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5-Hydroxytryptamine-Derived Alkaloids from Two Marine Sponges of the Genus *Hyrtios*

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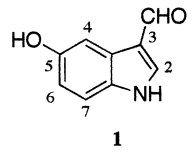
Indonesian specimens of the marine sponges *Hyrtios erectus* and *H. reticulatus* were found to contain 5-hydroxytryptamine-derived alkaloids. Their structures were determined on the basis of their spectral properties. *H. erectus* contained hyrtiosulawesine (**4**), a new β-carboline alkaloid, together with the already known alkaloids 5-hydroxyindole-3-carbaldehyde (**1**), hyrtiosin B (**2**), and 5-hydroxy-3-(2-hydroxyethyl)-indole (**3**). *H. reticulatus* contained the novel derivative 1,6-dihydroxy-1,2,3,4-tetrahydro-β-carboline (**11**) together with serotonin (**5**), 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (**7**), and 6-hydroxy-3,4-dihydro-1-oxo-β-carboline (**9**).

Marine sponges of the genus *Hyrtios* (Thorectidae, Dictyoceratida) have proven to be a rich source of secondary metabolites. Until now, three classes of secondary metabolites have been reported from this genus: terpenoids (mainly sesterterpenes[1,2] and sesquiterpene/quinones[3,4]), macrolides,[5] and tryptamine-derived alkaloids.[6,7] To evaluate the interest of these compounds as taxonomical markers for the genus *Hyrtios*, we have undertaken a chemical study of further species of this genus. Thus, recently, we reported the isolation of several sesquiterpene/quinones from *Hyrtios* sp. collected off the Seychelles Islands and from *H. tubulatus* collected off Curaçao.[4] In this paper, we report the isolation of several tryptamine-derived alkaloids from two further specimens of *Hyrtios*, *H. reticulatus* and *H. erectus* collected in South West Sulawesi (Indonesia).

The CH_2Cl_2/EtOH extract of the sponge *H. erectus* was subjected to successive flash column chromatographies to afford four Dragendorff positive compounds (**1**−**4**). 5-Hydroxyindole-3-carbaldehyde (**1**) and hyrtiosin B (**2**) are known compounds that have already been isolated from an Okinawan sample of *H. erectus*.[6] Their spectral properties (^{1}H NMR, HREIMS, UV, and IR) are identical to those reported.



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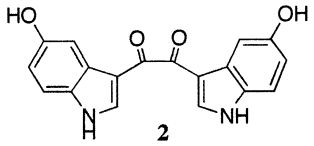

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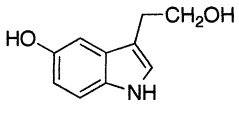

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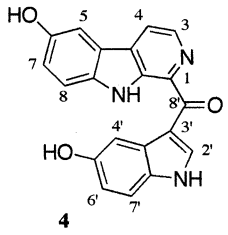
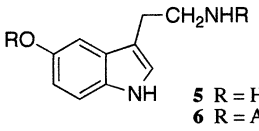


4

The spectral properties of compound **3** indicated that it is 5-hydroxy-3-(2-hydroxyethyl)indole, which is also a known natural compound. Indeed, it has been found in bovine pineal tissue, in the skin secretion of the toad *Bufo alvarius*, and in the urine of rats as one of the major metabolites of 5-hydroxytryptamine.[8] This is the first report of compound **3** from a marine invertebrate. Its HREIMS [*m/z* 177.0792 (37, M^+), calcd for $C_{10}H_{11}NO_2$ 177.0790] and ^{1}H NMR spectra are compatible with those reported by Battersby et al.[9] and Cheng and Dryhurst.[10] The identification was further confirmed by the previously unreported UV and ^{13}C NMR spectra. The ^{13}C NMR signal assignments were ascertained by 2D NMR experiments (^{1}H−^{1}H COSY, HMQC, and HMBC).

