[CONTRIBUTION FROM THE LABORATORY FOR THE STUDY OF HEREDITARY AND METABOLIC DISORDERS AND THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND MEDICINE, UNIVERSITY OF UTAH]

# Synthesis of *o*-Tyrosine and Related Phenolic Acids[1]

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Acetylglycine and salicylaldehyde were condensed in the presence of acetic anhydride and sodium acetate. The principal isolable product, designated by Dakin as 2-methyl-4-[*o*-acetoxybenzal]-5-oxazolone, was shown to be 3-acetamidocoumarin. 3-Chloroacetamido-dihydrocoumarin, rather than the oxazolone, was formed when N-chloroacetyl-*o*-tyrosine was subjected to the conditions of the Bergmann rearrangement. 3-Acetamidocoumarin was used to prepare DL-*o*-tyrosine, sodium DL-*o*-hydroxyphenyllactate, and 3-hydroxycoumarin (*o*-hydroxyphenylpyruvic acid lactone) in good yields.

DL-*o*-Tyrosine and some related *o*-hydroxyphenyl acids were required for studies of the metabolism of *o*-tyrosine and the formation of *o*-hydroxyphenylacetic acid in normal and phenylketonuric humans,[2,3] and also for reference compounds in an examination of the phenolic acids of human urine.[4] The reaction of salicylaldehyde with hippuric acid (I) had been used to prepare these *o*-hydroxy compounds by earlier investigators.[5,6,7] Aceturic acid (II) was employed in the present work, in order to avoid the troublesome formation of benzoic acid during subsequent processing.

The condensation of salicylaldehyde with I yields a mixture of 3-benzamidocoumarin (III) and 2-phenyl-4-[*o*-acetoxybenzal]-5-oxazolone (IV),[5,6,7] but with II, Dakin reported 2-methyl-4-[*o*-acetoxybenzal]-5-oxazolone (V) to be the exclusive product.[8] This difference between I and II in the course of the reaction has been stressed recently in review papers.[9,10] When Dakin's procedure was repeated, the primary isolable product was found to be 3-acetamidocoumarin (VI), in contrast to his report. It is probable that Dakin also obtained VI, since, in impure form, its physical properties closely resemble those ascribed to V; a similar confusion of oxazolone and coumarin products from 2-hydroxy-3-methoxy-benzaldehyde has been clarified recently.[11] Other routes to VI have included Beckmann rearrangement of 3-acetylcoumarin oxime[12] (75% yield), condensation of salicylaldehyde directly with glycine,[12] and partial hydrolysis and cyclization of the presumed V.[8]

In an attempt to obtain V, Dakin's procedure was subjected to several modifications. Pure acetylglycine was used as a starting material, since Dakin's method for preparing this compound *in situ* resulted in increased formation of tars. The product was isolated under anhydrous conditions to avoid the possible conversion of V to VI by action of water during isolation.[13] In other runs, the quantities of reactants and solvents, and the reaction time and temperature were varied widely. *o*-Acetoxybenzaldehyde was also tested in place of salicylaldehyde, and anhydrous potassium acetate or triethylamine instead of sodium acetate. VI was obtained as the main product in all cases, in a maximum yield of 25%. Attempts to obtain V by fractionation of the mother liquors from various runs were unsuccessful. The orange color of the reaction mixtures may provide an indication, however, that some V is formed along with VI.

The marked difference in products resulting from the condensation of salicylaldehyde with I and with II may occur as a result of two factors. In the first place, the formation of a greater proportion of IV than V in the condensation reaction may be expected because of the enhanced resonance stabilization of IV arising from the conjugation of two benzene rings with the oxazolone ring. Secondly, in the reaction with I, IV is less soluble than III and, therefore, is readily isolated,[6] whereas, in the reaction with II, VI crystallizes alone, and V, if formed, remains in the mother liquors with a complex mixture of other by-products.

An alternative route to V lay in the Bergmann rearrangement[14] of N - chloroacetyl - *o* - tyrosine (VIII). This compound was prepared in 72% yield by treatment of *o*-tyrosine (VII) with chloroacetyl chloride. VIII was refluxed with acetic anhydride, or was treated with acetic anhydride and pyridine or 2,6-lutidine at room temperature. Absence of color in the reaction mixtures indicated that no

(1) Supported by research grants from the National Institutes of Health, U. S. Public Health Service.

