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AMINE LEVELS IN MINERAL-DEFICIENT *HORDEUM VULGARE* LEAVES

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Key Word Index—*Hordeum vulgare*; Gramineae; barley; putrescine; spermidine; spermine; agmatine; arginine; potassium and magnesium deficiencies; dansyl reagent.

Abstract—In potassium-deficient barley leaves, on the basis of fresh weight, putrescine was 13·3-fold greater and in magnesium deficiency 3·8-fold greater than controls. Putrescine was found to be reduced in phosphorus (× 0·6), sulphur (× 0·5) and nitrogen (× 0·2) deficiencies and on substituting nitrate for ammonium (× 0·2). Calcium deficiency and high salt (KCl) increased putrescine by 1·5-fold. High KCl reduced spermidine levels (× 0·2) without a corresponding reduction in spermine levels. The agmatine content was enhanced in magnesium- (× 2·5) and potassium- (× 5) deficient plants, while the arginine was increased × 1·4 and × 2·0 respectively on a fresh weight basis. Compounds tentatively identified as diaminopropane and 1-(3-aminopropyl)pyrroline were increased significantly with high KCl and low calcium. No homospermidine or diaminodipropylamine could be detected in any of the extracts, but traces of cadaverine may be present.

INTRODUCTION

THE ACCUMULATION of the diamine putrescine is well established as a response to potassium deficiency in many plant species.[1] However, putrescine accumulation has been found in certain plants deficient in nutrients other than potassium. Takahashi and Yoshida[2,3] using a semi-quantitative method demonstrated putrescine accumulation in the leaves of tobacco plants grown with potassium, sulphur and phosphorus deficiencies. The incorporation rate of DL-ornithine-[2-^{14}C] and L-arginine-[U-^{14}C] into putrescine were both enhanced in potassium- and sulphur-deficient tobacco plants. Radioactive putrescine was degraded more slowly in both these deficiencies, though isotope dilution could account for this effect. The level of agmatine, which is the precursor of putrescine, has been found to be increased in barley leaves by deficiencies of potassium (× 10), phosphorus (× 3), calcium (× 3), sulphur (× 3), magnesium (× 2) and manganese (× 2)[4], but of these treatments apparently only potassium deficiency increased the level of putrescine significantly.

Since a preliminary investigation had indicated that magnesium deficiency increases putrescine level, the effect of a series of mineral deficiencies and also of high salt concentrations on putrescine level in barley leaves has now been studied. The polyamines spermidine and spermine were also estimated, since these are derived metabolically from putrescine.

RESULTS AND DISCUSSION

The results for the amine and potassium analysis and the percentage dry weights for the leaves of the barley plants grown with various nutrient treatments after 4 and 6 weeks'

[1] MURTY, K. S., SMITH, T. A. and BOULD, C. (1971) *Ann. Botany* **35**, 687.
[2] TAKAHASHI, T. and YOSHIDA, D. (1960)*Nippon Dojo-Hiryogaku Zasshi* **31**, 39.
[3] YOSHIDA, D. (1969) *Plant Cell Physiol.* **10**, 393.
[4] SINCLAIR, C. (1967) *Nature* **213**, 214.

growth are given in Tables 1 and 2. The amines were estimated by GLC[5,6] or, after dansylation by TLC[7–10] (Table 3). The estimates of putrescine based on gas chromatography were higher than those based on dansylation (ratio 1:0·86, s.e. 0·17). On the basis of fresh weight, the putrescine level almost doubled between the fourth and sixth weeks in the control (+ ammonium) plants. There was a significant reduction in putrescine with age, by comparison with the control, in the plants grown in a nitrate medium and in those deficient in phosphate. In the phosphate-, magnesium- and potassium-deficient plants there was a significant reduction of spermidine with age ($P = 1\%$), and in sulphur deficiency a rise with age ($P = 1\%$). In all treatments there was a consistent increase in spermine content with age. The apparent reduction in spermidine with potassium deficiency is not in agreement with the results found in a previous study. Histidine, which almost coincides with spermidine in the solvent used in the earlier work, could have caused an over-estimation of this polyamine in the extracts of the potassium-deficient leaves.

