SHIKIMIC ACID
(3,4,5-TRIHYDROXY-1-CYCLOHEXENE-1-CARBOXYLIC ACID)

BRUCE A. BOHM[1]

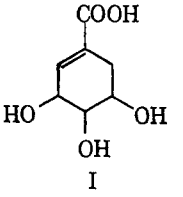
Department of Pharmacognosy, College of Pharmacy,
University of Rhode Island, Kingston, Rhode Island

Received December 18, 1964

CONTENTS

I. INTRODUCTION

Shikimic acid is shown as structure I. That such a seemingly unimpressive molecule should have the position as subject of a review points out the unpredictability of such studies as natural products and intermediary metabolism. Shikimic acid was reported to be a naturally occurring compound in 1885. Nearly 50 years elapsed before the total structure of the molecule had been determined. It is at this point that many naturally occurring compounds essentially vanish, but such was not to be the fate for shikimic acid. In the early 1950's, an impressive series of experiments showed



I

that the title compound was more than just another natural oddity. Shikimic acid was shown to occupy a major position in the formation of the essential aromatic amino acids. As a result of these leading investigations, many scientists, representing a wide variety of disciplines, examined shikimic acid and its derivatives from many points of view. The result is a considerable

(1) This work was supported in part by the National Science Foundation (GB-2200) for which the author expresses his appreciation.

literature. It is the purpose of this review to bring together the significant results of these many workers both past and present.

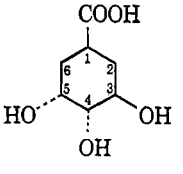
To complete the introduction, let us digress briefly. The first isolation of the subject compound was from the plant *Illicium religiosum* Sieb. It is from this plant's Japanese name, Shikimi-no-ki, that the term shikimic acid is derived.

II. CHEMISTRY OF SHIKIMIC ACID

A. NUMBERING OF THE SHIKIMIC ACID MOLECULE

According to the accepted rules governing nomenclature of organic compounds the proper name for shikimic acid is 3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid. At this point, no difficulty exists. To completely describe the molecule it would be necessary to indicate the stereochemistry of the three hydroxyl-bearing carbon atoms. This information is known with the name then being written 3α,4α,5β-trihydroxy-1-cyclohexene-1-carboxylic acid. In common usage, however, is a system which has the numbers of the hydroxy-bearing carbons reversed. In the incorrect usage, the number three is assigned to the carbon bearing the β-hydroxyl group.

The difficulty in the numbering of this molecule can be traced to an early structural problem, the location of the double bond. Early investigations showed that shikimic acid was a derivative of 3,4,5-trihydroxycyclohexanecarboxylic acid. The stereochemistry of the three hydroxyl groups was subsequently shown to be as is indicated in structure II. Insofar as derivatives of dihydroshikimic acid were used the location of the double bond was not immediately possible. The numbering shown in II was arbitrarily assigned. Had the reverse order been chosen at that time no difficulty would now exist. The unsaturation could have involved either carbons 1 and 2 or 1 and 6. The position was shown to be the latter. By the time that the double bond had been located, investigators had apparently become accustomed to the numbering system which gave the carbons bearing the *cis*-hydroxy groups the numbers 4 and 5. No effort was made to change the numbering to comply with the rule which requires, in effect, that such a compound should be numbered through the double bond and not away from it.



II

Prior to this review the author is aware of only two places where proper numbering of the shikimic acid molecule is to be found. One is *Chemical Abstracts;* the second is a recent paper by Hall (54) whose work on structural details of shikimic acid we shall encounter later on in the review. The remainder of the chemical literature and biochemical literature employ the officially incorrect system.

In order to avoid a good deal of confusion the numbering used in this review will coincide with that in the papers under considerations. The reader should be well aware of this situation and should be able to convert from one system to the other if the need arises.

B. PROPERTIES

Shikimic acid is a white solid for which melting points in the region of 190° have been recorded. The compound is levorotatory and has a specific rotation of −157° (87). A single maximum in the ultraviolet region at 213 mμ (ϵ 8900) has been reported (87).

The reactions of shikimic acid are characteristic of a compound possessing a carboxyl group, three hydroxyl groups, and a double bond. The stereochemistry of the molecule is such that intramolecular reaction between the carboxyl and one of the hydroxyl groups is possible under the appropriate conditions. Reactions of adjacent *cis*-hydroxyl groups also are observed. These features will be described in detail in the following sections.

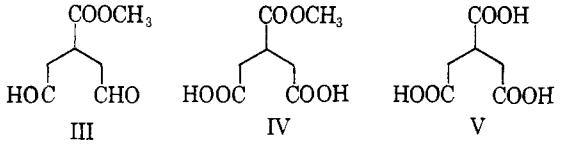
C. DEGRADATIONS AND STRUCTURE DETERMINATION

The first structural studies of the shikimic acid molecule were those of Eykman (25) who first described its isolation from *Illicium religiosum* Sieb. He reported a melting point of 183–184° and an elemental analysis corresponding to $C_7H_{10}O_5$. From these observations he suggested that the new compound resembled the known quinic acid and might indeed be the "anhydride" of quinic acid by which he must have intended the lactone. The relationship with quinic acid was substantiated by the observation that the products of dry distillation and alkali fusion of the two compounds were the same. Treatments of this sort would be expected to convert these compounds to aromatic derivatives, *e.g.*, protocatechuic acid, *p*-hydroxybenzoic acid, etc. Eykman recognized the new compound as an addition product of benzene and placed it in a group of compounds referred to by him as "benzénique."

Six years elapsed before the appearance of Eykman's second paper (26) which described a more detailed investigation of the nature of the shikimic acid molecule. He described the preparation of the triacetyl, tripropionyl, and tributyryl esters, a dibromide, a bromolactone, and dihydroxyshikimic acid. He reduced shikimic acid with sodium amalgam and recorded a change in optical rotation from −45 to −17.4° and showed that an acid-catalyzed decomposition product was *p*-hydroxybenzoic acid. Based on his observations he gave six possible polyhydroxycyclohexenecarboxylic acid structures which could behave as the unknown did.

One of the postulated structures was correct, but an unequivocal assignment of structure had to await a more detailed and systematic degradation study.

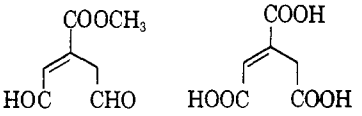
The detailed investigation appeared in the form of four papers by Fischer and Dangschat between 1934 and 1937. In these, the complete structure and configuration of shikimic acid, and hence many of its derivatives, were completely elucidated. In the first of these papers (28) the oxidation of methyl dihydroshikimate with periodic acid was described. The product was the dialdehyde III which was not isolated but was converted to the corresponding diacid IV with bromine



water. The dialdehyde was isolated as the bis-*p*-nitrophenylhydrazone for identification purposes. The methyl ester IV was hydrolyzed to give tricarballylic acid (V). Therefore, shikimic acid was a cyclohexenecarboxylic acid with hydroxyl groups at carbons 3, 4, and 5. The double bond must then be located between carbons 1 and 2 or between carbons 1 and 6.

Substantiation of the suggested structure was provided by the following. Dihydroshikimic acid was converted *via* the Curtius reaction into the corresponding amine. Titration of the amine with lead tetraacetate showed that 2 equiv. of the reagent were consumed. Methyl dihydroshikimate also consumed only 2 equiv. of lead tetraacetate. It was concluded from these observations that the amine function was not adjacent to a hydroxyl function, which is in agreement with the formation of the tricarballylic acid derivative by periodate oxidation of methyl dihydroshikimate.

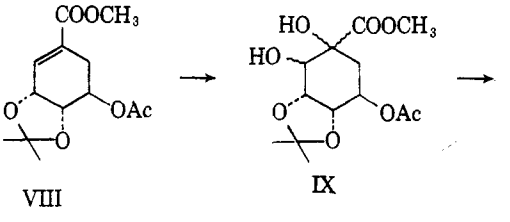
A further check on the above was afforded by the results of Fischer and Dangschat's (29) treatment of methyl shikimate with periodic acid. Carefully controlled oxidation gave aconitic acid methyl ester dialdehyde (VI). Without isolation the dialdehyde was converted to the corresponding diacid by oxidation with perpropionic acid followed by saponification of the ester function. The product was *trans*-aconitic acid

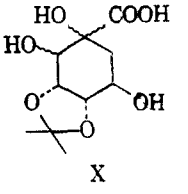


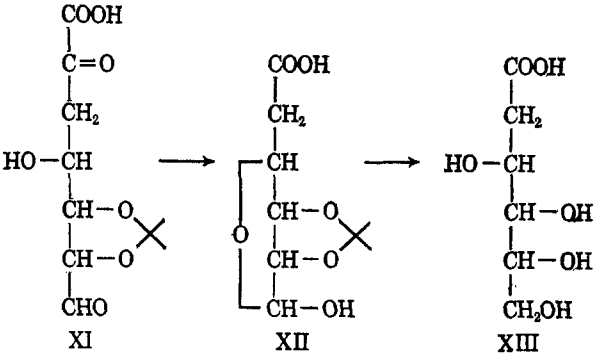
(VII). Thus, location of the three hydroxyl functions was established as shown in formula I. There still remained the tasks of locating the double bond, determining the relative configuration of the functional groups within the molecule, and establishing the relationship of the molecule to some substance whose stereochemical configuration was known.

The configuration of the functional groups with relation to each other was the subject of the third paper by Fischer and Dangschat (30). When dihydroshikimic acid was heated to 190° and then distilled at 220° (0.2 mm.), a lactone was formed. The lactone was unreactive toward lead tetraacetate indicating that adjacent, free hydroxyl groups did not exist. The lactone, thus, involved the hydroxyl function on carbon 4. It also indicated that the carboxyl and hydroxyl functions were *cis* with respect to each other. It was also demonstrated that an acetonide could be formed by treating the appropriate shikimic acid derivative with acetone under acidic or dehydrating conditions. Acetonide formation requires the presence of adjacent *cis*-hydroxyl functions; thus, one of the other hydroxyls in shikimic acid must be *cis* to the one at carbon 4.

The fourth of the series of papers (31) established the location of unsaturation and the stereochemistry. The degradation scheme employed is shown below.

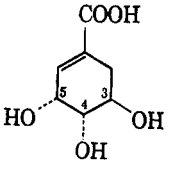






The methyl shikimate derivative VIII was hydroxylated with potassium permanganate to yield the pentahydroxy derivative IX. The ester functions were cleaved by treatment with sodium hydroxide to give X. Oxidation of compound X with periodic acid yielded the open-chain acid XI after consumption of a single equivalent of reagent. Mild oxidation by bromine in acetic acid resulted in the loss of CO_2 and formation of the hemiacetal acid XII. Reduction of XII with a nickel catalyst at 60 atm. pressure yielded glucodesonic acid (XIII). Glucodesonic acid prepared from shikimic acid

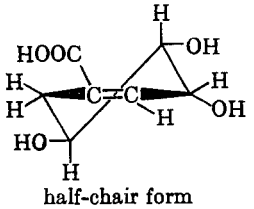
agreed in all respects with authentic material. This degradation establishes the double bond between carbons 1 and 6 and describes the hydroxyl functions at carbons 3, 4, and 5 as equivalent, stereochemically, to the hydroxyl functions at carbons 3, 4, and 5 in the glucose molecule. The structure is given as XIV.



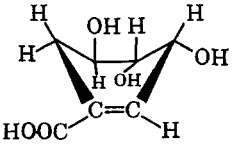
XIV

D. STEREOCHEMISTRY

The experiments of Fischer and Dangschat just described related the hydroxyl functions of shikimic acid to glucose hydroxyls on carbons 3, 4, and 5. There remained the problem of describing the geometry of the entire ring structure. In a recent paper Hall (54) presented the results of his study of the n.m.r. spectrum of shikimic acid. For a substituted cyclohexene system four extreme structures are possible, two with the half-chair form and two with the boat form. Two structures are possible for each form because of the possibility of axial and equatorial dispositions of the substituents. From the known stereochemistry of the molecule it is possible to write two forms, one half-chair and one boat, in which nonbonded interactions are minimized. These structures are represented below.



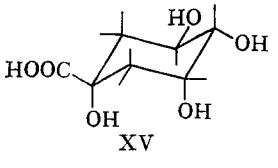
half-chair form



boat form

The spectrum observed for shikimic acid agreed with the existence of the molecule in a half-chair form with a minor contribution from the boat form.

There remains the related problem of geometry of the saturated derivative of shikimic acid, *i.e.*, quinic acid. Quinic acid represents a substituted cyclohexane system in which clear-cut boat and chair forms would be expected. Based on good precedent it would seem sensible to suggest the chair form for quinic acid. Hanson (55) has suggested that structure XV represents the most stable conformation of quinic acid. It would be of interest to see quinic acid as well as other members of the shikimic acid pathway examined by Hall's procedure.



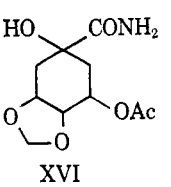
XV

E. SYNTHESIS

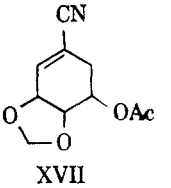
1. Interconversions of Similar Molecules

The resemblance of quinic acid and shikimic acid was noted by several of the early investigators. Of considerable interest to the entire structural picture of these compounds would be an unequivocal conversion of one to the other. Of even more interest would be conversions in both directions. These conversions have been accomplished thus establishing a very important stereochemical relationship.

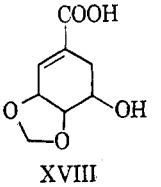
A brief description of a conversion of quinic acid to shikimic acid was published in 1938 by Dangschat and Fischer (18, 32). The amide of 3-acetyl-4,5-methylenequinic acid (XVI) was synthesized and then treated with *p*-toluenesulfonyl chloride in pyridine. This treatment accomplished three things: (1) dehydration of the amide function to a nitrile, (2) formation of the 1-*p*-toluenesulfonate ester, and (3) base-catalyzed elimination of the C-1 ester function to yield the unsaturated product XVII. Treatment of the nitrile with sodium hydroxide yielded 4,5-methyleneshikimic acid



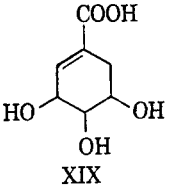
XVI



XVII



XVIII



XIX

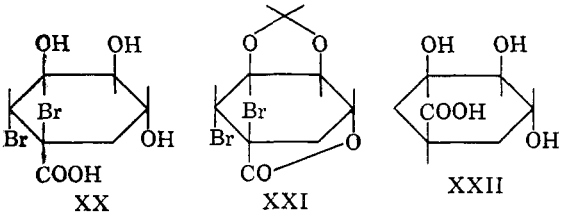
(XVIII) which was identical with authentic material prepared by treating shikimic acid (XIX) with formaldehyde and HCl.

The conversion of shikimic acid to quinic acid would involve addition of a molecule of water across the double bond of shikimic acid. Two approaches to the problem would involve: (a) direct addition of H_2O, or (b) addition of some reagent followed by replacement by a hydroxyl function. In either case, stereochemical considerations would be of paramount importance.

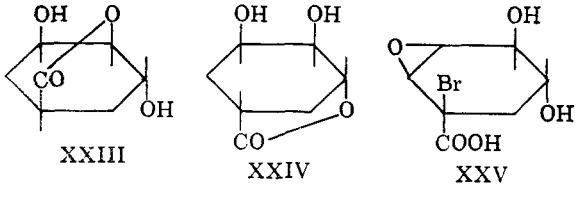
An excellent and detailed study of the desired conversion was published by Grewe and Lorenzen in 1953 (51). The route chosen involved the second alternative above and was based upon Eykman's (26) observation that bromine added readily to shikimic acid.

Two reaction sequences were carried out which unequivocally established the stereochemistry of the addition product. For our purposes it will be most convenient to show the correct structures and indicate how the experimental observations corroborate these conclusions pointing out possible trouble spots as the discussion pro-

ceeds. The dibromide was initially assigned the structure XX which corresponds to the stereochemistry of the quinic acid molecule. The resemblance to quinic acid was strongly indicated on the grounds that treatment of the dibromide XX with acetone in the presence



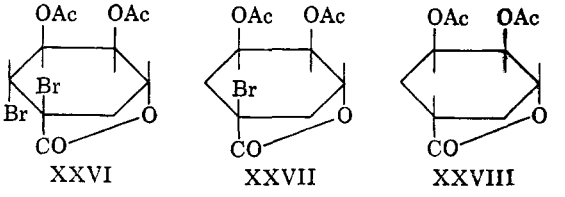
of dilute mineral acid resulted in the formation of the lactone acetonide XXI. A similar transformation is characteristic of quinic acid. When the dibromide was catalytically reduced in a buffered system, a bromine-free compound XXII was formed. Reduction of shikimic acid produced the same product. Therefore, the hydroxyl functions of the dibromide correspond to the original shikimic acid stereochemistry. Treatment of the trihydroxy acid XXII with acid yielded a lactone which was not reactive toward lead tetraacetate. This observation is consistent with the expected behavior of the lactone XXIII wherein the carboxyl function is β and thus *cis* to the hydroxyl function on C-4. If the carboxyl function had been in the α configuration, the lactone XXIV would have been formed. Lactone XXIV would be expected to be reactive toward lead tetraacetate. The carboxyl function in compounds XXII and XXIII must occupy the β or epi configuration.



The second reaction sequence was constructed on the knowledge that *trans*-halohydrins undergo cyclization to form epoxides upon treatment with base. If XX is the correct structure for the dibromide, epoxide formation involving the hydroxyl and bromine functions on C-5 and C-6, respectively, should occur under the appropriate conditions. Upon treatment with cold, dilute, aqueous base a bromine-containing epoxide was indeed formed. The structure is shown as compound XXV. Reduction of the epoxide, after absorption of 2 equiv. of hydrogen, gave a bromine-free product. Acetylation yielded a diacetyllactone identical with the diacetate of compound XXIII.

With the establishment of the stereochemistry of the dibromide as that of the quinic acid molecule attention could be turned to the next steps, replacement of the C-1 bromine by hydroxyl and replacement of the C-6 bromine by hydrogen. Several possibilities can be considered.

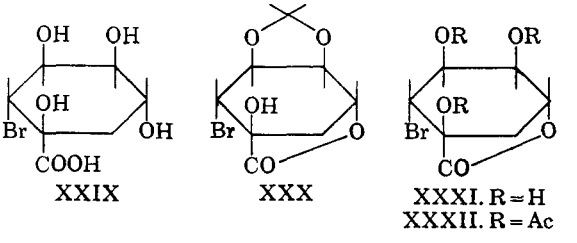
One possibility involved the diacetylbromolactone XXVII which was prepared from compound XXVI by



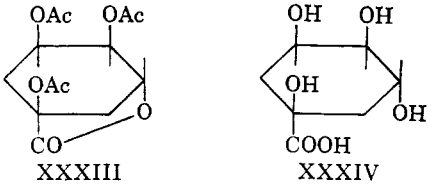
reduction in buffered solution. Unfortunately, the yields of XXVII were very small. The reduction product was very largely the completely debrominated XXVIII.

