# NOTES

# THE SYNTHESIS OF INDOLE-3-ACETYL-D,L-ASPARTIC ACID AND RELATED COMPOUNDS<sup>1</sup>

# By Norman E. Good

It has recently been shown that plants conjugate indole-3-acetic acid with aspartic acid (1, 4). Moreover unpublished work done in this laboratory indicates that the reaction is not confined to indoleacetic acid. Applications of indole-3-propionic acid and indole-3-butyric acid to plants result in the formation of indolepropionylaspartic and indolebutyrylaspartic acids. Consequently it is to be expected that such compounds will continue to interest biochemists and, since their preparation presents certain problems, a generally applicable method of synthesis has been developed. The classical method of amide formation via the acid chloride proved practically useless for derivatives of indoleacetic acid since the conditions necessary for the formation of indoleacetylchloride also led to its rapid decomposition. The mixed anhydride method of Boissonnas (2) which was used successfully in the preparation of other amides of indoleacetic acid (7) was unsatisfactory with aspartic acid, perhaps because of a competing formation of aspartic anhydride. Eventually it was found that esterification of aspartic acid with benzyl alcohol, condensation of the ester with indoleacetic acid through the use of N,N'-dicyclohexylcarbodiimide (6, 5), followed by reductive removal of the benzyl groups resulted in a good yield of indole-3-acetyl-D,L-aspartic acid. The same method when applied to 2,4-dichlorophenoxy-acetic, indole-3-propionic, and indole-3butyric acids gave comparable yields of the corresponding aspartic derivatives but, unfortunately, neither indolepropionylaspartic acid nor indolebutyrylaspartic acid has been crystallized as yet.

## EXPERIMENTAL

# Dibenzyl-D,L-aspartate Hydrochloride

Can. J. Chem. Downloaded from www.nrcresearchpress.com by DALHOUSIE UNIVER on 07/03/14 For personal use only.

The dibenzyl ester of aspartic acid was prepared by a modification of the azeotropic distillation method of Cipera and Nicholls (3). D,L-Aspartic acid (13.3 gm., 0.1 mole) was suspended in 100 ml. of benzyl alcohol (0.97 mole) containing 22.0 gm. benzenesulphonic acid (0.125 mole) in a round-bottomed flask. The flask was warmed for 5–10 min. on a steam bath by which time nearly all of the amino acid had dissolved. Hot carbon tetrachloride (300 ml.) was added and the cloudy reaction mixture was heated until about 10 ml. had distilled off. The flask was then fitted with a Soxhlet extractor containing a thimble filled with anhydrous magnesium sulphate (25 gm.) and the solution heated under reflux overnight. The mixture was allowed to stand at room temperature for several hours. The benzenesulphonic acid salt of dibenzyl-D,L-

<sup>1</sup>Contribution No. 77, Science Service Laboratory, Canada Department of Agriculture, University Sub Post Office, London, Ontario.

1356

#### GOOD: SYNTHESIS

aspartate was then removed by filtration, washed once with carbon tetrachloride and several times with dry ether. Residual solvent was removed under vacuum. The yield was 40 gm. (85%). (The salt of dibenzyl-L-aspartate is much more soluble in carbon tetrachloride and only precipitates after the addition of a considerable volume of ether.)

The benzenesulphonic acid salt (40 gm.) was suspended in about 300 ml. dry ether and 14 ml. triethylamine was slowly added with stirring. The suspension was allowed to stand for 0.5 hr. with occasional stirring and was then filtered. The precipitate was resuspended in dry ether containing a little triethylamine and the suspension again filtered. Ether and excess triethylamine were removed from the combined filtrates by distillation at reduced pressure and the residue was taken up in 500 ml. dry ether containing 10 ml. benzyl alcohol. Dry HCl was added slowly in considerable excess. The hydrochloride was then removed by filtration and washed with dry ether. The yield was 29 gm. (83% of the aspartic acid).

