The Micro-estimation and Origin of Methylamine in *Mercurialis perennis* L.

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The occurrence of methylamine in the tissues of higher plants appears to be very restricted. Schmidt (1878) isolated this amine from the shoots of *Mercurialis perennis* and *M. annua*. Trier (1912) reported the presence of methylamine in the rhizome of *Acorus calamus*, and Wakeman (1925) showed that it was present in the root of *Leptotaenia dissecta*. Other workers have shown that methylamine may be produced as the result of bacterial action on amino-acids and nitrogenous bases. Thus Ackermann & Schütze (1910, 1911) found methylamine in cultures of *Chromobacterium prodigiosum* (*Serratia marcescens*) on potato, and Hasebroek (1887) obtained this amine from anaerobic putrefaction of choline. Emmerling & Reiser (1902) isolated methylamine from cultures of *Pseudomonas fluorescens liquefaciens* on gelatin. Guggenheim (1940) gives references to papers reporting the occurrence of methylamine in algae. In animal tissues Kohn (1931) found methylamine as an intermediate in the degradation of glycine in the liver and Ratner, Nocito & Green (1944) showed that the glycine oxidase of pig kidney catalyses the oxidation of sarcosine to methylamine and glyoxylic acid. Klein & Steiner (1928) assessed the production of methylamine in *Mercurialis annua* and *M. perennis* by means of the dinitro- α -naphthol derivative, but up to the present time a specific method for the accurate estimation of small amounts of methylamine in plant tissues does not appear to have been developed. The aim of the present work was to devise a sensitive and specific method for the estimation of very small quantities of methylamine and to study the mode of production of this base in *M. perennis* by the infiltration of possible precursors into the leaves.

EXPERIMENTAL AND RESULTS

The isolation of methylamine from tissues of Mercurialis perennis

Flowering shoots (15 kg.) were harvested over a period of 17 days in batches of 1 kg.; each kg. was divided into portions of 50 g. which were immersed in ether for 10 sec., the ether allowed to evaporate and the material minced for 5 min. in a Waring blender with 100 ml. distilled water acidified to pH 4.5 with 0.01N-HCl. The mince was filtered through fine muslin and as much liquid as possible removed from the cell debris by squeezing. The filtrates from

each portion were combined, rapidly heated to 80° to coagulate protein and centrifuged. The supernatant was treated with excess of $Mg(OH)_2$ and steam-distilled into 0.1N-HCl (25 ml.) until 100 ml. of distillate had passed over. This procedure was repeated until 15 kg. of material had been extracted. NH_3 was removed at pH 7.4 from the combined distillates (1875 ml.) by the method of François (Pugh & Quastel, 1937), the filtrate from the François separation treated with excess of $Mg(OH)_2$ and distilled into 50 ml. of a solution of picrolonic acid (70 mg.) in ethanol. The picrolonate solution was concentrated to small bulk and allowed to crystallize. After two recrystallizations from aqueous ethanol, 35 mg. of picrolonate was obtained which melted at 242° (decomp.). The melting point of an authentic specimen of methylamine picrolonate was 243° (decomp.); mixed m.p., 242°. (Found: N, 23.50, calc. for $CH_5N \cdot C_{10}H_8O_6N_4$: N, 23.73%.)

