reaction mixture was collected by distillation which gave a positive Schiff test and also a positive test with 2,4-dinitrophenylhydrazine.

The reaction mixture was cooled and the mixture subsequently filtered. The crude material was recrystallized from a methanol–water pair to yield 0.13 g. (34.0%) of 1-chlorodibenzothiophene melting at 88°.

The infrared spectrum of this compound was almost identical with the spectra obtained from 1-bromodibenzothiophene.

Anal. Calcd. for $C_{12}H_7ClS$: C, 65.89; H, 3.23; Cl, 16.21; S, 14.66. Found: C, 65.93, 66.01; H, 3.23, 3.39; Cl, 16.29, 16.15; S, 14.51, 14.67.

(b).—Into a 125-ml. erlenmeyer flask equipped with a reflux condenser were placed 0.43 g. (0.00175 mole) of 1-nitro-2-aminodibenzothiophene, 20 ml. of concd. HCl and 20 ml. of absolute ethanol. The reaction mixture was refluxed for 1.5 hours at which time the reaction mixture was cooled, filtered and the residue recrystallized from a methanol–water pair to yield 0.11 g. (28.8%) of 1-chlorodibenzothiophene melting at 88° and which did not give a depression with the sample obtained from the treatment from 1-nitro-2-acetamidodibenzothiophene with concd. HCl and ethanol by the method of mixed melting points.

1-Chloro-2-(2-hydroxy-1-naphthaleneazo)-dibenzothiophene.—Into a 125-ml. erlenmeyer flask equipped with a reflux condenser were placed 0.8 g. (0.0028 mole) of 1-nitro-2-acetamidodibenzothiophene, 0.8 g. (0.0056 mole) of 2-naphthol, 40 ml. of absolute ethanol and 30 ml. of concd. HCl. The mixture was refluxed for a period of 20 min., allowed to cool, filtered and then recrystallized from glacial acetic acid twice to yield 0.41 g. (38.0%) of long, red needles melting at 257–258°.

Anal. Calcd. for $C_{22}H_{12}ClN_2OS$: Cl, 9.14; N, 7.22. Found: Cl, 8.95, 9.10; N, 7.09, 7.16.

1-Nitrodibenzothiophene.—To a suspension of 2.4 g. (0.0099 mole) of 1-nitro-2-aminodibenzothiophene in 40 ml. of absolute ethanol was added slowly 13 ml. of fuming sulfuric acid (15% SO_3). The reaction mixture was heated to approximately 80° at which time 6.0 g. of $NaNO_2$ was added over a period of 15 minutes. The mixture was allowed to cool, filtered and the resulting precipitate recrystallized from an ethanol–water pair, using Norit A to yield 1.21 g. (53.8%) of 1-nitrodibenzothiophene melting at 97°.

Anal. Calcd. for $C_{12}H_7NO_2S$: N, 6.11; S, 13.99. Found: N, 6.01, 6.02; S, 13.53, 13.57.

1-Aminodibenzothiophene.—Into a 125-ml. hydrogenation bottle were placed 1.21 g. (0.0053 mole) of 1-nitrodibenzothiophene, 30 ml. of absolute ethanol and approximately 0.4 g. of Raney nickel. The material was hydrogenated with an initial pressure of 50 p.s.i. for one hour in a mechanical shaker. The material was then filtered, the solution concentrated and finally recrystallized from an ethanol–water pair to yield 0.60 g. (57.0%) of 1-aminodibenzothiophene melting at 96°. This compound gives a depression in melting point of over 20° when admixed with 1-nitrodibenzothiophene.

Anal. Calcd. for $C_{12}H_9NS$: N, 7.03. Found: N, 6.62, 6.73.

1-Acetamidodibenzothiophene.—Into a 50-ml. erlenmeyer flask equipped with a reflux condenser were placed 0.40 g. (0.0020 mole) of 1-aminodibenzothiophene, 15 ml. of benzene and 1.0 ml. of acetic anhydride. The reaction mixture was refluxed for 10 minutes, allowed to cool, filtered and recrystallized from an ethanol–water pair to yield 0.40 g. (83.5%) of 1-acetamidodibenzothiophene melting at 227°.

