# ORGANIC AND BIOLOGICAL CHEMISTRY

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

## 5-Amino-4-imidazolecarboxamide Riboside from Inosine. Ring-opening Reactions of Purine Nucleosides

BY ELLIOTT SHAW[1]

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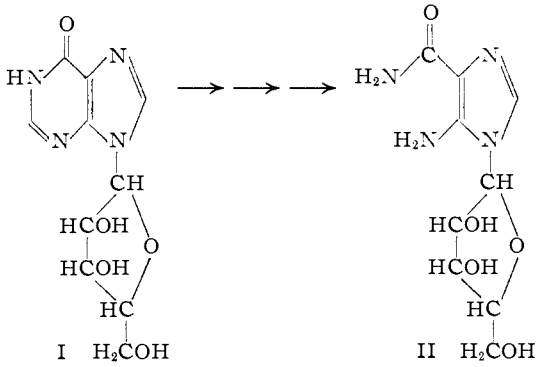
Aminoimidazolecarboxamide ribofuranoside (II), an intermediate in purine biosynthesis, has been obtained chemically from inosine (hypoxanthine ribofuranoside, I). The purine ring in the latter was made labile to alkaline hydrolysis by alkylation. Thus, 1-benzylinosine was prepared and shown to undergo hydrolysis to 5-amino-4-imidazole-N-benzylcarboxamide riboside by expulsion of carbon 2 of the purine ring. Debenzylation of this amide by sodium in liquid ammonia gave the desired imidazole nucleoside II.

The identification of a number of imidazole ribotides as intermediates in the biosynthesis of purines[2-4] has brought to light a hitherto unknown class of important natural compounds. These were obtained from bacteria or from mammalian enzymic reactions and have not heretofore been obtainable by chemical means.

The ready availability of purine nucleosides and nucleotides from natural sources makes it attractive to develop chemical methods for altering the nitrogenous part without affecting the ribofuranosidic part of the nucleosides. Thus a mild method for opening the pyrimidine ring in a purine ribofuranoside would provide a useful entry to a group of imidazole ribofuranosides. Such a method was sought. It would be of potential value, in addition, in the preparation of certain desoxyribonucleosides. Desoxyribofuranosides have not yet been amenable to synthetic approach.

In this paper is described the conversion of inosine (hypoxanthine riboside, I) to one of the naturally occurring imidazole nucleosides, aminoimidazolecarboxamide riboside[3] (II).



I II

To retain the glycosidic link intact, it seemed advisable to use alkaline conditions in an approach designed to render the purine ring labile to a hydrolytic opening of its pyrimidine ring. Those purine ribofuranosides accessible from nucleic acid are extremely stable to alkali and were, in fact, first isolated by autoclaving yeast nucleic acid for several hours at 145° in aqueous ammonium hydroxide.[5] However, Fischer had observed[6] that guanine and xanthine, when increasingly methylated at ring nitrogens, became more susceptible to hydrolysis by alkali. Although in these experiments the nature of the chemical change was not determined, the long known instability of caffeine (1,3,7-trimethylxanthine) to alkali was shown by Biltz and Rakett[7] to proceed by ring opening to an imidazole derivative, caffeidine.[8] These early findings suggested that alkylation of inosine might render the pyrimidine ring sensitive to alkaline hydrolysis. Such a result could be rationalized with the known acidic ionization of inosine providing a resonance-stabilized anion in which the negative charge may screen the adjacent carbon atoms from attack by the negative hydroxide ion. In an $N^1$-alkyl derivative such an ionization would be blocked.

In recent years a number of purines and purine ribosides not obtained from nucleic acids have been found to hydrolyze with alkali, however, with ring opening in a different sense from that described above and illustrated in the present work; these examples yield pyrimidine derivatives by cleavage of the C8–N9 bond.[9-11] While the effect of structure on the pathways of hydrolysis calls for further study, several influences of structure may be noted in a preliminary way. Those purines containing the –N–C– in the pyrimidine ring ionize

in alkali and are stable. Purines containing this grouping in the alkylated form, –N–C–, at least in

the 6-position, undergo hydrolytic attack directed preferentially into the pyrimidine ring, perhaps due to the importance of a form such as

(1) Department of Biochemistry, Tulane University Medical School, New Orleans 18, La. This paper was presented at the International Congress of Pure and Applied Chemistry, Paris, July, 1957.

