zein or casein. Thus, if gossypol altered the absorption of one or more amino acids from the protein of high quality cottonseed meal it could result in an alteration in overall absorption, a reduced absorption rate for many amino acids, a net decrease in nitrogen absorption, and thus a reduced protein value for the gossypol-containing meal.

Consideration must be given to at least two other factors besides an alteration of amino acid transport by gossypol which could cause the compound to reduce the total nitrogen removal from the intestine. Gossypol may increase the rate of passage of the chyme along the intestinal tract and reduce the time of exposure of the diet to digestion. The other possibility is that gossypol may have an action on the enzymes of protein digestion. Wong et al. (1972), Finlay et al. (1973), Lyman et al. (1959), and Tanksley et al. (1970) have shown that gossypol can react with the ϵ-amino groups of lysine in the pepsinogen, pepsin, and trypsin molecules, thus hindering their action. The above two actions of gossypol, coupled with alterations of amino acid transport, could produce a definitive decrease in amino acid absorption and explain the reduced nutritive value of gossypol-containing meals.

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Preparation of Optically Active 6-Chlorotryptophan and Tryptophan

Shigeki Yamada,* Masao Yamamoto, Chikara Hongo, and Ichiro Chibata

Optical resolution of racemic modifications of 6-chloro-DL-tryptophan and DL-tryptophan was studied in order to develop practical methods for the production of 6-chloro-D-tryptophan, a nonnutritive sweetening agent, and L-tryptophan, an essential amino acid. 6-Chloro-DL-tryptophan methanesulfonate and DL-tryptophan *p*-phenolsulfonate were resolved by preferential crystallization procedures. High yields of optically pure isomers of both amino acids were obtained. Industrial production of the isomers by these methods is considered promising if the appropriate synthetic methods for production of the racemic modifications are developed.

It has been reported that 6-chlorotryptophan (6-Cl-Trp) is useful as a nonnutritive sweetening agent (Kornfeld et al., 1970) and that it possesses interesting biological activities (McGeer et al., 1968; Peters, 1972; Pascalon et al., 1972). In the former report, the D isomer of 6-Cl-Trp was shown to be an exceedingly sweet compound. However, details of the preparation of the optically active isomer were not described and evaluations of the degree of sweetness were carried out with the DL form. The DL form was used in the latter biological studies because the optical resolution of 6-Cl-Trp was considered difficult. Fukuda et al. (1971) reported that 6-chloroindole is converted microbiologically into 6-Cl-L-Trp, but detailed physical data of the isolated 6-Cl-Trp were not included. Thus, neither a practical method for production of optically active 6-Cl-Trp nor

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its properties have been reported previously.

Likewise, L-tryptophan (L-Trp), an essential amino acid, is an important nutritional substance and its market has been expanding rapidly in recent years. L-Trp can be derived from DL-Trp by utilizing various optical resolution methods (Greenstein and Winitz, 1961). These conventional methods are laborious and unsatisfactory for commercial production. Recently, Stewart and Doherty (1973) have reported the complete resolution of DL-Trp by affinity chromatography. However, the chromatographic method is considered to be an expensive approach for practical purposes. It is therefore desirable to establish practical methods for the resolution of 6-Cl-DL-Trp and DL-Trp.

A preferential crystallization procedure is considered to be one of the most practical resolution methods, as the procedure can be easily accomplished by adding seed crystals of the desired isomer to a supersaturated solution of the racemic modification. In order to make this convenient method more generally applicable for optical resolution of racemic amino acids, we have previously proposed the resolution of amino acids as their aromatic sulfonates (Chibata et al., 1968; Yamada et al., 1973a–c) and have noted the many advantages of this simple resolution method. In this study, we prepared a wide variety of aromatic sulfonates of 6-Cl-DL-Trp and of DL-Trp, screened the salts, and selected the ones suitable for this resolution method. 6-Chloro-DL-tryptophan methanesulfonate (6-Cl-DL-Trp-MeS) and DL-tryptophan *p*-phenolsulfonate (DL-Trp-*p*-PhS) were resolved in good yield by a preferential crystallization procedure. In this report, on the basis of the relationships of liquid–solid phase equilibrium in the coexisting system of optically active and racemic forms, the conditions necessary for the reciprocal resolution of racemic modifications were investigated in detail and practical methods for resolution of 6-Cl-DL-Trp and DL-Trp were established. Furthermore, the optical rotation of the pure isomers of 6-Cl-Trp and their properties were determined.

