the dissociation of lithium fluoborate since at 300° the calculated dissociation pressure is *ca.* 675 mm.[5]

We have found it possible to prepare pure lithium fluoborate by using the same reactants given in eq. 1 but by carrying out the reaction in an ethereal solution at 35°. From measurements of the volume of gas evolved after each addition of small portions of boron trifluoride etherate to a slurry of lithium carbonate in anhydrous ethyl ether (Fig. 1), the stoichiometry of the reaction was ascertained to be

$$3Li_2CO_3 + 8BF_3 \xrightarrow{\text{ether}} 6LiBF_4 + 3CO_2\uparrow + B_2O_3 \quad (2)$$

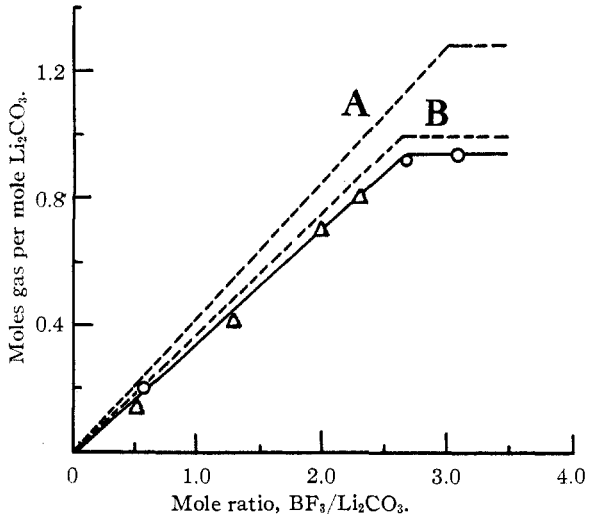

Fig. 1.—Gas evolution as a function of mole ratio of reactants: A, eq. (1); B, eq. (2).

From molecular weight determinations and vapor tension measurements (28 mm. at −111.8°) the gas was identified as (pure) carbon dioxide. The amount of boric oxide present as an end-product of the reaction was shown by titration to be in good agreement with that expected from eq. 2. Before dissolving the boric oxide all boron present as the fluoride complex was removed by ignition. The ignited solids were dissolved in water, the *p*H of the solution was adjusted to the phenolphthalein endpoint and the boric oxide was titrated with standard hydroxide after the addition of mannitol. Chemical analysis of the solid obtained by evaporation of the filtered ethereal solution indicated lithium fluoborate of 99.5% purity. The analysis consisted of measuring the loss in weight (boron trifluoride evolved) upon heating the solid, and then converting the resulting lithium fluoride to lithium sulfate.

The apparatus and techniques employed in this study have been described previously.[2] A typical experiment for preparing lithium fluoborate was as follows: To a slurry of 8.15 g. (0.110 mole) of lithium carbonate[6] in 400 ml. of dry ether was added dropwise 25 ml. (0.198 mole) of boron trifluoride etherate. The mixture was stirred vigorously and the ether refluxed during the addition of the boron trifluoride (0.5 hour) and for a period of three hours following the addition. After 20 minutes standing to permit settling of the solids, the supernatant liquid was transferred to a flask where the ether was evaporated under vacuum at room temperature. The lithium fluoborate was washed with a small quantity of ether and then dried, first, by passing dry nitrogen gas over the solid and, finally, by heating overnight in an oven at 80–90°. A further recovery of the lithium fluoborate remaining in the original solid was made by an extraction with ether. Six and one-half grams of lithium fluoborate was obtained from the original filtrate and one extraction. This quantity was only slightly less than that expected from the solubility of lithium fluoborate which was found by precipitation as nitron fluoborate[7] to be 1.3 g./100 ml. of ether at 25°.