(2) Armstrong, Shaw, and Robinson, *J. Biol. Chem.*, **213**, 797 (1955).

(3) Armstrong and Shaw, *J. Biol. Chem.*, **213**, 805 (1955).

(4) Armstrong, Shaw, and Wall, *J. Biol. Chem.*, **218**, 293 (1956).

(5) Plöchl and Wolfrum, *Ber.*, **18**, 1183 (1885).

(6) Erlenleyer, Jr., and Stadlin, *Ann.*, **337**, 283 (1904).

(7) Blum, *Arch. Exp. Pathol. Pharmakol.*, **59**, 273 (1908).

(8) Dakin, *J. Biol. Chem.*, **82**, 439 (1929).

(9) Carter, *Org. Reactions*, **3**, 198 (1946).

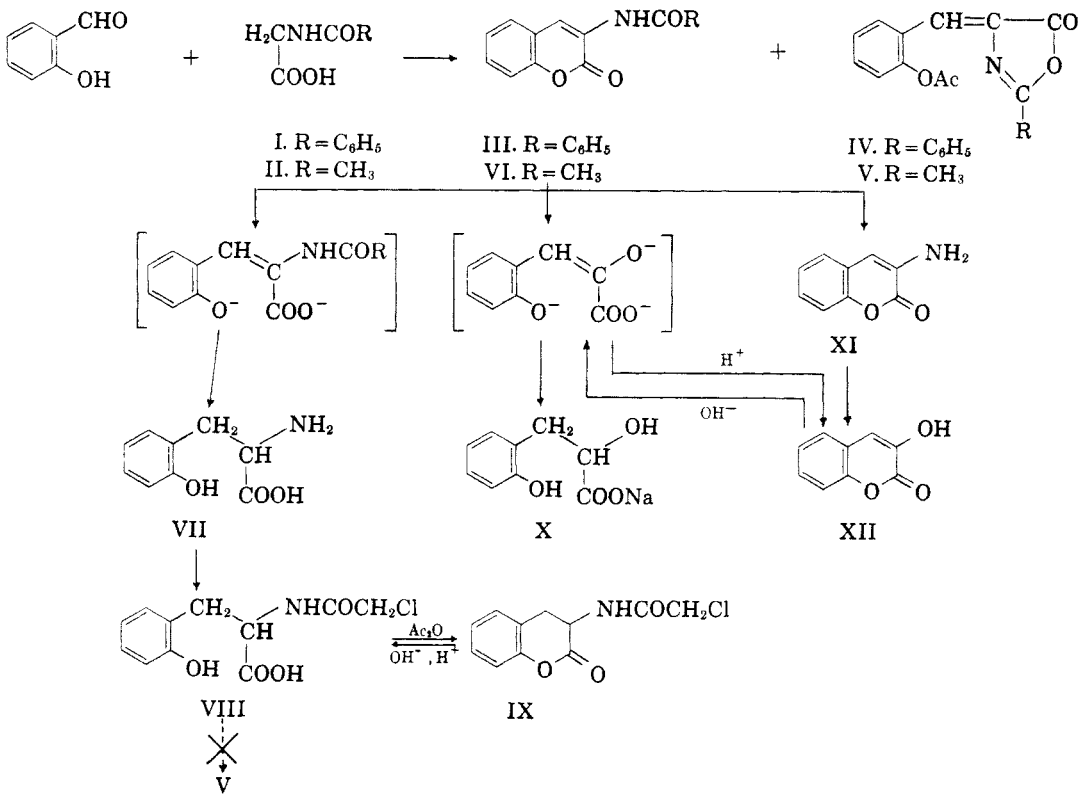
(10) Baltazzi, *Quart. Revs.*, **9**, 150 (1955).

(11) Lambooy, *J. Am. Chem. Soc.*, **76**, 133 (1954).

(12) Linch, *J. Chem. Soc.*, **101**, 1758 (1912).

(13) This possibility was suggested in connection with the condensation of 2,5-diacetoxybenzaldehyde with II. Lambooy, *J. Am. Chem. Soc.*, **71**, 3758 (1949).

(14) The scope of this reaction was extended and relevant literature reviewed recently. Sheehan and Duggins, *J. Am. Chem. Soc.*, **72**, 2475 (1950).




oxazolone was formed; instead, 3-chloroacetamidodihydrocoumarin (IX) was produced in 85% yield. The structure of IX was determined by analysis, color reactions, and by mild alkaline hydrolysis back to VIII.

