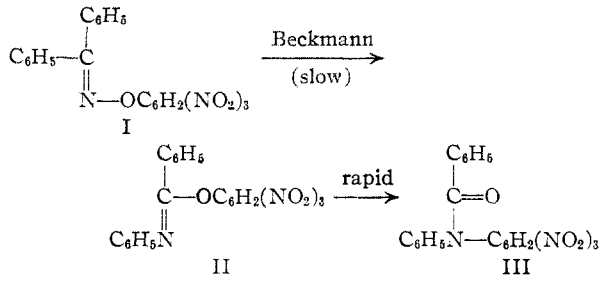
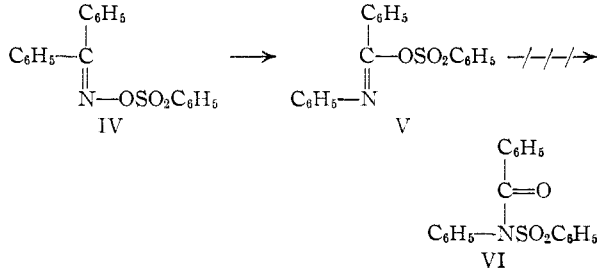
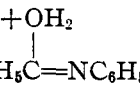


sulfonylbenzophenone oxime (IV) on standing or melting (m.p. 62°) probably had taken a similar course, and that the product, therefore, should be given structure VI rather than V, which had been



assigned by Kuhara, *et al.*[3] Since Kuhara, *et al.*, reported that their product *hydrolyzed rapidly* to benzanilide and benzenesulfonic acid on addition of water, Chapman and Howis[2a] apparently were assuming this behavior for VI, and by implication for III. Despite the fact that N-benzoylbenzenesulfonamides similar to VI have long been known to be unreactive toward water,[4] this suggestion of Chapman and Howis has been accepted in several reviews of the Beckmann rearrangement.[5] Recently Oxley and Short[6] have presented additional convincing evidence showing that Kuhara's intermediate has structure V rather than VI.

To clarify the situation further we have shown that N-picrylbenzanilide (III) is not readily hydrolyzed by water. When the rearrangement of I was carried out in aqueous acetone solution the Beckmann product, benzanilide, was formed instead of III. If II is formed under these conditions, it must, like V, react rapidly with water to give benzanilide. Actually, in anhydrous media there is no compelling evidence to indicate that the transformation of I to III involves a Beckmann rearrangement, since the intermediate II has not been isolated. It seems probable, however, by analogy with the rearrangement of IV that the transformation I → II → III suggested by Chapman and Howis[2a] does take place. On the other hand, in aqueous acetone solution the solvolysis of the picryl group by water and attack by water at the doubly bonded carbon of I may be simultaneous with the migration of the phenyl group. This concerted process could give rise to an imino-



carbonium ion,[7] $C_6H_5C{=}NC_6H_5$, and benzanilide could be formed under these conditions without the intermediate formation of II.[8]

Experimental

Following the procedure of Chapman and Howis,[2a] O-picrylbenzophenone oxime (I) was prepared by adding 51 ml. of 0.5 *N* aqueous sodium hydroxide and 6.3 g. of picryl chloride alternately in small portions to a solution of 5.0 g. of benzophenone oxime in 50 ml. of acetone. When the resulting heterogeneous mixture was heated to the boiling point, a solid was formed, which proved to be a mixture of benzanilide and N-picrylbenzanilide (III). In a second experiment sufficient acetone was used in the preparation to keep I in solution. The solution was warmed on an electric hot-plate and aliquots of water introduced, taking care that no oil separated. When the solution reached about 50% concentration, acetone was boiled off until oil droplets appeared, and a seed crystal of benzanilide added. The solid obtained on cooling was separated and crystallized from benzene to yield 2.9 g. (58%) of benzanilide, m.p. and mixed m.p. 158–159°.

When a solution of 1 g. of III in 25 ml. of acetone and 10 ml. of water was refluxed for two hours, the recovery of starting material was quantitative.

