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A NEW AMINO ACID, NICOTIANAMINE, FROM TOBACCO LEAVES

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In the course of our studies of nitrogen metabolism in tobacco plants, a new amino acid was isolated from, Nicotiana tabacum L. "Hicks".

We wish to report here the isolation and the structure elucidation of the amino acid tentatively named "nicotianamine" (represented as (I) in the following text).

Green tabacco leaves (70 Kg) were extracted 3 times with 70 % aqueous MeOH. The aqueous extract obtained by evaporating MeOH in vacuo was treated with charcoal, and the resultant clear solution was further treated with Dowex 50W-X4 resin. The cationic constituents thus obtained were subjected to displacement chromatography with a set of five columns of Dowex 50W-X4 resin according to the method of Partridge et al[1]. Fractions containing (I) were then submitted to cellulose column chromatography with n-BuOH: acetic acid: water (4:1:5) as solvent to yield almost pure compound(I). Recrystallization from water twice afforded pure 209 mg of (I).

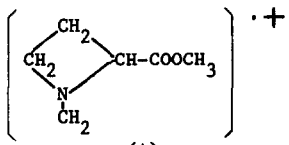
This compound (I), $[\alpha]_D^{23} = -60.5$ (C=2.7, in water), with $C_{12}H_{23}N_3O_7$ by elementary analysis, gradually darkened in colour above 240°C without melting, is labile to mineral acid and a test for α-amino acid[2] is positive. The elution peak with an amino acid analyzer in the usual physiological fluid analysis is overlapped with those of asparagine and glutamine.

In the NMR spectrum in D_2O (Fig. I), the following signals are observed: 4H(m), δ2.2; 2H(m), δ2.6; 2H(t), δ3.2; 2H(t), δ3.4; 4H(m), δ4.0. No signal assigned to methyl group is detected.

In the mass spectra of the methyl (Fig. 3) and the ethyl ester, parent peaks were observed at m/e 345 ($C_{15}H_{27}N_3O_6$) and m/e 387, base peaks at m/e 128 ($C_6H_{10}NO_2$) and m/e 142 respectively, indicating that (I) exists as a monohydrate and has three carboxyl groups. Since it absorbs no hydrogen atom on catalytic hydrogenation on platinum black, one ring system must be included in the molecule.

In a deutero labelling experiment[3], the parent peak of the methyl ester was shifted to m/e 348, but the base peak was not. These indicate that the methyl ester has three active hydrogen

atoms attached to basic nitrogen atoms and the nitrogen atom in the base peak has none of such hydrogen atoms. From the difference of 14 mass units between the base peak of the methyl ester and that of the ethyl ester, two oxygen atoms in the former are suggested to be attributed to a carbomethoxy group. On the other hand, $KMnO_4$ oxidation of (I) (1.1 μmole), yielded aspartic acid (0.4 μmole), β-alanine (0.09 μmole) and azetidine-2-carboxylic acid (0.2 μmole). The presence of azetidine-2-carboxylic acid moiety in (I) was further supported by the comparison of the NMR spectrumn of (I) with that of azetidine-2-carboxylic acid. In the latter, two 2H multiplets assigned to CH_2 at 3 and 4 position of the azetidine ring are observed at δ2.6 and δ4.0 respectively. Coupling pattern of the signal at δ2.6 is similar to that of (I) at δ2.6. Thus, 4H multiplet at 4.0 in (I) implies the 2H multiplet attributed to 4 position of the azetidine ring. In the both compounds the signal due to the proton attached to 2-position of carbon atom in the azetidine ring is hidden by the strong signal due to HDO at δ4.7. Considering the above results, and admitting that the base peak in the mass spectrum of the methyl ester is generated by primary fragmentation, the structure (A) is given for the base peak. The existence of the m/e 114 ($C_4H_8NO_2$) m/e 142 ($C_7H_{12}NO_2$) and m/e 156 ($C_8H_{14}NO_2$) peaks in addition to m/e 128, suggests that at least 3 CH_2 units combine linearly to the nitrogen atom of the azetidine ring.



