-rich 2,5-disubstituted furan ring, it was easily identified[21] as perlolyrin (**8**). This compound was known from plant origin[19] and was described very recently also as a bacterial metabolite.[20]

The ^{1}H NMR spectrum of a second yellow solid, **9**, with strong blue-green UV fluorescence at 366 nm was very similar to that of perlolyrin (**8**). Again a set of four protons for a 1,2-disubstituted benzene ring, two *ortho*-coupled protons of a pyridine ring, and an NH proton were observed as for **8** or other 1-substituted β-carbolines. The difference was in the replacement of the furan and the methylene proton signals of **8** by two protons of a *trans*-disubstituted double bond in conjugation with a carbonyl and a methoxy signal. A search in AntiBase[21] with the ^{1}H NMR data resulted in (*E*)-3-(β-carbolin-1-yl)propenoic acid methyl ester (**11**), whose data[22] were similar, however not identical.

The molecular weight *m*/*z* 280 of compound **9** was $\Delta m/z$ 28 (CO) higher than that of **11**. The supposed carbonyl group was confirmed by a signal at δ 189.2 in the ^{13}C NMR spectrum of **9**, which afforded two possible structures for the side chain, a 2-oxo- or a 4-oxo-butenoic acid methyl ester. HMBC couplings finally confirmed the structure: Both of the olefinic proton signals (δ 8.68 and 6.93) showed strong couplings to *both* carbonyl signals (δ 189.2 and 165.5). In case of the 2-oxo isomer, the cross signal between the proton at δ 8.68 and the ester carbonyl would be a ^4J coupling, which is, however, expected to be weak. Therefore, the strong coupling between the low-field olefinic proton and the ester carbonyl is better explained by a ^3J coupling in a 4-oxo-2-butenoic acid methyl ester side chain, resulting in structure **9**.

The ^{1}H NMR spectra of **9** and **10** were very similar, except for the missing methoxy signal in **10**. The molecular weight of *m*/*z* 266 (ESI and CIMS) was $\Delta m/z$ 14 (CH_2) lower than that of **9**, resulting in the final structure **10**.

1-Substituted β-carbolines can be obtained from tryptamine and aldehydes by a Pictet−Spengler reaction, which can easily occur under physiological conditions. As the strain GW21/1313 produced high concentrations of (hydroxymethyl)furfural (**1**), a formation during the isolation procedure is plausible at least for perlolyrin (**8**). The highly fluorescent **8** and the other β-carbolines were, however, detected by HPLC-MS in the crude extract; thus their generation under the workup conditions is less likely.

All isolated compounds were inactive in an agar diffusion test against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptomyces viridochromogenes* (Tü 57), and *Escherichia coli*, the fungi *Candida albicans* and *Mucor miehei*, and the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*. 2,5-Bis(hydroxymethyl)furan diacetate (**4**) exhibited weak inhibition of brine shrimp larvae[23] at a concentration of 6 μg/mL.

Experimental Section

General Experimental Methods. NMR spectra were measured on Varian Unity 300 (300.145 MHz), Varian Inova 500 (499.876 MHz), and Varian Inova 600 (599.740 MHz) spectrometers. ESI mass spectra were recorded on a Quattro triple quadrupole mass spectrometer with a nano-ESI API ion source. EI mass spectra were measured on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance for HREIMS. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer from KBr pellets; UV/vis spectra were obtained from a Perkin-Elmer Lambda 15 UV/vis spectrometer. Preparative HPLC was performed using an RP18 column (Eurochrom Eurospher RP 100-C18, 5 μm) with a diode array multiwavelength detector (195−640 nm, Jasco). Column chromatography was carried out on silica gel (230−400 mesh). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

Culture Media. M_2 Medium. Malt extract/yeast extract/glucose medium: Malt extract (10 g), yeast extract (4 g), and glucose (4 g) were dissolved in 1 L of tap water, and the medium was adjusted to pH 7.8 with 2 N NaOH and sterilized for 33 min at 121 °C. After sterilization, an end pH 7.0 of the medium is attained. **CYG Medium.** Calcium chloride (45 g), yeast extract (40 g), and glucose (5 g) were dissolved in 1 L of tap water, and the medium was adjusted to pH 6.5 with 2 N NaOH and sterilized for 33 min at 121 °C.

