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ESTERIFICATION OF TROPINE IN CULTURED TISSUES OF *DUBOISIA MYOPOROIDES*

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Key Word Index—*Duboisia myoporoides*; Solanaceae; biotransformation; callus; shoot; esterification; acetyltropine; butropine.

Abstract—Tropine was converted into acetyltropine in callus cultures of *Duboisia myoporoides*, while in shoot cultures butropine was found as an esterified product.

INTRODUCTION

It has been reported that *Datura innoxia* cell cultures are able to convert tropine into acetyltropine, whilst those of *Scopolia japonica* and *Atropa belladonna* are not able to esterify tropine with endogenous acetic acid [1].

Here we report on whether *Duboisia myoporoides* tissue cultures as well as *Datura innoxia* cell cultures have the ability to esterify tropine with endogenous acetic acid. Furthermore, we have determined that there is a difference between callus and shoot cultures with respect to esterification of tropine, although both cultured tissues did not produce tropane alkaloids [2]. The conversion of tropine in both callus and shoot cultures of *Duboisia* has not been previously reported.

RESULTS AND DISCUSSION

Esterification of tropine in callus cultures

Two alkaloids were detected from *Duboisia myoporoides* callus cultures supplied with tropine (100 mg/l.) by TLC and PC. One was tropine, which was easily distinguished by a purple coloured spot; usually tropane alkaloids are found as orange spots. The other was an alkaloid converted from tropine and found as an orange coloured spot. The alkaloid was expected to be acetyltropine from the papers published before on esterification of *Datura* cell cultures [1, 3] and by comparison with the R_f value of an authentic sample on TLC and PC. The alkaloids from callus cultures and authentic alkaloids were analysed by GC/MS and the alkaloids identified as tropine and acetyltropine from GC, R_t and mass spectrometry.

Romeike and Koblitz reported that tropine is esterified with endogenous acetic acid in *Datura* cell cultures but not in *Nicotiana* cell cultures [3]. The results of Hiraoka and Tabata also supported this [1]. As *Duboisia myoporoides* contains tobacco alkaloids as well as tropane alkaloids [4], it was interesting to examine whether such callus cultures can convert tropine into acetyltropine. Our results showed *Duboisia myoporoides* callus cultures have the ability to esterify tropine with endogenous acetic acid. *Duboisia myoporoides* callus and *Datura innoxia* cell cultures have the ability to esterify tropine, whilst those of *Scopolia japonica* and *Atropa belladonna* do not have this capability [3]. Thus, there are two different types with respect to esterification of tropine in cell (callus) cultures of tropane alkaloid bearing plants. It is not clear whether the esterification enzyme is absent or merely suppressed in cell cultures of *Atropa belladonna* and *Scopolia japonica*.

Esterification of tropine in shoot cultures

Two alkaloids were detected from *Duboisia myoporoides* shoot cultures supplied with tropine (100 mg/l.) by TLC and PC. One was tropine supplied from the medium, but the other was different from acetyltropine detected in the callus cultures. The alkaloid was not scopolamine or atropine. Therefore, we assumed the possibility that tropine was esterified with an aliphatic acid other than acetic acid. Alkaloids such as butropine, valtropine, tigloidine, poroidine and isoporoidine were reported in intact *Duboisia myoporoides* [5–7]; the alkaloids chromatographed together on TLC and PC. From GC/MS, the alkaloid from shoot cultures supplied with tropine was identified as butropine by comparison with an authentic sample. It was distinguished from *n*-butyryltropine found in *Anthocercis geneus* [8] by its different GC R_t although both compounds exhibit the same mass spectrum. The formation of butropine in shoot cultures has not been reported before.

In shoot cultures neither atropine nor scopolamine was found when tropine was supplied. Furthermore, isolated roots and adventitious roots from callus of *Duboisia myoporoides* produce both atropine and scopolamine [2, 9]. Therefore, it is suggested that endogenous tropic acid is biosynthesized in the roots but not in the shoots [10].

The present study shows that both callus and shoot cultures of *Duboisia myoporoides* have the ability to esterify tropine with endogenous aliphatic acids. Different aliphatic acids were, however, esterified in them, acetic acid in callus cultures and isobutyric acid in shoot cultures.

EXPERIMENTAL

Callus and shoot cultures of Duboisia myoporoides. Callus and shoot cultures were obtained as reported previously [2]. They

were maintained on Murashige–Skoog's basal agar medium [11] supplemented with 2,4-D (1 mg/l.) and kinetin (0.01 mg/l.) and with 6-benzylaminopurine (5 mg/l.), respectively. Calluses were cultured in the dark and shoots in the light (1000 lx) at 26°. Tropine (100 mg/l.) was added to the media before autoclaving, and calluses and shoots were inoculated on the media. After 1 month, they were removed from the flasks.

Extraction of alkaloids. Tissue cultures were lyophilized and then powdered. Samples (5 g) were extracted × 3 overnight with 100 ml 80% MeOH. The combined extracts were evaporated to dryness under red. pres. at below 50° and the residue was dissolved in 100 ml H_2O followed by filtration. The filtrate was extracted × 3 with 50 ml $CHCl_3$. The aq. layer was adjusted to pH 9 with 4% NH_4OH and extracted × 3 with 50 ml $CHCl_3$. The combined $CHCl_3$ layers were used for analysis after being dried (Na_2SO_4).

Analysis of alkaloids. Alkaloid fractions were analysed by silica gel TLC with the following solvent systems: (1) $CHCl_3$–EtOH–28% NH_4OH (85:14:1); (2) $CHCl_3$–Me_2CO–MeOH–28% NH_4OH (73:10:15:2), and by PC in 0.1 M citrate–HCl buffer (pH 3). Alkaloids were detected by Dragendorff's reagent. GC/MS was also used for identification of alkaloids. GC conditions were a 2.1 m × 3 mm glass column packed with 10% DC 550 coated on acid-washed and silanized Chromosorb W (80–100 mesh), column temp. 200°, injector temp. 230°, He as carrier at a flow rate of 30 ml/min, TIC detector and an ionizing energy of 70 eV. R_ts (min): tropine, 2.3; acetyltropine, 3.9; butropine, 6.7; *n*-butyryltropine, 8.1. MS fragments: tropine, MS *m/z* (rel. int.): 141 $[M]^+$ (34), 124 (29), 113 (22), 97 (24), 96 (68), 83 (73), 82 (100); acetyltropine, 183 $[M]^+$ (24), 143 (26), 124 (100), 96 (19), 94 (29), 83 (48), 82 (56); butropine, 211 $[M]^+$ (17), 140 (13), 124 (100), 96 (16), 94 (23), 83 (37), 82 (42); *n*-butyryltropine, 211 $[M]^+$ (21), 140 (17), 124 (100), 96 (18), 94 (24), 83 (49), 82 (52).

Authentic samples. Tropine was obtained commercially. Acetyltropine, butropine and *n*-butyryltropine were synthesized in our laboratory as reported previously [5, 7].

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