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

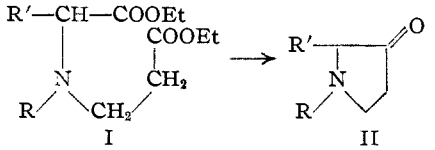
Only one 3-pyrrolidone, namely, 1-methyl-3-pyrrolidone (IIa), has been prepared previously *via* a Dieckmann or related ring closure,[1,2] and since interest in substituted 3-pyrrolidones has been revived,[3]

(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).

it seemed useful to record the satisfactory synthesis of two other 3-pyrrolidones (IIb, c) by the Dieckmann ring closure of amino diesters (Ib, c). Thus, 1-methyl-2-ethyl-3-pyrrolidone (IIb) was

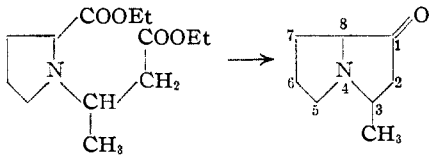


I II

a, R = CH_3, R′ = H; b, R = CH_3, R′ = C_2H_5; c, R = n-C_4H_9, R′ = H.

obtained in over-all yield of 65% starting with the addition of ethyl α-methylaminobutyrate to ethyl acrylate, followed by the ring closure of α-carbethoxypropyl-β′-carbethoxyethylmethylamine (Ib) using sodium ethoxide or potassium. 1-Butyl-3-pyrrolidone (IIc) was obtained in equivalent over-all yield by the condensation of ethyl chloroacetate with ethyl β-butylaminopropionate followed by the ring closure of β-carbethoxyethylcarbethoxymethylbutylamine (Ic) using sodium ethoxide. The ethyl β-butylaminopropionate was available in 88% yield by the addition of butylamine to ethyl acrylate.

In the closely related bicyclic series, 1-keto-3-methylpyrrolizidine (IV) was obtained *via* the ring closure of ethyl β-N-(2-carbethoxypyrrolidyl)-butyrate (III) using sodium hydride. The addition



III IV

of ethylproline to ethyl crotonate to give III (31% yield) did not proceed as readily as similar addition to ethyl acrylate.[4]

These five-membered ring compounds (II, IV) were of interest to us in connection with the study proceeding in this Laboratory on the Clemmensen reduction *cum* rearrangement of certain α-aminoketones.[5] While the similarly constituted six- and seven-membered ring α-aminoketones undergo ring contraction with reduction under Clemmensen conditions, the results we have obtained in the Clemmensen reduction of these five-membered ring compounds (II, IV) have been inconclusive.

The behavior of compound IIc at the dropping mercury electrode was found to be similar to that of the six-membered ring α-aminoketones: *i. e.*, an incipient wave appeared on the *I* (current) *vs.* *E* (voltage) curve just below hydrogen discharge (1.3–1.4 volts). There is reason to suspect that the incipient wave corresponded not to any reduction of the aminoketones, but rather to a catalyzed discharge of hydrogen.[6]

Experimental[7]

Methyl α-Methylaminobutyrate.—This ester was made by the same method as that employed for the preparation of the ethyl ester[8]; b.p. 36° (8 mm.), 64.5° (30 mm.); $n^{20}D$ 1.4180; d^{20}_4 0.950.

Anal. Calcd. for $C_6H_{13}NO_2$: C, 54.94; H, 9.99; MR_D, 35.32. Found: C, 55.25; H, 10.10; MR_D, 34.80.

Methyl α-Methylaminobutyrate Picrate.—The picrate was formed in ether solution and was recrystallized from anhydrous ether containing a few drops of methanol; yellow prisms, m.p. 94–95°.

Anal. Calcd. for $C_{12}H_{16}N_4O_9$: C, 40.00; H, 4.48; N, 15.55. Found: C, 40.17; H, 4.46; N, 15.52.

Methyl α-Methylaminobutyrate Picrolonate.—Prepared in ether and recrystallized from ethanol, the orange picrolonate melted at 174.5–175.5°.

Anal. Calcd. for $C_{16}H_{21}N_5O_7$: C, 48.60; H, 5.35; N, 17.72. Found: C, 48.56; H, 5.24; N, 17.65.

