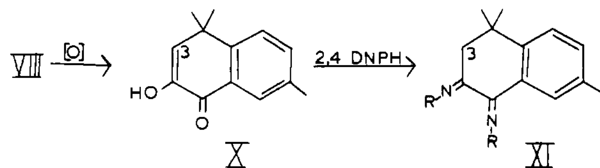

Figure 4. Oxidation product of 4,4,7-trimethyl-3,4-dihydro-2(1*H*)-naphthalenone and its 2,4-dinitrophenylhydrazone derivative.

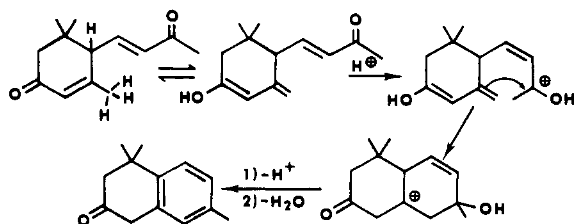

Figure 5. A suggested mechanism from 5-keto-α-ionone to 4,4,7-trimethyl-3,4-dihydro-2(1*H*)-naphthalenone.

11.0, 11.0 Hz) at δ 4.15, the axial proton at C_3 as a double doublet (J = 11.0, 12.5 Hz) at δ 1.65, the equatorial proton resonates as a double quartet (J = 2.0, 4.0, 12.5 Hz) at δ 1.94, the axial benzylic proton at C_1 as a double doublet (J = 11.0, 16.0 Hz) at δ 2.68, and the equatorial benzylic proton at C_1 as a double quartet (J = 2.0, 5.5, 16.0 Hz) at δ 3.08. As expected the benzoate (oil) of IX showed a downfield shift of the C_2 methine proton from δ 4.15 to 5.49. Dehydration of the secondary alcohol IX with warm aqueous formic acid, or better, with *p*-toluenesulfonic acid in benzene, gave the natural hydrocarbon II (Figure 3). The infrared spectrum of this compound corresponded to that published by Kemp et al. (1971) and to a spectrum obtained in our previous research (Stevens et al., 1976). The GLC retention time on a Igepal column (75 ft × 0.01 in.) was identical with that previously obtained. Although VIII does not appear to have been previously described, a compound with this structure was mentioned briefly without further description or detail as a minor by-product in a patented synthesis of 1-hydroxy-4-keto-α-ionone (Rowland, 1971).

When exposed to air or, better, treated with silver oxide, VIII is converted into the new product, $C_{13}H_{14}O_2$, mp 112–113°C (Figure 4, X). This product, λ_{max} (ϵ) 298 (9400) and 258 (11000) nm in ethanol, shows strong absorption bands in the infrared at 3430 (hydroxyl) and 1655 cm^{-1} (conjugated carbonyl). On the basis of its NMR spectrum, it is considered to be 4,4,7-trimethyl-1,4-dihydro-2-hydroxy-1-naphthalenone, i.e., the vinylic proton at C_3 appears as a sharp singlet at δ 6.21 and the hydroxyl as a broad 1 H peak at δ 6.46. High-resolution mass spectral analysis confirmed the molecular formula as $C_{13}H_{14}O_2$, while low resolution gave m/e 39 (100), 91 (92), 115 (89), 51 (82), 159 (77), 77 (60), 43 (58), 65 (54), 63 (53), and 27 (48). In further confirmation of this structural assignment, X reacts in its diketonic form with 2,4-dinitrophenylhydrazone to give a crystalline bis(2,4-dinitrophenylhydrazone) derivative (mp 255–257°C) of structure XI (Figure 4) (R = 2,4-$(NO_2)_2C_6H_3NH$–). In XI, the methylene protons at C_3 appear, as expected, as a 2 H singlet at δ 2.87.

The acid-catalyzed cyclization of 5-keto-α-ionone may be rationalized as shown in Figure 5.

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Identification of Salsolinol as a Major Dopamine Metabolite in the Banana

Ralph M. Riggin, Michael J. McCarthy, and Peter T. Kissinger*

A major pathway of dopamine metabolism in the banana involves reaction with endogenous acetaldehyde and condensation to form 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline. The process accelerates during advanced stages of ripening due to the increased production of acetaldehyde. The new metabolite was found using a combination of liquid chromatography and thin-layer electrochemistry. Structural confirmation was obtained by cyclic voltammetry, thin-layer chromatography, and gas chromatography–mass spectrometry.

During the course of an investigation of tyrosine metabolism in the banana using liquid chromatography with electrochemical detection (LCEC), an unidentified phenol appeared in high concentration during later stages of ripening. This species, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol), has apparently not been previously reported in plant matter.

A new technique (LCEC) has been under development

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907.

in our laboratory for the analysis of phenolic compounds (Kissinger et al., 1974; Riggin et al., 1975). Using this approach a routine clinical assay was devised for catecholamines (L-dopa, norepinephrine, epinephrine, and dopamine) in urine (Kissinger et al., 1976). Interest in possible dietary interferences with this clinical assay led us to investigate catecholamines in plant matter. Due to earlier reports of the presence of dopamine and norepinephrine in the banana (West, 1958; Udenfriend et al., 1959; Smith and Kirshner, 1960; Deacon and Marsh, 1971) this species was chosen for investigation.

