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COMMUNICATIONS

Biosynthesis of [^{14}C]Patulin by *Penicillium urticae*

Carbon-14-labeled patulin was biosynthesized by *Pencillium urticae* using sodium [1-^{14}C]acetate, sodium [2-^{14}C]acetate, or [U-^{14}C]glucose. Patulin yield ranged from 455 to 897 mg/100 mL of potato dextrose media after incubation at 20 °C for 14 days. The percentage conversion of carbon-14 label into patulin was 9.0–9.3% for sodium [2-^{14}C]acetate, 0.3–0.6% for sodium [1-^{14}C]acetate, and 6.0–7.6% for [U-^{14}C]glucose. Ten microCuries of sodium [2-^{14}C]acetate produced the highest specific activity of 0.189 μCi/mmol of patulin.

Patulin, 4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one, is a toxic secondary metabolite produced by several species of *Aspergillus*, *Penicillium*, and other related fungi isolated from food material (Buchanan et al., 1974; Ciegler et al., 1971; Scott, 1974). Patulin was originally isolated for its antibiotic activity (Katzman et al., 1944), but has since been demonstrated to be a carcinogenic (Dickens and Jones, 1961) and mutagenic mycotoxin (Mayer and Legator, 1969). The occurrence of patulin in foods and feeds (Buchanan et al., 1974; Harwig et al., 1973; Scott et al., 1972; Stott and Bullerman, 1975a) represents considerable environmental hazard to humans and animals.

Metabolism studies of patulin require labeled toxin of high specific activity. Several methods have been reported for possible incorporation of carbon-14 into patulin with *P. patulum* from readily available, inexpensive precursors, acetate and glucose (Bassett and Tannenbaum, 1960; Scott and Beadling, 1974).

MATERIALS AND METHODS

Media and Cultures. *Penicillium urticae* was obtained from the culture collection at Washington State University (Pullman, WA) and transferred twice on potato dextrose agar (Difco, Detroit, MI). A suspension was made from the mycelia. One-liter culture flasks containing 100 mL of potato dextrose media autoclaved at 120 °C for 45 min were inoculated with 10-mL suspensions of *P. urticae* according to the method of Norstadt and McCalla (1969). Ten microCuries of sodium [1-^{14}C]acetate, sodium [2-^{14}C]acetate, or [U-^{14}C]glucose was added to duplicate culture flasks. Flasks were placed on their sides for maximum surface area and incubated without agitation at 20 °C for 14 days.

Extraction and Semipurification. The media and mycelial mat were extracted three times with 100 mL of ethyl acetate in a large separatory funnel. Extracts were combined and rotary evaporated to 5 mL. Semipurification of the patulin was accomplished on an alumina column as described by Norstadt and McCalla (1969).

Quantitation of ^{14}C. The semipurified patulin samples were quantified using a fluorodensitometric thin-layer chromatography (TLC) method developed by Salem and Swanson (1976). Activated 250 μm TLC silica gel G plates were spotted with semipurified [^{14}C]patulin samples along with known concentration of patulin standard and run in benzene–methanol–acetic acid (90:5:5). Spots with a R_f of 0.20 were developed in concentrated ammonia fumes for 30 min and then quantified with a fluorodensitometer equipped with a TLC scanning unit. The maximum excitation wavelength was 254 nm and the maximum emission wavelength was 415 nm. Patulin spots were scraped, dissolved in Scinti Verse (Fisher Scientific Co., Fair Lawn, NJ), counted in a liquid scintillation counter (Packard Model 3255 Tri-Carb), and specific activity calculated.

Radiopurity. Radioactive patulin samples from TLC were run on a high-pressure liquid chromatograph (LC) to determine radiopurity. LC equipment consisted of a Waters Associates 6000A pump, Model U6K injector, and a Micrometrics Model 785 variable-wavelength detector set at 320 nm. Separation was achieved with a Lichrosorb ODS precolumn (4.6 mm i.d. × 5 cm), followed by a Zorbax ODS column (4.6 mm i.d. × 25 cm) using a solvent system of acidified acetonitrile (9 vol of acetonitrile to 91 vol of 0.5% formic acid) previously filtered through a 0.45-μm Millipore filter. Flow rate was 2.5 mL/min. Retention times were measured with a Spectra Physics Minigrator and compared with known patulin standards.

