FLAZIN, PERLOLYRIN, AND OTHER β-CARBOLINES FROM MARINE DERIVED BACTERIA

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ABSTRACT

From ethyl acetate extracts of the marine Streptomyces spp. isolates B1848, B6005, and the North Sea bacterium Bio215, we have isolated the antibiologically active β-carbolines, flazin, perlolyrin and 1-acetyl-β-carboline, previously isolated from plants, as well as the new 1-(9H-β-carbolin-1-yl)-3-hydroxy-propan-1-one, which was also isolated from the terrestrial Nocardia tenerifiensis GW39/1573, whose high antibiotic and phytotoxic activity was due to nargenicin B1. The 4-Hydroxy-2-keto-butyraldehyde, an intermediate in the Maillard reaction, found in mammalian cell cultures is proposed as potential precursor of 1-(9H-β-carbolin-1-yl)-3-hydroxy-propan-1-one. All structures were confirmed by spectroscopic analyses including mass spectra (ESI-MS, HR ESI/EIMS), 1D and 2D NMR experiments, and by comparison with related compounds.

Key word Index: Marine Streptomyceses, β-Carboline

RESUMEN

De los extractos de acetato de etilo de los aislados B1848 y B6005 de Streptomyces spp., y la bacteria del Mar del Norte Bio215, se obtuvieron las β-carbolinas con actividad antibiótica, flazina, perlolirina, y 1-acetil-β-carbolina, aisladas anteriormente de plantas, así como la nueva 1-(9H-carbolin-1-il)-3-hidroxi-propan-1-ona, la cual fue aislada también de Nocardia tenerifiensis GW39/1573, cuya actividad antibiótica y fitotóxica se debe a la nargenicina B1. El 4-hidroxi-2-ceto-butyraldehido, un intermediario en la reacción de Maillard encontrado en cultivos de células de mamíferos, se propone como el potencial precursor de la 1-(9H-carbolin-1-il)-3-hidroxi-propan-1-ona. Todas las estructuras fueron establecidas con base en sus datos espectroscópicos de EM (ESI-MS, HR ESI/EIMS), experimentos de RMN 1D y 2D y comparación con compuestos relacionados.

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INTRODUCTION

More than 140 \(\beta\)-carbolines have been isolated as plant metabolites (Chapman & Hall, 2004) some with simple, but others also having complex [e.g. kirondrine (Krebs et al., 1997), alangiobussinine (Diallo et al., 1995), alstonidine (Crow et al., 1970), kumujansine (Yang et al., 1988)], or even highly complex structures, like kauluamine, the manzamines (Ohtani et al., 1995; Sakai et al., 1987), or shishijimicin A (Oku et al., 2003). About 45 \(\beta\)-carboline derivatives were reported as microbial metabolites (Laatsch, 2007), e.g. lavendamycin, an antineoplastic antibiotic from \textit{Streptomyces lavendulae} (Doyle et al., 1981; Balitz et al., 1982). \(\beta\)-Carbolines were suggested as herbicidal and fungicidal agents (Barker et al., 1986), and their affinity to the benzodiazepine-receptor is of special interest (Bracher et al., 1993).

The microbial diversity of the marine biosphere offers enormous scope for the discovery of novel natural products. Marine bacteria, fungi, cyanobacteria and symbiotic microorganisms have been particularly productive sources of bioactive natural products. In our search for new microbial metabolites, the marine-derived isolates \textit{Streptomyces} spp. B1848, B6005, and the North Sea bacterium Bio215 afforded, amongst other metabolites, flazin (1a), perlolyrin (1b), 1-acetyl-\(\beta\)-carboline (2a), and the 1-(9H-\(\beta\)-carbolin-1-yl)-3-hydroxy-propan-1-one (2b). Some of these compounds (1a, 1b, 2a) were known as plant products, but have not been isolated from microorganisms before.