The micro-estimation of methylamine

Methylamine reacts quantitatively with ninhydrin in acid solution to give formaldehyde and NH_3 . Glycine and *N*-methylethanolamine also react with ninhydrin under similar conditions to give formaldehyde. In the case of *N*-methylethanolamine, the reaction is presumably due to the splitting off of methylamine. Alexander, Landwehr & Seligman (1945) have adapted this reaction for the estimation of glycine in blood by determining the liberated formaldehyde colorimetrically with chromotropic acid. The reaction can be made specific for methylamine, as this amine is readily volatile and can easily be separated from glycine and *N*-methylethanolamine by distillation at low temperature. *N*-methylethanolamine is slightly volatile in steam, but does not distil over if the distillation is carried out at a low temperature under reduced pressure. For the estimation of methylamine in tissues of *M. perennis*, the method of Alexander *et al.* (1945) for glycine was used with minor modifications. The intensity of the reddish violet colour given by formaldehyde with chromotropic acid was measured with the Hilger Spekker absorptiometer using the Ilford spectrum yellow filter. For the calibration curve, pure methylamine hydrochloride was used. Leaves (20–50 g.) were immersed in ether for 10 sec. and the ether allowed to evaporate. The leaves were minced in a Waring blender with 50–75 ml. distilled water acidified to pH 4.5 with 0.01N-HCl. The mince was filtered through fine muslin, squeezed out, and the cell debris washed twice with 10 ml. distilled water. The filtrate and washings were rapidly heated to 80°, centrifuged, the clear supernatant poured off and the residue washed once with 10 ml. distilled water. The extract was transferred to a distillation flask, treated with excess of $Mg(OH)_2$ and distilled *in vacuo* at 40° into 0.01N-HCl (5 ml.) until the volume was 15 ml. To concentrate the methylamine

solution further at low temperature, the microdiffusion technique of Conway & Byrne (1933) was used. Large dishes were constructed from glass capsules of 9.5 and 4.5 cm. diameter. A portion (10 ml.) of the distillate was placed in the outer chamber and 1 ml. 0.01 N-HCl in the inner vessel. Saturated K₂CO₃ (5 ml.) was added to the outer chamber and the dishes incubated at 30° for 16–18 hr. The methylamine solution was transferred quantitatively to a micro-distillation apparatus and the estimation carried out as for glycine (Alexander *et al.* 1945). The accuracy of the method was tested by adding a known amount of pure methylamine hydrochloride to the mince in the Waring blender. An average recovery of 95% was obtained. The method is sensitive to 3 µg. of methylamine.

Infiltration experiments

Wild plants of *M. perennis* and *Chenopodium album* L. were used; *C. vulvaria* L., *Beta vulgaris* L. and *Atropa belladonna* L. were raised from seed. Leaves (20–50 g.) of *Mercurialis perennis* were infiltrated with solutions of nitrogenous bases and amino-acids in an attempt to determine the source of methylamine in the tissues. Solutions of the bases (or their salts) and amino-acids were neutralized (pH 7.0) where necessary and made to 0.05 M. *N*-Methylethanolamine and dimethylethanolamine were prepared by the method of Knorr & Matthes (1898, 1901). *N*-Methylethanolamine and dimethylamine hydrochlorides were purified by means of the nitroso derivatives, and traces of methylamine found in the sample of trimethylamine hydrochloride were removed by treatment with nitrous acid and subsequent distillation. The vacuum infiltration method of Björkstén (1930) was employed, and, after infiltration and evaporation of excess water, the leaves were kept in a moist atmosphere in darkness for 40 hr., after which they were analysed for methylamine. In Table 1 the methylamine content of leaves of *M. perennis* removed from plants in April (plants in flower), June, July and August and infiltrated with solutions of nitrogenous bases and amino-acids is given.

acids, choline, and betaines. Thus trimethylamine present in *Chenopodium vulvaria* (Dessaignes, 1852) may arise as a breakdown product of choline, and methylamine may be formed as the result of the bacterial decomposition of glycine. Although volatile amines have been reported as occurring in a wide range of plants, their concentration is generally found to be low. However, according to Kapeller-Adler & Vering (1931) trimethylamine may reach 500 mg./100 g. dry weight in the red algae *Ceramium rubrum* and *Rhodomela subfusca*, and for methylamine a maximum of 40 mg./100 g. dry weight was recorded in the green algae investigated. Up to the present time little or no light has been thrown on the precise mechanism of formation of methylamine and trimethylamine in plants. Decarboxylation of glycine would seem to be the simplest and most obvious reaction leading to the formation of methylamine, but a glycine decarboxylase has not yet been described (Gale, 1948) and infiltration of glycine into the leaves of *Mercurialis perennis* does not lead to a significant increase of methylamine. The experiments described above show that breakdown of methylethanolamine with the formation of methylamine takes place readily in the leaves of *M. perennis*, but they do not prove conclusively that the methylamine normally found in the tissues is produced from methylethanolamine. These experiments do suggest, however, that methylethanolamine is the precursor of methylamine even though the mode of fission is not yet known. By analogy with the behaviour of choline on heating, breakdown may take place with the formation of ethylene glycol.

