INDOLES
PART TWO

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INDOLES

PART TWO

This is the twenty-fifth volume in the series

THE CHEMISTRY OF HETEROCYCLIC COMPOUNDS

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A SERIES OF MONOGRAPHS

ARNOLD WEISSBERGER and EDWARD C. TAYLOR

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Library of Congress Catalog Card Number: 76-154323

ISBN 0-471-37501-2

10 9 8 7 6 5 4 3 2 1

The Chemistry of Heterocyclic Compounds

The chemistry of heterocyclic compounds is one of the most complex branches of organic chemistry. It is equally interesting for its theoretical implications, for the diversity of its synthetic procedures, and for the physiological and industrial significance of heterocyclic compounds.

A field of such importance and intrinsic difficulty should be made as readily accessible as possible, and the lack of a modern detailed and comprehensive presentation of heterocyclic chemistry is therefore keenly felt. It is the intention of the present series to fill this gap by expert presentations of the various branches of heterocyclic chemistry. The subdivisions have been designed to cover the field in its entirety by monographs which reflect the importance and the interrelations of the various compounds, and accommodate the specific interests of the authors.

In order to continue to make heterocyclic chemistry as readily accessible as possible, new editions are planned for those areas where the respective volumes in the first edition have become obsolete by overwhelming progress. If, however, the changes are not too great so that the first editions can be brought up-to-date by supplementary volumes, supplements to the respective volumes will be published in the first edition.

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Preface

Indoles Part Two begins the detailed coverage of the preparation, properties, reactions and tabulation of compounds containing an indole nucleus. It starts with a chapter on indole biosynthesis since this was the first and only source of indole preparations during the early years of indole chemistry.

The editor is grateful to Mrs. Maria Fanlo and Mr. Siegfried Wahrmann for library assistance and to Miss Linda Heuser for typing a portion of the manuscript.

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Hanover, New Jersey

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INDOLES

PART TWO

CHAPTER III

Biosynthesis of Compounds Containing an Indole Nucleus

RONALD J. PARRY

University Chemical Laboratory, Cambridge, England

I. Introduction

Living plants produce an extraordinarily rich variety of chemical substances, many of which lack any apparent biochemical function. These

metabolites have often proved the delight (and the frustration!) of organic chemists for the challenges of structural and synthetic chemistry which they offer. With the advent of radioactive tracers and the development of more sensitive chemical and spectroscopic tools, the doors leading to a deeper understanding of the chemistry of the plant world have been opened: the investigation of natural product biosynthesis has begun. Of the fruits of a field yet in its infancy, those arising from an examination of the biosynthesis of the naturally-occurring indoles have proved among the most tantalizing, and much may be expected of the future. The present account summarizes our knowledge of the biosynthesis of compounds containing an indole nucleus, and covers the literature through November 1968.

II. Simple Indole Derivatives

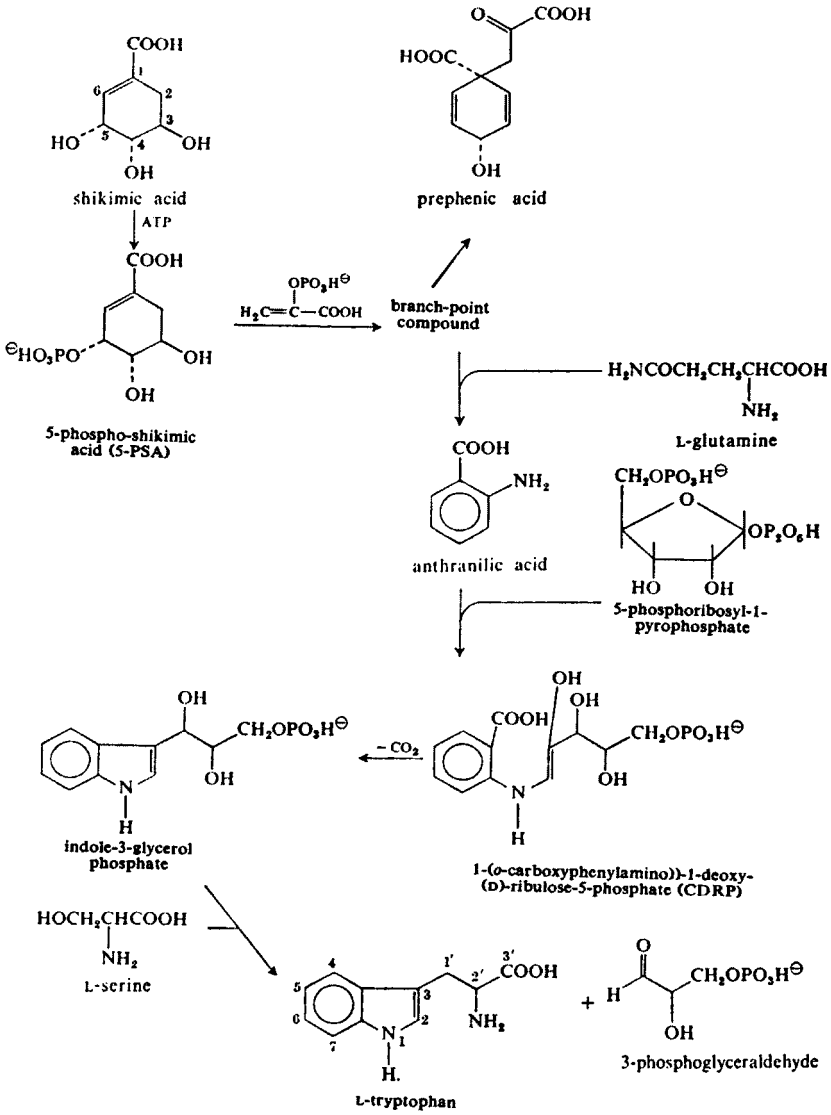
A. Tryptophan

By virtue of its ubiquitous distribution in plant and animal proteins, tryptophan may justifiably be regarded as the most important of the naturally-occurring indoles. Extensive explorations aimed at unraveling the tangled thread of its biosynthesis are a consequence of this importance. These investigations have been limited almost exclusively to microorganisms, and disappointingly few experiments have been conducted with fungi and higher plants.

The subject of tryptophan biosynthesis in microorganisms was carefully reviewed in 1960 by Doy[1]; Scheme 1 summarizes the metabolic picture presented by the experimental evidence available at that time. More recent work has supplied some of the significant detail absent from this picture.

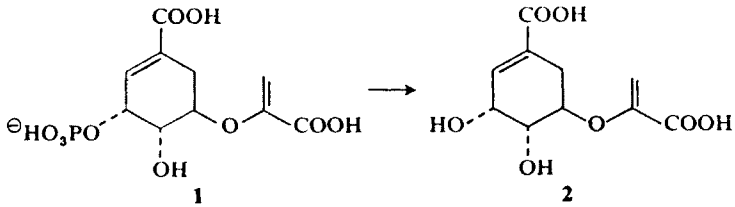
The enzyme which phosphorylates shikimic acid to 5-phosphoshikimic acid (5-PSA) has been isolated from *Escherichia coli* by Fewster.[2] Its optimum pH is 7.0 and it exhibits a requirement for divalent magnesium or manganese. Neither the formation of the enzyme nor its activity is affected by the ultimate products of the aromatic biosynthetic pathway. The same author also reported evidence for the presence of this enzyme system in a variety of microorganisms known to synthesize aromatic amino acids.

