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DIETHANOLAMINE, A SECONDARY AMINE FROM THE COMPOSITAE

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Improved analytical techniques have shown that ethanolamine, long known to be a common constituent of higher plants (1,2), is virtually universal (3). However, this is the first report that the corresponding secondary amine is also a natural product. The revelant basic nitrogen fractions, recovered from extracts with carboxylic ion-exchange substrates (4), were always reacted with 5-(dimethylamino)-naphth-1-yl sulfonyl chloride (dansyl Cl) before further analysis. Dansyl-diethanolamine (dansyl-2,2′-iminobisethanol) was recognized as a fluorescent spot migrating comparatively slowly on tlc plates: Rfs were 0.05, 0.06, 0.05, 0.08, 0.43, and 0.54 in solvents A,B,C,D,E, and F, respectively.

An isolate from *Senecio dunedin* (horticultural hybrid of *Senecio compactus* Kirk, *Senecio greyi* Hook f., and *Senecio laxifolius* Buchan) was initially characterized from its ^{1}H-nmr spectrum. Comparison with the spectra of synthetic standards showed that the dansyl group was represented by the singlet at δ 2.9 [$N(Me)_2$] and the signals in the δ 7.2-8.6 region (naphthalene ring protons). The absence of a signal for NH in the δ 4.2-5.0 range indicated that the amine originally dansylated was a secondary, not a primary amine. The chemical shift of the protons at δ 3.87 suggested an association with oxygen while the δ 3.87 and 3.48 signals were clearly coupled, suggesting the isolate was either dansyl-morpholine or dansyl-diethanolamine. The identification was confirmed to be the latter by comparison with synthetic dansyl-diethanolamine, which gave the same ^{1}H-nmr spectrum as the isolate and co-chromatographed with it in all six solvents (A-F) tested. Moreover, the mass spectrum of the isolate showed a molecular ion at the expected *m/z* value of 338.

An isolate from *Echinops exaltatus* Rchb. et Auct. was far too small for ^{1}H-nmr analysis, but it co-chromatographed with dansyl-diethanolamine in all six solvents tested. Its eims gave a distinct molecular ion at the calculated precise mass of 338.218±0.005, which increased in relative intensity with increasing temperature, confirming that it was really derived from the sample rather than from solvent residues.

Routine tlc analysis of the basic nitrogen fractions of 143 Compositae species showed that 24% of them gave a spot in the position of dansyl-diethanolamine on 2-D chromatograms. Such spots were found in members of the genera *Aspilla* (1 sp), *Aster* (1 sp), *Baeria* (1 sp), *Carthamus* (1 sp), *Catanache* (1 sp), *Centaurea* (1 sp), *Chamaemelum* (1 sp), *Chrysanthemum* (1 sp), *Commidendron* (1 sp), *Crepis* (2 sp), *Echinops* (1 sp), *Eupatorium* (1 sp), *Felicia* (2 sp), *Hieracium* (2 sp), *Kleinia* (6 sp), *Leontopodium* (1 sp), *Osteospermum* (1 sp), *Podolepis* (1 sp), *Pterocaulon* (1 sp), *Senecio* (5 sp), and *Veronia* (2 sp). The spot was sufficiently intense in seed extracts of *Baeria coronaria* Gray, *Crepis capillaris* (L.) Wallr., and *Crepis pulchra* L., and in leaf extracts of a *Kleinia* sp. and *Pterocaulon sphacelatum* Labill. for it to be eluted from the 2-D plate and co-chromatographed with the standard in solvents C and D.

Fluorimetric comparisons with standards suggested that the detection limit in this survey was ca 50 ng diethanolamine/g fresh weight. This approximated the level of the free amine in *E. exaltatus*, whereas the level was ca 20 times greater in *Senecio dunedin*. These

concentrations are fairly typical of the unknown amines now being revealed in plant extracts by the dansyl chloride technique.

Diethanolamine is mildly toxic to vertebrates (5-8), invertebrates (9), bacteria (10), and fungi (11,12) as well as inducing a number of physiological changes (13-16). However, it is unlikely to be effective against potential pathogens at the concentrations found in these composites if uniformly distributed in the tissues.

There has been only one previous example of the use of ^1H nmr to characterize a dansylated amine from a cell extract (17). The introduction of the fluorescent label obviously complicates the spectrum and often makes it necessary to use higher field instruments. However, this is offset by the advantages that dansylated amines can be detected at much lower levels than free amines, give much stronger molecular ions during eims, and are easier to purify, as they chromatograph cleanly and can be readily freed from salt by solvent extraction.

EXPERIMENTAL

PLANT MATERIAL.—*E. exaltatus* and *S. dunedin* were supplied by Westfield College, London, NW3 7ST, UK (where voucher specimens are currently deposited) and authenticated by Dr. G.J. Cunnell. *P. sphacelatum* (collector, H. Demarz) and the *Kleinia* sp. (authenticator, F.G. Davies) were obtained from the Royal Botanic Gardens, Kew, Surrey, TW9 3DS, UK. The remaining species were provided by the University of London Botanical Supply Unit, Elm Lodge, Eggham, Surrey, UK, and authenticated by J. Rees.

