THE USE OF ISOLATED ENZYMES IN HETEROCYCLIC CHEMISTRY.

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Current trends in the utilization of isolated enzymes in the synthesis and degradation of heterocyclic compounds are reviewed.

1. Introduction;
2. Heterocyclic amino acids;
3. Terpenoids;
4. Alkaloids;
5. Antibiotics;
6. Miscellaneous;
7. References.

1. INTRODUCTION

The utilization of microorganisms to effect particular structural modifications, especially in the area of steroidal transformations, paved the way for the current investigations into isolated enzyme systems. It has been possible to achieve a variety of reactions such as oxidation, reduction, hydrolysis, esterification, acylation, transglycosidation, methylation, condensation, cleavage of carbon bonds, decarboxylations, dehydration, amination, deamination, phosphorylation and halogenation using whole organisms. A lot of effort has been put into attempts to isolate the responsible enzyme

systems with a view of improving efficiency, stability and their more general utility to wider applications in organic chemistry.

These studies have met with success in certain limited fields, as can be gauged from some recent reviews of the use of enzymes in industry[1-7]. Much of this success can be attributed to the advent of immobilized enzymes[1,4,5,7], and research is now underway to improve these systems.

For example there is a recent report on the immobilization of enzymes using asymmetric hollow fiber membranes[8], the entrappment of enzymes into synthetic fibers which are then woven into cloth[1]and the adsorbtion of enzymes onto ion exchange beads[1]to name but a few recent developments. By 1980 it is estimated that the conversion of corn starch into syrup will be about 1 billion pounds and will account for about 30% of the normal sucrose market[1].

In some areas, however, progress has been slow, and this is especially true of the utilization of enzymes in the synthesis of highly priced heterocyclic pharmaceuticals. Some of this lack of progress has been due to the difficulty experienced in enzyme isolation from plant sources, and the recognition of the appropriate co-enzymes, co-factors and optimal operating conditions. On the other hand, the general concept of the important relationship between enzyme systems and drug action has led to interesting studies in which the role of enzymes is considered in relation to the designing of new drugs[9].

Another area of active study which holds much promise is the immobilizing of mitochondria on support such as porous silica beads[10]. W.S. Brinigar points to the fact that at present the only enzymes being used commercially are those catalyzing exergonic reactions, but if immobilized chloroplasts or mitochondria could be placed in the circulating system upstream from

immobilized biosynthetic enzymes, sunlight or the oxidation of simple organic compounds could provide the chemical free energy for the synthesis of particular complex compounds or food products[10].

Although progress has been slow in making significant utilization of the immobilization technique for the specific production of complex heterocyclic compounds, note should be made of an important event. Time magazine recently commented on the "Gene Makers"[11]. The Harvard University team of Efstratiades, Kafatos, Maniatis and Maxam have utilized the Temin-Baltimore enzyme, which is capable of reversing normal genetic processes, to trick RNA into making the DNA from which it itself has been produced.

It is proposed in this review to survey those isolated enzyme systems which have been utilized to either synthesise or degrade heterocyclic systems, such as certain aromatic amino acids, terpenoids, alkaloids and antibiotics. With the recording of achievements up to the present, hopefully one can gauge the more fruitful areas of investigations likely for the future in this particular field.

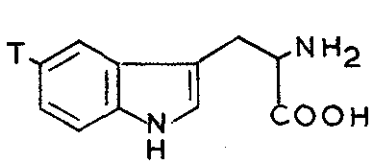
2. HETEROCYCLIC AMINO ACIDS

Tryptophan decarboxylase from Catharanthus roseus plays an important role in the early stages of indole alkaloid biosynthesis and has recently been partially purified by ammonium sulphate precipitation and gel filtration[12]. Similar enzymes have also been isolated from cucumber seedlings[13] and Phalaris tuberosa plants[14].

A new group of enzymes have been isolated from both wheat germ and rat liver and which is capable of oxidising the pyrrole ring of indoles to N-formamidophenylacyl derivatives. These enzymes called pyrroloxygenases, could use tryptophan, ethyl-N-acetyltryptophan, skatole, 3-indoleacetic

acid, 3-indolepropionic acid and indole as substrates[15].

Tritium labelled tryptophan (1) in the presence of tryptophan-5-hydroxylase gave the unexpected hydroxylated compound (2) in the now famous NlH-shift reaction[16].



