more propylguaiacyl polymers is certainly an oversimplification of the observed data.

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## Experiments on the Mechanism of the Urea–Urease Reaction

BY J. H. SINGLETON, E. R. ROBERTS AND E. R. S. WINTER

Although urease was the first enzyme to be isolated in a crystalline form,[1] in spite of the relative simplicity of its over-all reaction with urea and the detailed experiments of a number of workers, the reaction mechanism remains obscure. Now it has been shown elsewhere that the surface atoms of solid oxides possessing catalytic activity are very labile[2]: thus, using $O^{18}$ as a tracer, it was found that aluminum oxide readily undergoes exchange of its surface oxygen with water vapor at room temperature, while many oxides suffer exchange with both water vapor and gaseous oxygen at elevated temperatures. In view of these results, and because urea possesses the grouping $-CO-\underset{H}{N}-$ which is very common in protein, while so far as is known urease is wholly protein, we thought it worthwhile to examine whether the urea–urease reaction involves the chemical incorporation of all or part of the urea molecule into the enzyme, followed by the splitting out of the same structural elements from a neighboring part of the enzyme. Such a postulate can readily be tested by the use of isotopic urea: thus if $CO(N^{15}H_2)_2$ be used and either or both of the $-NH_2$ groups are incorporated into the enzyme, the first small amount of $NH_3$ evolved should contain less $N^{15}$ than the main product, since it should be diluted with $N^{14}H_3$ from the enzyme: as the reaction proceeds the $N^{15}$ content of the $NH_3$ evolved should approach that of the urea used. Similar remarks apply to the labeling of the urea with $C^{13}$ or $C^{14}$. If an exchange reaction can be demonstrated, then after the reaction with isotopic urea the enzyme could, for example, be hydrolyzed and the various hydrolysis products examined for the presence of isotopic nitrogen or carbon. An experiment of this type should throw considerable light on the nature of the enzyme reaction and of the reactive centers in the enzyme.

A preliminary examination along these lines is reported here, using urea containing about 30 atom % excess of $N^{15}$. Considerable exchange of nitrogen has been found by us during the enzyme reaction, but some at least of this is due to exchange between the ammonia and the enzyme material.

### Experimental

**Preparation of Urease.**—The enzyme was extracted from jackbean meal by the method of Sumner.[1] It was recrystallized from dilute acetone by addition of 0.5 *M* citrate buffer, *p*H 6.0.[3] The activity of the crystals, determined by the method of Van Slyke and Archibald,[4] was greater than $10^5$ units per gram of dry enzyme.

**Preparation of Urea.**—Urea containing excess $N^{15}$ was synthesized from ammonia and diphenyl carbonate by a modification of Henschel's[5] method.

**Exchange Experiments.**—In the first experiment 0.3 g. of the crystalline enzyme dissolved in distilled water was treated with 0.03 g. of urea. The middle fraction of the ammonia evolved was collected by aspiration in dilute hydrochloric acid.

In the second experiment, 0.3 g. of enzyme in 10 ml. of distilled water was treated with 0.006 g. of ammonia containing 30.5% $N^{15}$, passed by aspiration through the enzyme solution. The stream of air was continued until almost all of the ammonia had been collected in dilute hydrochloric acid. The enzyme reaction was then carried out on this pre-treated enzyme solution, using first 0.01 g. and then 0.02 g. of urea: almost complete recovery of ammonia being obtained from the first addition of urea before the second was added. The last runnings of the ammonia evolved from the second addition of urea were collected separately.

The ammonium chloride samples so obtained were converted to nitrogen, which was analyzed for $N^{15}$ content by mass spectrometer.

| | $N^{15}$, % Expt. no. 1 | $N^{15}$, % Expt. no. 2 |
|---|---|---|
| Original urea | 29.1 | 30.8 ± 0.9 |
| $N^{15}N_3$ before passage through enzyme | .. | 30.8 ± 0.9 |
| $N^{15}H_3$ after passage through enzyme | .. | 23.8 ± 0.3 |
| $NH_3$ evolved from 1st addition of urea | .. | 15.8 ± 5.0[a] |
| $NH_3$ evolved after 2nd addition of urea | 20.0 | 22.7 ± 1.1 |
| $NH_3$ evolved when reaction proceeds to completion | .. | 30.2 ± 1.3<br>30.0 |

[a] Sample contaminated with some air.

### Discussion

The results reported do not allow us to decide whether or not our original suggestion as to the mechanism of the enzyme reaction is correct, but the exchange found with ammonia alone is of interest. The exchangeable ammonia in the enzyme can hardly be present as $COONH_4$ groups since the preparation involved much manipulation in buffer solutions containing large excesses of sodium and potassium salts. It is possible that the enzyme contains aldehyde residues which form imino groups with ammonia and that these groups take part in the enzyme eaction proper (*cf.*, the pyridoxal–pyridoxamine transformation.[6] Some support for the suggestion that aldehyde residues are an essential part of the reactive centers is found in the negative temperature coefficient of sulfite inhibition.[7] A reversible formation of imino groups as above would probably explain also the inhibition by ammonia of the rate of reaction between urea and urease.[8] Work along these and allied lines, and a repetition and extension of the experiments reported here, are in progress.

