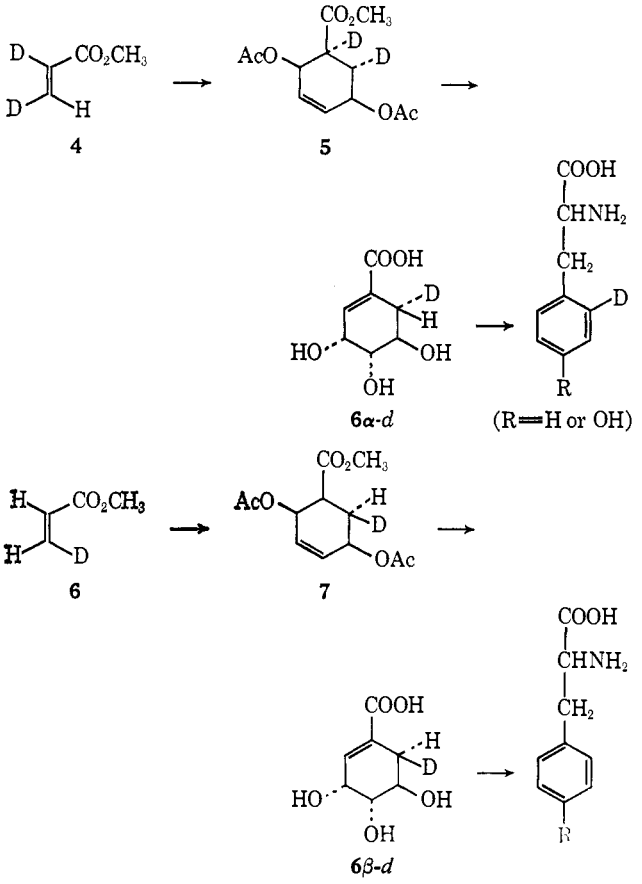
Assay of deuterium content by mass spectroscopy showed that the tyrosine and phenylalanine biosynthesized from **6α-*d*** retained at least 98% of the deuterium of the precursor, while the aromatic amino acids originating from **6β-*d*** were devoid of deuterium. The chorismate synthetase reaction is thus a stereospecific *trans*-1,4 elimination. Independent studies by Onderka and Floss[13] using a different approach have led to the same conclusion. These results suggest that this enzymatic elimination is probably not a concerted E2′ reaction.



Acknowledgments. We gratefully acknowledge the assistance of Miss Renee Gruber in culturing the bacterial mutants, and the generous cooperation and advice of Professors Charles Gilvarg and David B. Sprinson.

(13) D. K. Onderka and H. G. Floss, *J. Amer. Chem. Soc.*, **91**, 5894 (1969).

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Steric Course of the Chorismate Synthetase Reaction and the 3-Deoxy-D-*arabino*-heptulosonate 7-Phosphate (DAHP) Synthetase Reaction

Sir:

The reactions of the shikimic acid pathway,[1] the major route leading to the formation of aromatic compounds in plants and microorganisms, pose a number of interesting stereochemical questions. We report here data which establish the steric course of two of the reactions of the pathway, the chorismate synthetase reaction and the DAHP synthetase reaction. The formation of chorismate (VI) involves a 1,4-conjugate elimination of phosphoric acid (Scheme I) which could be either a *cis* or a *trans* process, *i.e.*, either the *pro-R* (*trans*) or the *pro-S* hydrogen (*cis*) could be removed from C-6. In the formation of DAHP (IV) from erythrose 4-phosphate (III) and phosphoenolpyruvate (II) we ask the question: from which side of the plane of the double bond is C-3 of phosphoenolpyruvate attacked?