The fourth Dragendorff positive compound of the extract is a novel compound that has been named hyrtiosulawesine (**4**). Its HREIMS gave a molecular ion at *m/z* 343.0957, while FABMS showed a $(M + H)^+$ ion at *m/z* 344. This indicated the formula $C_{20}H_{13}N_3O_3$ (calcd 343.0957), which implies 16 unsaturations. Comparison of the ^{1}H and ^{13}C NMR spectra of hyrtiosulawesine with those of hyrtiomanzamine[7] and manzamine X[11] indicated that these compounds possess the same 6-hydroxy-β-carboline moiety to



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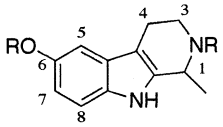
6 R = Ac



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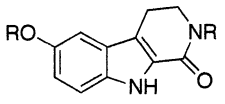
8 R = Ac



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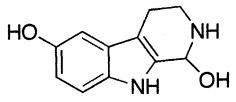
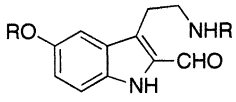
10 R = Ac



11



12 R = Ac
13 R = H

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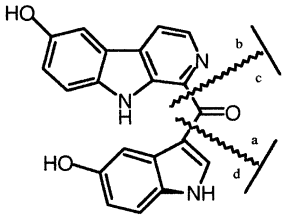
Table 1. HREIMS of Hyrtiosulawesine (**4**)



m/z measd	rel. int	*m/z* calcd	formula	ion structure
344.1016	70	344.1035	$C_{20}H_{14}N_3O_3$	$[M + H]^+$
343.0957	63	343.0957	$C_{20}H_{13}N_3O_3$	$M^{+\bullet}$
327.0990	100	327.1008	$C_{20}H_{13}N_3O_2$	$[M + H]^+ - OH^\bullet$
326.0936	76	326.0930	$C_{20}H_{12}N_3O_2$	$M^{+\bullet} - OH^\bullet$
315.0993	20	315.1008	$C_{19}H_{13}N_3O_2$	$[M + H]^+ - COH^\bullet$
314.0933	14	314.0929	$C_{19}H_{12}N_3O_2$	$M^{+\bullet} - COH^\bullet$
210.0426	7	210.0430	$C_{12}H_6N_2O_2$	fragment a − H
182.0479	44	182.0480	$C_{11}H_6N_2O$	fragment b − H
160.0399	30	160.0398	$C_9H_6NO_2$	fragment c
132.0447	12	132.0449	C_8H_6NO	fragment d

which a carbonyl group is attached at C-1. Moreover, comparison with the spectra of hyrtiosin B (**2**) suggested the presence in both compounds of a 5-hydroxyindole moiety substituted at C-3.[6] The ^1H and ^{13}C NMR signal assignments of hyrtiosulawesine (**4**) were ascertained by 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC). All these data together with the fragmentation in HREIMS (Table 1) and the UV [CH_3OH, λ_{max} 217 (145 000, sh. at 260), 305 (53 000, sh. at 325), 403 nm (22 700)] and IR spectra (3302 and 1640 cm^{-1}) led us to propose structure **4** for hyrtiosulawesine.

As for *H. erectus*, four Dragendorff positive compounds (**5**, **7**, **9**, **11**) were isolated from the MeOH extract of *H. reticulatus* after several flash column chromatographies. The first compound (**5**) was identified as the well-known neurotransmitter serotonin on the basis of its spectral properties and those of its diacetyl derivative **6** obtained by treatment of **5** with the mixture pyridine/acetic anhydride, 1:1. The spectral properties were compatible with those reported for N_b-acetylserotonin[12] and with those reported in part for serotonin by Battersby et al.[9] The assignments of the ^1H and ^{13}C NMR signals were confirmed by 2D NMR measurements ($^1H-^1H$ COSY and HMQC).

The spectral data of compound **7** were in complete accord with those expected for 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline. Moreover, the ^{13}C NMR spectrum of **7** was compatible with that reported by Zhang et al.[13] for a synthetic sample of the latter, and the ^1H NMR spectrum of the *N*,*O*-diacetyl derivative of **7** was identical to that of diacetylshepherdine (**8**). Shepherdine has been isolated by Ayer and Browne[14] from the roots of the Canadian tree *Shepherdia canadensis*. This alkaloid occurs also in brain, body fluids, and other tissues of mammals.[13]

