THE SYNTHESIS OF POTENTIAL PSYCHOPHARMACOLOGICAL AGENTS.

A Thesis submitted to the University of Glasgow for the degree of Doctor of Philosophy in the Faculty of Science by

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PREFACE

This thesis is a report of an investigation into the preparation of benzo-(b)-thiophen isosteres of physiologically active indole compounds.

In the introduction it is suggested that psychotomimetic and tranquillising drugs may exert their characteristic effects through an ability to interfere with the postulated neural basis of memory by a direct influence upon synaptic transmission within the central nervous system. Depending upon the locus of drug action this interference may either alter learned, perceptual discriminative ability thereby producing hallucinatory episodes, or else inhibit conditioned patterns of emotional behaviour. In support of this contention, current knowledge of the mode and site of action of certain psychopharmacological agents is reviewed. The need for drugs which can selectively interfere with the action of compounds suspected of being neurohormones is stressed. As a working hypothesis it is suggested that the benzo-(b)-thiophen isosteres of physiologically active indole derivatives may specifically affect the biological role of serotonin, a compound believed to be involved in neurohumoral transmission within the central nervous system.

The first part of the discussion examines approaches to the synthesis of the benzo-(b)-thiophen isostere of lysergic acid diethylamide, a potent psychotomimetic drug. Investigations into a novel approach to the synthesis of this and isosteres of other complex indoles are reported. These take advantage of the facile hydrolytic

cleavage of the cyclic amide linkage in the oxindole derivative of lysergic acid diethylamide. The free amino-group thus exposed can be diazotised and replacement of the diazonium function by a sulphur atom followed by ring closure would provide a benzo-(b)-thiophen derivative which could readily be reduced to the desired isostere.

The second part of the discussion deals with the synthesis of the benzo-(b)-thiophen isosteres of serotonin and derivatives of 5-hydroxy-gramine. Preliminary approaches to the synthesis of the psilocybin isostere are also discussed.

ACKNOWLEDGEMENTS

The author wishes to thank Professor J.B. Stenlake for the interest he has taken in the research herein described and also for providing the opportunity to work in his department. Appreciation must also be extended to Professor R.C. Garry, Institute of Physiology, University of Glasgow, and Professor P.L. Pauson, Department of Pure and Applied Chemistry, University of Strathclyde, for making available the facilities of their departments.

The author is delighted to have this opportunity of expressing his gratitude to Dr. M. Martin-Smith, Senior Lecturer in Medicinal Chemistry, University of Strathclyde, who suggested the nature of the research to be undertaken, for his continued guidance and enthusiasm which has been a welcome source of inspiration even in the face of "unchanged starting materials".

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To the Staff and Research Fellows of the Division of Medicinal Chemistry the author must remain indebted for valued advice and assistance. In particular, the help afforded by Mr. R. Nugent and

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CONTENTS.

INTRODUCTION.

Investigations into the pathogenesis of mental illness have been frustrated, until quite recently, by the inability of research workers to examine subjects, animal or human, where standardised states of mental aberration can be studied at will. Moreover, approaches such as those based on the analysis of the body fluids of schizophrenic patients in psychiatric wards may not permit of any significant conclusions since, in addition to the variable clinical picture between patients, there is the question of whether or not the subject is in any way disturbed at the time of examination. There are many aspects of this problem which do not require further amplification here.

The position has, however, been radically altered in the last few years by the advent of the psychotomimetic, or hallucinogenic, drugs which induce in the healthy subject a state of mind which bears a close resemblance to that of the schizophrenic - perceptual distortions and hallucinations often occurring. In animals these drugs cause behavioural changes which are suggestive of mental disorder. Accordingly, within the past fifteen years there has been a marked change in approach with heavy emphasis on attempts to elucidate the chemical basis, if any, of mental diseases. Much of this work, which has employed both intact animals and isolated tissue preparations, has been devoted to a search for natural metabolites, the physiological functions of which might be affected by hallucinogens. One such metabolite which has been singled out for particular attention is 5-hydroxytryptamine (syn. serotonin) since

many of the hallucinogenic drugs can be shown to interfere with the peripheral actions of this compound on tissues such as the rat uterus and the guinea pig ileum, thus suggesting that similar interference with the central actions of 5 - hydroxytryptamine could underlie their actions in the brain. Indeed the hypothesis has been extended to the contention that 5 - hydroxytryptamine is a neurohormone possessing a critical role in the functioning of the brain and that certain aberrent conditions, notably schizophrenia, stem from interference with the actions of 5 - hydroxyptamine in a manner similar to the interference produced by the hallucinogens. This theory, which is considered in more detail in a later section, thus contends that fundamental chemical changes are the cause, not the result, of certain mental illnesses, whilst the very high potency of some of the hallucinogens would serve to underline how minute the chemical changes may be.

This new emphasis on the chemical basis of mental disorders is also of considerable sociological importance since there can be little doubt that the absence of overt physical changes has contributed greatly to the irrational attitude adopted in the past by society towards sufferers from mental illness.

The Neural Basis of Memory.

New insight into the chemical basis of mental processes may also be possible through analogy with recent discoveries in the field of genetics. Since it is now

established that all genetic information is transmitted in the form of a chemical coding imprinted upon the nucleic acids, it might seem reasonable to suppose that memory and learning could also basically involve a chemical matrix. Whilst this does not imply that a code of the same type as the genetic code is necessarily operative in the cells of the brain, it does suggest the importance of a chemically based cybernetic system as a means by which the brain handles almost instantaneous messages with their emotional and aesthetic overtones.

The nervous system is organised into functional neural pathways by means of chemically mediated contact at the synapses. In 1949 Hebb [1] introduced a neurobiotactic theory of learning which proposed that under the influence of a learning stimulus new synaptic connections developed within the brain as a result of augmented neuronal growth. Shortly after this, Katz and Halstead [2] advanced a biochemical hypothesis to account for the proposed development of new functional pathways within the central nervous system. They suggested that the lattice structure of proteins within the neuronal membrane was so altered, after learning, as to facilitate synaptic transmission. Another hypothesis which evolved from the concept that facilitation of neurohumoral transmission could account for the development of new functional pathways was introduced by Milner.[3] He proposed that only a limited number of the sub-cellular vesicles, within which the neurohormone was stored, ruptured on arrival of the appropriate stimulus, whereas the remainder

only burst after these. Repetitive stimulation of the nerve cell, such as may occur during learning, it was suggested, might somehow sensitise the refractory vesicles thereby ensuring that on subsequent occasions an increased number of vesicles fire simultaneously, thus facilitating synaptic transmission. The ability of the severed tail ends of flatworms to remember previously conditioned situations after regeneration of their complete anatomy suggested to Thompson and McConnell [4] that in this species memory might involve the nucleic acids. Further evidence of this was afforded by the demonstration [5] that if the severed tails were allowed to regenerate in a medium containing the nucleic acid degrading enzyme ribonuclease, their ability to remember was destroyed. Apparently, no other group of workers have yet been able to repeat these interesting experiments. Nevertheless, there is some other evidence which could implicate ribonucleic acid (RNA) in the learning process.

Hyden [6] was able to detect changes in the base ratio of the nucleic acids extracted from vestibular nuclei of rats that had undergone training in rope climbing. Although these changes could indicate the deposition of a memory trace, it has been pointed out [7] that direct vestibular stimulation during climbing may have been responsible.

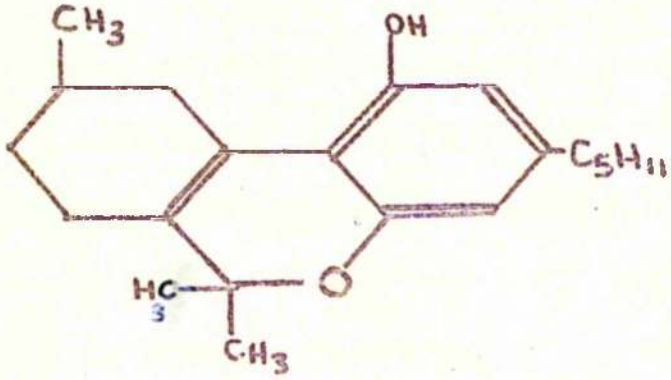
Rats premedicated with the RNA inhibitor, 8-azaguanine - $2C^{14}$, which is incorporated into RNA, were apparently slower in learning to solve maze problems than untreated rats, although memories consolidated prior to

medication were unaffected.[8] The claim has also been advanced that rats injected peritoneally with powdered RNA for several days were able to learn various tasks more quickly than untreated rats.[9]

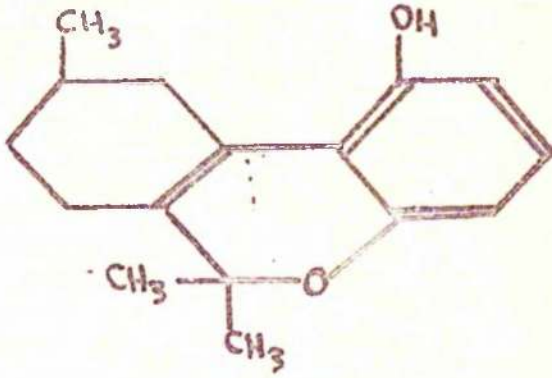
Changes within the ribonucleic acids will, of course, alter protein synthesis, hence these effects could be accommodated within the earlier theories of Hebb or Katz and Halstead, discussed above. Direct intracerebral injection of the antibiotic puromycin, which inhibits protein synthesis, has been shown to destroy learned responses conditioned three to six days earlier, although other learned responses were unaltered.[10]

A New Hypothesis of Drug Action within the Central Nervous System.

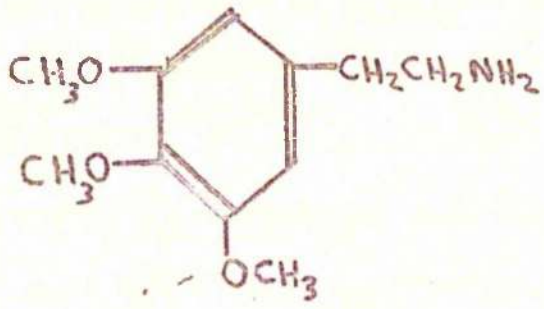
Although studies on the neural basis of memory are still in their infancy it is worthwhile considering the hypothesis that drugs which could facilitate or impede neurotransmission would, if favourably distributed within the brain, temporarily open or close functional nervous pathways thereby altering memory and learned responses. As will be explained below, many psychoactive drugs have pronounced effects upon neurotransmission. Indeed, it could be the case that the hallucinations induced by psychotomimetic drugs are due to such temporary changes in learned perceptual discrimination. This hypothesis may be extended to encompass the tranquillising drugs which affect emotional response. It is conceivable that these drugs act by inhibiting learned emotional patterns of behaviour.



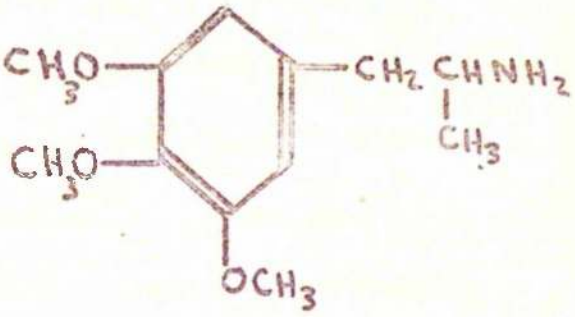
TETRAHYDROCANNABINOL



"SYNHEXYL"



MESCALINE



α-METHYL ANALOGUE OF MESCAL

Fig. 1

The Hallucinogens.

A brief outline of the chemical nature and history of the more important hallucinogens will help to indicate how the serotonin hypothesis arose, besides explaining why it has remained attractive despite criticism from several quarters. For a more detailed account, reference should be made to two excellent reviews by Downing.[11, 12]

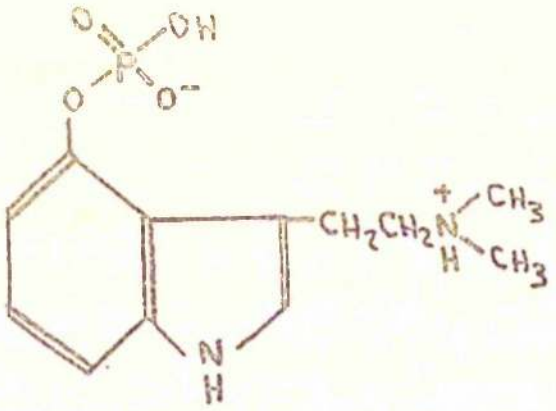
The earliest known hallucinogen which was well chronicled in the ancient herbals, is the green resin obtained from the female flowering tops of Cannabis sativa, known variously as Indian Hemp, Hashish, Marihuana, Bhang, Charas, Ganja, Dagga, or Kif. Still widely used, albeit illegally, throughout the world, it induces a state of euphoria that alternates with depression, time and spatial distortion, and what has loosely been called "double-consciousness".[13] Todd [14] has shown that tetrahydrocannabinol (Fig. 1), one of the resin's components, is capable of inducing a state of euphoria, although it seems likely that the total activity of the natural resin resides in a mixture of tetrahydrocannabinol isomers. A large number of homologues of tetrahydrocannabinol have been synthesised, many exhibiting greater potency than the parent compound, e.g. the derivative known as "Parahexyl" or "Synhexyl" (Fig. 1) which is psychotomimetic at oral dose levels ranging from 10 mg. to 200 mg.[15, 16]

Visiting Mexico in 1887, the German pharmacognocist, Lewis Lewin,[17] found that the Indians were in the habit of consuming mescal buttons, the dried tops of the cactus

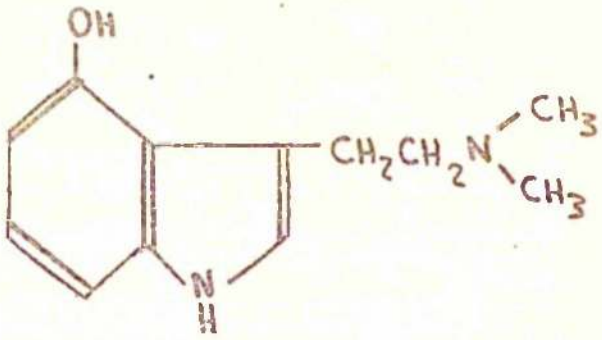
Lophophora williamsi, in order to initiate a ritual ecstatic state. The local name for mescal buttons was "peyotl". The cult of Peyotism is still practised by members of the Native American Church, the premier Christian organisation amongst the Indians of Western Canada and the United States.[18, 19] Here the sacramental consumption of peyotl replaces the bread and wine of orthodox Christianity.

The active constituent of L. williamsi appears to be mescaline (Fig. 1), at least nine syntheses of which have been reported.[20-22] In addition many analogues have been prepared,[23] although few of these compounds exhibit as high an activity as mescaline itself. A notable exception is the alpha-methyl analogue (Fig. 1) which produces hallucinations at dosage levels lower than the effective oral dose of mescaline (350 mg.)[24, 25] The mescaline-type of psychotomimetic produces visual hallucinations, depersonalisation, and time distortion, the effect varying with the individual and his environment.[26] Interestingly, radiotracer experiments with mescaline-C^{14} labelled in the side chain show that hallucinations begin after there is no longer any trace of the drug in the brain. Hence it has been suggested that mescaline is a pro-drug which undergoes biotransformation in the liver into an active metabolite.[27]

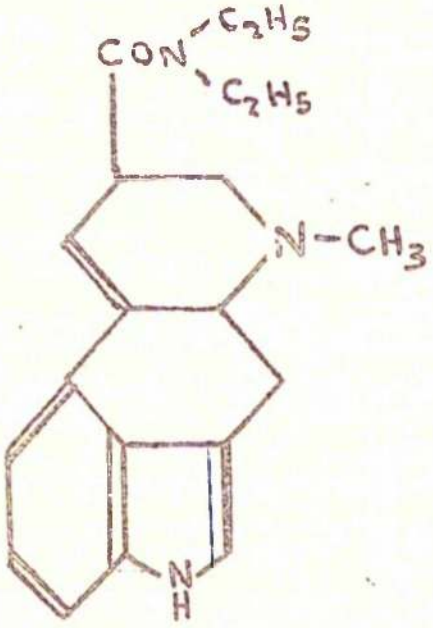
Nearly seventy years after Lewin's expedition, an American amateur ethnologist, Gordon Wasson, visited Mexico in an attempt to discover the mysterious teo-nanacatl ("God's flesh"), a mushroom attributed with divine powers, which was also mentioned in the ancient Spanish text where



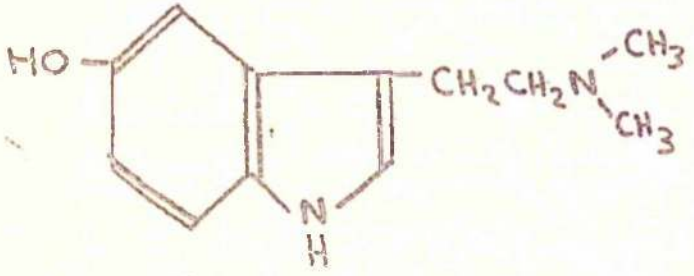
PSILOCYBIN



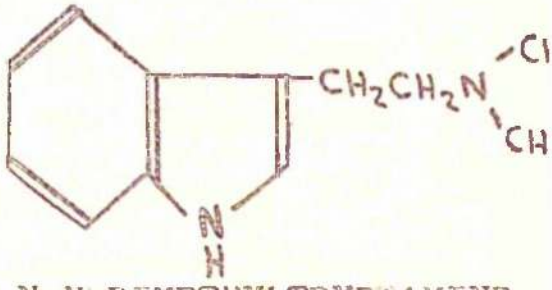
PSILOCIN



LYSERGIC ACID DIETHYLAMIDE



BUFOTENINE



N,N-DIMETHYLTRYPTAMINE

Fig. 2

Lewin first read of peyotl. On a second, successful visit, Wasson observed a native curandero (medicine man) consuming mushrooms in order to divine the outcome of a tribesman's illness.[28] Samples of this fungus were identified by Heim[29] as belonging to the Psilocybe species. Hofmann [30] then succeeded in isolating an active compound, psilocybin (Fig.2) from P. mexicana, Heim. In vivo, this compound is dephosphorylated into the corresponding phenol (Fig.2) known as psilocin, which is assumed to be the active drug.[31]

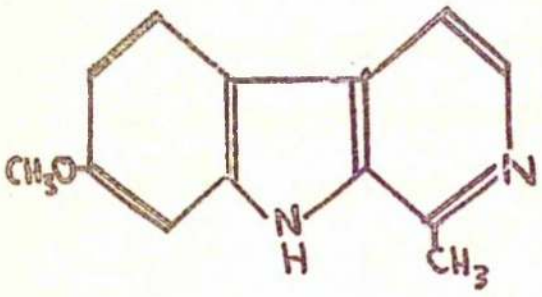
The isolation and identification of psilocybin by Hofmann is not without additional interest. Fifteen years earlier, in 1943, this same chemist had undergone a most remarkable experience in the course of an investigation of certain derivatives of lysergic acid, by then recognised as the common residue of the physiologically active ergot alkaloids used in obstetrics for the induction of parturition. In 1938, Hofmann and Stoll[32] synthesised the diethylamide of lysergic acid, at that time designated by the code name, LSD 25, which was later shortened to LSD (Fig.2). Although this compound proved almost as potent an oxytocic as the naturally occurring alkaloids, since it offered no distinct advantage it was neglected until the fateful afternoon in 1943 when Hofmann departed from his laboratory, feeling unwell while experimenting with LSD. On arrival at his home, he felt inebriated, then began to experience visual hallucinations which disappeared after a night's sleep.[33]

Hofmann later realised his experience could have been due to LSD. Knowing that satisfactory toxicity trials

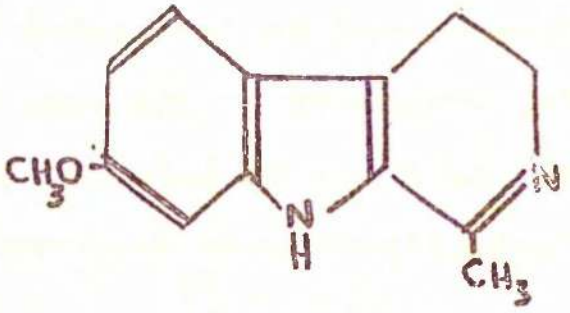
on the drug had been completed, he felt safe in deliberately swallowing 250 micrograms - a dose level much lower than the therapeutic dose of the common ergot alkaloids. He then underwent a very disturbing hallucinatory experience, the severity of which can be judged by the fact that later work showed the minimum effective dose to be as little as 25 micrograms.[34] Controlled clinical trials thereafter confirmed LSD as the most potent substance ever known to alter perception. Many lysergic acid amides were investigated for evidence of psychotropic properties, but only the 1 - acetyl derivative bore any comparison with the original drug.[35]

In 1954, another hallucinogen, bufotenine (Fig.2), was isolated by Stromberg [36] from the seeds of Piptadenia peregrina and P. macrocarpa which the natives of the West Indies and South America were known to grind into a snuff ("Cohoba") that was inhaled for its hallucinatory effect. Pachter [37] has also detected this compound in P. colubrina, a psychotomimetic Brazilian snuff. Previously bufotenine had been isolated by Phisalix and Bertrand [38,39] from the dried secretions of various toads, Bufo spp., this source being responsible for the name, and also by Wieland and his colleagues[40] who extracted it from certain toadstools belonging to the Amanita species which were reputed, in European folklore, to have mysterious attributes.

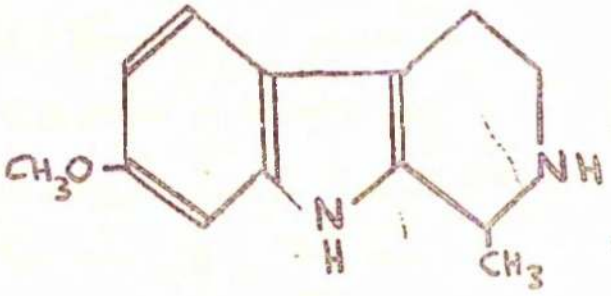
A further hallucinogen isolated [37, 41-44] from certain South American drinks was N, N-dimethyltryptamine (Fig.2). Here it is of interest that it has been



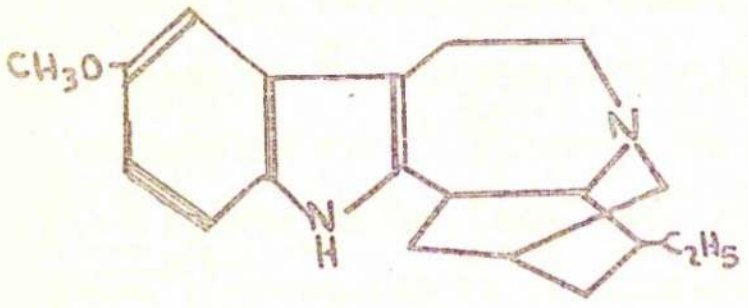
HARMINE



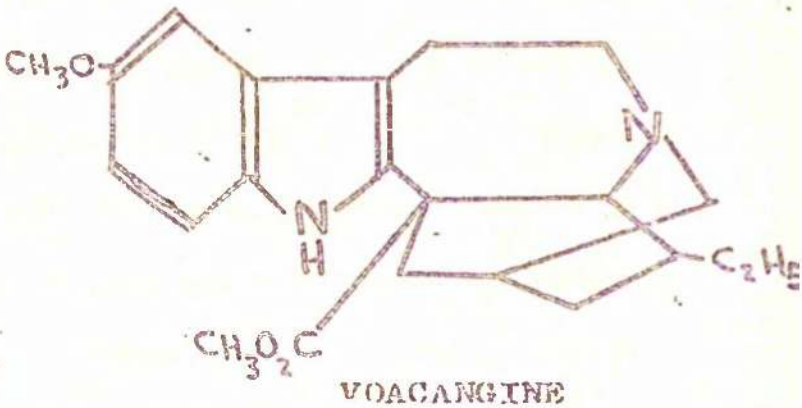
HARMALINE



TETRAHYDROHARMINE



IBOGAINE



VOACANGINE

Fig. 3

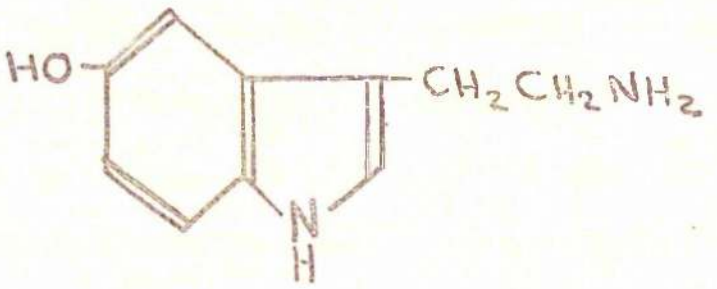
demonstrated that whereas the activity of the N, N-dimethyl and N, N-diethyltryptamines is similar to that of bufotenine, the 6-hydroxy derivative of N, N-diethyltryptamine is much more potent in initiating behavioural changes in animals. The intensity of the reaction to N, N-diethyltryptamine in man is found to parallel the quantity of 6-hydroxy - N, N-diethyltryptamine detected in the urine.[45]

Lewin[46] was also able to demonstrate that psychotomimetic activity was associated with harmine (Fig.3), an alkaloid isolated from Banisteriopsis caapi and other South American plants used in stimulating brews by the local Indians. However, pure harmine has been shown to exhibit a lower potency than the total crude extract, and it has been suggested[42] that harmaline (Fig. 3) and 1,2,3,4 - tetrahydroharmine (Fig. 3) contribute to the overall potency of the natural mixture. Recently an expedition[47] to the Upper River Negro, Amazonia, obtained samples of a liane used by the shaman (medicine man) of the Tukano and Tariana Indians. Thin layer chromatography demonstrated the presence of harmine, dihydroharmine, and tetrahydroharmine in these samples, while similar techniques showed[48] that bufotenine, dimethyltryptamine and their N-oxides occurred in epena, a snuff powder used by the Yanaoma Indians of the River Orinoco as a hallucinatory drug.

Natives of West Africa and the Congo are known to use an extract from the root of Tabernanthe iboga to increase their resistance to fatigue, many of them experiencing hallucinations after large doses.[49, 50] Animal experiments[51]

with one of the alkaloids present, ibogaine (Fig. 3), suggest that this compound could also be a psychotominetic agent. Voacangine, (Fig. 3), which can be hydrolysed and decarboxylated to yield ibogaine, is also found in T. iboga.[52] It does not appear to have been biologically evaluated however.

A further hallucinogenic preparation mentioned in ancient Spanish texts is ololiuqui. This product which is obtained from Rivea corymbosa Choisy is still used in Southern Mexico. Hofmann [53,54] has recently isolated various lysergic acid amides from it, while lysergamide and related compounds have been isolated [55] from the seeds of R. corymbosa ("morning glory") purchased from a market gardener in England, which is of interest since there have been reports in the British press that devotees were consuming "morning glory" seeds for their stimulant properties. However, studies with a number of varieties of "morning glory" which are commercially available to horticulturalists have shown that the quantity of lysergic acid-type alkaloids present in the seeds varies widely and this may account for the conflicting reports which have arisen concerning the potency of preparations derived from them. Further explanation of these discrepancies may lie in the fact that Kinross-Wright [56] who used alcoholic and ethereal extracts of ololiuqui in an unsuccessful attempt to induce psychosis in eight male subjects, was unaware that the alkaloids were present as the hydrochlorides. Vining and Taber,[57] who reported success, on the other hand, treated their samples with 10% ammonium hydroxide prior to ethereal extraction, while Taber and Heacock [58] utilised alkaline ether and so were working with the free bases which presumably



5-HYDROXYTRYPTAMINE
(SEROTONIN)

Fig. 4

would exhibit different distribution properties from the highly ionised salts.

Desmodium pulchellum, Benth ex Baker, a plant which grows throughout India has been used for many years in the Ayurvedic system of medicine to treat haemorrhage, poisoning, eye diseases and biliousness. Ghosal and Mukherjee [59,60] have recently isolated from it bufotenine, N, N-dimethyltryptamine and its N-oxide, 5-methoxy-N, N-dimethyltryptamine and its N-oxide, gramine, and 5-methoxy-N-methyltryptamine, but it is noteworthy that these authors offer no comment on any reputed psychogenic properties of this plant.

It becomes immediately apparent then, that consideration of the well-established hallucinogens reveals that, with the exceptions of the tetrahydrocannabinol series and the compounds related to mescaline (where there is evidence that biotransformation may be occurring [27]), all possess the common feature of being indole derivatives. Indeed, this fact has been of considerable significance in the consolidation of the hypothesis that hallucinogens act as antimetabolites of the natural indole derivative, 5-hydroxytryptamine or serotonin, as it is often called, (Fig.4), and this will now be discussed in some detail.

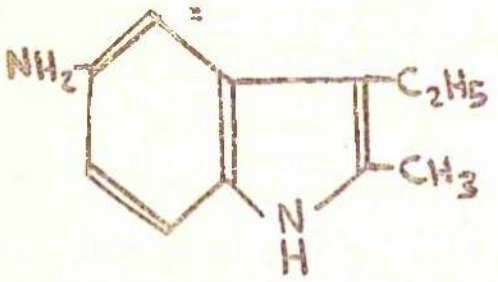
Serotonin and its Involvement in Mental Function.

The history of our knowledge concerning the biological properties and functions of serotonin is perhaps the most intriguing of any natural metabolite for, not only were its different peripheral roles discovered independently, but there has been a certain degree of conflict concerning

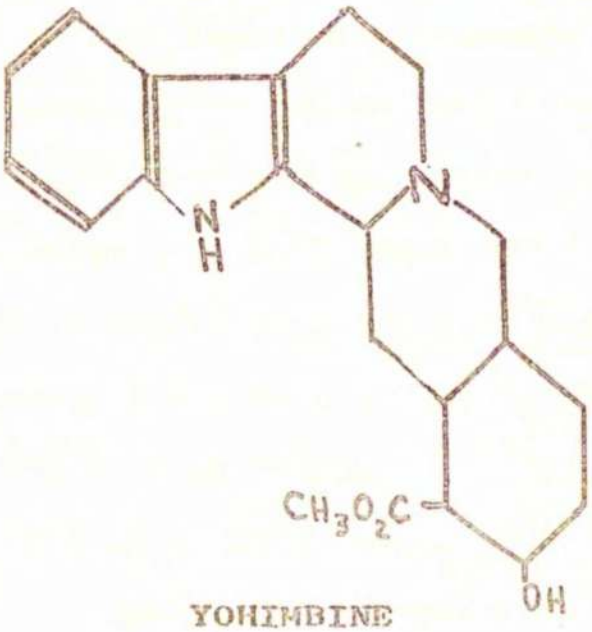
the priority of assignment of its significance in the central nervous system.

The compound was first isolated and fully characterised as a result of studies initiated by Rapport, Green, and Page, [61,62] who, in the knowledge that blood serum contained a powerful vasoconstrictor substance, set about the isolation of the pure principle from beef serum. Guided in this work by bioassays on the perfused excised rabbit ear, these workers were successful in isolating the active principle in crystalline form, and in 1949 Rapport [63] was able to identify it as the creatine sulphate of 3- (5-hydroxyindolyl) -ethylamine, i.e. 5-hydroxytryptamine. The synthesis of 5-hydroxytryptamine by Hamlin and Fischer [64] two years later then enabled Erspamer and Asero [65] to identify this compound with enteramine, the substance which they had shown was responsible for the characteristic staining of those gastrointestinal mucosal cells known as argentaffin cells because of their affinity for silver ions. In the course of his studies with enteramine Erspamer [66] had already shown that it would induce contraction in smooth muscle, and that it had distinctly different pharmacological properties from adrenaline.

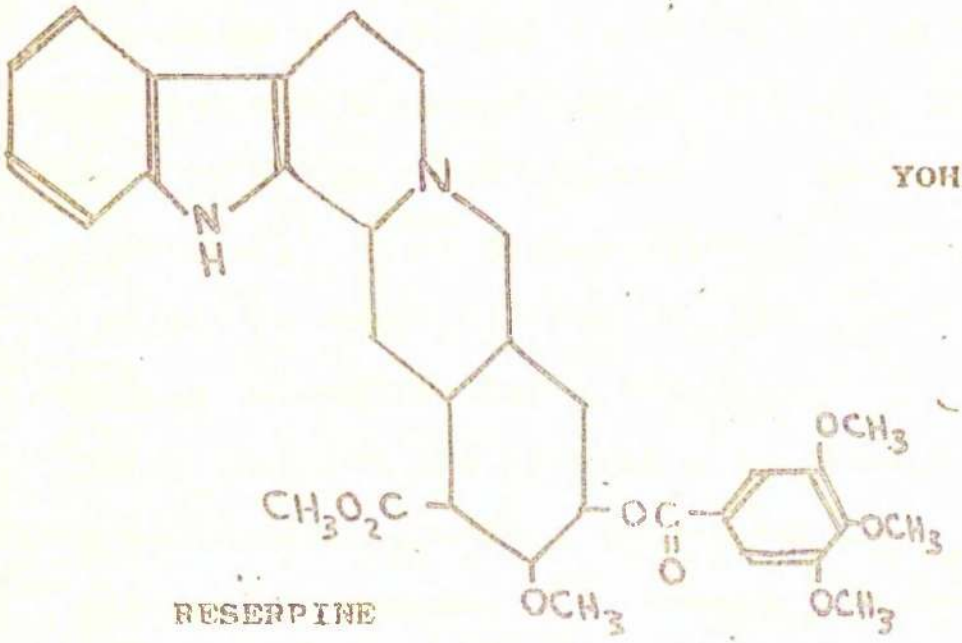
The occurrence of serotonin in the mammalian brain was conclusively demonstrated in 1953 by Twarog and Page [67] and by Gaddum and his coworkers [68] - the latter school then proceeding to map its distribution in nearly thirty anatomically discrete regions of the brain [69] - but the realisation of its probable significance in the central



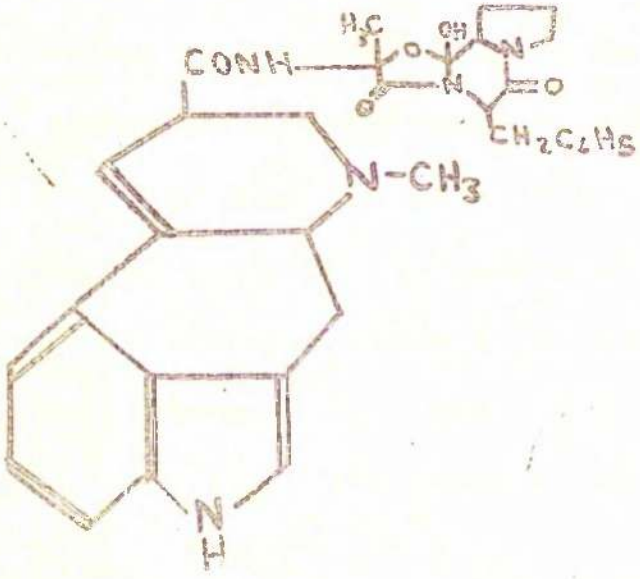
2-METHYL-3-ETHYL-5-AMINOINDOLE



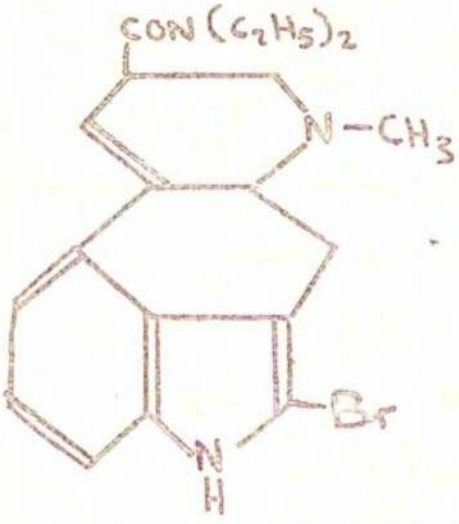
YOHIMBINE



RESERPINE



ERGOTAMINE



BOL

Fig. 5

nervous system depended heavily upon studies of antagonists to its peripheral actions by Woolley and Shaw in the United States and by Gaddum in Great Britain. This interest in antagonists of the peripheral actions of serotonin stemmed from the idea that the vasoconstrictor action of serotonin might be a dominant feature in essential hypertension, hence antimetabolites of this agent could conceivably be of value as therapeutic agents for the treatment of this condition.[70] The first such antimetabolite to be developed was 2-methyl-3-ethyl-5-aminoindole (Fig.5) which was synthesised by Woolley and Shaw[71] in 1952. Further studies[72,73] quickly indicated that yohimbine (Fig.5) and the ergot alkaloids possessed the ability to antagonise the peripheral actions of serotonin as did ergotamine (Fig.5) and lysergic acid diethylamide.[74,75] By far the most potent of these compounds was lysergic acid diethylamide[76,77] and the realisation[78] that the pronounced psychotomimetic properties of this drug would render it useless in the clinical treatment of essential hypertension together with the discovery that other less potent peripheral antimetabolites of serotonin undergoing clinical trial as hypotensive agents also temporarily induced mental changes similar to those characteristic of schizophrenia, inspired the ideas that these drugs were antagonising serotonin in the brain, and that schizophrenia was due in some way to changes in normal serotonin metabolism.[79,80]

Further evidence supporting a probable role for serotonin in influencing mental state was advanced when

Pletscher, Shore, and Brodie [81] were able to demonstrate that another indole derivative, the recently introduced tranquillising drug reserpine (Fig. 5), displaced serotonin from its bound form in certain animal tissues.

Originally it was considered that both schizophrenia and the schizoid symptoms induced by antimetabolites of serotonin resulted from the development of a serotonin deficiency in the brain, but later it became apparent, in the light of new experimental results, that these effects could in fact be due to an excess of serotonin (see below).

