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O-ACETYLETHANOLAMINE, A NATURAL PRODUCT FROM THE LEGUMINOSAE

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Key Word Index—*Lens culinaris*; Leguminosae; lentil; isolation: amines; ethanolamine, *O*-acetyl; dansyl chloride.

Abstract - *O*-Acetylethanolamine, studied as its dns derivative throughout, was isolated from *Lens culinaris* and identified by spectroscopy/synthesis. Chromatographic evidence indicated its presence in 12 other legumes.

INTRODUCTION

N-Acetylated derivatives of amines, like those of spermidine [1], putrescine [2], cadaverine [3], histamine [4], norepinephrine [5], serotonin [6], 5-methoxytryptamine [7] and tryptamine [8] are well known natural products, though, so far, only the latter has been found in a higher plant (*Prosopis nigra*). *O*-Acetylated amines are much rarer though acetylcholine occurs in *Viscum album* (mistletoe) [9], *Spinacea oleracea* [10], *Phaseolus vulgaris* [10], *Pisum sativum* [10], *Solanum nigrum* (black nightshade) [11] and *Ipomoea abutiloide* [12], as well as having an essential role in mammals.

Ethanolamine may well be a universal constituent of higher plants, probably due to its role in phospholipid metabolism, but its only known derivatives have been *N*-(γ-L-glutamyl)-ethanolamine, from the mushroom, *Agaricus bisporus* [13], and diethanolamine from a number of composite species [14]. We now add *O*-acetylethanolamine to this list of naturally occurring derivatives.

RESULTS

Here, all amines were first reacted with 5-dimethylamino-naphthalene-1-sulphonyl chloride (dns chloride) to make them both easier to detect and to isolate. Dns-*O*-acetylethanolamine (dns-ethanol-2-amino-acetate) was first recognized as a fluorescent spot running slightly slower than dns methylamine in both solvents on standard 2D-TLC-chromatograms: R_fs were 0.46, 0.35, 0.68 and 0.45 in solvents A, B, C and D, respectively.

After isolation from *Lens culinaris* Medic cv continental seeds the compound gave the ^{1}H NMR spectrum indicated in Table 1. Decoupling experiments revealed that the 4.96 ppm proton was coupled to the two protons at 3.20 ppm which were themselves coupled to the two protons at 3.98 ppm. These results suggested that the isolate was an ester of dns-ethanolamine. A mass spectrum produced a molecular ion with an *m/z* of 336 indicating that the isolate was dns-*O*-acetylethanolamine. A synthetic dns standard co-chromatographed with the isolate in all four TLC solvents tested (A–D) and had an identical ^{1}H NMR spectrum.

The *O*-acetylethanolamine isolated here might have been an artifact, produced when naturally occurring ethanolamine was evaporated in the aqueous acetic acid used to elute the amine fraction from an ion-exchange (CM52) column; this possibility was excluded first by eluting the CM52 with 0.5 M HCl in place of CH_3·COOH (evaporating the eluate immediately to avoid the decomposition that otherwise occurs) and secondly by dansylating a concentrated extract directly: a spot having a green fluorescence and co-chromatographing with inter-

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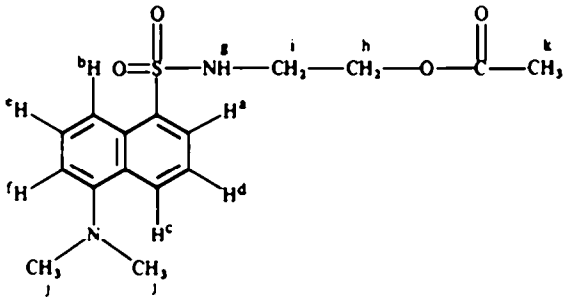
Table 1. ^{1}H NMR spectral data for the isolate

Proton	Chemical shift (ppm)	Off resonance pattern	Assignment
a	8.58	d	CH
b	8.28	d	CH
c	8.28	d	CH
d	7.60	t	CH
e	7.55	t	CH
f	7.22	d	CH
g	4.96	t	HN-dns
h	3.98	t	CH_2
i	3.20	q	CH_2
j	2.90	s	$N(CH_3)_2$
k	1.85	s	$CO-CH_3$



nal standards of dns-*O*-acetylethanolamine during 2D-TLC was still present in both cases.

The same criteria were used to show the presence of *O*-acetylethanolamine by direct dansylation in seed extracts of the following legumes: *Adansonia digitata* L., *Adenanthera pavonina* L., *Amphimas pterocarpoides* Harms, *Cassia siamea* Lam., *Lathyrus rotundifolius* Willd., *L. sativus* L., *L. sylvestris* L., *Parkia bicolor* Chevalier, *Phaseolus vulgaris* L., *Tephrosia platycarpa* Guill., *Vicia faba* L. and *Vigna radiata* L. Seed extracts of a further 23 species belonging to the following genera gave negative results: *Abrus*, *Bauhinia*, *Cassia*, *Cathormion*, *Erythrophleum*, *Glycine*, *Lathyrus*, *Lonchocarpus*, *Macrotyloma*, *Mucuna*, *Psophocarpus*, *Tetrapleura*, *Vicia* and *Voandzeia*.