Anal. Calcd. for $C_{14}H_{11}NO_2S$: C, 69.67; H, 4.60. Found: C, 69.73, 69.60; H, 4.79, 4.71.

Acknowledgments.—The authors wish to express their appreciation to Dr. Velmar A. Fassel and Mr. Marvin Margoshes for the infrared analyses.

AMES, IOWA

[CONTRIBUTION FROM THE DEPARTMENT OF BOTANY, CORNELL UNIVERSITY]

The Detection, Isolation and Identification of L(−)Pipecolic Acid in the Non-protein Fraction of Beans (*Phaseolus vulgaris*)[1,2]

BY R. M. ZACHARIUS,[3a] J. F. THOMPSON[3b] AND F. C. STEWARD[3c]

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The isolation and identification of pure (−)pipecolic acid from the green bean by procedures using chromatography and ion exchange resins is described. The wide occurrence of pipecolic acid in plants is recognized, though it occurs in quantity in the bean. The possible metabolic role of pipecolic acid is discussed.

The isolation of (−)pipecolic acid from beans (*Phaseolus vulgaris*) has already been reported in brief form.[4] This paper gives the complete account of the detection and isolation of pipecolic acid and presents the critical proof of its identity.

Detection and Isolation of Pipecolic Acid

Following the application of two-directional partition chromatography on paper[5] to the examination of the non-protein nitrogen compounds of the potato tuber by Dent, Stepka and Steward,[6] a survey was made of these compounds in a number of plant materials. This survey[7] revealed the frequent occurrence of a number of known, naturally occurring, amino acids and a number of ninhydrin-reacting compounds whose identity was not then known. Some of the results of this survey have been summarized by Steward and Thompson[7] by means of a map which records the position of the various substances that occur on two-directional chromatograms (phenol and collidine–lutidine) together with a key which describes the characteristics and occurrence of the unidentified compounds.

Prominent among the unidentified substances was one which is particularly abundant in the fruit of the common bean (*Phaseolus vulgaris*) and designated unknown 1 by Steward and Thompson.[7]

(1) This work began at the University of Rochester and was supported in part by a grant to one of us (F.C.S.) from the Nutrition Foundation. The completion of the work at Cornell University was supported by the Grasselli Grant to Cornell University for work directed by one of us (F.C.S.).

(2) Based on a portion of the dissertation presented by R. M. Zacharius in partial fulfillment of the requirements for the degree of Ph.D. at the University of Rochester, Rochester, N. Y.

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(4) R. M. Zacharius, J. F. Thompson and F. C. Steward, THIS JOURNAL, **74**, 2949 (1952).

(5) R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).

(6) C. E. Dent, W. Stepka and F. C. Steward, *Nature*, **160**, 682 (1947).

(7) F. C. Steward and J. F. Thompson, *Ann. Rev. Plant Physiol.*, **1**, 233 (1950).

Unknown 1 gives a violet-blue color with ninhydrin. This color is initially a different one than that obtained with α-amino acids and eventually turns to a gray-brown color. Unknown 1 occurs near valine and proline on phenol: collidine–lutidine chromatograms having an R_f value in phenol of 0.872 (mean of 28 determination) and an R_f value in collidine–lutidine of 0.372 (mean of 13 determinations).

Chromatograms of the non-protein nitrogen fraction of the common bean and other legumes which revealed unknown 1 showed no proline. Unknown 1 gives a gray-brown color with ninhydrin, after standing, which is somewhat similar to that obtained with proline. These facts plus the high R_f value in phenol all suggested a possible relationship to proline. With these points in mind, an isatin[8] test for piperidines and pyrrolidines was applied to a chromatogram. The isatin test was positive but it was still possible that this might have been caused by an undetected amount of proline, since their positions are close in phenol: collidine–lutidine chromatograms. Substitution of *n*-butyl–alcohol–acetic acid (9:1) for phenol resulted in a wide separation of added proline from unknown 1. This separation made possible the clear-cut proof that unknown 1 (free from proline) reacts with isatin and is, therefore, possibly a compound with a piperidine or pyrrolidine ring.