A second synthetic possibility involved carefully controlled, partial hydrolysis of the original dibromide XX. Conditions could not be found for this conversion, the sole product of the reaction was the bromoepoxide XXV.

Under the influence of silver carbonate, however, only the tertiary bromine atom was touched. The product was bromoquinic acid, here shown as compound XXIX. That no Walden inversion had occurred was shown by the facility with which the product formed the lactone acetonide XXX typical of quinic acid. The trihydroxybromolactone XXXI was then acetylated yielding the triacetate XXXII. Catalytic



reduction of the triacetate in dilute acid resulted in the formation of bromine-free triacetylquinide XXXIII which was identical in all respects with authentic material. Alkaline hydrolysis yielded quinic acid (XXXIV). The over-all conversion of shikimic acid to quinic acid was accomplished in 50% yield.



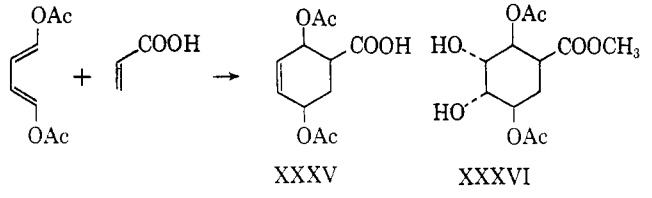
It is of interest to point out that Eykman, in his second paper (26), actually synthesized the 6-bromoquinide XXX by treating the dibromide addition product with water. His melting point, 235°, agrees well with the 228–234° reported by Grewe and Lorenzen (51). Although Eykman's reactions constituted the earliest reported conversion of a shikimic acid derivative to a quinic acid derivative, the structures of

the starting material and product were not known. The credit must go in full to Grewe and Lorenzen whose work was well planned and adeptly executed. These workers were at all times aware of the multitude of stereochemical pitfalls which plague work of this sort and handled the problems in such a way that the maximum amount of information was obtained.

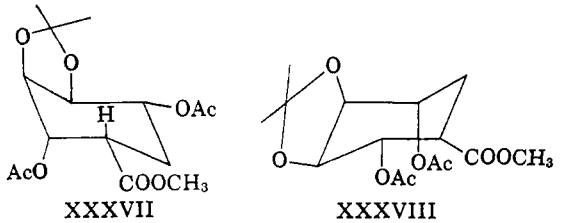
2. *Total Syntheses*

To date, four total syntheses of shikimic acid have been reported. In each case a Diels–Alder reaction was utilized for the formation of the six-membered ring. The products of these cyclizations either had oxygen at the necessary positions or possessed double bonds which were hydroxylated in subsequent operations.

The first successful synthesis of shikimic acid was that of McCrindle and co-workers (87). *trans,trans*-1,4-Diacetoxybutadiene was allowed to react with acrylic acid at 85–90° for 3 hr. to the yield the cyclohexene derivative XXXV. Treatment of XXXV with osmium tetroxide followed by diazomethane yielded structure XXXVI. The authors predicted the stereochemistry shown on the basis of the Alder rules. The oxygen functions at carbons 3, 4, and 5 are, respectively, α, α, and β, the configuration required for the shikimic acid molecule. In addition, the stereochemistry of the acetate function at C-6 and the hydrogen at C-1 make the next, critical step possible according to these workers. In preparation for the next step the acetonide was synthesized. Its structure is given as XXXVII and was rationalized on the grounds that fewer nonbonded interactions were present compared to the alternate structure XXXVIII. In the former a *trans* diaxial elimination of C-1 hydrogen and C-6 acetate should be possible. When heated to 290° with magnesium oxide, elimination occurred yielding the desired unsaturated product XXXIX. Elimination could also be brought about by treatment with sodium in anhydrous methanol. Hydrolysis of compound XXXIX with acid to remove the acetonide function and with base to cleave the ester functions yielded a product which was identical in all respects with authentic *dl*-shikimic acid. A satisfactory resolution using quinine methohydroxide salt of shikimic acid triacetate was accomplished.

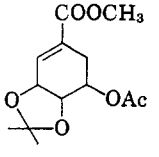


XXXV XXXVI



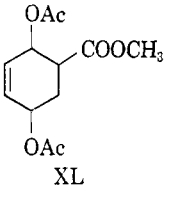
XXXVII

XXXVIII

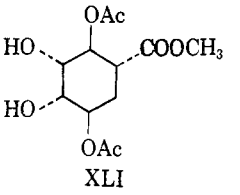


XXXIX

A similar synthesis was reported by Smissman and co-workers (118). According to these workers reaction between *trans,trans*-1,4-diacetoxybutadiene and methyl acrylate in boiling xylene for 37 hr. gave the adduct XL in 93% yield. Attempts to hydroxylate XL with potassium permanganate were unsuccessful, but osmium tetroxide converted XL to the dihydroxy derivative XLI. The stereochemistry assigned to this last product differs from that of the similar compound prepared by McCrindle, *et al.* (87), in that the carboxymethyl function is written α in contrast to the β assignment of the British workers.



XL

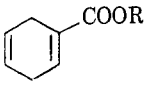


XLI

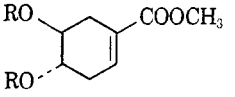
In the present work base-catalyzed elimination using a variety of reagents failed. Pyrolysis at 285° (0.007 mm.), however, brought about elimination of acetic acid to form the desired shikimic acid derivative in 95% yield. Hydrolysis and resolution using α-phenylethylamine yielded shikimic acid identical with an authentic sample.

A possible explanation for the stereochemical difference encountered above involves the somewhat different synthetic procedures used by the two groups of workers in the initial Diels–Alder reaction. McCrindle, *et al.*, used acrylic acid as the dienophile, a lower temperature, and a short reaction period, whereas Smissman, *et al.*, used methyl acrylate, higher temperature, and a long reaction time. Examination of the appropriate derivatives in the two sequences, however, indicates that the same compounds were ultimately used by the two groups for the elimination step. In each synthesis methyl 2,5-diacetoxy-3,4-dihydroxycyclohexane carboxylate was prepared. The ester from McCrindle, *et al.*, melted at 161–163° while that from the Americans melted at 166–167°. The acetonide of the former melted at 146–147° while that of the latter melted at 143–144°. It would seem unlikely that the compounds were isomers. However, it might be of interest to compare the compounds directly with regard to mixture melting point and spectral characteristics.

A recent synthesis has been reported by Grewe and Hinrichs (48) in which acetylenecarboxylic acid and butadiene were allowed to react at 130–140° under autogenous pressure to yield 85% of cyclohexadienecarboxylic acid (XLII). Esterification yielded XLIII. Treatment of the diene ester with performic acid followed by hydrolysis of the epoxide gave the *trans*-diol XLIV. The diol was acetylated in 80% yield with acetic anhydride in pyridine.

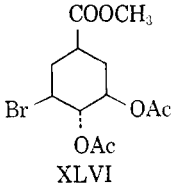


XLII. R = H
XLIII. R = CH_3

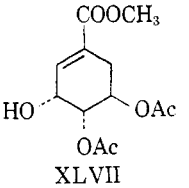


XLIV. R = H
XLV. R = Ac

The diacetate XLV was then dissolved in carbon tetrachloride and treated with N-bromosuccinimide. The product of the allylic bromination is shown as structure XLVI with the bromine atom *trans* to the adjacent acetate function and presumably lying in an equatorial conformation. Without purification the bromide was treated with silver acetate and an equiv-



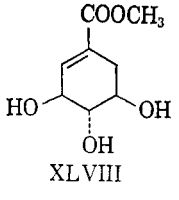
XLVI



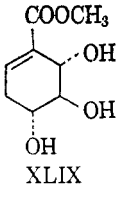
XLVII

alent amount of water to form the hydroxy derivative XLVII with inverted conformation at C-5. Removal of the acetate functions yielded *dl*-shikimic acid methyl ester identical with authentic material. The over-all conversion from XLII was 20%.

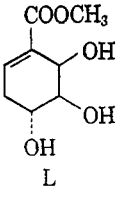
In addition to the desired compound three other related substances were found in the final reaction mixture. These, here listed with their yields, were *dl*-epishikimic acid methyl ester, 5%, *dl*-epiisoshikimic acid methyl ester, 1%, and *dl*-isoshikimic acid methyl ester, 7%, shown below as XLVIII, XLIX, and L, respectively.



XLVIII

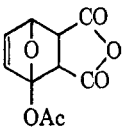


XLIX

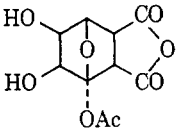


L

An unsuccessful attempt to synthesize shikimic acid was also reported by McCrindle and his co-workers (87). Reaction of 2-acetoxyfuran with maleic anhydride gave the Diels–Alder adduct LI. Hydroxylation by osmium tetroxide gave the diol LII, the assumed stereochemistry of which is shown. Conversion of this compound to the acetonide was accomplished with acetone and anhydrous copper sulfate. The next steps of

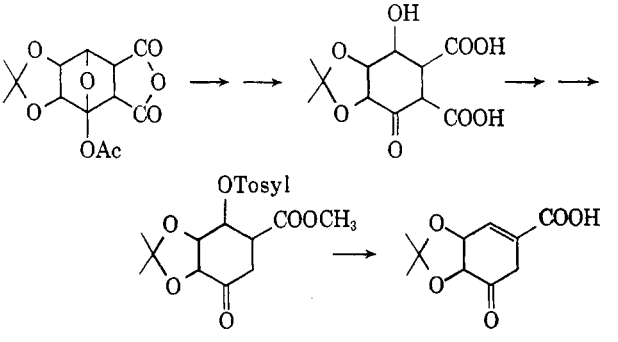


LI



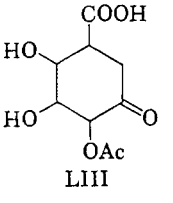
LII

the synthesis were to have been: cleavage of the hemiketal acetate, hydrolysis of the anhydride to the dicarboxylic acid, selective decarboxylation of the β-keto acid system, and elimination of the *p*-toluenesulfonic ester. The proposed synthesis is shown in the following sequence.

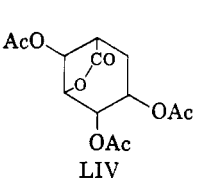


In practice these workers were unable to accomplish the cleavage of the hemiketal acetate without also bringing about aromatization.

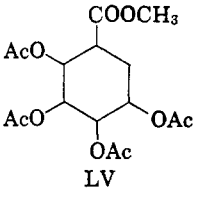
A successful synthesis based essentially upon the same sequence of reactions was accomplished by Doshi (24). The *exo-cis*-diol LII was stirred for 3 days with water which resulted in opening of the hemiketal acetate ring system, decarboxylation, and acyl migration to form the quinic acid derivative LIII in 66% yield. This diol yielded an acetonide indicating that the hydroxyl groups were *cis*. Reduction of the diol with sodium borohydride followed by treatment with acetic anhydride gave the lactone LIV indicating that the carboxyl function and the C-3 alcohol group were *cis*. The



LIII



LIV



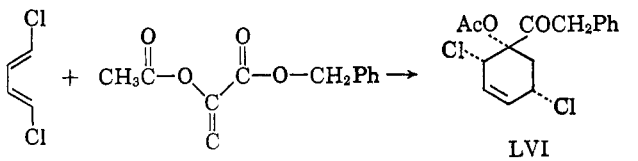
LV

lactone was converted to the methyl tetraacetoxycyclohexanecarboxylate LV by treatment with acidic methanol followed by acetic anhydride. The tetraacetate was converted in 92% yield to methyl 3,4,5-triacetylshikimate by heating under vacuum at 260° in the presence of soft glass powder. Alkaline hydrolysis yielded *dl*-shikimic acid in 75.5% yield (from the tetraacetate).

As pointed out above, quinic acid and shikimic acid differ only by the elements of water. Synthesis of quinic acid by an unequivocal route followed by dehy-

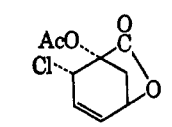
dration would give shikimic acid. Thus, a total synthesis of quinic acid would also fit into the present section. While no review of the synthesis of quinic acid is intended, the following synthesis fits into the current discussion insofar as it too is based upon a successful Diels–Alder reaction.

Smissman and Oxman (117) reported the synthesis based upon the condensation of *trans,trans*-1,4-dichlorobutadiene and benzyl α-acetoxyacrylate to form the cyclohexene derivative LVI. The initial adduct was converted into the chlorolactone LVII by heating at 150° for 22 hr. *cis*-Hydroxylation using osmium

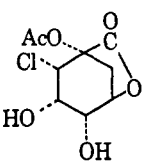


LVI

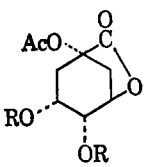
tetroxide gave 1-acetyl-6-chloroquinide (LVIII) in 46% yield. The chlorine was removed in excellent yield using a Raney nickel catalyst to give LIX. Con-



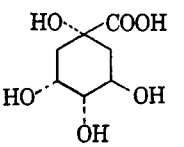
LVII



LVIII



LIX, R = H
LX, R = Ac



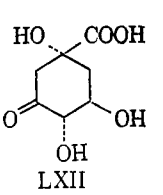
LXI

version of the thus formed 1-acetylquinide to the triacetyl compound LX was accomplished by reaction with acetic anhydride. Triacetylquinide was converted to quinic acid LXI by heating with aqueous potassium hydroxide followed by purification using ion-exchange chromatography.

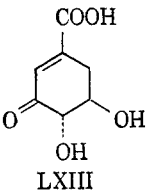
3. *Synthesis of Other Derivatives*

a. Synthesis of 5-Dehydroquinic and 5-Dehydroshikimic Acids

Grewe and Jeschke (50) have reported a very convenient synthesis of 5-dehydroquinic acid. Controlled oxidation of quinic acid with nitric acid yielded the product LXII in about 60% yield. The nature of the product was confirmed by chemical and biological tests. When 5-dehydroquinic acid was heated in the presence of acid, 5-dehydroshikimic acid (LXIII) was obtained. Thus, a variety of derivatives of these major pathway compounds becomes available as a result of simple synthetic procedures.



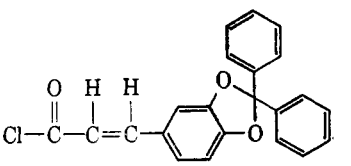
LXII



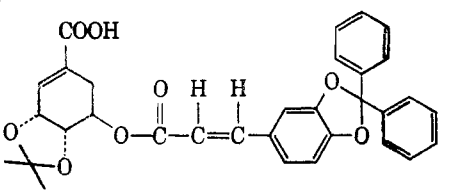
LXIII

b. Synthesis of 3-O-Caffeoylshikimic Acid

A synthesis of 3-O-caffeoylshikimic acid (dactylic acid) has been reported by Maier and co-workers (85). Diphenylmethylenedioxycaffeoyl chloride (LXIV) was condensed with shikimic acid acetonide to yield the protected product LXV. Hydrolysis with a mixture of acetic and hydrochloric acid afforded the product LXVI which was purified chromatographically. 3,4-Di-

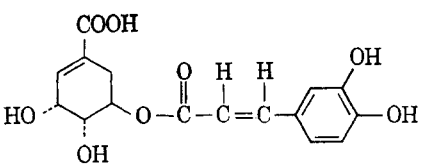


LXIV



LXV

methoxycinnamoyl chloride and methyl shikimate acetonide were condensed in a second synthesis to yield a compound which, after removal of the acetone function, was identical with the diazomethane reaction product of the original unknown.



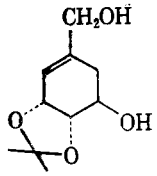
LXVI

F. REACTIONS

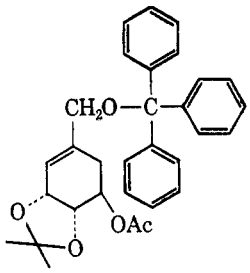
It is not within the scope of this review to discuss each of the reactions which shikimic acid undergoes. It is intended, however, to discuss some of the work which has been reported concerning major derivatives constructed on the shikimyl skeleton. Included in the discussion will be shikimyl alcohol, shikimaldehyde, shikimyl bromide, and shikimylamine.

The synthesis of shikimyl alcohol has been reported by Grewe and his group (49), who have studied a number of interesting reactions of this compound. Although shikimyl alcohol itself proved impossible to purify, many of its derivatives were crystalline compounds which allowed their preparation in a high de-

gree of purity. Reduction of methyl isopropylideneshikimate with lithium aluminum hydride gave the diol LXVII in excellent yield. Isopropylideneshikimyl alcohol was converted into a variety of derivatives in-



LXVII

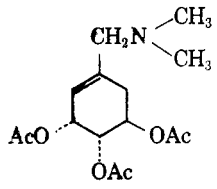


LXVIII

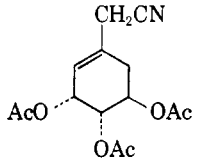
cluding the 7-benzoate and the 3-acetyl-7-trityl ether LXVIII.

Treatment of isopropylideneshikimyl alcohol with dilute mineral acid, in an attempt to remove the protecting group, yielded only acetone and benzaldehyde. When dilute acetic acid was used, some benzaldehyde was still formed but the product was optically active. Addition of sodium acetate to the reaction mixture repressed the formation of benzaldehyde to the extent that 90% yields of the alcohol could be obtained. Several derivatives were synthesized including the tri- and tetraacetates and the 7-trityl ether. 3,4,5-Triacetylshikimyl alcohol was converted to the corresponding bromide in excess of 80% yield by treatment with PBr_3. Treatment with dimethylamine converted the bromide into dimethyltriacetylshikimylamine (LXIX) in 74% yield. The amine was identical with the product obtained from N,N-dimethyl-3,4,5-triacetylshikimic acid amide by lithium aluminum hydride reduction followed by acetylation.

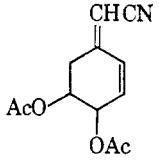
Treatment of the allylic bromide with potassium cyanide did not give the expected triacetylshikimyl cyanide (LXX). Instead there was formed a new compound which was shown to be 4,5-diacetoxycyclohex-2-enylidene acetonitrile (LXXI).



LXIX



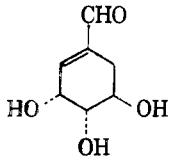
LXX



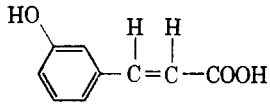
LXXI

Attempts to synthesize shikimaldehyde (LXXII) have been described by Grewe and Buttner (47). Neither Rosenmund reduction of triacetylshikimoyl chloride nor manganese dioxide oxidation of the shikimyl alcohol yielded the desired product. Successful reduction of the acid chloride was achieved using sodium trimethoxyborohydride at −80°. Under these conditions 85% yields of triacetylshikimaldehyde were realized. Saponification of triacetylshikimaldehyde with aqueous base yielded a new aldehyde and the products of a Cannizzaro reaction, *i.e.*, shikimyl alcohol and shikimic acid. Shikimaldehyde was obtained in good yield by saponification at −10° with sodium methoxide in methanol.

