

Fig. 2.

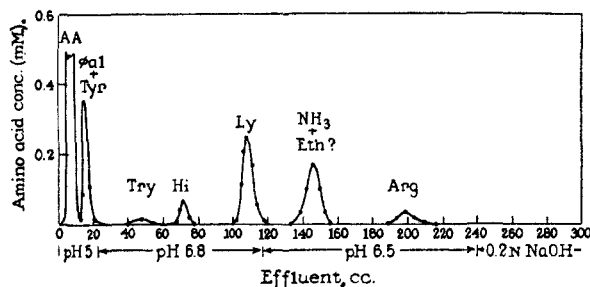


Fig. 3.

composition found by paper chromatography was confirmed and an as yet unidentified ninhydrin positive peak which seemed to be differentiated from the ammonia peak, appeared near the ethanolamine position on the 100-cm. column. On the short column this possible component may be included in the ammonia peak and the ammonia figure is therefore questionable. Cystine was found to be present in traces only, or not at all. The ammonia-arginine doublet was analyzed by evaporating the ammonia from every second tube.

Table I gives the amounts of the single amino acids in per cent. amino acid residue and per cent. nitrogen in the original protein, dried for 3 hours at 100°.

TABLE I

Amino acid	G. amino acid residue per 100 g. protein	Nitrogen, %
Aspartic acid	12.1	1.5
Alanine	4.9	1.0
Ammonia <sup>a</sup>	1.5	1.2
Arginine	3.3	1.2
Glutamic acid	9.7	1.1
Glycine	2.9	0.7
Histidine	2.2	0.7
Isoleucine	8.9	1.1
Leucine	6.0	0.8

Lysine	10.9	2.4
Methionine	1.3	0.1
Phenylalanine	6.2	.6
Proline	6.4	.9
Serine	3.1	.5
Threonine	4.8	.7
Tryptophan <sup>b</sup>	3.6	.5
Tyrosine <sup>c</sup>	6.0	.5
Valine	4.1	.6
Total	97.9	16.1
Found N (Kjeldahl)		16.2

<sup>a</sup> Tentative figure, perhaps including an additional component. <sup>b</sup> Determined spectrophotometrically by Kunitz. 2.5% was found chromatographically in the acid hydrolyzate. <sup>c</sup> This value checks closely with that of Kunitz, determined by the ultraviolet absorption method.

**Acknowledgment.**—I wish to thank Dr. M. Kunitz for providing the material for this investigation and Miss E. A. Jacobs for her technical assistance.

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### The Effect of $\alpha$ -Fluorine Atoms on $S_N1$ Reactivity<sup>1</sup>

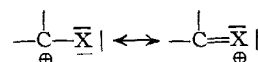
BY JACK HINE AND DONALD E. LEE

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It has been previously shown that in comparison to hydrogen, both chlorine and bromine atoms increase the  $S_N1$  reactivity<sup>2</sup> of other halogen atoms attached to the same carbon atom.<sup>3</sup> It is now found that the corresponding effect of  $\alpha$ -fluorine atoms is very much smaller than that of  $\alpha$ -chlorine or bromine and that it may even be of a deactivating nature.

In 50% aqueous acetone, benzodifluorochloride solvolyzes less than one-fifth as fast as benzyl chloride. If benzyl chloride, under the conditions used, hydrolyzes entirely by the  $S_N1$  (carbonium ion) mechanism, then  $\alpha$ -fluorine atoms do decrease the  $S_N1$  reactivity. However, there are several facts which suggest that this reaction may be at least partly  $S_N2$  (bimolecular displacement). One is the great reactivity of benzyl chloride in reactions known to be  $S_N2$  in mechanism. Another is the fact that while the replacement of the first chlorine of benzotrichloride by hydrogen causes a 33-fold decrease in reactivity, the replacement of the second causes only a 5-fold decrease in reaction rate (the situation with benzyl bromide is even more striking).<sup>3</sup> Hence the effect of  $\alpha$ -fluorine on  $S_N1$  reactivity in comparison to that of  $\alpha$ -hydrogen, while probably not large, is not discernible from the present work.