The ease of dehydration of VIII to lactone IX can be attributed both to a favorable steric relationship of the functional groups in VIII, and to the low solubility of IX. Since the formation of V is thus blocked completely, it is apparent that a free carboxyl group is required for the Bergmann rearrangement: conceivably, formation of a mixed anhydride with acetic anhydride is a first step towards oxazolone ring closure.

VI was converted to *o*-tyrosine (VII) in 81% yield by partial hydrolysis with dilute sodium hydroxide, reduction with sodium amalgam, and hydrolysis of the resulting N-acetyl-*o*-tyrosine. VII was first prepared from III and IV in the same way.[7] The reduction and hydrolysis of III and IV with red phosphorus and hydriodic acid at present offers the most convenient route to VII (2 steps, 36% yield).[15] Other methods have involved the condensation of salicylaldehyde, or its methyl ether, with hydantoin,[16] 2-thiohydantoin,[17] or 2,5-diketopiperazine.[18]

Hydrolysis of VI with concentrated alkali, followed by reduction with sodium amalgam yielded *o*-hydroxyphenyllactic acid. After several unsuccessful attempts to crystallize the free acid, it was isolated in 66% yield as its sodium salt (X), which is stable and readily purified. The calcium and zinc salts are described in the literature, but the free acid is listed as a syrup.[5]

*o*-Hydroxyphenylpyruvic acid is known only in the form of its calcium salt and its lactone, 3-hydroxycoumarin (XII), which have been obtained in unspecified yield by alkaline hydrolysis of III or IV.[5,6] In the present work, XII was prepared initially in 37% yield by alkaline hydrolysis of VI and lactonization of the resulting sodium salt by acidification. A superior product, however, was obtained in 89% yield by hydrolysis of VI with dilute acid. The possibilities of this route were apparent from its past application to the synthesis of 3-aminocoumarin (XI).[12] Contrary to the early report,[5] XII can be recrystallized from water without appreciable opening of the lactone ring or other decomposition, and is a convenient storage form of *o*-hydroxyphenylpyruvic acid.

## EXPERIMENTAL

*3-Acetamidocoumarin* (VI) (*Dakin's procedure for azlactone*[8]). A suspension of 37.5 g. (0.50 mole) of powdered glycine in 100 ml. (1.0 mole) of acetic anhydride (Mallinckrodt: 97%) and 75 ml. of glacial acetic acid was heated rapidly to the boiling point, and allowed to simmer until the glycine

(15) Lambooy, *J. Am. Chem. Soc.*, **78**, 771 (1956).
(16) Matsuura, Matsui, and Ichihara, *Med. J. Osaka U.*, **4**, 449 (1954).
(17) Johnson and Scott, *J. Am. Chem. Soc.*, **37**, 1846 (1915).
(18) Dickinson and Marshall, *J. Chem. Soc.*, 1495 (1929).

just dissolved (one minute).[19] The dark orange solution was treated immediately with 41.0 g. (0.50 mole) of anhydrous sodium acetate, 61.1 g. (0.50 mole) of salicylaldehyde, and 150 ml. of acetic anhydride, and heated on a boiling water-bath for two hours, with precautions to exclude moisture. The dark brown solution was cooled quickly to room temperature, mixed with 300 g. of ice and 300 ml. of ice-water for 30 minutes, and filtered. The product was washed three times with 50-ml. portions of cold water and then desiccated at 10 mm. A dark brown pasty solid (43.6 g.) was obtained, which smelled strongly of salicylaldehyde.

A portion (15.00 g.) of the crude product was allowed to simmer briefly with 120 ml. of toluene. A dark brown powder (1.25 g., not melted at 250°, insoluble in hot toluene) was removed by filtration. The solution was treated with 2.4 g. of charcoal, heated to the boiling point, and filtered. Following refrigeration, 4.61 g. of yellow-orange crystals were recovered, m.p. 183–193°. Three more crystallizations from benzene (charcoal) yielded 0.88 g. (2.5% yield based on glycine) of white needles, m.p. 206°,[20] undepressed upon mixing with authentic VI. Pure VI was obtained more readily from the same crude product by three crystallizations from benzene (3.7% yield), or by two crystallizations from 95% ethanol (9.6% yield) (charcoal with each solvent).

