

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, SYRACUSE UNIVERSITY]

Citrinin Studies. Hydroxy- and Methoxy-phenylglyoxylic Acids¹BY R. D. SPRENGER,² P. M. RUOFF AND A. H. FRAZER²

In the course of investigations³ into the chemistry of citrinin our interest centered at the time on 2,4-dihydroxy-3-ethyl-5-methylphenylglyoxylic acid (I) as a possible intermediate⁴ in the synthesis of the structure originally proposed by Coyne, Raistrick and Robinson.⁴ The strong dibasic properties of I prompted us to prepare a number of other hydroxy- and methoxyphenyl-

benzene by steam distillation from a strongly acid solution. In general the method is simple, employs readily available materials and gives good yields.⁶ Essentially, our procedure is a modification of the method described by Bouveault,⁷ Kindler, *et al.*,⁸ and others⁹ for the preparation of arylglyoxylic acids.

Table I summarizes our results.

TABLE I
PREPARATION OF HYDROXY- AND METHOXY-PHENYLGLYOXYLIC ACIDS^a, RCOCOOH

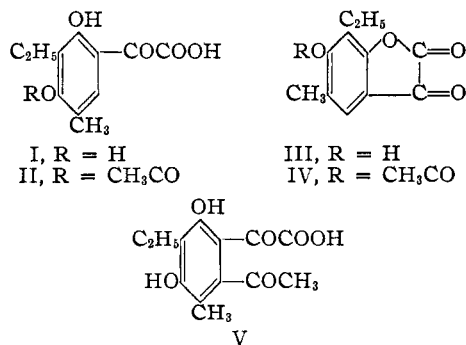
Reactant	R	Yield, %	M. p., °C.	Crystallization solvent
Phenol	4-Hydroxyphenyl	20	177.5–178 ^b	Ether–benzene–ligroin
Anisole	4-Methoxyphenyl ^c	80	93	Benzene
<i>p</i> -Cresyl methyl ether ^d	2-Hydroxy-5-methylphenyl ^{e,f}	60	107	Benzene
<i>p</i> -Cresyl methyl ether	2-Methoxy 5-methylphenyl ^g	70	116.5–117	Benzene
Resorcinol	2,4-Dihydroxyphenyl	72 ^h	167.5–168	Water
Orcinol	Lactone of 2,4-dihydroxy-6-methylphenyl	60 ⁱ	211–212	Ether–alcohol
2-Ethyl-4-methylresorcinol ^j	2,4-Dihydroxy-3-ethyl-5-methylphenyl (I)	70 ^k	137–138 ^l	Water

^a The 3-hydroxy- and 3-methoxy-phenylglyoxylic acids (previously unreported) required in this work were prepared from 3-hydroxyacetophenone as described in the experimental part. 2-Hydroxyphenylglyoxylic acid was prepared from isatin (Reichstein, *et al.*, *Helv. chim. acta*, **20**, 883 (1937)). ^b Fromherz, *Z. physiol. Chem.*, **70**, 351 (1911). ^c Bouveault, ref. 7, isolated intermediate ester. ^d Condensation with *p*-cresol failed. ^e Demethylation followed condensation upon heating reaction mixture for two hours at 85°. ^f Previously prepared by Fries and Finch, *Ber.*, **41**, 4282 (1908), by hydrolysis of 5-methyl-2-oximino-3-coumaranone. ^g New compound. *Anal.* Calcd. for C₁₀H₁₀O₄: C, 61.84; H, 5.19. Found: C, 61.55; H, 5.07. ^h Yield unreported using cyanoformate (refs. 6, 10). ⁱ Yield 53% using cyanoformate (ref. 6). ^j See ref. 3, footnote 3. ^k Yield 15% using cyanoformate (ref. 6). ^l M. p. 128–129.5° reported by Hunsberger and Amstutz (ref. 6).

glyoxylic acids in order to determine the effect of the position relative to the carbonyl group of the hydroxyl and methoxyl group on the physical properties of these acids.⁵

In the preparation of these acids we found that ethyl oxalyl chloride could be condensed satisfactorily with free phenols in the presence of aluminum chloride as well as with their corresponding methyl ethers. Furthermore, we found that it was not necessary to isolate the intermediate ethyl esters since they are easily hydrolyzed to the free acids during the removal of the nitro-

Compound I, as prepared by us, is of further interest. The position of the entering keto-carboxyl group (–COCOOH) *ortho* and *para*



(1) Taken from the theses of R. D. Sprenger and A. H. Frazer submitted in partial fulfillment of the requirements for the Ph.D. degree, June, 1946, and September, 1947, respectively. Presented in part before the Division of Organic Chemistry at the San Francisco meeting of the American Chemical Society, March, 1949.