RESULTS AND DISCUSSION

Flazin (1a) was obtained from strain Bio215 as a yellow solid with a greenish-yellow fluorescence on TLC at 366 nm, which showed no color change with sodium hydroxide. The \(^1\)H NMR spectrum exhibited a broad singlet of an acidic proton at \(\delta\) 11.58, signals of four aromatic protons of a 1,2-disubstituted aromatic ring and a singlet, whose downfield shift (\(\delta\) 8.83) indicated a heteroaromate (e.g. a pyridine system). Two 1H doublets with a small coupling constant (\(J = 3.5\) Hz) at \(\delta\) 7.41 and 6.62 were typical for a disubstituted five-membered heterocyclic ring, a 2H singlet at \(\delta\) 4.64 along with a hydroxyl proton at \(\delta\) 5.43 indicated a hydroxymethyl group. The \(^{13}\)C/APT NMR spectra displayed a carbonyl group at \(\delta\) 166.6, eight quaternary and seven methine carbon signals in the aromatic region, in addition to a signal at \(\delta\) 56.0, corresponding to the hydroxymethyl group.

The molecular weight of compound 1a was established as 308 amu according to the ESI mass spectra. EI MS delivered a main peak at \(m/z\) 264, due to the loss of \(\text{CO}_2\), which is indicative for a free carboxylic group; HR EIMS of the molecular ion delivered the formula \(\text{C}_{17}\text{H}_{12}\text{N}_2\text{O}_4\). The strong fluorescence and the NMR pattern suggested a structural similarity with \(\beta\)-carbolines, containing an additional carboxy group and an attached hydroxymethyl-furane moiety.
A compound with these properties had not been isolated from bacteria, however, the data resembled those of flazin (1a), a plant metabolite, previously isolated from soy sauce (Nakatsuka et al., 1986), sake (Japanese rice wine) (Higashi et al., 1936), Japanese rice vinegar, and other fermented soy bean products.

The structure of compound 1a was established by a detailed analysis of the HMBC and H,H COSY couplings. The 2D spectra confirmed the presence of the carboxyl group at C-3 of the β-carboline skeleton, and the 2-hydroxy methyl furan moiety must be linked to position 1 of the β-carboline system. The shifts of two quaternary carbons at δ 151.4 and 157.2 indicated their attachment to oxygen, and this confirmed the expected furan moiety. Hence, 1a was finally identified as 1-(5-hydroxymethyl-2-furyl)-β-carboline-3-carboxylic acid (flazin). It is most likely formed from tryptophane in a Pictet-Spengler reaction with furfural, which arises easily from glucose. As the sterile nutrient broth did not contain 1a, and as the concentration of 1a and all β-carbolines was increasing during fermentation, an artificial formation was unlikely.

Compound 1b was isolated as pale yellow solid from the Streptomyces sp. B6005 after PTLC followed by Sephadex LH-20. On TLC, it appeared as green UV fluorescent spot, which turned blue with Ehrlich’s reagent. The 1H NMR spectrum resembled that of 1a, however, instead of the aromatic singlet of H-4, a downfield-shifted AB system at δ 8.38 and 8.02 having the relatively small coupling constant of 5.1 Hz was observed. The EI mass spectrum showed a molecular ion peak at m/z 264, and HR EIMS revealed a molecular formula of C_{16}H_{12}N_{2}O_{2}, which corresponds to decarboxy-flazin.

The HMBC data allowed assembling of a 2,3-disubstituted indole unit and a 2,3,4-trisubstituted pyridine ring, similar to 1a. The residual proton signals at δ 7.22 and 6.54 must belong to a heterocyclic five-membered ring, according to their shifts and their small coupling constants (3.5 Hz). Based on the molecular formula and the shifts of the residual quaternary C atoms (δ 157.4, 154.7), a furan ring substituted at 2,5-positions was most likely. This confirmed the decarboxylation product of 1a, perlolyrin (1b). The latter was known as a plant metabolite from Raygras, Ginseng, and some Asiatic plants (Jeffreys, 1970, Takase et al., 1968; Yamazaki et al., 1977; Aoyagi et al., 1975; Kumagai et al., 1975), but was not known from bacteria. Perlolyrin (1b) was obtained synthetically from tryptophane and furfural under acidic conditions followed by dehydration (Bracher et al., 1993) or directly from tryptamine. A few related furyl-β-carbolines, the eudistomins, have been isolated from the tunicate Eudistoma olivaceum (Kobayashi et al., 1984).