One of the most fascinating problems in tryptophan biosynthesis, the nature of the so called branch point compound leading either to prephenic acid or to anthranilic acid, has yielded to the patience of the investigators. Early experimental evidence[3] suggested that at least one additional substance, called Z_1 and formulated[4] as the 5-enolpyruvyl ether of shikimic acid, was produced from 5-PSA before the branch point. Later work by Srinivasan[5]


shikimic acid
ATP
5-phospho-shikimic acid (5-PSA)
branch-point compound
prephenic acid
L-glutamine
anthranilic acid
5-phosphoribosyl-1-pyrophosphate
1-(o-carboxyphenylamino)-1-deoxy-(D)-ribulose-5-phosphate (CDRP)
indole-3-glycerol phosphate
L-serine
L-tryptophan
3-phosphoglyceraldehyde

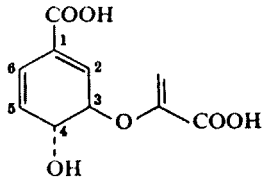
Scheme 1

indicated, however, that Z_1 was not in fact an intermediate in the conversion of 5-PSA to anthranilic acid in cellfree extracts of *E. coli*, and Levin and Sprinson[6] found that Z_1 is not converted to prephenic acid by extracts of the same organism. These authors present additional data suggesting that the first product formed from 5-PSA and phosphoenolpyruvic acid is 3-enolpyruvylshikimate-5-phosphate (**1**), which is then dephosphorylated to Z_1, proposed to be the 3-enolpyruvyl ether of shikimic acid (**2**). In the presence



of fluoride ion, the dephosphorylation reaction is inhibited, and Z_1-phosphate, **1**, accumulates. Since prephenate formation from 5-PSA and phosphoenolpyruvic acid could be demonstrated, it follows that Z_1-phosphate is probably the active intermediate leading to prephenate. Evidence implicating Z_1-phosphate as a precursor of anthranilic acid was also forthcoming. Cellfree extracts of *Aerobacter aerogenes*[7] converted shikimic acid or 5-PSA in the presence of phosphoenolpyruvic acid to a substance with properties identical to those of Z_1-phosphate as reported by Levin and Sprinson. By using mutants of the same organism which were unable to convert 5-PSA to Z_1-phosphate, to anthranilic acid, or to phenylpyruvic acids, the formation of these acids in a cell extract containing Z_1-phosphate could be detected. Treatment of the Z_1-phosphate containing extract with alkaline phosphatase followed by acid produced a substance supporting the growth of an *E. coli* mutant requiring shikimic acid. Neither treatment alone produced a growth factor, but either destroyed the substrate for anthranilic acid formation. Addition of fluoride ion improved the yield of anthranilic acid from the substrate. The role of Z_1-phosphate in anthranilic acid biosynthesis in *E. coli* has been studied by Rivera and Srinivasan.[8] Ammonium sulfate or protamine sulfate treatment of a crude anthranilate forming enzyme preparation from an *E. coli* mutant gave two fractions. One of these contained an enzyme, named 3-enolpyruvylshikimate 5-phosphate synthetase, that condensed 5-PSA and phosphoenolpyruvic acid to give Z_1-phosphate. This enzyme fraction further converted Z_1-phosphate to a new, unidentified substance which was itself converted to anthranilic acid by the second enzyme fraction in the presence of L-glutamine, divalent magnesium, nicotinamide adenine dinucleotide (NAD^+), and a nicotinamide adenine dinucleotide, reduced form (NADH) regenerating system. The second fraction was unable to convert Z_1-phosphate to anthranilate.

Gibson and Gibson[9, 10] also reported the presence of a new intermediate in aromatic ring biosynthesis in extracts of an *A. aerogenes* mutant. This substance could be converted by mild chemical treatment into prephenic acid, *p*-hydroxybenzoic acid, and phenylpyruvic acid; enzymically, it was transformed into anthranilic, prephenic, phenylpyruvic, *p*-hydroxyphenylpyruvic, and *p*-hydroxybenzoic acids. On the basis of this evidence, the substance was judged to be the elusive branch point compound, and was named chorismic acid (chorismic = separating). The obtention of a multiply-blocked auxotroph of *A. aerogenes* which accumulated the acid allowed its isolation[11] as the barium salt and formulation[11, 12] as the 3-enolpyruvyl ether of *trans*-3,4-dihydroxycyclohexa-1,5-diene carboxylic acid (**3**). Chorismic acid has also been isolated from a *Saccharomyces cerevisiae* mutant by Lingens and Luck.[13]



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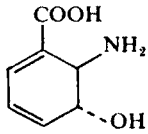
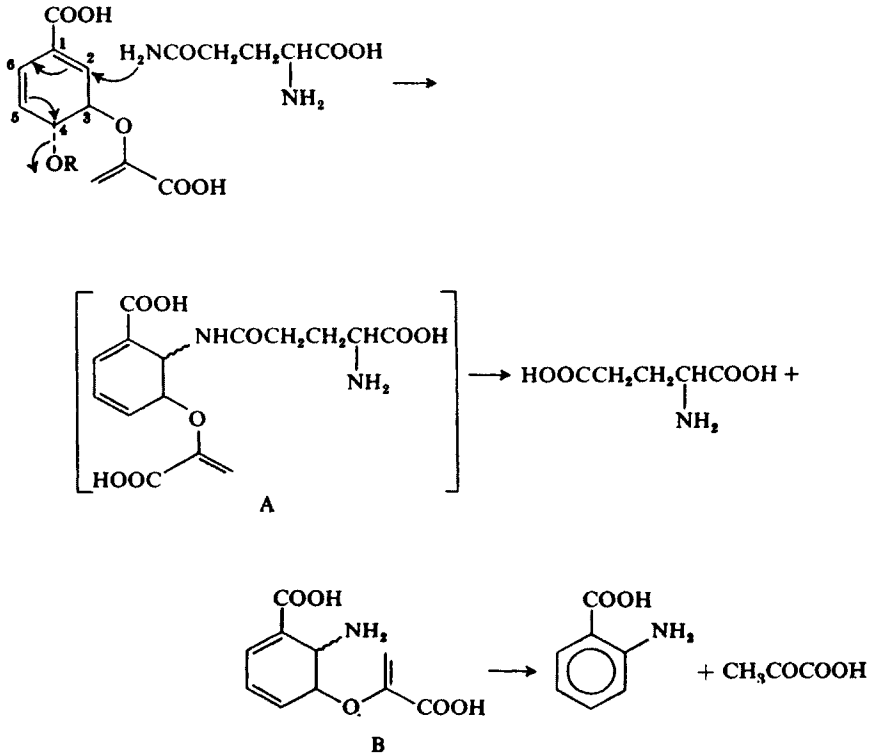
The problem of the conversion of chorismic acid into anthranilic acid is still under investigation. Srinivasan and Rivera,[14] working with *E. coli* mutants, demonstrated that an NADH regenerating system and either divalent magnesium or iron were required in addition to L-glutamine; the amino group of anthranilic acid was found to be derived from the amide nitrogen atom of glutamine. More recently, some very informative results have been obtained[15] by feeding 3,4-^{14}C-glucose to an *E. coli* mutant accumulating anthranilic acid. Earlier isotopic studies of the incorporation of 3,4 ^{14}C-glucose into shikimic acid established that the carboxyl carbon and carbons 3 and 4 become labeled.[16] Utilizing this information, degradation of the labeled anthranilic acid produced from the radioactive glucose showed that the carboxyl group of shikimic acid becomes the carboxyl group of anthranilic acid, and that the amination of chorismic acid occurs at C-2 rather than at C-6. Examination of *trans*-2,3-dihydro-3-hydroxyanthranilic acid (**4**), which has been isolated from *Streptomyces aureofaciens*,[17] as a



4

possible anthranilate precursor in cellfree extracts of *E. coli* gave negative results, indicating that the actual intermediate probably still bears the

enolpyruvyl moiety. These results were rationalized by the scheme shown in Scheme 2. In a recent report, Lingens et al. claim to have isolated a substance corresponding to A from a mutant of *Saccharomyces cerevisiae*.[18]

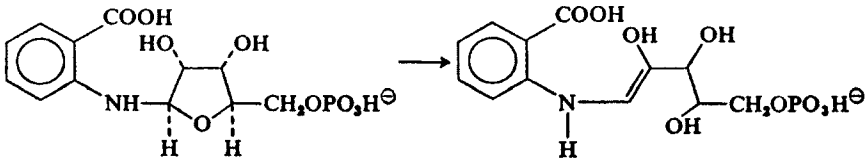


Scheme 2

DeMoss[19] has investigated the formation of anthranilic acid in *Neurospora crassa*. Cellfree extracts will convert shikimic acid into anthranilic acid after an inhibitor that is present has been removed by ammonium sulfate precipitation. The cofactors required for the transformations are identical to those of the bacterial systems studied except that nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) is needed in place of NADH. Omission of L-glutamine from the incubation mixture led to the accumulation of chorismic acid. The enzyme catalyzing the chorismate to anthranilate conversion was purified 83-fold and appeared to be homogeneous. Its activity was completely inhibited by low concentrations of L-tryptophan, and this inhibition was competitively reversed by chorismic acid, suggesting that the conversion of chorismic acid to anthranilic acid is specifically involved in tryptophan biosynthesis.

