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

RECEIVED JULY 25, 1955

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.

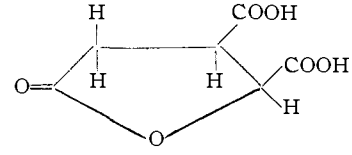
Potentiometric titration of DL-isocitric lactone yielded a pK_1' of 2.26 ± 0.03 and a pK_2' of 4.50 ± 0.03. Titration of DL-alloisocitric lactone yielded corresponding values of 2.13 ± 0.03 and 3.95 ± 0.03.

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]

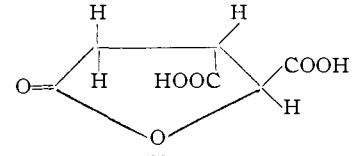
Applying this principle to the isocitric and alloisocitric lactones, the *cis* configuration may be assigned to DL-isocitric lactone, pK_1' being 180 times greater than pK_2' and the *trans* configuration to DL-alloisocitric lactone, pK_1' being 65 times greater than pK_2'.

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]



I



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.

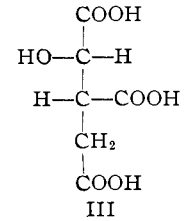
(5) G. E. K. Branch and M. Calvin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1949, p. 217.

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

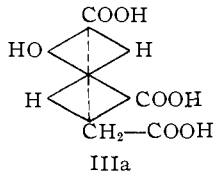
(7) We have slightly modified the nomenclature of Reference 2 for the purposes of this paper.

and II. Considering the β-carboxyl group as a substituent such as the β-hydroxyl of threonine, the β-carbon of I, then, by virtue of its carboxyl being *cis* to the α-carboxyl, is Ds and the β-carbon of II is Ls.

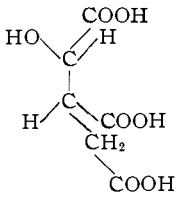
If the lactone ring of I is opened with base, isocitric acid of structure III or the equivalent structures IIIa, IIIb and IIIc is formed.



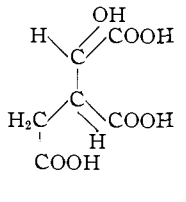
III



IIIa



IIIb



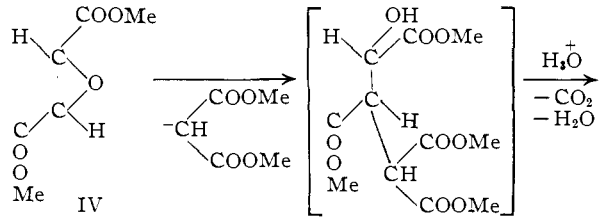
IIIc

Since it has been shown that *d*-isocitric acid, the naturally occurring isomer, has the α_{Ls} configuration and that this isomer constitutes one of the enantiomorphs of DL-isocitric lactone,[2] structures III, IIIa, IIIb and IIIc then represent this isomer and it may be designated as α_{Ls}-β_{Ds}-isocitric acid. The α_{Ls}-allo isomer may then be designated as α_{Ls}-β_{Ls}-alloisocitric acid. The corresponding lactones would then be α_{Ls}-β_{Ds}-isocitric lactone and α_{Ls}-β_{Ls}-alloisocitric lactone.

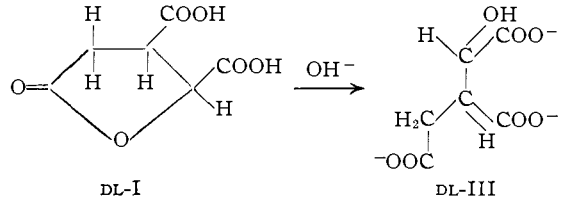
The above conclusions albeit reasonable, if not final, rest only upon the assignment of *cis* and *trans* configurations to the two racemates on the basis of the potentiometric data reported here. In view of this and in view of the biochemical implications, several of which are mentioned below, other proofs of *cis* and *trans* configuration should be sought.

