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Degradation of Phenylalanine and Tyrosine by *Sporobolomyces roseus*

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Ammonia-lyase activity for L-phenylalanine, *m*-hydroxyphenylalanine and L-tyrosine was demonstrated in cell-free extracts of *Sporobolomyces roseus*. Cultures of this organism converted DL-[*ring*-^{14}C]phenylalanine and L-[U-^{14}C]tyrosine into the corresponding cinnamic acid. Tracer studies showed that these compounds were further metabolized to [^{14}C]protocatechuic acid. Benzoic acid and *p*-hydroxybenzoic acid were intermediates in this pathway. Washed cells of the organism readily utilized cinnamic acid, *p*-coumaric acid, caffeic acid, benzoic acid and *p*-hydroxybenzoic acid. Protocatechuic acid was the terminal aromatic compound formed during the metabolism of these compounds. The cells of *S. roseus* were able to convert *m*-coumaric acid into *m*-hydroxybenzoic acid, but the latter compound, which accumulated in the medium, was not further metabolized. 4-Hydroxycoumarin was identified as the product of *o*-coumaric acid metabolism by this organism.

Among the wide variety of micro-organisms that can metabolize phenylalanine and tyrosine, the majority appear to degrade these amino acids via homogentisic acid with the initial formation of the corresponding phenylpyruvic acid, a route characteristic of mammals (Kluyver & Van Zijp, 1951; Evans, 1963; Rogoff, 1961; Treccani, 1963). The recent report on the occurrence of ammonia-lyases in fungi that catalyse the non-oxidative deamination of these aromatic amino acids to the corresponding cinnamic acids (Power, Towers & Neish, 1965) has stimulated us to investigate the metabolic fate of phenylalanine and tyrosine in fungi. Evidence for the conversion of phenylalanine into cinnamic acid and its subsequent transformation into protocatechuic acid was obtained in this Laboratory by a study of the metabolism of wood-destroying fungi, e.g. *Schizophyllum commune* (Moore, Subba Rao & Towers, 1967) and *Sporobolomyces roseus*. The present paper provides evidence for the degradation by *S. roseus* of phenylalanine and tyrosine via cinnamic acid, benzoic acid, *p*-hydroxybenzoic acid and protocatechuic acid. The metabolism of *m*-hydroxyphenylalanine, *m*-coumaric acid and *o*-coumaric acid was also examined.

MATERIALS AND METHODS

Chemicals. DL-[*ring*-^{14}C]Phenylalanine, L-[U-^{14}C]tyrosine, [1-^{14}C]benzoic acid and 1,4-bis-(5-phenyloxazol-2-yl)benzene were obtained from New England Nuclear Corp., Boston, Mass., U.S.A. [3-^{14}C]Cinnamic acid and [2-^{14}C]cinnamic acid were available commercially from Merck, Sharp and Dohme Inc., Rahway, N.J., U.S.A. Bacto-soytone, malt extract and yeast extract were purchased from Difco Laboratories, Detroit, Mich., U.S.A. 4-Hydroxycoumarin was obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks. DL-*o*-Hydroxyphenylalanine and DL-*m*-hydroxyphenylalanine were from Fluka A.-G., Buchs SG, Switzerland. 2,5-Diphenyloxazole was supplied by Nuclear-Chicago Corp., Des Plaines, Ill., U.S.A. All the other chemicals were the best grades available from commercial sources.

Chromatography. Solvent *A* (benzene–acetic acid–water; 10:7:3, by vol.) and solvent *B* (100% formic acid–water; 1:49, v/v) were used for the chromatographic separation of various cinnamic acids and benzoic acids on Whatman no. 1 filter paper. The spots were located on chromatograms by spraying with *p*-nitroaniline reagent (Ibrahim & Towers, 1960). The separation of amino acids was achieved by developing the paper chromatograms two-directionally in solvents *C* (phenol–water; 5:1, v/v) and *D* (butanol–acetic acid–water; 20:5:11, by vol.). For chromatograms on silicic acid (20 cm. × 20 cm. plates) solvent *E* (benzene–acetic acid; 9:1, v/v) was used.

Ultraviolet spectra. A Unicam SP. 800 recording spectrophotometer was used for obtaining ultraviolet spectra.

Infrared spectra. Infrared spectra were taken in a Unicam SP. 400 spectrophotometer, KBr disks being used.

