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IMIDAZOLE, A NEW NATURAL PRODUCT FROM THE LEGUMINOSAE

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Key Word Index—*Lens culinaris*; Leguminosae; lentil; amines; imidazole; dansyl chloride.

Abstract—Imidazole was isolated from *Lens culinaris* seeds as its dansyl derivative and shown to be identical to a synthetic standard by co-chromatography, ^{1}H NMR and EIMS. Chromatographic evidence indicated that it was also present in the seeds of 11 other legumes, 30% of those tested.

INTRODUCTION

Imidazole (glyoxaline, 1,3-diazole) has not previously been detected in higher plants or indeed isolated from any cell extract or physiological fluid. However, its 2-amino derivative is present in seeds of *Mundulea sericea* (Leguminosae) and a number of other species belonging to the tribe Tephroseae [1, 2]. More complex related plant products include 4-isovalerylaminomethylimidazole from *Dolichothele sphaerica* (Cactaceae) [3] and histamine [4, 5], which is more widely distributed than any of its nine recorded derivatives.

RESULTS

5-Dimethylamino-naphthalene-1-sulphonyl- (dansyl or dns) derivatives were used throughout because they were both easier to detect and to isolate than the parent compounds. Dansyl-imidazole was first recognised as a spot with an atypical orange/red fluorescence running with didns-tyramine in solvent B and substantially slower in solvent A: it occupies what is normally an empty region on standard 2D-chromatograms so is unlikely to be confused with any other plant product. R_fs were 0.33, 0.48, 0.79 and 0.45 in solvents A, B, C and D respectively (see Experimental).

After isolation from *Lens culinaris* Medic cv continental seeds, the compound gave the ^{1}H NMR spectrum indicated in Table 1. A mass spectrum produced a molecular ion with an *m/z* of 301. These results indicated that the isolate was dns-imidazole. A synthetic dns standard co-chromatographed with the isolate in all four TLC solvents (A–D) and had identical ^{1}H NMR and mass spectra, confirming the identification.

Table 1. ^{1}H NMR spectral data for the isolate

Proton	Chemical shift (ppm)	Off resonance pattern	Assignment
a	8.22	*d*	CH
b	8.4	*d*	CH
c	8.3	*d*	CH
d	8.15	*s*	CH
e	7.6	*t*	2 × CH
f	7.35	*s*	CH
g	7.25	*d*	CH
h	7.05	*s*	CH
i	2.9	*s*	$N(Me)_2$

Extracts of a number of other legume seeds gave a spot co-chromatographing with internal standards of dns-imidazole on 2D-plates: *Adenanthera pavonina* L., *Amphimas pterocarpoides* Harms, *Cathormion altissimum* Hutchinson, *Erythrophleum* sp., *Lathyrus rotundifolius* Willd., *L. sylvestris* L., *Macrotyloma uniflorum* (Lam.) Verdc., *Parkia bicolor* A. Chevalier, *Psophocarpus tetragonolobus* DC., *Tephrosia platycarpa* Guill. and *Vigna radiata* (L.) R. Wilczek. The spots from *P. bicolor* and *P. tetragonolobus* were sufficiently intense to show the characteristic orange/red fluorescence, which seems only to be shown by imidazole and its derivatives. Seed extracts of a further 24 legume species gave negative results: *Abrus precatorius* L., *Adansonia digitata* L., *Bauhinia* sp., *Cassia fistula* Herbb. ex Oliver, *C. nodosa* Buch.-Hom. ex Roxb. hort. Beng, *C. occidentalis* L., *C. siamea* Lam., *C. sieberiana* DC., *Glycine max* L., *Lathyrus latifolius* Vis. Stirt. Dalmat. *L. maritmus* Bigel., *L. niger* Bernh., *L. pratensis* L., *L. roseus* Phil., *L. sativus* L., *L. tingitanus* L., *L. tuberosus* L., *Lonchocarpus sericeus* H.B., *Mucuna deeringiana* Merrill., *Phaseolus vulgaris* L., *Tetrapleura tetraptera* Taub., *Vicia faba* L., *V. gerardi* Jacq. and *Voandzeia subterranea* Thou.

