CHORISMIC ACID. A BRANCH POINT INTERMEDIATE IN AROMATIC BIOSYNTHESIS

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Summary

The isolation and characterization of chorismic acid are described. The structure of the acid has been elucidated by spectroscopic methods and by chemical degradation as (IV). The relative and absolute stereochemistries have been established as identical with the corresponding centres in shikimic acid.

Chorismic acid has been shown to differ from a compound for which Lingens and Lück have claimed the structure (IV).

INTRODUCTION

The biosynthesis of a number of important aromatic compounds, including phenylalanine, tyrosine, tryptophan, *p*-hydroxybenzoic acid, and *p*-aminobenzoic acid, has been shown to involve shikimic acid (I) as a common intermediate. The steps by which shikimic acid is formed from β-D-glucose have been described.[1] It is also known that prephenic acid (V) (Fig. 1) is a precursor of phenylalanine and tyrosine, and that anthranilic acid is a precursor of tryptophan. The number of intermediates between shikimic acid and prephenic acid, which are also intermediates in the conversion of shikimic acid to anthranilic acid, has been the subject of a number of recent investigations.[2] Thus, it has been shown that shikimic acid 5-phosphate (II) and 3-enolpyruvylshikimic acid 5-phosphate, designated Z_1 phosphate (III), are involved in the pathway to prephenic acid and both these compounds have been suggested as the branch point between the prephenic acid and anthranilic acid pathways.[1,2] Final clarification of this region of aromatic biosynthesis has been provided by Gibson and his co-workers.

Rivera and Srinivasan,[3] and independently Gibson *et al.*[4] showed that Z_1 phosphate was a precursor of anthranilic acid as well as of tyrosine and phenylalanine. The latter workers pointed out that, since there existed bacterial mutants which accumulated Z_1 phosphate and which required the three aromatic amino acids as well as *p*-hydroxy- and *p*-amino-benzoic acids for growth, the branch point in the various pathways must lie beyond Z_1 phosphate.

Isolation of the branch point compound was subsequently made by Gibson and Gibson. Starting with a tryptophan auxotroph of *Aerobacter aerogenes* they were able to produce a new mutant (62–1), which also required tyrosine and phenylalanine

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[1] Sprinson, D. B., *Adv. Carbohyd. Chem.*, 1960, **15**, 235.

[2] Davis, B. D., In "Biochemist's Handbook." (Ed. C. Long.) p. 595. (Spon: London 1961.)

[3] Rivera, A. J., and Srinivasan, P. R., *Proc. Natn. Acad. Sci. U.S.A.*, 1962, **48**, 864.

[4] Gibson, M. I., Gibson, F., Doy, C. H., and Morgan, P. N., *Nature*, 1962, **195**, 1173.

