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METABOLISM OF [2-^{3}H]- AND [6-^{3}H]-NICOTINIC ACID IN INTACT *NICOTIANA TABACUM* PLANTS

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Abstract—Earlier observations of Dawson on the relative incorporation of [2-^{3}H]- and [6-^{3}H]-nicotinic acid into nicotine have been confirmed in intact *Nicotiana tabacum* plants. All the tritium in the nicotine derived from [2-^{3}H]-nicotinic acid was located at C-2 of the pyridine ring. However the radioactive nicotine derived from [6-^{3}H]-nicotinic acid was not labelled specifically at C-6 with tritium. By carrying out feeding experiments with [6-^{14}C, 2-^{3}H]- and [6-^{14}C, ^{3}H]-nicotinic acids, it was established that there was very little loss of tritium from C-2 and C-6 of nicotinic acid during 5 days of metabolism in the tobacco plant.

INTRODUCTION

DAWSON *et al.*[1-3] long ago established that nicotinic acid is a precursor of the pyridine ring of the tobacco alkaloid nicotine. This has been confirmed by others[4,5] who also showed that the point of attachment of the pyrrolidine ring of nicotine is at the site from which the carboxyl group is lost, that is at C-3. One experiment of Dawson which has been the subject of considerable discussion involved the use of nicotinic acid labelled around the pyridine ring with either tritium or deuterium.[2,3] The isotopically labelled nicotinic acids were administered to sterile excised root cultures of *Nicotiana tabacum* for 4 weeks. The incorporations of isotope from [2-^{3}H]-, [4-^{2}H]-, [5-^{3}H]- and [6-^{3}H]-nictonic acid were 11·3, 13·0, 14·2 and 1·1% respectively. The low incorporation of the [6-^{3}H]-nicotinic acid compared with the other labelled acids required an explanation. One way in which the tritium could be lost at this position would be by the formation of 6-hydroxynicotinic acid. However, this possibility was rendered unlikely when it was found that [^{15}N]-6-hydroxynicotinic acid failed to serve as a precursor of nicotine.[3] There was also no loss of tritium from the [6-^{3}H]-nicotinic acid whilst it was present in the nutrient solution. Nicotinic acid having the same specific activity as that administered 4 weeks previously was isolated from the culture medium. Another suggestion made by Dawson[3,6] was that the nicotinic acid was reduced to

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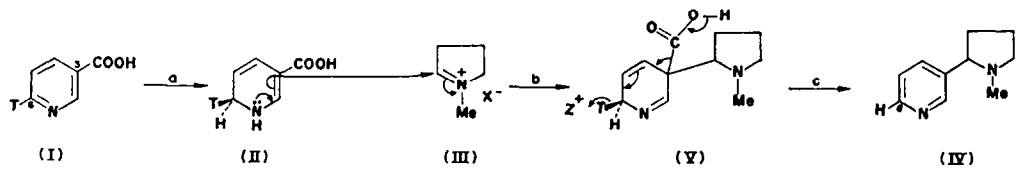
1,6-dihydronicotinic acid (II), a plausible intermediate which could react with the *N*-methyl-Δ^1-pyrrolinium salt (III), known to be a precursor of the pyrrolidine ring of nicotine.[7-9] This biogenetic scheme is illustrated in Scheme 1. In order to achieve loss of tritium from the position which becomes C-6 in nicotine it is necessary to postulate two stereospecific reactions, i.e. the hydrogen introduced at C-6 on reduction of nicotinic acid (reaction a) must have the opposite configuration from the hydrogen lost from this position in the final oxidative decarboxylation (reaction c). So far this hypothesis has not been tested.



SCHEME 1. BIOGENETIC PATHWAY.

RESULTS

We have repeated the work of Dawson feeding [2-^{3}H]- and [6-^{3}H]-nicotinic acids to intact *N. tabacum* plants, the roots growing in a hydroponic nutrient solution. The results of three separate experiments are recorded in Table 1. In agreement with Dawson we discovered that the [2-^{3}H]-nicotinic acid was a much more efficient precursor of nicotine than the [6-^{3}H]-acid. In the younger plants (Experiment 3), the labelled nicotinic acids were absorbed much more rapidly from the nutrient solutions, and the significantly higher incorporation of the [2-^{3}H]-nicotinic acid (1·64%) probably indicates that nicotine synthesis was proceeding at a more rapid rate in these plants. The radioactive nicotine obtained from these experiments was degraded according to Dawson's procedure (Scheme 2). The nicotine derived from [2-^{3}H]-nicotinic acid afforded 1-methyl-2-pyridone-5-carbonamide (VI) having almost the same specific activity (95%) as the nicotine, whereas the 1-methyl-2-pyridone-3-carbonamide (VII) had negligible activity (1·6%). This result, also in agreement with previous work,[3] indicates that essentially all the tritium was located at C-2 of the nicotine. Dawson did not apparently degrade the nicotine derived from [6-^{3}H]-nicotinic acid. We were surprised to discover that the pyridone VI obtained from this labelled nicotine contained 40–58% of the original activity of the nicotine. This result indicates that the tritium was not all located at C-6 of the pyridine ring of nicotine. However, almost all the tritium was located in the pyridine ring since the nicotinic acid, nicotinamide, and *N*-methylnicotinamide iodide (VIII) had essentially the same specific activity as the nicotine from which they were derived. Some light may be thrown on the mechanism of nicotine formation if the exact location of the tritium were known, and degradations are in progress to determine this.