The L-glutamine requirement does not appear to be obligatory. Gibson et al.[20] have recently recorded anthranilate biosynthesis that does not require glutamine. A strain of *E. coli* was obtained that required both glutamine and tryptophan. Cell suspensions of this organism were able to synthesize anthranilic acid by using glucose as the carbon source and ammonium ions as the only nitrogen source; addition of DON (6-diazo-5-oxo-6-norleucine), a glutamine antagonist to the cell suspensions, caused no inhibition of anthranilate formation. It was suggested that the un-ionized form of ammonia may be available for transfer reactions normally requiring glutamine.

The steps leading from anthranilic acid to tryptophan have been carefully scrutinized. Yanofsky proposed[21] that the first intermediate resulting from the reaction of anthranilic acid and 5-phosphoribosyl-1-pyrophosphate should be *N*-*o*-carboxyphenylribosylamine-5-phosphate (**5**), also known as *N*-(5′-phosphoribosyl)anthranilic acid or PRA. An Amadori rearrangement[22] of this substance to 1-(*o*-carboxyphenylamino)-1-deoxyribulose-5-phosphate (CDRP) (**6**) was postulated. Early failures to detect the presence of PRA[1, 23]



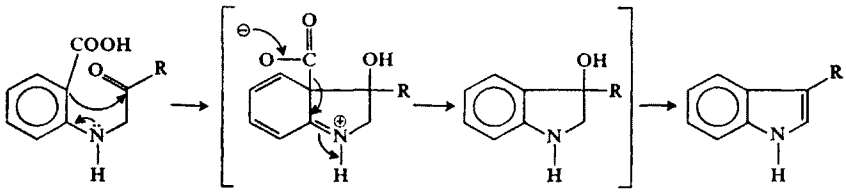
5 **6**

were ascribed to the lability of anthranilic acid glycosylamines.[1, 24] Evidence supporting this instability was subsequently provided by Doy and co-workers.[25] The half-life of synthetic PRA at 37°C in aqueous solution, pH 6, was found to be 6 min. The sensitivity of the substance increased with decreasing pH and decreased with increasing pH. This ease of hydrolysis predicts that mutants blocked between PRA and CDRP will appear to accumulate anthranilic acid unless special precautions are taken. An investigation by Doy et al.[25] confirms this prediction; extracts of certain mutant microorganisms (*E. coli*, *A. aerogenes*, *Salmonella typhimurium*), which in whole cell experiments accumulated anthranilic acid, were found to catalyze a reaction between anthranilic acid and 5-phosphoribosyl-1-pyrophosphate leading to an acid labile substance, less fluorescent than anthranilic acid, and readily hydrolyzing back to that compound. The substance was converted enzymically to indole-3-glycerol phosphate, and was surmized to be PRA. Doy[26] also reported similar behavior in two tryptophan auxotrophs of *Pseudomonas aeruginosa* which are phenotypically identical, i.e., both require indole or tryptophan for growth and accumulate anthranilic acid. The two strains, however, differ genotypically, because one is blocked between anthranilic acid and PRA while the other is blocked between PRA

and CDRP. The rapid hydrolysis of accumulating PRA results in the apparent accumulation of anthranilic acid by the second mutant.

1(-*o*-Carboxyphenylamino)-1-deoxyribulose, the dephosphorylated Amadori product, was originally detected in cell suspensions of *A. aerogenes* and in *E. coli* mutants.[27, 29] Its identity was based upon R_F values, color reactions, absorption spectra, and a DNP derivative as compared with synthetic material. On the basis of Yanofsky's scheme,[21] the substance is most reasonably considered as an artifact, derived from the actual tryptophan precursor by loss of the 5-phosphate group. Smith and Yanofsky[23] have since provided evidence for this by detecting what appears to be the phosphorylated compound in extracts of *E. coli* and *S. typhimurium* mutants; impure CDRP was obtained synthetically from anthranilic acid and the sodium salt of ribose-5-phosphate; its properties compared reasonably well with those of the naturally occurring substance. Both the synthetic and natural compounds were converted to indole-3-glycerol phosphate in the extracts.

The mechanism of the conversion of CDRP to indole-3-glycerol phosphate appears to have been little studied. Smith and Yanofsky[23] prepared the decarboxylated analog of CDRP, but found that it was not transformed into indole-3-glycerol phosphate by appropriate cell extracts. This implies that the decarboxylated substance is not a free intermediate in the reaction. Mechanistically, it seems reasonable to suppose that the decarboxylation may not occur until after ring closure has taken place, so that this biochemical inertness of the decarboxylated analog need occasion no surprise (Eq. 1).



(1)

Studies of the tryptophan synthetase enzyme system have elucidated some of the details of the last step in tryptophan biosynthesis. The enzyme obtained from *E. coli* has been shown to consist of two protein subunits, A and B,[28, 29] which catalyze three reactions[30] (Eqs. 2–4):

$$\text{indole} + \text{L-serine} \rightarrow \text{L-tryptophan} \tag{2}$$

$$\text{indole-3-glycerol phosphate} \rightleftharpoons \text{indole} + \text{3-phosphoglyceraldehyde} \tag{3}$$

$$\text{indole-3-glycerol phosphate} + \text{L-serine} \rightarrow \text{L-tryptophan} + \text{3-phosphoglyceraldehyde} \tag{4}$$

The B subunit will catalyze Reaction (2) in the absence of the A subunit,[31] and the A subunit will catalyze Reaction (3).[28] Reaction (4) only occurs in

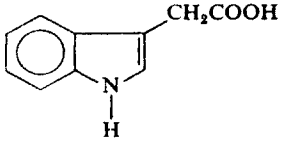
the presence of the AB complex, and indole is evidently not a free intermediate in the reaction.[28]

The A protein has been obtained crystalline,[32] characterized,[33, 34] and the complete amino acid sequence deduced.[35–39] The B subunit has likewise been purified;[40] it is of much higher molecular weight (ca. 108,000) than the A subunit (ca. 29,000) and requires pyridoxal phosphate as a cofactor to the extent of 2 moles of cofactor to each mole of B protein. The apoenzyme is unable to catalyze Reaction (2), but retains its ability to facilitate Reaction (4) when combined with the A protein. The evidence points to an AB complex containing two A protein units per molecule of B protein.

The tryptophan synthetase enzyme system of *N. crassa* appears to be quite similar to the *E. coli* system.[41] The *N. crassa* enzyme catalyzes the same three reactions discussed above, with a pyridoxal phosphate requirement for Reactions (2) and (3). The conversion of indole-3-glycerol phosphate to tryptophan by the enzyme does not appear to involve free indole as an intermediate.

B. 3-Indoleacetic Acid

The discovery of the remarkable plant hormonal properties of 3-indoleacetic acid (7) in 1934[42] and its subsequent isolation from a higher plant[43] have stimulated an extensive amount of research on the mode of action and metabolism of this substance. These investigations have been the subject of a number of detailed reviews,[44–50] so that the discussion here will be limited to the more relevant papers with emphasis placed on recent work.