The two last alkaloids of *H. reticulatus* were isolated after acetylation of the fraction containing derivatives **9** and **11**, to give derivatives **10** and **12**, respectively. TLC comparison of the fraction before and after acetylation indicated that the acetylated compounds were not present in the natural extract. Analysis of the spectral data of compound **10** (M^+ *m/z* 286.0956, calcd for $C_{15}H_{14}N_2O_4$ 286.0954) indicated that it was the *N*,*O*-diacetyl derivative of 6-hydroxy-3,4-dihydro-1-oxo-β-carboline. The NMR assignments were confirmed by 2D NMR measurements ($^1H-^1H$ COSY, HMQC, and HMBC). This is the first report of 6-hydroxy-3,4-dihydro-1-oxo-β-carboline (**9**) as a natural compound. It has already been reported as a synthetic derivative by Julia and Lallemand.[15]

The spectroscopic properties of compound **12** (M^+ *m/z* 288.1118, calcd for $C_{15}H_{16}N_2O_4$ 288.1110) indicated that it is 3-(2-acetylaminoethyl)-2-formyl-1*H*-indol-5-yl acetate. The NMR assignments were again confirmed by 2D NMR measurements ($^1H-^1H$ COSY, HMQC, and HMBC). The ^1H and ^{13}C NMR resonances of the formyl group at C-2 (δ_H 9.94 and δ_C 181.0) closely matched those of 3-methylindole-2-carbaldehyde (δ_H 10.18[16] and δ_C 180.5[17]), and comparison of the resonances of carbon atoms C-4 to C-7 (δ_C 113.7, 145.3, 122.8, and 113.5, respectively) with those of compound **10** clearly pointed to the presence in **12** of an indole ring hydroxylated at C-5. It follows that the compound present in the sponge extract before acetylation is most probably the dihydroxylated derivative **11**, as it is well known that amino aldehydes such as **13** quickly cyclize into the more stable cyclic isomer 1,6-dihydroxy-1,2,3,4-tetrahydro-β-carboline (**11**), which is a new compound.

In the present study, we have shown that specimens of the marine sponges *H. reticulatus* and *H. erectus* collected in South Sulawezi contain 5-hydroxytryptamine-derived alkaloids. Until now, about 23 sponge samples identified as *Hyrtios* and representing four different species [*H. erectus* (15 samples), *H. eubamma* (1 sample), *H. altum* (1 sample), and *H. tubulatus* (1 sample)] have been investigated for their secondary metabolite contents. In addition, five undetermined *Hyrtios* have also been studied. About 80% of these samples were found to contain terpenoid derivatives [sesquiterpene/quinones (5 samples), sesterterpenes (13 samples), or triterpenes (1 sample)]. Besides the two *Hyrtios* collections studied in this paper, only two specimens of *H. erectus* were found to contain 5-hydroxytryptamine-derived alkaloids.[6,7] The alkaloidal content of the Indonesian *H. erectus* we have studied is similar to that of the Okinawan samples studied by Kobayashi et al.,[6] as both samples contained hyrtiosin B and 5-hydroxyindole-3-carbaldehyde. Moreover, Kirsch et al.[18] have recently reported the isolation, in addition to bioactive sesterterpenes, of two β-carboline alkaloids from *H.* cf. *erectus*. Interestingly, several tryptamine derivatives were isolated from the saltwater culture of *Aspergillus niger* derived from *H. proteus*,[19,20] and trisindoline, an indole trimer, was found to be produced by a bacterium of the genus *Vibrio* separated from *H. altum*.[21] This suggests that the 5-hydroxytryptamine-derived alkaloids found in *Hyrtios* spp. could be of symbiotic origin.

Experimental Section

General Experimental Procedures. The IR spectra were obtained on a Bruker IFS 25 instrument as a film on a NaCl disk or as a KBr pellet. The UV spectra were recorded in MeOH on a Philips PU 8700 spectrophotometer using 1 cm quartz cells. Thin-layer chromatography analyses (TLC) were performed on 0.25 mm Polygram silica gel SILG/UV_{254} precoated plates (Macherey Nagel) and column chromatographies on Si gel (MN Kieselgel 0.04−0.063 mm), using the flash technique. HREIMS measurements were performed on a Micromass Autospec 3F instrument. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD at 600 and 150.87 MHz, respectively, using a Varian Unity 600 instrument, or at 300 and 75.4 MHz on a Bruker DA 300 instrument. Some ^1H NMR spectra were recorded at 250 MHz on a Bruker WM 250 spectrometer using TMS as internal standard.