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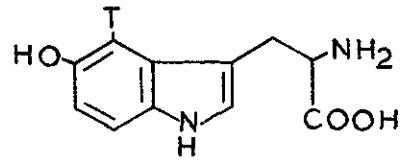

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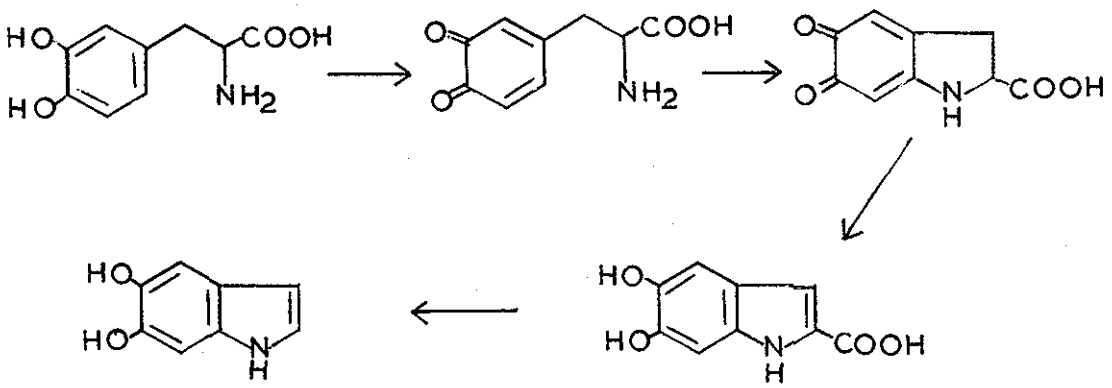
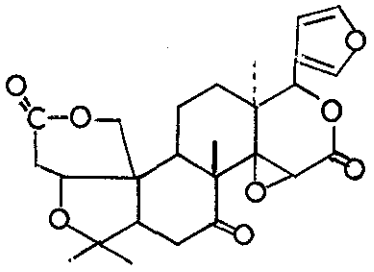
The isolated enzyme tyrosinase cast much light on the mode of formation of melanin. The Scheme 1 outlines the sequence from dihydroxyphenylalanine,[17-21] and more recent work with ^{3}H-labelled dihydroxyphenylalanine showed that the bulk but not all the links between indolequinone units occur through the 3,7-position[22].



Scheme I.

3. TERPENOIDS

In food chemistry, certain enzyme studies relate directly to heterocyclic systems. In one such example the limonin D-ring lactone hydrolases from grapefruit seeds and Pseudomonas sp.321-18 were compared[23]. Both enzymes had similar pH optima for both hydrolysis (pH 8.0) and lactonization (pH 6.0). Limonoate A-ring lactone was identified as the hydrolysis product of limonin (3) at pH 8 and limonoate D-ring lactone as the product of sodium limonoate at pH 6.0. These enzymes also catalysed the hydrolysis of obacunone (4), nomilin (5) and ichangin (6). One major area of difference was in their stabilities. Citrus hydrolase was markedly heat resistant but the bacterial hydrolase was almost completely inactivated at 60° after 5 minutes.



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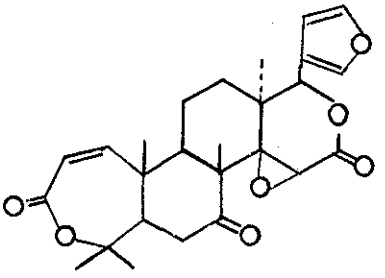

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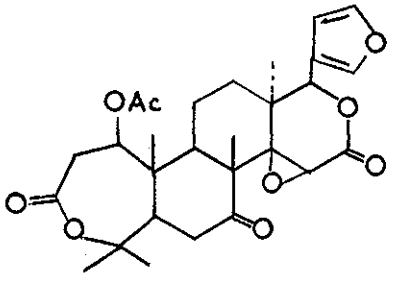

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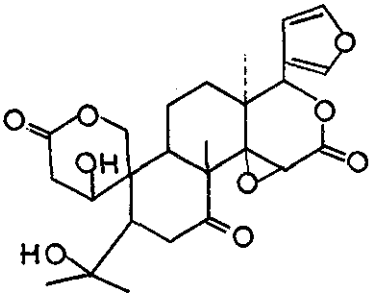

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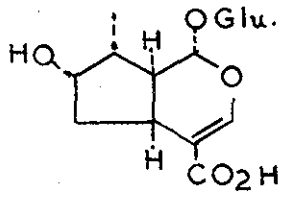
In the area of monoterpene chemistry there has been detailed investigations into the conversion of loganic acid (7) to loganin (8) and secologanic acid (9) to secologanin (10)[24-27].