Description of Strain GW21/1313. The terrestrial actinomycete strain GW21/1313 was Gram-positive, was nonacid fast, and grew aerobically with substrate and aerial mycelium. The well-developed aerial mycelium carried typical streptomycete-like long *spiral* chains of arthrospores. Neither aerial hyphae nor substrate mycelium showed fragmentation. Other morphological features such as sporangia, or motile spores, were not observed. The color of the aerial spore mass was light pink on yeast extract−malt agar and white on soil extract agar, and the substrate mycelium was brown on these media. Melanin pigments were not produced on tyrosine agar. The diaminopimelic acid isomer and the sugar composition of the whole cell hydrolysate indicated that the strain had cell walls of type I and belongs to the genus *Streptomyces*. The strain is deposited in the culture collection of actinomycetes at the Labor Grün-Wollny, Versaillerstrasse 1, D-35394 Giessen, Germany.

Description of Strain GW11/1695. The terrestrial strain GW11/1695 was also obtained from the strain collection of Labor Grün-Wollny. This strain was Gram-positive, aerobic, and nonacid fast, and produced a sterile nonfragmenting and highly branched aerial mycelium. Neither sporangia, or motile spores, nor any other special morphological structures were observed. Aerial hyphae and spore mass were gray on yeast extract−malt agar, oatmeal, and soil extract agar. The substrate mycelium was dark brown on most media. A red to brown diffusible pigment was produced on yeast extract−malt extract agar and on soil

extract agar. The strain formed melanin pigments on tyrosine agar slants. The presence of L,L-diaminopimelic acid and the absence of characteristic sugars in whole cell hydrolysate showed that this organism belonged to the cell wall chemotype I. On the basis of the chemotaxonomic properties, growth characteristics, and morphology, strain GW11/1695 can be grouped in the genus *Streptomyces*.

Fermentation, Extraction, and Isolation from GW21/1313. One hundred 1 L Erlenmeyer flasks, each containing 250 mL of CYG medium, were inoculated with a well-grown agar culture of *Streptomyces* sp. GW21/1313 and incubated for 4 days at 35 °C on a round shaker (110 rpm). The 25 L culture broth was worked up using our standard procedure,[24] which yielded 1.5 g of a brown, oily extract. The crude extract was subjected to size exclusion column chromatography on Sephadex LH-20 (CH_2Cl_2/50% MeOH). Fraction 1 contained mainly fats and fatty acid and was not further investigated. Further purification of the second fraction by PTLC and HPLC delivered 4-hydroxy-2-(5-(hydroxymethyl)furan-2-ylmethylene)-5-methylfuran-3-one (**5**, 25 mg), adenine (32 mg), and tyrosol (17 mg).

Workup of the 50 L of the culture broth obtained by culturing under the same conditions delivered 12.6 g of extract, which contained about 6.5 g of Niax added during the fermentation to avoid foaming. The extract was gel separated on Sephadex LH-20 (CH_2Cl_2/50% MeOH) to get two fractions. The first fraction contained solely Niax and was discarded. Fraction 2 was separated by a column chromatography on silica gel into subfractions I−VII with a stepwise gradient (CH_2Cl_2 to CH_2Cl_2/10% MeOH). Purification of subfraction II on Sephadex LH-20 yielded 2-(indol-3-yl)ethanol (13 mg). Subfraction III delivered by HPLC 5-(hydroxymethyl)furfural (**1**, 82 mg), **6** (32 mg), **7** (7 mg), and *p*-hydroxybenzoic acid (11 mg). Subfraction IV afforded 5-(hydroxymethyl)furfural (**1**, 950 mg) and **6** (20 mg). Similarly, subfraction V yielded 282 mg of tyrosol after size exclusion chromatography on Sephadex LH-20. Subfraction VI afforded perlolyrin (**8**, 13 mg), 3-(9*H*-*β*-carbolin-1-yl)acrylic acid methyl ester (**9**, 4 mg), and 3-(9*H*-*β*-carbolin-1-yl)acrylic acid (**10**, 2 mg) after purification by HPLC, Sephadex LH-20, and PTLC (CH_2Cl_2/7% CH_3OH). Uracil (180 mg) and adenine (459 mg) precipitated from subfractions VI and VII on adding CH_2Cl_2 to the solution in CH_3OH.

Fermentation, Extraction, and Isolation from GW11/1695. The *Streptomyces* strain GW11/1695 was cultivated in M_2 medium at 28 °C for 3 days and extracted with ethyl acetate. Purification of the ethyl acetate extract in a similar way to that for GW23/1313 yielded 2,5-bis(hydroxymethyl)furan monoacetate (**3**, 24 mg) as light yellow oil and 2,5-bis(hydroxymethyl)furan diacetate (**4**, 14 mg) as a colorless solid.

