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# SHORT REPORTS

## SACCHAROPINE FROM TOBACCO LEAVES

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**Key Word Index**—*Nicotiana tabacum*; Solanaceae; tobacco leaf; saccharopine.

In the course of a study of oligopeptides in tobacco leaves, several constituents which gave more than two ninhydrin-positive compounds by $KMnO_4$ oxidation were observed. One of these was isolated by sequential chromatography on Sephadex G-10, anion exchange resin and cellulose powder, and was identified as *N*-(2-glutaryl)-L-lysine (saccharopine). It has been reported that saccharopine is concerned with lysine metabolism in both yeast [1, 2] and animal tissue, but not in higher plants which are known to have the alternative diaminopimelate pathway. However, saccharopine was isolated from buckwheat seeds and its possible participation in lysine metabolism in higher plant was suggested [3]. The present finding suggests this compound may be more widely distributed in plants and may be of importance in relation to lysine metabolism.

### EXPERIMENTAL

*Ca* 60 kg of fr. tobacco leaves (*N. tabacum*, BY-4) was extracted with 200 l. of 70% MeOH. After conc, metal ions and neutral substances were removed with Dowex 50, $H^+$. The crude amino acid fraction (400 ml) was applied on a Sephadex G-10 (10 × 150 cm) and eluted with $H_2O$. Saccharopine, as well as many peptides, was eluted just before glutamic acid. The whole peptide fraction was applied to a column of Dowex 1 (AcOH form, 2.5 × 40 cm). After washing thoroughly with $H_2O$, the absorbed compounds were fractionated by stepwise elution with 0.1, 0.3, 1 and 2 M HOAc. The eluate with 0.3 M HOAc was collected, concd (4 ml) and applied to a cellulose column (Avicel, 2.5 × 90 cm) and eluted with BuOH–HOAc–$H_2O$ (12:3:5) at room temp. The fraction containing saccharopine (1–1.1 l.) was evapd to dryness at 45° *in vacuo* and dissolved into a small amount of hot $H_2O$. After keeping overnight at room temp., colorless needles appeared which were recrystallized 3 × giving, 40 mg. Anal. (Found: C, 46.82, H, 7.67, N, 9.92. Calcd. for $C_{11}H_{20}N_2O_6$: C, 47.82, H, 7.24, N, 10.14%); its parent peak in HDMS, *m/e* 277 ($M^+$, +1). PMR $\delta^{0.2\,N\,DCl}_{HDO\,(4.7)}$ 4.0 (2H, *tt*), 3.1 (2H, *t*), 2.5 (2H, *t*), 2.1 (2H, *m*), 1.5–1.8 (8H, *m*). CMR $\delta^{0\ 2\,N\,D\,Cl}_{dioxane\,(67.4)}$ 176.6 (*s*), 172.3 (*s*), 171.2 (*s*), 59.7 (*d*), 53.3 (*d*), 47.1 (*t*), 30.2 (*t*), 30.0 (*t*), 26.0 (*t*), 24.7 (*t*), 22.4 (*t*). The IR spectrum was identical with previous data [2–4]. Mp 257–259°, decomp. $[\alpha]_D^{23}$ +31.4° (*c* 1 in 0.5 N HCl).

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## METABOLISM OF 5-ACETOAMINO-2-HYDROXYVALERIC ACID IN TOBACCO LEAVES

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**Key Word Index**—*Nicotiana tabacum*; Solanaceae; tobacco leaf; 5-acetoamino-2-hydroxyvaleric acid; β-acetylornithine; metabolism.

**Abstract**—5-Acetoamino-2-hydroxyvaleric acid, (5-AHV) was metabolized to δ-acetylornithine in tobacco leaves. On the other hand, δ-acetylornithine fed to tobacco leaves was metabolized into at least 5 components, one major component being 5-AHV. These results show that tobacco plant has a reversible metabolic pathway between 5-AHV and δ-acetylornithine.

### INTRODUCTION

Recently we isolated a new compound, 5-acetoamino-2-hydroxyvaleric acid (5-AHV) from green tobacco leaves [1]. Preliminary experiments showed that not only young green leaves but also aged yellow leaves contain appreciable amounts of this compound. Since α-hydroxy

carboxylic acids are usually concerned with amino acid metabolism, we suspected that 5-AHV might be interconvertible into some amino acid which from its five carbon skeleton, belongs to the glutamate family. To confirm the above speculation, a tracer experiment using chemically synthesized radioactive 5-AHV was conducted and the results are reported herein.

