Equation 7 may be rearranged to give equation 8 and it follows that in a plot of $t/([\mathrm{S}]_0 - [\mathrm{S}]_t)$ *vs.* $(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))/([\mathrm{S}]_0 - [\mathrm{S}]_t)$ there will be obtained for various values of $[\mathrm{S}]_0$ a family of lines of slope $K_\mathrm{S}(K_\mathrm{P} + [\mathrm{S}]_0)/k_3[\mathrm{E}]K_\mathrm{P}$ with a common ordinate intercept of $(1 - K_\mathrm{S}/K_\mathrm{P})/k_3[\mathrm{E}]$.[9] For each of the lines of slope $K_\mathrm{S}(K_\mathrm{P} + [\mathrm{S}]_0)/k_3[\mathrm{E}]K_\mathrm{P}$ there is a point corresponding to $t = 0$ and as before[7] this point may be located by examination of the limits of the two parameters $t/([\mathrm{S}]_0 - [\mathrm{S}]_t)$ and $(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))/([\mathrm{S}]_0 - [\mathrm{S}]_t)$ as $t \to 0$. Since the limit of $t/([\mathrm{S}]_0 - [\mathrm{S}]_t)$ as $t \to 0$ is $1/-\mathrm{d}[\mathrm{S}]/\mathrm{d}t = 1/v_0$ and that of $(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))/([\mathrm{S}]_0 - [\mathrm{S}]_t)$ as $t \to 0$ is $1/[\mathrm{S}]_0$ it is evident that the points at which the lines of slope $K_\mathrm{S}(K_\mathrm{P} + [\mathrm{S}]_0)/k_3[\mathrm{E}]\ K_\mathrm{P}$ possess abscissa values equal to $1/[\mathrm{S}]_0$ will be the points where $t = 0$. Therefore, as the parameters of the points corresponding to $t = 0$ for various values of $[\mathrm{S}]_0$ are, respectively, $1/-\mathrm{d}[\mathrm{S}]/\mathrm{d}t = 1/v_0$ for the ordinate and $1/[\mathrm{S}]_0$ for the abscissa it follows[2] that a line drawn through these points will have a slope of $K_\mathrm{S}/k_3[\mathrm{E}]$ and an ordinate intercept of $1/k_3[\mathrm{E}]$. It will be recognized that this plot is similar to the first of the two plots proposed by Lineweaver and Burk,[2] *cf.*, Table I, but that in the present case the initial velocities need not be separately evaluated since they are given, in the form of their reciprocals, in terms of the ordinate $t/([\mathrm{S}]_0 - [\mathrm{S}]_t)$ for the condition that $t = 0$. Furthermore, K_P can be simultaneously determined either from the intercept $(1 - K_\mathrm{S}/K_\mathrm{P})/k_3[\mathrm{E}]$ or the slope $K_\mathrm{S}(K_\mathrm{P} + [\mathrm{S}]_0)/k_3[\mathrm{E}]K_\mathrm{P}$.

$$t/([\mathrm{S}]_0 - [\mathrm{S}]_t) = (K_\mathrm{P} - K_\mathrm{S})/k_3[\mathrm{E}]K_\mathrm{P} + \{K_\mathrm{S}(K_\mathrm{P} + [\mathrm{S}]_0)/k_3[\mathrm{E}]K_\mathrm{P}\}\{(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))/([\mathrm{S}]_0 - [\mathrm{S}]_t)\} \quad (8)$$

TABLE I

GRAPHICAL PROCEDURES FOR THE EVALUATION OF K_S AND k_3

Ref.	Ordinate	Abscissa	Ordinate intercept	Slope
2	$1/v_0$	$1/[\mathrm{S}]_0$	$1/k_3[\mathrm{E}]$	$K_\mathrm{S}/k_3[\mathrm{E}]$
..	$t/([\mathrm{S}]_0 - [\mathrm{S}]_t)$	$(\ln([\mathrm{S}]_0/[\mathrm{S}]_t)/([\mathrm{S}]_0 - [\mathrm{S}]_t)$		
2	$[\mathrm{S}]_0/v_0$	$[\mathrm{S}]_0$	$K_\mathrm{S}/k_3[\mathrm{E}]^a$	$1/k_3[\mathrm{E}]$
..	$t/(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))$	$([\mathrm{S}]_0 - [\mathrm{S}]_t)/(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))$		
3	v_0	$v_0/[\mathrm{S}]_0$	$k_3[\mathrm{E}]^b$	$-K_\mathrm{S}$
4	$([\mathrm{S}]_0 - [\mathrm{S}]_t)/t$	$(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))/t$		

