6.30; Br, 19.20; H_2O, 8.65. Found: C, 52.07; H, 6.24; Br, 18.82, 19.64; H_2O, 8.83.

1-Bromocodeine from 1-Bromocodeinone.—Under similar conditions, 101 mg. of 1-bromocodeinone[5] yielded 101 mg. of crude 1-bromocodeine, m.p. 158–160.5°, which on crystallization from ethyl acetate gave 72 mg. (71%) of pure 1-bromocodeine, m.p. 161–163°, whose mixed m.p. with authentic 1-bromocodeine[6] was undepressed.

Its **methiodide** melted at 262.5–264°[7] with decomposition, and did not depress the melting point of the methiodide obtained from authentic 1-bromocodeine.

Anal. Calcd. for $C_{19}H_{23}NO_3BrI$: C, 43.86; H, 4.46. Found: C, 43.89; H, 4.43.

(5) M. Gates and G. Tschudi, THIS JOURNAL, **74**, 1109 (1952).

(6) E. Speyer and H. Rosenfeld, *Ber.*, **58**, 1110 (1925).

(7) E. Vongerichten, *Ann.*, **297**, 204 (1897), has reported the melting point of this methiodide to be 242–244°.

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4-Alkyldiphenylketimine Hydrochlorides and Related Ketones

BY I. R. KAPLAN, H. N. PARTON AND J. VAUGHAN

RECEIVED MAY 19, 1953

In connection with another investigation which has since been discontinued, the ultraviolet absorption spectra of a series of 4-alkyldiphenylketimine hydrochlorides and the related ketones were determined. Of the compounds examined, four appear to be new. Ketimines were prepared by the method adopted by Pickard and Vaughan.[1] The hydrochlorides were precipitated from ethereal solution with dry hydrogen chloride, repeatedly recrystallized from chloroform solution and washed well with ether. Of the alkyl-substituted benzophenones, the methyl compound was prepared by the Friedel–Crafts reaction and the others were obtained by hydrolysis of the ketime hydrochlorides with 6 *N* hydrochloric acid. Spectral data were obtained with a Hilger "Uvispek" spectrophotometer and the range covered was 2100–3200 Å. Approximately 1×10^{-4} *M* solutions in methanol were used for all compounds. Absorption curves of the alkylated compounds have the simple shape shown by the parent bodies (*e.g.*, see Culbertson[2]).

TABLE I

	Ketone			Ketimine hydrochloride		
4-Substituent	M.p. or b.p., °C.	$\lambda_{max.}$ (Å.)	$\epsilon_{max.} \times 10^{-4}$	M.p.,[a] °C.	$\lambda_{max.}$ (Å.)	$\epsilon_{max.} \times 10^{-4}$
H	M. 48	2520	1.750	310	2755	1.665
Methyl	M. 58	2590	1.745	244	2855	1.570
Ethyl	B. 318–320	2535	1.555	264[b]	2820	1.660
Isopropyl	B. 338–340 (774 mm.)	2570	1.660	260[c]	2875	1.715
t-Butyl[d]	B. 198 (13 mm.)	2585	1.755	280–282[e]	2875	1.680

[a] Visible sublimation occurred to a greater or less extent with each salt, beginning 20–30° below recorded m.p. [b] *Anal.* Calcd. for $C_{15}H_{16}NCl$: N, 5.71; Cl, 14.47. Found: N, 5.58; Cl, 14.30. [c] Calcd. for $C_{16}H_{18}NCl$: N, 5.40; Cl, 13.66. Found: N, 5.44; Cl, 13.55. [d] Calcd. for $C_{17}H_{18}O$: C, 85.70; H, 7.56. Found: C, 85.86; H, 7.86; n^{19} 1.5762. [e] Calcd. for $C_{17}H_{20}NCl$: N, 5.13; Cl, 12.96. Found: N, 5.15; Cl, 12.88.

Analysis of the 4-*t*-butylbenzophenone was carried out by Dr. A. D. Campbell of Otago University.

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(1) P. L. Pickard and D. J. Vaughan, THIS JOURNAL, **72**, 876 (1950).

(2) J. B. Culbertson, *ibid.*, **73**, 4818 (1951).