*3-Acetamidocoumarin* (VI) *from* II (*aqueous precipitation*). A mixture of 61.1 g. (0.50 mole) of salicylaldehyde (Eastman # 225), 58.6 g. (0.50 mole) of acetylglycine (II),[21] 41.0 g. (0.50 mole) of anhydrous sodium acetate, 104 ml. of glacial acetic acid, and 250 ml. (2.5 moles) of acetic anhydride in a 2-liter flask with attached reflux condenser, was rapidly heated to 100° and was maintained at 100° (oil-bath) for 120 minutes. The dark red solution was cooled quickly to room temperature, and the resulting crystalline mass was fragmented, slurried with 300 g. of ice and 300 ml. of ice-water for 30 minutes, and filtered. The solid product was washed twice with 60 ml. of cold 40% aqueous acetic acid, twice with 60 ml. of ice-water, and then desiccated at 10 mm. A yellow powder (45.5 g.) was obtained, m.p. 142–195°.

A portion (8.00 g.) of the crude product was dissolved in 300 ml. of boiling benzene, treated with 1.5 g. of charcoal and filtered. The yellow filtrate was concentrated to dryness under reduced pressure, and the residue was crystallized from 145 ml. of boiling benzene; 3.50 g. (20% yield, based on II) of VI was recovered as faintly yellow long needles, m.p. 205–206°. A second recrystallization from benzene (or 95% ethanol) yielded white needles (87% recovery), m.p. 206°. (Lit. m.p. 203–204°,[8] 201.5°[12]).

*Anal.*[22] Calc'd for $C_{11}H_9NO_3$: C, 65.02; H, 4.47; N, 6.89. Found: C, 64.81; H, 4.43; N, 6.98.

The benzene filtrate from the first recrystallization was concentrated under reduced pressure. A second crop of 0.53 g. of faintly yellow short needles, m.p. 123–195°, and 3.42 g. of a light buff residual powder, melting at 95–170° and smelling of salicylaldehyde, thus were obtained. Attempts to obtain azlactone from these fractions were unsuccessful.

*3-Acetamidocoumarin* (VI) *from II* (*isolation under anhydrous conditions*). The same amounts of salicylaldehyde, II, and anhydrous sodium acetate noted in the preceding section were mixed with 150 ml. (1.5 moles) of acetic anhydride, rapidly heated to 100°, and maintained at this temperature for 90 minutes. Extensive formation of long needles, presumably VI, occurred suddenly after 60 minutes. The mixture was cooled quickly to room temperature, filtered, and the yellow filter cake was sucked as dry as possible. The crystal mass was simmered with 1200-, 200-, and 200-ml. volumes of benzene; the hot slurry was filtered each time. The undissolved residue consisted mainly of sodium acetate. The benzene extracts were stored at 5° for 18 hours, following which 25.3 g. (25% yield) of VI was recovered as long pale yellow needles, m.p. 205–206°. For the subsequent syntheses, no further purification is needed.

*o-Tyrosine* (VII). In a 300-ml. round-bottomed flask, 12.19 g. (0.060 mole) of VI was treated with 75 ml. of hot 2 *N* sodium hydroxide and heated rapidly to the boiling point. The clear yellow solution was quickly cooled to room temperature, and 184 g. (0.24 g.-atom) of freshly pulverized 3% sodium amalgam[23] was added in ten equal portions at five-minute intervals with high speed agitation. Agitation was continued for two hours more. The colorless aqueous supernatant was removed and the amalgam was washed with water. The aqueous phase was concentrated to 50 ml. and then was refluxed for 16 hours. The solution was diluted with water to 600 ml., acidified with concentrated hydrochloric acid to *p*H 5.6 (Hydrion paper), and heated to the boiling point. A minor amount of infusible flocculent material (presumably from the amalgam) remained undissolved. The solution was treated with 3.0 g. of charcoal, filtered, and concentrated to 500 ml. (One batch filtered so slowly that VII crystallized. In this case, the mixture was acidified to *p*H 1.5 and the filtrate was titrated subsequently to *p*H 5.6). Following overnight storage at 5°, 6.68 g. (61% yield) of VII was recovered, dec. pt. 262° (sample in bath at 260°, heating rate 3°/min.) (Lit.[7] m.p. 249–250°). A further 2.20 g. (20% yield), same dec. pt., was obtained by concentrating the filtrate. The decomposition point was raised 1° by recrystallization (charcoal) from water (91% recovery, two crops).

