ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 114, 576–584 (1966)

Preparation and Properties of *p*-Hydroxyphenylacetaldehyde and 3-Methoxy-4-Hydroxyphenylacetaldehyde

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Received December 27, 1965

A simple method for the preparation of the naturally occurring aldehydes, *p*-hydroxyphenylacetaldehyde and 3-methoxy-4-hydroxyphenylacetaldehyde, is presented. The aldehydes were prepared from synephrine and metanephrine, respectively, by treating the latter compounds with hot acid. The aldehydes were isolated and stored as their stable, crystalline, bisulfite addition products from which they could be conveniently obtained in a highly purified state. Identification of the aldehydes was made by functional group analysis, preparation of suitable derivatives, and by oxidation of the aldehydes to the corresponding acids by xanthine oxidase. Paper chromatography of the aldehydes and other identifying properties are presented.

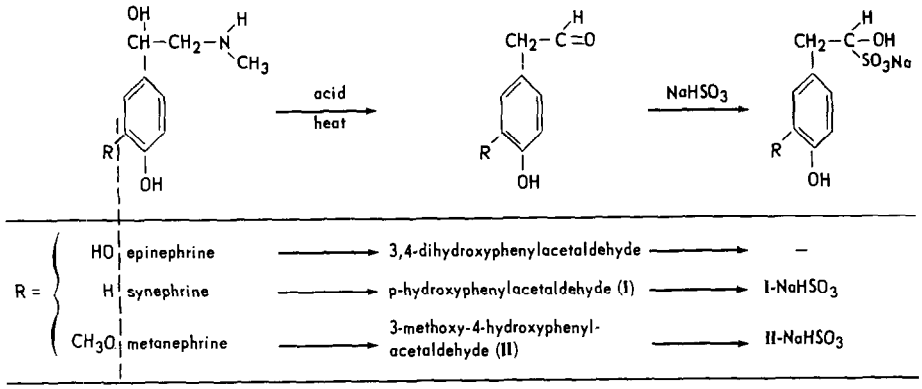
p-Hydroxyphenylacetaldehyde (I) and 3-methoxy-4-hydroxyphenylacetaldehyde (homovanillin) (II) are postulated intermediates in several biochemical reactions in plants and animals. I may be formed from tyramine by the action of a transaminase present in plants (1) or by an oxidative deamination in mammalian tissue (2, 3). It is possible that I may be a building block in the biosynthesis of some benzylisoquinoline alkaloids (4). There is evidence (5) that II is formed in animals from 3,4-dihydroxyphenylethylamine (dopamine) and that it may be oxidized or reduced to the corresponding acid or alcohol, respectively.

To my knowledge no authentic samples of these compounds have been used for biochemical studies despite the fact that methods for synthesizing them have been known for 50 years. In 1909 Langheld (6) synthesized I by treating tyrosine with sodium hypochlorite but was unable to collect the crystals formed and did not subject them to analysis. In 1915 Harries and Haarmann (7) and then Harries (8) synthesized II by the ozonolysis of eugenol. More recently Challis and Clemo (9) obtained II by the ozonolysis of *O*-carbethoxyeugenol. However, these syntheses of II required several steps and repeated fractional distillations. Apparently, neither I nor II has been prepared as a useful crystalline, sodium bisulfite addition compound from which the aldehyde could be obtained in a highly purified state. It was considered desirable, therefore, to prepare these naturally occurring aldehydes by a more convenient method and in a stable state and to study the biochemical reactions in which they are involved.

This paper reports a new and convenient synthesis of I and II, their chromatographic and other identifying characteristics, and the first useful preparation of their stable bisulfite addition compounds. An accompanying paper (10) presents evidence that these aldehydes can be oxidized to acids by a diphosphopyridine nucleotide-dependent aldehyde dehydrogenase of bovine liver.