## Indole-3-acetyl-D,L-aspartic Acid

Dibenzyl-D,L-aspartate hydrochloride (400 mgm., 1.14 mM.) was suspended in tetrahydrofuran (5 ml.), and triethylamine (0.16 ml., 1.14 mM.) was added slowly with stirring. The mixture was allowed to stand for a few minutes after which it was filtered and the precipitate washed with additional tetrahydrofuran. The combined filtrate and washings were then gently heated on a steam bath in a stream of air until nearly all the tetrahydrofuran had been removed. Indole-3-acetic acid (175 mgm., 1.0 mM.) and 1.0 ml. tetrahydrofuran were added. After the indoleacetic acid had dissolved, the viscous solution was cooled somewhat below room temperature and N,N'-dicyclohexylcarbodiimide (220 mgm., 1.05 mM.) in 0.5 ml. tetrahydrofuran was added with vigorous swirling over five minutes. Residual carbodiimide was washed into the reaction mixture with another 0.5 ml. tetrahydrofuran and the stoppered flask was left overnight at room temperature. Ethyl acetate (10 ml.) was added and N,N'-dicyclohexylurea (215 mgm.) was removed by filtration. The filtrate was extracted successively with 0.1 N HCl, 0.2 N NaHCO3, and water. The solvents were again removed by gently warming in an air stream. The residue was taken up in 50 ml. of *n*-butanol, and palladium catalyst (5% on charcoal) was added. The suspension was shaken under hydrogen (3 atm.) for four hours, whereupon the catalyst was filtered off and washed with more butanol. The filtrate was extracted once with 0.1 N HCl, once with water, and three times with 0.5 N  $NaHCO_3$  (5, 2.5, and 2.5 ml.). The pooled bicarbonate extracts were in turn extracted with ether and the dissolved ether removed by aeration. The aqueous solution was then acidified to pH 2.0 with 80% phosphoric acid. Indole-3-acetyl-D,L-aspartic acid (165 mgm., 57% of the indoleacetic acid) separated as pink crystals when the acidified solution was left overnight in the refrigerator, m.p. 187°-190° C. After treatment with norite and two recrystallizations from water the product was colorless and melted at 190°-191° C. Calc. for C<sub>14</sub>H<sub>14</sub>O<sub>5</sub>N<sub>2</sub>: C, 57.9; H, 4.83; N, 9.66. Found: C, 58.1; H, 4.94; N, 9.56%.

1357

1358

Can. J. Chem. Downloaded from www.nrcresearchpress.com by DALHOUSIE UNIVER on 07/03/14 For personal use only.

### CANADIAN JOURNAL OF CHEMISTRY. VOL. 34, 1956

When 2,4-dichlorophenoxyacetic acid was used in place of indole-3-acetic acid a 50% yield of 2,4-dichlorophenoxyacetyl-D,L-aspartic acid resulted. After one recrystallization from 50% alcohol the melting point was 212-214° C. Wood and Fontaine (8) give 217° C. as the melting point of the racemic derivative.

The author wishes to acknowledge the assistance of Mr. R. W. White.

- ANDREAE, W. A. and GOOD, N. E. Plant Physiol. 30: 380. 1955.
  BOISSONNAS, R. A. Helv. Chim. Acta, 34: 874. 1951.
  CIPERA, J. C. and NICHOLLS, R. V. V. Chemistry & Industry, 16. 1955.
  GOOD, N. E., ANDREAE, W. A., and VAN YSSELSTEIN, M. W. H. Plant Physiol. In press. 1976.
- 1956.

KHORANA, H. G. Chemistry & Industry, 1088. 1955.
 SHEEHAN, S. C. and HESS, G. P. J. Am. Chem. Soc. 77: 1067. 1955.
 WIELAND, T. and HÖRLEIN, G. Ann. 591: 192. 1955.
 WOOD, J. W. and FONTAINE, T. D. J. Org. Chem. 17: 891. 1952.

RECEIVED MAY 17, 1956. SCIENCE SERVICE LABORATORY, CANADA DEPARTMENT OF AGRICULTURE, UNIVERSITY SUB POST OFFICE, LONDON, ONTARIO.

# THE CALCULATION OF THE EXTENT OF ION-PAIR FORMATION IN SOLUTION

## By G. H. DEBUS\*

In recent publications (1, 2, 3) extensive use has been made of the Robinson-Stokes equation to evaluate the equivalent conductance of concentrated solutions of strong electrolytes. The agreement between theory and experiment is surprisingly good up to a concentration of about 3 moles per liter.

According to Wishaw and Stokes (6), when the observed conductance is found to be less than the calculated, this must be attributed to ion-pair formation. The results obtained for ammonium nitrate show reasonable constancy of K, the dissociation constant, but the method of Stokes and Wishaw involves laborious calculations; the degrees of dissociation,  $\alpha$ , could only be found by a series of approximations and the help of a graph. A graphical and more direct way consists in plotting the experimental and the calculated values (for  $\alpha = 1$ ) of  $\kappa \eta / \eta_0$  (product of the specific conductance and the relative viscosity) against the molarity. For equal values of the ordinate the degree of dissociation is obtained by the ratio of the corresponding concentrations.

The mathematical proof of this is as follows: The complete equation for the equivalent conductance,  $\Lambda$ , of a fully dissociated electrolyte is, according to Robinson and Stokes (5):

[1] 
$$\frac{1000\kappa}{c} = \Lambda = \left(\Lambda_0 - \frac{B_2\sqrt{c}}{1 + Ba\sqrt{c}}\right) \left(1 - \frac{B_1\sqrt{c}F}{1 + Ba\sqrt{c}}\right) \frac{\eta_0}{\eta}.$$

\*National Research Council Postdoctorate Fellow at the University of Manitoba, Winnipeg, Manitoba.