^{15}N - TOMATINE

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ABSTRACT

A method for preparative isolation of ^{15}N-tomatine from foliage of tomato plants grown hydroponically with ^{15}N-containing nutrient salts is described. Extractive workup of plant material gave a crude product which was chromatographed on Sephadex LH-20 to yield pure tomatine. Assay of ^{15}N content by mass spectrometry showed that isotopic purity was ca. 95%.

Key words: Tomato, Lycopersicon esculentum, tomatine

INTRODUCTION

Tomatine, a steroidal glycoalkaloid found in the tomato plant (Lycopersicon esculentum Mill.) and other Lycopersicon and Solanum species has been the subject of many reports that consider the alkaloid to confer host-plant resistance against a number of pests and pathogens. This substance is inhibitory to fungi[2,3] and is toxic to or is an antifeedant towards insects[3,4]. Tomatine may also play a role in the resistance of certain tomato varieties toward nematodes[5]. An account of a tritophic interaction[6] describes how accumulation of the alkaloid within larvae of a tomato leaf-feeding lepidopteran can inhibit development of or kill larvae of wasps which ordinarily would be able to successfully parasitize these caterpillars. Tomatine might also prove to be a useful precursor of steroidal pharmaceuticals[7]. A large number of analytical procedures for tomatine, all

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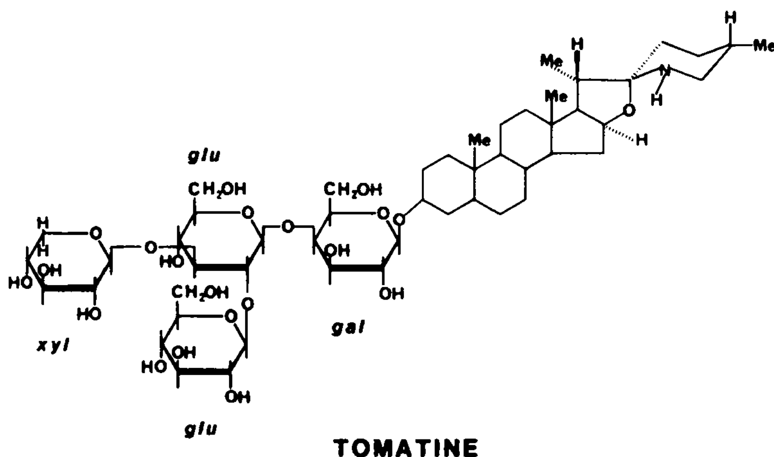

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of which suffer from one or more disadvantages, have been described[3]. These include the usual chromatographic procedures as well as colorimetric and gravimetric methods. It is clear that ^{15}N-tomatine could be used in mass spectral analyses of the alkaloid and may also be helpful in revealing the biochemical fate of this material in vivo.

This paper describes the preparative isolation of 95% isotopically pure ^{15}N-tomatine from plants of the Red Currant tomato, Lycopersicon pimpinellifolium, which was chosen for its high tomatine content.[1,7]

RESULTS AND DISCUSSION

Nitrogen-15 labelled plants were obtained by hydroponically growing L. pimpinellifolium on sand using a recirculating system containing the appropriate ^{15}N-containing nutrients. After 32 days under artificial illumination, the plants were ca. 75 cm high and exhibited minor chlorosis suggestive of expenditure of nitrogenous nutrients. At this point they were harvested and the total plant material was freeze-dried. Extractive workup, initially with chloroform to remove nonpolar material, followed by methanol containing a small amount of acetic acid yielded an extract which, after addition of water was rendered almost colorless by washing with chloroform. Concentration of the

aqueous methanol solution followed by adjustment of pH to about eight gave an insoluble, crude tomatine fraction which was carried through an additional precipitation stage followed by chromatography on Sephadex LH-20/methanol. Use of chromatography eliminated losses during tedious crystallization and gave pure material in one step. For mass spectral analysis a portion of product was converted to the aglycone, tomatidine, by acidic hydrolysis. Isotopic purity was estimated to be ca. 95%.