Radioactivity measurements. Suitable portions of radioactive samples in methanol were transferred to scintillation vials containing 15 ml. of scintillation fluid [1,4-bis-(5-phenyloxazol-2-yl)benzene, 4 g.; 2,5-diphenyloxazole, 50 mg.; toluene, 1 l.] and the radioactivity was measured in a Nuclear-Chicago 720 series liquid-scintillation spectrometer.

Organism. The culture of *S. roseus* (U.B.C. 901) was grown with shaking at 25° in a nutrient medium containing: malt extract, 3%; Bacto-soytone, 0·5%; yeast, 0·1%. The

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fungus was maintained on slants of the same medium supplemented with agar (1·5%). Subcultures were grown in 50ml. of the medium for 48hr. and used for inoculating large batches of medium distributed in 1l. flasks. After 72hr. the cells were collected by centrifuging at 10000*g* for 15min. and washed thrice with distilled water. The wet packed cells were used for studying the metabolism of various aromatic compounds.

Preparation of cell-free extracts. Wet cells (10g.) were homogenized in a mortar with an equal weight of aluminium oxide and extracted with 0·05M-tris–HCl buffer, pH8 (20ml.). The resulting slurry was centrifuged at 20000*g* for 20min. and the supernatant was used as the crude enzyme preparation.

Assay for the non-oxidative deamination of aromatic amino acids. The reaction mixture containing 0·5ml. of 0·05M-tris–HCl buffer, pH8·8, 0·5ml. of crude enzyme and 5μmoles of substrate (L-phenylalanine, L-tyrosine, DL-*m*-hydroxyphenylalanine, DL-*o*-hydroxyphenylalanine or DL-3,4-dihydroxyphenylalanine) in a final volume of 2ml. was incubated at 30°. After 1hr. the reaction mixture was acidified with 1ml. of N-HCl and extracted with 5ml. of peroxide-free ether. Suitable samples from the ethereal layer were evaporated to dryness under a stream of air and the amount of cinnamic acid or *p*-coumaric acid was determined spectrophotometrically (Koukol & Conn, 1961; Neish, 1961) after dissolving the residue in 3ml. of 0·05N-NaOH. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard.

Assay for catechol oxygenase and protocatechuate oxygenases. To study the disappearance of protocatechuic acid or catechol, 1ml. of crude enzyme was incubated at 30° with 2μmoles of substrate and 0·8ml. of 0·1M-sodium phosphate buffer, pH7, in a final volume of 2ml. After 30min. incubation at 30°, the reaction was stopped by the addition of 0·5ml. of 30% (w/v) trichloroacetic acid and the enzyme activity was assayed colorimetrically (Nair & Vaidyanathan, 1964).

Enzymic synthesis of [^{14}C]p-*coumaric acid.* The crude enzyme (18ml.) was treated with 2% protamine sulphate (2ml.) and the precipitate was removed by centrifugation and discarded. To the supernatant (20ml.) was added 10ml. of 0·05M-tris–HCl buffer and 20μc of L[U-^{14}C]-tyrosine (sp. activity 3·6mc/m-mole) and incubated for 3hr. at 30°. The reaction mixture was adjusted to pH2 with N-HCl and the [^{14}C]*p*-coumaric acid was extracted twice with equal volumes of peroxide-free ether. After shaking with anhydrous Na_2SO_4, the solvent was removed *in vacuo*. The residue was dissolved in 0·5ml. of 95% ethanol and subjected to thin-layer chromatography on silicic acid plates with solvent *E*. The radioactive band corresponding to *p*-coumaric acid was eluted with 95% ethanol, the eluent evaporated to dryness *in vacuo* and the residue redissolved in 5ml. of water. A sample was used for radioactivity determinations and a total of 4·3μc of radioactive *p*-coumaric acid was obtained.