The spacings and intensity of lines for powder diffraction data on lithium fluoborate are included here since these data apparently have not been published previously. The X-ray diffraction data were obtained with a cylindrical camera of 5.73 cm. radius using CuK_α radiation filtered through nickel foil. Line intensities were estimated visually as follows: 4.76 ms, 3.33 s, 3.19 s, 2.57 f, 2.39 s, 2.37 f, 2.27 f, 2.03 s, 1.89 vvf, 1.81 f, 1.73 f, 1.68 f, 1.59 f, 1.46 vvf, 1.43 vf, 1.36 vvf, 1.31 vvf, 1.28 vf, 1.22 vf, 1.18 vvf, 1.13 vvf, 1.06 vvf, 1.02 vvf, 0.994 vvf, 0.942 vvf, 0.931 vvf, 0.906 vvf, 0.849 vvf, 0.828 vvf.

Acknowledgment.—The authors are grateful to Dr. L. A. Burkardt for preparing and measuring the X-ray photographs.

(5) Calculated from the data of Klinkenberg as reported by H. S. Booth and D. R. Martin, "Boron Trifluoride and Its Derivatives," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 98.

(6) The purification of all reagents has been described in detail in ref. 2.

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CHEMISTRY DIVISION
U. S. NAVAL ORDNANCE TEST STATION
PASADENA, CALIFORNIA

Preparation of Isoasparagine by the Phthaloyl Method

BY STUART W. TANENBAUM[1]

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Recent methods developed for the syntheses of phthaloylglutamine[2] and of glutamine itself[3] based upon the γ-directive influence of the N-phthaloyl group coupled to the smooth procedures[4,5] for its removal, led us to attempt a similar approach for the aspartic acid homolog. This mode of asparagine formation has already been described in detail by King and Kidd.[6] Corollary to these experiments we had noted that the intermediary compound N-phthaloylaspartic anhydride (I), yields upon reaction with ammonia followed by dephthaloylation, asparagine (II), isoasparagine (III) or a mixture of the two; the direction of ring opening being dependent upon the nature of the solvent used during ammonolysis. It may be of some theoretical and practical interest to record conditions under which the different isomers are formed and to delineate the preparation and identification of isoasparagine.

The selective ring opening of phthaloylaspartic anhydride with ammonia in aqueous alcohol to give predominantly N-phthaloylisoasparagine is in contradistinction to what takes place with phthaloylglutamic anhydride under the same conditions.[2] Furthermore, ammonolysis in aqueous ether yields a mixture of N-phthaloylasparagine and N-phthal-

(1) Post-doctoral Fellow of the American Cancer Society recommended by the Committee on Growth of the National Research Council.

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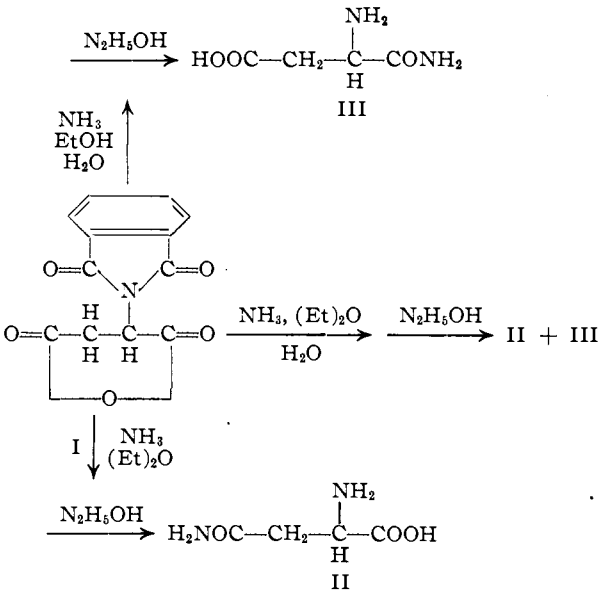

oylisoasparagine, whereas the use of anhydrous ether produces the asparagine derivative almost exclusively.[6] A somewhat similar example of the effect of solvent upon ring opening with ammonia has come to light recently.[7] In this case, β-propriolactone reacted with amines in water to give the hydracrylamides, while in some circumstances ring opening in ether favored the formation of β-alanine derivatives. For the ammonolysis of phthaloylaspartic anhydride it would appear then that two competing reactions are also involved and that under the influence of a polar solvent α-amide formation predominates. The hypothesis is offered that in the presence of water or of aqueous alcohol an acid-catalyzed attack by hydrogen or ammonium ion occurs at the α-carbonyl, whereas base-catalyzed attack takes place at the opposite end of the molecule.