Biological Material. *H. erectus* (reference no. 98/SS/APR29/BH/030) was collected by scuba diving at a depth of 11 m at the northwest side of Lankai Island, off Makassar, South West Sulawesi, Indonesia, on April 29, 1998, by Dr. B. W. Hoeksema. The specimen was a branching erect sponge

with blackish to dark-olive color and conulose surface. The branches were 10 cm or more in length and had a diameter of 2−3 cm. A few oscules of 2−3 mm in diameter were present along the branches, and one was located at the top of each branch. Conules (3 mm apart) of 1−2 mm high were also present. The skeleton consists of a reticulation of sand-filled fibers of 50−120 μm in diameter, making meshes of 250−600 μm in diameter, without a clear distinction of primary and secondary fibers. Amber-colored and laminated pieces of spongin are seen. The fiber reticulation is alternatively tighter meshed in the areas immediately underneath the conules and meshed more widely between conules. In places a finer fiber network of uncored fibers is present. When put into methanol, the sponge gives off a dark-colored exudate. The specimen conforms closely to Western Indian Ocean specimens and the original description of *H. erectus*. A voucher fragment is kept in the collections of the Zoological Museum Amsterdam under registration number ZMA POR 14477.

H. reticulatus (reference no. 98/SS/APR22/BH/007) was collected by scuba diving at a depth of 13 m at the west side of Bone Lola Reef, off Makassar, South West Sulawesi, Indonesia, on April 22, 1998, by Dr. B. W. Hoeksema. The specimen consisted of long orange-colored branches, up to 33 cm long and 2−3 cm in diameter, with side branches only very near the point of attachment. Several oscules up to 2 mm in diameter were present along the branches. Conules (3−4 mm apart) of 1−2 mm high were also present. The orange color was more enhanced at the apexes of the conules. The skeleton consists of an irregular reticulation of sand-filled fibers, 30−200 μm in diameter, with laminated ambre-colored spongin mostly visible. In areas underlying the conules the reticulation is rather tight, forming polygonal meshes of 300−450 μm in diameter; elsewhere it is lax and shows low spongin development. The specimen conforms to the original description from Ternate of *H. reticulatus*. A voucher fragment is kept in the Zoological Museum Amsterdam under registration number ZMA POR 14454.

Extraction and Isolation. Specimens of *H. erectus* (40.3 g dry weight) were repeatedly extracted with MeOH/CH_2Cl_2, 50:50, and the combined extracts were concentrated. The water content of the extract was adjusted to 200 mL before sequentially partitioning against CH_2Cl_2 and CH_2Cl_2/EtOH, 3:2. The organic layers were evaporated to dryness in vacuo to obtain the extracts EA (0.93 g) and EB (0.86 g), respectively. EA was mainly made of a mixture of 3β-hydroxysterols and of 5,8-epidioxy-3β-hydroxysterols (TLC and ^{1}H NMR), which were not further studied. EB was first flash chromatographed on a Si gel column using as eluent the mixture CH_2Cl_2/MeOH, 100:0 to 0:100, then on a Si gel column using as eluent toluene and increasing amounts of EtOAc. The chromatographies were monitored by TLC and the compounds visualized by UV and by spraying with Dragendorff reagent. This led to the isolation of 5-hydroxyindole-3-carbaldehyde[6] (**1**, 2.2 mg), hyrtiosin B[6] (**2**, 1.7 mg), 5-hydroxy-3-(2-hydroxyethyl)indole[9,10] (**3**, 1.6 mg), and hyrtiosulawesine (**4**, 4.1 mg), a new 6-hydroxy-β-carboline alkaloid.