Unknown 1 was stable to both acid and alkali and was found by the use of copper carbonate[9] in paper chromatography not to be an α-amino acid. This finding led to the isolation of unknown 1 by an alternative method which is described in a subsequent paper.

A preliminary attempt to precipitate unknown 1 by the use of mercuric acetate[10] in the presence of sodium carbonate and alcohol was made. Most of the unknown remained in solution. Similarly, attempts to precipitate unknown 1 with mercuric nitrate or phosphotungstic acid were unsuccessful.

Attention was then turned to methods of separation of nitrogen compounds using columns of ion exchange resins. An unsuccessful attempt at the isolation of unknown 1 was modeled after the techniques of Partridge and Westall.[11] The resin used was the cation exchange resin Zeo Rex rather than the recommended Zeo Karb 215 which was then unavailable in this country. These two resins were supposedly identical. A concentrated solution of a bean extract was put on the column and eluted with 0.10 *N* ammonium hydroxide. Tests of successive fractions from the column showed no adequate separation of unknown 1 from other amino acids.

The elution of bean extract from a column of Zeo Rex with hydrochloric acid as suggested by Stein and Moore[12] and then with ammonium hydroxide resulted in a number of fractions in which unknown 1 was the only ninhydrin-reactive substance. It was possible to crystallize unknown 1 as the hydrochloride and a total of 860 mg. was obtained in this way, of which a part was purified by recrystallization.

(8) R. Acher, C. Fromageot and M. Justisz, *Biochem. and Biophys. Acta*, **5**, 81 (1950).

(9) H. R. Crumpler and C. E. Dent, *Nature*, **164**, 441 (1949).

(10) C. Neuberg and J. Kerb, *Biochem. Z.*, **40**, 498 (1912).

(11) S. M. Partridge and R. G. Westall, *Biochem. J.*, **44**, 418 (1949).

(12) W. H. Stein and S. Moore, *Cold Spring Harbor Symp. on Quant. Biology*, **14**, 179 (1950).

Experimental

Material.—One hundred and seventy-five pounds of fresh green beans were obtained from the Beechnut Packing Company as they are prepared for use in proprietary foods. (In the light of knowledge that accrued later, the unknown 1 could have been more conveniently prepared from dried leguminous seeds: we were, however, interested to isolate the material that occurs in the fresh tissue.) The beans were chopped, washed in cold water and blanched at 210°F. for 2–3 minutes.

Extraction.—The beans were ground in a meat grinder and the liquid separated by centrifugation in a "bucket" centrifuge using a canvas bag. About 12 gallons of solution was obtained. The residue was extracted with water and centrifuged as before, yielding a further 14 gallons of solution. The extracts were concentrated by boiling off the water. Three separate batches of a dark brown concentrate were obtained. The approximate amino nitrogen of each batch was determined with ninhydrin[13] and the results are given in Table I.

TABLE I

NITROGEN CONTENT OF CONCENTRATED BEAN EXTRACTS

Bean extract	Volume, liters	Amino nitrogen, mg. N/ml.	Total N, g.
Batch A	1.5	6.28	9.42
Batch B	2.4	1.33	3.19
Batch C	2.0	3.54	7.08
		Total	19.69

Fractionation of Amino Acids.—Eight hundred grams of "Zeo Rex" (Permutit Company) was packed into a glass column 71.5 cm. long and 7.7 cm. in diameter with a sintered glass base-plate. The resin was cleaned by washing with excess of 2 *N* HCl, then excess of *N* NH_4OH. The column was then activated for use with 2 *N* HCl and washed with water until a minimum, constant, chloride test was obtained.

One liter of bean concentrate "B," containing 1.3 g. of amino nitrogen, was put on the resin column and washed with 12 liters of distilled water to remove sugar and other non-ionic materials. The water wash contained small amounts of aspartic and glutamic acids only.