Asparagine can readily be distinguished from isoasparagine either microbiologically, by microscopic examination, or by means of paper partition chromatography. By far the most critical and sensitive method for the detection of one isomer in admixture with the other is the latter technique. The biological assay, however, offers the possibility of being the simplest method for the quantitative micro-estimation of asparagine.

Experimental

Phthaloylaspartic Anhydride.—A mixture of L-aspartic acid (13.3 g., 0.1 mole) and phthalic anhydride (14.8 g., 0.1 mole) in 200 ml. of pyridine was refluxed for two hours. The solvent was distilled off at reduced pressure leaving a glassy yellow residue. This sirup was triturated with acetic anhydride at room temperature which resulted in the precipitation of phthaloylaspartic anhydride. After several hours in the cold the crude anhydride was filtered off, washed with dry ether followed by anhydrous dioxane to give 18.7 g. (76% yield) of product, m.p. 224–225°. Several recrystallizations from dioxane–ether raised the m.p. to 227–228°.

Anal. Calcd. for $C_{12}H_7O_5N$: C, 58.8; H, 2.9. Found: C, 58.8; H, 2.9.

N^{α}-Phthaloylisoasparagine.—The addition of 90 ml. of 1 *N* alcoholic ammonia (0.09 mole) to 10 g. (0.041 mole) of phthaloylaspartic anhydride resulted in a slightly exothermic reaction to give a pale yellow solution. The alcohol and excess ammonia were removed by evaporation *in vacuo* at a bath temperature not over 40°. After dilution with water the dropwise addition of 6 *N* hydrochloric acid to congo red precipitated N^{α}-phthaloylisoasparagine, m.p. 215–216°. The product was recrystallized twice from water; final yield 8.6 g. (80%), m.p. 220–222°.

Anal. Calcd. for $C_{12}H_{10}O_5N_2$: C, 55.0; H, 3.8. Found: C, 55.0; H, 3.8.

Isoasparagine.—In individual trial experiments the phthaloyl grouping was removed from N^{α}-phthaloylisoasparagine by refluxing with alcoholic hydrazine[4] and by the procedure of dissolving the compound in aqueous sodium carbonate followed by reaction with hydrazine hydrate at room temperature.[3] Acidification with hydrochloric acid precipitated phthalhydrazide in each case, and the filtrates were analyzed by paper chromatography. For rapid analyses at this point the horizontal filter paper method of Rutter[8] proved most convenient. It was found in this manner that although a small amount of asparagine was present in these filtrates, the predominant reaction product was isoasparagine, irrespective of the method used for removal of the phthaloyl substituent.

One and seven-tenths grams (0.0065 mole) of N^{α}-phthaloylisoasparagine was added to 0.51 ml. of 64% hydrazine hydrate in 100 ml. of absolute alcohol and the mixture refluxed for one hour. After distillation *in vacuo*, the remainder was dissolved in water and 6 *N* hydrochloric acid was added dropwise to *p*H 2.6. The resultant precipitate of phthalhydrazide was removed, and the filtrate was treated with Amberlite IR-4B resin (sodium salt) in a batchwise manner until *p*H 6 was attained. The neutral solution was then decanted from the settled resin, combined with washings, and slowly passed through a column 1 × 15 cm. of the same resin. The eluates were concentrated under reduced pressure, and the isoasparagine was precipitated by the addition of absolute alcohol. This precipitate gave equivocal growth response when tested auxanographically with *Neurospora* mutant S1007, indicating that some asparagine was present as a contaminant. Additional recrystallization from water–alcohol and from water–acetone produced needles (0.35 g., 36%) of pure isoasparagine hydrate, m.p. browned at 235°, but did not melt up to 285°. This preparation was now biologically inactive with the above test organism, and could not be distinguished chromatographically from an authentic sample of isoasparagine kindly supplied by Dr. J. P. Greenstein. A specimen dried at 100° was analyzed.

