Laboratory. The figures listed have been corrected for moisture and ash, the latter amounting to only 0.1%. Nitrogen was determined by the micro-Kjeldahl method, direct oxygen analyses were made by the Unterzaucher method, and sulfur was determined after Carius digestion. Only trace amounts (0.02%) of phosphorus were found. No carbohydrate could be detected by the orcinol method.[12]

TABLE 1

ELEMENTARY COMPOSITION OF α-LACTALBUMIN, %

N	15.86
C	53.32
H	7.01
O	21.56
S	1.91
Total	99.66

Tryptophan Analysis.—Tryptophan was determined by the Spies and Chambers method[13] on the protein in solid form. α-Lactalbumin was found to contain approximately 7% tryptophan. There is some uncertainty about the exact value because the minimum transmittancy of the color produced by this protein was at 620 mμ. Readings at 620 mμ gave a value of 7.2% tryptophan while at 590 mμ, a value of 6.7% was obtained. Sørensen and Sørensen[6] reported that "crystalline insoluble substance" contained 0.371–0.633 mg. tryptophan per mg. protein N (5.9–10.0% tryptophan, calculated on the basis of our determined nitrogen content of 15.86%).

Discussion

The method for preparing α-lactalbumin described above, which was worked out from the observations of Sørensen and Sørensen, has consistently yielded the same crystalline protein. It is of some importance, therefore, to attempt to correlate our findings with some of the earlier studies on the lactalbumin fraction of whey as has already been done with the ultracentrifuge data.

We have been able to prepare α-lactalbumin in crystalline form only in the presence of ammonium sulfate at *p*H 6.6. It has been mentioned that the crystals are easily soluble in water and that when acid is added to *p*H 4.6 to the aqueous solution, amorphous protein, only slightly soluble in water, is precipitated. It may be that the crystalline protein is actually some type of protein–salt combination or simply an ammonium salt and that the crystalline protein isolated by Kekwick[14] was likewise a water-soluble complex and hence called an albumin. The crystalline albumin prepared by Sjögren and Svedberg[15] by the method of Wichmann[16] (salting out with ammonium sulfate in the presence of dilute acid) was also soluble in water. However, the protein was not homogeneous and speculation concerning its solubility is unwarranted.

In regard to the electrophoretic characterization of α-lactalbumin, it is interesting to compare the observed mobility of −4.2 with the data of Smith.[17] In his investigation of the proteins of whey by the electrophoretic method, Smith found that one of the components comprising 12% of the total proteins, had a mobility of −4.5 (protein concentration = 1.23%, veronal buffer *p*H 8.6, ionic strength = 0.1). It seems reasonable to suggest that this component may now be identified as α-lactalbumin.

If the present work has been correlated correctly with Pedersen's ultracentrifuge data and Smith's electrophoretic experiments, it may be concluded that α-lactalbumin is a major component of whey which can be prepared in crystalline form without difficulty. Further studies on its properties and amino acid composition are in progress.

Acknowledgments.—We wish to thank T. L. McMeekin for much helpful advice and for the original suggestion that "crystalline insoluble substance" and α-lactalbumin might be identical. We are also indebted to J. H. Custer for the electrophoretic experiments.

(12) M. Sørensen and G. Haugaard, *Compt. rene. trav. lab. Carlsberg, Ser. chim.*, **19**, 1 (1933).

(13) J. R. Spies and D. C. Chambers, *Anal. Chem.*, **21**, 1249 (1949).

(14) R. A. Kekwick, unpublished; see K. O. Pedersen, *Biochem. J.*, **30**, 948 (1936).

(15) B. Sjögren and T. Svedberg, THIS JOURNAL, **52**, 3650 (1930).

(16) A. Wichmann, *Z. physiol. Chem.*, **27**, 575 (1899).

(17) E. L. Smith, *J. Biol. Chem.*, **165**, 665 (1946).

