ing to 0.01° in actual rotation with a concentration of $c = 0.02$) may be encountered. It is for this reason that we have listed in Table I not only the positions of the extrema[13] but also what we consider to be the last reliable measurement. It will be noted that the position of this last significant measurement covers quite a range, farther penetration into the ultraviolet being associated with higher rotation. Attention should be drawn to the threonine derivatives, which possess rather low rotations even in the experimentally significant region. As has been noted by Shellman[10] for threonine itself, this is probably due to the compensating effect of the second asymmetric center bearing the hydroxyl group.

We have already shown earlier that the sign of the Cotton effect of certain α-amino acid derivatives such as *N*-dithiocarbalkoxy[14] and *N*-thionocarbalkoxy[15] analogs can be used for purposes of attributing absolute configurations to α-amino acids or terminal amino acids in a peptide sequence. While these derivatives show true Cotton effects—in contrast to the possible ambiguities discussed above for phthalimido α-amino acids—the sign of the latter's dispersion curve can apparently be used equally effectively for stereochemical assignments. The use of *N*-phthaloyl derivatives has the advantage of employing intermediates which are of synthetic utility[5] rather than involving derivatives[14] which are prepared specifically for rotatory dispersion measurements because of their desirable spectral properties.

(13) In our opinion, it is rather difficult to decide at this point whether some of the extrema listed in Table I are in fact extrema of a Cotton effect or rather turning points where a Cotton effect of one sign overcomes the strong "background" rotation (for definition see p. 16 of ref. 7) of opposite sign. Such turning points are usually flat and rounded, yet the first extremum of III in Fig. 1, while showing such a shape, also appears to consist of fine structure and may, in fact, contain the first extremum of a positive Cotton effect.

(14) B. Sjöberg, A. Fredga, and C. Djerassi, *J. Am. Chem. Soc.*, 81, 5002 (1959).

EXPERIMENTAL[16]

All optical rotatory dispersion measurements were conducted in methanol or dioxane solution with 0.5- or 0.1-dcm. cells and concentrations in the range *c*, 0.1 (700–310 mμ)–0.02 (below 310 mμ) using a Rudolph automatically recording spectropolarimeter.[7,12] The results are summarized in Table I.

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(15) C. Djerassi, K. Undheim, R. C. Sheppard, W. G. Terry, and B. Sjöberg, *Acta. Chem. Scand., in press.*

(16) We are greatly indebted to Mrs. Ruth Records for technical assistance.

[CONTRIBUTION FROM THE ORGANIC CHEMISTRY LABORATORY, THE INSTITUTE OF PHYSICAL AND CHEMICAL RESEARCH]

Syntheses of DL-Isoleucine Based on the Darapsky and the Hofmann Degradations

ISABURO HORI, MINORU IGARASHI, AND HIROSHI MIDORIKAWA

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DL-Isoleucine was synthesized from ethyl 2-cyano-3-methyl-2-pentenoate *via* ethyl 2-cyano-3-methylvalerate in two ways—Darapsky's method (—$COOC_2H_5$ ⟶ —NH_2) and the Hofmann method (—CN ⟶ —$CONH_2$ ⟶ —NH_2). More isoleucine was found in the crude product made by Darapsky's method than by the Hofmann method.

Further, a similar experiment *via* ethyl 2-cyano-3-methylvalerate which was prepared from ethyl cyanoacetate and *sec*-butyl bromide was carried out. In this case, the crude product was richer in isoleucine by the Hofmann method than by Darapsky's.

Numerous syntheses of DL-isoleucine give alloisoleucine simultaneously. Doyle *et al.*[1] have prepared this amino acid by some classical and newer methods, and have conducted precise bioassays for isoleucine content in the crude products. It seems from their results that the stereospecific synthesis of DL-isoleucine was not fully accomplished.