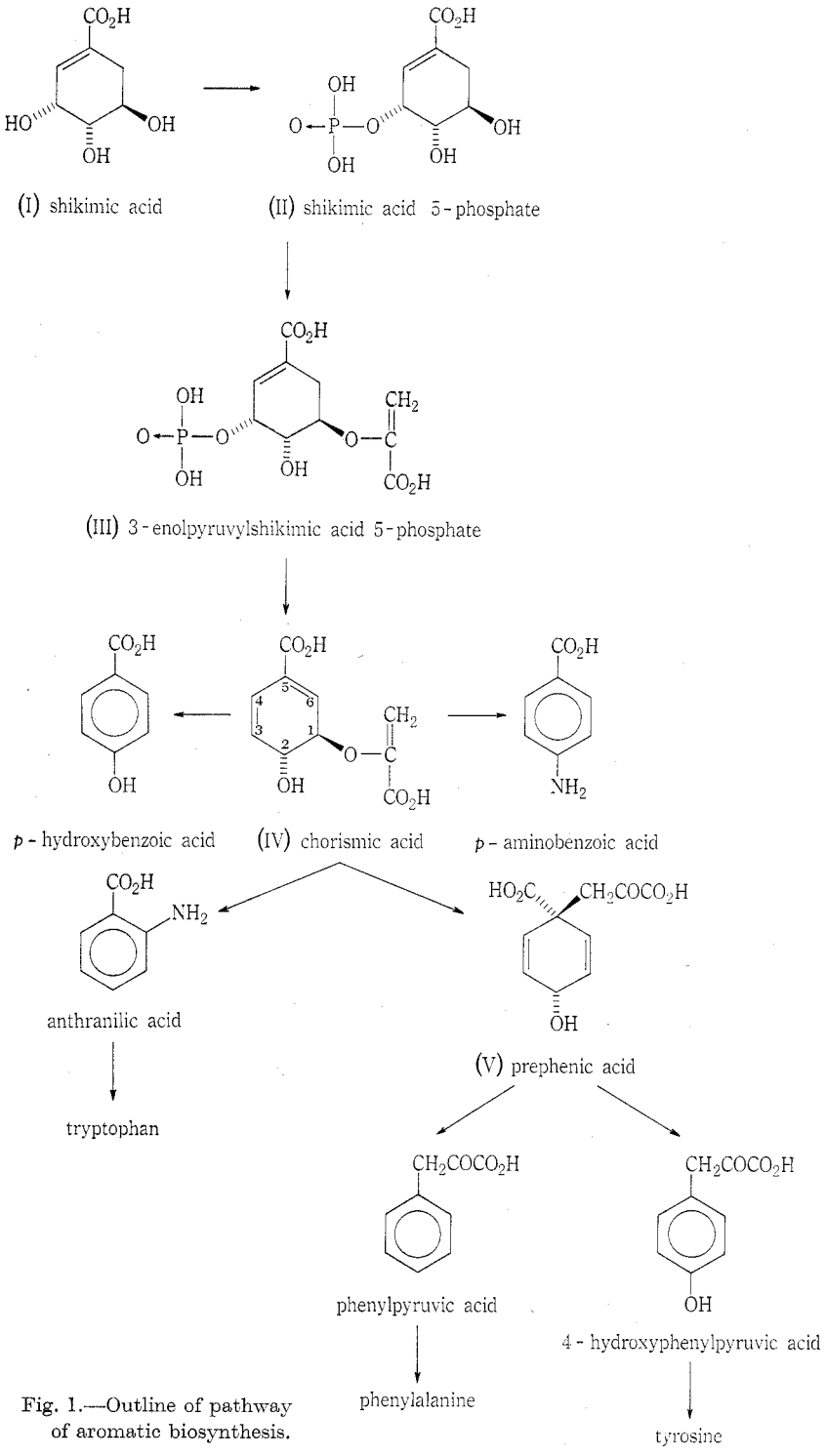


Fig. 1.—Outline of pathway of aromatic biosynthesis.

for optimum growth. The mutant 62–1, unless incubated in the presence of excess tryptophan, accumulates anthranilic acid. However, in the presence of excess tryptophan, production of anthranilic acid is subject to end-product inhibition and a new compound, which has been named chorismic acid (IV), accumulates. The isolation and preliminary characterization of the barium salt of chorismic acid and its enzymic conversion to prephenic acid, anthranilic acid, *p*-amino- and *p*-hydroxybenzoic acids have been described.[5,6]

We now describe chemical and physico-chemical studies which lead to the structure, and relative and absolute stereochemistry of chorismic acid.*

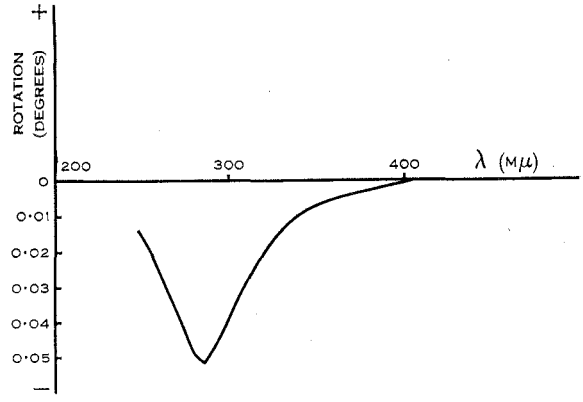


Fig. 2.—Optical rotary dispersion curve for chorismic acid in water.

Isolation and Characterization of Chorismic Acid

In his original publication on chorismic acid, Gibson describes its production by *A. aerogenes* 62–1 and its isolation as its barium salt. He characterized the barium chorismate by analysis ($C_{10}H_8BaO_6,3H_2O$), its ultraviolet spectrum [λ_{max} (H_2O) 272 mμ (ϵ 2700)] and its infrared spectrum.

We have slightly modified Gibson's procedure (see Experimental section) and have isolated the free acid.

Chorismic acid could be obtained as well-defined crystals from a number of solvent systems but showed a marked tendency to retain solvent molecules. Removal of solvents of crystallization was difficult because of the thermal instability of the acid (see below). For this reason it was difficult to obtain accurate analyses by the combustion technique. Samples analysing for a hemihydrate and a hydrate were obtained by recrystallization from moist solvents but these results were difficult to reproduce. The molecular formula of the acid was established as $C_{10}H_{10}O_6$ by high resolution mass spectrometry.

Chorismic acid is laevorotary and shows a negative Cotton curve (see Fig. 2).†

* A preliminary report of the n.m.r. spectrum and structure of chorismic acid has already been published (Gibson, F., and Jackman, L. M., *Nature*, 1963, **198**, 338).

† The optical rotary dispersion curve for chorismic acid has been obtained independently by Dr. U. Weiss of the National Institute of Arthritis and Metabolic Diseases, Maryland, and his results are in agreement with our own.

[5] Gibson, M. I., and Gibson, F., *Biochem. J.*, 1964, **90**, 248.

[6] Gibson, F., *Biochem. J.*, 1964, **90** 256.

The ultraviolet spectrum of aqueous solutions of the acid and its dianion are reproduced in Figure 3, and the infrared spectrum of the free acid is shown in Figure 4.

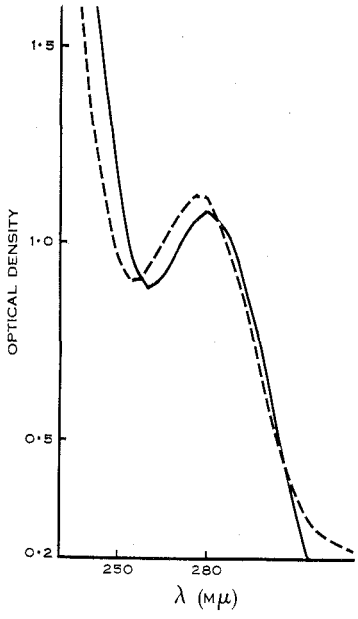


Fig. 3.—U.v. absorption spectra of chorismic acid and its dianion in water.
——— pH 2;
– – – – pH 9.