Experiments with radioactive compounds. Each radioactive compound was added under sterile conditions to a 25ml. flask containing 2g. (wet wt.) of freshly harvested cells of *S. roseus* suspended in 5ml. 0·01M-phosphate buffer, pH7. The flasks were incubated at 25° for 1–6hr. with constant agitation. The reaction was stopped by the addition of 1ml. of N-HCl and the cells were separated by centrifugation. The supernatant was collected and the residue was washed with 10ml. of distilled water and again centrifuged. The combined washings were extracted twice with 25ml. of peroxide-free ether. The ethereal layer was evaporated to dryness at room temperature after removal of traces of water with anhydrous Na_2SO_4. The residue was dissolved in 1ml. of methanol. A portion (0·1ml.) of the methanolic solution was transferred to a scintillation vial containing 15ml. of scintillation fluid and the radioactivity measured. The remaining methanolic solution was applied to Whatman no. 1 filter paper and developed two-directionally with solvent *A* in the first direction and solvent *B* in the second direction. Radioautographs were prepared with Kodak Medical X-ray film (Estar base), and the corresponding radioactive spots were cut out from paper chromatograms and placed in scintillation vials together with 15ml. of scintillation fluid for radioactivity determinations.

Experiments with non-radioactive compounds. Washed cells (3g. wet wt.) were added aseptically to 500ml. Erlenmeyer flasks containing 100mg. of substrate in 0·01M-sodium phosphate buffer, pH7 (100ml.), and incubated at 25° for 24hr. Samples (10ml.) were withdrawn at various intervals and acidified with N-HCl (1ml.), and the cells were separated by centrifugation. The disappearance of each substrate and formation of protocatechuic acid (*m*-hydroxybenzoic acid in the case of *m*-coumaric acid) were assayed as described below.

Cinnamic acid was determined spectrophotometrically (Koukol & Conn, 1961). Benzoic acid was determined by measuring the extinction at 229mμ after diluting suitable portions with 95% ethanol (Webley, Duff & Farmer, 1955). For the determination of *m*-coumaric acid, *p*-coumaric acid, caffeic acid, *m*-hydroxybenzoic acid and *p*-hydroxybenzoic acid, samples (10ml.) removed from the reaction mixture at various periods were extracted twice with 25ml. of peroxide-free ether. The solvent was removed *in vacuo* and the residue taken up in 1ml. of ethanol. Suitable samples were applied to Whatman no. 1 filter paper and developed in solvent *B*. The band corresponding to each compound was cut out, eluted with 95% ethanol and the concentration determined spectrophotometrically by measuring the extinction at 278, 333, 325, 300 and 258mμ respectively for *m*-coumaric acid, *p*-coumaric acid, caffeic acid, *m*-hydroxybenzoic acid and *p*-hydroxybenzoic acid. Standard graphs were prepared by subjecting different concentrations of authentic samples to similar manipulations. The colorimetric method of Nair & Vaidyanathan (1964) was used for the direct assay of protocatechuic acid. However, when cells were incubated with caffeic acid, the concentrations of protocatechuic acid at various time-intervals was followed spectrophotometrically at 260mμ after chromatographic separation with solvent *B* and elution with 95% ethanol.

Isolation of the metabolite from o-*coumaric acid.* Freshly harvested cells (60g.) were dispensed in 2l. of 0·01M-sodium phosphate buffer, pH7, containing 2g. of *o*-coumaric acid and incubated for 24hr. on a metabolic shaker at 25°. Samples (10ml.) were removed at 3hr. intervals for paper-chromatographic analysis of the ether-extractable metabolites with solvents *A* and *B*. The remaining medium was acidified after 24hr. and the cells were separated by centrifugation. The clear supernatant solution was concentrated *in vacuo* at room temperature to 100ml. and extracted twice with 200ml. portions of ether. The organic layer was separated out and evaporated to dryness. The residue, after

being taken up in hot water and treatment with charcoal, gave a white crystalline material that was further purified by recrystallization.

Studies on the fate of the side chain of [2-^{14}C]*cinnamic acid.* A suspension of wet cells (3g.) of *S. roseus* in 5ml. of 0·001M-sodium phosphate buffer, pH7, was incubated for 24hr. with 2μc of [2-^{14}C]cinnamic acid. The cells were collected by centrifugation, macerated in a mortar with 3g. of aluminium oxide and extracted with 0·1M-sodium phosphate buffer, pH7 (10ml.). The resulting slurry was centrifuged at 4100*g* for 15min. and the supernatant was placed on a column of Dowex 50 (H^+ form), washed with 1l. of distilled water and eluted with 4% (w/v) NH_3. The eluate was concentrated, applied to Whatman no. 1 chromatography paper and developed two-directionally in solvents *C* and *D*. A radioautograph was prepared from the chromatograms.