[a] Abscissa intercept $= -K_\mathrm{S}$. [b] Abscissa intercept $= k_3[\mathrm{E}]/K_\mathrm{S}$.

Equation 7 may also be rearranged to give equation 9 and for this situation a plot of $t/(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))$ *vs.* $([\mathrm{S}]_0 - [\mathrm{S}]_t)/(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))$ will give, for various values of $[\mathrm{S}]_0$, a series of parallel lines of slope $(K_\mathrm{P} - K_\mathrm{S})/k_3[\mathrm{E}]K_\mathrm{P}$.[10] It can be shown that the points corresponding to $t = 0$ for the lines of slope $(K_\mathrm{P} - K_\mathrm{S})/k_3[\mathrm{E}]K_\mathrm{P}$ are the points where the lines of slope $(K_\mathrm{P} - K_\mathrm{S})/k_3[\mathrm{E}]K_\mathrm{P}$ possess abscissa values of $[\mathrm{S}]_0$ and that a line drawn through these points, whose coördinates are $[\mathrm{S}]_0/-\mathrm{d}[\mathrm{S}]/\mathrm{d}t = [\mathrm{S}]_0/v_0$ and $[\mathrm{S}]_0$ when $t = 0$, will have a slope of $1/k_3[\mathrm{E}]$, an ordinate intercept of $K_\mathrm{S}/k_3[\mathrm{E}]$ and an abscissa intercept of $-K_\mathrm{S}$. It will be seen that this plot is similar to the second plot of Lineweaver and Burk,[2] *cf.*, Table I, but is superior to this plot in that for $t = 0$ the ordinate $t/(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))$ describes the initial velocities in terms of $[\mathrm{S}]_0/v_0$. Also K_P may be simultaneously evaluated by equating the observed slope of the parallel lines to the quantity $(K_\mathrm{P} - K_\mathrm{S})/k_3[\mathrm{E}]K_\mathrm{P}$.

$$t/(\ln([\mathrm{S}]_0/[\mathrm{S}]_t)) = K_\mathrm{S}(K_\mathrm{P} + [\mathrm{S}]_0)/k_3[\mathrm{E}]K_\mathrm{P} + \{(K_\mathrm{P} - K_\mathrm{S})/k_3[\mathrm{E}]K_\mathrm{P}\}\{([\mathrm{S}]_0 - [\mathrm{S}]_t)/(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))\} \quad (9)$$

While it appears that all three of the graphical procedures based upon equation 6 will be found to be superior to those based upon equations 2 and 3, because it is very probable that a large number of enzyme-catalyzed reactions are competitively inhibited by one or more of their reaction products, it is difficult to assert that any one of the procedures which are based upon equation 6 is superior to the other two for all cases that may be encountered. Therefore, for any particular situation it is suggested that all three be given consideration. In conclusion we wish to note that the procedures described in this communication are capable of extension to systems more complicated than those represented by equations 1, 4 and 5. However, we wish to defer discussion of such systems at this time.

CONTRIBUTION NO. 1989 FROM THE
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Nitration in the Presence of Polyphosphoric Acid

BY J. P. KISPERSKY AND K. KLAGER

RECEIVED MAY 20, 1955

Steinkopf and Supan previously reported that the nitration of the alkylmalonates required the use of fuming nitric acid–acetic anhydride mixture at 40 to 50°.[1] The authors cautioned against allowing the temperature of the reaction to rise over 50° presumably because an uncontrollable reaction sets in due to the exothermic nature of the nitration. It has been found that polyphosphoric acid may replace the acetic anhydride in the nitration mixture giving good yields of alkylnitromalonates and reducing the potential hazard of the reaction medium.

