


AN ABSTRACT OF THE THESIS OF

James Simpson Smyth for the M.S. in Chemistry

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Abstract Approved: 

(Major Professor)

Homogentisic acid (2,5-dihydroxyphenyl acetic acid, hydroquinone acetic acid) was desired for certain biochemical studies. A search of the literature revealed no mention of the subsequent verification or use of any of the five published methods of synthesis of the acid. Two were believed worthy of experimental investigation. The method of Osborne, a Friedel-Crafts alkylation of p-dimethoxy benzene with chloroacetic ester, followed by saponification and demethylation, was tried without success. The method of Hahn and Stenner, in which monobenzoyl hydroquinone allyl ether is rearranged to monobenzoyl allyl hydroquinone and further benzoylated to dibenzoyl allyl hydroquinone then ozonized to dibenzoyl homogentisic acid, was carried out. The over all yield, from hydroquinone, was 8.5 per cent.

Homogentisic acid was obtained by alkaline hydrolysis of the dibenzoyl derivative as crystals which melted sharply in the expected range but were discolored by traces of quinone compounds. All attempts to produce a colorless solid failed but it was found possible to decolorize water solutions by reducing with zinc and hydrochloric acid. The solutions regained color as soon as the reducing reagents were removed.

AN INVESTIGATION OF THE SYNTHESIS
OF HOMOGENITISIC ACID

by

JAMES SIMPSON SMYTH

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APPROVED:

[REDACTED]

Professor of Chemistry

[REDACTED]

Professor of Chemistry

In Charge of Major

[REDACTED]

Head of the Department of Chemistry

[REDACTED]

Chairman of School Graduate Committee

[REDACTED]

Chairman of State College Graduate Council

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AN INVESTIGATION OF THE SYNTHESIS
OF HOMOGENTISIC ACID

INTRODUCTION

A quantity of homogentisic acid (2,5-dihydroxyphenylacetic acid, hydroquinone acetic acid) was desired for certain biochemical studies in metabolism.

Preliminary studies indicated that investigators have obtained homogentisic acid from the urine of alcaptonuric patients in preference to synthesizing it. In fact, no report was found in which any of the published methods of synthesis had been confirmed or utilized by any one other than the original authors. Since alcaptonic urine was not readily available, an investigation of the synthesis was undertaken.

HISTORICAL

Homogentisic acid was first isolated from pathological urine by Marshall (2) in 1887, and named glycosuric acid. Walkow and Baumann (3) subsequently renamed it homogentisic acid, determined its chemical characteristics, and suggested the structural formula which was confirmed in 1884 by the synthesis of Baumann and Franckel (4) in which gentisic aldehyde was converted successively to dimethylgentisic aldehyde, -alcohol, -chloride, -nitrile, dimethylhomogentisic acid, homogentisic acid. The nitrile was very unstable and only enough homogentisic acid was obtained to establish its identity.

Osborne (5) in 1903, employed the Friedel-Crafts method to react chloroacetic ester with p-dimethoxybenzene, followed by saponification of the ester and demethylation with Hydrogen Iodide. He reported a yield of less than one per cent.

Neubauer and Flatow (6) in 1907, synthesized homogentisic acid through the following steps: isatin, isatic acid (2-aminophenylglyoxalic acid), 2-hydroxyphenylglyoxalic acid, 2,5-dihydroxyphenyl glycollic acid, homogentisic acid. Yields were not reported.

Hahn and Stenner (7) in 1929, utilized the steps: hydroquinone, monobenzoyl hydroquinone, monobenzoyl hydroquinone allyl ether, monobenzoyl allylhydroquinone, dibenzoyl allyl hydroquinone, dibenzoyl homogentisic acid, homogentisic acid. They reported an over all yield of 26 per cent.

NATURE OF THE INVESTIGATION

On the basis of their own reports the syntheses of Baumann and Franckel (4) and of Neubauer and Flatow (6) were not considered to be of preparative value.

Although Osborne (5) had reported a very low yield it was thought that his method might prove useful since it is known that the methoxy group favors alkylation of the benzene nucleus by the Friedel-Crafts reaction (8). Several attempts to utilize this method failed and attention was directed to the method of Hahn and Sterner (7).

TRIAL OF OXBORNE'S METHOD

Four attempts were made to alkylate p-dimethoxybenzene with chloroacetic ester, using equimolecular amounts of the reactants and a 50 per cent excess of aluminum chloride with carbon disulfide as solvent. The reaction mixtures were refluxed from two to five days. In each case a mixture of resinous tars resulted and the only pure organic material isolated was p-dimethoxybenzene, of which approximately one half the amount used was recovered from each of two batches. Red, brown, green, and black solutions, characteristic of the quinone derivatives, were obtained in attempts to separate the tars, leading to the belief that appreciable demethylation took place during the heating.