(2) Eaton Foundation Fellow.

(3) Sprenger and Ruoff, *J. Org. Chem.*, **11**, 189 (1946).

(4) Nuclear acylation of this compound followed by reduction to the dicarbinol, ring closure to the phthalan followed by oxidation, might conceivably lead to the old citrinin structure. The original structure of citrinin proposed by Coyne, Raistrick and Robinson, *Phil. Trans. Roy. Soc.*, **220**, 297 (1931), has been abandoned. For revised structures see, for instance, Cram, *THIS JOURNAL*, **70**, 4244 (1948); Robertson, *et al.*, *Nature*, **162**, 72 (1948). Robertson and co-workers, *J. Chem. Soc.*, 1563 (1949), have now reported in detail their synthesis of citrinin.

(5) The results of these studies will be forthcoming in another paper. Of particular interest were the dissociation constants of the acids and their behavior at the dropping mercury electrode. Absorption spectra studies were contemplated but were not included at that time.

to the phenolic groups is established by the fact that the lactone III was formed readily when I was kept at 120° for several hours. It should be

(6) Since this work was completed Hunsberger and Amstutz, *THIS JOURNAL*, **70**, 671 (1948), reported the preparation of a series of substituted 2,4-dihydroxyphenylglyoxylic acids by condensation of alkylresorcinols with ethyl cyanoformate in the presence of anhydrous hydrogen chloride.

(7) Bouveault, *Bull. soc. chim.*, [3] **15**, 1014 (1896); *ibid.*, **17**, 363 (1897).

(8) Kindler, *et al.*, *Ber.*, **76**, 308 (1943).

(9) See, for instance, Blicke and Geier, *THIS JOURNAL*, **65**, 1725 (1943), and Thomas, "Anhydrous Aluminum Chloride in Organic Chemistry," Reinhold Publishing Corp., New York, 1941, p. 252.

noted that our preparation of I formed a 2,4-dinitrophenylhydrazone in the conventional manner.¹⁰

Reaction of the phenylglyoxylic acid (I) with an excess of acetyl chloride in the presence of aluminum chloride with tetrachloroethane as a solvent gave a compound whose analysis corresponded to the expected 6-acetyl-2,4-dihydroxy-3-ethyl-5-methylphenylglyoxylic acid (V). Its chemical behavior,¹¹ however, indicated that it was not a nuclear acetyl derivative but a mono acetoxy compound. This was confirmed when the same compound was obtained by direct esterification of (I) with acetic anhydride. Several other attempts to acetylate the ring under different conditions were unsuccessful.

In establishing which hydroxy group was acetylated it was found that a lactone (IV) was formed when the acetyl derivative was kept for several hours at 110°. Since a lactone could form only if the *ortho* hydroxy group were free, and since no rearrangement seems likely under the conditions used, the acetylated phenylglyoxylic acid should have structure II. Hydrolysis of III and IV gave I in each case.

Testing of these compounds for bacteriostatic activity¹² against a variety of organisms showed that they may be classed as virtually inactive. No significant difference was noted with respect to the position relative to the carbonyl group of the hydroxyl group.¹³

Experimental

2,4-Dihydroxy-3-ethyl-5-methylphenylglyoxylic Acid (I).—The preparation of this acid illustrates the general procedure for all hydroxyphenylglyoxylic acids except 2-hydroxy-5-methylphenylglyoxylic acid. In that case, the reaction mixture was heated on a water-bath and kept at 85° for two hours before treating with hydrochloric acid and crushed ice in the usual manner. In the preparation of the methoxyphenylglyoxylic acids one and one-half moles of aluminum chloride per mole of reactant was used.