PHILADELPHIA 18, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, THE HEBREW UNIVERSITY]

Syntheses of DL-Aspartic Acid and DL-Asparagine *Via* Their N-Benzyl Derivatives

BY MAX FRANKEL, Y. LIWSCHITZ AND Y. AMIEL

RECEIVED JULY 14, 1952

New syntheses for the preparation of *dl*-aspartic acid and *dl*-asparagine through their N-benzyl derivatives have been worked out. α-Benzylamino-N-benzylsuccinamic acid has been prepared much more efficiently than reported previously.[1] A course for the reaction between maleic anhydride and benzylamine is proposed, differing from that postulated by Reppe and Ufer.[2]

In the course of work on syntheses of poly-α-amino acids we were in need of α-amino-N-benzylsuccinamic acid which had been prepared by McMillan and Albertson[1] by hydrogenolysis of α-benzylamino-N-benzylsuccinamic acid (II). Their synthesis of this latter compound is based on a procedure developed by Reppe and Ufer[2] and consists in the reaction of one mole of maleic anhydride with two moles of benzylamine in water at reflux temperature for 16 hours. On repeating the preparation of II we invariably found that the yield was only 18–25% as against 48% obtained by the above authors. On the other hand, from the reaction mixture another compound which proved to be the benzylamine salt of N-benzyl-*dl*-aspartic acid (III) was isolated in a yield of about 70%. III could be obtained quantitatively by a modified procedure with a reaction time of only 1.5 hours, by first allowing one mole of maleic

(1) F. H. McMillan and N. F. Albertson, THIS JOURNAL, **70**, 3778 (1948).

(2) W. Reppe and H. Ufer, U. S. Patent 2,200,220 (May 7, 1940).

anhydride to be completely hydrolyzed and then adding two moles of benzylamine.

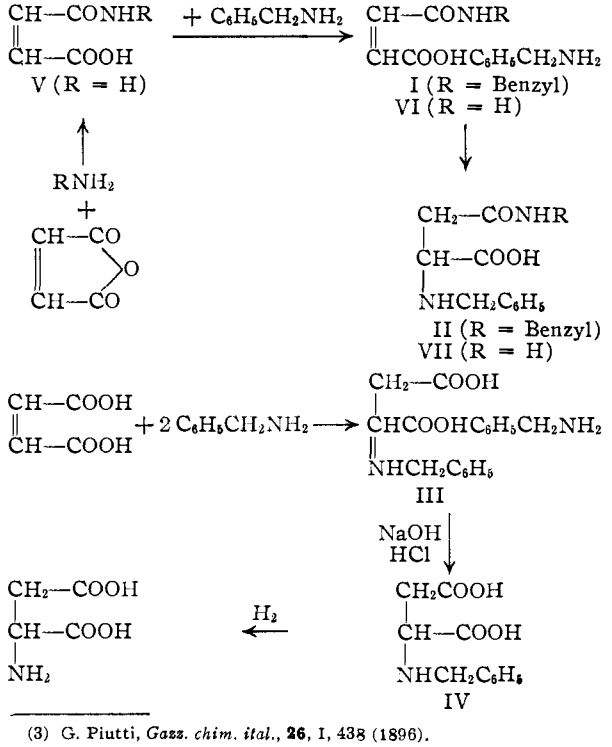
III yielded almost quantitatively N-benzyl-*dl*-aspartic acid (IV) on treatment with sodium hydroxide, extraction with ether of the liberated benzylamine and acidification with hydrochloric acid. Catalytic hydrogenolysis of IV resulted in a practically quantitative yield of *dl*-aspartic acid.

We found that α-benzylamino-N-benzylsuccinamic acid (II) can be prepared in over 70% yield by carrying out the reaction between maleic anhydride and benzylamine in dioxane (thereby precluding hydrolysis to maleic acid); the reaction time was reduced to about 1/2 hour as against 16 hours reported by McMillan and Albertson.

