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INDOLE PLANT-GROWTH INHIBITOR FROM *ABRUS PRECATORIUS* SEEDS

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Key Word Index—*Abrus precatorius*, Leguminosae, Jequirity bean, plant growth inhibitor, *N,N*-dimethyl-L-tryptophan

Abstract—The structure of a new plant growth inhibitor isolated from the seeds of jequirity bean (*Abrus precatorius*) has been shown to be *N,N*-dimethyl-L-tryptophan

INTRODUCTION

RECENTLY we reported the presence of a plant growth regulating material[1] in the seeds of jequirity bean (*Abrus precatorius*), which inhibited the growth of germinating lettuce and other seeds. This material appeared to be involved in many biochemical transformations and its biological responses were different from those of other known plant hormones. For example, the active principle[2] inhibited auxin-stimulated and kinetin-stimulated ethylene production and protein and RNA syntheses. Further, we provided evidence that the inhibitory material is a substituted indole.[1] In this communication, we describe the characterization of the growth regulating material and prove it to be different from abrine and hypaphorine, the indole alkaloids reported by others [3–14]

RESULTS AND DISCUSSION

The active fraction from the embryos of jequirity bean seeds was isolated as the methyl esters by chromatographic separation.[15] This fraction consisted of two compounds which were separated by TLC (R_f 0·55 and 0·75). The compound R_f 0·55 corresponded to abrine

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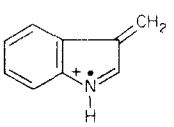
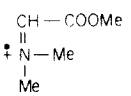
methyl ester[16,17] on the basis of cochromatography, NMR, and IR and MS analyses Abrine and its methyl ester were inactive in our bioassays

The methyl ester (R_f 0 75) in the active fraction, when examined by GC-MS, showed a M^+ at *m/e* 246 and a base peak at *m/e* 116 ($M^+ - 130$), which resulted from loss of a C_9H_8N fragment from the parent molecule.[18-21] This ester has a molecular formula of $C_{14}H_{18}N_2O_2$, which was deduced by comparing the intensities[22] of the M at *m e* 246 with those of M + 1 and M + 2 The UV spectrum showed that the ester is a β-substituted indole[23,24] The IR spectrum showed an ester carbonyl absorption (1725 cm^{-1}). The NMR spectrum indicated the presence of a side chain ($-CH_2$ $CHNMe_2COOMe$) on an indole nucleus Proof that the side chain was attached at C-3 was obtained by the appearance of a sharp indolic C-2 hydrogen singlet at δ 7 1 upon D_2O treatment. The ester had a base peak at *m/e* 116 ($M^+ - 130$), resulting from the loss of a C_9H_8N fragment, and another strong peak at *m/e* 130 ($M^+ - 116$), caused by the loss of $C_5H_{10}O_2N$ fragment, typical of β-substituted indoles[18,19] Tryptophan derivatives show major fragment ions resulting from fission of the activated bond β to the indole nucleus and α to the amino group Thus, the two fragments at *m/e* 116 and 130 corresponded to glycyl (fragment B) and indole (fragment A) moieties, respectively The other fragments at *m/e* 187 (third most abundant ion, $M^+-CO_2CH_3$) and 144 (derived from the protonation of the rearranged fragment after the loss of CO_2Me and *N,N*-dimethyl fragments), 93 5 and 58 (double charged peaks from *m/e* 187 and 116 respectively), 117, 102, 77, 56 and 42 clearly indicated the structure for this compound to be *N,N*-dimethyl-L-tryptophan methyl ester The structure was confirmed by comparing the spectral data with those of the authentic *N,N*-dimethyl tryptophan methyl ester[25] kindly furnished by Dr J A Lamberton The methyl ester showed similar biological activity to that of the acid, except that the acid is soluble in water, while the methyl ester is soluble only in organic solvents



Fragment A
(*m/e* 130)



Fragment B
(*m/e* 116)

Seeds of *Abrus precatorius* have aroused considerable interest in recent years[26-28] because of their poisonous nature, medicinal value, use in the assembly of necklaces and other jewelry, and also their sale by seed houses as a "weather plant" Apart from the toxic

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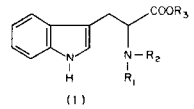
TABLE 1 PHYSIOLOGICAL RESPONSES OF LETTUCE SEEDLINGS TO TRYPTOPHAN ANALOGS

	Root growth*	IAA-stimulated ethylene†
Control	29	20
IAA	—	45
Tryptophan	19‡	40
N-Methyl tryptophan	32§	45
N,N-Dimethyl tryptophan	16‡	33
N,N-Dimethyltryptophan methiodide	30§	47
Hypaphorine	31§	46
Abrus inhibitor (crude)	5‡	29

