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# NEMATICIDAL HYDROCARBONS FROM *MUCUNA ATERRIMA*

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**Key Word Index**—*Mucuna aterrima*; Leguminosae; nematode; alcohol; ester.

**Abstract**—Two bioactive natural products were isolated from *Mucuna aterrima* leaves and stems. The bioassay was made *in vitro* with *Meloidogyne incognita* strain 3 and *in vivo* in the greenhouse using tomato plants. The products were an aliphatic alcohol (1-triacontanol) and an ester (triacontyl tetracosanate).

## INTRODUCTION

The velvetbean *Mucuna aterrima* is a legume commonly used in Brazil as cover crop, as green manure and as a forage. It can suppress nematode populations, especially those of the root-knot nematode *Meloidogyne incognita* [1]. In a preliminary study, crude extracts obtained from aerial (leaves and stems) and roots parts, using hexane, chloroform, ethyl acetate–acetone and ethanol–water, were tested for activity [2]. In this paper, the isolation and structural elucidation of two substances from crude hexane and ethyl acetate–acetone extracts of aerial parts of *M. aterrima* are reported. One substance, the ester triacontyl tetracosanate, is described for the first time as a natural substance. The alcohol 1-triacontanol was known previously, from plants in other families [3].

## RESULTS AND DISCUSSION

The crude hexane and ethyl acetate–acetone extracts of aerial parts of *M. aterrima* showing biological activity (Table 1) yielded **1** and **2** respectively. The $^{13}C$ NMR, $^{1}H$ NMR and IR data show that **1** is the aliphatic ester triacontyl tetracosanate and **2** is the aliphatic alcohol 1-triacontanol. The nematicidal activity of **1** and **2** was tested with *M. incognita* strain 3. The results *in vitro* valuation showed that with **1** the eclosion of juveniles remained low with some presenting reduced movements and others being paralysed. For **2** at 48 hours the eclosion of juveniles remained low, with some presenting reduced movements and others being paralysed. At 96 and 144 hours no eclosion of juveniles occurred (Table 2). The results of *in vivo* valuation was conducted to verify the nematostatic and/or nematicidal activity, using the tomato plant *Lycopersion esculentum* Mill. The results were subjected to the Taylor and Sasser scale [4]. These substances do not inhibit the eclosion of juveniles of *M. incognita* in relation to the control (Table 2). Nevertheless they can be considered to have a nematicidal effect causing paralysis and death of juveniles (Table 3).

$$CH_3-(CH_2)_{21}-CH_2-COO-CH_2-(CH_2)_{28}-CH_3$$

**1**

$$CH_3-(CH_2)_{28}-CH_2-OH$$

**2**

Table 1. Numbers of juveniles of *Meloidogyne incognita* strain 3 ecloded in crude hexane and ethyl acetate/acetate extracts of aerial parts of *Mucuna aterrima*

| | Hours | | |
|---|---|---|---|
| Crude extracts | 44 | 96 | 144 |
| Hexane | 478 | 497 | 691 |
| EtOAc–actone (4:1) | 0 | 230 | 341 |
| Control | 848 | 530 | 1165 |

Table 2. Numbers of juveniles of *Meloidogyne incognita* strain 3 ecloded in active substances. Average of five treatments

| | Hours | | |
|---|---|---|---|
| Substances | 48 | 96 | 144 |
| **1** | 1244 | 875 | 177 |
| **2** | 1209 | 0 | 0 |
| Control | 2227 | 1680 | 1644 |

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Table 3. Numbers of galls observed on roots of tomato plants after exposure to active substances. Average to five treatments

| Substances | No. of galls observed on roots | Taylor and Sasser scale |
|---|---|---|
| **1** | 5.0 | 2* |
| **2** | 10.0 | 2* |
| Control | 166.0 | 5† |

*3–10 galls on roots of tomato plants.
†More than 100 galls on roots of tomato plants.