We also proved that the reaction between maleic anhydride and benzylamine proceeds in two stages: the first consisting in opening of the anhydride and formation of benzylmaleamic acid[3] (or its benzylamine salt (I) when using two moles of benzylamine) at low temperature, and the second stage in an addition to the double bond of a second mole of benzylamine at elevated temperature leading to compound II. This reaction course does not conform to that proposed by Reppe and Ufer[2] according to whom imides are formed in the reaction between amines and maleic anhydride.

dl-Asparagine was synthesised by treating maleamic acid (V) with an amount of benzylamine slightly greater than equimolar at reflux temperature in dioxane. The salt (VI) which resulted was isolated and exposed to a higher temperature by reflux in xylene, yielding N-benzyl-*dl*-asparagine (VII) from which *dl*-asparagine was obtained by catalytic hydrogenolysis.[4]

TABLE I



(3) G. Piutti, *Gazz. chim. ital.*, **26**, I, 438 (1896).

(4) Direct treatment of V with benzylamine at a temperature above 138° resulted in the decomposition of this substance, whereas at lower temperatures addition to the double bond could not be effected.

Experimental

Microanalyses are by Drs. Weiler and Strauss. Melting points were determined in a Fisher–Johns apparatus.

α-Benzylamino-N-benzylsuccinamic Acid (II). (A) From Maleic Anhydride and Benzylamine.—9.6 grams of maleic anhydride was dissolved in 40 ml. of dry dioxane and 21.4 g. of benzylamine added portionwise, the reaction mixture becoming very hot. It was boiled under reflux for about 30 minutes, during which time a white voluminous precipitate settled in the flask. After cooling, the substance was filtered and washed several times with water and acetone. The yield was 24 g. (72%) of a material (melting at 215° on recrystallization from ethanol) whose m.p. was not depressed when mixed with the substance obtained by the procedure of McMillan and Albertson.[1]

Anal. Calcd. for $C_{18}H_{20}O_3N_2$: N, 9.0. Found: N, 8.7.

(B) From Benzylmaleamic Acid and Benzylamine.—Two grams of benzylmaleamic acid was dissolved in 15 ml. of dry dioxane (by warming) and 1 g. of benzylamine added. The mixture was boiled under reflux and after a few minutes a white crystalline precipitate settled. Reflux was continued for five more minutes and after cooling the substance was filtered and washed with water and acetone. It was identical with that obtained by the above procedure.

Benzylmaleamic Acid.—Four grams of maleic anhydride was dissolved in 100 ml. of dry ether and cooled in an ice-bath. Eight grams of benzylamine was now added dropwise with vigorous stirring. A white crystalline mass settled immediately. It was filtered off, washed with ether and dried. It weighed 11.7 g. (97.5%) and represented the benzylamine salt of benzylmaleamic acid (I), m.p. 94°.

Anal. Calcd. for $C_{18}H_{20}O_3N_2$: N, 9.0. Found: N, 8.7.

Eight grams of I was dissolved in a dilute sodium hydroxide solution and on acidification with hydrochloric acid a white crystalline precipitate was obtained which weighed 4.7 g. (90%) and melted at 138°.

Anal. Calcd. for $C_{11}H_{11}O_3N$: C, 64.4; H, 5.4; N, 6.8. Found: C, 64.4; H, 5.6; N, 6.7.

Benzylamine Salt of N-Benzyl-*dl*-aspartic Acid (III).—Forty-nine grams of maleic anhydride was boiled under reflux with 150 ml. of water for 1/2 hour. The reaction mixture was cooled and 107 g. of benzylamine cautiously added while cooling by immersing the flask in cold water. Heating under reflux was then continued for one more hour. After cooling, acetone was added until the substance was completely precipitated. The yield was quantitative, m.p. 180° (raised to 184° on recrystallization from ethanol).

Anal. Calcd. for $C_{18}H_{22}O_4N_2$: C, 65.5; H, 6.7; N, 8.4. Found: C, 65.5; H, 6.4; N, 8.5.

N-Benzyl-*dl*-aspartic Acid (IV).—Fifty grams of III was dissolved in 60 ml. of a 15% sodium hydroxide solution. The benzylamine was extracted several times with ether (and subsequently recovered), and the remaining alkaline solution was acidified with hydrochloric acid until acid to congo paper. On cooling and scratching the wall of the vessel, white crystals separated. Thirty-two grams of a substance melting at 194° (dec.) was obtained (95%).

Anal. Calcd. for $C_{11}H_{13}O_4N$: N, 6.3. Found: N, 6.1.

