tempts to prepare the peroxide of *cis*-stilbene-2-carboxylic acid were also unsuccessful. The above peroxides were desired as alternative routes to the *o*-benzoylphenyl radical and to the *o-cis-β*-styrylphenyl radical which are intermediates in certain reactions of the diazonium salts derived from 2-aminobenzophenone[8] and from *cis*-2-aminostilbene.[9]

Experimental

Preparation of Benzoyl Peroxide from the Urea–Hydrogen Peroxide Complex.—The urea–hydrogen peroxide complex was prepared according to the published method[6] except that the mixture of reactants, after being heated to 60°, was merely cooled in an ice-bath and the complex filtered and dried in the air. A mixture of 2.4 g. (25.5 mmoles) of the urea complex, 7.03 g. (50 mmoles) of benzoyl chloride and 50 ml. of absolute ether was stirred by means of a Hershberg stirrer and cooled in an ice-bath; then 4 g. (50.5 mmoles) of distilled pyridine was added and stirring continued in the cold for ten hours. (Later runs with other acid chlorides indicated that the reaction was complete in 3–4 hours if stirring was vigorous.) The colorless solid was filtered and then triturated first with water to remove pyridine hydrochloride, and then with methanol. This peroxide was combined with an additional amount of peroxide obtained by evaporation of the ether. The yield was 3.75 g. (62%), m.p. 104–106° dec. Analysis of the peroxide by the method of Kokatnur and Jelling[10] gave a peroxide content of 100.3%.

Benzoyl Peroxide from the Dicyclohexylamine–Hydrogen Peroxide Complex.—The complex between dicyclohexylamine and hydrogen peroxide was prepared by the method of Wagner-Jauregg.[4]

A solution of 7.05 g. (17.8 mmoles) of the complex (m.p. 92–94°) in about 175 ml. of ether was cooled to slightly below room temperature and then 5 g. (35.6 mmoles) of benzoyl chloride was added in 3–4 portions with constant swirling of the reaction flask. A white solid precipitated immediately. After standing in an ice-bath for about 0.5 hour the white solid (7 g.) was filtered and washed with a little ether. This solid was completely soluble in hot water and appeared to be the expected hydrochloride of dicyclohexylamine.

The ether filtrate on spontaneous evaporation overnight left a semi-solid mixture which smelled strongly of unreacted benzoyl chloride. The mixture was triturated with methanol and the white crystalline solid collected. The yield was 0.9 g. (21%), and a sample which was recrystallized from ethyl acetate–petroleum ether melted at 105–106° dec.

(8) D. F. DeTar and D. I. Relyea, THIS JOURNAL, **76**, 1680 (1954); D. I. Relyea and D. F. DeTar, *ibid.*, **76**, 1202 (1954).

(9) D. F. DeTar and Y. W. Chu, *ibid.*, **76**, 1686 (1954).

(10) V. R. Kokatnur and M. Jelling, *ibid.*, **63**, 1432 (1941).

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The Effect of Temperature on the Sorption of Polar Gases by Proteins[1]

BY SIDNEY W. BENSON AND R. SRINIVASAN

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In a series of recent papers from this Laboratory[2] it has been shown that the sorption of polar gases such as HCl, BF_3 and CH_3NH_2 by solid proteins is accompanied by the formation of a protein–gas complex of extremely low vapor pressure at room temperature (*i.e.* $\sim 10^{-5}$ mm.). The reproducibility of the composition of these complexes, particularly with HCl, was within ±5% in the range of ambient room temperatures (20–26°), for a very large number of proteins and was independent of sample source, presence of H_2O vapor and particle size (for sufficiently well-dispersed samples to assure fast diffusion).

On the basis of this reproducibility and the striking correlation between the composition of the complexes and the number of strongly-binding acid and basic groups in the proteins, it was proposed that the complex was a stoichiometric compound formed by acid–base interaction of the gas and protein substrate. Further evidence was adduced from the behavior of the complexes with NH_3, BF_3, etc., which indicated properties characteristic of back-titrations[3] and checked the original stoichiometry.