EXPERIMENTAL SECTION

Materials. 6-Cl-DL-Trp was prepared in our laboratory according to the method of Rydon and Tweddle (1955). A very small amount of 6-Cl-L-Trp used for initial seed crystals was prepared by optical resolution of *N*-Ac-6-Cl-DL-Trp by asymmetric hydrolysis with mold aminoacylase (Chibata et al., 1957). The optically active D- and L-6-Cl-Trp used for seed crystals were obtained by the preferential crystallization procedure. DL-, D-, and L-Trp were analytical standard grade amino acids manufactured by our company, Tanabe Seiyaku Co., Ltd. Methanesulfonic acid (MeS) and *p*-phenolsulfonic acid (*p*-PhS) were obtained from Tokyo Kasei Kogyo Co., Ltd., and were used without further purification.

Analyses. All samples were dried overnight in vacuo at 45–50°. Melting points were measured with a Yamato MP-21 melting point apparatus in an unsealed capillary tube and are uncorrected. Infrared spectra of samples were determined in KBr disks using a Shimazu infrared spectrophotometer, Model IR-27G. Optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter. Elemental analyses were performed with a Perkin-Elmer 240 elemental analyzer. Solubility was determined by approaching saturation equilibrium from both undersaturation and supersaturation. Solute concentration was measured with a Karl Zeiss immersion refractometer.

Preparation of 6-Cl-Trp-MeS. Methanesulfonic acid (50.9 g, 0.53 mol) and water (1000 ml) were added to 6-Cl-DL-Trp (119.4 g, 0.5 mol). The mixture was heated and treated with charcoal. The filtrate was concentrated to a volume of about 400 ml and allowed to stand in a refrigerator overnight. The resulting precipitate was collected, washed with cold water, and dried in vacuo. The initial crop of 6-Cl-DL-Trp-MeS (94.9 g) was obtained, mp 234–236° dec, and further crops were obtained by successive concentrations of the combined filtrates. The total yield was 159.0 g (95%). The products were used for resolution without further purification. Slow crystallization from water gave a racemic mixture, mp 237–238° dec; quick crystallization by agitation or scratch gave a racemic compound, mp 236–238° dec. Anal. Calcd for $C_{12}H_{15}O_5N_2ClS$: C, 43.05; H, 4.52; N, 8.37; Cl, 10.59; S, 9.58. Found: C, 43.14; H, 4.55; N, 8.39; Cl, 10.72; S, 9.73.

The solubility of the racemic mixture could not be determined because it changed into a racemic compound with agitation in water. The solubility of the racemic compound in water was 45.9 g/100 ml at 25°.

6-Cl-D- and -L-Trp-MeS were prepared in the same way as 6-Cl-DL-Trp-MeS. The L isomer had: mp 256–257° dec; $[\alpha]^{25}D$ +9.3°; $[\alpha]^{25}_{436}$ +26.2° (*c* 0.5, NHCl). Solubility in water was 30.8 g/100 ml at 25°. Anal. Found: C, 43.22; H, 4.60; N, 8.41; Cl, 10.52; S, 9.53.

The D isomer had: mp 256–257° dec; $[\alpha]^{25}D$ −9.3°; $[\alpha]^{25}_{436}$ −26.2° (*c* 0.5, NHCl).

Preparation of Trp-*p*-PhS. DL-Trp (204.2 g, 1 mol) was dissolved at room temperature under stirring in an aqueous solution (450 ml) containing *p*-PhS (179.4 g, 1.03 mol). The solution was cooled in a refrigerator overnight. The precipitate and further crops obtained by successive concentrations were collected, washed with cold water, and dried in vacuo. The total yield was 361.4 g (95.5%). The products were almost pure and could be used for resolution without further purification. To avoid decomposition of the salts, recrystallization was carried out from 0.25 *M* *p*-PhS aqueous solution. Colorless needles were obtained, mp 188–189° dec. Solubility in 0.25 *M* *p*-PhS (g/100 ml) at 25° was 46.9. Anal. Calcd for $C_{17}H_{18}N_2O_6S$: C, 53.96; H, 4.79; N, 7.40; S, 8.47. Found: C, 53.54; H, 4.92; N, 7.41; S, 8.48.