3-Phosphoglyceric-3-*t* acids (I) 3*R* and 3*S* were prepared from mannose-1-*t* and glucose-1-*t* using the glycolytic enzymes including phosphomannose isomerase or phosphoglucose isomerase, respectively, in the presence of arsenate. These phosphoglyceric acids upon incubation with phosphoglyceromutase and enolase give the two isomeric specimens of phosphoenolpyruvate tritiated asymmetrically at C-3. Preliminary evidence[2] indicates that the enolase reaction involves a *trans* elimination of the elements of water, *i.e.*, the phosphoenolpyruvate from (3*R*)-phosphoglycerate-3-*t* would have *E* configuration[3] (phosphate and tritium *trans* to each other). The formation of phosphoenolpyruvate was coupled to its further conversion, with nonlabeled erythrose 4-phosphate, into shikimate (V) using a cell-free extract of *E. coli* mutant 83-24. The two specimens of shikimic-6-*t* acid were chromatographically purified to radiochemical homogeneity. Aliquots were mixed with shikimic-7-^{14}C acid (total 1 μmol) and converted into chorismic acids using cell-free extracts of *Aerobacter aerogenes* mutant 62-1, and the changes in the T/^{14}C ratios were determined. By enzymatic conversion into anthranilate it was ascertained that all the remaining tritium of the chorismate samples was confined to the 6 position. (*R*,*S*)-Shikimic-1,6-^{14}C acid was added to another aliquot of the tritiated shikimic acids. These samples (0.75 μmol, 1.2 μCi of ^{14}C/μmol) were esterified and the methyl esters were ozonized, followed immediately by reduction with $NaBH_4$. The products were further degraded by periodate and bromine oxidation[4] to give (*R*,*S*)-malates, which after paper chromatographic purification were subjected to the fumarase reaction. Fumarate and malate were separated by tlc. There was a slight decrease in the T/^{14}C ratios (12%) during the degradation of the shikimates to malates, presumably due to some exchange by enolization during the work-up at the aldehyde stage.

The data from these experiments are summarized in Table I. Clearly, the two shikimic acids obtained from (3*R*)- and (3*S*)-phosphoglycerate-3-*t* are predominantly stereospecifically labeled at C-6, as observed earlier,[5] although there is some scrambling of the tritium. The hydrogen at C-6 of shikimate, which originates from the *pro*-3*S* hydrogen of phosphoglycerate, is the one that is eliminated in the chorismate synthetase reaction. This hydrogen is also the one which is eliminated in the con-

(1) For reviews *cf.*: (a) D. B. Sprinson, *Advan. Carbohyd. Chem.*, **15**, 235 (1960); (b) B. A. Bohm, *Chem. Rev.*, **65**, 435 (1965); (c) F. Lingens, *Angew. Chem. Intern. Ed. Engl.*, 7, 350 (1968).

(2) M. Cohn, J. Pearson, E. L. O'Connell, and I. A. Rose, unpublished results.

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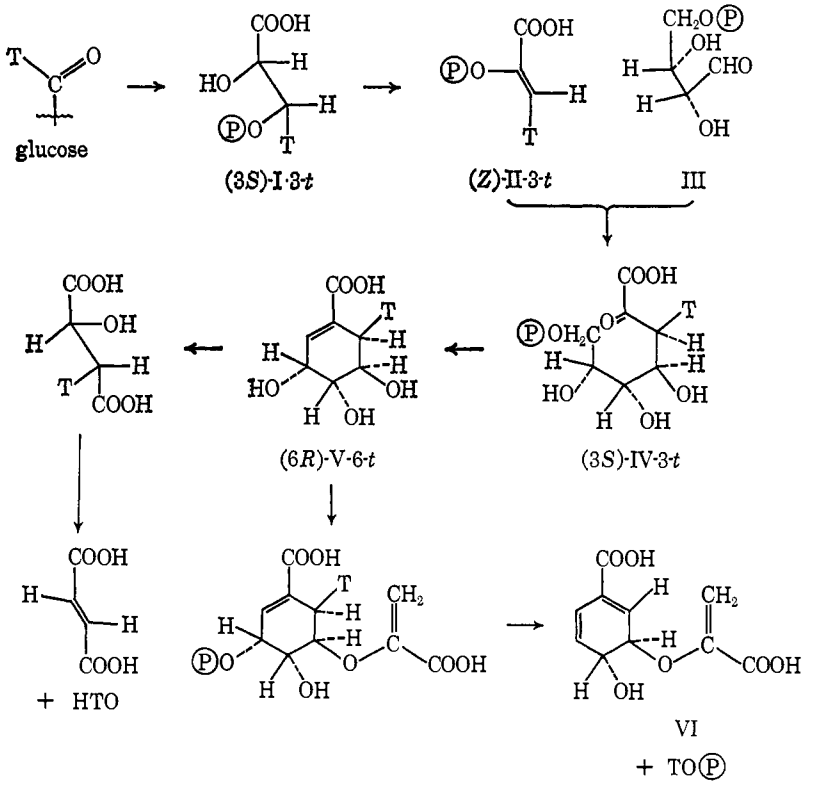
(4) K. R. Hanson and I. A. Rose, *Proc. Natl. Acad. Sci. U. S.*, **50**, 81 (1963).