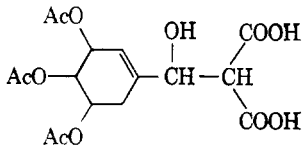
Shikimaldehyde was treated with malonic acid, pyridine, and piperidine in an attempt to form the Knoevenagel condensation product. The sole product from the reaction was *m*-hydroxycinnamic acid (LXXIII). The authors pointed out that the extensive elimination with concomitant aromatization can be explained by writing the Knoevenagel intermediate as LXXIV.



LXXII



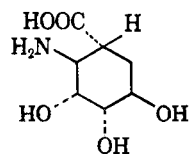
LXXIII



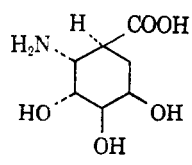
LXXIV

In this structure two oxygen-containing allylic functions are present which should readily undergo elimination. In addition the stereochemistry of the acetate functions would facilitate base-catalyzed eliminations. It was concluded that nucleophilic attack on shikimaldehyde or its derivatives would result in aromatization.

The knowledge of the biological conversion of shikimic acid to anthranilic acid prompted Pleininger and Schneider (100) to investigate the reaction between shikimic acid and ammonia. A mixture of shikimic acid and ammonium hydroxide was heated in a sealed container at 150° for 50 hr. A minor product, probably an unsaturated amino acid, was not investigated. The major product was shown to consist of two compounds of very similar properties. Based on the observations that both compounds could be converted into anthranilic acid by treatment with mineral acid and the knowledge that ammonia is known to add to α,β-unsaturated acids to give β-amino acids, the structures LXXV and LXXVI were proposed. The N-benzoyl derivatives were prepared and treated with acetone and anhydrous



LXXV



LXXVI

HCl. Compound LXXV formed an acidic acetonide agreeing with the postulated structure. Compound

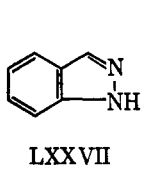
TABLE I
PAPER CHROMATOGRAPHIC BEHAVIOR OF SHIKIMIC ACID AND SOME OF ITS DERIVATIVES

System	R_f[a] SA	QA	DSA	PSA	Ref.
EtOH–H_2O (75:25)	52	...	...	...	90
EtOH–AmOH–1 *M* HOAc (2:1:1)	65	...	40	...	88
n-PrOH–NH_4OH–H_2O (75:1.5:23.5)	16	...	...	...	90
n-PrOH–HOAc–H_2O (75:1.5:23.5)	71	...	...	...	90
n-PrOH–35% NH_3–H_2O (6:3:1)	44	48	...	...	62
i-PrOH–H_2O (3:2)	68	57	...	...	65
i-PrOH–Pyr–H_2O–HOAc (8:8:4:1)	78	50	...	...	43
n-BuOH–HCOOH–H_2O (50:2.5:10)	43	...	46	...	90, 116
n-BuOH–HCOOH–H_2O (20:1:4)	43	...	...	...	78
n-BuOH–HOAc–H_2O (6:1:2)	77	...	...	...	68
n-BuOH–HOAc–H_2O (50:1.5:12.5)	38	...	...	...	90
n-BuOH–HOAc–H_2O (50:3:10)	37	...	...	02	21
n-BuOH–HOAc–H_2O (50:3:12.5)	42	...	22	...	98,[b] 116
n-BuOH–HOAc–H_2O (4:1:5)	46	24	...	...	65
n-BuOH–EtOH–borate buffer (1:1:1)	27	42	...	...	17[c]
t-BuOH–1 *M* HOAc (3:1)	45	...	10	...	88
t-BuOH–HCOOH–H_2O (70:15:15)	48	...	...	...	90
t-BuOH–BenzOH–*i*-PrOH–H_2O (1:3:1:1)	42	23	50	...	10, 62, 116
t-BuOH–88% HCOOH–H_2O (2:1:1)	75	...	10	...	88
n-AmOH–5 *M* HCOOH (1:1)	19	...	...	...	66
EtOAc–HOAc–H_2O (3:1:3)	50	36	...	...	71
Ether–acetone–HOAc–H_2O (6:3:3:1)	34	26	...	...	131
2% HOAc	84	94	...	...	65
Phenol–H_2O–98% HCOOH (3:1:0.04)	30	...	...	...	66, 98
Phenol–H_2O (3:1) plus 0.9% HCOOH	56	43	...	...	62
Phenol–H_2O (4:1)	55	...	60	...	63
Phenol–H_2O (3:1)	44	...	48	...	116
Phenol sat'd. with H_2O	40	...	...	09	21
Phenol–H_2O–HCOOH (3:1:1%)	58	50	73	...	10

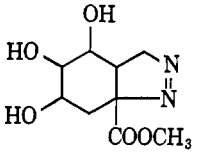
[a] R_f × 100 for shikimic acid (SA), quinic acid (QA), dehydroshikimic acid (DSA), and phosphoshikimic acid (PSA). [b] Whatman No. 3MM paper, ascending. [c] Whatman No. 4 paper pretreated with borate buffer, ascending.

LXXVI formed a neutral lactone which was in agreement with the stereochemistry of the carboxyl function and the hydroxyl group on C-3.

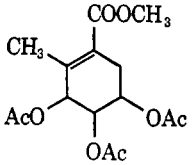
The reaction of shikimic acid and some of its derivatives with diazomethane has been investigated by Grewe and Bokranz (46). Reaction between diazomethane and shikimic acid itself yielded, after vacuum distillation, indazole (LXXVII) whose formation through the intermediate LXXVIII was postulated.



LXXVII



LXXVIII



LXXIX

Attempts to isolate the intermediate failed. When shikimic acid triacetate was used, the product LXXIX was that arising from an insertion reaction. Insertion products at C-6 were also observed when the isopropylidene and isopropylidene acetate derivatives of shikimic acid were employed.

III. ANALYTICAL METHODS

A. PAPER CHROMATOGRAPHIC BEHAVIOR

The simplest and by far the most common analytical device used in the study of shikimic acid and its derivatives is paper chromatography. This part of the analytical section deals with the various systems developed for chromatographic analysis and will present the solvent mixtures involved and the R_f values of the compounds studied. References to quinic acid are common in the literature and some will be included although no attempt to be thorough has been made. An occasional reference to the chromatographic behavior of other derivatives will be included.

In most instances Whatman No. 1 paper was used. Note will be made when a special technique was described. Minor differences in R_f values for a single compound from different laboratories can usually be explained as a temperature effect. (See Table I.)

B. DETECTION METHODS

Neither shikimic acid nor quinic acid are colored in visible light and neither fluoresce under ultraviolet light. To locate these compounds on paper chromatograms one must use a reagent which produces a color reaction. Available sites of reaction on the molecules include the carboxyl function, detectable using acid–base indicators, and the vicinal hydroxyl groups which are reactive toward several reagents. Listed in Table II are reagents which have been tested in several laboratories and generally found to give satisfactory and reproducible results.

TABLE II

CHROMATOGRAPHIC SPRAY REAGENTS USEFUL FOR SHIKIMIC ACID DERIVATIVES

Reagent	Color	Ref.
Potassium periodate, oversprayed with sodium borate–starch solution	White spot on blue background	89, 98
Bromocresol green	Yellow with acids	10
Sodium metaperiodate oversprayed with sodium nitroprusside and piperazine	Green-yellow with SA; orange-yellow with QA[a]	10, 11, 62
Alkaline silver nitrate	Black	17, 62
Periodic acid in acetone	Red with SA; white with QA	43
Potassium permanganate in acetone	White-yellow with SA; yellow with QA	43
Ferric ammonium sulfate	Yellow-green with SA and QA	65

[a] SA, shikimic acid; QA, quinic acid.

C. ISOLATION AND PURIFICATION

A considerable number of investigators have at one time or another isolated shikimic acid. Similarly, a considerable number of purification procedures have been used. In a later section methods for production of shikimic acid labeled with C-14 will be described. At the present time, it should suffice to mention some of the generally useful procedures and a few of the better sources.

Species of *Illicium* have been used by several people as sources of shikimic acid. Indeed, the original isolation of shikimic acid was from *I. religiosum*. In a study of the toxic principle of *I. anisatum* Lane and co-workers (80) isolated a large quantity of shikimic acid. After defatting, the crude drug was extracted with ethanol. Evaporation of the ethanol solution yielded a concentrated extract from which crude shikimic acid precipitated after several days standing. Twenty kilograms of defatted drug yielded approximately 600 g. of crude acid. No further purification was described.

Eucalyptus citriodora Hook. was shown by Anet and co-workers (1) to be a reasonable source of shikimic acid. Ten kilograms of young leaves yielded 3 g. of purified shikimic acid obtained in the following way. Zeocarb 225 resin was used to rid the ethanol solution of bases and amino acids. The organic acids were retained by passage through Amberlite IR-4B and displaced by elution with dilute HCl. The acids were eluted in the following order: shikimic, quinic, glutaric, succinic, malic, citric, and phosphoric.

A Dowex-1 column was used by Millican (90). A cell culture supernatant was passed through the column followed by elution with ammonium acetate solution. A second pass through a similar column using dilute acetic acid as the eluting agent gave shikimic acid of reasonably good purity. Successive separations using Dowex-50 and Dowex-2 were used by Simonart and Wiaux (116). Again acetic acid was the eluting solvent.

Zaprometov (153) utilized a Celite–charcoal column for purification of shikimic acid. He was also able to isolate shikimic acid 5-phosphate using the mixed column. Perkins and Aronoff (98) used an exclusively paper chromatographic method for purification of shikimic acid. For the first banding, Whatman No. 3MM paper was used with a butanol–water–acetic acid system. A second banding using a phenol–water mixture gave pure shikimic acid.

D. QUANTITATIVE METHODS

Several quantitative determinations of shikimic acid have been developed all of which involve, as the first step, oxidation by periodic acid. The glycol cleavage product is then treated with a chromogenic reagent and the concentration determined colorimetrically. The details of the procedures are sufficiently different to warrant a brief look at each.

The first procedure is that of Yoshida and Hasegawa (151). Samples were oxidized in an acetate buffer (pH 4.7) and the oxidation product treated with aniline to form the "polymethine pigment." Spectral measurements were made at 510 mμ, at which wave length the optical density is proportional to the concentration in the range of 40 to 200 μg./ml. of shikimic acid. Other common organic acids and quinic acid do not interfere in the reactions. The presence of excessive amounts of polyhydroxy compounds tends to obscure the results owing to reaction with the periodic acid. Treatment of an extract with an appropriate ion-exchange resin eliminated the interference. Aromatic hydroxy acids give a brown color with periodic acid, which can be corrected by running a control with water instead of aniline.

Gaitonde and Gordon (34) observed that an intense yellow color developed when sodium or barium hydroxide was added to the reaction mixture after shikimic acid had been treated with periodic acid (unbuffered). The color could be stabilized by the addition of glycine. The optical density was determined at 380 mμ (ϵ 4.76 $\times 10^4$, calcd. as shikimic acid) within 10 min. after addition of base. The intensity of the absorption begins to diminish as the solution ages. The lower limit for this procedure was about 0.3 μg./ml. of shikimic acid.

Quinic acid produces a pale yellow color in the reaction with sodium hydroxide, but the maximum absorption is not attained for about 20 min. Analyses for shikimic acid must then be done immediately after addi-

tion of the base to avoid quinic acid interference. Other compounds which interfere include gallic acid (3% of optical density of shikimic acid), tryptophan (2%), adrenaline (4%), NAD (4%), NADP (2%), and TDP (6%).

Two procedures have been published using thiobarbituric acid as the chromogenic reagent. The first was that of Saslaw and Waravdekar (112). Shikimic acid was oxidized with periodic acid in the presence of sulfuric acid. Excess periodic acid was removed with sodium arsenite and thiobarbituric acid added. The intensity of the blue solution, read at 660 mμ, must be determined immediately as the color fades on prolonged exposure to light.

The sensitivity of the assay is good; linear response was observed from 1.0 to 12.0 μg./ml. of shikimic acid. Reproducibility was about 5%. Quinic acid gave less than 3% of the absorbancy observed for shikimic acid. A periodate–thiobarbiturate assay for 2-keto-3-deoxyheptonic acid phosphate has been reported (142).

The second thiobarbituric acid assay for shikimic acid was described by Millican (91). Oxidation of shikimic acid was carried out with periodic acid using phosphoric acid as the reaction solvent. After removal of excess periodic acid with sodium arsenite, the thiobarbituric acid was added. The products of the reaction have peaks at 450, 535, and 660 mμ. The red pigment was extracted into cyclohexanone and measured at 535 mμ (ϵ 3.3 × 10^4, calcd. as shikimic acid). Absorption of the chromogen was linear with concentration throughout the range 0.01–0.06 μmole of shikimic acid.

Interference from deoxyribose derivatives and some shikimic acid derivatives was noted. For instance, adenosine gave a 300% color yield based on shikimic acid; deoxyribose gave a 200% yield. 5-Dehydroshikimic acid, quinic acid, and 5-dehydroquinic acid gave 60, 18, and 10% color yields, respectively. 5-Phosphoshikimic acid did not give any color under the conditions of the reaction.

IV. Biochemistry of Shikimic Acid

A. Concerning Naturally Occurring Shikimic Acid Derivatives

5-Dehydroquinic acid, 5-dehydroshikimic acid, shikimic acid 5-phosphate, and the enolpyruvyl ether of shikimic acid 5-phosphate are derivatives of shikimic acid dealt with in detail in the following section. Very few other derivatives of shikimic acid have been discovered in nature. Brief mention should be made of those that have, however.

Although the depside 3-O-caffeoylquinic acid (chlorogenic acid) has been recognized as an important naturally occurring phenolic compound for some time, the existence of similar compounds with shikimic acid remained uncertain until the work of Goldschmid and Hergert (42). In an examination of western hemlock for lignin precursors these workers detected compounds on paper chromatograms which were tentatively identified as *p*-coumaroyl-, caffeoyl-, and feruloylshikimic acids. No details of the structures were determined.

Further evidence for shikimic acid depsides was shown in Hanson and Zucker's (57) study of potato tuber metabolism. *p*-Coumaroylshikimic acid and caffeoylshikimic acid were shown to be possible metabolites. A crude enzyme preparation from potato cortex converted *p*-coumaroylshikimate to the caffeic acid derivative.

During studies of enzymic browning reactions in dates, Maier and co-workers (85) isolated a compound which yielded 1 equiv. each of caffeic acid and shikimic acid on hydrolysis. When the new compound, called dactylic acid, was treated with acetone and anhydrous HCl and then hydrolyzed, the products were isopropylidenecaffeic acid and isopropylideneshikimic acid. Thus, dactylic acid is 3-O-caffeoylshikimic acid. The synthesis of this compound was discussed above.

B. The Shikimate Pathway

The International Union of Biochemistry Commission on Enzyme Nomenclature has recommended that enzyme substrates be referred to in the ionic form if this is the form in which they occur under physiological conditions. Insofar as the ionized forms of the compounds are actually the functioning entities this is the logical choice. This reasoning can be followed for discussion of any substance existing in ionized form in a biological system. For the discussion to follow the ionized forms of the intermediate compounds will be used when appropriate.

Many workers refer to the "shikimic acid pathway" when they are in fact dealing with a sequence of reactions where shikimic acid does not occur *per se*. A considerable inconsistency in usage of this type is encountered in the modern literature. As an example dealing with the current topic one can find references to the "shikimic acid pathway" and the "acetate pathway" within the same work. Indeed, one can find these expressions within the same sentence. This is an inconsistency which is unnecessary. In time, perhaps, it can be eliminated.

In nature there are two sequences of reactions by which aromatic compounds are formed. In one a poly β-keto compound formed from acetate cyclizes to form an aromatic ring which usually retains several hydroxyl groups. This is the so-called "acetate pathway." The second method for forming aromatic compounds involves shikimate and some of its derivatives and derives its carbon from glucose metabolism.

In any discussion of a biological pathway one must decide at which point to start. Obviously, in the case

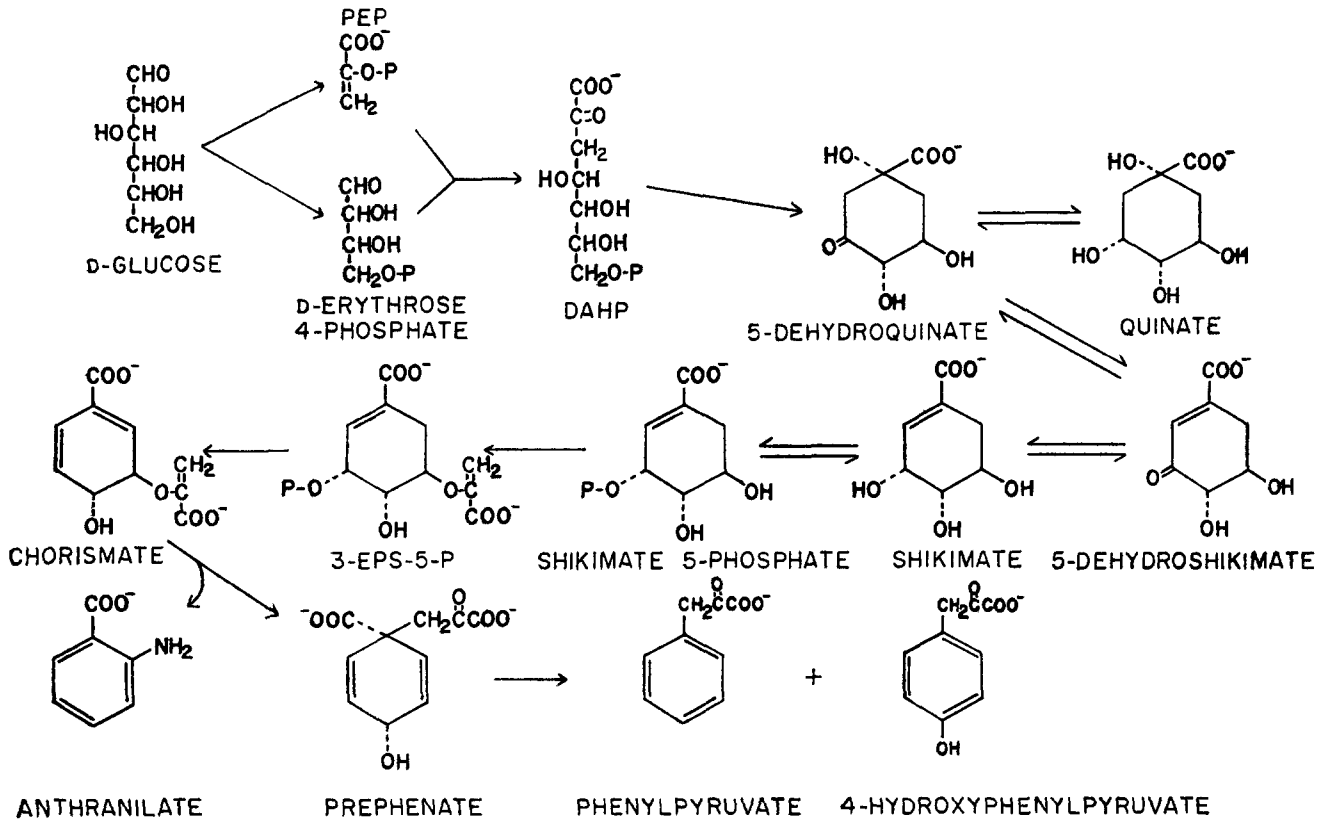


Figure 1.—The shikimate pathway from glucose to aromatic compounds. PEP is phosphoenolpyruvate; DAHP is 3-dexoy-D-*arabino*-heptulosonate 7-phosphate; 3-EPS-5-P is 3-enolpyruvylshikimate 5-phosphate.

of plants, it is possible to start at the very beginning and proceed from the early steps in photosynthetic fixation of carbon dioxide through the various carbohydrate transformations until the required "starting material" has been formed. In a number of organisms in which shikimate has been found photosynthesis does not occur. A more common starting material is required. Glucose has been shown to be a precursor for shikimate and will be taken as our starting point for the discussion to follow.