TABLE 1. DI- AND POLY-AMINE LEVELS IN THE LEAVES OF BARLEY PLANTS GROWN IN A VARIETY OF NUTRITIONAL CONDITIONS

	Amine nmol amine/g fr. wt								
	Putrescine Mean of GLC and TLC estimates			Spermidine TLC			Spermine TLC		
Age (weeks)	4	6	P %	4	6	P %	4	6	P %
Treatment									
+ Ammonium	157	292		160	129		34	64	
+ Nitrate	62	25	1	103	80	1	18	30	1
+ Salt (KCl)	367	321	5	27	29	1	30	46	>5
− Phosphorus	146	104	1	64	43	1	23	35	1
− Sulphur	77	132	1	47	77	1	20	44	1
− Nitrogen	71	38	1	34	44	1	16	29	5
− Calcium	409	349	1	50	67	1	16	43	1
− Magnesium	1001	701	1	128	76	1	29	32	5
− Potassium	3104	2850	1	87	57	1	14	28	1
Standard error of treatment mean	41 nmol			14 nmol			8 nmol		

P is the probability for a significant difference from the ammonium control for the mean of the combined 4 and 6 week values. Each estimate was the mean of two determinations.

On substituting the ammonium in the medium by nitrate (Table 4), the levels of putrescine, spermidine and spermine were all decreased ($P = 1\%$). This phenomenon has been established previously for putrescine in barley[11] and tobacco,[2] and for putrescine, spermidine and spermine in soya (*Glycine max*).[12] This reduction in amine level in nitrate medium

[5] SMITH, T. A. (1970) *Phytochemistry* **9**, 1479.
[6] SMITH, T. A. (1970) *Anal. Biochem.* **33**, 10.
[7] SEILER, N. and WIECHMANN, M. (1967) *Z. Physiol. Chem.* **348**, 1285.
[8] SEILER, N. and WIECHMANN, M. (1970) *Progress in Thin Layer Chromatography* (NIEDERWIESER, A. and PATAKI, G., eds), Humphrey, Ann Arbor.
[9] SEILER, N. (1970) *Methods of Biochemical Analysis* (GLICK, D., ed.), Vol. 18, p. 259, Wiley, New York.
[10] HERBST, E. J. and DION, A. S. (1970) *Federation Proc.* **29**, 1563.
[11] COLEMAN, R. G. and RICHARDS, F. J. (1956) *Ann. Botany* **20**, 393.
[12] LE RUDULIER, D. and GOAS, G. (1971) *Compt. Rend.* **273**, 1108.

might be attributed to antagonism of potassium uptake due to the ammonium ion, since the level of potassium in the dry weight is enhanced in the plants grown in the nitrate medium by comparison with the ammonium controls (Table 2). However, this hypothesis is unlikely to be correct, since high potassium (+ KCl) does not depress the putrescine level; on the contrary, the putrescine level is raised by 50% ($P = 5\%$) with high potassium levels. Perhaps a more likely explanation could be found in a reduced level of soluble nitrogen-containing compounds in the plants grown in the nitrate-containing medium.

TABLE 2. DRY WEIGHTS, AND POTASSIUM, DIAMINOPROPANE, 1-(3-AMINOPROPYL)PYRROLINE AND CADAVERINE CONTENT OF THE LEAVES OF BARLEY PLANTS GROWN IN A VARIETY OF NUTRITIONAL CONDITIONS

			Amine nmol amine/g fr. wt*				
	% dry wt	Potassium % of dry wt	Diaminopropane GLC		1-(3-aminopropyl) pyrroline GLC		Cadaverine GLC
Age (weeks)	6	6	4	6	4	6	6
Treatment							
+ Ammonium	11·8	5·9	3	8	4	5	1
+ Nitrate	9·9	7·1	2	—	5	4	1
+ Salt (KCl)	21·6	12·8	7	9	24	23	1
− Phosphorus	15·6	6·2	3	3	8	6	1
− Sulphur	10·9	6·4	3	6	9	7	—
− Nitrogen	19·2	4·5	—	—	5	9	—
− Calcium	14·8	5·7	6	11	23	37	5
− Magnesium	10·3	6·7	—	—	9	13	5
− Potassium	16·3	0·6	—	25	—	—	2

* Each estimate was the mean of two determinations. See Table 1 for putrescine, spermidine and spermine levels. (— = not determined).