EXPERIMENTAL SECTION

Banana fruit used for this work were purchased locally when still slightly green. They were allowed to ripen to a full yellow color at room temperature and then analyzed or permitted to overripen until an advanced stage (>14 days) when the peel was completely blackened and tissue breakdown was obvious. The whole banana (or parts thereof) was homogenized in 0.1 *M* $HClO_4$ (50 ml/g of material) in a blender and then centrifuged at 20000*g* for 10 min. The procedure used for determination of the catecholamines in the supernatant was identical with that described elsewhere for urine samples (Kissinger et al., 1976).

In brief, the catecholamines are specifically adsorbed on acid-washed aluminum oxide at pH 8.5, and then eluted into 1 *M* acetic acid. This preliminary cleanup serves to isolate basic catechols from other phenolic compounds. The catecholamines are then analyzed on a high performance liquid chromatograph using a sensitive thin-layer electrochemical detector (Bioanalytical Systems, Inc., Model LC-2). The instrumentation (Kissinger et al., 1974; Riggin et al., 1975) and assay procedure (Kissinger et al., 1976) have been fully described in earlier papers. Using this system with a 50 cm × 2 mm glass column packed with pellicular cation exchange resin (Vydac 232CX, The Separations Group), catecholamines can be easily determined at the nanogram level and often at lower levels depending on the origin of the sample.

During the course of our investigation we noticed, as had previous workers, that large quantities of dopamine and norepinephrine and traces of L-dopa were present in both the peel and the pulp of the banana. In addition, another more strongly retained compound was found in both parts. The amount of this compound increased dramatically as the banana ripened. Because this unknown compound was specifically adsorbed on alumina at high pH and released at low pH, it was suspected to be a catechol. In order to further substantiate this hypothesis a number of serial injections were made with the detector electrode set at different potentials. A plot of chromatographic peak current vs. electrode potential for both dopamine and the unknown metabolite provided reconstructed hydrodynamic voltammograms. Both voltammograms were found to have almost identical half-wave potentials (ca. +570 ± 10 mV vs. Ag|AgCl) under the chromatographic operating conditions used. This evidence strongly supported our contention that the unknown compound was a catechol, since most other electroactive moieties have either a much higher oxidation potential (e.g., monophenols, methoxyhydroxy compounds, and aromatic amines) or a much lower oxidation potential (e.g., *p*-hydroquinones and trihydroxy compounds).

The acetic acid eluents (5 ml) from several alumina extractions were combined and concentrated to ca. 50 μl by evaporation at 50°C under a stream of nitrogen. Thin-layer chromatography on cellulose (250 μm Avicel; Analtech, Inc.) using a butanol–acetic acid–water (70:15:15) solvent system showed, in addition to dopamine and norepinephrine, a compound with an R_f value of 0.55. The spots were developed using iodine vapor. Following preparative TLC of the same extract, the cellulose was removed from the R_f 0.50–0.60 region of the plate and extracted with ethanol. Two microliters of the ethanol extract was injected directly on the LCEC instrument and found to contain the unknown compound in high concentration.

Table I. Catecholamine Levels in the Ripe Banana Assessed by Liquid Chromatography (LCEC)[a]

	Climacteric		Postclimacteric[b]	
	Pulp	Peel	Pulp	Peel
Norepinephrine	5.8 ± 0.25[c]	81 ± 3.1	1.4 ± 0.1	27 ± 10
Dopamine	48 ± 1.8	720 ± 27	22 ± 0.8	210 ± 8
Salsolinol	<0.1	<0.1	40 ± 1.5	260 ± 14

[a] All values, micrograms per gram for wet tissue. [b] Ten days. [c] Precision (standard deviation) of repetitive assays, not species variation.

A portion of the ethanol extract from preparative TLC was evaporated to dryness and the residue dissolved in 200 μl of 1.0 *M* perchloric acid. Cyclic voltammetry (Bioanalytical Systems Inc., Model CV-1) at a small (0.16 cm diameter) carbon paste electrode using a scan rate of 350 mV/sec indicated a single chemically reversible redox couple with an anodic peak potential of +0.65 V and a cathodic peak potential of +0.44 V. Cyclic voltammograms of the unknown extract and authentic salsolinol (Aldrich Chemical Co.) matched perfectly.

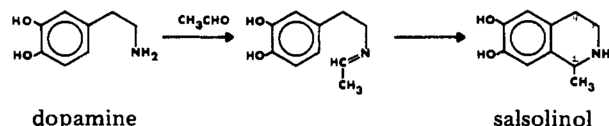
The remainder (100 μl) of the ethanol extract was evaporated to dryness and the residue derivatized with 200 μl of pentafluoropropionic anhydride (PFP) (Pierce Chemical Co.) at 80°C for 1 hr. The PFP was then evaporated under a stream of nitrogen and the residue taken up in 50 μl of benzene. Gas–liquid chromatography (Varian 2440, 6 ft × 1/8 in., 3% OV 101 on Chromosorb G 100–120 mesh, helium carrier at 30 ml/min, linear temperature program 10°C/min from 100°C for 3 min to 170°C) revealed a compound with a retention time (12.4 min) greater than that for either PFP–norepinephrine (11.4 min) or PFP–dopamine (10.4 min).