Proof of Structure

The u.v. spectrum is similar to that of 3,4-dihydrobenzoic acid and shows λ_{max} (H_2O) 275 mμ (ε 2630). Thus, Plieninger reports λ_{max} 276 mμ (ε 1950) for 3,4-dihydrobenzoic acid[7] and Bailey, Barclay, and Baylouny[8] quote λ_{max} 274–279

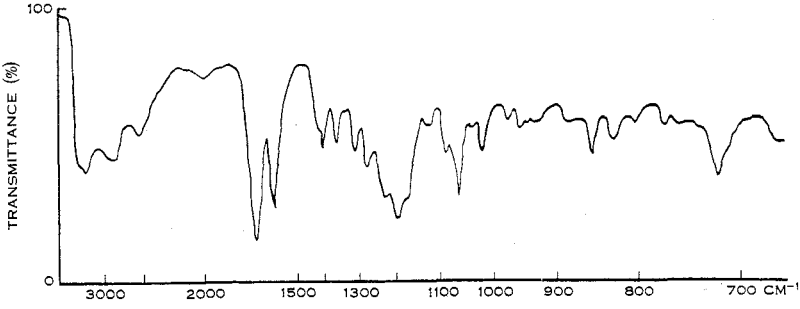


Fig. 4.—Infrared absorption spectrum of chorismic acid (KCl disk).

mμ (ε 2780) for its methyl ester. Chorismic acid therefore appears to be a derivative of 3,4-dihydrobenzoic acid.

[7] Plieninger, H., and Ege, G., *Chem. Ber.*, 1961, **94**, 2088.
[8] Bailey, W. J., Barclay, R., and Baylouny, R. A., *J. Org. Chem.*, 1962, **27**, 1851.

The infrared spectrum (KCl disk) exhibits the following characteristic absorptions: 3380 (OH) 1740–1690 ($-CO_2H$) and 1620 (C=C) cm^{-1}. Engelhardt, Plieninger, and Schreiber[9] report that absorptions at 1740 and 1620 cm^{-1} are characteristic of enol ethers of pyruvic acid. 3,4-Dihydrobenzoic acid absorbs at 1695 cm^{-1}. The spectrum of chorismic acid contains a band of medium intensity at 860 cm^{-1} which can be reasonably assigned to the out-of-plane deformation of the methylene group of the enol ether. Thus the infrared spectrum is consistent with the presence of the grouping $-OC(CO_2H)=CH_2$.

The structure of chorismic acid is partly established by consideration of its n.m.r. spectrum. Spectra of the free acid and its dianion in D_2O are reproduced in Figures 5 and 6. Conformation of the presence of the enol pyruvic acid side-chain is

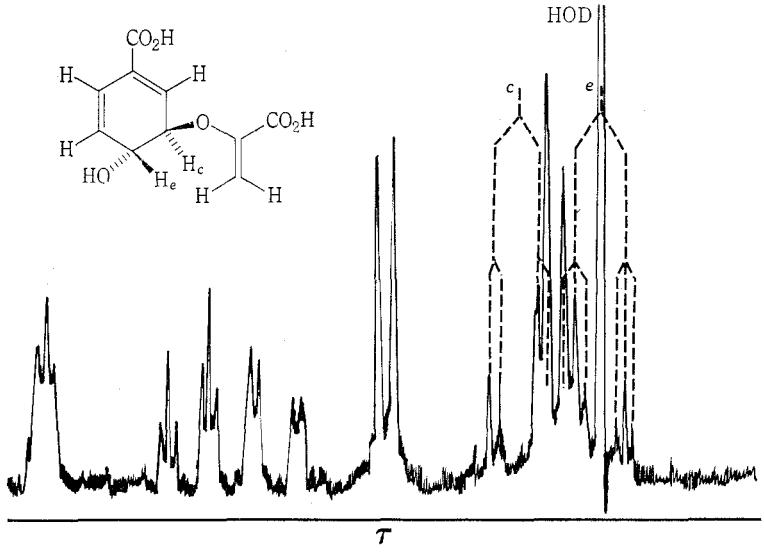


Fig. 5.—N.m.r. spectrum of chorismic acid (D_2O).

provided by the presence of a pair of doublets (J 2·8 c/s) at τ 5·46 and 4·79. These positions are approximately those anticipated from a consideration of the shifts of the methylene protons in methyl isopropenyl ether (6·2),[10] propene (*c.* 5·1),[11] and methyl methacrylate (4·0 and 4·5),[12] the absorption at lower field being assigned to the proton *cis* to the carboxyl group.[13] The absorption in the region 3·3–4·1 corresponds in intensity to three protons which are clearly olefinic in type. In this region there is a broad singlet at 3·38 and an AB quartet (J 9·75 c/s, τ 3·50, 3·97), the components of which show some additional fine structure.

[9] Engelhardt, M., Plieninger, H., and Schreiber, P., *Chem. Ber.*, 1964, **97**, 1713.
[10] Whipple, E. B., Goldstein, J. H., and Mandell, L., *J. Am. Chem. Soc.*, 1960, **82**, 3010.
[11] Banwell, C. N., and Sheppard, N., *Molec. Phys.*, 1960, **3**, 351.
[12] Jackman, L. M., and Wiley, R. H., *J. Chem. Soc.*, 1960, 2881.
[13] Jackman, L. M., and Wiley, R. H., *J. Chem. Soc.*, 1960, 2886.

The absorption at 3·38 can be assigned to a proton β to a carboxyl group;[12,13] and the AB system must be the protons of a *cis*-disubstituted olefin for which the coupling constant is usually of the order of 10 c/s.[14] These three protons are evidently associated with the conjugated diene system since the n.m.r. spectrum of 3,4-dihydrobenzoic acid shows similar features (broad singlet 3·36; AB quartet J 9·5 c/s 3·71 and 4·10).