The low level of spermidine in the plants fed with a medium containing a high concentration of potassium chloride is particularly significant. Only in these plants was the ratio of spermine to spermidine below unity (1:0·75). The spermine level in this treatment was not significantly affected. An increased putrescine content with high salt (NaCl) concentration in the medium has been found in bean (*Vicia faba*) by Strogonov.[13] However, in that work barley did not show a similar response to high salinity and in the present study only a slight increase in putrescine was found.

A high osmotic concentration in the medium has been shown to reduce the putrescine level in the bacterium *Escherichia coli* by causing excretion of this diamine. This effect is specifically dependent on the presence of potassium ions; sodium, ammonium, rubidium or magnesium ions would not substitute for potassium in this role.[14] In a mutant of *E. coli* which cannot retain potassium, on replacement of potassium by sodium, putrescine biosynthesis is greatly increased (8- to 10-fold) and the spermidine content depressed.[15] Rubenstein *et al.* suggest that when the bacterium is depleted of potassium some of the roles of spermidine in nucleic acid structure and function may be fulfilled by putrescine.[15]

[13] STROGONOV, B. P. (1962) *Physiological Basis of Salt Tolerance of Plants*. Translation by Israel Program for Scientific Translations, Jerusalem, 1964.
[14] MUNRO, G. F., HERCULES, K., MORGAN, J. and SAUERBIER, W. (1972) *J. Biol. Chem.* **247**, 1272.
[15] RUBENSTEIN, K. E., STREIBEL, E., MASSEY, S., LAPI, L. and COHEN, S. S. (1972) *J. Bacteriol.* **112**, 1213.

By comparison with the ammonium control, potassium deficiency increased the putrescine content of the barley leaves 13-fold, similar to the increase found previously in potassium-deficient barley.[5] In the present work, the increased putrescine content of the calcium-deficient leaves (× 1·7), and in particular of the magnesium-deficient leaves (× 3·8), was of considerable interest. Thus, while by far the greatest putrescine levels are found in potassium-deficient leaves, putrescine accumulation is not specifically caused by potassium deficiency. Regarding the severity of the symptoms found with the mineral deficiencies, plants grown in conditions of potassium deficiency were very small, and large areas of the leaves were necrotic. In contrast, calcium- and magnesium-deficient plants were larger although very pale by comparison with the controls. The growth reduction alone cannot be correlated with putrescine accumulation, since both phosphorus and nitrogen deficiencies produced plants which were as stunted as those found in conditions of potassium deficiency yet contained less putrescine than the controls. In view of the large differences in size between the potassium-deficient plants and those plants grown in media deficient in calcium and magnesium, it appears possible that with comparable severity the putrescine levels of the magnesium-deficient plants, and even the calcium-deficient plants, might approach those found in the potassium-deficient material.

TABLE 3. TLC R_f VALUES ON KIESELGEL G AND GLC RETENTION TIMES ON A 2 METRE CARBOWAX 20M/KOH COLUMN

Amine	TLC 1 $CHCl_3$ 5 triethylamine 1 single displacement 60 min	TLC 2 cyclohexane 1 ethyl acetate 1 double displacement 40 min each	TLC 3 cyclohexane 3 ethyl acetate 2 double displacement 40 min each	GLC 150°	GLC 200°
Agmatine*	0	0	0·03	> 40 min	> 40 min
1-(3-Aminopropyl)pyrroline*	–	–	–	4 min 50 sec	1 min 52 sec
Ammonia†	0·48	0·62	0·48	—	—
Cadaverine	0·62	0·63	0·44	5 min 30 sec	1 min 59 sec
Diaminodipropylamine	0·71	0·51	0·29	30 min	5 min 40 sec
Diaminopropane	0·53	0·58	0·39	2 min 30 sec	1 min
Ethanolamine	0·28	0·27	0·17	3 min 45 sec	1 min 15 sec
Homospermidine	0·74	0·56	0·30	> 15 min	11 min
Putrescine	0·55	0·60	0·38	3 min 30 sec	1 min 21 sec
Spermidine	0·73	0·55	0·28	> 15 min	> 8 min
Spermine	0·86	0·49	0·20	> 15 min	60 min

* Probably fragment on dansylation.
† Not detected by GLC.

