reflections (Tables II and IV), and lead to reasonable interatomic distances. The structure (Fig. 2) is an aggregate of K^+, octahedral $CbOF_5^=$, and HF_2^- ions; the double salt formula, $K_2CbOF_5 \cdot KHF_2$, is appropriate, therefore.

ITHACA, N. Y. RECEIVED OCTOBER 17, 1940

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL, AND THE DEPARTMENT OF PHYSICS, HARVARD UNIVERSITY]

Studies in the Physical Chemistry of Insulin. II. Crystallization of Radioactive Zinc Insulin Containing Two or More Zinc Atoms*

BY E. J. COHN, J. D. FERRY, J. J. LIVINGOOD AND M. H. BLANCHARD

I. Introduction

The molecular weight of insulin as estimated by the equilibrium ultracentrifuge is reported as 35,100,[1] and from results with the sedimentation ultracentrifuge[1] in combination with those from diffusion measurements[2] as 40,900. The molecular weight of insulin calculated from the dimensions of the unit cell revealed by X-ray measurements is 38,500 and as corrected for residual water, 37,400.[3-6]

The zinc content of insulin was given by Scott and Fisher[7] as 0.52% by weight for crystalline zinc insulin. The minimal molecular weight, assuming one atom of zinc, would thus be 12,570, or three atoms of zinc 37,710, a value in good agreement with that derived from X-ray or ultracentrifugal measurements.

Cohn, Ferry, Livingood and Blanchard[8] succeeded in crystallizing radioactive zinc insulin. Beginning with crystalline zinc insulin[9] they electrodialyzed the insulin free of zinc from acid solution. Amorphous, isoelectric insulin prepared in this manner has since been shown to contain a negligible amount of zinc, namely, less than 0.01%.[10] When they substituted the radioactive zinc (Zn^{65} isotope of half-life 250 days) for ordinary zinc, taking precautions to maintain the excess of zinc in solution at a low level, the radioactive zinc insulin crystals that separated rarely contained more than 0.31% although one preparation was obtained which contained 0.36% radioactive zinc.[11] If the radioactive zinc insulin that was crystallized contained but 2 atoms of zinc, which was assumed, the molecular weight on the basis of the lower zinc content reported would have been 42,200, and of the higher, 37,300, results which are also in good agreement with the molecular weights estimated from X-ray and ultracentrifuge studies.

Preparation of Radioactive Zinc.—The radioactive zinc that was available when these earlier measurements were carried out had been prepared by the cyclotron at the University of California and was supplied us by Dr. Livingood. Radioactive zinc has now been prepared by the Harvard cyclotron for the newer experiments that are here reported. Zinc was bombarded with 11-million volt deuterons for a total exposure of 500 microampere hours during January, February and March, 1940. The proportion of radioactive zinc atoms in the final product was of the order of one in ten billion; this was sufficiently concentrated so that analyses for radioactive zinc content could be carried out with reasonable accuracy on samples of 1 mg. of zinc, corresponding to zinc insulin samples of the order of 0.2 g. The bombarded zinc was treated chemically[12] to remove gallium and copper, and the zinc was prepared as the sulfide.

The zinc sulfide was transformed into the chloride for the crystallization of the zinc insulin.[13,7] Radioactivity, and therefore zinc content, was determined upon dried samples with a Lauritzen-type quartz fiber electroscope, using 2.3 mm. of aluminum filtration, so that only gamma rays were recorded. Comparison was made in each case with a weighed standard sample of the radioactive zinc sulfide. As a further analytical check, zinc sulfide was transformed into zinc ammonium phosphate[14] and then

* Presented at the Fifth Annual Symposium of the Division of Physical and Inorganic Chemistry of the American Chemical Society, Columbia University, New York, December 30, 1940, to January 1, 1941.

(1) T. Svedberg, *Nature*, **127**, 438 (1931); Sjögren and Svedberg, THIS JOURNAL, **53**, 2657 (1931).

(2) A. Polson, *Koll.-Z.*, **87**, 149 (1939).

(3) D. Crowfoot, *Nature*, **133**, 591 (1935).