Specimens of *H. reticulatus* (95.9 g dry weight) were repeatedly extracted with MeOH/CH_2Cl_2, 50:50, and the combined extracts were concentrated. The water content of the extract was adjusted to 300 mL before sequentially partitioning against CH_2Cl_2 (extract EA, 3.8 g) and CH_2Cl_2/EtOH, 3:2 (extract EB, 0.6 g). The residual aqueous layer was evaporated to dryness. The solid residue was treated with EtOH (2 × 100 mL), and after elimination of the insoluble material (extract EC, 15 g) by filtration through a ground-glass filter, the alcoholic solution was evaporated to dryness to yield extract ED (3.42 g). Part of ED (2 g) was chromatographed twice on a Si gel column using the mixture CH_2Cl_2/MeOH, 100:0 to 0:100, as eluent. This led to the isolation of 5-hydroxytryptamine[9] (≡serotonin, **5**, 33 mg) and 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline[13] (**7**, 8 mg), which were further characterized as their acetylated derivatives **6**[12] and **8**,[14] respectively (Ac_2O/pyridine, 1:1, for 20 h at room temperature). Fractions containing a mixture of 6-hydroxy-3,4-dihydro-1-oxo-β-carboline (**9**) and 1,6-dihydroxy-1,2,3,4-tetrahydro-β-carboline (**11**) were combined and acetylated with 2 mL of the mixture Ac_2O/pyridine, 1:1, for 20 h at room temperature. The reaction mixture was concentrated and further purified by flash chromatography on a Si gel column (eluent: stepwise gradient of MeOH in CH_2Cl_2) to yield the corresponding diacetyl derivatives **10** (4 mg) and **12** (7.5 mg), respectively.

5-Hydroxy-3-(2-hydroxyethyl)indole (3): UV (CH_3OH) λ_{max} 210 (14 400), 276 (4940), 300 nm (sh, 3450); ^{1}H NMR (250 MHz, CD_3OD) δ 2.89 (2H, t, J = 7, H-8), 3.77 (2H, t, J = 7, H-9), 6.64 (1H, dd, J = 8, 2, H-6), 6.91 (1H, d, J = 2, H-4), 7.00 (1H, s, H-2), 7.15 (1H, d, J = 8, H-7); ^{13}C NMR (150 MHz, CD_3OD) δ 151.3 (C-5), 133.4 (C-7a), 129.9 (C-3a), 124.7 (C-2), 112.9 (C-7), 112.5 (C-6), 112.2 (C-3), 103.8 (C-4), 63.9 (C-9), 30.2 (C-8); HREIMS m/z 177.0792 (37, M^+, calcd for $C_{10}H_{11}NO_2$ 177.0790), 146 (100), 117 (12), 91 (11).

Hyrtiosulawesine (4): UV (CH_3OH) λ_{max} 217 (145 000, sh. at 260), 305 (53 000, sh. at 325), 403 nm (22 700); IR 3302 and 1640 cm^{-1}; ^{1}H and ^{13}C NMR (600 MHz, CD_3OD) δ_H and δ_C 140.6 (C-1), 8.41 (d, J = 4.8) and 137.6 (HC-3), 8.14 (d, J = 4.8) and 118.8 (HC-4), 132.8 (C-4a), 122.9 (C-4b), 7.56 (d, J = 2.4) and 107.1 (HC-5), 152.9 (C-6), 7.13 (dd, J = 2.4, 8.5) and 120.1 (HC-7), 7.53 (d, J = 8.5) and 114.2 (HC-8), 137.8 (C-8a), 137.7 (C-9a), 8.88 (s) and 139.4 (HC-2′), 116.4 (C-3′), 130.2 (C-3′a), 8.01 (d, J = 2.4) and 108.3 (HC-4′), 154.7 (C-5′), 6.81 (dd, J = 2.4, 8.5) and 114.1 (HC-6′), 7.31 (d, J = 8.5) and 113.6 (HC-7′), 132.6 (C-7′a), 190.2 (C-8′); HREIMS m/z 343.0957 (M^+, calcd for $C_{20}H_{13}N_3O_3$ 343.0957); FABMS m/z 344.

5-Hydroxytryptamine (5): ^{1}H and ^{13}C NMR (300 MHz, CD_3OD) δ_H and δ_C 7.14 (s) and 125.6 (HC-2), 109.9 (C-3), 129.3 (C-3a), 6.98 (d, J = 2) and 103.7 (HC-4), 152.0 (C-5), 6.71 (dd, J = 2, 8) and 113.3 (HC-6), 7.22 (d, J = 8) and 113.7 (HC-7), 133.6 (C-7a), 3.08 (t, J = 8) and 25.0 (H_2C-8), 3.22 (t, J = 8) and 41.6 (H_2C-9); EIMS peaks at m/z 176 (18, M^+), 146 (100), and 133 (12).

