A sample of solid (I), allowed to come gradually to room temperature, decomposed completely, emitting a colorless gas and leaving a residue of acetic anhydride (anilide, m.p. 112.6–114°). The gas appeared to be a mixture of carbon dioxide and carbon monoxide. When it was passed through aqueous barium hydroxide, a portion of it was absorbed, forming a white precipitate which dissolved with effervescence when treated with hydrochloric acid; the remainder, unaffected by the barium hydroxide solution, burned with a blue flame.

Hydrolysis of (I).—Hydrolysis was accomplished by adding water dropwise, with stirring, to an ethereal solution of (I) at −10°, maintaining this temperature for two hours, and then allowing a slow rise to room temperature. Products were identified as acetic and oxalic acids.

Determination of Acetyl–Oxalyl Ratio.—A measured quantity of a 0.17 *N* solution of potassium hydroxide in 1:1 ethanol–water was cooled to −19° and added at the rate of one drop every five seconds, with stirring, to an ethereal solution of (I) at the same temperature. The mixture was allowed to stand for 18 hours at −19° and then for 2 hours at 3°. Total acid was determined by acidimetric titration of the excess base, oxalyl content by oxidimetric titration, and acetyl content by difference. Theoretical ratio of acetyl to oxalyl, 2.00; found, 2.23, 2.31.

Reaction with Aniline.—The concentration of (I) in an ethereal solution was estimated by the procedure just described. To a measured sample of this solution at −19° was added an ethereal solution of aniline at the same temperature, in the ratio of two moles of aniline to one mole of (I), at a rate of one drop per 10–15 seconds. The reactants then stood for 12 hours at −19°, followed by six hours at 3°. The precipitate was filtered, washed successively with ether and water, and dried under vacuum. It melted at 247.5–249°; its mixed melting point with a known sample of oxanilide was the same. It was further identified by nitrogen determination.[7]

Anal. Calcd. for $C_{14}H_{12}O_2N_2$: N, 11.66. Found: N, 11.47, 11.41.

Yield of oxanilide was theoretical in one run, 81% in another. No acetanilide was isolated, but in the second run there was a small amount of an unidentified oily by-product.

(7) Method of I. M. Kolthoff and V. A. Stenger, "Volumetric Analysis," Vol. II, Interscience Publishers, Inc., New York, N. Y., 1947, p. 173.

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Cyanomethylation of Indole with Diethylaminoacetonitrile

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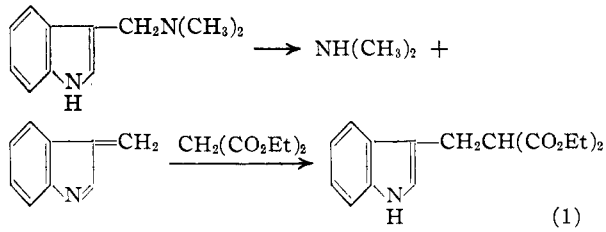
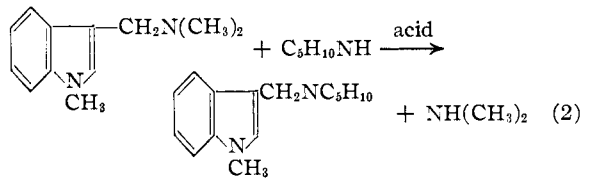
RECEIVED FEBRUARY 7, 1953

The extensive use of tertiary amines as alkylating agents[1] has prompted speculation on the mechanism of the alkylation reaction. It was at first assumed that alkylation proceeded by an elimination–addition mechanism[2] as illustrated for gramine in equation (1). Later, instances were found, however, where alkylations could not readily be explained by an elimination–addition mechanism, such as in the reaction of piperidine with 1-methylgramine[3] (equation 2) and with N-(2-nitroisobutyl)-

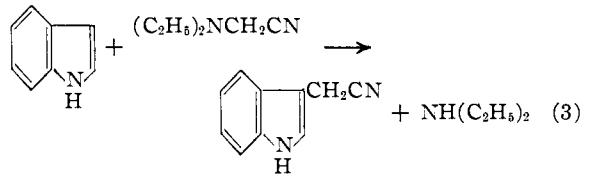


(1)

dimethylamine,[4] and of indole[5] and diethyl malonate[6] with the Mannich base of formamidomalonic ester.



