Phytochemistry, 1972, Vol. 11, pp. 1485 to 1488. Pergamon Press. Printed in England.

SHORT COMMUNICATION

SYNTHESIS OF INDOLE-3-ACETYLASPARTIC ACID

R. C. MOLLAN, D. M. X. DONNELLY and M. A. HARMEY

Departments of Botany and Chemistry, University College, Belfield, Dublin 4, Republic of Ireland

(*Received* 22 *November* 1971)

Abstract—A simple synthesis of indole-3-acetyl-D,L-aspartic acid and its L-isomer is described and their physical properties are listed.

INTRODUCTION

INDOLE-3-acetylaspartic acid (IAA-Asp) has been encountered in a range of plant species,[1–5] since its first detection and identification as a metabolite of exogenous indole-3-acetic acid (IAA) in pea seedlings.[1] In a few cases[2*,3] it is found in untreated plants but is more generally detected after feeding with IAA. In one instance,[3] the compound is described as indole-3-acetyl-D,L-aspartic acid (IAA-D,L-Asp) but is usually assumed to be L-isomer (IAA-L-Asp). However, no evidence is given for the different designations.

In the course of a study on the interaction of IAA with gibberellic acid in germinating barley seeds,[6,7] we observed that the seeds, when fed with carboxyl-^{14}C-labelled IAA, gave rise to a number of conjugates. The major compound we identified as IAA-L-Asp by comparison with an authentic sample synthesized by us.

Previously, the identification of the IAA conjugate has in almost all cases rested on R_f values (sometimes co-tested with a synthetic sample), colour tests on chromatograms

[1] W. A. ANDREAE and N. E. GOOD, *Plant Physiol.* **30,** 380 (1955).

[2] N. E. GOOD, W. A. ANDREAE and M. W. H. VAN YSSELSTEIN, *Plant Physiol.* **31,** 231 (1956); W. A. ANDREAE and M. W. H. VAN YSSELSTEIN, *Plant Physiol.* **31,** 235 (1956); W. N. DANNENBURG and J. L. LIVERMAN, *Plant Physiol.* **32,** 263 (1957); T. A. BENNET-CLARK and A. W. WHEELER, *J. Exptl Bot.* **10,** 468 (1959); S. C. FANG, P. THEISEN and J. S. BUTTS, *Plant Physiol.* **34,** 26 (1959); M. KUTACEK, K. ROKOSOVA and R. RETOVSKY, *Biol. Plant Acad. Sci. Bohemoslov.* **I,** 54 (1959); *Chem. Abs.* **53,** 17251j (1959); W. A. ANDREAE and M. W. H. VAN YSSELSTEIN, *Plant Physiol.* **35,** 225 (1960); *H. D. KLÄMBT, *Naturwissenschaften* **47,** 398 (1960); P. A. THURMAN and H. E. STREET, *J. Exptl. Bot.* **13,** 369 (1962); F. WIGHTMAN, *Can. J. Bot.*, **40,** 689 (1962); *G. M. WEAVER and H. O. JACKSON, *Can. J. Bot.* **41,** 1405 (1963); M. H. ZENK, *Régulateurs Naturels de La Croissance Végétale* (edited by J. P. NITSCH), pp. 241–249, CNRS, Paris (1964); M. IGARI, *Sci. Rept. Toboka Univ.* Fourth Series, **32,** 47 (1966); *Chem. Abs.* **65,** 7919j (1966); J. SUDI, *New Phyto.* **65,** 9 (1966); A. WINTER and K. V. THIMANN, *Plant Physiol.* **41,** 335 (1966); W. ESCHRICH, *Planta* **78,** 144 (1968); *H. OMAR OLNEY, *Plant Physiol.* **43,** 293 (1968); B. J. ROBINSON, M. FORMAN and F. T. ADDICOTT, *Plant Physiol.* **43,** 1321 (1968); B. P. LANTIGAN and R. M. MUIR, *Physiol. Plantarum (Kbh.)* **22,** 412 (1969); D. A. MORRIS, R. E. BRIANT and P. G. THOMPSON, *Planta* **89,** 178 (1969); H. VEEN and W. P. JACOBS, *Plant Physiol.* **44,** 1157 (1969); E. M. BEYER, JR. and P. W. MORGAN, *Plant Physiol.* **46,** 157 (1970); G. GUENTER, *Zesz. Nauk. Univ. Torunia, Nauki Mat.* **13,** 203 (1970); *Chem. Abs.* **74,** 41349s (1971); D. A. MORRIS, *Planta* **91,** 1 (1970); N. W. LEPP and A. J. PEEL, *Planta* **96,** 62 (1971); L. E. FELLOWS and E. A. BELL, *Phytochem.* **10,** 2083 (1971).

