NOTES

Some Derivatives of 4′-Hydroxydiphenylamine-4-carboxylic Acid

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In the course of studying various structural analogs of the thyroid hormones, a few diphenylamine derivatives were prepared with the hope that they might compete with oxidations of the hormones to quinonoid structures.[2] Their synthesis is reported below.

Experimental[3]

4-Benzoyloxydiphenylamine.—All attempts to prepare this compound with benzoyl chloride[4] furnished only the dibenzoyl derivative. Consequently, 260 g. (1.4 moles) of 4-hydroxydiphenylamine, 375 g. (1.65 moles) of benzoic anhydride and 250 ml. of dry pyridine were heated on a steam-bath for 6 hours, the cooled mixture was acidified with cold 50% sulfuric acid and filtered. The brownish residue was washed with 2% sodium hydroxide solution and water, excess benzoic anhydride was ethanolized, and the ethanolic solution was diluted with water. The resulting precipitate crystallized from dilute ethanol as pale-yellow leaflets, m.p. 112–114°. The yield was 330 g. (81%).

Anal. Calcd. for $C_{19}H_{15}NO_2$: C, 78.87; H, 5.26. Found: C, 78.73; H, 5.11.

4-Benzoyloxy-4′-cyanodiphenylamine.—A suspension of 40 g. (0.108 mole) of 4-benzoyloxy-4′-bromodiphenylamine[5] and 16 g. (0.172 mole) of cuprous cyanide in 240 ml. of dry quinoline was refluxed for 6 hours, the red solution was cooled and poured with rapid stirring into 200 ml. of ice-cold 37% hydrochloric acid. The precipitate was filtered, washed and recrystallized from benzene–ligroin. The almost colorless needles (17.5 g., 51%) had m.p. 178.5–180.5°.

Anal. Calcd. for $C_{20}H_{14}N_2O_2$: C, 76.41; H, 4.49. Found: C, 76.51; H, 4.50.

Hydrolysis with hot 5% ethanolic potassium hydroxide solution for 30 minutes gave a 71% yield of **4-cyano-4′-hydroxydiphenylamine**, m.p. 193–194.5° after recrystallization from dilute ethanol.

Anal. Calcd. for $C_{13}H_{10}N_2O$: C, 74.27; H, 4.79. Found: C, 74.37; H, 4.71.

4-Cyano-4′-methoxydiphenylamine, obtained with diazomethane, crystallized from aqueous acetone, m.p. 99–100°.

Anal. Calcd. for $C_{14}H_{12}N_2O$: C, 74.98; H, 5.40. Found: C, 74.82; H, 5.27.

4-Methoxydiphenylamine-4′-carboxylic acid was prepared in 43% yield by boiling the nitrile with 15% ethanolic potassium hydroxide for 20 hours. It crystallized from methanol, m.p. 165–167°. It was also obtained by hydrolysis of methyl 4-methoxydiphenylamine-4′-carboxylate with 10% sodium hydroxide solution.

Anal. Calcd. for $C_{14}H_{13}NO_3$: C, 69.12; H, 5.39. Found: C, 68.78; H, 5.78.

4-Hydroxydiphenylamine-4′-carboxylic Acid.—A solution of 5 g. of 4-cyano-4′-hydroxydiphenylamine in 40 ml. of ethylene glycol containing 6 g. of potassium hydroxide was refluxed for 3 hours, cooled and acidified. A brown precipitate was filtered and recrystallized from methanol with the aid of Darco. The colorless product weighed 3.56 g. (65%), m.p. 229–230° dec.[6] It turned pink in the air.

Anal. Calcd. for $C_{13}H_{11}NO_3$: C, 68.11; H, 4.83. Found: C, 67.81; H, 4.90.

Methylation with diazomethane gave **methyl 4-methoxydiphenylamine-4′-carboxylate**, which crystallized from ether–ligroin, m.p. 91.5–93.5°.

Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88. Found: C, 69.76; H, 5.94.

3,5-Dichloro-4-hydroxy-4′-cyanodiphenylamine.—When 0.1 mole of 4-hydroxy-4′-cyanodiphenylamine was treated with 0.4 mole of iodine monochloride according to the general procedure of Willgerodt and Arnold,[7] a pink powder was obtained which turned blue in the air. Repeated crystallization from ether–ligroin gave a 30% yield of almost transparent colorless needles, m.p. 215–216°.

