# *N*-(Indol-3-ylethyl)-2′-hydroxy-3′-methylpentanamide, a Novel Indole Derivative from *Xenorhabdus nematophilus*

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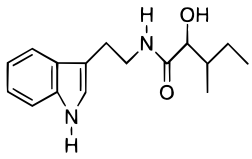
A new metabolite has been isolated for the first time from the culture broth of a symbiotic bacterium, *Xenorhabdus nematophilus*, and identified as *N*-(indol-3-ylethyl)-2-hydroxy-3-methylpentanamide (**1**) by spectroscopic methods.

Bacteria of the genus *Xenorhabdus* are known to exhibit antibacterial and antifungal activity[1−3] and to be symbiotically associated with insect pathogenic nematodes of the genus *Steinernema*.[4−6] Most species of *Xenorhabdus* exist in two phases, designated primary and secondary, when cultured *in vitro*. *Xenorhabdus* spp. primary phase, but not the secondary phase, produce metabolites that exhibit a wide spectrum of antibiotic activity.[1−3,6] These metabolites, which are active against plant, animal, and human pathogenic bacteria and fungi,[7,8] include indole derivatives, dithiolopyrrolones (xenorhabdins), and benzopyran-1-one derivatives (xenocoumacins).[7,8] Our continued interest in the metabolites of *Xenorhabdus* has led us to the discovery of a novel indole derivative from *X. nematophilus* strain BC1 (Enterobacteriaceae), and we report here its isolation and structural elucidation.

The culture broth was obtained by culturing *X. nematophilus* strain BC1 primary phase.[2] This broth was then subjected to solvent extraction with $CH_2Cl_2$ after removal of the cells by centrifugation. The $CH_2Cl_2$ extracts were concentrated and separated by Si gel chromatography with ethyl acetate:hexane (1:2) as eluent to give the novel compound **1**.




**(1)**

The presence of six low-field resonances observed between $\delta$ 7.05 and 8.04 in the $^1$H NMR spectrum and eight resonances observed between $\delta$ 136.5 and 111.3 in the $^{13}$C NMR spectrum suggest the presence of a 3-indolyl substituent in **1**. Resonances at $\delta$ 0.87 (3 H, t, $J$ = 7.4 Hz), 0.95 (3 H, d, $J$ = 6.7 Hz), 1.82 (1 H, m), 1.32 (1 H, m), and 1.14 (1 H, m) indicated the presence of a $CH_3CH(-R)CH_2CH_3$ unit, which is consistent with the $^1$H−$^{13}$C 2D NMR spectrum. The (low- and high-resolution) MS of **1** showed a molecular ion at $m/z$ 274 ($C_{16}H_{22}N_2O_2$) and the major principal daughter ion at $m/z$ 143 ($C_{10}H_9N$), which further indicated the presence of an indole ring and suggested a $-CH_2CH_2-$ unit directly attached to the ring. The signals at $\delta$ 3.66 (2 H, q, $J$ = 6.8 Hz) and 3.00 (2 H, td, $J$ = 6.8, 0.9 Hz) in the $^1$H NMR spectrum supported the assignment of a $-CH_2CH_2XH-$ unit. The peak at $\delta$ 173.0 in its $^{13}$C NMR spectrum and the absorbance at 1654 cm$^{-1}$ in the IR spectrum suggested the presence of a carbonyl group. The signals at $\delta$ 8.03 and 2.30 disappeared after the addition of $D_2O$, while the signal at $\delta$ 6.45 disappeared only after storage at room temperature for 2 days following the addition of $D_2O$. This suggested the presence of −OH and/or −NH groups (rapidly exchangeable protons) and a CONH group (slowly exchangeable proton), consistent with the strong absorbance at 3402 cm$^{-1}$ in its IR spectrum. On the basis of the above analysis, the structure *N*-(indol-3-ylethyl)-2-hydroxy-3-methylpentanamide was assigned for the component **1**. This assignment is fully supported by the $^1$H−$^{13}$C 2D NMR spectrum that also allowed the assignment of the $^1$H NMR and $^{13}$C NMR data. Further, the signals at $\delta$ 1.82 (H-3′), 1.32 (H-4′), and 1.14 (H-4′) suggested that **1** is either the 3′*S*,2′*R* isomer or the 3′*R*,2′*S* isomer.[9] As **1** appears to be derived from L-isoleucine, it is most likely to be *N*-(indol-3-ylethyl)-2′(*R*)-hydroxy-3′(*S*)-methylpentanamide . This is the first report of compound **1** from a natural source. Unlike the indole derivatives isolated previously from *Xenorhabdus*,[7] **1** did not inhibit growth of the bacterium, *Bacillus subtilis*, when tested at a concentration of 100 $\mu$g/mL.