[3] V. V. ROW, W. W. SANFORD and A. E. HITCHCOCK, *Contr. Boyce Thompson Institute* **21,** 1 (1961).

[4] H. D. KLÄMBT, *Planta* **56,** 309 (1961).

[5] F. H. KENDALL, C. K. PARK and C. L. MER, *Ann. Bot.* **35,** 565 (1971).

[6] A. MURRAY and M. A. HARMEY, *Sci. Proceed. Royal Dublin Soc.* **2B,** 275 (1970).

[7] D. M. X. DONNELLY, M. A. HARMEY, R. C. MOLLAN and A. MURRAY. Unpublished results.

and, in a few instances, biological activity. A UV spectrum (identical with that of IAA) of the conjugate, and the hydrolysis of the IAA-Asp to IAA and aspartic acid is recorded.[1,5]

A literature survey revealed that IAA-D,L-Asp has been synthesized[8] by dicyclohexylcarbodiimide coupling of IAA and the dibenzyl ester of D,L-aspartic acid, followed by

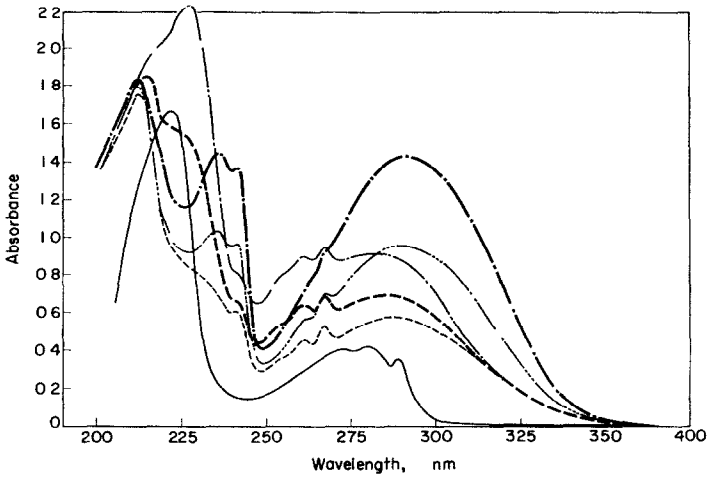

FIG. 1. UV SPECTRA OF INDOLE-3-ACETYLASPARTIC ACID (-L- OR -D,L-).
In MeOH, conc. $6{\cdot}5 \times 10^{-5}$ M, ——. In 12 M H_2SO_4* at 30° conc. $4{\cdot}3 \times 10^{-4}$ M, — · — 2 min; — · · · — 6 min; - - - - - 30 min; — — 3 hr; — - - — 7 hr.

hydrogenolysis. A m.p. and elemental analysis are published. This method is applied to the synthesis of the L-isomer[9] but no details are recorded. A synthesis of IAA-L-Asp under Schotten-Baumann conditions from indole-3-acetyl chloride is claimed[10] and the product described as a crystalline material m.p. 164–5°(dec), $[\alpha]_D^{27°}$ −4·51°. A third synthesis of IAA-L-Asp via the mixed anhydride method[4,11] is on record and reference is made to attempted purification by its barium salt:[4] again no informative details are published.

It is evident from the summary of physical properties that the product synthesized by Weller and Sell[10] is not the same as our IAA-L-Asp. The few properties of either isomer recorded in the literature emphasise the need for a comprehensive description. Furthermore, in the light of more recent knowledge,[12,13] a simpler, better synthesis can be devised in order to make available a ready supply of the free acid for further biological study.

RESULTS

Synthesis of IAA-Asp

We report here a simple, high yield, synthesis of IAA-D,L-Asp and IAA-L-Asp using well established peptide synthetic principles;[12] and we list the physical properties of the

* We have found that the spectral behaviour of 3-substituted indole compounds in 12 M H_2SO_4 provides a useful guide to their structure. Details of this work are to be published shortly.

[8] N. E. GOOD, *Can. J. Chem.* **34**, 1356 (1956).
[9] O. HUTZINGER, *J. Chrom.* **40**, 117 (1969).
[10] L. E. WELLER and H. M. SELL, *J. Org. Chem.* **23**, 1776 (1958).
[11] M. D. ARMSTRONG, K. N. F. SHAW, M. H. GORTATOWSKI and H. SINGER, *J. Biol. Chem.* **232**, 17 (1958).
[12] M. BODANSKY and M. A. ONDETTI, *Peptide Synthesis*, Interscience, New York (1966).
[13] S. W. CHIEN, Ph.D. Thesis, p. 77, Massachusetts, Institute of Technology (1967).