A third β-carboline was isolated from the fast moving phase of fraction II of the marine Streptomyces sp. B1848 as a faint yellow solid with a strong blue UV fluorescence at 366 nm, similar to that of 1a and 1b. The CI and EI mass spectra determined the molecular weight as 210 Dalton, and an additional peak at m/z 168 confirmed the
heterocycle. Dereplication (Chapman & Hall, 2004) revealed the identity with 1-acetyl-β-carboline (2a), a metabolite unknown from bacteria so far. Also this compound was, however, reported previously as plant metabolite, although with wrong assignment of the NMR data (Bracher et al., 1993; Dillman et al., 1991).

Harman, the simplest 1-substituted β-carboline alkaloid, occurs in plants (Allen et al., 1980), fungi, microorganisms and marine animals (Blackman et al., 1987; Yomosa et al., 1987), inhibits monoamineoxidase, the cAMP phosphodiesterase (Hopp et al., 1976), and shows antimicrobial activity (Prinsep et al., 1991).

Compound 2b, a further β-carboline with green UV fluorescence, was obtained from the North Sea Flavobacterium strain Bio215, which was isolated from a biofilm. The same compound, together with nargenicin B1 (3), was found in Nocardia tenerifiensis (Kämpfer et al., 2004). The 1H NMR spectrum of 2b exhibited four downfield proton signals of an o-disubstituted ring; two signals at δ 8.49 and 8.38 showed small ortho-couplings, similar to 1b and 2a. Two aliphatic 2H triplets (δ 4.05, 3.55) of an ethanediyl group indicated oxygen and nitrogen or an sp2 carbon atom, respectively, as neighbours. The EI mass spectrum of 2b afforded a molecular weight of 240 Dalton, with a base peak at m/z 211, due to the loss of an aldehyde group; the signal at m/z 168 corresponded again to the β-carboline skeleton. From these data and from the molecular formula C14H12N2O2 (HR EIMS), two structures were resulting, 1-β-carbonyl-3-propionic acid (2c), and 1-(9H-β-carbolin-1-yl)-3-hydroxy-propan-1-one (2b). As methylation brought no change in the spectroscopic data and as synthetic 2c (Steglich et al., 1984) showed different data, the latter was excluded. Structure 2b was further confirmed by comparison with the closely related oxopiraline G (2d) (Abe et al., 1993). The β-carboline 2b could be formed from tryptamine and 4-hydroxy-2-keto-butyraldehyde via a Pictet-Spengler reaction. The latter aldehyde is a naturally occurring antibiotic from human tissue cultures and an intermediate in Maillard reactions (Weenen et al., 1998; Nedvidek et al., 1992).
Nargenicin B1 (3) was highly active against bacteria and micro-algae. It has been isolated previously from *Nocardia argentinensis* I (ATCC 31438) (Celmer et al., 1980). As no details are available in the literature, we report the spectroscopic data here (see Experimental). They are close to the published values of nargenicin A1.

**EXPERIMENTAL**

The NMR spectra were measured on a Bruker AMX 300 (300.135 MHz), a Varian Unity 300 (300.145 MHz) and a Varian Inova 600 (150.820 MHz) spectrometer. ESI MS was recorded on a Finnigan LCQ with quaternary pump Rheos 4000 (Flux Instruments). El mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosine as reference substance for HR EIMS. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer from KBr pellets. UV-VIS spectra were measured on a Perkin-Elmer Lambda 15 UV/VIS spectrometer. Flash chromatography was carried out on silicone gel (230-400 mesh). *R*<sub>f</sub>-values were measured on Polygram SIL G/UV<sub>254</sub> (Macherey-Nagel & Co.). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

**M<sub>2</sub> Medium:** Malt extract (10 g), yeast extract (4 g), and glucose (4 g) were dissolved in tap water (1 liter). Before sterilization, the pH was adjusted to 7.8 by addition of 2N NaOH. For M<sub>2</sub> medium, tap water/seawater 1:1 was used.