(4) D. Crowfoot, *Proc. Roy. Soc.* (London), **A164**, 580 (1938).

(5) Crowfoot and Riley, *Nature*, **144**, 1011 (1939).

(6) I. Fankuchen, *Annals N. Y. Acad. of Sci.*, in press.

(7) Scott and Fisher, *Biochem. J.*, **29**, 1048 (1935).

(8) Cohn, Ferry, Livingood and Blanchard, *Science*, **90**, 183 (1939).

(9) Furnished through the kindness of Eli Lilly and Co., Indianapolis, Indiana.

(10) We are indebted to Dr. Vincent du Vigneaud and to Dr. Julian Rachele of the Department of Biochemistry of the Cornell University Medical College for proving that our amorphous insulin was zinc-free on the basis of polarigraphic measurements; and for the results of analysis of the ordinary zinc insulin crystals.

(11) In a paper presented to the 100th meeting of the American Chemical Society, September 12, 1940, L. C. Maxwell and R. F. Feldkamp reported that they had confirmed these results by obtaining insulin crystals with a zinc content of 0.355%.

(12) We are indebted to Dr. A. K. Solomon for carrying out this chemical separation.

(13) D. A. Scott, *Biochem. J.*, **28**, 1592 (1934).

(14) P. Artmann, *Z. anal. Chem.*, **62**, 8 (1923).

into zinc pyrophosphate.[15] The results were in good agreement with the original zinc sulfide standard, both chemically and as analyzed by radioactivity. The zinc sulfide has therefore been employed as the standard throughout.

Preparation of Amorphous Insulin.—Crystalline zinc insulin[9] was the starting material for all these experiments. The zinc contents of two preparations of this material were determined polarigraphically[10] as follows: no. T-1115, 0.57% zinc; no. 972519, 0.61% zinc. The amorphous samples were prepared from crystalline material, in every case, by dissolving in dilute hydrochloric acid (15 cc. 0.1 *N* per gram protein), diluting to about 40 cc. per gram protein, electrodialyzing in the Pauli electrodialyzer for two hours against flowing *N*/300 hydrochloric acid (40 liters in all) and then electrodialyzing in a Brintzinger electrodialyzer for at least twelve hours against distilled water, thereby removing all acid and precipitating the amorphous insulin.

Crystallization of Radioactive Zinc Insulin.—The electrodialyzed amorphous insulin was crystallized with the zinc chloride prepared with the radioactive zinc isotope in the presence of acetate buffers, phosphate buffers, or phosphate buffers containing sodium chloride, following the method of Scott[13,7] or from buffer-free solutions containing 0.1 *N* sodium chloride.

II. Influence of Excess of Zinc upon Crystallization

It seemed important first of all to discover why the radioactive zinc insulin prepared by us during 1939 had a lower zinc content than that reported by Scott. One of the factors which we believed might have brought this about was the small excess of zinc employed in our earlier experiments. Accordingly, 1.5 g. of zinc insulin no. 972519 was electrodialyzed, divided into two equal portions, and crystallized from acetate buffer with radioactive zinc (Experiment XIV). Sample A contained 30% excess of zinc (calculated on the basis of 3 atoms of zinc per molecule). Sample B contained a fivefold excess of zinc. Both samples yielded a mixture of crystals and amorphous material. These were separated by differential centrifuging and washing until no amorphous material could be detected in the crystalline fractions by examination under the microscope. The radioactivities of the separated amorphous material, as well as of the crystals, after careful drying in a desiccator, were then determined. The material that appeared to be largely amorphous contained 0.28% zinc. The values for the content of radioactive zinc in the crystallized insulin were appreciably higher. Sample A was estimated to contain 0.43%, sample B 0.42%, indicating that the zinc content of the insulin crystallized under these conditions from acetate buffer solutions was independent of the amount of zinc in the solution in which the crystals were formed.