The column was eluted with 7 liters of 2 *N* HCl collecting 172 fractions of 40 ml. each. Each fraction was tested for ninhydrin activity as follows: One drop of each fraction was placed on filter paper and allowed to dry. Any excess HCl was neutralized by placing the paper in an atmosphere of ammonia. The ammonia was allowed to evaporate and the ninhydrin activity was observed as a color produced by heating the paper at 60° after spraying the sheet with a 0.2% solution of ninhydrin in 95% alcohol. Ninhydrin activity appeared at fraction 22 and continued to fraction 172 with no pronounced peaks. An aliquot of each even numbered fraction was chromatographed one-directionally on paper with phenol. Fractions 55, 80, 120, 130 and 172 were chromatographed two-directionally and the results are given in Table II.

TABLE II

THE RELATIVE AMINO ACID COMPOSITION[a] OF SELECTED FRACTIONS OF A BEAN EXTRACT CHROMATOGRAPHED ON AN ION EXCHANGE RESIN COLUMN WITH 2 *N* HCl

Amino acid	Fraction number 55	80	120	130	172
Aspartic acid	++++	++++	++++	++++	++
Glutamic acid	++	++	++	+++	+
Serine	++	+++	++	++	Trace
Asparagine	++	+++	++	++	Trace
Threonine	+	+	+	−	−
Alanine	++	+++	++	++	Trace
Valine	−	−	+	−	−
β-Alanine	Trace	+	+	+	−
γ-Amino-butyric acid	−	+	++	+++	++++

[a] Based on reaction with ninhydrin on paper.

(13) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176** 367 (1948).

The one and two directional chromatograms clearly showed that most of the known amino acids (except the basic ones) had been eluted from the column. The principal remaining amino acids were γ-aminobutyric acid, unknown 1 and the basic amino acids. After washing the resin column with 3 liters of water to remove HCl, the remaining amino acids were removed with 6 liters of 0.5 *N* NH_4OH and 2 liters of 1 *N* NH_4OH. Elution with ammonia resulted in 180 fractions of 40 ml. each. A test of ninhydrin activity showed no activity for 130 fractions. With fraction 132, amino acids, ammonia and a dark brown color began to come from the column. (Considering the recent work of Hulme[14] the dark color may be caused by chlorogenic acid.) One directional chromatograms in phenol showed that unknown 1 was the most prominent constituent of the ammonia eluate while γ-aminobutyric acid was the next in amount. There were small amounts of aspartic and glutamic acids (probably from amide decomposition on the column) and of serine, threonine and alanine.

Crystallization.—Fractions 132–180 (of ammonia eluate) were combined, evaporated to a small volume on a steam-bath and dried in a stream of air at room temperature. The residue was dissolved in 100 ml. of water and decolorized to a pale yellow color with activated charcoal (Darco G-60).

Attempts to obtain crystals by the use of ethanol were unsuccessful. Unknown 1 was crystallized as the hydrochloride from concentrated HCl. No attempt was made to achieve a quantitative yield of unknown 1. Quantitative methods are being described separately. However, from the total isolated batch of 55 to 60 mg. of pure crystals was obtained. These crystals were found to be unknown 1 and to be free of contaminants (Fig. 1) by paper chromatography in three different solvents (phenol, collidine–lutidine 3:1 and *n*-butyl alcohol–acetic acid 9:1). The amino acids that showed clearly on the one directional chromatogram (Fig. 1) of the initial extract are, in sequence from the origin, aspartic acid, glutamic acid, serine, asparagine, threonine, alanine, γ-aminobutyric acid and valine, and the unknown 1. **Properties of the crystalline isolate of unknown** 1: the hydrochloride of unknown 1 melted with decomposition at 258–259° (uncor.). Synthetic DL-pipecolic acid, by catalytic reduction of α-picolinic acid, in the form of its hydrochloride, melted at 259–261°. The specific rotation of the isolated material was found to be $[\alpha]^{23}D$ −10.5° ± 0.5 in water at a concentration of 9.8 g. per 100 ml. This is a higher value than that reported by King, *et al.*,[15] of $[\alpha]^{18}D$ −5.0. The reason for this discrepancy is not known.