Coleman and Richards[11] suggested that the organic cation putrescine is formed in the leaves of potassium-deficient plants to replace the inorganic cation potassium. The induction in barley seedlings of the enzymes concerned in putrescine biosynthesis by acid feeding suggested a homeostatic mechanism for putrescine formation in the control of cellular pH.[16] In the control plants (ammonium medium) potassium and magnesium were about 6 and 0·25% of the dry weight and in the deficient plants 0·6 and 0·1% of the dry weight respectively. In terms of the fresh weight, the potassium level is reduced to 14% and the magnesium level to 35% of the normal by deficiencies of these nutrients. Since magnesium deficiency does not induce a low potassium status (Table 2) and as magnesium represents only a relatively small proportion of the total cations, putrescine formation in magnesium deficiency is unlikely to be attributable to a pH shift due to ionic imbalance as appears likely in potassium deficiency.

[16] SMITH, T. A. and SINCLAIR, C. (1967) *Ann. Botany* **31**, 103.

A more plausible explanation for the increased putrescine level in magnesium deficiency may be found in the relationship between magnesium and nucleic acids. It is already well established that *in vitro* magnesium causes aggregation of 30*S* and 50*S* ribosomes to give 70*S* ribosomes which are active in protein synthesis,[17,18] and it appears that this function of magnesium *in vivo* is fulfilled, at least in part, by spermidine in *E. coli*[18,19] and probably by spermidine and/or putrescine in higher plants.[20] In *E. coli*, spermidine bound to ribosomes varies inversely with the ribosome-bound magnesium on varying the magnesium level in the growth medium, while ribosome-bound putrescine levels are unaffected.[19] In the fungus *Neurospora crassa* a 10-fold decrease in magnesium level in the medium almost doubled the spermidine and spermine levels in the mycelium on a dry weight basis.[21] The putrescine content of this fungus is low and was not determined. It was suggested that the polyamines may here be interchangeable with the divalent cation magnesium in nucleic acid synthesis.

TABLE 4. COMPOSITION OF NUTRIENT SOLUTIONS IN MILLIMOLAR CONCENTRATIONS*

	NH_4	NO_3	+KCl	−P	−S	−N	−Ca	−Mg	−K
K_2SO_4	2·0	—	2·0	2·0	—	2·0	2·0	2·0	—
$MgSO_4$	1·5	1·5	1·5	1·5	—	1·5	1·5	—	1·5
$CaCl_2$	4·0	—	4·0	4·0	4·0	4·0	—	5·5	4·0
NaH_2PO_4	—	1·33	—	—	—	1·33	—	—	—
$NH_4H_2PO_4$	1·33	—	1·33	—	1·33	—	1·33	1·33	1·33
$(NH_4)_2HPO_4$	0·07	—	0·07	—	0·07	—	0·07	0·07	0·07
NH_4NO_3	6·0	—	6·0	6·0	6·0	—	6·0	6·0	6·0
$MgCl_2$	—	—	—	—	1·5	—	4·0	—	—
KCl	—	—	100	—	4·0	—	—	—	—
Na_2SO_4	—	—	—	—	—	—	—	—	2·0
KNO_3	—	4·0	—	—	—	—	—	—	—
$Ca(NO_3)_2$	—	4·0	—	—	—	—	—	—	—

Micronutrients (see Experimental) were added in each case.

Moreover, spermidine concentration in magnesium-deficient *E. coli* is increased 20-fold,[22] and spermidine and spermine supplied exogenously will partially overcome the reduction in growth due to magnesium deficiency in this bacterium.[23] The apparent increase of putrescine while the spermidine level remains unchanged in magnesium-deficient barley was therefore unexpected. However, although putrescine will not cause the aggregation of *E. coli* ribosomes, putrescine is effective in activating ribosomes isolated from a pseudomonad. The amount of ribosomal putrescine and hydroxyputrescine (here substituting for spermidine, which is absent) varies inversely with the magnesium concentration in the medium.[24] Putrescine may therefore be formed in the magnesium-deficient barley plants to substitute for this divalent ion in its role in the regulation of nucleic acid metabolism and function.