TABLE 1. PHYSICAL PROPERTIES OF IAA-D,L-ASP

m.p.		189–190°
IR cm^{-1}	KBr disc	3470; 3399; 2920 (major peak in 3060–2300 region); 1732; 1699; 1616; 752; 748
UV nm (ϵ)	MeOH (conc. $6{\cdot}5 \times 10^{-5}$ M)*	289 (5200); 280 (6300); 273 (6000); 222 (25000) See Fig. 1
UV	12 M H_2SO_4	See Fig. 1
NMR τ	D_2O conc. 25 mg/ml†	7·18 (d. J = 6Hz) amino acid —CH_2—; 6·25 (s) IAA—CH_2—; 2·3–3·0 (m, major peak 2·70) 5 aromatic protons
MS	300°‡	Small peak at M-18, 272

* ϵ, and to a lesser extent the wavelength of the 222 nm band maximum, is concentration dependent.
† Supersaturated solution, s—singlet; d—doublet; m—multiplet. Me_4Si external standard.
‡ Compound very involatile—peaks less intense than general background level.

compounds (Tables 1 and 2). The method should be applicable to a variety of indole-3-acetamides.

Several 'active esters' of IAA have been prepared.[14] We find that the *p*-nitrophenyl ester reacts with the tetramethylammonium salt of D,L-aspartic acid in dimethylsulphoxide (DMSO)[13] to give good yields (84%) of IAA-D,L-Asp.

The same method gives good yields of the non-crystalline L-isomer (79%) but the product contains a greater or lesser amount of DMSO which is extremely persistent. A better product is obtained using the tetramethylguanidine salt of L-aspartic acid[13] with aqueous methanol as solvent. Although the yield is lower (48%), the latter method has the added advantage of having a simpler work-up procedure.

The product of the aqueous methanol synthesis is shown, by TLC on silica in isopropanol–ammonia–water (8:1:1), to be free of 'active ester', *p*-nitrophenol and IAA. UV light shows two trace impurities of greater polarity than IAA-L-Asp, one of which

TABLE 2. PHYSICAL PROPERTIES OF IAA-L-ASP

$[\alpha]_D^{25°}$	c. 0·64 water c. 0·58 *n*-butanol	+8·5° +27°
IR cm^{-1}	KBr disc	3400; 3050; 2930; 1725; 1630; 1525; 740
UV nm (ϵ)	MeOH (conc. $6{\cdot}4 \times 10^{-5}$ M)*	289 (5150;) 280 (6250); 273 (5900); 222 (25000). See Fig. 1
UV	12M H_2SO_4	See Fig. 1
NMR τ	D_2O conc. 36 mg/ml Me_4Si external standard	7·20 (d, J = 6 Hz) amino acid—CH_2—; 6·26 (s) IAA—CH_2—; 2·3–3·0 (m, major peak 2·70) 5 aromatic protons
	D_2O conc. 130 mg/ml	7·31 (d, J = 6 Hz); 6·42 (s); 2·5–3·2 (m. major peak 2·91)
	H_2O conc. 130 mg/ml	Additional peaks at 2·20 (broad d) —NH—; 0·20 (very broad) —CO_2H
MS	300°	M-18, 272 (% abundance 5); 176 (4); 175 (29); 131 (14); 130 (100); 129 (5); 128 (7); 103 (11); 102 (5)

* ϵ, and to a lesser extent the wavelength of the 222 nm band maximum, is concentration dependent.

[14] J. C. HART, E. M. MATHESON and O. HUTZINGER, *Can. J. Chem.*, **48**, 177 (1970).

remains on the base line and is iodine positive and faintly Erlich positive. The other is iodine and Erlich negative. TLC on cellulose in isopropanol–formic acid–water (20:1:5)[15] shows the product to be free of aspartic acid. NMR in D_2O shows no unexpected peaks, and the product gives a satisfactory elemental analysis. The IAA-L-Asp was not improved on attempted purification by salt formation, thick layer and paper chromatography.

EXPERIMENTAL

M.ps are uncorrected. Only significant bands from IR are quoted. Optical rotations were measured on a Perkin-Elmer Model 141 Polarimeter.