The assignment of a stoichiometry to a protein–HCl complex determined in the above fashion is somewhat arbitrary,[4] resting as it does on the pumping speed of the vacuum line, an arbitrarily selected sensitivity of weighing and some implicit assumptions about the vapor pressure characteristics of the "complex." In the apparatus used for the aforementioned work the arbitrary criterion employed is that the vapor pressure of HCl in equilibrium with complex is less than $\sim 10^{-5}$ mm. in the temperature range studied.[5] Important assumptions implicit in the assignment of stoichiometry are that: (1) the vapor pressures of HCl added to all other groups in the molecule are at least 1–2 orders of magnitude higher than that for the strongly binding groups considered[6]; (2) the vapor pressures of HCl on the strongly binding groups remain significantly less than 10^{-5} mm. in the temperature range used; (3) diffusion through the solid protein particles is sufficiently fast to allow vapor pressure equilibrium to be maintained.

The last assumption regarding diffusion will break down if samples are not well dispersed and we have reported spurious effects caused by slow diffusion.[2b,3] Such effects, however, are detectable and can in principle be avoided. The first and second assumptions are more serious ones. It is clear that at sufficiently low temperatures the vapor pressures of "weakly" binding sites must fall below the arbitrary 10^{-5} mm. Conversely, at sufficiently high temperatures the vapor pressures of "strongly" binding sites must rise above this value.

These observations have been confirmed in recent studies made in our laboratories on the effect of temperature on the formation of protein complexes with HCl and BF_3. For egg albumin the amount of permanently bound HCl decreases smoothly from 0.91 mmole HCl/g. protein at 22° to zero at 80°, while for *β*-lactoglobulin the values at the same temperatures are 1.23 and 0.15, respectively. At the higher temperature equilibrium was attained as quickly as at room temperature, but

(1) This work has been supported by a Research Grant (G-3541-C2) from the Public Health Service, National Institutes of Health, Bethesda, Md.

(2) (a) S. W. Benson and J. M. Seehof, THIS JOURNAL, **73**, 5053 (1951); (b) **75**, 3925 (1952).

(3) S. W. Benson and J. M. Seehof, *ibid.*, **77**, 2579 (1955).

(4) This has been discussed briefly in ref. 3 (see especially footnotes 13, 18).

(5) *I.e.*, this is about what the criterion of less than 0.1 mg. change in weight per day would correspond to for the pumping system used, assuming diffusion through the solid is not important in the rate process. Slow diffusion in the solid would raise the estimated vapor pressures.

(6) A difference of 2–3 kcal. in binding energy would be enough to account for such differences in vapor pressure, entropy effects being assumed the same for strongly and weakly binding groups.

the egg albumin was found to be degraded by the treatment. Similar results have been obtained by Reyerson and Peterson working with insulin.[7]

Similarly, in the cases of the proteins egg albumin, β-lactoglobulin, lactalbumin, zinc insulin, fibrin, casein and bovine plasma albumin the amount of BF_3 bound permanently varied with temperature in the range 0° to 80°. In the first four cases, the amount of BF_3 bound decreased to zero at 80°. Equilibrium at the higher temperatures was achieved only after several days, and at the same time the proteins were observed to undergo degradation and loss in weight. Details of these studies will be published separately.

These results indicate that despite the striking correlations obtained between functional groupings and protein–HCl complexing, the agreements may be fortuitous. This question can only be resolved by a very careful study of the desorption isotherms of the protein–HCl complexes in the low pressure region over an extended temperature range.

To study this point further, isotherms for the desorption of HCl from egg albumin were studied at 32 and at 52°. HCl at a pressure of 10 cm. was sorbed on a sample of spray-frozen egg albumin and the desorption was followed stepwise using a versatile range, combination gas buret and McLeod gage (range 3 to 10^{-5} cm.) to measure gas volumes and pressures. Equilibrium was reached in 6–10 hours. At pressures below 10^{-4} cm. the outgassing of the walls became troublesome and for this reason the isotherms were not followed below this pressure.