We are indebted to Mr. J. Blears of Metropolitan-Vickers, Ltd., for the isotope analyses, which were carried out in the spring of 1947. Two of us (E.R.R. and E.R.S.W.) acknowledge

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## Infrared Data on the Carbonyl Group in Substituted Acetophenones

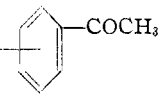
BY ALBERT H. SOLOWAY AND S. L. FRIESS[1]

In an extension of a study[2] involving the reaction of meta- and para-substituted acetophenones with perbenzoic acid, it was considered of some interest to determine the characteristic infrared frequencies of the carbonyl groups in these compounds. Since previous work[2,3] indicated the existence of an ordered relationship between the nature of ring substituents present in acetophenones and the reactivity of their carbonyl function toward addition reactions, it was anticipated that a similar correlation might exist between structure-sensitive $\lambda$ values for the carbonyl bands and some index of chemical reactivity of the ketone groupings.

Accordingly, a series of twelve acetophenones from the previous study with *m*- and *p*-substituents ranging in character from strongly electron-supplying to electron-withdrawing was carefully purified, and the spectrum of each member scanned over the region of the sharply defined carbonyl band. Each solid ketone was investigated as a finely ground mull in Nujol, while ketones liquid at room temperature were run directly without added solvent. The results of these infrared measurements are given in Table I, together with a tabulation of Hammett's $\sigma$ substituent constants[4] and a comparison column of carbonyl reactivity as measured by the rate constants for the same acetophenones in the peracid reaction.[2]

TABLE I

DATA ON MONOSUBSTITUTED ACETOPHENONES



| Substituent, X | λ (in μ) for carbonyl peak | Hammett's σ value[4] for X | Rate constant in peracid reaction,[a] (l./mole sec.) × $10^5$ |
|---|---|---|---|
| | A. Nujol mulls | | |
| *p*-$NH_2$ | 6.12 | −0.66 | |
| *p*-OH | 6.11 | | 11.5[b] |
| *p*-$OCH_3$ | 6.03 | − .27 | 4.42 ± 0.10 |
| *p*-$NHCOCH_3$ | 6.02 | | |
| *p*-Br | 6.01 | + .23 | |
| *p*-$OCOCH_3$ | 6.00 | | |
| *m*-$NO_2$ | 5.95 | + .71 | |
| *p*-$NO_2$ | 5.93 | +1.27 | |
| | B. Pure liquids | | |
| *p*-$CH_3$ | 5.97 | −0.17 | 3.20 ± 0.16 |
| *m*-$OCH_3$ | 5.95 | + .12 | 2.42 ± 0.18 |
| *p*-Cl | 5.93 | + .23 | |
| *m*-Br | 5.93 | + .39 | |

[a] Rate runs in chloroform solution of perbenzoic acid at 29.9°. Constants are given for those acetophenones which obey second order kinetics, since it is only for this type of ketone that $k_2$ measures carbonyl addition reactivity. [b] Value must be regarded as approximate because of a side reaction producing color in solution, presumably by nuclear attack.

From the data of Table I it is seen that as the character of the substituent meta or para to the acetyl function changes progressively from electron-supplying to electron-withdrawing, the value of $\lambda$ for the carbonyl band drops in magnitude.[5] Although the available data do not indicate a complete linear correlation between $\lambda$ values and $\sigma$ substituent constants, it is to be noted that a rough parallelism between these factors does exist, and that a similar degree of correspondence is found between $\lambda$ values and rate-constants for the second order peracid reaction of variously substituted acetophenones.

These observations would imply that some of the same energy factors that determine a $\sigma$ value for a substituent, as it affects reactivity of a functional group attached to a meta or para position, are operative in altering fundamental vibrational frequencies within groups at these positions.

### Experimental

Small samples of the acetophenones previously purified for rate work[2] were either recrystallized or redistilled before scanning of their infrared spectra.[6]

Sample and blank tracings were obtained using a Perkin-Elmer single beam recording infrared spectrometer (model 12 AB), at maximum sensitivity over a 2 μ range including the carbonyl band. A cell of 0.025 mm. thickness was used in all runs.

(1) Naval Medical Research Institute, Bethesda, Md.

(2) S. L. Friess and A. H. Soloway, THIS JOURNAL, **73**, 3968 (1951).

(3) R. P. Cross and P. Fugassi, *ibid.*, **71**, 223 (1949).

(4) For $\sigma$ values as indices of relative electron supply or withdrawal see L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chap. 7.

(5) For a similar effect on the carbonyl band of unconjugated esters, in which electronegative $\alpha$-substituents like the acetoxy or the cyano group cause a lowering of the wave length for carbonyl stretching relative to that for the unsubstituted ester, see R. S. Rasmussen and R. R. Brattain, THIS JOURNAL, **71**, 1073 (1949).

(6) We are indebted to Mr. Carl Whiteman for obtaining the machine tracings in all runs.

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## Organic Peracid–Sodium Bisulfite Mixtures as Acylating Agents[1]

BY A. H. SOLOWAY[2] AND S. L. FRIESS[3]

In the course of work on the peracid degradation of certain aromatic ethers, it was observed that either aqueous peracetic acid solution or dilute aqueous perbenzoic acid solution, upon treatment with sodium bisulfite in the usual procedure to destroy the oxidizing power of the peracid, results in a solution which contains an active acylating agent for certain amines. Each amine which does react with the peracid–bisulfite mixture furnishes a single product in good yield *via* the over-all reaction.

$$RCO_3H \xrightarrow[2,\ R'NH_2]{1,\ NaHSO_3} RCONHR'$$

R = $CH_3$, $C_6H_5$

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(2) Beaunit Mills Fellow in Chemistry, 1950–1951.

(3) Naval Medical Research Institute, Bethesda, Md.