(15) (a) G. Pattenden and B. C. L. Weedon, *J. Chem. Soc. C*, 1984 (1968); (b) L. Crombie, P. Hemesley, and G. Pattenden, *Ibid.*, 1016 (1969); L. Crombie, P. Hemesley, and G. Pattenden, *ibid.*, 1024 (1969); (c) H. J. Bestmann, P. Range, and R. Kunstmann, *Chem. Ber.*, **104**, 65 (1971).
(16) (a) E. J. Corey and G. T. Kwiatkowski, *J. Am. Chem. Soc.*, **88**, 5653 (1966); (b) A. S. Kovaleva, V. M. Bulina, L. L. Ivanov, Yu. B. Pyatnova, and R. P. Evstigneeva, *Zh. Org. Khim.*, **10**, 696 (1974); (c) H. J. Bestmann, O. Vostrowsky, and H. Platz, *Chem.-Ztg.*, **98**, 161 (1974); (d) H. J. Bestmann, O. Vostrowsky, and A. Plenchette, *Tetrahedron Lett.*, 779 (1974); H. J. Bestmann and W. Stransky, *Synthesis*, 798 (1974); (e) M. Fetizon, unpublished method quoted in ref 12a; p 16.
(17) Although the Wittig olefin synthesis is usually described as proceeding via initial betaine formation, this may not be the situation in most cases in nonpolar solvents. It has been shown that, in tetrahydrofuran, an aldehyde and a nonstabilized alkylide react to give directly an oxaphosphetane intermediate (e.g., 11): E. Vedejs and K. A. J. Snoble, *J. Am. Chem. Soc.*, **95**, 5778 (1973); see also W. P. Schneider, *Chem. Commun.*, 785 (1969); in some cases in the presence of lithium salts or of polar solvents, the betaines (e.g., **10**) are presumably involved to some extent as intermediates in the Wittig reaction. The direct formation of the *erythro*-oxaphosphetane via a cycloaddition process satisfactorily explains the predominant formation of cis-alkene product.
(18) M. Schlosser and K. F. Christmann, *Angew. Chem., Int. Ed. Engl.*, **5**, 126 (1966); E. J. Corey and H. Yamamoto, *J. Am. Chem. Soc.*, **92**, 226 (1970); M. Schlosser and K. F. Christmann, *Synthesis*, 38 (1969).
(19) The formation of analogous hydroxyphosphonium ylides as intermediates has been postulated to explain the partial loss of stereochemistry, which occurs under protic conditions, when epoxides are deoxygenated with lithium diphenyl phosphide (followed by methyl iodide): E. Vedejs, K. A. J. Snoble, and P. L. Fuchs, *J. Org. Chem.*, **38**, 1178 (1973); see also D. E. Bissing and A. J. Speziale, *J. Am. Chem. Soc.*, **87**, 2683 (1965); S. Trippett, *Q. Rev., Chem. Soc.*, **17**, 406 (1963).
(20) If the lithium diisopropylamide (1.1 equiv) in this experiment was consumed to form predominantly ester enolate, then it is possible that, if ≥ 2 equiv of $LiN(CHMe_2)_2$ were used, some equilibration might occur.
(21) Recently the reaction of an aldehyde with an α-silylalkyllithium (metalated silane) reagent has been reported to give a ca. 1:1 mixture of cis and trans olefins: T. H. Chan and E. Chang, *J. Org. Chem.*, **39**, 3264 (1974).
(22) C. R. Enzell and B. R. Thomas, *Acta Chem. Scand.*, **19**, 1875 (1965).
(23) P. E. Eaton, G. F. Cooper, R. C. Johnson, and R. H. Mueller, *J. Org. Chem.*, **37**, 1947 (1972).
(24) R. J. Anderson, V. L. Corbin, G. Cotterrell, G. R. Cox, C. A. Henrick, F. Schaub, and J. B. Siddall, *J. Am. Chem. Soc.*, **97**, 1197 (1975).