(2) G. R. Greenberg, THIS JOURNAL, **74**, 6307 (1952).

(3) G. R. Greenberg and E. L. Spilman, *J. Biol. Chem.*, **219**, 411 (1956); E. D. Korn, F. C. Charalampous and J. M. Buchanan, THIS JOURNAL, **75**, 3610 (1953).

(4) J. M. Buchanan in "Chemistry and Biology of the Purines," Ciba Foundation Symposium, Little, Brown and Co., Boston, Mass., 1957, pp. 233–256.

(5) P. A. Levene and L. W. Bass, "Nucleic Acids," The Chemical Catalog Co. (Reinhold Publ. Corp.), New York, N. Y., 1931, p. 163.

(6) E. Fischer, *Ber.*, **31**, 3266 (1899).

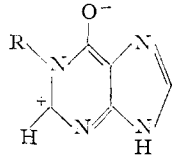
(7) H. Biltz and H. Rakett, *ibid.*, **61**, 1409 (1928).

(8) Another example in which treatment of a purine derivative with alkali very likely involves opening to an imidazole is the unexplained formation of 7-methylguanine from 6-amino-2-chloro-7-methylpurine; *cf.* E. Fischer, *Ber.*, **31**, 542 (1898). This reaction is now being investigated. *Cf.* also observations of G. Elion, ref. 4, p. 39.

(9) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

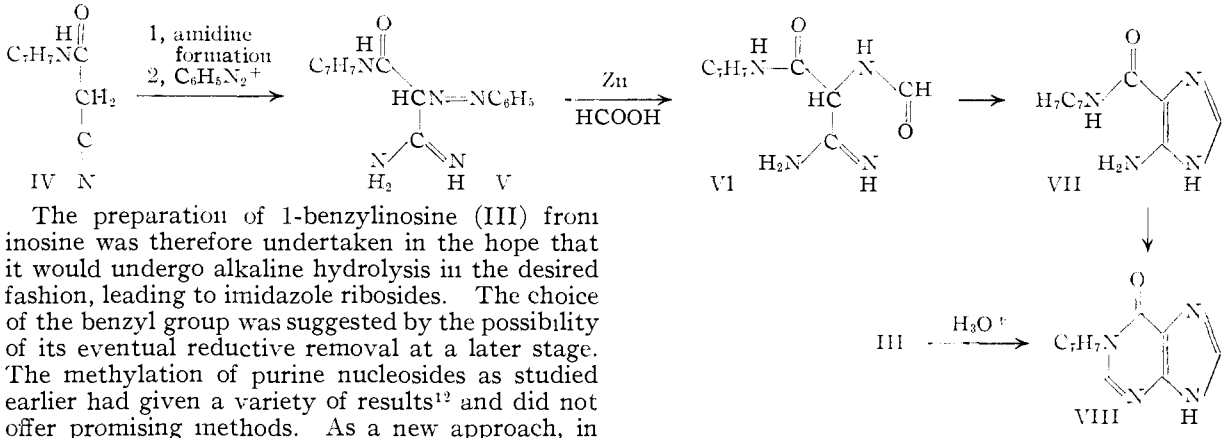
(10) M. P. Gordon, V. S. Weliky and G. B. Brown, THIS JOURNAL, **79**, 3245 (1957), and related papers cited therein.

(11) D. I. Magrath and G. B. Brown, *ibid.*, **79**, 3252 (1957).