This experiment demonstrated not only (a) that the amount of zinc in crystalline zinc insulin was under these conditions independent of the amount of zinc in solution, but also (b) that amorphous insulin bound some zinc, an observation consistent with that previously reported[8] regarding radioactive zinc insulin protaminate. Crystallization in the above experiment was carried out from acetate buffers near *p*H 6. The zinc content of these crystals was higher than that previously reported by us, but lower than that determined by Scott and Fisher.[7] It therefore seemed desirable to crystallize radioactive zinc insulin from systems in which the *p*H was more rigorously controlled.

III. Influence of *p*H upon Crystallization

For most of the *p*H range in which crystallization of insulin has generally been carried out phosphate solutions are far better buffers than acetate solutions. Accordingly, 1.3 g. of electrodialyzed insulin, recovered from previous experiments, was crystallized with radioactive zinc from phosphate buffer solutions varying in composition and in *p*H. Insulin was present in all systems to the extent of 0.16% and the total phosphate was approximately 0.02 *M*. Acetone, to the extent of 7.2%, was added to facilitate crystallization. After standing in the cold for two days, aliquot parts were equilibrated by rotating at 5° for three days, the *p*H and insulin concentration of the supernatant solutions determined, and the precipitates examined. The results are recorded in Table I.

Systematic variation of *p*H revealed a progressive change in the nature of the precipitate from the almost completely amorphous material present in the more acid reactions to almost completely crystalline material at reactions alkaline to *p*H 6.4. In certain of the experiments the form of the crystals appeared, upon superficial observation, to vary with *p*H, the tendency being for star-shaped crystals to predominate at the more acid, and cube-shaped crystals at the more alkaline, reactions.

The total precipitate was analyzed in the earlier measurements (Experiment XVI) for zinc content. In three experiments, when the *p*H was

(15) Treadwell and Hall, "Analytical Chemistry," John Wiley and Sons, Inc., New York, N. Y., Vol. II, p. 142, 1928.

5.78, 6.06 and 6.09, the dry insulin precipitates analyzed for 0.51, 0.52, and 0.52% radioactive zinc, in excellent agreement with the earlier measurements of Scott and Fisher.[7] In the more alkaline reactions, where no amorphous material was observed, insulin crystals containing more zinc than 0.52% were noted. In two experiments at *p*H 6.29, and 6.40, zinc contents of 0.59% and 0.60% were determined. The low figure for zinc content at the acid end of the range, 0.26%, may have been due, as in the previous experiment, to admixture of amorphous material with the crystals or to the presence of crystals containing less than 2 atoms of zinc per molecule.

TABLE I

ZINC CONTENT OF INSULIN CRYSTALLIZED FROM 0.0202 MOLAR PHOSPHATE BUFFERS VARYING IN *p*H AND IONIC STRENGTH

Expt.	*p*H	Ionic strength, Γ/2	Insulin in solution, g./l.	Zinc in insulin precipitate, %	Zinc in crystalline insulin, %
XVIII	5.06	0.023	0.080		0.33
XVI	5.22	.023	.036	0.26	
XVIII	5.35	.024	.040		.35
XVIIA[a]	5.51	.025	.049		.34
XVI	5.78	.025	.028	.51	
XVI[b]	6.06	.034	.046	.52	
XVI	6.09	.027	.046	.52	
XVI	6.29	.029	.104	.59	
XVI	6.40	.031	.291	.60	
XVIII	6.41	.030	.220		.63
XVIII	6.53	.032	.369		.65

[a] The phosphate concentration in this experiment was 0.0223. [b] The phosphate concentration in this experiment was 0.0263.

In order to determine whether the crystals from a more acid reaction contained less than 3 atoms of zinc per molecule, the crystals were separated as far as possible from the amorphous precipitate. In Experiments XVIIA and XVIII, 4.5 g. of Lilly crystalline zinc insulin no. 972519 was electrodialyzed and rendered zinc-free and then crystallized from phosphate buffers with radioactive zinc as in the previous experiment. After standing in the cold room for three days aliquots were equilibrated for four days by rotating at 5° and the determinations of *p*H and of the concentration of insulin in solution are recorded in Table I. The precipitates, excepting as in the previous experiments at reactions alkaline to *p*H 6.4, contained clearly visible amorphous material as well as well-formed crystals. Moreover, the precipitates contained more crystals and less amorphous material the more alkaline the reaction.