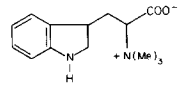
* Root growth was measured in mm after 4 days
† Ethylene was measured in nl 4 hr after adding IAA and analogs to 24-hr seedlings
‡ 33 μg/ml
§ 100 μg/ml

protein (abrin), the seeds are a rich source of alkaloids. Among these, abrine (*N*-methyl-L-tryptophan) (**1b**), hypaphorine (**2**), methyl ester of *N,N*-dimethyl-tryptophan methocation (**3**), precatorine (**4**), choline, and trigonelline (**5**) have been fully characterized.[3–14] Abrine (**1b**) and hypaphorine (**2**) occur in some members of other Leguminosae, e g., *Desmodium tiliifolium*[29] Methyl esters of some indole compounds are also found as the major alkaloids in the same Leguminosae family. The methyl esters of *N*-methyl- and *N,N*-dimethyl-L-tryptophan are reported in *Pultenaea altissima* (tall bush pea),[25] *Aotus subglauca*[16] and *Gastrolobium callistachys.*[17] To our knowledge, *N,N*-dimethyl-L-tryptophan as a free acid (**1d**) has not been isolated from a natural source However, the synthesis of this compound as a racemate has been reported earlier[30] *N,N*-dimethyl-L-tryptophan methyl ester (**1e**) undergoes partial racemization upon alkaline hydrolysis

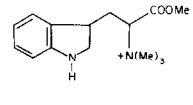


(1)

(a) $R_1 = R_2 = R_3 = H$
(b) $R_1 = R_3 = H$, $R_2 = Me$
(c) $R_1 = H$, $R_2 = R_3 = Me$
(d) $R_1 = R_2 = Me$, $R_3 = H$
(e) $R_1 = R_2 = R_3 = Me$



(2)



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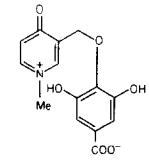

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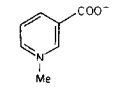

(5)

We have now shown that the acid (**1d**) is a plant-growth inhibitor but different from other known plant-growth inhibitors in chemical structure and mode of action. The other indole compounds from jequirity bean, namely, abrine, hypaphorine or the quaternary salt, *N,N*-dimethyl-L-tryptophan methiodide, were not biologically active (Table 1). Only the *N,N*-dimethyl-L-tryptophan base (**1d**) and its methyl ester (**1e**) were active in our tests. Biosynthesis of **1d** is probably from tryptophan, which is converted into the *N,N*-dimethyl-L-tryptophan through abrine by *N*-alkylation. Our results also reveal structural similarities between the plant-growth inhibitor, *N,N*-dimethyl-L-tryptophan, and the promoter, indole-3-acetic acid (IAA) Because the inhibitor and the promoter, IAA, may be derived from the same amino acid precursor, the question arises as to whether some physiological processes of the plant may be controlled in part by a balance between these two substances

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EXPERIMENTAL

M ps are uncorrected NMR spectra were recorded at 60 M Hz using $CDCl_3$ as the solvent and TMS as internal standard MS were measured at 70 eV The compounds were introduced directly into the ion chamber or after GLC separation on a 0 75% SE 30 column

Isolation and separation of N-*methyl- and* N,N-*dimethyl tryptophan methyl esters* Extraction of the embryos from jequirity bean seeds, following the previously described procedure [15] yielded an active principle as a colorless powder ν_{max}^{KBr} 3410 (OH and, or NH) and 1585 cm^{-1} (carbonyl C=O) This material was treated with ethanolic HCl to give an alcohol-soluble product, which was then treated with CH_2N_2 The resulting methyl esters were separated by column chromatography on silica gel and the active portion was eluted with C_6H_6 MeOH (9 1) GLC analysis on a 3% SF-30 column showed that this fraction consisted of two compounds, which were then separated by TLC on silica gel using $CHCl_3$ MeOH (9 1)

Abrine methyl ester (R_f 0 55) IR $\nu_{max}^{CCl_4}$ 3500 (N H) and 1735 cm^{-1} (ester C=O) NMR δ 2 34 (3 H, s NMe) 1 81 (1-H s NH Me), and 3 6 (3-H s CO_2 Me), MS *m e* 232 (M^+) 173 (M^+ COOMe) 130 (base peak) 117 115, 103 102, 86 5 and 77

N N-*Dimethyl tryptophan*, m p 97 98 (hexane), R_f 0 75, UV λ_{max}^{EtOH} 203 (ϵ, 19680) 222 (ϵ 35670) 276 (ϵ 6150), 283 (ϵ 6890) and 290 nm (ϵ 5840), IR ν_{max}^{KBr} 3160 (NH) and 1725 cm^{-1} (ester C=O), NMR δ 2 45 (6-H, s, N Me_2) 3 62 (3-H s CO_2Me) 3 2 (2-H, *d* $-CH_2-C-N$) 3 5 (1-H, *t*, $-CH$), 7 0 7 8 (5-H *m* indole) and 8 15 (1-H broad s, disappearing on deuteration N-H) MS *m e* 246 (M^+), 187 (M^+-CO_2Me) 144 130 117 116 (base peak) 102, 93 5, 77, 58, 56, and 42

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