combined and evaporated leaving a yellow solid. Recrystallization from EtOH afforded an orange–yellow crystalline compound (689 mg; 0.19% of dry plant material). This was homogeneous on TLC in three different systems, $CHCl_3$–EtOH–HOAc (19:9:1) $CHCl_3$–MeOH (17:3), and EtOH–2N HCl (9:1). The isolated compound had mp 205–206°; UV (H_2O at pH 3): λ_{max} 214 (ε 16000), 277 (6000), 355 nm (2700); IR: 2700–2500 (several, $-{}^+NH_3$), 1610, 1565, 1500 ($-{}^+NH_3$), 1535, 1345 ($-NO_2$), 825, 805, 760 (trisubstituted benzene ring); PMR (HOAc-d_4:TMS): δ 3.08, 3.30 (AA'BB' system, Ph–CH_2–CH_2–$^+NH_3$), 7.15 (*d*, *J* = 8 Hz), 7.59 (*dd*, J = 8, *J* = 2 Hz), 8.04 (*d*, *J* = 2 Hz) (aromatic protons of a 1,2,4-trisubstituted benzene ring); MS: (*m/e*) 182 (M-18, 8%), 152 (182–30, 3%), 135 (182–HNO_2, 29%), 106 cf. [13] (182–30–NO_2, 11%), 105 (182–30–HNO_2, 16%), 83 (182–30–28–41, *o*-nitro effect, 16%), cf. [13] 77 (Ph^+, 20%), 51 ($C_4H_3^+$, 13%), 30 ($^+CH_2$–NH_2, 100%). Found: C, 47.86; H, 6.01; N, 13.90. $C_8H_{12}N_2O_4$ (MW 200.19) requires: C, 47.99; H, 6.04; N, 13.99%.

Preparation of the nitrate and the hydrochloride. The compound (40 mg) in H_2O (5 ml) was treated with conc HNO_3 (0.5 ml) and the soln evaporated to dryness. The residue was recrystallized from MeOH yielding the nitrate, mp 215–216° decomp. The compound (40 mg) was dissolved in boiling MeOH (15 ml) and treated with a few drops of conc HCl. The soln was evaporated and residue recrystallized from EtOH affording the hydrochloride, mp 214–215°.

Synthesis of 3-nitro-4-hydroxy-phenethylammonium nitrate. Tyramine (500 mg) was suspended in H_2O (3.5 ml) and treated at 0° with 56% HNO_3 (1.75 ml). The mixture was stirred at the same temp for 8 hr and then kept at 0° for 24 hr. The solid was filtered, washed with cold H_2O and MeOH, and dried. Recrystallization from MeOH gave 3-nitro-4-hydroxy-phenethylammonium nitrate (449 mg) mp 214–216° decomp (lit. [6] 217° decomp). The mp was not depressed on admixture with the nitrate from the natural compound.

Synthesis of 3-nitro-4-hydroxy-phenethylammonium hydrochloride. The synthetic product (100 mg) was dissolved in boiling MeOH, the soln treated with a few drops of conc HCl, and the whole evaporated to dryness. Recrystallization of the residue from EtOH afforded 3-nitro-4-hydroxy-phenethylammonium hydrochloride mp 213–214° (lit. [6] 214.5°). The mp was not depressed on admixture with the hydrochloride from the natural compound.

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HOMOSPERMIDINE IN *RHIZOBIUM* AND LEGUME ROOT NODULES

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Key Word Index—*Lupinus*; *Phaseolus*; *Pisum*; *Vicia*; Leguminosae; *Rhizobium*; lupin, pea and bean root nodules; homospermidine; polyamines.

