

4-Hydroxy-Piperidine-2-Carboxylic Acid in Green Plants

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We recently isolated a new piperidine-carboxylic acid from *Rhapis flabelliformis* and identified it as 5-hydroxy-piperidine-2-carboxylic acid¹. By paper chromatography this substance was found also in *Acacia* species. In this case, however, also another spot in connection with the spot of 5-hydroxy-piperidine-2-carboxylic acid could be seen on the paper chromatogram (Fig. 1). The colour produced with ninhydrin of this spot was at first yellow but turned after heating gradually sky blue, while the spot given by the 5-hydroxy acid was blue tinged with violet. Without this difference in colour it had been difficult at first to observe that two different substances together formed the large spot.

We have now isolated this other amino acid from a 70 % alcohol extract of *Acacia pentadena*. The solution of 655 g of fresh plants (= 346 g dry material, 764 mg soluble N) was first run through an Amberlite IR-120 column (1.5 × 43 cm, 80 ml resin). The amino acids remained in the column and were eluted with 1 N ammonia whereby

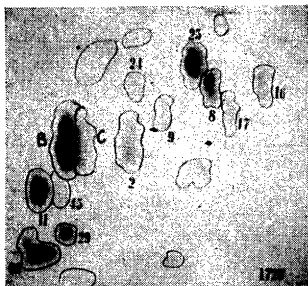


Fig. 1. Two-dimensional paper chromatogram (butanol-acetic acid + phenol-NH₃) from a 70 % alcohol extract of *Acacia pentadena*. 2 = ala, 8 = ser, 9 = threo, 11 = pro, 16 = asp, 17 = glu, 24 = glu-NH₂, 25 = asp-NH₂, 29 = γ -aminobut. acid, 45 = ethanolamine, 60 = piperidine-2-carboxylic acid (pipercolic acid), B = unknown amino acid, C = 5-hydroxy-piperidine-2-carboxylic acid.

all amino acids came out in fractions Nos. 4–19 of 17 ml each. Ammonia was evaporated *in vacuo* from these fractions. The syrupy residue was dissolved in 10 ml of 1.5 N HCl and the solution obtained was passed into a Dowex 50 column (4 × 64 cm, 800 ml resin). The resin had previously been treated with 1.5 N HCl. The amino acids were eluted with 1.5 N HCl. 400 fractions of 9 ml were collected. The unknown amino acid came out in fractions Nos. 201–260. Fractions 201–220 contained besides α -amino acids (serine, threonine and asparagine) only the unknown amino acid, fractions 221–260 also 5-hydroxy-piperidine-2-carboxylic acid. Fractions 201–220 were therefore used for the isolation of the unknown amino acid. In order to destroy the α -amino acids they were deaminated with nitrogen oxides. The solution was then passed through an Amberlite IR-120 column and the amino acid in question, which was not deaminated, was eluted with ammonia. After evaporation to a small volume the unknown amino acid crystallized in fine flakes. The yield was 180 mg corresponding to about 18 mg N (2.4 % of the soluble N in *Acacia*). When the amount of this amino acid in the mother liquid and in fractions 221–260 are taken into account about 2.7 % of the soluble N in *Acacia* belongs to the unknown amino acid. Analysis of the recrystallized substance; Found: C 49.25; H 7.67; N (Dumas) 9.63. Calc. for hydroxy-piperidine-carboxylic acid C₆H₁₁O₃N: C 49.65; H 7.59; N 9.66. M. p. determination: turned brown at 250° C, decomposed at 270° C.

This amino acid could be clearly distinguished from 5-hydroxy-2-piperidine-carboxylic acid by paper chromatography, because it moved somewhat faster in phenol. *R_F*-values (Whatman No. 4 paper):

	Phenol-NH ₃	Butanol-acetic acid
5-hydroxy-piperidine-2-carboxylic acid	0.783	0.254
4-hydroxy-piperidine-2-carboxylic acid (unknown amino acid)	0.826	0.232
α -alanine	0.609	0.247

The new amino acid did not reduce alkaline permanganate solution. Upon reduction with 66 % HI (*d* 1.96) and red phosphorus at 140° C piperidine-2-carboxylic acid (pipercolic acid) was formed (Fig. 2).

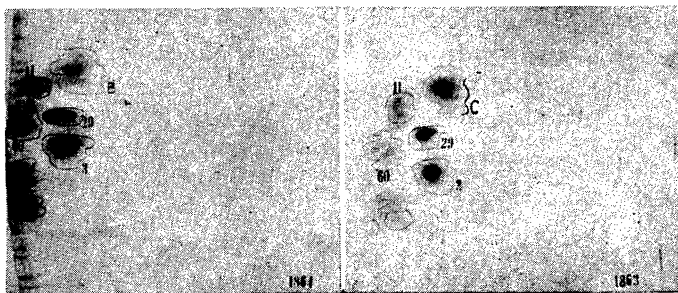


Fig. 2. Products formed from unknown amino acid B and 5-hydroxypiperidine-2-carboxylic acid C by reduction with HI and P. B = unknown amino acid which partly remained unchanged, C = 5-hydroxy-piperidine-2-carboxylic acid which partly remained unchanged, 60 = pipercolic acid formed from B and C; amino acids 3, 11, and 29 added for comparison. Two lower spots on the chromatograms are unknown, probably I containing substances.

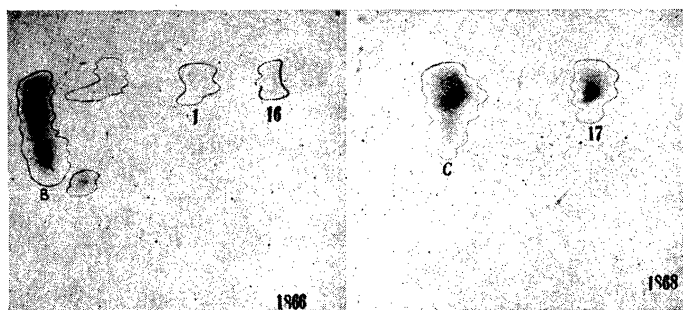
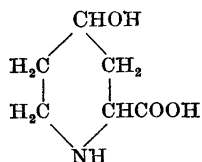


Fig. 3. Products formed from unknown amino acid B and 5-hydroxypiperidine-2-carboxylic acid by oxidation with KMnO_4 in H_2SO_4 -solution. B = unknown amino acid which partly remained unchanged, C = 5-hydroxypiperidine-2-carboxylic acid which partly remained unchanged, 16 = asp, 17 = glu, 1 = glu.

Accordingly, some hydroxy-piperidine-2-carboxylic acid was in question, having its OH group in another position than 5. On oxidation with potassium permanganate in sulphuric acid solution the substance gave, in addition to two substances not closer investigated, aspartic acid and a smaller amount of glycine, but no glutamic acid (Fig. 3). In parallel experiments our 5-hydroxy-piperidine-2-carboxylic acid gave only glutamic acid. Accordingly the unknown amino acid is 4-hydroxypiperidine-2-carboxylic acid.



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In our previous paper we advanced the hypothesis that 5-hydroxy-piperidine-2-carboxylic acid could be formed from δ -hydroxylysine. Correspondingly 4-hydroxy acid could be formed from γ -hydroxylysine. This acid has not yet been found in nature.

In addition to *Acacia pentadena*, *Acacia retinoides* and *Strelitzia reginae* contained both 4- and 5-hydroxy-piperidine-2-carboxylic acid. *Albizia lophantha* again contained only 4-hydroxypiperidine-2-carboxylic acid.

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