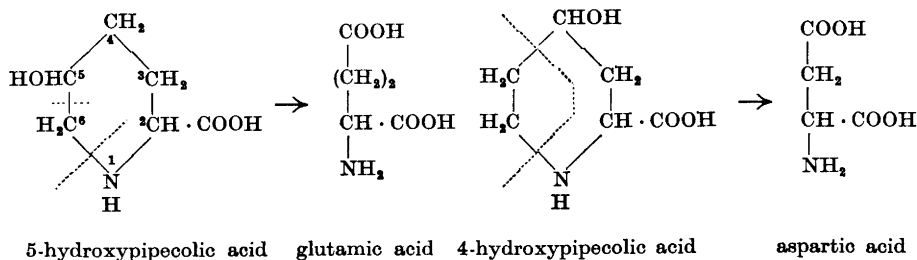


On the Structure of 4-Hydroxypiperidic Acid Isolated from Green Plants

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Some years ago Virtanen and Kari¹ isolated from some *Acacia*-species a new cyclic imino acid and identified it as 5-hydroxy-piperidine-2-carboxylic acid (5-hydroxypiperidic acid). From the same plants also another cyclic imino acid could be isolated². This was characterized as 4-hydroxypiperidic acid because it was reduced with hydrogen iodide and red phosphorus to piperidic acid and was oxidized with potassium permanganate to aspartic acid. Glycine and two other amino acids, not nearer characterized, were also formed then, but no glutamic acid which was the main or only oxidation product of 5-hydroxypiperidic acid. The positions of the hydroxyl group in the isolated hydroxypiperidic acids were concluded from the oxidation products



Witkop and Foltz³ later confirmed the structure of our 5-hydroxy compound through synthesis and presented evidence for the *trans* arrangement of the two functional groups.

Last year Fowden⁴ described the isolation of an imino acid from *Armeria maritima* which he identified with the 4-hydroxypiperidic acid isolated by Virtanen and Kari. As oxidation products with KMnO_4 he could identify β -alanine, glycine, γ -aminobutyric acid, and aspartic acid. Comparisons with synthetic 3- and 4-

hydroxypiperidic acids indicated that the amino acid may not be 4-hydroxypiperidic acid, but 3-hydroxypiperidic acid. A strictly identical behaviour of natural and synthetic 3-hydroxypiperidic acid could not be demonstrated by Fowden, since the two substances differed in their stereoisomeric composition.

Because the formation of aspartic acid as an oxidation product of a hydroxypiperidic acid is difficult to understand if the hydroxyl group is not in 4-position, we have reinvestigated the structure of the imino acid which according to Virtanen and Kari would be 4-hydroxypiperidic acid. The acid was now isolated from the seeds of *Acacia willardiana* and *Lysiloma bahamense*. Analysis of the preparation. Found: C 49.91; H 7.48; N 9.35; Calc. for $\text{C}_6\text{H}_{11}\text{O}_3\text{N}$: C 49.65; H 7.46; N 9.65. On the basis of IR-spectra, paper chromatographical behaviour, and colour reactions, it was identical with the 4-hydroxypiperidic acid isolated by Virtanen and Kari from fresh plants of *Acacia pentadena* and *Albizia lophantha*. The acid could be found also in different *Armeria*-species.

Optical rotation of 4-hydroxypiperidic acid:

$$\begin{aligned}
 [\alpha]_{\text{D}}^{25} &= -12.5^\circ \text{ in } \text{H}_2\text{O} \\
 &» = +0.34^\circ \text{ in } 1 \text{ N HCl} \\
 &» = -18.5^\circ \text{ in } 1 \text{ N NaOH}
 \end{aligned}$$

As principal products of acidic (H_2SO_4) and alkaline (NaOH) permanganate oxidation of the imino acid β -alanine and aspartic acid were identified (Fig. 1). In addition small amounts of glycine and some unidentified substances, coloured by ninhydrin, which possibly were intermediate products of the oxidation, were found on the paper chromatogram. γ -Aminobutyric acid was

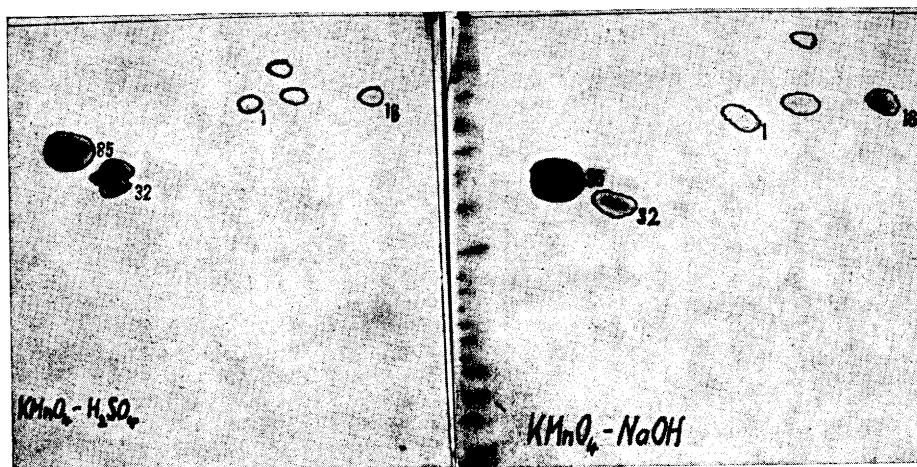


Fig. 1. Two-dimensional paper chromatogram of oxidation products of 4-hydroxypipercolic acid. Left: oxidation with KMnO_4 in 20 % H_2SO_4 solution; right: oxidation with KMnO_4 in NaOH solution. 1 gly, 16 asp, 32 β -ala, 85 unchanged 4-hydroxypipercolic acid. Solvents: butanol-acetic acid-water and phenol- NH_3 .

never found as an oxidation product. Excluding this important difference, the oxidation products were qualitatively the same as Fowden's. On the chromatogram of Virtanen and Kari³ β -alanine was present as a "not nearer characterized" spot (cf. Fig. 3 in their paper).

