THE SYNTHESIS OF NEW PURINE ANALOGUES

A THESIS

PRESENTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN THE

ORGANIC CHEMISTRY DEPARTMENT

OF THE

UNIVERSITY OF ADELAIDE

By

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FEBRUARY, 1965

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SUMMARY

Although many purine analogues and derivatives have been found to possess tumour-inhibiting activity, the structural features necessary for such activity are still far from clear. The present investigation has been concerned with the synthesis of derivatives of 5-azaindole, pyrazolo[4,3-c]pyridines, pyrazolo[3,4-b]pyridines and pyrazolo[3,4-d]pyrimidines for assessment of their tumour-inhibiting activities.

Unsuccessful attempts have been made to extend the Reissert indole synthesis and the Harley-Mason indole synthesis to the preparation of 5-azaindoles. Satisfactory methods for the preparation of 4-nitronicotinic acid 1-oxide, 4-anilinonicotinic acid 1-oxide and 4-methylaminonicotinic acid 1-oxide, necessary intermediates in the above syntheses, have been devised. As part of this investigation the reduction of 4-nitro-3-picoline 1-oxide and similar N-oxides has been studied. This work has been reported in two publications (G.M. Badger and R.P. Rao, Aust.J.Chem., 1964, 17, 1399 and R.P. Rao, Aust.J.Chem., 1964, 17, 1434).

3-Hydroxy-1H-pyrazolo[4,3-c]pyridine, 3-hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine, 3-hydroxy-1-methyl-1H-pyrazolo[4,3-c]-pyridine, and their 5-oxides, have been successfully prepared and these have been submitted to the Sloan-Kettering Institute for screening. Lactam-lactim tautomerism in these compounds is described. This work is accepted for publication (G.M. Badger and R.P. Rao, Aust.J.Chem., 1965, 18, in press).

Analogous derivatives of the alternative diazaindole, pyrazolo[3,4-*b*]pyridine have also been prepared. In addition 4-mercapto-3-methylpyrazolo[3,4-*d*]pyrimidine; 4-amino-6-hydroxy-(and 6-mercapto-) 3-methylpyrazolo[3,4-*d*]pyrimidines; and 4,6-dihydroxy- and 4-hydroxy-6-mercapto-3-methylpyrazolo[3,4-*d*]-pyrimidines have been prepared. This work will shortly be submitted as a paper for publication in the Aust.J.Chem.

STATEMENT

The present work incorporates no material previously submitted for a degree in any University, except where due reference has been made.

(Ravindra Pratap Rao)

Adelaide,
February, 1965

ACKNOWLEDGMENTS

I would like to express my deep appreciation of the stimulating and encouraging guidance of Professor G.M. Badger, to whom this work owes its inception, and whose wide knowledge of the chemistry of heterocyclic compounds was always at my disposal.

I should also like to thank other members of the staff for their co-operation.

The support of the University of Adelaide Anti-Cancer Foundation is also gratefully acknowledged.

(Ravindra Pratap Rao)

Adelaide,
February, 1965.

INTRODUCTION

Cancer is no ordinary disease and it has long been shrouded in mystery. Peyrilhe, writing in the latter part of the eighteenth century (Dissertation Academique Sur Le Cancer, Paris, 1776) claimed that every attempt to cure a cancer, by any method which is to restore the diseased tissues to a healthy state, is not only vain but absurd. To some extent this statement can be justified even in the light of our present day knowledge of the origin and treatment of cancer. Unfortunately even the most modern methods of curing cancer are pitifully inadequate. These facts provide ample justification for the most painstaking and persistent pursuit for finding a cure for cancer.

Cancer is a neoplastic (Greek, neos new, and plasma formation) disease. It is a group of more or less similar disorders in which a mass of new tissue grows independently and invades surrounding structures and organs. This outgrowth has no physiological function and is referred to as a cancerous growth. This definition[1] refers to the solid tumours which, in the main, are divided into sarcomas (made up of a substance like embryonic connective tissue) and carcinomas (new growths made up of epithelial cells). The leukaemias are diseases of the blood-forming organs, characterised by a marked increase in the number of leucocytes (white blood cells) and their precursors in the blood stream, together with enlargement and proliferation of the lymphoid tissue of the spleen, lymphatic glands

and bone marrow.

