Phytochemistry, 1973, Vol. 12, pp. 443 to 446. Pergamon Press. Printed in England.

IDENTIFICATION OF CADAVERINE IN *PISUM SATIVUM*

J. N. ANDERSON and R. O. MARTIN*

Department of Biochemistry, University of Saskatchewan, Saskatoon, Canada

(*Received* 7 *August* 1972. *Accepted* 1 *September* 1972)

Key Word Index—*Pisum sativum*; Leguminosae; diamine; cadaverine; mass spectrum.

Abstract—Cadaverine has been identified in normal young peas and in saline grown (necrotic) peas by GLC and MS. Its concentration was at least of 5 μg/g fresh plant in both cases. Analysis of the alkaloidal extracts failed to reveal any lupinine, anabasine or other pyridine alkaloids in normal or necrotic, salt grown, peas.

INTRODUCTION

CADAVERINE, $NH_2(CH_2)_5NH_2$, the decarboxylation product of lysine has long been implicated in the biosynthesis of the quinolizidine, piperidine and other types of alkaloids.[1–4] However, the only reported occurrences of cadaverine in plants has been as a minor component among many amines and diamines produced by certain Arum lilies during anthesis[5] in the germ of grains[6] in old (necrotic) peas[7] and young necrotic peas grown in saline conditions.[8] In all cases, direct chemical evidence has been lacking, with identification being based on behaviour in one or several PC systems at the most.

On the other hand, peas, not normally considered to be alkaloid forming plants, contain lysine decarboxylase activity[9] as well as diamine oxidase which oxidizes cadaverine to the corresponding amino aldehyde which spontaneously cyclizes to Δ^1-piperideine.[10] The latter is a highly reactive enimine which can undergo autocondensation to the dimeric tetrahydroanabasine and further polymeric products.[11] Finally on the basis of their behaviour in a single PC system, the alkaloids lupinine, anabasine, trigonelline and other 'pyridine condensates' have been reported in this plant grown under saline conditions.[8]

In the present study peas were examined for cadaverine and lupinine preparatory to examination of the role of cadaverine in the formation of such alkaloids in several *Lupinus* species.[12]

* Supported by grants from the National and Medical Research Councils of Canada.

[1] R. ROBINSON, *The Structural Relations of Natural Products*, Clarendon Press, Oxford (1955).
[2] I. D. SPENSER, *Comprehensive Biochem.* **20**, 231 (1968).
[3] E. LEETE, in *Biogenesis of Natural Products* (edited by P. BERNFELD), 2nd Edn, p. 953, Pergamon Press, Oxford (1967).
[4] H. R. SCHÜTTE, in *Biosynthese der Alkaloide* (edited by K. MOTHES and H. R. SCHÜTTE), p. 324, VEB. Deut. Verlag. Wiss., Berlin, D.D.R. (1969).
[5] B. N. SMITH and B. J. D. MEEUSE, *Plant Physiol.* **41**, 343 (1966).
[6] G. MORUZZI and C. M. CALDARERA, *Arch. Biochem.* **105**, 209 (1964).
[7] J. K. MIETTINEN, *Ann. Acad. Sci. Fennicae*, **AII**, 520 (1955); *Chem. Abs.* **49**, 84051 (1955).
[8] L. S. PRIKHOD'KO and L. K. KLYSHEV, *Tr. Inst. Botan. Akad. Nauk. Kaz. SSSR* **20**, 166 (1964); *Chem. Abs.* **64**, 16283b (1965).
[9] K. HASSE, O. T. RATYCK and J. SALNIKOW, *Z. Physiol. Chem.* **348**, 843 (1967).
[10] P. J. G. MANN and W. R. SMITHIES, *Biochem. J.* **61**, 89 (1955).
[11] C. SCHOPF, H. ARM and F. BRAUN, *Chem. Ber.* **85**, 937 (1952).
[12] J. N. ANDERSON and R. O. MARTIN, *J. Am. Chem. Soc.* to be published.