Determination of evolution of $^{14}CO_2$. Fresh cells (2g. wet. wt.) were dispensed into a flask containing 1μc of DL-[*ring*-^{14}C]phenylalanine in 5ml. of 0·01M-sodium phosphate buffer, pH7. The $^{14}CO_2$ produced during a total incubation period of 6hr. at 25° was collected in a solution of Hyamine hydroxide (10ml.) and the radioactivity measured in a liquid-scintillation spectrometer.

RESULTS

Enzymic formation of cinnamic acids. Cell-free preparations from *S. roseus* deaminated L-phenylalanine, DL-*m*-hydroxyphenylalanine and L-tyrosine to the corresponding cinnamic acids (Table 1). There was no ammonia-lyase activity when the crude preparations were incubated with DL-*o*-hydroxyphenylalanine or DL-3,4-dihydroxyphenylalanine.

Degradation of DL-[^{14}C]*phenylalanine to* $^{14}CO_2$. Fig. 1 shows the time-course of $^{14}CO_2$ evolution when growing cultures of the organism were given DL-[*ring*-^{14}C]phenylalanine. It is apparent that the aromatic ring of phenylalanine can be degraded to carbon dioxide by *S. roseus*.

Feeding experiments with radioactive compounds. When DL-[*ring*-^{14}C]phenylalanine was administered to washed-cell suspensions of *S. roseus*, the radioactivity was incorporated into cinnamic acid, benzoic acid, *p*-hydroxybenzoic acid and protocatechuic acid (Table 2). Radioautography revealed the presence of four radioactive spots on chromatograms corresponding to these acids. In view, however, of the poor resolution of cinnamic acid and benzoic acid on paper chromatograms the radioactivities of these two compounds are added together in Table 2. Under the experimental conditions, neither [^{14}C]phenylpyruvic acid nor [^{14}C]*p*-hydroxyphenylpyruvic acid could be detected. Further, these compounds when added to the washed cells along with the [^{14}C]phenylalanine failed to trap any radioactivity.

The conversion of cinnamic acid into benzoic acid, *p*-hydroxybenzoic acid and protocatechuic acid was established by using [3-^{14}C]cinnamic acid.

Table 1. *Enzymic formation of cinnamic acids in the presence of cell-free preparations from* S. roseus

Experimental details are given in the text.

Substrate	Product	Specific activity (μmole of product formed/mg. of protein/hr.)
L-Phenylalanine	Cinnamic acid	0·87
m-Hydroxyphenylalanine	*m*-Coumaric acid	0·30
L-Tyrosine	*p*-Coumaric acid	0·16

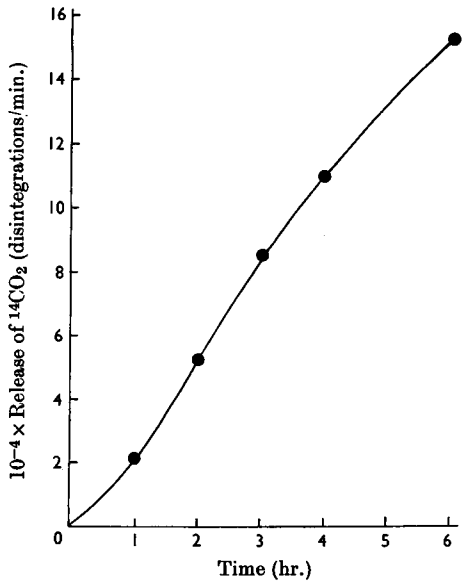


Fig. 1. Release of $^{14}CO_2$ from DL-[*ring*-^{14}C]phenylalanine in the presence of washed cells of *S. roseus*. Experimental details are given in the text.

No other radioactive spot could be detected on radioautographs. Table 2 shows that, when [1-^{14}C]benzoic acid (ring-labelled) was given to washed whole cells of *S. roseus*, it was completely utilized in 1hr.: 18% of the radioactivity was detected in $^{14}CO_2$, whereas 9·4% was recovered in *p*-hydroxybenzoic acid and protocatechuic acid. The remaining radioactivity was present in the aqueous fraction probably incorporated into various organic and amino acids.

p-Coumaric acid, *p*-hydroxybenzoic acid and protocatechuic acid were intermediates in the degradation of tyrosine as evidenced by the changes in radioactivity of L-[U-^{14}C]tyrosine during a 6hr. period. The distribution of radioactivity in

Table 2. *Metabolism of radioactive phenylalanine, cinnamic acid and benzoic acid by the cells of* S. roseus

Experimental details are given in the text.