At reactions alkaline to *p*H 5 a quantity of crystals could always be obtained by differential sedimentation, which appeared under the microscope to be free of amorphous material. The radioactivity of these crystalline samples, after drying, was determined and is recorded in the last column of Table I, calculated as per cent. of zinc. The crystals separating from phosphate buffers of ionic strength 0.023 to 0.025 and *p*H 5.06 to 5.51 contained essentially the same per cent. of zinc, namely, 0.34 ± 0.01. On the other hand, the crystals separating from phosphate buffers of the same concentration, 0.0202, at *p*H 6.41 and 6.53, and having ionic strengths 0.030 and 0.032, contained, respectively, 0.63 and 0.65%, or nearly twice as much as the crystals separating at reactions acid to 5.5. If crystalline insulin separating at acid reactions contains 2 atoms of zinc per molecule, the crystals separating at the alkaline reactions investigated appeared to contain more than 3 atoms of zinc per molecule. It remained possible, however, that the high zinc content of these crystals might be due to occlusion of zinc phosphate. These results are graphically represented in Fig. 1, in which the zinc contents of the separated crystals are plotted against the *p*H.

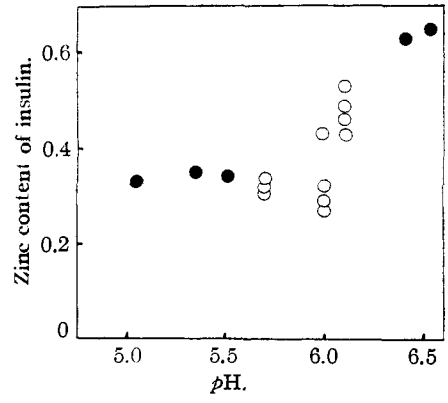

Fig. 1.—Zinc content of insulin crystals separated at low ionic strength ● and after extraction with water O, plotted as a function of *p*H.

IV. Influence of Ionic Strength upon Crystallization

The concentration of phosphate in the buffer system from which the insulin was crystallized was retained essentially constant throughout these measurements. As a result the ionic strength varied with the *p*H, being greater the more alkaline the reaction.[16] In order to determine the influence of the ionic strength upon the

(16) E. J. Cohn, THIS JOURNAL, 49, 173 (1927).

crystallization of insulin, further experiments were undertaken (XIX, XX and XXI), in which sodium chloride was added to the phosphate buffer to increase the ionic strength. Acetone to the extent of 7.2% was added, as before, to facilitate crystallization. The *p*H of the systems investigated varied from 4.91 to 6.26.

The yield of crystals formed at *p*H 4.91 was so small that they could not be satisfactorily separated from the large amount of amorphous material. The crystals that separated at 5.40 analyzed for a zinc content of 0.38% whereas those separating at reactions alkaline to *p*H 5.8 all analyzed for over 0.62% zinc. In a single experiment a zinc content as high as 0.77% has been estimated. The results thus far obtained suggest that the insulin crystals separated from the systems more concentrated in chloride and alkaline to *p*H 5.8 contained slightly more zinc. Experiments to demonstrate whether this zinc was present as zinc insulin containing 4 or more atoms per molecule, or as occluded zinc phosphate, are being continued.

V. Influence of Ionic Strength upon Solubility

The solubility of the insulin in these systems increased appreciably with increase in ionic strength. The solubilities of insulin were determined after equilibration in the above systems for some days at 5° and are also reported in Tables I and II. The systems are more complex than those that we have generally investigated and the results somewhat less accurate, but they none the less reveal two effects. The graph in Fig. 2 indicates (a) that solubility is higher at the higher ionic strength and besides (b) that the solubility at each ionic strength was minimal in the neighborhood of *p*H 5.5. This is close to the *p*H of electrodialyzed amorphous insulin, the solubility of which we have previously investigated both in water and in glycine solutions at 5°. The result reported[8] for water of approximately 0.01 g. per liter is lower than the measurements here reported for systems with appreciable ionic strengths, in addition to acetone. The higher solubility in the presence of ions may be compared with our previous study of the influence of the dipolar ion, glycine, on amorphous insulin at the same temperature.[8]