α-Carbethoxypropyl-β′-carbethoxyethylmethylamine.—A solution of 6.4 g. (0.044 mole) of ethyl α-methylaminobutyrate,[8] 20.6 g. (0.21 mole) of freshly distilled ethyl acrylate, and a trace of hydroquinone was heated 60 hours at 105–110°, protected from moisture. The product was fractionally distilled; b.p. 88–89° (0.5 mm.); $n^{20}D$ 1.4387; yield 8.6 g. (80%).

Anal. Calcd. for $C_{12}H_{23}NO_4$: C, 58.75; H, 9.45; N, 5.71. Found: C, 58.50; H, 9.52; N, 5.70.

α-Carbomethoxypropyl-β′-carbomethoxyethylmethylamine.—The same method was used for the dimethyl ester as that described above for the diethyl ester; b.p. 118° (10 mm.); $n^{20}D$ 1.4403.

Anal. Calcd. for $C_{10}H_{19}NO_4$: C, 55.28; H, 8.81; N, 6.45. Found: C, 55.49; H, 8.99; N, 6.30.

1-Methyl-2-ethyl-3-pyrrolidone.—The Dieckmann ring closure of either the diethyl or the dimethyl ester (see above) by means of sodium ethoxide[1,8] or potassium,[8,9] followed by hydrolysis and decarboxylation, afforded 1-methyl-2-ethyl-3-pyrrolidone in a maximum yield of 82%. The colorless liquid, b.p. 64–64.5° (20 mm.), $n^{20}D$ 1.4450, which decomposed rapidly, was converted immediately to derivatives. **The hydrochloride**, obtained by passing dry hydrogen chloride through an ethereal solution of the aminoketone, crystallized as colorless needles, m.p. 147.5–148.5°.

Anal. Calcd. for $C_7H_{14}ClNO$: C, 51.37; H, 8.62; N, 8.56. Found: C, 51.48; H, 8.45; N, 8.66.

The picrate, formed in ether and recrystallized from benzene–ethanol, separated as yellow plates, m.p. 130–132°, with decomposition.

Anal. Calcd. for $C_{13}H_{16}N_4O_8$: C, 43.82; H, 4.53; N, 15.73. Found: C, 44.02; H, 4.69; N, 15.55.

The picrolonate, formed in ether and recrystallized from ethanol, melted at 187–188°, with decomposition.

Anal. Calcd. for $C_{17}H_{21}N_5O_6$: C, 52.17; H, 5.41. Found: C, 52.24; H, 5.42.

Ethyl β-Butylaminopropionate.—To a solution of 123 ml. (91.4 g., 1.25 moles) of *n*-butylamine in 150 ml. of absolute ethanol was added dropwise with stirring 100.1 g. (1.00 mole) of freshly-distilled ethyl acrylate stabilized with a trace of hydroquinone. During the addition the reaction mixture was maintained at 20–25°; following the addition, at 60° for two hours. Ethanol and unreacted starting materials were removed by distillation *in vacuo*, and the residual oil was fractionated; b.p. 65° (1.4 mm.); $n^{20}D$ 1.4290; d^{20}_4 0.912; yield 154 g. (88%).

Anal. Calcd. for $C_9H_{19}NO_2$: C, 62.41; H, 11.06; MR_D, 49.16. Found: C, 62.39; H, 11.18; MR_D, 48.94.

β-Carbethoxyethylcarbethoxymethylbutylamine.—The amino diester was made by a method similarly applied by Clemo, Morgan and Raper,[10] the reaction of equimolar quantities of ethyl chloroacetate and ethyl β-butylaminopropionate in the presence of anhydrous potassium carbonate; b.p. 92–94° (*ca.* 0.1 mm.); $n^{20}D$ 1.4397; d^{20}_4 0.975; yield 90%.

Anal. Calcd. for $C_{13}H_{25}NO_4$: C, 60.21; H, 9.73; MR_D, 69.64. Found: C, 60.07; H, 9.86; MR_D, 70.08.

(4) Dr. M. Carmack, private communication.

(5) For leading reference, see N. J. Leonard and Eric Barthel, Jr., THIS JOURNAL, **72**, 3632 (1950).

(6) The authors are indebted to **Dr. H. A. Laitinen for his help** and advice concerning the polarography of these compounds.

(7) All melting points are corrected.

(8) N. J. Leonard and W. V. Ruyle, THIS JOURNAL, **71**, 3094 (1949).

(9) R. Adams and N. J. Leonard, *ibid.*, **66**, 257 (1944).