The L-isomer had: mp 214–215° dec; $[\alpha]^{25}D$ +3.4°; $[\alpha]^{25}_{365}$ 38.0° (*c* 2, NHCl). Solubility in 0.25 *M* *p*-PhS (g/100 ml) at 25° was 15.6. Anal. Found: C, 53.52; H, 5.00; N, 7.40; S, 8.28.

The D isomer had: mp 214–215° dec; $[\alpha]^{25}_{365}$ −38.0° (*c* 2, NHCl). Anal. Found: C, 53.72; H, 4.92; N, 7.44; S, 8.49.

Recrystallization of the optically active Trp-*p*-PhS from water gave the hemisulfonate as needles, mp 225–226° dec. Anal. Calcd for $C_{11}H_{12}N_2O_2 \cdot 0.5(C_6H_6O_4S) \cdot H_2O$: C, 54.36; H, 5.54; N, 9.06; S, 5.18. Found: C, 54.48; H, 5.61; N, 9.05; S, 5.16.

Infrared Spectra of 6-Cl-Trp-MeS and Trp-*p*-PhS. The infrared spectra of D-, L-, and DL-Trp-*p*-PhS in KBr were identical. The spectrum of the racemic mixture of 6-Cl-Trp-MeS was identical with those of the D and L isomers but the spectra of the racemic compound of 6-Cl-Trp-MeS were different; see Figure 1.

Optical Resolution of 6-Cl-DL-Trp-MeS. A typical experiment for the resolution was carried out as follows. 6-Cl-DL-Trp-MeS (47.50 g) and 6-Cl-L-Trp-MeS (3.00 g) were dissolved in water (50 ml) at elevated temperature. The mixture was cooled to 25°, seeded with 6-Cl-L-Trp-MeS (0.05 g), and stirred at the same temperature. By refractometric and polarimetric measurements of liquid phase it was observed that crystallization of the L isomer occurred, while the D isomer remained in the solution. After 110 min, the precipitated crystals were collected by filtration and washed with a small amount of cold water. Drying the precipitated crystals yielded 6-Cl-L-Trp-MeS (5.88 g): $[\alpha]^{25}_{436}$ +24.1° (*c* 0.5, NHCl); optical purity, 92.0%.

After the separation of the L isomer in the above experiment, 6-Cl-DL-Trp-MeS (6.25 g) and a small amount of water were added to the mother liquor so that the gross concentration might be the same as that of the previous resolution. This supersaturated solution was seeded with 6-Cl-D-Trp-MeS (0.05 g) at 25° and stirred for 80 min. The precipitated crystals were filtered to give 6-Cl-D-Trp-MeS

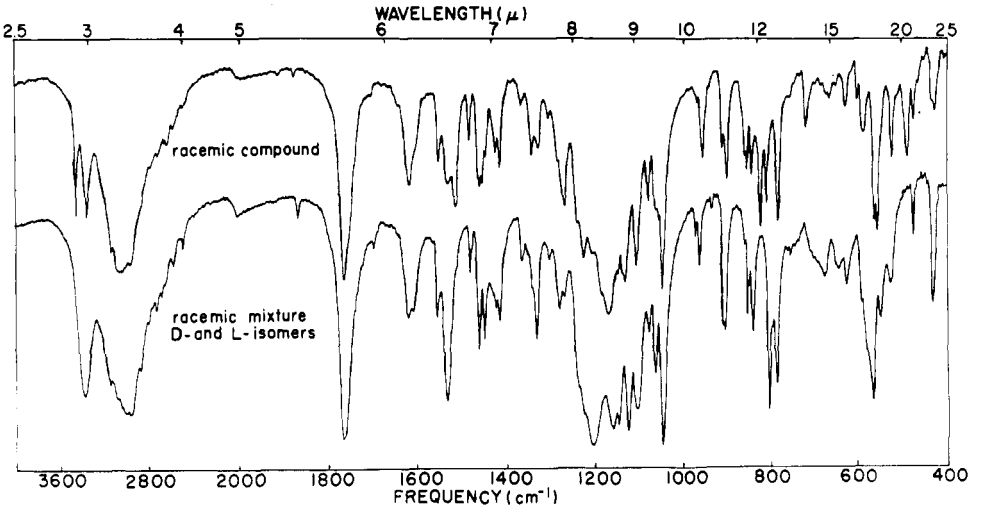


Figure 1. Infrared spectra of optically active and racemic 6-Cl-Trp-MeS.