In connection with this, preliminary results of a synthesis[8] designed to yield one of the two racemates may be mentioned here.

When DL-*trans*-dicarbomethoxyethylene oxide (IV) was treated with sodiomalonic ester and followed by acid saponification, decarboxylation and lactonization, a semi-crystalline mass was obtained from which a barium salt was isolated. Analysis of this salt showed it to contain 51.6% barium and 18% DL-isocitrate[9]; calculated for tribarium DL-isocitrate, 52.8% barium and 47.2% DL-isocitrate. While the reaction pathway, assuming a *trans* opening of the oxide ring[10] as can be seen from the accompanying flow sheet, is expected to lead to DL-isocitrate, the low yield of this product does not permit any definite stereochemical conclusions at present.

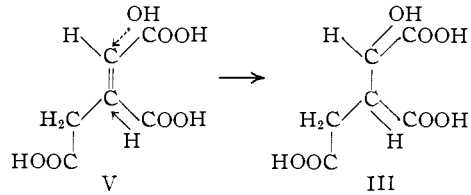


IV



DL-I DL-III

One of the biochemical implications of structure III as representing the natural isomer, *d*-isocitric acid, is to be found in the *cis*-aconitase system. As yet, it is not known if hydration of *cis*-aconitic acid to *d*-isocitric acid proceeds *via cis* or *trans* addition of water across the double bond. In order to obtain structure III from *cis*-aconitic acid (V) a hydroxyl group must approach from the rear and a proton from the front. Accordingly, the hydration catalyzed by *cis*-aconitase must proceed in a *trans* fashion.



V III

Of further biochemical interest in this connection is the recently reported isocitritase-catalyzed condensation of glyoxylic acid and succinic acid to yield isocitric acid.[11] The configuration about the two assymmetric carbon atoms formed in this condensation now can be seen to be the same as the configuration of the two asymmetric carbons formed by the well-known aldolase catalyzed condensation of dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate to yield fructose-1,6-diphosphate.

Experimental

DL-Isocitric Lactone.—This compound, prepared by the procedure of Kato and Dickman,[12] melted at 160–161° and gave a satisfactory neutral equivalent.

DL-Alloisocitric Lactone.—This compound was prepared by the epimerization procedure of Senear[13] using identical quantities as outlined in this author's paper. Employing twice recrystallized DL-isocitric lactone, m.p. 160–161°, the pyridinium salt of DL-alloisocitric lactone crystallized upon scratching, after standing for 24 hours in the refrigerator. Subsequent steps gave the same results obtained by Senear,[13] although three recrystallizations were necessary to give

(8) This synthesis was originally designed to yield one of the four stereoisomers of the lactones by starting with one of the enantiomorphs of DL-*trans*-dicarboxyethylene oxide; O. Gawron, K. C. Schreiber and A. Manyak, unpublished work. Support of this project by research grant G3590 from the National Institutes of Health, U. S. Public Health Service, is herein acknowledged.

(9) Analyzed as the potassium salt for *d*-isocitrate by the enzymatic method of A. Meister and C. G. Baker, *Arch. Biochem. Biophys.*, **31**, 460 (1951).

(10) Malonate anion opening of oxide rings proceeds via a *trans* opening of the ring. S. Winstein and R. B. Henderson in R. C. Elderfield's "Heterocyclic Compounds," John Wiley and Sons, Inc., New York, 1950, vol. 1, p. 31.

(11) R. A. Smith, J. J. R. Campbell and I. C. Gunsalus, Abstracts of the 126th Meeting of the American Chemical Society, September, 1954, p. 75c.

(12) H. P. Kato and S. R. Dickman in "Biochemical Preparations," John Wiley and Sons, Inc., New York, N. Y., 1953, Vol. 3, p. 52.