Compound administered	Incubation time (hr.)	Total radioactivity of ether extract (mμc)	Compounds in ether extract: [^{14}C]Cinnamic acid + [^{14}C]benzoic acid (mμc)	Compounds in ether extract: *p*-Hydroxy[^{14}C]-benzoic acid (mμc)	Compounds in ether extract: [^{14}C]Protocatechuic acid (mμc)
DL[*ring*-^{14}C]Phenylalanine (1 μc)	2	7·1	2·1	3·6	1·4
	4	7·8	2·2	3·5	2·1
	6	9·4	3·0	4·2	2·2
[3-^{14}C]Cinnamic acid (1 μc)	1	140	27	99	14
	2	120	10	84	26
	4	74	3	57	14
	6	53	2	28	23
[1-^{14}C]Benzoic acid (1 μc)	1	94	0	69	25
	2	84	0	60	24
	3	69	0	42	27
	6	49	0	24	25

Table 3. *Metabolism of radioactive tyrosine and* p-*coumaric acid by the cells of* S. roseus

Experimental details are given in the text.

Compound administered	Incubation time (hr.)	Total radioactivity of ether extract (mμc)	Compounds in ether extract: [^{14}C]*p*-Coumaric acid (mμc)	Compounds in ether extract: *p*-Hydroxy[^{14}C]-benzoic acid (mμc)	Compounds in ether extract: [^{14}C]Protocatechuic acid (mμc)
L-[U-^{14}C]Tyrosine (1 μc)	1	7	5·4	1·6	0
	2	9·8	5·6	1·6	2·6
	3	9·7	4·0	2·4	3·3
	6	7·9	1·6	2·9	3·4
[U-^{14}C]*p*-Coumaric acid (0·86 μc)	1	160	123	21	16
	2	150	106	24	20
	4	140	93	25	22
	6	130	68	37	25

the various acids is summarized in Table 3. Incubation of washed-cell suspensions of the organism with [U-^{14}C]*p*-coumaric acid also resulted in its conversion into *p*-hydroxybenzoic acid and protocatechuic acid.

Metabolism of non-radioactive cinnamic acids and phenolic acids by S. roseus. The pathway leading to the oxidation of phenylalanine and tyrosine to protocatechuic acid was further established by giving non-radioactive compounds. Incubation of washed cells of *S. roseus* under sterile conditions with cinnamic acid, *p*-coumaric acid, benzoic acid or *p*-hydroxybenzoic acid resulted in a steady utilization of these compounds in a 24 hr. period. In each case, the formation and disappearance of protocatechuic acid was unequivocally established (Fig. 2). The concentration of protocatechuic acid invariably reached an optimum after 9 hr. and approached zero at the end of the experimental period. Cell-free preparations of the fungus also rapidly destroyed protocatechuic acid. However, under the same experimental conditions, catechol was not metabolized.

m-Coumaric acid was oxidized to *m*-hydroxybenzoic acid. The latter compound, which accumulated in the medium, however, was not further metabolized (Fig. 3).

Although DL-3,4-dihydroxyphenylalanine was not deaminated by *S. roseus* the corresponding cinnamic acid, caffeic acid, was readily converted into protocatechuic acid by washed cells (Fig. 4).

Metabolic fate of o-*coumaric acid.* *o*-Coumaric acid was not degraded to salicylic acid. On the contrary, it served as a precursor for the biosynthesis of 4-hydroxycoumarin. The latter compound was isolated from incubation media in pure crystalline