The control of cancer is a problem in applied science that remains handicapped by the fact that the relevant frontiers of knowledge have not yet been adequately explored by pure science. Cancer research therefore proceeds in many different ways, developing and applying what is already known or seeking along the outer margins of fundamental knowledge for new facts that may be applicable to its problems. Clinical research, together with certain kinds of laboratory research immediately related thereto, is concerned with improvements in methods of diagnosis and prognosis, with more effective eradication by surgery and radiotherapy, with the testing of chemotherapeutic methods which might retard or check the growth of certain kinds of cancer, and also with the amelioration of the suffering of incurable cancer. In spite of the fact that the approaches have been empirical in the sense that a complete understanding of the biology of cancer has not been reached, progress continues, and the advances that have been made in these directions have been substantial. The rational treatment and cure of cancer must await a complete understanding of the biology of cancer.[2] Such an understanding can only be acquired by experimental laboratory research, organised and conducted with this objective in mind. Nevertheless, cancer research is no longer groping in the dark;[cf.2] up to a point, the general nature of cancer is clear, and its

origin can be related to many possible causes. Thus the main directions in which cancer research must be pursued are clear enough. The quest calls for the present and future resources of many scientific disciplines and specialities, ranging from physics and chemistry, through biochemistry and sister sciences, to many branches of biological and medical science.

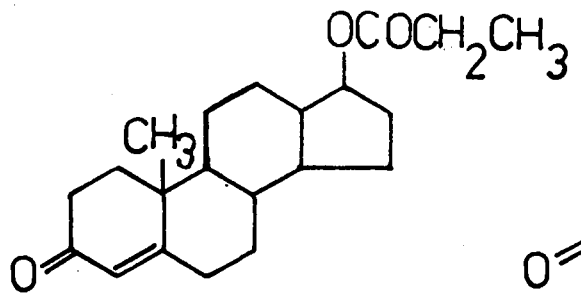
CHEMOTHERAPY OF CANCER

Records which date back to the earliest days in the history of medicine refer to incurable and fatal conditions which may well have been cancer. References to cancer have been found in earliest recorded Roman, Greek and Sanskrit writings. In the Sanskrit language it is known as "ARBUD". Through the ages man has attempted to cure the disease through the use of empirically concocted "nostrums" and autochthonous drugs, mostly plant and herbal preparations. Progress in cancer research over the past two decades has surpassed that of the previous two centuries. Clinical control of malignant disease may be approached by a number of possible routes, one of the more hopeful of which is cancer chemotherapy. The modern developments in the chemotherapy of cancer stem from two main sources, one through established knowledge and the other through accidental findings.

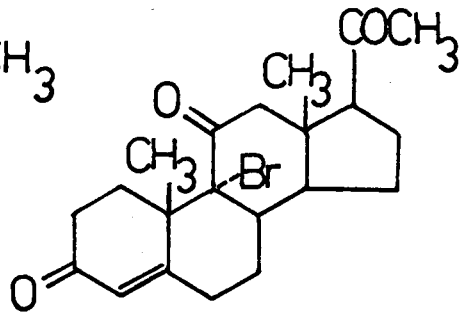
HORMONE THERAPY

The first source comprises the pioneer work of Lacassagne and, later, Huggins,[3] who established the relationship of male sex hormones (androgens) to the genesis of cancer of the prostate in men; and the finding of Beatson[4] (as long ago as 1896) that some mammary carcinomas regressed following removal of the ovaries. This ultimately resulted in the establishment of the relation between female sex hormones (oestrogens) and cancer of the breast in women. The result of the above findings was the development of what is known as the 'Hormonal Therapy' or simply 'Hormone Therapy'. This consists of controlling the growth of cancer cells by stopping the supply of certain hormones. In men, the chief sources of androgens are the gonads, and two methods have been developed to alter the endocrine environment of the tumour; the first is castration (orchiectomy) and the second is neutralisation of the male hormone by administering female sex hormones (in practice the synthetic ones e.g. stilboestrol, diethylstilboestrol and hexoestrol are used). According to Nes[5] the application of diethylstilboestrol against disseminated neoplasms marked the beginning of the chemotherapy of cancer. On the other hand, a whole group of hormones produced in the ovaries, the adrenals and the pituitary are known to regulate the activities of the female breast, i.e., its growth during puberty and its preparation for lactation during