The synthesis of I and II is based upon a method devised by Fellman (11, 12) who showed that 3,4-dihydroxyphenylacetaldehyde can be synthesized from epinephrine (Table I) presumably by a pinacol-pina-

TABLE I
SYNTHESIS OF SOME 3-(SUBSTITUTED)-4-HYDROXYPHENYLACETALDEHYDES AND THEIR SODIUM BISULFITE ADDITION PRODUCTS



colone type of rearrangement which occurs when epinephrine is heated in acid solution. The starting materials which were used in the preparation of I and II are synephrine and metanephrine, respectively, compounds which differ structurally from epinephrine only in the substitution on the third carbon atom of the aromatic ring (Table I).

EXPERIMENTAL METHODS AND RESULTS

Preparation of the aldehyde-bisulfite addition compounds. Four gm of the starting material (synephrine[1] for the preparation of I or *dl*-metanephrine hydrochloride[2] for the preparation of II) is mixed with 60 ml of 85% phosphoric acid in a 125-ml Erlenmeyer flask which is immersed in a mineral oil bath at 170–180°. A clear, yellow solution is obtained by the time the contents of the flask have reached 80–90°. The temperature of this solution is allowed to rise to 120°, at which point the flask is removed from the bath. After standing for 15–20 seconds, the hot solution is poured into 720 ml of distilled water at room temperature. The resulting tan solution is allowed to stand for 90 minutes during which time it shows an increasing capacity to give a positive Schiff's reaction and to form a yellow precipitate with 2,4-dinitrophenylhydrazine. The solution is then saturated with sodium chloride and extracted, successively, with 120-, 70-, and 50-ml portions of peroxide-free ether. The resulting 240 ml ether extract is combined with another 240 ml ether extract which has been simultaneously and similarly prepared from another 4 gm of the starting material. This pooled ether extract is then shaken for 10 minutes with 40 ml of sodium phosphate buffer (10^{-1} M, pH 7.6) saturated with sodium chloride. The ether layer is decanted and evaporated under a stream of dry nitrogen. A yellow liquid is obtained which is carefully layered and left undisturbed on the surface of 60 ml of a saturated solution of sodium bisulfite in a 125-ml Erlenmeyer flask. Within a few minutes white crystals of the aldehyde-bisulfite addition compound form at the interface between the aqueous bisulfite phase and the yellow liquid containing the aldehyde. If the crystals do not grow downward into the aqueous phase but remain at the interface, a stirring rod is used to push the crystals downward, thereby permitting more of the bisulfite-saturated aqueous phase to come into contact with the overlying aldehyde.

After 1–2 hours the formation of white crystals is essentially complete. At this time only a small amount of a brownish-yellow liquid remains on the surface of the aqueous phase and in contact with some of the uppermost crystals. Much of this material may be removed with an absorbent paper. The crystals are collected on a Buchner funnel and are sucked almost dry. They are washed successively with absolute ethanol and ether.

The dried crystals are ground to a powder and suspended in a large volume of absolute ethanol. The mixture is filtered, and the cake is washed with more alcohol and ether. If the powder is not completely white, it is resuspended in alcohol, and the resulting mixture is then filtered. The alcohol-cleaned, ether dried cake is ground to a powder, suspended in a large volume of ether, collected on

[1] Regis Chemical Co., Chicago, Illinois.

[2] Calbiochem, Los Angeles, Calif.; Sigma Chemical Co.; St. Louis, Mo.; Winthrop Laboratories, New York, New York.

a funnel, and dried. The cake obtained is dissolved in the smallest possible amount of 45° water, and the resulting solution, which is less than 20 ml, is filtered through Whatman No. 1 filter paper directly into 400–600 ml of absolute ethanol. The total volume of alcohol is increased to 1000 ml, and the mixture is allowed to stand for several hours or overnight at room temperature. Crystals usually form immediately. They are collected by filtration, washed with ether, sucked dry, and ground to a fine powder.

One hundred per cent yield and analysis[3] of I-$NaHSO_3$ calcd. for $C_8H_9O_5NaS$: 11.5 gm with 40.00% C. Found: 1.6 gm with 32.33% C (11% yield); 2.2 gm with 21.72% C (11% yield).

One hundred per cent yield and analysis of II-$NaHSO_3$ calcd. for $C_9H_{11}O_6NaS$: 9.2 gm with 40.00% C. Found: 2.7 gm with 35.77% C (27% yield); 2.8 gm with 35.66% C (27% yield).