Although increasing ease of double bond formation and the resultant increased carbonium ion



(1) From an M.S. thesis submitted by Donald E. Lee to the Graduate School of the Georgia Institute of Technology.

(2) For the significance of the terms,  $S_N1$  and  $S_N2$ , see I. Dostrovsky, E. D. Hughes and C. K. Ingold, *J. Chem. Soc.*, 173 (1946), and earlier papers.

(3) J. Hine and D. E. Lee, *This Journal*, **73**, 22 (1951).

stability may overrule the slight increase in electro-negativity found on going from bromine (2.8 on Pauling's scale) to chlorine (3.0), it does not appear capable of surmounting the large increase in electro-negativity found in fluorine (4.0).

#### Experimental

The benzodifluorochloride used had the following properties:  $n_D^{24}$  1.4622,  $d_4^{20}$  1.2397; molar refractivity calcd. 35.59, found 36.03.

First order rate constants for hydrolysis in 50% (by volume) aqueous acetone, determined as described previously,<sup>1</sup> yielded the following results

at 36°,  $k = (0.0830 \pm 0.0023) \times 10^{-4} \text{ min.}^{-1}$   
 at 45°,  $k = (0.2232 \pm 0.0023) \times 10^{-4} \text{ min.}^{-1}$   
 at 60°,  $k = (0.9880 \pm 0.0048) \times 10^{-4} \text{ min.}^{-1}$   
 at 30°,  $k = 0.0419 \times 10^{-4} \text{ min.}^{-1}$  (extrapolated from the data above to facilitate comparisons with Table I, reference 3)

The average activation energy was 21.2 kcal./mole.

**Acknowledgment.**—The authors wish to express their thanks to The Hooker Electrochemical Company for the gift of the benzodifluorochloride used in this investigation.

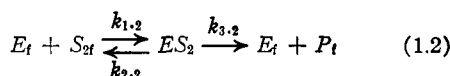
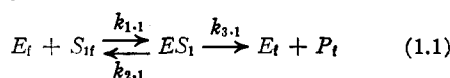
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### The Determination of the Relative Affinities of a Series of Specific Substrates from their Respective $K_S$ and $k_3$ Values<sup>1</sup>

BY DAVID S. HOGNESS AND CARL NIEMANN<sup>2</sup>

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For the systems



where  $K_{S_1} = (k_{2,1} + k_{3,1})/k_{1,1}$  and  $K_{S_2} = (k_{2,2} + k_{3,2})/k_{1,2}$  it has been argued<sup>3-5</sup> that  $k_{2,1}/k_{1,1} > k_{2,2}/k_{1,2}$  when  $K_{S_1} > K_{S_2}$  and  $k_{3,1} < k_{3,2}$ , thus implying that in certain cases it is possible to order the relative affinities of a series of specific substrates solely on the basis of their respective  $K_S$  and  $k_3$  values. While this conclusion was at one time accepted by workers in this Laboratory<sup>6</sup> it is now recognized that the argument is fallacious and that an unambiguous ordering of affinities cannot be achieved by the above procedure. That this latter conclusion is correct is apparent from the fact that  $k_2/k_1 = K_S(1 + k_3/k_2)^{-1}$  and that

$$\frac{k_{2,1}/k_{1,1}}{k_{2,2}/k_{1,2}} = \frac{K_{S_1}(1 + k_{3,2}/k_{2,2})}{K_{S_2}(1 + k_{3,1}/k_{2,1})} \quad (2)$$

Thus if no restriction is placed upon the values of  $k_{2,1}$  and  $k_{2,2}$ , except that they be positive, then  $k_{2,1}/k_{1,1}$  can be less than, equal to, or greater than  $k_{2,2}/k_{1,2}$  regardless of the relative values of  $K_{S_1}$  and  $K_{S_2}$  and of  $k_{3,1}$  and  $k_{3,2}$ . Since the determination of

$K_S$  and  $k_3$  does not give any information relative to the magnitude of  $k_2$  it follows that the evaluation of only these two constants, *i.e.*,  $K_S$  and  $k_3$ , for two or more specific substrates cannot under any circumstance lead to an unambiguous ordering of their respective enzyme-substrate dissociation constants.