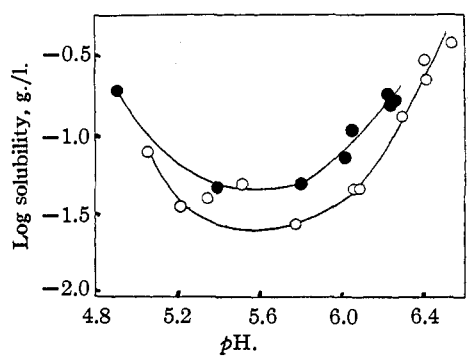

Fig. 2.—Solubility of insulin at 5° in phosphate buffers of ionic strength 0.023–0.034 ○, and 0.066–0.080 ●, plotted as a function of *p*H.

TABLE II

ZINC CONTENT OF INSULIN CRYSTALLIZED FROM 0.0202 MOLAR PHOSPHATE BUFFERS, VARYING IN *p*H AND IONIC STRENGTH

Expt.	*p*H	Ionic strength, Γ/2	Insulin in solution, g./l.	Zinc in crystalline insulin, %
XIX	4.91	0.078	0.197	
XX	5.40	.078	.0474	0.38
XX	5.81	.078	.0505	.62
XX	6.02	.078	.0728	.69
XXI	6.05	.066	.116	.75
XIX	6.23	.080	.154	.62
XXI	6.23	.066	.185	.73
XX	6.26	.078	.167	.77

VI. Solubility and Zinc Content of Crystalline Insulin upon Repeated Equilibration with Water

The solubilities recorded in Tables I and II must represent the sum of both amorphous and crystalline insulin in these systems. The solubility of crystalline zinc insulin has generally been considered lower than that of amorphous zinc insulin. It seemed possible therefore that the contribution of the crystalline insulin to that sum might be very small. It further seemed important to determine the zinc content of the crystals after repeated equilibration with large volumes of water and the *p*H of the solution in equilibrium with the crystals.

Accordingly, to determine these points, the crystals of insulin which had been formed in phosphate buffers, acid to *p*H 5.51, were combined, transferred to a 250-cc. double glass-stoppered vessel of the type employed in our solubility studies,[17] repeatedly washed with conductivity water, two to three-day intervals generally being allowed for each equilibration. Comparably, the insulin crystals that had been separated at reactions alkaline to 6.4 were combined and treated in the same way. Three washings sufficed to free the crystals of measurable amounts of chloride and phosphate. Thereafter the solubility and the

(17) Ferry, Cohn and Newman, THIS JOURNAL, **58**, 2370 (1936).

*p*H at each equilibration were measured. The results are recorded in Table III. The solubility of the crystalline insulin in water at 5° was of the same order as the result previously reported for amorphous insulin under the same conditions.[8] Both for the crystals which contained two and those which contained more atoms of zinc per molecule, solubility appeared to vary considerably with repeated washings in the manner frequently observed for such very slightly soluble proteins in unbuffered solutions. No large difference between the solubilities of the two samples of the crystalline zinc insulins has been thus far demonstrated.

TABLE III

SOLUBILITY OF INSULIN CRYSTALS IN WATER AT 5°

Equilibrations	Insulin containing 0.33–0.35% zinc *p*H	solubility, g./l.	Insulin containing 0.64–0.65% zinc *p*H	solubility, g./l.
4	5.8	0.007	6.1	0.008
5	5.7	.010	6.0	.007
6	5.7	.008	6.0	.006
7	5.7	.005	6.1	.004
8	5.7		6.1	
9	5.8	.009	6.2	.006
10	5.9	.010	6.2	.005
11	5.7	.013	6.0	.008
12	5.8	.008	6.1	.003

The very low solubility of the crystalline zinc insulin rendered it impossible to determine *p*H by accurate electromotive force measurements. Colorimetric estimates were, however, made and are reported in Table III. The *p*H of distilled water in equilibrium with atmospheric carbon dioxide and saturated with the crystals which separated at reactions acid to 5.51, after being freed from acetone, chloride and phosphate, tended to a more nearly neutral reaction near *p*H 5.8. On the other hand, the *p*H of such distilled water saturated with the insulin crystallized at reactions more alkaline than 6.4 tended to a more acid reaction near *p*H 6.0.

