with a sealed stirrer, a special dropping funnel (Ace Glass Cat. No. 7347), and a condenser with an attached calcium chloride tube. However, the reactants were exposed for a short time to the laboratory atmosphere when they were transferred to the reaction vessel. Moreover, the amine hydrochloride formed in the reaction was removed by filtration without any attempt to prevent the ingress of moisture. It appears that the phosphonamidic anhydrides must be formed by traces of water inadvertently introduced from the laboratory atmosphere. The following reaction sequence is suggested

$$p\text{-}O_2NC_6H_4POCl_2 + 2R_2NH \longrightarrow p\text{-}O_2NC_6H_4PO(NR_2)Cl + R_2NH_2Cl \quad (1)$$

$$2\ p\text{-}O_2NC_6H_4PO(NR_2)Cl + H_2O + 2R_2NH \longrightarrow [p\text{-}O_2NC_6H_4PO(NR_2)]_2O + 2R_2NH_2Cl \quad (2)$$

This hypothesis is consistent with the fact that we have obtained widely varying yields of anhydride in duplicate runs. However, the deliberate introduction of water (0.5 mole per mole of phosphonic dichloride) during the condensation has given us materials which we have been unable to purify. The addition of water 0.5 hour or 20 hours after the reaction mixture had been refluxed failed to give larger yields of phosphonamidic anhydrides. With diethylamine and diisopropylamine the reaction has been studied under a variety of experimental conditions. With diethylamine a total of 16 and with diisopropylamine a total of 24 reactions were run. We have not found reaction conditions whereby the phosphonamidic anhydrides can be prepared in reproducible yields.

When *p*-nitrophenylphosphonic dichloride was condensed with diethylamine in acetone, we did succeed in obtaining the corresponding diamide in two experiments (*cf.* Table I). However, in other experiments in which acetone was used, we isolated only the phosphonamidic anhydride. Further work obviously is required to determine why different results are obtained under apparently identical reaction conditions. The reaction between *p*-nitrophenylphosphonic dichloride and dipropylamine, diisopropylamine, diisobutylamine or 2-methylpiperidine yielded phosphonamidic anhydrides; no diamide was ever isolated from these reactions.

Experimental

The preparation of *p*-nitrophenylphosphonic dichloride has been described previously.[3] Diisopropylamine was kindly furnished by Carbide and Carbon Chemicals Co. Ethylenimine was purchased from the Chemirad Corporation. The other amines were obtained from the Eastman Kodak Co. With the exception of ethylenimine, all the amines were dried over Drierite and fractionated before use. The solvents used in the condensations were also dried over Drierite and fractionated.

The Condensation of *p*-Nitrophenylphosphonic Dichloride with Secondary Amines.—The method used for preparing both the diamides and the phosphonamidic anhydrides was similar to procedure 1 as previously described.[3] An example of the preparation of a phosphonamidic anhydride (N,N-diethyl-P-(*p*-nitrophenyl)-phosphonamidic anhydride) is given below. The synthesis of P-(*p*-nitrophenyl)-phosphonic diaziridide and N,N-diisopropyl-P-(*p*-nitrophenyl)-phosphonamidic anhydride differs somewhat from the general procedure; the preparation of these two compounds is described below in detail.

P-(*p*-Nitrophenyl)-phosphonic Diaziridide.—A solution of 4.70 g. of *p*-nitrophenylphosphonic dichloride in 150 ml. of carbon tetrachloride was added to an ice-cold solution of 4.5 g. (excess) of anhydrous ethylenimine in 50 ml. of carbon tetrachloride. When all the acid chloride had been added, the mixture was allowed to stand overnight at room temperature. A white precipitate was removed by filtration, washed with carbon tetrachloride and discarded. When the filtrate and washings were concentrated *in vacuo* to about 10 ml., crystals were obtained which were washed with about 10 ml. of ether. These crystals after drying in a desiccator gave satisfactory analytical values for the desired diamide.

N,N-Diethyl-P-(*p*-nitrophenyl)-phosphonamidic Anhydride.—To a stirred solution of 8.9 ml. of diethylamine in 25 ml. of acetone was added 5.18 g. of *p*-nitrophenylphosphonic dichloride dissolved in 100 ml. of acetone. The mixture was refluxed gently for 1 hour and then allowed to stand overnight at room temperature. The diethylamine hydrochloride was removed by filtration and washed with acetone. The combined filtrate and washings were evaporated to dryness (either on the steam-bath or at room temperature), and the residue was recrystallized from aqueous alcohol.