*Anal.* Calc'd for $C_9H_{11}NO_3$: N, 7.73; Found: 7.70.

*N-Chloroacetyl-o-tyrosine* (VIII). VII (5.45 g., 0.030 mole) was dissolved in 60 ml. of water by adding 30.0 ml. of 1 *N* NaOH. The solution was agitated vigorously and maintained at 5 ± 2° while 2.50 ml. (0.033 mole) of chloroacetyl chloride was added dropwise during 60 minutes. The solution was kept at *p*H 10.0 ± 0.2 (Beckman Model G meter) by concurrent addition of 33.0 ml. of 1 *N* NaOH. After agitation for ten more minutes, 75 ml. of ethyl acetate was added and the mixture was acidified to *p*H 1.7 with concentrated hydrochloric acid. The aqueous phase was extracted with five more 75-ml. portions of ethyl acetate. The ethyl acetate extracts were dried over sodium sulfate and were concentrated under reduced pressure to 7.3 g. of an amber gum. The gum was redissolved in 40 ml. of ethyl acetate and 30 ml. of petroleum ether (b.p. 30–60°) was added. The clear solution was seeded with a rubbed aliquot. Following storage at 5° for four hours, 3.84 g. (50% yield) of white crystals was recovered, m.p. 136–137°. A second crop of 1.71 g. (22% yield), m.p. 137–139°, was obtained by working up the filtrate in the same manner as the original extracts. After recrystallization from acetonitrile, then ethyl acetate, VIII was obtained as tetragonal crystals, m.p. 149° (dec.) A crude product has been reported to sinter at 121°.[24]

*Anal.* Calc'd for $C_{11}H_{12}ClNO_4$: C, 51.27; H, 4.69; N, 5.44; Cl, 13.76. Found: C, 51.23; H, 4.52; N, 5.40; Cl, 13.60.

*Attempted Bergmann rearrangement of* VIII *to* V: *3-chloroacetamido-dihydrocoumarin* (IX). Crude VIII (2.58 g.,

(19) When only 50 ml. of acetic anhydride was used,[8] solution of the glycine was incomplete after boiling for 20 minutes, and decomposition was extensive.

(20) Melting points are uncorrected and were taken in open capillary tubes.

(21) Herbst and Shemin, *Org. Syntheses*, Coll. vol. 2, 11 (1943).

(22) Analyses were performed by the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

(23) Fieser, *Experiments in Organic Chemistry*, 2nd ed., D. C. Heath and Co., New York, 1941, p. 418.

(24) Abderhalden and Schairer, *Fermentforschung*, 12, 295 (1931) [*Chem. Abstr.*, 25, 2693 (1931)].

0.010 mole) suspended in 7.50 ml. of acetic anhydride was treated with 2.50 ml. of pyridine (distilled over barium oxide) and the mixture was allowed to stand at room temperature for 30 minutes. Rapid solution occurred and the temperature of the mixture rose to 39°. No appreciable color developed. Crushed ice (25 g.) and 25 ml. of ice water were added. The mixture was shaken for 20 minutes and filtered. After washing with water and desiccation at 10 mm., 2.04 g. (85% yield) of white granules was recovered, m.p. 122–123°. Long colorless needles, m.p. 125°, were obtained by recrystallization from acetone-cyclohexane (1:4) (charcoal). IX gave negative tests with ferric chloride and with ninhydrin; the *p*H of an aqueous suspension was 4.0. Absence of free carboxyl, amino, and phenolic hydroxyl groups was indicated.

*Anal.* Calc'd for $C_{11}H_{10}ClNO_3$: C, 55.13; H, 4.21; N, 5.85; Cl, 14.80; Mol. wt., 240. Found: C, 55.18, H, 4.17; N, 5.71; Cl, 15.10; Mol. wt., 215.

Similar results were obtained when the pyridine was replaced by 3.50 ml. of 2,6-lutidine, or when VIII was refluxed with the acetic anhydride for ten minutes prior to the addition of pyridine.