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[18] COHEN, S. S. (1971) *Introduction to the Polyamines*, Prentice-Hall, Englewood Cliffs, New Jersey.
[19] HURWITZ, C. and ROSANO, C. L. (1967) *J. Biol. Chem.* **242**, 3719.
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[22] HURWITZ, C. (1966) cited by COHEN, S. S. and RAINA, A. (1967) in *Organizational Biosynthesis*, p. 157, Academic Press, New York.
[23] MORUZZI, M. S. and BORGATTI, M. A. (1971) *Boll. Soc. Ital. Biol. Sper.* **47**, 839.
[24] ROSANO, C. L. and HURWITZ, C. (1969) *Biochem. Biophys. Res. Commun.* **37**, 677.

In contrast with the effects of phosphorus and sulphur deficiencies in tobacco,[2,3] these deficiencies resulted in putrescine, spermidine and spermine levels in barley leaves which were significantly lower than those in the controls. In barley, not all cation deficiencies induce putrescine. Iron and manganese deficiencies caused a loss of 50% in putrescine, while the spermidine and spermine levels were relatively unaffected.

TABLE 5. DRY WEIGHTS, ARGININE, AGMATINE AND PUTRESCINE CONTENT OF THE LEAVES OF NORMAL, MAGNESIUM- AND POTASSIUM-DEFICIENT BARLEY PLANTS, AGED 6 WEEKS

Treatment	Dry Wts		Arginine		Agmatine		Putrescine	
	%	Ratio	nmol/ g fr. wt	Ratio	nmol/ g fr. wt	Ratio	nmol/ g fr. wt	Ratio
Control	8·5	1	132	1	227	1	195	1
− Magnesium	10·0	1·18	185	1·40	524	2·51	1258	6·45
− Potassium	18·7	2·20	260	1·97	1080	4·77	2447	12·55

Standard error of the means is 9%; each estimate was the mean of two determinations.

Putrescine is known to be formed from arginine in barley leaves; the arginine is first decarboxylated to agmatine, and putrescine is formed from the agmatine by a two-stage hydrolysis.[25] In view of this, it was of interest to determine the change in the levels of arginine, agmatine and putrescine induced by magnesium and potassium deficiency (see Table 1 in Ref. 26). As 50% of the arginine in the 5% trichloroacetic acid (TCA) extracts was adsorbed to the Dowex-50 resin in the technique used for the preparation of amine fractions, the arginine and agmatine were separated on IRC-50 resin by a modification of a method originally developed for agmatine assay[27] (see Experimental). The results are presented in Table 5. The arginine content of the potassium-deficient leaves was found to be approximately doubled, while the putrescine content was increased by a factor of 12·6. The agmatine level was increased 4·8-fold confirming the mechanism previously proposed,[25,27] in which the enhanced putrescine content of potassium-deficient barley leaves is attributed to the increased activity of the enzymes converting arginine to putrescine. An analogous relationship between arginine, agmatine and putrescine was found in the magnesium-deficient barley plants, suggesting a similar enhancement of these enzymes in the absence of magnesium.

The peak attributable to diaminopropane was difficult to determine in those extracts containing large amounts of putrescine, i.e. from magnesium- and potassium-deficient plants. There was a correlation between the amount of presumed diaminopropane and of 1-(3-aminopropyl)pyrroline, as might be expected from their common origin as the oxidation products of spermine.[28] Both amines were increased in calcium deficiency and with potassium chloride feeding (Table 2), and this result was again obtained on repeating the experiment. The reason for the increase in these amines under these conditions is at present obscure.

[25] SMITH, T. A. (1971) *Biol. Rev. Cambridge Phil. Soc.* **46,** 201.
[26] SINCLAIR, C. (1969) *Plant Soil* **30,** 423.
[27] SMITH, T. A. (1963) *Phytochemistry* **2,** 241.
[28] SMITH, T. A. (1972) *Phytochemistry* **11,** 899.