In the present experiment, ethyl 2-cyano-3-methyl-2-pentenoate (I), obtained by condensing methyl ethyl ketone with ethyl cyanoacetate, was a starting material. If I is a mixture of *cis* and *trans* isomers (Ia and Ib), ethyl 2-cyano-3-methylvalerate (II), given by catalytic hydrogenation of I, should consist of two racemic diastereoisomers (IIa and IIb). There are two methods to prepare an α-amino acid in this case. The first is to make the required compound by Darapsky's method[2] (a modified Curtius's reaction) which converts the ethoxycarbonyl group into an amino group *via* an hydrazinocarbonyl group and the cyano group

(1) F. P. Doyle, D. O. Holland, W. Marflitt, J. H. C. Nayler, and (Miss) C. M. O'Connor, *J. Chem. Soc.*, 1719 1955).

(2) A. Darapsky and D. Hillers, *J. prakt. Chem.*, 92, 297 (1915); A. Darapsky, *J. prakt. Chem.*, 146, 250 (1936).

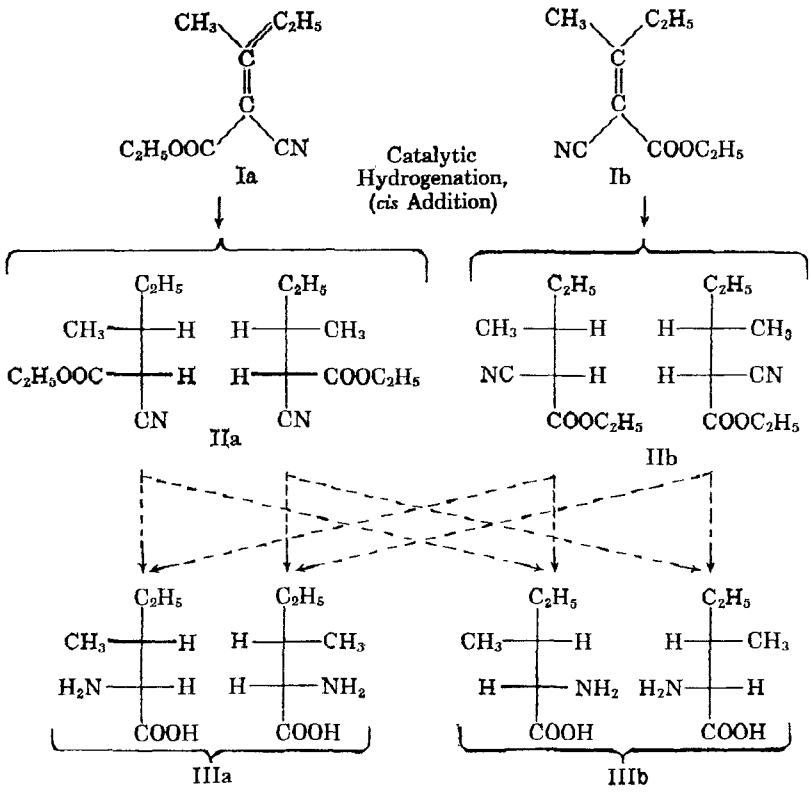

Fig. 1. Darapsky method ——————→
Hofmann method - - - - - - - - - - - - - - →

into a carboxyl group by hydrolysis. The second is to convert the cyano group into an amino group by amidation and subsequent Hofmann degradation, and the ethoxycarbonyl group into a carboxyl group by hydrolysis.

Provided that the catalytic hydrogenation of the double bond occurs by *cis* addition[3] and the amidation and the hydrolysis are accompanied by no inversion, all reaction processes are illustrated in Fig. 1.

If I, as the starting material, contains one of the two *cis-trans* isomers predominantly or only one isomer, the two crude products obtained by Darapsky's and the Hofmann methods respectively should be considerably different from each other in their isoleucine content.

In this experiment, Darapsky's method gave a crude product having about 60% isoleucine, while the Hofmann method gave a crude product having about 40% isoleucine.

Moreover, with regard to the *N*-benzoyl compound of the crude product obtained by Darapsky's method, on recrystallization from 30% aqueous ethanol, it was easier to elevate its melting point to over 130°[4] than the corresponding compound obtained by the Hofmann method.

I, synthesized by means of various condensing agents,[5,6] respectively gave nearly similar results, as shown in Table I.