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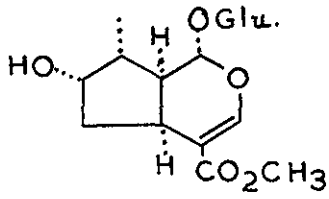

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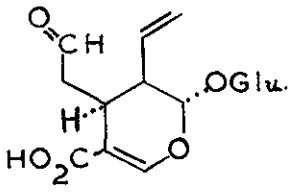

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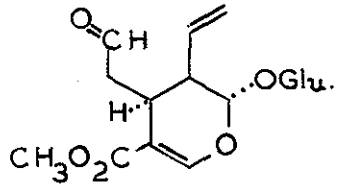


(10)

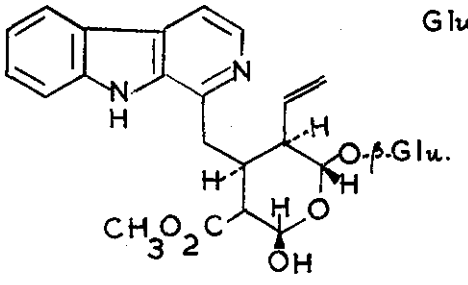
It has been shown that a partially purified enzyme from Catharanthus roseus was able to catalyse the transfer of the methyl group of S-adenosyl-L-methionine to loganic acid, thus forming loganin, an intermediate in the biosynthesis of indole alkaloids of this plant. Secologanic acid could similarly be converted to secologanin. Under the same assay conditions no significant methylation of 7-deoxyloganic, 7-epiloganic or genipic acids were observed[27].

4. ALKALOIDS

In recent times there has been a fair amount of activity involving the utilization of enzymes in alkaloid chemistry, and there is high hope for a major break-through in the use of immobilised enzymes for the

synthesis of commercially important alkaloids.

Extensive use has been made of β-glucosidase in the structural elucidation of a variety of indole alkaloids. Some recent cases are exemplified by palinine (11)[28], 10-β-D-glucosyl-oxyvincoside lactam (12)[29] and rubenine (13)[30] which had their glucose moieties removed to facilitate structural analysis.



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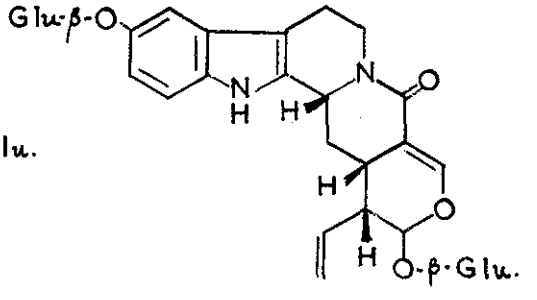

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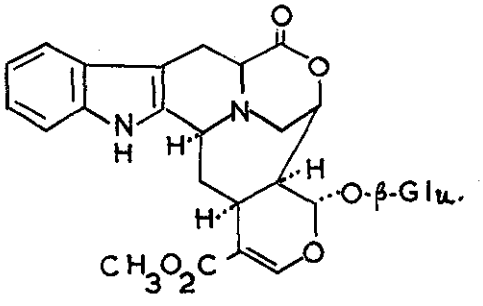
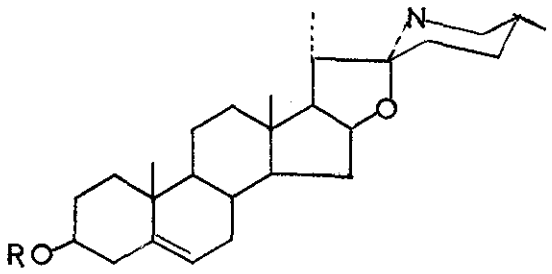


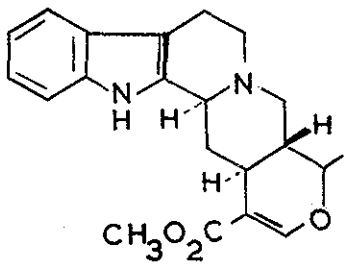
(13)

It is possible to use an enzyme preparation to achieve the reverse process, namely converting an alkaloid to a glycoside. Such was the case for solasodine (14) which in the presence of UDP-glucose was converted to the 3β-glucoside (15) by using an enzyme extracted from Solanum laciniatum[31].



(14) R = H
(15) R = Glucose

An exciting report concerns the use of enzymes from 5-7 day old Catharanthus roseus seedlings to convert a mixture of ^{14}C-tryptamine, secologanin, FAD and NADPH to ajmalicine (16) and geissoschizine (17), albeit, in low yields[12].



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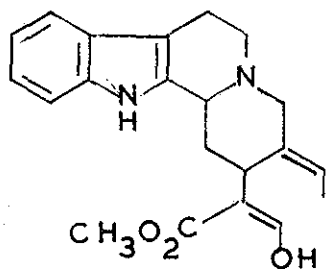
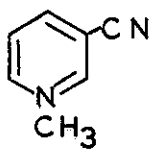


(17)

However, when enzymes from calluses were used, a 18% conversion to ajmalicine and a 1% to geissoschizine was observed. Ajmalicine could also be formed from geissoschizine in a 7.7% yield. Scott commented that purification and immobilization of selected enzymes of this pathway are now feasible and such an approach to the production of anti-leukaemic alkaloids such as vincaleukoblastine and leurocristine will no doubt be attempted.