Figure 1 shows the isotherms at 32 and 52°. The latter shows clearly an isobaric region in the neighborhood of 0.1 mm. which can be interpreted as evidence for compound formation. Since only one point was taken in this region, the extent of the isobar cannot be determined accurately. While there is no such isobar present in the 32° desorption isotherm, its presence can be reasonably inferred from the shape of the curve and the knowledge (from other work) that an asymptote exists at about 10^{-4} to 10^{-5} cm. and 0.95 ± 0.05 mmole HCl/g. protein.

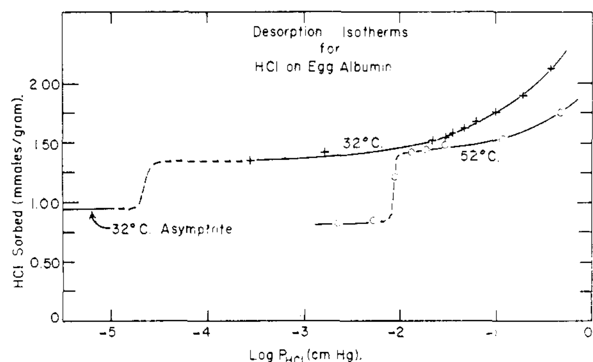

Fig. 1.—Desorption isotherms for HCl on egg albumin: +, experimental points at 32° (upper curve); solid line at left of upper curve is experimental asymptote at 32°; dashed line is hypothetical; ⊙, refer to experimental points at 52°; dashed line is hypothetical.

Using the Clausius–Clapeyron equation, the partial molal heats of binding are found to have values of 15.8 kcal./mole for the HCl sorbed above the isobaric region and a minimum value of 43 kcal./mole for the HCl bound below the isobaric region. This latter value indicates that the "permanently" bound HCl which has been attributed to strongly binding basic groups is bound as strongly as the HCl in such hydrochlorides as NH_4Cl. This lends further support to the identification of this fraction as stoichiometrically bound HCl.

This preliminary work appears very promising since a study of the desorption isotherms appears to offer an unambiguous method of investigating the nature of the compounds of HCl with proteins. It is possible that the free basic groups can be distinguished among themselves in this manner. The chief difficulty with the method which is not insuperable is the long equilibration time. In principle, the method can be extended to lower pressures by correcting properly for the outgassing from the walls.

(7) L. H. Reyerson and L. Peterson, *J. Phys. Chem.*, **59**, 1117 (1955).

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Preparation of Flavanone-2-C[14] by an Exchange Reaction[1]

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The preparation of flavanones by the isomerization of the corresponding 2′-hydroxychalcones is a well-established procedure.[3] Polyhydroxychalcones usually have been prepared by the condensation of the appropriately substituted benzaldehyde with the required *o*-hydroxyacetophenone in the presence of a basic catalyst.[4] Although the isomerization reaction is usually quite efficient,[5] the condensation reaction to produce the chalcone is frequently poor for the more highly hydroxylated compounds.[6]

Since work here and elsewhere[7] has demonstrated the reversibility of the benzaldehyde–acetophenone condensation, a simple path to C^{14}-labeled flavanones, which might avoid the low yields in the condensation step, appeared to be the exchange of the appropriate carbonyl-labeled benzaldehyde with the desired non-radioactive chalcone followed by ring-closure of the recovered labeled chalcone.

As a test, equimolar amounts of benzaldehyde-α-C^{14} and *o*-hydroxybenzylideneacetophenone were

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(3) (a) S. v. Kostanecki and W. Szabranski, *Ber.*, **37**, 2635 (1904); (b) F. Mayer and A. H. Cook, "The Chemistry of Natural Coloring Matters," Reinhold Publishing Corp., New York, N. Y., 1943, p. 164.

(4) (a) C. Feuerstein and S. v. Kostanecki, *Ber.*, **31**, 715 (1897); (b) T. Emilewicz and S. v. Kostanecki, *ibid.*, **28**, 2014 (1898).

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(7) (a) R. L. Frank and R. P. Seven, THIS JOURNAL, **71**, 2629 (1949); (b) M. Weiss, *ibid.*, **74**, 200 (1952).