Anal. Calcd. for $C_{13}H_8Cl_2NO$: C, 55.93; H, 2.89; Cl, 25.41. Found: C, 55.64; H, 3.00; Cl, 25.25.

This unexpected chlorination with iodine chloride has its counterpart in the chlorination of 2,6-dinitro-4-methyl-4′-hydroxydiphenylamine with the same reagent.[6]

(1) National Institutes of Health Fellow, 1952–1953.

(2) C. Niemann and C. E. Redeman, THIS JOURNAL, **63**, 1549 (1941); C. Niemann and J. F. Mead, *ibid.*, **63**, 2683 (1941).

(3) All melting points are corrected. All hydrolyses were carried out in an inert atmosphere.

(4) A. E. Smith and K. J. P. Orton, *J. Chem. Soc.*, **93**, 314 (1908).

(5) A. E. Bradfield, L. H. N. Cooper and K. J. P. Orton, *ibid.*, 2854 (1927).

(6) This acid had been prepared by R. C. Cookson, *ibid.*, 643 (1953), by a different route.

(7) C. Willgerodt and E. Arnold, *Ber.*, **34**, 3343 (1901).

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The Evaluation of the Kinetic Constants of Enzyme-catalyzed Reactions by Procedures Based upon Integrated Rate Equations. II[1]

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Enzyme-catalyzed reactions that can be represented by equations 1, 2 and 3 are of sufficient

$$E_f + S_f \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E_f + P_{1f} + P_{2f} \cdots \quad (1)$$

$$E_f + P_{1f} \underset{k_5}{\overset{k_4}{\rightleftharpoons}} EP_1 \quad (2)$$

$$E_f + P_{2f} \underset{k_7}{\overset{k_6}{\rightleftharpoons}} EP_2 \quad (3)$$

general interest as to encourage the continued development of more reliable and convenient methods for the evaluation of the kinetic constants of such reactions.

For zone A conditions[3–5] a reaction represented by equations 1, 2 and 3 can be formulated in terms of equation 4 where $k_3' = k_3K_P/(K_P - K_S)$,

$$-d[S]/dt = k_3'[E][S]/(K_S' + [S]) \quad (4)$$

$K_S' = K_S(K_P + [S]_0)/(K_P - K_S, K_S = (k_2 + k_3)/k_1, K_P = 1\Big/\sum_{j=1}^{n} 1/K_{Pj}, K_{P1} = k_5/k_4$ and $K_{P2} = k_7/k_6$.

Definite integration of equation 4 to time t followed by rearrangement gives equation 5. It is seen from equation 5 that a

$$\left(\int_0^t [S]dt\right)\Big/([S]_0 - [S]_t) = ((2K_S' + [S]_0)/2k_3'[E]) + ([S]_t/2k_3'[E]) \quad (5)$$

(1) Supported in part by a grant from Eli Lilly and Co.

(2) To whom inquiries regarding this article should be sent.

(3) O. H. Straus and A. Goldstein, *J. Gen. Physiol.*, **26**, 559 (1943).

(4) A. Goldstein, *ibid.*, **27**, 529 (1944).

(5) R. J. Foster and C. Niemann, THIS JOURNAL, **77**, 1886 (1955).

plot of $\left(\int_0^t [S]dt\right)/([S]_0 - [S]_t)$ *vs.* $[S]_t$ will lead to a series of lines of slope $1/2k_3'[E]$ and ordinate intercept $(2K_S' + [S]_0)/2k_3'[E]$ for various values of $[S]_0$, *cf.*, Fig. 1.