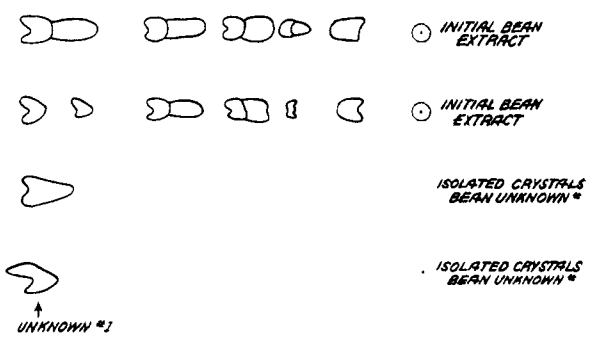

Fig. 1.

Identification of Unknown 1.—Using bean extract, it has been established that unknown 1 is not an α-amino acid and that it is a derivative of pyrrolidine or piperidine. The ultraviolet absorption spectrum of unknown 1 did not indicate conjugated double bonds and it showed a general similarity to that of proline (general absorption at about 220 millimicrons).

Unknown 1 first reacts with ninhydrin to give a compound with an absorption maximum at 570 mμ, reminiscent of the color produced by α-amino acids and ninhydrin.[16] When the reaction of unknown 1 and ninhydrin is performed on paper after collidine chromatography and the product is allowed to stand, the blue-violet color changes to a yellow-brown color, the absorption spectrum of which (Fig. 2a) is similar to that of piperidine (Fig. 2b) and of proline,[16] with strong absorption maximum at about 350 mμ and a less pronounced one at about 560 mμ. Chromatography of unknown 1 with pipecolic acid, nipecotic acid, isonipecotic acid and baikiain[15] (1,2,3,6-tetrahydropyridine-2-carboxylic acid) established the presumption that unknown 1 was pipecolic acid. This was determined by R_f values (Table III) in several solvents.

TABLE III

R_f VALUES OF UNKNOWN 1 AND CERTAIN PIPERIDINE DERIVATIVES AFTER CHROMATOGRAPHY IN THREE SOLVENTS

	Solvents		
Compound	Phenol	Collidine–lutidine (1:3)	Butanol–acetic acid (9:1)
DL-Pipecolic acid	0.895	0.384	0.214
DL-Nipecotic acid	.882	.270	.171
DL-Isonipecotic acid	.878	.254	.178
Baikiain	.878	.374	.192
Unknown 1	.895	.384	.214

Baikiain gives a yellow-brown color with ninhydrin immediately, whereas the other compounds give a bright blue-violet color. Isonipecotic acid is less sensitive to ninhydrin than the other compounds.

Unknown 1 and pipecolic acid were mixed and chromatographed in the three solvents system mentioned above. In every case only a single spot was obtained.

The calculated elementary composition for pipecolic acid hydrochloride is C, 43.51; H, 7.30; N, 8.46. This agrees well with the values as found: namely, C, 43.40; H, 7.30; N, 8.54. These values would fit equally well for three isomeric piperidinecarboxylic acids. However, the melting point for pipecolic acid hydrochloride (256–258°) agrees better with the melting point of unknown 1 (258–259°) than the melting points of nipecotic acid hydrochloride (m.p. 237°) and isonipecotic acid hydrochloride (m.p. 280°).

Final proof of the identity of unknown 1 and pipecolic acid was obtained by infrared spectra. The infrared spectrum for the optically active (−)pipecolic acid hydrochloride, which was prepared by reduction from an authentic sample of baikiain (kindly supplied by Dr. King) was found to be identical with that of unknown 1 in the form of its hydrochloride (Fig. 3). The spectra were also identical in a halocarbon mull which reveals the parts obscured by nujol. As confirmatory evidence, the infrared absorption spectra of substances isomeric with pipecolic acid such as nipecotic (piperidine-β-carboxylic acid), and isonipecotic acids (γ-pipecolic or piperidine-γ-carboxylic acid) were obtained. Also infrared spectra of closely related compounds showing the effects of different degrees of reduction of the pyridine nucleus were obtained (α-picolinic acid and baikiain). As shown by Fig. 3, the infrared absorption spectra readily distinguish these substances from each other and from pipecolic acid.