TRIAL OF HAHN AND STENNER'S METHOD

MONOBENZOYL HYDROQUINONE. 80 grams (0.73 moles) of hydroquinone and 160 grams of sodium carbonate were dissolved in 2.4 liters of cold water in a 3 liter flask and 105 grams (0.75 moles) of benzoyl chloride added dropwise over a period of 5 hours, with rapid stirring to minimize formation of the dibenzoyl derivative. The mixture was stirred an additional hour and the solids collected by suction filtration, washed with sodium carbonate solution and water, dried in a vacuum oven and recrystallized from methanol. Results are summarized in table I.

TABLE I MONOBENZOYL HYDROQUINONE

Batch number	Grams solid products	Grams monobenzoyl hydroquinone	Melting point	Per cent yield
1	135	83.5	164-5	54
2	170	*		
3	156	*		
4	178	*		
5	136	*		
6	161	*		
2-6		501	164-6	64
Reported by Hahn and Sterner			162-3	68

*Crude products combined for purification.

MONOBENZOYL HYDROQUINONE ALLYL ETHER. Monobenzoyl hydroquinone, allyl bromide, and anhydrous potassium carbonate in molar ratios of 1.0, 1.15, and 1.17 respectively, were mixed with acetone and shaken in the cold. Glass beads were used to prevent formation of large aggregates of solids in the mixture. The reaction mixture was poured into ten volumes of cold water made slightly alkaline with sodium carbonate and allowed to stand several hours. The solids were removed by suction, dried in the vacuum oven at a temperature below 50 degrees, and recrystallized from high boiling petroleum ether or Stoddard's solvent. Conditions and results are summarized in table II. Higher yields obtained with the last four batches are believed due to special purification and drying of the reagents.

Several attempts to substitute allyl chloride for allyl bromide failed. The reaction was tried at the reflux temperatures of acetone and of dioxane but only traces of the ether were obtained and the major portion of the monobenzoyl hydroquinone was recovered in all cases.

TABLE II MONOBENZOYL HYDROQUINONE ALLYL ETHER

Batch number	Grams monobenzoyl hydroquinone	Hours shaken	Crude products		Recrystallized allyl ether		Per cent yield
			grams	M.P.	grams	M.P.	
1	20	24	20	60-3	*		
2	20	24	21	62-4	*		
3	20	24	19	61-5	*		
4	20	24	20	61-5	*		
5	20	24	22	61-5	*		
6	20	24	23	61-5	*		
1-6					73.3	63.5	34
7	60	24	41		*		
8	120	40	130		*		
7-8					93	62.4	43
9	120	38	*				
10	80	48	*				
9-10			192		157	66-8	44
	Reported by Hahn and Stenner		-		-	71.2	70
	*Crude products combined for purification						

DIBENZOYL ALLYL HYDROQUINONE. Monobenzoyl hydroquinone allyl ether was caused to undergo the Claisen rearrangement by heating without solvent to its reflux temperature at reduced pressure. The apparatus used consisted of a 200 cc. retort connected by means of a ground glass joint to a 125 cc. distilling flask. A manometer, trap, and water pump were connected to the side arm of the distilling flask. The retort was partially immersed in a metal bath of lead, tin, antimony, and bismuth, having a melting point of about 190 degrees, and so arranged that the apparatus could be changed from reflux to distillation position without cooling or changing pressure. Glass wool in the retort was found helpful in reducing spill-over from excessive bumping. After refluxing several hours the material was distilled, transferred to a flask and mixed with a little ether to lower the viscosity and allow crystallization.

A disproportionation of the benzoyl groups gave a product consisting of a mixture of allyl hydroquinone, monobenzoyl allyl hydroquinone, and dibenzoyl allyl hydroquinone. The dibenzoyl product partially crystallized out and was separated by suction and washed with a little ether. The ether filtrate was shaken with one normal sodium hydroxide to remove the phenolic compounds, dried over anhydrous sodium sulfate, and the ether evaporated to recover the remainder of the dibenzoyl product. The alkali solution was immediately acidified with hydrochloric acid and extracted with ether and the ether extract dried over sodium sulfate and evaporated. The residue, consisting of allyl hydroquinone and monobenzoyl allyl

hydroquinone, was dissolved in pyridine, transferred to a flask fitted with a reflux condenser, and a calculated excess of benzoyl chloride added. The mixture was refluxed for 15 minutes, cooled and poured into a large volume of warm water.. The water was decanted and renewed several times and the last volume allowed to cool and stand for several hours. Solids were removed by suction, dried in warm air or the vacuum oven at low temperature, and recrystallized from methanol. The total weight of dibenzoyl allyl hydroquinone obtained by disproportionation and by rebenzoylation is reported as yield for the rearrangement.