A third group, also un-ionized in alkali, may be rather unstable to alkali, but lacking the directive influence attributed to the oxygen atom, cleaves in the smaller ring.[10]

SCHEME 1




The preparation of 1-benzylinosine (III) from inosine was therefore undertaken in the hope that it would undergo alkaline hydrolysis in the desired fashion, leading to imidazole ribosides. The choice of the benzyl group was suggested by the possibility of its eventual reductive removal at a later stage. The methylation of purine nucleosides as studied earlier had given a variety of results[12] and did not offer promising methods. As a new approach, in view of the convenient solubility of inosine in N,N-dimethylformamide, the nucleoside was treated at 100° in that solvent with benzyl chloride in the presence of sodium bicarbonate. While chromatography on paper revealed the formation of a number of substances, the major product was a monobenzyl derivative eventually obtained crystalline in a yield of 50%. This was shown to be 1-benzylinosine (III) by acid hydrolysis to 1-benzylhypoxanthine (VIII) which was characterized by an unambiguous synthesis. The benzylation of inosine was also conveniently achieved by treatment of its triacetate with sodium hydride and benzyl chloride in N,N-dimethylformamide.

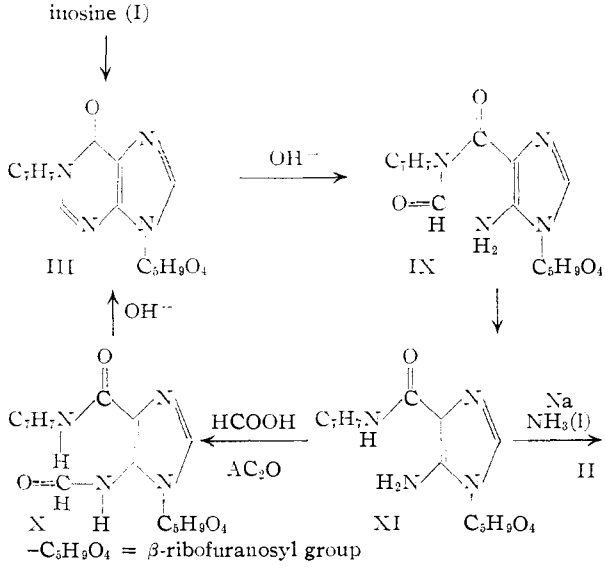
The unambiguous synthesis of 1-benzylhypoxanthine (VIII) proceeded by an extension of methods previously devised in this Laboratory.[13,14] Cyano-N-benzylacetamide (IV) was converted to the amidine whose phenylazo derivative V readily underwent reductive formylation to VI. The latter cyclized on heating to 4(5)-amino-5(4)-imidazole-N-benzylcarboxamide (VII). The N-formyl derivative of this amine, in turn, ring-closed when heated to yield 1-benzylhypoxanthine (VIII).

When 1-benzylinosine was refluxed with aqueous alcoholic sodium hydroxide, a diazotizable amine group soon was detected,[15] indicating that ring opening was taking place. After two hours, a 70% yield of a crystalline product was obtained, shown by analysis to have lost a carbon atom. Since acid hydrolysis of this riboside liberated 4(5)-amino-5-(4)-imidazole-N-benzylcarboxamide (VII) identical with a synthetic sample, the pyrimidine ring had been opened as anticipated with formation of the aminoimidazole-N-benzylcarboxamide riboside (XI).

The action of other nucleophilic agents on 1-benzylinosine, *viz.*, ethanolic hydroxylamine or hydrazine, did not give evidence of easy ring opening as found with aqueous alkali.

The course of the ring opening reaction is thought to be attack of hydroxide ion at the 2-position, but the available information does not permit a choice between the two likely intermediates (IX and X). The formamidoamide X was prepared and shown to cyclize back in part to 1-benzylinosine under alkaline conditions.

SCHEME 2




There remained the debenzylation of the N-benzylamide (XI) to obtain the desired aminoimidazolecarboxamide riboside. Palladium and other catalysts gave no debenzylation with hydrogen unless the solvent contained acids, in which case splitting of the glycosidic link occurred to a serious degree. With dissolved sodium in liquid ammonia, however, appreciable formation of the desired product was achieved. The yield obtainable with this reagent was limited by reduction in the imidazole ring which proceeded rapidly even

(12) Reviewed by H. Bredereck, *Ber.*, **80**, 401 (1947). The 1-methyl derivative claimed to have been formed from guanosine has been found to be a 7-methyl isomer (unpublished results).