**LB Medium:** Trypton (10 g), yeast extract (4 g), glucose (4 g), NaCl (10 g) were dissolved in artificial seawater (0.5 liter) and tap water (0.5 liter). Before sterilization, the pH was adjusted to 7.8 by addition of 2N NaOH.

Description of the strains: The actinomycete strain B1848 has been derived from muddy sediment collected from the North Atlantic Ocean off the Shetland Islands at a depths of 58 m and was isolated on Marine Agar 2216 (Difco, BD Diagnostic Systems, USA) at 18 °C. The almost complete 16S rRNA gene sequence of the strain B1848 shows 100% similarity to the gene sequence of *Streptomyces champavatii* but also to *Streptomyces coelicolor*.

Strain B1848 forms a yellow-brown substrate mycelium and a yellow aerial mycelium with straight to flexuous (Rectiflexibles) spore chains on yeast extract-malt extract agar (Weyland, 1981). Melanin pigment is neither produced on peptone-yeast-iron agar nor on tyrosine agar (Shirling et al., 1966). The optimum temperature for strain growth is at about 30 °C. The strain does neither grow at 4 °C nor at 45 °C. Growth occurs in media from 0% up to 7% NaCl-salinity. Chitin, starch, caseis gelatine and esculin are degraded. The strain does not reduce nitrate.

The utilization of carbon sources was tested with SFN-Biolog plates (Hayward, CA, USA) using BMS as basal medium (Helmke et al., 1984). Good growth was obtained with: dextrin, N-acetyl-D-galactosamine, N-actyl-D-glucosamine, L-arabinose, D-arabitol, cellobiose, D-fructose, D-galactose, gentobiose, D-glucose, lactose, maltose, D-manitol, D-mannose, α-methyl-D-glucoside, D-trehalose, α-keto butyric acid, α-keto glutaric acid, D,L-lactic acid, succinic acid, D alanine, aspartic acid, L-glutamic acid. glycy1-L-glutamatic acid, L-leucine, L-ornithine, L-phenylalanine, L-proline, γ-amino butyric acid, putrescine, glycerol.

The strain B6005 originates from coastal sediment of the Golf of Papua sampled south of Port Moresby. The strain was isolated on oatmeal agar (Shirling et al., 1966; ISP 3 medium) with 50% natural seawater at 18 °C.

The almost complete 16S rRNA gene sequence of the strain B6005 shows 99.9% similarity to the gene sequence of *Streptomyces variabilis* (strain NRRL B-3984T). The substrate mycelium is yellow-brown and the aerial mycelium gray with spiral
spore chains (Spirales). Diffusible pigments are not produced. Melanin pigment is not produced. Optimum growth temperature is at about 30 °C.

The reference culture of B1848 as well as of B6005 is kept on yeast extract-malt extract agar (Weyland, 1981) in the collection of marine actinomycetes at the Alfred-Wegener-Institute for Polar and Marine Research in Bremerhaven.

Bio215. Strain Bio215 was isolated from a biofilm, which developed on a glass plate exposed to the North Sea for 2 weeks at a depth of 0.5 m. Sequencing of the 16S rRNA showed that it is a Flavobacterium spec.

Fermentation and Isolation: 12 of 1 liter Erlenmeyer flasks, each containing 250 ml M₂⁻ medium were inoculated with slant agar cultures of S. spp. B1848 and B6005, respectively, and incubated for 3 days at 28 °C. Bulk fermentation was carried out in a 25-litre jar fermentor under similar conditions. The culture broth was separated by filtration; both, filtrate and mycelium were extracted repeatedly with ethyl acetate. Silica gel column chromatography of both extracts delivered UV-fluorescent fractions, which were re-chromatographed by PTLC followed by Sephadex LH-20, giving two pale yellow compounds: S. sp. B6005 delivered 4.2 mg of the greenish-yellow fluorescent perlolyrin (1b), while isolate B1848 delivered 10 mg of the blue fluorescent 1-acetyl-β-carboline (2a).