EXPERIMENTAL

General: Column chromatography was performed on Sephadex LH-20 from Pharmacia Co. Thin layer chromatography was on 0.25 mm thickness silica gel F-254 (E. Merck) and the chromatograms were visualized by anisaldehyde spray[9]. All solvents were reagent grade. Circulation of hydroponic solution and metering of methanol for chromatographic elution was by means of a CMP-3 pump (Milton-Roy Co.). Mass spectral analyses were carried out on a Micromass 7070F instrument (V.G. Micromass, Ltd.). Nitrogen-15 calcium nitrate and potassium nitrate were obtained from Icon Services, Inc., Summit N.J.

Plant material: Seeds of L. pimpinellifolium, LA1245, were obtained from the Tomato Genetics Stock Center, University of California, Davis and were subjected three successive times to 30 min. etchings with half strength household bleach to permeablize the seed coat[8] and maintenance between moistened blotter disks for 5-7 days. After the third period of treatment, germination occurred. The sprouted seeds (14 individuals) were planted in a 2-inch deep layer of sand (0.5-1 mm dia) contained within a 8 x 10" box having a perforated bottom lined with filter paper. A nutrient solution (Table 1), 2000 ml in total volume, was circulated constantly through the sand by means of a metering pump at 300 ml per hour using standard irrigation drippers on top of the sand bed with return to the pump reservoir by means of a catch basin beneath the growing box. The plants were held in a chamber at 22° with a 16 hr photoperiod per 24 hrs having illumination from six F40CW fluorescent tubes supplemented by four 100 W incandescent bulbs. To compensate for transpiration, water in the pump

reservoir was adjusted to the initial volume on alternate days. After 32 days the plants were separated from most adhering sand by washing under a gentle stream of water and were freeze-dried to give 31 g of anhydrous material.

Hydroponic Solution for L. pimpinellifolium

Substance	Concn. (Millimolar)
$Ca(^{15}NO_3)_2$	6
$K\ ^{15}NO_3$	10
KH_2PO_4	1
$Mg\ SO_4$	0.5
Fe^{+3} EDTA, Na Salt	1×10^{-2}
KCl	2.5×10^{-2}
H_3BO_3	1.25×10^{-2}
$MnSO_4$	2.5×10^{-3}
$ZnCl_2$	1×10^{-3}
$CuSO_4$	2.5×10^{-4}
$(NH_4)_6Mo_7O_{24}$	7.5×10^{-6}

Extraction and Fractionation: The dry plant material was ground with two 400 ml portions of $CHCl_3$ (discarded) followed by three 300 ml portions of methanol/0.1% HOAc using a one quart Waring Blendor and filtering by suction. The combined methanol extracts were concentrated to 50 ml *in vacuo*, 100 ml of water was added, and the mixture was washed with four 100 ml portions of $CHCl_3$ to give an almost colorless aqueous solution. After further concentration to about 80 ml, the aqueous solution was made basic with excess NH_4OH to give a flocculant precipitate in a strongly yellow solution (rutin). After centriguation at 7000 x g/10min the resulting yellow pellets were redissolved in 20 ml of 0.5 N HOAc. The solution was again made basic and the pale yellow precipitate (0.32 g) was collected by centrifugation, applied in 10 ml MeOH to a 950 mm x 50 mm dia. LH-20 column and chromatographed using methanol at 300 ml/hr. Tomatine in the eluate was detected by spotting one microliter portions from each 25 ml tube onto a silica gel TLC plate and visualizing with anisaldehyde spray. Tomatine forms a strong blue-green color under this treatment and was thereby shown to be present in an elution volume of 1225 to 1550 ml. The colorless product, 0.285 g, was shown to be nearly homogeneous tomatine by TLC[10].

Hydrolysis and Mass Spectral Analysis: A 1 mg portion of ^{15}N-tomatine was treated with 0.5 ml of 1 N HCl at 100° for 90 min. The cooled solution was made basic with 0.6 ml of 1 N NaOH solution, and the free aglycone, ^{15}N-tomatidine, was extracted into ethyl acetate. This solution was used for mass spectral analysis. Estimation of ^{15}N content was facilitated by comparison of the intensity of the fragment ion of m/z 138 known to be formed by cleavage of the nitrogen containing ring in unlabelled tomatine[11] with the intensity of the corresponding ion, m/z 139 for the ^{15}N-containing substance. The incorporation of ^{15}N was about 95%.

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