RESULTS AND DISCUSSION

Freshly cut shoots of 13-day-old peas grown on either water or 1% NaCl were immediately extracted for polyamines. Electrophoresis of an aliquot of this extract revealed a major component with electrophoretic mobility and ninhydrin color indistinguishable from authentic cadaverine (Table 1). GLC of both salt- and water-grown samples showed that the R_t of the major component was identical to that of authentic cadaverine. The MS of samples (50 μg) collected from GLC and of authentic cadaverine were identical, confirming the presence of cadaverine in normal and necrotic peas. This is the first unequivocal report of this diamine as a natural constituent in the vegetative parts of a normal plant. The level of cadaverine was 7 μg/g fresh wt in water-grown pea shoots and 5·5 μg/g fresh wt in the shoots of salt-grown peas.

TABLE 1. GLC AND ELECTROPHORETIC BEHAVIOUR OF POLYAMINES

Polyamine	GLC retention*	Electrophoretic mobility†	Polyamine	GLC retention*	Electrophoretic mobility†
Putrescine	2·5	−8·9	Hexamethylenediamine	8·0	−6·5
Cadaverine	4·5	−7·5	Spermidine	11·0‡	—

* Carbowax 20 M column at 100° isothermal.
† Cm from center of 20 cm plate after 90 min.
‡ At 150° isothermal.

These studies do not prove that cadaverine is produced in the shoots and it is possible that it is synthesized in the cotyledons which contain the majority of the lysine decarboxylase and diamine oxidase activity[13] and is then transported to the shoots. Though the age chosen here is close to that when diamine oxidase activity is reported to be maximal[13] it would be of interest to determine whether there is any correlation between its appearance and the concentration of cadaverine.

Four minor components were observed in the polyamine extract but were not further examined. One had a GLC R_t close to that of putrescine, one between cadaverine and hexamethylene diamine, and two longer than hexamethylene diamine. Extraction of cadaverine by the procedure described here does not appear to have been previously reported and offered certain advantages over that of steam distillation, principally that of lower losses permitting recoveries of amounts less than 100 μg. Unfortunately, the requirement for super heated steam, though predictable[14] is not indicated in the relevant literature.[15] This procedure overcomes the interference from polyphenols encountered during attempted ion-exchange separation of the diamines from the aqueous extract.

TLC of the alkaloid extract of normal peas (whole plants) revealed no alkaloidal material prior to 5 or 6 days of growth. Separate analysis of the roots, shoots and cotyledons of 600 g of 12-day-old plants showed that the cotyledons contained >90% of the alkaloidal material with the roots and shoots containing very small amounts of only four constituents (R_fs 0·82, 0·10, 0·07, 0·03). The major alkaloid (R_f 0·22) of the cotyledons, absent in the roots and shoots was very volatile. Comparison with lupinine, anabasine, nicotine and

[13] R. H. KENTEN and P. J. G. MANN, *Biochem. J.* **50**, 360 (1952).
[14] K. B. WIBERG, *Laboratory Techniques in Organic Chemistry*, p. 71, McGraw-Hill, New York (1960).
[15] H. R. SCHÜTTE, D. KNÖFEL and O. HEYER, *Z. Pflanzen Physiol.* **55**, 110 (1966).

isotripiperideine (a polymer of Δ^1-piperideine) revealed no such components to be present in the pea extracts. Konig's reaction using cyanogen bromide for the presence of pyridines[16] was also negative. The alkaloid levels were very low, such that the total extract from a minimum of 10 g of fresh peas was required for minimal visualization of the alkaloids by iodoplatinate reagent after TLC.

GLC of the pea alkaloids revealed two groups of peaks, the first and major component eluting within 3 min at 25° followed by a rather complex group eluting between 180 and 230°. The reference alkaloids, anabasine, lupinine and sparteine eluted between 120 and 160° where none appeared from the pea extract.

Similarly GLC and TLC analysis of the alkaloidal extracts of 400 g of 13-day-old peas grown in 1% NaCl failed to reveal any lupinine, anabasine, or pyridines. In this case, however, the concentration of alkaloid in the plant appeared to be approximately 2–3 times that of the water-grown peas. While no further attempt was made to characterize the components of the alkaloidal material from either, it was concluded that lupinine, anabasine or other pyridine condensates are not found in detectable amounts in normal or necrotic peas, contrary to the previous report of their occurrence in salt-grown peas[8] which was based on a single PC system.