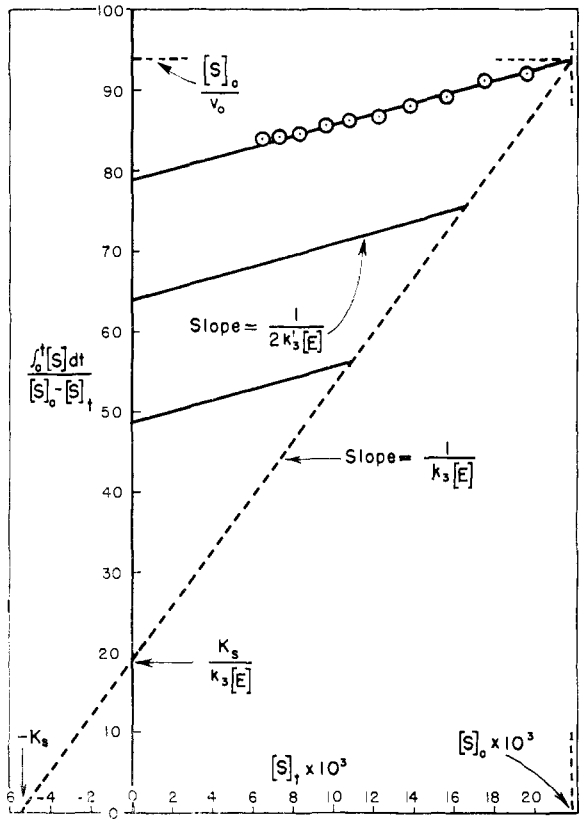

Fig. 1.—α-Chymotrypsin-catalyzed hydrolysis of L-tryptophanhydroxamide in aqueous solutions at 25° and *p*H 6.92 and 0.3 *M* in the THAM component of the THAM–HCl buffer; [E] = 0.0932 mg. protein nitrogen/ml.; $[S]_0$ = 21.8 × 10^{-3} *M*; Δt = 5 min.; $\int_0^t [S]\,dt/([S]_0 - [S]_t)$ in minutes; $[S]_t$ in *M* × 10^3, *i.e.*, moles per liter × 10^3.

For each of the lines of slope $1/2k_3'[E]$ and ordinate intercept $(2K_S' + [S]_0)/2k_3'[E]$ there is a point corresponding to $t = 0$, *i.e.*, when $[S]_t = [S]_0$. These points may be located as before[6-8] by the determination of the limits of the two parameters $\left(\int_0^t [S]dt\right)/([S]_0 - [S]_t)$ and $[S]_t$ as $t \to 0$. Since the limit of $\left(\int_0^t [S]dt\right)/([S]_0 - [S]_t)$ as $t \to 0$ is $[S]_0/(-d[S]/dt) = [S]_0/v_0$ and that of $[S]_t$ as $t \to 0$ is $[S]_0$ it is evident that the points at which the lines of slope $1/2k_3'[E]$ possess abscissa values of $[S]_t = [S]_0$ will be the points where $t = 0$. Furthermore, the initial velocities, *i.e.*, the values of v_0, will be given in terms of $[S]_0/v_0$ by their ordinate parameter $\left(\int_0^t [S]dt\right)/([S]_0 - [S]_t)$ for the condition that $t = 0$.

As the coördinates of the points corresponding to $t = 0$ are, respectively, $[S]_0/v_0$ and $[S]_0$ it follows[8] that a line drawn through these points will describe the behavior of the reaction system when $t = 0$. This line will have a slope of $1/k_3[E]$, an ordinate intercept of $K_S/k_3[E]$ and an abscissa intercept of $-K_S$. With K_S and k_3 so determined K_P may be evaluated from the slope and ordinate intercepts of the parallel lines of slope $1/2k_3'[E]$ and ordinate intercept $(2K_S' + [S]_0)/2k_3'[E]$ and the relation $K_P = k_3'K_S/(k_3' - k_3) = K_S(K_S' + [S]_0)/(K_S' - K_S)$.

The definite integral in equation 5 can be evaluated in several ways. However, it has been found that approximate integration through the use of Simpson's Rule is generally satisfactory when it is used in the form given in equation 6 where *m* is one-

$$\int_0^{2m} [S]dt = (h/3)([S]_0 + 4[S]_1 + 2[S]_2 + 4[S]_3 + 2[S]_4 + \cdots + 4[S]_{2m-1} + [S]_{2m}) - m[S]^{(4)}h^5/90 \quad (6)$$

half the number of intervals over which the integral is being evaluated, *h* is the time interval between successive observations and $[S]^{(4)}$ is a value of the fourth derivative of [S] with respect to *t* at some point between $[S]_0$ and $[S]_t$.[9] In practice the definite integral $\int_0^t [S]dt$ is first evaluated without regard to the contribution of the remainder term of equation 6. From this approximate value of $\int_0^t [S]dt$ for a particular value of $[S]_0$ values of k_3' and K_S' are obtained from a $\left(\int_0^t [S]/dt\right)/([S]_0 - [S]_t)$ *vs.* $[S]_t$ plot. With these values of k_3' and K_S' and the corresponding values of $[S]_0$ and [E] the quantity $m[S]_0^{(4)}h^5/90$ is evaluated to give a maximum estimate of the difference between the actual area under the experimental curve and the area given by equation 6 without regard to the remainder term. If this difference is within the limits of experimental error, as is frequently the case, the above values of k_3' and K_S' may be taken as the final values. However, if the above difference is observed to be greater than that ascribable to experimental error the definite integral is again evaluated with the inclusion of the remainder term obtained as above.