***dl*-Aspartic Acid.**—Five grams of IV was suspended in 110 ml. of glacial acetic acid and 0.3 g. of $PdCl_2$ on carbon catalyst (containing 30% $PdCl_2$) added. The reduction was carried out in a Parr low pressure hydrogenation apparatus (initial pressure 50 p.s.i.) for three hours at about 60°. The cold reaction mixture was filtered; the portion of the material which adhered to the catalyst was extracted with cold formic acid. On separate evaporation of both solvents *dl*-aspartic acid was obtained almost quantitatively.

Anal. Calcd. for $C_4H_7O_4N$: N, 10.5. Found: N, 10.6.

Maleamic Acid (V).—Three grams of maleic anhydride was dissolved in 25 ml. of dry dioxane and gaseous ammonia passed through under stirring. A white crystalline precipitate formed immediately. The reaction was continued until the theoretical amount of ammonia was taken up. The substance was filtered, washed with dioxane and dried. It melted at 142°.

Benzylamine Salt of Maleamic Acid (VI).—Three grams of V was suspended in 50 ml. of dioxane and refluxed with 3 g. of benzylamine. Most of the material dissolved after a few minutes, but later on a mass of white crystals of VI precipitated. Reflux was continued for 35 minutes alto-

gether and the substance filtered after cooling. It weighed 5.5 g., and still gave reactions for double bonds. It melted at 222°.

Anal. Calcd. for $C_{11}H_{14}O_3N_2$: N, 12.6. Found: N, 12.5.

N-Benzyl-*dl*-asparagine (VII).—Five grams of VI was heated under reflux in 50 ml. of xylene for one hour. The resulting material did no longer show reactions for double bonds. It melted at 216° on recrystallization from water. The over-all yield from V was about 70%.

Anal. Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.5; H, 6.3; N, 12.6. Found: C, 59.6; H, 6.4; N, 12.0.

***dl*-Asparagine.**—Four grams of VII was dissolved in a mixture of 50 ml. of glacial acetic acid and 20 ml. of water and 0.3 g. of $PdCl_2$ on carbon catalyst (30%) added. The reduction was carried out similarly to that of N-benzyl-*dl*-aspartic acid (see above). After about three hours the reduction was complete and on filtration and evaporation of the solvent the practically theoretical amount of *dl*-asparagine was recovered.

Anal. Calcd. for $C_4H_8O_3N_2 + H_2O$: N, 18.7. Found: N, 18.4.

JERUSALEM, ISRAEL

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

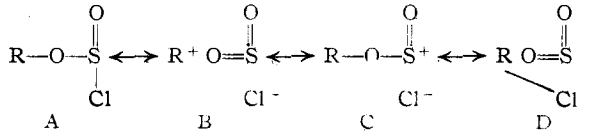
Studies in Stereochemistry. XVI. Ionic Intermediates in the Decomposition of Certain Alkyl Chlorosulfites

BY DONALD J. CRAM

RECEIVED MAY 24, 1952

The reaction of thionyl chloride with the stereoisomers of 3-phenyl-2-butanol, 2-phenyl-3-pentanol and 3-phenyl-2-pentanol has been studied. Decomposition of the initially formed chlorosulfite derivatives of these secondary alcohols in thionyl chloride, dioxane or formic acid produced phenylalkyl chloride products which were subsequently reduced to the corresponding alkylbenzenes. The structures and configurations of the individual components as well as the distributions of products in these alkylbenzene mixtures were determined through polarimetric and spectral techniques. From the stereochemical and structural relationships between the starting alcohols and the final hydrocarbon products, the following conclusions regarding the mechanism of the reaction are reached. (1) Bridged ion-pairs (phenonium chlorosulfite, phenonium chloride or both) occur as discrete intermediates in these reactions. (2) These ion-pairs collapse to give both rearranged and unrearranged products. (3) The reactions are highly stereospecific in the 3-phenyl-2-butyl and *threo*-2-phenyl-3-pentyl and *threo*-3-phenyl-2-pentyl systems, but in the *erythro*-2-phenyl-3-pentyl and *erythro*-3-phenyl-2-pentyl systems about one third of the product arises through a simple substitution reaction. The general mechanism of the S_Ni reaction is discussed in the light of these results.