(25) The acetates **1a** and **2a** were only partially resolved by GLC (4-m, 3% PDEAS, 160°) with retention times of 30.0 and 28.8 min, respectively. Our synthetic products were identical with samples obtained from Farchan Division, Story Chemical Corporation. The corresponding benzoates of **1c** and **2c** are more readily resolved by GLC analysis than the acetates, with retention times of 36.8 and 35.2 min respectively (4-m, 3% PDEAS, 210°) (cf. ref 6c).
(26) M. Tamura and J. Kochi, *Synthesis*, 303 (1971); M. Tamura and T. K. Kochi, *J. Organomet. Chem.*, **42**, 205 (1972).
(27) The earlier workers (ref 23) reported that this reagent (**14b**) "was less useful" than the corresponding organolithium **14a**.
(28) E. J. Corey and G. H. Posner, *J. Am. Chem. Soc.*, **90**, 5615 (1968); C. R. Johnson and G. A. Dutra, *Ibid.*, **95**, 7777 and 7783 (1973).
(29) The crude diene ester products from these Wittig reactions were very clean by TLC and GLC analysis. An excess of ylide (1.17 to 1.5 equiv) was generally used, but the isolated yield of diene esters, based on **6**, was never >70%. It is possible that, in the presence of alcohols, some side reactions are occurring giving undetected polar phosphorus-containing products; see, for example, E. E. Schweizer, D. M. Crouse, T. Minami, and A. T. Wehman, *Chem. Commun.*, 1000 (1971); J. W. Rakshys, Jun. and S. V. McKinley, *Ibid.*, 1336 (1971); D. J. H. Smith and S. Trippett, *J. Chem. Soc., Chem. Commun.*, 191 (1972); S. Trippett and B. J. Walker *J. Chem. Soc. C*, 887 (1966); E. E. Schweizer, T. Minami, and D. M. Crouse, *J. Org. Chem.*, **36**, 4028 (1971); however, the yields were no higher when the Wittig reaction was carried out in nonpolar solvents (Table I).
(30) The isomer ratio was determined by GLC analysis (2-m, 3% PDEAS, 180°) of the diepoxide derivatives of **8a** and **9a** (retention times of 21.6 and 18.8 min, respectively). The ratio was confirmed in several cases by GLC analysis of the derived acetates **1a** and **2a** (and the corresponding benzoates)[25] and also by GLC and HPLC analysis of the benzoates of **8b** and **9b** (prepared with benzoyl chloride in ether–pyridine at room temperature). The diene benzoates were resolved by GLC on 4-m, 3% PDEAS, at 200° with retention times of 29.8 and 28.8 min, respectively. HPLC analysis of the benzoates was carried out in hexane–heptane (1:1) saturated with ethylene glycol on a 6-ft Corasil II–0.8% $AgNO_3$–1.7% ethylene glycol column. The *Z,Z* and *Z,E* isomers were completely resolved with retention times of 38.5 and 24.8 min, respectively. In no case were the diastereomers of the diepoxides of **8a** and **9a** resolved by GLC in contrast to the reported ready GLC separation of the diastereomeric diepoxides of the conjugated diene, 2,4-hexadiene: G. E. Heasley, R. V. Hodges, and V. L. Heasley, *J. Org. Chem.*, **39**, 1769 (1974).
(31) The cuprate complex was formed at −20°. At the temperature previously reported (−60 to −70°),[23] the formation of the ate complex was generally incomplete after 2 hr.
(32) H. Gilman and F. Schulze, *J. Am. Chem. Soc.*, **47**, 2002 (1925).