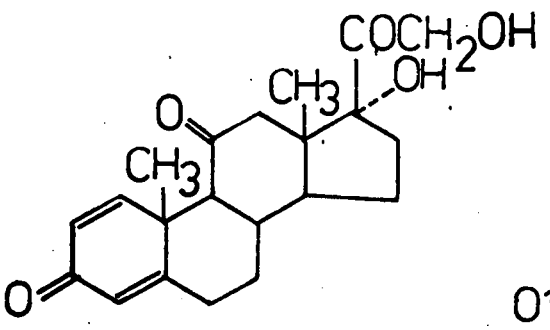
PLATE 1
HORMONES



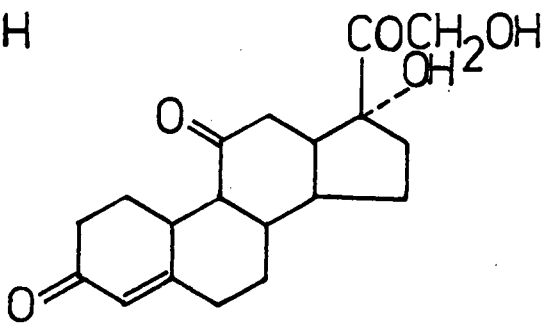
Testosterone propionate



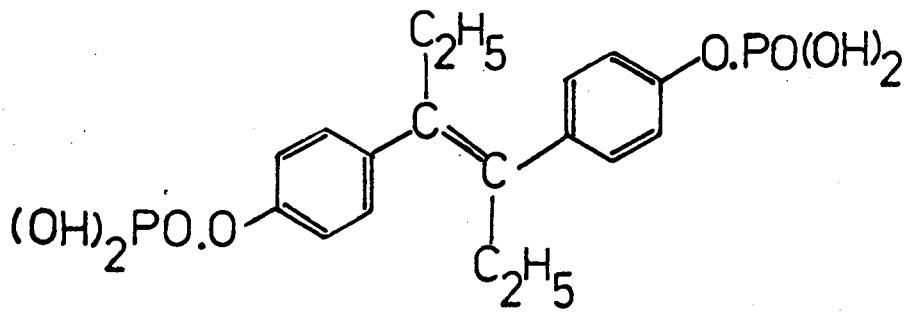
Broxorone, BOP



Prednisone



Cortisone



Diethylstilboestrol diphosphate

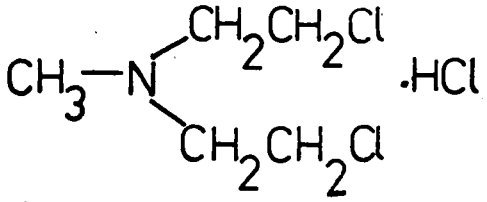
pregnancy. Cancer of the breast can be cured by undoing the effect of some or all of these hormones. Reference has already been made to the observations of Beatson, on the palliative effect of ovariectomy (removal of the ovaries) in breast cancer. This subject has been further exploited and now-a-days either ovariectomy and adrenalectomy (removal of the adrenals) or hypophysectomy (removal of the pituitary) are used. The second line of approach is hormone therapy; it involves giving male testicular hormone (testosterone) to women suffering from breast cancer, and is therefore analogous to the hormone therapy of prostatic cancer in men. In addition to these, cortisone and its derivatives and ACTH are used in management of acute and chronic lymphatic leukaemia[6] and malignant lymphomas.[7] The mechanistic knowledge of the action of all these substances used in hormone therapy is still inadequate, inspite of the fact that this type of therapy has been in use for well over twenty years. The difficulties[8] encountered in hormone therapy of malignant tumours necessitate further improvements in this therapy to make it more effective.