TABLE I

ISOLEUCINE CONTENT AND MELTING POINT OF THE *N*-BENZOYL DERIVATIVES OF THE CRUDE PRODUCTS PREPARED BY VARIOUS METHODS

Starting Material (Condensing Agent)	Darapsky's Method		Hofmann Method	
	DL-Isoleucine %[a]	M.p. of *N*-benzoyl deriv.[b]	DL-Isoleucine %[a]	M.P. of *N*-benzoyl deriv.[b]
I (Ammonium acetate)[5]	60.5	118–120	40.2	112–114
I (Piperidine)[5]	60.4	116–119	41.5	112–114
I (Potassium fluoride)[6]	60.8	118–120	45.3	112–114
II[c]	42[d]		61.2	118–121

[a] Doubled the percentage of L-isoleucine given by bioassay. [b] Recrystallized once from benzene. [c] Prepared by the procedure of Gagnon *et al.* See ref. 9. [d] See ref. 1.

The hydrogenation was carried out over palladium. The isolation of the crude amino acid mix-

(3) It has been generally considered that *cis* addition is involved in the catalytic hydrogenation of ethylenic bonds [K. N. Campbell, and B. K. Campbell, *Chem. Revs.*, **31**, 77 (1942); R. L. Burwell, Jr., *Chem. Revs.*, **57**, 895 (1957); A. Farkas and L. Farkas, *Trans. Faraday Soc.*, **33**, 837 (1937)].

(4) Pure DL-*N*-benzoylisoleucine, m.p. 136–138°; pure DL-*N*-benzoylalloisoleucine, m.p. 126–127° [ref. (1), p. 1721].

(5) A. C. Cope, C. Hofmann, C. Wyckoff, and E. Hardenbergh, *J. Am. Chem. Soc.*, **63**, 3452 (1941).

(6) M. Igarashi, H. Midorikawa, and S. Aoyama, *Sci. Papers Inst. Phys. Chem. Research (Japan)*, **52**, 151 (1958).

ture was effected by treating alcoholic solutions of the hydrochlorides with triethylamine, and the L-isoleucine assay was carried out microbiologically, as Doyle *et al.*[1] did, in order to compare the results.

The methods employed in this experiment contain a few indefinite nonstereospecific processes—hydrogenation, amidation, and hydrolysis. However, one of the two *cis-trans* isomers (Ia and Ib) is presumed to exist predominantly in I.[7] The degree of inversion caused by nonstereospecific processes will be confirmed by definite starting material consisting of a pure isomer.[8]

On the other hand, Gagnon *et al.*[9] have already synthesized the amino acid by the Darapsky method from II which was prepared by condensing ethyl cyanoacetate and *sec*-butyl bromide. This crude product contained about 42% isoleucine in the case of the experiment of Doyle *et al.*[1]

In the present experiment, Hofmann's method afforded the crude product containing about 60% of isoleucine, as expected.

EXPERIMENTAL[10]

The L-isoleucine assays were carried out by the method of Hood and Lyman.[11] *Leuc. mesenteroids* P-60 was employed as the organism.

Ethyl 2-cyano-3-methyl-2-pentenoate (I). I was prepared by condensing methyl ethyl ketone with ethyl cyanoacetate, while ammonium acetate,[5] piperidine,[5] and potassium fluoride,[6] respectively, were used as a condensing agent. All the condensed products were subjected to careful distillation, to obtain a colorless mobile oil, b.p. 114–115°/10 mm.

Ethyl 2-cyano-3-methylvalerate (II). (a) II was derived from I by catalytic hydrogenation in quantitative yield. I (83.6 g., 0.5 mole) was dissolved in two volumes of ethanol and shaken with hydrogen at room temperature and pressure in the presence of 5 g. of kieselguhr containing 0.1 g. of palladium. After the theoretical quantity of hydrogen was absorbed, the solution freed from the catalyst was distilled at 100–102°/12 mm.

(b) II was prepared also by the method of Gagnon *et al.*,[9] in which ethyl cyanoacetate and *sec*-butyl bromide were added to an alcoholic solution of sodium ethoxide, and the whole refluxed for 20 hr. It boiled at 92–93°/5 mm.