Generation of free aldehydes from the bisulfite addition compounds. The following procedure is employed to prepare approximately 5 mg quantities of I and 10 mg quantities of II. Twenty mg of the aldehyde-bisulfite addition compound is placed in a small test tube and dissolved in a few drops of water. The solution is saturated with sodium chloride, and the resulting mixture is overlaid with peroxide-free ether. Four drops of a 10% sodium carbonate solution are then added. The test tube is sealed with aluminum foil and shaken for one minute. The ether layer is removed, and the sample is again extracted with approximately 1.5 ml of ether. The ether extracts, combined in a small, preweighed test tube, are heated in a 40° water bath and taken to dryness under a stream of dry nitrogen, leaving the free aldehyde in a clear, thin film of liquid. For the preparation of larger quantities of aldehyde a similar procedure is employed in which, for every 400 mg of the aldehyde-bisulfite compound, 6 ml of water, saturating amounts of sodium chloride, and 4 ml of 10% sodium carbonate solution are used. The free aldehyde is extracted with 2 or 3 portions of peroxide-free ether which is then evaporated.

The aldehydes are obtained in the form of clear, slightly straw-colored liquids. I has a slightly pungent and irritating odor; II has an odor resembling that of vanillin.

When one to two mg quantities of either aldehyde were dissolved in 0.5 ml of water or 1 N hydrochloric acid, clear, colorless solutions were obtained, aliquots of which gave a strong reaction with 2,4-dinitrophenylhydrazine in acid solution. Dissolving the aldehydes in 1 N sodium hydroxide or in 10% sodium carbonate resulted in yellow solutions which gave very weak reactions with 2,4-dinitrophenylhydrazine in acid solution. I-$NaHSO_3$ and II-$NaHSO_3$ have been stored at room temperature for several months without any apparent change in their white color, consistency or capacity to yield free aldehyde.

Anal. I calcd. for $C_8H_8O_2$: C, 70.57; H, 5.92; O, 23.50%. Found (after drying *in vacuo* over phosphorus pentoxide): C, 70.60; H, 5.98; O, 23.33%.

II calcd. for $C_9H_{10}O_3$: C, 65.04; H, 6.07; O, 28.88; CH_3O, 18.68%. Found (after drying *in vacuo* over phosphorus pentoxide): C, 65.14; H, 6.34; O, 28.57; CH_3O, 18.44%.

Classification and Identifying Tests

Carbonyl group. Both I and II formed precipitates when added to a sodium acetate-buffered solution of semicarbazide hydrochloride and to acidic solutions of 2,4-dinitrophenylhydrazine and *p*-nitrophenylhydrazine.

Aldehydic group. An intense wine-purple color formed when milligram quantities of I or II were added to Schiff's reagent. Another highly specific test for aldehydes, the "methone test" (13), was also positive, for 5.0-mg quantities of I or II in 1.0 ml of water produced a milky suspension within 2 minutes upon the addition of 3 drops of a 5% solution of 5,5-dimethylcyclohexane-1,3-dione in ethanol.

Reducing properties. Both I and II rapidly reduced 1% potassium permanganate solution and Fehling's solution at room temperature.

Monohydroxyphenolic group. Five mg of I was added to 1.0 ml of Millon's reagent. After the latter was heated to boiling, a deep red color developed, as expected from a monohydroxy phenol having at least one ortho position open (14). The same color was obtained when 5.0 mg of *p*-hydroxyphenylacetic acid or synephrine was substituted for I.

Other identifying tests. Experiments were conducted to determine the colors produced

[3] All microanalytical determinations were performed by the Clark Microanalytical Laboratory, Urbana, Ill.

