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Short Communications

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

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70 % alcoholic extracts of different *Acacia* species and *Rhapis flabelliformis* gave on a two-dimensional chromatogram (solvents: butanol-acetic acid and phenol-NH_3), after treatment with ninhydrin a very strong blue spot C (Fig. 1) which did not disappear after hydrolysis with 6 *N* HCl.

The fluorescence of the blue spot was very similar to that of the corresponding spot of pipecolic acid (piperidine-2-carboxylic acid). When treated with isatin the colour of the spot C is sky blue, and that of pipecolic acid greenish blue. Both pipecolic acid, proline, and the unknown C react with *p*-nitrobenzoylchloride, the amino group in compound C is accordingly secondary. This is also shown by the fact that compound C does not become deaminated when treated with oxides of nitrogen.

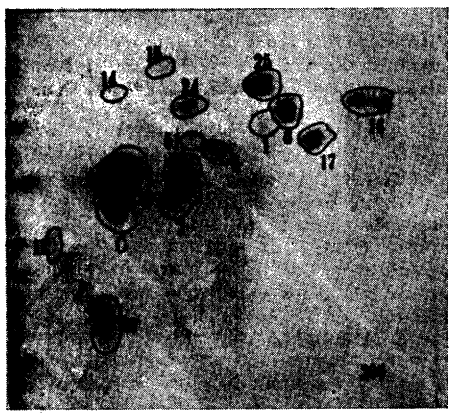


Fig. 1. Two-dimensional paper chromatogram of the unhydrolyzed 70 % alcohol extract of Rhapis flabelliformis. 1 = gly, 2 = ala, 8 = ser, 9 = threo, 11 = pro, 14 = arg, 15 = lys, 24 = glu-NH, 25 = asp-NH, 29 = α-aminobut. acid, 51 = homoser., C = unknown spot.

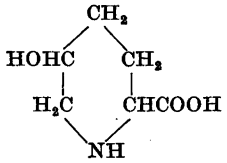
All these findings suggested that the substance C is a derivative of piperidinecarboxylic acid. By comparing the chromatograms of piperidine-2-, 3- and 4-carboxylic acids with that of substance C it could be noticed that these carboxylic acids moved with butanol-acetic acid appreciably faster than substance C. It seemed therefore most likely that the unknown compound was a hydroxy-piperidine-carboxylic acid.

The substance C was isolated from the alcohol extract of *Rhapis flabelliformis* (650 g fresh material = 290 g dry substance) using an Amberlite IR—120 column. Amino acids were displaced with 1 *N* ammonia taking 16 fractions of 16—17 ml. Fractions 8—12 were mixed and evaporated to 2 ml. The syrup crystallized partly in an icebox, and the crystals washed with abs. alcohol appeared to represent the compound C.

The syrup with crystals was dissolved in 25 ml of 1.5 *N* HCl, and using a Dovex 50 column 276 fractions of 10 ml were taken. Fractions 181—210 and 211—233 were concentrated. Upon cooling beautiful crystals (prisms) were formed. They were filtered off and washed with 70 % alcohol. The substance gave on the paper chromatogram only spot C. M. p. 225—230° (decomp.). (Found: C 40.14; H 6.81; N 7.58. Calc. for $C_6H_{11}O_3NHCl$: C 39.68; H 6.66; N 7.71.)

A comparison of our unknown compound with synthetic 5-hydroxy-piperidine-2-carboxylic acid showed that both substances moved with the same velocity on the paper chromatogram (butanol-acetic acid

and phenol-NH_3). Because we do not know to what extent the position of the OH group in the ring influences the R_F-value we do not consider the identical chromatogram given by compound C and 5-hydroxy-piperidine-2-carboxylic acid a conclusive proof for the identity of these substances. Oxidation with permanganate in 20 % sulphuric acid solution at 10° C gave mainly glutamic acid and aspartic acid in smaller amounts. On the basis of this we may conclude that in the hydroxy-piperidine-carboxylic acid which we have isolated the OH group actually is in the position 5 and the carboxyl group in the position 2. The formation of glutamic acid cannot be explained otherwise.



It is possible that the new amino acid is formed from δ-hydroxylysine.

We are very grateful to Professor T. J. King, Nottingham, for a preparation containing some 5-hydroxy-pipecolic acid, to Dr. G. Curzon, Stanmore, England, for the *iso*nipecotic acid (piperidine-4-carboxylic acid), to Mr. O. Oja for helping us in the preparation of nipecotic acid (piperidine-3-carboxylic acid), and to Professor A. Kalela for the plant material we got from the University Botanical Garden.

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Triterpenoids in Lichens
II. Taraxerene, a Naturally Occurring Triterpene

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During our researches on lichens a sample of *Cladonia deformis* Hoffm. was investigated. The origin of the sample has been reported previously [1]. The general procedure for extraction with ether and working up of the extract has been reported in part I of this series [2].

The neutral part of the ether extract, when chromatographed on alumina furnished a petroleum (b. r. 40—70°) eluate, which contained a hydrocarbon mixture (0.3 g) that did only partly melt on the boiling water-bath. The high-melting part of the fraction was much less soluble in petroleum than the low-melting one, and by taking advantage of this fact a sample of m. p. 230—233°, $[\alpha]_D$ +4° (*c*, 0.83) was obtained *. A much better separation, however, was achieved by careful chromatography: The hydrocarbon, dissolved in petroleum, was brought on a column of 30 g of alumina, 17 cm high, 1.5 cm diameter, and fractions of 5 ml were collected. The high-melting hydrocarbon was retained more tenaciously than the low-melting one, and thus a satisfactory separation could be obtained. For further purification the hydrocarbon was treated with petroleum, and finally the substance was crystallised from ether, m. p. 237—238°, $[\alpha]_D$ + 1° (*c*, 0.81). The total amount of hydrocarbon was about 15 mg from 2.9 kg of dry lichen.

The manner in which the substance was obtained seemed to justify the assumption that it was a hydrocarbon, and the high m. p. suggested a polycyclic one. A triterpene ** was not ruled out, and inspection of the literature indicated that identity with skimmiene IIa[3] might exist. The chemistry of taraxerol (skimmiol) is presently being investigated by Dr. C. J. W. Brooks, compare [4]. Dr. Brooks incidentally learned of this possible identity through Dr. P. de Mayo, and very kindly provided a sample of taraxerene, which, although, strictly speaking, it has not been proved, is very likely identical with skimmiene IIa,*** and which he had recently prepared by Wolff-Kishner reduction of taraxerone, and for which he reported m. p. 238—240° (strong sublimation), $[\alpha]_D$ + 3°, very sparingly soluble in chloroform, in agreement with our observations on the hydrocarbon from *Cl. deformis*. Taken at the same time the hydrocarbon from the lichen,

* All rotations in chloroform in a 1 dm tube.

** For nomenclature compare Barton, D.H.R. in Simonsen, John, *The Terpenes*, Vol. III, Cambridge 1952, p. 328.

*** Note added in proof: Weissenberg diagram of skimmiene II a, very kindly provided by Dr. K. Takeda, showed identity with taraxerene.