Although cadaverine has been found in *Glycine*, *Pisum* and *Vicia* plants,[12,13,29,30] a GLC peak with a retention time corresponding to this diamine was barely detectable in the amine fractions of the barley leaf tissue, and the maximum level of cadaverine was about 1 nmol/g fresh weight (Table 2). The upper level for cadaverine is therefore about 0·5% of the putrescine in the normal plants, although it may rise to 5 nmol/g fresh weight in the calcium- and magnesium-deficient plants. Neither *sym*homospermidine[31–33] nor diaminodipropylamine[34] could be found in the barley amine extracts.

EXPERIMENTAL

Plant material. Barley seed (*Hordeum vulgare* L., cv. Zephyr) was sown in sand in polythene pots in a greenhouse on 31 August 1972 (Tables 1 and 2) or 6 November 1972 (Table 5). The sand was watered daily with the nutrients shown in Table 3. In addition, the following micronutrients were included as the compounds shown: FeEDTA, 5·6 ppm; $MnSO_4.4H_2O$, 0·5 ppm; $CuSO_4.5H_2O$, 0·06 ppm; $ZnSO_4.7H_2O$, 0·07 ppm; H_3BO_3, 0·66 ppm; $Na_2MoO_4.2H_2O$, 0·05 ppm; NaCl, 1·45 ppm; $CoSO_4$, 0·01 ppm; and $NaVO_3$, 0·01 ppm. Barley plants grown in the absence of iron and manganese were also investigated. Duplicate samples (10 g) of the upper fully expanded leaves were extracted in 80 ml of 5% TCA for putrescine, spermidine and spermine estimation. Similar 10 g samples were extracted in duplicate in 80 ml of 80% EtOH for arginine and agmatine determination. Other samples (5 g) were taken for dry wt, K and Mg estimations.

Amine fractions were prepared from the TCA extracts by the ion-exchange resin method of Smith.[5] Using this technique, the amines in the 10 g leaf samples were isolated and dissolved in 1 ml 0·1 N HCl. Duplicate estimates (by GLC) of recovery of 2 mg putrescine (2 HCl) by the resin procedure in the presence of a TCA extract of 10 g barley leaves were 86 and 90%. On removal of the TCA by Et_2O extraction (1 vol.) prior to treatment with the resin, the recovery was 94%. Recoveries of spermidine and spermine in the presence of plant material by the resin technique have been studied previously.[5]

Estimation of putrescine, spermidine and spermine by the dansyl technique. Putrescine, spermidine and spermine were estimated by a modification of the 'dansyl' method.[7–10] The amine fraction (0·1 ml) was placed in 5 ml glass stoppered tubes with 50 mg $NaHCO_3$ and 0·2 ml 1-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl chloride) (300 mg in 10 ml acetone) for 16 hr at room temp. 15 mg proline dissolved in 0·1 ml H_2O was then added, and after 30 min the dansylated amines were extracted into 2·5 ml toluene by vortex mixing for 15 sec. The tubes were then centrifuged and 2–10 μl samples of the toluene layer were applied to origins 1·5 cm apart on a 20 × 20 cm Kieselgel G thin layer plate (250 μm thick) which had been activated at 100° for 2 hr.

In early experiments (Tables 1 and 2), dansyl-putrescine was isolated by solvent 1 (Table 3) and dansyl-spermidine and -spermine were isolated with solvent 2. Subsequent work (Table 5) showed that better separation could be obtained by running the plates with a pad of Whatman No. 1 paper (wt 50 g) fastened to the upper edge of the plate. The solvent soaking into the pad enabled the putrescine derivative to travel 80% of the length of the plate within 2·5 hr. Dansyl-putrescine was isolated in solvent 3. The progress of the spots was monitored by their fluorescence observed on brief exposure to a UV lamp during chromatography.

Immediately on removal from the tank, the plate was sprayed with 25 ml of a mixture of triethanolamine–isoPrOH (1:4) in a horizontal band about 10 cm in height to cover the area of the plate containing the dansylated amines to be estimated. The spray was applied progressively during a period of about 15 min to avoid the formation of 'puddles'.