In conclusion, a novel indole derivative (**1**) has been isolated and identified from *X. nematophilus* BC1. This is the first report of an indole derivative from this species of *Xenorhabdus*. This discovery further demonstrates the diversity of metabolites produced by the symbiotic bacteria, *Xenorhabdus* spp., and the potential of these bacteria as sources for lead chemicals for pharmaceuticals and agrochemicals.

## Experimental Section

**General Experimental Procedures.** The NMR spectra, low-resolution MS, high-resolution MS, IR spectra, and HPLC analysis were performed on the same instruments as those described in an earlier paper on *Xenorhabdus* metabolites.[7]

**Culture Conditions.** *X. nematophilus* strain BC1 and its nematode symbiont *Steinernema feltiae* used in this study were collected from soil in British Columbia, Canada, and identified by Chen[2] and the bacterium deposited in the American Type Culture Collection (ATCC700168). The procedures and conditions for the

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isolation, maintenance, and culture of the bacterium are the same as those described in an earlier paper.[7]

**Extraction and Isolation.** The cell-free material (3 L) was extracted with $CH_2Cl_2$ four times, and the combined extracts were dried with anhydrous $Na_2SO_4$ and filtered through glass wool. The filtrate was concentrated on a rotary evaporator below 30 °C under vacuum to yield a brown oil. The crude extracts were processed through a Si gel (200g Si gel 60, 40 × 5 cm, EM Science, Darmstadt, Germany) chromatographic column with 33% ethyl acetate in hexane as the eluent to give the major UV-detectable, solid metabolite **1** (7.4 mg): $[\alpha]^{25}_D$ −0.2° (*c* 0.32, $CHCl_3$); EIMS (70 eV) *m*/*z* $[M]^+$ 274 (5), 144 (17), 143 (100), 131 (6), 130 (47), 115 (4), 103 (3), 81 (2), 77 (6), 69 (6); HRMS 274.1676 (calcd for $C_{16}H_{22}N_2O_2$ 274.1681), 143.0740 (calcd for $C_{10}H_9N$ 143.0735, 100); IR (KBr) $\nu_{max}$ 3402 (s), 2964, 1655, 1534, 1460, 744 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 8.03 (1 H, br s, NH), 7.62 (1 H, dd, $J$ = 7.8, 1.0 Hz, H-4), 7.37 (1 H, dd, $J$ = 8.4, 0.8 Hz, H-7), 7.22 (1 H, td, $J$ = 8.1, 1.1 Hz, H-5), 7.13 (1 H, td, $J$ = 7.9, 1.0 Hz, H-6) 7.05 (1 H, d, $J$ = 2.3 Hz, H-2), 6.45 (1 H, m, CONH), 3.94 (1 H, t, $J$ = 4.2 Hz, H-2′), 3.66 (2 H, q, $J$ = 6.8 Hz, H-11), 3.00 (2 H, td, $J$ = 6.8, 0.9 Hz, H-10), 2.30 (1H, d, $J$ = 5.2 Hz, OH), 1.82 (1 H, m, H-3′), 1.32 (1 H, m, H-4′), 1.14 (1 H, m, H-4′), 0.95 (3 H, d, $J$ = 6.7 Hz, Me-3′), 0.87 (3 H, t, $J$ = 7.4 Hz, H-5′); $^{13}C$ NMR ($CDCl_3$) $\delta$ 173.0 (s, CO), 136.5 (s, C-9), 127.4 (s, C-8), 122.3 (d, C-5), 122.0 (d, C-2), 119.6 (d, C-6), 118.7 (d, C-4), 113.1 (s, C-3), 111.3 (d, C-7), 76.4 (d, C-2′), 39.4 (t, C-11), 38.9 (d, C-3′), 25.5 (t, C-10), 23.2 (t, C-4′), 15.5 (q, Me-3′), 11.8 (q, C-5′).

## References and Notes

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