TABLE II

COLOR REACTIONS OF *p*-HYDROXYPHENYLACETALDEHYDE (I) AND 3-METHOXY-4-HYDROXYPHENYLACETALDEHYDE (II)[a]

Spray reagents	I	II
1. 2% aqueous ferric chloride	Pale violet	Light grey
2. 2% aqueous phosphomolybdic acid followed by dilute ammonia[b]	Bluish-grey	Bluish-grey
3. 2,4-dinitrophenylhydrazine saturated in 1 N hydrochloric acid	Yellow	Yellow
4. diazotized *p*-nitroaniline[c] followed by cold 20% potassium carbonate	Dark pink	Pale violet[d]
5. Schiff's reagent	Immediately blue on a colorless background, quickly becoming wine-purple on a pink background	Same as I
6. 1 % aqueous potassium permanganate	Immediately yellow on a brown background, becoming brown on purple background with continued spraying	Same as I
7. 0.4% *p*-nitrobenzenediazonium fluoroborate in 50% ethanol followed by dilute ammonia[b]	Yellow after fluoroborate, becoming intense yellow on a red background after dilute ammonia	Orange after fluoroborate, turning brown on a red background after dilute ammonia

[a] 5 μl of an ethanolic solution of I or of II (each at a concentration of 10 μg/μl) was applied as a spot ¼ inch in diameter to Whatman No. 1 filter paper. The spots were air dried and then sprayed.

[b] Concentrated aqueous ammonia (25%) diluted with an equal volume of water.

[c] 1 ml 5% sodium nitrite added to a solution made by diluting 5 ml of a stock solution of 0.2% *p*-nitroaniline in 1 N hydrochloric acid with 50 ml of 0.1 N hydrochloric acid.

[d] With 10 μl rather than 50 μl the color is light grey.

when I and II, applied to chromatography paper, were sprayed with several commonly employed detecting reagents. The results are presented in Table II.

Preparation of Derivatives

I-p-Nitrophenylhydrazone. Attempts to prepare this derivative with *p*-nitrophenylhydrazine dissolved in hydrochloric acid proved unsuccessful, since an apparently noncrystalline precipitate formed which rapidly turned black. A similar difficulty was experienced by Langheld (6). To avoid these difficulties, approximately 100 mg of I was mixed with a solution consisting of 150 mg of *p*-nitrophenylhydrazine dissolved in 24 ml of acetic acid. A yellow precipitate formed immediately which increased in amount when the mixture was heated gently. After several minutes the precipitate began to darken and become tan, but it became yellow again upon the addition of 2–3 volumes of distilled water. The mixture was allowed to stand for 10 minutes with occasional swirling. It was then filtered, yielding a gummy material which stuck to the filter paper. This material was washed with water, dissolved in warm ethanol, and filtered.

After the filtrate was diluted with water and cooled in ice, a tan precipitate was obtained which was composed of tiny needles which became bright yellow when washed on the funnel with cold ethanol. The yield was 90 mg, m.p.[4] 158.5–159.5° dec. (sintering from 155.0–158.5°) (lit. ref. (6), m.p. 158°).

Anal. I-*p*-Nitrophenylhydrazone calcd. for $C_{14}H_{13}O_5N_3$: C, 61.98; H, 4.84; N, 15.49%. Found (after heating *in vacuo* for 4 hours at 60°): C, 61.88; H, 5.00; N, 15.08%.

I- and II-Semicarbazones. The free aldehyde generated from 400 mg of the appropriate bisulfite addition compound was added to 20 ml of 50%

[4] All melting points were determined with a Fisher-Johns melting point apparatus and are corrected.

ethanol containing 1.0 gm of semicarbazide hydrochloride and 1.5 gm of sodium acetate. After heating the resulting solution at 70° for 8 minutes, 1–2 volumes of water were added, and the solution was cooled in the refrigerator. The precipitate which formed overnight was collected and washed with water.

I-Semicarbazone was recrystallized twice from ethanol-water to yield 114 mg of white crystals, m.p. 187.5–189° dec. (sublimation at 186°).

Anal. I-Semicarbazone calcd. for $C_9H_{11}O_2N_3$: C, 55.95; H, 5.74; N, 21.75%. Found (after drying to constant weight *in vacuo* at 80°): C, 56.10; H, 5.65; N, 21.67%.

II-Semicarbazone was recrystallized 3 times from methanol-water and twice from absolute methanol to yield 71 mg of hard, tan needles, m.p. 170.0–171.5° dec. (sublimation at 168.0°) (lit. ref. (7), m.p. 173°; (9), m.p. 173°, uncorrected).