(13) A. E. Senear, THIS JOURNAL, **77**, 2564 (1955). We wish to thank Dr. Senear for correspondence concerning this procedure.

material melting at 154–156°.[14,15] After neutralization of the free carboxyls, a neutral equivalent of 177 was obtained, calculated 174.1.

Potentiometric Titrations.—Fifty to 100 mg. of a particular lactone was dissolved in 10 ml. of water and titrated at 28° under nitrogen with standard 0.1 N base, using a model G Beckman pH meter and external electrodes.

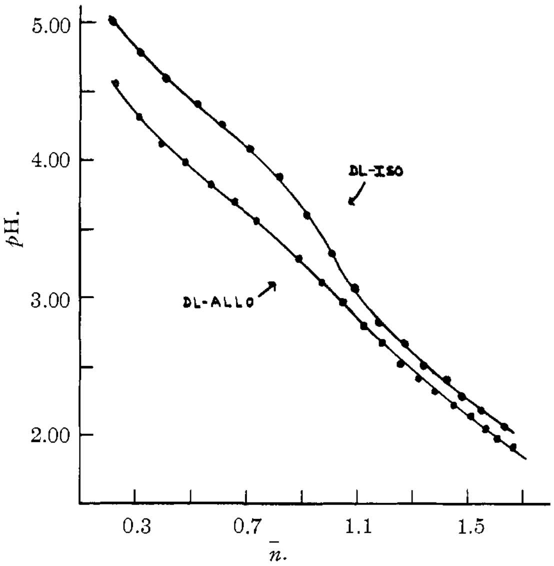


Fig. 1.—Potentiometric titration of 59.1 mg. of DL-alloisocitric lactone (neut. equiv. 88.2, theory 87.1) and of 64.8 mg. of DL-isocitric lactone (neut. equiv. 86.3, theory 87.1).

pK_A values were obtained from a plot (Fig. 1) of pH $vs.$ $\bar{n}$, $\bar{n}$ being defined as[16]

$$\bar{n} = \frac{2(\mathrm{TA}) - (\mathrm{NaOH}) - (\mathrm{H}^+) + (\mathrm{OH}^-)}{(\mathrm{TA})} = \frac{(K_1/(H^+)) + 2}{1 + \frac{K_1}{(\mathrm{H}^+)} + \frac{K_1K_2}{(\mathrm{H}^+)^2}}$$

where

(TA) = concentration of added lactone
(NaOH) = concentration of added base
(H^+) = hydrogen ion concentration as calculated from the pH measurement
(OH^-) = hydroxyl ion concentration as calculated from K_W and the hydrogen ion concentration

At $\bar{n}$ equal to 1.5, the pH is equal to pK_1 and at $\bar{n}$ equal to 0.5, pH is equal to pK_2.

(14) Senear[13] reports 157–158.5°.

(15) Greenstein, *et al.*,[1] report 153°.

(16) Using the Bjerrum treatment and considering H^+ as the ligand. For details of this treatment see A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds," Prentice-Hall, Inc., New York, N. Y., 1952, p. 78.

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Dialkoxyalkanenitriles. III. Hydrogenation to α-Amino Acetals

BY JOHN G. ERICKSON,[1] W. H. MONTGOMERY AND OLE RORSO

RECEIVED AUGUST 11, 1955

Of the several methods described for the preparation of acetals, the one involving reaction of an acetal of an α-haloaldehyde with ammonia or amines usually has been employed.[2] No ketone acetals appear to have been used in this reaction. The reaction also has been carried out with alkali metal derivatives of aniline and alkylanilines.[3] One disadvantage of the synthesis from ammonia and chloroacetal is the considerable amount of corrosion which may accompany the reaction unless the vessel is constructed of the proper material. It may also be difficult to obtain a pure product, since chloroacetal, aminoacetal and hydroxyacetal cannot be separated very well by distillation.