The reactions of thionyl chloride,[1] phosgene,[2] phosphorus pentachloride in liquid SO_2[3] or dry hydrogen bromide (at −80°)[4] with certain secondary alcohols to give halides whose configurations are the same as those of the starting materials have been classified as examples of the S_Ni reaction.[5] Hughes, Ingold and co-workers[5] suggested that the reactions involving thionyl chloride occurred by the internal decomposition of an intermediate chlorosulfite, and although not stated explicitly, these authors imply that only one transition state intervenes between the alkyl chlorosulfite and the alkyl chloride product. This reaction has been studied kinetically by Lewis and Boozer[1c] who state that if the decomposition of the chlorosulfite intermediate does occur by the concerted mechanism, then structures A–D should all be considered as contributing to the transition state. In a study more similar to that at hand, Lucas and Gould[6] demonstrated that optically active *erythro*-3-chloro-2-butanol reacts with thionyl chloride to give a 16% yield of *meso*-2,3-dichlorobutane, whereas optically active *threo*-3-chloro-2-butanol with the same reagent produces a 20% yield of racemic *d,l*-2,3-dichlorobutane. The authors interpreted these steric results in terms of ethylene chloronium ion intermediates.



The present paper reports the results of a study of the mechanism of the decomposition of the chlorosulfites of the 3-phenyl-2-butyl, 2-phenyl-3-pentyl and 3-phenyl-2-pentyl systems. The symmetry properties associated with the first of these systems and the structural relationships between the two latter systems have already proved of value in studies of the mechanisms of the solvolytic Wagner–Meerwein rearrangement reaction,[7] the E_1 reaction,[8] and the reaction of the *p*-toluenesulfonate esters with lithium aluminum hydride.[9] A similar approach has been used in the current study in which the stereochemical relationships between the products and reactants are used to define the mechanistic requirements for the reaction.

Methods

The reactions were carried out utilizing the optically pure diastereomers of 3-phenyl-2-butanol[7a,c] (I), 2-phenyl-3-pentanol[7b,8b] (III) and 3-phenyl-2-pentanol[7b,8b] (IV) and thionyl chloride as starting materials, and excess thionyl chloride, dioxane or formic acid (the chlorosulfite was preformed) as solvents. The phenylalkyl chloride products

(1) (a) P. A. Levene and L. A. Mikeska, *J. Biol. Chem.*, **75**, 587 (1927); (b) J. Kenyon, A. G. Lipscomb and H. Phillips, *J. Chem. Soc.*, 415 (1930); (c) J. Kenyon, H. Phillips and F. Taylor, *ibid.*, 382 (1932); (d) E. S. Lewis and C. E. Boozer, THIS JOURNAL, **74**, 308 (1952).

(2) A. H. J. Housa and H. Phillips, *J. Chem. Soc.*, 108, 1232 (1932).

(3) E. D. Hughes, C. K. Ingold and I. C. Whitfield, *Nature*, **147**, 206 (1941). See also reference 1a.

(4) P. A. Levene and A. Rothen, *J. Biol. Chem.*, **127**, 237 (1939).

(5) This term was introduced by W. A. Cowdrey, E. D. Hughes, C. K. Ingold, S. Masterman and A. D. Scott [*J. Chem. Soc.*, 1267 (1937)] to designate those nucleophilic displacement reactions which occur with retention of configuration. This designation is limited to those reactions that do not appear to involve the participation of some neighboring group [see S. Winstein and R. E. Buckles, THIS JOURNAL, **64**, 2780 (1942)].

(6) H. J. Lucas and C. W. Gould, Jr., *ibid.*, **63**, 2541 (1941).

(7) (a) D. J. Cram, *ibid.*, **71**, 3863 (1949); (b) **71**, 3875 (1949); (c) **74**, 2129 (1952).

(8) (a) D. J. Cram, *ibid.*, **74**, 2137 (1952); (b) **74**, 2159 (1952).

(9) (a) D. J. Cram, *ibid.*, **74**, 2119 (1952); (b) **74**, 2152 (1952).