Anal. II-Semicarbazone calcd. for $C_{10}H_{13}O_3N_3$: C, 53.80; H, 5.87; O, 21.50; N, 18.82%. Found: C, 53.72; H, 5.75; O, 20.93; N, 18.61%.

Chromatography

Apparatus and solvents. Descending chromatography was performed on Whatman No. 1 filter paper in cylindrical chromatogram jars 18 inches high and 6 inches in diameter. The six solvent systems used were those described by Reio (15) and were prepared and stored essentially as he suggests. Solvents A, B, C, and D consisted of the clear organic phase obtained after 1 hour of shaking the following: A, 1000 ml of methyl isobutyl ketone shaken with 100 ml of 3.6% formic acid; B, 1000 ml of chloroform shaken with a 200-ml solution of methanol:water: 90% formic acid (100:96:4); C, 100 ml of 1.8% formic acid shaken with a 1000-ml mixture of benzene:ethyl methyl ketone (900:100); D, 1000 ml of benzene shaken with 100 ml of 1.8% formic acid. Solvents E and F consisted of a mixture of the following: E, ethyl methyl ketone-water-diethylamine (921:77:2); F, ethyl methyl ketone:acetone: water:90% formic acid (80:4:12:2). Some solvent was placed in the bottom of the appropriate jar, and filter paper was extended upward from it several inches along the inside of the tank to keep the atmosphere saturated. A 50-ml beaker containing 2% formic acid was placed on the bottom of the jars containing solvent systems A, C, D and F,[5] and contained a filter paper wick which extended upward along the inside of the jar to aid in saturation of the atmosphere. The chromatograms were removed from the jars when the solvent had descended 25 cm from the origin, a migration taking approximately 2¼ hours. The temperature of the room was not controlled and varied somewhat from day to day. All chromatograms were air dried and sprayed within 15 minutes thereafter.

Chromatographic purity of I and II. The chromatographic properties and purity of I and II were investigated by chromatographing up to 125 and 250 μg quantities of I and II, respectively, in each of the six solvent systems (Fig. 1). In each chromatogram only one major component can be seen.

In order to determine whether this component was responsible for the reactions with the various spray reagents recorded in Table II, the following experiment was performed. Six chromatogram sheets were prepared, each containing separately placed applications of approximately 10 μg of the same aldehyde. Each sheet was then developed in one of the six solvent systems. From this quantity of either I or II only one spot could be detected after chromatography in any one of the six solvent systems, and the R_f of the spot found with any one of the detecting reagents was identical to that found with the others. All seven reagents in Table II were used to detect I, while only the reagents numbered 3–6 were used for II.

Schiff's reagent and diazotized *p*-nitroaniline (followed by 20 % potassium carbonate) were the spray reagents employed in an attempt to detect impurities in the chromatograms of the larger quantities of I and II developed in each of the six solvent systems. Only one trace impurity could be detected in I, and this was located by both detecting reagents on the chromatograms developed in solvent systems B and C in both of which it migrated ahead of I (Fig. 2). However, its concentration was apparently so low and its rate of color formation so slow that it was not possible to determine whether it gave the blue cast characteristic of aldehydes when sprayed with Schiff's reagent. The determination of the exact color was further complicated by the pink background coloration assumed by the chromatography paper within 20 seconds after spraying with the Schiff's reagent.

The chromatograms of II indicated the

[5] In the jar containing solvent system B the aqueous phase was used in place of the formic acid.