*Hydrolysis of* IX *to* VIII. A suspension of 0.480 g. (0.0020 mole) of IX in 50 ml. of 10% aqueous acetone was stirred for ten minutes, while 3.80 ml. of 1.00 *N* sodium hydroxide was added dropwise. The resulting solution was filtered, acidified to *p*H 1.7 (Hydrion paper) with concentrated hydrochloric acid, and extracted with five 50-ml. portions of ethyl acetate. The extracts were treated with charcoal, dried over sodium sulfate, and concentrated to dryness under reduced pressure. Upon crystallizing the residue from ethyl acetate, 0.31 g. (60% yield) of VIII was recovered as colorless tetragonal crystals, m.p. 149° (dec.).

*Sodium o-hydroxyphenyllactate* (X). A mixture of 6.09 g. (0.030 mole) of VI and 50 ml. of 10 *N* sodium hydroxide was refluxed under nitrogen for five hours. After two hours, 25 ml. of water were added to curtail bumping, caused by a small crystalline precipitate. The hydrolysate was cooled to room temperature, diluted with water to 200 ml., and 77 g. (0.10-g. atom) of freshly pulverized 3% sodium amalgam was added in eleven equal portions at five-minute intervals with high speed agitation. Agitation was continued for one hour more, then the mixture was stored at 5° overnight. The colorless aqueous supernatant was removed and the amalgam was washed with water. The aqueous phase was acidified with concentrated hydrochloric acid to *p*H 1.6 (Hydrion paper). The solution was filtered (very slow) to remove a gelatinous precipitate (0.43 g. after drying; infusible). The filtrate was extracted with five 100-ml. volumes of ethyl acetate. The extracts were dried over sodium sulfate, treated with 2.5 g. of charcoal, and concentrated to dryness under reduced pressure. The resulting pale brown syrup of *o*-hydroxyphenyllactic acid (4.3 g., 79% yield) was quite soluble in water, and in most organic solvents from which it again precipitated as an oil upon addition of petroleum ether or cyclohexane. Attempts to obtain the compound in crystalline form were unsuccessful.

The syrup was dissolved in 40 ml. of water and the solution was adjusted to *p*H 8.0 (Hydrion paper) with 1 *N* sodium hydroxide. The solution was shaken with 0.40 g. of charcoal to remove a few droplets of an insoluble brown oil, filtered, and concentrated under reduced pressure to dryness. The residue was taken up in 100 ml. of absolute ethanol and again was concentrated to dryness. The resulting white solid was dissolved in 300 ml. of boiling 95% ethanol. The solution was concentrated to 150 ml. at atmospheric pressure, treated with 0.75 g. of charcoal, and filtered to remove a small white flocculent precipitate. The filtrate was concentrated further to 100 ml. and, still clear, was stored at 5° overnight. Following filtration, washing with cold 95% ethanol, and drying in air, 3.16 g. (52% yield) of X was recovered as hard white crystal nodules, dec. pt. 241°. A further 0.86 g. (14% yield), dec. pt. 241°, was obtained by concentrating the filtrate. The compound was recrystallized from 95% ethanol with no change in properties.

*Anal.* Calc'd for $C_9H_9NaO_4$: C, 52.95; H, 4.44; Na, 11.26. Found: C, 53.03; H, 4.64; Na, 11.26.

*3-Hydroxycoumarin* (XII). A mixture of 6.10 g. (0.030 mole) of VI and 100 ml. of 3 *N* hydrochloric acid was refluxed under nitrogen for two hours. VI dissolved slowly during the first half hour and a small amount of a light yellow fine solid precipitated gradually. The reaction mixture was chilled to 5° in an ice-bath, whereupon extensive crystallization occurred. Following filtration and washing with cold water, 4.31 g. (89% yield) of light yellow crystals was obtained, m.p. 148–152°. The crude product was simmered with 325 ml. of water and 0.26 g. of yellow powder, m.p. 197–235° was removed by filtration. The filtrate was allowed to simmer with 1.6 g. charcoal, filtered, and stored at 5° overnight. XII was recovered (83%) as long colorless needles, m.p. 153–154°. The melting point was 154° after a second recrystallization from water. (Lit. m.p. 152–153°[5], 152°[6]). The *p*H of a saturated aqueous solution was *ca.* 5.0 (Hydrion paper).

*Anal.* Calc'd for $C_9H_6O_3$: C, 66.67; H, 3.73; Found: C, 66.47; H, 3.96.

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