The strain Nocardia tenerifiensis GW39/1573 was cultivated in the same way on M₂ medium. The extract of a 20 L culture delivered by preparative HPLC 3.1 mg cyclo-(isoleucylprolyl), 5.6 mg cyclo-(leucylprolyl), 3.8 mg cyclo-(phenylalanylprolyl), 0.7 mg (2b), and 151 mg nargenicin B1 (3).

Cultivation of the North Sea bacterium Bio215 using LB-medium afforded intensively yellow colored colonies on agar. Inoculation of a 25-litter jar fermentor delivered a yellow culture broth, which gave two polar yellowish-green fluorescent compounds. PTLC followed by Sephadex LH-20 (MeOH) yielded indolyl-3-acetic acid methyl ester (5 mg), cis-cyclo-(prolylvalyl) (6 mg), p-hydroxyphenylacetic acid (4 mg), adenine (30 mg), flazin (1a) (200 mg), and 1-(9H-β-carbolin-1-yl)-3-hydroxypropan-1-one (2b) (2 mg).

Flazin (1a): Obtained as a yellowish-green solid (200 mg) from fraction IV (0.81 g) by applying to Sephadex LH-20 (MeOH), PTLC (20 x 20 cm, 10% MeOH/CHCl₃) and again Sephadex LH-20 (CHCl₃/MeOH 6:4). Rᵢ = 0.23 (CHCl₃ / 10% MeOH), green fluorescence under UV, yellow with anisaldehyde/sulphuric acid after heating. UV (MeOH): λₘₐₓ = 204, 273, 286, 367 (sh), 377 nm. IR (KBr): ν = 3445, 2927, 1717, 1701, 1645, 1626, 1497, 1458, 1244, 1129, 1027, 744 cm⁻¹. ¹H NMR ([D₆] DMSO, 300 MHz): δ = 11.58 (s br, 2 H, NH, OH), 8.83 (s, 1 H, 4-H), 8.41 (d, 3J = 8.2 Hz, 1 H, 5-H), 7.82 (d, 3J = 8.1 Hz, 1 H, 8-H), 7.62 (t, 3J = 8.1 Hz, 1 H, 7-H), 7.41 (d, 3J = 3.5 Hz, 1 H, 4'-H), 7.32 (t, 3J = 8.2 Hz, 1 H, 6-H), 6.62 (d, 3J = 3.5 Hz, 1 H, 3'-H), 5.43 (s br, 1 H, OH), 4.64 (s, 2 H, 2'-CH₂). – ¹³C/APT-NMR ([D₆] DMSO, 75 MHz): δ = 166.6 (C=O, 3-COOH), 157.2 (C-2), 151.4 (C-5'), 141.5 (C-8a), 132.5 (C-1), 132.0 (C-9a), 130.0 (C₃⁻), 129.0 (CH-7), 122.0 (CH-5), 121.0 (2 C-3a/a/4b), 120.7 (CH-6), 115.7 (CH-4), 112.9 (CH-8), 111.2 (CH-4'), 109.4 (CH-3'), 56.0 (2'-CH₂). – (+)-ESI MS: m/z (%) = 354 ([M – H + 2 Na⁺], 100), 332 ([M + Na⁺], 83), 310 ([M+H⁺], 10). – (-)-ESI MS: m/z (%) = 308 ([M - H]). – DCl (NH₃): m/z (%) = 309 ([M + H⁺]). – EI MS (70 eV): m/z (%) = 308 ([M⁺], 60), 264 ([M - COO⁻], 100), 247 (28), 205 (34), 177 (12), 91 (54), 44 (42), 41 (40). – HR EIMS: m/z = 308.0803 (calcd. 308.0797 for C₁₇H₁₄N₂O₂).