## EXPERIMENTAL

The air-dried leaves and stems (1 kg) of *M. aterrima* collected in April 1992 from the experimental garden of the Phytopathology Department of the Federal University of Viçosa, were successively extracted with hexane, chloroform, ethyl acetate–acetone (4:1) and methanol–water (4:1). The nematicidal crude extracts hexane (3 g) and ethyl acetate–acetone (4:1) (4 g) were submitted to silica-gel (70–230 mesh) column chromatography. The nematicidal constituent of crude extracts hexane **1** 30 mg, was eluted with hexane and was recrystallized from acetone. The nematicidal constituent of crude extract ethyl acetate–acetone **2** 10 mg was eluted with chloroform.

To evaluate substances **1** and **2**, egg masses obtained from pure cultures of the root-knot nematode *M. incognita* strain 3 kept at the Phytopathology Department was used. The substances were suspended in distilled water at a concentration of 1% together with 2 drops of Tween 20. Portions of each substance were added (3 ml) in eclosion chambers which were made of Petri plates with 1 mm i.d. nylon screens and covered with facial tissue upon which were deposited 10 egg masses. Five repetitions per treatment were performed. The eclosion chambers were kept at 25° in the dark. The juvenile counting was done at 48, 96 and 144 hours after exposure to the substances, using a Peter counting chamber. The *in vivo* test was conducted in greenhouse using tomato plants. The solutions from the last evaluation with the juveniles inside were added to the pot with tomato seedlings and 30 days later roots galls were counted in each pot. Five repetitions per treatment were performed. The values obtained were submitted to the Taylor and Sasser scale [4], that consist in five values of 0, 1, 2, 3, 4 and 5 to the plants that show respectively 0; 1–2; 3–10; 11–30; 31–100 and more than 100 galls for each radicular system.

The NMR spectra of **1** and **2** were recorded at 400 MHz for $^1H$ and at 100 MHz for $^{13}C$ with a JEOL EX 400 spectrometer.

The physicochemical data of **1** are shown below: Amorphous powder, m.p. 71–74°. IR $\nu_{max}$ (KBr) $cm^{-1}$ 1736 (C=O), 1463 ($CH_2$), 1377 ($CH_3$), 1073 (C–O), 720–730 ($[CH_2]_n$). $^1H$ NMR ($CDCl_3$): $\delta$ 0.87 (6H, triplet, $J = 6.78$ Hz $CH_3$ terminal), 1.25 (80H, singlet, $CH_2$), 1.48 (1H, singlet), 1.62 (2H, multiplet), 2.30 (2H, triplet, $J = 7.52$ Hz Me–$CH_2$–COO), 4.20 (2H, triplet, $J = 6.78$ Hz O–$CH_2$–ME); $^{13}C$ NMR ($CDCl_3$): $\delta$ 33.8 (C-1), 25.3 (C-2), 30.3 (C-3), 29.5 (C-4), 29.8 (C-5), 29.9 (C6-19), 29.8 (C-20), 29.3 (C-21), 32.0 (C-22), 22.8 (C-23), 13.7 (C-24), 64.8 (C-1), 28.9 (C-2), 29.3 (C-3), 29.8 (C-4), 30.1 (C-5), 30.2 (C6-25), 29.8 (C-26), 29.5 (C-27), 32.0 (C-28), 22.8 (C-29), 13.7 (C-30).

The physicochemical data of **2** are shown below: Amorphous powder, m.p. 76–78°. IR $\nu_{max}$ (KBr) $cm^{-1}$ 3424 (OH), 1463 ($CH_2$), 720–730 ($[CH_2]_n$). $^1H$ NMR ($CDCl_3$): $\delta$ 0.87 (3H, triplet, $J = 6.78$ Hz $CH_3$ terminal), 1.25 (40H, singlet, $CH_2$), 1.58 (1H, singlet, OH), 3.64 (2H, triplet, $J = 6.60$ Hz $CH_2OH$); $^{13}C$ NMR ($CDCl_3$): $\delta$ 13.7 (C-30), 22.8 (C-29), 29.5 (C-27), 29.8 (C-26), 29.9 (C25-6), 29.8 (C-5), 29.5 (C-4), 27.3 (C-3), 32.8 (C-2), 61.7 (C-1).

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