(13) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **181**, 89 (1949).

(14) E. Shaw, *ibid.*, **185**, 439 (1950).

(15) A. C. Bratton and E. K. Marshall, *ibid.*, **128**, 537 (1939).

at −60°. The optimal conditions for obtaining the imidazole riboside were found empirically and, following column chromatography, a 20–23% yield of the pure riboside was obtained. While the yield in this step is somewhat disappointing in view of the success of the earlier ones, aminoimidazolecarboxamide riboside can now be conveniently obtained from inosine and it is felt that further exploration of such ring-opening methods, now underway, will result in improvement and extensions.

The labilization of the pyrimidine ring in inosine to hydrolysis which resulted when its acidic ionization at N1 was blocked suggests that the mechanism of action of the enzyme inosinicase,[4] which catalyzes a similar ring-opening reaction, involves an activation of its substrate inosinic acid in some bound form at N1 prior to the ring-opening step.

**Acknowledgment.**—The technical assistance of Miss Inge Koehelik is gratefully acknowledged.

## Experimental[16]

**1-Benzylinosine** (III).—Inosine (1.0 g.) was dissolved with heating in N,N-dimethylformamide (30 ml.) in a small round-bottomed flask. Sodium bicarbonate (1.0 g.) and benzyl chloride (1.0 ml.) were added. An air condenser was attached and the mixture was heated for 6 hours in a boiling water-bath with occasional stirring. The solvent was then removed at 15 mm. pressure with intermittent heating in the water-bath. To eliminate unreacted benzyl chloride, water (35 ml.) was added to the residue and, after trituration, was removed by evaporation to dryness under reduced pressure. The process was repeated.

The first batch of crystalline material was obtained by chromatography. Samples of the insoluble residue were dissolved in ethanol and chromatographed on paper sheets developed ascendingly in 1-butanol–1 *N* $NH_4OH$ (7:1) overnight. 1-Benzylinosine appeared as the major product, $R_f$ 0.45–0.50, visualized with an ultraviolet lamp. It was eluted with hot 50% aqueous ethanol, and crystallized from concentrated 95% ethanol. The availability of seed crystals permitted subsequent isolations without chromatography.

The residue from the benzylation was seeded with 1-benzylinosine and stirred occasionally under a layer of water until granulation was complete. The crude product was filtered and triturated with cold 0.2 *N* NaOH (25 ml.) to extract unreacted inosine. The insoluble residue was filtered, washed with water and dried. Recrystallization from 95% ethanol gave 0.63 g., m.p. 219–222°, a yield of 47%.

*Anal.* Calcd. for $C_{17}H_{18}N_4O_5$: C, 56.98; H, 5.06; N, 15.64. Found: C, 57.46; H, 5.30; N, 15.82.

**5-Amino-4-imidazole N-Benzylcarboxamide β-Ribofuranoside** (XI).—A. 1-Benzylinosine (1.5 g.) was refluxed for three hours in 95% ethanol (450 ml.) and 6 *N* sodium hydroxide (10 ml.). The solution was brought to *p*H 8 with aqueous hydrochloric acid and taken to dryness under reduced pressure. The residue was extracted with several 50-ml. portions of hot absolute ethanol and the combined extracts were concentrated to about 25 ml. Water was added slowly to bring the volume to about 100 ml., whereupon the solution was seeded and left at 4°. The initial crop of crystals together with a recrystallized second crop gave a 70% yield of material, m.p. 171–172°.

*Anal.* Calcd. for $C_{16}H_{20}N_4O_5$: C, 55.16; H, 5.79; N, 16.08. Found: C, 55.38; H, 5.85; N, 16.09.