TLC.—Tlc was 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, $CHCl_3$-BuOAc (8:3); D, $CHCl_3$-C_6H_6-MeOH (2:17:1); E, $CHCl_3$-Et_3N (8:3); and F, *n*-BuOH-H_2O-HOAc (90:29:10).

ANALYSIS OF PLANT BASES.—Routine analysis of plant basic nitrogen fractions involved extracting 0.5 g seed or leaf material with 25 ml 70% MeOH and evaporating to dryness in vacuo at 50°. After dissolving the residue in 25 ml H_2O and filtering, it was applied to a 5 cm×1 cm diameter column of CM 52 (carboxymethylcellulose, Whatman) in the H^+ form. The column was washed with 10 ml followed by 100 ml H_2O and eluted with 25 ml 0.5 M HCl, the flow rate being 42±5 ml/h at every stage. Excess HCl was removed by evaporating the eluate to dryness as before and twice redissolving the residue in 10 ml H_2O and re-evaporating.

Dansylation was accomplished by a modification of a published procedure (18). The aqueous sample (0.2 ml) was mixed with 0.4 ml dansyl Cl (5 mg/ml in Me_2CO) and incubated for 15 h at 20±5° in a darkened, sealed tube in the presence of sufficient solid $NaHCO_3$ to saturate the mixture. Aqueous 15% (w/v) proline (0.6 ml) was then added, and, after leaving for 2 h, the derivatized products were extracted by vortex mixing with 2×2.5 ml EtOAc. The organic phase was evaporated in a stream of air at 50°, and the residue was redissolved in 0.2 ml EtOAc before 2-D tlc, first in solvent A and then in B. The spots were examined under 366 nm uv light.

LARGE SCALE ISOLATIONS FROM *S. dunedin* AND *E. exaltatus*.—Mature leaves and stems of *S. dunedin* (1 kg, fresh weight), harvested just before flowering, were extracted with 2.5 liters MeOH, followed by 750 ml 70% aqueous MeOH. The extracts were pooled, filtered, and applied to a column of CM52 in the Na^+ form, 57 cm×3 cm diameter, which had been pre-equilibrated with 70% MeOH. The flow rate was limited to 50 ml/h during loading and elution to maximize recovery. The column was washed with 2 liters 70% MeOH and 5 liters H_2O, followed by elution with 2 liters 2 M HCl. The acid elute was evaporated to dryness in vacuo at 50°, redissolved in 100 ml H_2O, and re-evaporated 15 times to remove residual HCl.

The basic nitrogen fraction so obtained was redissolved in 100 ml H_2O, and 2-ml aliquots were derivatized with 4 ml dansyl Cl, using the standard recipe scaled up 10 times. Each reaction mixture was extracted with 6×5 ml EtOAc, which was evaporated as usual. The products of the 50 derivatizations were pooled and separated by 2-D tlc on 100 plates run successively in solvents A and B. The relevant spots were removed, and the pooled silica gel was extracted once with 30 ml Me_2CO-H_2O (10:1) and twice with 30 ml Me_2CO, each time for 2 days. The Me_2CO soluble material was further purified, first by 1-D tlc in solvent C (10 plates) and then on 0.25-mm, thick layers of Kieselgel 60 HR (Merck) in solvent E (4 plates), before being dried in vacuo over P_2O_5. The Me_2CO and the components of solvent E were of analytical reagent grade.

Mature stems and leaves of *E. exaltatus* (1 kg, fresh weight) were treated identically except that MeOH was replaced by industrial methylated spirits at all stages, and the column used for isolating the basic fraction was packed with the Na^+ form of Duolite 436 (60-120 mesh).

Fluorimetric comparisons with the standard indicated that 3 mg of dansyl-diethanolamine was isolated from *S. dunedin* and 0.15 mg from *E. exaltatus*.

^{1}H nmr of the *S. dunedin* isolate (100 MHz, $CDCl_3$, TMS) δ ppm 2.9 (6H, s), 3.48 (4H, t, J=5 Hz), 3.87 (4H, t, J=5 Hz), 7.2 (1H, d, J=8 Hz), 7.5 (1H, t, J=7 Hz), 7.6 (1H, t, J=7 Hz), 8.15 (1H, d, J=7 Hz), 8.37 (1H, d, J=8 Hz), 8.57 (1H, d, J=8 Hz).

The *S. dunedin* isolate was examined conventionally with eims. Only a little material was obtained from *E. exaltatus*; thus, it was chromatographed in C_6H_6-HOAc (9:1) on a 15×15 cm polyamide layer, prewashed in MeOH-HOAc (3:1 v/v), and introduced directly into the spectrometer still adsorbed to the polyamide particles (19); *m/z* (rel. int.) 338 (M, 25), 307 (9), 171 (47), 170 (100), 169 (8), 168 (15), 155 (9), 154 (14), 128 (10), 127 (12).

SYNTHESIS OF AUTHENTIC DANSYL-DIETHANOLAMINE.—Derivatization was usual except that diethanolamine (10 μl in 10 μl H_2O) was reacted with 6 ml dansyl Cl, followed by 9 ml proline, and the products were extracted with 2×37.5 ml EtOAc. Purification was by 1-D tlc in solvent B (5 plates) and then on Kieselgel 60 HR in solvent E (5 plates), as for the isolates.

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