2,5-Bis(hydroxymethyl)furan monoacetate (3): light yellow oil; UV/vis (MeOH) λ_{max} (log ε) 223 (4.48) nm; IR (KBr) ν_{max} 3440 (OH), 2945, 2875, 1747, 1561, 1437, 1378, 1233, 1022, 963, 923, 802, 763 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.36 (1H, d, ^3J = 3.4 Hz, H-3), 6.27 (1H, d, ^3J = 3.0 Hz, H-4), 5.03 (2H, s, CH_2-OAc), 4.06 (2H, s, CH_2OH), 2.08 (3H, s, CH_3), 1.96 (1H, br s, OH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.7 (C, CO), 154.8 (C, C-5), 149.2 (C, C-2), 111.4 (C, C-3), 108.5 (C, C-4), 58.1 (CH_2, *C*H_2OAc), 57.4 (CH_2, *C*H_2OH), 20.8 (CH_3); EIMS (70 eV) *m/z* (%) 170 $[M]^+$ (25), 152 $[M - H_2O]^+$ (22), 110 (100), 43 $[COCH_3]^+$ (70); HREIMS (70 eV) *m/z* 170.0629 (calcd for $C_8H_{10}O_4$, 170.0579).

2,5-Bis(hydroxymethyl)furan diacetate (4): colorless solid; UV/vis (MeOH) λ_{max} (log ε) 221 (4.11); IR (KBr) ν_{max} 3113, 2958, 1736, 1569, 1438, 1381, 1358, 1246, 1025, 998, 963, 917, 819, 765 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.38 (2H, s, H-3,4), 5.04 (4H, s, 2 CH_2), 2.10 (6H, s, 2 CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.6 (2 C, 2 CO), 150.2 (2 C, C-2,5), 111.6 (2 C, C-3,4), 58.1 (2 CH_2, 2 *C*H_2-OAc), 20.9 (2 CH_3, 2 CO*C*H_3); EIMS (70 eV) *m/z* (%) 212 $[M]^+$ (4), 169 $[M - COCH_3]^+$ (5), 153 $[M - OCOCH_3]^+$ (36), 110 (100), 94 (10), 83 (12), 43 $[COCH_3]^+$ (94); CIMS (NH_3) *m/z* 247 $[M + NH_4 + NH_3]^+$, 230 $[M + NH_4]^+$; HREIMS (70 eV) *m/z* 212.0679 (calcd for $C_{10}H_{12}O_5$, 212.0684).

4-Hydroxy-2-(5-(hydroxymethyl)furan-2-ylmethylene)-5-methylfuran-3-one (5): orange solid; λ_{max} (log ε) 248 (3.63), 365 nm (4.38); ^1H NMR (CD_3OD, 300 MHz) δ 7.03 (1H, d, ^3J= 3.4 Hz, H-4), 6.63 (1H, s, CH-6), 6.52 (1H, d, ^3J = 3.4 Hz, H-3), 4.56 (2H, s, CH_2OH) 2.33 (3H, s, CH_3-11); ^{13}C NMR (CD_3OD, 75 MHz) δ 182.8 (C, C-8), 164.2 (C, C-10), 159.9 (C, C-2), 149.3 (C, C-5), 144.0 (C, C-7), 137.2 (C, C-9), 119.9 (CH, C-4), 111.9 (CH, C-3), 102.3 (CH, C-6), 57.5 (CH_2, *C*H_2-1), 12.2 (CH_3, *C*H_3-11); (+)-ESIMS *m/z* (%) 369 [3 M + Na]$^+$ (22), 467 [2 M + Na]$^+$ (100), 245 [M + Na]$^+$ (10), 223 [M + H]$^+$ (12); (−)-ESIMS *m/z* (%) 1929 [8 M − 8H + 7Na]$^-$ (7), 1685 [7 M − 7H + 6Na]$^-$ (20), 1441 [6 M − 6H + 5Na]$^-$ (40), 1197 [5 M − 5H + 4Na]$^-$ (60), 953 [4 M − 4H + 3Na]$^-$ (80), 709 [3 M − 3H + 2Na]$^-$ (54), 465 [2 M − 2H + Na]$^-$ (27), 221 [M − H]− (100); EIMS (70 eV) *m/z* (%) 222 $[M]^+$ (100), 204 $[M - H_2O]^+$ (24), 191 $[M - CH_2OH]^+$ (18), 176 (8), 151 (16), 133 (55), 121 (36), 105 (28), 79 (8), 43 $[CH_3CO]^+$ (32); CIMS (NH_3) *m/z* (%) 257 $[M + NH_4 + NH_3]^+$ (4), 240 $[M + NH_4]^+$ (100), 223 $[M + H]^+$ (28).