(10) G. R. Clemo, W. McG. Morgan and R. Raper, *J. Chem. Soc.*, 1743 (1935).

1-Butyl-3-pyrrolidone.—Dieckmann ring closure using sodium ethoxide in xylene at 120°[1] followed by hydrolysis and decarboxylation gave the aminoketone in 73% yield; b.p. 38° (0.5 mm.); $n^{20}D$ 1.4510; d^{20}_4 0.918.

Anal. Calcd. for $C_8H_{15}NO$: C, 68.04; H, 10.71; MR_D, 41.06. Found: C, 67.81; H, 10.70; MR_D, 41.38.

The picrate of 1-butyl-3-pyrrolidone was obtained as fine yellow needles, m.p. 157.5–158°, with decomposition, after formation in ethanol and recrystallization from methanol.

Anal. Calcd. for $C_{14}H_{18}N_4O_8$: C, 45.41; H, 4.90. Found: C, 45.33; H, 5.17.

Ethyl β-N-(2-Carbethoxypyrrolidyl)-butyrate.—A solution of 25 g. (0.175 mole) of ethylproline and 100 g. (0.877 mole) of ethyl crotonate was heated at 100° for 48 hours.[9] The excess ethyl crotonate was removed by distillation at atmospheric pressure and the diester was obtained as a colorless liquid, b.p. 99–100° (0.3 mm.); $n^{20}D$ 1.4566; d^{20}_4 1.064; yield 13.8 g. (31%).

Anal. Calcd. for $C_{13}H_{23}NO_4$: C, 60.69; H, 8.98; N, 5.44. Found: C, 60.52; H, 8.96; N, 5.62.

1-Keto-3-methylpyrrolizidine.—The procedure of McElvain and Rorig[11] employing sodium hydride was used for the Dieckmann ring closure of ethyl β-N-(2-carbethoxypyrrolidyl)-butyrate. The aminoketone obtained in 50% yield after hydrolysis and decarboxylation was converted directly and no attempt was made to isolate both of the possible racemates. The hydrochloride was hygroscopic. **The picrate,** prepared in ether and recrystallized from methanol, separated as long yellow needles, m.p. 189–190°, with decomposition.

Anal. Calcd. for $C_{14}H_{16}N_4O_8$: C, 45.65; H, 4.37; N, 15.21. Found: C, 45.79; H, 4.43; N, 15.24.

The picrolonate, prepared in ether and recrystallized from ethanol, formed orange leaflets, m.p. 206–207°, with decomposition.

Anal. Calcd. for $C_{18}H_{21}N_5O_6$: C, 53.59; H, 5.25; N, 17.36. Found: C, 53.86; H, 5.32; N, 17.07.

Wolff–Kishner Reduction of 1-Keto-3-methylpyrrolizidine. 3-Methylpyrrolizidine.—A solution of 0.20 g. of 1-keto-3-methylpyrrolizidine, 1.0 g. of hydrazine hydrate (85%), and 1.0 g. of potassium hydroxide in 10 ml. of triethylene glycol was boiled under reflux for one hour. The solution was then distilled until the distillate was no longer basic. The distillate was saturated with potassium carbonate and extracted with ether. The ethereal solution was dried and treated with picric acid in ether. **The picrate** thus obtained crystallized from methanol as yellow elongated plates, m.p. 251–252°, with decomposition.

Anal. Calcd. for $C_{14}H_{18}N_4O_7$: C, 47.46; H, 5.12; N, 15.81. Found: C, 47.62; H, 5.12; N, 15.83.

The Clemmensen reduction of 1-keto-3-methylpyrrolizidine gave results which were inconclusive, however; the crude product yielded a small amount of $C_8H_{15}N$ picrate, m.p. 251–252° (dec.), which was found to be identical with the 3-methylpyrrolizidine picrate described above by mixed melting point and infrared absorption spectra determinations.

(11) S. M. McElvain and K. Rorig, THIS JOURNAL, **70**, 1820 (1948).

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The Optical Rotation of 2-Chlorobutane

BY ROBERT L. LETSINGER, LUCIEN G. MAURY AND ROBERT L. BURWELL, JR.