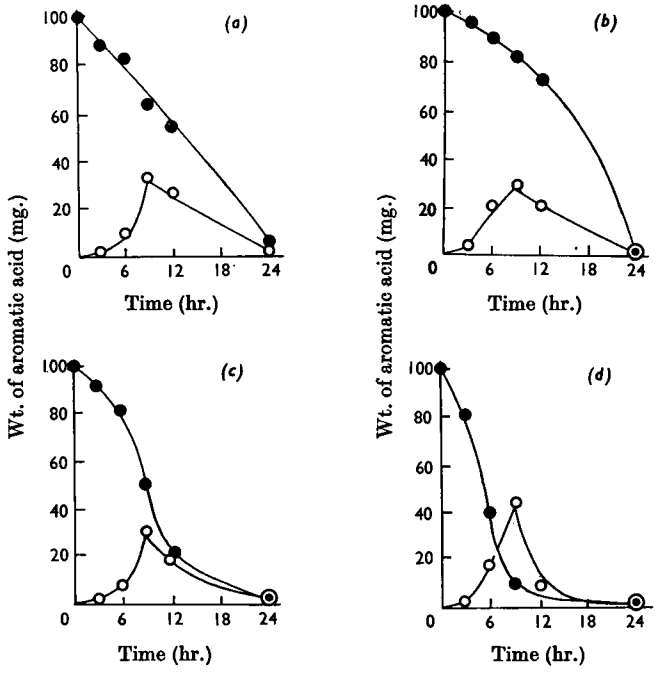


Fig. 2. Time-course of the disappearance of substrate (●) and formation of protocatechuic acid (○) in the presence of washed cells of *S. roseus*. The substrates used were: (*a*) cinnamic acid; (*b*) benzoic acid; (*c*) *p*-hydroxybenzoic acid; (*d*) *p*-coumaric acid. Experimental details are given in the text.

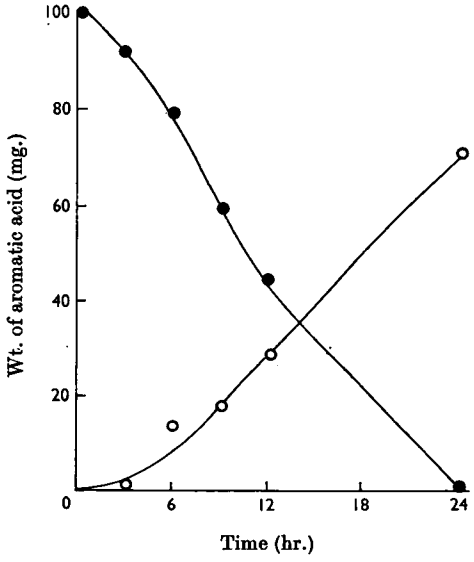


Fig. 3. Time-course of the conversion of *m*-coumaric acid (●) into *m*-hydroxybenzoic acid (○) catalysed by the cells of *S. roseus*. Experimental details are given in the text.