Conditions and results are summarized in table III.

Temperatures reported are those of the metal bath.

It is evident that the rearrangement was not complete in batches 4 - 8 since the large amounts of material melting at 65 - 70 degrees represent unchanged monobenzoyl hydroquinone allyl ether.

TABLE III DIBENZOYL ALLYL HYDROQUINONE

Batch number	Grams monobenzoyl hydroquinone allyl ether	Hours heated	Pressure mm. Hg.	Bath temp.	Grams distillate	Grams dibenzoyl allyl hydroquinone by		Grams total	Per cent yield
						Disproportionation M.P.	Rebenzoylation M.P.		
1	33	2	8-10	160-280	27.2	2 104-5	2 104-5	4	8.6
2	34	2	8	220-280	24.5	*			
3	34	2	24	220-280	25.3	*			
2-3						10 107	9.5 105-6	19.5	21.7
4	50	2.5	10	240-280	38	1.6 69-70			
5	50	3	10	240-280	34	4.1 69-70			
6	50	3	10	240-280	28	*			
7	50	3.3	10	240-280	41	*			
8	80	4	10	240-280	65	*			
4-8						65 65-8	20 104-6	20	5.1
9	50	4	10-70	240-330	40	1.0 104-6	9.2 106-7	10.2	14.5
10	31	5	37-55	240-300	28	2.0 106-7	17.6 106-7	19.6	45
						107-8	107-8	82	

Reported by Hahn and Stenner

*Crude products combined for purification

DIBENZOYL HOMOGENTISIC ACID. Ozone was passed through a solution of dibenzoyl allyl hydroquinone in glacial acetic acid and the ozonide decomposed by adding water to the acid solution and boiling, resulted in dibenzoyl homogentisic acid.

An ozone generator was constructed as an adaptation of the apparatus described by Smith (9), Henne (10), and Whitmore (11). Three ozone tubes, constructed according to Henne's specifications, were large jacketed test tubes 50 cm. in length, the inner and outer walls sealed together at the open end and the inner tube terminating in a throat of smaller diameter projecting 10 cm. from the seal. The outer jacket was 4 cm. in diameter with a spacing of 6-7 mm. between the walls. The three tubes were mounted by means of clamps in a vertical position side by side on a single large ring stand and immersed, throat upward, to a depth of about 52 cm. in a large water bath made by removing one head from a 40 gal. steel barrel. The jackets of the three tubes were connected, the lower end of one to the upper end of the next, by means of sealed-in glass delivery tubes fitted with ground glass connections. Lead wires dipping into the sulfuric acid served as one electrode while the steel barrel was used as the other. A potential of 10,000 volts was maintained with a transformer of the type used in neon signs. Oxygen, dried by passing through sulfuric acid and phosphorous pentoxide towers, was passed through the outer jackets of the three tubes, entering at the upper end of the first and leaving at the lower end of the third through a glass delivery tube rising above the top of the bath. The delivery tube was fitted with a three-way stopcock to permit the gases to

either be passed into an ozonizing vessel or exhausted through an ozone destroying tube which consisted of a heavy pyrex glass tube 20 cm. long packed with manganese dioxide and heated to a dull red with a bunsen flame. Ozone decomposes rubber almost instantly so that all connections were made with ground glass joints, gummed paper wrapping, or mercury seals. The latter are advantageous in allowing flexibility and ease of changing. The paper seals are satisfactory for holding the gas but are difficult to remove. A stopcock was placed at the oxygen intake of the first ozone tube to protect the rubber oxygen delivery tube from ozone which would otherwise diffuse back while the generator was not in operation.

The performance of the ozone generator was determined by the method of Smith (9), passing a gas sample through a solution of sodium iodide which was then acidified and the liberated iodine titrated with standard thiosulfate. The volume of the exhausted oxygen was measured through a wet-test meter and the time recorded by a stop watch. The ozone required to liberate the iodine was calculated and reported as per cent ozone by volume. The results are summarized in table IV.

It may be noted that the concentration of ozone remained approximately constant within limits of error of the analysis while the rate of flow was varied from 4 to 40 liters per hour.