Early work by Robinson with a crude enzyme system from Ricinus communis seedlings showed that 1-methylnicotinonitrile (18) could be oxidised to the corresponding 4- and 6-pyridones[32], and more recently he and his co-workers have resolved this crude mixture into three components, all of which catalysed the oxidation of 3-cyano-1-methylpyridinium perchlorate to the pyridones (19) and (20).



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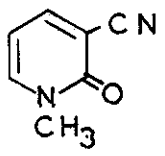

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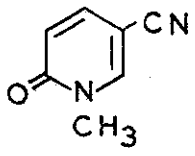

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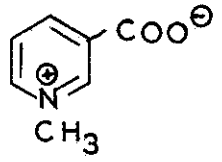
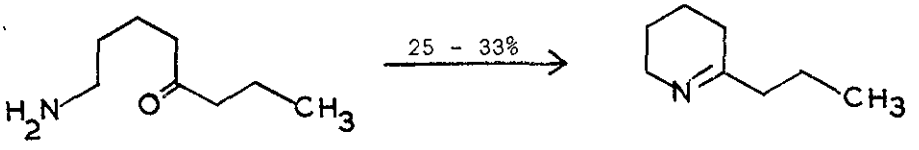


(21)

These enzymes were non-specific, and 3-formyl-1-methylpyridinium iodide, 3-cyano-1-ethylpyridinium iodide, 3-nitro-1-methylpyridinium iodide, 3-acetyl-1-methylpyridinium iodide and 1-benzyl-3-cyanopyridinium chloride were all oxidised. N-Methylnicotinamide, trigonelline (21) sulphate, 1-methylpyridinium iodide, nicotinic acid, 1-methylquinolinium iodide and

3-cyanopyridine were not oxidised to any appreciable extent[33].

A transaminase in hemlock (Conium maculatum) was shown to convert 5-ketooctanal (22) to γ-coniceine (23) utilizing L-alanine.[35]



(22) (23)

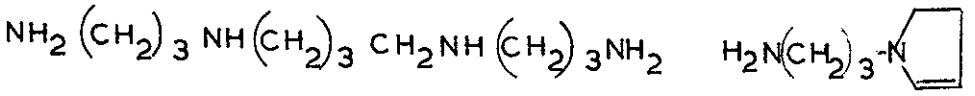
A particulate fraction from the roots of Nicotiana rustica catalysed the decarboxylation of nicotinic acid (24);[35] while the reversible hydroxylation of nicotinic acid to 6-hydroxynicotinic acid (25) has been

(24) (25)

accomplished by an enzyme purified from a fermenting Clostridia[36].

A crude homogenate from the cotyledon of Sarothamnus scoparius converted (-)sparteine (26) to (+)-lupanine (27) in a 30% yield;[37]

(26) (27)



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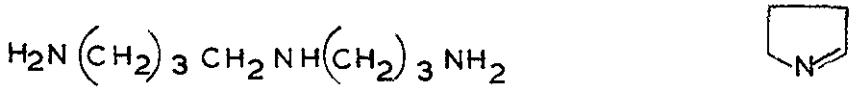
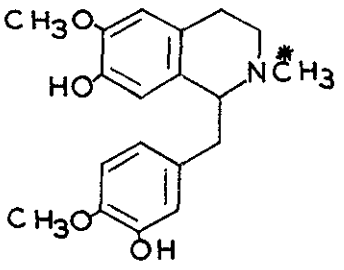


(30) (31)

and a particulate fraction from barley yielded an amine oxidase which converted spermine (28) into 1,3-diaminopropane and 1-(3-aminopropyl)-Δ^2-pyrroline (29) and spermidine (30) to Δ^1-pyrroline (31). This amine oxidase was also found in oats (Avena sativa), maize (Zea mays), wheat (Triticum vulgare) and rye (Secale cereale)[38].

Enzymes from animal sources have also been utilized. Homogenized rat liver was used to transform (±)-[N-$^{14}CH_3$]-reticuline (32) to coreximine (33) in a 0.083% yield[39].