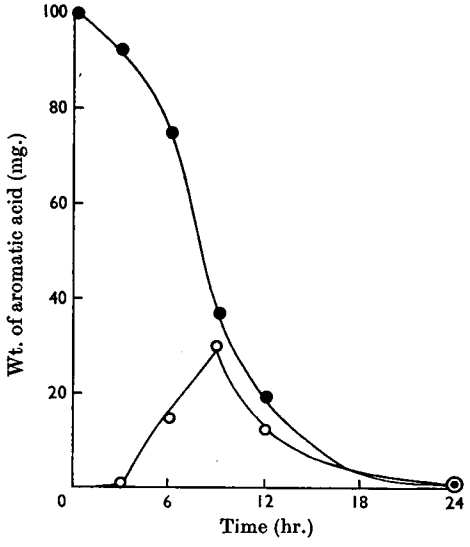
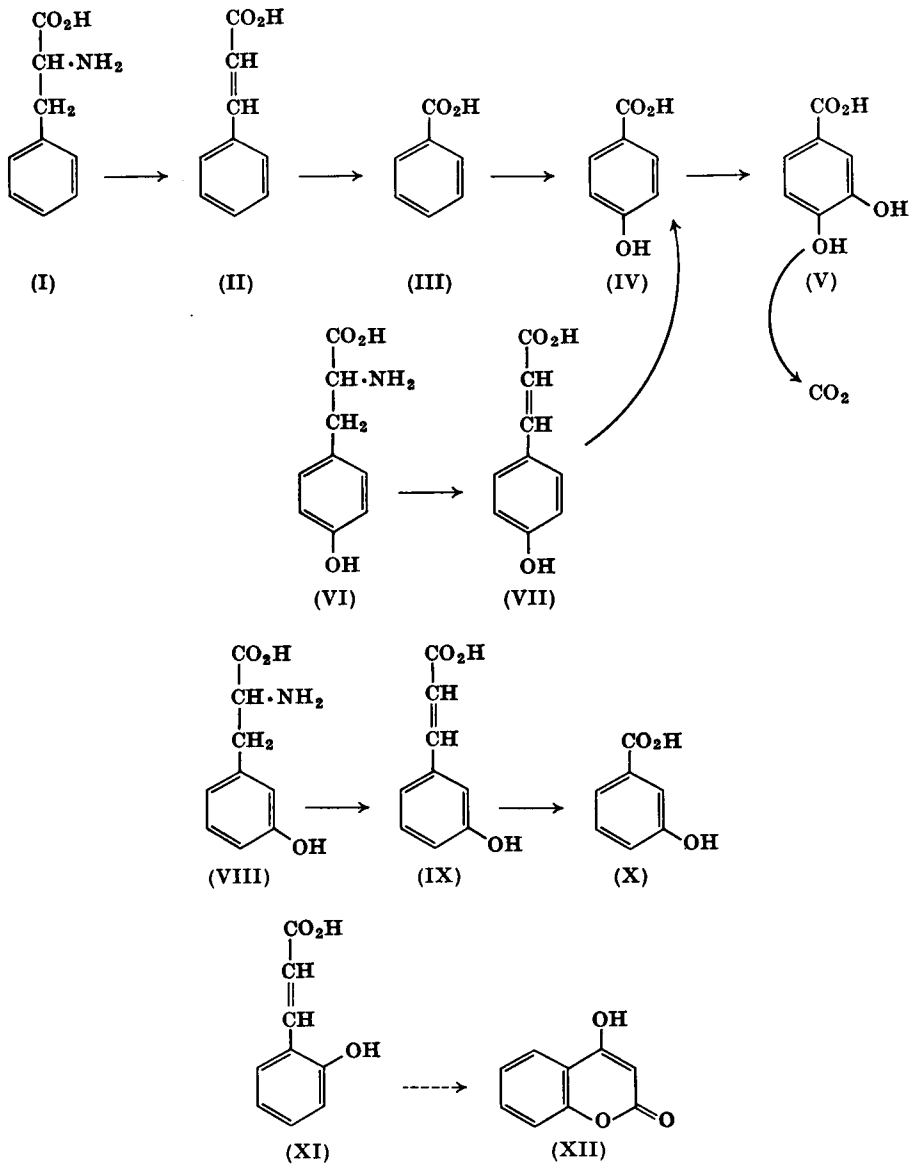


Fig. 4. Time-course of the disappearance of caffeic acid (●) and formation of protocatechuic acid (○) in the presence of washed cells of *S. roseus*. Experimental details are given in the text.



Scheme 1. Pathways for the degradation of phenylalanine and tyrosine by *S. roseus*. (I) Phenylalanine; (II) cinnamic acid; (III) benzoic acid; (IV) *p*-hydroxybenzoic acid; (V) protocatechuic acid; (VI) tyrosine; (VII) *p*-coumaric acid; (VIII) *m*-hydroxyphenylalanine; (IX) *m*-coumaric acid; (X) *m*-hydroxybenzoic acid; (XI) *o*-coumaric acid; (XII) 4-hydroxycoumarin.

form and its identity established by analysis and by comparing its ultraviolet, infrared and nuclear-magnetic-resonance spectra and melting point with those of an authentic sample.

Metabolism of the side chain of cinnamic acid. To follow the metabolic changes in the side chain of cinnamic acid, the organism was incubated with [2-^{14}C]cinnamic acid. Analysis of the aqueous layer, after separation of the ether-soluble fraction, gave ninhydrin-positive spots when subjected to

paper chromatography. Radioautography revealed that most of the radioactivity was confined to glutamic acid.

DISCUSSION

Pathways for the degradation of phenylalanine and tyrosine by *S. roseus* are depicted in Scheme 1. The initial step involves the formation of the corresponding cinnamic acid (Tables 1, 2 and 3) by enzymes similar to those present in vascular plants (Koukol & Conn, 1961; Neish, 1961). The pathways reported hitherto differ from those reported for mammals (Henderson, Gholson & Dalgliesh, 1962) and micro-organisms (Evans, 1963; Rogoff, 1961; Treccani, 1963) where the initial step in phenylalanine and tyrosine degradation is the formation of phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid respectively.

Whiting & Carr (1959) observed that *Lactobacillus pastorianus* was able to reduce the side chain of cinnamic acid and hydroxycinnamic acid with subsequent decarboxylation. A non-oxidative decarboxylase specific for cinnamic acids bearing a 4-hydroxyl group was reported by Finkle, Lewis, Corse & Lundin (1962).