(5.83 g), which had 93.9% of optical purity; $[\alpha]^{25}_{436}$ −24.6° (*c* 0.5, NHCl). By repeating these procedures, L and D isomers were successively obtained.

Purification of Optically Impure 6-Cl-Trp-MeS. The impure 6-Cl-L-Trp-MeS (5.50 g, optical purity 92.0%) obtained by the above procedure was dissolved in 0.2 *M* MeS (8 ml) at elevated temperature and the solution was allowed to stand in a refrigerator overnight. The resulting crystals were collected, washed with cold water, and dried, giving colorless needles: mp 256–257° dec; $[\alpha]^{25}_{436}$ +26.2° (*c* 0.5, NHCl); yield, 76% based on L isomer in the original optically impure 6-Cl-Trp-MeS. No change in melting point and specific rotation was observed by further recrystallization. In the same manner, the optically impure 6-Cl-D-Trp-MeS (optical purity, 93.9%) was recrystallized two times from 0.2 *M* MeS, giving colorless needles: mp 256–257° dec; $[\alpha]^{25}_{436}$ −26.2° (*c* 0.5, NHCl). Anal. Calcd for $C_{12}H_{15}O_5N_2ClS$: C, 43.05; H, 4.52; N, 8.37. Found: C, 43.24; H, 4.60; N, 8.43.

Preparation of Optically Active 6-Cl-Trp. The 6-Cl-D-Trp-MeS (3.00 g) obtained above was dissolved in hot water (15 ml), adjusted to pH 6 with 5 *N* ammonium hydroxide, and placed in a refrigerator overnight. The precipitate was collected, and a second crop was obtained by concentration of the combined filtrates. The total yield of 6-Cl-D-Trp was 2.03 g (95%). Recrystallization from 50% MeOH (v/v) afforded colorless needles, mp 254–255° dec. Anal. Calcd for $C_{11}H_{11}O_2N_2Cl$: C, 55.35; H, 4.65; N, 11.74; Cl, 14.86. Found: C, 55.17; H, 4.86; N, 11.77; Cl, 15.12.

In the same way, 6-Cl-L-Trp was obtained: mp 254–255° dec. Anal. Found: C, 55.15; H, 4.74; N, 11.76; Cl, 14.58.

The product was shown to contain less than 0.1% of the D isomer when assayed with D-amino acid oxidase (Meister et al., 1951). The optical rotation of the pure 6-Cl-Trp thus obtained is shown in Table I.

Successive Resolution of DL-Trp-*p*-PhS. The resolution of DL-Trp-*p*-PhS was carried out in the same manner as described for 6-Cl-Trp-MeS. A supersaturated solution containing DL-Trp-*p*-PhS (75.00 g), L-Trp-*p*-PhS (6.50 g), and 0.25 *M* *p*-PhS (100 ml) was seeded with finely pulverized L-Trp-*p*-PhS crystals (0.10 g), and crystallization of the L isomer occurred. After 80 min the crystals were collected by filtration, washed with water (3 ml), and dried. The yield of separated L-Trp-*p*-PhS was 13.42 g, optical purity 96.3%.

After the separation of the L isomer in the above experiment, DL-Trp-*p*-PhS (13.98 g; the sum of separated L isomer, 13.42 g, and operation loss, 0.56 g) and a small amount of water were added to the filtrate so that the gross concentration was the same as that of the previous resolution.