Anal. Calcd. for $C_4H_8O_3N_2$: C, 36.4; H, 6.1; N, 21.2; amide N, 10.6. Found: C, 36.7; H, 5.8; N, 20.5; amide N, 10.6.

Mixture of the Amides; Asparagine.—Amidation of phthaloylaspartic anhydride in ether without the exclusion of water followed by working up as detailed above for isoasparagine afforded a 60% yield of the N^{α}-phthaloylamides, m.p. 219–224°. Chromatographic analyses of the aspartic amides obtained from this mixture indicated both asparagine and isoasparagine to be present in roughly equal quantity.

Strict adherence to the procedure of King and Kidd for the ammonolysis, followed by removal of the phthaloyl group and isolation of the amide as outlined above gave prisms of asparagine hydrate in 40% yield. It contained but a trace of isoasparagine when examined chromatographically and was fully biologically active.

Anal. Calcd. for $C_4H_8O_3N_2 \cdot H_2O$: H_2O, 12.0; α-amino N (after drying), 10.6. Found: H_2O, 11.8; α-amino N (after drying), 10.4.

The optical properties of the amides formed by these procedures were not examined. Conditions for the synthesis of stereochemically pure peptides by the phthaloyl method have recently been described by Sheehan and co-workers.[9]

Paper Chromatography.—Butanol–water–acetic acid mixture proved to be a satisfactory solvent for separating the two compounds (Table I). Both substances are ninhydrin positive, asparagine giving yellow to tan colored spots which turn purple after several days standing, while isoasparagine

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(9) J. C. Sheehan, D. W. Chapman and R. W. Roth, THIS JOURNAL **74**, 3822 (1952).

gives an immediate wine colored reaction. In this solvent system, excess or unreacted hydrazine appears as a bright yellow spot superimposed upon the isoasparagine spot following ninhydrin treatment.

TABLE I

R_f VALUES AND COLOR REACTIONS OF ASPARAGINE AND ISOASPARAGINE IN BUTANOL–WATER–ACETIC ACID (40:50:10) AND IN WATER-SATURATED PHENOL

18 hour ascending chromatograms; spray reagent 0.25% ninhydrin in butanol

	Phenol		Butanol-water-acetic acid	
	R_f	Color	R_f	Color
Isoasparagine	0.39	Wine[a]	0.14	Wine
Asparagine	.40	Yellow-tan	.095	Yellow-tan

[a] Unless the phenol is allowed to air dry for 24 hours, even pure samples of isoasparagine will show a tan-colored halo around the wine spot due to interaction with the residual phenol at 100°.

Biological Assay.—Since only asparagine is active in supporting growth of *Neurospora* mutant S1007, the auxanographic plate technique[10] is applicable for differentiating between asparagine and isoasparagine on a semi-micro scale. Here, the addition of a crystal of test substance to a minimal agar plate heavily seeded with the microörganism followed by incubation at 30° for eighteen hours indicates the presence of asparagine by a zone of growth. Both D- and L-isomers can serve to fulfill this nutritional requirement. The quantitative determination of asparagine by means of measuring growth of the organism in liquid cultures will be described elsewhere.

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DEPARTMENT OF BIOLOGICAL SCIENCES
STANFORD UNIVERSITY
STANFORD, CALIFORNIA

Copolymerization of Vinyl Acetate with a Cyclic Disulfide

BY W. H. STOCKMAYER, R. O. HOWARD AND J. T. CLARKE

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In the course of an extensive study of chain transfer in the free-radical polymerization of vinyl acetate, the high reactivity of disulfides as compared with monosulfides was observed. For example, the following transfer constants C were obtained at 60°: di-*n*-butyl sulfide, 0.026; di-*n*-butyl disulfide, 1.0; diethyl dithioglycolate, 1.5. These figures are the slopes of plots[1] of reciprocal number-average polymerization degree P_n^{-1} against the mole ratio S/M of transfer agent to monomer, for low-conversion polymers initiated by benzoyl peroxide or azo-bis-isobutyronitrile. The values of P_n were obtained viscometrically with the aid of a viscosity–molecular weight relation to be described elsewhere.