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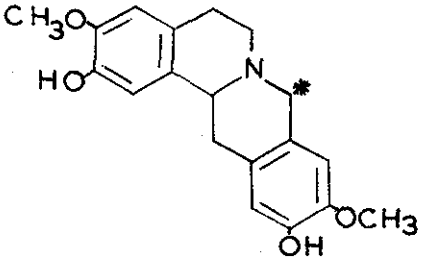

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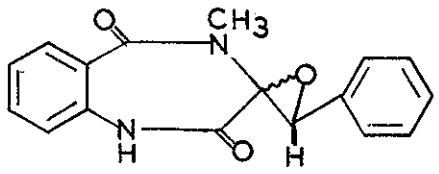
A mixed function oxygenase, cyclopenin M-hydroxylase was shown to be able to transform cyclopenin (34), one of the two major alkaloids of

Penicillium cyclopium into cyclopenol (35)[40]. NADP, ascorbic acid, tetrahydropteridine and molecular oxygen were co-substrates, and with the exception of viridicatin (36) all compounds structurally related to cyclopenin which were tested gave hydroxylated products.



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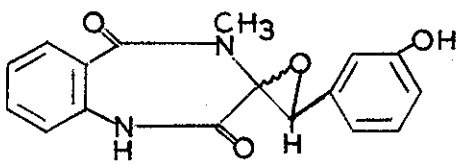

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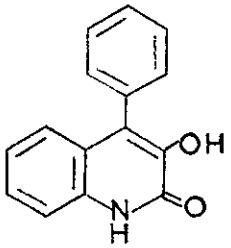

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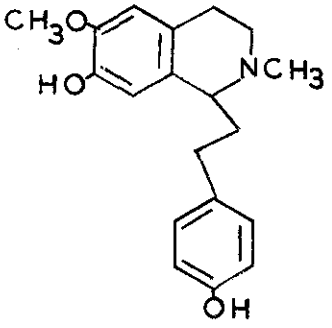
Peroxidase-type enzymes, especially the horseradish peroxidase, has been used extensively to accomplish a variety of oxidative coupling reactions in the field of alkaloids. Since the early aspects of the use of this enzyme have been reviewed on previous occasions[41,42], only the recent highlights will be mentioned in this review. Horseradish peroxidase consists of a protein (apoenzyme) together with an ironporphyrin compound, protohematin, as coenzyme. The iron is thought to be surrounded octahedrally by the prophyrin, (four planar positions) the protein and another ligand (e.g. H_2O_2)[43]. When laudanosoline methobromide was oxidised with peroxidase, an aporphine compound was the product[43], while Inubushi and his co-workers observed that in the case of the oxidation of quaternary ammonium bromide and iodides, halogenation of the phenolic nucleus occurred predominantly.[44]

As expected, the oxidation of phenethylisoquinoline (37) resulted in a head to tail coupling to produce the diphenyl ether promelanthioidine (38)[45].



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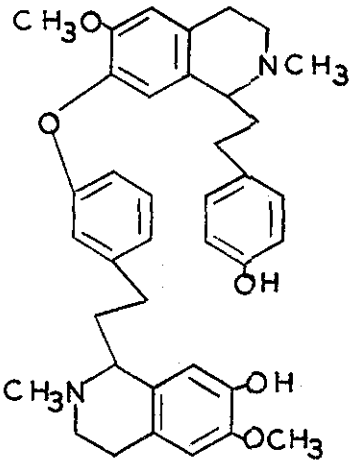

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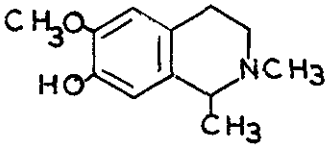
Imbushi and his group have also examined the oxidation of a number of isoquinoline alkaloids[44]. dl-N-Methylisosalsoline (39) yielded on oxidation, followed by diazomethane treatment, compounds (40) and (41), while similar reaction conditions with dl-lophocerine (42) gave O-methylisopilocereine (43) and its diastereoisomer.



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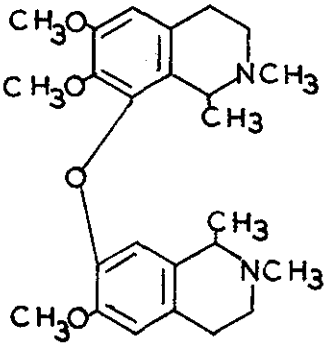

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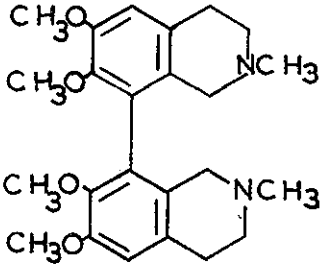

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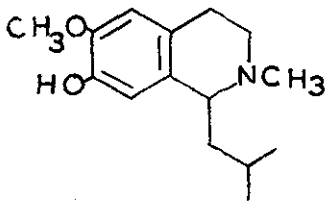