Table I. Specific Rotations of Optically Active 6-Cl-Trp

	Sp. rot. (deg) for 6-Cl-D-Trp at mp 254–255° dec, $[\alpha]^{25}\lambda$		Sp. rot. (deg) for 6-Cl-L-Trp at mp 254–255° dec, $[\alpha]^{25}\lambda$	
λ, nm	*c* 0.5, H_2O	*c* 1, NHCl	*c* 0.5, H_2O	*c* 1, NHCl
589	+20.7	−11.0	−20.8	+11.0
578	+21.5	−11.6	−21.4	+11.8
546	+23.7	−14.4	−23.8	+14.4
436	+33.6	−33.5	−33.5	+33.3
365	+31.8	−79.8	−31.9	+79.5

This adjustment was carried out simply by adding the appropriate amounts of the racemic modification and water determined by refractometric measurement. The concentration of optically active isomer was not corrected. Thus, almost the same composition as in the previous operation was obtained, except that the predominant isomer was the opposite antipode of the previous mixture. Calculated compositions of the solution are shown in parentheses in Table II. By seeding this supersaturated solution with 0.10 g of D isomer, the preferential crystallization of D isomer was carried out as described previously. The process was controlled by a crystallization time of 80 min. Table II shows the results obtained by the four reciprocal resolutions. The isomers obtained by this procedure had an average optical purity of about 95%. When the optical purity is unsatisfactory further purification can be accomplished as described in the following example.

Optical Purification of Optically Impure Trp-*p*-PhS. Crude L-Trp-*p*-PhS (12.00 g, optical purity 80.5%) was mixed with 0.25 *M* *p*-PhS (5 ml) and 0.25 *M* *p*-PhS saturated with DL-Trp-*p*-PhS at 25° (50 ml) and dissolved at elevated temperature. The mixture was then stirred for 5 hr at 25°; the resulting crystals were filtered off, washed with a small amount of cold water, and dried. By this operation, L-Trp-*p*-PhS (9.28 g, optical purity 99.5%) was obtained; $[\alpha]^{25}_{365}$ +37.8° (*c* 2, NHCl).

Preparation of Optically Active L-Trp. The optically active L-Trp-*p*-PhS (8.00 g) obtained above was dissolved in hot water (40 ml). The solution was adjusted to pH 6 with 5 *N* ammonium hydroxide and allowed to stand in a refrigerator overnight. The resulting precipitate was collected and washed with water, and a second crop was obtained by concentration of the combined filtrate. The total

Table II. Successive Resolutions of DL-Trp-*p*-PhS

Expt. no.	Amount of addition		Composition of solution		Separated crystals	
	DL form, g	Active form, g	DL form, g	Active form, g	Yield, g	Optical purity, %
1	75.0	6.50	75.0	6.50	13.42	96.3
2	13.98		(75.18)	(6.32)	13.65	94.7
3	14.37		(74.99)	(6.51)	13.48	95.0
4	14.04		(75.30)	(6.20)	13.31	93.8
Mean	14.13		(75.12)	(6.38)	13.47	95.0

yield of L-Trp was 4.09 g (95%); $[\alpha]^{25}D$ −32.5° (*c* 1, H_2O).

Racemization of D-Trp. D-Trp (40.00 g) was suspended in water (80 ml) and the mixture was heated in an autoclave for 15 hr at 180°. One hundred and twenty milliliters of 1.6 *N* HCl was added to the reaction mixture, and the mixture was stirred for 20 min at 80° and stored in the refrigerator overnight. The insoluble product (1.20 g) was removed by filtration and discarded. The filtrate was treated with charcoal and adjusted to pH 6 with 5 *N* ammonium hydroxide, and the precipitate was collected. This DL-Trp was identical with authentic DL-Trp except that it had a minute optical rotation: 38.20 g; 95.5%; $[\alpha]^{25}_{436}$ −0.4° (*c* 1, 4 *N* HCl).

RESULTS AND DISCUSSION

It is well known that resolution by preferential crystallization is possible when the solubility of each of the pure optical isomers is less than that of the racemic modification and that resolution is more easily performed when the racemic modification forms a racemic mixture. Synthetic 6-Cl-DL-Trp and its ordinary salts such as the hydrochloride and sulfate are not suitable for this resolution method. Therefore, we prepared a wide variety of aromatic sulfonates of 6-Cl-DL-Trp and screened the salts forming racemic mixtures by comparing the infrared spectrum, melting points, and solubility relationships of the racemic modifications and the optically active isomers.