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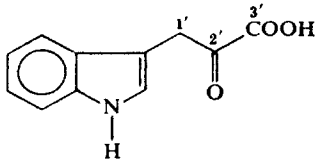


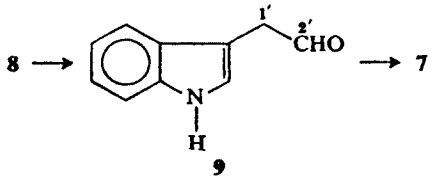
8

1. *Biosynthesis in Higher Plants*

The evidence currently available regarding 3-indoleacetic acid biosynthesis in higher plants strongly suggests that the substance is derived from tryptophan. Murakami and Hayashi,[51] for example, have shown that the juice from immature rice-grains will convert tryptophan into 3-indoleacetic acid, and that the conversion is stimulated by α-ketoglutaric acid. More recently, Libbert[52] demonstrated the conversion of tryptophan to 7 by pea sprouts in

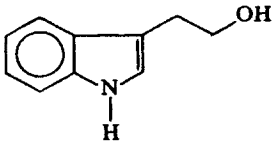
the presence of α-ketoglutaric acid, and isolated[53] an enzyme system from the plants which effects the same conversion.

There have been various proposals as to the nature of the intermediates involved in 3-indoleacetic acid biosynthesis. One intermediate frequently suggested[54–58] is 3-indolepyruvic acid (**8**), the product resulting from transamination of tryptophan. The requirement for α-ketoglutarate noted in the transformations above supports this suggestion. Subsequent decarboxylation of **8** would produce 3-indoleacetaldehyde (**9**), oxidation of which would yield



(5)

3-indoleacetic acid. The possible intermediacy of **9** in the biosynthesis derives credibility from its detection[50, 59] in a number of plants, and from the occurrence of an enzyme system in plants[60] which will convert **9** into **7** (Eq. 5). The detection of 3-indolepyruvic acid in plant extracts proves more difficult as the substance decomposes readily during chromatography,[50, 61] though the patterns of decomposition are apparently quite characteristic.[58] 3-Indolepyruvic acid has been tentatively identified in maize,[56, 62] and in watermelon tissue[63] after the feeding of tryptophan. In a careful chromatographic analysis carried out by Libbert and Brunn[64] on the products from the metabolism of tryptophan by an 3-indoleacetic acid synthesizing enzyme from pea plants, 3-indolepyruvic acid and tryptophol (**10**) were detected.



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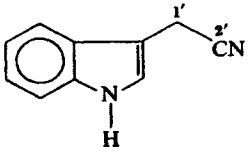
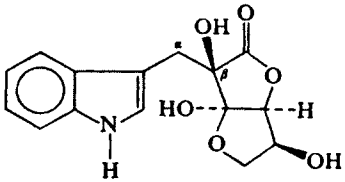


11

Another intermediate along the path from tryptophan to **7** may well be 3-indoleacetonitrile (**11**). This substance was originally isolated from cabbage (*Brassica oleracea*)[65] and evidence presented for its occurrence in other members of the *Cruciferae*. A variety of other simple indoles including **7**, 3-indolecarboxaldehyde, 3-indolecarboxylic acid, and ascorbigen (**12**), were also found to occur in *B. oleracea*. Libbert and Ballin[66] have since detected **11** in a variety of higher plants and found evidence in many for its enzymatic hydrolysis to 3-indoleacetic acid. Earlier work by Thimann[67] and by Seeley and coworkers[68] also provides evidence for the presence of an enzyme system effecting the conversion of the nitrile to 3-indoleacetic acid.

An interesting problem resides in the mechanism of the conversion of 3-indolepyruvic acid into 3-indoleacetonitrile. A number of proposals have been made[50, 56, 65, 69] and the most attractive of these involve the intermediacy of oximes. Dannenburg and Liverman[63] postulated the decarboxylation of 3-indolepyruvic acid to **9** followed by oximation and dehydration to **11**. Evidence favoring this hypothesis has been reported by Mahadevan and coworkers[70, 71] who observed the conversion of 3-indoleacetaldehyde oxime into 3-indoleacetonitrile in banana-leaf tissue and in cellfree preparations of cabbage leaves. Furthermore, Underhill[72] has recently discovered the conversion of phenylalanine into phenylacetaldehyde oxime in *Tropaeolum majus*, and Kindl et al. have stated[73] that 3-indoleacetaldehyde oxime is a product of tryptophan metabolism in *B. oleracea*. Alternatively, Stowe[50] has offered the oxime of 3-indolepyruvic acid as a possible biosynthetic intermediate. The principal support that can be mustered for this idea derives from the *in vitro* conversion of this oxime to the nitrile under simulated physiological conditions,[69] from the known occurrence of α-ketoacid oximes in plant tissue,[74] and from the discovery of a transoximase system catalyzing the transfer of an oximino moiety between α-ketoacids.[75, 76]

Further indications of a biochemical thread running from tryptophan through 3-indoleacetonitrile to **7** have been supplied by Wightman.[77] Radioactive 2′-^{14}C-tryptophan (see Section II.A for numbering) was incorporated by 15-week old cabbages into 3-indoleacetonitrile and 3-indoleacetic acid, as well as into 3-indolecarboxaldehyde, 3-indolecarboxylic acid, and ascorbigen. These substances were also obtained radioactive when 1′-^{14}C-3-indoleacetonitrile was administered to the cabbages. It is of some interest that no evidence could be found for the intermediacy of 3-indolepyruvic acid or its oxime in these transformations; additional tracer experiments indicated that neither 2′-^{14}C-tryptamine(tryptophan numbering) nor 1′-^{14}C-3-indoleacetaldehyde were converted to radioactive **11** in the cabbage tissue. More recent investigations, dealing with ascorbigen, discussed in Section II.D, complicate the interpretation of these results; it now appears[78, 79] that ascorbigen is an artifact produced during the extraction of the cabbage tissue by enzymic hydrolysis of the mustard-oil glucoside, glucobrassicin (**13**). This



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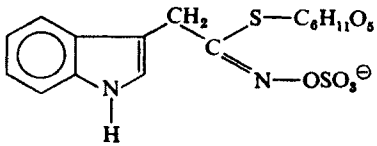

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hydrolysis also produces **11**, and the feeding of 1′-^{14}C-tryptophan to cabbages apparently does not produce any radioactive **11** or ascorbigen when the

proper precautions are taken during the subsequent extraction. The natural occurrence of **11** in cabbage tissue must therefore be questioned.

As indicated earlier, evidence does exist for the presence of 3-indoleacetonitrile in plants other than *B. oleracea*, and an enzyme catalyzing the conversion of the nitrile to 3-indoleacetic acid has been found in various plants, isolated, and purified.[80] It is active in the absence of oxygen and is not deactivated by sulfhydryl or heavy metal reagents. Interestingly enough, 3-indoleacetamide, the presumed intermediate in the conversion, is not liberated in detectable concentration during the enzymic hydrolysis, and is itself not readily attacked by the enzyme. Complete hydrolysis thus appears to take place before the enzyme–substrate complex dissociates. This behavior may perhaps explain the observation of Eifert and Eifert[81] when testing the growth stimulating activity of various substances on the vine, *Vitis vinifera*. Tryptophan, **8**, **9**, **11**, and tryptamine all produced stimulation while 3-indoleacetamide had an inhibitory effect; the amide probably competes with 3-indoleacetonitrile for the hydrolyzing enzyme, and blocks it by virtue of the difficulty with which it is hydrolyzed.

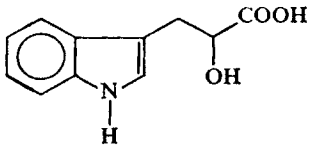
The transformation of tryptophan into 3-indoleacetamide in the presence of horse-radish peroxidase and pyridoxal phosphate has been observed by Riddle and Mazelis,[82] and by Kleambt.[83] The reaction is very similar to the conversion of methionine into 3-methylthiopropionamide by the same enzyme and cofactor as reported by Mazelis, Ingraham, and Weston.[84] In a more detailed study,[85] Riddle and Mazelis report that cabbage seedling homogenates contain natural inhibitors of peroxidase activity which are removable by dialysis. After such treatment, the homogenates will convert tryptophan into 3-indoleacetamide with a small amount of 3-indoleacetic acid also being produced. Whole cabbage seedlings were shown to convert 1′-^{14}C-tryptophan into radioactive **11** and **7**, but 3-indoleacetamide could not be detected. In view of the work described earlier, the occurrence of **11** in cabbage seedlings should be questioned. On the other hand, infiltration of 1′-^{14}C-indoleacetamide (numbered as **11**) into the cabbage seedlings resulted in significant hydrolysis to 3-indoleacetic acid after 12 hr. Homogenates of cabbage seedlings were also capable of the same transformation unless dialyzed. These experiments point to the presence of 3-indoleacetamide, which may or may not be derived from 3-indoleacetonitrile, as an intermediate leading to 3-indoleacetic acid in cabbages.