The compounds thus prepared are listed in Table I along with the yield, b.p., refractive index and analysis. On the basis of the data presented it is evident that branching on the carbon atom attached to the malonic ester moiety reduces the yield on nitration.

We are indebted to the Office of Naval Research for the funds which supported this work.

Experimental

Inasmuch as the same experimental procedure was used in the preparation of all of the compounds listed in Table I a general method will be described.

Fifty grams of technical 100% nitric acid was added to 80 g. of polyphosphoric acid[2] contained in a 200-ml. three-necked flask equipped with stirrer, thermometer and dropping funnel. The mixture was heated to 60° and, when homogeneous, 0.14 mole of diethyl alkylmalonate was added dropwise over a period of 15 to 30 minutes. After a small quantity of ester had been added the mixture required slight cooling to maintain a temperature of 60°. After the addition of the ester the reaction was stirred for one

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[9] It will be noted that this intercept is the reciprocal of that obtained in the case of a $([\mathrm{S}]_0 - [\mathrm{S}]_t)/t$ *vs.* $(\ln([\mathrm{S}]_0/[\mathrm{S}]_t))/t$ plot.

[10] It is seen from this relation that when $K_\mathrm{P} > K_\mathrm{S}$ the slope will be positive, when $K_\mathrm{P} = K_\mathrm{S}$ the lines will parallel the abscissa and when $K_\mathrm{P} < K_\mathrm{S}$ the slope will be negative.

(1) W. Steinkopf and A. Supan, *Ber.*, **43**, 3239 (1910).

(2) Obtained from the Victor Chemical Works, Chicago 4, Illinois.

TABLE I

NITRATION OF SUBSTITUTED MALONIC ESTERS, $R—\overset{NO_2}{C}(CO_2C_2H_5)_2$

R	Yield	B.p. °C.	Pressure	$n^{25}D$	Analyses,[a] % Calcd. C	H	N	Found C	H	N
Isopropyl	60	83–84	0.3 mm.	1.4337	48.6	6.89	5.67	47.06	6.76	5.58
Isobutyl	78	88–89	0.5 mm.	1.4351	50.6	7.29	5.37	51.43	7.35	5.04
n-Butyl	75	93–94	0.5 mm.	1.4340	50.6	7.29	5.37	50.03	7.48	5.11
Cyclohexyl	15	110–120	5μ	1.4597	54.4	7.31	4.88	54.47	7.42	4.73
n-Decyl	97[b]	120–130	14μ	1.4450	59.2	9.05	4.06	59.38	9.30	4.56

[a] Analysis by the Elek Microanalytical Laboratories, Los Angeles, Calif. [b] Yield based on total weight recovered.

hour at 60° then cooled and poured onto 200 g. of chipped ice. After the ice had melted the oily layer was extracted with ether, and washed with 5% sodium bicarbonate solution until neutral, then with water. After drying, the ether was removed by distillation and the remaining liquid distilled through a small Vigreux column. The diethyl alkylnitromalonates were obtained as colorless oily liquids.

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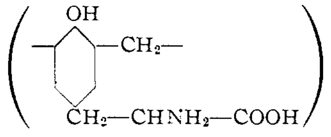
Reaction of Tyrosine with Formaldehyde in Acid Solution

BY HAROLD S. OLCOTT

RECEIVED MAY 31, 1955

In a study of protein reactions,[1] we wished to have available, as one type of model, a non-dialyzable substance containing a number of free amino groups. It occurred to us that tyrosine should react with formaldehyde in acid solution[2] to form such a polymer. The authors of the only previous studies on this system[3,4] believed that they had obtained isoquinoline derivatives. We have prepared products by the methods they described and also under other reaction conditions and find that they are not isoquinoline derivatives but that instead they display the properties to be expected of acid-catalyzed tyrosine–formaldehyde polymers.

