BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 2554—2558 (1970)

The Resolution of *N*-Benzyloxycarbonyl-DL-amino Acids Using Ephedrine*1

Katsue Oki, Kuniyoshi Suzuki, Seiichi Tuchida, Tomoo Saito and Hiroshi Kotake

Department of Chemistry, Faculty of Science, Kanazawa University, Kanazawa

(Received February 10, 1970)

The use of ephedrine has here been proposed to be of value in the direct resolution of racemic *N*-benzyloxycarbonylamino acids. By using a half equi-amount of natural (−)-ephedrine, *N*-benzyloxycarbonyl derivatives of *β*-methyl DL-aspartate, DL-leucine, DL-methionine, and *O*-benzyloxycarbonyl-DL-serine resulted, each in turn producing a salt of the respective D-isomer. On the other hand, DL-isoleucine, *erythro*-*β*-methyl-DL-leucine, *threo*-*N*,*β*-dimethyl-DL-leucine, DL-valine, and DL-phenylalanine each produced a salt of each L-isomer with the same base. It should be better to protect, if possible, the third functional groups of three functional amino acids before the resolution. Each antipode of the optically active *N*-benzyloxycarbonylamino acid obtained above was also conveniently produced, in a high optical purity, by using (+)-ephedrine alternately. However, a few limitations on the desirable method of resolution have also been observed.

Varieties of *N*-protecting groups for amino acids have recently been developed by splendid studies of peptide syntheses and have been used well. The *N*-benzyloxycarbonyl group, explored by Bergmann and Zervas in 1932[1], is, however, still one of the most favorable *N*-protecting groups for the sequential synthesis of peptides. The direct resolution of *N*-benzyloxycarbonyl derivatives of racemic amino acids would, therefore, be a much more convenient way of preparing optically active *N*-benzyloxycarbonylamino acids than the route of incorporating the protecting group onto optically active amino acids obtained *via* the appropriate resolution of temporary derivatives of racemic amino acids, *e.g.*, *N*-acetyl ones.

In this paper, the resolution of racemic *N*-benzyloxycarbonylamino acids by using (−)- and (+)-ephedrine alternately and a survey of the resolution will be described. In these experiments, a half equi-amount of ephedrine in relation to racemic *N*-benzyloxycarbonylamino acids was used principally. Although ethyl acetate could be employed as a predominant solvent in many cases, ethyl acetate-petroleum ether (2 : 3v/v) for *N*-benzyloxycarbonyl-DL-leucine and ether for *N*-benzyloxycarbonyl-DL-isoleucine and *N*-benzyloxycarbonyl-DL-phenylalanine were also effectively used in concentrations of *N*-benzyloxycarbonylamino acid and ephedrine of 0.45—4.68 and 0.25—2.58 M respectively. The selection of a suitable solvent for the aforementioned resolution is important, especially in the case of phenylalanine; usually only a racemate or a partially-resolved salt was deposited from a solution in a solvent such as ethyl acetate, benzene, or chloroform, though when ether was used the resolution was favored. The results obtained with salts with natural (−)-ephedrine are listed in Table 1. Of all the amino acids used, *N*-benzyloxycarbonyl derivatives of amino acids without a *β*-methyl side group—*β*-methyl DL-aspartate, DL-leucine, DL-methionine, and *O*-benzyloxycarbonyl-DL-serine—deposited to afford predominantly, if not exclusively, salts of the D-isomer of amino acids with natural (−)-ephedrine. Unlike the above cases, however, the same derivatives of amino acids with a *β*-methyl side group—DL-isoleucine, *erythro*-*β*-methyl-DL-leucine, *threo*-*N*, *β*-dimethyl-DL-leucine, and DL-valine—afforded salts bearing a reverse configuration, L, at the α-carbon atom of the amino acids. Entirely opposite results yielding each antipode were attained when (+)-ephedrine was used instead of (−)-base. Thus, it is possible to choose either antibode of ephedrine before the resolution, because it is possible, as a rule, to predict the configutarion of the amino acid to be obtained as an insoluble salt, depending on the presence of a *β*-methyl side group. This would be especially useful for the resolution of a new amino acid, as we have shown successfully in a preceding paper.[2] In consonance with this view, the preceding observation, made by

*1 Presented, in part, at the 22nd annual meetings of the Chemical Society of Japan, held at Tokyo, April 1969. Copies of the paper are available from T.S. or H.K. on request.

1) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

2) H. Kotake, T. Saito and K. Ōkubo, This Bulletin, **42**, 1367 (1969).

TABLE 1

Salt of *N*-Benzyloxycarbonylamino Acid with (−)-Ephedrine	Molecular Formula	Analysis Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	Yield (%)	Mp (°C)	$[\alpha]_D$** (deg)
β-Methyl D-aspartate	$C_{23}H_{30}O_7N_2$	61.87	6.77	6.27	61.84	6.74	6.60	71	141	−30.3
D-Leucine	$C_{24}H_{34}O_5N_2$	66.95	7.96	6.51	66.44	8.06	6.44	78	92— 94	−12.3
D-Methionine	$C_{23}H_{32}O_5N_2S$	61.59	7.19	6.25	61.40	7.29	5.96	89	129—130	−28.1
O-Benzyloxycarbonyl-D-serine	$C_{29}H_{34}O_8N_2$	64.67	6.36	5.20	64.87	6.61	5.17	95	133—134	−33.8
L-Isoleucine	$C_{24}H_{34}O_5N_2$	66.96	7.96	6.51	66.68	7.95	6.69	89	138	−17.2
erythro-β-Methyl-L-leucine*	$C_{25}H_{36}O_5N_2$	67.54	8.16	6.30	67.50	8.09	6.28	45	124—125	−17.6
threo-*N*, β-Dimethyl-L-leucine*	$C_{26}H_{38}O_5N_2$	68.09	8.35	6.11	68.26	8.24	6.27	94	160—161	−75.0
L-Valine	$C_{23}H_{32}O_5N_2$	66.32	7.74	6.73	66.27	7.83	6.43	78	145	−20.3
L-Phenylalanine	$C_{27}H_{32}O_5N_2$	69.80	6.94	6.03	69.97	7.04	6.28	85	139—140	+11.3

* Represented from our preceding paper.[2]
** All optical rotation values were measured in a solution of absolute alcohol, at 22—27°C.

Overby and Ingersoll,[3] that a salt of *N*-benzyloxycarbonyl-D-alanine with (−)-ephedrine was produced as a less soluble product when *N*-benzyloxycarbonyl-DL-alanine was allowed to react with natural ephedrine in ethyl acetate, also supports our above conclusion. An exception to the rule was, however, observed in the case of phenylalanine, which bears a β-aromatic group but no β-methyl group; thus, a salt of the L-isomer with natural (−)-ephedrine was deposited preferentially. In order to determine the effect of a β-aromatic side group on the resolution, further attempts have been made to resolve *N*-benzyloxycarbonyl-DL-tyrosine and -DL-tryptophan into their respective isomers, but these attempts have so far been unsuccessful. Even if the base was used in a half- or equi-molar amount ratio in various solvents, neither *N*-benzyloxycarbonyl-DL-aspartic acid nor *N*-benzyloxycarbonyl-DL-glutamic acid have provided favorable results. A successful resolution of DL-aspartic acid was, however, achieved by protecting β-carboxyl as its methyl ester. An additional attempt to resolve the γ-methyl DL-glutamate was unsuccessful, though. Similarly, the resolution of DL-serine was performed successfully only when its *N*,*O*-dibenzyloxycarbonyl derivative was employed. Thus, one recommendation, that the third functional groups of amino acids should be protected for the favorable resolution, can be made on the basis of our experiments involving aspartic acid and serine. Each antipode of *N*-benzyloxycarbonylamino acid with an entirely optical purity resulted, when (+)-ephedrine was used alternately, from a solution of the same solvent used in the corresponding case (Table 2), though it could also be produced in an optically active state from the mother liquor obtained after the removal of the

TABLE 2

Salt of *N*-Benzyloxycarbonylamino Acid with (+)-Ephedrine	Analysis Found C	Found H	Found N	Yield (%)	Mp (°C)	$[\alpha]_D$** (deg)
β-Methyl L-aspartate	62.31	6.81	6.29	53	141—142	+31.0
L-Leucine	66.43	8.01	6.47	68	91—93	+12.3
L-Methionine	61.27	7.27	6.09	82	130—131	+28.2
O-Benzyloxycarbonyl-L-serine	64.50	6.39	5.36	91	132	+33.0
D-Isoleucine	67.06	8.00	6.36	83	139	+15.7
erythro-β-Methyl-D-leucine*	67.60	8.14	6.32	50	124—125	+17.7
threo-N, β-Dimethyl-D-leucine*	67.97	8.31	6.16	89	159—160	+73.5
D-Valine	65.98	7.73	6.82	96	145	+19.1
D-Phenylalanine	69.44	7.05	6.28	85	139—140	−11.1

* Represented from our preceding paper.[2]
** All optical rotation values were measured in a solution of absolute alcohol, at 22—27°C.