Dibenzoyl allyl hydroquinone was dissolved in glacial acetic acid in the ratio of one gram per 16 cc. The solution, contained in a distilling flask, was held at 80 degrees centigrade while ozone-oxygen was passed at a rate of about ten liters per hour. After ozonization

the solution was transferred to a large evaporating dish, water added, and the mixture evaporated to dryness, repeating to the absence of an odor of acetic acid. The dry residue was ground with a little ether and filtered and the solid reported as dibenzoyl homogentisic acid. Results are summarized in table V.

The crude dibenzoyl homogentisic acid was recrystallized from absolute ethanol. Results are not included in the table, since the product from several ozonizations were combined in each case.

The ether filtrate, containing a mixture of dibenzoyl homogentisic, monobenzoyl homogentisic, homogentisic, and benzoic acids, was evaporated and the residue treated separately to recover the homogentisic acid.

TABLE IV PERFORMANCE OF THE OZONIZER

Run	Seconds time	cc. Thio. 0.0890 N.	Moles ozone	Liters gas per hour	Per cent ozone
1	1020	37.0	0.00165	4.2	3.2
2	1020	35.5	0.00158	4.2	2.9
3	720	35.0	0.00160	6.2	2.9
4	430	35.3	0.00157	10.4	2.9
5	435	35.7	0.00159	10.4	2.9
6	270	40.3	0.00178	16.7	3.2
7	272	39.0	0.00174	16.7	3.2
8	120	36.7	0.00163	38.6	3.0
9	108	33.3	0.00149	41.3	2.7

Sample volume, 0.05 cubic feet, 0.055 moles at S.T.P.

TABLE V DIBENZOYL HOMOGENTISIC ACID

Batch number	Grams dibenzoyl allyl hydroquinone	Hours of ozonization	Dibenzoyl homogentisic acid grams	M.P.	Grams solids in filtrate
1	5	4	1.4	170-7	2.6
2	10	22	2.9	170-4	4.7
3	10	8.5	3.9	168-75	4.9
4	7.5	3.5	1.1	166-74	4.4
5	10	11	3.6	162-76	4.1
6	7.4	15	3.6	155-70	2.3

HOMOGENENTISIC ACID. Cold two normal sodium hydroxide was mixed with dibenzoyl homogentisic acid in a ratio of 10 cc. per gram in a distilling flask under nitrogen. The mixture was heated in a boiling water bath for 10-15 minutes longer than the time required to effect solution, then cooled in an ice bath and made acid with concentrated hydrochloric acid. The precipitated benzoic acid was removed by suction filtration and the filtrate extracted with ether. The ether extract was dried over sodium sulfate, concentrated to small volume and about twice the volume of chloroform added. The precipitated homogentisic acid was filtered and dried.

DISCUSSION

The yields of monobenzoyl hydroquinone were satisfactory. Monobenzoyl hydroquinone allyl ether was made in useful quantity from allyl bromide but conditions were not found in which allyl chloride could be utilized. The Claisen rearrangement was favored by higher reaction temperatures and longer time of reflux but the gain was partially offset by formation of a larger proportion of tar which remained in the retort after distillation. The use of ozone as an oxidizing reagent is particularly advantageous since it introduces no foreign material and destroys the traces of quinone derivatives which are inevitably present as colored impurities throughout the series of reactions.

The yield obtained in the ozonization is difficult to estimate accurately since a mixture of dibenzoyl homogentisic acid, monobenzoyl homogentisic acid, benzoic acid, and unreacted dibenzoyl allyl hydroquinone were present in the ether filtrate and were subjected, without separating, to the hydrolysis reaction for the recovery of the homogentisic acid. By disregarding entirely the material in the filtrate a yield of 51 per cent was found (Batch number 6, table V) while by assuming that half of the solids present in the filtrate were dibenzoyl homogentisic acid the best yield would be 67 per cent. Hahn and Stemmer reported 83 per cent.

The over all yield from hydroquinone to dibenzoyl homogentisic acid was 8.5 per cent.

SUMMARY

Homogentisic acid was desired for biochemical purposes. Five methods of synthesis have been reported in the literature, but no mention of the verification or subsequent use of any of them was found (4, 5, 6, 7, 12). The Friedel-Crafts alkylation of p-dimethoxybenzene with chloroacetic ester, reported by Osborne (5), was tried without success. The method of Hahn and Stenner (7), involving a Claisen rearrangement of monobenzoyl hydroquinone allyl ether and benzoylation of the products to dibenzoyl allyl hydroquinone which was ozonized to dibenzoyl homogentisic acid, was verified experimentally with an over all yield of 8.5 per cent from the hydroquinone. Homogentisic acid was produced satisfactorily by alkaline hydrolysis of the dibenzoyl derivative but attempts to obtain a perfectly colorless solid were unsuccessful.

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