Thus one is led to conclude that most of the zinc is bound to two rather than to one imidazole group. One simple model is to suppose that all of the imidazole groups may be divided into binding sites, two per insulin monomer, each site consisting of two groups. These two groups may be on the same monomer unit, or on adjacent ones.[24,25]

As before, equation 1 represents the hydrogen ion equilibria of the dissociable groups. However, for each zinc ion bound, two imidazole groups (rather than one) are now removed from participation in this equilibrium, *i.e.*, it applies to $(4 - 2\bar{\nu}_{Zn})$ of the imidazole groups. In place of equation 2 we now have

$$\log \frac{\bar{\nu}_{Zn}}{\bar{\nu}_{F.S.}} = \log C_{Zn^{++}} + \log K_{Zn} - 1.736jwZ' \quad (7)$$

where $\bar{\nu}_{F.S.}$ represents the number of free sites per insulin monomer, *i.e.*, the number of sites with both imidazole groups free of both H^+ and Zn^{++}. The number of sites without zinc is $2 - \bar{\nu}_{Zn}$; the fraction of these entirely free from H^+ is x^2_{IM}, *i.e.*, $\bar{\nu}_{F.S.}$ is equal to $x^2_{IM}(2 - \bar{\nu}_{Zn})$. All the other parameters have the same meaning as before, but the value of log K_{Zn} should now have about twice its previous value, *i.e.*, it is equal to 6.0.[26]

Substituting for $\nu_{F.S.}$ and, as before, for $C_{Zn^{++}}$, equation 7 becomes

$$\log \frac{\bar{\nu}_{Zn}}{(2 - \bar{\nu}_{Zn})(1 - \bar{\nu}_{Zn})} = 2 \log x_{IM} + \log P + \log K_{Zn} - 1.736jwZ' \quad (8)$$

The value of r is now

$$r = 2\bar{\nu}_{Zn} + (4 - 2\bar{\nu}_{Zn})x_{IM} + \Sigma n_i x_i \quad (9)$$

the summation again extending over all groups other than imidazole groups. Equations 5 and 6 are unchanged.

The titration curve computed in this way is shown by curve 3 of Fig. 3; the computed polarographic curve by curve 3 of Fig. 2. Both curves are in reasonable agreement with the experimental data. This does not, of course, indicate that the simple model on which the calculation is based is correct, but it leaves little doubt as to the conclusion that most of the zinc ions, even on the reversed curve, are chelated to *two* imidazole groups.

The above calculations have not taken into account the dissociation of hydrogen ions from the hydration sphere of the zinc ion. If they are extended to higher *p*H values a curve is obtained which becomes identical with the curve for zinc-free insulin above *p*H 7.

In actual fact, the reversed curve of Fig. 1 differs from the curve for zinc-free insulin by nearly 2 groups at *p*H 6.75. This difference is far too large to be accounted for by dissociation of hydrogen ions from the hydration sphere of the zinc ion, if the first intrinsic dissociation constant of $(-1m)_2Zn(H_2O)_2^{++}$ is taken to be equal to Diehl's value[12] for the first dissociation constant of $Zn(H_2O)_4^{++}$, *i.e.*, log $K = -8.0$. A much larger value, log $K = -6.5$, must be used instead.[27] If this value is used, a value of *ca.* 0.16 must be assigned to jw to fit the titration curve above *p*H 7 to *p*H 9. For zinc-free insulin, for the most reasonable pK values, a constant value of jw of 0.10, corresponding to an actual molecular weight of 12,000, had to be used in this *p*H range. The value of 0.16, corresponds to the existence of insulin as a trimer in this *p*H range, in agreement with observed values of the molecular weight.[28,29] This suggests the possibility that the molecular weight of insulin on the alkaline side may depend on whether or not zinc is present.

Direct Curve.—The crystalline precipitate of zinc-insulin probably consists of a closely-knit array of monomer units, in contrast to the loosely-connected structure of the amorphous precipitate. The passage of ions in and out of the crystals is likely to be difficult. It is this which causes the flattening of the titration curve in the isoelectric region. Passage of ions is, however, not completely inhibited, for, if it were, the direct titration curve would fall along curve 4 of Fig. 3, and, since only dissolved insulin could contribute to the polarographic current, the direct polarographic data of Fig. 2 would fall along the solubility curve below *p*H 5.6, instead of above it.