***N*$_b$,*O*-Diacetylserotonin (6):** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (s, HC-2), 7.05 (d, J = 2, HC-4), 6.92 (dd, J = 2, 8, HC-6), 7.34 (d, J = 8, HC-7), 2.93 (t, J = 6.5, H_2C-8), 3.56 (dt, J = 6.5, 6.5, H_2C-9), 1.94 (s, $NCOCH_3$), 2.34 (s, $OCOCH_3$), 8.28 (br s, HN-1), 5.58 (br s, HN-10); EIMS m/z 260 (9, M^+), 201 (55), 188 (23), 159 (100), and 146 (95).

Shepherdine (7): ^{1}H and ^{13}C NMR (300 MHz, CD_3OD) δ_H and δ_C 4.79 (q, J = 8) and 49.8 (HC-1), 3.45/3.70 (m) and 41.7 (H_2C-3), 3.02 (m) and 18.6 (H_2C-4), 105.0 (C-4a), 130.7 (C-4b), 6.84 (d, J = 2) and 102.4 (HC-5), 150.9 (C-6), 6.71 (dd, J = 2, 9) and 111.8 (HC-7), 7.18 (d, J = 9) and 112.3 (HC-8), 132.0 (C-8a), 127.0 (C-9a), 1.72 (d, J = 8) and 16.8 (H_3C-10); EIMS m/z 202 (17, M^+), 187 (30), 173 (18), 146 (16), 79 (37), 60 (100).

***N*$_b$-Acetyl-6-acetoxy-3,4-dihydro-1-oxo-β-carboline (10):** UV (CH_3OH) λ_{max} (27 200) and 311 nm (22 200); IR (film) 3302, 1760, 1697, 1665 cm^{-1}; ^{1}H and ^{13}C NMR (300 MHz, $CDCl_3$) δ_H and δ_C 162.0 (C-1), 4.34 (t, J = 7) and 44.5 (H_2C-3), 3.04 (t, J = 7) and 21.8 (H_2C-4), 124.5, 125.8, 128.4, 136.8 (C-4a, C-4b, C-8a, C-9a), 7.37 (d, J = 2) and 113.8 (HC-5), 145.7 (C-6), 7.12 (dd, J = 2, 9) and 122.0 (HC-7), 7.44 (d, J = 9) and 113.7 (HC-8), 2.36 (s) and 21.5 ($OCOCH_3$), 2.67 (s) and 27.9 ($NCOCH_3$), 170.8 ($OCOCH_3$), 173.8 ($NCOCH_3$), 9.01 (bs, HN-9); HREIMS m/z 286.0956 (36, M^+, calcd for $C_{15}H_{14}N_2O_4$ 286.0954), 244 (100), 185 (24), 173 (45), 145 (34).

3-(2-Acetylaminoethyl)-2-formyl-1*H*-indol-5-yl acetate (12): UV (CH_3OH) λ_{max} 313 nm (13 950); IR (film) 3320, 1755, 1662, 1604 cm^{-1}; ^{1}H and ^{13}C NMR (300 MHz, $CDCl_3$) δ_H and δ_C 135.7, 134.2, 128.1, 125.8 (C-2 + C-3 + C-3a + C-7a), 7.45 (d, J = 2) and 113.7 (HC-4), 145.3 (C-5), 7.12 (dd, J = 2, 9) and 122.8 (HC-6), 7.40 (d, J = 9) and 113.5 (HC-7), 9.94 (s) and 181.0 (HOC-8), 3.29 (t, J = 6) and 24.1 (H_2C-9), 3.57 (dt, J = 6, 6) and 41.3 (H_2C-10), 2.35 (s) and 21.5 ($OCOCH_3$), 1.95 (s) and 23.6 ($NCOCH_3$), 170.9 ($OCOCH_3$), 170.7 ($NCOCH_3$), 9.23 (bs, HN-1), 5.58 (bt, OCNH); HREIMS m/z 288.1118 (21, M^+, calcd for $C_{15}H_{16}N_2O_4$ 288.1110), 246 (7), 229 (62), 217 (8), 187 (100), 175 (80), 174 (49), 159 (72), 146 (18).

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