Many of the psychotomimetic drugs, as has already been pointed out, are indole derivatives bearing a certain chemical similarity to serotonin, and this has been taken as further support for the serotonin hypothesis about mental diseases. It should, perhaps, be emphasised, nevertheless that psilocybin, ibogaine, and bufotenine were in fact discovered to have psychotomimetic properties only after the enunciation of the hypothesis, whereas those of yohimbine, the ergot alkaloids, LSD, the harmala alkaloids, and two very active synthetic antimetabolites, viz. 2-methyl-3-ethyl-5-dimethylaminoindole (medmain) and 2-methyl-3-ethyl-5-nitroindole, were already known by 1954.

Objections to the Serotonin Hypothesis.

One of the earliest objections to the serotonin hypothesis as just outlined arose from the discovery by Cerletti and Rothlin [82,83] that although the drug 2-bromo-lysergic acid diethylamide (BOL) (Fig. 5) was a powerful peripheral antagonist of serotonin as evidenced by

experiments on the isolated rat uterus, it was completely devoid of psychogenic activity. Moreover it was shown by these workers that BOL was present in the brain thus apparently eliminating the explanation (valid in the case of 1-benzyl-2-methyl-5-methoxytryptamine (BAS) [84] that inability to pass through the hypothetical blood-brain barrier underlay the absence of central activity. BAS, which found limited therapeutic use as an antagonist of the peripheral actions of serotonin in hypertension,[85] is capable of producing marked behavioural changes only if injected directly into the lateral ventricles of the mouse brain.[86] However, the possibility that BOL does not reach the locus of hallucinogenic activity within the brain, despite having negotiated the blood-brain barrier, does not appear to have been commented upon.

The original experiments with LSD and serotonin showed this drug to act as a serotonin antagonist when tested on arterial slices [87] or the isolated rat uterus,[88] but later experimentation demonstrated that in certain other biological preparations LSD was capable of mimicking the action of serotonin. Thus, Marrazzi and Hart [89] found that both LSD and serotonin are capable of inhibiting transmission of electrical impulses from the optical pathway through the corpus callosum from one side of the brain to the other. Serotonin mimicry by LSD was also observed with tests on the clam heart,[90] canine blood pressure,[91] and contraction of liver flukes.[92] The use of a specific serotonin anti-metabolite such as BAS to inhibit these effects of LSD also confirms that LSD may act as a serotonin substitute. A

further convincing demonstration of pro-serotonin activity with LSD is afforded by experiments [93] which showed that it could mimic serotonin by causing isolated tissue cultures of oligodendroglia cells (found between the cerebral blood capillaries and neurons) to contract tetanically.

Whether LSD exerts a pro- or anti-serotonin effect may depend on the actual dose level. In tests on the clam heart only mimicry is observed, but large doses of LSD can protect dogs against subsequent injection of serotonin although small doses have a pro-serotonin effect.[91] Careful control of dosage can enable a degree of serotonin-like activity to be observed with LSD on the isolated rat uterus.[94]

Drug-Receptor Interaction.

To understand the apparent paradox as to why LSD should exert pro- or anti-serotonin effects on different tissues it is necessary to consider modern, molecular level drug-receptor theory.

Current concepts of drug action envisage the drug to interact with a receptor whose physical disposition is believed to be complementary to that of the drug. The drug-receptor complex which is the product of this interaction is then considered to be the initiator of the sequence of biochemical events that culminate in the observed response.

With certain exceptions (e.g. the phosphorus containing anticholinesterases), drugs do not normally form permanent covalent bonds with their receptors, as evidenced by the ease with which they can be removed from animal tissue by dialysis or solvent extraction. It thus appears that

complex formation is generally due to what Ariens and Van Rossum [95] contend is essentially an interaction of fields of forces originating in the drug molecule and the tissue. These forces are considered to involve electrostatic attractions, hydrophobic interactions, hydrogen bonds, and Van der Waals forces. The apposition of a drug to its receptor must be very close before such tenuous short-range forces can come into play, hence the molecular conformation of the drug when it successfully complexes with the receptor will need to be such that the spatial disposition of its atoms are complementary to those of the receptor. Regions of high electron density in the receptor will be opposed to those of low electron density in the drug, and projecting regions of one component will be aligned with recessed areas on the other. In many cases drugs are capable, by virtue of structural similarity, of competing with physiologically important substrates for their normal receptors thereby forming a new receptor complex, but either mimicry or antagonism of the action of the natural substrate can occur. Mimicry is considered to result from what Belleau [96] postulates is a unique, specific conformational perturbation in the receptor which leads to the biological response, whilst antagonism of the action of the natural metabolite is regarded as the result of an inert receptor complex which, although unable to produce a biological response, prevents the natural substrate from exerting its characteristic effect. Drugs acting in this latter manner are often termed antimetabolites. Evidence for the displacement of a natural metabolite may sometimes be gained

by demonstrating an increased concentration of the metabolite or its breakdown products in the urine. Partial antagonism may also occur and this is considered to be a direct result of the ability of the drug-receptor complex to produce both the specific conformational perturbation needed to Elicit the response concurrently with non-specific perturbations. The overall result will thus depend upon the statistical probability of one or the other event occurring. Both mimicry and antagonism can offer therapeutic possibilities, depending upon the system under consideration, and an insight into the biochemical pathways involved may sometimes be gained by quantitative studies.

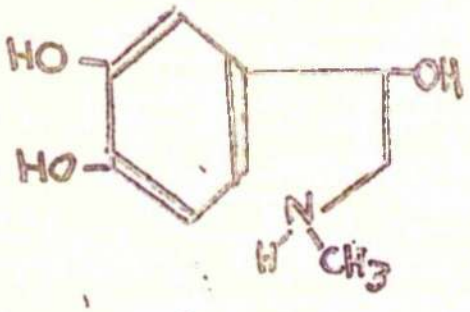
Thus, the serotonin antimetabolites which have been discussed can be considered as capable of forming drug receptor complexes with the serotonin receptor. In the case of those with only anti-serotonin activity the resultant drug-receptor complex will not involve the specific conformational perturbation which leads to the positive response. In some cases, however, as with LSD, for example, a pro-serotonin effect will result when specific conformational perturbations are induced in the receptor, and anti-serotonin activity will result when only non-specific conformational perturbations are possible due to variations between different receptors. Again, minor changes in the LSD molecule may permit only non-specific conformational perturbations at all serotonin receptors, or even prevent receptor combination altogether.

Modifications of the Serotonin Hypothesis.

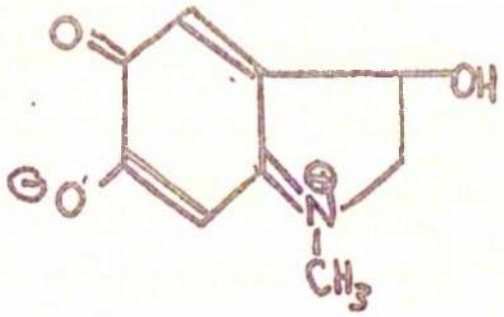
In answer to criticism from Cerletti and Rothlin,[82]

Woolley and Shaw[91] emphasized the ability of LSD to mimic serotonin in certain preparations, an attribute which was not known to be conferred on BOL. They claimed that LSD acted by exerting a pro-serotonin effect. This, of course, leaves the criticism that some effect on psychological function should occur since the normal role of serotonin, now seen as excitatory, is being thwarted by the true antimetabolite BOL. In reply to this, Woolley cites the recent finding of Page[97] that BOL produces mild depression in normal individuals. Further, Ginzel and Mayer-Gross[98] were able to confer protection against an LSD induced psychosis by pre-treatment with BOL for several days prior to LSD administration whereas if BOL were taken after LSD there was no effect on the LSD experience. The possibility of cross-tolerance between LSD and BOL should not be ruled out in this case (although not commented upon in the literature). However, it does seem fair to argue in terms of receptor theory that LSD has a greater affinity for the serotonin receptor than has BOL, thereby competitively occupying the receptor unless BOL has been given in advance whence it has the opportunity to form an inert, stable receptor complex which effectively removes the receptor from LSD's sphere of activity.

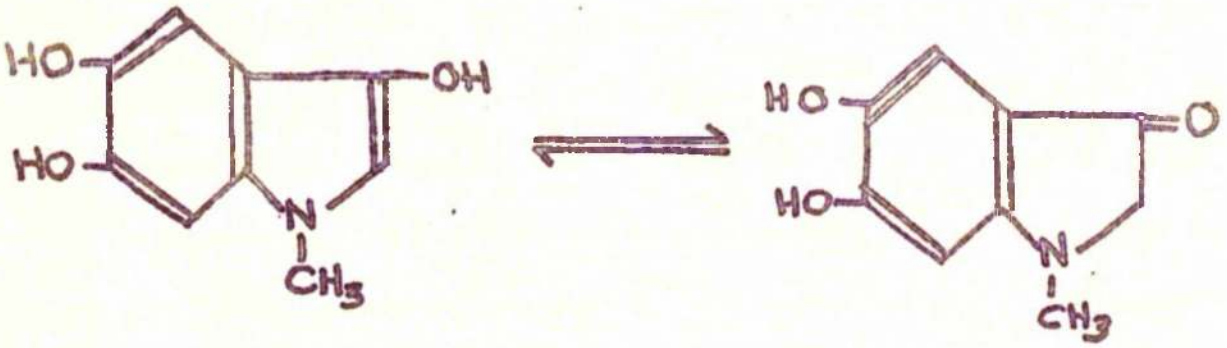
Although tissue culture experiments on oligodendroglia cells (which could conceivably be the site of serotonin's action <u>in vivo</u>) indicate a pro-serotonin action from LSD there is no conclusive evidence that LSD does indeed act in this manner in the mammalian brain. Suggestions have been advanced[99] to account for the normal pulsating



Adrenaline



Adrenochrome



Adrenolutin

Fig. 6

movements of oligodendroglia which LSD alters in pure culture. The brain is poorly vascularised compared to other organs, hence it may require some compensatory mechanism such as pumping pulsations from oligodendroglia to agitate the blood and extra-cellular fluid, thereby accelerating interchange of nutrient and waste products between the cells and blood. By upsetting the action of the oligodendroglia LSD could then cause accumulation of toxic waste products or deprivation of essential nutrient. Geiger [100,101] has studied the effect of serotonin and LSD on pure cultures of neurons and glial cells with time-lapse, phase-contrast microcinematography finding that LSD at concentrations of 0.0002 to 0.0010 micrograms per millilitre caused nuclear granules to be dispersed into the cytoplasm of the neuron within fifteen to twenty minutes, accompanied by cellular contraction and slowing of the terminal boutons on the cell body at the synapse. Nuclear movement increased, becoming irregular. The overall effects were similar to those after stimulation by metrazol or electric current. When LSD was washed out the observed effects were reversed in half-an-hour. Serotonin caused extrusion of cellular material after contraction, but of particular importance is the observation by Geiger that adrenaline, nor-adrenaline and adrenochrome have a similar effect. Adrenochrome, the most active substance in these experiments, has been shown by Hoffer, Osmond and Smythies [102] to arise from the degradation of adrenaline <u>in vivo</u> (Fig.6). It is an indole derivative which may be psychotomimetic, although not in the usual sense, elevated blood levels being found, by some workers, in schizophrenics.

The evidence that LSD and the hallucinogens which interfere with serotonin's role in the brain do so by acting as agonists or partial agonists (i.e. drugs capable of exerting an effect identical to the natural substrate, varying in degree according to ability to induce the conformational perturbations mentioned above) of serotonin is purely speculative.

If the hallucinogens are partial agonists of serotonin then raised levels of serotonin in the biophase should produce some sort of psychosis. To test this idea, Himwich and Costa [103] artificially boosted brain serotonin levels by administering the biogenetic precursor of serotonin, 5-hydroxytryptophan, to dogs pre-treated with amine-oxidase inhibitors which delay the breakdown of serotonin and, indeed, gross behavioural changes were observed.

It is pertinent at this stage to give detailed consideration to the true justification for equating the psychotogenic effects of hallucinogens with pro-serotonin activity and the action of tranquillising drugs with anti-serotonin action, or vice-versa. That tranquillisers may interfere with the normal metabolism of serotonin is not disputed, since, indeed, the discovery of the effect of reserpine on serotonin release remains one of the landmarks in the development of the serotonin hypothesis. Chlorpromazine, too, antagonises the action of serotonin, [104] but it is not a true antimetabolite of serotonin since it does not act at the same receptor. Szent-Györgyi [105] has suggested that, being a strong electron donor, chlorpromazine could FORM CHARGE TRANSFER COMPLEXES WHICH WOULD INTERFERE WITH THE

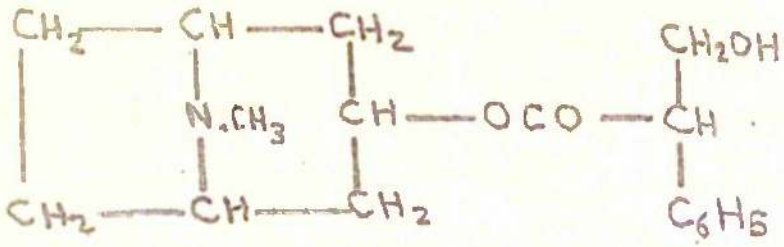
transport of metallic ions through cellular membranes, thus disturbing the normal sequence of electrical events. Yet to believe that a conceptual switching on and off of a biological system - that involving serotonin - by means of drugs produces simply excitement or depression would seem somewhat naive. The brain is an extremely complex organ and blockage of a nervous impulse by means of a given biochemical mechanism in one cranial region could produce an entirely different effect to blockage by an identical mechanism elsewhere. Indeed, the situation pertinent to the action of drugs on the central nervous system has been succintly summarised by Domino, Fox, and Brady [106] who wrote as follows, "The usual definition of a 'stimulant' is an agent that increases a functional activity, and that of a 'depressant' is an agent that decreases functional activity. In the complex organisation of the central nervous system it is difficult to determine whether a compound is a true 'stimulant' or 'depressant' on the basis of gross observations in the intact animal. A drug may exert its effects at a variety of central sites. It may act at the neuronal level either to excite or depress; it may act at the synapse or other portions of the neuronal arc, or it may influence facilitatory or inhibitory neurons which feed into the common nervous pathway. Thus a pharmacological agent that stimulates inhibitory neurons might be an overt depressant, whereas an agent that selectively depresses inhibitory neurons may cause an overt excitatory response." The distinction between the layman who considers alcohol to be a stimulant and the pharmacologist who calls it a depressant aptly illustrates

this point. Further, in the case of serotonin, Amin, Crawford and Gaddum [69] have established that wide variations in serotonin levels exist throughout the brain. So serotonin antagonists distributed to one region could easily exert opposite effects to those shown by antagonists in another region since anti-metabolite activity will always depend upon the amount of natural substrate present. The anatomical distribution in the brain therefore will introduce a variable which must be taken into account for all centrally-acting drugs. Not only do serotonin levels vary but so also do those of other possible neurohormones including nor-adrenaline acetylcholine, and gamma-aminobutyric acid.

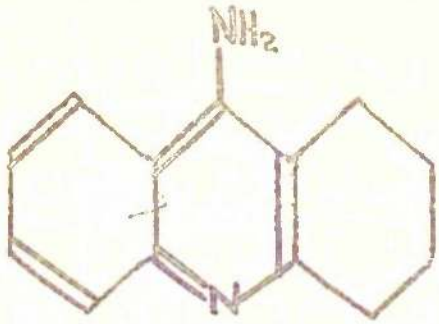
The major drawback to general acceptance of the serotonin hypothesis is the uncertainty surrounding the precise normal physiological role of this compound. The evidence presented above certainly suggests that interference with the action of serotonin can lead to mental disturbance, but to attempt to adduce from this the exact part which serotonin plays in brain function is fraught with hazard in view of the fact that many of the compounds concerned also interfere with the action of other hormones and enzymes.

The Effect of Psychopharmacological Agents on Acetylcholine Metabolism.

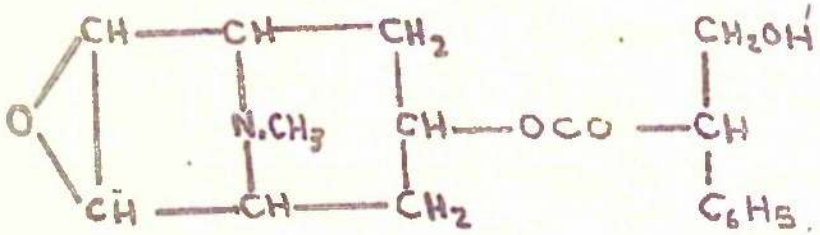
Goldberger, [107] using histochemical techniques, found that LSD inhibited true cholinesterase in rat brain sections, although it had no effect on pseudo-cholinesterase. These findings were, however, in sharp contrast to those of Thompson, Tickner, and Webster [108,109] who found significant



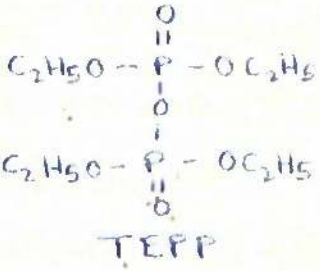
Atropine



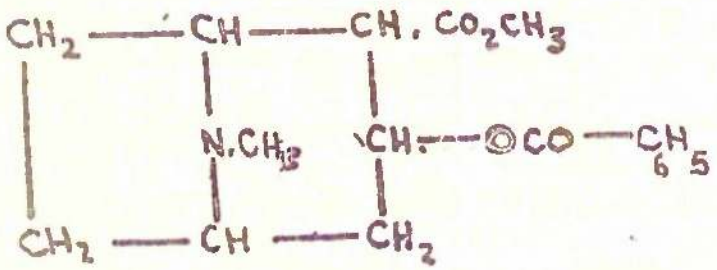
1,2,3,4-Tetrahydro-aminacrine



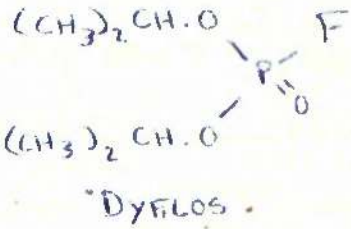
Hyoscine



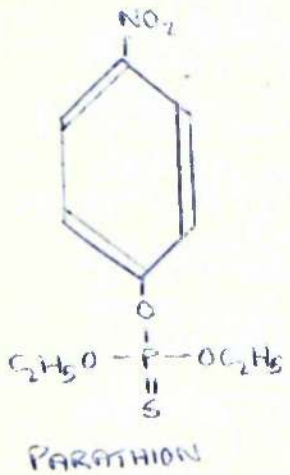
TEPP



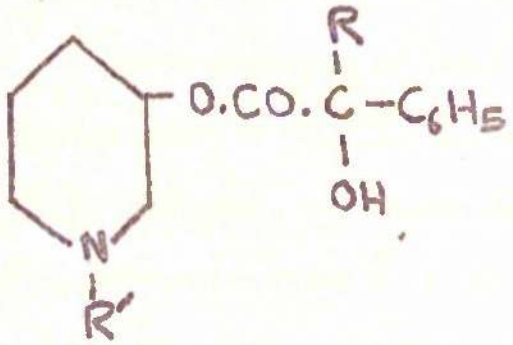
Cocaine



"Dyfos"



PARATHION



Piperidyl Glycollate

Fig. 7

inhibition of human plasma cholinesterase, but not true cholinesterase. Other workers [110] found small doses of LSD actually potentiated cholinesterase activity in rat brain, while Zsigmond, Foldes, and Foldes [111,112] claimed that plasma cholinesterase was inhibited more than true cholinesterase from grey matter, although small doses of LSD did not accelerate hydrolysis of acetylcholine. Zehnder and Cerletti [113] have observed that BOL is a less powerful esterase inhibitor than LSD.

These findings help to account for the increased acetylcholine levels in the brain found after LSD administration by Poloni and Maffezzoni.[114] Rothlin [115] has observed that LSD produces parasympathetic effects including salivation, lacrimation, nausea, retching, and vomiting.

The knowledge of the role of acetylcholine in the autonomic nervous system has naturally led to the suggestion that it could also be a neurohormone in the central nervous system.[116,117] The distribution of bound acetylcholine in the brain is very similar to that of serotonin, adrenaline, and nor-adrenaline, greatest amounts being detected in the caudate nucleus, amygdaloid hippocampus, and hypothalamus.[118]

The ability of Datura stramonium and Atropa belladonna to cause behavioural disturbances has been known since mediaeval times. The principal active constituents of these plants are the anti-cholinergic compounds (-) hyoscyamine, its racemic form atropine, and (-) hyoscine (Fig.7). Large doses of atropine (5 to 8 mg. orally) bring about changes in mood, depersonalisation, and hallucinations.[119,120] The chemically related alkaloid, cocaine, (Fig.7), will also

produce hallucinations [121] in doses greatly in excess of those required to elicit the euphoric effect well known to the Indians of Peru and Bolivia who chew the leaves of Erythoxylum coca, and which is responsible for addiction to cocaine.

Abood [122] has tested an extensive series of anti-cholinergic compounds, based on the atropine nucleus, which exhibit enhanced psychotomimetic activity. These are the piperidyl glycollates (Fig.7) in which the tropic acid moiety of atropine has been replaced by substituted glycollic acids, the position of attachment being meta instead of para to the heterocyclic ring. Oral doses of 5 mg. can produce effects reminiscent of schizophrenia, the effects lasting up to 14 hours.[119]

Structure-action studies [119] have indicated that there is little correlation in this series between toxicity, mydriasis, and antispasmodic activity, although only compounds with pronounced anticholinergic activity are centrally active.

The central effects of the piperidyl glycollates are reversed by the action of 1,2,3,4-tetrahydroaminacrine, a cholinesterase inhibitor which allows acetylcholine to accumulate, thereby enabling it to overcome the antagonism of the piperidyl glycollates. This tends to confirm that these drugs act by interfering with acetylcholine. The potent, toxic anticholinesterase phosphorus compounds parathion, tetraethyl pyrophosphate (TEPP) [124,125] and dyflos [126-128] (Fig.7) also result in the manifestation of behavioural disorders. The high levels of acetylcholine,

resulting from the inhibition of its hydrolysis by acetylcholinesterase, could result in a prolonged depolarisation of the dendritic membrane, thereby rendering it indifferent towards subsequent electrical impulses. The net result of both anticholinergic compounds and anticholinesterase drugs will, therefore, be similar, as indeed is found experimentally.

Direct intraventicular injection of acetylcholine in cats produces agitation and catatonia.[129] This may seem surprising in view of the rapid rate of hydrolysis by acetylcholinesterase which normally occurs elsewhere in the body, but would support the above explanation.

Biel, Abood and colleagues [130] have suggested that acetylcholine and nor-adrenaline are inter-related in the lower brain in the same way as they are in the peripheral nervous system, and that acetylcholine or a similar substance may be the chemical mediator of central depressant functions. Thus a case can be presented for the psychotomimetic activity of LSD being considered a function of its ability to inhibit cholinesterase, thereby producing acetylcholine accumulation and subsequent failure of depolarisation with blockage of synaptic transmission, leading to dominance of central excitatory function. Further work with other hallucinogens is necessary before any valid conclusions can be drawn, although psilocybin has been shown to inhibit cholinesterase.[131]

Sympathetic Effects of LSD.

Besides producing parasympathetic effects, LSD can elicit sympathetic manifestations such as mydriasis, piloerection, increase in blood sugar levels, and hyperthermia,

responses which, in some animals, are reversed by administration of ganglion blocking agents or adrenergic blocking agents. The suggestion, made by Hoagland, Rinkel, and Hyde,[132] in 1955, that LSD might owe its psychotomimetic properties to an ability to interfere with adrenaline metabolism is certainly supported by the knowledge[133] that after ingestion of this drug adrenaline blood levels rise and then fall before eventually returning to normal. Hoffer[134] states that the adrenal gland is activated by LSD, both the medulla and cortex being involved as measured by increased production of adrenaline and 17-hydroxycorticosteroids.[135,136]

Adrenochrome Involvement in Drug Action.

Connected with the role of LSD on adrenaline metabolism is the issue of its effect on adrenochrome, (Fig.6) a compound first characterised by Green and Richter[137] who demonstrated that it was responsible for the red colour imparted to solutions of adrenaline that had been allowed to oxidise. Thus when Hoffer, Osmond, and Smythies[138] were attempting to integrate the role of adrenaline with the apparent importance of the indole ring in psychotomimetic activity, this oxidation product seemed to be the link they were seeking. Indeed they made the claim that parenteral administration of adrenochrome evoked psychotic manifestations, but unfortunately, some other workers[139-142] have had difficulty in reproducing these results. Later Smythies[143] conceded that adrenochrome may not be a hallucinogen in the usual sense. Nevertheless, Hoffer[144] remains convinced of the psychotogenic properties of adrenochrome. He claims

that after injection of LSD adrenochrome levels in the blood rise,[145] although Szara [140] has been unable to detect adrenochrome in the blood at all. Difficulties in obtaining pure, stable samples of adrenochrome [146] have now been overcome and it will be interesting to follow further developments in this field.

The possible <u>in vivo</u> conversion of adrenaline to adrenochrome is one example of what Osmond and Smythies [147] postulate to be a biochemical equivalence of phenylethylamines and indoles, the former being readily oxidised to the latter. These authors have also suggested that the hallucinogenic drug mescaline might be converted to an indole which was the active entity. O-methylation of adrenaline (or related compounds) has also been considered as a possible route leading to the production of an endogenous psychotomimetic agent involved in the aetiology of mental disease [148] and this in turn has given rise to the proposal [143] that mescaline could produce its effect by acting as an antimetabolite of adrenaline. In this connection it is noteworthy that mescaline has long been known to antagonise the pressor and vagal effects of adrenaline, causing a fall in canine blood pressure.[149]

Melander and Martens [150] have found that LSD potentiates the action of adrenochrome and adrenolutin (a further oxidation product) in man and lower animals, whereas compounds capable of interfering with the adrenochrome psychosis, such as nicotinic acid in large doses [151] have been found by other workers [152] to inhibit the LSD

experience. Sanker [153] and his colleagues have recently proved that LSD inhibits the conversion of nor-adrenaline into nor-metadrenaline (nor-metanephrine). This conversion which is catalysed by catechol O-methyl transferase, represents the first stage in the main pathway for the metabolism of circulating nor-adrenaline.[154,155] If LSD acts by inhibiting catechol O-methyl transferase, not only will nor-adrenaline metabolism be altered, but so also will that of other catecholamines [156] including adrenaline which is formed from nor-adrenaline by the action of phenyle-thanolamine N-methyl transferase.[157] Consideration must therefore be given to the possibility that LSD and mescaline, which Heath and Leach [158] claim increase the conversion of adrenaline into adrenolution in plasma, acts by blocking the normal metabolic pathway for adrenaline (or any other catecholamine) thereby allowing the adrenochrome pathway to develop along with other less harmful metabolic routes. This could also explain the protective action of ceruloplasmin against LSD in animals.

Ceruloplasmin.

Ceruloplasmin, a blue copper containing protein found in blood plasma, is an enzyme which oxidises the benzenoid ring of serotonin, various amino-phenols, and phenylene diamines. It appears to be identical with the soluble amine-oxidase of blood.[159] After the discovery by Akerfeldt [160] that the oxidase activity of ceruloplasmin was raised in the blood of schizophrenics, controversy developed over the issue of whether or not this could be the basis of

a diagnostic test.[161-163] It appears that the oxidase activity was in some way dependent upon the dietary intake of ascorbic acid, and Saunders and Chipkiewicz[161] took cognisance of this in their controlled investigation which confirmed Akerfeldt's original work. Woolley[164] has pointed out that these studies on ceruloplasmin measured the oxidase activity or copper content of samples to indicate the amount of ceruloplasmin present - indirect measurements which could conceal the presence of some undetermined factor.

Winter and Flataker[165] produced symptoms akin to those of LSD after administering plasma from schizophrenics to trained rats. This supports the contention[166] that schizophrenia is due to abnormal ceruloplasmin activity or the presence of another abnormal globulin, designated "taraxein". Globulin from healthy subjects failed to produce the abnormal behavioural and electroencephalogram changes in monkeys and human volunteers which "taraxein" plasma produced.[167,168] The implications of these findings for blood transfusion in clinical practice do not appear to have been discussed.

Significantly, the major substrate of ceruloplasmin, according to Woolley,[164] is serotonin. Raised blood levels of the globulin, he believes, could result in a localised deficiency of serotonin which would lead an effect similar to that of those antimetabolites which possessed no serotonin - like activity, namely depression. There does not appear to be any evidence that whole body serotonin levels in schizophrenics are altered, but it must be remembered that only 5% of the serotonin in the human body is found in the brain, hence assessment of brain levels is difficult in human

subjects. The drawback to this suggestion of Woolley's lies in his conclusion that depression will occur as a result of raised ceruloplasmin levels. Schizophrenics might not seem to be depressed patients, but Woolley has strongly advocated Strecker's [169] work supporting the view that the mentally ill introvert will elaborate the syndrome of schizophrenia, whereas the extrovert will exhibit a manic depressive psychosis. Woolley asks whether the manic phase is symptomatic of excess serotonin and the depressed phase indicative of a serotonin deficiency?

The protective action of ceruloplasmin against LSD must also be explained. Melander and Martens [150] claim that adrenolutin is irreversibly bound to ceruloplasmin thereby rendering it unable to induce psychosis. If Hoffer's view that LSD acts by raising adrenochrome - hence adrenolutin-levels is accepted, the protective role of ceruloplasmin is understandable. Indeed, this role has been advanced as evidence for the involvement of adrenolution in LSD's mode of action.

The Indirect Action Hypothesis.

Several of the above findings have prompted Hoffer[134] to advance an "indirect action hypothesis" to explain the high potency of LSD as a hallucinogenic agent. Since many of the properties of LSD mentioned above are exhibited by other drugs (e.g. anticholinesterase action, inhibition of catechol O-methyl transferase, adrenergic activity, etc.), it would appear that the combination of all these properties in a single compound results in a drug with unique biological

effects. Hoffer's hypothesis involves the entire autonomic nervous system. By inhibiting cholinesterase, LSD will increase acetylcholine levels thereby producing parasympathetic domination which causes the adrenal medulla, and storage sites in the brain, to secrete nor-adrenaline and adrenaline. At the same time as adrenaline and noradrenaline levels are artificially boosted, LSD is inhibiting catechol O-methyl transferase thereby denying access of these compounds to their normal metabolic pathways, resulting in augmented levels of adrenochrome and adrenolutin. The resultant combination of high acetylcholine, high adrenaline, and raised adrenochrome levels, Hoffer believes, could account for the characteristic effects of LSD medication.

The indirect action hypothesis is primarily concerned with explaining the unique effects of LSD, but it enables the action of other hallucinogens to be understood insofar as they can be seen as lacking the high potency of LSD while possessing psychotropic activity through the ability to elicit some of the biochemical effects of LSD.

Hoffer's hypothesis is a development of Nandy and Bourne's[170] suggestion that LSD blocks synaptic transmission by enzyme inhibition, while simultaneously raising levels of acetylcholine, adrenaline, and adrenochrome in the blood. These authors also believe that by inhibiting true cholinesterase and monoamine oxidase of the spinal ganglia and dorsal horn, LSD would facilitate transmission of spinal impulses thereby disrupting the delicate balance of cortical function by giving undue prominence to these

afferent signals.

Hoffer has not assigned any role to serotonin in his indirect action hypothesis. In view of the interplay of endogenous amines discussed by Daly and Witkop in a recent review article,[171] this might appear to be an unfortunate omission, particularly because of the occurrence of serotonin together with its biosynthetic and metabolic accoutrement in brain tissue. Serotonin is antagonised by nor-adrenaline, and histamine[172] while it may itself antagonise or be synergistic with nor-adrenaline.[173,174] Further, histamine (traces of which are found in certain regions of the brain) releases catecholamines[175] while itself being released by adrenaline[176] and serotonin.[177]

In passing, it should be noted that the indirect action hypothesis raises wider issues for the medicinal chemist. The "Theory of Biological Relativity", first expounded by Martin,[178] sets out to demonstrate that no one drug is ever completely specific in its biological action (side effects or untoward effects always being present to a greater or lesser degree). Nor is any one compound ever found to be the sole agent capable of producing a particular biological response. Martin has couched his theory in language designed to draw a parallel with the theory of physical relativity - the main tenet being that, "There are no one hundred per cent specificeties in biological systems." The indirect action hypothesis clearly indicates one direction in which the medicinal chemist can advance if he wishes to prepare useful therapeutic agents. Taking advantage of the inherent lack of specificity of drug action

he can seek agents with multiple biochemical effects each of which alone is unable to produce the desired biological response, but integration of which will produce a novel effect. Other drugs besides LSD owe their characteristic effects to such factors. Daly and Witkop [171] have summarised the means by which drugs can interact with endogenous amines and the enzymes that synthesise, inactivate, and degrade them. A given drug may block the active transport of an amine or its precursor, inhibit a crucial enzyme, or compete for storage sites or receptors. Drugs generally act in all three ways to different degrees.

The Role of Serotonin in the Central Nervous System.

Studies on the mode of action of psychotropic drugs led Brodie and Shore [179] to suggest, in 1957, that serotonin was the chemical transmitter at synapses in a hypothetical central parasympathetic (tropotrophic) nervous system, while nor-adrenaline acted as a sympathetic (ergotropic) mediator. The signs of sympathetic dominance after administration of LSD convinced Brodie and Shore that this compound antagonised the central parasympathetic transmitter, serotonin, thereby allowing sympathetic control to dominate. The outcome of this sympathetic dominance was excitation, whereas the parasympathetic dominance due to the action of reserpine resulted in tranquillisation. The stimulation consequent upon the administration of monoamine oxidase inhibitors was believed to be a result of the accumulation of serotonin which would inhibit normal parasympathetic transmission.

That large doses of serotonin could block its normal action had been clearly demonstrated by Gaddum [180] who rendered the isolated rat uterus insensitive to serotonin with doses a hundredfold greater than normal, and also by Shaw and Woolley [181] who showed that ten times the normal dose of serotonin failed to raise canine blood pressure besides preventing any rise after a subsequent small dose. The inhibitory effects dissipated fairly quickly, presumably because of enzymatic destruction of the excess.

Monoamine oxidases are mitochondrial enzymes [182] that can oxidatively deaminate intracellular catecholamines and serotonin to the corresponding aldehydes which are immediately converted to acids or alcohols depending on whether aldehyde oxidase or alcohol dehydrogenase supervenes.[183] Under normal circumstances the main pathway for serotonin metabolism is oxidative deamination catalysed by monoamine oxidase, but after administration of monoamine oxidase inhibitors injected serotonin is metabolised at a rapid rate by an unknown alternative route.[184] The major metabolic pathway for circulating catecholamines, on the other hand, is via catechol O-methyl transferase although oxidative deamination through monoamine oxidase plays an important minor role.[183] Catechol O-methyl transferase cannot O-methylate monophenols such as serotonin.[185]

Crout, Creveling, and Udenfriend [186] found that monoamine oxidase activity was greater than that of catechol O-methyl transferase in the rat brain. Further, it had previously been shown that antidepressant drugs such as iproniazid and various substituted hydrazines which inhibit

monoamine oxidase raise the levels of nor-adrenaline in brain and other tissues,[187,188] although they fail to prolong the response to injected catecholamines.[189] Belleau,[190] using radiotracer techniques, demonstrated that monoamine oxidase played no part in the inactivation of nor-adrenaline at the effector cell level.

After the administration of monoamine oxidase inhibitors a marked increase in the excretion of meta-drenaline occurrs, indicating that metabolism via catechol O-methyl transferase is being augmented,[191] but no alteration in the rate of disappearance of catecholamines is observed.[192]

Axelrod [157] has collated a considerable amount of information on catecholamine metabolism which has led him to postulate that when catecholamines are released from their protected bound form within the cell they are exposed to the action of monoamine oxidase, some catecholamine being released into the extra-cellular fluid while the rest is metabolised within the nerve ending before leaving it as an inactive deaminated metabolite. The catecholamine which is released unchanged from the cell is exposed to the action of catechol O-methyl transferase. Should monoamine oxidase be inhibited the released catecholamine will accumulate within the nerve cell, gradually escaping to be metabolised extra-cellulary. Thus, the increased tissue levels of catecholamines detected by analysis,[187,188] represent catecholamines which do not necessarily exert any physio-logical effects unless they are released from the cell.

Injected catecholamines are not normally exposed to significant amounts of monoamine oxidase, hence it is not surprising that inhibitors of this enzyme have no effect on the response to them. Although serotonin is also stored intracellulary in a bound form it does not yet appear to be known whether or not monoamine oxidase inhibitors affect its metabolism in an analogous manner to that of the catecholamines. Until this information is forthcoming it is difficult to accept unreservedly Brodie and Shore's views on the significance of monoamine oxidase inhibitors in the light of their hypothesis concerning the role of serotonin in the central nervous system. Further, the effect of monoamine oxidase inhibitors on catecholamines complicates the issue of the action of such drugs on the metabolism of serotonin.

Brodie and Shore [179] suggested that the tranquillisation induced by reserpine was due to its ability to release serotonin from an inert bound form at such a rate that persistent stimulation of the central parasympathetic system (producing an overall depression) took place before monoamine oxidase was able to destroy the neurohormone. This certainly would not be inconsistent with the experimental observations of Pletscher, Shore, and Brodie [193,194] who clearly demonstrated that reserpine could drastically reduce tissue levels of serotonin, that of the brain falling to as little as 10 per cent of its original value, while greatly increased urinary levels of 5-hydroxyindoleacetic acid were detected. Further, it was found that other alkaloids of Rauwolfia serpentina which failed to displace serotonin from tissues also failed to act as tranquillisers.