EXPERIMENTAL

Plant material. Pisium sativum L. var. 'Homoesteader' were soaked overnight in either tap H_2O or 1% NaCl solution, then planted in vermiculite. The plants were grown in a controlled environment chamber at 90% relative humidity with a 16-hr light period at 32° and an 8-hr dark period at 25°. Plants were watered as required using tap H_2O or 1% NaCl respectively.

Extraction. Fresh plants were washed, the cotyledons removed, and immediately macerated in 80% MeOH. After filtering under vacuum, the solids were resuspended in EtOH, filtered and the combined filtrates acidified (pH < 2) with conc. HCl. The MeOH was evaporated *in vacuo*, the residue taken up in 0·5 N HCl and filtered under vacuum using Celite to reduce clogging of the filter. This aq. acid extract was then washed once with CH_2Cl_2. Pollyamines were recovered by making the acidic extract basic (pH > 11) with 10 N NaOH and extracting with 9 × 1·5 vol. CH_2Cl_2. This basic extract was subsequently dried over Na_2SO_4, acidified with HCl gas, and dried *in vacuo*. The residue was transferred in MeOH to vials, reduced to dryness under N_2 and stored at 5°. Recoveries of 20–100 μg each of putrescine, cadaverine and hexamethylenediamine were 33, 60 and 90% respectively. Attempted recovery of cadaverine by steam distillation as previously reported[15] was poor (< 10%). Only by use of superheated steam (330°) could recoveries approaching those obtained by the extraction procedure be obtained but only for amounts > 1 mg. Alkaloids were recovered from the acid extract as described previously.[17] Prior to removal of CH_2Cl_2 from the final extraction, it was acidified with anh. HCl to prevent the loss of any volatile alkaloids.[18]

Electrophoresis. Amines were separated by thin-layer electrophoresis, using Silica gel G (500 μm thick), and pyridine–HOAc–H_2O (5:1:95) buffer (pH 6). A voltage gradient of 40 V/cm was applied for 60–90 min. Amines were visualized with 0·2% ninhydrin in acetone.

TLC. Alkaloid extracts were separated on Silica gel G, using a freshly prepared solvent composed of $CHCl_3$–MeOH–conc. NH_3 (85:14:1).[19] Alkaloids were revealed with iodoplatinate reagent;[20] pyridine type components were detected by exposing the plates to cyanogen bromide vapour followed by spraying with 1% *p*-aminobenzoic acid in MeOH.[16]

GLC. GLC was carried out with a hydrogen flame detector on 0·6 m × 4 mm i.d. U-shaped Pyrex glass columns. For polyamines the columns were filled with Chromosorb W (AW only) coated first with 20% KOH then 20% carbowax 20 M. Both coatings were dissolved in a minimum amount of MeOH, the Chromosorb added, and the MeOH removed *in vacuo*. Columns were conditioned at 180° for 3 hr, then operated at 100° to resolve cadaverine, putrescine and hexamethylene-diamine. Diamines were collected from the column effluent in a capillary tube[17,19] with the tube bent to allow immersion in ice. The ends of the capillary tubes, immediately sealed with a flame were stored at 0° until introduction into the MS. Alkaloid extracts were analyzed using a 10% QF-1 column prepared as previously reported[17,18] and programmed

[16] C. F. Huebner, *Nature, Lond.* **167,** 119 (1951).
[17] Y. D. Cho and R. O. Martin, *Can. J. Chem.* **49,** 265 (1971).
[18] S. M. C. Dietrich and R. O. Martin, *Biochemistry* **8,** 4163 (1969).
[19] Y. D. Cho and R. O. Martin, *Anal. Biochem.* **44,** 49 (1971).
[20] R. Mumer and M. Macheboeuf, *Bull. Soc. Chem. Biol.* **144,** 31 (1949).

at 5°/min from 25 to 230°. Prior to injection, the extracts, stored as their HCl salts, were converted to the free base form with anh. ammonia after dissolving in 0·5 ml MeOH.[18] Though the HCl salts could be injected directly, the useful life of the column was considerably shortened. MS were determined using an A.E.I. MS12 instrument at 70 eV. Samples contained in capillary tubes were inserted by means of a probe directly into the ion source.

Reference compounds. Lupinine was a gift from Professor E. Wenkert, Indiana University. Anabasine was a gift of Professor H. Rapoport, University of California. Isotripiperideine was synthesized from piperidine.[11]