The remaining absorption, which is equivalent to two protons, is a complex band in the region τ 5–6. Comparison of the spectrum of the free acid with that of its dianion reveals that this absorption is essentially an AB quartet in which the A

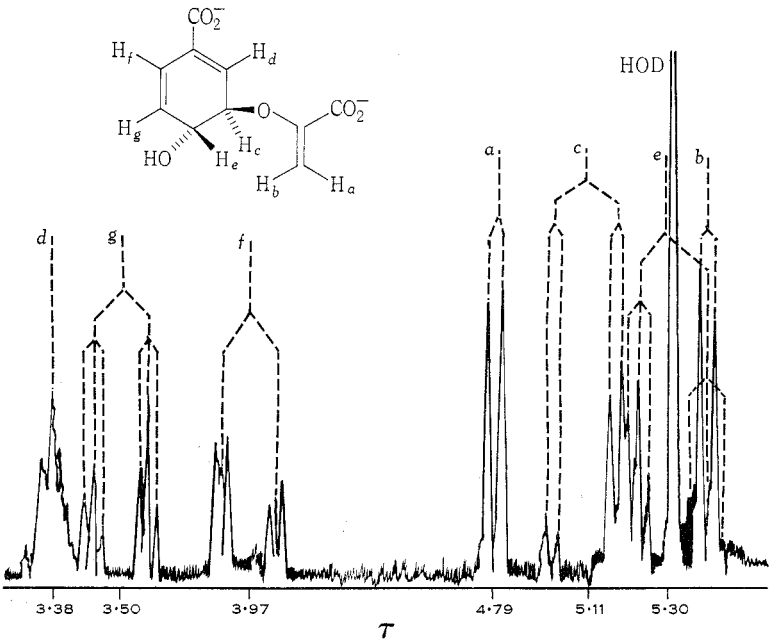


Fig. 6.—N.m.r. spectrum of sodium chorismate (D_2O).

lines are further split as doublets and the B lines as triplets. These additional splittings are clearly due to coupling with the olefinic protons and the AB quartet must therefore be assigned to protons which are allylic to the diene system. However, the chemical shifts, 5·11 and 5·30, indicate that these two protons are also adjacent to oxygen atoms and thus constitute the system

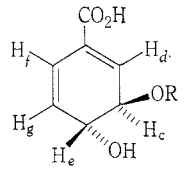
$$=C-\underset{|}{CH}-\underset{|}{CH}-C=$$
$$\quad\quad O \quad\quad O$$

The AB coupling constant, 11·7 c/s, is characteristic of a vicinal interaction for which the dihedral angle is close to 180°.[15]

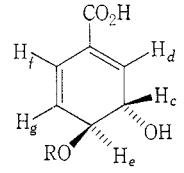
[14] Jackman, L. M., "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry." p. 85. (Pergamon Press: London 1959.)

[15] Karplus, M., *J. Phys. Chem.*, 1961, **64**, 1793.

Chorismic acid must therefore have the structure [A] or [B].*



[A]



[B]

The additional multiplicity of the AB quartet can be explained in terms of these structures. The A absorption is assigned to the proton *c*, since this will be deshielded by the $-M$ effect of the 5-carboxyl group. The A components are further split as doublets by spin–spin coupling (J 1·9 c/s) between protons *c* and *d*. The B components, which are assigned to the proton *e*, are further split as triplets and this is attributed to equal spin–spin coupling (J 1·9 c/s) of proton *e* with protons *g* and *f*. The n.m.r. spectrum does not distinguish between the structures [A] and [B].

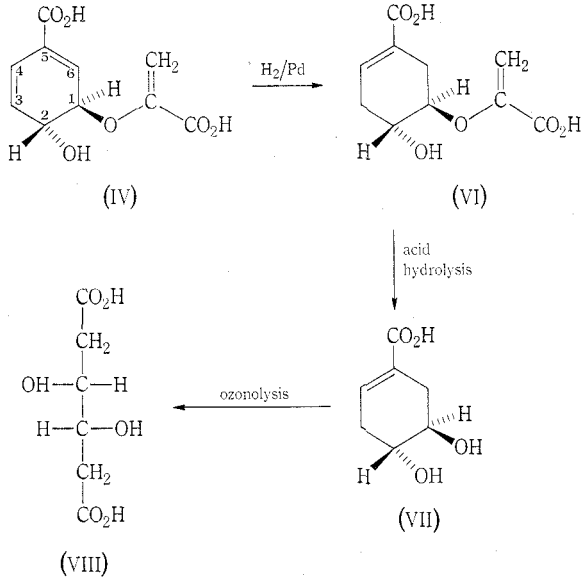


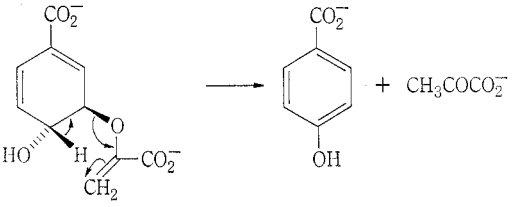
Fig. 7.—Degradation products of chorismic acid leading to determination of absolute stereochemistry.

Confirmation of the correctness of structures [A] and [B] is provided by chemical degradation (Fig. 7). Chorismic acid over 10% palladized charcoal rapidly absorbed 1 mole of hydrogen to yield a dihydro compound which lacks the light absorption of the diene system. The n.m.r. spectrum of the crude dihydrochorismic acid shows the presence of the enolpyruvic acid side-chain and of a trisubstituted unsaturated acid. However, the spectrum indicates the presence of four allylic protons, and two protons

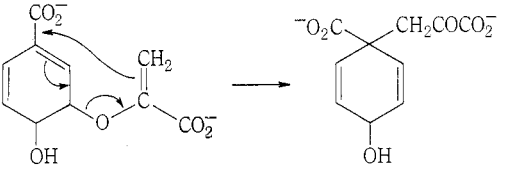
* The numbering of this formula is chosen to coincide with the subsequent formulation of chorismic acid as α-(5-carboxy-1,2-dihydro-2-hydroxyphenoxy)acrylic acid.