Woolley [164] has criticised these early papers of Brodie and his colleagues in which they considered the action of reserpine to be due to the displaced serotonin rather than to reserpine itself, and maintains that the reserpine is acting as a true antimetabolite of serotonin by competing with it for the target receptors at which initiation of the biological response takes place. Thus the two schools take diametrically opposed views in that Brodie and Shore assign an overall depressant action to serotonin, whilst Woolley contends that serotonin has an overall excitatory role in the brain. The fact that monoamine oxidase inhibitors which are known to produce an increase in serotonin levels in the brain are also excitatory would seem to fit in more simply with the Woolley hypothesis than with that of Brodie and Shore although these latter workers have suggested that in high concentration serotonin shows a reversal of its normal action, in order to account for this.

However, it must be borne in mind, that since reserpine is also known to displace nor-adrenaline from the brain and from nerve endings, [195] it could well be that the tranquillising action depends upon an effect on nor-adrenaline- not serotonin.

A major criticism [196] of Brodie and Shore's hypothesis has been that it presupposed the existence of a very high central sympathetic tone (uncovered by the action of antiserotonin drugs) balanced by an equally powerful parasympathetic tone in which serotonin plays an activating role. In reply to this criticism Brodie [197] has suggested that as LSD is a phenylethylamine derivative it might

conceivably stimulate adrenergic receptors at the same time as it inhibits central parasympathetic transmission. Certainly the ergot alkaloids and yohimbine, which are closely related chemically to LSD, have pronounced action at adrenergic receptors.[198,199]

A more satisfactory hypothesis is that put forward by Hoffer[134] which explains the sympathetic effects of LSD in a satisfactory manner. Also, by making allowance for the ability of reserpine to release bound nor-adrenaline as well as serotonin, its effects are seen as due to interference with both the central sympathetic and parasympathetic systems. The assignment of an excitatory role to 5-hydroxytryptophan, however, remains a dubious factor since it is devoid of sympathetic properties.

Although Woolley[164] believes that the role of serotonin in the brain is excitatory he has not assigned to it a role as chemotransmitter in any specific portion of the central nervous system. However, this has been done by Olds[200] who claims that sympathin (a mixture of adrenaline and nor-adrenaline) is the excitatory transmitter in both the central sympathetic and parasympathetic systems, whereas serotonin is the inhibitory transmitter in both. On the other hand, Marrazzi[201-206] has suggested that nor-adrenaline and serotonin are both inhibitory transmitters, LSD, mescaline, and adrenochrome being psychotomimetic by virtue of an ability to inhibit central synaptic transmission. Marrazzi believes that the central excitatory transmitter is acetylcholine or a related compound.

Histamine in the Central Nervous System.

Bunag and Walaszek [207] have recently suggested that the inhibition of serotonin-induced arterial pressure changes by lysergic acid derivatives is due to their ability to prevent histamine release. Sankar [153] has also shown that LSD reduces blood histamine levels by 26% in the rabbit. Histamine is found in the basal areas of the brain, particularly in the hypothalamus,[208,209] but its role is as yet unresolved. Histamine is stored intracellularly in a bound form, and it may be of some significance that morphine and pethidine are capable of producing its release.[210]

Injection of histamine into the septal region of the brain causes behavioural changes in animals.[211] Electroencephalogram changes arising from this region after histamine injection into the third ventricle are abolished by septal lesion.[212]

Marrazzi [213] demonstrated that histamine was as potent a synaptic inhibitor as serotonin, while tripelennamine (an anti-histamine drug) overcame this inhibition. The sedative properties of antihistaminic drugs are widely recognised, and indeed constitute one of the main side-effects of this group of drugs. Moreover, chlorpromazine besides being a major tranquilliser is one of the most potent anti-histaminics known. Trendelenburg [214] has shown that histamine has a direct action upon central ganglia. It has also been claimed [215] that histamine infusion moderates the effects of LSD in humans.

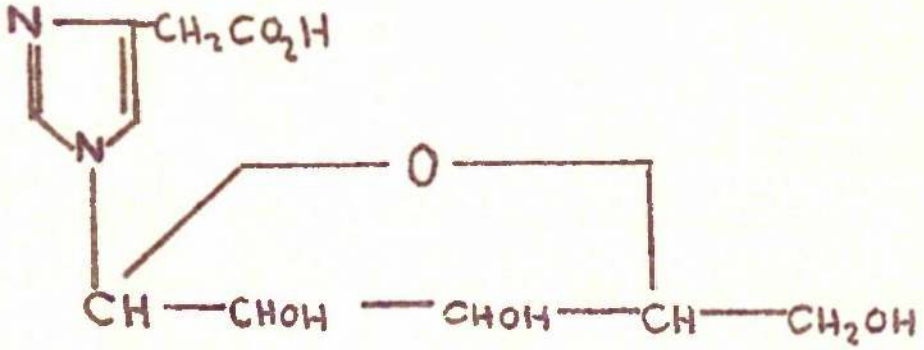
However, as long as the investigations into the role

of histamine in the brain remain incomplete, speculation on the possibility that certain psychotropic drugs owe their effects to an ability to interfere with histamine are probably premature. It should be noted though that if histamine is at all involved, the role of the hypothalamus in brain function must be emphasised since this organ could then be the primary site of action of these drugs since it is the major source of brain histamine. Indeed Brodie and Shore [179] considered this to be the site of action of LSD and reserpine.

It is known, however, that the hypothalamus is responsible for the integration of the autonomic peripheral nervous system by sending efferent projection fibres downward to the bulbar mechanisms of respiration, heartbeat, and glandular regulation, and even further downward to the preganglionic spinal neurons, thereby regulating local autonomic functions.[216] Fibres run from the hypothalamus to the pituitary gland which mediates the body's hormonal balance. The hypothalamus is itself controlled by the cerebral cortex from which it receives descending fibres. The organ is also concerned with emotional response. (See below).

Thus, drugs acting on the hypothalamus would be expected to produce profound effects. If this organ were capable of controlling the hypothetical central autonomic nervous system as well as the peripheral one, the effects of psychotropic drugs could indeed be understood in terms of hypothalamic activity, but until supporting evidence for this theory, which is not without attraction, is forthcoming, it must be relegated to the realms of speculation.

Feldberg and Myers [217,218] have recently proposed



Imidazole-4-acetic Acid Riboside

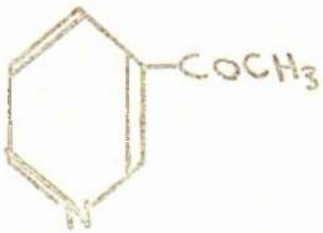
Fig 8.

that serotonin and adrenaline or nor-adrenaline are involved in the hypothalamus in the regulation of body temperature through the medium of autonomic control. Excess of serotonin is believed to induce hyperthermia, whereas excess of catecholamine is thought to lower body temperature. It is to be noted in this connection that chlorpromazine can also lower body temperature.

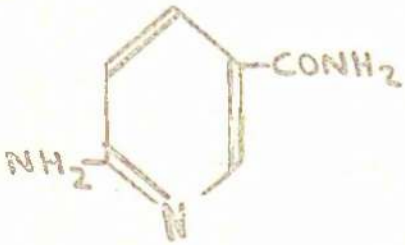
The demonstration by Alivisatos [219] that one of the key steps in the conversion of histamine into its urinary metabolite imidazole-4-acetic acid riboside [220] (Fig.8) involves reaction with the ribose-phosphate of adenylic acid liberated from nicotinamido-adenine dinucleotide (NAD, formerly DPN) by the action of nicotinamido-adenine dinucleotidase (NAD-ase), may be of great significance since the brain is known to contain an unusually large amount of NAD-ase. This NAD-ase cleavage also results in the liberation of a hydrogen ion which could conceivably be involved in electrical processes at the cell membrane where cationic exchange reactions occur. Since the NAD-ase reaction is extremely fast it could provide the necessary speed for nerve action. This NAD-ase in the brain could, therefore, either provide hydrogen ions to interchange with sodium or potassium ions, or else act as the terminator of histaminic action. [221]

The NAD-ase reaction, of course, cannot occur in the absence of NAD. This nucleotide is the coenzyme for an extensive range of enzymes which promote oxidations and reductions throughout the body. Also involved in these reactions is nicotinamido-adenine dinucleotide phosphate

NICOTINIC ACID NICOTINAMIDE



3-ACETYLPYRIDINE



6-AMINONICOTINAMIDE

(NADP, formerly TPN) which is formed by the reaction of NAD with adenosine triphosphate. NAD and NADP are involved in reactions concerned with glycolysis, amino acid metabolism, fat metabolism, and numerous other reactions including the oxidation of tryptophan to 5-hydroxytryptophan, of dopamine to nor-adrenaline, and the transamination of gamma aminobutyric acid, suspected of being an inhibitory neurohormone in the brain. Burton [222] has found NAD levels in the brain of the rat to be increased after treatment with tranquillisers such as reserpine or chlorpromazine, whereas they are decreased by stimulating drugs such as iproniazid.

Nicotinamide in Mental Processes.

NAD is formed from nicotinamide or nicotinic acid, shown to be vitamins of the B group by Elvehjem, Madden, Strong, and Woolley [223] in 1937. The acid and amide are interconvertible in vivo (Fig.9). Avitaminosis B leads to the disease known as pellagra, characterised by glossitis, skin rashes, and, frequently, mental abnormalities which at one time necessitated the institutionalisation of sufferers. When it was discovered that the mental ravages of the disease could be permanently reversed by administration of nicotinamide, a landmark in the chemotherapy of mental diseases had been reached and indeed Woolley [224] has pointed out that the idea of a chemical factor being responsible for a mental illness was totally alien to many psychiatric workers at the time, Freudian views on the origins of such diseases being generally accepted.

Although nicotinamide is an essential constituent

of the normal diet, in man it can also be synthesised from tryptophan if enough of this essential amino acid is provided in the diet [209] and indeed tryptophan can cure pellagra in man. Serotonin is also synthesised from tryptophan, but this pathway only utilises about one per cent of the dietary intake of the amino acid. [224]

In 1957, Hoffer [225] claimed that daily doses of three to five grams of nicotinamide or nicotinic acid could cure schizophrenic patients provided therapy was initiated in the early stages of the disease. After further experience extending over five years in which nicotinic acid therapy has been combined with administration of tranquillisers, Hoffer [226] has reported that the readmission rate of such patients is significantly lower than those treated with tranquillisers alone. Surprisingly, other workers do not seem to have repeated these experiments although Woolley [227] demonstrated that large doses of nicotinic acid induced tranquillisation in mice, as well as affording protection against the diarrhoea normally associated with subsequently injected 5-hydroxy-tryptophan, twenty-five milligrams of nicotinic acid counter-acting 0.4 milligrams of 5-hydroxytryptophan.

Agnew and Hoffer [228] pretreated normal volunteers with three grams of nicotinic acid daily for three days prior to LSD administration. They then found that a different type of psychosis occurred, in which there was a close resemblance to the schizoid state. Perceptual disorders were moderated, whereas affective disturbances were intensified. Administration of nicotinic acid during the LSD psychosis markedly reduced the symptoms in individuals

who were not premedicated, although affective disorders remained. Other workers now use nicotinic acid either to moderate or terminate the LSD experience.[229-234] It has also been claimed [151] that nicotinic acid affords protection against the adrenochrome psychosis.

Antimetabolites of Nicotinamide.

Administration of a nicotinamide antimetabolite, 3-acetylpyridine (Fig.9), to nicotinamide deficient dogs accelerated the development of pellagra. Marked behavioural changes were also noted.[235,236] Prior administration of nicotinamide prevented the disease, but failed to reverse its development once it had begun. Eventually, Kaplan [237] showed that when NAD-ase cleaved NAD to release a proton, the antimetabolite competed with nicotinamide for the available ribose-phosphate adonylic acid. If tissue levels of nicotinamide were not high enough the 3-acetylpyridine analogue of NAD was formed. This analogue subsequently acted as an antagonist of NAD inside the brain cells.

Some time later, Coggeshall and MacLean [238] traced the ataxia and behavioural changes associated with 3-acetylpyridine administration to lesions in the hippocampus, which could be observed histologically. These permanent lesions were also observed to be associated with the ataxia and psychiatric disturbances caused by 6-amino-nicotinamide given to humans as an antimetabolite in the treatment of cancer.[239,240] It was shown that the 6-amino-nicotinamide analogue of NAD was formed by the action of NAD-ase on NAD.[241]

The action of these antimetabolites of nicotinamide suggests that nicotinamide, or, more likely, NAD (or NADP) are of importance in the normal functioning of the hippocampus. They certainly indicate that mental aberration follows chemical changes in the hippocampus.

The hippocampus behaves functionally as part of the limbic system, the complex system which incorporates the cerebral cortex, certain subcortical structures, the hypothalamus, and the hippocampus.[242] On an evolutionary basis this part of the brain, the rhinoncephalon, appears to have been closely involved in the self-preservation of the species, according to MacLean [242] but it is now best considered as being involved in emotional response.

The Physiological Basis of Emotion.

Two aspects of emotion have to be recognised. The first is the affective, sensory component, and the second is the expressive, behavioural component. The latter is, of course, readily amenable to laboratory study by utilising nerve ablation techniques, electrophysiological recording, and objective behavioural studies. That this outward expression of emotion, be it fear, anger, or pleasure, is highly complex, requiring central co-ordination, would seem to be self evident. Moreover, it is of great evolutionary significance since any individual member of a given species unable to make the appropriate emotional response to a given situation will be at a disadvantage as compared to his emotionally mature fellows. Survival of the fittest, it can be assumed, will ensure there is continued development of

desirable emotional characteristics of ever increasing complexity.

Early theories of emotion have been discussed by Gellhorn [243] and Morgan. [244] The first general theory attempted to interrelate emotional behaviour and emotional experience. The James-Lange theory postulated that the involuntary viscerosomatic changes that occurred are associated with an emotional stress and were the cause, not the result, of this emotional experience. This rather unlikely hypothesis has been somewhat contemptuously dismissed by Morgan who has aptly quoted James as saying, "We are afraid because we run, we do not run because we are afraid." Today although it is accepted that the viscerosomatic changes are not the initiator of emotional experience, it is nevertheless believed that they may help to sustain the general level of central activity necessary for the full development of emotional expression.

Utilisation of neuro-surgical techniques available to physiologists in the first quarter of the century enabled Cannon [245] and Bard [246] to propose that emotional response resulted when afferent impulses from the thalamus bombarded the cerebral cortex which instantly engaged the hypothalamus in the management of the expressive response. Lashley [247] criticised this suggestion on the basis that there was no evidence to suggest that emotional experience arose from the thalamus which was primarily concerned with the propagation of sensory information and the perception of pain. That the hypothalamus does play a role is, nevertheless, generally accepted today.

Electroencephalogram studies during the post-war era indicated the existence of a functional activation centre in the brain, the activity of which was associated with alertness. The centre responsible is now known to be within the reticular formation (see below). Lindsley [248] taking into account the obvious fact that the somnolent or sedated animal exhibits little emotive behaviour, suggested that activation of the reticular system is a *sine qua non* which precedes further emotional activity. One weakness of this suggestion, also recognised by Lindsley himself, arises from the knowledge that the hypothalamus includes stimulation of the reticular alerting centre amongst its diverse functions. Hence the necessary degree of arousal can be obtained once cortical signals have delivered their "instructions" to the hypothalamus.

Papez [249] proposed, in 1937, that the structures of the rhinencephalon were functionally unified in a system that mediated the affective and expressive facets of emotion. However, it was not until 1952 that this concept became generally accepted when MacLean [250] renamed the rhinencephalon as the limbic system. Since then a considerable volume of experimentation has helped to elucidate the role of the various components of the limbic system.

Electrode implantation into the hypothalamus can elicit the expression of fear, anxiety, irritation, rage, aggressiveness, or curiosity [251,252] and these different modes of emotional behaviour have been correlated with distinct loci within the hypothalamus.[253]

The lowered threshold to emotional arousal which

is observed in decorticate animals indicates that the cortex normally provides inhibitory control over the lower centres. However, the facile arousal which occurs is of brief duration indicating that the cortex may also prolong emotional response.[254] On the other hand, partially decorticate animals in which the cortical projection of the limbic system - the cingulate gyrus - remains untouched are demonstrably placid.[255] It would thus appear that the cingulate gyrus of the neocortex is an inhibitory centre for emotional activity. Pribram and Kruger [256] consider this to be the structure through which awareness of emotional reaction is attained.

Conflicting reports appear in the literature with regard to the bilateral extirpation of the amygdaloid complex, the adjacent pyliform lobe, and the hippocampus, but Himwich [257] believes the consensus of opinion is veering towards the original conclusions of Kluver and Bucy [258] that these structures provide the excitatory influence on emotional behaviour. Himwich [257] believes that the variable results reported may arise from differing environmental influences including the manner of handling the animals, which certainly could profoundly influence the initial emotional state of the animals. Indeed two groups of workers have attempted to analyse the discordant results on this basis.[259,260]

Although studies on the neurophysiological correlates of emotion are far from complete it is possible to consider the action of tranquillising and excitant drugs in terms of the limbic system. The site of action of such

drugs need not necessarily lie within this system. Indeed, there is a considerable body of evidence to suggest that many drugs arouse or depress, or otherwise modify, the reticular formation of the mid-brain (see below). Although the contention that reticular arousal precedes emotional response may not be correct for normal physiological conditions, it may well be that drug-induced arousal (or depression) does indeed potentiate (or mitigate) the sequence of events leading to emotional expression. Thus, in the presence of excitant drugs, stimuli which normally fail to induce a significant emotional reaction may become capable of so doing by virtue of a lowered threshold consequent upon reticular arousal. The opposite would apply for depressant drugs.

The Electroencephalogram in Neurophysiological Experimentation.

For many years neurophysiologists have studied the electrical waves that can be recorded at the surface of the head. These are generated by the aggregation of minute electrical potentials developed by individual brain cells. In the resting subject whose eyes are closed, in quiet surroundings, a regular pattern of sine waves is recorded on the electroencephalogram. This alpha-rhythm consists of a regular sequence of medium amplitude waves recurring at frequencies of 8 to 12 cycles per second. When the subject opens his eyes or engages in purposive activity the regularity disappears due to a redirection of potential changes as new nervous pathways are activated. The electroencephalographic waves are now said to be desynchronised. On the other hand,

should the subject fall asleep a different rhythm developes, the delta rhythm, in which the waves are of increased amplitude and diminished frequency. Although the physiological basis of the electroencephalogram is insufficiently understood, the instrument nevertheless provides considerable information about the levels of activity of different cerebral structures either under normal conditions or in the presence of psychotropic drugs.

The Reticular Formation.

In 1949, Moruzzi and Magoun [261] discovered that electrical stimulation of the diffuse brain stem structure known as the reticular formation resulted in a desynchronisation of the cortical electroencephalogram in the cat. The new pattern of more frequent, lower amplitude waves which resulted indicated that the animal had been aroused from its previous state of relaxation. These experiments on cats were followed by others which demonstrated that sleeping cats [262] or monkeys [263] were readily aroused by mild reticular stimulation, whereas lesions within this area produced electroencephalographic and behavioural changes indicative of somnolence. Subsequent experimentation by numerous workers leaves little doubt that the "waking centre" of the brain lies within the reticular formation.

The Mesodiencephalic Activating System.

Several years before the discovery of the reticular activating system, Morison and Dempsey [264] found that electrical stimulation of thalamic nuclei which project

diffusely to the cortex, resulted in a recruiting response characterised by an ever increasing cortical responsiveness consequent upon serial stimulation. Later, Moruzzi and Magoun [261] showed that the midbrain reticular formation acted in conjunction with this diffuse thalamic projection system, the integrated unit forming the mesodiencephalic activating system. Nevertheless, the distinction which exists between the two portions of this system should be noted since drugs and hormones may selectively influence one or the other. The activation produced by stimulation of the diffuse thalamic projection system is less persistent than that produced from reticular stimulation.[265] A finding which could be of considerable pharmacological importance is that a reduced frequency of stimulation in the diffuse thalamic projection system can induce sleep.[266]

The mesodiencephalic activating system is strongly influenced by other regions of the brain. Sensory information is carried from the peripheral receptors along the classical afferent pathways through the lemnisci to the sensory thalamic nuclei where the signals are analysed and directed to the requisite cortical projection area. However, collateral fibres from the lemnisci carry afferent impulses into the reticular formation, whence they ascend to the thalamic nuclei.[267] From here they pass to a wide area of the cortex via the diffuse thalamic paths.[268,269] Thus, "the mesodiencephalic activating system affords the emotional cloak which accompanies some kinds of stimulation of the body".[257] Electroencephalogram studies have enabled a distinction to be drawn between the time of arrival at the

cortex of impulses from the long, classical afferent pathways and the later signals from the reticular formation.[270]

Besides sending signals to the cortex, the reticular formation is influenced by feedback from the cortex, efferent descending impulses being introduced in a manner similar to the afferent impulses.[271-273]

The reticular formation does not indiscriminately pass on all incoming sensory signals, but rather appears to exert a considerable degree of selectivity. In animals with a highly developed sensory apparatus some form of selective discrimination to restrict the perception of unwanted information is essential for efficient functioning. Any task would be difficult to perform if all minor disturbances were apportioned the same degree of attention as the task itself. On an evolutionary basis it is clear that selective perception will become highly developed. The belief that hallucinogenic drugs interfere with the functional neural pathways upon which learned, perceptual discrimination depends is supported by the knowledge that LSD acts upon the reticular formation (see below) as this may well be the centre responsible for maintaining such discriminative ability.

There is good reason to believe that the sensory information reaching the cortical projection areas via the classical afferent pathways has no significance to the organism until the projection areas have been aroused by corticopetal signals from the reticular formation. Indeed when the reticular formation is naturally depressed, as during sleep, sensory information is not perceived, unless an alerting

signal arouses the individual. Similarly, during anaesthesia, when the reticular formation is artifically depressed, sensory information makes no impact.

Thus, consideration must be given to the suggestion[263] that the presence or absence, to a greater or lesser degree, of corticopetal signals from the reticular formation to the cortical projection areas provides the basis of selective perceptual awareness, i.e. the focus of attention. There is some experimental support for this contention, based on the demonstration of decreased reaction times after alerting stimuli,[274-276] and the discovery of the ability of the reticular formation to selectively inhibit afferent stimuli. Using chronically implanted electrodes, Hernandez-Peon, Scherrer, and Jouvet [277] showed that the discharge evoked in the dorsal cochlear nucleus of the cat by serially repeated clicks became markedly reduced in amplitude when the cat's attention was distracted by the appearance of a mouse. Similar results followed other distractions. Other workers have demonstrated a suppression of auditory nerve impulses in response to reticular formation stimulation.[278-281] Jouvet,[282] using electroencephalographic techniques to study the response of the occipital cortex in the presence of flashes of light, found that when the attention of human subjects was diverted, the cortical responses were drastically reduced. Ogawa [283] has also demonstrated that reticular activation clearly augments the activity in the lateral geniculate nucleus which carries optical relay signals to the visual cortex. It will be of interest to learn whether further studies by Ogawa using his technique of microelectrode

implantation into the neurons of the lateral geniculate nucleus will demonstrate that the reduction in cortical response following distraction of attention is due to effects mediated at the lateral geniculate nucleus. Finally, it should be mentioned that several investigators using microelectrodes have discovered neurons in sensory areas of the cortex which only respond when the animals concerned have their attention aroused with regard to the sensory modality under investigation.[284]

The above discussion concerning the limbic system, and the reticular formation and its probable role in the focussing of attention has been included since there is evidence to suggest that psychotropic drugs may exert their primary effect on these systems.

The Sites of Action of Psychopharmacological Agents.

Before the appreciation of the significance of the reticular formation, it was generally believed that the cerebral cortex was metabolically more active than the lower parts of the central nervous system, and so was more susceptible to the action of drugs.[285,286] In 1954, however, Arduini and Arduini [287] demonstrated that the effects of afferent stimulation to the reticular formation were disturbed by drugs or metabolic changes more readily than similar effects carried to the cortex by the classical afferent pathways. Actually this was in agreement with the earlier experiments of French, Verzeano, and Magoun.[288] Accordingly it was suggested [287] that the complexity of internal organisation within the reticular formation was at least as

great as that of the cortex. Certainly, drugs, which are generally assumed to exert their psychotropic effects at the synaptic level would seem likely to select polysynaptic structures for their site of action within the brain.

The major tool for investigating the site of action of psychopharmacological agents remains the electroencephalogram but studies on human beings are largely restricted to recordings from the scalp, whereas with animals electrodes are usually implanted within the brain. Brazier [289] has recently reviewed the effects of drugs on the electroencephalogram in man, while the results of electroencephalographic experimentation in lower animals have been collated by Longo.[290]

Caton [291] who first demonstrated electrical activity in the brain, showed that changes were induced by the administration of chloroform. Berger, [292] the father of modern electroencephalography, confirmed the early results more than half a century later. The effects of anaesthetics on the electroencephalogram (E.E.G) in animals [293] and in man [294-296] have been summarised in recent publications.

Recordings from the scalp in man show that, in general, as loss of consciousness developes there are corresponding changes in the electrical activity of the brain. These are seen as an initial increase in frequency, followed by regular or irregular slow waves, then finally intermittent electrical silence in the E.E.G. ("burst suppression") characteristic of a state of deep surgical anaesthesia. These changes represent the changing balance between excitation and inhibition in the major subcortical systems

influencing the activity at the cortex.[289] The minor differences which occur with different drugs reflect the relative susceptibilities of various structures within these systems to the individual drugs. There is a close similarity between the E.E.G. changes induced by the volatile anaesthetics and the barbiturates[289] which is of particular interest to the medicinal chemist in view of attempts to divide drugs into the somewhat arbitrary groups of structurally-specific and structurally non-specific drugs[297,298] since barbiturates are often considered to belong to the former group and the volatile anaesthetics to the latter. Such attempts to divide drugs into the structurally-specific and the structurally non-specific arose from the fact that whereas the biological activities of some drugs are profoundly modified by very minor changes in chemical structure (as in morphine, for example, where the dextro-rotatory enantiomorph possesses none of the analgesic properties of the laevo-rotatory compound), in other instances (as with the volatile anaesthetics) considerable differences in chemical structures have little effect on biological behaviour. Although the terminology strictly refers to the dependence or otherwise of a particular pharmacological effect upon specialised molecular features, it is also true that structurally specific drugs tend to exhibit a narrower spectrum of biological actions than do structurally-non-specific drugs which usually exert an effect on a wide variety of biological phenomena.

Structurally Non-specific Drugs.

In the case of structurally non-specific drugs it

is generally considered that the biological activity displayed is primarily dependent upon certain favourable physical properties, and a number of attempts have been made to identify the particular physical property in question. Martin-Smith and Khatoon,[299] however, have pointed out that such an approach does not necessarily give a fundamental distinction from structurally specific drugs since very few drugs are known which form covalent bonds with tissue constituents (i.e. act "chemically"). Notable exceptions are the heavy metals, penicillin, the nitrogen and sulphur mustards, and various organo-phosphorus compounds.[199]

Such attempts as have been made to interpret the biological properties of structurally non-specific drugs in terms of vapour pressure or boiling point, distribution coefficient, ability to lower surface tension, van der Waal's constants, square root of molecular weights, hydrated microcrystal formation, or "iceberg" formation have been matched by attempts to relate the biological activity of such structurally specific drugs as the carcinogenic aromatic hydrocarbons to K electron density, the tranquillisers to their ability to lower the surface tension of Ringer's solution[300] (showing a close correlation with clinical efficacy) and a variety of structurally specific nitrogen-containing drugs to $f(K_p)$. Most of the attempted correlations just discussed are treated in detail by Albert[199] and Burger.[301] A detailed account of structurally non-specific drugs has been given by Ferguson,[302] who sought a relationship between biological activity and thermodynamic activity in those cases where drug action appeared dependent

primarily upon a favourable phase distribution and concluded that substances which are present at the same proportional saturation in a given medium exhibit the same degree of biological activity.

After the demonstration of anaesthesia of mice with the inert gases,[303] operations on human patients were successfully performed in the United States utilising xenon as the sole anaesthetic.[304] Albert [199] believes that its lack of dipole moment and its spherically symmetrical structure indicate that xenon, and hence narcotics in general, act in the bulk phase rather than by adsorption at a surface. As long ago as 1921 Warburg [305] suggested that depressant drugs form a layer on the outer surface of cells thereby inhibiting oxidative processes since hypnotics injected intracellularly proved devoid of activity. Some years later Quastel [306] showed that only certain oxidative processes were inhibited and hence he concluded that the spatial relationships of consecutive enzymes in an oxidative chain were disturbed. However, Butler [307] believes that the reduction in cellular respiration may be a result, and not a cause, of diminished nervous activity. Albert [199] wisely states that all that can be safely said at present is that narcosis by inert molecules takes place as soon as a constant fraction of the total volume of some non-aqueous phase of the nerve-cell is occupied by foreign molecules.

The major objection to classifying the barbiturates as structurally non-specific drugs arises from the fact that certain minor changes in chemical constitution, e.g. methylation of both nitrogen atoms, or lengthening of the

5-alkyl group [199] can change the activity to convulsant and not depressant. Since the barbiturates can be regarded as pyrimidine derivatives it is possible that they may act as anti-metabolites of some naturally occurring pyridimidine. It is known that barbiturates inhibit respiration by interfering with the link between NADH and flavoprotein with uncoupling of the phosphorylation.[289]

Beneficial Effects of Barbiturates in Painful Conditions.

Work by Magoun and his associates [288] has shown that, in animals, the area of the brain most susceptible to the barbiturates is the ascending reticular system of the midbrain. Actually the response of the cortex to sensory information via the classical afferent pathways is enhanced, although the motor cortex is depressed.[308] Hence it is not surprising that barbiturates have little effect upon the pain threshold except in doses sufficient to impair consciousness.[309] Nevertheless, it has been found clinically that the administration of barbiturates to patients suffering pain may have beneficial effects. Wolf and Ripley [310] concluded that these effects occurred either because of alterations in the reaction of the subject to the painful experience or from interruption of the mechanism responsible for the noxious stimulus. The latter mechanism would probably be due to the peripheral effects of the barbiturates on the autonomic nervous system. [13] However, in the light of the suggestions that sensory information reaching the cortex has no significance until the projection area has been

aroused by corticopetal impulses from the reticular formation, it seems likely that barbiturates owe any analgesic properties they have to a depressant effect on the pain perception arousal mechanism. Suitable experimental design which eliminates the possibility of arousal during the testing of analgesics is hard to attain, but the results obtained if this could be achieved would be most interesting. At present there is considerable doubt whether analgesics such as morphine, methadone, or pethidine have any effect on the pain threshold.[311,312] Beecher and his colleagues [313] believe the reaction to pain is of more importance than the actual pain threshold and have suggested that analgesics should be tested in a clinical situation by assessing their effects on naturally occuring pain.

The likelihood of barbiturate anaesthetics leaving cortical function undisturbed while selectively depressing the reticular formation raises wider issues that can only be briefly discussed here. As Brazier [289] has indicated, the fully conscious state consists of more than the arrival at the cortex of sensory information via the classical afferent pathways and its mediation by the reticular activating system since another important function supervenes, namely memory. Yet no memory of painful stimuli troubles patients after their recovery from barbiturate anaesthesia. When it was believed that such anaesthetics depressed the cerebral cortex this absence of the memory of pain seemed quite natural. Now that it appears that the cortex is not depressed the conclusion to be drawn is that sub-cortical structures are involved in

the memory process. Brazier[289] believes that there is some evidence suggesting the involvement of the limbic system, particularly the hypothalamus since, when this is stimulated electrically, certain effects are observable on the memory process. Detailed results are to be published by Brazier in two papers currently in press.[314,315] There is, however, other evidence which seems to indicate the involvement of sub-cortical structures in memory.

Memory and Conditioning.

A useful indication of an efficient memory in animals would appear to be their ability to exhibit conditioned responses since in the absence of a memory conditioning could not take place. Conditioning has been demonstrated in decorticate dogs by Polytrew and Zeliony.[316] These Russian workers, however, did not perform post-mortem studies to ensure that decortication was complete, although this was later done by several American investigators[317,318] who confirmed the Russian results. In considering the results of these experiments on decorticate animals allowance must be made for the effect of increased emotional response. Similar consideration must also be given to the effects of selective ablation studies on lower centres where, for the most part, only quantitative changes in the rate of conditioning are noted. Lesions within the diffuse thalamic projection system, however, markedly impair both the retention of previously learned conditioned avoidal responses as well as the subsequent ability to relearn these.[319] It can be concluded that if conditioning is a reliable measure of

memory function then memory does exist in the decorticate animal. In 1937, Girden [320] and Culler [321] demonstrated that responses conditioned while an animal was curarised with crude curare extract disappeared when the effects of the drug were off, whereas conditioning successfully elicited before giving the curare could not be observed while the effects of the drug lasted. It was found that the curare depressed the motor cortex and possibly the whole cortex was depressed. Culler [321] suggested, on this basis, that normal conditioning involved the cortex whereas under curare principally sub-cortical structures were involved. It should be noted that the pure principle, d-tubocurarine, does not depress the cerebral cortex.

Considerable caution must be exercised when interpreting the results of experiments designed to test the effects of drugs on conditioning. It is necessary to make allowance for impairment of sensory, motor, or emotional function before valid conclusions can be drawn from behavioural studies. Failure of an animal to respond to the conditioned stimulus while still showing the ability to respond to the unconditioned stimulus when pre-medicated with a psychopharmacological agent indicates that there is a specific block of the conditioned response, whereas failure to respond to either stimulus is indicative of a non-specific block of the conditioned response. Cook and Weidley [322] found that chlorpromazine, reserpine, and morphine had a specific effect in blocking the conditioned response whereas there was a non-specific effect with the sedatives

methylparafynol, meprobamate, and the barbiturates at toxic dose levels. It was claimed that serotonin produced a 50 per cent inhibition of the conditioned response - a somewhat surprising result since this compound which is generally believed unable to pass the blood brain barrier was administered by parenteral injection as were the other drugs. High doses of LSD specifically blocked the response whereas the only effect of low doses was to antagonise the blocking action of other drugs. This is of significance insofar as it shows a dependence of overt behavioural effects on the dose level of the drug. Among the drugs antagonised by LSD were reserpine and chlorpromazine. Mescaline had no significant effects. Other investigators have also studied the inhibition of conditioned responses by tranquillising agents and there would appear to be a general agreement that this technique offers a useful procedure for the evaluation of new drugs.[323] The literature on this subject is too vast to be considered here; a review article has been published by Brady.[324]

Tranquillising Agents.

In clinical practice, the barbiturates are often used as tranquillising agents and, indeed, before the advent of the modern tranquillisers they represented one of the main weapons in the physician's armoury for combatting emotional disturbances. It is possible that any beneficial effects they have in this connection are due to the diminution of arousal of the limbic system by the reticular formation.

It might be expected that drugs which effectively tranquillise without causing sedation act primarily on the limbic system without depressing the reticular formation. Nevertheless, it was found that after administration of chlorpromazine to rabbits the alerting response in the E.E.G. associated with reticular arousal disappeared.[286] Subsequent animal experimentation suggests that this absence of arousal is due to inhibition of impulses traversing the collateral fibres from the classical afferents to the reticular formation.[325-327] Indeed, it has been proved that transmission in the classical afferents is not inhibited.[328] Recordings from the scalp show that, in man, sub-cortical changes occur before any effect on cortical activity can be seen.[329] It appears that as the changes in the level of activity of the reticular formation are indirect, no depression of this centre occurs, hence there are no sedating properties associated with chlorpromazine. Since inhibition of sensory transmission to the reticular formation takes place it would seem likely that cortical feedback into this centre could attain an increased significance in the absence of distracting stimuli. As the drug is an effective tranquilliser this probably does not occur, hence it must be assumed that the feedback process is also inhibited.

Chlorpromazine, like the barbiturates, finds a role in clinical practice as an analgesic although no effect on pain threshold is demonstrable and, as has just been stated, no inhibition of the classical afferents takes place. Himwich[257] believed that this could be accounted for, in

part, by the improvement in the patient's emotional state as a result of the tranquillising effect, but some other factor had to be taken into consideration since reserpine was devoid of analgesic properties although effective as a tranquilliser. Himwich suggested that this factor might be depression of the reticular formation since reserpine was shown to be capable of arousing this system with concomitant E.E.G. alerting.[330] The analgesic effect can now be accounted for by a similar explanation to that pertaining to the situation with barbiturates, the actual locus of interference with the sensory arousal process being the collateral fibres leading to the reticular formation rather than this structure itself.

The absence of depressant action on the reticular formation with reserpine indicates that tranquillising ability is not directly related to effects therein. As already suggested, it might be expected, in the light of current knowledge, that the site of action for effective tranquillisation is the limbic system.

The early researches on chlorpromazine and reserpine indicated that these drugs inhibited hypothalamic function, particularly that of the posterior hypothalamic nuclei which exert central control over sympathetic innervation of the viscera and blood vessels.[331] The clinically observed bradycardia, hypotension, papillary constriction, and increased intestinal motility following administration of reserpine is characteristic of sympathetic inhibition.[332] Superimposed upon the central effects of reserpine, however, is the characteristic emptying of catecholamine depots

throughout the body. Thus, even if central inhibition at the hypothalamic level is only partial, descending afferent impulses impinging upon sympathetic nerves will evoke a limited response since post-ganglionic adrenergic function will be depressed.[257] The general parasympathetic dominance observed after administration of chlorpromazine can be attributed to the hypothalamic inhibition and to the peripheral action of the drug on sympathetic nerves controlling blood-pressure. A characteristic feature of chlorpromazine medication is the tachycardia due to impairment of the parasympathetic innervation of the heart.[333]

There is reason to believe that the region of the brain most susceptible to the action of tranquillisers is the amygdala. E.E.G. observations showed that after five days of medication with chlorpromazine in cats "spiking" could be detected in the amygdala (but not elsewhere in the brain) twenty hours after injection.[334] Spontaneous, seizure-like waves have been recorded from the limbic system of the cat after treatment with reserpine, the seizures spreading from the amygdala to the hippocampus and thence to higher rhinencephalic structures with the notable exception of the cingulate gyrus which is the cortical projection of the limbic system.[335] A similar spread of activity is found after administration of large doses of chlorpromazine where seizure activity can occur if the electrical disturbance spreads to the motor cortex.[334] Penfield [336] suggested, in 1950, that psychomotor epilepsy might originate within the amygdala, thereafter ascending to the cortex via the centrencephalic integrating system.