To complete this rather complex picture of 3-indoleacetic acid biosynthesis, an alternative pathway needs mention. There is indirect evidence indicating that tryptamine may serve as an 3-indoleacetic acid precursor in higher plants. The presumed pathway involves decarboxylation of tryptophan to tryptamine, transamination of the latter to 3-indoleacetaldehyde, and oxidation of the aldehyde to the corresponding acid. In support of this

hypothesis, the natural occurrence of a variety of tryptamines[86] in higher plants speaks for the presence of a tryptophan decarboxylase system in the Angiosperms. In addition, Skoog[87] has demonstrated an auxin activity for tryptamine in oats, and Winter[88] has found that tryptamine produces a marked stimulation of growth in *Avena sativa* coleoptiles, which stimulation was inhibited by the addition of amine oxidase inhibitors. Curiously, tryptophan showed no growth stimulating properties when applied to the coleoptiles. The case is given further strength by the isolation of an amine oxidase from peas which converts tryptamine to 3-indoleacetaldehyde,[89] and by Libbert's report[90] of the formation of tryptamine from tryptophan in crude enzyme preparations from pea plants. On the other hand, Gordon has stated[57] that, in some plant tissues, inhibitors of amine oxidase do not affect 3-indoleacetic acid formation from tryptophan, and he concludes that tryptamine is not a normal intermediate. Libbert's detection of the production of tryptamine from tryptophan has also recently been questioned[91] on the grounds that no precautions were taken to exclude bacterial contamination. The question of the role of tryptamine in 3-indoleacetic acid biosynthesis thus does not appear to be settled as yet, and its importance as a precursor may in fact vary with different plants.

2. *Biosynthesis in Lower Plants*

The occurrence of 3-indoleacetic acid in the lower plants seems to be rather widespread,[44, 92] and the biosynthetic pathways, in so far as they have been elucidated, generally parallel those existing in the higher plants. Srivastava and Shaw[93] have shown, for example, that the fungus *Melampsora lini* will convert 2′-^{14}C-tryptophan into **7**; 3-indoleacetaldehyde and probably 3-indolepyruvic acid appeared to be intermediates in the process while tryptamine and 3-indoleacetonitrile were not. The metabolism of tryptophan by *Taphrina deformans* also appears to produce **8** as well as **7**, **10**, and 3-indolelactic acid (**14**),[91] but no tryptamine, as had been originally reported.[94]



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Cellfree preparations of *Acetobacter xylinum* are also said[95, 96] to metabolize tryptophan to tryptophol (**10**) and 3-indoleacetic acid, with 3-indoleacetaldehyde being trapped when sodium bisulfite is added to the medium;

α-ketoglutaric acid was required and the production of all three indoles was stimulated by the addition of pyridoxal phosphate. Still another example is provided by *Endomycopsis vernalis*[97] which converts tryptophan to **7**, **8**, **14**, **10**, 3-indolecarboxaldehyde, and 3-indolecarboxylic acid; when cellfree preparations were utilized, 3-indoleacetaldehyde could be detected. In the case of the crown-gall organism, *Agrobacterium tumefaciens*, the reported production of **8** during the biosynthesis of **7**[58] is fortified by a subsequent isolation of an amino transferase[98] exhibiting broad specificity in transferring an amino group from tryptophan, valine, leucine, or isoleucine to phenylpyruvic acid; the tryptamine pathway is apparently absent in this organism.

An unusual pathway to 3-indoleacetic acid has been discovered in *Pseudomonas solanacearum*.[99] A wild, pathogenic strain converted 1′^{14}C-tryptophan into 3-indoleacetic acid which was only weakly labeled; under the same circumstances, the chain-labeled tryptophan was efficiently incorporated into cellular protein. When ring-labeled tryptophan was employed, the resulting 3-indoleacetic acid displayed a considerably higher level of radioactivity, and radioactive products of the kynurenine pathway for tryptophan metabolism were formed. The results led to the suggestion that the organism may synthesize **7** through the kynurenine pathway rather than by the usual routes. Strangely, a mutant, nonpathogenic strain of the same organism employed the more conventional routes to **7** as both ring- and chain-labeled tryptophan led to radioactive acid.

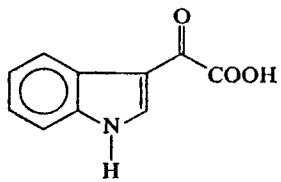
C. 3-Indolecarboxaldehyde and 3-Indolecarboxylic Acid

The biosynthesis of these substances has been discussed by Stowe,[50] and only the more recent developments are mentioned here.

3-Indolecarboxaldehyde and the corresponding acid seem to be formed biologically from either 3-indoleacetic acid or 3-indoleacetonitrile. Flowering heads of cauliflower,[100] for example, will metabolize both **7** and **11** to 3-indolecarboxaldehyde and the corresponding acid; the acid appears to be produced from the aldehyde as the aldehyde is itself converted to the acid by cauliflower tissue. A 3-indoleacetic acid oxidase system isolated[101] from the root tips of *Lens culinaris* converts ring-labeled **7** into labeled 3-indolecarboxaldehyde. Another 3-indoleacetic acid oxidase system is present in *Lupinus albus*[102] and, when coupled to a cytochrome oxidase system, will also transform **7** into 3-indolecarboxaldehyde. The intermediate in this conversion may be presumed to be 3-indoleglyoxylic acid (**15**), though its presence in these systems was not ascertained. The glyoxylic acid has been tentatively identified in cabbages[77] as a product of tryptophan metabolism, and young

tomato plants have been observed[103] to convert **7** into **15**, 3-indoleglycolic acid (**16**), 3-indolecarboxaldehyde, and 3-indolecarboxylic acid.

The conversion of 3-indoleacetonitrile to 3-indolecarboxaldehyde and



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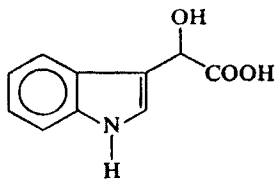

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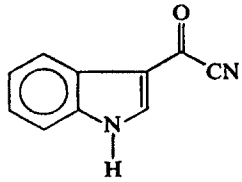
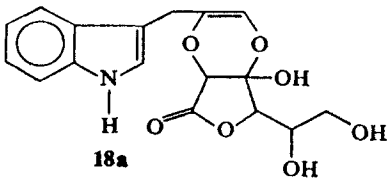


17

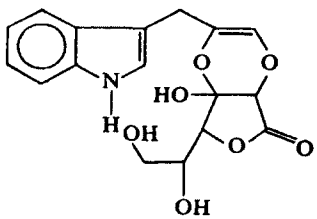
3-indolecarboxylic acid in pea and wheat tissue has been reported.[104] The reaction was suggested to proceed by α-oxidation of the nitrile to either the acyl cyanide (**17**) or the corresponding cyanohydrin; neither intermediate could be detected, however. The conversion of 3-indolecarboxaldehyde into the corresponding acid in the same tissues was noted. A crude enzyme preparation transforming **11** into 3-indolecarboxylic acid has also been isolated from pea seedlings.[105] Schiewer and Libbert[106] have observed the conversion of **11** into 3-indolecarboxaldehyde and the acid by three species of brown algae; no 3-indoleacetic acid was detected.