(4) See, for example, Gerhardt and Chiozza, *Ann. chim. phys.*, [3] **46**, 151 (1856); Wolkowa, *Z. Chem.*, 579 (1870); or note the slow hydrolysis of saccharin, Suter, "Organic Chemistry of Sulfur," John Wiley and Sons, Inc., New York, N. Y., 1944, p. 623.

(5) Jones, *Chem. Revs.*, **35**, 335 (1944); Sidgwick, "The Organic Chemistry of Nitrogen," Oxford University Press, 1945, p. 191. Alexander, "Principles of Ionic Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1950, pp. 72, 75.

(6) Oxley and Short, *J. Chem. Soc.*, 1514 (1948).

(7) Jones, *Nature*, **157**, 519 (1946); Smith, THIS JOURNAL, **70**, 371 (1948); Harvill, Roberts and Herbst, *J. Org. Chem.*, **15**, 58 (1950).

(8) This possibility also exists, of course, for the hydrolysis or ammonolysis[6] of the O-benzenesulfonyl oximes.

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Purity Determination with the Electron Microscope

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An important and extremely difficult problem in the chemistry of proteins and other substances with large molecules is the determination of purity. The methods in current use, such as ultracentrifugation, electrophoresis, solubility determination, etc., have been reviewed and criticized on numerous occasions. There is fairly general agreement that no completely satisfactory solution to the problem has yet been developed.

A fundamentally different approach to the estimation of purity was recently described by Williams and Backus.[2] In conjunction with the demonstration of a new technique for determining molecular weight, they used the electron microscope to determine the purity of bushy stunt virus protein preparations. The purity assay consisted of a careful examination of a large number of electron microscope fields for presence or absence of foreign particles.

As the authors pointed out, it is obvious that particles with size below the limit of resolution of the electron microscope go undetected by this method. Furthermore, particles present in small number, even though they be very large, might be overlooked. The authors also discussed this latter limitation; nevertheless, they concluded from the use of the electron microscope that the contamination of their preparations by particles of relatively large size was less than 1%.

(1) Publication No. 2 of the Department of Biophysics.

(2) R. C. Williams and R. C. Backus, THIS JOURNAL, **71**, 4052, 1949. See also Williams, Backus and Steere THIS JOURNAL **73**, 2062 (1951).

The thesis of the present communication is that the electron microscope, or, in fact, any other device which counts particles in a limited sample, is in principle not capable of providing adequate evidence of absence of large amounts of impurity in the total body of material. This point of view is contingent upon the premise that the useful criterion of purity is the relative mass of contaminant. No criticism is intended of the new method of determining molecular weight, provided adequate criteria of purity are satisfied.

According to the Poisson law,[3] the probability, P, that a particle will not be detected when examination is made of a total volume of material expected to contain, on the average, n such particles, is given by the expression $P = e^{-n}$. In practice, this means that, if a sample large enough to contain ν macromolecules is examined, the probability is 0.37 that a contaminant present in a number ratio relative to the particles of the primary component of $1/\nu$ will go undetected. If it happened that the ratio of the mass of the contaminant particle to the mass of the primary particle was numerically about equal to ν, this would mean that even preparations containing on a mass basis equal amounts of contaminant and primary component would seem about one time in three not to be impure.

Williams and Backus examined fields containing a total of five million virus particles and found no bacteria. If it is assumed that bacteria weigh 3×10^{-13} g. on a dry basis, or 20,000 times[4] as much as bushy stunt virus particles, it can be calculated on the basis of the above formula that the probability is $^{11}/_{12}$ that their preparation does not contain more than 1% by mass of such bacteria. However, suppose that the bacterial particles weigh 3×10^{-12} g. In such a case, their experiment would indicate a probability of $^{11}/_{12}$ that the contamination does not exceed 10% and only $^{2}/_{9}$ that it does not exceed 1%.