The role in clinical medicine of reserpine, chlorpromazine, and numerous other drugs developed from them through minor structural modifications has been critically examined by Trouton and Eysenck.[337] Improvement in the condition of deteriorated chronic schizophrenics after treatment with reserpine has been confirmed by several British workers,[338-341] although chlorpromazine is possibly more useful, with fewer side effects.[342] Beyond this there does not yet appear to be any justification for the current widespread prescribing of such drugs both in hospital and general practice. A carefully controlled, double-blind clinical trial comparing the effects of chlorpromazine, a barbiturate, and a placebo in 142 chronic, psychotic in-patients revealed no statistically significant difference in the results obtained.[343] Sargant [344] believes that the greater enthusiasm, matched by appropriate clinical evidence, for the tranquillising drugs in the United States is a consequence of the relative neglect of the psychiatric patient in the grossly understaffed mental hospitals of that country, coupled with an immature psychiatric outlook dominated by psychoanalytical ideas. As a result, improvements obtainable in Britain without resort to drugs are hailed as therapeutic advances consequent upon tranquilliser administration in the United States.

The Mode of Action of Stimulant Drugs.

Both adrenaline and nor-adrenaline produce electrophysiological activation in cats when administered parenterally.[345] It is believed that there exists a portion

of the mesencephalic activating system which is highly sensitive to adrenaline, and it is thought that amphetamine (and related compounds) can mimic the action of adrenaline at the receptors therein.[346] Dexamphetamine sulphate has been used as a euphoriant in the treatment of certain psychopaths.[347] There is a vast literature on the behavioural effects of the amphetamines and this has been discussed by Trouton and Eysenck.[337] The stimulant drug methylphenidate also causes reticular arousal but certain qualitative differences indicate a different site of action to that of amphetamine.[348] Unlike the situation with amphetamine the stimulation produced by methylphenidate leads to directed activity and does not interfere with attention.[349]

Still another mode of action must be ascribed to caffeine where stimulation can not be due solely to reticular arousal since the electrophysiological activation caused by it can occur even after lesions have been made in the reticular system.[350] It is believed to be capable of suppressing the diffuse thalamic projection system, although the main site of action may be the cerebral cortex. The actions and uses of caffeine have been reviewed.[13,337]

Strychnine increases the responsiveness of the reticular system,[287] but the main site of action is on the spinal cord where it suppresses the function of inhibitory neurons which dampen the activity of the motor neurons.[350]

Electrophysiological Effects of LSD.

The literature on the electrophysiological effects of LSD is somewhat confusing although it now seems to be

accepted that arousal of the reticular system takes place in animals.[289] Results in man are vague. Delay and his colleagues [351,352] found that E.E.G. activation was not blocked by LSD, whereas Purpura [353] showed that axodendritic activity within the reticular formation was inhibited although a facilitatory effect on cortical potentials from the classical afferents could be detected. On the other hand, Bradley and Elkes [354,355] used implanted electrodes to demonstrate an alerting effect in cats after LSD administration. Rinaldi and Himwich [356-358] using curarised (sic) rabbits confirmed these results and also found a reversal of effect occurred with doses of 20 to 60 micrograms per kilogram (normal dose is 1 to 5 micrograms per kilogram). It would be interesting to know whether this reversal is due to physiological inhibition or biochemical hyperpolarisation. Evarts [359] confirmed Bradley and Elkes' report [355] that LSD could overcome the depressant effects of barbiturates on the E.E.G. In contrast to these workers, Killam and Killam [360] reported that in curarised cats doses of LSD up to 100 micrograms per kilogram, intravenously, caused slight increases in the threshold of arousal response of the E.E.G. to sciatic nerve and reticular formation stimulation. Rinaldi and Himwich [358] had found doses of 1 to 5 micrograms per kilogram lowered the threshold for reticular stimulation.

Bradley and Elkes [354,355] discovered that even large doses of LSD were without effect on the E.E.G. of spinal (encephale isolé) decerebrate (cerveau isolé) cats and hence they concluded that the effects of the drug are

dependent upon spinal connections. The ability of azacyclonol to prevent the alerting effects of LSD but not of amphetamine [356-358] (known to stimulate the reticular formation) also indicated a site of action other than within the reticular system. Bradley [361] finally concluded that the reticular arousal which LSD caused was due to facilitation of the impulses from the classical afferents traversing the collateral fibres from the lemnisci to the reticular formation.

LSD may, however, have a direct action on one portion of the mesendiencephalic activating system. Purpura [353] found that LSD in doses from 10 to 30 micrograms per kilogram depresses the recruiting responses in cats immobilised with succinylcholine. In contrast, the Killams [360] found only a slight reduction in the responses from the diffuse thalamic projection system. These differences may be explained by the observation [353] that shorter intervals between the pentobarbitone sodium injection (used by Purpura to prepare his animals for subsequent experimentation) and initiation of LSD medication favoured increased inhibition of recruiting responses.

The effects of LSD on recordings of human cortical activity taken from electrodes placed on the scalp are very slight. Rinkel and his associates [362] observed a small increase in alpha frequency, a result confirmed by Gastaut, Ferrer, and Castello [363] and Bradley, Elkes, and Elkes.[364,365] The only study on large numbers of subjects which appears to have been reported, is that carried out by Ferrer and Goldner[366] who were unable to detect any significant E.E.G. changes in psychotic subjects who had received up to 2 micrograms of LSD

per kilogram. Schwartz, Bickford, and Rome [367] found only minimal E.E.G. changes in 13 subjects each given a total dose of 50 micrograms LSD, whereas in 7 subjects there was some reduction in the alpha-rhythm. Using techniques which measured total electrical output at the scalp Pfeiffer, Goldstein, and Murphree [368,369] observed that in normal subjects LSD reduced the mean energy and diminished E.E.G. variability; this effect resembled that of amphetamine. When LSD was administered to chronic schizophrenic patients, however, there was no change in energy content but there was an increase in variability which reached a maximum 1½ hours after administration of LSD.

Monroe [370] has carried out studies with electrodes placed deep within the human brain. He and his colleagues were thus able to demonstrate pathological changes in the hippocampal, amygdaloid, and septal areas of chronic schizophrenics which could not be detected with electrodes placed at the scalp. When LSD was administered to these patients the marked increase in psychotic behaviour which occurred corresponded to the appearance of paroxysmal activity in the hippocampus, amygdala and septal region.

Himwich and Rinaldi [358] found that the effect of mescaline on the curarised rabbit was similar to LSD, the alerting pattern being reversed by the tranquilliser azacyclonol. Chweitzer, Geblewicz, and Liberson [371,372] found that a decrease in the amplitude of alpha activity in man occurred, with blocking of alpha-activity during visual hallucinations. The decrease in alpha-activity

persisted for several days after the cessation of symptoms. Variable effects were found by several other workers.[373-378]

Electrophysiological Studies with other Hallucinogens.

There would appear to have been fewer studies of the electrophysiological effects of other hallucinogens. Adrenochrome was shown to have no effect on scalp recordings taken from normal human beings although changes were detected with epileptic patients.[379] However, intraventricular injection in cats of adrenolutin or adrenochrome has been reported to induce slow, low amplitude waves in the E.E.G. which dissipated on arousal.[380] It has recently been shown [381] that bufotenine acts by stimulating an area between the first cervical segment and the midbrain in rabbits. Neither Indian Hemp nor "Synhexyl" produced effects which could be related to E.E.G. activity in man.[382,383] Himwich [381] has advanced the claim that, in rabbits, electrophysiological arousal is due to a direct action of 5-hydroxytryptophan on the midbrain.

However, results of electrophysiological studies, which are often contradictory, must be treated with caution. For example, there is believed to be a species difference between man and the cat with respect to the E.E.G. response to amphetamine administered intravenously.[384] Nevertheless, electrical studies can be of considerable value to the medicinal chemist who must ascertain that various drugs are acting at the same site before he can consider one compound as a possible antimetabolite of another.

Personality and Drug Action.

The eminent psychologist William McDougall accepted the existence of a personality continuum corresponding to the factor of extraversion-introversion which had been proposed by Jung.[385] McDougall[386] believed that the distribution of personalities on this continuum could best be explained by the influence of an endogenous chemical factor acting upon the nervous system. He was puzzled by the problem of whether the chemical factor was introverting or extraverting, but arbitrarily decided it was extraverting. McDougall considered the introvert to be the man in whom the lower, primitive levels of the nervous system are constantly subjected to a high degree of inhibition by the higher cortical activities. The affective, emotional function he considered to be the most important of the lower processes. As the child developed, according to McDougall, there would be an increase in introversion corresponding to the increased cortical dominance. Nature, however, provided a compensatory mechanism in the form of a hormone with extraverting effects to overcome the tendency to introversion. Personality on the extravert-introvert continuum would be determined by the amounts of the hormone secreted.

McDougall considered alcohol to be capable of depressing the higher functions, thereby releasing the primitive behaviour which the cortex normally suppressed. He observed that extraverts succumbed to the effects of this drug more easily than introverts and concluded that this was because only a small amount was required to augment the cortical inhibition produced by circulating extraverting

hormone thereby producing a primitive state in drunkeness. He assumed that the synapses at the higher levels of the brain were more susceptible to chemical interference by strychnine, alcohol, ether, chloroform, or extraverting hormone. Since the introvert was deficient in the extraverting hormone he would be susceptible to disorders of continuing conflict in the absence of this hormone's dissociative influence and would thereby be prone to neurasthenia, schizophrenia, and insomnia. The extravert, on the other hand, would be exposed to dissociative disorders such as functional paralysis, anaesthesias, amnesia, hypnosis, and somnolence.

Eysenck [387] considers that there are a variety of reasons for the total neglect of McDougall's hypothesis. Firstly, McDougall suggested no objective measure of placement on the extraversion-introversion continuum either before or after administration of drugs. When this was eventually carried out by Shagass [388,389] the observation that extraverts were highly susceptible to alcohol was readily confirmed by measuring the amounts of alcohol required by extraverts before any effects were detected. A further weakness in McDougall's ideas was the vague nature of his extraverting hormone. McDougall was primarily interested in this hormone and only introduced the concept of depressant drugs having an extraverting action to illustrate his views on the extraverting hormone.

The results of a neglected experiment by Hull [390] which showed that the stimulant drug caffeine caused an increase in anticipatory reactions to a questionnaire given

after the stimulant drug caffeine were interpreted by Eysenck [391] as indicating that the drug eliminated the internal inhibition which normally held anticipatory reactions in check. In 1957 Eysenck [392] postulated that, "Depressant drugs increase cortical inhibition, decrease cortical excitation and thereby produce extraverted behaviour patterns. Stimulant drugs decrease cortical inhibition, increase cortical excitation and thereby produce introverted behaviour patterns."

Eysenck's ideas on the effects of drugs are part of his wider approach to the problem of personality. It would be unfair to him to attempt to discuss here the widespread implications his views have for psychology. In grossly over-simplified terms, it may be said that he views the extravert as being relatively insulated from his environment by virtue of a high degree of cerebral inhibition which raises the threshold to the experiencing of afferent stimulation. This reflects itself in behavioural patterns whence it can be demonstrated successfully that extraverts require a greater degree of stimulation in order to achieve a hypothetical hedonistic level that introverts attain with minimal stimulation. Thus the extravert tends to lead a boisterous existence forever in search of stimulation. Among other sources of stimulation he may well turn to tobacco, alcohol, or drugs whereas the introvert is more likely to spurn these chemical comforts. Paradoxically, the consumption of alcohol will have a depressant effect that will take the extravert still further away from the hedonistic level he seeks (unknowingly?) to attain unless only minimal

quantities are absorbed. The correlation between smoking and carcinoma of the lung may reflect a tendency for extraverts to lead an existence which predisposes them towards this fatal disease.[393]

If depressant drugs have an extraverting effect and excitant ones an introverting effect it should be possible to prove this experimentally by observing their influence on the objective criteria that Eysenck and his school have established in order to assess the standing of individuals on the extravert-introvert scale. Early studies in this field which have now been published in a book edited by Eysenck[394] may be said to confirm, in general, his propositions.

It must be pointed out that Eysenck's ideas have not proved acceptable to many psychiatric workers. They have been referred to here not because they are believed to be correct but only because of the possibility, however remote, that neurophysiologists may on some future occasion correlate the concepts of cortical excitation and inhibition with the activities of the non-specific brain mechanisms elaborated within the reticular, limbic, and possibly other as yet unknown systems. The antagonism which McDougall[386] visualised as existing between the higher cortical activities and primitive emotion may even prove to reflect antagonism between the reticular and limbic systems.

Interdisciplinary Considerations.

The value of an interdisciplinary approach to the

problems of psychopharmacology cannot be overemphasised. The major hypotheses that have been advanced to explain the mode of action of psychotropic drugs at the biochemical level were introduced during a period when rapid advances in the understanding of non-specific brain mechanisms were taking place. Now that neurophysiologists and pharmacologists are able to assess the contribution of the other's discipline it can confidently be expected that future hypotheses will attempt to localise drug action so that no longer will authors talk of drugs as stimulant or depressant merely because of overt behavioural effects. In their 1957 paper [179] Brodie and Shore expressed the opinion that competing "serotonergic" and "adrenergic" systems could exist within the hypothalamus where the greatest amounts of these two hormones are found. Much of their evidence was adduced from a consideration of the effects of the tranquillisers reserpine and chlorpromazine on the autonomic nervous system which they believed was controlled by the balance between the competing hypothalamic systems. Electro-physiological studies have confirmed that these drugs do indeed act on the hypothalamus although their actions are probably not limited to this structure alone.

The Transportation of Drugs within the C.N.S.

A recent review [395] of the physiological transport of drugs concludes that almost nothing is known about the localisation of drugs in the brain beyond the facts that N-acetyl-4-amino-antipyrine (a compound which is not appreciably bound to body tissues) is distributed evenly throughout the gross areas of the brain, [396] isonicotinic

acid hydrazide is localised in the hippocampus,[397] and acetazolamide is concentrated within the caudate nucleus, hippocampus, and hypothalamus.[398] Certainly neurophysiological techniques can help to indicate the major sites of action of drugs but the overall distribution can only be determined by direct chemical methods which unfortunately at present are not wholly reliable. Even so there is still the problem that the distribution of a drug within the brain may not reflect its primary site of action.

Schanker [395] states that studies with a wide array of drugs indicate that the blood-brain barrier exhibits the characteristics of a lipid-like boundary. The early studies of Ferguson with the volatile anaesthetics, referred to above, have now been matched with studies on barbiturates which show [399,400] that there is a relationship between their rate of entry into the brain and their oil to water partition ratio at pH 7.4. This has also been confirmed with other drugs.[396] The finding of the relationship between the clinical effectiveness of a series of tranquillising agents and their ability to lower the surface tension of Ringer's solution (see above) is presumably a reflection of the importance of favourable lipid-water partition coefficients.

The now classical investigations of Albert [401,199] into the role of ionisation in biological systems in general have been extended by other workers to the blood-brain barrier. In accordance with the principles elegantly established by Albert, Waddell and Bather [402] showed that drugs which were salts of weak acids became distributed between brain tissue and plasma according to their pKa value and the pH differential

between these two liquid phases. When the pH of the plasma was lowered the plasma level of the drug decreased while the brain level increased. The converse also held true. Since it is the lipid soluble unionised species which passes through the lipid barrier that constitutes the blood-brain barrier, the lipid solubility of this species is the critical factor. The pKa value of the drug will determine what proportion of it is present in the unionised form at the physiological pH. It appears that the blood-brain barrier is either the brain capillary wall or its surrounding layer of glial cells.[395]

Once a drug has successfully negotiated the blood-brain barrier it is presented with yet another obstacle - the membrane of the cell. Those nerve fibres which are covered with a thick, lipoidal, myelin sheath (white matter) are known to be less readily penetrated by urea or phenobarbitone[398,403,404] than are the non-myelinated fibres and cell bodies of grey matter.

Although many of the factors which affect the pKa or lipid solubility of organic compounds are understood it is not possible to predict a priori what degree of lipid solubility or what pKa will ensure the distribution of a drug to a desired area of the brain. The drug may also undergo undetectable biochemical changes in the presence of enzymes or it may be bound to plasma proteins to such an extent that it cannot be present in sufficient quantity at the desired site of action. Although it is likely that drugs are capable of being bound to naturally occurring lipids, mucopolysaccharides, and other high molecular weight compounds, only binding to plasma proteins has been studied in any detail.[405,406]

Rational Approaches to the Syntheses of New Psychopharmacological Agents.

To his intense discomfort, the medicinal chemist is well aware that very few of his inspired, rational syntheses result in the production of biologically active compounds of any real value. Nonetheless, continuing researches have enabled his art to reach a level where at least much wastage of effort can be avoided and the undesirable characteristics of many drugs can be overcome by molecular modifications. Very often the optimum efficacy can be attained within a series of compounds by a careful theoretical evaluation of the variables affecting drug action. Amongst the factors taken into consideration in this optimisation process is the possibility of altering the chemical structure of the drug so as to improve its binding to its receptor. Since many receptors are believed to be enzymes, proteins, or other macromolecules, it seems likely that the very factors the medicinal chemist uses to improve the drug-receptor binding will also serve to increase the possibility of plasma protein binding or adsorption at other sites of loss.

So remote is the likelihood of attaining a balance between the factors determining drug transport to the desired site of action, binding to macromolecules, resistance to undesired metabolism, high selectivity of action, freedom from undesired effects such as over-prolonged activity, and numerous other factors which are discussed in introductory texts, that the medicinal chemist is compelled to resort to empiricism in his search for new psychotropic compounds.

The empirical approach has been greatly simplified by the realisation that relatively large molecular units are

often associated with certain types of pharmacological action. Thus, hydroxylated β-phenylethylamine systems frequently display adrenergic activity, the thiazide nucleus is often associated with diuretic action, the anthraquinone ring system is present in many purgatives, and tranquillising properties are encountered in many phenothiazines, benzhydrols, and substituted glycerols. Similarly certain features have been proposed as essential for analgesic activity, acetylcholine-like action, or anti-cholinesterase activity.

The knowledge that many psychotomimetic drugs and certain tranquillisers contain the indole nucleus indicates that this molecular unit could serve as a useful sub-structure upon which to build potential psychopharmacological agents. Many variations on this nucleus have been, and will continue to be introduced. This present thesis, however, is concerned with one particular type of variation, namely, the isosteric replacement of the indolic nitrogen by sulphur.

Bio-isosterism.

The concept of isosterism was introduced by Langmuir[407] nearly fifty years ago to explain common features in the physical properties of simple molecules such as carbon monoxide and nitrogen, which possess identical electronic dispositions in their outer valency shells. It has been elaborated by other workers to include larger molecules whose layers of peripheral electrons are identical, e.g., benzene, pyridine, furan, pyrrol and thiophen which each have six π electrons.[301]

The similarity in the physical properties of isosteric compounds has encouraged the medicinal chemist to synthesise new compounds bearing an isosteric relationship to known physiologically active compounds, thereby minimising the introduction of new physico-chemical properties which would probably interfere both with the biological transportation to the ultimate site of action and also with the drug-receptor interaction at this site. The idea that isosteres should possess similar biological properties to those of their analogues is inherent in the term "bio-isosterism" introduced by Friedman.[408] It should be noted, however, that three basic requirements must be satisfied before bio-isosteres can mimic the actions of their analogues. Firstly, they must possess the ability to negotiate the numerous obstacles which impede their progression to the desired site of action. These obstacles are many and diverse. Some are physico-chemical, e.g. adsorption at sites of loss or inactivation, and passage through biological membranes, while others are biochemical, e.g. enzymic inactivation. It is unlikely that the bio-isosteres will react to all these barriers in an identical manner to their analogues, hence some variation in biological activity is very likely to occur. This may be desirable or undesirable depending on the nature of the variation which cannot be known in advance. However, the possibility of a reduction in side-effects or improved transportation to the desired site of action ensures that the bio-isosteric approach will be considered worthwhile.

The second requirement which bio-isosteres must satisfy is the ability to successfully complex with the

receptor at the site of action. Since both the topology and electronic disposition of the bio-isostere will closely resemble that of the active analogue there is a strong possibility that a drug-receptor complex will be formed, i.e. the affinity for the receptor will be high. The third requirement to be met is the ability to induce the specific perturbation leading to the biological response. Since the drug-receptor complex may either have a high or a low intrinsic activity [95] bio-isosteres may mimic or antagonise the action of the analogue they are modelled upon. It is not yet possible to predict the outcome a priori, but either type of activity could lead to useful agents.

Object of the Present Research.

One feature of the bio-isosteric approach that may be of some value is the potential ability it affords the medicinal chemist of improving the selectivity of drug action. A major obstacle to understanding the mode of action of the hallucinogens, the tranquillisers, and other psychoactive compounds is their lack of selectivity. As already discussed, not only do those compounds interfere with the role of serotonin, but they also affect the role of the catecholamines and sometimes even acetylcholine. Isosteric replacement of their indole ring by the benzo-(b)-thiophen nucleus might conceivably render them unable to satisfy the requirements of catecholamine or acetylcholine receptors while still retaining the ability to complex with serotonin receptors. It may be argued that the opposite effect is just as likely to occur,

but there is reason to believe that this is not necessarily so. The lack of specificity of these psychopharmacological agents can be seen as a reflection of their ability to satisfy the requirements of adrenergic and acetylcholine receptors by virtue of a structural similarity to the normal substrates. By modifying their chemical structures so that they bear less resemblance to these normal substrates, the probability of successful interaction with such receptors will be reduced. If the modification is one which, however, is designed to meet the requirements of the serotonin receptor an increase in selectivity may be attained. As a working hypothesis, it is suggested here that the replacement of the nitrogen atom in the indole ring by a sulphur atom will meet the above criteria. A research programme aimed at the synthesis of the benzo-(b)-thiophen isosteres of physiologically active indole alkaloids was initiated in the Department of Experimental Pharmacology, Glasgow University,[409,410] and has been continued in this department, and the present thesis represents an extension of this earlier work.

Failure to achieve this desired selectivity of action need not detract from the value of this research programme since lack of selectivity in itself may prove capable of inducing novel biological effects, such as may occur with LSD. The possibility of uncovering a therapeutically useful response must always be considered. It would, nonetheless, be of considerable value to the physiologist if an effective drug could be prepared which specifically mimicked or antagonised the action of serotonin, or else specifically interfered with the biosynthesis, release,

or metabolism of it.

If such isosteres of the indole alkaloids are shown to be biologically active it may be possible to speculate on the nature of the receptor itself. Nevertheless, conclusions regarding the nature of a receptor which have been deduced from a consideration of the shape of non-rigid molecules must remain purely speculative since there is no reason to assume that the thermodynamically preferred conformation of the active molecule in solution is actually adopted at the receptor site. Further, the receptor itself could conceivably be non-rigid and therefore able to alter its characteristics to suit the steric and electronic requirements of the drug molecule.[411] Indeed, a recent approach towards understanding the fundamental nature of drug action - Belleau's macromolecular perturbation theory - [96] considers the nature of hydrophobic and electrostatic interactions between drug and receptor which will determine whether a specific or non-specific conformational perturbation of the receptor will be induced by the approach of the drug. While only certain molecular characteristics will be capable of producing the single specific perturbation which leads to the biological response, a wider range of molecular features will distort the normal receptor conformation thereby preventing the normal response, that is on the assumption that there is a close enough structural similarity between the normal substrate and the antagonist for it to approach close enough to the receptor. In the light of Belleau's ideas it would appear that the bio-isosteric approach has much to commend it since there is only a minimal change in the hydrophobic/hydrophilic nature of the molecule, while

electrostatic forces are altered quantitatively rather than qualitatively.

A Unified Hypothesis of Drug Action within the C.N.S.

The hypothesis advanced at the beginning of this Introduction, namely that psychotomimetic and tranquillising drugs may exert their characteristic effects through an ability to interfere with the physiological basis of memory, does not appear to be inconsistent with current knowledge of the mode and site of action of psychopharmacological agents. The framework upon which it rests - the hypothetical neural basis of memory - is far from securely established, and until this is so there can be scant justification for its acceptance even were no other objections to be offered.

The contention that hallucinogens alter learned, perceptual discrimination whereas tranquillisers inhibit conditioned patterns of emotional behaviour assumes that these qualitative differences are reflections of the sites of action of these drugs. The general anaesthetics, which can depress cortical function, may indeed destroy the more familiar type of conscious memory, but since they also depress the reticular formation the subjective realisation of this is not feasible. The possibility of discovering an experimental drug which selectively depresses (or arouses) regions of the cortex without affecting other parts of the brain is worthy of investigation.

The likelihood of an early experimental proof of the hypothesis advanced here would appear to be remote. Nevertheless, it is generally accepted that changes in the electrical

potentials recorded from the brain by the electroencephalogram are indicative of a reorientation of impulses within the central nervous system. Thus, it may be argued, the hypothesis is supported by the knowledge that psychotropic drugs produce characteristic changes in the electroencephalogram. However, until more is known about the theoretical basis of the electroencephalographic response considerable caution must be exercised here.

Not only do drugs interfere with synaptic processes, but so also do normal constituents of the body. Although the situation within the brain remains confused, it can surely be accepted that variations in tissue levels of neurohormones or the apparatus concerned with their biosynthesis and metabolism might affect the development of new neuronal pathways during learning and conditioning, as well as the integrity of existing pathways which act as the basis of memory (both conscious and functional), if the postulates mentioned above are accepted.

By considering each of the diverse sub-structures within the brain to have elaborated a functional memory appropriate to its physiological role, it can be seen that localised chemical variations, too obscure, perhaps, to be detected by contemporary techniques, might initiate profound behavioural changes. The serotonin hypothesis, which is concerned with the examination of one type of chemical variation, has occasioned the best documented study of such factors. Since the major obstacle to a clear understanding of the role serotonin fulfils in the brain is the lack of specificity of the drugs at present available, attempts to

prepare specific psychopharmacological agents are of considerable importance.

Insofar as memory is a reflection of personal experience, different individuals, it may be claimed, will elaborate widely differing networks of functional, neural pathways. The ability of his inherited biochemical character, which has developed over countless years of evolution, to permit the creation of new synaptic connections and elicit those previously formed will probably vary from individual to individual, as do most biochemical functions. Herein may lie the basis of personality differences. Indeed, the inhibition of which Eysenck has written (see above) might, perhaps, reflect these biochemical differences which could, it is now suggested, affect the neural basis of memory.

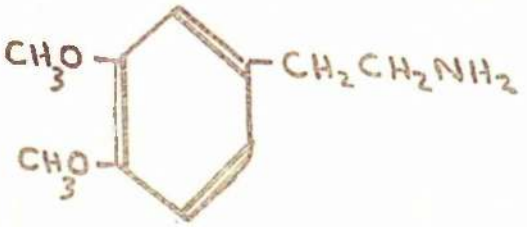
Interference with neurohormonal function, whether arising endogenously or from drug administration, could conceivably occasion those personality changes designated as abnormal. It is not necessary, however, to postulate the existence of a single chemical substance to account for such interference. Variation in any one of the factors concerned with synaptic transmission might be sufficient to assail the integrity of neuronal pathways. That such changes should occur in apparently normal individuals under stress, when hormonal changes are demonstrably evident, or in individuals with certain types of personality, might not seem surprising. Nevertheless, why certain types of experience should give rise to stress in some individuals remains a question for the psychiatrist.

The Genetic Basis of Schizophrenia.

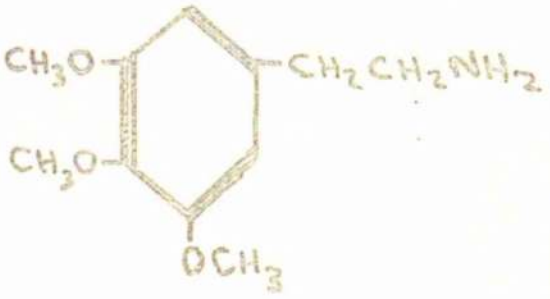
Although it is not necessary to postulate the formation of a single psychotomimetic metabolite to account for the development of mental diseases, there is, nevertheless, good reason to believe that an enzymic malfunction, which could lead to the formation of such a metabolite, exists in schizophrenics. This is based on current knowledge of the genetic background of the disease and the successful isolation of such a metabolite from the urine of schizophrenics.

It is known that schizophrenia is usually based on a single partially dominant gene with low penetrance. Such is the penetrance of this Sc gene that it only induces schizophrenia in about one quarter of the cases where it is present in the genotype. The non-manifestation of the disease in the remaining 75% of Sc carriers has been attributed both to the genetic environment where minor genes affect manifestation, and also to the external environment where familial, social, cultural, and physical factors are involved.[412-414]

The available statistics show the incidence of manifest schizophrenia to be at least 1% in all racial and ethnic types and probably in all social classes.[415] Since there is general agreement among evolutionary geneticists[416,417] that all genetic characters which exist in a population at a higher frequency than can be maintained solely by mutation must involve morphism,[418] Huxley, Mayr, Osmond,



3,4-DIMETHOXYPHENYLETHYLAMINE



MESCALINE

Fig. 10

and Hoffer[419] suggest that the Sc gene must be in morphic balance, and must confer certain selective advantages to compensate for its obvious disadvantages which include a 30% reduction in fertility. These advantages are believed to include a high resistance to surgical and wound shock, to otherwise dangerous concentrations of insulin and other hormones, histamine, etc., and to various allergies and infections.

Huxley and his colleagues[419] believe that the Sc gene causes an error of metabolism, resulting in the formation of some substance capable of interfering with the normal integration of perception. Since the publication of Huxley's paper unequivocal confirmation of the existence of a psychotomimetic metabolite in the urine of schizophrenics, but not of normal controls, has been presented and this will now be briefly discussed.

A Biochemical Abnormality Associated with Schizophrenia.

In 1962, Friedhoff and van Winkle[420] reported the isolation of 3,4-dimethoxyphenylethylamine (Fig. 10) from the urine of 15 out of 19 schizophrenics, but not from that of 14 normal individuals. This metabolite was detectable by chromatographic procedures as a pink spot on the chromatogram. A large scale investigation, planned with exemplary statistical control, has very recently (October, 1965) confirmed these findings. Thus Bourdillon, Clarke, Ridges, Sheppard, Harper, and

Leslie [421,422] have found that of 265 entirely healthy, mentally normal people only one excreted the relevant metabolite, and inconstantly at that. Further, no pink spot could be observed in chromatograms derived from 126 mentally normal hospital in-patients suffering from a variety of conditions including liver disease, chronic neurological states, and pre- and post-operative cases. Experiments were next conducted on mentally abnormal hospital in-patients some of whom were schizophrenic and some otherwise disturbed. Of 101 such patients pink spots were absent from 16 out of the 17 patients later assessed as non-schizophrenic. Of the remaining cases, where a subsequent diagnosis of schizophrenia was made, the pink spot was present in 46 cases, absent in 27 and impossible to assess in a further 11.

Of particular significance was the high association of pink spots with the so-called "non-paranoid" type of schizophrenia, typified by hallucinations, thinking aloud, and catatonia. The pink spot was observed in 80% of such cases, although the association in "paranoid" schizophrenia was only of the order of 10%.

In a further experiment, 296 mentally abnormal people were examined using a "double-blind" technique where the samples were delivered to the biochemist without his being aware of what type of person they had been collected from. Results paralleled those of the earlier experiments.

Since 3,4-dimethoxyphenylethylamine appears to

be an abnormal metabolite in schizophrenics it suggests the presence of a 4-O-methyltransferase in these patients.[420] It has already been shown that 3,4-dihydroxyphenylethylamine is metabolised by the action of 3-O-methyltransferase in brain homogenates[423] and can be 4-O-methylated by liver homogenates in the presence of S-adenosyl-methionine.[424]

While it is tempting to believe that the presence of 3,4-dimethoxyphenylethylamine leads to schizophrenia it must be borne in mind that, in humans, tolerance develops to all known hallucinogens. The nature of this tolerance is such that chronic administration eventually fails to produce a biological response unless administration of the drug is interrupted for several days; thereafter, a normal response can be attained.[425-427] Mescaline is no exception and, presumably, the same state of affairs will apply so far as 3,4-dimethoxyphenylethylamine is concerned. The problem that now arises is that if this compound causes schizophrenic elaborations why does tolerance not develop since it is always present in non-paranoid schizophrenics? Presumably the nature of tolerance to psychotomimetics could somehow be concerned with biological transport mechanisms. Alternatively, the 3,4-dimethoxyphenylethylamine might not be the cause, but rather the result of abnormal, gene-linked, enzymic activity. This serves to emphasise the difficulty, stressed at the outset, of obtaining significant

information from the analysis of body fluids. Nevertheless, it is clear that this recent work may represent a rational basis upon which the medicinal chemist can design potential therapeutic agents. Surely no other field can offer the medicinal chemist a greater challenge than this?

Sociological Implications of Recent Trends in Psychopharmacology.

Despite the promising trends discussed above, this introduction must end on a note of caution. The late Aldous Huxley first wrote of a universal panacea, which he called "soma", in "Brave New World",[428] published in 1932. In this novel Huxley described life in a society that had introduced a euphoric drug devoid of undesirable side effects, and readily available to all who sought it. He showed how this drug became a powerful instrument in the hands of a dictatorship which exploited the dependance of the masses on the fantasy world of "soma". By the beginning of this decade Huxley was convinced that chemists were near to developing such a drug. He expressed the view[429] that recent advances in pharmacology marked the dawn of a fateful era in which technology would progress from control of man's environment to control of man's mind.

In a detailed sociological survey of the use and users of LSD, which has recently been published, Blum[430] warns that in the United States its use "has

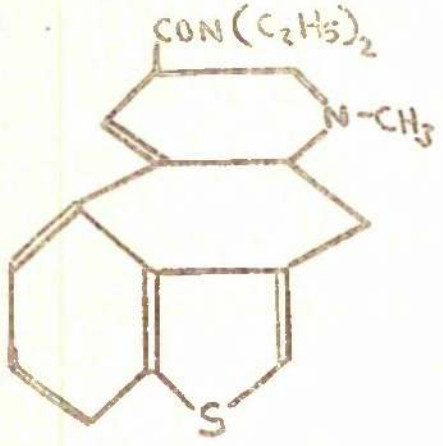
become institutionalised as a drug movement among intellectuals and professional people who resort to the drug not primarily for therapeutic or research purposes but as a means to psychedelic experiences that may be religious, mystical, aesthetic, philosophical, or emotional in nature. This turning towards the values of the inner life suggests a rejection of, or at least a withdrawal from, the goals and rewards of a competitive, acquisitive, and ambitious society by the very group that may most easily achieve and exploit them."

It may be that so long as the chemist is aware of the moral issues that can arise from his work all is well. But can he be certain that in the future his allegiance to science, and his duty to the sick, will not conflict with the wider interests of humanity at large?

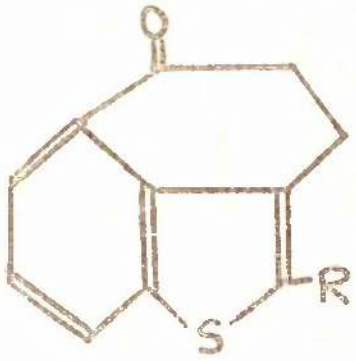
DISCUSSION.