adjacent to oxygen but not to a double bond. It therefore appears that hydrogenation involved the 1,4-addition of hydrogen to the conjugated diene. Hydrolysis of the crude dihydro acid (VI) yielded pyruvic acid and an optically active acid $C_7H_{10}O_4$, $[\alpha]_{5890}^{25}$ $-78{\cdot}5\pm1°$. The latter has an n.m.r. spectrum consisting of broad multiplets at 7·8 (4 protons), 6·4 (2 protons), and 2·5 (1 proton), and on treatment with acetic anhydride in pyridine it yielded a diacetate, $C_{11}H_{14}O_6$, $[\alpha]_{5890}^{25}$ $-352\pm20°$. Accordingly, the acid can be formulated as 4,5-dihydroxycyclohex-1-ene-1-carboxylic acid (VII). In fact, the diacetate was found to have an infrared spectrum (CCl_4) which was identical with that of synthetic (±)-*trans*-4,5-diacetoxycyclohex-1-ene-1-carboxylic acid. Preparation of the racemic acid diacetate was carried out by performic acid hydroxylation of cyclohexa-1,4-diene-1-carboxylic acid,[16] followed by acetylation. Grewe and Hinrichs have described the preparation of the methyl ester of (VII) by a similar route.[17]

That chorismic acid has the structure [A] rather than [B] follows from the previously reported observation that it is converted by heating for 1 hr at 70° and pH 8 into prephenic and *p*-hydroxybenzoic acids.[5] The conversion to *p*-hydroxybenzoic acid under alkaline conditions requires the elimination of the pyruvate anion from the 1-position presumably by a cyclic mechanism in which aromaticity is developed in the transition state.



The rearrangement to prephenic acid is also explicable in terms of structure [A] since both structure and stereochemistry (see below) permit a *S*-i′ rearrangement.*



* Hill and Edwards (*Tetrahedron Letters*, 1964, **44**, 3239) have objected to our classification of this rearrangement as an S_Ni' reaction and state that it is simply a Claisen rearrangement. We are of course aware that it is an example of the Claisen rearrangement but in our preliminary communication (*Nature*, 1963, **198**, 388) we preferred to give it the more general classification. Rearrangements such as those investigated by Hurd and Pollack, Cope and Hardy, and Mumm and Möller (*J. Am. Chem. Soc.*, 1938, **60**, 1905; *J. Am. Chem. Soc.*, 1940, **62**, 441; *Ber. dt. chem. Ges.*, 1937, **70**, 2214) as well as the Claisen rearrangement itself, are formally very similar to intramolecular anionotropic rearrangements of allylic carboxylic esters. Ingold ["Structure and Mechanism in Organic Chemistry." p. 598. (Cornell University Press: Ithaca, N.Y., 1953)] has pointed out this similarity and suggested that they be classified either as S_Ni' or simply *S*-i′ reactions.

[16] Emerman, S. L., and Meinwald, J., *J. Org. Chem.*, 1956, **21**, 375.

[17] Grewe, R., and Hinriche, I., *Chem. Ber.*, 1964, **97**, 443.

Additional evidence for some of the structural features of chorismic acid is provided by mass spectral studies. The mass spectrum of chorismic acid is shown in Figure 8(*a*). The ion 208 can be formulated as arising by loss of water from chorismic acid (see Fig. 9) to give the ion (IX). The strong transition at *m/e* 164 appears to be due to the parent ion of phenylpyruvic acid (X) which could arise from rearrangement with loss of CO_2 and H_2O from the chorismic acid radical ion. The possibility that this fragment arises entirely from phenylpyruvic acid produced thermally from chorismic acid appears unlikely as comparison of the chorismic acid spectrum with that (Fig. 8(*b*)) of phenylpyruvic acid indicates that the ions *m/e* 118 (XI) and 91 [the tropylium ion (XII)] are relatively much less abundant in the chorismic acid spectrum. The ion *m/e* 138 corresponds to the parent of *p*-hydroxybenzoic acid.

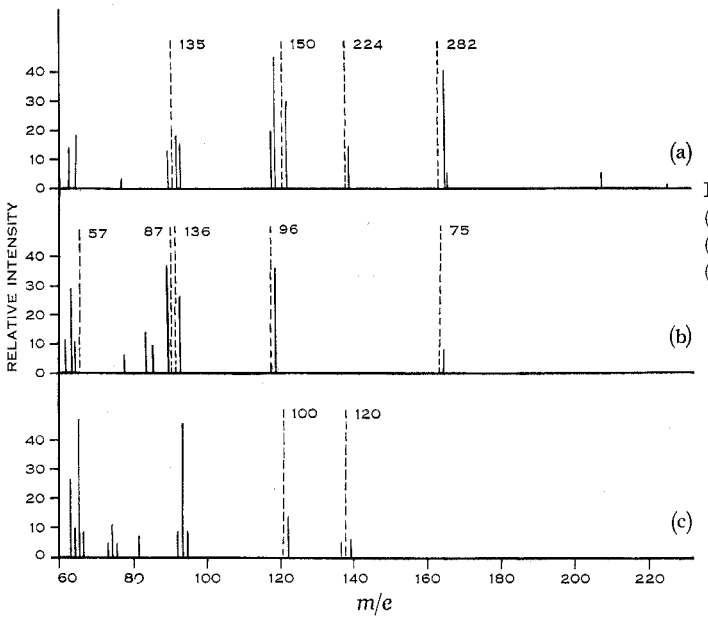


Fig. 8.—Mass spectra of (*a*) chorismic acid, (*b*) phenylpyruvic acid, (*c*) *p*-hydroxybenzoic acid.