It was found that benzenesulfonate of 6-Cl-DL-Trp (6-Cl-DL-Trp-BS), crystallized from water, always forms a racemic mixture and that 6-Cl-DL-Trp-MeS forms a racemic mixture only when crystallization is carried out slowly by seeding with the racemic mixture. Both salts were resolvable using the method described in the previous report on lysine *p*-aminobenzenesulfonate (Lys-*p*-ABS) (Yamada et al., 1973b). With this method, seeding a supersaturated solution of racemic modification with the crystals of the desired isomer brought about preferential crystallization of that isomer, while the nonseeded isomer remained in the mother liquor in a supersaturated state. 6-Cl-DL-Trp-MeS described in the Experimental Section was more suitable for practical resolution than 6-Cl-DL-Trp-BS because the latter is much less soluble and the degree of resolution per unit volume was very low.

6-Cl-Trp-MeS has two crystal forms, a racemic mixture and a racemic compound, and the former changes into the latter with agitation in water. Therefore, it was difficult to observe a typical characteristic of a racemic mixture in a liquid–solid phase equilibrium in the mixture of the optically active isomer and the racemic modification. This differs from the case of Lys-*p*-ABS reported previously. Thus, optical purification by the method which had been applied to Lys-*p*-ABS was unsuccessful in the case of 6-Cl-Trp-MeS. However, optically impure 6-Cl-Trp-MeS could be easily purified by ordinary recrystallization. In our evaluation of the relative sweetness, 6-Cl-D-Trp was approximately one thousand times sweeter than sucrose, while 6-Cl-L-Trp was slightly bitter.

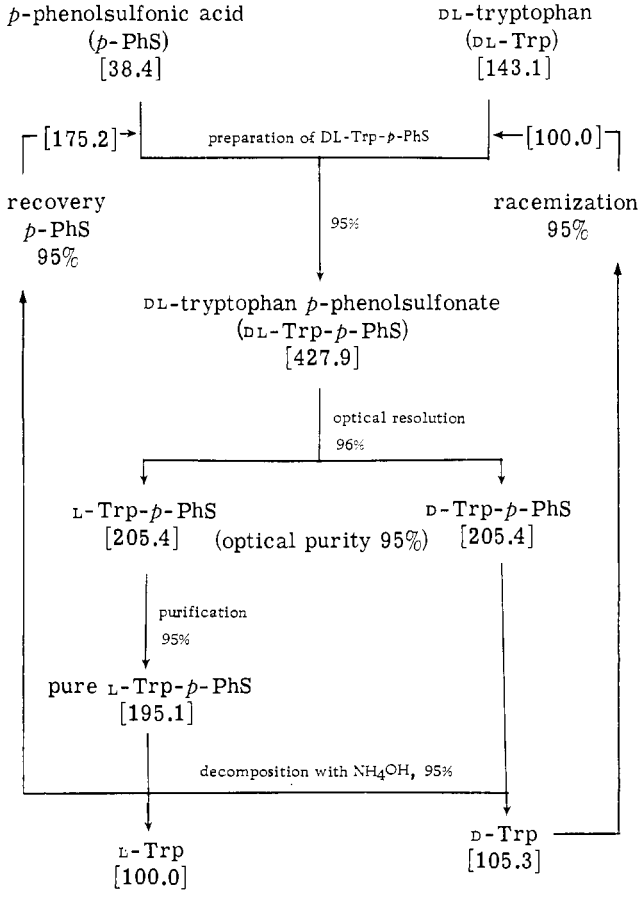
With respect to optical resolution of DL-Trp, we have already reported that DL-Trp can be resolved as a salt with benzenesulfonic acid (Trp-BS) (Yamada et al., 1973c). However, the industrial production of L-Trp by this method seemed to be impractical because DL-Trp-BS was not very soluble and the degree of resolution per unit volume

Table III. Comparison of Resolution Results for DL-Trp-BS and DL-Trp-*p*-PhS[a]

	Trp-BS	Trp-*p*-PhS
Crystallization time, min	50	80
Yield, *W*, g/100 ml	2.59	13.47
Optical purity, *P*, %	92	95
Yield of resolved isomer, *Y*, g/100 ml	1.23	6.32
Resolution rate, *R*, %	15.4	16.8

[a] *W*, the total weight of the separated crystals. *P*, the optical purity of the separated crystals. *Y*, $WP - A - S$. *A*, the weight of the excess isomer existing initially in the original solution. *S*, the weight of seed crystals added to the solution. *R*, *Y*/*B*. *B*, the weight of the seeded isomer in the original racemic modification.