## RESULT AND DISCUSSION

Infiltrated (±)-5-AHV was metabolized to the extent of 3–6% in tobacco leaf disks. Under the conditions used all the radioactivity was found in 70% MeOH soluble fraction which when separated on the amino acid analyser showed label only in δ-acetylornithine (δ-AO). Recrystallisation with cold δ-AD confirmed this finding. If the conversion of 5-AHV to δ-AO occurs in the usual manner one might expect to find 5-AcN-2-ketovaleric acid among the products but this keto acid could not be identified because it behaved like 5-AHV on the columns or TLC used.

The metabolism of δ-AO in tobacco leaves was next examined. In this case 10–40% of the radioactive α-AO infiltrated into the leaf disks was converted into other products, mainly in the 70% MeOH soluble fraction. This fraction was separated on a Dowex 1 column, and 3 radioactive peaks were eluted. Three compounds were expected in the first peak; these are 5-AHV, 5-AcN-2-ketovaleric acid and α,δ-diacetylornithine which has been reported to be a direct metabolite of δ-AO [2]. These compounds are difficult to separate by TLC so the whole fraction was treated with 2,4-DNP and extracted with EtOAc at pH 2.2. An appreciable amount of radioactivity was transferred into the organic layer suggesting that 5-AcN-2-ketovaleric acid, may well be present. The $H_2O$ layer was subjected to acid hydrolysis and the products separated by an amino acid analyser. The results showed that the major radioactive component in the overall fraction was 5-AcN-2-HV. The radioactive compounds in the other fractions from the Dowex 1 column have not been identified.

Previously, Brown and Fowden reported that δ-AO is easily biosynthesized from ornithine, although the reverse reaction is negligible [2]. If this reaction occurs in tobacco leaves, the above pathway between δ-AO and 5-AHV is a link with it. Brown and Fowden also reported that when labelled δ-AO was supplied to mung bean or sainfoin, proline became labelled although no label was detected in ornithine itself [2]. Our results are of interest here to the biosynthesis of proline in tobacco leaves. If 5-AcN-2-ketovaleric acid formed from 5-AHV is deacetylated, it will give 5-amino-2-ketovaleric acid which cyclizes nonenzymatically to give $\Delta^1$-pyrroline-2-carboxylic acid, already known to be reduced to proline enzymatically in plant tissues [3, 4]. Although proline was not detected in our feeding experiments, this may be dur to addition of excess water supply to leaves which is known to strongly retard proline biosynthesis [5].

## EXPERIMENTAL

*Chemical synthesis of 5-AHV.* To a soln (0.01 N HCl, 2.5 ml) of (±)-Orn-5-$C^{14}$ (250 μCi, sp. act. 7.28 mCi/mmol) were added $Ag_2CO_3$ (15 mg) and $CuCO_3$ (10 mg). The soln was heated at 100° for 1 hr and the ppt. discarded. To the filtrate were added $Ba(OH)_2.8H_2O$ (14 mg) and $Ac_2O$ (10 μl) with cooling by ice over a period of 30 min. After keeping at room temp. for 30 min, the soln was passed through a column of Dowex 50w × 4 (2 ml, 100–200 mesh). The cations were eluted with 2N $NH_4OH$. Crude δ-AO was further purified by an amino acid analyser (Beckman 120C) [1]. About 100 μCi of the pure δ-AO was obtained. To the radioactive δ-AO dissolved in 1 ml of HOAc was added 30% $NaNO_2$ (4 ml) and the whole allowed to stand for 5 min. After drying *in vacuo*, the residue was dissolved in a small amount of $H_2O$ and applied to a column of AG Dowex 1 × 8 (0.8 × 150 cm, < 400 mesh). 5-AHV was eluted at 65 min with 0.2N HCl (flow rate, 0.92 ml/min). More than 90% of the radioactivity was recovered as 5-AMV.

*Infiltration of the radioactive materials into leaf disks.* Infiltration of the radioactive 5-AHV or δ-AO into tobacco leaf disks (*N. tabacum*, BY 4, about 40 days after transplantation) was carried out according to the method of ref. [6]. The methanolic extract was dried, dissolved in $H_2O$ and separated on an amino acid analyser (JEOL 6AH) or a column of AG Dowex 1 as described above. The elution was monitored by a Packard Liquid Scintilation Counter.

*Identification of the radioactive products.* In addition to the columns mentioned above 2-D TLC (Avicel SF) was used for identification of the radioactive products. Solvent systems used were as follows; BuOH–AcOH–$H_2O$ (4:1:5) and phenol–$H_2O$ (4:1), BuOH–$HCO_2H$–$H_2O$ (7:3:12) and PrOH–$NH_4OH$ (2:1). Sakura film (Macro autoradiography $H^3$ type) was used for autoradiography. Exposures, 1–14 days.

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