There are two less important methods of preparing α-amino acetals. One is the reduction of glycine esters with sodium amalgam and subsequent conversion of the aminoacetaldehyde so formed into its acetals.[4] The other is the reduction of acetals of nitroacetaldehyde with sodium and alcohol.[5]

We have found that hydrogenation of 2,2-dialkoxyalkanenitriles gives α-amino acetals (I) in good yields. Since the nitrile group is converted

$$(\mathrm{RO})_2\underset{\mathrm{R'}}{\underset{|}{\mathrm{C}}}\mathrm{CN} \xrightarrow{\mathrm{H_2}} \mathrm{H_2NCH_2}\underset{\mathrm{R'}}{\underset{|}{\mathrm{C}}}(\mathrm{OR})_2$$

I. R = alkyl
R' = H, alkyl or aryl

to an aminomethyl group, this method can give acetals of aminoacetaldehyde and a wide variety of α-aminomethyl ketones. We used it to prepare acetals of aminoacetaldehyde, aminoacetone and α-aminoacetophenone. By hydrogenation in the presence of primary and secondary amines, N-substituted α-amino acetals could be prepared. This phase has not been studied thoroughly.

It has been shown[6] that the cyano group in dialkoxyalkanenitriles is more reactive toward dicyandiamide than it is in most other nitriles. This appears to be true in hydrogenations also. The hydrogenation reaction takes place readily under moderate conditions, giving, in general, good yields of amino acetals—from 65 to 90%. No unreacted nitrile survives to complicate the purification of the amino acetal. When no ammonia is added to the reaction mixture, the usual

(1) Research Department, General Mills, Inc., Minneapolis, Minn.

(2) (a) A. Wohl, *Ber.*, **21**, 616 (1888); **39**, 1951 (1906); (b) L. Wolff, *ibid.*, **21**, 1481 (1888); (c) W. Marckwald, *ibid.*, **25**, 2354 (1892); (d) R. Burtles, F. L. Pyman and J. Roylance, *J. Chem. Soc.*, **127**, 581 (1925); (e) F. A. Mason, *ibid.*, **127**, 1032 (1925); (f) W. H. Hartung and H. Adkins, THIS JOURNAL, **49**, 2517 (1927); (g) J. Boeseken and B. B. C. Felix, *Ber.*, **62**, 1311 (1929); (h) J. S. Buck and S. N. Wrenn, THIS JOURNAL, **51**, 3612 (1929); (i) A. Kirrmann, M. Goudard and M. Chahidzadeh, *Bull. soc. chim.*, **2**, 2143 (1935); (j) H. Albers, R. Kallischnigg and A. Schmidt, *Ber.*, **77**, 617 (1944); (k) R. B. Woodward and W. E. Doering, THIS JOURNAL, **67**, 860 (1945); (l) J. P. Fourneau and S. Chantalou, *Bull. soc. chim.*, **12**, 845 (1945); (m) H. H. Richmond and G. F. Wright, *Can. J. Research*, **23D**, 158 (1945); (n) E. F. J. Janetzky, P. E. Verkade and W. Meerburg, *Rec. trav. chim.*, **66**, 312 (1947); (o) C. F. H. Allen and J. H. Clark, *Org. Syntheses*, **24**, 3 (1944); (p) I. A. Kaye and I. Minsky, THIS JOURNAL, **71**, 2272 (1949); (q) R. S. Sweet, U. S. Patent 2,490,385 (Dec. 6, 1949); (r) R. G. Jones, E. C. Kornfeld, K. C. McLaughlin and R. C. Anderson, THIS JOURNAL, **71**, 400 (1949); (s) S. Senda, *J. Pharm. Soc. Japan*, **71**, 601 (1951).

(3) E. F. J. Janetzky, P. E. Verkade and W. Meerburg, *Rec. trav. chim.*, **66**, 317 (1947).

(4) E. Fischer, *Ber.*, **41**, 1019 (1908).

(5) M. S. Losanitsch, *ibid.*, **42**, 4044 (1909).

(6) V. P. Wystrach and J. G. Erickson, THIS JOURNAL, **75**, 6345 (1953).