STUDIES ON THE METABOLISM OF HYOSCYAMINE

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Abstract

(-)-Hyoscyamine infiltrated into alkaloid-free ***Solandra grandiflora*** scions on tomato ***(Lycopersicon esculentum*** Mill.) stocks and aerial parts of a normal tomato plant was transformed to atropine, noratropine and tropine. These alkaloids were also isolated from a tomato scion raised on a ***Solandra grandiflora*** stock. The results are discussed in relation to the site of synthesis and secondary transformation of (-)-hyoscyamine in ***S. grandiflora.***

Introduction

(-)-Hyoscyamine, the principal alkaloid of the roots, does not occur in the aerial parts of ***Solandra grandiflora*** Sw. (Family Solanaceae). Instead they contain atropine as the major alkaloid (1). It is thus assumed that hyoscyamine is synthesised in the roots and translocated to the aerial parts where it is eventually transformed to atropine and other alkaloids through mediation of some enzyme systems. In order to further examine this assumption and to investigate the metabolic changes of (-)-hyoscyamine in the aerial parts of various plants, two ***S. grandiflora*** scions grown on Tomato ***(Lycopersicon esculentum*** Mill.) stocks and the aerial parts of a normal young Tomato plant (a non-tropane alkaloid-solanaceous plant) were infiltrated with (-)-hyoscyamine -U-^{14}C and their metabolic changes studied. At the same time the alkaloid spectrum of a Tomato scion raised on a ***S. grandiflora*** stock was also investigated. The results of these investigations, a part of which was briefly reported elsewhere(2), are reported in this paper.

Experimental

Construction of the grafts: Reciprocal grafts were constructed between young ***S. grandiflora*** and Tomato plants grown in a temperate green house. Five months later metabolic studies were initiated selecting only the healthier grafts for infiltrating the alkaloid solutions. One of the healthy Tomato scions was not treated, but harvested at that time for analysis.

Preparation and infiltration of the alkaloids: Two samples of pure unlabelled (-)-hyoscyamine base (30 mg each) in ethanolic solution were neutralised with 0. 1N-sulphuric acid (ca. 1 ml each). The solutions were then evaporated to dryness and the residues dried ***in vacuo*** for 4 hours before dissolving in distilled water (2 m' ach) for infiltration. One of the solutions was fec an alkaloid-free ***Solandra***

grandiflora scion and the other to a normal young Tomato plant by the "Wick method". Both of them were harvested for analysis 21 days after the feeding.

A sample of labelled (-)-hyoscyamine-U-14C base (5.6 mg), recovered from (-)-hyoscyamine-U-^{14}C picrate (10 mg), specific activity 17.7 x 10^5dpm/mM, was similarly prepared and fed to an alkaloid -free ***S. grandiflora*** scion. This scion was also harvested for analysis 21 days after the feeding.

Analysis of the harvested plant materials: All the harvested plant materials were dried at 50° and powdered separately. The powdered plant material was mixed with lime (one-fifth the weight of the powder), moistened with sufficient water and macerated with ether for an hour in a closed percolator and finally exhausted with more ether. Removal of the solvent afforded the crude extract. The extract was fractionated and refractionated (where required) by Partition column chromatography(3) at pH 6.8 eluting the column successively with petroleum ether (bp 40 – 60°), ether, chloroform and chloroform-ammonia. The individual fractions were examined by TLC (system I: alumina gel G, ether - ethanol (1:1); system II: silica gel G. chloroform - diethylamine (9:1) and PC (Whatman No. 1 filter paper, petroleum ether (bp 60 - 80°) - glacial acetic acid - amyl alcohol - distilled water (1:3:3:3).

Preparation of tigloyl esters: Tigloyl chloride (prepared by refluxing tiglic acid (4 g) with phosphorous trichloride (3 g) at 70-80° for two hours on an oil-bath and distilling off the upper yellowish layer under reduced pressure at 64°) was added dropwise to the dried alkaloid sample, The mixture was heated very gently over a microburner till bubbling started when it was removed from the flame until reaction ceased. The process was repeated until there was a slight excess of the acid chloride. The reaction mixture was refluxed at 90-100° for 2.5 hours, acidified with dil. Sulphuric acid and the excess acid chloride (as acid) removed with ether. The tigloyl esters were then extracted from the basified (with ammonia solution) aqueous layer with chloroform.

