means of essentially the same procedure which has been used for the hydrogenation of II to 2,6-dimethylcyclohexanol,[6] the conversion of I to 2,6-dimethylcyclohexanol in one operation could not be accomplished in good yield in the presence of this catalyst. Better yields of the dimethylcyclohexanol were realized from I when the intermediate II was isolated and purified before its hydrogenation was attempted.

For purposes of identification, some samples of 2,6-dimethylcyclohexanol produced from I in a single operation and some samples obtained by the hydrogenation of II were converted to their phenylurethans, all of which melted at 158°. Application of the same hydrogenation procedure to a sample of II obtained from the Shell Development Co. has been observed[6] to give a 2,6-dimethylcyclohexanol, whose phenylurethan melts at 132°. Anziani and Cornubert[7] have shown that the 132° and 158° phenylurethans are derived from stereoisomeric forms of 2,6-dimethylcyclohexanol. The foregoing observations indicate that small changes in reaction conditions, the nature of the Raney nickel catalyst and small amounts of impurities present in the xylenol may exert a profound effect upon the stereochemical course of the reaction. There appears to be no recorded evidence that mixtures of stereoisomeric modifications of 2,6-dimethylcyclohexanol ever have been formed by any of the several available preparative methods. One isomer always is produced to the exclusion of the other.[6,7,8]

Experimental

2-Methyl-6-dimethylaminomethylphenol (I).—A stirred mixture of 60 g. of *o*-cresol (Eimer and Amend, Technical) and 90 g. of 25% aqueous dimethylamine (du Pont, Technical) was kept at 15–20° while 50 g. of 30% formalin was added. The aqueous layer was saturated with sodium chloride, separated and extracted with three 60-ml. portions of ether. The ether extracts were combined with the organic layer from the reaction mixture, and the temperature of the resulting solution was maintained below 30° during extraction with two 500-ml. portions of 15% hydrochloric acid. Neutralization of the ice-cold acid extracts to *p*H 9 with 25% aqueous sodium hydroxide effected the separation from the solution of an oil. The aqueous layer was extracted with three 60-ml. portions of ether, and the extracts were combined with the oil. Removal of the ether left 54 g. (60%) of crude Mannich base (I), which gave 39 g. (44%) of distillate, b. p. 91–95° (2 mm.), $n^{25}D$ 1.5195. The reported b. p. is 110–112° (13 mm.).[5]

Anal. Calcd. for $C_{10}H_{15}NO$: C, 72.69; H, 9.15. Found: C, 72.95; H, 8.96.[9]

Picrate, yellow needles from methanol, m.p. 153–154°. *Anal.* Calcd. for $C_{16}H_{18}N_4O_8$: C, 48.73; H, 4.60; N, 14.21. Found: C, 48.91; H, 4.60; N, 13.78.[9]

Because distillation of crude I was always attended by the evolution of dimethylamine and the formation of resins (through intermolecular condensations of I), greater overall yields of II were realized when crude I was used without purification.

2,6-Xylenol (II).—A mixture of 54 g. of crude I and about 8 g. of Raney nickel was treated at 200° with hydrogen at 1800 lb./sq. in. pressure until one mole of hydrogen per mole of I had been consumed. A benzene solution of the reaction mixture was shaken with 15% hydrochloric acid and then with five 100-ml. portions of 15% aqueous sodium hydroxide. Acidification of the cold alkaline extract with 20% hydrochloric acid brought about the separation of the xylenol (II) as an oil, which was combined with the ether extracts of the residual aqueous solution and dried over "Drierite." Distillation of the oil remaining after removal of the ether gave 24 g. (60%) of II, b. p. 197–202°.[10] On redistillation, this crude xylenol yielded a solid condensate, m. p. 35–40°,[11] which formed an α-naphthylurethan, white needles, m. p. 178°,[12] from petroleum ether (b. p. 66–71°).

(6) Carlin, THIS JOURNAL, **67**, 928 (1945).

(7) Anziani and Cornubert, *Compt. rend.*, **217**, 233 (1943).

(8) Skita, *Ber.*, **56B**, 2284 (1923).

(9) These samples were prepared for analysis by Mr. Gerald W. Larson.

(10) Gattermann, *Ann.*, **357**, 327 (1907), gives the b. p. 203°.

(11) Noelting, *Ber.*, **21**, 2829 (1888), reported the m. p. 49°.

(12) Hurd and Pollack, THIS JOURNAL, **58**, 181 (1936), reported the m. p. 176.5°.

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On Long Range Forces in Solution

BY B. CARROLL

In looking for long range forces[1] in solution we followed the procedure of trying to coat the bovine serum albumin molecule by the adsorption of simple dye anions. The effect of a proteolytic enzyme on the coated protein was then determined.

Calculations based on such data as molecular weight, diffusion coefficient, viscosity, and dielectric dispersion[2] lead to a value of about 25,000 sq. Å. for the surface area of the serum albumin molecule if a prolate ellipsoid is used as a model. Other models such as a flat disc 20 Å. thick, as suggested by spreading experiments, yield a somewhat smaller value.

Orange I was used for building up the "screen" or "blanket" on the protein molecule. The area covered by this dye molecule may be 60 to 150 sq. Å. depending on whether the dye molecule is cube-shaped, or is spread flat over the surface of the albumin molecule. If the average is taken on these two extreme values, it will be seen that some 250 dye molecules on the surface of an albumin molecule may be considered a unimolecular layer.