(24) The model used represents, of course, a tremendous oversimplification. The number of possible arrangements in a randomly oriented precipitate is virtually infinite. Even on insulin in solution (in the *p*H range under consideration it exists largely as tetramer) it is most unlikely that all of the imidazole groups can be conveniently paired off, as we have assumed. The assumption that Zn^{++} is bound only to two imidazole groups, is, of course, also oversimplified. Undoubtedly some zinc ions are bound to only one imidazole group; others may be bound to as many as three or four.

(25) Structural considerations suggest that the two groups must be on adjacent monomer units. However, the simple equilibrium constants written are independent of the location of the groups.

(26) In the case of zinc, in contrast to cadmium and most other metals, the standard free energy change accompanying bonding to a second complexing group is approximately equal to that accompanying bonding to the first such group. *Cf.* ref. 13.

(27) The second dissociation constant has been assigned a value one fourth that of the first, allowing for the usual statistical factor. Electrostatic interaction with the charged protein molecule has been taken into account in making the calculations.

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NOTES

Reactions of *p*-Dimethylaminochalcones with Acetic Anhydride

BY GERALD BRANCH AND JEROME F. THOMAS

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Katzenellenbogen and Branch[1] measured the spectra of the isomeric *p*-dimethylaminochalcones, *p*-$C_6H_5COCH{=}CHC_6H_4N(CH_3)_2$ and *p*-$(CH_3)_2NC_6H_4COCH{=}CHC_6H_5$, in methanol, in methanol 0.1 N with respect to hydrochloric acid, in acetic anhydride with 0.4% by volume of sulfuric acid and in concentrated sulfuric acid. Intense bands were found at 419 and 387 mμ in methanol, 294 and 315 mμ in acidified methanol, 483 and 490 mμ in acidified acetic anhydride and 425 and 396 mμ in sulfuric acid. The first of each pair of wave lengths is for the first-mentioned dimethylaminochalcone.

From each of these solutions the unchanged base was recovered by simple means. Assuming that the easy recoveries of the chalcones were simple formations of bases from salts, it was concluded that the four spectra from each chalcone were those of the free base and its ions with one, two and three charges. This would mean that the bands at 483 and 490 mμ were due to $C_6H_5CO^+HCH{=}CHC_6H_4N^+H(CH_3)_2$ and $(CH_3)_2\overset{+}{N}HC_6H_4C\overset{+}{O}HCH{=}CHC_6H_5$.

However, the peak of the principal band of C_6H_5-

(1) E. Katzenellenbogen and G. Branch, THIS JOURNAL, **69**, 1615 (1947).

$\overset{+}{C}OHCH{=}CHC_6H_5$ is at 390 mμ in acetic acid with 20% by volume of sulfuric acid.[2] Further the λ_{max} values for the *p*-dimethylaminochalcones in sulfuric acid agree fairly well with that for the positive ion of chalcone, considering the bathochromic solvent effect of sulfuric acid on the principal bands of positive ions of unsaturated aldehydes and ketones. One must conclude that the bands observed at 483 and 490 mμ are not due to the second ions, but those observed at 425 and 396 mμ are.

In a solution of the singly charged positive ion of a tertiary amide, the tautomeric equilibrium $R_2\overset{+}{N}H\text{-}(R)C{=}O \rightleftarrows R_2\overset{+}{N}{=}C(R)OH$ is established. The interpolation of a phenyl or styryl group between the carbonyl and amino groups does not exclude this tautomerism, though it should greatly reduce the relative amount of the imidol tautomer, for its formation requires a change from a benzenoidal to a quinoidal structure. This quinoidal tautomer should absorb light in the visible. Hence the spectra obtained in acidified methanol show that the first ions of the *p*-dimethylaminochalcones are almost completely amido in methanol. The positive ion of *p*-nitrosodimethylaminobenzene is an example where the imidol or quinoidal tautomer is the more stable of the two.

Acetic anhydride would acetylate the quinoidal tautomer, thereby stabilizing a quinoidal structure. The formulas of the resulting acetates of the quinoidal forms of the first ions are $C_6H_5(CH_3CO_2)C{=}CH{-}CH{=}C_6H_4{=}\overset{+}{N}(CH_3)_2$ and $(CH_3)_2\overset{+}{N}{=}C_6H_4{=}C(O_2CCH_3)CH{=}CHC_6H_5$.