(2)



(3)

To these interesting examples of direct alkylation with tertiary amines should now be added the alkylation of indole with diethylaminoacetonitrile (equation 3). This alkylation proceeds at *ca.* 170° in the absence of a solvent to produce indole-3-acetonitrile in moderate (33–44%) yield. The nitrile was identified by comparison of its infrared spectrum with that of an authentic sample,[7] by the melting point of its picrate[7] and by hydrolysis to the acid in excellent yield. This identification incidentally indicates that the nitrile obtained by the "cyanomethylation" reaction was a very pure sample, and that the method here developed is a convenient one for the preparation of indole-3-acetonitrile on a small scale. Unfortunately attempts to scale up the preparation resulted in diminishing yields; this is probably due to the great sensitivity of the cyanomethylation reaction to temperature fluctuations. At temperatures below 160° very little reaction ensues at all, while around 180° tar formation supervenes.

While this work was in progress, a publication appeared[6] describing unsuccessful attempts at alkylating diethyl formamidomalonate with diethylaminoacetonitrile and piperidinoacetonitrile. We were likewise unable to alkylate acetophenone, 2-naphthyl methyl ether or 2-naphthol with diethylaminoacetonitrile; in the former two cases the starting materials were recovered while in the latter case phenolic polymers constituted the bulk of the reaction product.

Experimental

Indole-3-acetonitrile.—A mixture of 11.8 g. (0.1 mole) of indole[8] and 22.4 g. (0.2 mole) of diethylaminoacetonitrile[9]

(1) For a review, see J. H. Brewster and E. L. Eliel in R. Adams "Organic Reactions," Vol. 7, John Wiley and Sons, Inc., New York, N. Y., 1953, chapter 3.

(2) K. Auwers, *Ber.*, **36**, 1878 (1903); *Ann.*, **344**, 131 (1906); K. von Auwers and Ph. Bullmann, *Ber.*, **59**, 2719 (1926); C. Mannich, W. Koch and F. Borkowsky, *ibid.*, **70**, 355 (1937); H. R. Snyder and E. L. Eliel, THIS JOURNAL, **70**, 1703 (1948); H. R. Snyder and J. H. Brewster, *ibid.*, **70**, 4230 (1948); C. E. Dalgliesh, *ibid.*, **71**, 1697 (1949).

(3) H. R. Snyder and E. L. Eliel, *ibid.*, **70**, 4233 (1948).

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(5) A. Butenandt, H. Hellmann and E. Renz, *Z. physiol. Chem.*, **284**, 175 (1949).

(6) H. Hellmann and E. Brendle, *ibid.*, **287**, 235 (1951).

(7) J. Thesing and F. Schülde, *Ber.*, **85**, 324 (1952).

(8) We are indebted to E. I. du Pont de Nemours and Co., Inc., for a generous gift of indole.

(9) C. F. H. Allen and J. A. VanAllan, *Org. Syntheses*, **27**, 20 (1947).

was maintained at an internal temperature close to 170° under nitrogen for six hours in a flask equipped with a reflux condenser. The excess diethylaminoacetonitrile was then removed at water pump pressure and the residue was submitted to distillation at the oil pump. After a small forerun of indole, indoleacetonitrile was collected at 144–152° (0.03 mm.) or 160–165° (0.2 mm.) (lit.[7] 157° (0.2 mm.)) as a viscous, slightly cloudy liquid weighing 5.2–6.9 g. (33–44%). The picrate[7] melted at 128–129° without recrystallization (lit.[7] 127–128°) and hydrolysis of the nitrile with 20% aqueous potassium hydroxide[7] yielded the acid, m.p. 162–164° (dec.) in 85% yield (lit.[7] m.p. 164–165° (dec.), yield 86%). We were unable to find a solvent for the reaction, aromatic hydrocarbons or aliphatic alcohols having been proved unsuitable. Attempts to scale up the preparation led to a drop in yield.