Comparison of chromatograms of plant extracts with standard dns-imidazole suggested that the parent compound was present in the seeds of *L. culinaris* at a concentration of *ca* 10 ng/g fresh weight. It must be present at a minimum concentration of 5 ng/g fresh weight in the other species mentioned to allow its detection.

Imidazole is mildly biologically active, having been used in cosmetics to reduce baldness [6] and in animal shampoos to kill ectoparasites [7] but is unlikely to have any physiological activity at the levels found in these legumes. It is more likely to be an intermediary metabolite, perhaps present in most tissues in trace amounts. The transfer of an amino acid side chain derived from serine to several aromatic nuclei like indole [8], pyrazole, pyridine and pyrimidine [9, 10] has been established. Imidazole and histidine could be interconverted by a parallel reaction. The imidazole found here is unlikely to be an artifact: histidine its only derivative found ubiquitously in plants, gives no detectible dns-imidazole when dansylated either by itself or when mixed with plant amine fractions.

There is only one previous report of imidazole as a natural product [11]. Unfractionated, sonicated preparations of the salivary glands of *Octopus vulgaris* give ^{1}H NMR spectra showing resonances matching those of the compound with respect to chemical shifts and pK_a. However, this conclusion could not be confirmed by dansylation/TLC. Application of our standard method to extracts of the gland showed no detectible dns-imidazole, though this was clearly visible after spiking the homogenate with standard at 1.5 μg/g fresh weight [Price, N. personal communication].

EXPERIMENTAL

Plant material and its extraction. Lens culinaris seeds were purchased from a retail source, all the others were obtained through The Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, U.K., where they were authenticated by Mr G. P. Lewis. Ground seed material, 2 g, was routinely extracted twice overnight with 20 ml 70% MeOH. The combined filtrates were evapd to dryness *in vacuo* at 50° and, after dissolving the residue in 0.4 ml water, this was dansylated and the products chromatographed two-dimensionally in solvent A, followed by solvent B.

Amine dansylation and TLC. The procedure was as previously described [12], the dansylated amines being chromatographed on silica gel in one of the following solvents, before examination under 366 nm UV light: A, C_6H_{12}–EtOAc (2:3); B, C_6H_6–Et_3N (5:1); C, $CHCl_3$–Et_3N (8:3); D, $CHCl_3$–BuOAc (6:4); all by vol.

Isolation of imidazole as dns derivative from L. culinaris. The amines from 6 kg ground seed material were isolated on CM52 (microgranular carboxymethylcellulose, Whatman) and divided into 100 × 2 ml fractions. Each fraction was reacted with 2 ml dns-chloride (30 mg/ml in acetone) followed by 2 ml proline (30% w/v in H_2O). Other details were as for the isolation of *O*-acetylethanolamine [12]. The yield was estimated to be 0.15 mg.

Synthesis of authentic dns-imidazole. Derivatisation was as usual except that imidazole (10 mg in 0.5 ml H_2O) was reacted with 1 ml dns-chloride, followed by 1 ml proline and the products were extracted with 3 × 2 ml EtOAc. Purification was by 1-D TLC in solvent B (1 20 cm sq. plate) and then on Kieselgel 60 HR in solvent C (1 plate) as for the isolate.

Spectroscopy. EIMS, *m/z* (rel. int.): 301 $(M)^+$ (25), 237 (30), 203 (39), 170 (25), 94 (35), 68 (100), 67 (23), 66 (23), 57 (49), 55 (47), 44 (42). NMR spectra were recorded by a Bruker WH 400 instrument with reference to $CHCl_3$ at 7.27 ppm.

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