3) L. R. Overby and A. W. Ingersoll, *J. Amer. Chem. Soc.*, **82**, 2067 (1960).

salts with natural ephedrine. All the salts obtained herein were treated with 2N hydrochloric acid to remove the basic moieties and to produce optically active *N*-benzyloxycarbonylamino acids. Each amino acid was then regenerated in an optically pure state by the removal of the benzyloxycarbonyl

Table 3

	N-Benzyloxycarbonyl derivative			Free amino acid regenerated
	Yield (%)	Mp (°C)	$[\alpha]_D$** (deg)	$[\alpha]_D$*** (deg)
β-Methyl D-aspartate	88	98—99	+17.8[a]	
D-Leucine	100	oil		−15.9 (lit, −15.1[11,e])
D-Methionine	95	70—71 (lit, 69—70[4])	+17.2 (lit, +18.2[7])	−26.0 (lit, −23.9[12,e])
O-Benzyloxycarbonyl-D-serine	96	83—84	−4.3, −23.5[b]	−14.0[f] (lit, −14.3[13])
L-Isoleucine	100	oil		+40.8 (lit, +40.7[11,14])
erythro-β-Methyl-L-leucine*	100	oil		+38.9[e]
threo-*N*, β-Dimethyl-L-leucine*	92	98—99	−75.9	+38.3[e]
L-Valine	96	66—67 (lit, 66—67[4] 57—61[8])	+ 0.7 (lit, +0.1[4] −4.3[8,c])	+29.5 (lit, +28.8[14])
L-Phenylanine	94	86—89 (lit, 88—89[4])	+ 8.4 (lit, +5.1,[4] +5.3[9,c])	−31.9[g] (lit, −34.8[14,g])
β-Methyl L-aspartate	90	96 (lit, 96—98[5])	−16.5[a] (lit, −17.4[5,a])	
L-Leucine	100	oil		+14.6 (lit, +14.9[14])
L-Methionine	93	69—70 (lit, 68—69[4])	−19.4 (lit, −16.6[10])	+23.4 (lit, +23.5[14])
O-Benzyloxycarbonyl-L-serine	95	83—84	+4.4, +23.6[b]	+13.9[f] (lit, +14.5[14,f])
D-Isoleucine	100	oil		−40.3 (lit, −40.7[11])
erythro-β-Methyl-D-leucine*	100	oil		−39.4[e]
threo-*N*, β-Dimethyl-D-leucine*	93	98—99	+75.3	−39.3[e]
D-Valine	93	66—67 (lit, 60—62[6])	−0.53 (lit, +5.0,[6,d] +4.2[8,c])	−28.3 (lit, −26.4[12,e])
D-Phenylalanine	91	86—87 (lit, 88—89[4])	−8.3 (lit, −4.6[4,c])	+32.4[g] (lit, +33.9[12,e])

* Represented from our preceding paper.[2]
** Measured in solution of absolute alcohol unless stated otherwise, at 22—26°C.
*** Measured in a solution of 6N hydrochloric acid unless stated otherwise, at 22—25°C.
a) Measured in pyridine. b) Measured in ether. c) Measured in glacial acetic acid. d) Measured in methanol. e) Measured in 5N hydrochloric acid. f) Measured in 1N hydrochloric acid. g) Measured in water.

4) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2 John Wiley & Sons Inc., New York (1961), pp. 892—894.
5) H. Schwarz, F. M. Bumpus and I. H. Page, *J. Amer. Chem. Soc.*, **79**, 5701 (1957).
6) R. Sargers and B. Witkop, *ibid.*, **87**, 2020 (1965).
7) N. F. Albertson and F. C. McKay, *ibid.*, **75**, 5323 (1953).
8) E. Schröder, *Ann. Chem.*, **692**, 241 (1966).
9) D. W. Clayton, J. A. Farrington, G. W. Kenner and J. M. Furner, *J. Chem. Soc.*, **1957**, 1398.
10) K. Hofmann, A. Jöhl, A. E. Furlenmeier and H. Kappeler, *J. Amer. Chem. Soc.*, **79**, 1636 (1957).
11) W. A. H. Huffman and A. W. Ingersoll, *ibid.*, **73**, 3366 (1951).
12) J. R. Parikh, J. P. Greenstein, M. Winitz and S. M. Birnbaum, *ibid.*, **80**, 953 (1958).
13) E. Fischer and W. A. Jacobs, *Ber.*, **39**, 2942 (1906).
14) S. Akabori and S. Mizushima (Eds.), "Protain Chemistry" (Tanpakushitu Kagaku) Vol. 1 Kyoritsu Shuppan K.K., Tokyo (1952), pp. 112.

group using hydrogen bromide in glacial acetic acid,[15] followed by neutralization with triethylamine, with the exception of the deprotection of *N,O*-dibenzyloxycarbonyl serine. In the latter case, the protecting groups were removed by catalytic hydrogenation, using palladium-black as a catalyst in ethyl alcohol, in order to avoid any *O*-acylation, which is likely caused by the action of hydrogen bromide in glacial acetic acid.[16] The specific rotations of resolved *N*-benzyloxycarbonylamino acids and free amino acids are listed in Table 3, and their values are compared with those reported in the literature. The specific rotation value of β-methyl *N*-benzyloxycarbonyl-D-aspartate is comparable in magnitude to that of its L-antipode. The value of *N*-benzyloxycarbonyl-L-phenylalanine, along with that of the D-isomer, is relatively higher in magnitude than those reported. A striking difference is found to exist in the optical-rotation values of benzyloxycarbonyl valine reported in the literature; there are only a few values compatible with that of benzyloxycarbonyl valine obtained in this experiment. However, the two isomers of benzyloxycarbonyl valine afforded optically pure L- and D-valine respectively by debrocking.

Experimental

All the melting points are uncorrected. The optical rotation values were measured with a Jasco DIP-SL-type polarimeter.

Starting Materials. *A) (−)- and (+)-Ephedrine Hemihydrate Used Throughout This Work.* Commercially-available (−)-ephedrine hydrochloride, with a specific rotation value of −33.8° in water, was treated with a slight excess of N sodium hydroxide, and the organic base thus liberated was immediately taken up into ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness *in vacuo* to afford a crystalline material. This was then recrystallized from petroleum ether and dried spontaneously under an atmosphere to give (−)-ephedrine hemihydrate as needles; the melting point was 39—40°C, $[\alpha]_D = -5.8°$ in water. Found: C, 68.80; H, 9.14; N, 8.09%. Calcd for $C_{10}H_{15}ON \cdot 1/2H_2O$: C, 68.93; H, 9.26; N, 8.04%. Similarly, from (+)-ephedrine hydrochloride ($[\alpha]_D = +33.1°$ in water), (+)-ephedrine hemihydrate was obtained; mp 40°C, $[\alpha]_D = +5.9°$ in water. (Found: C, 69.29; H, 9.27; N, 8.21%).

B) N-Benzyloxycarbonyl Derivatives of Racemic Amino Acids. The compounds were prepared, in excellent yields, by the generally-applicable Schotten-Baumann method. Recrystallization from appropriate solvents afforded pure products as follows [name of *N*-benzyloxycarbonyl-DL-amino acid, mp°C, (lit, mp°C).]: β-methyl DL-aspartate, 110—112°C, (112°C).[17] DL-Isoleucine, 64—66°C, (48°C[18]). *erythro*-β-Methyl-DL-leucine, 62—64°C.[2] *threo-N,*β-Dimethyl-DL-leucine, 79°C.[2] DL-Leucine, 53—54°C, (52—55°C[4]). DL-Methionine, 112°C, (112°C[4]). DL-Phenylalanine, 102°C, (103°C[4]). *O*-Benzyloxycarbonyl-DL-serine, 93°C, (94°C[4]). DL-Valine, 74—75°C, (76—78°C[4]).