In the tissues of *Chenopodium album*, *C. vulvaria*, *Beta vulgaris* and *Atropa belladonna*, methylethanolamine does not appear to be broken down with

Table 1. *Methylamine content of leaves of Mercurialis perennis after infiltration with various possible precursors*

Solution infiltrated	Methylamine (µg./100 g. wet wt., to nearest µg.)						
	April				June	July (Mean value of 2 samples)	August
	(1)	(2)	(3)	Mean			
Tap water	40	45	44	43	7	Trace	Trace
Ethanolamine	62	54	55	57	16	Trace	Trace
<i>N</i> -Methylethanolamine	135	146	127	136	93	76	60
Dimethylethanolamine	46	48	43	46	7	Trace	Trace
Choline (chloride)	46	50	50	49	9	Trace	Trace
Betaine (hydrochloride)	40	44	45	43	8	Trace	Trace
Dimethylamine (hydrochloride)	47	46	43	45	8	Trace	Trace
Trimethylamine (hydrochloride)	46	42	40	43	6	Trace	Trace
Glycine	53	49	50	51	12	Trace	Trace
Sarcosine	42	39	42	41	8	Trace	Trace

DISCUSSION

In living tissues the lower aliphatic amines are generally regarded as fission products of more complex nitrogenous substances such as amino-

liberation of methylamine. If fission does take place in these tissues, the methylamine formed must immediately be removed. Recent work on the biosynthesis of choline summarized by Jukes (1947) has provided strong evidence for the formation of choline

by the stepwise methylation of ethanolamine, and Artom & Cornatzer (1948) have shown that administration of ethanolamine, methylethanolamine and dimethylethanolamine to rats stimulates the formation of total phospholipins. In higher plants experimental evidence in support of this mode of synthesis of choline is lacking. Attempts in this laboratory to isolate methylethanolamine from the tissues of *Mercurialis perennis* have so far failed, but there is at least presumptive evidence for its existence in this plant if the methylamine normally present is derived from it. The slight rise in methylamine content resulting from infiltration of glycine and ethanolamine may be explained on the assumption that these substances can give rise to methylethanolamine in the tissues. The following scheme is put forward to show possible reactions leading to the formation of the methylated amines in plants:

Choline and betaine act as methyl donors in the tissues of the rat (Stetten, 1941; Du Vigneaud,

Chandler, Simmonds, Moyer & Cohn, 1946; Du Vigneaud, Simmonds, Chandler & Cohn, 1946), but one methyl group only is labile. As a result of partial demethylation dimethylethanolamine and dimethylglycine are formed respectively. These substances cannot act as methyl donors and their breakdown, together with that of methylethanolamine and sarcosine, involves either the splitting off of the methyl group in combination with nitrogen as dimethylamine and methylamine; or, in the case of dimethylglycine and sarcosine, oxidative demethylation may take place with the formation of formaldehyde and glycine (Handler, Bernheim & Klein, 1941).

SUMMARY

1. Methylamine has been isolated from the tissues of *Mercurialis perennis*.
2. A sensitive and specific micromethod for the estimation of methylamine in plant tissues has been developed.
3. Infiltration of the leaves of *M. perennis* with methylethanolamine leads to a substantial increase in the content of methylamine.
4. Methylethanolamine is regarded as the probable precursor of methylamine in *M. perennis*.

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