Note

Isolation of Harman and Norharman from *Nocardia* sp. and Their Inhibitory Activity against Plant SeedlingsKen YOMOSA,[†] Akira HIROTA,
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Numerous studies on the metabolites from *Streptomyces* have been reported, while reports about metabolites from *Actinomycetes*, except for *Streptomyces*, are comparatively few. Generally speaking, *Actinomycetes* isolated from soil samples are mostly *Streptomyces* and, in addition, *Actinomycetales* and *Actinoplanaceae* grow slowly.

In the course of screening for new bio-active metabolites produced by rare *Actinomycetes*, the strain P-9 was noticed to produce several substances, positive to Dragendorff's reagent, which had inhibitory activity against lettuce seedlings. The P-9 strain was isolated from a soil sample and determined to belong to *Nocardia* sp. judging from its cell wall type and morphological features. This strain was cultivated for 96 hr at 30°C by a jar-fermentor in the following medium: glucose, 15 g; yeast extract, 2 g; polypepton, 2 g; meat extract, 1 g; and adecanol, 0.1 g, in 1,000 ml of distilled water.

The culture filtrate was adjusted to pH 9 with 2N NaOH and extracted with ethyl acetate. The organic layer was then extracted with 0.1N HCl solution. This layer was adjusted to pH 10 with aqueous ammonia and extracted with ethyl acetate. The organic layer was then concentrated *in vacuo* and was twice subjected to silica gel column chromatography (a benzene-acetone) solvent system). The substances (9-B and 9-C) positive to Dragendorff's reagent were eluted with benzene-acetone (80:20), and this eluate was subjected to silica gel column chromatography (chloroform saturated with aqueous ammonia). The fraction containing the 9-B substance (I) was first eluted and was subjected to Sephadex LH-20 column

chromatography (methanol). I was crystallized from cold acetone as a yellow powder, mp 230~232°C (decomposition), and had UV absorption maxima (in methanol) at 234, 286 and 346 nm. The fraction containing the 9-C substance (II) was also subjected to Sephadex LH-20 column chromatography (methanol). II was obtained as a white powder by crystallization from cold acetone, mp 196~198°C (decomposition), and had UV absorption maxima (in methanol) at 232, 286 and 347 nm. The yield of I and II from a 200 liter broth was 20 and 15 mg, respectively.

By high resolution mass spectrometry, the molecular formula of I was determined to be C₁₂H₁₀N₂ (M⁺ m/z 182.0877; calcd. 182.0845). The 400 MHz ¹H-NMR spectrum of I (in (CD₃)₂CO) indicated that I was harman (1-methyl-β-carboline, Fig. 1): δ 2.78 (3H, s, 1'-H₃), 7.25 (1H, ddd, J=8.0, 7.0 and 1.0 Hz, 6-H), 7.52 (1H, ddd, J=8.2, 7.0 and 1.0 Hz, 7-H), 7.60 (1H, br. dd, J=8.2 and 1.0 Hz, 8-H), 7.89 (1H, br. d, J=5.5 Hz, 4-H), 8.19 (1H, br. dd, J=8.0 and 1.0 Hz, 5-H), 8.26 (1H, d, J=5.5 Hz, 3-H) and 10.62 (1H, br. s, 9-H). Furthermore, the ¹³C-NMR spectrum of I (in (CD₃)₂CO) supported this identification:¹⁾ δ 21.1 (C-1'), 113.2 (C-4), 113.7 (C-8), 120.8 (C-7), 122.8 (C-5), 123.1 (C-11), 127.4 (C-12), 129.1 (C-6), 136.2 (C-10), 139.5 (C-3), 142.1 (C-13) and 143.6 (C-1). The high resolution mass spectrum of II determined the molecular formula of II to be C₁₁H₈N₂ (M⁺ m/z 168.0736; calcd. 168.0688). II was identified as norharman (β-carboline, Fig. 1) by the 400 MHz ¹H-NMR spectrum (in (CD₃)₂CO): δ 7.21 (1H, ddd, J=8.0, 7.0 and 1.0 Hz, 6-H), 7.50 (1H, ddd, J=7.5, 7.5 and 1.0 Hz, 7-H), 7.60 (1H, dd, J=8.2 and 1.0 Hz, 8-H), 8.00 (1H, br. d, J=5.5 Hz, 4-H), 8.28 (1H, br. d, J=8.0 Hz, 5-H), 8.33 (1H, d, J=5.5 Hz, 3-H), 8.90 (1H, br. s, 1-H) and 10.63 (1H, br. s, 9-H).²⁾

Harman (I) has already been isolated from plants,³⁾ sake,⁴⁾ mushroom,⁵⁾ *Streptomyces*⁶⁾ and marine dinoflagellate.⁷⁾ It is known that I has monoamine oxidase-inhibiting activity⁸⁾ and cAMP phosphodiesterase-inhibiting activity.⁹⁾ Norharman (II) has also been isolated from *Streptomyces*⁶⁾ and marine dinoflagellate,⁷⁾ and it inhibits monoamine oxidase.⁸⁾ However, the biological activity of I and II against plants has not previously been reported and,

FIG. 1. Structures of Harman (I), Norharman (II) and Carbazole (III).

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therefore, the biological activity of **I** and **II** against rice and lettuce seedlings was tested. **I** reduced the root elongation of rice seedlings (*Oryza sativa* L., cv. Nihonbare) by 10% at 100 ppm, and **II** reduced it by 20% at 100 ppm. **I** also reduced the root elongation of lettuce seedlings (*Lactuca sativa* cv. Great Lakes 366) by 50% at 40 ppm, and **II** reduced it by 50% at 20 ppm. Interestingly, carbazole (**III**), an analogue of norharman, promoted the root elongation of lettuce seedlings by 30% at 50 ppm. Accordingly, the tertiary amine group which reacts with Dragendorff's reagent seems to have played an important role in the inhibitory activity of **I** and **II** in the lettuce seedlings assay.

As already noted, the metabolites from *Actinomycetes*, except for *Streptomycetaceae*, have not been studied much. Although *Nocardia* sp. has been studied for many years, small numbers of antibiotics from *Nocardia* sp. have been discovered¹⁰⁾ and biologically active substances from *Nocardia* sp., except for antibiotics, have been less reported.

Consequently it is significant that biologically active substances, harman and norharman, were isolated from a strain of *Nocardia* sp.

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