N,N-Diisopropyl-P-(*p*-nitrophenyl)-phosphonamidic Anhydride.—This compound was prepared by a procedure similar to that used for the other phosphonamidic anhydrides. Acetone was used as the solvent for the condensation. (No phosphonamidic anhydride was obtained when carbon tetrachloride was used as the solvent.) After the reaction mixture was allowed to stand overnight, the precipitate was removed by filtration and washed with water until the washings were free of chloride ion. The residue consisted of the analytically pure phosphonamidic anhydride. A second crop was obtained by evaporating the acetone mother liquors to dryness and recrystallizing the residue from 95% ethanol.

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Quinoxaline Studies. VII. A Quinoxaline Analog of Pteroic Acid

BY JOHN DRUMHELLER[1] AND HARRY P. SCHULTZ

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Martin, *et al.*,[2] found N-(4-[(4-quinazolyl)-amino]-benzoyl)-glutamic acid to be a microbiological growth factor with a potency of from 0.01 to 0.1 that of pteroylglutamic acid. More recently Leese and Rydon[3] reported the synthesis of two quinoxaline analogs of pteroic acid, 4-[2-quinoxalyl)-methylamino]-benzoic acid and 4-[(3-hydroxy-2-quinoxalyl)-methylamino]-benzoic acid. The purpose of this note is to report the synthesis of 4-[(2-quinoxalyl)-amino]-benzoic acid.

2-Hydroxyquinoxaline was prepared by the methods of Goldweber and Schultz[4] as well as Gowenlock, *et al.*[5] This was transformed into 2-chloroquinoxaline according to the procedure of Gowenlock, *et al.*[5] Attempts to prepare 2-chloroquinoxaline in one step by direct reaction between

(1) Abstracted from the M.S. thesis of John Drumheller, The University of Miami.

(2) G. J. Martin, J. Moss and S. Avakian, *J. Biol. Chem.*, **167**, 737 (1947).

(3) C. L. Leese and H. N. Rydon, *J. Chem. Soc.*, 308 (1955).

(4) M. Goldweber and H. Schultz, THIS JOURNAL, **76**, 287 (1954).

(5) A. H. Gowenlock, G. T. Newbold and F. S. Spring, *J. Chem. Soc.*, 622 (1945).

chloral and *o*-phenylenediamine in non-aqueous media, such as ethanol, benzene and carbon tetrachloride, failed to give the desired product. However, reaction between chloral hydrate and *o*-phenylenediamine in water gave 2-chloroquinoxaline in 2% yield.

All attempts to obtain 4-[(2-quinoxalyl)-amino]-benzoic acid by condensing 2-chloroquinoxaline with *p*-aminobenzoic acid failed. Condensation was effected between ethyl *p*-aminobenzoate and 2-chloroquinoxaline in acidic aqueous solution to give ethyl 4-[(2-quinoxalyl)-amino]-benzoate, which was saponified to the desired 4-[(2-quinoxalyl)-amino]-benzoic acid.

The latter material was found to contain less than 0.01% of folic acid activity per mg., as determined by the *S. faecalis* titrimetric assay.[6]

Experimental Procedures

Ethyl 4-[(2-Quinoxalyl)-amino]-benzoate.—A mixture of 0.66 g. (0.004 mole) of 2-chloroquinoxaline, 0.66 g. (0.004 mole) of ethyl *p*-aminobenzoate and 0.4 ml. of concentrated hydrochloric acid in 35 ml. of water was refluxed for 0.5 hour, then cooled.

After cooling and acidifying to *p*H 1, the precipitate was filtered and dried to give 0.68 g. of crude, orange material melting at 208–214°. This material was twice recrystallized from ethanol–water (1:1), treating with charcoal, to give 0.45 g. (38.4% of theory) of pale yellow needles melting at 220–221°.

Anal. Calcd. for $C_{17}H_{15}O_2N_3$: N, 14.32. Found: N, 14.48, 14.43. Absorption maxima, mμ, and molecular extinction coefficient ($\epsilon \times 10^{+3}$) 95% ethanol: 237, inf. (15.4); 306 (38.2); 374 (11.2).

4-[(2-Quinoxalyl)-amino]-benzoic Acid.—One hundred milliliters of 2 *N* sodium hydroxide solution and 0.45 g. (0.00153 mole) of ethyl 4-[(2-quinoxalyl)-amino]-benzoate were refluxed for four hours, until all the ester had dissolved. The solution was treated with charcoal and filtered hot. The filtrate was adjusted to *p*H 4 with acetic acid and the yellow, gelatinous precipitate filtered and washed with hot water. The material was twice reprecipitated from dilute sodium carbonate solution to give 0.35 g. (86% of theory) of orange powder, decomposing at 340°, with some sublimation at 290°.