D. Ascorbigen

This curious substance was originally isolated from *Brassica oleracea* and one of the two alternative structures, **18a** or **18b**, allotted to it.[107] Subsequent



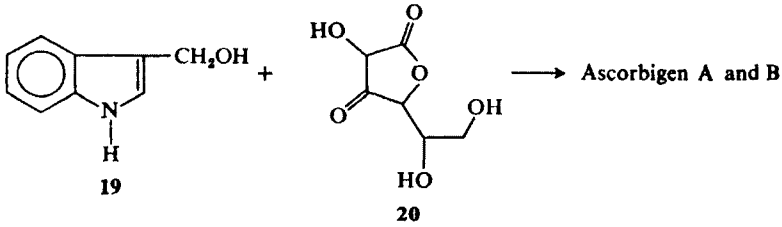
18a



18b

work by Gmelin and Virtanen[78] and by Kutacek and coworkers[79] has revealed that the substance is in fact an artifact produced during the isolation process, and casts doubt on the original structural proposal. A boiling methanolic extract of intact cabbage leaves was found to yield no ascorbigen, but only the mustard-oil glucoside, glucobrassicin (**13**). Myrosinase, an enzyme present in cabbage tissue, hydrolyzes this glucoside to 3-hydroxymethylindolo (**19**), **11**, and various other products whose relative proportions are pH dependent. If the enzymic hydrolysis is conducted in the presence of ascorbic

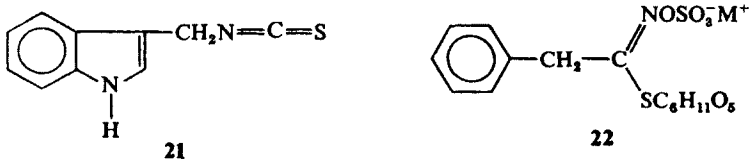
acid (**20**), which is also present in cabbage tissue, ascorbigen results, and indeed, an excellent yield of ascorbigen can be produced synthetically by the near room temperature reaction of ascorbic acid and 3-hydroxymethylindole.[108] Feeding 1′-^{14}C-tryptophan to the cabbage plants yielded only radioactive glucobrassicin; no radioactive ascorbigen or 3-indoleacetonitrile was detected. Furthermore, α-^{14}C-ascorbigen and 1′-^{14}C-3-indoleacetonitrile were not incorporated into glucobrassicin. These results contrast with the earlier report by Kutacek and coworkers[109] where, care not being taken to avoid enzymic hydrolysis, radioactive ascorbigen and 3-indoleacetonitrile were produced from 1′-^{14}C-tryptophan while no glucobrassicin, either active or inactive, was detected. The data led Gmelin and Virtanen[78] to suggest that the original structural proposal was incorrect, and the structure has in fact been reexamined.[110] The product resulting from the reaction of 3-hydroxymethylindole and ascorbic acid is formulated as a mixture of epimers and ascorbigens A and B, differing only at the configuration of the β-carbon; ascorbigen A (**12**) is the naturally occurring isomer (Eq. 6).



(6)

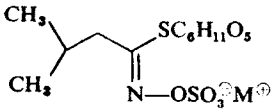
E. Glucobrassicin

Glucobrassicin (**13**) is a representative of a large group of unusual thioglucosides possessing the common property of hydrolyzing to glucose, sulfuric acid, and an isothiocyanate in the presence of the enzyme myrosinase. The chemistry and botanical distribution of this interesting class of natural products has been reviewed.[111, 112] Recent biosynthetic work suggests that glucobrassicin is derived from tryptophan. 1′-^{14}C-Tryptophan is incorporated into the skeleton of the thioglucoside by *B. oleracea*,[79] and ^{35}S-sulfur dioxide labels the thioether moiety when administered to cauliflower plants[113]; hydrolysis of the radioactive thioglucoside labeled in this fashion gives the radioactive isothiocyanate **21**. Schraudolf and Bergmann[114] similarly observed



the incorporation of Ar-^{14}C-DL-tryptophan into **13** by *Sinapis alba*; ^{35}S-sulfate was rapidly utilized in the formation of the thioether group; only L-tryptophan was transformed into **13**; D-tryptophan was converted into D-*N*-malonyl-tryptophan.

Tracer experiments on the related thioglucoside glucotropaeolin (**22**) shed some light on possible intermediates in glucobrassicin biosynthesis. 2′-^{14}C-DL-phenylalanine is an efficient precursor of the thioglucoside aglucone[115–117] and ^{14}*C*-^{15}*N*-L-phenylalanine is incorporated as a unit, except for the loss of *C*-3′ (tryptophan numbering).[118] Subsequent experiments[72, 117] indicated that phenylacetaldehyde oxime is a more efficient precursor of glucotropaeolin aglycone than phenylalanine, and the conversion of phenylalanine to the aldehyde oxime in *Tropaeolum majus* was demonstrated.[72] It is stated that analogous experiments in *B. oleracea* detected 3-indoleacetaldehyde oxime as a product of L-tryptophan metabolism.[73] Furthermore, isobutyraldehyde oxime and 3-phenylpropionaldehyde oxime act as efficient precursors of the mustard-oil glucosides, glucoputranjivin (**23**) and gluconasturtiin (**24**),[72] respectively. Thus, a common biosynthetic sequence for the formation of these thioglucosides appears to be operating in a variety of plants, and 3-indoleacetaldehyde oxime is probably the precursor of glucobrassicin in *B. oleracea*. Studies of the nature of the amino acid to oxime conversion for glucotropaeolin suggest that the *N*-hydroxyamino acid is an intermediate.[119]



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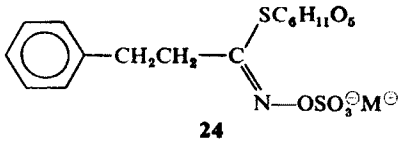

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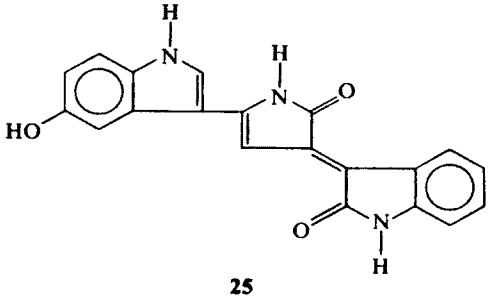
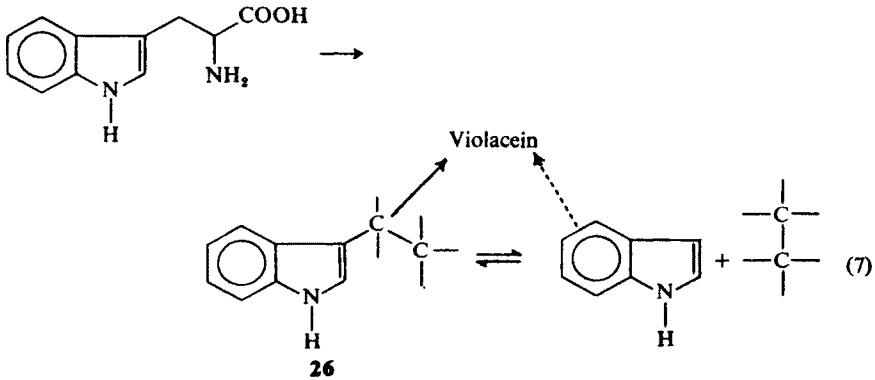
F. Violacein

This unusual pigment of structure **25** is obtained from *Chromobacterium violaceum*.[120] Its biosynthesis has been studied by two groups. DeMoss and



25

Evans[121, 122] found that L-tryptophan was required as the sole carbon source for nonproliferating cells of *C. violaceum*, synthesizing the pigment; oxygen was also required, while D-tryptophan was not converted to the pigment. The incorporation of L-tryptophan labeled with ^{14}C in either carbons 1′, 2′, or 3′ (Scheme 1) of the side chain proceeded with loss of the carboxyl carbon and retention of the two remaining carbon atoms of the side chain though their activity was diluted somewhat by an unknown endogenous carbon source. 5-Hydroxytryptophan, which is reported[123] to be formed by *C. violaceum*, was not incorporated. Sabek and Jäger[124] also reported the conversion of L-tryptophan into violacein by *C. violaceum*; in addition, they found that lyophilized preparations of the washed cells synthesized indole from tryptophan, and that washed, nonlyophilized cells incubated in an atmosphere of indole vapors rapidly produced violacein. From these results, they concluded that tryptophan is converted to violacein through the intermediacy of indole. This conclusion is incompatible with DeMoss and Evan's results and appears to be unwarranted. The results of both groups may be explained by assuming that tryptophan loses its carboxyl carbon to give an intermediate (**26**) which