Under the conditions which usually obtain in studies of the α-chymotrypsin-catalyzed hydrolysis of L-tryptophanhydroxamide and acetyl-L-tyrosinhydrazide it has been observed[10] that when Δt is of the order of two to five minutes the remainder term of equation 6 is generally less than 0.5% of the total and therefore is well within the limits of experimental error. Finally, it should be noted that if the use of Simpson's Rule is contemplated for the approximate integration of the integral $\int_0^t [S]dt$ the experimental observations should be made at a constant and closely spaced time interval.

The data which are presented in Fig. 1 relate to the α-chymotrypsin-catalyzed hydrolysis of L-tryptophanhydroxamide in aqueous solutions at 25° and *p*H 6.92 and 0.3 *M* in the THAM[11] component of a THAM–HCl buffer for the condition

(6) R. J. Foster and C. Niemann, *Proc. Natl. Acad. Sci.*, **39**, 999 (1953).

(7) T. H. Applewhite and C. Niemann, THIS JOURNAL, **77**, 4923 (1955).

(8) R. R. Jennings and C. Niemann, *ibid.*, **77**, 5432 (1955).

(9) W. E. Milne, "Numerical Calculus," Princeton University Press, Princeton, N. J., 1949, p. 121.

(10) Unpublished observations of K. A. Booman and W. Lands.

(11) Tris-(hydroxymethyl)-aminomethane.

that [E] = 0.0932 mg. protein-nitrogen/ml., $[S]_0 = 21.8 \times 10^{-3}$ M and $\Delta t = 5.0$ minutes with the reaction as represented by equations 1 to 3 inclusive being allowed to proceed to an extent of approximately 70%. These data when evaluated *via* a $\left(\int_0^t [S]dt\right)\Big/([S]_0 - [S]_t)$ *vs.* $[S]_t$ plot, in which $\int_0^t [S]dt$ was approximated through the use of equation 6 without regard for the remainder term of this equation, gave a value of $k_3' = 7.86 \times 10^{-3}$ M/min./mg. protein nitrogen/ml., a value of $K_S' = 4.69 \times 10^{-3}$ M and a value of $v_0 = 2.32 \times 10^{-4}$ M/min. From the above values of k_3' and K_S' and the other known parameters of the system the quantity $m[S]_0^{(4)}h^5/90$ was evaluated and found to be but 6.86×10^{-6}% of the total area thus providing complete justification for ignoring the remainder term of equation 6 in the evaluation of $\int_0^t [S]dt$ in this particular instance.

It will be recognized that the plot of $\left(\int_0^t [S]dt\right)\Big/([S]_0 - [S]_t)$ *vs.* $[S]_t$ described in this communication has many points in common with the plot of $t/(\ln([S]_0/[S]_t))$ *vs.* $([S]_0 - [S]_t)/(\ln([S]_0/[S]_t))$ described in an earlier communication from these laboratories[8] and that both of these plots are related to the $[S]_0/v_0$ *vs.* $[S]_0$ plot of Lineweaver and Burk.[12] However it should be noted that the latter plot requires the separate evaluation of the initial velocities and even if this operation is performed in an objective manner[13] this plot can be used only for the evaluation of data obtained in the initial stages of a reaction represented by equations 1 to 3 inclusive provided that K_P is substantially greater than K_S. As this latter information is not disclosed by a $[S]_0/v_0$ *vs.* $[S]_0$ plot, or by either of its two variants,[8] it is clear that the use of these three plots is accompanied by some uncertainty in the absence of knowledge of the relative magnitudes of K_P and K_S particularly since it has been observed[14] that with certain but not all specific substrates of α-chymotrypsin K_P may be substantially less than K_S when K_P is evaluated from experiments conducted in the absence of added hydrolysis products.