Anal. Calcd. for $C_{15}H_{11}O_2N_3$: N, 15.85. Found: N, 15.81, 16.03. Absorption maxima, mμ, and molecular extinction coefficients ($\epsilon \times 10^{+3}$), 95% ethanol: 237, inf. (12.7); 303 (30.6); 377 (14.2); 0.1 *N* sodium hydroxide: 237, inf. (14.4); 296 (28.4); 379 (12.1).

(6) The authors are grateful to Dr. K. Folkers and his associates of Merck and Co., Inc., for assaying the 4-[(2-quinoxalyl)-amino]-benzoic acid.

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On the Stereochemistry of the Isocitric and Alloisocitric Lactones

BY OSCAR GAWRON AND ANDREW J. GLAID, III

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The stereochemistry of the α-carbon of the isocitric and alloisocitric lactones recently has been elucidated by Greenstein and co-workers.[1,2] As yet, however, no stereochemical information is available concerning the β-carbon of these lactones.

As part of a program on the stereochemistry of these lactones, pK' values have been determined for DL-isocitric lactone and for DL-alloisocitric lactone. In this publication we are reporting these values and the stereochemical conclusions which can be drawn from them.

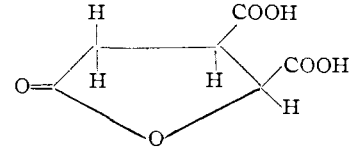
Potentiometric titration of DL-isocitric lactone yielded a pK_1' of 2.26 ± 0.03 and a pK_2' of 4.50 ± 0.03. Titration of DL-alloisocitric lactone yielded corresponding values of 2.13 ± 0.03 and 3.95 ± 0.03.

cis and *trans* configurations of adjacent carboxyls on a ring system or about a double bond may be differentiated on the basis of relative difference in pK_1 and pK_2 values, the *cis* configuration being assigned to that isomer for which the pK_2 value differs most from the pK_1 value.[3,4]

Applying this principle to the isocitric and alloisocitric lactones, the *cis* configuration may be assigned to DL-isocitric lactone, pK_1' being 180 times greater than pK_2' and the *trans* configuration to DL-alloisocitric lactone, pK_1' being 65 times greater than pK_2'.

Since the α-carboxyls are closest to the electronegative lactone grouping, the pK_1' values might be assigned to these carboxyls. Consequently, the pK_2' values would be assigned to the β-carboxyl groups. This assignment is usual[5] for electronegative substituents. The possibility of hydrogen bonding between the α-carboxyl and the ether oxygen of the lactone ring altering this assignment has not been considered here since the argument with regard to *cis* and *trans* configuration is not altered by assignment of a particular pK_a value to a particular carboxyl group.

On the basis of the *cis* configuration of the carboxyl groups of DL-isocitric lactone, structure I can be considered as a perspective drawing of a Fisher–Hirschfelder model[6] of one of the antipodes of DL-isocitric lactone, the α_{Ls}-isomer. Structure II, perforce, is a perspective drawing of α_{Ls}-alloisocitric lactone.[7]



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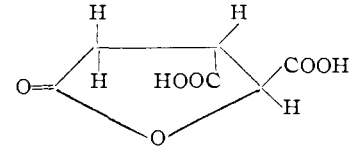

II

An assignment of optical configuration may now be made with regard to the β-carbon of structures I

(1) J. P. Greenstein, N. Izumiya, M. Winitz and S. M. Birnbaum, THIS JOURNAL, **77**, 707 (1955).

(2) M. Winitz, S. M. Birnbaum and J. P. Greenstein, *ibid.*, **77**, 716 (1955).

(3) L. F. Fieser and M. Fieser, "Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1950, p. 281.

(4) For example, R. Kuhn and A. Wasserman, *Helv. Chim. Acta*, **11**, 50 (1928), found a pK_1 of 4.34 and a pK_2 of 6.76 for *cis*-1,2-dicarboxycyclohexane and for *trans*-1,2-dicarboxycyclohexane a pK_1 of 4.18 and a pK_2 of 5.93.

(5) G. E. K. Branch and M. Calvin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1949, p. 217.

(6) With the usual models, the lactone rings are quite strained.

(7) We have slightly modified the nomenclature of Reference 2 for the purposes of this paper.