Again, this must be produced in an ion reaction as well as thermally since examination of the mass spectrum (Fig. 8(*c*)) of *p*-hydroxybenzoic acid reveals peaks which are of reduced relative intensity in the spectrum of chorismic acid. Figure 9 summarizes the probable structures of the fragment ions of chorismic acid.

Relative and Absolute Stereochemistry

Chorismic acid possesses two asymmetric centres, the relative stereochemistry of which is established by the degradation of dihydrochorismic acid to (—)-*trans*-4,5-dihydroxycyclohex-1-ene-1-carboxylic acid (VII).

The absolute configuration of chorismic acid has been established by conversion of (—)-*trans*-4,5-dihydroxycyclohex-1-ene-1-carboxylic acid to (+)-β,β'-dihydroxyadipic acid (VIII). Posternak and Susz[18] have converted this dibasic acid to

[18] Posternak, Th., and Susz, J.-Ph., *Helv. Chim. Acta*, 1956, **39**, 2032.

(+)-malic acid, which has the *R*-configuration. It follows that the two asymmetric centres in (+)-β,β'-dihydroxyadipic acid are of the *S*-configuration. On the numbering system (IV) adopted for chorismic acid, both asymmetric centres have the *S*-configuration. This is in agreement with the absolute configuration which has been established for naturally occurring (−)-shikimic acid.[19]

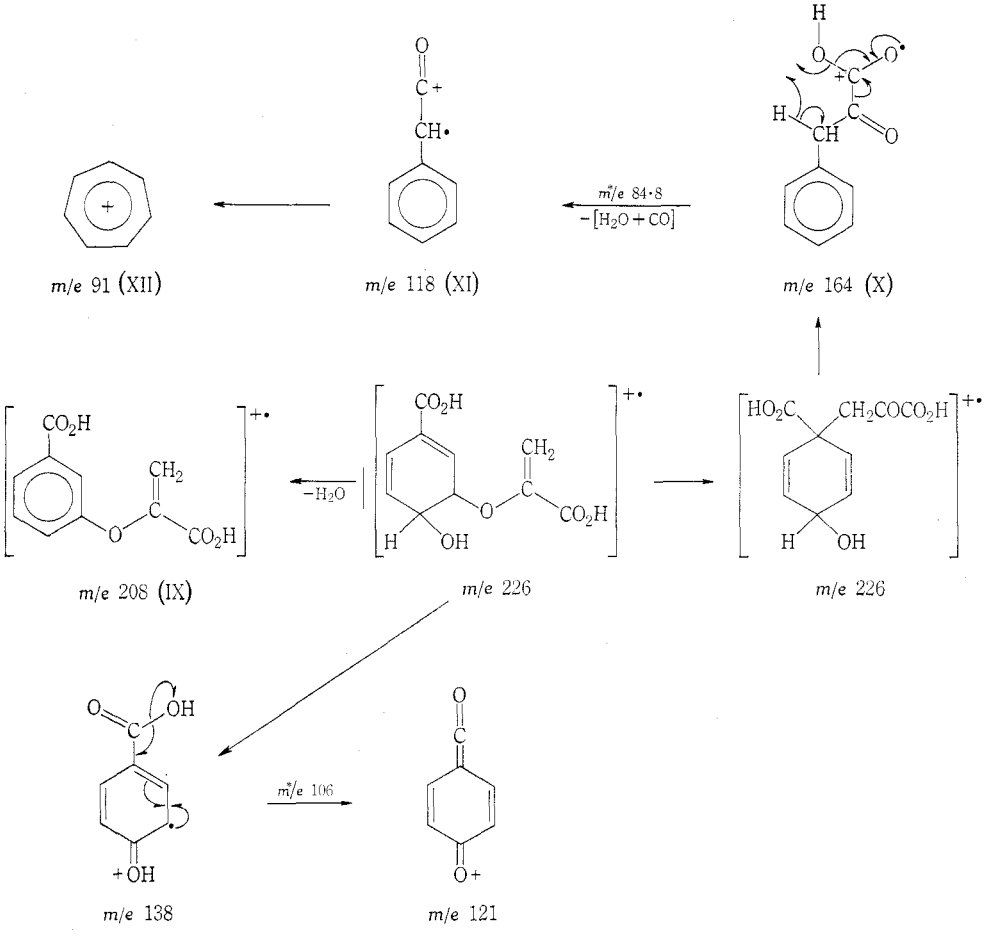


Fig. 9.—Proposed scheme for the ion reaction of chorismic acid.

The n.m.r. spectrum confirms the *trans*-configuration, and the magnitude of the coupling constant between the protons adjacent to oxygen as well as of the allylic coupling constants[20] suggests the extensive population of the conformation in which the two substituents are quasi-equatorial. This conformation is not suitable for the *S*-i′ rearrangement and presumably the role of the enzyme (chorismate mutase)

[19] Fischer, H. O. L., and Dangschat, G., *Helv. Chim. Acta*, 1937, **20**, 705, and Hanson, K. R., *J. Chem. Educ.*, 1962, **39**, 419.

[20] Sternhell, S., *Rev. Pure Appl. Chem.*, 1964, **14**, 15.

which brings about the rearrangement is to invert the conformation of the ring as well as to orientate the side-chain correctly (see Fig. 10).