We found that the adsorption of (2.0×10^{-5} *M*) Orange I by bovine albumin in 0.01 *M* hydrochloric acid indicated values above 200 dye molecules per protein molecule, when the protein concentration was two parts per million or less. As the protein concentration was increased, the number of dye molecules per protein molecule dropped gradually. At an albumin concentration of 10 parts per million, the number of dye molecules per protein molecule was 110. The

(1) Rothen, THIS JOURNAL, **70**, 2732 (1948).

(2) Alexander and Johnson, "Colloid Science," Vol. I, Oxford University Press, 1949.

spectrophotometric method was used to observe the extent of adsorption.[3]

To determine the effect of proteolytic enzymes on the dye-coated substrate at high dilutions, a new method was developed for measuring proteolytic activity.[4] Klotz[5] has shown that denaturing agents such as heat or strong alkali, will cause the bovine serum albumin molecule to gradually lose its binding capacity for simple dye anions. We found that proteolytic enzymes had the same effect on the protein molecule. Thus, the action of the enzymes was followed by the change in the spectral property of the dye.

This spectral method was found so sensitive that the effect of one part per billion of pepsin upon the albumin molecule could be observed. However, when a coated albumin molecule having more than 200 dye anions on its surface was used as the substrate, even a concentration as high as 0.1% pepsin had no effect. When the substrate molecule contained less than a hundred dye anions, the substrate could be attacked and made to lose its binding capacity completely for dye anions. Thus, the incomplete unimolecular layer offered very little protection to the substrate.

The thickness of the "blanket" which inhibits the proteolytic action of pepsin on bovine albumin is a few Å. units. In view of these results, Rothen's interpretation of his experimental data is open to question.[6]

The author is indebted to Prof. A. W. Thomas for his interest and suggestions during the course of this work.

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(3) Klotz, THIS JOURNAL, **68**, 2299 (1946).

(4) Carroll, *Science*, in press.

(5) Klotz, THIS JOURNAL, **70**, 2935 (1948).

(6) Two points of criticism have been raised by the reviewer: (1) does a unimolecular layer form or do the dye molecules cluster about "certain sites," (2) is not the cessation of enzymatic activity, due to the conversion of the substrate from a positively to a negatively charged particle.

In answer to the first point, it may be stated that adsorption in 0.01 *M* hydrochloric acid appears to be a two-step process. Within one minute after the mixing of dye and albumin about 100 to 130 dye anions (of diversified structures) are taken up by a single albumin molecule. Since there are 110 cationic groups per protein molecule it is reasonable to assume that these groups are the loci of attachment for the dye anions. Further uptake of dye is more deliberate. Aging for several weeks was required to attain a coating of 250 dye anions. Whether these additional dye molecules take up vacant places on the surface of the albumin or whether two dye molecules cluster about every one of the 110 sites does not change the argument very much in favor of the existence of a unimolecular layer.

In regards to the question of the charge of the substrate, it would appear that the very criticism would be an additional reason why complex formation between enzyme and substrate is required for enzymic activity. It may be mentioned that the pepsin–albumin reaction has a half life of eight minutes[4] even when the system is at a *p*H 5.4. However the strongest argument that steric and not electrostatic effects are responsible for the cessation of enzymic activity will be published shortly. In this work the inhibiting effect of equivalent numbers of different dye anions on the surface of an albumin molecule are compared.

Further Applications for Egloff's Boiling Point Equation

BY W. D. ENGLISH AND R. V. V. NICHOLLS

In our investigations of the hydrides of Group IV elements we were interested in deriving boiling point relationships. Because of similarity to hydrocarbons, Egloff's equation was considered.[1]

$$T = a \ln (n + b) + k$$

T is b. p. in °K., n is the number of central (carbon) atoms, and a, b and k are empirical constants.

Previous investigators found that a and b were constant for all hydrocarbons except for widely varying structures,[2] while k varied with structure.

The classes of compounds investigated were the silanes, germanes, mono-*n*-alkyl silanes (terminal Si) and poly-*n*-alkyl silanes (internal Si). There were two different structural types for the poly-*n*-alkyl silanes (Tables V and VII).

We have found that equations similar to those developed by Egloff for the hydrocarbons are valid for these other series. The new equations (Tables II, III, IV, V and VII) show a root mean square deviation of the observed from the calculated boiling points of 0.68°, compounds in parentheses being omitted.

The tables include for comparison abstracts of two tables (nos. I and VI) from Egloff's paper.[1] It may be seen from Tables I, II and III that compounds which contain only one central atom have a practically constant deviation of −18° from the calculated boiling point. In Table VIII is a comparison of the various values of a, b and k for the different series of compounds: k has the value −416.3 for all normal compounds and −424.5 for iso compounds; a and b vary from class to class, but have the same values when confined to different structures of the same class. Note the very similar values of a and b for the two classes of normal alkyl silanes.

As the values reported for a and b were calculated from a small number of compounds in most cases, they may be changed slightly when more compounds become known. However, the good agreement between calculated and experimental boiling points indicated the constants are close to their true values.

TABLE I

ALKANES[1]

$T = 323.73 \ln (n + 4.4) - 416.31$

Name	No. central atoms	T, °K. Obsd.	T, °K. Calcd.	ΔT
(Methane)	(1)	(111.55)	(129.63)	(−18.08)
Ethane	2	184.6	184.6	0.0
Propane	3	230.9	231.6	−0.7
Butane	4	272.6	272.7	−0.1

[1] Abstracted from Table 2, ref. (1).

(1) Egloff, Sherman and Dull, *J. Phys. Chem.*, **44**, 730 (1940).

(2) Corbin, Alexander and Egloff, *Ind. Eng. Chem.*, **38**, 156 (1946).