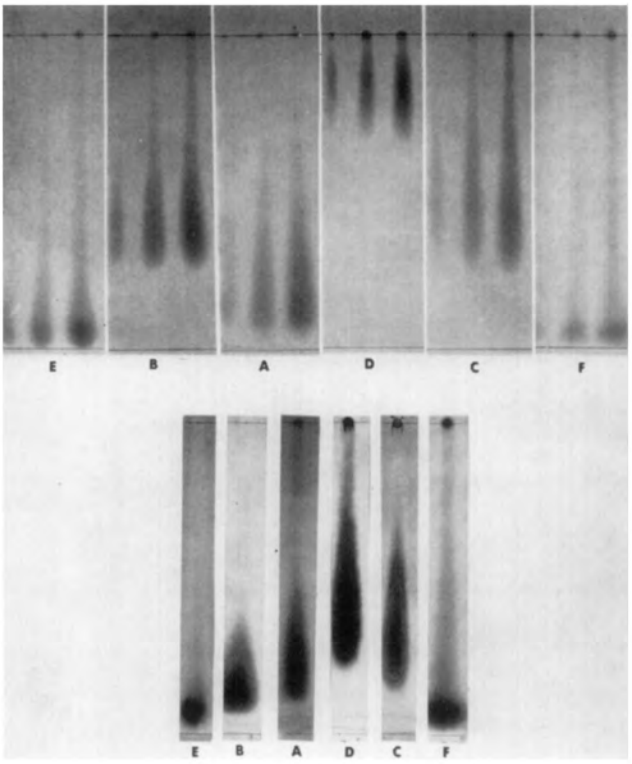


FIG. 1. Descending paper chromatography of I and II. *Upper plate:* Each chromatogram contained three different quantities of I (25, 50, and 125 μg) and was developed in the solvent system indicated by the appropriate letter. Detection of the spots was accomplished by spraying with diazotized *p*-nitroaniline followed by cold 20% potassium carbonate. For details and identification of solvent systems, see text. *Lower plate:* Chromatograms of 250-μg quantities of II developed in the indicated solvent systems and then sprayed with Schiff's reagent. The pencil lines just behind the solvent front indicate the location of a very faint purple band which develops upon spraying Whatman No. 1 paper with Schiff's reagent after the paper has been developed in any of the six solvent systems even if no samples have been applied to the paper. For details and identification of solvent systems, see text.

presence of at least one impurity. This impurity was seen at and just beneath the origin on the chromatograms developed in solvent systems C and D (see penciled markings there in Fig. 1, lower plate). It gave a blue color with Schiff's reagent and a purple color with the diazotized *p*-nitroaniline reagent. There was the suggestion of a second impurity on some chromatograms developed in solvent system B; it appeared to stain weakly with the diazotized *p*-nitroaniline but was not detected with Schiff's reagent.

Oxidation of I and II by Xanthine Oxidase

An ammonium sulfate suspension of a purified milk xanthine oxidase preparation[6] was diluted 1:40 with distilled water. One-half ml aliquots of the resulting enzyme solution were placed in small test tubes, and 1 ml of phosphate buffer (10^{-2} M, pH 7.5) containing 400 μg of I or II was added. Controls were run in which the enzyme solution was replaced either by distilled water or by enzyme solution which had been heated in boiling water for 3 minutes. In another control, substrate was absent. The tubes were incubated in a water bath at 37°C with occasional shaking. After 90 minutes the solutions were saturated with sodium chloride and extracted twice with 1.5-ml portions of ether. The aqueous phase was then acidified by

[6] Worthington Biochemical Corp., Freehold, New Jersey.

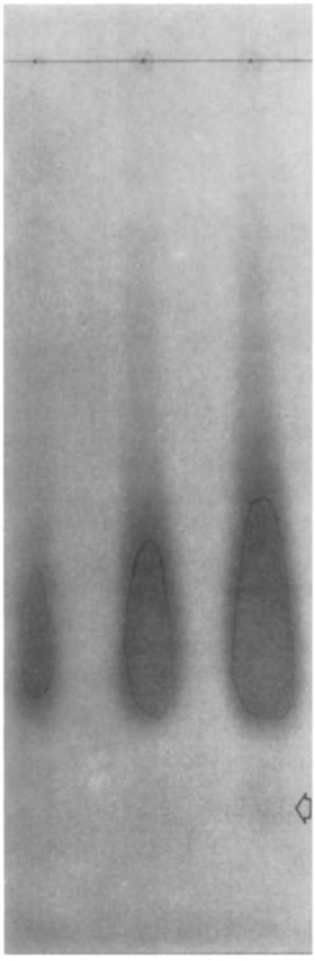


FIG. 2. Chromatogram of 25, 50, and 125 μg quantities of I developed in solvent system B and then sprayed with Schiff's reagent. A faint spot marked by the arrow is visible migrating ahead of the 125 μg quantity of I. The faint darkening along the solvent front is due to Schiff-positive material of the chromatography paper (see legend for lower plate of Fig. 1).