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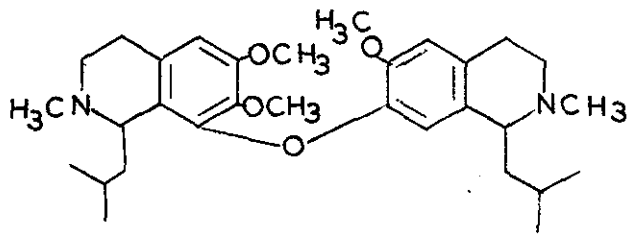
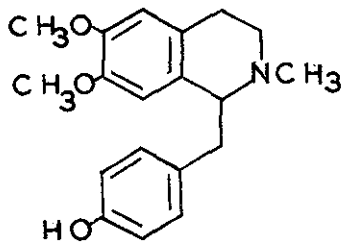


(43)

dl-Armepavine (44) underwent fragmentation to (45) and (46), the latter isolated as O-methylcorypalline (47) after sodium borohydride reduction.



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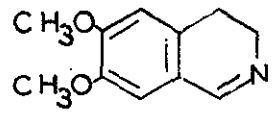

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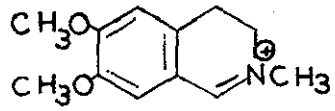

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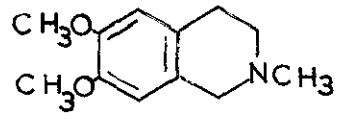
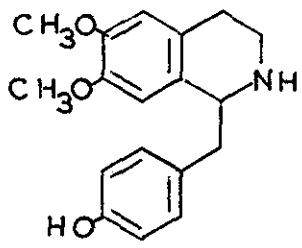


(47)

dl-N-Norarmepavine (48) produced (49) and (50), the latter isolated after $NaBH_4$ treatment as O-methylsendaverine (51).



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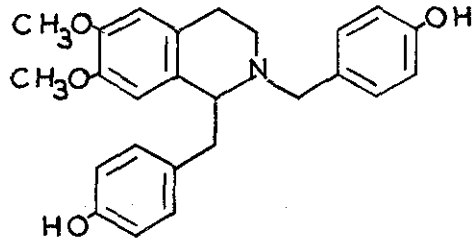

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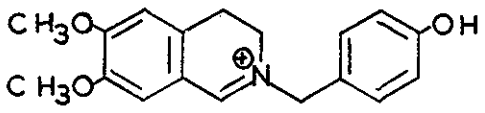

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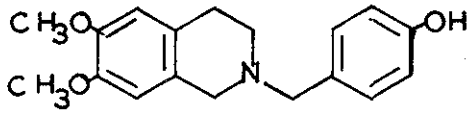
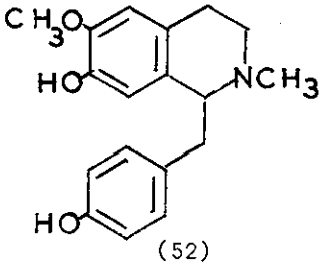


(51)

dl-N-Methylcoclaurine (52) yielded (53) and two other products identified after $NaBH_4$ reduction, as compounds (54) and (55), the latter after additional treatment with CH_2N_2.



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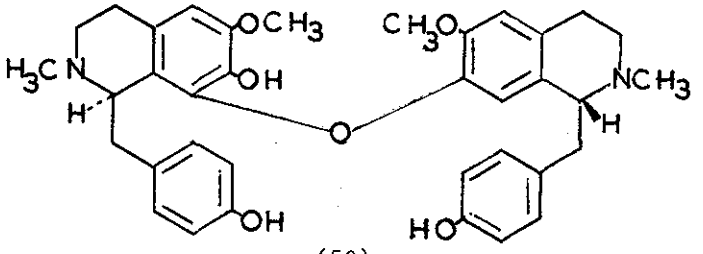
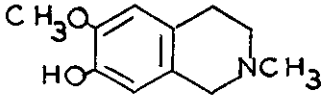
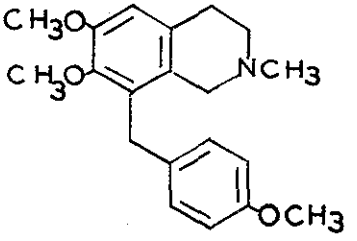


(53)



(54)

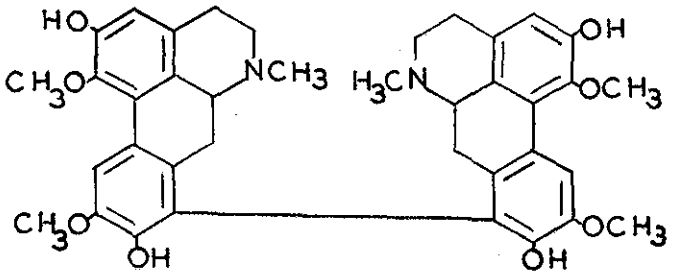


(55)

A possible mechanism involving an unstable quinoid type compound was proposed to explain the interesting rearrangement products (50) and (55)[44]. Compounds (56) and (57) were the major products after (s)-boldine (58) was oxidised with peroxidase and H_2O_2[46].