A gas chromatograph–mass spectrometer–computer (GC–MS–COM) system based on the LKB 9000 with a 70-eV electron impact source was used in the repetitive scanning mode to obtain a mass spectrum for the PFP derivative of the unknown metabolite. The mass spectrum as well as the GC retention time were identical with the same data for authentic salsolinol. A molecular ion at 617 and large peaks at M − 15 (602), M − 50 (567), and M − 65 (552) characterize the spectrum with major fragmentations involving loss of methyl (15), C_2F_5 (119), and the PFP (147) groups, and combinations thereof.

Besides the GC–MS results, confirmation of the salsolinol structure was also clear from comparative TLC, LCEC, and cyclic voltammetry data and there is little room for doubt that this is a correct assignment for the unknown metabolite. Estimations of norepinephrine, dopamine, and salsolinol levels in banana tissue by LCEC are given in Table I.

DISCUSSION

The obvious route to salsolinol biosynthesis in the banana involves the classical Pictet–Spengler condensation whereby a phenylethylamine couples with acetaldehyde forming an imine which subsequently cyclizes to the tetrahydroisoquinoline as shown below (Whaley, 1951). Although salsolinol has been found in unripe bananas, its



synthesis is greatly accelerated during later stages of the ripening process. This observation is consistent with the above reaction. Acetaldehyde may be enzymatically generated from ethanol produced during the postclimacteric phase. Tressel and Jennings (1972) have examined volatile compounds in the ripening banana by gas chromatography and they reported relative ethanol production as a function of storage. Both ethanol and acetaldehyde have been noted in bananas for many years in studies of volatile flavor components. This work has been reviewed by Hultin and Proctor (1961) and by Wick et al. (1966). Compounds related to salsolinol have been recently found in the seeds of the legumes *Mucuna mutisiana* (Bell et al., 1971) and *Mucuna deeringiana* (Daxenbichler et al., 1972).

If the above reaction is an accurate picture of the biosynthetic pathway, we would also expect to find a corresponding metabolite (4-hydroxysalsolinol) for norepinephrine (α-(aminomethyl)-3,4-dihydroxybenzyl alcohol). Thus far we have not found evidence for substantial amounts of 4-hydroxysalsolinol. This can be rationalized from two known facts. Norepinephrine is present in unripe bananas at a level about 11% of that for dopamine (Table I). Perhaps more important is the fact that the Pictet–Spengler reaction is known to proceed at a much slower rate for catecholamines with a benzylhydroxyl group (Robbins, 1968).

We envision three competitive degradation pathways for dopamine and norepinephrine in the banana. The first involves a one-electron oxidation leading to free-radical coupling products. The second involves a two-electron oxidation to the orthoquinone followed by 1,4-addition of the side-chain amine to the ring, leading to an indoline which subsequently oxidizes (more easily than the original catecholamine) and then rearranges to a 5,6-dihydroxindole. The latter product is extremely unstable and readily oxidizes and polymerizes to a melanin polymer. This second pathway has been implicated by others in the "browning reaction" of the banana (Deacon and Marsh, 1971).

The third likely route entails the coupling with acetaldehyde (or other aldehydes), cyclization to a tetrahydroisoquinoline, and oxidation of the latter to a quinone which couples with the melanin responsible for the black appearance of overripe bananas.

If the metabolism of dopamine in the banana were entirely analogous to that in man, we would expect 3,-4-dihydroxyphenylacetaldehyde to be a product (Axelrod, 1971). This product has been shown to couple with dopamine itself leading to the isoquinoline alkaloid tetrahydropapaveroline by the same route as that proposed for salsolinol (Sandler et al., 1973). This appears not to be a significant pathway in the banana. Tetrahydropapaveroline and salsolinol have been reported as urinary metabolites of L-dopa in Parkinson's patients on drug therapy (Sandler et al., 1973) and have also been postulated as possible active agents in a theory of alcohol addiction (Davis and Walsh, 1970).

Possible fourth and fifth metabolic pathways for the catecholamines would be O-methylation and side-chain oxidation via a monoamine oxidase (MAO). Both reactions constitute the major pathway in the mammalian system (Axelrod, 1971), but there is no evidence to date indicating that they take place in the banana.

The power of electrochemical reactions for both qualitative and quantitative studies of natural products is not generally appreciated. The present example illustrates the useful information which can be made available by modern electrochemical techniques (e.g., hydrodynamic and cyclic voltammetry) particularly when combined with thin-layer and high performance liquid chromatography. In the future we hope to explore more fully the quantitative aspects of salsolinol formation and degradation using this new approach to natural product chemistry.

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