Stereochemistry of β-Replacement Reactions Catalyzed by Tyrosine Phenol-Lyase

Seiji Sawada,[1] Hidehiko Kumagai,[2] Hideaki Yamada,[2] and Richard K. Hill*[3]

Contribution from the Department of Chemistry, Kyoto University of Education, and The Research Institute for Food Science, Kyoto University, Kyoto, Japan, and the Department of Chemistry, University of Georgia, Athens, Georgia 30602. *Received August 5, 1974*

Abstract: The stereochemistry of one of the β-replacement reactions catalyzed by tyrosine phenol-lyase has been elucidated by studying the reaction of tyrosine (stereospecifically deuterated at C-3) with resorcinol, catalyzed by the purified enzyme from *Escherichia intermedia*. The configuration of deuterium in the product, 2,4-dihydroxy-L-phenylalanine, was elucidated by nmr comparison with a stereospecifically synthesized sample. The results show that the exchange occurs with retention of configuration at C-3. A mechanism involving an enzyme-bound aminoacrylate moiety is discussed.

Tyrosine phenol-lyase (L-tyrosine phenol-lyase (deaminating) EC 4.1.99.2, formerly known as β-tyrosinase) is a pyridoxal phosphate-dependent multifunctional enzyme which catalyzes α,β elimination (eq 1–4),[4,5] β replacement (eq 5 and 6),[6,7] racemization (eq 7),[8] and the reverse of α,β eliminations (eq 8 and 9)[9,10] to form L-tyrosine or its derivatives from pyruvic acid, ammonia, and phenols.

L-tyrosine + H_2O ⟶ phenol + pyruvic acid + NH_3 (1)

S-methyl-L-cysteine + H_2O ⟶ methylmercaptan + pyruvic acid + NH_3 (2)

L-cysteine + H_2O ⟶ H_2S + pyruvic acid + NH_3 (3)

L-serine ⟶ pyruvic acid + NH_3 (4)

L-tyrosine + pyrocatechol ⟶ 3,4-dihydroxyphenyl-L-alanine + phenol (5)

S-methyl-L-cysteine + resorcinol ⟶ 2,4-dihydroxyphenyl-L-alanine + phenol (6)

L(D)-alanine ⟶ DL-alanine (7)

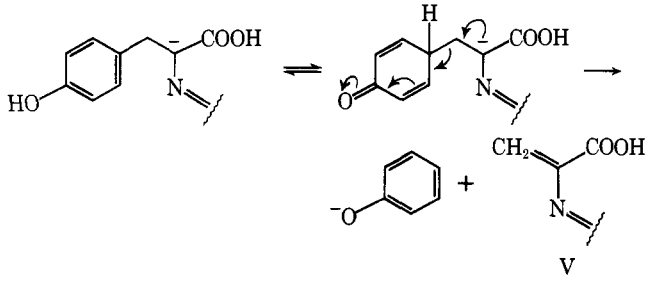
phenol + pyruvic acid + NH_3 ⟶ L-tyrosine + H_2O (8)

pyrocatechol + pyruvic acid + NH_3 ⟶ 3,4-dihydroxyphenyl-L-alanine (9)

The function of tyrosine phenol-lyase including the synthesis of 2,4-dihydroxyphenyl-L-alanine from tyrosine and

resorcinol can be accounted for[5,11] (Figure 1) by adopting the general mechanism for pyridoxal phosphate-dependent reactions proposed by Braunshtein and Shemyakin[12] and by Metzler, *et al.*[13] Pyridoxal phosphate binds through an azomethine linkage between the formyl group of the coenzyme and the ϵ-amino group of lysine residues in the holoenzyme[5] (I, Figure 1) to give the activated enzyme. The first step for the catalysis by tyrosine phenol-lyase is the interaction of the holoenzyme with substrate II by a transaldimination reaction[14] which replaces the azomethine linkage between coenzyme and apoenzyme with a similar bond between coenzyme and substrate (III, Figure 1). The second step for the catalysis by the enzyme is a labilization of the hydrogen atom at C-2 of the substrate.[11]

The resulting carbanionic intermediate IV, a resonance hybrid of such structures as IVa and IVb, is now capable of eliminating the group R provided it can be released as an anion. In the cases of serine, cysteine, and *S*-methylcysteine, where R = OH, SH, and SCH_3, this is obviously feasible; for tyrosine, release of a *p*-hydroxyphenyl carbanion is unlikely, and it seems more reasonable to postulate an initial tautomerism which will allow elimination of the phenolate anion:



V

The resulting intermediate V, an enzyme-bound α-aminoacrylate–pyridoxal phosphate complex, may again be considered a resonance hybrid of such structures as Va and Vb. The final step in this sequence varies depending on the enzymatic reaction conditions. In the case of the α,β-elimination reactions (eq 1-4), the α-aminoacrylate moiety of V is simply hydrolyzed to yield pyruvate and ammonia, regenerating tyrosine phenol-lyase. Alternatively, in the presence of phenol homologs such as resorcinol, a Michael-type addition to V can occur, giving rise to the β-replacement product, 2,4-dihydroxyphenyl-L-alanine (VI).