I wish to report the identification of *sym*-homospermidine ($NH_2(CH_2)_4NH(CH_2)_4NH_2$) in legume root nodules and in the bacterial genus *Rhizobium*. This triamine, which is closely related structurally to the widely occurring polyamine, spermidine, has previously only been found in the free state in the leaves of the sandalwood tree (*Santalum album* L.) in which it comprises 0.5–1.5% of the dry weight [1–3]. In *Solanum tripartitum* homospermidine occurs as amide conjugates with fatty acids which have tumour inhibitory properties [4]. Other closely related polyamines occur rarely. *N*-3-Aminopropyl-1,5-diaminopentane has been found in a mutant of *Escherichia coli* [5]. Diaminodipropylamine ($NH_2(CH_2)_3NH(CH_2)_3NH_2$) has been reported in turnip yellow mosaic virus [6, 7], though this was subsequently disputed [8].

In the present work, using the dansyl technique, homospermidine has been demonstrated in root nodules of lupin, broad bean, runner bean, and pea, though it could not be found in the nodule-free roots of pea and broad bean (Table 1). Identification is based on TLC R_f, GLC R_t, and MS. The amine was detected by dansylation and TLC in the symbiotic nitrogen fixing bacteria, *Rhizobium* spp (Rothamsted strain 1045, *R. leguminosarum*, host *Pisum* and in strain 3824, *R. phaseoli*, host *Phaseolus*) grown on agar plates for 11 and 17 days resp. at 25°. Neither putrescine, spermidine, nor spermine could be detected in the bacteria. No polyamines were found in the agar gel. This is the first record of *sym*-homospermidine in bacteria.

Ions at *m/e* 168 in the MS of the TFA derivatives of spermidine and homospermidine indicate the presence of $—(CH_2)_4NH_2$ in both amines. Ions at *m/e* 154 ($—(CH_2)_3NHCOCF_3$) are present in TFA-spermidine

Table 1. Polyamine levels (approximate) determined by dansylation in legume roots and root nodules, and in the symbiotic nitrogen fixing bacteria, *Rhizobium spp.* In the absence of sufficient authentic homospermidine, figures for this triamine are based on the spermidine standards

Source	nmol/g. fr. wt			
	Putrescine	Spermidine	Homospermidine	Spermine
Lupinus nodules	350	150	130	35
Phaseolus nodules	100	200	200	<20
Vicia nodules	930	290	840	<20
Vicia roots	350	60	<20	<20
Rhizobium leguminosarum	<20	<20	200	<20
Rhizobium phaseoli	<20	<20	500	<20

but absent from TFA-homospermidine. Absence of this ion in the sample of the nodule amine suggests that this polyamine is not $NH_2(CH_2)_3NH(CH_2)_5NH_2$, a triamine isomeric with *sym*-homospermidine. This conclusion is supported by the absence of *m/e* 182 (—$(CH_2)_5$-$NHCOCF_3$). Similarly the small abundance of *m/e* 196 and 210 indicates that the unknown is not an isomer of homospermidine having the structures $NH_2(CH_2)_2NH$-$(CH_2)_6NH_2$ or $NH_2CH_2NH(CH_2)_7NH_2$. MS (direct probe) of the dansyl derivative of homospermidine from pea root nodules at 275° showed a major ion at *m/e* 304.126 (—$(CH_2)_4$NH-dans). A further prominent peak at *m/e* 250 confirmed the presence of primary amino groups. The parent ion was below the limits of detection at 275°.

EXPERIMENTAL

Plants of *Lupinus albus* L. (Kievskij mutant), *Pisum sativum* L. var Meteor, *Phaseolus coccineus* L., and *Vicia faba* L., were obtained locally. *Rhizobium* was grown on yeast mannitol agar. (K_2HPO_4, 0.5 g; $MgSO_4$ 7 H_2O, 0.2 g; NaCl, 0.1 g; $CaCO_3$, 3 g; mannitol, 10 g; yeast extract (Oxoid L20 paste), 1 g; agar, 15 g; made up to 1 l., the pH adjusted to 6.8, and autoclaved at 120° for 15 min). Nodules and root tissues were washed in H_2O, macerated in 5% TCA (3 vol) and centrifuged. *Rhizobium* taken from the agar with a spatula, was added to 3 vol 5% TCA and the suspension used after 24 hr. After removal of TCA by shaking with Et_2O, the dansyl amines were prepared and estimated by the method of ref. [9].