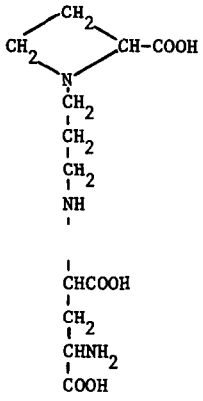
(A)

By $KMnO_4$ oxidation of azetidine-2-carboxylic acid (0.17 μmole), aspartic acid and β-alanine were obtained, yielding 0.005 μmole and 0.03 μmole respectively. Comparing these results with the yields of the oxidation products of (I), most of aspartic acid formed in the oxidation of (I) is assumed not to be derived from azetidine-2-carboxylic acid moiety, but from the other moiety which is easily oxidized to aspartic acid.

The U.V. spectrum of the DNP derivative of (I) has λmax at 360 mμ with $\varepsilon = 1.6 \times 10^4$, showing that one NH_2 group exists in (I). Thus, the three nitrogen atoms in (I) are supposed to exist in one primary, one secondary and one tertiary amine. $KMnO_4$ oxidation of the DNP derivative afforded DNP-aspartic acid. These indicate that the NH_2 group in (I) exists in the moiety which yields aspartic acid by $KMnO_4$ oxidation.

By the double resonance experiment irradiated at δ2.2, triplets at δ3.4 and δ3.6 changed to two 2H singlets and two 1H singlets appeared near δ4.0 remaining the unchanged multiplet signals due to the two protons in 4 position of the azetidine ring. This indicates that either two $-CH_2CH_2CH-$, or one $-CH_2CH_2CH_2-$ and one $-CHCH_2CH-$ groups are present in the molecule where the pro-

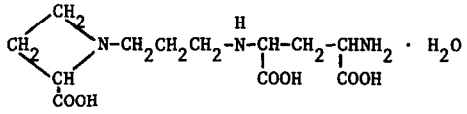
tons attached to the both terminal carbons are deshielded by adjacent nitrogen atoms, carboxyl groups, or both of them. In considering the presence of the m/e 128, 142 and 156 peaks in the mass spectrum of the methyl ester, this supports the presence of an isolated propylene group, which is combined to two nitrogen atoms, one of which is the tertiary nitrogen atom of azetidine ring and the other is secondary one. Thus, the partial structure of this moiety would be given as (B). Accordingly, the remaining $-C_5H_8NO_4$ moiety, from which aspartic acid is formed by $KMnO_4$ oxidation, would have a partial structure $-CHCH_2CH-$. As one of the two carboxylic acid and one NH_2 group in the moiety is situated in α position to each other, the partial structure (C) is given for this moiety.



(B)

(C)

In the high resolution mass spectrum of the methyl ester, the existence of the m/e 257 ($C_{12}H_{21}N_2O_4$, M-$CHNH_2COOCH_3$), m/e 243 ($C_{11}H_{19}N_2O_4$, M-$CH_2CHNH_2COOCH_3$), m/e 171 ($C_8H_{15}N_2O_2$, M-$CHCOOCH_3CH_2NH_2COOCH_3$) and m/e 174 ($C_7H_{12}NO_4$, $CHCOOCH_3CH_2CHNH_2COOCH_3$) peaks established the structure of (I) as 1-[3'-(γ-amino-α,γ-dicarboxy propylamino)-propyl]-azetidine-2-carboxylic acid.