While the elimination step in this sequence is a well-recognized reaction of the pyridoxal phosphate family, *e.g.*, in the conversion of homoserine to threonine, the striking ability of tyrosine phenol-lyase to catalyze Michael additions to the aminoacrylate intermediate makes it of special theoretical and practical significance. It seemed of particular interest to elucidate the stereochemistry at the β carbon of the amino acid as a clue to the mechanism of this enzymatic process. If the elimination of phenolate ion from tyrosine were stereospecific, the prochirality at C-3 of the original substrate would be conserved in the intermediate V, which has a σ plane through the C-3 atom. Then if the Michael step is also stereospecific, the prochirality at C-3 would persist to the β-replacement product VI. Since it is reasonable to expect that both the nucleophile eliminated to form V and that added to V should be oriented perpendicular to the plane of the extended π system of Va, it seemed likely that the β replacement should be stereospecific. The outgoing and incoming nucleophiles might be oriented on the same side of the plane of Va, leading to overall retention at C-3, or on opposite sides of the plane, leading to inversion at C-3. It is recognized that there are many opportunities for loss of configurational specificity: nonstereospecific elimination of phenolate ion from IV, random addition of the nucleophile

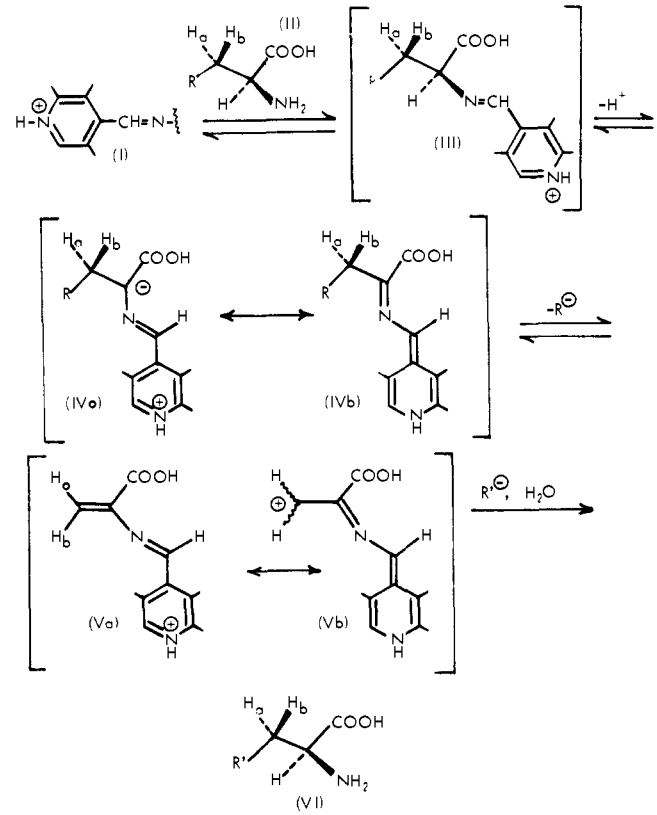


Figure 1.

from either face of Va, or sufficient contribution of the resonance contributor Vb to allow free rotation about the bond between C-2 and C-3; any of these would destroy the nonequivalence of the diastereotopic hydrogens at C-3 and lead to overall racemization at that site. This paper deals with a study of the stereochemical change at C-3 during the tyrosine phenol-lyase catalyzed reaction of tyrosine with resorcinol to yield 2,4-dihydroxyphenyl-L-alanine.

Experimental Section

Stereospecifically Deuterated Amino Acids. Two diastereomers of tyrosine chirally labeled with deuterium at C-3 were prepared[15] *via* the azlactones by the method of Kirby and Michael[16] (see Table I): (−)-(2*S*,3*R*)-[3-^{2}H]tyrosine (VIIb), which was resolved with chymotrypsin, and (±)-(2*S*,3*S*;2*R*,3*R*)-[2,3-$^2H_2$]-tyrosine (VIIc), used as the racemate. Their nmr data are listed in Table I.

An authentic sample of (±) 2,4-dihydroxyphenylalanine (IXa) was synthesized by the method of Lambooy.[17] Deuterium was stereospecifically introduced at C-3 by repeating the synthesis with 2,4-$(CH_3O)_2C_6H_3CDO$, using again the method of Kirby and Michael,[16] to give (±)-(2*S*,3*R*;2*R*,3*S*)-[3-^{2}H]-2,4-dihydroxyphenylalanine (IXb). Their nmr data are listed in Table I.