To a solution of 6 g. (0.04 mole) of 2-ethyl-4-methyl-resorcinol³ (m. p. 100–101°) and 5.4 g. (0.04 mole) of ethyl oxalyl chloride⁸ in 50 ml. of nitrobenzene cooled to 0°, 10.5 g. (0.08 mole) of anhydrous aluminum chloride was added gradually with stirring. After addition of the aluminum chloride was completed the mixture was allowed to stand for twenty-four hours at room temperature under protection of a drying tube. At the end of this period the red complex was decomposed in the usual manner with crushed ice and hydrochloric acid. The nitrobenzene was removed by steam distillation and the cooled

residue¹⁴ extracted with ether. The ether solution was washed three times with one-half its volume of saturated sodium bicarbonate solution and the combined washings acidified with dilute hydrochloric acid to give the free acid. Recrystallization from water gave yellow plates melting at 137–138°; yield, 6 g. (70%). *Anal.* Calcd. for C₁₁H₁₂O₅: mol. wt. 224; C, 58.93; H, 5.38. Found: neut. equiv. 225, 228; C, 58.77; H, 5.68.

Hunsberger and Amstutz⁸ obtained a 15% yield of a light orange product, m. p. 128–129.5°.

Lactone of 2,4-Dihydroxy-3-ethyl-5-methylphenylglyoxylic Acid (III).—The acid, m. p. 137–138°, when heated at 120° for six hours, partially sublimed and changed to an orange solid with a m. p. of 188–189°. Three determinations of the neutral equivalent gave a value of 210¹⁵; calculated for C₁₁H₁₀O₄ is 206. An alkaline solution of this compound on acidification gave the original acid melting at 137–138°. *Anal.* Calcd. for C₁₁H₁₀O₄: C, 64.06; H, 4.89. Found: C, 64.16; H, 4.98.

2,4-Dihydroxy-3-ethyl-5-methylphenylglyoxylic Acid 2,4-Dinitrophenylhydrazone.—To 25 ml. of a saturated methanol solution of 2,4-dinitrophenylhydrazone was added 100 mg. of 2,4-dihydroxy-3-ethyl-5-methylphenylglyoxylic acid. The solution was brought to boiling, a drop of hydrochloric acid added and the boiling resumed for one to two minutes. Water was added until a faint turbidity was produced and the solution cooled. The precipitate was recrystallized from 75% alcohol giving red needles melting at 264–265° with decomposition. *Anal.* Calcd. for C₁₇H₁₆O₈N₄: C, 50.51; H, 4.00. Found: C, 50.23; H, 4.29.

4-Acetoxy-3-ethyl-2-hydroxy-5-methylphenylglyoxylic Acid (II).—Two grams (0.013 mole) of 2,4-dihydroxy-3-methyl-5-methylphenylglyoxylic acid, 50 ml. of tetrachloroethane, and 4.5 g. (0.06 mole) of acetyl chloride were placed in a 200 ml. round-bottomed flask and the mixture cooled to 0°. Ten and one-half grams (0.08 mole) of anhydrous aluminum chloride was gradually added to the cooled solution and the mixture allowed to warm to room temperature. After fifty-two hours the deep red complex was decomposed with ice and hydrochloric acid. The ether extract was washed twice with an equal volume of 10% sodium bicarbonate. Upon careful acidification an oil separated which solidified after standing for twenty-four hours in the refrigerator. Recrystallization from benzene gave approximately 0.3 g. of yellow needles, m. p. 117–118°. A mixture with the original acid melted at 90–95°. *Anal.* Calcd. for C₁₃H₁₄O₆: C, 58.64; H, 5.30. Found: C, 58.40; H, 5.69.

The *p*-nitrophenylhydrazone was prepared and crystallized in the usual manner from glacial acetic acid giving orange needles, m. p. 250–252°.