(I)

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Reference

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(2) P. O. Larsen and A. Kjaer; Biophys. Biochem. Acta, 38, 148 (1960)

(3) H. Budzikiewicz, C. Djerassi and D. H. Williams; Structure Elucidation of Natural Products by Mass Spectrometry, vol. 1, 17

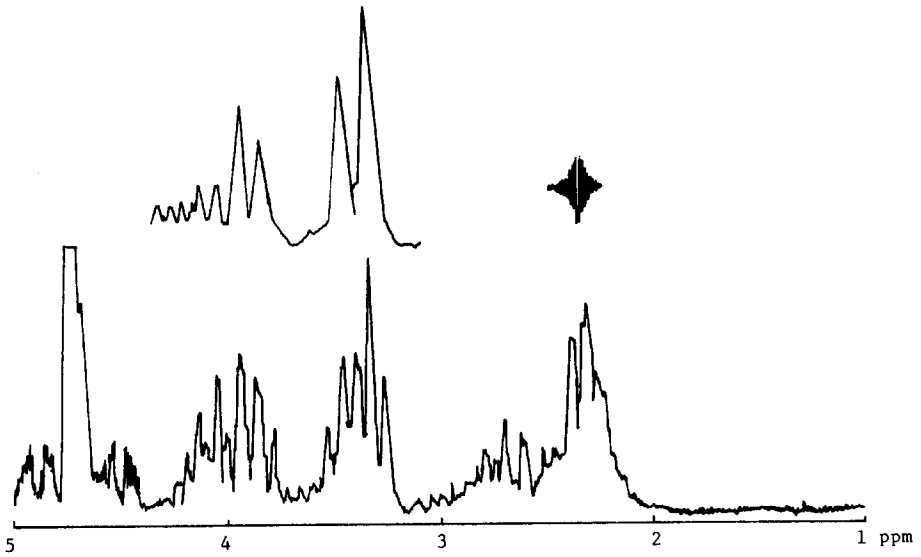

Fig. (1) NMR spectrum of (I)

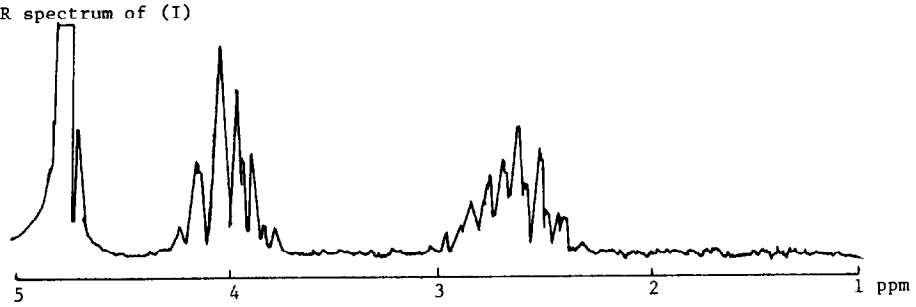

Fig. (2) NMR spectrum of azetidine-2-carboxylic acid.

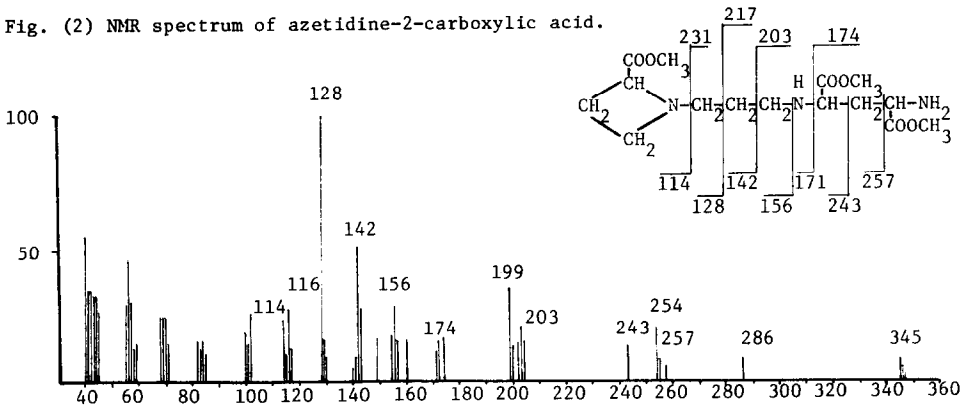

Fig. (3) Mass spectrum of the methyl ester.