Phytochemistry, Vol. 25, No. 3, pp. 617–619, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

THE SITE OF SYNTHESIS AND SECONDARY TRANSFORMATION OF HYOSCYAMINE IN *SOLANDRA GRANDIFLORA*

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(*Revised received* 6 *June* 1985)

Key Word Index—*Solandra grandiflora*; Solanaceae; hyoscyamine; synthesis; secondary transformation.

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

INTRODUCTION

Although (−)-hyoscyamine occurs as the principal alkaloid in the roots of *Solandra grandiflora* Sw., it was reported absent from the aerial parts of the plant. Instead, these parts were found to contain atropine as the principal alkaloid [1]. It is thus assumed that hyoscyamine is synthesized in the roots of the plant and translocated to its 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 *S. grandiflora* Sw. two alkaloid-free scions of *S. grandiflora* Sw. grown on *Lycopersicon esculentum* Mill. (tomato) (Solanaceae) stocks were infiltrated with (−)-hyoscyamine and labelled (−)-hyoscyamine-U-^{14}C respectively. For supporting evidence the aerial parts of a normal young tomato plant (a non-tropane alkaloid-producing plant) were also fed with a similar dose of (−)-hyoscyamine. The metabolic changes of the alkaloid were simultaneously studied in these plants. For evidence of the site of synthesis of the alkaloid, the alkaloid spectrum of an untreated tomato scion raised on a *S. grandiflora* stock was also investigated at the same time. The results of these investigations, a part of which was the subject of a preliminary communication [2], are reported in this paper.

RESULTS AND DISCUSSION

The results of the experiments are summarized in Table 1. Isolation of atropine instead of the injected (−)-hyoscyamine from the *S. grandiflora* scions and also from the treated tomato plant (see Table 1) indicates that the aerial parts of both the plants possess an enzyme system that causes immediate racemization of (−)-hyoscyamine. By experience it was established that the methods of extraction, isolation and characterization used in these experiments do not cause racemization, demethylation or hydrolysis of (−)-hyoscyamine. Other alkaloids (noratropine and tropine) are probably demethylated and hydrolysed products respectively of the injected (−)-hyoscyamine or its racemate atropine. A similar phenomenon was observed when (−)-hyoscine was fed to the aerial parts of *S. grandiflora* [3].

Recovery of up to 69.6% (atropine, 42.33%; noratropine, 24.33% and tropine, 2.97%) and more than 83.13% (atropine, 83.13%; noratropine, trace quantity and tropine, unquantified amount) of the injected alkaloid (as its metabolites) from the *S. grandiflora* scion and the tomato plant respectively (see Table 1) shows that the rate of degradation of the alkaloid in its modified forms is very slow in these plants. In contrast, in a similar feeding experiment on the metabolism of atropine, Hamon and Youngken [4] recorded as much as 59.4% degradation of the alkaloid per day in sexually mature plants of *Datura innoxia* Mill.

Isolation of radioactive atropine with 99.4% activity (see Table 1) of the infiltrated radioactive (−)-hyoscyamine-U-^{14}C clearly shows that the scions were free of hyoscyamine/atropine or any other related alkaloids. Thus isolation of noratropine from the originally alkaloid-free scions and also from the non-tropane alkaloid-producing tomato plant (see Table 1) definitely indicates that it has originated from the injected (−)-hyoscyamine by racemization 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 (see Table 1). Detection of noratropine in the injected tomato plant (see Table 1) also suggests that the enzyme system that mediates demethylation of (−)-hyoscyamine may also be present in this plant.

Tropine isolated from the injected scions and tomato plant (see Table 1) must also have originated from the injected alkaloid by hydrolysis before or after racemization. This is further supported by the isolation of radioactive tropine from the (−)-hyoscyamine-U-^{14}C-fed *S. grandiflora* scion. These findings suggest that tropine and pseudotropine present in the aerial parts of normal

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Table 1. Results of the feeding experiments to *Solandra grandiflora*

Plant sample analysed and wt (g)	Type of treatment	Exposure time	Alkaloids*					
			Atropine		Noratropine		Tropine	
			wt (mg)†	%‡	wt (mg)†	%‡	wt (mg)†	%‡
S. grandiflora scion on tomato stock (6.7)	Infiltrated with unlabelled (−)-hyoscyamine (30 mg)	21 days	12.7	42.33	7.3	24.33	0.89	2.97
S. grandiflora scion on tomato stock (6.1)	Infiltrated with labelled (−)-hyoscyamine-U-^{14}C (5.6 mg)	21 days	1.7	30.36	0.76	13.6	Unquantified§	
			Sp. act. = 17.6×10^5 dpm/mM (99.4%)‖		Sp. act. = 8.7×10^5 dpm/mM (49.15%)‖		Sp. act. = 1.1×10^5 dpm/mM (6.2%)¶	
Normal *Lycopersicon esculentum* (tomato) (10.1)	Infiltrated with unlabelled (−)-hyoscyamine (30 mg)	21 days	24.94	83.13	Trace		Unquantified§	
Tomato scion on *S. grandiflora* stock (6.5)	Untreated	5 months	2.2	—	Trace		Unquantified§	

*Isolated by partition column chromatography and characterized by co-chromatography, mp, mmp, IR spectra and elemental analyses of their picrates.
†Calculated as hyoscyamine by equivalence of 0.005 N H_2SO_4 required for neutralization.
‡Percentage of the weight of the infiltrated alkaloid.
§Also characterized as its tigloyl ester.
‖Percentage activity of that of the infiltrated labelled alkaloid.
¶Percentage activity when diluted with unlabelled dried technical tropine (3.002 mg).

plants may be produced by hydrolysis of their ester alkaloids [cf. 3].

