Phytochemistry, Vol. 30, No. 8, pp. 2607–2609, 1991
Printed in Great Britain.

0031-9422/91 $3.00+0.00

8-THIABICYCLO[3.2.1]OCTAN-3-ONE AS A BIOCHEMICAL TOOL IN THE STUDY OF TROPANE ALKALOID BIOSYNTHESIS

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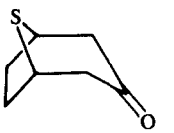
(*Received in revised form* 18 *December* 1990)

Key Word Index—*Datura stramonium*; Solanaceae; biosynthesis; inhibitor; tropane alkaloids; tropine; pseudotropine; 8-thiabicyclo[3.2.1]octan-3-one.

Abstract—8-Thiabicyclo[3.2.1]octan-3-one, the sulphur analogue (replacing the methylaza bridge) of tropinone, was fed to *Datura stramonium* transformed root cultures. The compound was metabolized to a large degree. Notable products included the reduction product, 8-thiabicyclo[3.2.1]octan-3-ol, analogous to tropine, and also the derived 3-*O*-acetyl ester. In addition, tropane alkaloid synthesis was perturbed. Hyoscyamine levels, and in particular tropine and 3α-acetoxytropane levels, were reduced. Pseudotropine, on the other hand, was found to increase in concentration. It is concluded that 8-thiabicyclo[3.2.1]octan-3-one may be a good biochemical tool in the study of tropane alkaloid biosynthesis.

INTRODUCTION

In recent years there has been a resurgence of interest in the biochemistry of tropane alkaloids. This is due in part to the availability of both normal and transformed root cultures which are easy to handle and show high levels of alkaloid synthesis (e.g. [1–5]). Many aspects of the biosynthetic pathway are now well understood, and the relevant enzymes identified [4, 6–8]. Certain areas have, however, been slower to yield results. One problem is that there are few inhibitors of the tropane alkaloid biosynthetic pathway. The chemical synthesis of substrate analogues is one approach to the development of such inhibitors. In the present paper we describe the effects of the tropinone analogue 8-thiabicyclo[3.2.1]octan-3-one (TBON, 1) on alkaloid synthesis in transformed roots of *Datura stramonium* L., and also investigate the metabolism of this compound by the cultures.



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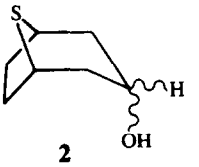

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RESULTS AND DISCUSSION

Effects of TBON on tropane alkaloid biosynthesis

Treatment of *D. stramonium* root cultures with 0.6 mg ml^{-1} TBON for 14 days resulted in some reduction in growth, control cultures growing from 0.2 to 9.0 g, but treated cultures only reaching a biomass of 3.9 g. Although hyoscyamine remained the dominant alkaloid, absolute levels were reduced in treated roots (Table 1). Changes were also seen in the levels of some of the other alkaloids (Table 1).

Part of the effect on alkaloid biosynthesis could be a non-specific consequence of the growth-inhibition observed, since in these cultures hyoscyamine levels increase several-fold throughout the growth cycle [9, 10]. Not all effects can, however, be explained in this manner, for the full pattern of changes in alkaloid levels observed does not resemble that seen during the development of untreated root cultures [10]. Rather, the nature of the changes seen in the levels of the various alkaloids indicates that TBON is also having specific effects on alkaloid metabolism in the tropinone/tropine/pseudotropine area. Thus, hygrine levels remain high, while the levels of tropine and its esters fall dramatically. (Tropinone levels were too low to be measured accurately). Interestingly the reduction in hyoscyamine observed is less than that seen for tropine, supporting the suggestion [8, 10] that in control cultures the synthesis of hyoscyamine may to some extent be limited by the supply of the tropoyl residues for esterification rather than by the supply of tropine. That 3α-acetoxytropane levels decline so dramatically in treated roots may indicate that this compound is acting as some sort of 'spill-over' pool for tropine, only being synthesized in significant quantity when high levels of free tropine accumulate.

In addition to the inhibition of tropine and hyoscyamine accumulation, TBON was also observed to increase pseudotropine levels within the root (Table 1), with this compound now becoming a relatively major constituent. It is unclear as to whether this is a separate effect, or whether it might result from an increased availability of tropinone for pseudotropine production following a possible inhibition of tropine synthesis from tropinone. There is evidence [7, 11] that separate enzymes are indeed responsible for the reduction of tropinone to pseudotropine and tropine.

Table 1. The effect of TBON on alkaloid levels in *Datura stramonium* transformed root cultures (treatment was with 0.6 mg ml^{-1} TBON for 14 days)

Alkaloid	Content per g fr. wt as per cent of control value	Percentage contribution to total alkaloid (with control values in parentheses)
Hygrine	77	4.9 (0.9)
Tropine	5.0	2.7 (7.1)
Pseudotropine	*ca* 300	9.6 (trace)
3α-Acetoxytropane	1.5	0.8 (7.6)
Hyoscyamine	15	68.0 (71)

The hyoscyamine content of control tissues is typically *ca* 2 μmol g^{-1} fr. wt.