In principle a $\left(\int_0^t [S]dt\right)\Big/([S]_0 - [S]_t)$ *vs.* $[S]_t$ plot should be equivalent to a $t/(\ln([S]_0/[S]_t))$ *vs.* $([S]_0 - [S]_t)/(\ln([S]_0/[S]_t))$ plot,[8] a $t/([S]_0 - [S]_t)$ *vs.* $(\ln([S]_0/[S]_t))/([S]_0 - [S]_t)$ plot,[8] or a $([S]_0 - [S]_t)/t$ *vs.* $(\ln([S]_0/[S]_t))/t$ plot.[6,7] Therefore, it is of interest to compare the values of k_3', K_S' and v_0 obtained from a $\left(\int_0^t [S]dt\right)\Big/([S]_0 - [S]_t)$ *vs.* $[S]_t$ plot with the comparable values obtained from a $([S]_0 - [S]_t)/t$ *vs.* $(\ln([S]_0/[S]_t))/t$ plot[6,7] using in each instance the same experimental data. It was noted above that the experimental data represented in Fig. 1 gave, on the basis of a $\left(\int_0^t [S]dt\right)\Big/([S]_0 - [S]_t)$ *vs.* $[S]_t$ plot, a value of $k_3' = 7.86 \times 10^{-3}$ M/min./mg. protein nitrogen/ml., a value of $K_S' = 46.9 \times 10^{-3}$ M and a value of $v_0 = 2.32 \times 10^{-4}$ M/min. When the same experimental data were evaluated through the use of a $([S]_0 - [S]_t)/t$ *vs.* $(\ln([S]_0/[S]_t))/t$ plot[6,7] it was found that $k_3' = 8.09 \times 10^{-3}$ M/min./mg. protein nitrogen/ml., $K_S' = 48.8 \times 10^{-3}$ M and $v_0 = 2.33 \times 10^{-4}$ M/min.

In practice it has been observed that for reactions which may be represented by equations 1 to 3 inclusive the plot based upon equation 5 is better suited for treating data that have been obtained during the initial stages of a given reaction, *i.e.*, for a lesser extent of reaction, than are the three alternative plots which are derived by indefinite integration and rearrangement of the common differential rate equation. In addition there is less numerical work involved in evaluating experimental data with the first plot than with the other three. However, as has been noted previously[8] this latter factor of convenience may be outweighed by other considerations.

As all of the plots considered above are useful only when the reaction in question has been allowed to proceed to an extent compatible with the evaluation of K_P, which will be determined not only by the magnitude of K_P but also by the relative magnitudes of K_P and K_S we are now engaged in exploring the possible use of methods involving numerical differentiation since such methods would be useful and desirable in those cases where reactions proceed only at very low velocities and where extended times of observation are not desirable.

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(12) H. Lineweaver and D. Burk, THIS JOURNAL, **56**, 658 (1934).
(13) R. R. Jennings and C. Niemann, *ibid.*, **75**, 4687 (1953).
(14) Unpublished observations of W. Lands and R. Lutwack.

The *t*-Butylbenzenes. II. A High Melting Hydrocarbon from Friedel–Crafts Alkylation of 1,3,5-tri-*t*-butylbenzene with *t*-Butyl Chloride[1]

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In part I[3] we reported a compound, $C_{22}H_{34}$, m.p. 218.5–219°, obtained by Friedel–Crafts alkylation of 1,4-di-*t*-butylbenzene with *t*-butyl chloride below 0°. The empirical formula $C_{22}H_{34}$, the ultraviolet absorption spectrum, and the high melting point indicated the presence of at least one alicyclic ring in this aromatic hydrocarbon. In this communication experimental evidence is presented which elucidates the structure of this compound.

Bartlett and co-workers[4] alkylated 1,4-di-*t*-butylbenzene with *t*-butyl chloride to form 1,3-di-*t*-butylbenzene, 1,3,5-tri-*t*-butylbenzene and a compound, m.p. 209–210°, which is probably the same as our high melting hydrocarbon.

1,1,4,4,5,5,8,8-Octamethyl-1,2,3,4,5,6,7,8-octahydroanthracene.—The high melting hydrocarbon was dehydrogenated with palladium at about 400° in a sealed tube and the ultraviolet spectrum of the product clearly showed the presence of anthracene or an anthracene derivative. Among the possible

(1) Taken in part from the M.Sc. thesis of Eileen E. Betts.
(2) Recipient of a National Research Council of Canada Bursary.
(3) L. R. C. Barclay and E. E. Betts, *Can. J. Chem.*, **33**, 672 (1955).
(4) P. D. Bartlett, M. Roha and R. M. Stiles, THIS JOURNAL, **76** 2349 (1954).