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### An Improved Synthesis for $\alpha$ -Methyl- $\Delta, \alpha, \beta$ -butenolide<sup>1,2</sup>

BY WILLIAM H. HOUFF AND HAROLD M. SELL

RECEIVED DECEMBER 22, 1951

#### Introduction

In a study on the effects of inhibition of respiration in germinating seeds by  $\alpha, \beta$ -unsaturated lactones it was necessary to synthesize  $\alpha$ -methyl- $\Delta, \alpha, \beta$ -butenolide. The reported method of synthesis,<sup>3</sup> which involves the condensation of form-aldehyde and acetone, could not be employed because of the great tendency for butanol-1-one-3 to polymerize to yield other compounds. This note is a report of a modified method which will produce  $\alpha$ -methyl- $\Delta, \alpha, \beta$ -butenolide in good yields.

#### Experimental

**1-Chlorobutanone-3.**—Into a 3-necked flask equipped with a dropping funnel, stirrer, and downward condenser was placed 32.5 g. (0.3 mole) of 1-chlorobutanol-3. The solution was heated to 125° and a mixture of 60 g. of potassium dichromate in 300 ml. of dilute 1-1 sulfuric acid was added dropwise until the temperature increased and the oxidizing mixture no longer gave the green color of chromous ion. The ketone, as it was formed, was permitted to distil over with the water. The two layers were separated by means of a separatory funnel and the aqueous portion was extracted three times with ether. The product was combined with the ether extracts and the solution was dried over anhydrous sodium sulfate for 12 hours. The ether was distilled off and the fraction distilling at 52-53° at 15 mm. was collected. The yield was 24 g. or 80%.

**$\alpha$ -Hydroxy- $\alpha$ -methylbutyrolactone.**—To 24 g. (0.22 mole) of 1-chlorobutanone-3 in 100 ml. of water was added 100 ml. of a solution containing 20 g. of potassium cyanide. The solution was cooled to 15° and the 100 ml. of a 25% solution of hydrochloric acid was added slowly with stirring. An additional quantity of 200 ml. of concentrated hydrochloric acid was added and the solution stirred vigorously for four hours. The reaction mixture was extracted continuously with ether in a liquid-liquid extraction unit for eight hours. After drying the ethereal extract over anhydrous sodium sulfate, the ether was removed under vacuum. The crude product distilled with an evolution of hydrogen chloride. The yield of the fraction distilling at 109-110° at 6 mm. was 14 g. or 54%; m.p. of the *p*-nitrobenzoate of the hydroxylactone 161-161.5°, reported<sup>3</sup> m.p. 162°.

**$\alpha$ -Methyl- $\Delta, \alpha, \beta$ -butenolide.**—The same procedure described by Cavallito, *et al.*,<sup>3</sup> was used. The product distilling at 82° at 7 mm. was collected. The yield was 3.5 g. or 30%;  $n_D^{20}$  was 1.465.

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(2) The research was supported by a grant from the Rockefeller Foundation.

(3) C. J. Cavallito and T. H. Haskell, *THIS JOURNAL*, **68**, 2332 (1946).

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

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

(3) H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 69 (1950).

(4) J. E. Snoke and H. Neurath, *Arch. Biochem.*, **21**, 351 (1949).

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(6) H. T. Huang and C. Niemann, *THIS JOURNAL*, **73**, 1541 (1951).