Table 2. Sulphur-containing metabolites found in the chloroform extract of *Datura stramonium* root cultures treated with 0.6 mg ml^{-1} TBON for 14 days

Identification*	Mass spectrum†	Approximate relative abundance (%)
TBON	142 (100) 114 (53) 99 (25) 85 (80) 67 (62)	15
3-Mercapto-cycloheptanone	144 (100) 110 (57) 95 (28) 85 (55) 67 (50)	5
TBOL	144 (100) 126 (27) 116 (16) 87 (75) 85 (65) 67 (65)	15
3-*O*-Acetyl TBOL	186 (18) 126 (100) 98 (23) 85 (35) 67 (30)	1.5
TBON-sulphoxide	158 (12) 142 (47) 114 (50) 109 (45) 81 (100) 67 (55)	3
?TBOL-sulphoxide A‡	[202§ (10)] 160 (17) 142 (16) 111 (15) 93 (100) 67 (34)	
B	[202§ (5)] 160 (10) 142 (22) 111 (11) 93 (100) 67 (22)	60‖
C	160 (25) 142 (16) 111 (18) 93 (100) 67 (41)	

*Listed in order of elution on GC. Compounds elute between tropine and apoatropine.
†GC-MS 70 eV, *m/z* (rel. int.).
‡Two asymmetric centres are present.
§Probably due to impurities.
‖Not well resolved.

Metabolism of TBON

Chloroform extracts of treated root samples were found to contain not only the starting material, but also several other TBON-derived compounds when examined by GC-MS. (Sulphur-containing compounds were readily identified by MS, as their M_rs are 3 units greater than for the normal tropane derivatives, and also by virtue of their characteristic isotopic abundance—95% ^{32}S, 1% ^{33}S, 4% ^{34}S.) These are listed in Table 2, along with their approximate relative abundance. As the partition characteristics of the various compounds are unknown, these apparent abundances need not, however, totally reflect the true pattern of metabolism. Notable metabolites assigned were 8-thiabicyclo[3.2.1]octan-3-ol (TBOL, 2) and its 3-*O*-acetyl derivative, the former of which showed a mass spectral fragmentation pattern closely similar to that of tropine. No tropoyl ester equivalent to hyoscyamine was found. A number of putative sulphoxides were detected, but these probably arise non-specifically, through non-enzymatic oxidation of the various sulphur-containing metabolites present.

Applications

Clearly TBON can perturb alkaloid production *in vivo*, in a manner which should help throw light on the biochemical pathways involved, and upon their regulation. Because of the *in vivo* nature of the present experiments, and the extensive metabolism of TBON, it is impossible to define precisely the mechanisms of the perturbations which have occurred and the nature of the active species. The formation of TBOL, however, suggests that TBON may be a substrate for the recently described tropinone reductases [7, 11]. It is likely, therefore, that TBON at least will be a competitive inhibitor of tropinone reduction, with apparently the formation of the α-isomer, tropine, being particularly sensitive to inhibition (see above).

Our results strongly suggest that TBON will prove a useful tool in understanding the biosynthesis of tropine and pseudotropine. The observed formation of 3-*O*-acetyl TBOL, but not of 3-*O*-tropoyl TBOL, suggests that TBOL could also be a useful tool for understanding the esterification of tropine.

EXPERIMENTAL

Root cultures. Root cultures of *D. stramonium* var. *inermis* transformed with *Agrobacterium rhizogenes* LBA 9402 were initiated and maintained as described in ref. [10]. This line is referred to as line D15/5.

Synthesis of TBON. TBON was prepd using an adaptation of

the method in ref. [12]. Iodomethane (5.5 g, 38.7 mmol) was added dropwise to a stirred soln of tropinone (4.06 g, 29.2 mmol) in EtOH (25 ml) and stirring continued for 2 hr. Et_2O (75 ml) was added and the ppt. formed was recrystallized from aq. MeOH to give tropinone methiodide (7.51 g, 26.7 mmol, 91%) mp 276–279° (decomp.) (lit. [13] mp 263–265°); IR ν_{max}^{KBr} 1725 cm^{-1}. Tropinone methiodide (5.6 g, 20 mmol) and $Na_2S \cdot 9H_2O$ (5.6 g, 23.3 mmol) were stirred in H_2O (425 ml) at 85° under N_2 for 2 hr. On cooling, the soln was extracted with Et_2O and the extracts washed with 0.1 M HCl, with satd NaCl to neutrality, dried and evapd. The resulting yellow solid was decolourized by passing in Et_2O through a short column of basic alumina and recryst. from aq. MeOH to give TBON (1.57 g, 11 mmol, 55%), mp 155–157° (lit. [12] mp 155–156°); IR ν_{max}^{KBr} 1717 cm^{-1}; $\delta(CDCl_3$, 90 MHz) 1.8–2.3 (4H, *m*, C (6,7) H_2), 2.7 (4H, *m*, C(2,4) H_2) and 3.8 (2H, *m*, C (1,5) H).

Feeding of TBON. Roots (0.2 g) were subcultured into 50 ml fresh medium. After 4 days growth, 4 ml of a filter-sterilized stock of aq. TBON was added to give a final TBON concentration of 0.6 mg ml^{-1}. After a further 14 days the roots were harvested and analysed.

Analytical methods. Roots were extracted as described in ref. [1], then after taking up the residue in MeOH, tropane alkaloids were determined by GC and GC-MS as described in ref. [8]. With GC, a nitrogen detector was used so that the sulphur-containing metabolites were not detected. For analysis of the sulphur compounds GC-MS was employed, using the same GC conditions as for the tropane alkaloids. Individual components were identified chemically by comparison of their GC behaviour with that of commercially available standards (where available), and from their GC-MS fragmentation patterns. The fragmentation behaviour of tropane-type compounds is well-characterized [14], and the behaviour of various sulphur-containing molecules including heterocycles, sulphoxides and mercaptans is also described in ref. [15].

Acknowledgements—We thank Dr J. Eagles (IFR) for kindly helping with the GC-MS analysis, and Ms A. C. J. Peerless (IFR) for excellent technical assistance. Professor D. J. Robins and Mr A. Watson (Glasgow) provided helpful discussion and encouragement.

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