Before we begin the discussion of the shikimate pathway another point should be brought out. The pathway, even from glucose, involves several steps. These steps were not discovered in their natural order. Some of the intermediate compounds were not isolated and identified until several years after the need for such compounds was realized. Hence, a considerable amount of jumping about has occurred in the efforts to elucidate the entire pathway. For this reason a chronological discussion of the pathway would tend to be confusing to most readers. Consequently, the discussion will follow the stepwise conversion of starting material through the various intermediates in the pathway and will terminate when the aromatic compounds have been formed. The entire pathway is represented in Figure 1.

1. *Carbohydrate Reactions*

Growth of *Escherichia coli* on a medium containing glucose-C^{14} as the sole source of carbon resulted in the formation of labeled shikimate (126). The distribution of label in shikimate suggested that it had been formed from a fusion of a three-carbon intermediate of glycolysis and a tetrose phosphate from the pentose phosphate pathway. Support for this contention came from additional work of Srinivasan and co-workers (122, 123). It was shown that dehydroshikimate synthesis from erythrose-4-phosphate and phosophoenolpyruvate was not inhibited by either fluoride (enolase inhibitor) or iodoacetate (triose phosphate dehydrogenase inhibitor) but that synthesis from sedoheptulose was completely blocked. The inhibitions could be reversed by the addition of phosphoenolpyruvate (or 3-phosphoglyceraldehyde in the case of iodoacetate inhibition). These observations suggest that glycolysis reactions involving triose phosphate and phosphoenolpyruvate participate in the conversion of sedoheptulose to dehydroshikimate. These reactions are summarized below.

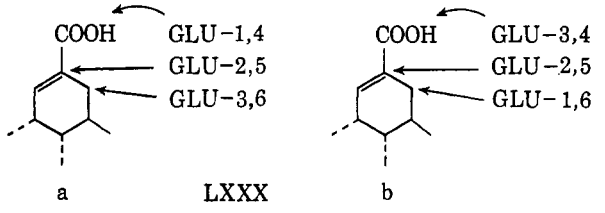
sedoheptulose ⟶ erythrose-4-P + dihydroxyacetone-P (Eq. 1)

dihydroxyacetone-P ⟶ 3-P-glycerate (Eq. 2)

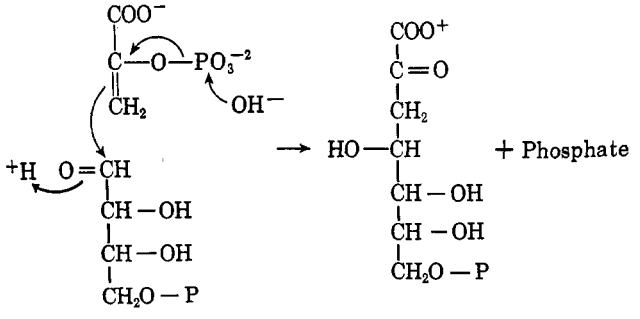
3-P-glycerate ⟶ P-enolpyruvate

P-enolpyruvate + erythrose-4-P ⟶ dehydroshikimate (Eq. 3)

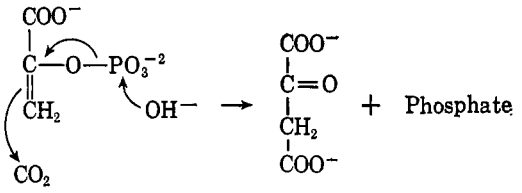
Carbohydrates other than glucose have been investigated as shikimate precursors. Kalan, *et al.* (78), reported incorporation of glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate in the range of 2.4 to 4.6%. 6-Phosphogluconate was not incorporated. Sedoheptulose was incorporated to the extent of about 7%. In the following paper (128), experiments with sedoheptulose-1,7-diphosphate were described. An incorporation of 81% in 2 hr. was observed. This striking observation raised the obvious question as to whether sedoheptulose diphosphate was the immediate precursor of the cyclic pathway intermediates. The question was resolved in the following manner. The only known formation of sedoheptulose-1,7-diphosphate is through condensation of a triose phosphate and a tetrose phosphate. Carbons 1, 2, and 3 of the product are derived, *via* the triose, from C's (1,6), (2,5), and (3,4) of glucose, respectively. Thus, if cyclization of intact sedoheptulose-1,7-diphosphate occurred the origin of the carbons of shikimate would be given by LXXXa. In fact, however, the reverse pattern LXXXb was observed with the label from glucose-1-C^{14} or glucose-6-C^{14} appearing at C-2 in the shikimate. Therefore, carbons 1, 2, and 3 of sedoheptulose-1,7-diphosphate must be detached and inverted before cyclization occurs.



It would appear from the foregoing that the erythrose-4-phosphate and phosphoenolpyruvate formed by cleavage of sedoheptulose-1,7-diphosphate must be utilized in the formation of a new seven-carbon compound. Presumably, this would then be the acyclic precursor for the first cyclic member of the pathway. Srinivasan, *et al.* (122, 123), suggested the formation of such a compound according to the following mechanism. The authors pointed out that this reaction is analogous to the reaction of phosphoenolpyruvate and carbon dioxide



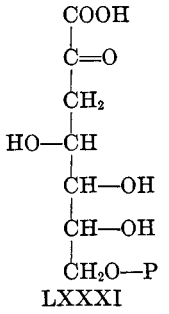
to yield oxaloacetate and inorganic phosphate as shown below.



While studying the metabolism of ribose-5-phosphate in an *E. coli* mutant, Weissbach and Hurwitz (142) observed the appearance of an unknown compound which they identified as 2-keto-3-deoxyheptonic acid. They noted that removal of phosphate from the 2-keto-3-deoxy-7-phosphoheptonic acid of Srinivasan and coworkers (122) should give a compound similar to theirs. Removal of the phosphate function with potato phosphatase yielded a compound which was spectrally and chromatographically identical with their isolated 2-keto-3-deoxyheptonic acid.

Further studies (143) showed that enzyme fractions from *E. coli* catalyzed the formation of 2-keto-3-deoxyheptonic acid from ribose-5-phosphate or erythrose-4-phosphate and phosphoenolpyruvate. Significantly, it was also observed that their enzyme preparation contained phosphatase activity.

The problem of the existence of the seven-carbon sugar derivative was solved by the work of Srinivasan and Sprinson (127). First, the compound was shown to be 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate[2] (LXXXI). Second, an enzyme preparation was ob-



LXXXI

tained which catalyzed the condensation of D-erythrose-4-phosphate and phosphoenolpyruvate to form the heptulosonic acid 7-phosphate and inorganic phosphate. The yields of product molecules were quantitative, and the process appeared to be irreversible.

The enzyme was purified 60-fold using, successively, ammonium sulfate precipitation, acetone precipitation, and chromatography on a DEAE–cellulose column. The preparation appeared to be homogeneous in the ultracentrifuge. With phosphate buffer the pH optimum was 6.4 while with trismaleate buffer an optimum range of 6.4 to 7.4 was observed. The Michaelis constants were 1.2×10^{-3} *M* for D-erythrose-4-phosphate and 3.5×10^{-3} *M* for phosphoenolpyruvate.

(2) This name agrees with the "Rules of Carbohydrate Nomenclature," *J. Org. Chem.*, **28**, 28 (1963), and replaces the older "2-keto-3-deoxy-D-*arabo*-heptonic acid 7-phosphate."

D-Erythrose-4-phosphate could not be replaced by D-erythrose, D-glyceraldehyde-3-phosphate, ribose-5-phosphate, glucose-6-phosphate, glucosamine 6-phosphate, or N-acetylglucosamine 6-phosphate. Phosphoenolpyruvate could not be replaced by either pyruvate or pyruvate plus ATP. The enzyme was not inhibited by Co^{+2}, Zn^{+2}, or Mg^{+2} (at 2×10^{-3} *M*), EDTA (4×10^{-4} *M*), fluoride, arsenite, azide, or iodoacetate. *p*-Chloromercuribenzoate (2×10^{-5} *M*) completely inhibited the enzyme, with complete reversal of inhibition observed upon addition of cysteine. Inhibition was also observed with sedoheptulose-1,7-diphosphate, fructose-1,6-diphosphate, and 3-deoxy-D-*arabo*-heptonic acid 7-phosphate.

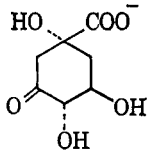
Phenylalanine and tyrosine reduce the accumulation of shikimate in *E. coli* mutant 83–24 (33). At the level of 0.1 μmole/ml., both compounds reduced accumulation of shikimate to about 1% of the control. The addition of neither phosphoenolpyruvate nor erythrose-4-phosphate increased the production of shikimate in the presence of the two aromatic amino acids. A detailed study of the problem has been published by Smith and coworkers (120). Ammonium sulfate fractionation yielded two 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate synthetases. One of the synthetases was inhibited by L-phenylalanine and the other by L-tyrosine. In addition it was shown that low concentrations of L-tyrosine repressed the formation of the L-tyrosine-sensitive synthetase whereas this amino acid had no effect on the L-phenylalanine-sensitive enzyme synthesis. Relatively high concentrations of L-phenylalanine suppress the formation of either synthetase.

In a continuation of their work on this topic the authors (119) studied the effect of phenylalanine and tyrosine analogs on the synthesis and activity of the two synthetases. The binding sites on the synthetases occupied by phenylalanine and tyrosine are relatively sensitive to the nature of the 4-position on the aromatic ring. In the case of phenylalanine analogs a vacant 4-position enhances binding whereas with tyrosine analogs the presence of a 4-hydroxyl function greatly increases the degree of binding to the enzyme surface. Experiments indicated that the structure requirements for suppression of synthetase formation are less specific than those for enzyme inhibition.

The synthesis of barium 5,7-O-ethylidene 2-keto-3-deoxyheptonate has recently been reported (97). For the successful synthesis 2,4-O-ethylidene-D-erythrose was condensed with oxalacetic acid.

2. 5-Dehydroquinate

The earliest known cyclic precursor in the shikimate pathway is 5-dehydroquinate (LXXXII). Early indication of the existence of such an intermediate was discussed by Davis (19). The acid was isolated by Weiss and co-workers (139) from *E. coli* mutant 170–27 in

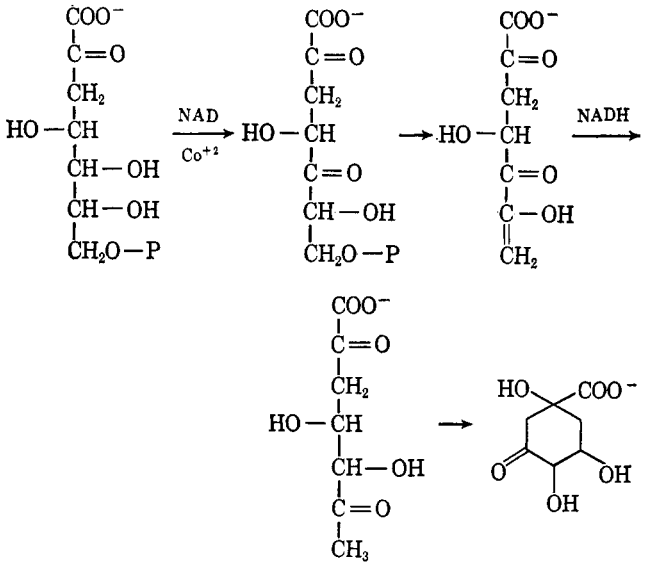


LXXXII

yields of about 0.2 mg./ml. of culture fluid. Analytical data indicated a difference of one molecule of water from 5-dehydroshikimic acid. Two possibilities existed for the position of the hydroxyl group, but aqueous ferric chloride reagent gave a positive test indicating the presence of an α-hydroxycarboxylic acid. Acidic conditions converted the compound into 5-dehydroshikimic acid. Catalytic hydrogenation producted quinic acid.

Srinivasan and his group (125) have reported a study of the enzyme responsible for the conversion of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate to 5-dehydroquinate. The enzyme was obtained from *E. coli* mutant 83–24 and was purified 20-fold by two successive precipitations using ammonium sulfate followed by a single precipitation with acetone. Cofactor studies showed requirements for Co^{+2} and NAD. Optimum synthetic activity occurred between pH 7.4 and 8.4. Inhibition was observed with NADase, EDTA, tris(hydroxymethyl)aminomethane, and 5,5-diethylbarbiturate.

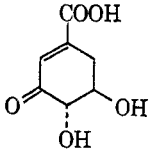
Experiments with 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate-1-C^{14} showed only two radioactive compounds, precursor and cyclic product, even after only 2-min. incubation. After 30 min. incubation all label was in 5-dehydroquinate. These data were in perfect agreement with the kinetic data which showed that the rate of appearance of 5-dehydroquinate and inorganic phosphate was the same as the rate of disappearance of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate.



It could be safely predicted on structural grounds that the conversion from the heptulosonic acid to 5-dehydroquinate would occur through a stepwise process. However, the failure to detect radioactive intermediates and the equality of the rates of precursor disappearance and product appearance suggest that a concerted process is in operation. Though several molecular transformations are undoubtedly involved in the synthesis, all intermediates must be retained on the enzyme surface until the product is formed. The authors suggested the above mechanism as a possible explanation for the process. This mechanism explains the requirement for catalytic amounts of NAD and has precedent for the phosphate elimination step from similar eliminations of phosphate esters of 3-hydroxyaldehydes and 3-hydroxy esters.

3. *5-Dehydroshikimate*

5-Dehydroshikimic acid was isolated initially by Salamon and Davis (111) from *E. coli* mutant 83–2. The compound possessed about 75% of the biological activity of shikimic acid. A melting point of 152° was observed but when heated further the melt resolidified and remelted at 202°. Protocatechuic acid (3,4-dihydroxybenzoic acid) was identified as the product. The isolated compound gave carbonyl derivatives and a methyl ester diacetate in agreement with the structure LXXXIII.



LXXXIII

Mitsuhashi and Davis (92) isolated an enzyme preparation from *E. coli* which they termed "5-dehydroquinase."[3] The enzyme was purified by a factor of eight by successive treatments with manganese chloride, calcium phosphate gel, and ammonium sulfate. Specificity tests showed that only 5-dehydroquinate was utilized as a substrate; the system had no effect on quinate, malate, isocitrate, or citrate. The enzyme was partially inhibited by iodoacetamide, Cu^{+2}, Zn^{+2}, and Fe^{+2}, had a pH optimum of about 8, and a Michaelis constant for dehydroshikimate at pH 7.4 of 4.4×10^{-4} *M*. No cofactor requirements could be demonstrated. Ignoring the concentration terms for water a K_{eq} value of 15 was calculated for the reaction. Distribution studies for 5-dehyroquinase were run. Results of these studies are presented in Table III.

Additional work concerning the distribution of 5-dehydroquinase in higher plants has appeared. Nandy and Ganguli (94) prepared a cell-free extract of *Phaseolus aureus* seedlings and tested the ability of this system to synthesize 5-dehydroshikimate from various supplied precursors. Of the sugar derivatives tested glucose-6-phosphate was the best precursor followed in decreasing order by glucose plus ATP plus magnesium chloride, fructose-6-phosphate, and glucose. Fructose-1,6-diphosphate and ribose-5-phosphate were not utilized. A mixture of phosphoenolpyruvate and erythrose-4-phosphate was a good precursor and gave approximately twice the 5-dehydroshikimate than when either phosphoenolpyruvate or erythrose-4-phosphate was tested separately. 3-Phosphoglycerate, dihydroxyacetone phosphate, and glyceraldehyde 3-phosphate were not converted to product. An interesting observation was the fact that conversion of glucose-6-phosphate to product required both microsomal and supernatant portions of the extract whereas the conversion of phosphoenolpyruvate and erythrose-4-phosphate to product could be obtained by using the supernatant fraction alone.

TABLE III

DISTRIBUTION OF 5-DEHYDROQUINASE ACTIVITY

Organism	Activity[a]
Escherichia coli (ATCC 9637)	18
Aerobacter aerogenes	15
Saccharomyces cerevisiae	2.1
Euglena gracilis	5.3
Peas	2.4
Spinach leaves	1.5
Liver (guinea pig)	0.03

[a] Expressed as units $\times 10^{-3}$/mg. of protein.

Balinsky and Davies (4) described an enzyme preparation from cauliflower buds which resembled the *E. coli* 5-dehydroquinase. A purification of 7.6-fold was accomplished by two precipitations with ammonium sulfate. The system was apparently specific for its natural substrate, there was no dialyzable cofactor, and a wide pH range was observed. There was no inhibition by sulfhydryl reagents or by compounds related structurally to 5-dehydroquinic acid or 5-dehydroshikimic acid.

Hanson and Rose (56) have demonstrated, using tritium labeling, that the addition of water to 5-dehydroshikimate to form 5-dehydroquinate is stereospecifically *cis*. The dehydroquinase preparation used for the study was obtained from *Aerobacter aerogenes* A 170–143.