**B. From Inosine Triacetate.**—Inosine triacetate[12] (6.9 g.) was added to a suspension of sodium hydride (0.5 g.) in N,N-dimethylformamide (80 ml.). After the initial hydrogen evolution ceased, benzyl chloride (2.1 ml.) was added and the mixture was maintained at 60° for 15 hours. Absolute alcohol (5 ml.) was added to ensure decomposition of the excess hydride and all volatile material was removed at reduced pressure. The residue was then taken up in 95% ethanol and transferred to a larger flask for ring opening in this solvent (1 l.) and 6 *N* sodium hydroxide (40 ml.). From this point the procedure was that of the preceding preparation and provided a yield of 45% for the two steps.

**C. Reconversion to 1-Benzylinosine.**—A solution of 5-amino-4-imidazole N-benzylcarboxamide riboside (0.25 g.) in 98% formic acid (1.2 ml.) and acetic anhydride (6 ml.) was heated for one hour in a water-bath, taken to dryness *in vacuo*, and the residue desiccated over solid KOH. The formylation product was then dissolved in ethanol (10 ml.) and 3 *N* NaOH (1 ml.). After two days at room temperature, the solution was neutralized with acetic acid, concentrated, diluted with water and seeded. A 50% yield of 1-benzylinosine was obtained, m.p. 221–223°, when taken alone or mixed with authentic 1-benzylinosine.

**α-Guanyl-N-benzylacetamide**[17] **(N-Benzylmalonamidine).**—N-Benzylcyanoacetamide[18] in dry chloroform (40 ml. per g.) containing the theoretical amount of absolute ethanol was converted to an imino ether hydrochloride by treatment with hydrogen chloride at 0°. After standing overnight at 4°, the mixture was taken to a viscous oil under reduced pressure below 40° and the residue was desiccated *in vacuo* over potassium hydroxide pellets. To remove unreacted starting material, the crude imino ether hydrochloride was stirred with a chloroform–ether mixture (2:1) and filtered with suction. The insoluble portion, after drying, was converted to the amidine when stirred overnight with a 20% excess of 0.6 *N* ethanolic ammonia. The ethanolic solution was concentrated to a small volume and filtered to remove a small amount of ammonium chloride. The filtrate was then freed of solvent under reduced pressure and the residue stirred into warm water (20 ml./g., 40°) to dissolve the amidine hydrochloride. Residual starting material and neutral by-products were removed at this point by filtration. After concentration of the filtrate to dryness and thorough dehydration, the crystalline residue obtained was amidine hydrochloride of adequate purity for the next step. The yield was 65%. An analytical sample was obtained from alcohol and ether and had m.p. 148–149°.

*Anal.* Calcd. for $C_{10}H_{14}N_3OCl$: C, 52.74; H, 6.20; N, 18.46. Found: C, 52.73; H, 6.20; N, 18.65.

**α-Formamido-α-guanyl-N-benzylacetamide Hydrochloride**[17] **(VI).**—The phenylazo-derivative of the substituted malonamamidine V was prepared as follows: aniline (0.95 ml.) in 3 *N* hydrochloric acid (12 ml.) was diazotized with aqueous sodium nitrite (0.73 g. in 5 ml.) in the usual way and added to a solution of α-guanyl-N-benzylacetamide (2.28 g. in 50 ml.). The *p*H was brought to 4 with concentrated aqueous sodium acetate. The product separated as a yellow oil which subsequently crystallized, was filtered with suction and washed with water, yielding 2.4 g. This was not purified for the next step.

The crude phenylazo derivative (8.0 g.) in 98% formic acid (100 ml.) was reduced with zinc dust (5.0 g.) at 40–45°.[13] After 0.5 hour, a second addition (2.5 g.) of zinc dust was made for complete decolorization. Finally, the reaction mixture was filtered and the filtrate plus formic acid washings of the insoluble part were combined and taken to a sirup at reduced pressure with gentle heating. The process was repeated with several 50-ml. additions of water to remove formic acid. To the residue in water (100 ml.) was added 0.027 mole of hydrochloric acid and the resultant mixture was extracted with benzene to remove formanilide. The aqueous layer was adjusted to *p*H 4 with pyridine and the zinc was removed by means of hydrogen sulfide. When the filtrate was taken to dryness, the residue crystallized. It was thinned with absolute alcohol and ether for filtration and provided 4.0 g., 58%, m.p. 169–171°, of product. An analytical sample obtained as long needles from aqueous alcohol had a m.p. of 171–172°.