Reported Isolation of Chorismic Acid from a Saccharomyces cerevisiae Mutant

Lingens and Lück[21] have isolated a compound formed by a mutant of *Saccharomyces cerevisiae* requiring tryptophan for growth. They claim this compound has the structure of chorismic acid (IV). Their compound has m.p. 178–182° (decomp.); its u.v. spectrum has maxima at 224 and 274 mμ. It is converted to pyruvic acid and phenol by pyrolysis. In these respects it differs markedly from chorismic acid described here. Thus, we have crystallized chorismic acid under a wide range of conditions, but we have never obtained a sample melting above 149°. The spectrum of our compound does not contain a maximum at 224 mμ.*

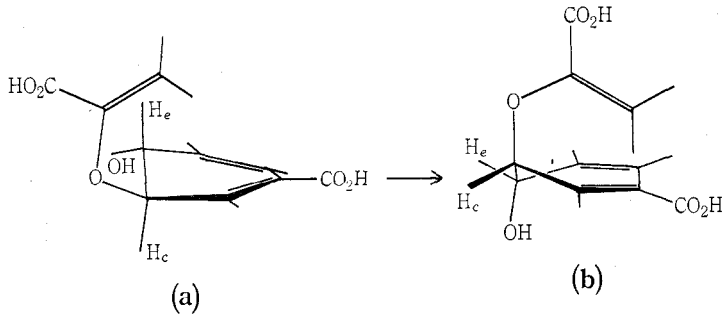


Fig. 10.—Conformation of chorismic acid. (*a*) Predominant conformation. (*b*) Conformation necessary for the *S*-i′ rearrangement.

Finally we have examined the products formed when chorismic acid is heated *in vacuo*. These are largely *p*-hydroxybenzoic, phenylpyruvic, and pyruvic acids. No phenol could be detected.

It therefore appears that the compound described by Lingens and Lück does not have the structure (IV) and that their experimental findings require an alternative interpretation.

Experimental

Microanalyses were carried out by the Australian Microanalytical Service, Melbourne. Melting points were determined on the Kofler block. The ultraviolet absorbtion spectra were determined with a Cary II recording spectrophotometer, the infrared absorbtion spectra with a Perkin–Elmer Infracord 137 spectrometer, the n.m.r. spectra with a Varian Associates HR60 spectrometer, and the optical rotatory dispersion spectrum with a Jasco ORD/UV5 recorder. The optical rotations were determined with a ETL-NPL 143A automatic polarimeter. The mass spectra were determined with an A.E.I. MS9 high resolution mass spectrometer.

* We are grateful to Dr. Lingens for supplying a copy of the u.v. spectrum of his compound.

[21] Lingens, F., and Lück, W., *Angew. Chem. (Int. Ed.)*, 1964, **3**, 66.

Preparation of Chorismic Acid

The previously described preparation[2] of chorismic acid was modified in the following way. After incubation, the cultures of 62-1 were immediately centrifuged and resuspended in the accumulation medium without a saline wash. The mixture was incubated at 30° for 16 hr, centrifuged immediately, made alkaline (pH 8·5), and chromatographed. The solution (50 ml) of ammonium chorismate obtained from the chromatography was acidified with 1N HCl to pH 1·5 and extracted with ether until the u.v. spectrum of the aqueous layer showed > 90% extraction. The ether extracts were combined, washed once with water and dried over $MgSO_4$ at 4°. After concentration of the extract to 30 ml by rotary evaporation at 20°, light petroleum (b.p. 60–80°) was added until a faint precipitate persisted. The mixture was then cooled in an ice/ethanol bath. The resulting slightly yellow crystals were dried (wax-P_2O_5) under vacuum. This process yielded 0·8 g, m.p. 116–119° (decomp.), from 2 l. of accumulation medium. The crude acid was 93% pure when assayed by conversion to anthranilic acid[5] and contained 0·1% prephenic acid.[5]

Chorismic acid monohydrate was obtained by recrystallization from ethyl acetate/light petroleum (b.p. 60–80°). The solution in ethyl acetate was made at room temperature, and cooled to −10° to give colourless crystals, m.p. 148–149° (decomp.) (Found: C, 49·3; H, 5·1. Calc. for $C_{10}H_{10}O_6,H_2O$: C, 49·2; H, 5·0%). The acid was 100% pure by conversion to anthranilic acid. $[\alpha]_{5890}^{25}$ −295·5±3° (0·2% water), $[\alpha]_{330}$ −1410°, $[\alpha]_{283}$ min. −5440°, $[\alpha]_{260}$ −2940° (*c*, 0·0092 in water). Light absorption (water) max. 275 mμ (ε 2630). ν_{max} 3380m (OH), 1740–1690s (CO_2H), 1620s (C=C), 1208s (=C–O–) and 862m (=CH_2) cm^{-1} (KCl disk). Other solvent systems gave: from ether/light petroleum (b.p. 60–80°) m.p. 112° (decomp.) (C, 51·4; H, 5·0. Calc. for $C_{10}H_{10}O_6,\frac{1}{2}H_2O$: C, 51·2; H, 4·7%) and from ethyl acetate/carbon tetrachloride a solid, m.p. 115° (decomp.), analysing for the anhydrous acid +10% CCl_4.

Determination of Molecular Formula

The exact mass of the molecular ion from chorismic acid was determined relative to the fragment C_4F_9 (mass 218·9856) in the spectrum of heptacosafluorotributylamine and was found to be 226·0479. $C_{10}H_{10}O_6$ requires 226·0477. The error in this determination is estimated to be less than 5 p.p.m.

3,6-Dihydrochorismic Acid (VI)

A solution of chorismic acid (1·5 g) in distilled water (20 ml) was hydrogenated over 10% palladized charcoal (0·5 g). One mole of hydrogen was rapidly absorbed (20 min). The n.m.r. spectrum of a concentrated solution of crude dihydro-acid in deuterium oxide showed the following absorptions: broad singlet, 3·4 (1H); doublet, 4·8 (*J* 2·8, 1H); doublet 5·3 (1H); broad multiplet 6·0 (2H); broad multiplet 7·8 (4H).