O-Acetylethanolamine is probably quite widely distributed. Albert [15] found that 52 out of 140 composite species gave a spot running in a similar position to dns-*O*-acetylethanolamine on 2D-TLC-chromatograms. However, when this work was done, no standard was available. The new compound is always present at very low concentrations. Fluorimetric comparisons with standards suggested that the free compound was present in seeds of *L. culinaris* at a concentration of *ca* 250 ng/g fr. wt. The amine must be present at a minimum concentration of 100 ng/g fr. wt in the other species mentioned to allow its detection. Metabolically plant *O*-acetylethanolamine is probably formed either by decarboxylation of relatively rare *O*-acetylserine [16–18] or by the acetylation of the much more common ethanolamine.

O-Acetylethanolamine has anti-inflammatory properties [19] and accelerates the heart beat of *Periplaneta americana* (cockroach) [20] but is unlikely to have any physiological activity at the levels found in these legumes.

EXPERIMENTAL

Amine dansylation and TLC. Dansylation was by a modification of a published procedure [21]. The aq. sample (0.4 ml) was mixed with 0.8 ml dns chloride (5 mg/ml in acetone, Sigma) and incubated for 16 hr at 20° in a darkened, sealed tube in the presence of sufficient solid $NaHCO_3$ to saturate the mixture. Aq. 15% (w/v) proline (0.8 ml) was then added, and, after leaving for 1 hr, the derivatized products were extracted by vortex mixing with 2 × 2.5 ml EtOAc. The combined organic phases were evaporated in a stream of air at 50°, and the residue was redissolved in 0.2 ml EtOAc before TLC on 20 × 20 cm, 0.25 mm layers of silica gel (Kieselgel 60G Merck) in one of the following solvents: A, C_6H_{12} EtOAc (2:3); B, C_6H_6–Et_3N (5:1); C, $CHCl_3$ Et_3N (8:3); D, $CHCl_3$ BuAc (6:4). The spots were examined under 366 nm UV light.

Routine analysis of plant extracts. Ground seed material (2 g) was extracted twice overnight with 20 ml 70% MeOH. The combined filtrates were evaporated to dryness *in vacuo* in a rotary film evaporator at 50°. At this stage extracts were either dissolved in 10 ml H_2O, filtered and applied to an 8 × 1 cm diameter column of CM52 (microgranular carboxymethylcellulose, Whatman) in the Na^+ form. After washing with 5 × 10 ml H_2O, bound amines were normally eluted with 50 ml 0.5 M CH_3COOH or where stated with 50 ml 0.5 M HCl (flow rate 2 ml/min throughout). Free *O*-acetylethanolamine apparently decomposes in 0.5 M HCl within 15 hr at 20°. The eluants after evaporation to dryness *in vacuo* as before, followed by re-evaporation with 2 × 10 ml H_2O to remove excess acid, were dansylated and chromatographed two-dimensionally in solvent A followed by solvent B.

Isolation of O-acetylethanolamine as dns derivative from L. culinaris. Seed material, 6 kg, ground to a fine powder, was extracted overnight 2 × with 7 l. 70% MeOH. The extracts were filtered, combined and applied in aliquots to 12 columns of CM52 in the Na^+ form (35 cm × 3 cm i.d.), each of which had been pre-equilibrated with 1 l. 70% MeOH. Each column was washed with 2 l. 50% MeOH followed by H_2O until the effluent gave a negative ninhydrin reaction (500 ml). The amines were then eluted with 1 l. 0.5 M HOAc, the flow rate being 0.5 ml/min at every stage. The eluants were pooled and acid was removed by evaporation *in vacuo* as before followed by re-evaporation 3 × with 50 ml H_2O. This amine fraction was redissolved in H_2O and divided into 100 × 2 ml fractions. Each was reacted with 2 ml proline (30% w/v in H_2O) other details being as for the standard procedure. The dns compounds from each reaction mixture were extracted into 4 × 2 ml EtOAc, the organic layers pooled and evaporated *in vacuo*. The residue redissolved in 2 ml EtOAc was chromatographed one-dimensionally in solvents A, B and C used in this order. Each time the fluorescent bands were eluted with acetone, concentrated *in vacuo* and re-chromatographed in the next solvent. The final separation was done on kieselgel 60HR in 'Aristar' solvents. The purified sample was desiccated over P_2O_5 for 2 days.

Synthesis of dns-O-acetylethanolamine. Free *O*-acetylethanolamine is not readily available, so the required derivative was prepared by acetylating dns ethanolamine. Ethanolamine, 20 mg in 0.5 ml H_2O was reacted with 1 ml dns chloride (5 mg/ml in acetone), followed by 1 ml proline, otherwise following the usual procedure. Dns ethanolamine after extraction in 4 × 2 ml EtOAc was purified by one-dimensional TLC in solvent A (details as for the *L. culinaris* isolate). The product was dissolved in 2 ml HOAc and heated at 60° in a sealed tube for 2 hr before

removing the acid in a stream of air at 50°. Chromatography in solvent A showed two bands with R_fs of 0.29 and 0.49, the slower corresponding to unreacted dns ethanolamine. The faster component was purified in this solvent taking the same precautions as for the final stage of isolate purification. The estimated yield was 30% based on dns-ethanolamine.

Spectroscopy. EIMS, *m/z* (rel. int.): 336 $[M]^+$ (100), 171 [M − 165] (95), 170 [M − 166] (69), 169 [M − 167] (13), 168 [M − 168] (35), 155 [M − 181] (12), 154 [M − 182] (18), 128 [M − 208] (10), 127 [M − 209] (16), 126 [M − 210] (11). NMR spectra were recorded by a Bruker WH 400 instrument with reference to $CHCl_3$ at 7.27 ppm.

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