All the above observations, which supplement similar observations [5–8] with other genera, were also supported by the detection and isolation of atropine, noratropine and tropine from the untreated tomato scion grown on *S. grandiflora* stock (see Table 1). Since the normal tomato plant does not produce any tropane alkaloid, these tropane alkaloids must have originated from an alkaloid primarily synthesized in the roots of the *S. grandiflora* stock. Thus, from this observation and the observation that (−)-hyoscyamine is racemized to atropine in the aerial parts of tomato plant (see above), and from the fact that the roots of *S. grandiflora* contain (−)-hyoscyamine (*not* atropine), it can be interpreted that (−)-hyoscyamine synthesized in the roots of the stock was translocated to the *untreated* tomato scion where these secondary transformations (racemization, demethylation and hydrolysis) took place.

These results therefore further support the views that (i) hyoscyamine is synthesized in the roots, and (ii) atropine, noratropine and tropine in the aerial parts of *S. grandiflora* arise from (−)-hyoscyamine [2].

EXPERIMENTAL

Construction of the grafts. Reciprocal grafts were constructed between young *Solandra grandiflora* and *Lycopersicon esculentum* plants (grown from seeds of cultivated plants) in a temperate green house. Five months later metabolic studies were initiated selecting only the healthier grafts for infiltration of alkaloid samples.

Preparation of the alkaloid samples. (i) Two samples of pure unlabelled (−)-hyoscyamine base (30 mg each) in ethanolic soln were neutralized with 0.1 N H_2SO_4 (*ca* 1 ml each). The solvent was removed at low temp by a current of forced air and the residues dried *in vacuo* for 4 hr before dissolving in distilled water (2 ml each) for infiltration. (ii) A sample of labelled (−)-hyoscyamine-U-^{14}C base (5.6 mg), recovered from labelled (−)-hyoscyamine-U-^{14}C picrate (10 mg), specific activity 17.7 × 10^5 dpm/mM, was similarly prepared.

Method and plan of feeding. The alkaloid solns were infiltrated into the plants in the following way: the main stem of the scion or the plant, as the case may be, was pierced by a big sewing needle through its conducting tissues on two opposite sides. A thread of cotton yarn was passed through one hole and returned through the other. The two ends of the thread were then dipped into the alkaloid soln in a small container. The soln moved up to the conducting tissues due to capillary action of the cotton thread and was ultimately taken up into the plant by the transpiration stream.

The following plan was adopted for feeding experiments: (i) One of the solns of the unlabelled (−)-hyoscyamine samples was fed to an alkaloid-free *S. grandiflora* scion and the other to a normal young tomato plant. (ii) The labelled (−)-hyoscyamine-U-^{14}C was infiltrated into an alkaloid-free *S. grandiflora* scion. The three treated plants were harvested for analysis 21 days after feeding. (iii) One of the healthy tomato scions growing on a well-developed *S. grandiflora* stock was *not treated* and allowed to grow. It was harvested when the graft was 5 months old.

Analysis of the harvested plant materials. The harvested plant materials were dried at 50° and powdered separately. The powdered plant material (see Table 1 for individual weight) was mixed with lime (one-fifth the weight of the powder), moistened with sufficient H_2O and macerated with Et_2O for 1 hr in a closed percolator. The extract was drained off and the marc was finally extracted exhaustively with more Et_2O. Removal of the solvent afforded the crude extract. The extract was fractionated and refractionated (where required) by partition column chromatography using kieselguhr (Hyflo Super Cel grade) as adsorbent [9] at pH 6.8 and eluting the column successively with petrol (bp 40–60°), Et_2O, $CHCl_3$ and ammoniacal $CHCl_3$ ($CHCl_3$ shaken with NH_3 soln). Individual fractions were examined by TLC (system I: alumina, Et_2O–EtOH (1:1), visualized with a satd soln of I_2 in CCl_4; system II: silica gel G, $CHCl_3$–Et_2NH (9:1), visualized with iodoplatinate reagent) and PC (Whatman No. 1 filter paper, petrol (bp 60–80°)–HOAc–*n*-AmOH–H_2O (1:3:3:3), visualized with Dragendorff's reagent).

Preparation of tigloyl esters. Tigloyl chloride was added dropwise to the dried alkaloid sample. The mixtue 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 hr, acidified with dil. H_2SO_4 and the excess acid chloride removed (as acid) with Et_2O. The tigloyl esters were then extracted from the basified (with NH_3 soln) aq. layer with $CHCl_3$.

Measurement of radioactivity. All measurements and calculations were carried out as previously described [10].

Acknowledgements—The author is indebted to Dr. W. C. Evans and Dr. V. A. Woolley of the University of Nottingham Pharmacy Department for their valuable help during the progress of the work.

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