Part I

Synthetic Approaches to the Benzo-(b)-thiophen Isostere of Lysergic Acid Diethylamide



I



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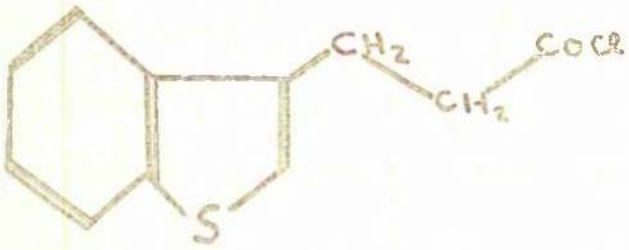

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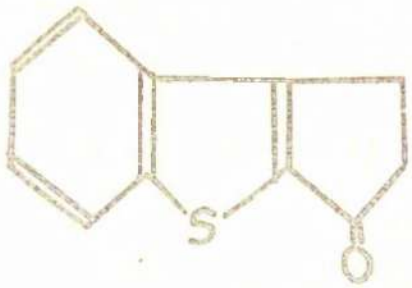

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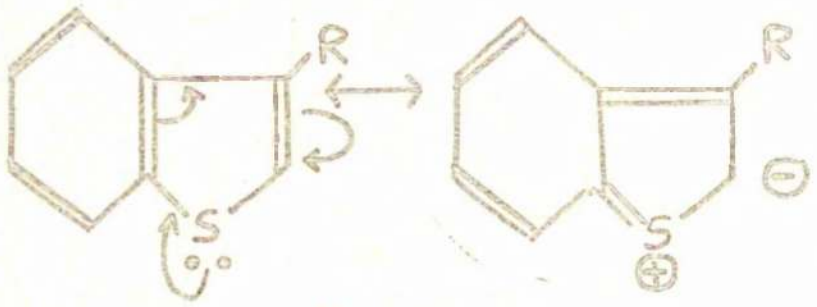
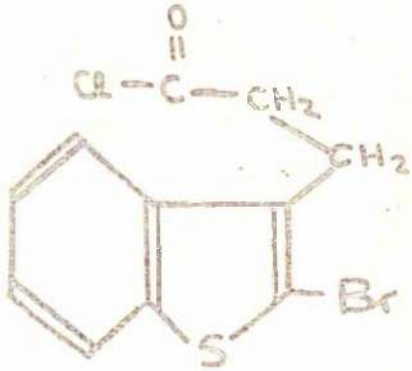


V



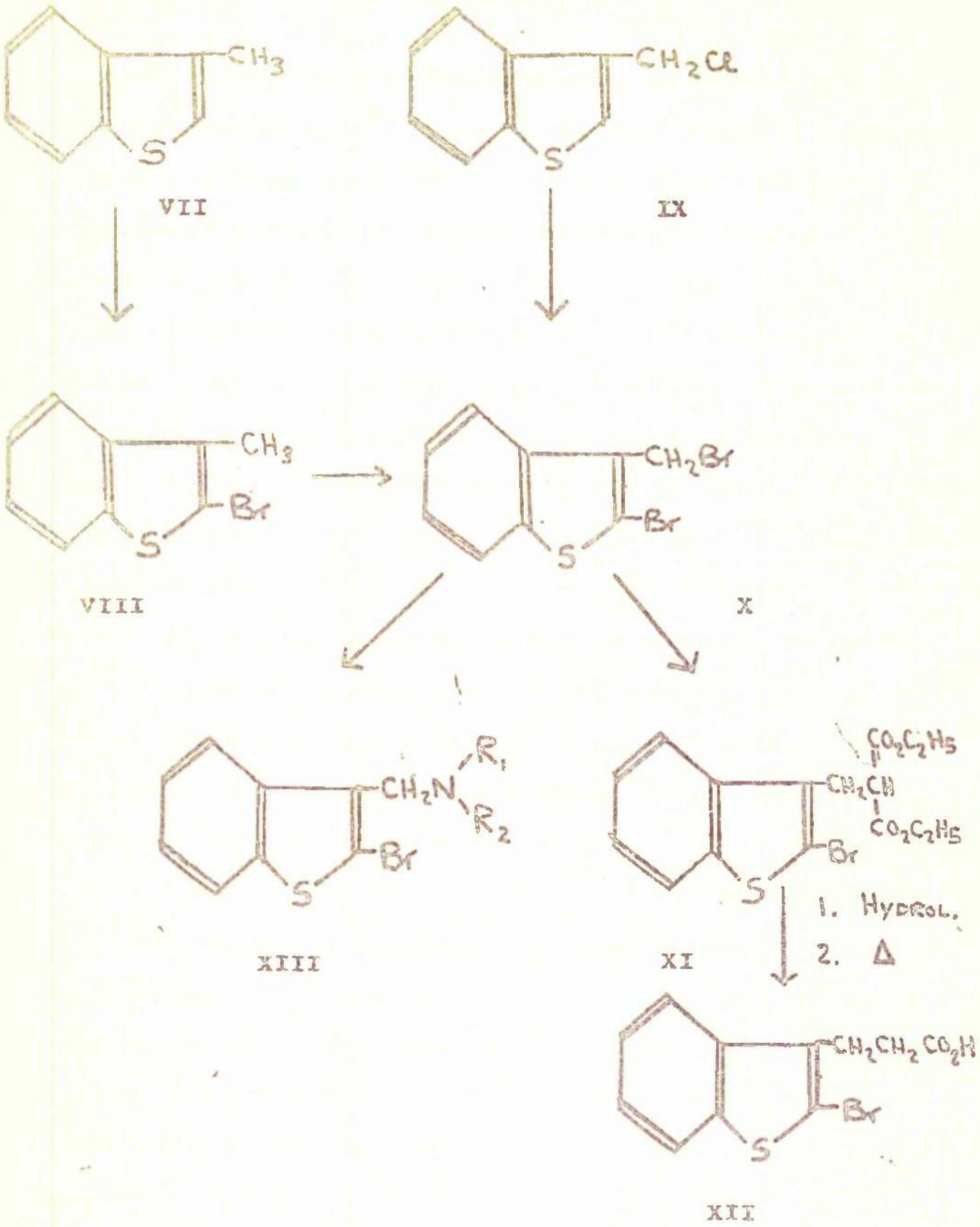
VI

The first line of approach towards a synthesis of the benzo-(b)-thiophen isostere (I) of lysergic acid diethylamide to be undertaken hinged upon attempts to prepare an intermediate tricyclic ketone of type II, in which the benzo-(b)-thiophen nucleus had already been incorporated.

Since it had been established[431] that application of the Friedel-Craft reaction to benzo-(b)-thiophen-3-propionyl chloride (III) gave ring closure solely to the 2-position to yield IV, in accordance with the expected activation to electrophilic attack of this position (V), an investigation was undertaken of the behaviour under Friedel-Craft conditions of a benzo-(b)-thiophen-3-propionyl chloride possessing a blocked 2-position, in order to ascertain whether or not ring closure would occur to the 4-position in this type of compound. In view of its ease of preparation the 2-substituted benzo-(b)-thiophen-3-propionyl chloride used in these experiments was the 2-bromo-compound (VI). Its synthesis, which utilised 2-bromo-3-bromomethylbenzo-(b)-thiophen (X) as a key intermediate, is outlined in Scheme I.

Compound X, had previously been prepared by Gaertner[432,433] who converted 3-methylbenzo-(b)-thiophen[434] (VII) into 2-bromo-3-methylbenzo-(b)-thiophen (VIII) which, in turn, was treated with N-bromosuccinimide in carbon tetrachloride solution to furnish X.

In a new route to X developed in the present work,

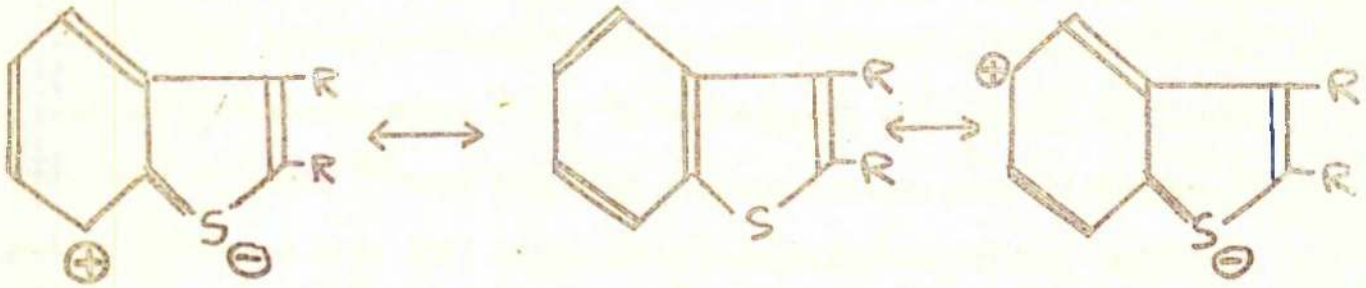

CH3
S
VII
CH2Cl
S
IX
CH3
Br
S
VIII
CH2Br
Br
S
X
CH2N
R1
R2
Br
S
XIII
CO2C2H5
CH2CH
CO2C2H5
Br
S
1. Hydrol.
2. Δ
XI
CH2CH2CO2H
Br
S
XII

Scheme I

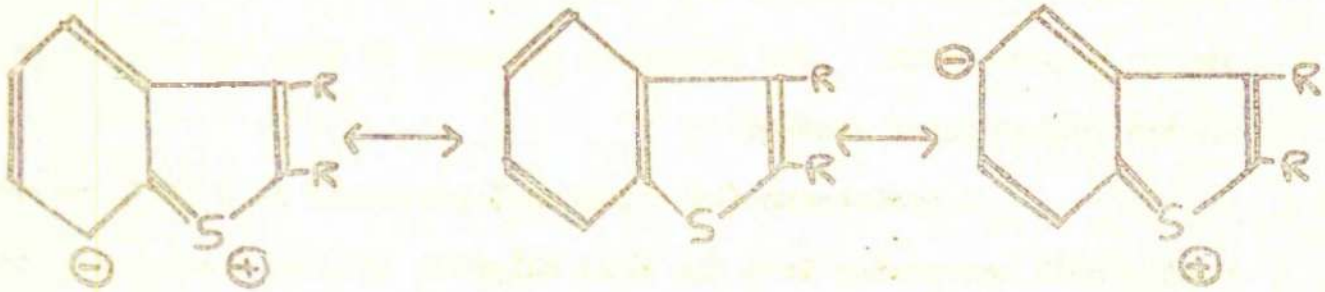
benzo-(b)-thiophen was chloromethylated by a modification of the procedure described by Blicke and Sheets,[435] and the resulting 3-chloromethylbenzo-(b)-thiophen (IX) treated with bromine in acetic acid. Under these conditions not only did electrophilic substitution by bromine occur in position 2 of the benzo-(b)-thiophen nucleus, but also nucleophilic substitution of chlorine by bromine occurred in the benzylic position to give compound X in one stage. Compound X was then converted into 2-bromobenzo-(b)-thiophen-3-propionic acid (XII) by means of a malonic ester synthesis modelled on the procedure of Cagniant.[431] An attempt to synthesise XII via an alternative route through direct bromination of benzo-(b)-thiophen-3-propionic acid was unsuccessful insofar as a partially brominated product only resulted.

In view of the success reported by Harley-Mason[436] in the preparation of several indole-3-propionic acids through heating certain indoles with β-propiolactone, similar experiments were conducted with benzo-(b)-thiophen, but these resulted only in recovery of starting material.

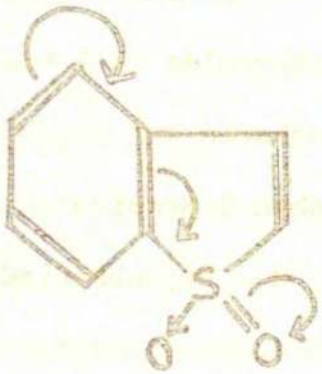
The 2-bromo-3-bromomethylbenzo-(b)-thiophen (X), prepared as shown in Scheme I, was also employed as an intermediate for the preparation of a number of 2-bromo-substituted gramine isosteres (XIII) analogous to certain compounds prepared by Reid.[409] Since the compounds described by Reid were conspicuously lacking in specificity of pharmacological action,[437] the possibility of preparing new derivatives less able to interact with certain receptors as a result of the



XIII



XIV



XV

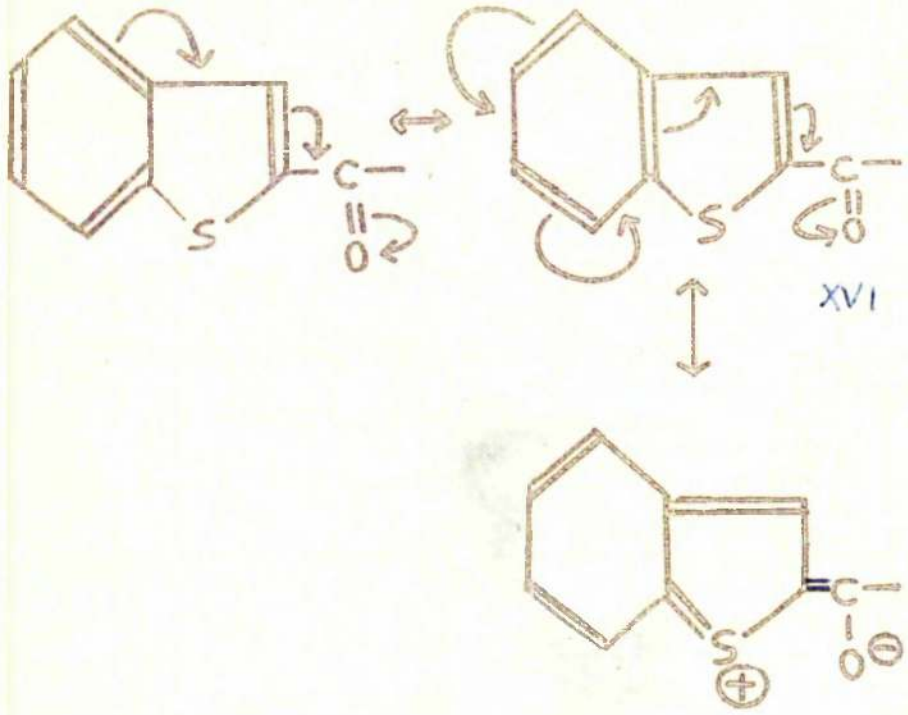
introduction of a bromine atom at the 2-position seemed attractive enough to justify the preparation of the 2-bromo-gramine analogues described in this present work. Although Reid[409] found it necessary to reflux 3-chloromethylbenzo-(b)-thiophen (IX) with secondary amines in the presence of sodamide in order to prepare his gramine-like compounds, Chapman, Clarke, and Iddon[438] reported the preparation of several tertiary amines through direct condensation of 5-substituted-3-bromomethylbenzo-(b)-thiophens with the appropriate amines in dry benzene, and application of this milder method was found to be adequate in the present work. The compounds prepared in this way are described in the experimental section.

2-Bromobenzo-(b)-thiophen-3-propionic acid (XII) underwent smooth conversion into the acid chloride (VI) on treatment with thionyl chloride. However, employing a variety of different reaction conditions (including use of the catalysts aluminium chloride, stannous chloride, and boron tribromide on 2-bromobenzo-(b)-thiophen-3-propionyl chloride, and polyphosphoric and sulphuric acids on the corresponding 2-bromobenzo-(b)-thiophen-3-propionic acid), in no case was intramolecular acylation to II, R = Br, observed. Recovery of variable yields of 2-bromobenzo-(b)-thiophen-3-propionic acid from these experiments would suggest the possibility of intermolecular acylations taking place to yield polymeric material, although in no case was such polymeric material characterised. Any such occurrence of intermolecular acylations without intramolecular cyclisation to the 4-position on

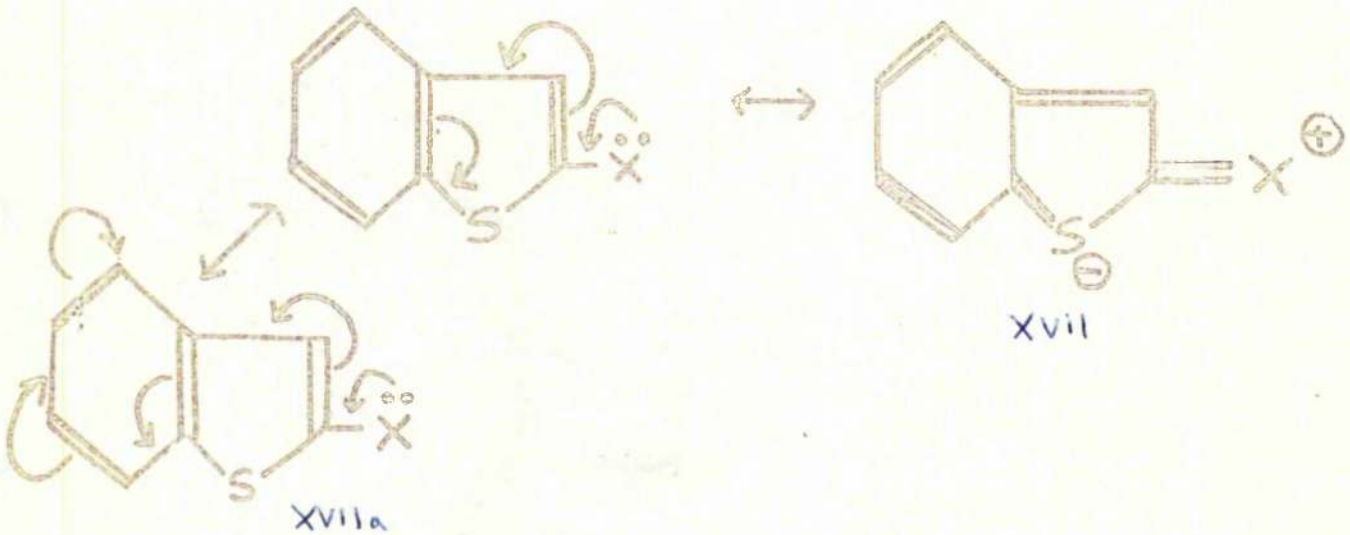
exposure

of 2-bromobenzo-(b)-thiophen-3-propionyl chloride to Friedel-Craft reaction conditions could be readily rationalised in terms of the known behaviour of 2,3-disubstituted benzo-(b)-thiophens to electrophilic reagents, where it is established[439,440] that attack occurs predominately at the 6-position thus establishing the 4-position as a less favoured site of electrophilic attack.

The favoured nature of the 6-position as the prime point of electrophilic attack in 2,3-disubstituted benzo-(b)-thiophens is of some considerable interest insofar as it indicates that canonical forms involving expansion of the sulphur atom octet (XIII) which leave positions 4 and 6 richer in electron density than positions 5 and 7 (i.e. the sulphur atom is acting as a meta-directing group) are preferred to canonical forms involving ortho- or para-quinonoid systems electron deficient on sulphur (XIV)[441] which would be expected to give rise to electrophilic substitution in the 7- or 5-positions. The ability of the octet of electrons on the sulphur atom to expand to a decet (or even a duodecet) is due to the ease with which two of its electrons can occupy pd^2 orbitals, thus facilitating conjugation with π-electrons contributed by the carbon atoms.[442,443] The existence of peri-interaction between substituents in positions 3 and 4 of the benzo-(b)-thiophen nucleus would explain why electrophilic attack does not take place at the 4-position but occurs virtually exclusively at the 6-position (which is also the position of electrophilic substitution in benzo-(b)-thiophen-1,1-dioxide (XV)[444]) since both positions 4 and 6 would appear to have



XVI



XVII

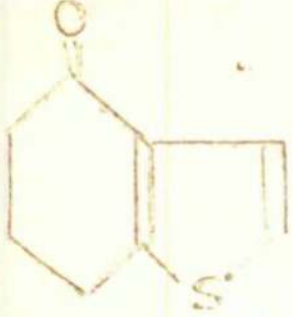
XVIIa

identical electronic activations.

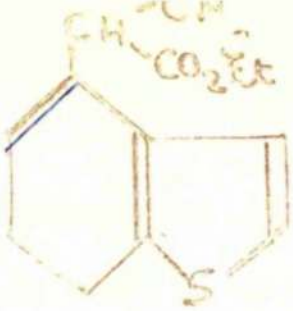
The fact that positions 4 and 6 suffer identical electronic activations (or deactivations) with electrophilic attack being favoured at position 6 would also imply that little was to be gained by preparing further 2-substituted benzo-(b)-thiophen-3-propionyl chlorides possessing a substituent other than bromine in the 2-position in attempts to facilitate cyclisation at position 4. In any case, if a strong meta-directing group were to replace the mild ortho- para-directing bromine atom in the 2-position deactivation would be expected at position 4 (and 6) - see XVI - whilst a strong ortho- para-directing group could also deactivate position 4 through suppression of XIII by XVII, although canonical form XVIIa would be expected to favour attack at position 4.

With the failure to achieve cyclisation of VI to yield II, an alternative approach to the synthesis of I was sought.

The ready availability of 4-oxo-4,5,6,7-tetrahydrobenzo-(b)-thiophen (XVIII) via a four stage synthesis from thiophen[445] made a route (such as that shown in Scheme II) based upon this compound appear attractive. Indeed, utilising a method introduced by Cope[446] this ketone was successfully condensed with cyanoacetic ester in the presence of acetic acid and ammonium acetate, the water which was eliminated as the reaction proceeded being removed by continuous azeotroping with benzene. The problem of determining whether the

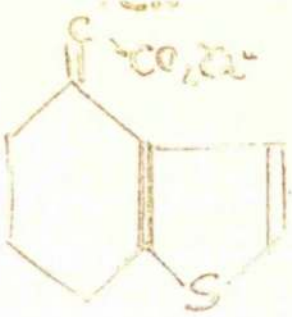


XVIII

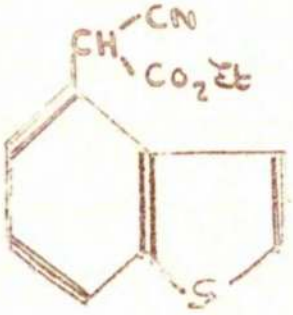


XX a

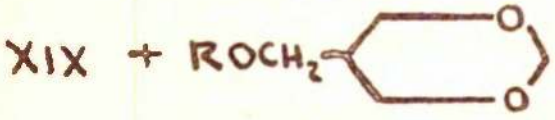
OR



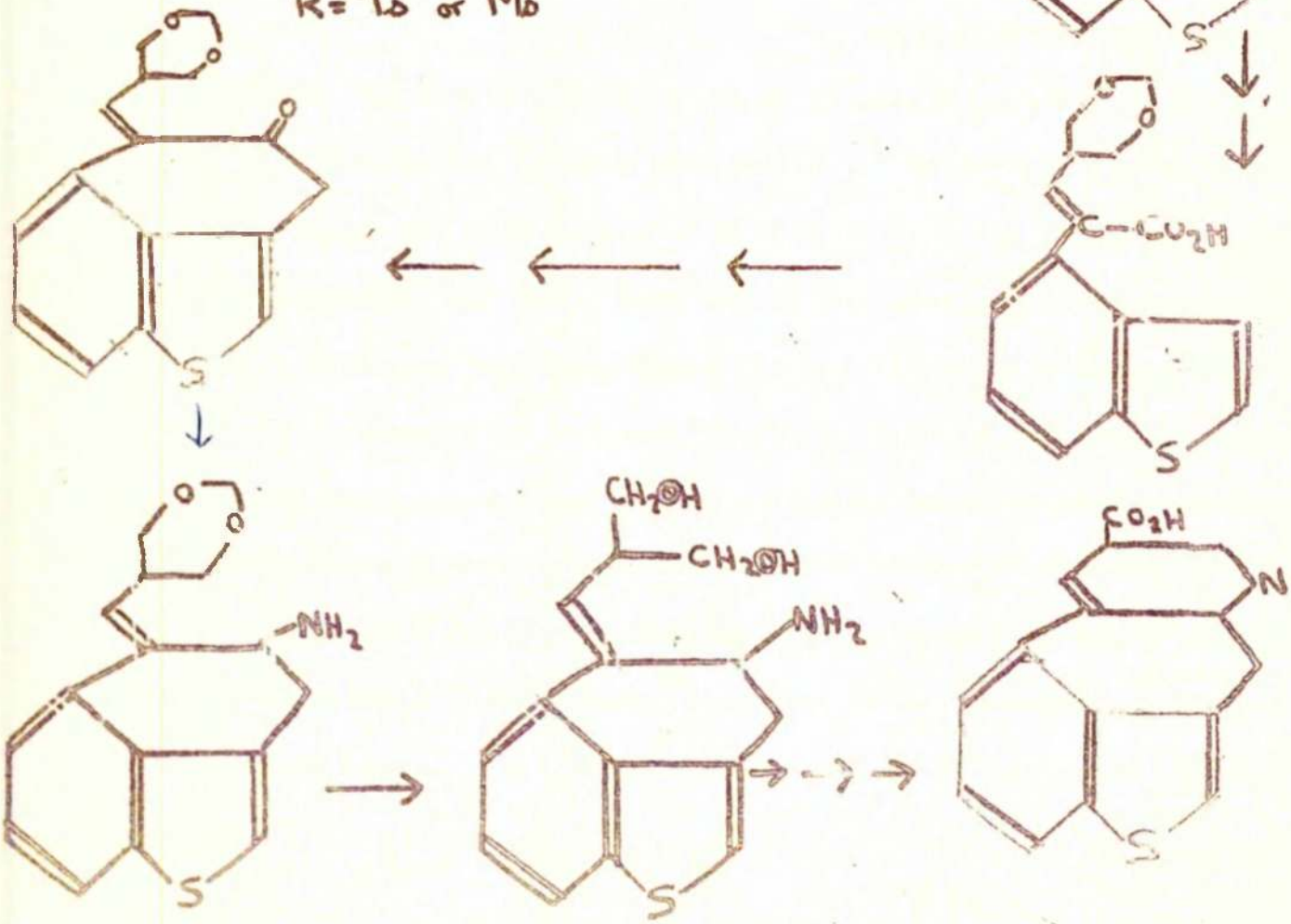
XX b



XIX



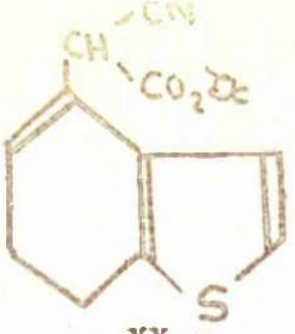
R= Tb or Mb



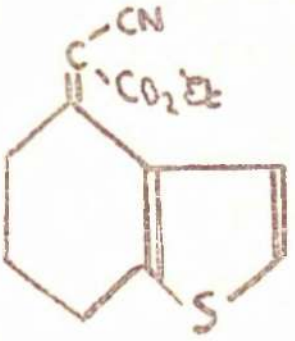
Scheme II

resultant condensation product contained an endocyclic (XXa) or an exocyclic (XXb) double bond was resolved by means of nuclear magnetic resonance spectroscopy which indicated the presence of but 2 vinylic protons, i.e. vinylic protons were present on the 2- and 3-, but not 5-, positions of the reduced benzo-(b)-thiophen nucleus. Further, the protons on the 2- and 3-positions gave the expected AB quartet with $J_{2,3}$ = 5.5 C.P.S. and ν_{H_2} = 2.82τ, ν_{H_3} = 1.77τ. Thus it is seen that the signal originating from H_3 is well downfield from that of TMS. Since deshielding by the carboethoxy group is believed to be much greater than that by the cyano group,[447] the full structure of the compound is likely to be that shown in XXc where the cyano group is trans to the thiophen ring.

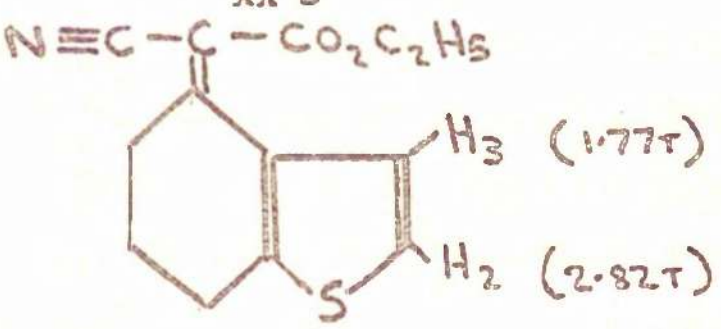
The condensation product (XXc) resisted all attempts at dehydrogenation to yield XIX using both sulphur and chloranil.[448] Difficulty might indeed have been anticipated with the double bond in the exocyclic position but it had been hoped that establishment of an equilibrium between XXa and XXb would have been possible at high temperatures, thus permitting dehydrogenation to proceed. The projected route outlined in Scheme II was not investigated further but was abandoned in favour of attempts to achieve direct replacement of the -NH- group of oxindoles by a sulphur atom since development of a successful conversion would open up a general route applicable to the synthesis of benzo-(b)-thiophen isosteres of many complex indole



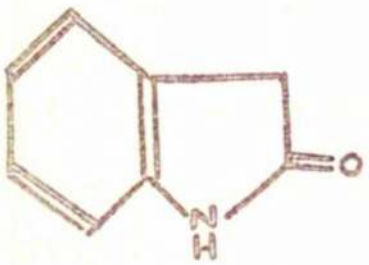
XX a



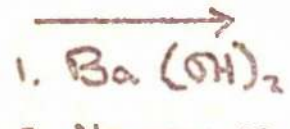
XX b

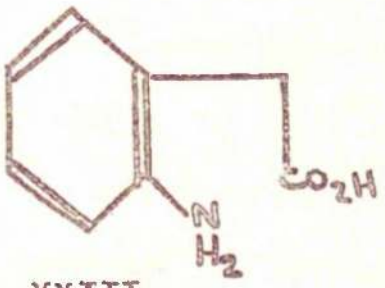


XX c

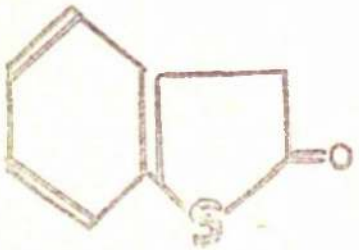


XXII

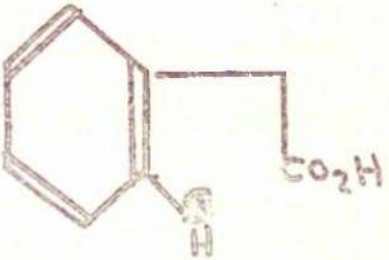




XXIII



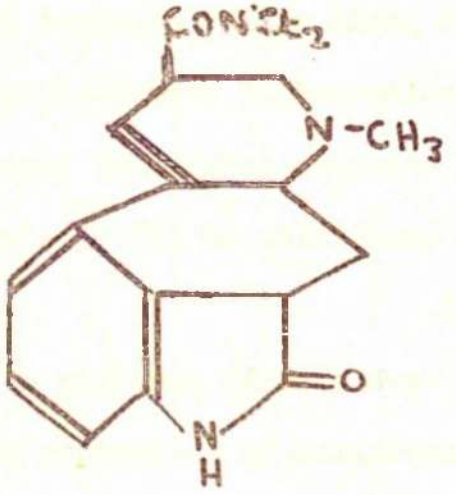
XXI



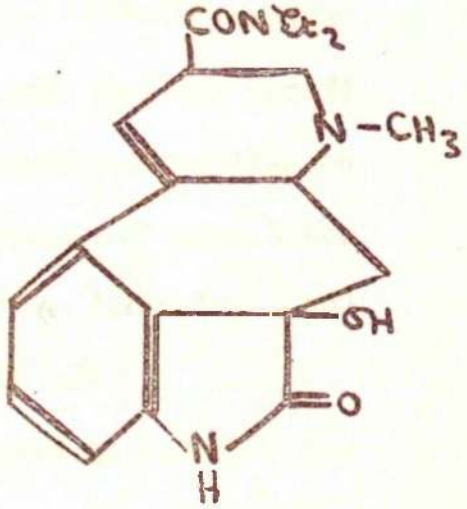
XXIV

derivatives, so avoiding lengthy individual syntheses for each isostere. That such a direct displacement of the -NH- group by sulphur might be feasible was suggested by experiments reported in the literature. Firstly, in 1912, Marschalk[449,450] reported the preparation of thio-oxindole (XXI) by means of alkaline hydrolysis of oxindole (XXII), diazotisation of the exposed primary amine (XXIII), and conversion of the diazonium salt into o-mercaptophenylacetic acid (XXIV) before ring closure to XXI. The original procedure gave low yields of thio-oxindole as a result of inefficient ring closure in the o-mercaptophenylacetic acid but a modification reported by Glauert and Mann[451] permits facile ring closure to give a 90% yield of thio-oxindole. Secondly, in 1912, Friedländer and Woroschzow[452] synthesised 2H-naphtho-(1,8)-thiophen-2-one from the corresponding oxindole by a similar method. Another example of replacement of the oxindole -NH- by S-, which may be pertinent, is the conversion of oxindole into thio-oxindole by the action of phosphorus trisulphide.[453]

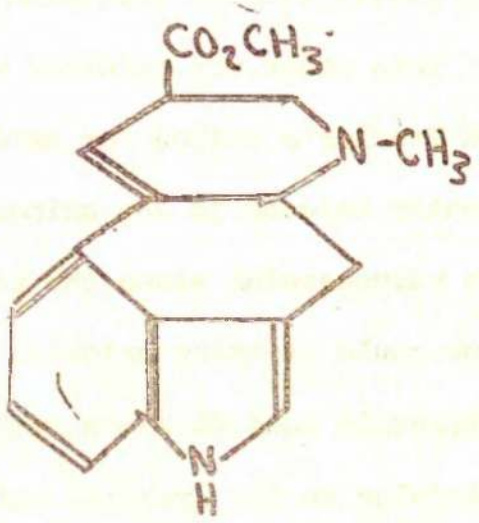
The possibility of applying an approach based on these findings to the syntheses of isosteres of complex indole alkaloids received additional impetus from the report that the oxindole (XXV) prepared from lysergic acid diethylamide could be hydrolysed by heating in dilute sodium hydroxide solution, and the free amino group so liberated diazotised and coupled with β-naphthol.[454,455] Moreover, oxindole XXV, which is a metabolic product able to be recovered after



XXV



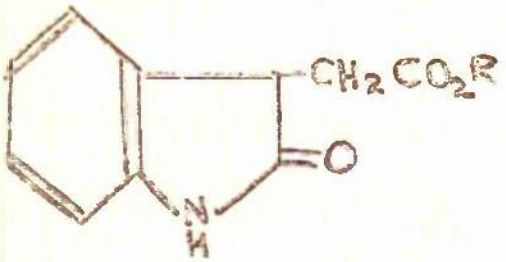
XXVII



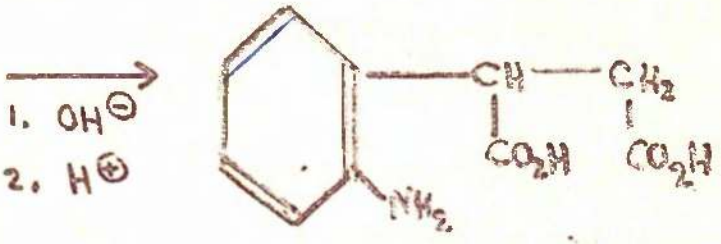
XXVI

LSD administration, has been synthesised from LSD by two groups of workers.[456,457,458] Although the yield of XXV obtained from these syntheses was low, the recent development of fermentation processes permitting the preparation of kilogram batches of lysergic acid[459,460] would seem to secure adequate quantities of XXV as a starting material for a potential synthesis of I.

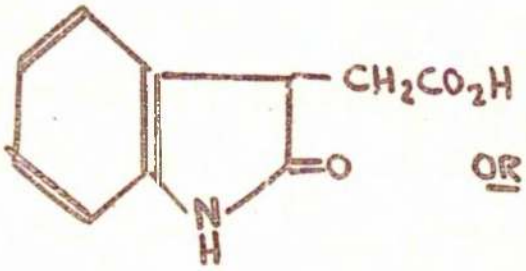
For the present work a small quantity of lysergic acid was obtained by hydrolysing ergotoxine in methanolic potassium hydroxide, according to the method introduced by Jacobs and Craig.[461] Since the conversion of lysergic acid into LSD presents considerable technical difficulties,[462] the available lysergic acid was converted into its methyl ester (XXVI) through treatment with diazomethane by an established method.[461] This ester was oxidised with hypochlorite solution by analogy with Troxler's method for oxidising LSD into a dioxindole which was subsequently reduced to the oxindole XXV. This present experiment, however, was unsuccessful since the required dioxindole could not be isolated, nor could starting material be recovered. A similar failure to oxidise lysergic acid α-hydroxyethylamide suggests that the preparation of dioxindoles in the lysergic acid field is highly sensitive to substituents and probably reaction conditions. Indeed, Dr. Troxler[463] has intimated that lysergic acid methyl ester would only be converted to an oxindole with difficulty since it can be expected that such an oxindole would be extremely sensitive and unstable. It is clear that



XXVIII



XXIX



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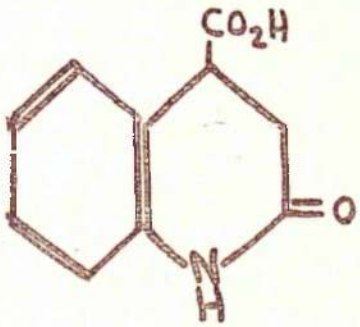
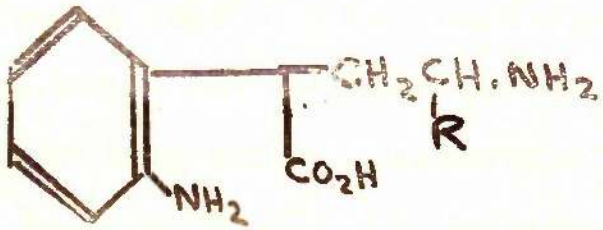
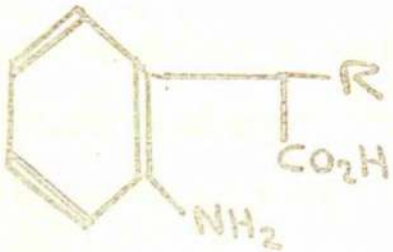


XXX a

XXX b



XXXI

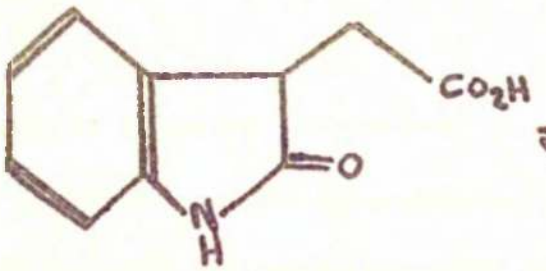


XXXII

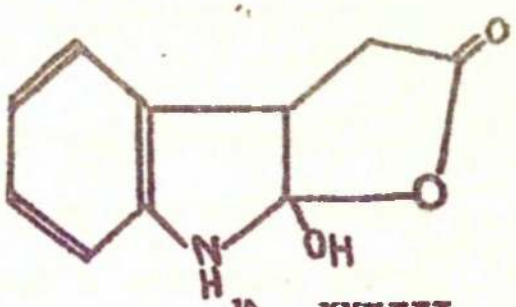
any further work in this field is highly dependent upon a supply of XXV being available.

Turning to the possibility of replacement of the -NH- in simpler indole derivatives via a diazotisation procedure on the corresponding oxindoles, it is readily apparent that such an approach is somewhat restricted in application. For example, in those cases where a suitably disposed electrophilic group is present in the side chain attached to the 3-position of the oxindole nucleus, the nitrogen atom of the liberated amino function will be susceptible to intramolecular attack. Moreover, even if diazotisation of the amino acid and replacement of the amino group by a sulphydryl function were achieved, in such cases without prior attack on the nitrogen atom occurring, the possibility of similar electrophilic attack on sulphur is a further limitation. On the other hand, the presence of a nucleophilic group in the side chain could lead to intramolecular nucleophilic attack in the diazonium salt once this had been formed.