Scheme I[a]



[a] Numbers in brackets are weights.

and the optical purity of the product were unsatisfactory. In the present method these disadvantages could be largely avoided. Comparisons of resolution results for DL-Trp-BS and DL-Trp-*p*-PhS are summarized in Table III. The undesired isomer of Trp could be racemized easily by dissolving it in water and heating at 180°. Scheme I shows the flow sheet of L-Trp production.

The optical resolution methods now presented are very advantageous because they require neither an optically active resolving agent nor conversion of amino acids into complicated derivatives, the yield per unit volume is very high, and the operation is very simple. Industrial productions of optically active 6-Cl-Trp and Trp by this method are considered to be very promising if combined with a proper synthetic method for 6-Cl-DL-Trp and DL-Trp.

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Binding of Mg(II) by the 11S Fraction of Soybean Proteins

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Binding of Mg(II) by the 11S fraction of soybean proteins has been measured in 0.1 *M* borate buffer of pH 7.8 by equilibrium dialysis. The binding sites are possibly the imidazole groups of the histidine residues of the protein molecule. NaCl at 0.5 *M* concentration suppresses binding. Addition of urea or prior treatment of the protein with EDTA reduces binding. EDTA treatment causes dissociation of the protein which is partly reversed by the addition of Mg(II). No conformational change occurs in the protein due to the binding of Mg(II). The protein, both before and after EDTA treatment, is quantitatively precipitated by 1×10^{-2} *M* Mg(II). NaCl at 0.5 *M* concentration suppresses precipitation. Addition of Mg(II) to the water extract of defatted soybean yields a precipitate which consists almost entirely of the 11S fraction.

Calcium salts have been used to precipitate the 11S fraction of soybean proteins (Koshiyama, 1965). This protein binds Ca(II) ions (Appu Rao and Narasinga Rao, 1975). Mg(II) does not appear to have been used for such precipitation. In this investigation the binding of Mg(II) by the 11S fraction has been measured and compared with Ca(II) binding. The effect of Mg(II) binding on the physicochemical properties of the protein has also been determined. Addition of Mg(II) to unfractionated soybean proteins yields a precipitate which consists almost entirely of the 11S fraction.

EXPERIMENTAL SECTION

Preparation of 11S Fraction. Improved Pelican variety soybeans were used. The 11S fraction was obtained by the method of Wolf et al. (1962). Homogeneity of the preparation was determined by analytical ultracentrifugation. The sedimentation velocity pattern in 0.05 *M* phosphate buffer (pH 7.9) containing 0.5 *M* NaCl indicated the presence of a small amount of 7S fraction which did not amount to more than 2–3% of the total (Figure 1). The protein was free from phosphorus and carbohydrate impurities.

Protein Concentration. This was determined by measuring the absorbance at 280 nm and using a value of 9.2 for $E_{1cm}^{1\%}$ (Wolf and Briggs, 1959).

Equilibrium Dialysis. Aliquots (2.5 ml) of 1% protein solution in 0.1 *M* borate buffer (pH 7.8) were dialyzed against 5 ml of buffer solution containing varying amounts of $MgCl_2$, for 48 hr at 30°. Corresponding "blanks" containing only the buffer solution were also run. An interval of 48 hr was found sufficient for equilibrium to be attained. At the end of the period, the concentration of Mg(II) of the outside solutions was estimated. From the difference in Mg(II) concentration, the number of Mg(II) ions bound by 100,000 g of protein was calculated. When the experiments were done in the presence of urea, all the solutions were prepared in 0.1 *M* borate buffer containing 8 *M* urea.

Estimation of Mg(II). The colorimetric method of Smith (1955) was used. The color was developed with 0.1% Eriochrome Black T. The dye solution was prepared freshly for each set of measurements. A calibration curve of absorbance at 520 nm vs. Mg(II) concentration was constructed. Beer's law was obeyed over a concentration range of 0 to 5×10^{-4} *M* Mg(II). A separate calibration

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