Ethyl 2-carbamoyl-3-methylvalerate. II(10 g.) was dissolved in 20 g. of concd. sulfuric acid and left to stand for 24 hr. at room temperature or heated on a water bath at 60–70° for 30 min. After being cooled, the reaction mixture was poured on ice, and white crystals were separated and recrystallized from benzene, to obtain colorless plates, m.p. 101.5–102.5°. The yield was 11 g. (90%).

Anal. Calcd. for $C_9H_{17}O_2N$: N, 7.48. Found: N, 7.56.

Darapsky's degradation of II. A mixture of 4.2 g. (0.025 mole) of II and 1.5 g. of 80% aqueous hydrazine hydrate was dissolved in absolute ethanol. After the solution was left overnight, the reaction mixture was freed from the solvent in a vacuum desiccator. The residual product (hydrazide) was used for subsequent reaction without further purification. A solution of the crude hydrazide in 50 ml. of 15% aqueous hydrochloric acid was cooled to 0° and covered with 40 ml. of ether. Then the hydrazide was converted into azide by adding 5 g. of sodium nitrite in a little water. The ethereal layer was dried, mixed with 40 ml. of absolute ethanol, and heated on a water bath to expel the ether. After being refluxed for 1 hr., the ethanol was evaporated under reduced pressure. The residue (urethane) was hydrolysed by refluxing for 20 hr. with 100 ml. of 20% aqueous hydrochloric acid. After filtration, the solution was decolorized with charcoal and evaporated to dryness under vacuum. The excess hydrochloric acid was expelled by repeatedly dissolving the residue in water and evaporating to dryness under vacuum. The residue was dissolved in ethanol and adjusted to *p*H 6 with triethylamine. The resulting colorless precipitate, the crude product of isoleucine, was collected and washed with ethanol. The yield was about 55%.

Hofmann degradation of ethyl 2-carbamoyl-3-methylvalerate. Bromine (1.6 g.) was added drop by drop to a solution containing 3.2 g. of sodium hydroxide in 30 ml. of water at 0°. The clear yellow solution was immediately mixed with 1.8 g. of ethyl 2-carbamoyl-3-methylvalerate and stirred to dissolve the amide. After prolonged stirring for 30 min., the reaction mixture was then warmed for 30 min. at 60–70° on a water bath. The yellow solution became colorless on heating. Then it was cooled to room temperature, neutralized with dilute hydrochloric acid and evaporated to dryness under vacuum. Again the residue was dissolved in concentrated hydrochloric acid, separated from the insoluble material by filtration, and evaporated to dryness under vacuum. Further, by the similar treatment to Darapsky's degradation, the residue gave a crude product of isoleucine as a colorless precipitate. The yield was about 50%.

The crude products of isoleucine obtained through various methods, were assayed microbiologically for their isoleucine content and benzoylated by the usual Schotten Baumann method. These results are listed in Table I.

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KOMAGOME, BUNKYO-KU, TOKYO, JAPAN

(7) The configuration of isoleucine (IIIa in Fig. 1) has been established recently by chemical and x-ray evidence [S. Ställberg-Stenhagen and E. Stenhagen, *Arkiv. Kemi. Mineral Geol.*, 24B, No. 9, 1 (1947); J. Trommel, *Proc. Konikl. Nederl. Akad. van Wetenschappen*, B56, 272 (1953); 57, 364 (1954)]. Consequently, it may be expected that Ia (Fig. 1) is predominant.

(8) From this point of view, it will be desirable to start from a crystallized material. The authors (M. I. and H. M.) already reported that, on repeated redistillations, I was obtained as crystals melting at 27° [ref. (6)]. But the crystallization of I could not be reproduced in this experiment.

(9) P. E. Gagnon, K. Savard, R. Gaudry, and E. M. Richardson, *Can. J. Research*, 25B, 28 (1947).

(10) All melting points and boiling points are uncorrected.

(11) D. W. Hood and C. M. Lyman, *J. Biol. Chem.*, 186, 195 (1950).