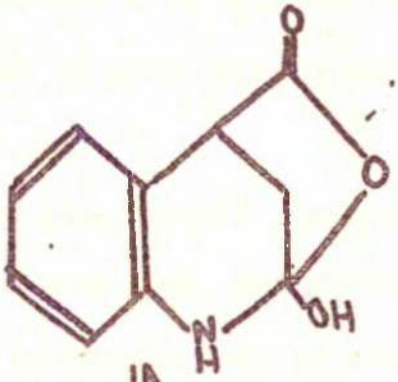
Consideration of the hydrolytic product (XXIX) obtained from the 3-substituted oxindole (XXVIII) shows that ring closure to either a 5-membered (XXXa) or 6-membered ring (XXXb) may occur. Aeschlimann[464] investigated this situation and concluded that in those cases where either the quinolone or oxindole structure can be formed upon ring closure the former would be preferentially produced, in agreement with the generalisation that 6-membered rings are more stable

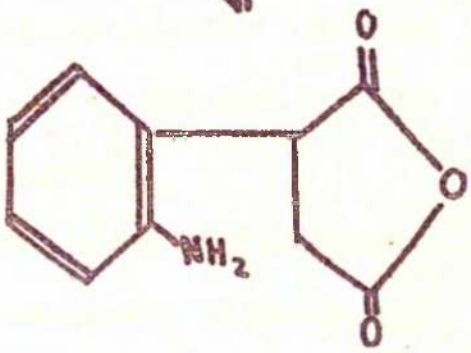


XXX a

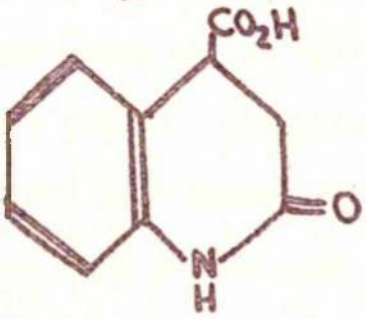


XXXIII

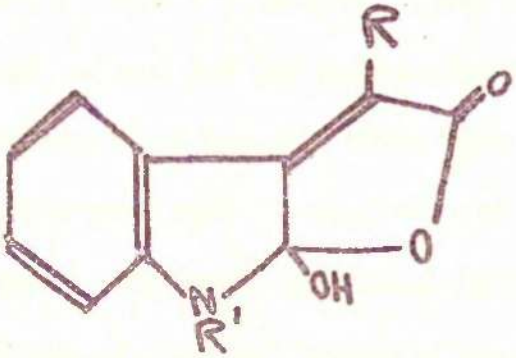




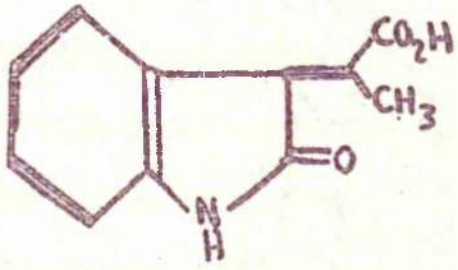
XXXIV



XXXV



XXXVI



XXXVII

than 5-membered rings as a result of diminished internal strain. Hence, compound XXXb will be formed in preference to XXXa. However, in the case of the conversion of oxindole into thio-oxindole the absence of a side chain at the 3-position eliminates the possibility of undesired ring expansion. Similarly, ring expansion of the pyrrole ring in the oxindole derived from LSD is not possible.

An examination of the literature reveals only two instances where the open chain amino acid intermediates have been detected upon hydrolysis of oxindoles potentially capable of ring expansion to quinoline derivatives.[465,466] This suggests that the energy barrier to such ring expansion is so small as to limit the ability of the amino acid to lead an independent existence in the reaction mixture. On the other hand, the greater internal strain developed in the 5-membered ring system may ensure that in those compounds where this is the only system that can be formed upon ring closure, isolation of the open chain amino acid is feasible since the energy barrier towards ring closure is comparatively greater.

Confusion about the stability of o-aminophenylacetic acids (XXXII) exists in the literature. Baeyer,[467] who believed that o-aminophenylacetic acid could only exist in the lactam form as oxindole, reduced o-nitrophenylacetic acid with tin and hydrochloric acid and indeed isolated oxindole as his product. Subsequently several other workers synthesised substituted oxindoles from phenylactic acids, thereby apparently corroborating Baeyer's belief in the non-existence of

o-aminophenylacetic acids. Despite earlier demonstrations that the primary amines liberated by baryta hydrolysis of oxindole and of a tricyclic oxindole (see above) could be successfully diazotised in acid solution,[449,450,452] Baeyer's contention was generally accepted until as late as 1922 when Neber[468] actually isolated o-aminophenylacetic acid. The significance of such diazotications appears to have been overlooked both by Neber and his contemporaries, as well as by subsequent reviewers. Neber himself reported both in his original paper[468] and in a later publication,[469] that o-aminophenylacetic acid could be successfully diazotised and coupled with β-naphthol to produce a red dyestuff. Yet he made the claim that this o-aminophenylacetic acid (prepared by reduction of o-nitrophenylacetic acid with ferrous sulphate and ammonium hydroxide, then acidification in the cold with exactly one mole of acid) was quantitatively converted to oxindole on treatment with excess acid. In view of the reported diazotisation in acid solution it is clear that, contrary to general belief, o-aminophenylacetic acid (and those derivatives of it which can be diazotised) can indeed exist in solutions of low pH. It may be significant that the diazotisations were performed at a low temperature (as is usual) since it has been observed that o-aminophenylsuccinic acid (XXIX) is converted into 2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid (XXXb) upon standing in vacuo at room temperature for 48 hours, or by heating at 100° for two hours.[464] Although neutralisation or acidification of the salt formed by alkaline hydrolysis of an oxindole is an essential prerequisite

for ring closure, it would appear that a source of (thermal) energy might have to be provided before condensation is possible in certain instances, as with compounds which cannot form the thermodynamically favoured 6-membered ring. It is possible that the heat of neutralisation of the alkaline reaction mixture provides the necessary energy. Neber's statement[468] that neutralisation of the barium salt of o-aminophenylacetic acid has to be performed in the cold, lends support to this contention.

A further objection to the selection of oxindoles where rearrangement via the intermediate amide cleavage product can occur, arises from uncertainty of the constitution of such compounds since hydrolysis and rearrangement may have taken place undetected during their synthesis. Indeed, this has been a source of considerable confusion in the literature. For example, the compound originally believed[470] to be oxindole-3-acetamide is now, in fact, known to be 2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxamide, hydrolysis of the oxindole ring having taken place in the course of the synthesis, with subsequent ring closure to the thermodynamically preferred 6-membered ring system.[471] Although the ethyl ester of oxindole-3-acetic acid (XXVIII, R = C_2H_5) had been prepared,[470,472] early attempts to prepare the parent acid (XXXa) resulted in the production of 2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid (XXXb). This was not realised by the original workers, but it was later pointed out that their syntheses involved such vigorous hydrolysis, either by acids or bases,

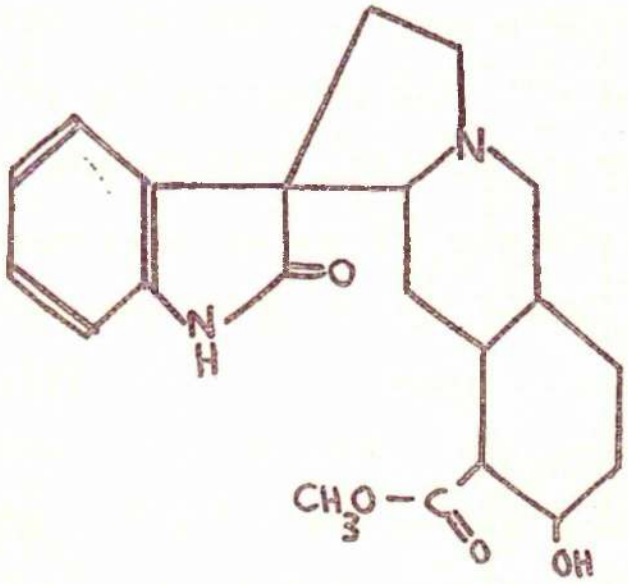
that any oxindole-3-acetic acid which formed would certainly have been converted into o-aminophenylsuccinic acid (XXIX) which, in turn, would ring close to the quinolone (XXXb).[471] In fact, Sumpter[473] actually stated later that oxindole-3-acetic acid could not exist, but Julian, Printy, Ketcham, and Doone[474] finally removed all doubt by preparing authentic oxindole-3-acetic acid utilising reaction conditions which precluded ring cleavage. Similarly, Kendall, Osterberg, and MacKenzie[475] were able to prepare oxindole-3-propionic acid although another group of workers,[476] employing reaction conditions which would have permitted hydrolytic cleavage, also claimed to have synthesised this acid. Their product, however, was not identical with that obtained by Kendall and his associates.

The susceptibility of certain oxindole derivatives, such as oxindole-3-acetamide, oxindole-3-acetic acid, or oxindole-3-propionic acid, to either alkaline or acid hydrolysis is surprising in view of the vigorous conditions necesaary for cleavage of the amide linkage in oxindole itself. Further, the contention which has been presented here, namely that warming o-aminophenylacetic acid derivatives in acid solution will effect ring closure, would appear to be contradicted by the ability of the oxindole derivatives in question to be acid hydrolysed, as evidenced by their rearrangement to quinolone derivatives. This paradox, however, is satisfactorily resolved by reference to a paper by Wenkert and Reid[477] who showed that the side chain attached to the oxindole

nucleus at the 3-position facilitated the hydrolysis. For example, oxindole-3-acetic acid (XXXa) possesses a side chain which can interact with the carbonyl of the lactam (a process analogous to that occurring with γ-ketoacids) thereby yielding the pseudo-acid (XXXIII). This can then rearrange to a pseudo-anhydride (XXXIV) which, in turn, can give rise to the quinolone (XXXV). Attainment of overall equilibrium can be catalysed by both acids and bases with the possibility existing of any of the reaction intermediates being hydrolysed to o-aminophenylsuccinic acid under the correct experimental conditions, although this is not necessarily an essential precursor of the quinolone.

Wenkert and Reid[477] also suggest that the presence of steric factors which may restrict rotation of the side chain carboxyl group and hold it rigidly in close proximity to the lactam linkage would diminish the stability of the oxindole nucleus, thereby explaining the susceptibility of substituted carboxymethylene oxindoles to hydrolysis. The short-lived intermediate carboxymethylene compounds would be expected to exist exclusively in their pseudo-acid form (XXXVI). This internal interaction can only occur if the carboxyl and amide groups are initially cis towards each other. If R is equal to, or larger than carboxyl this will certainly be the case, but in (XXXVII) the carboxyl group is trans to the lactam, thus apparently explaining the observed resistance of this compound to hydrolysis.[474]

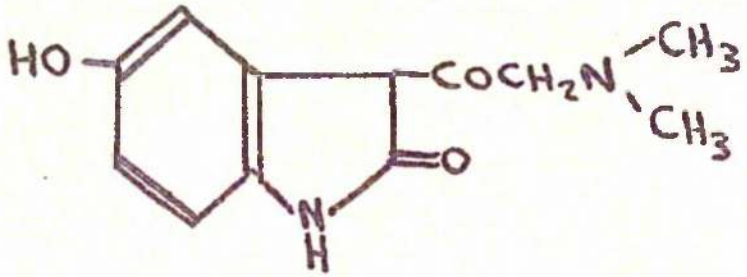
Wenkert and Reid[477] have also developed the concept that



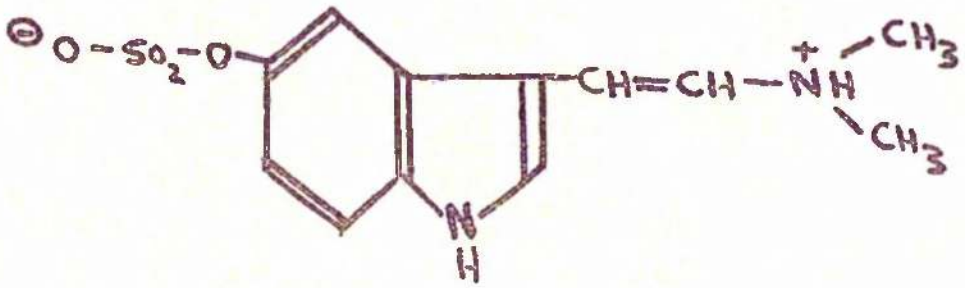
XXXVIII

oxindoles are intrinsically resistant to hydrolysis, but that the presence of appropriate substituents can overcome this resistance. This concept helps to explain the difficulties which arose in the present work when the oxindole (XXXVIII) could not be hydrolysed. This compound was prepared from yohimbine by a recently introduced method.[478] It had been hoped that application of the diazonium replacement sequence would have opened up a useful route to complex benzo-(b)-thiophen isosteres when applied to this oxindole. Despite the use of a variety of hydrolytic techniques (utilising concentrated barium hydroxide solutions, alcoholic potassium hydroxide, Claisen's alkali,[479] or anionic exchange resins loaded with hydroxyl ion) ring cleavage was not observed. As a test for ring cleavage and consequent exposure of a primary aromatic amine, the reaction mixtures were treated with sodium nitrite, then acidified at 0^{o} by the dropwise addition of acid before testing for the presence of a diazonium function with β-naphthol or resorcinol.

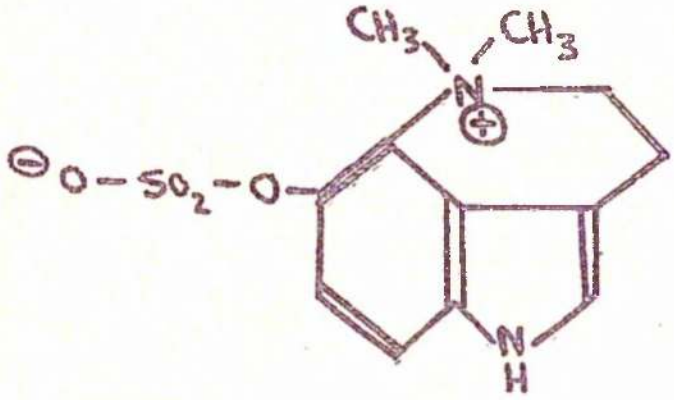
The failure to hydrolyse the oxindole derived from yohimbine prompted consideration of yet another oxindole system upon which to try the diazonium replacement route to benzo(b)-thiophens. A compound seemingly well suited to this work appeared to be the degradation product, which had been assigned structure XXXIX,[480] derived from the alkaloid bufothionine[481,482] (thought to have structure XL[480])- especially since successful basic hydrolysis and diazotisation of this degradation product had been reported.[480] Accordingly, work


HO
COCH2N
CH3
CH3
O
N
H

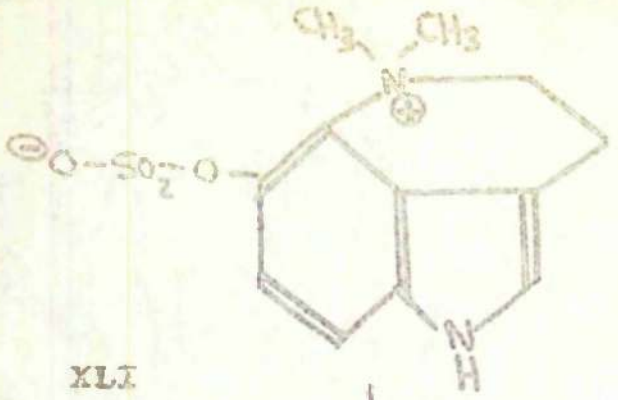
XXXIX


⊖O-SO2-O
CH=CH-NH
+
CH3
CH3
N
H

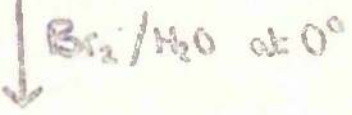
XL

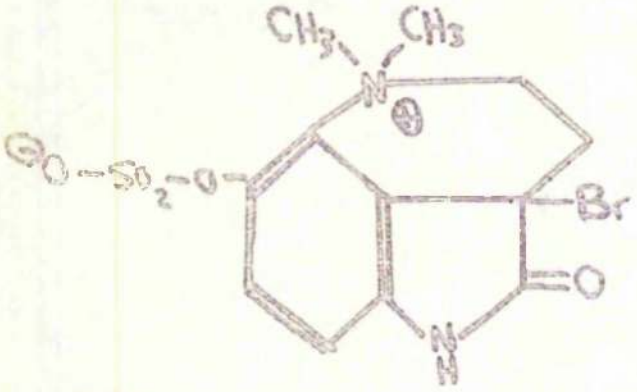

CH3
CH3
N
+
⊖O-SO2-O
N
H

XLI

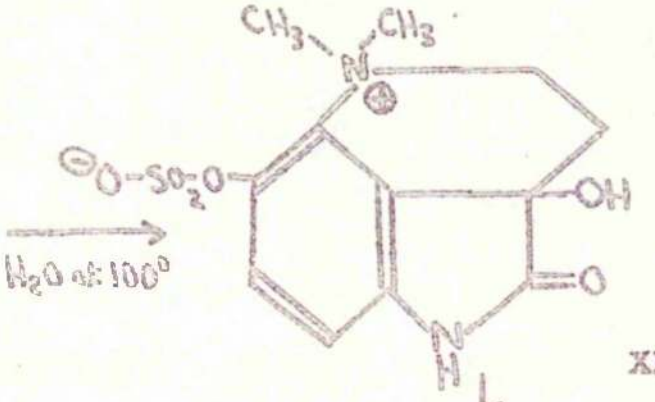


XLI

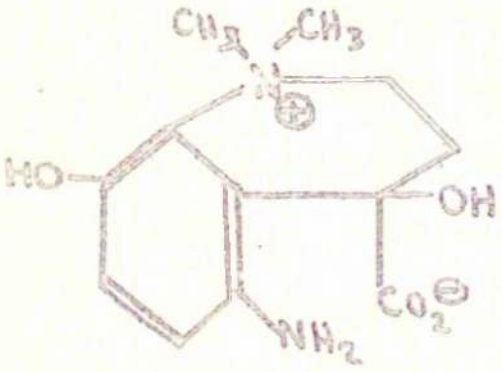




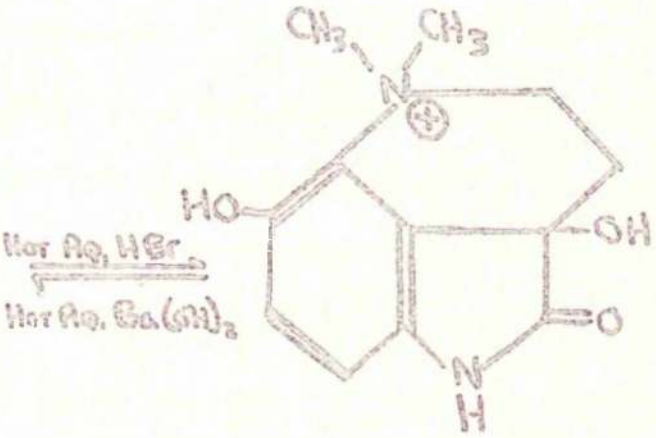
XLII



XLIII



XLV



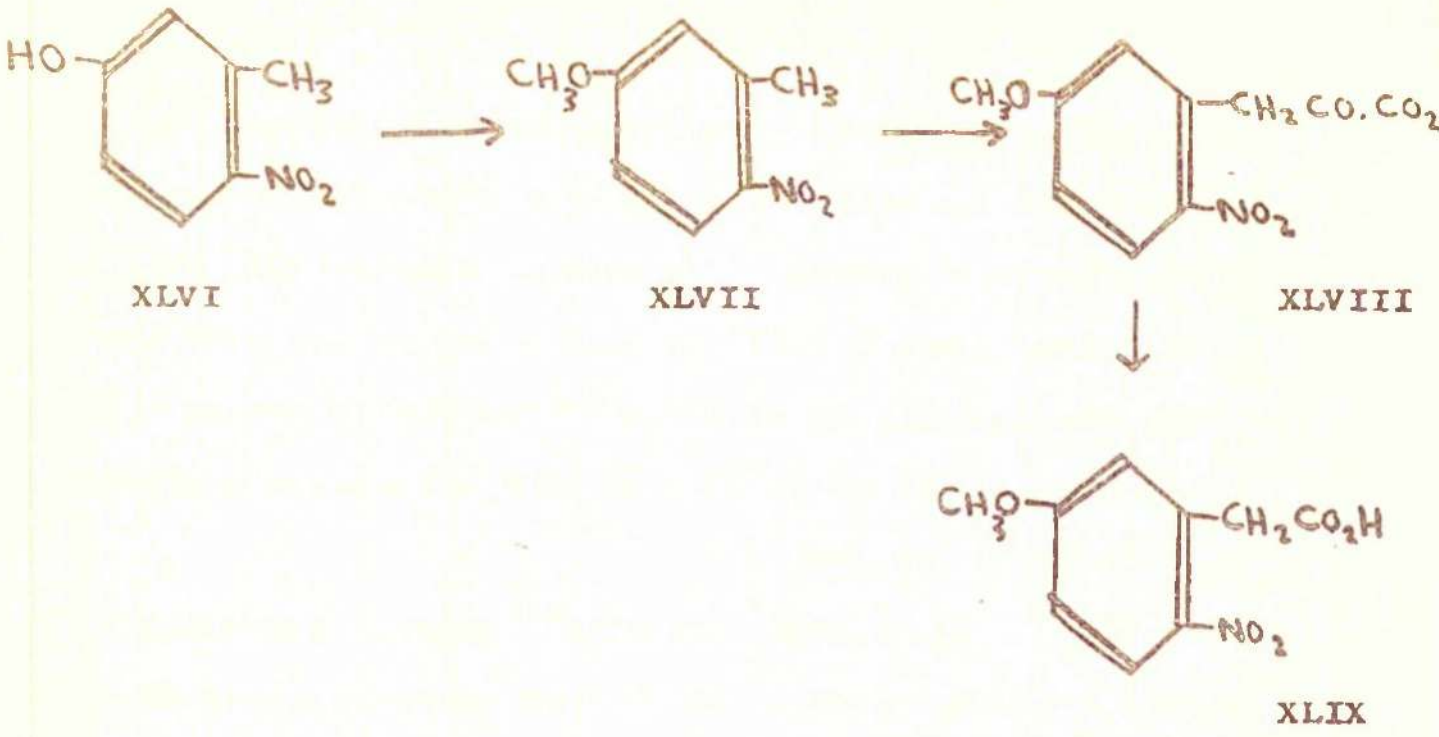
XLIV

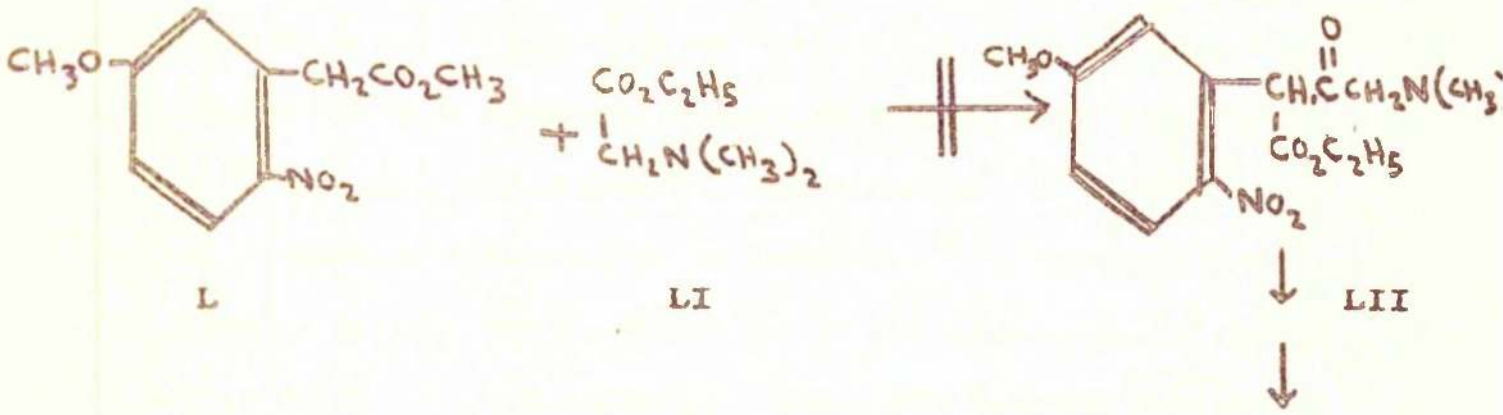
Scheme XXI

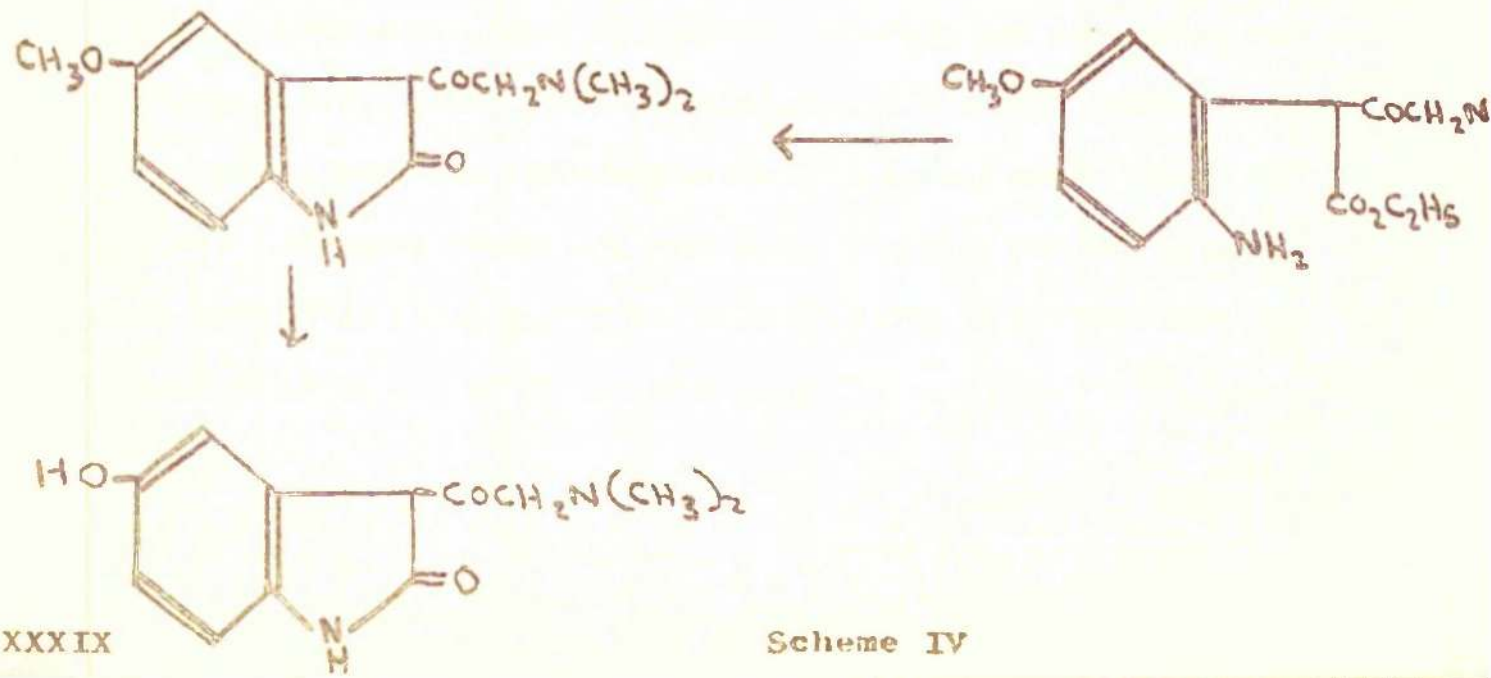
was initiated towards the synthesis of XXXIX.

However, with the demonstration based on nuclear magnetic resonance spectroscopy[483] that bufothionine had, in actual fact, structure XLI and not structure XL, re-interpretation of the nature of the degradation product was necessary. Clearly, it must have structure XLIV. The derivation of this compound from bufothionine is shown in Scheme III.

In the attempted synthesis of XXXIX advantage was taken of Baeyer's route[467] to substituted oxindoles which utilises the ready availability of substituted o-nitrophenylacetic acids which can be prepared by condensation of o-nitrotoluenes with ethyl oxalate and conversion of the resulting o-nitrophenylpyruvates into the o-nitrophenylacetic acids.[484] The route followed here is illustrated in Scheme IV. The desired 2-nitro-5-methoxyphenylacetic acid XLIX, which did not appear to have hitherto been reported in the literature, was readily prepared by decarbonylation of the known 3-(2-nitro-5-methoxyphenyl)-pyruvic acid (XLVIII) through application of the general method developed by Reissert[485] involving treatment with hydrogen peroxide in dilute alkaline solution. Compound XLVIII was obtained by condensing ethyl oxalate with 3-methyl-4-nitroanisole (XLVI). This compound in turn had previously been prepared[485] by treatment of the silver salt of 3-methyl-4-nitrophenol (XLVI) with methyl sulphate, but in the present work it was found more convenient to alkylate the phenol with methyl


HO
CH3
NO2
CH3O
CH2CO.CO2
CH2CO2H
XLVI
XLVII
XLVIII
XLIX

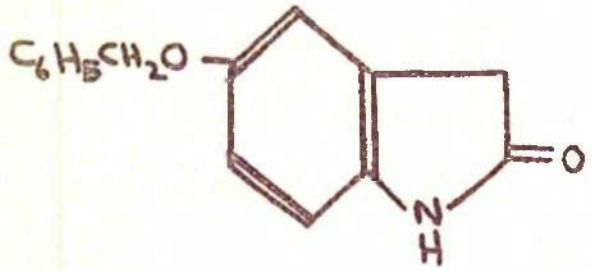

CH3O
CH2CO2CH3
NO2
CO2C2H5
CH2N(CH3)2
O
CH3O
CH2CCH2N(CH3)2
CO2C2H5
NO2
L
LI
LII


CH3O
COCH2N(CH3)2
O
N
H
CH3O
COCH2N
CO2C2H5
NH2
HO
COCH2N(CH3)2
O
N
H
XXXIX

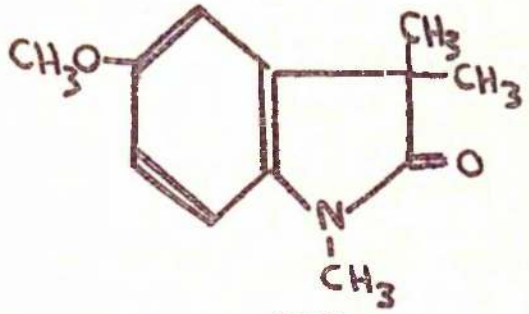
Scheme IV

iodide in the presence of potassium carbonate. The acid XLIX was then converted into its methyl ester (L) by treatment with hydrogen chloride in the presence of methanol. An attempt to prepare LII, which would offer a direct route to XXXIX, by condensation of the ester L with N,N-dimethylglycine ethyl ester (LI)[486] met with no success. However, the synthesis of the benzyl ether of XXXIX was achieved by another route. This will now be described.

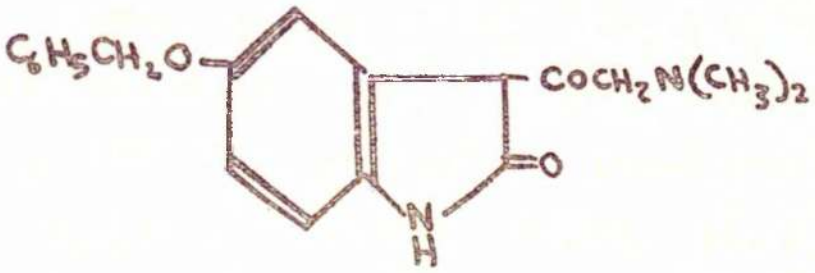
Julian, Pikl, and Wantz[487] obtained 1-methyl-3-dimethylaminoacetyloxindole and its 5-ethoxy derivative by the condensation of N,N-dimethylglycine ethyl ester (LI) with N-methyloxindole or 5-ethoxy-N-methyloxindole. These authors did not investigate condensations with oxindole itself since the keto-enol tautomerism would have complicated their studies which were concerned with the reduction of acyloxindoles to alkyloxindoles. However, ethyl acetate[488] and malonic acid diethyl ester[489] are known to condense with oxindole. On this basis the condensation of an appropriately substituted oxindole with N,N-dimethylglycine ethyl ester was investigated. Since it was necessary to protect the phenolic function in 5-hydroxyoxindole so that condensation with N,N-dimethylglycine ethyl ester could be attempted, the benzyl ether (LIII) of 5-hydroxyoxindole was synthesised. This compound did not appear to have been previously prepared. In view of the formation of 5-methoxy-1,3,3-trimethyloxindole (LIV) when previous workers[488] added even a slight excess of methyl sulphate to 5-hydroxyoxindole



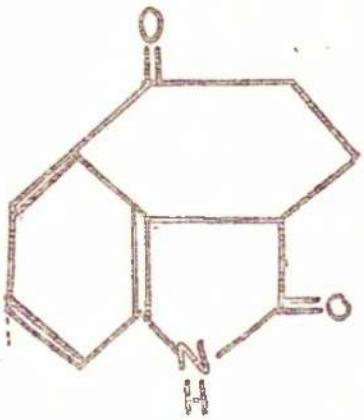
LIII



LIV



LV



LVI

in acetone, the product obtained after treatment of 5-hydroxyoxindole with exactly one equivalent of redistilled benzyl chloride in sodium ethylate solution was carefully examined. Both analytical figures and mass spectrometry confirmed that this compound was indeed 5-benzyloxyoxindole (LIII). The 5-hydroxyoxindole was prepared by a modification of an established method.[488]

Attempts to condense 5-benzyloxyoxindole with N,N-dimethylglycine ethyl ester met with no success until a high reaction temperature was employed. Under these conditions 3-dimethylaminoacetyl-5-benzyloxyoxindole (LV) was obtained. Hydrolysis of LV by refluxing in 48% aqueous hydrobromic acid failed to yield the expected 3-dimethylaminoacetyl-5-hydroxyoxindole (XXXIX), and starting material could not be recovered. This is, of course, in agreement with the known instability of 3-acyloxindoles.[471] Wieland and Wieland[480] found that the oxindole which they believed had the structure XXXIX was able to withstand refluxing in concentrated hydrobromic acid. Since it is now known that the stable oxindole of Wieland and Wieland possesses structure XLIV all discrepencies are eliminated.

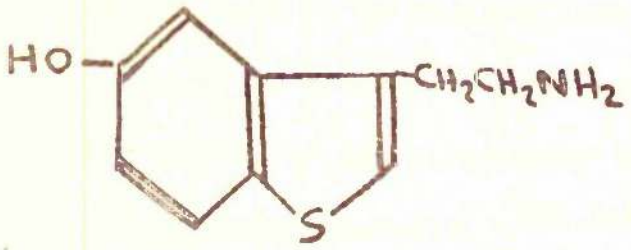
At the beginning of this section attempts to prepare the tricyclic ketone II were described. The diazonium replacement reaction would appear to offer a possible route to this important intermediate. Should it not prove possible to prepare the benzo-(b)-thiophan isostere of lysergic acid diethylamide from 2-oxo-lysergic acid diethylamide, it may become necessary to synthesise this tricyclic ketone. An appropriate

route to this may now be available in view of the synthesis of 5-oxo-2a,3,4,5-tetrahydronaphthostyril (LVI)[490] which could be hydrolysed and subjected to the diazonium replacement route.

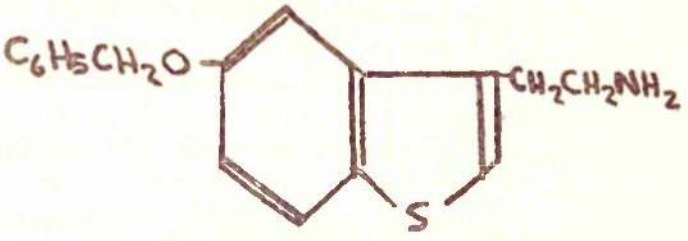
Although little success can be reported here, the diazonium replacement route may still be of value in certain circumstances. Not only does it offer an approach to the synthesis of benzo-(b)-thiophens, but it could also serve as a route to benzofuran isosteres through replacement of the diazonium nitrogen by hydroxyl. The diazonium route would seem to remain attractive for a possible synthesis of the benzo-(b)-thiophen isostere of LSD provided that a supply of 2-oxo-lysergic acid diethylamide (XXV) is made available.

Part 2

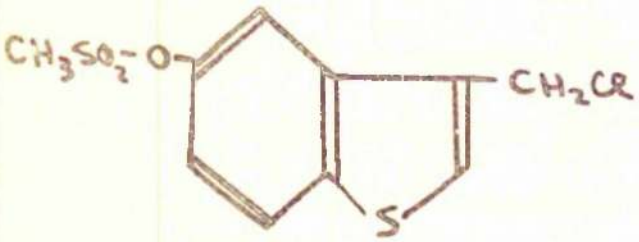
The Synthesis of the Benzo-(b)-thiophen Isosteres of Serotonin and Related Compounds.