We were concerned that perhaps the administered [6-^{3}H]-nicotinic acid was not radiochemically pure. However, a similar degradation carried out on this precursor yielded the pyridone (VI) having less than 1% of the initial activity of the nicotinic acid, indicating that all the tritium was located at C-6. In order to determine whether the tritium was being lost from the nicotinic acid prior to its incorporation into nicotine, nicotinic acid labelled with

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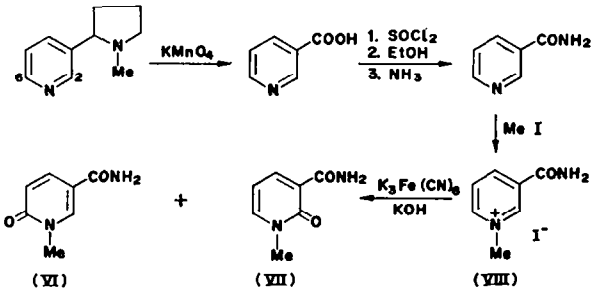
TABLE 1. FEEDING OF [2-^{3}H]- AND [6-^{3}H]-NICOTINIC ACID TO *N. tabacum* PLANTS

Compound	Experiment 1		Experiment 2		Experiment 3	
Nicotinic acid	[2-^{3}H]	[6-^{3}H]	[2-^{3}H]	[6-^{3}H]	[2-^{3}H]	[6-^{3}H]
Sp. act. (dpm/mmole)	$6{\cdot}44 \times 10^8$	$5{\cdot}05 \times 10^8$	$6{\cdot}44 \times 10^8$	$5{\cdot}05 \times 10^8$	$4{\cdot}68 \times 10^8$	$1{\cdot}83 \times 10^8$
Amount fed (mg)	20	20	21·5	20·1	21·2	23·2
Age of plants (weeks)	12		12		6	
Feeding period (days)	8		6		6	
Nicotine dipercholarate						
Sp. act. (dpm/mmole)	$2{\cdot}24 \times 10^6$	$2{\cdot}15 \times 10^5$	$4{\cdot}74 \times 10^6$	$3{\cdot}75 \times 10^5$	$7{\cdot}66 \times 10^6$	$8{\cdot}51 \times 10^5$
Sp. incorp. (%)	0·35	0·043	0·74	0·074	1·64	0·047
Sp. incorp. of [2-^{3}H]-acid / Sp. incorp. of [6-^{3}H]-acid	8·1		10		35	

^{14}C at C-6 and with ^{3}H at C-2 or C-6 was administered to tobacco plants. Since the amount of free nicotinic acid in tobacco plants is quite small,[10] carrier nicotinic acid was added to the plants when they were harvested. There was very little loss of tritium relative to the ^{14}C during 5 days of feeding. The recovered [6-^{14}C, 2-^{3}H]-nicotinic acid retained 93% of the ^{3}H, and the [6-^{14}C, ^{3}H]-acid retained 89%.



SCHEME 2. DEGRADATION OF THE NICOTINE.

EXPERIMENTAL

General methods. Radioactive compounds were assayed in a Nuclear Chicago Mark II Liquid scintillation counter, using as solvents either dioxane–EtOH or toluene, with the usual scintillators.[11]

Administration of the labelled nicotinic acids to tobacco. [2-^{3}H]- and [6-^{3}H]-nicotinic acids were prepared by the method of Dawson.[3] The *N. tabacum* plants were grown in soil for 1–2 months and then transferred to a hydroponic nutrient solution[12] through which air was bubbled. The labelled nicotinic acid, dissolved in H_2O containing a little NH_3, was added to this nutrient solution. In 5–6 days most of the radioactivity was absorbed by the tobacco plants, which were harvested shortly after (see Table 1). The nicotine was isolated as previously described[13] and purified by TLC on silica gel.[14]

Feeding of [6-^{14}C, 2-^{3}*H*]- *and* [6-^{14}*C*, ^{3}*H*] *nicotinic acid to tobacco and reisolation of the nicotinic acid.* [6-^{14}C]-Nicotinic acid was purchased from Amersham-Searle, Illinois. [6-^{14}C, 2-^{3}H]-Nicotinic acid (49·7 mg, ^{14}C: $5{\cdot}99 \times 10^7$ dpm/mmol; 3H/^{14}C = 7·81) was fed to 5 tobacco plants (2 months old). After 5 days the plants were harvested and maserated with 80% EtOH which contained inactive nicotinic acid (65·4 mg). The homogenate was boiled for 30 min and filtered. The filtrate was then processed as described by Byerrum[10] affording nicotinic acid (45 mg, having a ^{14}C-activity of $1{\cdot}26 \times 10^5$ dpm/mmol (^{3}H/^{14}C = 7·35). Similarly [6-^{14}C, ^{3}H]-nicotinic acid (72·6 mg, ^{14}C: $7{\cdot}16 \times 10^7$ dpm/mmol; ^{3}H/^{14}C = 25·6) was fed to 6 plants for

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5 days. Carrier nicotinic acid (61·1 mg) was added to the harvested plants, and the reisolated nicotinic acid had a ^{14}C-activity of $4{\cdot}50 \times 10^5$ dpm/mmol ($^3H/^{14}C = 22{\cdot}9$).

Degradation of the labelled nicotine. The following data refers to a sample of nicotine derived from [2-^{3}H]-nicotinic acid. Activities reported in parentheses are dpm/mmol $\times 10^{-4}$. Nicotine diperchlorate (4·80) was oxidized with $KMnO_4$ affording nicotinic acid (4·65), which was esterified yielding ethyl nicotinate. Reaction of this ester with ammonia yielded nicotinamide (4·68), which on treatment with MeI yielded *N*-methylnicotinamide iodide[15] (4·69). Oxidation of this quaternary salt with alkaline $K_3Fe(CN)_6$[16] yielded a mixture of 1-methyl-2-pyridone-5-carbonamide (4·57) and 1-methyl-2-pyridone-3-carbonamide (0·079) separated by chromatography on alumina.[3]

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