The formation of these acetates should be highly reversible. The positive charge of the ion is distributed by resonance over many atoms including the oxygen atom to which the acetyl group is attached. This makes these ions high energy acetates. The reformation of the benzenoidal tautomer also tends to reverse the acetylation. Hence acetic acid should react with these acetates to reform acetic anhydride to an anomalous extent. These expected reversals are shown in the published spectra. The bands of the benzenoidal ions (at 294 and 315 mμ) and those of the acetates of the quinoidal ions (at 483 and 490 mμ) appear on the same spectra.

The values of λ_{max} are reasonable for the structures suggested. The value of λ_{max} for the related compound $p\text{-}(CH_3)_2\overset{+}{N}{=}C_6H_4{=}C(C_6H_5)_2$ is 462 mμ in acetic acid. The replacement of a phenyl group by a styryl group would be highly bathochromic, while the replacement of the other group by an acetate group would be hypsochromic. Hence 490 mμ is a reasonable expectation for λ_{max} of $(CH_3)_2\overset{+}{N}{=}C_6H_4{=}C(O_2CCH_3)CH{=}CHC_6H_5$. The main cause of the color in both isomers is the conjugate system $\overset{+}{N}{=}C_6H_4{=}C{-}C{=}CC_6H_5$, so it is natural that the λ_{max} values of the two isomers are not very different.

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The Reaction of Fluorine with Titanium, Zirconium and the Oxides of Titanium(IV), Zirconium(IV) and Vanadium(V)[1]

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Titanium reacts appreciably with fluorine above 150°, the vigor of the reaction depending on the size of the metal particles. Conversion is complete above 200°. Experimental quantities of titanium(IV) fluoride could be synthesized effectively by fluorination of massive titanium, preheated to 350° in the reactor.

Fluorine does not react appreciably with titanium(IV) oxide below 250°. Conversion is about 80% at 285° and complete at 350°. The titanium(IV) fluoride vaporizes and can be condensed.

Titanium(IV) fluoride is extremely hygroscopic; so much so that handling even in a dry-box becomes difficult. Apparently because of this the literature on X-ray powder patterns[2] is unreliable. The reported pattern is not that of titanium(IV) fluoride but is that of one or more reaction products of the fluoride with water. Work on this problem is in progress.

Fluorine reacts with zirconium metal above 190°. Fluoride coating of metal prevents complete conversion; the maximum obtained was about 90% at 420°. At higher temperatures product was lost.

Zirconium(IV) oxide does not react with fluorine at 100°, but converts to zirconium(IV) fluoride above 250°. Conversion is about 80% at 400°, 95% at 450° and complete at 525°. All lines in the X-ray powder patterns of the products could be attributed to oxide or fluoride. Comparison with prepared zirconium(IV) oxyfluoride showed its absence, which is in accord with its formation from oxide and fluoride only above 550°.[3]

Vanadium(V) oxide reacts with fluorine at 475° to form volatile vanadium oxytrifluoride, VOF_3, a reaction analogous to that reported for bromine trifluoride and the oxide.[4]

Experimental

The fluorinations were carried out in a manner similar to that previously reported.[5] An L-shaped nickel reactor was used with the titanium and vanadium oxides, the short side being cooled with Dry Ice. Titanium metal could be fluorinated successfully in glass, the volatilized fluoride being collected in an extension of the reactor tube. All product transfers were made in the dry-box.

Stock chemicals were used for the titanium and zirconium reactions. Vanadium(V) oxide was prepared by thermal decomposition of ammonium metavanadate.

Titanium was determined by ignition to the oxide with nitric and sulfuric acid or by precipitation with 8-quinolinol after removal of fluoride.[6]

Anal. Calcd. for TiF_4: Ti, 38.7. Found: ($Ti + F_2$) Ti, 38.5, 38.7, 39.1; ($TiO_2 + F_2$) Ti, 38.6, 39.2, 38.9.

(1) Research supported by the Research Corporation and the Atomic Energy Commission.

(2) X-Ray Diffraction Data Cards, American Society for Testing Materials, Philadelphia, Pa.

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