Measurement of radioactivity: – All measurements were carried out in a Labgear 4II counting chamber, D4126 and a Philips electronic counter, PW4032; timer, PW4052; high tension unit/amplifier, PW4022, operating in the Geiger-Muller region (1350 volts). The counter was used with a 2-chamber geometry using a gas mixture of helium and butane (1.3:98.7). The geometrical efficiency of the 2 II counting chamber (46%) was determined by using a reference polystyrene disc (2.5cm x 0.2cm), No. CFR3 (Amersham), specific activity 2.1 x 10^3 dpm/cm^2. Samples (picrates of the alkaloids:) were counted for 10 x 1000 counts at a thickness of 1mg (- 0.05mg) per 1.131cm^2 (the area of the dimple planchet on which the samples were spread as transpgrant films using drops of acetone-water). All samples were recrystallised and respread until a constant count was obtained. Self absorption losses (14%) were compensated for by multiplying the recorded counts per minute by 100 x 100) 2.528.
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Results and Discussion

The results of the experiments are summarised in Table I. The isolation of atropine instead of the injected (-)-hyoscyamine from the ***S. grandiflora*** scions and the normal tomato plant (Table I) indicates that the aerial parts of both the plants possess an enzyme system which mediates immediate racemisation of (-)-hyoscyamine. Other alkaloids (noratropine and tropine) are probably demethylated and hydrolysed products respectively of the racemised alkaloid, atropine. A similar phenomemon was observed when (-)-hyoscine was fed to the aerial parts of ***Solandra grandiflora*** Sw.(4).

The recovery of upto 69.6% of (-)-hyoscyamine as its metabolites (atropine, 42.33%; noratropine, 24.33% and tropine, 2.97%) from the ***S. grandiflora*** scions and more than 83.13% from the normal tomato plant (Table I) shows that degradation of (-)-hyoscyamine in its racemised form is very slow in these plants. But in a similar feeding experiment on the metabolism of atropine (in Datura innoxia Mill.) Hamon and Youngken (5) recorded as much as 59.4% degradation per day in sexually mature plants. The above finding also indicates that the racemised products are well accumulated by the aerial parts of ***S. grandiflora*** Sw. (cf. 1).

Table I: Results of the feeding experiments

Plant sample analysed	Type of treatment	Exposure time	Alkaloids[a]					
			Atropine		Noratropine		Tropine	
			wt. (mg)	b %	wt (mg)	b %	wt. (mg)	b %
S grandiflora scion on tomato stock	Infiltrated with unlabelled(-)-hyoscyamine amine (30 mg)	21 days	12.7	42.33	7.3	24.33	0.89	2.97
S. grandiflora scion on tomato stock	Infiltrated with labelled(-)-hyoscyamine-U-^{14}C (5.6 mg)	21 days	1.7 Sp. activity = 17.6×10^5 dpm/mM	30.36 (49.15%[d])	0.76 Sp. activity= 8.7×10^5 dpm/mM	13.6 (49.15%d)	Unquantified[c] Sp. activity= 1.1×10^5 dpm/mM	(6.2%[e])
Normal tomato plant	Infiltrated with unlabelled (-)-hyoscyamine (30mg)	21 days	24.94	83.13	Trace		Unquantified[c]	
Tomato scion on *S. grandiflora* stock	Untreated	5 months	2.2	—	Trace		Unquantified[c]	

a = Isolated by Partition column chromatography and characterised by co-chromatography and mp, mixed mp, ir spectra and elemental analysis of their picrates.
b = Per cent. of the infiltrated alkaloid.
d = Also characterised as it tigloyl ester.
d = Per cent. activity of the infiltrated labelled alkaloid.
e = Per cent. activity when diluted with un-labelled dried technical trophine (3.002 mg).

Isolation of radioactive atropine with 99.4% activity of the infiltrated alkaloid clearly shows that the scions were free of hyoscyamine/atropine or any other related alkaloids. Thus isolation of noratropine from the orignally alkaloid-free scions and the non-tropane alkaloid producing Tomato plant definitely indicates that it has originated from the injected (-)-hyoscyamine by racemisation followed by demethylation or *vice versa*. This observation was further supported by the isolation of radioactive noratropine from the *S. grandiflora* scion fed with (-)-hyoscyamine-U-^{14}C (Table I). Detection of noratropine in the normal Tomato plant also suggests that the enzyme which mediates demethylation of hyoscyamine may also be present in this plant.

All the above observations, which supplement similar observations (5–9) with other genera, were also supported by the detection and isolation of atropine, noratropine and tropine from the Tomato scion grown on *S. grandiflora* stock (Table I). Since the normal Tomato plant does never produce any tropane alkaloid, these transformed alkaloids must have originated from some alkaloid primarily synthesised in the roots of the *S. grandiflora* stock. Thus it can be interpreted that (-)-hyoscyamine synthesised in the roots of the stock(1) was translocated to the Tomato scion where these secondary transformations (racemisation, demethylation and hydrolysis) took place. These results further support the views: (i) that hyoscyamine is synthesised in the roots, and (ii) that in the aerial parts of *Solandra*, atropine, noratropine and tropine can arise from (-)-hyoscyamine(2)

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