AUXIN-INDUCED CONJUGATES OF BENZOIC ACID

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Summary: An inducible metabolite of benzoic acid in pea tissues, formerly identified as benzoylaspartic acid, has now been shown to consist largely or entirely of benzoylmalic acid. Auxins, but not physiologically inactive analogs, induce in addition the synthesis of small amounts of a second conjugate with similar chromatographic properties. This minor product is identical with authentic benzoylaspartic acid.

Pea tissues are able to conjugate indoleacetic acid (IAA) and naphthaleneacetic acid (NAA) with L-aspartic acid to form the N-acylaspartates (1,2). Synthesis of these conjugates is greatly enhanced by pretreatment of the tissue with auxins, while chemically related but physiologically inactive analogs are without effect (3). The inducing action is not dependent on auxin-stimulated growth (4). Andreae and Good reported (5) that peas also conjugate benzoic acid with aspartic acid and the ability to synthesize the putative benzoylaspartate has been shown to be similarly induced by auxin treatment and to be dependent on RNA and protein synthesis (6). In extending this work however, it was found that unlike the formation of IAA- and NAA-aspartates, synthesis of the benzoyl conjugate is also enhanced by pretreatment with numerous aromatic carboxylic acids which are inactive physiologically (Venis, manuscript in preparation). This finding implied either that there are at least 2 inducible acylaspartate synthetases in peas, or that the benzoate metabolite is not in fact benzoylaspartic acid. Since the original report (5) identified this metabolite solely on the basis of R_f value and hydrolysis products, it was evident that a recharacterization was warranted.

Stem segments (ca. 2 cm) of 7 day etiolated peas (Pisum sativum, var.

Alaska) were shaken for 16 hr in autoclaved solutions containing 20 mg/l benzoic acid and 20 mg/l 2,4-dichlorophenoxyacetic acid (as inducer). They were then transferred for 4 hr to ^{14}C-carboxyl-benzoic acid (20 mg/l; 3 μc/l). All solutions were buffered in 5 mM maleate pH 5.5 and contained 50 mg/l penicillin G. Following incubation the tissue was macerated in a blendor with 80% ethanol (1.5 vols.) and filtered through 2 layers of Miracloth. Ethanol was removed on a rotary evaporator at 40° and the aqueous residue saturated with ammonium sulfate. After addition of infusorial earth, the precipitated protein was filtered off, the clear filtrate adjusted to pH 6 and extracted 3 times with ether. These extracts were discarded and the aqueous phase was re-extracted with ether at pH 3. This ether extract was reduced in volume, streaked on Whatman 3 MM paper and developed in i-propanol:ammonia:water, 8:1:1 (ascending, 16 hr). The radioactive and UV-quenching region of R_f ca. 0.15-0.25 was eluted in 80% ethanol and re-chromatographed on Whatman No. 1 paper in n-butanol:acetic acid: water (60:15:25). The radioactive region (R_f ca. 0.8-0.9) was eluted once more and subjected to TLC (Camag Kieselgel DF-5) in toluene:propionic acid:water (2:2:1, upper phase). This system resolved the main metabolite (R_f 0.6, ca. 90%; yield 20 mg from 2 kg tissue) from a minor metabolite (R_f 0.25, yield 3.5 mg from 5 kg tissue).

The major metabolite was crystallized from $CHCl_3$. It was identified as O-benzoyl-L-malic acid by comparison with an authentic specimen by m.p., mixture m.p., UV, IR (KBr disc) and NMR spectra. The authentic specimen, prepared according to Freudenberg and Noe (7), had m.p. 142°, although m.p. 163° had been reported by these authors. However, analysis (calcd: C 55.47, H 4.23; found C 55.26, H 4.00) and the characteristic NMR spectrum in CD_3OD: δ 8.27-7.30 (m, 5H, benzoyl), 5.65 (m, X part of ABX system, 1H, $>CH.OCO.C_6H_5$) and 3.03 (m, AB part of ABX system, 2H, $-CH_2-$) left no doubt that the compound had the expected structure. It was optically active with $[\alpha]_D^{23°}$ - 5.7 (10% solution in acetone).

The minor metabolite, which was chromatographically identical with benzoylaspartic acid, was methylated using diazomethane in ether. After

purification by TLC, the product yielded NMR ($CDCl_3$) and IR($CHCl_3$) spectra indistinguishable from those of authentic N-benzoyl-L-aspartic acid (5) similarly methylated.

In the original report on benzoylaspartate formation (5), the tissue was fed with benzoic acid alone, without an auxin inducer. We find that under these conditions the acidic conjugation product consists entirely of benzoylmalate. Benzoylaspartic and benzoylmalic acids are not separated in the ammoniacal solvent system used by Andreae and Good (5) and their report of aspartic acid as a hydrolysis product probably reflects insufficient purification of the conjugate prior to hydrolysis.

Synthesis of benzoylmalate is greatly increased by pretreatment of the tissue with both auxins and inactive analogs, but only auxins are able to induce the synthesis of benzoylaspartic acid. Under no circumstances have we been able to detect the formation of malic acid conjugates of IAA or NAA in peas. Pea tissues thus appear to possess an N-acylaspartate synthetase, acting on IAA, NAA and benzoic acid and induced specifically by auxins, and a benzoylmalate synthetase which is induced by a variety of aromatic carboxylic acids, including compounds with auxin activity. Details of the induction specificity and other characteristics of these systems will be published elsewhere.

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