Incubation Experiments. The enzyme was purified from *Escherichia intermedia* grown on a tyrosine-containing medium according to the method described by Kumagai, *et al.*[4] Instead of employing pyrocatechol for the replacing substrate (eq 5), the incubation was carried out in the presence of resorcinol (*cf.* eq 6) since 2,4-dihydroxyphenyl-L-alanine (VIII) was more easily accessible and purified in the free state than the 3,4-isomer. Both substrates VIIb and VIIc were treated with tyrosine phenol-lyase, and the products were purified according to the method previously reported[7] with the following modification. Incubation was carried out at 30° for 24 hr in a reaction mixture containing deuterated tyrosine (3.0 mmol), resorcinol (3.7 mmol), pyridoxal phosphate (0.02 mmol), sodium sulfite (1.5 mmol), EDTA (0.5 mmol), potassium phosphate buffer (10 mmol, pH 7.8), and 10 mg of the crystallized enzyme in a total volume of 200 ml. The reaction was terminated by heating at 80° for 20 min, and denatured protein was removed by filtration. The filtrate was applied to an Amberlite

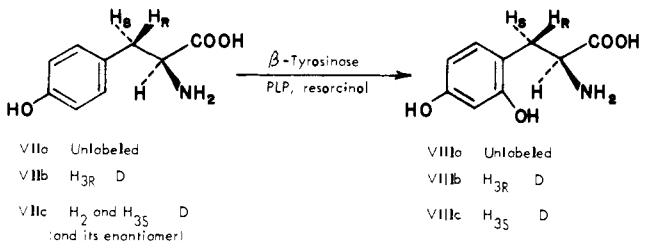


Figure 2.

CG-120 column (40 × 2.5 cm, H^+ form), and the column was eluted with a gradient of hydrochloric acid in a concentration from 0.6 to 2.0 *N*. The fraction containing the product was further purified on aluminum oxide and recrystallized from aqueous ethanol. The optical rotations and nmr data of both products, VIIIb and VIIIc, are listed in Table I, together with those of the authentic nondeuterated specimen VIIIa. All the enzymatic products were levorotatory in conformity with the L-configurational series of naturally occurring amino acids in general. As indicated in Table I, the nmr spectrum of the biosynthetic amino acid VIIIa was identical with that of the synthetic sample IXa, while the nmr spectrum of the monodeuterated biosynthetic sample VIIIb was identical with that of the monodeuterated synthetic amino acid IXb.

Mass Spectra. The synthetic product IXb and both enzymatic products VIIIb and VIIIc showed the largest fragment peak at *m/e* 180. On the other hand, the unlabeled authentic specimens VIIIa and IXA, whether obtained enzymatically or by synthesis, gave spectra with similar fragmentation patterns and a maximum fragment at *m/e* 179. The loss of water from the parent amino acid to form the major fragments at 179 or 180 suggests cyclodehydration to the dihydrocoumarin upon electron impact.[18] The mass spectrum also shows that one atom of deuterium is lost during conversion of VIIc to VIIIc, and the nmr spectrum makes it clear that the deuterium at C-2 is lost.

Discussion

An exchange reaction (Figure 2) between L-tyrosine and resorcinol, catalyzed by tyrosine phenol-lyase, takes place readily to form 2,4-dihydroxy-L-phenylalanine (VIIIa), in which the phenol ring has been replaced by the resorcinol ring. The product is identical with that formed by the same enzyme-catalyzed exchange between *S*-methyl-L-cysteine and resorcinol,[7] and the structure is verified not only by analysis of its spectroscopic properties but also by direct comparison with an authentic synthetic sample.[17] The nmr spectrum, in which the couplings between the three side-chain protons are easily discernible, makes possible a study of the stereochemical result at C-3 during the exchange process.

Use of (−)-(2*S*,3*R*)-[3-^{2}H]tyrosine (VIIb)[15,16] as substrate led to a product (VIIIb) containing one atom of deuterium, as shown by the mass spectrum. The product was evidently a single diastereomer since the nmr spectrum showed the proton at C-2 and a single proton at C-3, each appearing as a doublet, with a coupling constant of 4.5 Hz. The absence of the H_{3R} signal, as well as the 2*S*,3*R* and 3*R*,3*S* couplings, taken with the coincidence of the $J_{2,3}$ coupling constant of 4.5 Hz with that of $J_{2S,3S}$ = 5.0 Hz in the substrate VIIb of proved configuration, establishes unambiguously that the deuterium in VIIIb has the 3*R* configuration, and that the exchange reaction has taken place with complete retention of configuration at C-3. As final proof, a stereospecifically labeled sample IXb of racemic (2*S*,3*R*; 2*R*,3*S*)-[3-^{2}H]-2,4-dihydroxyphenylalanine was synthesized by the method of Kirby and Michael,[16] and it proved to have ir, nmr, and mass spectra identical with those of VIIIb.