Some bacteria[5] and some yeast particles[6] weigh as much as 10^{-9} g. Thus, the virus preparations could have had more bacteria or yeast than virus, on a mass basis, in spite of the negative finding with the electron microscope. The point to these calculations is not that there is substantial doubt concerning the absence of extreme bacterial contamination in the virus preparations studied. Independent considerations indicate otherwise. Rather, the point is that, even if very heavy contamination had been present, it could have been missed entirely by the electron optical examination.

A fundamental difficulty with examination of small samples as a method of purity assay is that one can set no upper limit to the size of sample which must be examined to establish reasonable evidence of absence of impurity, unless one knows the upper limit of mass of the contaminant particles which must be sought. This information cannot be obtained by any method which involves the mere counting of particles in a limited volume.

On the basis of the foregoing, it is concluded that procedures based on counting small numbers of contaminant particles in a limited sample, such as electron optical examination, are incapable of assaying preparations of macromolecules for large size impurities. This incapacity is in addition to the obvious inability of the method to determine amounts of impurities whose particles are too small to be resolved.

Other criteria of purity, such as electrophoretic mobility, sedimentation rate, solubility, etc., also have their limitations. Obviously, each can determine lack of uniformity only with respect to some particular property. Also, sensitivity is limited, especially when the experiments are not carried out with the greatest possible precision. However, these methods, in contrast with electron optical examination, have the advantage of measuring directly properties very closely related to the integrated masses of the various components per unit volume of solution. With these methods one can easily avoid missing large quantities of materials which differ substantially from the primary component with respect to the criterion used.

In spite of the serious limitation discussed above, electron optical examination can provide useful evidence of absence of impurity when used in conjunction with other criteria, such as ultracentrifugation, electrophoresis, etc. For example, sedimentation experiments, particularly when carried out in several media of different densities, can provide substantial evidence of absence of high concentrations of very large particles. Naturally, when bacteria are the contaminant, this applies only to the time the sedimentation experiment was carried out. Such experiments are also efficient in separating the primary component from large amounts of much smaller particles. However, ultracentrifugation techniques are relatively insensitive to small amounts of contaminants which differ from the primary component by only an order of magnitude in size. They are also insensitive to small differences in morphology. When the primary component has particles in the proper size range, electron optical examination is well adapted to the detection of impurities of these sorts.

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(3) T. C. Fry, "Probability and its Engineering Uses," D. Van Nostrand, Co., Inc., New York, N. Y., 1928, p. 214 ff.

(4) The figure used by Williams and Backus.

(5) According to R. St. John-Brooks in "A System of Bacteriology" I p. 164 (1930) (Medical Research Council), bacteria range in size from micrococci 0.2 μ in diameter to rods 5 μ in diameter and 80 μ long. Typical bacteria are rods 1 μ in diameter and 6 μ long. On the assumption that bacteria are composed of 10–20% solids, the range of dry mass is from 10^{-15} g. to 10^{-9} g. with 10^{-12} g. as a typical value.

(6) N. F. Conant in "Bacterial and Mycotic Infections of Man," pp. 588 ff. (Lippincott, 1948, edited by Dubos) refers to various yeast-like forms with diameters varying from 5 μ to 20 μ. Calculations similar to those for bacteria lead to dry weights from 10^{-11} to 10^{-9} g.

Some Five-membered Ring α-Aminoketones

BY NELSON J. LEONARD, FRANCIS E. FISCHER, ERIC BARTHEL, JR., JOHN FIGUERAS, JR., AND WILLIAM C. WILDMAN

Only one 3-pyrrolidone, namely, 1-methyl-3-pyrrolidone (IIa), has been prepared previously *via* a Dieckmann or related ring closure,[1,2] and since interest in substituted 3-pyrrolidones has been revived,[3]

(1) E. A. Prill and S. M. McElvain, THIS JOURNAL, **55**, 1233 (1933).

(2) A. H. Cook and K. J. Reed, *J. Chem. Soc.*, 399 (1945).

(3) P. L. Southwick, D. I. Sapper and L. A. Pursglove, THIS JOURNAL, **72**, 4940 (1950),