General Procedure for the Resolution of *N*-Benzyloxycarbonyl-DL-amino Acids. To a solution of 0.100 mol of *N*-benzyloxycarbonyl-DL-amino acid in ethyl acetate at a suitable concentration, depending on the amino acid employed, as will be shown below individually, 0.055 mol of (−)-ephedrine hemihydrate was added; then the solution was allowed to stand at room temperature (or stored in a refrigerator in some cases) until there was an adequate amount of a precipitate (this took from several hours to several days, depending on the materials employed.). The precipitate was collected by filtration and washed with ethyl acetate. An almost pure product was recrystallized from ethyl acetate to afford a fine salt of optically active *N*-benzyloxycarbonylamino acid with (−)-ephedrine (Table 1).

The suitable solvents and concentrations for *N*-benzyloxycarbonylamino acid favored for the resolution were as follows [name of *N*-benzyloxycarbonylamino acid, solvent (ethyl acetate was used unless stated otherwise), concentration in mole (M)]: β-methyl DL-aspartate, 0.45. DL-Isoleucine, ether, 2.49. *erythro*-β-Methyl-DL-leucine, ether, 2.00. *threo-N,*β-Dimethyl-DL-leucine, 1.00. DL-Leucine, ethyl acetate - petroleum ether (2 : 3 V/V), 2.62. DL-Methionine, 2.46. DL-Phenylalanine, ether, 0.17. *O*-Benzyloxycarbonyl-DL-serine, 1.00. DL-Valine, 4.68.

The above mother liquor was washed with a small amount of N hydrochloric acid and then water. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*. The residue thus obtained was redissolved in a half volume of the same solvent as was used in the initial resolution stage, and 0.055 mol of (+)-ephedrine was added. The solution was then allowed to stand. The deposition of salt was usually much faster than in the initial case. The precipitate was filtered and washed with ethyl acetate. Recrystallization from ethyl acetate gave pure antipodal salt (Table 2).

Liberation of Optically Active *N*-Benzyloxycarbonylamino Acids from Salts. A suspension of the salt of optically active *N*-benzyloxycarbonylamino acid with ephedrine in ethyl acetate was treated with a slight excess of 2N hydrochloric acid, and the organic layer was washed with a small amount of water. The solution of ethyl acetate was then dried over anhydrous sodium sulfate. After filtration, the filtrate was evaporated to dryness *in vacuo* to give an almost optically pure material. Recrystallization from an appropriate solvent was performed to afford pure materials (Table 3).

Regeneration of Free Amino Acids Bearing Entirely Optical Activities. *A)* According to the usual way, optically active *N*-benzyloxycarbonylamino acid was dissolved in threefold equi-amounts of 30% hydrogen

15) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952); D. Ben-Ishai, *ibid.*, **19**, 62 (1954).

16) K. Okawa, This Bulletin, **30**, 977 (1957); J. Noguchi, T. Saito, T. Hayakawa and Y. Hayashi, *Nippon Kagaku Zasshi*, **80**, 299 (1959).

17) C. H. Bamford, A. Elliot and W. E. Hanby, "Synthetic Polypeptides," Acad. Press, New York (1956), p. 46.

18) Yu. I. Khurgin and M. G. Dmitrieva, *Tetrahedron*, **21**, 2305 (1965).

bromide in glacial acetic acid. After about one hour, much anhydrous ether was added and deposition was collected by fitration. A solution of the resulting hydrobromide in ethyl alcohol was neutralized with triethylamine to deposit free amino acid. This was recrystallized from water or water–ethyl alcohol to give optically pure amino acids (Table 3).

B) In the case of serine, optically active *N,O*-dibenzyloxycarbonylserine was dissolved in ethyl alcohol and a palladium-black catalyst was added. Hydrogenolysis was carried out under slight pressure for 6 hr at room temperature with the aid of an efficient shaking machine. Then the catalyst, with the substance adhering to it, was filtered and the product was separated from the catalyst by extraction with hot water. To the aqueous solution, dioxane was added to complete the precipitation. Recrystallization from water and acetone afforded optically active serine (Table 3).

The authors wish to express their gratitude to Dr. Eishin Kyuno of this Department and to Fuji Pharmaceutical Industries Co., Ltd., for their kind donation of (+)-ephedrine.