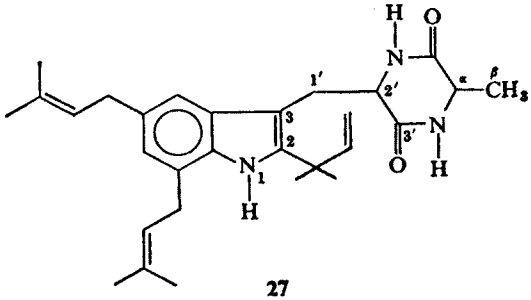


is in equilibrium with indole and a two-carbon fragment. The dilution of the activity of the side-chain carbons observed by DeMoss and Evans might then be a consequence of the incorporation of an inactive, endogenous two-carbon unit into the intermediate **26** via its equilibration with indole (Eq. 7). Both groups of authors tested the ability of *C. violaceum* to convert a wide variety of likely precursors into violacein; negative results were obtained in every case. The nature of the steps leading from tryptophan to the pigment thus remains a mystery.

G. Echinulin

The mold metabolite echinulin (**27**) was first isolated and investigated by Quilico and his school.[125] Birch and his collaborators[126] subsequently employed

tracer studies as an aid to the elucidation of the structure of the molecule. Feeding 2-^{14}C-mevalonic lactone (**28**) to *Aspergillus amstelodami* produced



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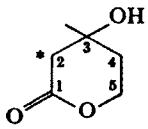

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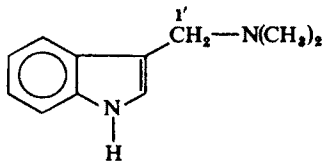


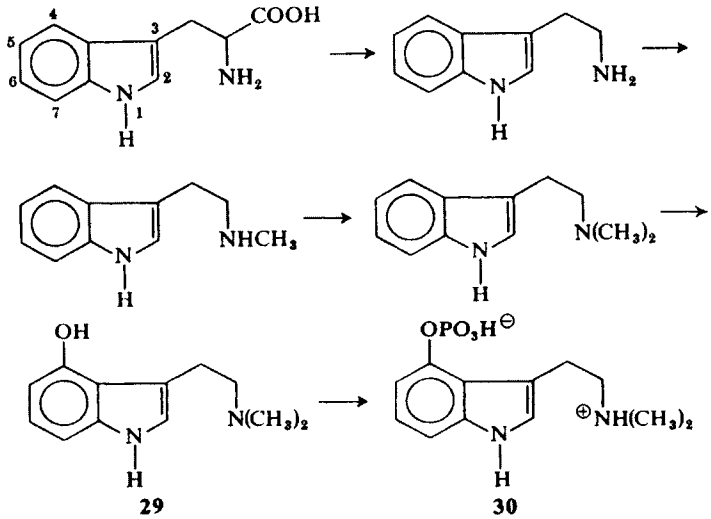
31

radioactive echinulin, degradation of which indicated the presence of three isoprene units in the metabolite. β-^{14}C-DL-Alanine was shown to be efficiently incorporated into the alanine-derived portion of the diketopiperazine moiety. Birch and Farrar[127] also found significant incorporation of 1′-^{14}C-DL-tryptophan into echinulin, a result taken to indicate that isoprenylation occurs at a stage later than tryptophan in echinulin biosynthesis. MacDonald and Slater[128] have since verified this result and have shown that the incorporation of ^{14}C-DL-tryptophan labeled at the 2-position of the indole ring or in either the 2′ or 3′ positions of the side chain proceeds as anticipated. 1′-^{14}C-L-Tryptophan was found to be incorporated about twice as efficiently as the D-isomer. This last result is in support of a very recent ORD study[129] which concluded, on the basis of the Cotton effects exhibited below 250 mμ by echinulin and a series of model diketopiperazines, that echinulin contains an L-tryptophan unit. This conclusion is at variance with that derived earlier from ORD studies limited to the region above 290 mμ.[130]

H. Psilocybin

The biosynthesis of psilocybin (**30**), the active principle in certain Mexican hallucinogenic fungi of the genus *Psilocybe*,[131] has only recently been scrutinized. The incorporation of labeled tryptophan into psilocybin was first

recorded by Hofmann's group,[132] and subsequently confirmed by Agurell and coworkers[133, 134]; the latter authors also investigated the utilization of other likely precursors by *Psilocybe cubensis*. Tryptamine, which is synthesized from tryptophan by the same fungus, was found to be a more efficient precursor than the amino acid, even when it was assumed that only the L-amino acid was utilized. *N*-Methyltryptamine was a still more efficient precursor, while *N*,*N*-dimethyltryptamine was relatively inefficient; this last result was attended with some ambiguity however, as the amine was poorly absorbed by the fungus. 4-Hydroxytryptophan also proved to be an inefficient progenitor, suggesting ring hydroxylation at a later stage in the biosynthesis; psilocin (**29**), which also occurs in the fungus, was readily converted into psilocybin. The authors summarized the data in terms of the diagram presented in Scheme 3.



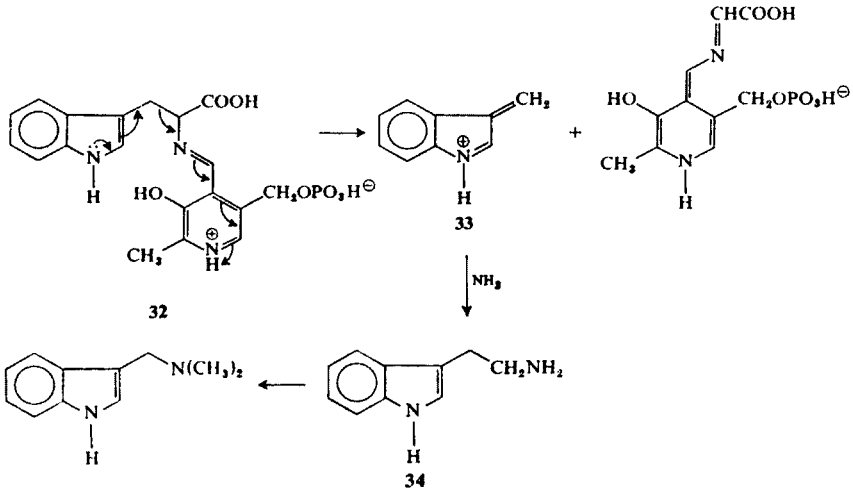
Scheme 3

III. Indole Alkaloids

A. Gramine

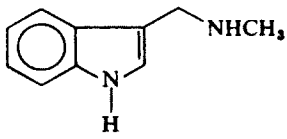
The simple indole alkaloid gramine (**31**) is present in the sprouting barley plant (*Hordeum vulgare*).[135, 136] Initial work[137, 138] on its biosynthesis revealed the ability of sprouting barley plants to convert 1′-^{14}C-tryptophan into gramine; a continuation of the investigations[139] verified the incorporation

of the indole ring and C-1′ as a unit. By feeding a mixture of DL-tryptophan labeled at C-2 of the indole ring and DL-tryptophan labeled at C-1′, gramine was obtained which was labeled solely at the expected positions and with a ratio of activities identical to that in the original tryptophan mixture. *S*-Methyl-^{14}C-methionine has been shown to be the source of the *N*-methyl groups in gramine.[140] An examination of various plausible intermediates[141] between tryptophan and gramine indicated that 1′-^{14}C-3-indolepyruvic acid and 1′-^{14}C-3-indoleacrylic acid could act as precursors. The incorporations of these acids were specific, but quite poor. 3-Indoleacetic acid, 3-indoleglyoxylic acid, 3-indolecarboxaldehyde, and 3-indoleacetamide each failed to give rise to gramine. In the light of more recent work, 3-indoleacrylic acid can be dismissed as an intermediate. Very low incorporation was found when the labeled acid was fed to excised barley shoots,[142] and the level of radioactivity was in fact higher in the isolated tryptophan than in the gramine. This suggests prior conversion of the acrylic acid to the amino acid before incorporation. O'Donovan and Leete[143] have published more conclusive evidence. Administration of a mixture of 1′-^{3}H-DL-tryptophan and 1′-^{14}C-DL-tryptophan to intact barley seedlings yielded radioactive gramine labeled only at C-1′, and with the same $^{3}H/^{14}C$ ratio as the original tryptophan mixture. Since no loss of tritium was observed, the 1′-methylene group of tryptophan must maintain its integrity during the conversion of the amino acid to gramine. The result may also invalidate 3-indolepyruvic acid and 3-indoleacetic acid as possible precursors since the tritium atoms in these substances would be born by a carbon atom adjacent to a carbonyl function and might therefore be exchanged.