Pipecolic Acid in *Phaseolus vulgaris*: Evidence on its Derivation from Lysine

BY N. GROBBELAAR[1] AND F. C. STEWARD

RECEIVED JULY 7, 1953

After the discovery of pipecolic acid as a prominent constituent of the green bean (*Phaseolus vulgaris*) and of other plants, the question of its origin and metabolic relationships arises. Advantage has been taken of the availability of lysine containing radioactive carbon to test the possibility that it may arise from, or be interconvertible with, lysine by a process of ring closure and loss of ammonia. The lysine used in this experiment was prepared synthetically by Dr. R. W. Helmkamp of the University of Rochester and was made available to us through the courtesy of Dr. Leon Miller, also of the University of Rochester.

The lysine was labelled in the ϵ-position and was made available to us dissolved in dilute salt solution. The specific activity of the lysine was 0.85 microcurie per milligram and 10 mg. of L-lysine was dissolved in 0.65 ml. of 0.9% sodium chloride solution.

The plants selected for the experiments were grown in pots and had fruits approximately 10 cm. in length. The morphology of the specimen selected for the first experiment is shown in Fig. 1. It will be noted that there were two developing fruits in the axil of the same leaf which was removed (X in Fig. 1). The main branch bearing the fruits was also decapitated (Y in Fig. 1). The method was to inject with a hypodermic needle, 0.25 ml. of the autoclaved lysine solution into the cavities surrounding the two lower ovules of fruit A (Fig. 1).

After the elapse of an appropriate period (55 hours) the tissue of the injected fruit was dissected and sampled and also the tissue of the adjacent fruit in the same leaf axil (B in Fig. 1).

In sampling the material for analysis, the ovules and carpel walls were treated separately and all the rest of the tissue of the plant examined as a whole. The weights of the organs analyzed are given in Table I.

TABLE I

FRESH WEIGHTS OF TISSUES EXTRACTED

Material	Weight, g.
First Experiment	
Injected fruit	2.468
carpel wall	2.229
ovules	0.239
Uninjected fruit	4.501
carpel wall	3.530
ovules	0.971
Rest of shoot	15.521
Second Experiment	
Stem tissue (F–F)	0.446
Fruit	8.098
carpel wall	5.395
ovules	2.703
Rest of shoot	37.600

(1) Predoctoral Rockefeller Foundation Fellow at Cornell University.

At the time of harvesting the material all of the injected solution had been absorbed from the carpel cavities. It was found that the ovules of the two fruits had sizes as indicated in Fig. 1.

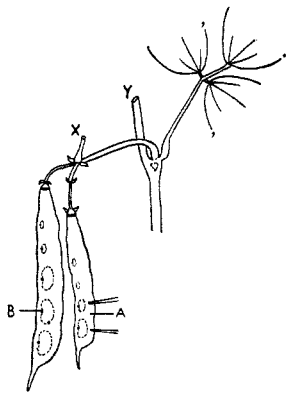

Fig. 1.

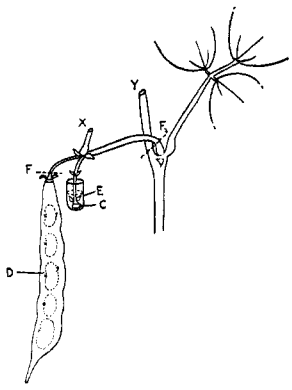

Fig. 2.

The sampled plant material was treated as follows: (1) The carpel walls and ovules were treated with 5 times their weight of 70% ethanol in a Potter glass homogenizer. (2) The rest of the shoot was also treated with 5 times its weight of 70% ethanol in a Waring Blendor. The material so treated was centrifuged to separate the ethanol extract and the insoluble material re-extracted with a similar volume of alcohol. The combined alcoholic extracts were evaporated to dryness at room temperature in a stream of air and used for two-directional paper chromatographic analysis. The insoluble residues were hydrolyzed with 6 *N* HCl for 18 hours at 120° in sealed ampules, evaporated to dryness, re-dissolved in water and this solution also used for chromatographic examination.

The paper chromatographic procedure adopted was as follows: Recognizing that the amounts in question might be small, the papers used were restricted to 5 inches by 5 inches of Whatman #1. Chromatographic cabinets (American Museum jars) of the dimensions 4.5 × 6 × 6 inches were used. These were already fitted with heavy ground glass covers. The papers were suspended vertically and the solvent allowed to ascend. The solvents were firstly aqueous phenol and secondly collidine–lutidine (3:1) saturated with water. The approximate distance of travel of the solvent was about 5 inches and these procedures required approximately 2.5 hours in each direction.