With the commercial appearance of peroxidase as an insolubilized reagent (e.g. ENZITE-peroxidase, which has a carboxymethyl cellulose

support and attachment accomplished by the Curtius azide method)[47], it is likely that we will see further application of this enzyme in the not too distant future.



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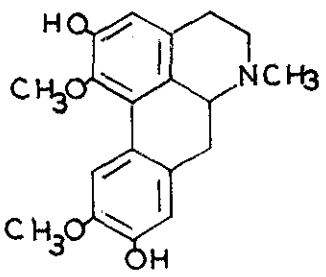
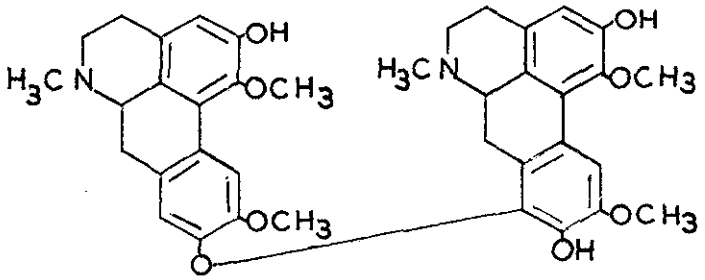


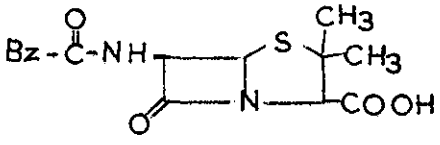
(58)



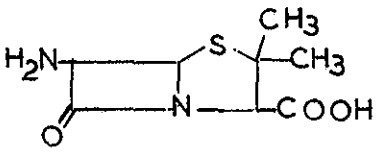
(57)

5. ANTIBIOTICS

Most of the commercially valuable applications of enzymes in the area of antibiotics has been confined mainly to the cleavage of benzylpenicillin (59) by penicillin amidase to yield 6-amino-penicillanic acid (60).[48-50]



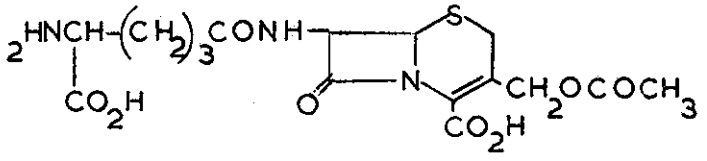
(59)



(60)

By using a crude enzyme extract from pigeon liver, penicillanic acid in aqueous acetone and with α-phenylglycine added, can be converted back to benzylpenicillin[51].

The first report of the enzymic modification of the D-α-aminoadipyl side chain of cephalosporin (61) by using D-aminooxidase has appeared[52].

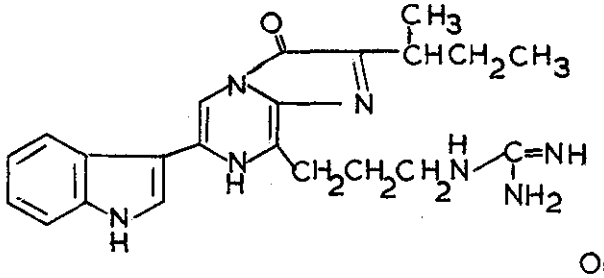


(61)

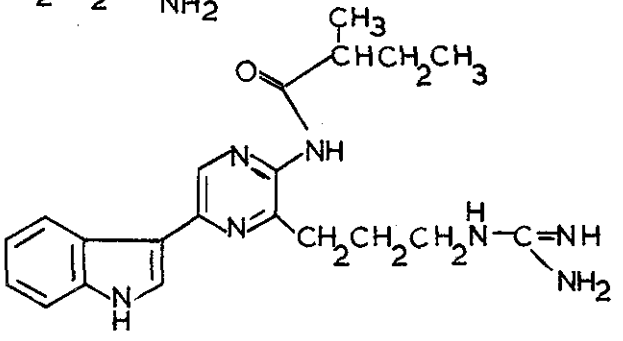
6. MISCELLANEOUS

a) Bioluminescence.

Luciferase converts luciferin (62) to oxyluciferin (63) in the presence of oxygen with the production of light and CO_2[53]. The commercial potential of this reaction has not yet been fully realised.