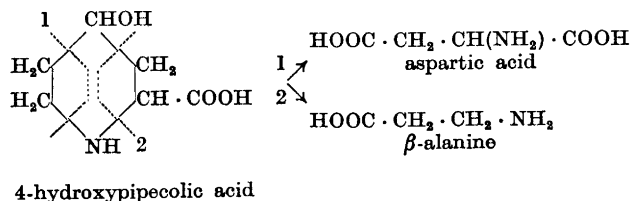
The formation of aspartic acid and also of β -alanine as oxidation products from 4-hydroxypipercolic acid is reasonable, but not from 3-hydroxypipercolic acid.

Because γ -aminobutyric acid was not found as an oxidation product, there was no evidence for the 3-hydroxypipercolic acid.

In order to elucidate the properties of 3-hydroxypipercolic acid we have prepared this acid in the following way. The new antibiotic, etamycin, isolated by Heine-*mann et al.*⁵ and Bartz *et al.*⁶ is according

to Sheehan *et al.*⁷ a cyclopeptide which contains 3-hydroxypyridine-2-carboxylic acid (3-hydroxypipercolic acid) as one member. 2.1 g of etamycin was hydrolyzed with 6 N HCl at 110°C for 24 h. The concentrated hydrolysate was dissolved in 1 l of water and passed through a column containing 100 ml of Dowex 50 (200 mesh) in the H-form. On elution with 1 % ammonia 3-hydroxypipercolic acid emerged before the amino acids. The fraction was evaporated *in vacuo* and 3-hydroxypipercolic acid was crystallized three times from methanol-ether. Yield 134 mg. M.p. 214–215°C. (Found: C 51.70; H 3.83; N 10.41. Calc. for $\text{C}_8\text{H}_9\text{O}_3\text{N}$: C 51.81; H 3.63; N 10.08).

100 mg of 3-hydroxypipercolic acid was dissolved in 20 ml of water after addition of some drops of diluted HCl and was 48 h catalytically hydrogenated (50 mg PtO_2 as



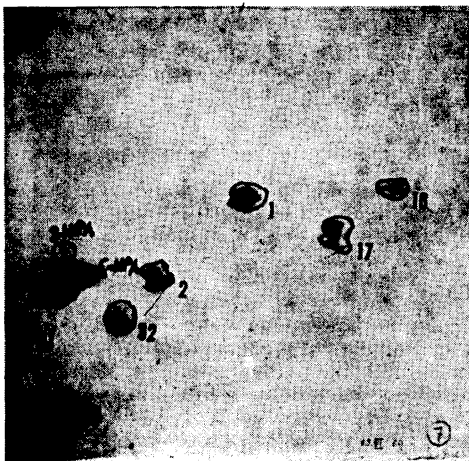


Fig. 2. Two-dimensional paper chromatogram of different amino acids including 3-, 4-, and 5-hydroxypipelic acids. 1 gly, 2 ala, 11 pro, 16 asp, 17 glu, 29 γ -aminobut, 32 β -ala, 60 pipercol, 3-HPA, 4-HPA, and 5-HPA the corresponding hydroxypipelic acids. Solvents as in Fig. 1.

catalyst). About seven ninhydrin-positive spots could be found on the paper chromatogram. The amino acids formed through ring splitting were removed with HNO_3 and the imino acids regenerated with HCl . After running through an Amberlite IR-120 column three compounds could be found: 3-hydroxypipelic acid, pipelic acid, and an unknown substance which on the basis of its R_F -values was possibly tetrahydro-3-hydroxypipelic acid. The 3-hydroxypipelic acid could be obtained in crystalline form by addition of acetone to a water solution. After recrystallization from water-acetone the substance was paper chromatographically pure. Yield 8.5 mg. Decomposition from about 250°C . (Found: N 9.96. Calc. for $\text{C}_6\text{H}_{11}\text{O}_3\text{N}$: N 9.66).

The position of the spots of 3-, 4-, and 5-hydroxypipelic acid on a two-dimensional paper chromatogram can be seen in Fig. 2. As appears from the figure 3-, 4-, and 5-hydroxypipelic acids can be separated from each other by paper chromatography using butanol-acetic acid-water and phenol-water- NH_3 as solvents. These imino acids also give different colours with ninhydrin, isatin, and naphthoquinonesulphonic acid (e.g. 3-hydroxy compound with isatin no colour, 4-hydroxy compound a very weak green colour, and 5-hydroxy compound a strong greenblue colour).

On the basis of optical rotation it is probable that the natural 4-hydroxypipelic acid belongs to the L-series.

All known facts are in accordance with the structure Virtanen and Kari gave to the imino acid (4-hydroxypipelic acid) they isolated from *Albizia lophantha* and *Acacia pentadena*.

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