4. *Shikimate*

The first indication that shikimate occupied a significant biological position came from the work of Davis (20). Using strains of *E. coli* which needed the addition of aromatic amino acids for growth, he tested a variety of compounds, 66 in all, for possible action as aromatic precursors. Shikimate gave a growth rate 62% of that of the wild strain. The amount utilized

(3) Systematic name: 5-dehydroquinate hydroylase (4.2.1.10).

was roughly equivalent to the total weight of the four or five aromatic requirements. A combination of shikimate, phenylalanine, and tyrosine gave a growth rate closely approximating that of the wild type. Other effective compounds were the α-keto acids corresponding to phenylalanine and tyrosine, indole, and anthranilic acid.

The enzymatic formation of shikimate from 5-dehydroshikimate was investigated by Yaniv and Gilvarg (148). *E. coli* (strain W) yielded a preparation which was shown to be substrate specific and NADP specific. A ninefold purification was accomplished using manganese chloride and ammonium sulfate precipitations and calcium phosphate gel chromatography. A pH maximum of about 8.5 was observed, and Michaelis constants were determined to be 5.5×10^{-5} *M* for shikimate and 3.1×10^{-5} *M* for NADP. Distributional studies showed the presence of enzyme activity in several strains of *E. coli*, *A. aerogenes*, *Euglena gracilis*, *Saccharomyces cerevisiae*, spinach, and peas.

A dehydroshikimic acid reductase[4] system has been obtained from etiolated pea epicotyls by Balinsky and Davies (2). Precipitations by ammonium sulfate and ammonium citrate followed by chromatography on a calcium phosphate–cellulose column gave a 78-fold purification of the enzyme. The enzyme was NADP specific. Quinate, protocatechuate, malate, and lactate were not acted upon by the enzyme in the presence of NADP, suggesting a specificity similar to that observed by Yaniv and Gilvarg (148) with their preparation from *E. coli*. There was no metal requirement as shown by experiments with potassium cyanide and EDTA. *p*-Chloromercuribenzoate inhibition was observed suggesting a sulfhydryl protein. Cysteine addition resulted in restoration of 92% of the initial activity. Iodoacetamide inhibited the enzyme to the extent of 16%. A pH maximum of 10.0 was observed.

The second paper in the series by Balinsky and Davies (3) discussed the mode of attachment of the substrate to the surface of dehydroshikimate reductase. Under the conditions of the experiments the inhibition data presented in Table IV were observed. For the sake of the present discussion the authors assumed the half-chair conformation for shikimate which is in agreement with the more recent work of Hall (54). This structure was given above in section IID.

The presence of 4-hydroxyl groups in the natural substrates and in the inhibitors suggests that this functional group may play a role in the attachment to the enzyme surface. Phenol itself showed no inhibitory character indicating that some other functional group is necessary for action. The other possible groups are the remaining two hydroxyl functions at C-3 and C-5 and the carboxyl function at C-1. Results with gallic acid show that this may be the case. Three points of attachment are possible with gallate, the carboxyl group and the hydroxyls on C-3 and C-4. Attachment at the C-5 hydroxyl is not likely as the group is axial in shikimate and absent in 5-dehydroshikimate. Protocatechuate is also effective as an inhibitor. Pyrogallol and catechol both inhibit the enzyme system; hence two adjacent hydroxyl groups seem to be sufficient, but they are still less effective than *p*-hydroxybenzoate. It would appear, then, that either a 3-hydroxyl or a 1-carboxyl group is necessary in addition to the 4-hydroxyl. Guaiacol showed that an attachment through the methoxy group was possible, and in vanillin the methoxy attachment as well as a strong aldehyde function attachment was observed. Thus, positions 1 and 3 are necessary but not entirely specific while position 4 is specific. It was also demonstrated that the amine function could not replace the hydroxyl group.

TABLE IV

INHIBITION STUDIES ON THE SYSTEM DEHYDROSHIKIMATE REDUCTASE

Compound	Concn., m*M*	Inhibition, %	Inhib. const.
Pyrogallol	10	100	...
Catechol	5	100	1.9
Guaiacol	5	91	11.0
Vanillin	0.5	100	0.093
Gallic acid	5	100	0.38
Protocatechuic acid	1	23	0.75
p-Hydroxybenzoic acid	5	100	0.74

Carvajal and co-workers (12–14) have demonstrated that a number of phenolic compounds inhibit shikimate utilization in *E. coli* and *A. aerogenes* mutants. Possessing moderate activity were the ethyl esters of 2,4-dihydroxy- and 2,3,4-trihydroxybenzoic acids. Ethyl gallate possessed higher activity than these but was itself surpassed by ethyl 3,4-dihydroxybenzoate and ethyl 3,5-dihydroxybenzoate.

Balinsky and Davies (5) have also reported information concerning the distribution of dehydroshikimate reductase and dehydroquinase activities in higher plants. In cauliflower buds about 8% of the total enzyme activity was in the mitochondria, while the remaining 92% was in the supernatant fraction. In peas 30% of the activity was found in the mitochondrial fraction, the remainder being in the supernatant fraction. The possibility that the smaller mitochondrial activity of the cauliflower buds could be due to absorption was mentioned. No attempts were made to obtain a microsomal fraction in these studies.

Other activities detected in the supernatant fraction included pentose phosphate and glycolysis reactions and NADPH production. These observations support the functioning of a dehydroshikimate reductase system.

It was shown that the enzymes were synthesized in seedlings and increased in concentration as the plant aged. The ratio of dehydroshikimate reductase–de-

(4) Systematic name: shikimate: NADP oxidoreductase (1.1.1.25).

hydroquinase was determined for several plants. The results are shown in Table V. Little enzyme activity was observed in storage tissues, *e.g.*, beets and potatoes.

TABLE V

THE RATIO DEHYDROSHIKIMATE REDUCTASE/DEHYDROQUINASE FOR SEVERAL PLANTS

Plant	Ratio
Zea mays	3.7
Avena sativa	8.0
Triticum sp.	7.6
Lupinus sp.	11.3
Pisum sativum	5.0

A study of the biosynthesis of shikimate in *Pinus resinosa* Ait. seedlings was recently published by Yoshida and Towers (150). Of the compounds tested the most effective precursor was pyruvate. In order of decreasing effectiveness other precursors were: glucose and ribose, sucrose and fructose, and with very much lower incorporations, formate, acetate, malonate, and succinate.

When uniformly labeled glucose was administered, 13% of the activity in the resulting shikimate was found in the carboxyl carbon. Theory requires 14% based on conversion of glucose-U.L.-C^{14} to shikimate-U.L.-C^{14}. Ribose would be expected to provide only the triose portion for the synthesis of the heptulosonic acid and would give shikimate with 33% of its label in the carboxyl function. In the present work 27% was observed. Cold erythrose diluted the label in shikimate when administered with glucose-U.L.-C^{14}. These results are generally consistent with the pathway observed in microorganisms.

5. *Shikimate 5-Phosphate*

Davis and Mingioli (21) observed that several organisms accumulate compounds which occupied positions beyond shikimate in the pathway. The substances, termed "Z1" and "Z2," were completely without nutritive value for the organisms. Both, however, gave rise to shikimate upon acid hydrolysis. "Z2" was subsequently shown by Weiss and Mingioli (141) to be 5-phosphoshikimic acid. *A. aerogenes* strain A170–40 accumulated 500 to 1000 mg. of the unknown per liter of culture medium. The compound was sensitive to phosphatase thus eliminating the possibility of a mixed anhydride. Rapid consumption of 1 equiv. of periodic acid eliminated the 4-position as the point of attachment. The compound failed to give an isopropylidene derivative. These observations suggest that the phosphate group is at position 5. This was confirmed by synthesis of 3-phosphoshikimic acid which was shown to be different from the isolated compound. The authors demonstrated that the extraction and purification procedures did not alter the structure of the compound.

Fewster (27) has studied the phosphorylation process in cell-free extracts of several microorganisms. Shikimate and ATP were added to cell-free extracts, and the disappearance of the reactants measured. The results are given in Table VI.

TABLE VI

PHOSPHORYLATION OF SHIKIMATE BY CERTAIN MICROORGANISMS

Organism	Activity[a]
E. coli 518 (minimal medium)	135
E. coli 83–1 (minimal medium, shikimic acid)	140
E. coli 83–1 (minimal medium, aromatic mixture)	130
E. coli 83–24 (minimal medium, aromatic mixture)	120
Acetobacter suboxydans 621	80
A. aerogenes A 170–40	165
Arthrobacter globiformis 8602	130
Cornynebacterium erythrogenes 142	160
Pseudomonas ovalis Chester	60
Leuconostoc mesenteroides	None
Lactobacillus arabinosus	None

[a] Millimicromoles of acid disappearing/mg. of protein/hr.

The enzyme system was not purified but some of its characteristics were determined. At neutrality the preparation had its maximum activity. Either Mn^{+2} or Mg^{+2} was required by the system. The enzyme was inhibited to the extent of 55% by 3 m*M* iodoacetate, but was not inhibited by 0.2 m*M* phenylmercuric acetate or by the products of the aromatic biosynthetic pathway.

Recently, Srinivasan and Rivera (124) described experiments concerning conversion of shikimate 5-phosphate to anthranilate. *E. coli* mutant B-37, which is blocked between anthranilate and indoleglycerol 3′-phosphate, was used for the studies. The enzyme system was not purified. A NADH regenerating system and either Mg^{+2} or Fe^{+2} were required for activity. Only shikimate 5-phosphate and glutamine served as precursors. Shikimate plus ATP gave 10% conversion in contrast to the nearly quantitative consumption of the 5-phosphate. The kinase suggested by this observation was not investigated.

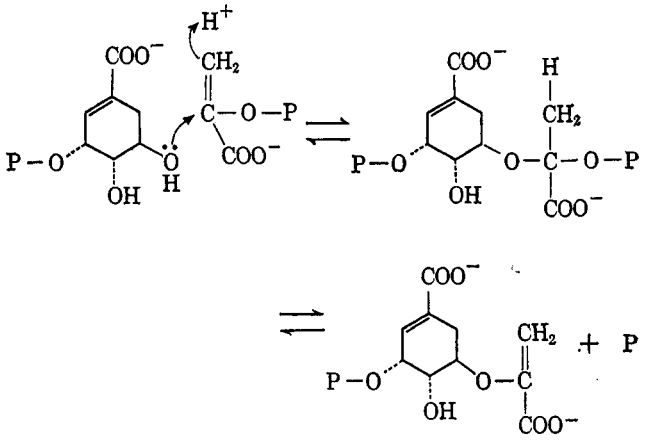
6. *3-Enolpyruvylshikimate 5-Phosphate*

Before discussing the formation and metabolism of 3-enolpyruvylshikimate 5-phosphate it is desirable to look at compound "Z1." The unknown could be cleaved into equimolar quantities of shikimic acid and pyruvic acid with acid (41). Cleavage with periodic acid gave products suggesting substitution on the 3-carbon of shikimic acid. Thus, a 3-enolpyruvylshikimic acid was a likely possibility. The likelihood of "Z1" occupying a position in the pathway was also considered. In fact, incubation of shikimate 5-phosphate and phosphoenolpyruvate with certain of the bacterial extracts resulted in the formation of a substance having the chromatographic and chemical properties of "Z1"

A further study of these reactions was reported by Levin and Sprinson (82) who showed that a partially purified extract could extensively hydrolyze shikimate 5-phosphate to shikimate and inorganic phosphate. When equivalent amounts of shikimate 5-phosphate and phosphoenolpyruvate were added to the enzyme preparation, however, "Z1" and 2 equiv. of inorganic phosphate were formed. The addition of fluoride to the reaction medium inhibited the dephosphorylation and allowed 3-enolpyruvylshikimate 5-phosphate to accumulate.

A complete report of this work has appeared recently (83). Using *E. coli* K12 mutant 58–278 these workers were able to isolate a quantity of the ether phosphate. The compound reacted as expected for an enol ether and gave an infrared spectrum which agreed with the proposed structure.

The enzyme necessary for synthesis of the enol ether had no observable requirement for heavy metals or cofactor. Optimum synthetic activity occurred at pH 5.4 to 6.2. The equilibrium was calculated to lie 75–80% toward the products but a relatively large phosphate concentration was strongly reversing for the reaction. K_m values were 2.4×10^{-4} *M* for phosphoenolpyruvate and 3.4×10^{-4} *M* for shikimate 5-phosphate. The following mechanism for the reaction was proposed.



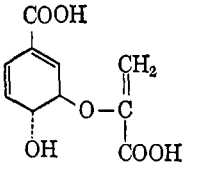
The role of 3-enolpyruvylshikimate 5-phosphate in the biosynthesis of anthranilate has been studied by Rivera and Srinivasan (110). Ammonium sulfate precipitations of an extract from *E. coli* B-37 yielded two enzyme fractions. Fraction 1 possessed the ability to consume shikimate 5-phosphate without producing anthranilate. The presence of 3-enolpyruvylshikimate 5-phosphate was demonstrated indicating that the corresponding synthetase must be contained in fraction 1. Addition of fraction 2 to fraction 1 resulted in the formation of anthranilate. It was suggested that fraction 1 contained an enzyme system capable of converting the enol ether phosphate into a new substance which in turn could be converted to anthranilate. This was indicated by the requirement of glutamine as amino donor for the formation of anthranilate.

Other observations with bacterial mutants argue for another intermediate after the enol ether phosphate. Early observations of Davis and Mingioli (21) on the occurrence of mutants which accumulated 3-enolpyruvylshikimate and required the full complement of aromatic amino acids suggested such an intermediate. Furthermore, observations of Gibson, *et al.* (39), with *Aerobacter aerogenes* would indicate the same thing, a "branch point compound." In the presence of glutamine the newly suggested intermediate would be converted to anthranilate, while in the absence of the amino donor prephenate would be formed. This intermediate is the subject of the next paragraphs.

7. *Chorismate*

The first concrete evidence for the existence of the postulated intermediate came from studies of *Aerobacter aerogenes* strain 62–1 by Gibson and Gibson (37). When a limiting tryptophan medium was used, shikimate was metabolized to anthranilate, but, when glutamine was omitted from the medium, a new compound was formed which could be extracted from the medium with ether. Chemically, the compound was converted into a mixture of *p*-hydroxybenzoic acid and phenylpyruvic acid under acidic conditions and to prephenic acid by treatment with pH 8 buffer at 37°. Acidic chromatographic solvents transformed the compound into phenolic substances while neutral solvents had no chemical effect but gave poor separations.

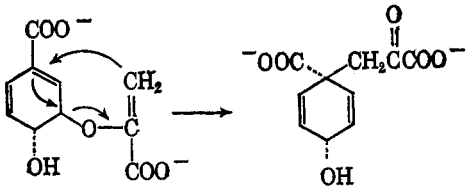
Two papers appeared recently which describe in detail the isolation and metabolism (38) and the chemical and physical properties (36) of the new substance. Using *A. aerogenes* 62–1 and tryptophan it was possible to obtain quantities of the compound, named chorismic acid, for study. The compound was very labile in either acid or base but was isolable as its barium salt. Most structural information was obtained from spectral studies. In the ultraviolet region a cyclohexadienecarboxylic acid chromophore was evident. The infrared spectrum showed an enol ether band and a terminal methylene function both of which disappeared on conversion to prephenic acid. A n.m.r. study (40) confirmed the structure which is shown below as LXXXIV. The



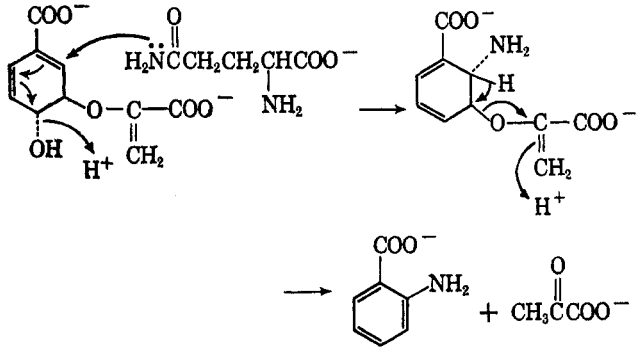
LXXXIV

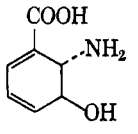
stereochemistry was assigned on the basis of the stereochemistry of the shikimic acid molecule.

The formation of anthranilate in certain bacterial mutants from shikimate derivatives has already been mentioned. Under other conditions, especially the absence of glutamine, prephenate is formed. Mechanisms for these reactions have been postulated, the prephenate formation is shown below. In essence the reaction involves attack of the methylene carbon of the enol ether function on C-1. Concomitant shift of the C-1, C-2 double bond satisfies the electronic requirements of the process with the formation of prephenate. The stereochemistry of the prephenic acid molecule has been discussed by Pleininger (99).



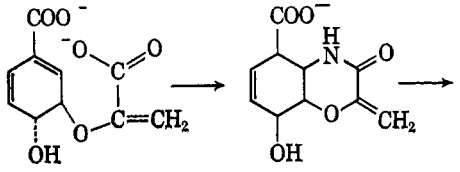
In the presence of glutamine the product observed is anthranilate. A mechanism to account for this transformation has been proposed by Leven and Sprinson (83) and is represented below. It is of interest to mention the isolation (86) of compound LXXXV at this point. This molecule, isolated from *Streptomyces aureofaciens*, bears a remarkable similarity to the proposed intermediate above. A study of the origin of this hydroxyamino acid in its parent organism would be of interest.



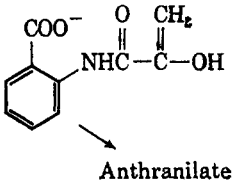


LXXXV

An interesting idea has recently been advanced by Ratledge (106) based upon his observations concerning aromatic biosynthesis in *Mycobacterium smegmatis* and *Aerobacter aerogenes*. Using a mutant of *A. aerogenes* blocked immediately after anthranilate he was able to demonstrate the presence of an N-acylanthranilate derivative. The nature of the acyl function was not determined although the possibility of the N-formyl derivative was eliminated. Two obvious functions for the compound are: (1) precursor and (2) metabolite. The possibility as a precursor suggested involvement of a heterocyclic compound, here represented as LXXXVI, derived from chorismate. Assuming such an intermediate a simple extension would give an N-acylanthranilate derivative LXXXVII. Hydrolysis of compound LXXXVII would give anthranilate. Hydrolysis of the heterocyclic intermediate LXXXVII could conceivably result in the formation of salicylate, found in *M. smegmatis* in this study, or 2,3-dihydroxybenzoate found in the *A. aerogenes* mutant. Further work on these possibilities would be most welcome.



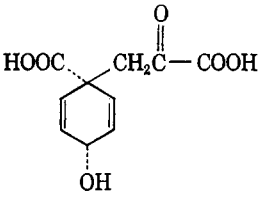
LXXXVI



LXXXVII

8. *Prephenate and Beyond*

E. coli strain 83–5 was shown (140) to require phenylalanine and accumulate a nonaromatic substance capable of being converted to phenylpyruvic acid, under mildly acidic conditions. The substance was isolated and purified and its structure determined through chemical and spectral examination. Prephenic acid is shown as LXXXVIII.