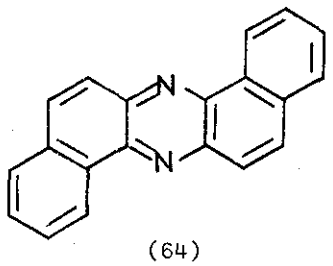
(62)



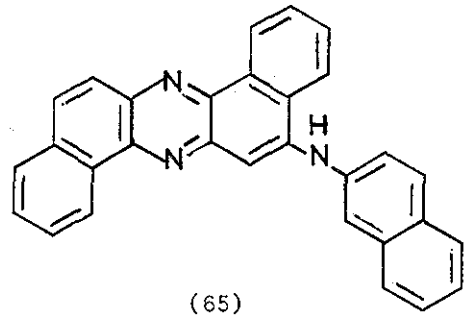
(63)

b) Phenazines.

The oxidation of 2-naphthylamine using peroxidase and H_2O_2 produced dibenzo (a,h)-phenazine (64) and a compound which appears to be 5-(2-naphthylamine)-dibenzo[a,h]-phenazine (65).[54]

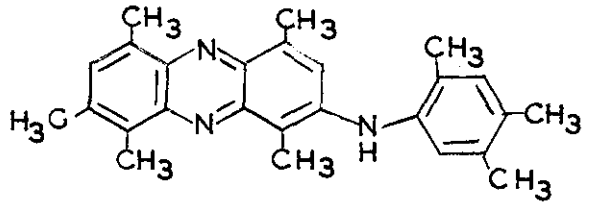


(64)



(65)

Although phenazines are rarely isolated from the peroxide oxidation of di- and trimethylated anilines since further oxidation to polymeric compounds usually takes place, compound (66) was obtained from 2,4,5-trimethylated aniline because it is sterically hindered and oxidises only very slowly[54].



(66)

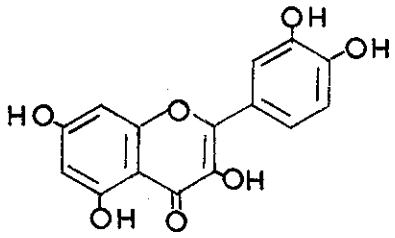
c) Nucleotides

A recent review has appeared dealing with these compounds[55]. In a study by Gassen, use was made of the synthetic properties of ribonuclease covalently bound to a highly substituted carboxymethyl cellulose to

produce the trinuclotides UAA, UAG and UGA[56]. These compounds had previously been obtainable only by difficult chemical synthesis.

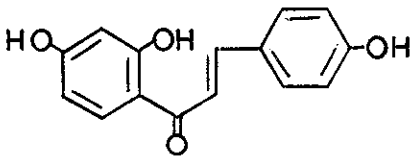
d) Flavanones and Related Compounds

The enzyme quercetinase from Aspergillus flavus degraded quercetin (67) to CO and a depside, 2-protocatechnoylphloroglucinol carboxylic acid. This enzyme was able to degrade 10,800 moles of (67) /hr./mg protein, and was shown to have a MW of 111,000[57].

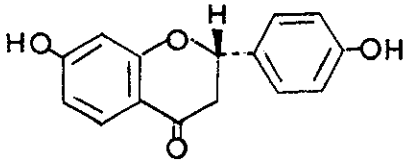


(67)

Soy bean seeds have yielded an enzyme which can catalyse the conversion of chalcones to the corresponding flavones[58]. For example, 2',4,4'-trihydroxychalcone (isoliquiritigenin) (68) was converted to (-)4',7-dehydroxyflavone (liquiritigenin) (69) in a 52% yield.



(68)



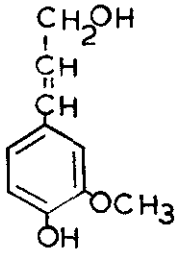
(69)

Oxidation experiments on flavans involving laccase[59] and tyrosinase[60,61] have also been reported. A review on the oxidative coupling of natural polyhydroxyflavanes as it relates to the fermentation of green and black teas has appeared[62], and the formation of theaflavin in an unspecified

tea from the condensation of epicatechin and epigallocatechin, probably by a tyrosinase type enzyme has been studied[63].

e) Other Uses of Peroxidase and Laccase

Scott in a review in 1965, made reference to the use of laccase and peroxidase in the production of geodin, dehydrogriseofulvin and the classical oxidation compound, Pummerer's ketone[64]. Harkin and his co-workers investigated ligin-like polymers resulting from the peroxidase oxidation of compound (70)[65,66].



(70)

It is very evident from the data available, that the results so far obtained are not commensurate with the efforts put into this field of investigation. Hopefully, this situation will be remedied shortly.

ADDENDUM: Attention is drawn to a review by C. J. Suckling and K.E. Suckling (Chem. Rev. 1974, 3, 387) which covers some topics not mentioned in this review as well as a very recent article on enzymes by J. L. Meers (Chemistry in Britain, 1976, 12, 115).

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