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IDENTIFICATION OF THE TOXIC PRINCIPLE OF *CNESTIS GLABRA* AS METHIONINE SULPHOXIMINE

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Key Word Index—*Cnestis glabra*; Connaraceae; neurotoxin; glabrin; structural determination; toxic amino acid; methionine derivative; methionine sulphoximine.

Abstract—Glabrin, the toxic principle of *Cnestis glabra* isolated from root bark, was identified as *S*-(3-amino-3-carboxypropyl)-*S*-methyl sulphoximine (methionine sulphoximine) by spectroscopic and chemical means. The natural occurrence of this toxic methionine derivative is reported for the first time.

INTRODUCTION

The toxic principle of *Cnestis glabra*, a neurotoxic compound occurring in all parts of the plant and temporarily named glabrin, was isolated from root barks in 0.4% yield [1] and some of its physicochemical and biological properties were studied [1, 2]. We found that glabrin, appearing as a white, solid compound, has a M_r less than 500. It is heat-stable, soluble in water, but not in usual organic solvents. The compound has no aromatic group in its structure (no retention on activated charcoal and no absorption in UV). It reacts positively with ninhydrin and behaves as methionine on an amino acid analyser. These physicochemical properties preclude glabrin from belonging to some classes of well-known toxic natural compounds of low M_r occurring in the plant kingdom, such as alkaloids. Glabrin could be a non-aromatic amino acid, possibly a methionine derivative. This hypothesis was partially supported by the fact that a number of unusual toxic amino acids are well known in higher plants [3, 4]. In the present paper we report the spectral and chemical data which led us to the identification of glabrin as methionine sulphoximine.

RESULTS AND DISCUSSION

Mass spectrometry showed glabrin to have $[M+1]^+$ at m/z 181, in agreement with the formula $C_5H_{12}N_2O_3S$ obtained from high-resolution mass spectrometry. The carbon atoms gave rise to five signals in the ^{13}C NMR spectrum: δ27.18 (*t*, J = 130 Hz), 44.08 (*q*, J = 140 Hz), 54.98 (*t*, J = 145 Hz), 56.00 (*d*, J = 145 Hz), 175.96 (*s*). The last signal is indicative of a C=O function, probably that of a carboxyl group, as was suggested by the broad absorption between 2250 and 3500 cm^{-1} in the IR spectrum.

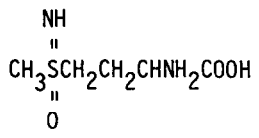
The ^1H NMR spectrum of glabrin exhibited a one-proton triplet at δ3.89 (J = 7.5 Hz), a two-proton multiplet centred at 2.37, a two-proton multiplet centred at 3.43, and finally a methyl singlet at 3.17. This pattern is similar to that of methionine, the conspicuous difference being the chemical shifts of the signals and especially that of the methyl group, which suggested a new environment for the sulphur atom in the natural compound.

No change was observed on an amino acid analyser when performic acid treatment was attempted to oxidize the sulphur atom, thus suggesting a tetracoordinate

sulphur atom in the structure of glabrin. Indeed careful inspection of the IR spectrum revealed the presence of two intense absorptions at 1200 and 1020 cm^{-1} which could be attributed to S→O and S–N linkages, respectively. Thus, glabrin may be identical to methionine sulphoximine (**1**).

Although not previously reported as a natural product, methionine sulphoximine was originally discovered to be the toxic factor in 'agenised' flour producing hysteria in dogs [5, 6], and later synthesized [7–12]. Physical data run on a commercially available sample are consistent with those recorded for glabrin.

Methionine sulphoximine seems to be characteristic of the toxic Connaraceae since it was also isolated from *Cnestis polyphylla* and *Rourea orientalis* [13].



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EXPERIMENTAL

General procedure. NMR spectra were taken in D_2O at 25° using d_4-Na trimethylsilyl propionate as internal standard, ^{13}C NMR at 50.32 MHz and ^1H NMR at 200 MHz. MS (70 eV) were run using direct insertion in a chemical ionization system.

Plant material. Roots were collected in January from low-altitude forests in the north-east of Madagascar.

Isolation of the toxin. Full details of the isolation procedure have been described in ref. [1]. The isolated compound has the following characteristics: CIMS *m/z* (rel. int.): 181 $[M+1]^+$ (100); IR ν_{max}^{KBr} cm^{-1}: 2500–3500, 2140, 1600, 1200, 1020. ^1H NMR and ^{13}C NMR: the signals are given in the text.

Performic acid oxidation. 2 μl of aqueous toxin soln was incubated for 1 hr at room temp. with 200 μl of performic acid mixture (1 vol. 30% H_2O_2 and 9 vol. conc. HCOOH). The mixture was then cooled to −10° for 3 hr and finally dried under red. pres.

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