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The Estimation of the Relative Activities of a Series of Specific Substrates[1]

BY ROBERT J. FOSTER AND CARL NIEMANN[2]

RECEIVED MARCH 6, 1953

It is generally recognized that when the rate of disappearance of the specific substrate S_t in the system

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

is given by equation (2), where $K_S = (k_2 + k_3)/k_1$, a plot of $\ln [S]_0/[S]_t$ *versus* t will at particularly

$$-d[S]/dt = k_3[E][S]/(K_S + [S]) \quad (2)$$

low concentrations of $[S]_0$ when K_S is large relative to $[S]_0$ approximate that of a first-rrder reaction, that a plot of $([S]_0 - [S]_t)$ *versus* t will at particularly high concentrations of $[S]_0$ when K_S is small relative to $[S]_0$ approximate that of a zero-order reaction.

Despite the apparent success in relating the activities of several series of specific substrates on the basis of their respective approximate first-order constants determined at a single and arbitrary initial specific substrate concentration, *i.e.*, on the basis of so-called first-order proteolytic coefficients,[3–6] it is clear from the investigations of Neurath and his co-workers[7–9] that this practice is basically unsound and should be abandoned.

In an attempt to devise a more rational procedure for the comparison of the activities of a series of specific substrates Neurath, *et al.*,[7–9] suggested that the approximate first-order constants be extrapolated to zero initial specific substrate concentrations, *i.e.*, where the so-called maximum first-order proteolytic coefficient C^1_{max} is defined by equation (3).

$$2.3\, C^1_{max} \doteq k_3/K_S \quad (3)$$

There are two limiting cases for equation (3): I, where $k_3 \gg k_2$, $K_S \doteq k_3/k_1$ and $C^1_{max} \doteq k_1$; and II, where $k_3 \ll k_2$, $K_S \doteq k_2/k_1$ and $C^1_{max} = k_1k_3/k_2$. It is obvious that in case I C^1_{max} is in no way related to the susceptibility of ES to subsequent reaction being clearly the constant for the reaction depicted in equation (4). Thus, if

$$E_f + S_f \xrightarrow{k_1} ES \quad (4)$$

it can be shown that for all specific substrates being compared $k_3 \gg k_2$ then values of C^1_{max} can be used to compare the rates with which these specific substrates will combine with a given enzyme present in a particular reaction system recognizing of course that independent evidence must be provided to show that all of the specific substrates are reacting with the same catalytically active site if the results are to be interpreted in this manner.

For case II C^1_{max} is directly proportional to k_3, which in many instances, but not in all, can be taken as an index of the suceptibility of ES to subsequent reaction, and inversely proportional to the dissociation constant k_2/k_1 of ES. Thus, in this case C^1_{max} can only lead to a somewhat ambiguous estimate of the relative activity of a series of specific substrates and cannot be used to estimate on one hand the affinity of the enzyme for a particular specific substrate, or set of specific substrates, and on the other the susceptibility to subsequent reaction of the corresponding enzyme–substrate complexes. An example of the confusion that can arise through the use of C^1_{max} values to estimate the relative activity of a series of specific substrates where $k_3 \ll k_2$ is given immediately below.

If it is assumed with some justification[10,11] that the molecular weight of α-chymotrypsin is *ca.* 22,000 and its nitrogen content is *ca.* 16%, then for acetyl-L-tryptophanamide[12] $k_3 = 0.029$ sec.$^{-1}$, $K_S = 0.0053$ M and $C^1_{max} = 5.5$ M^{-1} sec.$^{-1}$, and for acetyl-L-phenylalaninamide[13] $k_3 = 0.047$ sec.$^{-1}$, $K_S = 0.034$ M and $C^1_{max} = 1.4$ M^{-1} sec.$^{-1}$ For these two specific substrates it is probable that in both instances $K_S \doteq k_2/k_1$[13,14] and consequently C^1_{max} is to be interpreted in terms of case II above. The fact that C^1_{max} for acetyl-L-phenylalaninamide is but *ca.* 1/4 of that for acetyl-L-tryptophanamide is not due to a greater susceptibility to hydrolysis of the enzyme–substrate complex arising from acetyl-L-tryptophanamide than that arising from acetyl-L-phenylalaninamide since the respective k_3 values, *i.e.*, 0.029 and 0.047 sec.$^{-1}$ actually predict the reverse situation, but rather to the far greater affinity of the enzyme for acetyl-L-tryptophanamide than for acetyl-L-phenylalaninamide as indicated by the K_S values of 0.0053 and 0.034 M, respectively. Thus in the absence of any knowl-

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

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

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