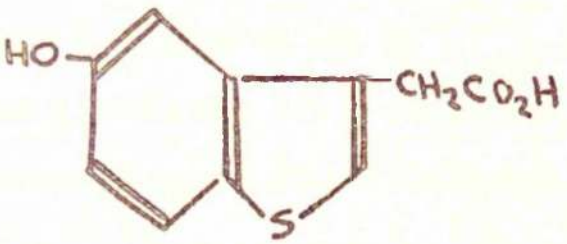
LVII



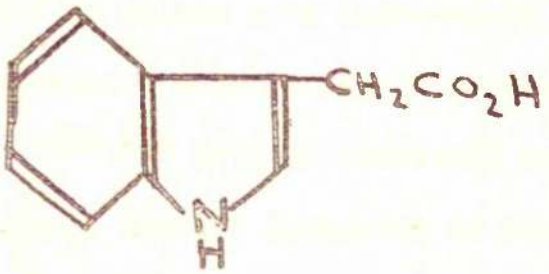
LVIII



LIX



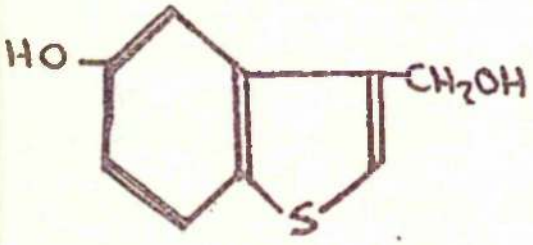
LX



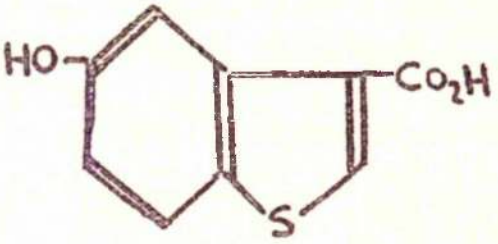
LXI

This present project is a continuation of studies initiated in the Department of Experimental Pharmacology, University of Glasgow, in the course of which several benzo-(b)-thiophen isosteres of simple indole derivatives were successfully prepared.[409,410] Extensive attempts to synthesise the benzo-(b)-thiophen isostere (LVII) of serotonin, however, met with no success although Reid[409] did succeed in preparing the corresponding benzyl ether (LVIII) which, unfortunately, could not be cleaved to the phenol (LVII) by either reductive or hydrolytic techniques. The route chosen for the preparation of LVIII did not permit of prior removal of this refractory protective group.

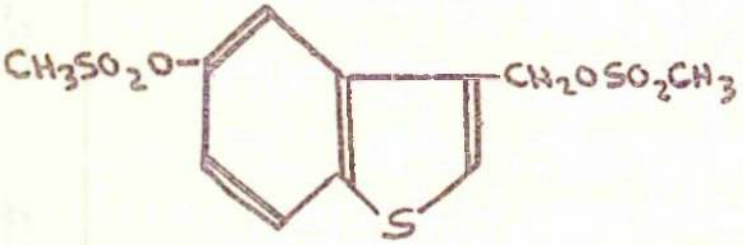
In the present work a successful synthesis of the desired serotonin isostere was achieved by employing the key intermediate 3-chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen (LIX). In point of fact this represented a second total synthesis of compound LVII, since before the present work was entirely completed publication announcing a synthesis of this compound was made by Neiss.[491] Besides serving as a precursor in the synthesis of the serotonin isostere (LVII), compound LIX permitted the preparation of a series of 5-hydroxybenzo-(b)-thiophens analagous to the series of substituted 3-aminomethylbenzo-(b)-thiophens prepared as gramine isosteres by Reid.[409,437] Certain other hitherto unreported compounds of potential further synthetic value were obtained from LIX including 5-hydroxybenzo-(b)-thiophen-3-acetic acid (LX) which is of interest because of its relationship to the plant heteroauxin,



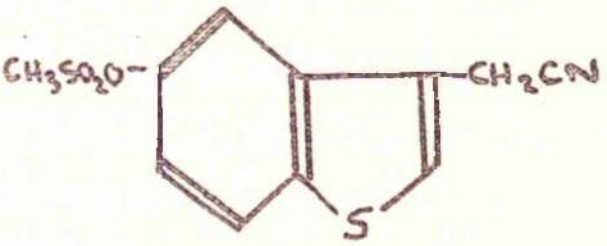
LXII



LXIII



LXIV



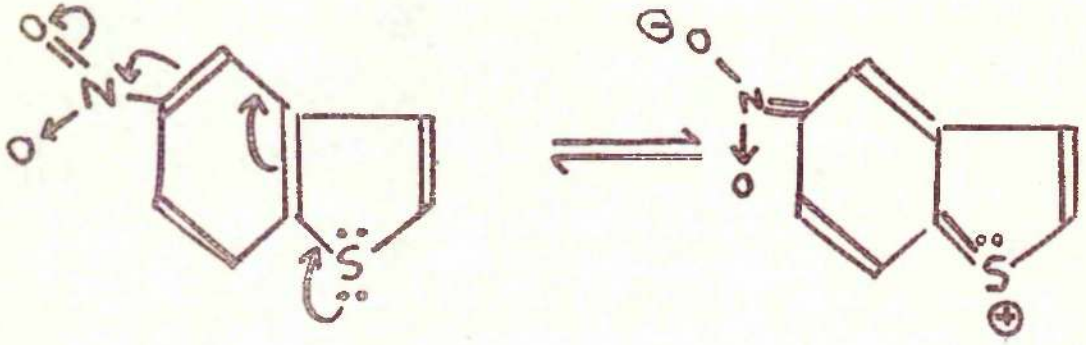
LXV

indole-3-acetic acid. Synthetic analogues of indole-3-acetic acid are of considerable agricultural importance since they are used in selective weed control to induce weeds to outgrow their food reserves.[199] At the molecular level indole-3-acetic acid acts by increasing the rate of enzymic methylation of pectin, a complex carboxylic acid whose insoluble calcium salt endows the plant cell wall with its characteristic rigidity. This calcium salt can, of course, no longer form once the carboxyl group is esterified hence enlargement of the softened cell wall ensues.[492] The structural requirements for a substance to exhibit indole-3-acetic acid activity have been formulated by Koepfli, Thimann and Went.[493] These requirements are satisfied by compound LX. In this connection it is of considerable interest that benzo-(b)-thiophen-3-acetic acid is known to exhibit plant growth hormonal activity, although it is markedly less potent than indole-3-acetic acid.[494-496]

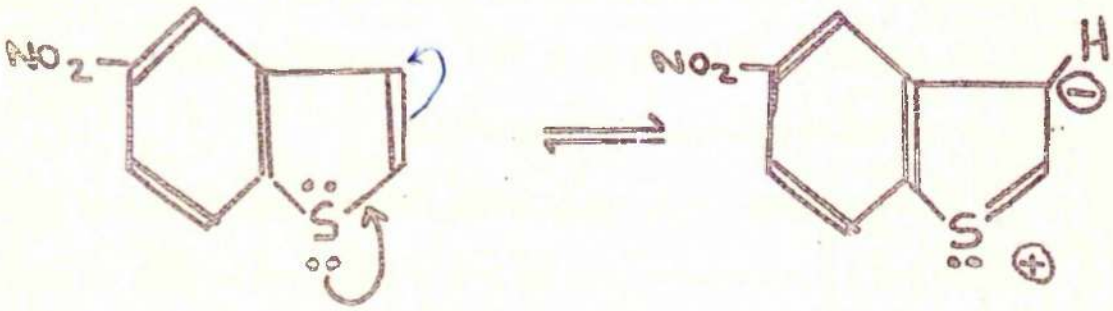
As a result of earlier studies several nuclear substituted benzo-(b)-thiophens were available as precursors for the synthesis of the key intermediate LIX[409,441,497,498] or analogous compounds possessing alternative good leaving groups in the benzylic position. Of these, 3-methanesulphonyloxymethyl-5-methanesulphonyloxybenzo-(b)-thiophen (LXIV) was prepared by Brown[410] from 3-hydroxymethyl-5-hydroxybenzo-(b)-thiophen (LXII) which Reid[409] had obtained by reduction of the carboxylic acid LXIII. Conversion of this dihydroxy compound into the dimethanesulphonate ester (LXIV) was achieved under anhydrous conditions. Treatment of the diester with sodium cyanide in dimethyl sulphoxide, however, produced

a complex mixture showing not only split cyanide absorption in the infra-red, but also carbonyl absorption. The split carbonyl absorption was considered to arise from an alternative S_N2 type displacement of the benzylic methanesulphonyloxy group (cf. [491,499]), whilst carbonyl absorption was considered[410] to arise from some type of Hoesch reaction involving the cyanide and a liberated free phenolic compound.[500] Thus Brown was unsuccessful in converting the dimethanesulphonate ester (LXIV) into the nitrile LXV. The successful synthesis of this compound via the key intermediate LIX in the present work is described below.

Consideration was given to the possibility of direct chloromethylation of an appropriately 5-substituted benzo-(b)-thiophen to obtain the corresponding 3-chloromethyl derivative. As has been pointed out in the first part of this discussion, benzo-(b)-thiophen is readily chloromethylated at the activated 3-position upon treatment with formaldehyde solution in the presence of gaseous hydrogen chloride.[435] However, in order to induce such electrophilic substitution at the 3-position of a 5-substituted benzo-(b)-thiophen it was essential that no strongly electron releasing group be present in the 5-position since it has been established that in such compounds electrophilic substitution is directed, in the first instance, into the 4-position and then, subsequently into the 6-position.[409,497,501] Hence it was clearly necessary to have in the 5-position a deactivating group capable of subsequent conversion into a phenolic hydroxyl group. Since it was known that attempts to chloromethylate 5-nitrobenzo-(b)-thiophen had been unsuccessful (starting material



LXVI a

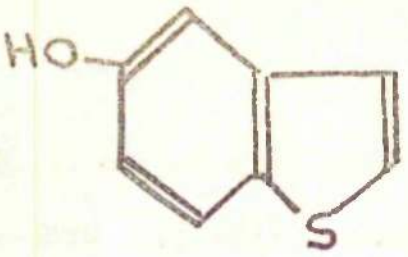


LXVI b

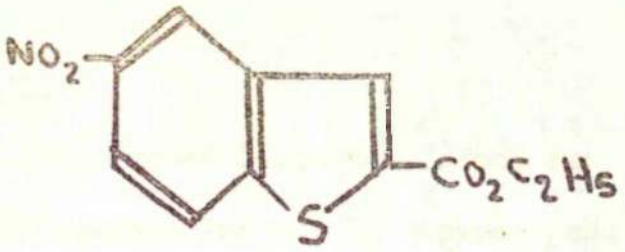
being recovered in almost quantitative yield),[502] it was decided to avoid compounds having a strong electron withdrawing group at the 5-position. Even though "through resonance" between a 5-substituent and the 3-position is not possible, the contribution of canonical form LXVIa presumably restricts that of LXVIb which is responsible for the activation of position 3 to electrophilic attack, via suppression of the availability of the p electrons on the sulphur atom.

Protection of the 5-hydroxy group as a carboxylic ester during chloromethylation was shown to be ruled out by Reid[502] since, under the acid conditions of the reaction, hydrolysis of such esters occurred with the free phenol undergoing substitution in the benzene ring with concomitant polymerisation. Later, in an attempt to take advantage of Bordwell's observation[501] that 5-acetoxybenzo-(b)-thiophen undergoes electrophilic substitution at the 3-position, Neiss[491] recovered a small amount of 5-hydroxybenzo-(b)-thiophen and intractable tar after an attempted chloromethylation of the 5-acetoxy derivative.

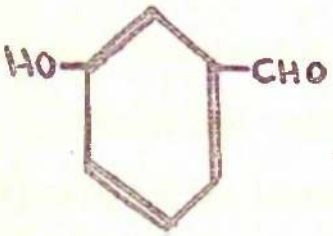
The need for an alternative, mildly deactivating group that could withstand acid hydrolysis led to the selection in the present work of sulphonate esters as protective functions of the phenolic 5-hydroxy group. The particular choice of ester was influenced by the circumstances under which it was expected that the protective group would be removed. Although sulphonate esters can be hydrolysed by alkali[503] or removed by reduction with lithium aluminium hydride,[504] it seemed desirable to select an ester



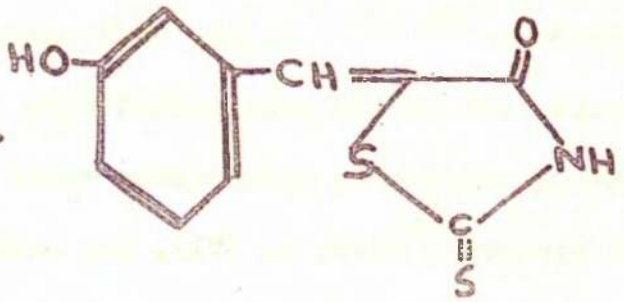
LXVII



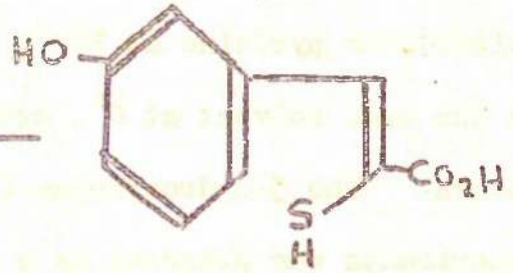
LXVIII



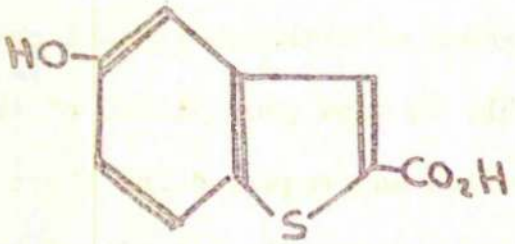
LXIX



LXX



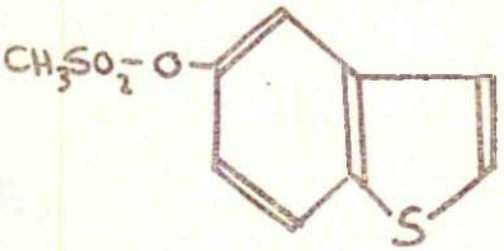
LXXI



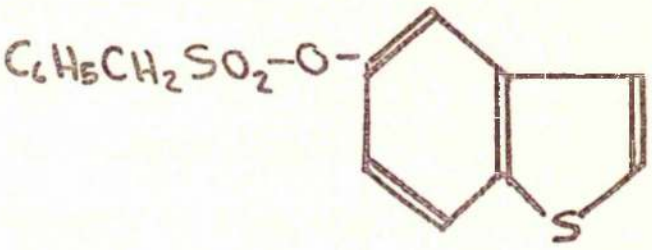
LXXII

that could be removed under non-aqueous conditions in order to facilitate the recovery of the zwitterionic serotonin isostere (LVII). Such an ester was available in the form of the benzylsulphonic acid ester which undergoes S-O fission upon hydrogenation in the presence of Raney nickel to liberate the phenol.[505,506] This is in marked contrast to the behaviour of aryl esters of p-toluenesulphonic or methanesulphonic acids which undergo C-O fission under similar conditions thereby generating aromatic hydrocarbons.[505,506] In the early stages of the present work benzylsulphonic acid esters were indeed used, but after the discovery that the protecting sulphonate ester group could be removed at the penultimate stage of the synthetic route to LVII, the methanesulphonate esters were employed.

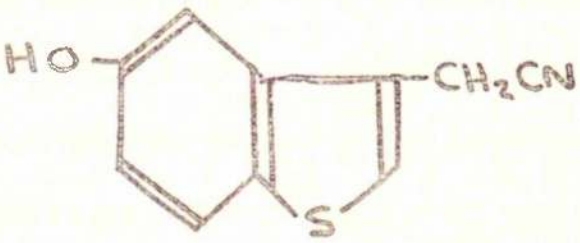
The preparation of sulphonate esters at the 5-position of the benzo-(b)-thiophen nucleus presented no difficulty. Treatment of 5-hydroxybenzo-(b)-thiophen (LXVII) with one equivalent of benzylsulphonyl chloride in pyridine at 100°, or with an excess of methanesulphonyl chloride in the same solvent at 0°, proceeded smoothly to give good yields of the esters. The 5-hydroxybenzo-(b)-thiophen which was required for these experiments was prepared by a modification of a synthesis introduced by Hemmecke[507] and since improved by several other workers.[497,501,507-510] The modification employed in the present work involved the application of a new method of preparing 2-carbethoxy-5-nitrobenzo-(b)-thiophen (LXVIII) developed by Rossi and Trave[511] in the course of their work directed towards the synthesis of analogues of the antibiotic chloramphenicol. This new



LXXIII



LXXIV



LXXV

method more than doubled previously reported overall yields of 5-hydroxybenzo-(b)-thiophen, but it should be noted that Weiss[512] adopted a new procedure, introduced by Campaigne,[513,514] for the synthesis of substituted benzo-(b)-thiophens thus securing 5-hydroxybenzo-(b)- thiophen from a 4-stage synthesis in yields similar to those attained with the modified older method. This 4-stage synthesis involves, firstly, condensation of m-hydroxybenzaldehyde (LXIX) with rhodanine to produce 5-(3-hydroxybenzylidene)-rhodanine (LXX) which is hydrolysed to β-3-hydroxyphenyl-α-mercaptoacrylic acid (LXXI) on treatment with base. Ring closure of this acid is effected by the use of iodine in dioxan, thus producing 2-carboxy-5-hydroxybenzo-(b)-thiophen (LXXII) which is decarboxylated by the method of Martin - Smith and Gates.[497]

As anticipated, both 5-methanesulphonyloxybenzo-(b)-thiophen (LXXIII) and 5-benzylsulphonyloxybenzo-(b)-thiophen (LXXIV) were found in the present work to undergo conversion into their 3-chloromethyl derivatives in good yields on treatment with gaseous hydrogen chloride in 37% aqueous formaldehyde solution. It was found that by allowing the reaction to continue for several hours in the cold the product crystallised from the reaction mixture in a pure state. An alternative procedure in which extraction of the reaction mixture with benzene was carried out, afforded a product contaminated with starting material, only removed with difficulty on repeated recrystallisation from ethanol.

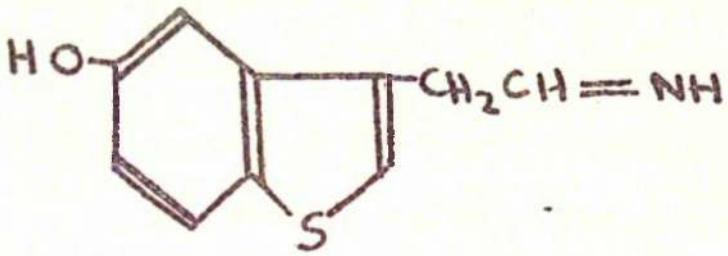
Before proceeding further with the projected synthesis of the serotonin isostere it was necessary to prove that chloromethylation

had taken place at the 3-position. This was, in fact, done by converting the chloromethyl compounds into 3-hydroxymethyl-5-hydroxybenzo-(b)-thiophen (LXII) by way of their 3-acetoxymethyl derivatives. Comparison of the product obtained in each case with an authentic sample of 3-hydroxymethyl-5-hydroxybenzo-(b)-thiophen, kindly supplied by Dr. S. T. Reid of the Department of Experimental Pharmacology, Glasgow University, confirmed that the chloromethyl groups were, indeed, attached to the benzo-(b)-thiophen nucleus at the 3-position.

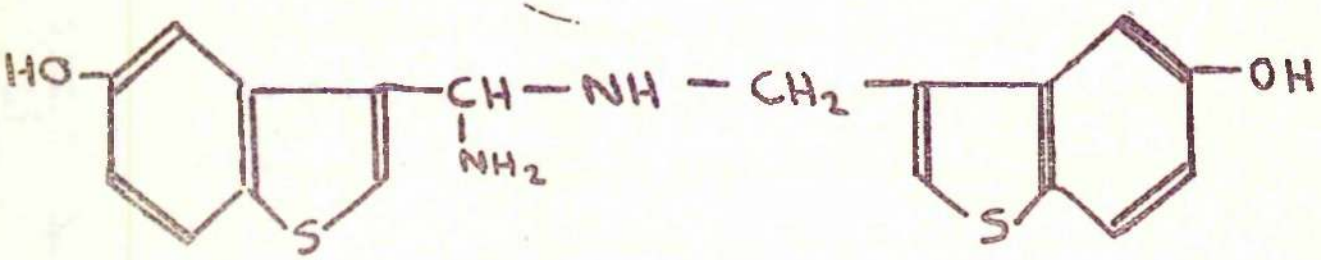
Conversion of the chloromethyl derivatives into cyanomethyl compounds initially presented some difficulty insofar as low yields were obtained. However, after several attempts it was found that good yields were possible if this S_N2 reaction was carried out in aqueous acetone rather than ethanol, aqueous ethanol, or dimethylsulphoxide. In this manner a supply of 3-cyanomethyl-5-methanesulphonyloxybenzo-(b)-thiophen (LXV) was obtained from LIX.

Attempts to prepare 3-cyanomethyl-5-hydroxybenzo-(b)-thiophen (LXXV) by alkaline hydrolysis of LXV were unsuccessful since hydrolysis of the nitrile also occurred thereby forming 5-hydroxybenzo-(b)-thiophen-3-acetic acid (LX). As has been mentioned above, this compound is of interest in view of its relationship to indole-3-acetic acid.

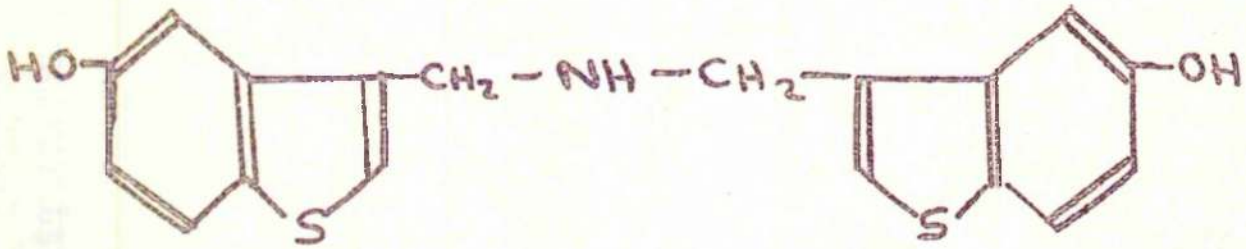
3-Cyanomethyl-5-hydroxybenzo-(b)-thiophen (LXXV) was, however, obtained by treatment of LXV with two equivalents of sodium in dry ethanol. An excess of sodium would probably have reduced the nitrile, but since isolation of LXXV was desirable only two equivalents of sodium were employed.



LXXVI



LXXVII

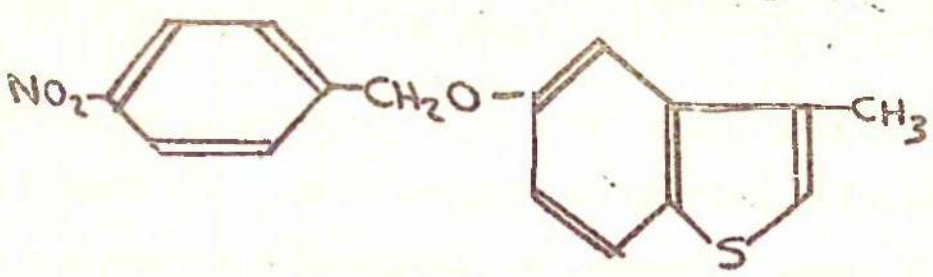


LXXVIII

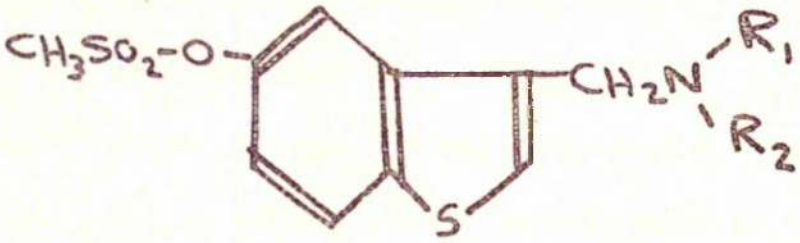
The infra-red spectrum of the phenol which was isolated from this reaction showed cyanide absorption at 2250 cm.$^{-1}$ It was also observed that 5-methanesulphonyloxybenzo-(b)-thiophen was converted into 5-hydroxybenzo-(b)-thiophen under analogous conditions.

Hydrogenation of 3-cyanomethyl-5-hydroxybenzo-(b)-thiophen (LXXV) in the presence of Raney nickel proceeded smoothly to give the benzo-(b)-thiophen isostere (LVII) of serotonin. The reduction was conducted at normal temperature and pressure in methanol saturated with ammonia, by analogy with the method employed for the preparation of β-phenylethylamine from benzyl cyanide.[515] As reduction proceeds the aldimine (LXXVI) is formed. This is then reduced to the primary amine (LVII). However, unless precautions are taken, it would be expected[516] that some of the primary amine will react with the aldimine to form the anino-diamine (LXXVII) which on further reduction eliminates ammonia to form the secondary amine (LXXVIII). The presence of a high concentration (about 10 $\bar{N}$) of ammonia in the methanolic reaction solution is known to repress the formation of the secondary amine.[517]

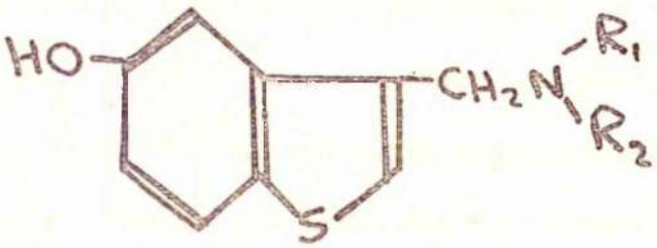
In view of the non-aqueous conditions that were employed, recovery of the serotonin isostere from the hydrogenation mixture presented little difficulty. After removal of the Raney nickel by filtration the ammoniacal solvent was distilled under reduced pressure, leaving a grey residue. No attempt was made to characterise this residue in view of the hygroscopic nature of certain other phenolic benzo-(b)-thiophen aliphatic amines prepared in the present work (see below) and also by



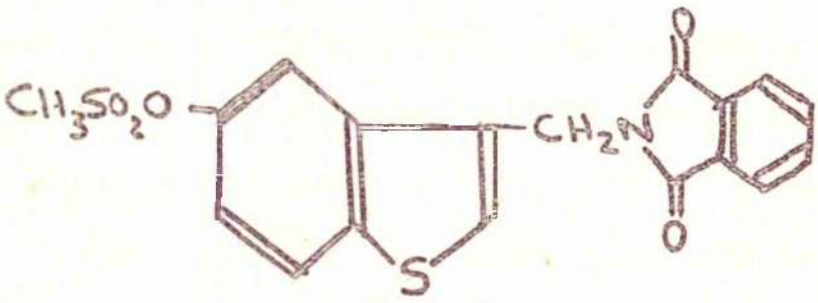
LXXIX



LXXX



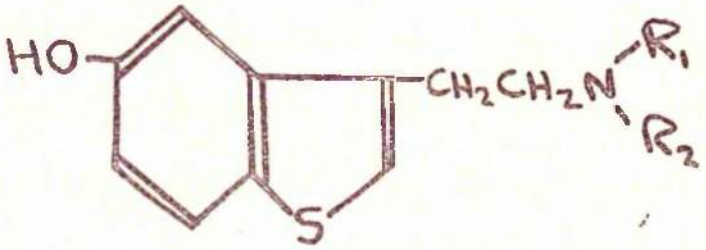
LXXXI



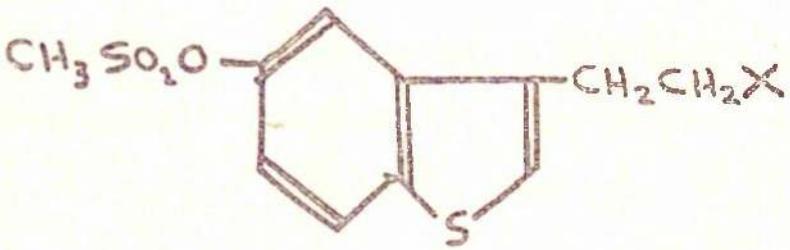
LXXXII

Neiss,[491] but addition of ether failed to dissolve the product, thereby distinguishing it from the starting material. The residue did not dissolve in water. However, on addition of a few drops of dilute hydrochloric acid, solution was readily effected. This might indicate that the product was a phenolic nickel salt. Treatment of the acidified solution with a slight excess of saturated sodium picrate solution enabled the serotonin isostere to be isolated and characterised as its picrate (cf. the isolation of 5-hydroxytryptamine as its picrate[518]). The infra-red spectrum of this picrate bore a marked resemblance to that of 3-piperidinomethyl-5-hydroxybenzo-(b)-thiophen whose preparation is described in the experimental section. All earlier attempts to secure the free base had been unsuccessful, presumably because of the sensitive nature of this product. Indeed, Neiss[491] found that he could not obtain a correct carbon and hydrogen analysis for his free base, but by allowing for absorption of carbon dioxide from the atmosphere the anomalous figures were explained.

The condensation of several amines with the key intermediate LIX from the serotonin isostere synthesis permitted the preparation of the methanesulphonate esters (LXXX) of benzo-(b)-thiophen isosteres of 5-hydroxy-gramine derivatives. These esters were hydrolysed in alkali to give the corresponding free phenols (LXXXI) which were characterised as their picrates. In an attempt to prepare 3-dimethylamino-5-hydroxybenzo-(b)-thiophen, compound LIX was successfully condensed with potassium phthalimide in dimethylformamide to give 3-phthalimidomethyl-5-



LXXXIII

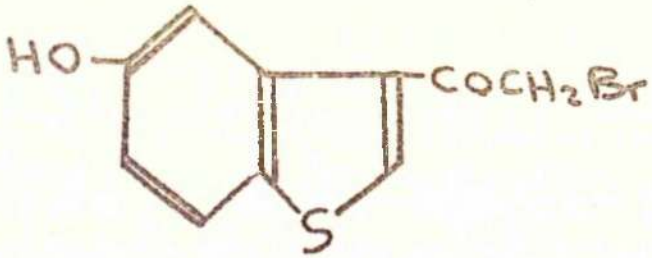


LXXXIV

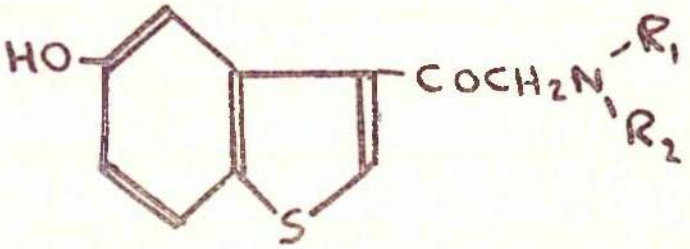
methanesulphonyloxybenzo-(b)-thiophen (LXXXII). An attempt to convert this into the primary amino derivative by treatment with hydrazine hydrate in ethanol was unsuccessful.

Attempted preparations of tertiary amines of type LXXXIII in the present work were unsuccessful. Such amines should be of considerable interest in view of their structural resemblance to bufotenine. Facile syntheses of these would presumably have been achieved if it had been possible to prepare a 3-(β-haloethyl)-5-hydroxybenzo-(b)-thiophen or its methanesulphonate ester (LXXXIV).

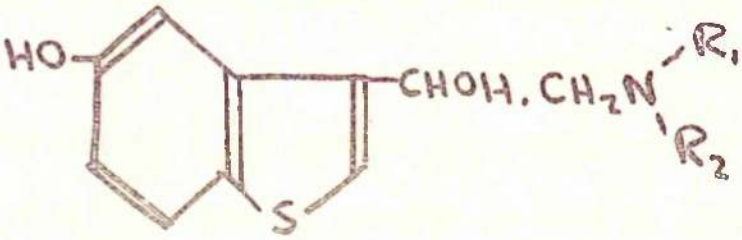
Olah and Kuhn[519] have successfully haloethylated benzene, toluene, m-xylene, and mesitylene with monofluoro-monohaloethanes in the presence of various boron trihalides, thus overcoming the difficulties[519] associated with attempted haloethylations using vinyl chloride or ethylene dichloride in the presence of aluminium chloride or related catalysts. Accordingly, preliminary attempts to prepare LXXXIV employing 1-chloro-2-fluoroethane in the presence of boron tribromide on 5-methanesulphonyloxybenzo-(b)-thiophen (LXXIII) were undertaken in the present work, but unchanged starting material was the only product to be isolated. In this connection it may be noted that further work employing boron tri-iodide (a stronger catalyst) and 1-bromo-2-fluoroethane might profitably be undertaken, as might experiments employing the complex formed between nitromethane and aluminium chloride or that formed between nitromethane and ferric chloride as catalysts since these could conceivably be strong enough to permit haloethylation without inducing secondary alkylation.[519]



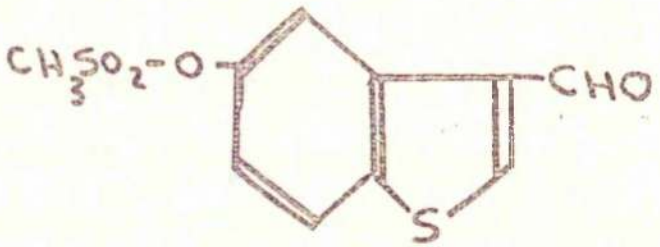
LXXXV



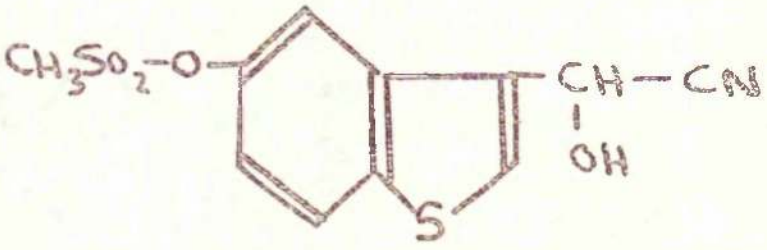
LXXXVI



LXXXVII



LXXXVIII

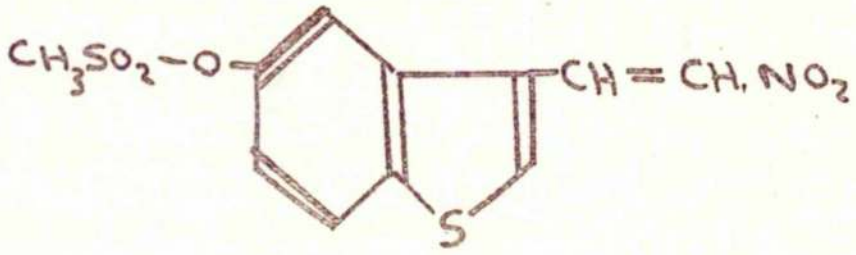


LXXXIX

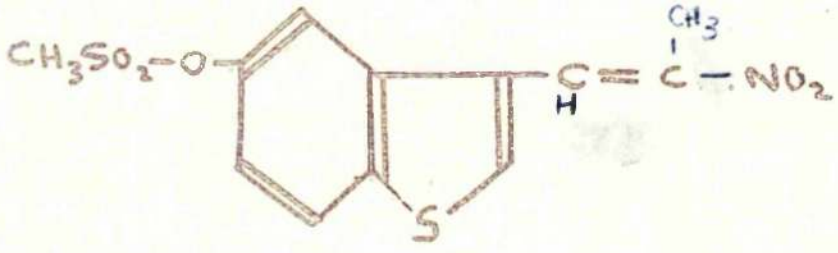
Another possible approach to the syntheses of 3-(β-aminoethyl)-5-hydroxybenzo-(b)-thiophens is suggested by the recent discovery[520] that a suspension of cupric bromide in chloroform-ethyl acetate reacts with nuclear hydroxylated acetophenones to give the corresponding ω-bromo ketones; bromination occurring solely in the side chain in quantitative yield. Application of this reaction to 3-acetyl-5-hydroxybenzo-(b)-thiophen, which was successfully synthesised by Brown,[410] should result in the formation of 3-bromoacetyl-5-hydroxybenzo-(b)-thiophen (LXXXV) which could then be subjected to condensation with various amines to form the substituted acetylamino compounds of type LXXXVI. These could subsequently be converted into either the 3-β-ethylamine derivatives of type LXXXIII, or into α-hydroxy compounds of type LXXXVII, by correct selection of reduction conditions. These latter compounds should be of interest in view of their structural relationship to noradrenaline and adrenaline.

Another route towards the synthesis of hydroxy-amines of type LXXXVII would appear to be available as a result of the successful synthesis of 3-formyl-5-methanesulphonyloxybenzo-(b)-thiophen (LXXXVIII) in connection with this present work. This aldehyde was prepared by oxidation of 3-chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen with cupric nitrate in nitric acid. Treatment of the aldehyde with hydrogen cyanide should provide the cyanohydrin (LXXXIX) which, on reduction, could yield the α-hydroxyethylamine derivative.