When the acetoxy compound, m. p. 117–118° (II), was dissolved in 5% sodium hydroxide, warmed briefly on a water-bath and then acidified with hydrochloric acid, yellow crystals, m. p. 137–138°, were obtained. A mixed melt with 2,4-dihydroxy-3-ethyl-5-methylphenylglyoxylic acid showed no depression. This behavior indicated an acetoxy derivative rather than the expected nuclear acetylated product, and further proof of this fact was obtained in the following manner: One gram of the phenylglyoxylic acid, m. p. 137–138°, was added to 10 ml. of pyridine. One gram of acetic anhydride was added and the mixture refluxed for ten minutes. After cooling and pouring into ice water the solid was removed, washed and dried. Recrystallization from benzene gave yellow needles, m. p. 117–118°. A mixed melt with the acetylated product obtained in the attempted Friedel-Crafts acylation gave no depression.

Numerous other attempts were made to obtain a nuclear acetylated compound by various modifications of the Friedel-Crafts reaction but only the starting compound

(14) In some cases the crude acid was filtered and combined with acid obtained from the ether extracts.

(15) The neutral equivalent of this compound as well as that of 2,4-dihydroxy-3-ethyl-5-methylphenylglyoxylic acid was determined satisfactorily by titration using a Beckmann pH meter.

(10) Although Hunsberger and Amstutz⁸ did not specifically mention the suppression of the carbonyl reactions of their preparation of I, they did cite the failure of 5-*n*-hexyl-2,4-dihydroxy-phenylglyoxylic acid to form the hydrazone derivative. Their result was in accord with the findings of Karrer and Ferla, *Helv. chim. acta*, **4**, 203 (1921), who were unable to form either an oxime or a phenylhydrazone from 2,4-dihydroxyphenylglyoxylic acid. This failure was attributed to an interaction of the carbonyl and orthohydroxy groups, an explanation recently recalled by Robertson and co-workers, *J. Chem. Soc.*, 1563 (1949), from an earlier paper, *ibid.*, 2196 (1927).

(11) The characterization of compound II was completed by one of us (RDS) in the chemical laboratories of the Department of Chemistry, College of Puget Sound, Tacoma, Washington.

(12) We are indebted to Dr. D. L. Cramer, formerly with the Norwich Pharmacal Co., for testing these compounds.

(13) See Cavallito and co-workers, *This Journal*, **71**, 1210 (1949).

or small amounts of the acetoxy compound could be isolated.

Lactone of 4-Acetoxy-3-ethyl-2-hydroxy-5-methylphenylglyoxylic Acid (IV).—When either of the acetylated products, m. p. 117–118°, was heated for six hours at 110° the material partially sublimed and changed to an orange solid, m. p. 174–175°. A solution of this compound in saturated sodium bicarbonate gave on acidification the original compound (II), m. p. 117–118°.

3-Methoxyphenylglyoxylic Acid.—This acid, previously unreported, was prepared in 60–65% yields by the careful oxidation of 3-methoxyacetophenone with alkaline permanganate. As noted by Mauthner¹⁶ all attempts to hydrolyze 3-methoxyphenylglyoxylonitrile¹⁷ with concentrated sulfuric acid were unsuccessful. When the hydrolysis was attempted with concentrated hydrochloric acid,¹⁸ 3-methoxybenzoic acid was the only product isolated. Oxidation of 3-acetoxy- and 3-benzoylacetophenone with neutral and alkaline permanganate failed as did the oxidation of 3-methoxyacetophenone in neutral solution.

In a 2-l. three-necked flask equipped with a mechanical stirrer, 3.5 g. (0.22 mole) of potassium permanganate was dissolved in 500 ml. of water and 25 ml. of 3 *N* potassium hydroxide solution. To this solution cooled to 0° was added with stirring 24 g. (0.28 mole) of 3-methoxyacetophenone¹⁹ along with 50 g. of crushed ice. During the reaction, crushed ice was added throughout so that at all times ice was in the reaction mixture. After the permanganate was decolorized, the solution was filtered and the manganese dioxide was washed thoroughly with cold water. The filtrates were combined and concentrated to a volume of approximately 250 ml. The hot solution was treated with decolorizing charcoal. The filtrate was acidified with dilute hydrochloric acid and extracted three times with ether. The combined ethereal extracts were washed three times with one-half volume of a saturated sodium bicarbonate solution. Acidification of the cooled washings with dilute hydrochloric acid gave a white precipitate which was characterized as 3-methoxybenzoic acid. The filtrate was extracted three times with ether, and these combined ethereal extracts were dried over anhydrous sodium sulfate. Removal of the ether left an oily residue which solidified on cooling. Recrystallization from a benzene-ligroin mixture gave 11.2 g. (60%) of the crystalline 3-methoxyphenylglyoxylic acid melting at 69–70°.