In order to determine the zinc contents of the repeatedly washed crystals they were again dried and yielded, respectively, 0.34 and 0.53% zinc, after these repeated triturations with conductivity water. Upon still further washing, the zinc content was still further reduced (Table IV).

In order to determine whether the insulin crystallized from higher ionic strengths and separated by sedimentation from amorphous material would contain less zinc upon being washed free of salt and of excess zinc, whether present as chloride or phosphate, the dried crystals of Experiment XX were repeatedly washed, dried and reanalyzed. The results are also recorded in Table IV, and render it certain that the insulin remaining undissolved contained at most 2 zinc atoms per molecule. After the repeated equilibrations with water the form of the crystals was no longer perfect and amorphous material may have been present.

TABLE IV

ZINC CONTENTS OF INSULIN CRYSTALS AFTER REPEATED EXTRACTION WITH WATER

Equilibrations	*p*H at crystallization: 5.06–5.35	5.40	5.81	6.02	6.26	6.41–6.53
	Zinc content of crystals, %					
0	0.35	0.38	0.62	0.69	0.77	0.65
	.33					.63
1		.33	.48	.51	.55	
2		.32	.43	.46	.49	
6		.26	.29	.32	.29	
8	.34					.53
12	.31					.44

Whether the loss of zinc from the crystals which originally appeared to contain over 3 atoms of zinc per molecule represents (1) removal of occluded zinc, (2) solution of zinc from the crystal lattice, (3) dissociation of zinc insulin into zinc and amorphous insulin, (4) dissociation into zinc and crystalline insulin containing two atoms per molecule or (5) the existence of a more soluble zinc insulinate is being further investigated.

Summary

1. Insulin has been crystallized from solutions containing radioactive zinc under a variety of conditions.

2. The amount of zinc in the crystals separated from acetate buffer at *p*H 5.9–6.0 was independent of the amount of zinc in the solution in which the crystals were formed.

3. The solubility of insulin in the phosphate systems in which the crystals were formed passed through a minimum near *p*H 5.5 and was lower at lower ionic strengths.

4. The precipitates consisted almost completely of crystalline insulin at reactions alkaline to *p*H 6.4, and contained more amorphous material the more acid the reaction.

5. The crystals separated from the precipitates in systems of low ionic strength acid to *p*H 5.5, contained close to 0.34 per cent. radioactive zinc; those separating at reactions alkaline to *p*H 6.4 contained larger amounts.

6. The solubilities in water at 5° of the zinc insulin were of the order of 0.01 g. per liter, after repeated equilibration with conductivity water.

7. Insulin repeatedly equilibrated in this way with conductivity water never contained more than two atoms of zinc per molecule.

BOSTON, MASS. RECEIVED OCTOBER 17, 1940

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, NO. 782]

The Isomeric 2,3-Epoxypentanes and 2-Pentenes. The Extent to which Mixtures of Diastereomers Are Formed in Reactions of Some Pentane Compounds

BY H. J. LUCAS, M. J. SCHLATTER AND R. C. JONES

The isomeric *cis* and *trans*-2-pentenes have been the subject of numerous investigations.[1-6] Their preparation from the isomeric 2,3-epoxypentanes analogous to the preparation of *cis* and *trans*-2-butene from *trans* and *cis*-2,3-epoxybutane,[7] and from *cis* and *trans*-2,3-epoxybutane[8] has been accomplished. In addition, a study has been made of the extent to which a single reaction only takes place during a given chemical change, that is to say, if the change takes place with complete retention of configuration or with complete inversion of configuration, whichever the case may be. This could not be done for any single chemical change, however, but only for a group of two or more changes.