(5) D. K. Onderka and H. G. Floss, *Biochem. Biophys. Res. Commun.*, **35**, 801 (1969).

Scheme I. Formation of Shikimic Acid from Phosphoglycerate-3-*t*, Its Degradation, and its Conversion into Chorismic Acid



version of the malate, obtained by degradation of this shikimate, into fumarate by fumarase. Since the enzyme fumarase is known[6,7] to catalyze the removal of the

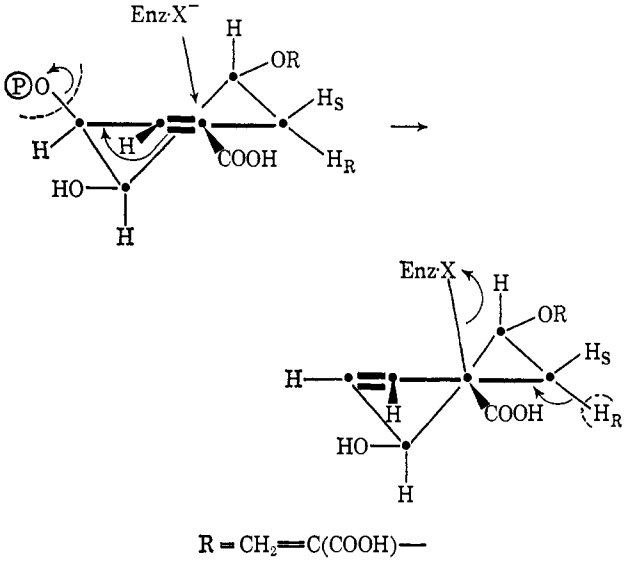
Table I. Determination of the Configuration of the Labeled Hydrogen Atoms in Shikimic Acids Tritiated Stereospecifically at C-6 and of Their Fate in the Chorismate Synthetase Reaction

	Material obtained from (3*R*)-Phosphoglycerate-3-*t*		Material obtained from (3*S*)-Phosphoglycerate-3-*t*	
Compound	T/^{14}C	T retention, %	T/^{14}C	T retention, %
Enzymatic Conversion of Shikimate				
Shikimate	14.3, 5.9		14.0, 12.6	
Chorismate	11.7, 5.2	82, 88	2.5, 2.3	18, 18
Anthranilate	0.06, 0.03	0.4, 0.5	0.1, 0.7	0.7, 6
Degradation of Shikimate				
Shikimate	2.93		2.56	
(*R,S*)-Malate	2.54	87	2.26	88
Fumarate	2.10	83[a]	0.64	28[a]

[a] Relative to malate.

hydroxyl group and the *pro*-3*R* hydrogen of (2*S*)-malate, it follows that the hydrogen eliminated in the chorismate synthetase reaction is a *pro*-6*R* hydrogen. Therefore, the elimination of phosphate and the proton is from opposite sides of the plane of the ring or *trans*. The same result was obtained independently by Hill and Newkome[8] using a different approach. Although chemical studies on the steric course of 1,4-conjugate eliminations have given somewhat conflicting results,[9,10] it has been pointed out[11,12] that on theoretical grounds a concerted process (E2′) should involve a *cis* elimination of the substituents. The observed *trans* stereochemistry of chorismate formation would thus render a synchronous, one-step mechanism for this reaction less likely. As one alternative, a two-stage X-group mechanism[13] as shown in Scheme II could be considered.