Perlolyrin (1b): Yellow solid, Rᵢ = 0.72 (CHCl₃/MeOH 9:1), blue with Ehrlich’s reagent. – UV (MeOH): λₘₐₓ = 204, 251 (sh), 285, 310, 366 (sh), 377 nm. – IR (KBr): ν = 3442, 2927, 1722, 1705, 1645, 1608, 1568, 1517, 1496, 1456, 1429, 1384, 1321, 1283, 1237, 1129, 1021 cm⁻¹. – ¹H NMR
1-Acetyl-β-carboline (2a): Faint yellow solid (10 mg) from fraction II (1.50 g) by PTLC (20 x 20 cm; CHCl₃/5% MeOH), followed by Sephadex LH-20 (CHCl₃/MeOH 6:4); R₅ = 0.90 (CHCl₃/10% MeOH) with a blue UV fluorescence, yellow by anisaldehyde/sulfuric acid or Ehrlich’s reagent. – UV (MeOH): λmax = 203, 261, 284, 377 nm. – IR (KBr): ν = 3443, 2926, 1717, 1701, 1581, 1384, 1041 cm⁻¹. – H NMR ([D₆]acetone, 300 MHz): δ = 10.96 (s, br, 1H, 9-NH), 8.58 (d, 3J = 4.9 Hz, 1H, 10-H), 8.18 (d, 3J = 4.9 Hz, 1H, 4-H), 8.29 (dd, 3J = 8.1 Hz, 3', H, 5-H), 7.86 (dd, 3J = 8.1 Hz, 4J = 1.1 Hz, 1H, 8-H), 7.61 (td, 3J = 8.1 Hz, 4J = 1.2 Hz, 1H, 6-H), 7.33 (t, 3J = 8.1 Hz, 1H, 7-H), 4.05 (t, 3J = 6.2 Hz, CH₂-3'), 3.55 (t, 3J = 6.2 Hz, CH₂-2'); (CDCl₃, 600 MHz) δ 11.50 (s, br, 1H, 1-H/D exchangeable, 1H, NH), 8.52 (d, 3J = 4.9 Hz, 1H, 3-H), 8.19-8.16 (m, 2H, 4-H, 5-H), 7.62 (m, 2H, 7-H, 8-H), 7.00 (m, 1H, 6-H), 6.24 (m, 2H, 9-H, 10-H), 5.85 (dd, 3J = 9.0 Hz, 3J = 6.8 Hz, 4J = 1.7 Hz, 1H, 6-H), 5.53 (dd, 3J = 9.0 Hz, 3J = 2.9 Hz, 1H, 5-H), 5.46 (dq, 3J = 7.0, 4J = 1.0 Hz, 1H, 15 H), 5.22 (dd, 3J = 8.6 Hz, 3J = 6.4 Hz, 1H, 17-H), 5.16 (t, 3J = 4.9 Hz, 1H, 9-H), 4.30 (t, 3J = 4.4 Hz, 1H, 2-H), 4.22 (d, 3J = 4.9 Hz, 1H, 8-H), 3.97 (m, 3H, one of them H/D exchangeable, OH, 13-H, 11-H), 3.62 (dd, 3J = 9.0 Hz, 3J = 6.1 Hz, 1H, 10-CH₂OCH₃), 3.46 (m, 4H, two of them H/D exchangeable, 2OH, 10-CH₂OCH₃, 19-H). – HR EIMS: m/z (%) = 240 ([M + H]^+), 211 ([M + H]^+), 100. – (+)-ESI MS: m/z (%) = 211 ([M + H]^+), 182 ([M–CO]^–), 168 ([M–(COCH₃)]^+, 92), 140 (22). – CI MS (NH₃): m/z = 211 ([M + H]^+), 100.