Occurrence and Metabolic Significance of Pipecolic Acid.—Although the whole fruit of the bean was the source of the extract from which pipecolic acid was isolated, it is more abundant in the seed than in the ovary wall. The presence of pipecolic acid has been determined chromatographically in several legumes: *viz.*, peas (*Pisum sativum*), white lupine (*Lupinus albus*), the lima bean (*Phaseolus limensis*) and the honey locust (*Gleditsia tricanthos*). The occurrence of pipecolic acid in legumes is confirmed by the isolation of pipecolic acid from clover[17] (*Trifolium repens*). The isolation of pipecolic acid from apples[18] and hops[19] also has been reported.

The occurrence of pipecolic in other families than

(14) A. C. Hulme, *Biochem. J.*, **53**, 337 (1953).

(15) F. E. King, T. J. King and A. J. Warwick, *J. Chem. Soc.*, 3590 (1950).

(16) J. F. Thompson, R. M. Zacharius and F. C. Steward, *Plant Physiol.*, **26**, 375 (1951).

(17) R. I. Morrison, *Biochem. J.*, **53**, 474 (1953).

(18) A. C. Hulme and W. Arthington, *Nature*, **170**, 659 (1952).

(19) G. Harris and J. R. A. Pollock, *J. Inst. Brewing*, **59**, 28 (1953

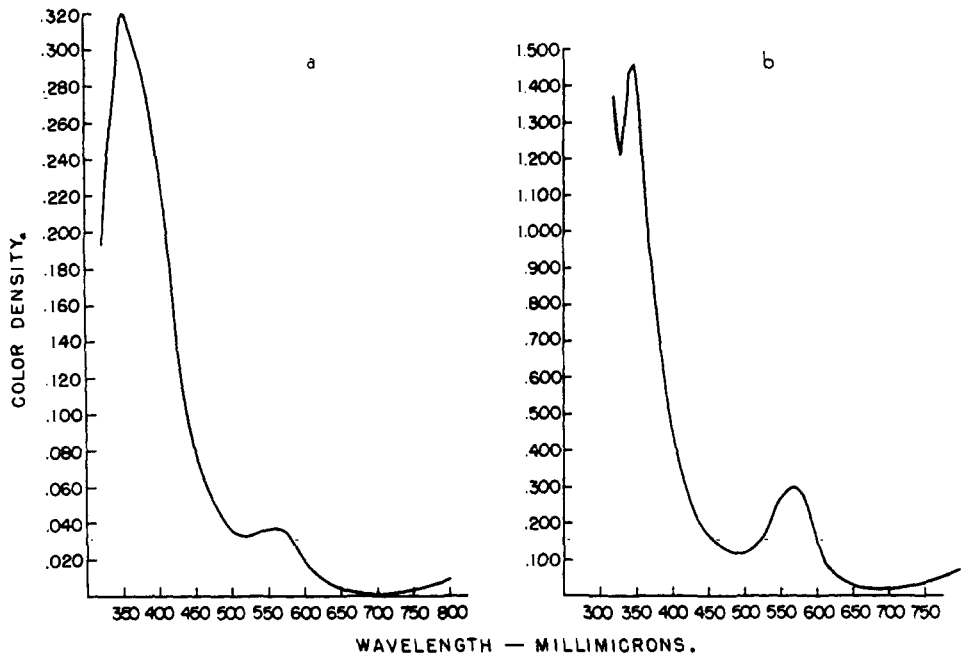

Fig. 2.—Left: absorption spectrum of the brown delayed product of the reaction of ninhydrin with unknown 1 on paper. Right: absorption spectrum of the product of the reaction of ninhydrin with piperidine.

the leguminosae is also evident. Unknown 24 in the chromatogram of Dent, Stepka and Steward[6] on an extract of potato has been shown to be chromatographically identical with pipecolic acid, for it was inseparable from pipecolic acid after paper chromatography in three solvents. Plants in which the presence of pipecolic acid has been observed are listed in Table IV.