Scheme II. Possible Mechanism of the Chorismate Synthetase Reaction



Assuming the preliminary assignment[2] of the steric course of the enolase reaction to be correct, the data also establish the over-all steric course of the reaction at C-3 of phosphoenolpyruvate in the formation of DAHP. Phosphoenolpyruvate-3-*t* of *Z* configuration which re-

(6) O. Gawron and T. P. Fondy, *J. Amer. Chem. Soc.*, **81**, 6333 (1959).
(7) F. A. L. Anet, *ibid.*, **82**, 994 (1960).
(8) R. K. Hill and G. R. Newkome, *ibid.*, **91**, 5893 (1969).
(9) H. D. Orloff and A. J. Kolka, *ibid.*, **76**, 5484 (1954).
(10) S. J. Cristol, W. Barasch, and C. H. Tieman, *ibid.*, 77, 583 (1955).
(11) K. Fukui, *Tetrahedron Lett.*, 2427 (1965).
(12) N. T. Anh, *Chem. Commun.*, 1089 (1968).
(13) J. W. Cornforth, *Angew. Chem. Intern. Ed. Engl.*, **7**, 903 (1968).

sults from (3*S*)-phosphoglycerate-3-*t* gives rise to (6*R*) shikimate-6-*t*. Therefore, the DAHP formed in the reaction is labeled in the *pro*-3*S* hydrogen,[14] *i.e.*, the attack at C-3 of phosphoenolpyruvate is at the side of the plane viewed at which the substituents phosphate, carboxyl, methylene appear in counterclockwise order (*si* face[15]). This information is, of course, of no mechanistic significance, but it may be of interest from the viewpoint of enzyme evolution. Rose and his coworkers[16] recently studied different phosphopyruvate carboxylases (EC 4.1.1.31, EC 4.1.1.32, EC 4.1.1.38) and found that in all three cases the reaction involves *si* attack at C-3 of phosphoenolpyruvate.

Acknowledgments. We are indebted to Dr. B. D. Davis, Harvard Medical School, for providing us with a culture of *E. coli* mutant 83-24 and in particular to Dr. Howard Zalkin, Department of Biochemistry, Purdue University, for gifts of bacterial cultures as well as enriched preparations of DAHP synthetase and anthranilate synthetase. Thanks are due to Dr. I. A. Rose, Institute for Cancer Research, Philadelphia, Pa., for communicating to us his unpublished results. Partial support of this work by U. S. Public Health Service Grant No. FR 05586 is gratefully acknowledged.

(14) Note that the 3*S* hydrogen of DAHP is sterically equivalent to the 6*R* hydrogen of shikimate. The possibility that an inversion takes place at this carbon atom during the conversion of DAHP into shikimate, although not strictly excluded, is considered to be extremely remote.

(15) K. R. Hanson, *J. Amer. Chem. Soc.*, 88, 2731 (1966).

(16) I. A. Rose, E. L. O'Connell, P. Noce, M. F. Utter, H. G. Wood, J. M. Willard, and T. G. Cooper, unpublished results.

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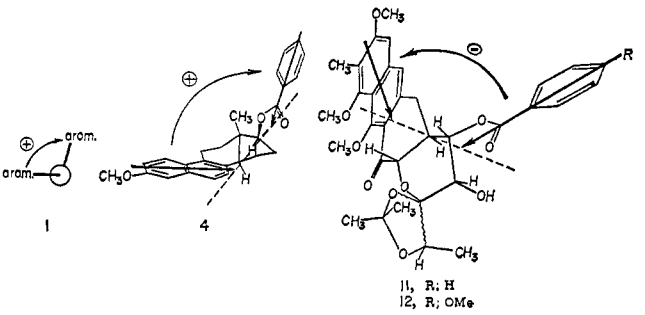
A Method for Determining the Chirality of Two Aromatic Chromophores and the Absolute Configurations of Chromomycin A_3 and Related Antibiotics

Sir:

We have recently developed a method for determining the chiralities between hydroxyl groups[1,2] which is based on the splitting of the original Cotton effect associated with the benzoate chromophore. Thus, the isolated benzoate group shows a Cotton effect at 225 nm ($\Delta\epsilon \sim 3.5$),[3,4] whereas, in the case of interacting benzoate groups, this is subject to a Davydov splitting[5,6] and is characterized by two Cotton effects centered at 233 and 219 nm ($\Delta\epsilon \sim 10$–15); the sign of the longer wavelength Cotton effect (first Cotton) is in accord with the screwness of the benzoate groups, which is defined as positive when represented as in **1** (right-handed screw).