The solution from the hydrogenation experiment was treated with a solution of barium acetate (4·4 g) in water (15 ml). Addition of methanol (160 ml) gave a white solid which on reprecipitation from water gave pure *barium 3,6-dihydrochorismate dihydrate* (Found: C, 30·3; H, 3·6; Ba, 34·2. $C_{10}H_{10}O_6Ba,2H_2O$ requires C, 30·0; H, 3·5; Ba, 34·4%).

(−)-trans-*4,5-Dihydroxyclohex-1-ene-1-carboxylic Acid (VII)*

A solution of dihydrochorismic acid (from 1·6 g of chorismic acid) prepared above was heated at 60° for 1 hr, cooled, and continuously extracted with ether for 4 days. After removal of the solvent the crude product (0·52 g) was recrystallized from dioxan/benzene to give pure (−)-trans-*4,5-dihydroxycyclohex-1-ene-1-carboxylic acid* as colourless crystals, m.p. 187–188°; $[\alpha]_{5890}^{25}$ −78·5±1°; ν_{max} (KCl) 3333s (OH), 2890m (CH), 1686vs (C=O), 1639s (C=C), 1250s and 1064s (C–O) cm^{-1} (Found: C, 53·1; H, 6·8. $C_7H_{10}O_4$ requires C, 53·2; H. 6·4%).

The dihydroxy-acid (0·30 g) was treated with acetic anhydride (2 ml) in pyridine (7 ml). Working up in the usual way and crystallization from light petroleum (b.p. 90–100°) gave the *diacetate* as colourless crystals, m.p. 96–97°: $[\alpha]_{5890}^{25}$ −352±20°; ν_{max} (KCl) 1736vs (ester C=O), 1672s (acid C=O), 1639m (C=C), 1242s and 1036s (C–O) cm^{-1} (Found: C, 54·5; H, 5·9. $C_{11}H_{14}O_6$ requires C, 54·5; H, 5·8%).

Ozonolysis of (−)-trans-*4,5-Dihydroxyclohex-1-ene-1-carboxylic Acid*

A solution of (−)-*trans*-4,5-dihydroxyclohex-1-ene-1-carboxylic acid (200 mg) in methanol (5 ml) was treated with ozone at a rate of 0·7 mmole/hr of ozone for 1·75 hr. The solvent was removed and the residue was boiled with hydrogen peroxide (1 ml; 100 vol.) and formic acid (1 ml; 90%) until no further evolution of gas occurred. The solution was concentrated and ethanol added to precipitate the crude product (28 mg, 16%). Recrystallization from ethyl acetate afforded the pure (−)-γ,γ'-dilactone of 3,4-dihydroxyadipic acid, m.p. 125–126° (lit.[18] 122–123°); $[\alpha]_{5890}^{25}$ +145±8° (lit.[18] $[\alpha]_D^{19}$ +143±2·5°). The dilactone was converted to the free acid, $[\alpha]_{5890}^{25}$ +20±6° (lit.[18] $[\alpha]_D^{20}$ +19·3±1·3°), and the dihydrazide, m.p. 205–208° (lit.[18] 216–217°), $[\alpha]_{5890}^{25}$ +49·5±3° (lit.[18] $[\alpha]_D^{18}$ +44·5±6°).

(+)-trans-*4,5-Dihydroxycyclohex-1-ene-1-carboxylic Acid*

2,5-Dihydrobenzoic acid, m.p. 120°, was prepared from butadiene and propiolic acid in 50% yield by the method of Emerman.[16]

Hydrogen peroxide (2·7 ml; 100 vol.) was added to a stirred solution of 2,5-dihydrobenzoic acid (2 g) in formic acid (50 ml, 90%) over a period of 1 hr at 30°. The resulting solution was maintained at 40° for a further 4 hr; after removal of the solvents the residue was heated at 100° with water (50 ml) for 1 hr and then evaporated to dryness. The resulting product was crystallized from dioxan/benzene to yield (±)-trans-*4,5-dihydrocyclohex-1-ene-1-carboxylic acid* (1·20 g) as colourless crystals, m.p. 186–187° (Found: C, 53·5; H, 6·5. $C_7H_{10}O_4$ requires C, 53·2; H, 6·4%).

The dihydroxy-acid was converted, by the method described for the active acid, to its *diacetate*, m.p. 95–96° (light petroleum, b.p. 40–60°) (Found: C, 54·4; H, 5·9. $C_{11}H_{14}O_6$ requires C, 54·5; H, 5·8%). Treatment of the diacetate with ethereal diazomethane yielded the methyl ester as colourless crystals from light petroleum (b.p. 90–100°), m.p. 61–62° (lit.[17] 61°); ν_{max} (KCl) 1739s (acetate C=O), 1715m (ester C=O), 1645w (C=C), 1258s and 1242 (C–O) cm^{-1} (Found: C, 56·7; H, 6·5. Calc. for $C_{12}H_{16}O_6$: C, 56·3; H, 6·3%).

3,4-Dihydrobenzoic Acid

This acid was prepared by the method of Plieninger.[7] After purification by chromatography over Dowex-1 anion exchange resin the acid had m.p. 24–26°.

Thermal Decomposition of Chlorismic Acid

Chorismic acid was heated at 210° and 0·4 mm. The decomposition products either sublimed or were condensed in a liquid air trap. A small unidentified residue remained after heating. The major products, which sublimed, were *p*-hydroxybenzoic acid, m.p. 213°, λ_{max} 253, λ_{infl} 273, 283 mμ, and phenylpyruvic acid, m.p. 156° (decomp.), detected by its change in light absorption at 320 mμ with change in pH. Pyruvic acid was detected as its 2,4-dinitrophenylhydrazone. No phenol could be detected in any of the fractions.

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