We have determined limiting values for the rotation of optically pure 2-chlorobutane by means of a displacement reaction with benzylsodium. The use of this reagent was suggested by the characteristics of its reaction with (+)2-bromobutane.[1]

Benzylsodium and 2-chlorobutane ($\alpha^{25}D$ −10.93°) combined to give 1-phenyl-2-methylbutane with $\alpha^{25}D$ + 3.26°. On the basis of $\alpha^{25}D$ +9.9° ($[\alpha]^{25}D$ +11.6°) as the maximum rotation for this hydrocarbon,[1] therefore, $\alpha^{25}D$ 33.2° is an upper limit for the rotation of optically pure 2-chlorobutane. The actual value may be somewhat lower since 9.9° is probably a little high for the hydrocarbon and some loss of asymmetry may have occurred in the benzylsodium condensation.

Of reported cases, the preparation of 2-chlorobutane which involved the least racemization was the conversion of 2-butanol ($[\alpha]^{25}D$ −5.54°) to 2-chlorobutane ($[\alpha]^{25}D$ + 13.70°).[2] Calculation of these data to the basis of optically pure alcohol ($[\alpha]^{25}D$ 13.64°)[3] gives $\alpha^{25}D$ 29.2° (or $[\alpha]^{25}D$ 33.8°) as a rotation of 2-chlorobutane experimentally obtainable. The rotation for the pure enantiomorph must therefore lie between $\alpha^{25}D$ 29.2° and 33.2°.

Experimental

The symbol, $\alpha^{25}D$, designates the homogeneous rotation in a 1 dm. tube.

2-Chlorobutane.—2-Butanol (5.66 g., $\alpha^{25}D$ +7.48°, $n^{25}D$ 1.3944) was saturated with hydrogen chloride gas at zero degrees. The solution was then sealed in a tube and heated at 75° for 72 hours, during which time it separated into two layers. After cooling, the tube was opened and the contents washed successively two times with water, three times with concentrated hydrochloric acid, three times with concentrated sulfuric acid, and finally three times again with water. The 2-chlorobutane thus obtained was dried over calcium chloride; 6.8 g. product (96%); $\alpha^{25}D$ −12.24°, $n^{25}D$ 1.3942. Two samples prepared in this manner were combined to give a product with $\alpha^{25}D$ 10.93°; $n^{25}D$ 1.3942.

1-Phenyl-2-methylbutane.—Butylsodium was prepared as previously described[1] from *n*-butyl chloride (23.1 g., 0.25 mole) and sodium (11.5 g., 0.5 g. atom) with hexane as a solvent. To ensure complete reaction of the halide, the butylsodium was stirred two hours at 25° and then permitted to stand at room temperature for 20 hours. At the end of this time, toluene (160 cc.) was added and the mixture stirred for an additional six hours at 25°. Metalation was comparatively rapid as evidenced by the change from blue to green in less than ten minutes, and then to yellow in about half an hour.

A solution of 2-chlorobutane (8.67 g., 0.0937 mole, $\alpha^{25}D$ −10.93°) in 10 cc. of hexane was added over a period of five minutes to the rapidly stirred suspension of benzylsodium, which was maintained at 15°. After an additional ninety minutes of stirring, the mixture was carbonated on powdered Dry Ice. The hydrocarbon portion was separated by conventional procedures and the 1-phenyl-2-methylbutane isolated by distillation; 7.63 g. (55%); b.p. 90–91° (27 mm.); $\alpha^{25}D$ +3.26°, d^{25}_4 0.8544, $n^{25}D$ 1.4848.

(1) R. L. Letsinger, THIS JOURNAL, **70**, 406 (1948).

(2) J. F. Lane and S. E. Ulrich, *ibid.*, **72**, 5132 (1950).

(3) R. H. Pickard and J. Kenyon, *J. Chem. Soc.*, **99**, 45 (1911). This value was interpolated from rotations measured at 20° and 50°.

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An Analysis of the Electron Diffraction Data for Decaborane

BY CHARLYS M. LUCHT

Silbiger and Bauer[1] have presented their electron diffraction data for the molecular structure of decaborane, $B_{10}H_{14}$. They had tried all the molecular models which had been proposed in the literature up to that time and also calculated many others which might have been possible. The arrangement of planar hexagons suggested by Pitzer[2] and several

(1) G. Silbiger and S. H. Bauer, THIS JOURNAL, **70**, 115 (1948).

(2) K. S. Pitzer, *ibid.*, **67**, 1136 (1945).