This "dibenzoate chirality rule" can now be extended to encompass other aromatic chromophores for which directions of the two interacting transitions are known; it therefore provides an extremely powerful method for determining the absolute configurations or conformations of natural products which already possess or are readily convertible into derivatives possessing suitable chromophores.



The nonempirically calculated absolute signs of Cotton effects resulting from interacting benzoate chromophores agree with the chirality of the two aromatic groups.[1,7] It was thus expected that the treatment employed in the case of interacting benzoate groups could be extended to other groups as well. This is confirmed by the CD datum of 17β-dihydroequilenin 3-methyl ether 17-benzoate (**3**), mp 167–169°[8] (Figure 1). The free 17β-ol **2** exhibits Cotton effects centered at 330, 280, and 226 nm corresponding to the uv maxima of the methoxynaphthalene group. The $^1A \rightarrow {}^1B_b$ transition (long axis) at 230 nm[9] (Figure 2) is strongly coupled with the benzoate transition at 230 nm, and consequently the CD spectrum has two strong Cotton effects at 235 and 220 nm (Figure 1). Significantly, the positive sign of the first Cotton effect is in agreement with the chirality between the long axis of the naphthalene moiety and the benzoate group (or to a first approximation, direction of the C_{17}–O bond; see **4**). The two other transitions, $^1A \rightarrow {}^1L_a$ (short) at 280 nm and $^1A \rightarrow {}^1L_b$ (long) at 330 nm, were not affected by the dipole–dipole coupling with the benzoate groups (Figure 1) because (a) the bands are located far from the 230-nm benzoate transition, and (b) their electric transition moments are relatively small, and therefore the rotational strength which is proportional to the electric transition moment is also small.[1]

The present method was next applied to chromomycin A_3 (**5**) belonging to the chromomycin[10] and olivomycin[11,12] group of antitumor antibiotics, the absolute configuration of which remains to be established.[13]

(1) N. Harada and K. Nakanishi, *J. Amer. Chem. Soc.*, 91, 3989 (1969).

(2) M. Koreeda, N. Harada, and K. Nakanishi, *Chem. Commun.*, in press.

(3) N. Harada, Mo. Ohashi, and K. Nakanishi, *J. Amer. Chem. Soc.*, 90, 7349 (1968).

(4) N. Harada and K. Nakanishi, *ibid.*, 90, 7351 (1968).

(5) A. S. Davydov, "Theory of Molecular Excitons," translated by M. Kasha and M. Oppenheimer, Jr., McGraw-Hill Book Co., Inc., New York, N. Y., 1962.

(6) J. A. Schellman, *Accounts Chem. Res.*, 1, 144 (1968).

(7) See also S. F. Mason in "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," G. Snatzke Ed., Heyden, London, 1967, Chapter 4.

(8) Prepared by benzoylation of the 3-methyl ether: W. E. Bachmann and A. S. Dreiding, *J. Amer. Chem. Soc.*, 72, 1323 (1950). The 17β configuration has been confirmed by X-ray analysis for estradiol 3-*p*-bromobenzoate: Y. Tsukuda, T. Sato, M. Shiro, and H. Koyama, *J. Chem. Soc., B*, 1387 (1968).

(9) H. H. Jaffé and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," John Wiley & Sons, Inc., New York, N. Y., 1962.

(10) M. Miyamoto, Y. Kawamatsu, K. Kawashima, M. Shinohara, K. Tanaka, S. Tatsuoka, and K. Nakanishi, *Tetrahedron*, 23, 421 (1967), and previous papers.

(11) G. P. Bakhaeva, Yu. A. Berlin, O. A. Chuprunova, M. N. Kolosov, G. Yu. Peck, L. A. Piotrovich, M. M. Shemyakin, and I. V. Vasina, *Chem. Commun.*, 10 (1967), and previous papers.

(12) The olivomycins differ from chromomycin in that they lack the 7-Me group in the aglycone. The various chromomycins and olivomycins differ in the number and type of sugar residues attached to C-2 and C-6.

(13) Previous studies are not well grounded and have led to conflicting results. The absolute configuration of the chromomycins[14] rested