The principal constituents, before the rupture of the benzene ring of *trans*-cinnamic acid by a soil pseudomonad, were *o*-hydroxyphenylpropionic acid and 2,3-dihydroxyphenylpropionic acid (Coulson & Evans, 1959). Blakley & Simpson (1964), in a study of the metabolism of cinnamic acid by a pseudomonad, suggested that the first step is the reduction of the double bond in the side chain, giving rise to phenylpropionic acid, which undergoes hydroxylation in either the *ortho*- or *meta*-position. *Achromobacter* degrades β-phenylpropionic acid by a pathway involving *o*-hydroxyphenylpropionic acid and 2,3-dihydroxyphenylpropionic acid as intermediates (Dagley, Chapman & Gibson, 1965). Neither phenylpropionic acid nor its hydroxylation products were identified as possible intermediates in the degradation of cinnamic acid by *S. roseus*. Under the experimental conditions described in the text, this organism converted cinnamic acid into benzoic acid (Table 2).

Webley, Duff & Farmer (1955, 1956, 1957, 1958), Henderson & Farmer (1955) and Henderson (1961) reported that cinnamic acid and related compounds could be converted by micro-organisms into the corresponding benzoic acids by a process involving β-oxidation. Zenk (1965, 1967) and Zenk & Müller (1964) have provided evidence for the conversion of cinnamic acids into the corresponding benzoic acids by the leaves of *Salix* and *Catalpa* in a process involving the removal of a C_2 fragment from the side chain as acetate. These workers suggested that benzoic acid, salicylic acid and *p*-hydroxybenzoic acid are formed from the parent cinnamic acid by β-oxidation in a manner analogous to fatty acid degradation (Stumpf, 1965). Similar assumptions were made by Vollmer, Reisener & Grisebach (1965), who demonstrated the incorporation of [1-^{14}C]-acetic acid into glutamic acid in wheat when [1-^{14}C]*p*-coumaric acid was administered. The formation of radioactive glutamic acid from [2-^{14}C]cinnamic acid by *S. roseus* also suggests that acetate may be a product of the reaction involving the conversion of cinnamate into benzoate.

Although *p*-coumaric acid and *o*-coumaric acid may be produced by *Aspergillus niger* supplied with cinnamic acid on prolonged incubation (Bocks, 1967*a*), there was no evidence for the hydroxylation of cinnamic acid by *S. roseus* in the present study. Benzoic acid, however, was readily hydroxylated in the *para*-position. There was never any detectable formation of either *o*- or *m*-hydroxybenzoic acid. Protocatechuic acid may be converted into catechol by *Aerobacter aerogenes* before ring-cleavage (Pittard, Gibson & Doy, 1962). Although protocatechuic acid was a conspicuous metabolite of phenylalanine, benzoic acid or *p*-hydroxybenzoic acid in *S. roseus*, catechol was never detected among the products. The disappearance of protocatechuic acid, but not catechol, with cell-free preparations of the organism is further evidence that protocatechuic acid is the terminal aromatic compound in the degradation of phenylalanine or tyrosine.

Evidence presented in this study showed that the degradation of *m*-tyrosine proceeded via the formation of *m*-coumaric acid and ended with the accumulation of *m*-hydroxybenzoic acid (Fig. 3). Bocks (1967*b*) reported that *m*-hydroxybenzoic acid can be converted into protocatechuic acid and ultimately into catechol by *Aspergillus*, but attempts to demonstrate the further metabolism of this compound by *S. roseus* were unsuccessful.

S. roseus was also able to convert *o*-coumaric acid into 4-hydroxycoumarin. This is similar to the results of Bocks (1967*a*) and Mead, Smith & Williams (1958) with other organisms.

The system capable of oxidizing the side chain of cinnamic acid seems to be rather unspecific, since *m*-coumaric acid, *p*-coumaric acid and caffeic acid were readily converted into the corresponding benzoic acids. The hydroxylating system involved in the conversion of *p*-hydroxybenzoic acid into protocatechuic acid appears to be quite specific, since *m*-hydroxybenzoic acid did not serve as substrate. A hydroxylating system for *p*-coumaric acid is not evident in *S. roseus*, in contrast with *Lentinus lepideus*, in which caffeic acid is a metabolite of *p*-coumaric acid (Power *et al.* 1965).

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