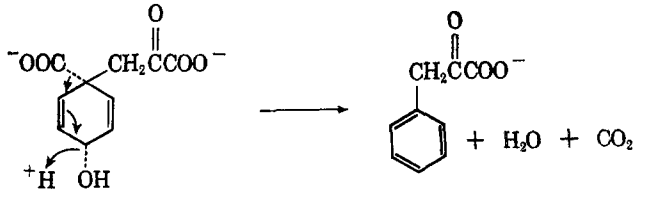
LXXXVIII

Cell-free extracts of *E. coli* wild type possessed the ability to transform prephenate to phenylpyruvate. Similar extracts from strain 83–5 could not perform this reaction. This study constituted the first direct observation of aromatization in the pathway. No detailed study of prephenate aromatase from microorganisms has been reported. Such a study would be a welcome addition to the literature in this area.

The conversion of prephenate to phenylpyruvate involves loss of one molecule each of water and carbon dioxide. A reasonable mechanism for the conversion is shown.

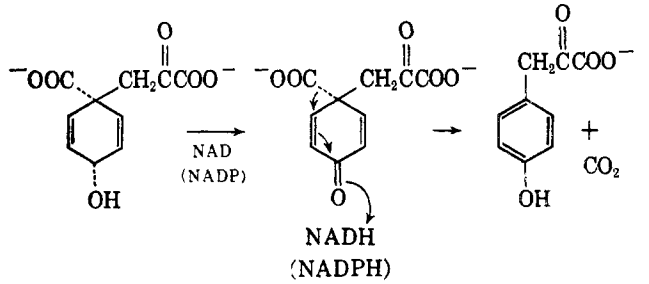
TABLE VII
AROMATIC PRECURSOR STUDIES WITH *Phaseolus aureus* EXTRACTS

Precursor tested	Cofactor	Products
Phenylpyruvate glutamate	None	Phenylalanine
4-Hydroxyphenylpyruvate glutamate	None	Tyrosine, *p*-hydroxyphenylacetate
Prephenate glutamate	NADP	Phenylalanine, tyrosine
Prephenate glutamate	NAD	Phenylalanine
Prephenate	NADP	*p*-Hydroxyphenylpyruvate, *p*-hydroxyphenylacetate, phenylpyruvate



An enzyme preparation from *Phaseolus aureus* Roxb. capable of forming aromatic compounds from prephenate has been described by Gamborg and Simpson (35). The cell-free extract was partially purified by passage through a Sephadex column. Results of this study, including cofactor requirements, are presented in Table VII.

The results show the presence of transaminase, prephenate aromatase, and prephenate dehydrogenase activities in the extract. Only when glutamate was incubated with the substrate and extract were the amino acids formed. The observation that conversion of prephenate to tyrosine required NADP agrees with the mechanism postulated for the reaction by Sprinson (121) on similar observations with microorganisms.



A cell-free extract from *E. coli* strain 83–5 capable of converting prephenate to *p*-hydroxyphenylpyruvate has been described by Schwinck and Adams (114). Owing to the instability of the enzyme no purification was attempted. Some characteristics of the system were determined however. A wide pH optimum existed between pH 6.5 and 9.0. The approximate K_m for *p*-hydroxyphenylpyruvate was calculated to be 2×10^{-3} *M* at pH 9.0. A requirement for NAD was demonstrated.

In addition to *p*-hydroxyphenylpyruvate, *p*-hydroxyphenyllactate was found as a product under certain conditions. It was demonstrated, however, that free *p*-hydroxyphenyllactate was not an intermediate in the reaction, since it was not oxidized to *p*-hydroxyphenylpyruvate under the same conditions which converted prephenate to this product. The requirement for NAD (NADP) was rationalized by Sprinson (121) and is shown in the mechanism above.

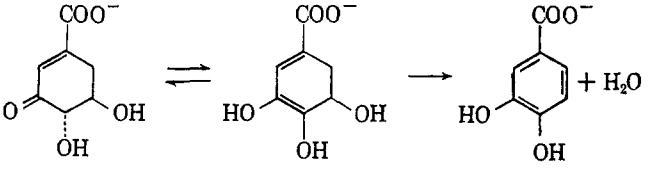
9. *Aromatization in Neurospora*

In 1954, Tatum, *et al.* (133), reported studies of a mutant of *Neurospora crassa* which accumulated large amounts of protocatechuate along with relatively small amounts of 5-dehydroshikimate. A block between 5-dehydroshikimate and shikimate was postulated with protocatechuate being formed directly from 5-dehydroshikimate. A similar conclusion was reached by Metzenberg and Mitchell (88) also using *Neurospora.*

Incorporation of label from glucose into protocatechuate was reported by Tatum and Gross (132) using *Neurospora.* The incorporation pattern was similar to that observed by Davis in the early work with *E. coli* suggesting two fragments from glucose arising *via* two different pathways.

A partially purified enzyme preparation from *Neurospora* has been obtained by Gross (53) which catalyzed the formation of protocatechuate from 5-dehydroshikimate. The enzyme had maximum activity at pH 7.4–7.6, a K_m 6.0 $\times$ 10^{-4} *M* for 5-dehydroshikimate, and was totally inhibited by chloromercuribenzoate. Reduced glutathione reversed the inhibition. However, when the system was incubated with the inhibitor for 10 min., the inhibition could be reversed only to the extent of about 10% with reduced glutathione.

The exact nature of the intermediate (or intermediates) in the reaction has not been determined but a likely mechanism has been proposed (53). The elimination of oxygen from C-3 was proved by the use of labeled compounds. Elimination of water from the compound in the enol form would seem a reasonable reaction. The reaction is of course aided by the reso-



nance stabilization of the aromatic ring in protocatechuate.

The conversion of shikimate and quinate to protocatechuate and gallate in *Bacillus cereus* has been studied by Ishikawa and Oki (75). It was found that the

formation of protocatechuate and gallate from shikimate, quinate, or 5-dehydroshikimate was completely blocked by mercuric *p*-chlorobenzoate, copper sulfate, or mercuric acetate. The formation of aromatic amino acids from these precursors was only partially blocked by these inhibitors. The authors conducted that 5-dehydroshikimate was serving as a direct precursor of protocatechuate. Conversion of protocatechuate to gallate was not studied.

The possibility of conversion of 5-dehydroshikimate to protocatechuate in *Pseudomonas ovalis* S-5 has been discussed by Yano and Arima (149). It has also been postulated by Bassett and Tanenbaum (6) that *p*-hydroxybenzoate arises directly from shikimate in *Penicillium patulum*.

Two interesting papers concerned with phenolic acid formation in *Phycomyces* appeared in 1962 which throw light on an aspect of phenol biosynthesis not often considered by most workers. In the first paper Hashem and Brucker (61) reported 2% conversion of shikimate to gallate and 80% conversion of shikimate to protocatechuate. Different biosynthetic pathways could explain these observations. Protocatechuate could be formed by a mechanism similar to that just described above. The low incorporation into gallate suggests that hydroxylation of protocatechuate in not an important biosynthetic process in this particular organism, but that gallate might be formed *via* the acetate pathway.

The second paper by these investigators (8) presented their data on the effect of certain metabolic inhibitors on the shikimate and acetate pathways in twelve strains of *Phycomyces blakesleeanus*. The inhibitors which would be expected to affect the shikimate acid pathway had little effect on the formation of phenolic compounds whereas compounds which inhibited acetyl-CoA function greatly influenced accumulation of phenolic compounds. Thus, a very considerable proportion of the phenolic compounds accumulated were formed by way of the acetate pathway.

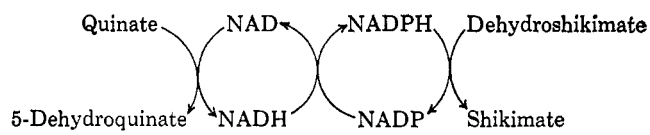
10. *The Role of Quinate in Aromatic Biosynthesis*

Quinic acid has been isolated from many higher plants, often in considerable quantity. Owing to the similarity of quinic acid and shikimic acid one might consider quinic acid as a possible contributor to formation of aromatic compounds. An early suggestion that this might be the case came from the work of Gordon, *et al.* (44), who found that a *Neurospora* mutant could grow on a synthetic medium supplemented with quinate.

Davis and Weiss (22) demonstrated that an *Aerobacter aerogenes* mutant responded to quinate as well as it did to 5-dehydroquinate. Significantly, *E. coli* mutants which did not respond to 5-dehydroquinate did not respond to quinate or accumulate it. A search was made for mutants of *A. aerogenes* blocked between 5-dehydroquinate and quinate but without success. These observations would suggest that quinate is not in the direct aromatic pathway. Support for this conclusion comes from the observation that quinate dehydrogenase was absent from several organisms which were capable of synthesizing their own aromatic amino acids.

A purification of quinate dehydrogenase[5] obtained from an *Aerobacter aerogenes* mutant has been accomplished by Mitsuhashi and Davis (93). Treatment of a cell-free extract, successively, with manganese chloride, calcium phosphate gel, and ammonium sulfate gave a purification factor of about 8. Cofactor studies showed a requirement for NAD. A wide pH range with a maximum at about 9.8 was reported. The system was specific for quinate and had a K_m value of 4.9×10^{-4} *M* for the substrate and 1.4×10^{-5} *M* for NAD at pH 9.4.

In the initial extracts a coupled system was operating; the NAD specific oxidation of quinate to 5-dehydroquinate was coupled to the NADP specific reduction of 5-dehydroshikimate to shikimate. The system is represented below.



From data obtained in these studies a 5-dehydroshikimate/5-dehydroquinate ratio of 15 was calculated which lies well in the direction required for aromatic biosynthesis.

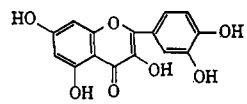
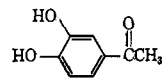
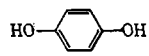
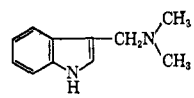
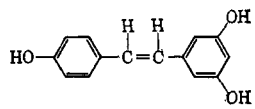
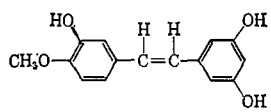
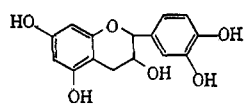
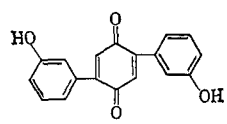
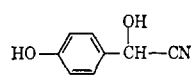
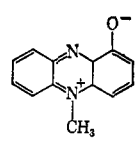
The question still existed as to the metabolic involvement of quinate in higher plants. Some light was thrown on the subject by Weinstein and co-workers (136) who administered quinic acid-C^{14} to rose plants and studied the distribution of label after a period of metabolism. Shikimate was the most highly labeled product, but phenylalanine and tyrosine were also labeled. Label also occurred in asparagine, aspartate, glutamate, succinate, fumarate, malate, and citrate with the largest proportion of label being in the latter two. The presence of quinate dehydrogenase was suggested.

A further study by these workers (137) confirmed the observations that metabolism of quinate occurred in higher plants. *Phaseolus vulgaris, Nicotiana tabacum, Pyrus malus, Persea americana, Rumex acetosa, Kalanchoe* sp., as well as corn, oats, and tomatoes, were examined and found to convert considerable quantities of administered quinate to phenylalanine and tyrosine. A relatively large amount of labeled carbon dioxide was respired in most cases. In *Persea americana* no shikimate was observed among the labeled products.

(5) Systematic name: quinate: NAD oxidoreductase (1.1.1.24).

TABLE VIII

STUDIES IN WHICH SHIKIMIC ACID HAS BEEN USED AS A PRECURSOR

Compound	Structure	Organism	Ref.
Quercetin		*Fagopyrum tataricum*	135
Pungenin aglycone		*Picea pungens*	96
Arbutin aglycone		*Pyrus communis*	52
Gramine		*Grevillea robusta* *Hordeum vulgare*	146
Piceid aglycone		*Eucalyptus sideroxylon*	70
Rhapontin aglycone		*E. sideroxylon*	70
Catechin		Tea sprouts	154
Volucrisporin		*Volucrispora aurantiaca*	107
Dhurrin aglycone		*Sorghum vulgare*	79
Pyocyanine		*Pseudomonas aeruginosa*	84
Tetracyclines	.....	*Streptomyces viridifaciens*	45
Tetracyclines	.....	*Streptomyces sayamaensis*	115

C. MISCELLANEOUS BIOSYNTHETIC STUDIES WITH SHIKIMIC ACID

With the considerable interest in the chemistry and metabolism of shikimic acid and some of its derivatives came the obvious need for sources of supply of the compounds. For the chemical studies simple extraction from an appropriate source as outlined above in section IIIC could be done. For biochemical examinations where a radioactivity labeled compound was necessary methods had to be devised whereby the compound was obtained with label in the desired position or positions. The obvious choice of labels was carbon-14.

A common organism for preparation of shikimic acid-C^{14} is *E. coli* mutant 82–24. Methods can be found in the early papers by Davis and his group for shikimic acid-C^{14} preparation. An example of a later method is that of Millican (90) who simply passed the broth supernatant from this organism through a Dowex-1 column (acetate form) and eluted with ammonium acetate. The eluate was passed through a second column of the same resin and the free acid eluted with acetic acid. The source of C-14 for these preparations is glucose. Uniformly labeled glucose affords uniformly labeled shikimic acid. Choice of appropriately labeled glucose can give shikimic acid labeled in a variety of ways so that a degree of flexibility is available for researchers.

Further examples of production of labeled shikimic acid include Perkins and Aronoff's (98) paper chromatographic purification procedure on material from the *E. coli* mutant and Simonart and Wiaux's (116) isolation from *Penicillium griseofulvum* and purification using columns of Dowex-50 (chloride form) and Dowex-2 (acetate form).

A major objection to the use of microorganisms for the production of C-14 labeled shikimic acid is the high loses of label in the conversion of glucose (the most commonly used precursor) to the product. It was virtually impossible to obtain high specific activities required for careful metabolic studies. This difficulty was overcome by the procedure developed by Weinstein and co-workers (138). Shoots of *Ginkgo biloba* L. were allowed to metabolize for several days in an atmosphere of radioactive CO_2. The plant material was extracted with ethanol–water and the extract passed through a column of Dowex-50-X4 (H^+ form) to remove basic substances. The effluent was passed next through Dowex-1-X8 (acetate form). Gradient elution with 2.5 *N* acetic acid into water gave excellent separation of shikimic acid and quinic acid. A final passage through Dowex-1-X8 (acetate form) yielded the compounds in forms pure enough for immediate use. Yields of 2.12 of shikimic acid and 0.593 g. of quinic acid were obtained from 287 g. of fresh leaves.

Underhill (134) has employed a modification of the above procedure in which both *Ginkgo* shoots and rose shoots are grown together in the radioactive atmosphere. Working up of the total extract of both plants yields a higher quantity of quinic acid than when *Ginkgo* is used alone.

Although this review is supposed to concern itself with shikimic acid, we have followed the aromatic biosynthetic pathway through compounds which resemble shikimic acid only superficially to the actual point of aromatization. This is probably justifiable on the basis of the significance of the over-all pathway. Once aromatic compounds have been formed the story changes and so must the teller. However, many references occur in the literature concerning aromatic biosynthesis where only the nature of the precursor and the product molecule are under major consideration. Insofar as shikimic acid has been used as a precursor in a number of these studies mention should at least be made of this fact in a review dealing with shikimic acid. Listed in Table VIII are some studies in which shikimic acid has been employed simply as a possible aromatic precursor.

Shikimic acid has also been used in precursor studies of lignin. A discussion of this topic is also beyond the scope of this review. For a recent discussion of lignin and tannin biosynthesis the interested reader is referred to Brown (7).

D. PHYSIOLOGICAL CONSIDERATIONS

With knowledge on hand concerning the biosynthesis and metabolism of shikimic acid and some of its derivatives attention can be turned to the physiological aspects of the pre-aromatic pathway. In comparison to chemical and biochemical studies the physiology of the pathway has received considerably less notice to date. A few questions have been asked and a few answers have been offered.

Shikimic acid has been found to occur in a variety of tissues in the many plants which have been studied. An idea of the diversity of location can be formed from Table IX.

TABLE IX

LOCATION OF SHIKIMIC ACID IN VARIOUS PLANT TISSUES

Plant	Organ or part	Ref.
Iris pseudoacorus	Rhizome	66
Helianthus tuberosus	Tuber	76
Picea pungens	Whole twigs	95
Picea glauca	Whole twigs	95
Eucalyptus sieberiana	Cambium	69
Eucalyptus regnans	Cambium	69
Eucalyptus sieberiana	Phloem	68
Thuja plicata	Phloem	129
Phoenix dactylifera	Fruit	85
Dahlia	Tuber	77
Apple	Peel	73
Pear	Peel and pulp	74
Wheat	Nodes and internodes	15
Many plants	Leaves	

A study of the quantitative distribution of shikimic acid in the organs of various plants has been reported by Hasegawa and co-workers (59). The results of their study are shown in Table X.

TABLE X

DISTRIBUTION OF SHIKIMIC ACID IN ORGANS OF VARIOUS PLANTS

Plant	Root	Bark	Stem	Leaf	Seed
Pinus densiflora[a]	93[b]	114	...	13.5	...
Pinus Thunbergii	...	119.7	...	55.6	...
Illicum anisatum	...	397.7	...	98.6	...
Magnolia grandiflora	...	19.2	...	32.0	...
Houttuynia cordata	61.2	...	97.7	83.3	91.4
Saxifraga stolonifera	0	...	56.8	36.0	...

[a] Seedlings. [b] Expressed as μg./g. of fresh weight.