1-(9H-β-Carbolin-1-yl)-3-hydroxy-propan-1-one (2b): Yellowish-green solid (2 mg) from fraction IV (0.81 g) by applying to Sephadex LH-20 (MeOH), PTLC (20 x 20 cm, 10% MeOH/CHCl₃) and finally Sephadex LH-20 (CHCl₃/MeOH 6:4); R₅ = 0.29 (CHCl₃/10% MeOH), red on spraying with anisaldehyde/sulfuric acid. – UV (MeOH): λmax = 203, 215, 261, 3443, 2926, 1717, 1701, 1581, 1384, 1041 cm⁻¹. – H NMR ([D₆]acetone, 150 MHz): δ = 10.31 (s, 1H, 9-NH), 8.58 (d, 3J = 4.9 Hz, 1H, 10-H), 8.18 (d, 3J = 4.9 Hz, 1H, 4-H), 8.29 (dd, 3J = 8.1 Hz, 3', H, 5-H), 7.86 (dd, 3J = 8.1 Hz, 4J = 1.1 Hz, 1H, 8-H), 7.61 (td, 3J = 8.1 Hz, 4J = 1.2 Hz, 1H, 6-H), 7.33 (t, 3J = 8.1 Hz, 1H, 7-H), 4.05 (t, 3J = 6.2 Hz, CH₂-3'), 3.55 (t, 3J = 6.2 Hz, CH₂-2'); (CDCl₃, 600 MHz) δ 11.50 (s, br, 1H, 1-H/D exchangeable, 1H, NH), 8.52 (d, 3J = 4.9 Hz, 1H, 3-H), 8.19-8.16 (m, 2H, 4-H, 5-H), 7.62 (m, 2H, 7-H, 8-H), 7.00 (m, 1H, 6-H), 6.24 (m, 2H, 9-H, 10-H), 5.85 (dd, 3J = 9.0 Hz, 3J = 6.8 Hz, 4J = 1.7 Hz, 1H, 6-H), 5.53 (dd, 3J = 9.0 Hz, 3J = 2.9 Hz, 1H, 5-H), 5.46 (dq, 3J = 7.0, 4J = 1.0 Hz, 1H, 15 H), 5.22 (dd, 3J = 8.6 Hz, 3J = 6.4 Hz, 1H, 17-H), 5.16 (t, 3J = 4.9 Hz, 1H, 9-H), 4.30 (t, 3J = 4.4 Hz, 1H, 2-H), 4.22 (d, 3J = 4.9 Hz, 1H, 8-H), 3.97 (m, 3H, one of them H/D exchangeable, OH, 13-H, 11-H), 3.62 (dd, 3J = 9.0 Hz, 3J = 6.1 Hz, 1H, 10-CH₂OCH₃), 3.46 (m, 4H, two of them H/D exchangeable, 2OH, 10-CH₂OCH₃, 19-H). – HR EIMS: m/z (%) = 240.0897 for C₁₆H₁₂N₂O₂.
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10-CH₂OCH₃), 3.31 (d, ²J = 9.8 Hz, ³J = 7.1 Hz, 1 H, 19-H₆), 3.16 (s, 3 H, 19-OCH₃), 3.09 (q, ³J = 6.8 Hz, 1 H, 16-H), 2.70 - 2.54 (m, 4 H, 3-H₅, 4 H, 7, 10-H), 2.50 (d, ³J = 2.0 Hz, 1 H, 12-H), 1.81 (s, 3 H, 14-CH₃), 1.55 (ddd, ²J = 16.4 Hz, ³J = 6.4 Hz, 1 H, 3-HB), 1.29 (d, ³J = 6.8 Hz, 3-H, 16-CH₃).

− ¹³C/APT NMR ([D₆]acetone, 150 MHz): δ = 176.2 (C₉-1), 160.3 (C₉-6'), 136.1 (C₈-14), 135.5 (CH-5), 131.1 (CH-15), 127.7 (CH-6), 124.4 (CH-2'), 123.2 (C₅-5), 116.2 (CH-4'), 110.6 (CH-3), 90.4 (C₂-13), 81.7 (CH-2), 75.72 (CH-17), 75.69 (CH₂₋₁₉), 73.3 (CH-11), 72.6 (10 CH₂OCH₃), 71.2 (CH-9), 70.2 (CH-8), 69.4 (CH-18), 59.0 (10-CH₂OCH₃), 58.8 (19-OCH₃), 50.0 (CH-12), 42.1 (CH-4), 40.0 (CH-10), 39.4 (CH-7), 36.1 (CH₂₋₃), 33.4 (CH-16), 17.5 (15 CH₃), 16.1 (16-CH₃).

− (+)-ESI-MS: m/z (%) 1145.3 ([2×M+Na]+, 100), 584.7 ([M+Na]+, 84); HR EIMS 561.2565 (calcd for C₂₉H₃₉NO₁₀, 561.2566).

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