The changes investigated are shown in Fig. 1. Here only one of the two antipodes of *dl*-mixtures is shown. The configurations have been assigned on the basis that they are analogous to the configurations of the corresponding butane derivatives, for no evidence is available from these reactions or from the physical properties of the pentane derivatives themselves. This assignment of configuration to the pentenes, however, agrees in general with those made previously.[1,3,4,5b,6c,9] Walden inversions are indicated by the conventional arrow and circled shaft. The dotted arrows indicate that the reaction was not tried, but is predicted.

Figure 1 shows how each isomeric 2,3-epoxypentane can be converted into *cis* and *trans*-2-pentene by two different paths. One path involves the following steps: oxide -O→ glycol ⟶ diacetate -O→ dibromide -O→ 2-pentene. The other path is: oxide -O→ bromohydrin ⟶ dibromide -O→ 2-pentene. In the first case three inversions are involved and in the second, two inversions. It is possible to pass from any one compound shown to any other compound (for this purpose each bromohydrin mixture is regarded as a compound) if it can be assumed that hypobromous acid adds to 2-pentene with one inversion, as it does to 2-butene.[8] The step involved here, *viz.*, 2-pentene -O→ bromohydrin, was not investigated. Although it would be possible to prepare the *cis* oxide from the *trans* oxide, and *vice versa*, and also the *cis*-2-pentene from the *trans*-2-pentene, and *vice versa*, no product would be entirely free of its isomer, as discussed later.

The starting materials for the cycle were the *cis*- and *trans*-2,3-epoxypentanes, which were obtained as 100% and 98% pure products, respectively, by fractional distillation at 200 mm. of a mixture of the two isomers.[10] This mixture was obtained from a mixture of the isomeric 2-pentenes[11] through the chlorohydrins by the procedure employed with the corresponding C_4 compounds.[7] The much greater separation of the boiling points of the oxides (4.9 at 200 mm. and 5.2° at 748 mm., Table II) as compared to 0.6° in the case of the 2-pentenes, shows the advantage of fractionating the oxides.

The lower boiling oxide, approximately 75% of the total, was assigned the *trans* configuration and the higher boiling oxide the *cis* configuration, analogous with the butene oxides.[7,12]

The stereochemical relationship between *trans*-2-pentene, *trans*-2,3-epoxypentane, and the intermediate chlorohydrin presumably is correctly

(1) (a) Bourguel, *Bull. soc. chim.* [4] **41**, 1475 (1927); (b) Bourguel, Grédy and Piaux, *Compt. rend.*, **195**, 129 (1932); (c) Grédy, *Bull. soc. chim.*, [5] **2**, 1029 (1935).

(2) Clark and Hollonquist, *Trans. Roy. Soc. Can.*, [3] **24**, Sect. 3, 1115 (1930).

(3) Kharasch, Walling and Mayo, THIS JOURNAL, **61**, 1559 (1939).

(4) Lauer and Stodola, *ibid.*, **56**, 1215 (1934).

(5) (a) Lucas and Moyse, *ibid.*, **47**, 1459 (1925); (b) Lucas and Prater, *ibid.*, **59**, 1682 (1937).

(6) (a) Sherrill, Otto and Pickett, *ibid.*, **51**, 3023 (1929); (b) Sherrill, Baldwin and Haas, *ibid.*, **51**, 3034 (1929); (c) Sherrill and Matlock, *ibid.*, **59**, 2134 (1937); (d) Sherrill and Launspach, *ibid.*, **60**, 2562 (1938).

(7) Wilson and Lucas, *ibid.*, **58**, 2396 (1936).

(8) Winstein and Lucas, *ibid.*, **61**, 1576 (1939).

(9) Carr and Stücklen, *ibid.*, **59**, 2138 (1937).

(10) The authors are indebted to Mr. Herbert Sargent for his assistance in carrying out this separation.

(11) From secondary amyl alcohol, by the method of Norris and Reuter, THIS JOURNAL, **49**, 2624 (1927).

(12) Brockway and Cross, *ibid.*, **59**, 1147 (1937).