The change in concentration of shikimic acid with age of a plant has been observed by several investigators. Henshaw and co-workers (66), studying the rhizome of *Iris pseudoacorus*, found that the concentration of shikimic acid fell from 739 μg./g. of fresh weight in February to 545 μg/g. in the following June while the over-all concentration of quinic acid remained essentially unchanged although an increase was noted in March. The authors commented that the shikimic acid concentration dropped significantly following leaf formation suggesting its utilization in metabolic processes. In con-

TABLE XI

PLANTS EXAMINED FOR SHIKIMIC ACID

	Species	Test[a]	Ref.
	A. Bryophyta		
Marchantiaceae	*Marchantia polymorphia* L.	S	59
	Polytricum sp.	–	59
	B. Pteridophyta		
Plagiogyriaceae	*Plagiogyria japonica* Nakai	S	59
	P. Matsumuraeana Mak.	S	59
Polypodiaceae	*Adiantum monochlamys* Eat.	S	59
	Crytomium Fortunei J. Sm. var. *clivicola* Tagawa	S	59
	Dryopteris uniformis Mak.	S	59
	Lemnaphyllum microphyllum Presl.	S	59
	Onoclea sensibilis L.	S	59
	Onychium japonicum Kze.	S	59
	Woodwardia orientalis Sw.	S	59
	Athyrium niponicum Hance	–	59
	Diplazium Wichurae Diels	–	59
	Lepisorus Thunbergianus Ching	–	59
	Pteridium aquilinum Kuhn var. *japonicum* Nakai	–	59
	Rumohra amabilis Ching	–	59
Gleicheniaceae	*Dicranopteris glauca* Robinson	S	59
Osmundaceae	*Osmunda japonica* Thunb.	S	59
Isoetaceae	*Isoetes japonica* Al. Br.	S	59
Lygodiaceae	*Lygodium japonicum* Sw.	–	59
Salviniaceae	*Salvinia natans* All.	–	59
Equisetaceae	*Equisetum arvense* L.	–	59
Lycopodiaceae	*Lycopodium obscurum* L.	–	59
	L. clavatum L. var. *nipponicum* Nakai	–	59
Selaginellaceae	*Selaginella uncinata* Spring	–	59
	C. Gymnospermae		
Cycadaceae	*Cycus revoluta* Thunb.	–	64
Ginkgoaceae	*Ginkgo biloba* L.	S	64
Pinaceae	*Pinus excelsa*	SQ	101
	P. Laricio Poir. var. *corsicana*	S	101
	P. montana Mill.	SQ	101
	P. nigra Arn. var. *austriaca*	SQ	101
	P. parviflora Sieb. et Zucc.	S	101
	P. Pinaster Sol.	SQ	101
	P. pinea L.	SQ	101
	P. strobus L.	SQ	101
	P. sylvestris L.	Q	101
	P. densiflora Sieb. et Zucc.	S	64
	P. Thunbergii Parl.	S	64
	P. resinosa Ait.	S	150
	Picea alba Link	Q	101
	P. asperata Mast.	–	101
	P. excelsa Link	SQ	101
	P. mariana B.S.P.	–	101
	P. orientalis Carr.	SQ	101
	P. glauca	S	95
	P. pungens Engelm.	S	95
	Tsuga canadensis Carr.	Q	101
	T. Sieboldii Carr.	SQ	101
	T. heteophylla	S	42
	Pseudotsuga Douglasii Carr.	SQ	101
	P. japonica Beissner	Q	64
	Abies cephalonica Link	Q	101
	A. cilicica Carr.	SQ	101
	A. concolor Lindl.	Q	101
	A. Nordmanniana Spach	SQ	101
	A. Pinsapo Boiss.	SQ	101
	A. Mayriana Miyabe et Kudo	Q	64
	Larix europaea DC	Q	101
	L. leptolepis Gord.	–	64

Table XI (*Continued*)

	Species	Test[a]	Ref.
	Pseudolarix Kaempferi Gord.	Q	101
	Cedrus atlantica Manetti	Q	101
	C. Deodora Loud.	SQ	101
	C. Libani Barr. var. *Deodora*	Q	64
	Keteleeria Davidiana Beissner	S	64
Taxodiaceae	*Sequoia gigantea* Torr.	Q	101
	S. sempervirens Endl.	SQ	101
	Metasequoia glyptostroboides Hu et Cheng	S	64
	Cunninghamia lanceolata Hook.	S	64
	Cryptomeria japonica D. Don	S	64
	Taxodium distichum Rich.	S	64
Cupressaceae	*Cupressus arizonicum* Greene	–	101
	C. Benthamii Endl.	S	64
	Juniperus communis L.	–	101
	J. chinensis L.	S	64
	J. virginiana L.	S	64
	Thuja Standishii Carr.	S	64
	T. plicata	S	129
	Thujopsis dolabrata Sieb. et Zucc	S	64
	Libocedrus formosana Florin	S	64
	Chamaecyparis obtusa Endl.	S	64
	Biota orientalis Endl.	S	64
Podocarpaceae	*Podocarpus Purdieana* Hook.	S	101
	P. macrophylla D. Don.	–	64
	P. Nageia Zoll. et Moritzi	–	64
Cephalotaxaceae	*Cephalotaxus drupacea* Sieb. et Zucc.	S	64
Auricariaceae	*Auricaria imbricata* Pav.	S	64
Taxaceae	*Taxus cuspidata* Sieb. et Zucc.	S	64
	T. baccata L.	S[b]	64
	Torreya nucifera Sieb. et Zucc.	S[b]	64
	D. Angiospermae Monocotyledoneae		
Hydrocharitaceae	*Elodea densa* Casp.	–	59
Gramineae	*Lolium perenne*	S	109
	L. multiflorum Lam.	S	130
	L. temulentum L.	S	59
	Festuca elatior L. subsp. *arundinaceae* Hack.	S	59
	Hordeum sativum Jessen var. *hexastichon* Hack.	S	59
	Bromus uniloides H. B. & K.	–	59
	Eleusine indica Gaertn.	–	59
	Miscanthus sinensis Anders.	–	59
	Poa acroluca Steud.	–	59
	Triticum vulgare var.	S	15
Cyperaceae	*Carex brunnea* Thunb. var *Nakiri* Ohwi	–	59
Palmae	*Phoenix dactylifera* L.	S	85
	Trachycarpus excelsus Wend.	–	59
Araceae	*Colocasia antiquorum* Schott var. *esculentum* Schott	–	59
Commelinaceae	*Commelina communis* L.	S	59
Liliaceae	*Lilium japonicum* Houtt.	–	59
	Allium monanthum Maxim.	S	59
	Yucca recurvifolia Salisb.	–	59
	Aspidistra elatior Bl.	–	59
	Tricyrtis hirta Hook. var. *parviflora* Masamune	–	59
Amaryllidaceae	*Narcissus Tazetta* L. var. *suisen* Sieb.	S	59
	Lycoris radiata Herb.	–	59
	Agave americana L. var. *variegata* Nicholson	–	59
Dioscoreaceae	*Dioscorea japonica* Thunb.	–	59
Iridaceae	*Iris pseudoacorus* L.	S	66
	Dicotyledoneae Archichlamydeae		
Saururaceae	*Houttuynia cordata* Bueck	S	59
Chloranthaceae	*Sarcandra glabra* Nakai	S	59
Salicaceae	*Salix babylonica* L.	S	59
Myricaceae	*Myrica rubra* Sieb. et Zucc.	S	59

Table XI (*Continued*)

	Species	Test[a]	Ref.
Juglandaceae	*Petrophiloides strobilaceae* Reid et Chandler	S	59
Betulaceae	*Carpinus Tschonoskii* Maxim.	S	59
Fagaceae	*Castanea crenata* Sieb. et Zucc.	–	59
	Corylus heterophylla Fisch. var. *japonica* Koidz.	S	59
	Cyclobalanopsis gilva Oersten	S	59
	C. paucidentata Kudo et Masamune	S	59
	C. acuta Oerst.	–	59
	Kuromatea glabra Kudo	S	59
	Quercus phylliraeoides A. Gray	S	59
	Q. variabilis Bl.	S	59
	Shiia cuspidata Mak.	S	59
	S. Sieboldi Mak.	S	59
	S. Sieboldi Mak. var. *rotundifolia* Mak.	S	59
Ulmaceae	*Ulmus Davidiana* Planch. var. *japonica* Nakai	–	59
Moraceae	*Morus bombycic* Koidzumi	–	59
	Humulus japonica Sieb. et Zucc.	–	59
Utricaceae	*Boehmeria longispica* Steud.	–	59
Aristolochiaceae	*Heterotropa nipponica* F. Maekawa	–	59
Polygonaceae	*Polygonum Yokusaianum* Mak.	–	59
Chenopodiaceae	*Spinacia oleraceae* L.	–	59
Amaranthaceae	*Euxolus ascendens* Hara	–	59
Nyctaginaceae	*Mirabilis Jalapa* L.	–	59
Phytolaccaceae	*Phytolacca Americana* L.	–	59
Portulacaceae	*Portulaca oleraceae* L.	–	59
Trochodendraceae	*Euptela polyandra* Sieb. et Zucc.	S	59
Cercidiphyllaceae	*Cercidiphyllum japonicum* Sieb. et Zucc.	S	59
Ranunculaceae	*Thalictrum Thunbergii* DC var. *hypoleucum* Nakai	S	59
Lardizabalaceae	*Akebia trifoliata* Koidz	S	59
Berberidaceae	*Mahonia Fortunei* Fedde	S	59
Magnoliaceae	*Illicium anisatum* L.	S	59
	I. religiosum Sieb.	S	25
	Kadsura japonica Dun.	S	59
	Liriodendron tulipifera L.	S	59
	Magnolia denudata Desr.	S	59
	M. grandiflora L.	S	59
	M. Kobus DC	S	59
	M. liliflora Desr.	S	59
	M. obovata Thunb.	S	59
	Michelia compressa Sarg.	S	59
Calycanthaceae	*Calycanthus glaucus* Wield	–	59
Lauraceae	*Cinnamonum camphora* Sieb.	S	59
Papaveraceae	*Macleya cordata* R. Br.	S	59
Cruciferae	*Raphanus sativus* L. var. *acanthiformis* Mak.	–	59
Crassulaceae	*Sedum bulbiferum* Mak.	–	59
	S. oryzifolium Mak.	–	59
Saxifragaceae	*Saxifraga stolonifera* Nemoto	S	59
Hamamelidaceae	*Distylium racemosum* Sieb. et Zucc.	S	59
	Liquidambar styraciflua L.	S	103
	Hamamelis japonica Sieb. et Zucc.	–	103
	H. mollis Oliv.	–	103
	H. vernalis Sarg.	–	103
	H. virginiana L.	–	103
	Corylopsis glabrescens Franch. et Sav.	–	103
	C. pauciflora Sieb. et Zucc.	–	103
	C. platypetala Rehd. et Wils.	–	103
	C. sinensis Hemsl.	–	103
	C. spicata Sieb. et Zucc.	–	103
	C. Wilmottiae Rehd. et Wils.	–	103
	Parrotia persica C.A. Mey.	–	103
	Parrotiopsis Jacquemontiana Rehd.	–	103
	Fothergilla major Lodd.	–	103
	Sycopsis sinensis Oliv.	–	103
Eucommiaceae	*Eucommis ulmoides* Oliv.	–	59
Platanaceae	*Platanus occidentalis* L.	S	59
Rosaceae	*Aruncus silvester* Kostel. var. *americanus* Maxim.	S	59

Table XI (*Continued*)

	Species	Test[a]	Ref.
	Cydonia vulgaris Pers. var.	S	145
	Eriobotrya japonica Lindl.	S	59
	Prunus yedoensis Matsumura	–	59
	Pyrus communis var.	S	145
	P. malus var.	S	145
	Ribes Grossularia var.	S	145
	Rubus corchorifolius L.	–	59
	R. sp.	S	145
Leguminosae	*Caesalpinia coriaria*	S	105
	C. spinosa (Mol.) Ktze., O.	S	72
	Lespedeza bicolor Turcz. var. *japonica* Nakai	S	59
	Vicia faba L.	S	113
Geraniaceae	*Geranium Thunbergii* Sieb. et Zucc.	S	59
Oxalidaceae	*Oxalis violacea* L.	–	59
Rutaceae	*Skimmia japonica* Thunb.	–	59
Meliaceae	*Melia Azedarach* L. var. *japonica* Mak.	–	59
Euphorbiaceae	*Aleurites cordata* Steud.	–	59
	Daphniphyllum macropodum Miq.	–	59
Anacardiaceae	*Poupartia axillaris* King et Prain	–	59
	Pistacia Lentiscus L.	SQ	102
	P. atlantica Desf.	SQ	102
	P. Terebinthus L.	SQ	102
	P. vera L.	SQ	102
	Schinus dependens Orteg.	SQ	102
	S. latifolius Engl.	SQ	102
	Lithraea Araeirinha March.	SQ	102
Aquifoliaceae	*Ilex integra Thunb.*	–	59
	I. latifolia Thunb.	–	59
Celastraceae	*Euonymus japonicus* Thunb.	S	59
Staphyleaceae	*Staphylea Bumalda* DC	–	59
Aceraceae	*Acer distylum* Sieb. et Zucc.	S	59
Hippocastanaceae	*Aesculus turbinata* Bl.	S	59
Sapindaceae	*Sapindus Mukurossi* Gaertn.	S	59
Balsaminaceae	*Impatiens balsamina* L.	–	59
Rhamnaceae	*Rhamnus Frangula* L.	–	59
Vitaceae	*Cayratia japonica* Merrill	–	59
	Vitus sp.	S	9
Malvaceae	*Hibiscus mutabilis* L.	–	59
Sterculiaceae	*Firmiana platanifolia* Schott et Endl.	–	59
Theaceae	*Thea sinensis* L. var. *bohea* Szyszylowicz	–[d]	59
	Sakakia ochnacea Nakai	S	59
Guttiferae	*Caulophyllum antillanum*	–	104
	Clusia rosea	–	104
	Hypericum calycinum	–	104
	H. chinense	S	151
	H. monanthum nigro-punctatum	S	59
	Mammea americana L.	S	104
	Mesua ferrea	–	104
	Ternstroemia japonica Thunb.	S	59
Thymelaeaceae	*Daphne odora* Thunb.	–	59
Elaeagnaceae	*Eleagnus pungens* Thunb. var. *variegata*	S	59
Lythraceae	*Lagerstroemia subcostata* Koehne var. *hirtella* Koehne	–	59
Combretaceae	*Anogeissus latifolia*	S	108
	Terminalia chebula	S	105
Myrtaceae	*Eucalyptus globulus* Labill.	S	59
	E. Citriodora Hook.	S	1
	E. nitens	S	58
	E. regnans	S	69
	E. sieberiana F. Muell.	S	69
Onagraceae	*Oenothera Lamarkiana* Ser.	–	59
Araliaceae	*Hedera Tobleii* Nakai	S	59
Cornaceae	*Aucuba japonica* Thunb.	S	59
	Sympetalae		
Clethraceae	*Clethra barbinervis* Sieb. et Zucc.	–	59
Ericaceae	*Pieris japonica* D. Don	S	59

Table XI (*Continued*)

	Species	Test[a]	Ref.
Ebenaceae	*Diospyros Kaki* Thunb. var. *domestica* Mak.	–	59
Symplocaceae	*Bobua myrtacea* Sieb. et Zucc.	–	59
Styracaceae	*Halesia tetraptera* L.	–	59
Oleaceae	*Ligustrum lucidun* Ait.	S	59
	Syringa vulgaris L. var. *alba* Ait.	S	59
	Osmanthus ilicifolius Standish	–	59
Gentianaceae	*Gentiana scabra* Bunge var. *Buergeri* Maxim.	–	59
Apocynaceae	*Nerium odorum* Soland.	–	59
Convolvulaceae	*Ipomoea Batates* Lam. var. *edulis* Mak.	–	59
Polemoniaceae	*Phlox paniculata* L.	S	59
Boraginaceae	*Ehretia Dicsoni* Hance var. *japonica* Nakai	–	59
Verbenaceae	*Vitex cannabifolia* Sieb. et Zucc.	S	59
Labiatae	*Perilla fructescens* Britten var. *crispa* Decne. form *purpurea* Mak.	–	59
Solanaceae	*Nicotiana tabacum* L.	–	59
	Solanum lycopersicum L. var.	S	16
Scrophulariaceae	*Paulownia tomentosa* Steud.	–	59
Gesneriaceae	*Conandron ramondioides* Maxim.	–	59
Plantaginaceae	*Plantago asiatica* L.	–	59
Rubiaceae	*Gardenia jasminoides* Ellis var. *grandiflora* Nakai	S	59
	G. jasminoides Ellis var. *ovalifolia* Nakai	S	59
	Rubia Akane Nakai	S	59
Caprifoliaceae	*Viburnum Awabucki* K. Koch	S	59
	V. japonicum Spr.	S	59
	Weigela grandiflora Fortune	S	59
Valerianaceae	*Patrina villosa* Juss.	S	59
Cucurbitaceae	*Trichosanthes cucumeroides* Maxim.	–	59
	Lagenaria leucantha Rosby var. *clavata* Mak.	S	131
Compositae	*Chrysanthemum morifolium* Rumatuelle	–	59
	Helianthus tuberosus L.	S	76
	Dahlia sp.	S	81

[a] Explanation of symbols: S, shikimic acid present; –, shikimic acid absent; SQ, both shikimic acid and quinic acid present; Q, quinic acid present. [b] Reported absent by Plouvier (101). [c] Reported absent by Plouvier (103). [d] Reported present by Zaprometov (152).

trast Neish (95) showed that the concentration of shikimic acid in both year-old leaves and new growth leaves of *Picea pungens* remained relatively constant over a period of study of 4 to 6 months. Raffinose disappeared with the onset of warm weather and the concentration of pungenin (3,4-dihydroxyacetophenone glucoside) decreased during the summer months.

Hulme (73) has reported an increase in shikimic acid concentration with increase in age in the peel of apples. He (74) also observed a net increase in shikimic acid and a net decrease in quinic acid in the peel of pears kept at 15° for 100 days. The author suggested that shikimic acid as an active metabolic intermediate occurs free in tissues in which metabolic processes are slowing down. Whiting (145) observed that the concentration of shikimic acid in gooseberry fruits remained practically constant from under-ripe to fully ripe which is in contrast to Hulme's observations with apples and pears. Shikimic acid in grape leaves disappears during maturation (23).

Shikimic acid has been found (69) to be present in the cambium of *Eucalyptus sieberiana* and *E. regnans*. A trace was found in 1- to 2-year-old sapwood, none in 3- to 8-year-old sapwood, and none in the heartwood. In a later paper Hillis (67) demonstrated the presence of shikimic acid in the leaves of *E. sieberiana* and showed that a maximum concentration was attained when the leaves were at full size. As the leaf aged further the concentration dropped. Translocation to the cambium was suggested but no direct evidence is available to chose between this possibility and the alternative possibility, localized cambial synthesis of shikimic acid. The formation of phenolic compounds at the heartwood boundary from starch and glycosides present in sapwood cells and from translocated carbohydrates was discussed by Hillis and Carle (68).

The seasonal variation in shikimic acid content in *Ginkgo biloba* and *Pinus Thunbergii* has been studied by Hasegawa and Tateoka (60). With *Ginkgo* a maximum concentration was observed in August in both inner bark and leaf tissue. A peak in July was observed in outer sapwood. With the pine a concentration peak was observed in July of the second year with leaf tissue. General increases of concentration with age are apparent with inner bark and outer sapwood, but considerable variation was observed throughout both. The results with outer sapwood, with the concentration being essentially zero, agrees with the fact that spring wood and autumn wood are formed in this region.

Tsuga heterophylla cambium and sapwood were examined for phenolic precursors by Goldschmid and Hergert (42). *p*-Coumaric acid, caffeic acid, and ferulic

acid derivatives of shikimic acid were found in the cambium. Shikimic acid has been reported in the newly formed inner bark of *Pseudotsuga Douglasii* by Holmes and Kurth (71) and in the bark phloem of *Thuja plicata* by Swan (129).