After about 20 min, when the isoPrOH had evaporated, the plate was dried over silica gel under vacuum for 16 hr. The plates were then equilibrated in the room used for scanning for 1 hr, and after marking the line of each separation with spots of rhodamine B (0·5% in EtOH) above and below the dansylated amines, the fluorescent dansyl spots were scanned using the Vitatron TLD 100 universal densitometer (mode, lin + II; level e; zero C6; span 10·0; slit 0·25 mm; amplitude 1 cm). The light emission at 507 nm was determined on irradiation with light of 365 nm. Response was linear with at least up to 5 mM spermine or spermidine and 10 mM putrescine in the amine fraction. At 50 mM putrescine, the calibration line was low by 30%, and

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[30] WANG, L. C. and SELKE, E. (1973) *Plant Physiol.* **51**, 432.
[31] KUTTAN, R., RADHAKRISHNAN, A. N., SPANDE, T. and WITKOP, B. (1971) *Biochemistry* **10**, 361.
[32] KUTTAN, R. and RADHAKRISHNAN, A. N. (1972) *Biochem. J.* **127**, 61.
[33] KUPCHAN, S. M., DAVIES, A. P., BARBOUTIS, S. J., SCHNOES, H. K. and BURLINGAME, A. L. (1969) *J. Org. Chem.* **34**, 3888.
[34] JOHNSON, M. W. and MARKHAM, R. (1962) *Virology* **17**, 276.

at 100 mM by 50%. As standards, 10 μl of a solution containing 10 μmol/ml of the amine in 0·1 N HCl were made up to 0·1 ml with 0·1 N HCl prior to dansylation. Standards were run on all plates used for the quantitative estimation of the amines. The ratio of fluorescence for equimolar quantities of putrescine, spermidine and spermine were 1:1·15:1·38 respectively.

2-D chromatography in solvent 2 followed by solvent 1 indicated that dansyl-spermidine and -spermine were not coincident with any other spots in solvent 2. Dansyl-putrescine was isolated by solvent 1 for all treatments except high salt and low calcium in which it was superimposed by an unknown yellow fluorescing spot. In this solvent, the dansyl-putrescine spot became rather elongated. In solvent 2, dansyl-putrescine was difficult to estimate due to its proximity to the dansyl derivative of ammonia, and in plant extracts it is superimposed by a red fluorescing spot (probably dansyl *N*-methyl tyramine). In solvent 3, putrescine forms a very discrete spot and coincides only with diaminopropane which is present in relatively very small amounts. An unidentified fluorescent spot was superimposed on dansyl-spermine in this solvent. Although more than one spot was found on dansylation of agmatine, contrary to an earlier report,[8] dansyl-putrescine was not detected as one of the products. 1-(3-Aminopropyl)pyrroline also fragmented on dansylation, the major fluorescent products being found at R_f 0·24 and 0·02 in solvent 3, with a minor component at R_f 0·18.

GC. The column parameters of Smith[5,6] were used, putrescine being estimated at 200° and diaminopropane, 1-(3-aminopropyl)pyrroline and cadaverine at 150°. The retention times are given in Table 3. The 1-(3-aminopropyl)pyrroline, used as standard, was formed by the stoichiometric oxidation of spermidine by pea seedling diamine oxidase.[28]

Determination of arginine and agmatine (Table 5). HOAc (10 ml) was added to the EtOH leaf extracts to prevent possible adsorption of the agmatine to carboxyl and phosphate groups in cell debris. After centrifuging, the supernatant was evaporated to dryness under vacuum. The residue was dissolved in 10 ml of 0·1 N NH_4OH and shaken with 1 ml (wet vol.) of IRC-50 resin (H^+) for 30 min. The resin was then washed in 10 ml H_2O for 30 min. The arginine was eluted on shaking the resin with 10 ml of 4 N NH_4OH for 30 min. After washing the resin again with 4 N NH_4OH (10 ml) for 30 min and discarding this solution, the agmatine was eluted on shaking the resin for 30 min in 10 ml of sat ammonium carbonate. The solutions containing the arginine and agmatine were evaporated to 1 ml by boiling, and the residues made up to 2 ml with H_2O. Estimations were made on 50–200 μl samples by the Sakaguchi reagent[27] at 490 nm.

Potassium and magnesium were estimated by flame photometry and atomic absorption spectrophotometry respectively after digestion of 100 mg samples of the dried ground leaf material in 2 ml conc. H_2SO_4 with H_2O_2 as oxidant.

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