*Anal.* Calcd. for $C_{11}H_{15}N_4O_2Cl$: C, 48.80; H, 5.59. Found: C, 48.45; H, 5.85.

**4(5)-Amino-5(4)-imidazole-N-benzylcarboxamide (VII).**—The formamido-compound VI (0.05 g.) was cyclized thermally at 185–190° and 15 mm. pressure for 15 minutes.[13] The cooled melt was dissolved in water (10 ml.) and filtered free of a blue impurity. Addition of aqueous potassium bicarbonate to the filtrate precipitated the product in crystalline form; washed with water and dried, the yield was 0.3

(16) Melting points are uncorrected. Microanalyses were performed by S. Theodore Bella.

(17) The term "guanyl" is used for the amidine group in accordance with "Chemical Abstracts" usage which has some convenience in naming N-substituted malonamidines.

(18) O. C. Dermer and J. King, *J. Org. Chem.*, **8**, 121 (1943).

g., m.p. 123–125°. The base recrystallized readily from either aqueous alcohol or from ethyl acetate, but the m.p., though sharp, was not easily reproducible and fell in the range 120–130°. A characteristic picrolonate was obtained in aqueous alcohol with m.p. 243–244°.

*Anal.* Calcd. for $C_{21}H_{20}O_6N$: C, 52.50; H, 4.20. Found: C, 52.72; H, 4.34.

The same substance (identified by mixed m.p. of bases and picrolonates) was obtained from the riboside XI after refluxing with 5 *N* ethanolic hydrogen chloride followed by appropriate isolation.

**1-Benzylhypoxanthine** (VIII).—4(5)-Amino-5(4)-imidazole-N-benzylcarboxamide was converted to the intermediate formamido derivative by heating the amine (0.15 g.) in 98% formic acid (2 ml.) and acetic anhydride (0.5 ml.) for three hours in a water-bath, removing the reagents *in vacuo*, and crystallizing the residue by addition of water. There was obtained 0.14 g., m.p. 175–177°. The formamido compound was refluxed for 90 minutes in formamide (3 ml.). When the major portion of the solvent was removed *in vacuo*, the residual sirup crystallized, was thinned with water and filtered, yielding 69 mg. of product. Recrystallization from 95% ethanol had little effect on the m.p., 268–270°.

*Anal.* Calcd. for $C_{12}H_{10}N_4O$: C, 63.70; H, 4.46; N, 24.77. Found: C, 63.52; H, 4.73; N, 25.25.

The same base (mixed m.p.) was obtained from 1-benzylinosine (III) after refluxing one hour in 5 *N* ethanolic hydrogen chloride followed by appropriate isolation.

**5-Amino-4-imidazolecarboxamide β-Ribofuranoside** (II).—It is advisable to adhere to the following conditions closely or over-reduction will occur with considerable loss of material. A piece of metallic sodium (0.65 g.) was cut into small chips which were added all at once to liquid ammonia (100 ml.) at −60° in an apparatus which provided stirring and protection from moisture. After 15 minutes, 5-amino-4-imidazole-N-benzylcarboxamide riboside (1.0 g.) was added to the blue solution. Six minutes later, powdered ammonium chloride (1.0 g.) was introduced to discharge the blue color. After evaporation of the ammonia, the residue was desiccated *in vacuo* over concd. sulfuric acid to remove last traces of the solvent and finally dissolved in water (40 ml.) for chromatography. This purification was based on the isolation of this substance by Greenberg and Spilman.[3] One-half of the solution was applied to a column (3 × 34 cm.) of Dowex 50 (200–400 mesh, 4% cross-linked) ammonium form, which had been washed with water. Elution was continued with water. After an alkaline band[19] (first 200 ml.), the eluate became neutral and the product, detectable by the Bratton–Marshall test,[15] emerged in a sharp band of about 100-ml. volume. The column retained some starting material which could be eluted with 0.01 *N* ammonium hydroxide and recovered if desired (about 20%).