The aldehyde (LXXXVIII) had, in fact, been prepared with



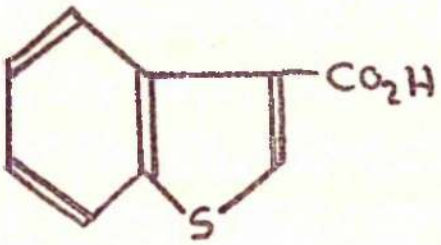
LXC



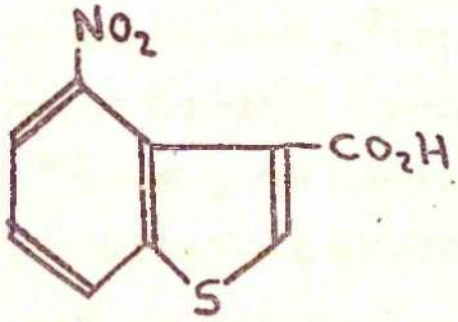
LXCI

the intention of condensing it with nitromethane or nitroethane in order to obtain nitrovinyl intermediates (LXC and LXCI respectively) which on reduction would have provided ethylamino- and ∝-methyl ethylamino- compounds. However, attempts to effect the necessary condensations have so far been unsuccessful. Similar failure to condense 3-formyl-5-hydroxybenzo-(b)-thiophen with nitromethane was reported by Brown[410] who quotes extensive evidence concerning the difficulties in the condensations between aldehydes and nitroalkanes or other compounds with reactive methylene groups.[521-525] In order to obtain an acceptable yield of the nitrovinyl derivative a series of control reactions with a variety of condensing agents may have to be run.[524] Further, good yields result only where the nitrovinyl compound is sufficiently insoluble to precipitate from the reaction mixture, or else trimeric compounds will be formed.[524,526]

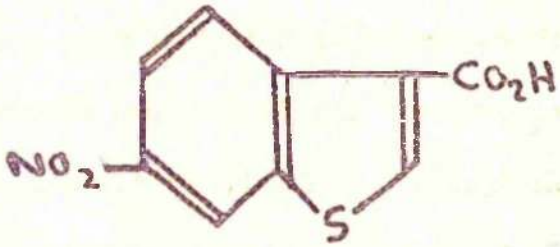
In the introductory section of this thesis attention was drawn to the biological role of 5-hydroxytryptophan. It is clear that the benzo-(b)-thiophen analogue of this natural metabolite would be of considerable interest insofar as like its natural isostere, it might pass the blood-brain barrier. The possibility that it may antagonise 5-hydroxytryptophan decarboxylase thereby leading to a deficiency of serotonin within parts of the brain renders this compound worthy of investigation. The successful preparation of both 3-chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen (LIX) and 3-formyl-5-methanesulphonyloxybenzo-(b)-thiophen (LXXXVIII) suggests ready routes to the 5-hydroxytryptophan isostere by analogy with methods



LXCII



LXCIII



LXCIV

already employed to synthesise the benzo-(b)-thiophen isostere of trypto-phan employing 3-chloromethylbenzo-(b)-thiophen[527] and 3-formylbenzo-(b)-thiophen[528] as starting materials. In this connection it is worthy of note that a malonic ester synthesis leading to the preparation of 5-hydroxybenzo-(b)-thiophen-3-propionic acid from 3-chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen was successfully achieved in the present work.

With the successful synthesis of the benzo-(b)-thiophen isosteres of 6-hydroxytryptamine by Reid,[409] and of 5-hydroxytryptamine (this thesis and Weiss[491]), there remains the problem of the synthesis of the 4-hydroxytryptamine (psilocin) isostere. Indeed, preliminary investigations were carried out by Brown[410] and Reid.[529] Thus, Brown[410] nitrated benzo-(b)-thiophen-3-carboxylic acid (LXCII) and obtained a mixture of products. He expected the major product would be 4-nitrobenzo-(b)-thiophen-3-carboxylic acid (LXCIII) (a compound which was to serve as a key intermediate in his projected synthesis of the psilocin isostere) since 3-nitrobenzo-(b)-thiophen had been reported[508] to give predominately 3,4-dinitrobenzo-(b)-thiophen upon nitration, suggesting that a meta directing group in the 3-position favoured 4-substitution in the benzo-(b)-thiophen field. Indeed, by decarboxylation of his crude product Brown was able to isolate a small quantity of 4-nitrobenzo-(b)-thiophen (a known compound[508]). Reid[529] re-investigated the nitration of benzo-(b)-thiophen-3-carboxylic acid and found that fractional crystallisation of the product yielded two separate components. These

were designated component A, m.p. 263° - 265°, (the major component), and component B, m.p. 219° - 220°. Decarboxylation of component A to give a nitrobenzo-(b)-thiophen, followed by treatment with hydrogen peroxide solution in acetic acid gave a compound identical with authentic 6-nitrobenzo-(b)-thiophen-1,1-dioxide.[444] This clearly indicated that component A was 6-nitrobenzo-(b)-thiophen-3-carboxylic acid (LXCIV). Further, component B was decarboxylated to yield a compound identical with authentic 4-nitrobenzo-(b)-thiophen. However, there appeared to be a discrepancy between these findings and those of Brown[410] insofar as the latter's recrystallised product, believed to be the 4-isomer, had the same melting point as the compound which Reid characterised as the 6-isomer.

In view of the importance of fully characterising any intermediates to be used in a synthesis of 3-(β-aminoethyl)-4-hydroxybenzo-(b)-thiophen, work was undertaken in the course of this present study to resolve the anomaly arising from the observations of Brown[410] and Reid.[529] Accordingly, nitration of benzo-(b)-thiophen-3-carboxylic acid followed by fractionation of the products as described by Reid[529] was performed. To ensure that components A and B were each pure compounds these products were converted into methyl esters by treatment with diazomethane. Pilot studies indicated that a mixture of both esterified products gave but two pure compounds after column chromatography on alumina, with carbon tetrachloride eluting ester I which corresponded to component B. Subsequent elution with benzene brought off ester II. Thus, the methyl esters were readily purified.

Each ester was saponified to afford the corresponding acid. Ester II yielded a pure acid of m.p. 263° - 265° corresponding to Reid's component A. Further, this latter acid has once more been characterised as 6-nitrobenzo-(b)-thiophen-3-carboxylic acid by conversion into 6-nitrobenzo-(b)-thiophen-1,1-dioxide identical with authentic material. Ester I also yielded a pure acid of m.p. 219° - 220° corresponding to Reid's component B. Thus Reid's findings are confirmed. It would, therefore, seem that in the course of purification for analysis of the product which had been decarboxylated for characterisation Brown[410] incidentally achieved a fractionation which selectively removed 6-nitrobenzo-(b)-thiophen, whilst purification of the product of nitration of benzo-(b)-thiophen-3-carboxylic acid selectively afforded 6-nitrobenzo-(b)-thiophen-3-carboxylic acid. This, in point of fact, is in accordance with the observed solubilities and melting points of these compounds.

With the removal of the discrepancy between the results of Reid[529] and Brown,[410] the way to a synthesis of the benzo-(b)-thiophen isostere of psilocin would now seem to be open since these workers had converted the proven 4-nitrobenzo-(b)-thiophen-3-carboxylic acid into 4-hydroxybenzo-(b)-thiophen-3-carboxylic acid, a valuable intermediate for such a synthesis.

EXPERIMENTAL.

2-Bromo-3-bromomethylbenzo-(b)-thiophen (X)

Fused sodium acetate (4.5 g.) was added to 3-chloromethylbenzo-(b)-thiophen[435] (10.0 g, 0.055 mole) dissolved in the minimum volume of glacial acetic acid. Bromine (8.75 g, 0.005 mole) was added slowly to this stirred solution which was then warmed on a steam bath until the colour of the bromine had been discharged. On pouring into cold water crystals of 2-bromo-3-bromomethylbenzo-(b)-thiophen were obtained. These were dried in a desiccator before recrystallisation from light petroleum (b.p. 60° - 80°). White needles were thus obtained (5.88g; 41%), m.p. 92° - 95° (lit.,[432] 97.5° - 98.5°). No melting point depression was observed on admixture with an authentic sample.

2-Bromo-3-dimethylaminomethylbenzo-(b)-thiophen (XIII, $R_1 = R_2 = CH$

2-Bromo-3-bromomethylbenzo-(b)-thiophen (0.978g, 0.003 mole) and dimethylamine (0.270g, 0.006 mole) were dissolved in dry benzene (15 ml.). The solution was set aside at room temperature for 6 hours and then boiled for 30 minutes. Ether (15 ml.) was added, and the mixture was washed with water to remove the dimethylamine hydrobromide and unchanged dimethylamine. The solution was dried over anhydrous sodium sulphate, then the solvent was removed on a rotary evaporator. The oily residue was taken up in ether and converted into the hydrochloride by passing in hydrogen chloride gas. The hydrochloride was extracted into water (20 ml.). Treatment of the aqueous solution

with an excess of saturated sodium picrate solution produced a copious yellow precipitate of 2-bromo-3-dimethylaminomethylbenzo-(b)-thiophen picrate which was twice recrystallised from methanol. The crystalline picrate had m.p. 184° - 188° (Found: C, ; H, $C_{17}H_{14}N_4O_7S$ Br requires C, 40.1; H, 2.81%).

The following three amine picrates were also prepared by the above procedure:

a) 2-Bromo-3-morpholinomethylbenzo-(b)-thiophen picrate, m.p. 219° - 222° (decomp.) (Found: C, 42.2 ; H, 3.43. $C_{19}H_{17}N_4O_8S$ Br requires C, 42.1 ; H, 3.14%).

b) 2-Bromo-3-piperidinomethylbenzo-(b)-thiophen picrate, m.p. 205° - 207° (Found: C, 45.1 ; H, 3.73. $C_{20}H_{19}N_4O_7S$ Br requires C, 44.5 ; H, 3.53%).

c) 2-Bromo-3-pyrrolidinomethylbenzo-(b)-thiophen picrate, m.p. 134° - 137° (Found: C, 43.4 ; H, 3.55 $C_{19}H_{17}N_4O_7S$ Br requires C, 43.4 ; H, 3.24%).

2-Bromobenzo-(b)-thiophen-3-propionic acid (XII)

Diethyl malonate (40.8g) was added to a suspension of sodium sand (5.1g) in dry benzene (100 ml.). Once the sodium had completely reacted, 2-bromo-3-bromomethylbenzo-(b)-thiophen (25.5g) in dry benzene (400 ml.) was added. After 5 hours stirring and refluxing, water was added and the organic layer separated, washed, and dried over sodium sulphate.

The diester (XI) which was obtained as an oil upon removal of the solvent was slowly added to a lukewarm solution of potassium hydroxide (31.2g) in water (26 ml.), whereupon the reaction solidified to a white mass consisting mainly of the potassium salt of the monoester. By heating under reflux, hydrolysis of the mixture was completed. On pouring the cooled reaction into dilute hydrochloric acid, crystals of the dicarboxylic acid were obtained, m.p. 173° (benzene). After drying, these crystals were decarboxylated by heating at 175°. The brown residue (11.2g, 47%) was then sublimed at 140° at 0.2 mm. Hg. An analytical sample of 2-bromobenzo-(b)-thiophen-3-propionic acid thus prepared had m.p. 115° - 117.5°. (Found: C, 46.2 ; H, 3.24. $C_{11}H_9SO_2Br$ requires C, 46.3; H, 3.16%). The acid chloride (VI) was obtained by heating this acid at 70° in the presence of a slight excess of thionyl chloride.

Attempted Condensation of Benzo-(b)-thiophen with β-propiolactone.

Benzo-(b)-thiophen (2.68g, 0.020 mole) and β-propiolactone (1.80g, 0.025 mole) were heated together for three hours at 110°. On the addition of ether (100 ml.) to the cooled reaction, a white crystalline precipitate separated. After washing with 10% sodium carbonate solution, then water, this precipitate was dried and identified as unchanged starting material. Acidification of the alkaline washings and subsequent extraction with organic solvents failed to produce any of the desired benzo-(b)-thiophen-3-propionic acid.

4,5,6,7-Tetrahydrobenzo-(b)-thiophen-4-ylidene ethyl cyanoacetate (xxiv).

4-Oxo-4,5,6,7-tetrahydrobenzo-(b)-thiophen (9.5g, 0.0625 mole) and ammonium acetate (0.96g) were dissolved in a solution of ethyl cyanoacetate (7.1g, 0.1250 mole) in acetic acid (3 ml.) and benzene (12.5 ml.). The reaction mixture was refluxed overnight in a liquid-liquid continuous extractor which enabled the water which azeotroped off with the benzene during the course of the reaction to be separated. On washing the cooled reaction mixture with water, crystals of 4,5,6,7-tetrahydrobenzo-(b)-thiophen-4-ylidene ethyl cyanoacetate were precipitated. After drying, broad needles were obtained from light petroleum (b.p. 60° - 80°), m.p. 78° - 80°. (Found: C, 64.1 : H, 5.58. $C_{13}H_{13}SO_2N$ requires C, 64.0 ; H, 5.28%). (See p. 103 for NMR data).

3-Methyl-4-nitroanisole (XLVII)

A stirred mixture of m-nitrocresol (2.0g), redistilled methyl iodide (5 ml.), anhydrous potassium carbonate (3.2g), and acetone (10 ml.) was refluxed for three hours. The cooled reaction was filtered at the pump, and the material in the Buchner funnel was washed with acetone, the washings being added to the filtrate. The acetone in the filtrate was replaced with petroleum ether (b.p. 80° - 100°) from which long needles of 3-methyl-4-nitroanisole separated. These were recrystallised from a small volume of petroleum ether (b.p. 80° - 100°) with charcoaling, m.p. 52° - 53° (lit.,[485] 55°). The yield was 1.72g (79%).

2-Nitro-5-methoxyphenylacetic acid (XLIX)

One hundred volume hydrogen peroxide (3.5 ml.) was added to a solution of 2-nitro-5-methoxyphenylpyruvic acid[485] (2.0g) in 2% aqueous sodium hydroxide (50 ml.) thereby discharging the red colour from the alkaline solution. The reaction was terminated after 15 minutes by the slow addition of dilute hydrochloric acid, a strong evolution of carbon dioxide taking place. The 2-nitro-5-methoxyphenylacetic acid which was precipitated was collected at the pump and washed with water. The washed precipitate was taken up in methanol and converted into the methyl ester by saturating this methanolic solution with hydrogen chloride gas. After standing overnight the methanol was boiled down to a small volume and the remaining solution was poured into dilute sodium carbonate solution. Extraction with ether followed by drying over sodium sulphate and removal of the ether gave an oily residue. This was crystallised from methanol to yield white needles of the methyl ester of 2-nitro-5-methoxyphenylacetic acid. These were recrystallised from ether, m.p. 75°. (Found: C, 53.1; H, 4.60. $C_{10}H_{11}NO_5$ requires C, 53.3; H, 4.89%).

5-Hydroxyoxindole

N-Chloroacetyl-p-anisidine[488] (25.0g) was added to a vigorously stirred melt of aluminium chloride (100g) and sodium chloride (20g) at 140°. Rapid heating to 235°-240° induced a brisk evolution of hydrogen chloride which slackened after five minutes. Care was taken to prevent

the reaction temperature from rising above 245° since this would have caused decomposition of the desired product. The hot melt was poured on to a marble slab, allowed to solidify, then pulverised before being added to crushed ice containing some dilute hydrochloric acid. The resulting suspension was kept at 3° - 5° overnight before collecting the precipitate at the pump. This precipitate was purified via its sodium salt (charcoal). A sample recrystallised from ethyl acetate melted at 268° - 269° when heated rapidly (lit.[488] 270° decomp.). The yield of 5-hydroxyoxindole was 10.36g (55%) (lit.[488] 45%).

5-Benzyloxy-oxindole (LIII). 5-Hydroxyoxindole (4.2g, 0.028 mole) was added to dry ethanol (15 ml.) in which sodium wire (0.644g, 0.028 mole) had been dissolved. Redistilled benzyl chloride (3.50g, 0.028 mole) was added to this solution which was then refluxed overnight. After cooling, the reaction mixture was precipitated by pouring into water. The precipitate was taken up in benzene, filtered and allowed to crystallise. Fine, white crystals of 5-benzyloxy-oxindole were thus obtained, m.p. 169° - 171°. Mass spectrometry showed these to have a molecular weight of 239, as calculated. (Found: C,75.3; H, 5.20. $C_{15}H_{13}NO_2$ requires C, 75.3; H, 5.44%).

3-(N,N-Dimethylaminoacetyl)-5-benzyloxy-oxindole. (LV)

5-Benzyloxy-oxindole (1.0g, 0.0042 mole) and N,N-dimethylglycine ethyl ester (1.0g, 0.0076 mole) were added to a refluxing solution of sodium ethoxide prepared from sodium wire (0.1g, 0.0043 mole) and ethanol (2.5 ml.). The temperature of the oil bath in which the reaction was heated was then raised from 120° to 135° for five hours during which time the solution first clarified and then later deposited the sodium salt of the desired product. After cooling the sodium salt was collected and washed with hot methanol before being dissolved in cold, glacial acetic acid. On pouring the acetic acid solution into water a white precipitate was obtained. This was collected, dried, and recrystallised from benzene (charcoal) to give white needles of 3-(N,N-dimethylaminoacetyl)-5-benzyloxyoxindole, m.p. 221° - 222° (decomp.). (Found: C, 70.2 ; H,6.07; N, 8.01. $C_{19}H_{20}N_2O_3$ requires C, 70.4 ; H, 6.17; N, 8.64).

This compound gave a purple colour with ferric chloride solution as does 3-acetyl-5-methoxy-oxindole.

5-Methanesulphonyloxybenzo-(b)-thiophen (LXXIII)

Methanesulphonyl chloride (25 ml.) was added dropwise over a period of 10-15 minutes to a well-stirred solution of 5-hydroxybenzo-(b)-thiophen[497] in dry pyridine (100 ml.) at 0°. The reaction was stirred at this temperature for a further 1 hour before being poured into ice water (500 ml.). The solid which soon separated was collected at the pump

and was thoroughly washed with dilute hydrochloric acid and water. After drying overnight over phosphorus pentoxide this material was taken up in benzene and chromatographed on Woelm neutral alumina, grade I, prepared in this same solvent. The fraction eluted with benzene was recrystallised from ethanol. Crystals of 5-methanesulphonyloxy-benzo-(b)-thiophen, m.p. 81° - 82°, were thus obtained (5.9g, 70%). (Found: C, 47.8; H, 3.78. $C_9H_8S_2O_3$ requires C, 47.8; H, 3.54%).

5-Benzylsulphonyloxybenzo-(b)-thiophen (LXXIV)

5-Hydroxybenzo-(b)-thiophen (1.34g, 0.010 mole) and benzylsulphonyl chloride (2.10g, 0.011 mole) were heated in dry pyridine (10 ml.) for one hour at 100°, then the reaction was cooled and poured into water. The aqueous suspension was extracted with ether, and the ethereal layer washed with dilute hydrochloric acid and water before being dried over sodium sulphate. The residue obtained upon evaporation of the solvent was chromatographed on Woelm neutral alumina, grade I, prepared in ethanol. The material eluted with ethanol was recrystallised from this same solvent to give white crystals of 5-benzylsulphonyloxybenzo-(b)-thiophen (3.7g, 77%), m.p. 86° - 86.5°. (Found: C, 59.3; H, 4.18. $C_{15}H_{12}S_2O_3$ requires C, 59.2; H, 3.95%).

3-Chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen (LIX)

37% Aqueous formaldehyde solution (75 ml.) was saturated with hydrogen chloride gas. To this rapidly stirred warm solution was added 5-methanesulphonyloxybenzo-(b)-thiophen (3.2g), in small portions. Stirring was continued while the gas was passed in for a further two hours during which time white crystals of 3-chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen separated. These were recrystallised once from ethanol (100 ml.) to give shiny needles, m.p. 125° - 125° (2.2g, 51%). (Found: C, 43.4 ; H, 3.34. $C_{10}H_9S_2O_3Cl$ requires C, 43.4 ; H, 3.26%). Extraction of the reaction mixture with benzene enabled a further mixture of this product and starting material to be recovered.

3-Chloromethyl-5-benzylsulphonyloxybenzo-(b)-thiophen

5-Benzylsulphonyloxybenzo-(b)-thiophen (1.68g) was added in small portions to a rapidly stirred 37% aqueous formaldehyde solution (50 ml.) at 70°, saturated with hydrogen chloride. Stirring was continued while hydrogen chloride was passed in for a further hour, then the reaction mixture was diluted with water (50 ml.) before extraction with benzene (3 x 25 ml.). The benzene extracts were thoroughly washed with water (2 x 50 ml.), sodium bicarbonate solution (1 x 50 ml.) and more water (1 x 50 ml.) and dried over sodium sulphate before removal of the solvent on a rotatory evaporator. The yellow oil thus obtained was crystallised from methanol to give needles of 3-chloromethyl-5-benzylsulphonyloxybenzo-(b)-thiophen contaminated with unchanged starting material (1.66g). On dissolving

these needles in a small volume of petroleum ether (b.p. 80° - 100°) most of this starting material could be separated by filtration. The filtrate was evaporated to dryness and the residue was recrystallised twice from methanol to give white needles (1.07g, 55%), m.p. 104.5°-107° (Found: C, 54.99; H, 3.40. $C_{16}H_{13}S_2O_3Cl$ requires C, 54.47; H, 3.69%).

3-Acetoxymethyl-5-methanesulphonyloxybenzo-(b)-thiophen

3-Chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen (0.20g) and sodium acetate (0.10g) were refluxed in anhydrous acetic acid (2.5 ml.) for two hours, then the hot solution was filtered. The residue obtained after removal of the acetic acid on a rotatory evaporator was shaken with water (5 ml.) and benzene (15 ml.). The benzene layer was separated, dried, and evaporated to leave 3-acetoxymethyl-5-methanesulphonyloxybenzo-(b)-thiophen (0.11g) as a colourless oil which could not be crystallised. Thin layer chromatography on silica gel using ether as solvent showed this to be a single species. The infra-red spectrum showed absorption at 1715 cm^{-1} characteristic of an acetate function. The compound was not characterised further. 3-Acetoxymethyl-5-benzylsulphonyloxybenzo-(b)-thiophen was similarly prepared.

Hydrolysis of 3-acetoxymethyl-5-methanesulphonyloxybenzo-(b)-thiophen

3-Acetoxymethyl-5-methanesulphonyloxybenzo-(b)-thiophen (0.10g), potassium hydroxide (0.50g), water (2 ml.) and ethanol (1.5 ml.) were refluxed

for three hours, cooled, acidified with dilute hydrochloric acid, then extracted with ether. This extract was dried over sodium sulphate and the residue obtained upon removal of the solvent was found to be identical with authentic 3-hydroxymethyl-5-hydroxybenzo-(b)-thiophen (LXII) supplied by Dr. S. Reid of the Department of Experimental Pharmacology at the University of Glasgow. Thin layer chromatography on silica gel also confirmed the absence of any 2-hydroxymethyl-5-hydroxybenzo-(b)-thiophen - an authentic specimen of which had also been made available by Dr. Reid.

3-Acetoxymethyl-5-benzylsulphonyloxybenzo-(b)-thiophen was treated in a similar manner to the above ester. Again the product was found to be identical with authentic 3-hydroxymethyl-5-hydroxybenzo-(b)-thiophen.

3-Cyanomethyl-5-methanesulphonyloxybenzo-(b)-thiophen (LXV)

3-Chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen (1.0g) dissolved in acetone (6 ml.) was added to a solution of potassium cyanide (0.5g) in water (2 ml.). The suspension was stirred under reflux for 16 hours then cooled and poured into water (100 ml.). The resulting white suspension soon deposited crystals of 3-cyanomethyl-5-methanesulphonyloxybenzo-(b)-thiophen which were collected at the pump and were thoroughly washed with water. After air drying these were crystallised from ethanol (0.6g, 65%), m.p. 128° - 130°. (Found: C, 49.3 ; H, 3.43. $C_{11}H_9S_2O_3N$ requires C, 49.5; H, 3.38%). The infra-red spectrum showed characteristic cyanide absorption at 2235 $cm.^{-1}$

3-Cyanomethyl-5-benzylsulphonyloxybenzo-(b)-thiophen

A solution of 3-chloromethyl-5-benzylsulphonyloxybenzo-(b)- thiophen (500 mg.) in ethanol (25 ml.) was added to potassium cyanide (500 mg.) dissolved in water (1 ml.). The resulting suspension was refluxed for two hours, then filtered before addition of water (25 ml.) and removal of the ethanol by distillation. The resulting aqueous solution was extracted with n-butanol (3 x 25 ml.). The butanol extract was shaken with N-hydrochloric acid in order to hydrolyse any isocyanide present and then it was dried over sodium sulphate. The residue obtained upon removal of the solvent was crystallised twice from ethanol to give white needles of 3-cyanomethyl-5-benzylsulphonyloxybenzo-(b)-thiophen, m.p. 148° (120 mg, 25%). (Found: C, 59.3; H, 3.64. $C_{17}H_{13}S_2NO_3$ requires C, 59.5; H, 3.79%).

3-Morpholinomethyl-5-methanesulphonyloxybenzo-(b)-thiophen

3-Chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen (0.50g, 0.0018 mole) and morpholine (0.16g, 0.0018 mole) were added to a suspension of sodamide (0.22g, 0.0054 mole) in dry toluene (10 ml.). The mixture was refluxed for 18 hours, then the excess of sodamide was destroyed by the addition of water. The organic layer which separated was washed with water then dried over sodium sulphate. After removal of the solvent the desired product was obtained as a dark oil. This was taken up in anhydrous ether and converted into the hydrochloride by treatment with gaseous hydrogen

chloride. The precipitate thus formed was extracted into a small volume of water. This aqueous solution of the hydrochloride was then treated with a slight excess of saturated sodium picrate solution whereupon a copious yellow precipitate of 3-morpholinomethyl-5-methanesulphonyloxybenzo-(b)-thiophen picrate was obtained. Fine yellow needles were obtained on recrystallisation from ethanol, m.p. 189°-190° (Found: C, 43.5; H, 4.21. $C_{20}H_{20}S_2O_{11}N_4$, C_2H_5OH requires C, 43.8; H, 4.32%).

The following two amines were similarly prepared.

a) 3-Piperidinomethyl-5-methanesulphonyloxybenzo-(b)-thiophen picrate, m.p. 204°-205°. (Found: C, 45.8.; H, 3.93. $C_{21}H_{22}S_2O_{10}N_4$ requires C, 45.5; H, 3.97%).

b) 3-Pyrrolidinomethyl-5-methanesulphonyloxybenzo-(b)-thiophen. This compound was not characterised, but was used directly for the next stage of the synthetic project.

3-Phthalimidomethyl-5-methanesulphonyloxybenzo-(b)-thiophen

3-Chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen (0.50g, 0.0018 mole) was dissolved in dimethyl formamide (10 ml.) and heated under reflux with potassium phthalimide (0.33g, 0.0018 mole) for 2 hours, during which time crystals of potassium chloride separated. The cooled reaction was poured into ethyl acetate and the resulting solution was thoroughly washed with water. The organic layer was dried over sodium sulphate and the solvent was removed to leave a residue which was twice recrystallised from ethanol. A sample for analysis of 3-phthalimidomethyl-5-methanesulphonyloxybenzo-(b)-thiophen thus obtained had m.p. 143°-144°.

(Found: C, 55.4; H, 3.64. $C_{18}H_{13}S_2O_5N$ requires C, 55.8; H, 3.36%).

3-Morpholinomethyl-5-hydroxybenzo-(b)-thiophen

3-Morpholinomethyl-5-methanesulphonyloxybenzo-(b)-thiophen (0.35g) dissolved in ethanol (10 ml.) was added to a 2.5% aqueous solution of sodium hydroxide (20 ml.). The mixture was heated on a steam bath for one hour, the ethanol being allowed to boil off. The cooled solution was acidified with dilute hydrochloric acid then dilute ammonium hydroxide solution was added until the pH was between 8 and 9. The resulting cloudy solution was extracted with ether and the ethereal layer was washed with water then dried over sodium sulphate. On removal of the solvent an oil was obtained. This was crystallised from ethyl acetate to give cubes of 3-morpholinomethyl-5-hydroxybenzo-(b)-thiophen m.p. 162°, (Found: C, 62.6; H, 5.80. $C_{13}H_{15}NO_2S$ requires C, 62.6; H, 6.02%). The amine was dissolved in ether and converted into the hydrochloride by treatment with hydrogen chloride. The precipitate thus formed was extracted into water and the aqueous extract was treated with a slight excess of saturated sodium picrate solution. The copious yellow precipitate of 3-morpholinomethyl-5-hydroxybenzo-(b)- thiophen picrate thus formed was recrystallised from methanol, m.p. 251.5°. (Found: C, 47.3; H, 3.85. $C_{19}H_{18}N_4O_9S$ requires C, 47.8; H, 3.77%).

The following two amines were similarly prepared.

a) 3-Piperidinomethyl-5-hydroxybenzo-(b)-thiophen picrate, m.p. 210°-211°. (Found: C, 50.2; H, 4.18. $C_{20}H_{20}N_4O_8S$ requires C, 50.4; H, 4.20%).

b) 3-Pyrrolidinomethyl-5-hydroxybenzo-(b)-thiophen picrate, m.p. 171° - 172°

3-Formyl-5-methanesulphonyloxybenzo-(b)-thiophen (LXXVIII)

3-Chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen (0.225 g) suspended in water (1 ml.) was refluxed with copper nitrate (0.090g) and concentrated nitric acid, S.G. 1.3 (0.150g) for 2½ hours, with vigorous stirring. As the reaction proceeded the initial blue colour from the cupric ion turned green as cuprous ion was formed. After cooling, the yellow precipitate which had formed was collected at the pump and recrystallised from benzene, m.p. 219°-223°. (Found: C, 46.8; H, 3.60. $C_{10}H_8S_2O_4$ requires C, 46.9; H, 3.13%). The infra-red spectrum showed absorption characteristic of aldehydes (1685 cm^{-1}) and sulphonate esters (1320 cm^{-1}).

5-Hydroxybenzo-(b)-thiophen-3-acetic acid (LX)

3-Cyanomethyl-5-methanesulphonyloxybenzo-(b)-thiophen was heated in a 5% sodium hydroxide solution on a steam bath for one hour. The cooled solution was acidified with 10% hydrochloric acid solution, and extracted with ether. The ethereal extract was dried over sodium sulphate, then the solvent was removed to leave a residue of 5-hydroxybenzo-(b)-thiophen-3-acetic acid m.p. 174°-177° which was crystallised from benzene in which it was slightly soluble. (Found: C, 57.8; H, 3.99. $C_{10}H_8SO_3$ requires C, 57.8; H, 3.83%).

3-Cyanomethyl-5-hydroxybenzo-(b)-thiophen (LXXV)

3-Cyanomethyl-5-methanesulphonyloxybenzo-(b)-thiophen (0.37g, 0.0014 mole) was dissolved in refluxing dry ethanol (15 ml.). Sodium (0.064g, 0.0028 mole) was added to the refluxing solution and as it dissolved a precipitate of sodium methanesulphinate appeared. After 15 minutes the reaction was cooled, filtered, and poured into a 5% solution of hydrochloric acid whereupon a white suspension was obtained. This was quickly extracted with ether. The ether extract was shaken with a 5% sodium hydroxide solution which was immediately separated and acidified with dilute hydrochloric acid. The white suspension thus obtained, which was no longer contaminated with traces of unchanged starting material, was extracted with ether. The extract was dried over sodium sulphate, and on replacement of the ether by petroleum ether (b.p. 40°-60°) white crystals of 3-cyanomethyl-5-hydroxybenzo-(b)-thiophen were obtained, m.p. 128°-130° (0.11g, 52%). (Found: C, 63.7 H, 3.93 $C_{10}H_7SON$ requires C, 63.5; H, 3.70%).

Hydrogenolysis of 5-methanesulphonyloxybenzo-(b)-thiophen

A few milligrams of 5-methanesulphonyloxybenzo-(b)-thiophen were dissolved in dry ethanol and treated with an excess of sodium. After all the sodium had dissolved the solution was filtered and acidified with dilute hydrochloric acid. The resulting suspension was extracted with chloroform. The chloroform extract was dried over sodium sulphate and on removal of the solvent a residue which could be recrystallised from petroleum ether

(b.p. 80°-100°) was obtained. This was identified as 5-hydroxybenzo-(b)-thiophen through comparison (T.L.C., mixed m.p.) with an authentic specimen.

3-β-Ethylamino-5-hydroxybenzo-(b)-thiophen (LVII)

3-Cyanomethyl-5-hydroxybenzo-(b)-thiophen (0.10g) was dissolved in methanol (25 ml.) saturated with ammonia. Approximately 0.4g of Raney nickel was added to this solution. Hydrogenation was then performed at normal temperature and pressure. The amount of hydrogen absorbed could not be calculated since ammonia was being released from the reaction mixture. After 2 hours the hydrogenation was terminated and the reaction was filtered. On removal of the solvent on a rotatory evaporator a pale grey solid residue was obtained. On addition of ether (25 ml.) it was observed that this residue did not dissolve. The subsequent addition of water (25 ml.) also failed to dissolve an appreciable amount of the residue, but on adding a few drops of dilute hydrochloric acid solution the residue immediately dissolved in the aqueous layer which developed a pale green colour. The aqueous layer was separated and treated with an excess of saturated sodium picrate solution. On standing overnight a slight precipitate was obtained. By reducing the volume of solution on a rotatory evaporator a copious yellow precipitate of 3-β-ethylamino-5-hydroxybenzo-(b)-thiophen picrate was obtained. This was recrystallised from a small volume of water to give yellow crystals, m.p. 191°-193°. (Found:

C, 45.4; H, 3.39. $C_{16}H_{14}N_4SO_8$ requires C, 45.5; H, 3.32.)

5-Hydroxybenzo-(b)-thiophen-3-propionic acid

3-Chloromethyl-5-methanesulphonyloxybenzo-(b)-thiophen (1.00g, 0.00036 mole) dissolved in hot xylene (10 ml.) was added to a solution of sodio-malonic ester prepared from sodium (0.083g, 0.00036 mole) and diethyl malonate (0.609g, 0.00038 mole) in xylene (5 ml.). The mixture was refluxed for three hours then poured into cold water. The xylene layer was separated and the aqueous layer was extracted with ether. The organic layers were combined and dried over sodium sulphate. Removal of the solvents left a brown oil (1.29g, 89%). The presence of peaks characteristic of sulphonate ester absorption at 1380 cm^{-1} in the infra-red spectrum of this oil (liquid film) indicated that it was 5-methanesulphonyloxybenzo-(b)-thiophen-3-β-carboxy-propionic acid diethyl ester; this diester was hydrolysed by refluxing overnight in a solution of potassium hydroxide (2.5g) in water (5 ml.). The cooled reaction was then poured into 10% hydrochloric acid solution and the resulting suspension was extracted with ether. The extract was dried over sodium sulphate, and on removal of the ether 5-hydroxybenzo-(b)-thiophen-3β-carboxy-propionic acid (0.43g, 64%) was obtained as a pale brown oil. This oil was decarboxylated by heating at 180° in a stream of nitrogen. The hard brown glass obtained on cooling was sublimed at 160° at 0.1 mm. Hg to give white crystals of 5-hydroxybenzo-(b)-thiophen-3-propionic acid, m.p. 181.5°-182.5° (0.19g, 53%). (Found: C, H, $C_{11}H_{10}O_3S$ requires

C, 59.5; H, 4.50%).

Nitration of Benzo-(b)-thiophen-3-carboxylic acid

Benzo-(b)-thiophen-carboxylic acid (10.0g) was dissolved in glacial acetic acid (100 ml.), and the resulting solution heated to 60°. Concentrated sulphuric acid (10 ml.) was added and then concentrated nitric acid (3.6 ml.) was added dropwise. The reaction mixture was kept at 60° for one hour during which time crystalline material began to appear. After cooling, the crystalline product was collected by filtration, and after one recrystallisation from ethanol had m.p. 237°-242° (6.4g). Repeated recrystallisation from ethanol or acetic acid, or purification via the methyl ester (see below), gave as pale yellow needles of 6-nitrobenzo-(b)-thiophen-3-carboxylic acid, m.p. 263° - 265° (Found:[502] C, 48.43; H, 2.72. $C_9H_5NO_4S$ requires C, 48.43; H, 2.26%).

The mother liquor remaining after collection of the crystalline material (above) was poured into water, the crystalline precipitate collected and recrystallised from ethanol to give cubic crystals of 4-nitrobenzo-(b)-thiophen-3-carboxylic acid, m.p. 219° - 220° (2.5g). (Found:[502] C, 48.60; H, 2.09. $C_9H_5NO_4S$ requires C, 48.43; H, 2.26%).

Purification of Nitrobenzo-(b)-thiophen-3-carboxylic acids

Conversion of the 4- and 6-nitrobenzo-(b)-thiophen-3-carboxylic acids into their methyl esters was carried out by treatment of an ethanol-ether solution of the acid with excess of diazomethane. Removal of the solvents and extraction of the residue into ether, followed by washing with sodium bicarbonate solution and water, then removal of the ether, gave the desired esters.

4-Nitrobenzo-(b)-thiophen-3-carboxylic acid methyl ester was dissolved in carbon tetrachloride solution and passed down a Woelm neutral alumina II column prepared in the same solvent. Elution with carbon tetrachloride gave crystals of m.p. 77.5° - 78° (pet. ether, b.p. 40° - 60°).

6-Nitrobenzo-(b)-thiophen-3-carboxylic acid methyl ester was dissolved in a large volume of carbon tetrachloride solution which was then added to a Woelm neutral alumina II column prepared in the same solvent. Thorough elution with carbon tetrachloride removed traces of the 4-nitro isomer. Elution of the 6-isomer with benzene gave crystals of m.p. 167° - 168° (benzene).

The methyl esters were hydrolysed by heating in 10% aqueous potassium hydroxide to which sufficient ethanol to effect solution was added. The acids thus obtained had the same melting points as indicated above.

6-Nitrobenzo-(b)-thiophen[529]

6-Nitrobenzo-(b)-thiophen-3-carboxylic acid was heated in quinoline in the presence of copper bronze for one hour, under nitrogen. The cooled reaction was diluted with ether, filtered, and the ether removed. On pouring the quinoline solution into 6N-sulphuric acid a solid was precipitated. On recrystallisation from ethanol needles of 6-nitrobenzo-(b)-thiophen were obtained, m.p. 83° - 84° (lit.[529] 84° - 85°).

6-Nitrobenzo-(b)-thiophen-1,1-dioxide[444]

6-Nitrobenzo-(b)-thiophen (0.05g) was heated in acetic acid (2.5 ml.) and 30% hydrogen peroxide solution (1.5 ml.) on a steam bath for one hour. Water was added and the solution was extracted with chloroform. The extract was dried over sodium sulphate and on removal of the solvent a residue was obtained. This was recrystallised from ethanol to give crystals of m.p. 185° - 186° which did not depress the melting point of authentic[444] 6-nitrobenzo-(b)-thiophen-1,1-dioxide.

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