It was then possible to confirm this result with a substrate of opposite chirality at C-3, racemic (2*S*,3*S*;

Table I. Optical Activity and Nmr (Aliphatic Protons,[a] 60 MHz) Data

Tyrosine (VII)	Nmr in D₂O–OD⁻ Chemical shift, ppm	Nmr in D₂O–OD⁻ Coupling const, Hz	$[\alpha]^{26}D$ in 5 *N* HCl	2,4-Dihydroxyphenylalanine (VIII)	Nmr in D₂O Chemical shift, ppm	Nmr in D₂O Coupling const, Hz	$[\alpha]^{25}D$ in H_2O
(VIIa); (−)-(2*S*)-	H_2 = 3.37 q H_{3R} = 2.64 q H_{3S} = 2.82 q	$J_{2,3R}$ = 7.0 $J_{2,3S}$ = 5.0 $J_{3R,3S}$ = 13.5	−10.0[b]	(VIIIa); (−)-(2*S*)- and IXa	H_2 = 4.02 q H_{3R} = 3.00 q H_{3S} = 3.25 q	$J_{2,3R}$ = 8.0 $J_{2,3S}$ = 4.5 $J_{3R,3S}$ = 14.2	−13.5 *c* 2.0
(VIIb); (−)-(2*S*,3*R*)-[3-^{2}H]-	H_2 = 3.37 d H_{3S} = 2.80 d	$J_{2,3S}$ = 5.0	−9.8 *c* 1.1	(VIIIb); (−)-(2*S*,3*R*)-[3-^{2}H]- and IXb	H_2 = 4.07 d H_{3S} = 3.20 d	$J_{2,3S}$ = 4.5	−14.0 *c* 2.0
(VIIc); (−)-(2*S*,3*S*)-[2,3-$^2H_2$]- and its enantiomer	H_{3R} = 2.67 bs		Racemate	(VIIIc); (−)-(2*S*,3*S*)-[3-^{2}H]-	H_2 = 4.01 d H_{3R} = 2.95 d	$J_{2,3R}$ = 8.0	−14.5 *c* 1.7

[a] Aromatic proton signals of VII and VIII appeared around 6.50–7.18 and 6.30–7.25 ppm, respectively. [b] Literature $[\alpha]^{22}D$ −10.6 in 1 *N* HCl, *c* 4.0.

2*R*, 3*R*)-[2,3-$^2H_2$]tyrosine (VIIc), also prepared by Kirby's method. Although tyrosine phenol-lyase has been shown to have racemase activity with alanine,[8] we were able to show in simultaneous runs with D- and L-tyrosine, stopped after short incubation periods, that D-tyrosine was inactive under conditions which produced VIIIa from L-tyrosine in the tyrosine phenol-lyase catalyzed exchange with resorcinol, and that consequently it is safe to conclude that only the natural (2*S*, 3*S*) component of the racemic mixture takes part in the reaction. The product of this exchange reaction (VIIIc) contained only one atom of deuterium, and the $J_{2,3}$ coupling constant of 8.0 Hz showed clearly that the deuterium occupies the 3*S* configuration. This supplemental result provides supporting evidence that the β-replacement reaction is not only completely stereospecific but occurs with retention of configuration at C-3.

The loss of deuterium from C-2 during the enzymatic transformation of VIIc to VIIIc suggests, of course, the loss of chirality at that position and is in full agreement with the postulated role of the coenzyme in forming intermediate IV (Figure 1). A final stereospecific protonation at C-2 results in formation of the "natural" 2*S* configuration in 2,4-dihydroxyphenyl-L-alanine.