the addition of 4 drops of 1 N hydrochloric acid and extracted with 2 or 3 1.5-ml portions of ether. The extracts were evaporated almost to dryness under a stream of dry nitrogen and then chromatographed. Other incubations were conducted and extracted similarly but with appropriately larger volumes of all reagents.

p-Hydroxyphenylacetic acid (Spot 5, Fig. 3) was formed in incubations containing I and the enzyme and was extracted into ether only from the acidified incubation fluid. It was identified by paper chromatography in all six solvent systems in each of which it had an R_f identical to that of

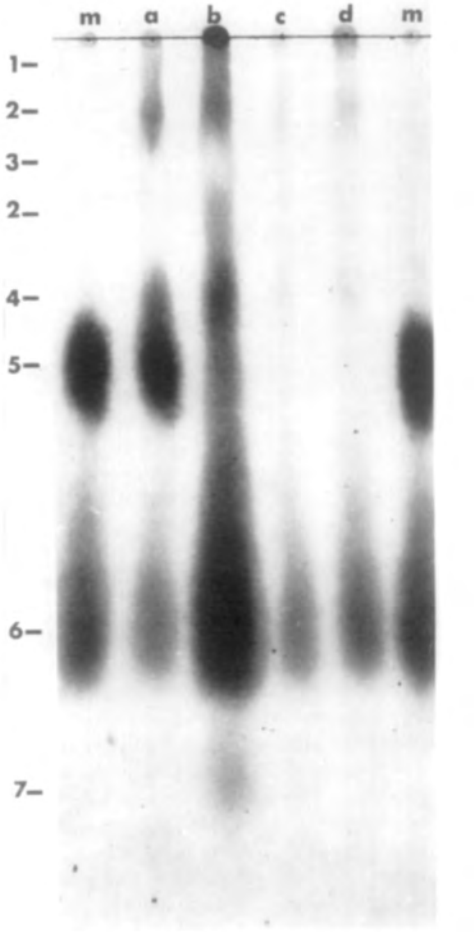


FIG. 3. Chromatogram showing the formation of *p*-hydroxyphenylacetic acid (Spot 5) from I (Spot 6) by xanthine oxidase. Whatman No. 1 paper; solvent system B; diazotized *p*-nitroaniline spray followed by 20% potassium carbonate. m, 2 μl of an ethanolic solution of I (approximately 5 μg/μl) and *p*-hydroxyphenylacetic acid (2.5 μg/μl) as reference compounds; a and b, ether extracts from an incubation fluid containing I and xanthine oxidase obtained when fluid was basic (b) and after its acidification (a); c, ether extract from an acidified incubation fluid containing boiled enzyme; d, ether extract from an acidified incubation fluid containing no enzyme. In the cases of c and d, the ether extractions obtained while the fluids were basic, prior to their acidification, were discarded. See text for further details.

authentic acid. It gave a blue color similar to that of the authentic acid when sprayed with diazotized *p*-nitroaniline followed by 20% potassium carbonate. No *p*-hydroxyphenylacetic acid was detected in incubations containing boiled enzyme or in incubations lacking either I or enzyme.

From Fig. 3 it can be seen that at least four new compounds (Spots 1–4) were detected in incubations containing I. These unidentified compounds migrated behind *p*-hydroxyphenylacetic acid, were extracted into ether largely from the basic incubation fluid, and did not appear if I was omitted from the incubation fluid. Their formation does not require enzyme, for they are present in incubations containing boiled or no enzyme. The spot migrating just ahead of I (Spot 7, Fig. 3) is apparently the trace contaminant previously noted to be present in samples of I (Fig. 2).