Antibiotic Activity. In addition to chemical analyses, biological assays were used to determine the antibiotic activity of patulin using streak plate methods (Geiger and Conn, 1945) or disc assay methods (Stott and Bullerman, 1975b). Blank Bacto half-inch sterile sensitivity discs were immersed in TLC purified solutions of [^{14}C]patulin and a known standard (3 mg/mL) until saturated. Discs were immediately placed on a violet red bile agar plate which was inoculated with a lawn of *Escherichia coli*. The plates were incubated at 37 °C for 48 h and zones of inhibition were observed.

RESULTS AND DISCUSSION

The yield of patulin obtained from the potato dextrose cultures ranged from 455–897 mg/100 mL of medium (Table I). This represents an increase of two–three times that reported previously by Norstadt and McCalla (1969) with *P. urticae*. This discrepancy may be due to the method of purification. In this study, TLC rather than crystallization was used to purify patulin. Crystallization techniques often result in the loss of some product. LC demonstrated that the TLC purified [^{14}C]patulin had a

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Table I. Incorporation of ^{14}C from Different Substrates into Patulin

labeled compound[a]	patulin yield (mg/100 mL)	sp act. dpm/mg	sp act. μCi/mmol	% label conversion
sodium [1-^{14}C]acetate	455	0.16	0.011	0.338
	708	0.19	0.014	0.622
sodium [2-^{14}C]acetate	739	2.72	0.189	9.059
	872	2.39	0.166	9.387
[U-^{14}C]glucose	897	1.90	0.132	7.676
	691	1.93	0.134	6.023

[a] Obtained from New England Nuclear, Boston, MA.

Table II. Antibiotic Activity of [^{14}C]Patulin as Determined by Disc Assay[a]

patulin sample obtained from:	zones of inhibition, mm
standard	12
sodium [1-^{14}C]acetate	11
	11
sodium [2-^{14}C]acetate	9
	12
[U-^{14}C]glucose	11
	11

[a] Method of Stott and Bullerman (1975) using *E. coli*.

R_f comparable to known patulin standards. Recycle chromatography failed to show any evidence of an impurity.

The results shown in Table I indicate that sodium [2-^{14}C]acetate was the preferred labeling source with approximately 9% of the radioactivity incorporated into patulin. The preference of sodium [2-^{14}C]acetate over sodium [1-^{14}C]acetate was 15 to 1 and may be due to the conversion of the C-1 carbon to CO_2 by way of the Krebs tricarboxylic acid cycle. The preference of sodium [2-^{14}C]acetate over [U-^{14}C]glucose may exist for the same reason, or may be the result of specific utilization of glucose for growth rather than for toxin production.

The antibiotic activity of [^{14}C]patulin compared to a standard patulin sample is shown in Table II. All samples exhibited antibiotic activity comparable to the standard, suggesting that there was very little degradation of the biological activity of [^{14}C]patulin by the alumina column clean-up and TLC purification.

Based upon the results obtained from this study, the use of sodium [2-^{14}C]acetate incorporates a much greater efficiency of label into [^{14}C]patulin than does the use of other precursors (Bassett and Tannenbaum, 1960; Tannenbaum and Bassett, 1959; Nip and Chu, 1977). It is therefore concluded that large quantities of [^{14}C]patulin can be successfully biosynthesized with *Penicillium urticae* and sodium [2-^{14}C]acetate in a potato dextrose medium.

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Inhibition of [^{14}C]Glucose Uptake into Rat Liver Glycogen by Dietary Cyclotetraphosphate

A dietary intake of 0.1% P as cyclotetraphosphate (sodium tetrametaphosphate) for 2 weeks was shown to significantly decrease the uptake of [^{14}C]glucose into liver glycogen of fasted rats. Total liver glycogen was 39% less in these animals when compared to that from animals fed a comparable level of orthophosphate.

The cyclic phosphates, cyclotriphosphate and cyclotetraphosphate, comprise approximately 7–9% of the phosphorus of hexametaphosphate, one of the food additive condensed phosphates currently used (Martens and

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