V. The Distribution of Shikimic Acid in Nature

The purpose of this section is to list plants which have been examined for the presence of shikimic acid. It is conceivable that the absence of shikimic acid in a particular organism might be of significance to some investigators; hence, species in which no test for shikimic acid was obtained are included in the list. In a few cases the presence of quinic acid is indicated. In most cases only shikimic acid was under investigation.

The plants are organized in the following manner. The lower organisms are presented as they appeared in the compilation by Hasegawa and co-workers (59). The Gymnospermae are presented in the order of West (144) and the Angiospermae according to Engler as given by Willis (147). Several family names were changed to correspond to the usage in Willis (147). Other than this the plants are presented as they appeared in the original references.

VI. Rererences

(1) Anet, E. F. J. L., Birch, A. J., and Massy-Westropp, R. A., *Australian J. Chem.*, **10**, 93 (1957).
(2) Balinsky, D., and Davies, D. D., *Biochem. J.*, **80**, 292 (1961).
(3) Balinsky, D., and Davies, D. D., *Biochem. J.*, **80**, 296 (1961).
(4) Balinsky, D., and Davies, D. D., *Biochem. J.*, **80**, 300 (1961).
(5) Balinsky, D., and Davies, D. D., *J. Exptl. Botany*, **13**, 414 (1962).
(6) Bassett, E. W., and Tanenbaum, S. W., *Biochim. Biophys. Acta*, **28**, 247 (1958).
(7) Brown, S. A., "Biochemistry of Phenolic Compounds," Academic Press Inc., New York, N. Y., 1964, p. 361.
(8) Brucker, W., and Hashem, M., *Ber. Deut. Botan. Ges.*, **75**, 3 (1962).
(9) Carles, J., and Alquier-Bouffard, A., *Compt. rend.*, **254**, 925 (1962).
(10) Carr, J. G., Pollard, A., Whiting, G. C., and Williams, A. H., *Biochem. J.*, **66**, 283 (1957).
(11) Cartwright, R. A., and Roberts, E. A. H., *Chem. Ind.* (London), 230 (1955).
(12) Carvajal, G., and Carvajal, E. J., *Rev. Latinoam. Microbiol.*, **1**, 41 (1958).
(13) Carvajal, G., and Carvajal, E. J., *Rev. Latinoam. Microbiol.*, **2**, 133 (1959).
(14) Carvajal, G., Penna, S., and Carvajal, E. J., *Rev. Latinoam. Microbiol.*, **5**, 45 (1962).
(15) Coic, Y., Lesaint, C., and LeRoux, F., *Ann. Physiol. Veg.*, **3**, 33 (1961).
(16) Coic, Y., Lesaint, C., and LeRoux, F., *Compt. rend.*, **254**, 4193 (1962).
(17) Coulson, C. B., and Evans, W. C., *J. Chromatog.*, **1**, 374 (1958).
(18) Dangschat, G., and Fischer, H. O. L., *Biochim. Biophys. Acta*, **4**, 199 (1950).
(19) Davis, B. D., *J. Bacteriol.*, **64**, 729 (1952).
(20) Davis, B. D., *J. Biol. Chem.*, **191**, 315 (1951).
(21) Davis, B. D., and Mingioli, E. S., *J. Bacteriol.*, **66**, 129 (1953).
(22) Davis, B. D., and Weiss, U., *Arch. exptl. Pathol. Pharmakol.*, **220**, 1 (1953).
(23) Delmas, J., Poitou, N., and Levadou, B., *Ann. agron.*, **14**, 951 (1963).
(24) Doshi, M. M., *Dissertation Abstr.*, **24**, 3998 (1964).
(25) Eykman, J. F., *Rec. trav. chim.*, **4**, 32 (1885).
(26) Eykman, J. F., *Ber.*, **24**, 1278 (1891).
(27) Fewster, J. A., *Biochem. J.*, **85**, 388 (1962).
(28) Fischer, H. O. L., and Dangschat, G., *Helv. Chim. Acta*, **17**, 1200 (1934).
(29) Fischer, H. O. L., and Dangschat, G., *Helv. Chim. Acta*, **18**, 1204 (1935).
(30) Fischer, H. O. L., and Dangschat, G., *Helv. Chim. Acta*, **18**, 1206 (1935).
(31) Fischer, H. O. L., and Dangschat, G., *Helv. Chim. Acta*, **20**, 705 (1937).
(32) Fischer, H. O. L., and Dangschat, G., *Naturwiss.*, **26**, 562 (1938).
(33) Fradejas, R. G., Ravel, J. M., and Shive, W., *Biochem. Biophys. Res. Commun.*, **5**, 320 (1961).
(34) Gaitonde, M. K., and Gordon, M. W., *J. Biol. Chem.*, **230**, 1043 (1958).
(35) Gamborg, O. L., and Simpson, F. J., *Can. J. Biochem.*, **42**, 583 (1964).
(36) Gibson, F., *Biochem. J.*, **90**, 256 (1964).
(37) Gibson, M. I., and Gibson, F., *Biochim. Biophys. Acta*, **65**, 160 (1962).
(38) Gibson, M. I., and Gibson, F., *Biochem. J.*, **90**, 248 (1964).
(39) Gibson, M. I., Gibson, F., Doy, C. H., and Morgan, P., *Nature*, **195**, 173 (1962).
(40) Gibson, F., and Jackman, L. N., *Nature*, **198**, 388 (1963).
(41) Gilvarg, C., reference in Davis, B. D., *Federation Proc.*, **14**, 691 (1955).
(42) Goldschmid, O., and Hergert, H. L., *Tappi*, **44**, 858 (1961).
(43) Gordon, H. T., Thornburg, W., and Werun, L. N., *Anal. Chem.*, **28**, 849 (1956).
(44) Gordon, M., Haskins, F. A., and Mitchell, H. K., *Proc. Natl. Acad. Sci. U. S.*, **36**, 427 (1950).
(45) Gourevitch, A., and Lein, J., U. S. Patent 2,712,517; *Chem. Abstr.*, **49**, 14278 (1955).
(46) Grewe, R., and Bokranz, A., *Ber.*, **88**, 49 (1955).
(47) Grewe, R., and Buttner, H., *Ber.*, **91**, 2452 (1958).
(48) Grewe, R., and Hinrichs, I., *Ber.*, **97**, 443 (1964).
(49) Grewe, R., Jensen, H., and Schnoor, M., *Ber.*, **89**, 898 (1956).
(50) Grewe, R., and Jeschke, J.-P., *Ber.*, **89**, 2080 (1956).
(51) Grewe, R., and Lorenzen, W., *Ber.*, **86**, 928 (1953).
(52) Grisdale, S. K., and Towers, G. H. N., *Nature*, **188**, 1130 (1960).
(53) Gross, S. R., *J. Biol. Chem.*, **233**, 1146 (1958).
(54) Hall, L. D., *J. Org. Chem.*, **29**, 297 (1964).
(55) Hanson, K. R., *J. Chem. Educ.*, **39**, 419 (1962).
(56) Hanson, K. R., and Rose, I. A., *Proc. Natl. Acad. Sci. U. S.*, **50**, 981 (1963).
(57) Hanson, K. R., and Zucker, M., *J. Biol. Chem.*, **238**, 1105 (1963).
(58) Hasegawa, M., and Higuchi, T., *J. Japan. Forestry Soc.*, **42**, 305 (1960).
(59) Hasegawa, M., Nakagawa, T., and Yoshida, S., *J. Japan. Forestry Soc.*, **39**, 159 (1957).
(60) Hasegawa, M., and Tateoka, T., *J. Japan. Forestry Soc.*, **42**, 224 (1960).
(61) Hashem, M., and Brucker, W., *Flora*, **152**, 57 (1962).

(62) Hathway, D. E., *Biochem. J.*, **63**, 380 (1956).
(63) Hattori, S., Yoshida, S., and Hasegawa, M., *Arch. Biochem. Biophys.*, **74**, 480 (1958).
(64) Hattori, S., Yoshida, S., and Hasegawa, M., *Physiol. Plantarum*, **7**, 283 (1954).
(65) Hennig, K., and Burkhardt, R., *Weinberg Keller*, 542 (1958).
(66) Henshaw, G. G., Coult, D. A., and Boulter, D., *Nature*, **194**, 579 (1962).
(67) Hillis, W. E., *J. Exptl. Botany*, **10**, 87 (1959).
(68) Hillis, W. E., and Carle, A., *Biochem. J.*, **74**, 607 (1960).
(69) Hillis, W. E., and Carle, A., *Holzforschung*, **12**, 136 (1958).
(70) Hillis, W. E., and Hasegawa, M., *Chem. Ind.* (London), 1330 (1962).
(71) Holmes, G. W., and Kurth, E. F., *Tappi*, **44**, 893 (1961).
(72) Horler, D. F., and Nursten, H. E., *J. Chem. Soc.*, 3786 (1961).
(73) Hulme, A. C., *Nature*, **178**, 991 (1956).
(74) Hulme, A. C., *Qualitas Plant. Mater. Vegetabiles*, **3–4**, 468 (1958).
(75) Ishikawa, H., and Oki, T., *Bull. Agr. Chem. Soc. Japan*, **23**, 451 (1959).
(76) Jolivet, E., and Nicol, M. Z., *Compt. rend.*, **254**, 721 (1962).
(77) Jolivet, E., and Nicol, M. Z., *Compt. rend.*, **254**, 2056 (1962).
(78) Kalan, E. B., Davis, B. D., Srinivasan, P. R., and Sprinson, D. B., *J. Biol. Chem.*, **223**, 907 (1956).
(79) Koukol, J., Miljanich, P., and Conn, E. E., *J. Biol. Chem.*, **237**, 3223 (1962).
(80) Lane, J. F., Koch, W. T., Leeds, N. S., and Gorin, G., *J. Am. Chem. Soc.*, **74**, 3211 (1952).
(81) Lesaint, C., Tendille, C., and Papin, J.-L., *Compt. rend.*, **255**, 1002 (1962).
(82) Levin, J. G., and Sprinson, D. B., *Biochem. Biophys. Res. Commun.*, **3**, 157 (1960).
(83) Levin, J. G., and Sprinson, D. B., *J. Biol. Chem.*, **239**, 1142 (1964).
(84) MacDonald, J. C., *Can. J. Microbiol.*, **9**, 809 (1963).
(85) Maier, V. P., Metzler, D. M., and Huber, A. F., *Biochem. Biophys. Res. Commun.*, **14**, 124 (1963).
(86) McCormick, J. R. D., Reichenthal, J., Kirsch, U., and Sjolander, N. O., *J. Am. Chem. Soc.*, **84**, 3711 (1962).
(87) McCrindle, R., Overton, K. H., and Raphael, R. A., *J. Chem. Soc.*, 1560 (1960).
(88) Metzenberg, R. L., and Mitchell, H. K., *Biochem. J.*, **68**, 168 (1958).
(89) Metzenberg, R. L., and Mitchell, H. K., *J. Am. Chem. Soc.*, **76**, 4187 (1954).
(90) Millican, R. C., *Biochim. Biophys. Acta*, **57**, 407 (1962).
(91) Millican, R. C., *Anal. Biochem.*, **6**, 181 (1963).
(92) Mitsuhashi, S., and Davis, B. D., *Biochim. Biophys. Acta*, **15**, 54 (1954).
(93) Mitsuhashi, S., and Davis, B. D., *Biochim. Biophys. Acta*, **15**, 268 (1954).
(94) Nandy, M., and Ganguli, N. C., *Biochim. Biophys. Acta*, **48**, 608 (1961).
(95) Neish, A. C., *Can. J. Botany*, **36**, 649 (1958).
(96) Neish, A. C., *Can. J. Botany*, **37**, 1085 (1959).
(97) Nigam, V. N., *Can. J. Biochem.*, **42**, 1561 (1964).
(98) Perkins, H. J., and Aronoff, S., *Can. J. Biochem. Physiol.*, **37**, 149 (1959).
(99) Pleininger, H., *Angew. Chem. Intern. Ed. Engl.*, **1**, 367 (1962).
(100) Pleininger, H., and Schneider, K., *Ber.*, **92**, 1587 (1959).
(101) Plouvier, V., *Compt. rend.*, **249**, 1563 (1959).
(102) Plouvier, V., *Compt. rend.*, **250**, 1721 (1960).
(103) Plouvier, V., *Compt. rend.*, **252**, 599 (1961).
(104) Plouvier, V., *Compt. rend.*, **258**, 2921 (1964).
(105) Rao, J. B., Rajadurai, S., Satry, K. N. S., and Nayudamma, Y., *Bull. Central Leather Res. Inst. Madras* (India), **8**, 66 (1961); *Chem. Abstr.*, **56**, 8890 (1962).
(106) Ratledge, C., *Nature*, **203**, 428 (1964).
(107) Read, G., Vining, L. C., and Haskins, R. H., *Can. J. Chem.*, **40**, 2357 (1962).
(108) Reddy, K. K., Sastry, K. N. S., Rajadurai, S. and Nayudamma, Y., *Bull. Central Leather Res. Inst. Madras* (India), **8**, 64 (1961); *Chem. Abstr.*, **56**, 8890 (1962).
(109) Richardson, A. J., and Hulme, A. C., *Nature*, **175**, 43 (1955).
(110) Rivera, A., Jr., and Srinivasan, P. R., *Biochemistry*, **2**, 1063 (1963).
(111) Salamon, I. I., and Davis, B. D., *J. Am. Chem. Soc.*, **75**, 5567 (1953).
(112) Saslaw, L. D., and Waravdekar, V. S., *Biochim. Biophys. Acta*, **37**, 367 (1960).
(113) Schramm, R. W., and Pialtkouska, M., *Acta Soc. Botan. Polon.*, **30**, 381 (1961).
(114) Schwinck, I., and Adams, E., *Biochim. Biophys. Acta*, **36**, 102 (1959).
(115) Sekizawa, I. Y., *Meiji Shika Kenkyu Nempo* (Yakuhin Bumon), 66 (1959); *Chem. Abstr.*, **54**, 25025 (1960).
(116) Simonart, P., and Wiaux, A., *Nature*, **186**, 78 (1960).
(117) Smissman, E. E., and Oxman, M. A., *J. Am. Chem. Soc.*, **85**, 2184 (1963).
(118) Smissman, E. E., Suh, J. T., Oxman, M., and Daniels, R., *J. Am. Chem. Soc.*, **84**, 1040 (1962).
(119) Smith, L. C., Ravel, J. M., Lax, S. R., and Shive, W., *Arch. Biochem. Biophys.*, **105**, 424 (1964).
(120) Smith, L. C., Ravel, J. M., Lax, S. R., and Shive, W., *J. Biol. Chem.*, **237**, 3566 (1962).
(121) Sprinson, D. B., *Advan. Carbohydrate Chem.*, **15**, 235 (1960).
(122) Srinivasan, P. R., Katagiri, M., and Sprinson, D. B., *J. Am. Chem. Soc.*, **77**, 4943 (1955).
(123) Srinivasan, P. R., Katagiri, M., and Sprinson, D. B., *J. Biol. Chem.*, **234**, 713 (1959).
(124) Srinivasan, P. R., and Rivera, A., Jr., *Biochemistry*, **2**, 1059 (1963).
(125) Srinivasan, P. R., Rothschild, J., and Sprinson, D. B., *J. Biol. Chem.*, **238**, 3176 (1963).
(126) Srinivasan, P. R., Shigeura, H. T., Sprecher, M., Sprinson, D. B., and Davis, B. D., *J. Biol. Chem.*, **220**, 477 (1956).
(127) Srinivasan, P. R., and Sprinson, D. B., *J. Biol. Chem.*, **234**, 716 (1959).
(128) Srinivasan, P. R., Sprinson, D. B., Kalan, E. B., and Davis, B. D., *J. Biol. Chem.*, **223**, 913 (1956).
(129) Swan, E. P., *Tappi*, **46**, 245 (1963).
(130) Synge, R. L. M., and Wood, J. C., *Biochem. J.*, **70**, 321 (1958).
(131) Tachi, I., and Sato, A., *Bull. Agr. Chem. Soc. Japan*, **24**, 633 (1960).
(132) Tatum, E. L., and Gross, S. R., *J. Biol. Chem.*, **219**, 797 (1956).
(133) Tatum, E. L., Gross, S. R., Ehrensvard, G., and Garnjobst, L., *Proc. Natl. Acad. Sci. U. S.*, **40**, 271 (1954).
(134) Underhill, E. W., private communication.
(135) Underhill, E. W., Watkin, J. E., and Neish, A. C., *Can. J. Biochem. Physiol.*, **35**, 219 (1957).
(136) Weinstein, L. H., Porter, C. A., and Laurencot, H. J., Jr., *Contrib. Boyce Thompson Inst.*, **20**, 121 (1959).
(137) Weinstein, L. H., Porter, C. A., and Laurencot, H. J., Jr., *Contrib. Boyce Thompson Inst.*, **21**, 201 (1961).

(138) Weinstein, L. H., Porter, C. A., and Laurencot, H. J., Jr., *Contrib. Boyce Thompson Inst.*, **21**, 439 (1962).
(139) Weiss, U., Davis, B. D., and Mingioli, E. S., *J. Am. Chem. Soc.*, **75**, 5572 (1953).
(140) Weiss, U., Gilvarg, C., Mingioli, E. S., and Davis, B. D., *Science*, **119**, 774 (1954).
(141) Weiss, U., and Mingioli, E. S., *J. Am. Chem. Soc.*, **78**, 2894 (1956).
(142) Weissbach, A., and Hurwitz, J., *J. Biol. Chem.*, **234**, 705 (1959).
(143) Weissbach, A., and Hurwitz, J., *J. Biol. Chem.*, **234**, 710 (1959).
(144) West, R. G., "Classification of Plants," Cambridge University Press, Cambridge, 1961.
(145) Whiting, G. C., *Nature*, **179**, 531 (1957).
(146) Wightman, F., Chisholm, M. D., and Neish, A. C., *Phytochemistry*, **1**, 30 (1961).
(147) Willis, J. C., "A Dictionary of Flowering Plants and Ferns," 6th Ed., Cambridge University Press, Cambridge, 1960.
(148) Yaniv, H., and Gilvarg, C., *J. Biol. Chem.*, **213**, 787 (1955).
(149) Yano, K., and Arima, K., *J. Gen. Appl. Microbiol.*, **4**, 241 (1958).
(150) Yoshida, S., and Towers, G. H. N., *Can. J. Biochem. Physiol.*, **41**, 579 (1963).
(151) Yoshida, S., and Hasegawa, M., *Arch. Biochem. Biophys.*, **70**, 377 (1957).
(152) Zaprometov, M. N., *Biokhimiya*, **26**, 373 (1961).
(153) Zaprometov, M. N., *Biokhimiya*, **26**, 597 (1961).
(154) Zaprometov, M. N., *Biokhimiya*, **27**, 312 (1962).