The incubations of II with enzyme formed 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid), which, together with an unknown compound, was extracted into ether only from the acidified incubation fluid. The homovanillic acid was formed enzymically and was identified by means of its R_f in all six solvent systems and by the grey color it formed when sprayed with diazotized *p*-nitroaniline followed by 20% potassium carbonate. The unknown compound gave a purple color when sprayed and was not detected in the absence of active enzyme. Both homovanillic acid and the unknown compound had almost identical R_f's in solvent systems A, D, E, and F but were clearly separated in solvent systems B and C. Two unidentified components were found in the basic ether extracts from all incubations containing II even in the absence of enzyme or in the presence of boiled enzyme.

DISCUSSION

In the present experiments, I and II have been obtained in a highly purified state and have been stored for several months as their bisulfite addition salts which have shown no evidence of deterioration. Consequently, no attempt has been made to obtain I or II in crystalline form. It should be noted, however, that Harries (8), who synthesized II by the ozonolysis of eugenol, obtained crystals of II with a sharp melting point but only with great difficulty and after repeated fractional distillations. Challis and Clemo (9) did not crystallize II which they obtained by ozonolysis of *O*-carbethoxyeugenol. Langheld (6), who synthesized I by treating tyrosine with sodium hypochlorite, reported obtaining crystals of I but did not analyze the crystals or determine their melting point.

The preparation of the crystalline bisulfite addition compounds of I and II has made it possible to purify these aldehydes from most organic impurities. Harries and Haarmann (7) reported that II formed a bisulfite addition compound but that the chemical analysis of this compound did not agree with the calculated values. In the present experiments the carbon content of I-$NaHSO_3$ and of II-$NaHSO_3$ was considerably below the calculated theoretical values. However, this discrepancy was expected because it is not possible to prevent contamination of the addition compounds with sodium bisulfite when they are collected by filtration from the saturated sodium bisulfite solution in which they are formed. During this filtration the filter cake of the addition compound is wet with the saturated sodium bisulfite solution and becomes contaminated with sodium bisulfite which is precipitated onto the cake when it is washed with alcohol. However, when the aldehydes are to be used in biochemical and other studies, they are generated from their bisulfite addition compounds in aqueous solution and are then extracted into ether. During this process, salt impurities remain in the aqueous phase, as indicated by results of the microanalysis of the free aldehydes. The values which were found closely approached the theoretical values calculated for the pure aldehydes.

It is of interest that while I and II rapidly formed crystalline bisulfite addition compounds, the aldehyde obtained by treating epinephrine with hot acid failed to form a crystalline bisulfite addition compound on the two attempts made in this laboratory. The aldehyde used, presumably 3,4 - dihydroxyphenylacetaldehyde (see Table I), had been shown by Fellman (11)

to be formed by treating epinephrine with hot acid. He did not mention any attempt to form this aldehyde's bisulfite addition compound. The failure so far to obtain a crystalline bisulfite addition product from what appears to be 3,4-dihydroxyphenylacetaldehyde is being investigated. Apparently, substituting the hydroxyl group on the third carbon atom of the aromatic ring with either a hydrogen atom (as in I) or a methoxy group (as in II) permits formation of stable, crystalline bisulfite addition products (see Table I).

At least one trace impurity was noted in the chromatographed samples of I and II. It is possible that other impurities were present which failed to be separated adequately from the main component despite the use of the six different solvent systems. Such impurities would be difficult, if not impossible, to detect because of the tendency of I and II to produce trailing during their chromatography on paper particularly when large quantities are chromatographed.

It is not known whether the impurities found were present originally as crystalline bisulfite addition compounds or whether they were formed during the generation of the free aldehydes from their addition compounds in the presence of 10% sodium carbonate in which the aldehydes are unstable. If the latter is the case, it may be possible to generate the aldehydes without formation of these impurities by using a less alkaline buffer and by extracting the generated aldehyde immediately into the ether phase. If the impurities were present as crystalline bisulfite addition compounds which may have formed, for example, from a contaminating aldehyde or a methyl ketone, fractional crystallization of the bisulfite addition compounds from various concentrations of alcohol may be a useful purification procedure.

ACKNOWLEDGMENTS

This work was carried out at the suggestion and under the direction of Dr. H. Weil-Malherbe to whom I wish to express my gratitude. I am also indebted to Drs. D. D. Clarke, L. Ornstein and H. Posner for their advice and encouragement throughout.

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