The preparations were soluble in dilute acid and alkali but insoluble in the neutral range. Depending upon the conditions used in the polymerization, 40–70% was retained in dialyzing bags (Visking tubing) after extensive dialysis. Free amino nitrogen (by Van Slyke) was equivalent to 75–93% of the total nitrogen. These observations, together with the known reactions of other substituted phenols with formaldehyde,[5] indicate that the polymer is composed for the most part of units with the structure



The low total nitrogen and amino nitrogen analyses in all likelihood reflect the presence of additional methylol groups and methylene cross-links involving amino nitrogen in parts of the polymer.

Experimental

Preparation.—Tyrosine (20 g., 0.11 mole) was dissolved in 12.7 *N* sulfuric acid (17.5 ml., 0.11 mole) and to the solution, which rapidly solidified, was added formalin (40% formaldehyde, 8.4 ml., 0.11 mole). This mixture was autoclaved at 20 lb. pressure for 17 hours,[6] dissolved in water and thoroughly dialyzed.[7] The product tended to precipitate in the dialyzing bag but could be redissolved by dialyzing for a short time against dilute sodium carbonate solution. The final solution contained 48% of the original nitrogen (by Kjeldahl). The product was precipitated by the addition of dilute sulfuric acid to *p*H 4.1, washed with water, alcohol and ether, and air-dried at room temperature. It contained 12.2% water.

Anal. Calcd. for $C_{10}H_{11}O_3N$: N, 7.2; amino N, 7.2. Found (dry basis): N, 6.8; amino N, 5.4.

A product prepared according to Wellisch[4] with methylal and concentrated hydrochloric acid was 44% retained by extensive dialysis. The amino nitrogen content of the final product amounted to 70% of the total nitrogen.

Found (dry basis): N, 6.8; amino N, 4.8.

Properties.—The polymer gave a strong positive ninhydrin test, but quantitatively[8] the color was approximately only 70% of that to be expected from the Van Slyke amino nitrogen analyses (15-minutes reaction period). Strong color tests were also given by the Folin color reagent for free phenolic groups.[9] The product was not attacked by tyrosine decarboxylase (from *S. faecalis*), nor did it inhibit the rate of decarboxylation of tyrosine by tyrosinase. Its reaction with neutral 30% glucose solution was described previously.[1]

Acknowledgment.—The tyrosinase tests were performed by B. E. Axelrod with a preparation furnished by J. C. Lewis.

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(1) A. Mohammad, H. Fraenkel-Conrat and H. S. Olcott, *Arch. Biochem.*, **24**, 157 (1949).

(2) The polymer formed from tyrosine and formaldehyde in alkaline solution has been described by A. E. Brown, THIS JOURNAL, **68**, 1011 (1946). It contains no free amino nitrogen.

(3) A. Pictet and T. Spengler, *Ber.*, **44**, 2030 (1911).

(4) J. Wellisch, *Biochem. Z.*, **49**, 173 (1913).

(5) Reviewed in J. F. Walker, "Formaldehyde," 2nd Ed., Reinhold Publ. Corp., New York, N. Y., 1953.

(6) The polymerization probably does not require this extended reaction time. A somewhat similar product was obtained after 4 hours of autoclaving.

(7) Provision should be made for the considerable increase in volume which occurs.

(8) V. J. Harding and R. M. MacLean, *J. Biol. Chem.*, **24**, 503 (1916).

(9) R. M. Herriott, *J. Gen. Physiol.*, **19**, 283 (1935).

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The Pyridylethylation of Active Hydrogen Compounds. IV. The Acid-catalyzed Pyridylethylation of Primary Amines

BY HENRY E. REICH[1] AND ROBERT LEVINE

RECEIVED MAY 19, 1955

In previous papers from this Laboratory the pyridylethylation of ketones[2,3] and of secondary amines[4] was discussed.

The present report is concerned with the con-

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(1) American Cyanamid Company Research Fellow, 1952–1954.

(2) R. Levine and M. H. Wilt, THIS JOURNAL, **74**, 342 (1952).

(3) M. H. Wilt and R. Levine, *ibid.*, **75**, 1368 (1953).

(4) H. E. Reich and R. Levine, *ibid.*, **77**, 4913 (1955).