5-[(5-(Hydroxymethyl)furan-2-yl)methoxymethoxymethyl]furan-2-carbaldehyde (6): light yellow oil; UV/vis (MeOH) λ_{max} (log ε) 214 (sh, 4.05), 279 (4.01) nm; $[\alpha]_D^{20}$ −4.95 (*c* 1.01 mg/mL, MeOH); IR (KBr) ν_{max} 3419, 2928, 2850, 1680, 1667, 1524, 1383, 1281, 1195, 1104, 1022, 806 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz) δ 9.61 (1H, s, C*H*O), 7.37 (1H, d, ^3J = 3.4 Hz, H-3), 6.65 (1H, d, ^3J = 3.4 Hz, H-4), 6.39 (1H, d, ^3J = 3.0 Hz, H-4′), 6.25 (1H, d, ^3J = 3.0 Hz, H-3′), 5.59 (1H, s, C*H*-1′), 4.56, 4.51 (2H, AB, J_{AB} = 13.4 Hz, CH_2O-6), 4.49 (2H, s, CH_2O-6′), 4.30 (1H, br s, H/D exchangeable, CH_2O*H*), 3.35 (3H, s, OCH_3); ^{13}C NMR (acetone-d_6, 75 MHz) δ 178.3 (CH, *C*HO-1), 158.7 (C, C-5′), 156.7 (C, C-5), 153.8 (C, C-2), 150.9 (C, C-2′), 123.3 (CH, C-3), 112.3 (CH, C-3′), 110.1 (CH, C-4), 108.1 (CH, C-4′), 97.8 (CH, C-1′), 59.6 (CH_2, C-6), 57.2 (CH_2, C-6′), 53.5 (CH_3, O*C*H_3); (−)-ESIMS *m/z* (%) 289 $[M + Na]^+$; HREIMS (70 eV) *m/z* 266.0798 (calcd for $C_{13}H_{14}O_6$, 266.0790).

5-({5-[(5-(hydroxymethyl)furan-2-yl)methoxy(methoxymethyl)]-furan-2-yl}methoxy(methoxymethyl))furan-2-carbaldehyde (7): light yellow oil; UV/vis (MeOH) λ_{max} (log ε) 280 (4.50), 218 (sh, 4.00) nm; $[\alpha]_D^{20}$ −1.95 (*c* 1.54 mg/mL, MeOH); IR (KBr) ν_{max} 3417, 2927, 2850, 1680, 1668, 1522, 1385, 1280, 1193, 1074, 1023, 810, 778 cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz) δ 9.60 (1H, s, C*H*O-1), 7.36 (1H, d, ^3J =3.2 Hz, H-3), 6.64 (1H, d, ^3J = 3.2 Hz, H-4), 6.43 (1H, d, ^3J = 3.3 Hz, H-4"), 6.38 (1H, d, ^3J = 3.2 Hz, H-4′), 6.35 (1H, d, ^3J = 3.4 Hz, H-3′), 6.24 (1H, d, ^3J =3.2 Hz, H-3″), 5.62 (1H, s, H-1″), 5.52 (1H, s, H-1′), 4.66, 4.62 (2H, AB, J_{AB} = 13.3 Hz, CH_2O-5), 4.54, 4.50 (2H, AB, J_{AB} = 13.1 Hz, CH_2O-5′), 4.48 (2H, d, ^3J = 6.0 Hz, CH_2OH-5″), 4.32 (1H, t, ^3J = 6.0 Hz H/D exchangeable, CH_2O*H*-5″), 3.36 (3H, s, OCH_3), 3.30 (3H, s, OCH_3); ^{13}C NMR (CD_3OD, 75 MHz) δ 178.3 (CH, *C*HO), 158.7 (C, C-5″), 156.6 (C, C-5′), 153.8 (C, C-5), 152.7 (C, C-2), 152.8 (C, C-2′), 151.2 (C, C-2″), 123.3 (CH, C-3), 112.3 (CH, C-3′), 110.7 (CH, C-3″), 110.2 (CH, C-4′), 109.8 (CH, C-4), 108.0 (CH, C-4″), 97.8 (CH, C-1′), 97.3 (CH, C-1″), 59.9 (CH_2, C-6′), 59.6 (CH_2, C-6), 57.2 (CH_2, C-6″), 53.6 (CH_3, OCH_3), 53.0 (CH_3, OCH_3); some shifts were tentatively assigned; (+)-ESIMS *m/z* 429 $[M + Na]^+$; HREIMS (70 eV) *m/z* 406.1259 (calcd for $C_{20}H_{22}O_9$, 406.1264).