TABLE IV

PLANTS OBSERVED TO CONTAIN PIPECOLIC ACID

Plant	Part
Phaseolus vulgaris	Seed, fruit and flower
Phaseolus limensis	Fruit
Pisum sativum	Fruit
Lupinus albus	Root, stem and leaves
Gleditsia tricanthos	Seed
Trifolium repens[17]	Leaves and stems
Pyrus malus[18]	Fruit
Humulus lupulus[19]	Fruit
Solanium tuberosum	Tuber
Nicotiana tabacum	Leaf
Brassica rapa	Root and leaf
Ficus pumila	Shoot
Capsicum grossum	Fruit
Asparagus officinalis	Shoot
Tulipa gesneriana	Bulb
Apium graveolens	Petiole and blade
Agaricus campestris	Fruiting body

This widespread occurrence of pipecolic acid in the plant kingdom would seem to indicate that pipecolic acid probably has somewhat general metabolic role. Pipecolic acid has not been found in proteins. The fact that pipecolic acid occurs in highest concentration in seeds indicates that it may serve as a reserve form of nitrogen particularly when the amides, asparagine and glutamine are low in amount.

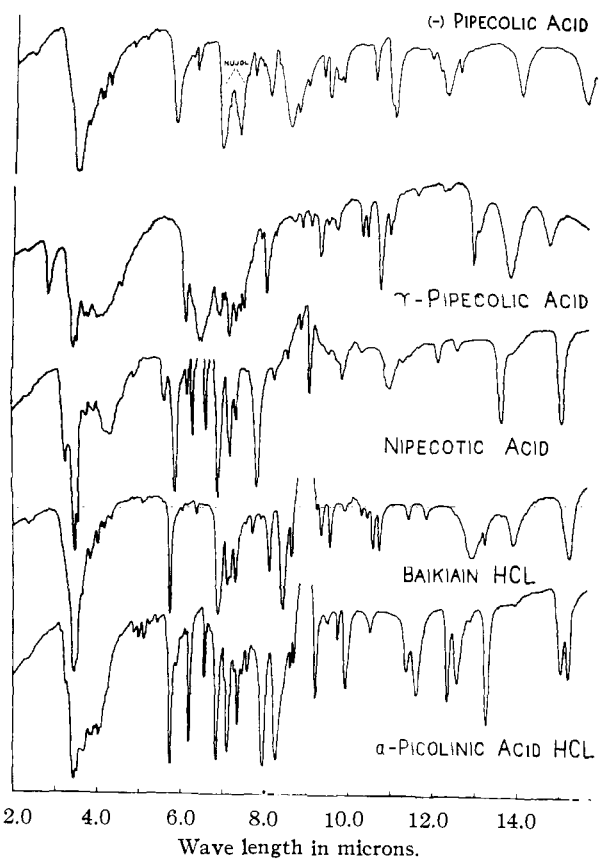

Fig. 3.—Infrared absorption spectra of compounds related to pipecolic acid in a nujol mull.

Several possible roles for pipecolic acid have been suggested. Willstätter[20] thought that pipecolic

(20) R. Willstätter, *Ber.*, **34**, 3168 (1901).

acid arises as a product of oxidation of the alkaloids. Guggenheim[21] has suggested that homarin, the betaine of α-picolinic acid, may be formed from pipecolic acid by dehydrogenation and methylation. Guggenheim's concept is that pipecolic acid should be derived from lysine by cyclization with the loss of ammonia. In fact the conversion of lysine to pipecolic acid in the developing fruit of the bean has now been established by Grobbelaar and Steward[22] and the same conversion has been proved in the rat by Rothstein and Miller.[23] It is evident, therefore, that "free" lysine is low in legumes where pipecolic acid is present. However, Stevens and Ellman[24] failed to replace lysine by pipecolic acid in the nutrition of the rat, in *Streptococcus faecalis* or *Leuconostoc mesenteroides*. With present knowledge the full role of pipecolic acid in metabolism remains obscure, though it has been suggested that it is a step en route from lysine to α-aminoadipic acid.