Nitrophenyl ester of IAA. IAA (11·6 g, 0·066 M) and *p*-nitrophenol (9·5 g, 0·066 M) were dissolved in EtOAc (250 ml) and the mixture was cooled to 0–5°. Dicyclohexylcarbodiimide (13·7 g, 0·066 M) in EtOAc (50 ml) was added and the mixture was stirred at 0–5° for 1 hr and at room temp. for a further hr. The dicyclohexylurea was filtered off; the solution was concentrated (to 50 ml), cooled and refiltered. Removal of the remaining solvent gave crystalline *p*-nitrophenyl ester m.p. 100–103°. Recrystallization from EtOAc/light petroleum (b.p. 60–80°) gave a yellow solid m.p. 106–107° (lit.[14] m.p. 110°) (16·3 g 79%). IR (KBr disc) 3400; 1775; 1614; 1590 cm^{-1}. NMR ($CDCl_3$) τ 5·69 (singlet —CH_2—); 2·70–2·88 (6 aromatic protons); 2·28 (multiplet, indole 2H proton), 1·82 (superimposed Doublets J = 9Hz, *p*-nitrophenol *meta* protons); 1·82 (multiplet —NH—).

*Indole-3-acetyl-*D,L*-aspartic acid.* D,L-Aspartic acid (0·665 g, 50 mM) was dissolved in a 25% aq. solution of tetramethylammonium hydroxide (3·65 g, 100 mM) and the mixture was lyophilized (0·39 g H_2O remained) The salt was suspended in DMSO (25 ml) and solution took place when IAA-*p*-nitrophenyl ester (1·48 g, 50 mM) was added. The mixture was stirred overnight. The DMSO was removed *in vacuo* and the product was taken up in $NaHCO_3$ (50 ml; 5%) and Et_2O (50 ml). The aqueous phase was extracted with Et_2O (50 ml), then acidified to pH 5 with conc. HCl, and again extracted with Et_2O (2 × 50, 1 × 25 ml). The aqueous phase was concentrated *in vacuo* (to 30 ml), subsequently acidified to pH 2 and kept at 5° for 12 hr. IAA-D,L-Asp crystallized out as a pale pink product m.p. 186–188° (1·22 g 84%). Recrystallization from water gave a colourless product m.p. 189–190° (lit.[8] m.p. 190–191°). For physical properties see Table 1. IAA-D,L-Asp is stable when stored in a dark bottle.

*Indole-3-acetyl-*L*-aspartic acid. 1st method.* As for indole-3-acetyl-D,L-aspartic acid. The aqueous phase (pH 5) was further acidified to pH 1 and extracted with butanol (2 × 25 ml). The butanol phase was washed with 0·1 N HCl (25 ml) and with H_2O (10 ml) and the butanol was removed *in vacuo* to give a pale pink glass (1·14 g, 78·5%). (Found: C, 57·66; H, 4·85; N, 9·36). $C_{14}H_{14}O_5N_2$ requires: C, 57·93; H, 4·86; N, 9·65%.) TLC showed the aqueous phase and washings to contain further product. *2nd Method.* L-Aspartic acid (0·33 g, 25 mM) and tetramethylguanidine (0·58 g, 50 mM) was dissolved in aq. MeOH (10 ml; 50%) and the mixture was treated with finely ground IAA-*p*-nitrophenyl ester (0·74 g; 25 mM). The suspension dissolved slowly as it was stirred for 48 hr. H_2O (50 ml) was added and the mixture was extracted with Et_2O (2 × 50 ml). The aqueous phase was acidified to pH 5 with conc. HCl and extracted with Et_2O (3 × 50 ml). It was further acidified to pH 1 and extracted with butanol (1 × 40, 1 × 10 ml). The butanol phase was washed with H_2O (2 × 15, 2 × 10 ml) and was concentrated to dryness to give a pale pink glass (0·35 g; 48%). (Found: C, 58·30; H, 4·94; N, 9·76. $C_{14}H_{14}O_5N_2$ requires: C, 57·93; H, 4·86; N, 9·65%.) Again TLC showed the aqueous phase and washings to contain further product. IAA-L-Asp is stable when stored desiccated in a dark bottle.

Acknowledgements—We are indebted to the National Science Council of Ireland for the award of a Fellowship (to R. C. M.); to Dr. P. H. Boyle of Trinity College, Dublin for mass spectral analysis. The microanalyses were carried out by Mrs. E. M. Carey of this Chemistry Department.

[15] K. JONES and J. G. HEATHCOTE, *J. Chromatogr.* **24**, 104 (1966).

Key Word Index—Indole-3-acetylaspartic acid; synthesis; D,L and L forms.