Purification of nodule amines. An extract of 100 g of *Phaseolus* nodule tissue in 300 ml of 5% TCA was centrifuged and applied to a column (2 × 3 cm) of AG 50 W × 2, 200–400 mesh (H^+ form) without prior removal of TCA. Amino acids and salts were then eluted by a sequence modified from ref. [10]. After washing the column with 60 ml H_2O, the column was washed with NaPi buffer (60 ml) prepared by titrating together NaH_2PO_4 (0.1 M) and Na_2HPO_4 (0.1 M) to pH 8 and then adding NaCl to 0.25 M. The column was then washed in 60 ml of 0.5 M HCl and the amines were eluted with 60 ml of 6 M HCl. After evaporation to dryness amines were dissolved in 2 ml 0.1 M HCl. TLC of the dansylated amine fraction in cyclohexane–EtOAc (5:4) on Si gel G [9] showed spermidine (mobility relative to ammonia, R_{am} 0.60) and the unknown (homospermidine) (R_{am} 0.63), together with dansyl putrescine (R_{am} 0.80) and dansyl spermine (R_{am} 0.40). TLC of the dansylated amine fraction in $CHCl_3$–Et_3N (5:1) showed putrescine (R_f 0.51), spermidine (R_f 0.71), unknown (homospermidine) (R_f 0.74) and spermine (R_f 0.84). The unknown from the root nodules was identical with the authentic homospermidine on co-chromatography in both solvents.

GC–MS of nodule amines. The amine fraction was reapplied to the column of AG 50 W (H^+ form) and eluted in sequence with 0.5, 1, 2.5, 3, 4 and 6 M HCl (60 ml of each). After concentration by boiling, residue was made up to 2 ml with H_2O. TLC of dansyl derivatives of these fractions showed that spermidine and the unknown were eluted together in the 2.5 M HCl fraction. GLC was effected by a modification of the methods in refs. [11, 12]. A sample of the 2.5 M HCl fraction (0.1 ml) was evaporated to dryness under N_2 at 100° in a glass tube (1 × 10 cm) having a screw top with a PTFE seal. Acetonitrile (0.25 ml) and trifluoroacetic anhydride (TFAA) (0.25 ml) were added, the tube sealed and heated at 100° for 5 min. After cooling, the solvent and excess TFAA were removed at room temp under a stream of N_2, and the residue taken up in EtOAc (0.5 ml). GC–MS of 10 μl samples was effected on 2% OV-17 on Chromosorb-W HP (100–120 mesh) in a 4 mm i.d. × 1 m stainless steel column using He (15 ml/min) as carrier with a jet separator at 70 eV. The column was programmed at 10°/min from 100° to 250°. The following R_fs were found for TFA amine derivatives. 1,3-Propane diamine, 8.5 min; putrescine 10.5 min; cadaverine 11.5 min; spermidine 17 min; unknown (homospermidine) 18 min; spermine 23 min. GC–MS of the authentic and natural tri-TFA spermidine gave *m/e* 433, M^+ (2), 364, M − CF_3 (34); 336, M − CF_3CO (42); 320 (18); 307 (23); 293 (20); 279, M − $CF_3CONH(CH_2)_3$ (29); 223 (27); 168, $(CH_2)_4NHCOCF_3$ (34); 166 (61); 154, $CF_3CONH(CH_2)_3$ (100). GC/MS of the authentic and presumed tri-TFA *sym*-homospermidine gave *m/e* 447, M^+ (2); 378, M − CF_3 (30); 350, M − CF_3CO (30); 334 (16); 321 (27); 293 (53); 281 (7); 275 (25); 223 (28); 180 (11); 168, $(CH_2)_4NHCOCF_3$ (70); 166 (100).

Cellulose TLC. Co-TLC of the underivatized amine fraction on cellulose (CC41) in buffered phenol [13] with ninhydrin as chromogenic reagent showed homospermidine (R_f 0.33) and spermidine (R_f 0.17) in the amine fraction from the *Phaseolus* nodules, eluted in 2.5 M HCl. Spermine, which was not detected in this fraction, had R_f 0.11.

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