Because of the danger of losing the labelled carbon as carbon dioxide during ninhydrin treatment, in case it had been converted to a carboxyl group in the plant, radioautographs of the chromatograms were first prepared. The procedure here was as follows: Before exposing the film to the chromatogram, the time period necessary was estimated by scanning the chromatogram with a Geiger–Müller counter and measuring the activity of the radioactive spots. A piece of X-ray No-Screen Film, 5 × 5 inches, was then placed immediately behind the chromatogram. The chromatogram and the film were held in place by two sheets of stout cardboard and rubber bands. A needle was pierced through the cardboard, film and chromatogram at two places in order to permit the chromatogram and developed radioautograph to be exactly superimposed. Exposure times of the order of one week were found adequate to produce strong spots on the radioautographs.

The chromatograms were then sprayed with ninhydrin and the color developed in air at 60° for about ten minutes. Clear separation of the principal free amino acids in the plant material was obtained as shown in Fig. 3.

On these chromatograms lysine and pipecolic acid could be readily recognized when present, the latter by its distinctive color, position and crimson fluorescence in ultraviolet light.

The first results observed were as follows: The injected lysine was absorbed by the carpel wall of the injected fruit (A in Fig. 1). The activity passed however via the vascular system predominantly to the ovules contained in the carpel B (Fig. 1).

TABLE II

TOTAL RADIOACTIVITY OF PLANT FRACTIONS

Material	Radioactivity in counts/second Alcohol soluble	Alcohol insoluble
First Experiment		
Injected fruit	947[a]	452[a]
carpel wall	806	339
ovules	141	113
Uninjected fruit	240[a]	167[a]
carpel wall	33	0
ovules	207	167
Rest of shoot	30	...
Second Experiment		
Stem tissue (F–F)	2008	477
Fruit	1525[a]	1063[a]
carpel wall	271	392
ovules	1254	671
Rest of shoot	0	...

[a] Summation of radioactivity of carpel wall and ovule fractions.

As shown in Table II the activity of these ovules was much greater than the activity of the ovules of the injected fruit. The carpel wall of fruit B had a negligible amount of activity whereas both the alcohol-soluble and alcohol-insoluble extracts of the carpel wall of fruit A showed strong activity. The activity present in the ovules of the fruit B was found to be present in both the alcohol-soluble fraction and also in the alcohol-insoluble fraction.

Examination of the paper chromatograms and the radioautographs immediately showed the following results. The carpel wall that had initially absorbed the lysine (fruit A) contained a rather small amount of pipecolic acid in the alcohol-soluble extract. This, however, was labelled with C^{14} in quite high specific activity. Some (lysine and a spot in the position of glutamic acid) of the remaining amino acids of this same extract also contained radioactivity but in very much smaller specific activity. In the alcohol-soluble extract of the seeds of fruit A, pipecolic acid was weakly labelled. The remaining radioactivity of this extract was contained in material (probably alcohol-soluble proteins) that remained at the origin of the chromatogram.

However, in the alcohol-soluble extract of the ovules from the adjacent fruit B, which had received their lysine via the vascular system, the activity occurred *exclusively* in the pipecolic acid. As mentioned above, a negligible amount of radioactivity was present in the carpel wall of this fruit. The exact superposition of the ninhydrin-reactive spot of pipecolic acid and the spot corresponding to radioactivity for these samples is indicated in Fig. 3.

These results clearly demonstrate the transformation of lysine to pipecolic acid in the developing carpel wall and ovules of the bean. Since the entry of lysine into the developing fruit is at a distance from the point of application and since the material inevitably enters the fruit via the vascular system, it seems quite clear that pipecolic acid represents one of the first conversion products of the lysine.

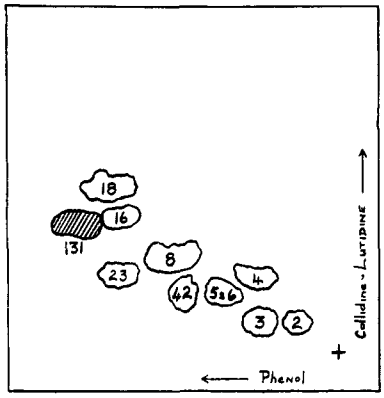

Fig. 3.—Two-directional chromatogram of the alcohol-soluble extract of the ovules of fruit B (Fig. 1). Spots are labelled: aspartic acid, 2; glutamic acid, 3; serine, 4; asparagine, 5; glycine, 6; alanine, 8; valine, 16; leucine, 18; γ-aminobutyric acid, 23; peptide, 42; pipecolic acid, 131. The shaded spots were radioactive.