4-(9*H*-*β*-Carbolin-1-yl)-4-oxobut-2-enoic acid methyl ester (9): light yellow solid; no fluorescence under UV at 366 nm; UV/vis (MeOH) λ_{max} (log ε) 206 (4.43), 235 (sh 3.97), 306 (3.52), 408 (3.36) nm; IR (KBr) ν_{max} 3426, 3367, 2924, 2853, 1729, 1708, 1657, 1614, 1458, 1432, 1382, 1318, 1282, 1251, 1227, 1211, 1197, 1064, 1042, 1018, 995, 840, 795, 738, 635, 596, 562, 516, 427 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.12 (1H, s, H/D exchangeable, NH), 8.68 (1H, d, ^3J = 16.2 Hz, H-11), 8.59 (1H, d, ^3J = 4.9 Hz, H-3), 8.51 (1H, d, ^3J = 4.9 Hz, H-4), 8.32 (1H, d, ^3J = 8.0 Hz, H-5), 7.82 (1H, dd, ^3J = 8.3 Hz, ^4J = 0.8 Hz, H-8), 7.62 (1H, ddd, ^3J = 8.2, 7.1 Hz, ^4J = 1.1 Hz, H-7), 7.33 (1H, ddd, ^3J = 8.0, 7.2 Hz, ^4J = 1.2 Hz, H-6), 6.93 (1H, d, ^3J = 16.2 Hz, H-12), 3.82 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 189.2 (C, C-10), 165.5 (C, C-13), 141.9 (C, C-8a), 137.8 (CH, C-3), 136.1 (CH, C-11), 135.1 (C, C-9a), 135.0 (C, C-1), 131.3 (C, C-4a), 130.0 (CH, C-12), 129.1 (CH, C-7), 121.9 (CH, C-5), 120.5 (CH, C-6), 120.2 (CH, C-4), 119.9 (C, C-4b), 113.1 (CH, C-8), 52.2 (CH_3, OCH_3); (+)-ESIMS *m/z* (%) 303 $[M + Na]^+$, 281 $[M + H]^+$; HREIMS (70 eV) *m/z* 280.0856 (calcd for $C_{16}H_{12}N_2O_3$, 280.0848).

4-(9*H*-*β*-Carbolin-1-yl)-4-oxobut-2-enoic acid (10): yellow solid; no (!) fluorescence under UV at 366 nm; UV/vis (MeOH) λ_{max} (log ε) 206 (4.49), 235 (sh 4.10), 301 (3.72), 400 (3.54) nm; IR (KBr) ν_{max} 3423, 2925, 2853, 1651, 1634, 1620, 1576, 1558, 1542, 1526, 1494, 1458, 1433, 1421, 1382, 1318, 1285, 1255, 1207, 1131, 1022, 800, 738, 636, 472, 432 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 12.02 (1H, s, H/D exchangeable, NH), 8.55 (1H, d, ^3J = 4.9 Hz, H-3), 8.45 (1H, d, ^3J = 4.9 Hz, H-4), 8.30 (1H, d, ^3J = 7.9 Hz, H-5), 8.23 (1H, d, ^3J = 15.8 Hz, H-11), 7.81 (1H, d, ^3J = 8.3 Hz, H-8), 7.59 (1H, ddd, ^3J = 8.3, 7.2 Hz, ^4J = 1.1 Hz, H-7), 7.30 (1H, dd, ^3J = 7.9, 7.2 Hz, ^4J = 1.1 Hz, H-6), 6.95 (1H, d, ^3J = 15.8 Hz, H-12); (+)-ESIMS *m/z*

267 $[M + H]^+$; (−)-ESIMS *m/z* (%) 553 $[2\ M + Na - 2H]^-$ (34), 265 $[M - H]^-$ (100); HREIMS (70 eV) *m/z* 266.0679 (calcd for $C_{15}H_{10}N_2O_3$, 266.0691).

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Supporting Information Available: ^{1}H NMR spectra of **5**, **6**, and **7**; HSQC and HMBC spectra of **5** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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