Scheme 4

This evidence is compatible with an attractive hypothesis proposed by Wenkert[144] for the conversion of tryptophan to gramine. Tryptophan was postulated to condense with pyridoxal phosphate to yield the Schiff's base **32** which could undergo fragmentation to the protonated 3-methylene-indolenine **33**; addition of ammonia to this highly reactive entity would yield 3-aminomethylindole (**34**), methylation of which would afford gramine (Scheme 4). Support for this scheme derives from two sources. First, 3-aminomethylindole and 3-methylaminomethylindole (**35**) have in fact been



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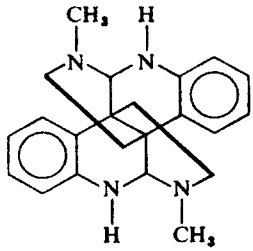

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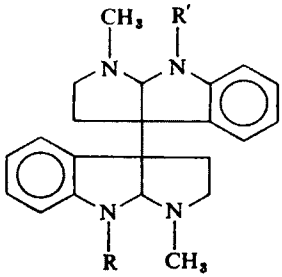
isolated from barley seedlings and an enzyme preparation obtained from barley shoots which methylates 3-aminomethylindole to **35** and gramine.[145] Second, Gower and Leete[146] have prepared 2-^{14}C-3-aminomethylindole and 2-^{14}C-3-methylaminomethylindole and administered them to excised barley shoots. The incorporations of the two amines were quite high, being 14.2 and 24.5% respectively, and the radioactive gramine so obtained was labeled exclusively at C-2.

B. Calycanthus Alkaloids

Biosynthetic work on the Calycanthus alkaloids[147] appears to be limited to a single investigation. Schutte and Maier[148] report the incorporation of



37; R = H; R′ = CH_3

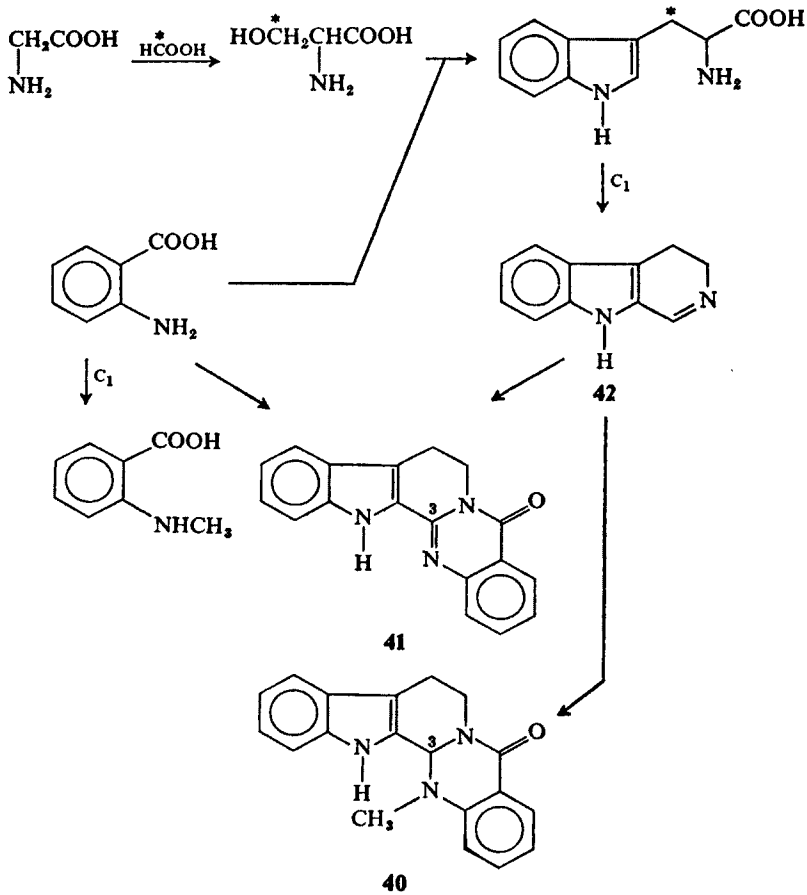
38; R = R′ = H

39; R = R′ = CH_3

1′-^{14}*C*-tryptophan into calycanthine (**36**), calycanthidine (**37**), chimonanthine (**38**), and folicanthine (**39**) by *Calycanthus floribunda*, though the identifications of the alkaloids were uncertain. Additional work on these interesting alkaloids is clearly needed.

C. Evodia Alkaloids

Two papers by Yamazaki and coworkers[149, 150] explore the formation of alkaloids in the fruit of *Evodia rutaecarpa*.[151] 1′-^{14}*C*-Tryptophan led to radioactive evodiamine (**40**) and rutaecarpine (**41**) whose degradation indicated that most of the activity resided in the tryptamine portion of the bases.



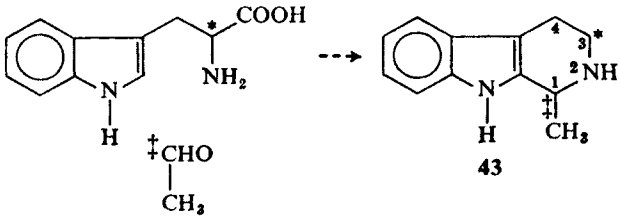
Scheme 5

^3H-Anthranilic acid was utilized in the formation of both **40** and **41**, but the radioactive alkaloids were not degraded. Sodium ^{14}C-formate was incorporated primarily into C-3 of rutaecarpine with the remainder of the activity localized in the tryptamine portion of the molecule, a result attributed to *in vivo* condensation between the labeled formate and glycine to give radioactive serine which subsequently transformed into tryptophan. In the case of evodiamine, the formate label was located primarily at C-3 and in the *N*-methyl group. Methyl-^{14}C-methionine supplied radioactivity exclusively to C-3 and the *N*-methyl group of evodiamine, and solely to C-3 of rutaecarpine. The specific activity of the evodiamine formed when these C_1 donors were fed was lower in comparison with that of rutaecarpine than would have been anticipated on the basis of the presence of two C_1-derived carbons in the former alkaloid. This was interpreted to mean that evodiamine does not arise from *N*-methylation of a rutaecarpine-like precursor, but rather by the introduction of a C_1 unit at an earlier stage of the biosynthesis to give *N*-methylanthranilic acid which could be diluted by nonlabeled, endogenous *N*-methylanthranilic acid. The biosynthetic network illustrated in Scheme 5 was proposed by the authors, though no evidence was provided for dihydronorharman (**42**) being an intermediate.

D. Carboline Alkaloids

The known carboline bases constitute a group of alkaloids derived from simple variations in the oxidation state of the β-carboline ring system.[152] These alkaloids have long been a subject of biosynthetic speculation, beginning with the farsighted proposal by Perkin and Robinson in 1919[153] that they arise *in vivo* from a Mannich condensation between a tryptamine derivative and acetaldehyde. Only three papers have thus far appeared that provide experimental data on carboline alkaloid biosynthesis.

O'Donovan and Kenneally[154] examined the formation of eleagine (**43**), the



(8)

simplest member of the series, in *Elaeagnus angustifolia*. 2′-^{14}C-DLTryptophan and sodium 1-^{14}C-acetate were incorporated to give radioactive alkaloid