A further experiment was performed with the following modifications. Recognizing that the most useful information had been obtained from the developing fruit and ovules which had received pipecolic acid via the vascular system, the following procedure was adopted.

A plant very similar to the one used in the previous experiment was again selected and trimmed as before. It also bore two fruits in the same leaf axil. One of the fruits (C in Fig. 2) was severed and the pedicel immersed in 0.4 ml. of aqueous solution containing the lysine (E in Fig. 2). The plant was allowed to absorb this solution directly. The total amount of this solution was absorbed over a period of approximately 72 hours. After 96 hours the plant was sampled in the manner already described and similar extracts and determinations were made. In this case, the piece of stem tissue indicated between letters F and F on Fig. 2 was separately extracted.

The sizes of the seeds of fruit D were found to be as indicated in Fig. 2. The weights of the tissues extracted are given in Table I while the total activities of the final extracts and hydrolysates are given in Table II.

In principle the results were the same as in the earlier experiment. The alcohol-soluble fraction of the ovules of fruit D in Fig. 2 was labelled exclusively and strongly in pipecolic acid. The alcohol-soluble fraction of the carpel wall of fruit D contained very little radioactivity but again it all occurred in the small amount of pipecolic acid present.

In the absorbing peduncle C (Fig. 2) and the adjacent stem tissue (F–F in Fig. 2) the alcohol-soluble fraction was strongly radioactive and all its radioactivity occurred in two compounds. The one compound appeared on the chromatograms in the position occupied by glutamic acid while the other superimposed with a purple ninhydrin spot below alanine (X in Fig. 4). These tissues contained no detectable pipecolic acid.

The hydrolysates of the alcohol-insoluble fractions of the ovules, carpel wall and tissue F–F all yielded the same results. All the radioactivity was always found in two spots on the radioautographs. The one spot superimposed with a purple spot in the position normally occupied by glutamic acid. The second spot superimposed with the purple spot (Y in Fig. 5) on the chromatogram. The position of this latter spot is very close to that of ε-amino-

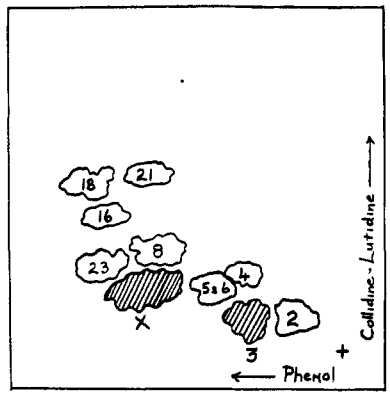

Fig. 4.—Two-directional chromatogram of the alcohol-soluble extract of stem tissue F–F (Fig. 2). Spots are labelled as in Fig. 3. Additional spots are: tyrosine, 21 unidentified spot, X. The shaded spots were radioactive.

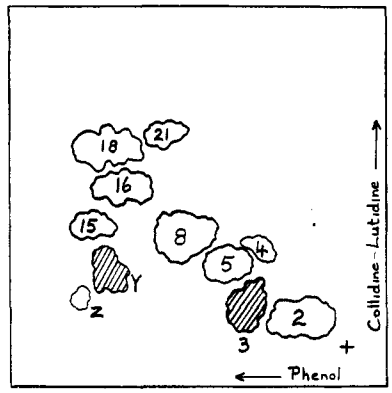

Fig. 5.—Two-directional chromatogram of the acid hydrolysate of the alcohol-insoluble fraction of the stem tissue F–F (Fig. 2). Spots are labelled as in Figs. 3 and 4. Additional spots are: proline, 15; unidentified spots, Y and Z. The shaded spots were radioactive.

hexanoic acid[2] or δ-aminovaleric acid.[2,3] The behavior of this compound on $CuCO_3$-treated paper also seems to indicate that it is not an α-amino acid.[4]

These experiments establish the transformation of lysine to pipecolic acid in the developing green bean. Further work will be necessary to determine completely the intermediate steps. The radioactive compounds X, Y and "glutamic acid" might act as intermediates in this transformation. It would, however, seem more probable that they act as intermediates during the incorporation of the carbon of lysine into the markedly lysine-deficient protein moiety. Inasmuch as radioactivity also appears in the alcohol-insoluble fractions, use can be made of this technique to investigate protein synthesis.

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