The crucial finding of this study is that the β-replacement reaction of tyrosine with resorcinol takes place with complete retention of configuration at C-3, and this is presumably true of other β replacements catalyzed by tyrosine phenol-lyase. After our work had been completed, two results of related enzymatic studies appeared: (1) Floss, *et al.*, reported[19] that the synthesis of tryptophan from serine and indole, catalyzed by tryptophan synthetase, occurs with retention of configuration at C-3 of serine, and (2) Fuganti, *et al.*,[20] reported that the synthesis of tyrosine from serine and phenol, catalyzed by tyrosine phenol-lyase, takes place with retention of configuration at C-3 of serine. Thus, the growing family of pyridoxal phosphate-dependent enzymatic reactions which carry out α,β eliminations and their reversals through an enzyme-bound α-aminoacrylate–pyridoxal phosphate complex appear to share a common stereochemical course. This leaves no doubt that the functional or binding groups in the enzyme interact with the aminoacrylate complex to keep it relatively planar (as in Va), maintaining the prochirality at C-3 and the distinction between H_{3S} and H_{3R} throughout the reaction.

In 1971, Dunathan summarized the elegant work of his and other groups on the stereochemistry of pyridoxal phosphate-catalyzed reactions[21] and concluded that in all reactions examined to that date all bond breaking and bond making took place on only one side of the cofactor–substrate imine. It is striking that the results reported in this paper and other recent work extend this generalization to the α,β eliminations and their reverse reactions as well. The subtle yet powerful stereochemical conclusions of this sort are clearly laying the groundwork for more detailed understanding of pyridoxal phosphate catalysis.

Acknowledgment. We gratefully acknowledge the support of this research by the National Institutes of Health through Research Grant GM-16944.

References and Notes

(1) Kyoto University of Education.
(2) The Research Institute for Food Science.
(3) University of Georgia.
(4) H. Kumagai, H. Yamada, H. Matsui, H. Ohkishi, and K. Ogata, *J. Biol. Chem.*, **245**, 1767 (1970).
(5) H. Kumagai, H. Yamada, H. Matsui, H. Ohkishi, and K. Ogata, *J. Biol. Chem.*, **245**, 1773 (1970).
(6) H. Kumagai, H. Matsui, H. Ohkishi, K. Ogata, H. Yamada, T. Ueno, and H. Fukami, *Biochem. Biophys. Res. Commun.*, **34**, 266 (1969).
(7) T. Ueno, H. Fukami, H. Ohkishi, H. Kumagai, and H. Yamada, *Biochim. Biophys. Acta*, **206**, 476 (1970).
(8) H. Kumagai, N. Kashima, and H. Yamada, *Biochem. Biophys. Res. Commun.*, **39**, 796 (1970).
(9) H. Yamada, H. Kumagai, N. Kashima, H. Torii, H. Enei, and S. Okumura, *Biochem. Biophys. Res. Commun.*, **46**, 370 (1972).
(10) H. Enei, H. Nakazawa, H. Matsui, S. Okumura, and H. Yamada, *FEBS lett.*, **21**, 39 (1972).
(11) H. Kumagai, H. Ohkishi, N. Kashima, and H. Yamada, *Hakko To Taisha*, **27**, 11 (1973).
(12) A. E. Braunshtein and M. M. Shemyakin, *Biokhimiya*, **18**, 393 (1953); *Chem. Abstr.*, **48**, 4603 (1954).
(13) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Amer. Chem. Soc.*, **76**, 648 (1954).
(14) E. E. Snell, *Vitam. Horm. (New York)*, **16**, 77 (1958); *Brookhaven Symp. Biol.*, **15**, 32 (1962).
(15) S. Sawada, H. Kumagai, H. Yamada, R. K. Hill, Y. Mugibayashi, and K. Ogata, *Biochem. Biophys. Acta*, **315**, 204 (1973).
(16) G. W. Kirby and J. Michael, *Chem. Commun.*, 187, 415 (1971).
(17) J. P. Lambooy, *J. Amer. Chem. Soc.*, **76**, 133 (1954).
(18) Largely on the basis of the mass spectrum, the product of the tyrosine phenol-lyase catalyzed exchange between *S*-methyl-L-cysteine and resorcinol was initially regarded as 3,4-dihydro-3-amino-7-hydroxycoumarin[7] and should now be revised to the parent amino acid (VIIIa).
(19) G. E. Skye, R. Potts, and H. G. Floss, *J. Amer. Chem. Soc.*, **96**, 1593 (1974).
(20) C. Fuganti, D. Ghiringhelli, D. Giangrasso, P. Grasselli, and A. Santopietro, Abstracts, Ninth IUPAC Symposium on the Chemistry of Natural Products, Ottawa, Canada, June 26, 1974, p 12C.
(21) H. C. Dunathan, *Advan. Enzymol. Relat. Areas Mol. Biol.*, **35**, 79 (1971).