It should be mentioned that the disulfides caused considerable retardation of the polymerization of vinyl acetate; for example, at a benzoyl peroxide concentration of 10^{-2} M the addition of 5.5×10^{-3} M and 2.7×10^{-2} M dibutyl disulfide reduced the polymerization rate to about 40 and 1.5%, respectively, of the value for pure vinyl acetate. Under such conditions, the true transfer constant C may be less than the slope of P_n^{-1} against S/M. At worst, however, this slope becomes $2C$, so that the values given above still display the high reactivity of the disulfides.

The above figures suggest that the transfer reaction in disulfides involves the scission of the disulfide link (compare the "induced decomposition" of benzoyl peroxide[2])

$$R'M_n\cdot + RSSR \longrightarrow R'M_nSR + RS\cdot \qquad (1)$$

The lability of this bond under more extreme conditions is of course well known. If reaction (1) correctly depicts the transfer process, a *cyclic* disulfide would be capable of copolymerization with vinyl monomers

$$R'M_n\cdot + \overset{\frown R \frown}{S\text{——}S} \longrightarrow R'M_nSRS\cdot \qquad (2)$$

provided the resulting radical were sufficiently reactive to propagate the chain

$$R'M_nSRS\cdot + M \longrightarrow R'M_nSRSM\cdot\text{, etc.} \qquad (3)$$

If this scheme holds, a cyclic disulfide would give an apparently *low* transfer constant by the molecular-weight method, but the polymer would contain a large amount of combined sulfur.

We have demonstrated that the reaction actually follows this course to a considerable extent, using for this purpose the cyclic disulfide 1-oxa-4,5-dithiacycloheptane,[3] $\overline{\lfloor SC_2H_4OC_2H_4S \rfloor}$. An impure but adequate sample of this material was prepared[3] by cracking of the related disulfide polymer; from its properties (45.1% S, n^{25}D 1.5711, d^{25}_{25} 1.261) the purity of the product is about 90% if the sole contaminant is the related monosulfide, *p*-oxathiane. The properties of a series of low-conversion vinyl acetate polymers prepared at 60° in the presence of various concentrations of the cyclic disulfide are given in Table I. The values of P_n^{-1} fall on a fair straight line against S/M, yielding an apparent transfer constant of about 0.25. However the high sulfur content (7.07% S) of the last polymer corresponds to a mole ratio 0.11 of disulfide to vinyl acetate in the polymer, and therefore to an actual transfer constant of about 2.5. Since P_n for this sample is about 90, there are on the average about nine disulfide units per polymer molecule, so that the predicted copolymerization is clearly substantiated. We may remark, in view of the impurity of our disulfide and of a retardation comparable to that found with dibutyl disulfide, that the transfer constants given above are only approximate.

TABLE I

POLYMERIZATION[a] OF VINYL ACETATE IN PRESENCE OF A CYCLIC DISULFIDE, $\overline{\lfloor SC_2H_4OC_2H_4S \rfloor}$, AT 60°

S/M	$[\eta]$[b]	P_n	% S[c]
0	1.24	2200	
0.010	0.31	310	
.023	.26	250	
.035	.15	110	
.045	.12	90	7.07

[a] Initiator, 5×10^{-3} M azo-bis-isobutyronitrile. [b] Limiting viscosity number of low-conversion polymer in acetone, 25°. [c] Weight per cent. of sulfur in the polymer.

Obviously this reaction could in principle be used to prepare certain "block" copolymers by polymerizing vinyl monomers in the presence of polymeric disulfides. However, retardation such as that evi-

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