Anal. Calcd. for $C_9H_8O_4$: C, 59.99; H, 4.47. Found: C, 59.88; H, 4.58.

When heated with alkaline permanganate, 3-methoxyphenylglyoxylic acid gave 3-methoxybenzoic acid.

3-Hydroxyphenylglyoxylic Acid.—This acid was prepared by demethylation and hydrolysis of the methyl ester of 3-methoxyphenylglyoxylic acid.

In a 500-ml. three-necked flask equipped with a mercury-sealed mechanical stirrer, a reflux condenser fitted with a drying tube, and an addition tube for anhydrous aluminum chloride was placed a solution of 2 g. (0.01 mole) of methyl *m*-methoxyphenylglyoxylate²⁰ in 200 ml.

of nitrobenzene. To the stirred solution was added 3 g. (0.02 mole) of anhydrous aluminum chloride in small portions. The flask was then heated for six hours with stirring on a water bath at 85°. The mixture was cooled and added to 25 ml. of concentrated hydrochloric acid and 200 g. of crushed ice. Nitrobenzene was removed by steam distillation and the residual solution was extracted three times with ether. The combined ethereal extracts were washed three times with one-half of their volume of a saturated sodium bicarbonate solution. The cold bicarbonate washings were acidified with dilute hydrochloric acid, and the acidic solution was extracted three times with ether. The combined ethereal extracts were dried over anhydrous sodium sulfate. Removal of the ether gave a brown tar which was extracted with boiling benzene. A yellow solid (9 g. 60%) separated on cooling the benzene solution. Recrystallization from benzene gave the pure 3-hydroxyphenylglyoxylic acid, m. p. 104–105°. *Anal.* Calcd. for $C_8H_6O_4$: C, 57.84; H, 3.64. Found: C, 57.37, H, 3.57. Oxidation of this acid with alkaline permanganate gave 3-hydroxybenzoic acid.

2-Hydroxyphenylglyoxylic Acid.—After several unsuccessful attempts to obtain this acid by modified Friedel-Craft reactions or Fries rearrangements, it was finally prepared from isatin²¹ by isolating the 2,3-coumarandione by distillation of the crude acid. This added step gave a very pure product which could then be hydrolyzed to the desired acid on warming with sodium carbonate. Recrystallization of the acid from benzene-ligroin gave pure yellow *o*-hydroxyphenylglyoxylic acid, m. p. 56–57° (lit. 56–57°); yield 20% based on isatin.

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Summary

1. A convenient method is described for the preparation of hydroxy- and methoxy-phenylglyoxylic acids using ethyl oxalyl chloride.

2. 2,4-Dihydroxy-3-ethyl-5-methylphenylglyoxylic acid prepared by this method has been characterized and its properties compared with those of the compound recently prepared in another manner.

3. Attempts to prepare the nuclear acetyl derivative of 2,4-dihydroxy-3-ethyl-5-methylphenylglyoxylic acid were unsuccessful.

4. The preparation of 3-hydroxy- and 3-methoxyphenylglyoxylic acid has been described.

5. Bacteriostatic testing showed that the hydroxy- and methoxy-phenylglyoxylic acids prepared may be classed as virtually inactive.

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(21) Reichstein, Titoff and Müller, *Helv. Chim. Acta*, **20**, 883 (1937).

(16) Mauthner, *Ber.*, **42**, 192 (1909).

(17) The nitrile was also prepared by the method of Oakwood and Weisgerber, "Organic Syntheses," **24**, 17 (1944), from 3-methoxybenzoyl chloride.

(18) See Reichstein, *et al.*, *Helv. chim. Acta*, **20**, 883 (1937).

(19) Prepared by methylation of 3-hydroxyacetophenone (obtained from Dow Chemical Co.) with dimethyl sulfate.

(20) The ester was prepared in 70% yield (b. p. 152–154° (10 mm.)) using anhydrous methanol and gaseous hydrogen chloride.