The chromatographic fractions containing aminoimidazolecarboxamide riboside from both halves of a reduction product were combined and taken to dryness. The residual glass eventually crystallized; 0.12–0.14 g. was obtained, a yield of about 20%, based on unrecovered N-benzylamide. The recrystallized nucleoside did not depress the m.p. of an authentic sample kindly provided by Dr. G. R. Greenberg[3]; in addition, the infrared spectra of the samples were identical.

(19) Products of further reduction were apparently contained in this fraction. The presence of dihydro-imidazoles was suggested by a study of the ultraviolet absorption spectra to be described later.

NEW YORK, N. Y.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, RUTGERS, THE STATE UNIVERSITY]

# Preparation of a Substituted 1,2-Benzofluorenone: An Unusual Perkin Reaction[1a]

BY JOHN C. GODFREY[1b] AND RODERICK A. BARNES

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The preparation, properties and proof of structure of the δ-lactone of 3-hydroxy-4-(2′-carboxyphenyl)-1,2-benzofluorenone are described. A study of the mechanism of the reaction by which it is produced from phthalic anhydride and homophthalic acid is reported.

## Discussion

The preparation of 2,2′-dicarboxydesoxybenzoin by the pyrolysis of phthalic anhydride and homophthalic acid in the presence of a catalytic amount of sodium acetate has been described by Ephraim,[2] but in these laboratories the yield was found not to exceed *ca.* 50% although a variety of catalyst ratios, temperatures and rates of heating were investigated. Furthermore, the product was invariably accompanied by a small amount of orange by-product which gave a deep red color to the solution obtained by the hydrolysis of the whole product with warm, dilute potassium hydroxide.

In the hope of obtaining an improved yield of uncontaminated product, the procedure employed by Buckles and Bremer[3] in the preparation of stilbene was applied to the present problem. When phthalic anhydride was condensed with homophthalic acid in the presence of acetic anhydride and triethylamine, no 2,2′-dicarboxydesoxybenzoin was obtained. The product which formed in good yield was bright orange needles, m.p. 250°. The structure I was assigned to this compound on the basis of the following evidence.

Elemental analyses were in excellent agreement with the empirical formula $C_8H_4O$. The compound was neutral, and its infrared spectrum exhibited carbonyl absorption at 5.75 and 5.89 μ. Its complex ultraviolet spectrum, $\lambda_{max}$ 226 mμ (log ε 4.60), 267 (4.62), 320 (4.32) and 397 (3.60), suggested a polynuclear aromatic system. Compound I slowly dissolved on refluxing with dilute, aqueous potassium hydroxide, giving an intensely purple solution. Acidification of the hydrolysate precipitated red-orange prisms (II), which reverted to I above 170°. The infrared spectrum of II showed general, diffuse absorption in the 3 μ region characteristic of carboxylic acids and phenols, and a single, intense carbonyl band at 5.95 μ. The two expected carbonyl absorptions are undoubtedly superimposed. Potentiometric titration of II gave $pK_1$ 6.75 and $pK_2$ 9.75, and an equivalent weight of 187 (calcd. 183 for II). Comparison of the $pK$ values with a large number of dicarboxylic acids and phenols[4]

(1) (a) Presented before the Division of Organic Chemistry, Second Delaware Valley Regional Meeting, American Chemical Society, Philadelphia, Pa., February 5, 1958. (b) Smith, Kline and French Postdoctoral Fellow, 1955–1957.

(2) J. Ephraim, *Ber.*, **24**, 2820 (1891).

(3) R. E. Buckles and K. Bremer, *Org. Syntheses*, **33**, 70 (1953).

(4) N. A. Lange and G. M. Forker, "Handbook of Chemistry," 8th edition, Handbook Publishers, Inc., Sandusky, Ohio, 1952, pp. 1229–1233.