Acknowledgments.—The authors are indebted to the Beechnut Packing Company for the beans used in this work. The Grasselli Chemicals Division of E. I. du Pont de Nemours and Co. kindly supplied certain samples of piperidine carboxylic acids which were used. The authors are grateful to Dr. H. Posvic, then of the Chemistry Department of Cornell University, for assistance with the infrared absorption spectra.

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(22) N. Grobbelaar and F. C. Steward, THIS JOURNAL, **75**, 4341 (1953).

(23) M. Rothstein and L. L. Miller, *ibid.*, **75**, 4371 (1953).

(24) C. M. Stevens and P. B. Ellman, *J. Biol. Chem.*, **182**, 75 (1950).

ITHACA, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF BOTANY, CORNELL UNIVERSITY]

The Bulk Isolation of L(−)Pipecolic Acid from *Phaseolus vulgaris* and its Quantitative Determination[1]

BY N. GROBBELAAR,[2] R. M. ZACHARIUS AND F. C. STEWARD

RECEIVED JANUARY 11, 1954

Following the identification of L(−)pipecolic acid in the common bean (*Phaseolus vulgaris*) a procedure has been devised by which it may be isolated in high yield. This uses a method for the isolation of non-α-amino acids that was previously used for the isolation of γ-aminobutyric acid. After preliminary purification of the aqueous extract of beans, by absorbing all the soluble nitrogen compounds on a resin and then displacing them, the nitrogenous compounds were freed from other soluble products. The fractionation of the extract leading to the isolation of pure pipecolic acid was achieved by chromatographing the copper complexes of the nitrogen compounds on successive columns of mixed copper carbonate and alumina. Final recrystallization from pyridine–water yielded 13.4 g. of pure pipecolic acid, in the form of the free acid, from about 150 lb. of green beans. The preparation and characteristics of this material are described. The quantitative determination of pipecolic acid on paper chromatograms is described.

Pipecolic acid was first detected on paper chromatograms of extracts of *Phaseolus vulgaris*[3] as an unknown spot which was later identified.[4] It has also been isolated from extracts of *Trifolium repens*[5,6] and *Humulus lupulus* and identified.[7] The presence of pipecolic acid also has been observed in several legumes, in the edible mushroom, in potato tuber, green pepper, tulip, celery, asparagus,[8] Rhodesian teak,[9] barley[10] and coconut milk.[11] This compound is therefore of widespread occurrence in plants.

Thus far, pipecolic acid has been isolated in milligram quantities only by rather laborious methods.[4] The present paper describes a convenient method for the isolation of the compound in bulk and provides information on the quantitative determination of the compound by paper chromatography. As the metabolic reactions and nutritional role of this substance are investigated, access to stocks of the natural and optically active form of the compound become necessary, even though the racemic mixture may be readily synthesized by reduction of α-picolinic acid.[12] The isolation of the natural (−)pipecolic acid in bulk is therefore of importance for such studies.

The bulk isolation in high yield was from an extract from 175 lb. of green beans (*Phaseolus vulgaris*) made as described in the preceding paper.[13] The extract as made (sap + aqueous extract = 26 gallons) was concentrated to about 6 liters, which contained soluble compounds approximately equivalent to 20 g. of amino-N. Part of this extract was used in the first isolation and in the critical identification of a pure product.[13] After this had been done, the remainder was available for the attempt to isolate the bulk of the pipecolic acid in good yield. For this latter purpose the amount of extract available corresponded to about 147 lb. of beans, and consisted of 3.5 liters of the concentrated aqueous extract. The viscous dark-brown concentrate was found to contain about 16.5 g. of amino nitrogen as determined by the method of Moore and Stein.[14] The solution was centrifuged and the bulky pre-

(1) This work was supported by the Grasselli Grant to Cornell University for work being directed by one of us (F.C.S.).

(2) During the course of this work, N. Grobbelaar held a Fellowship awarded by the Rockefeller Foundation.

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