BIOLOGICAL ALKYLATING AGENTS

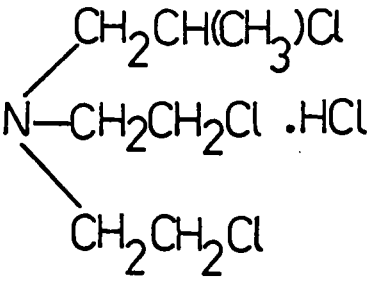
The second source was the research on chemical warfare conducted during the First World War (1914-18)[9] and later; it led to the finding that exposure to mustard gas (bis-2-chloroethylsulphide) is capable of producing systemic effects on the haemopoietic and

PLATE 2

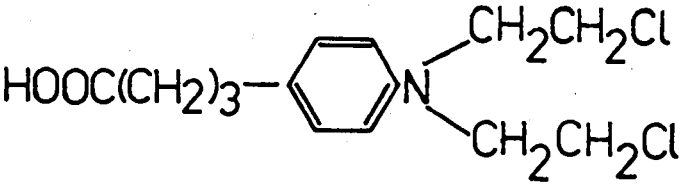
BIOLOGICAL ALKYLATING AGENTS



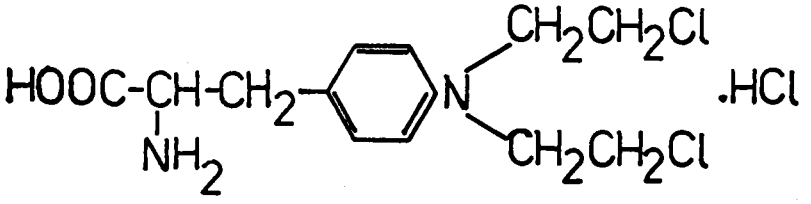
Nitrogen mustard, HN_2,
Embichin



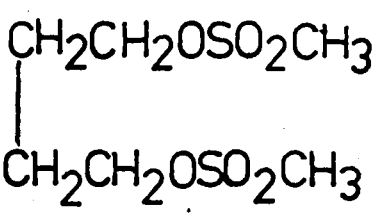
Novembichin



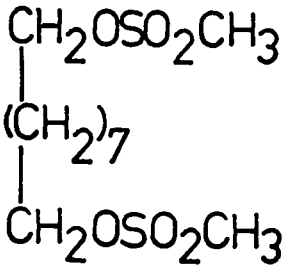
Chlorambucil



Melphalan



Myleran



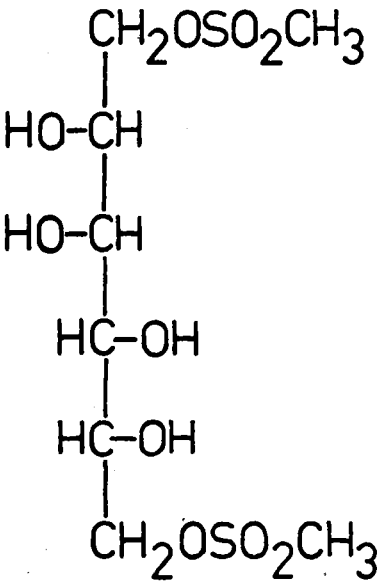
Nonane

especially the leucopoietic tissues and on the gastrointestinal tract.[10] Since leukopenia (a profound depression of white blood cells) would be an indication of improvement in leukaemia, bis-chloroethylsulphide was tested in leukaemic patients, but because of its toxicity and vesicant action its use was abandoned. During the Second World War many new compounds were developed for the purposes of chemical warfare. These were the nitrogen analogues of mustard gas or so-called "nitrogen mustards". Prominent among these were the bis- and tris-2-chloroethylamines. It was soon recognized that these, like mustard gas itself, were not merely contact-vesicants, but could induce cytotoxic effects in a wide variety of tissues, and especially in those which are in a state of active proliferation.[11] Bis-2-chloroethylmethylamine hydrochloride (code name HN2) was the second of these compounds. It is still the drug of choice for the control of Hodgkin's disease. It can be reasonably said that the information gathered during the studies related to the physiological action of HN2 on a large variety of neoplasms forms the basis on which 'cancer chemotherapy' now stands. Since that time a number of nitrogen mustards and related compounds have been developed and found to be useful in the chemotherapy of cancer. These drugs have been shown to produce their biological activity by chemical reactions (characterized as alkylation) with essential functional groups in tissues. The drugs are therefore known as biological alkylating agents, and the most important are:

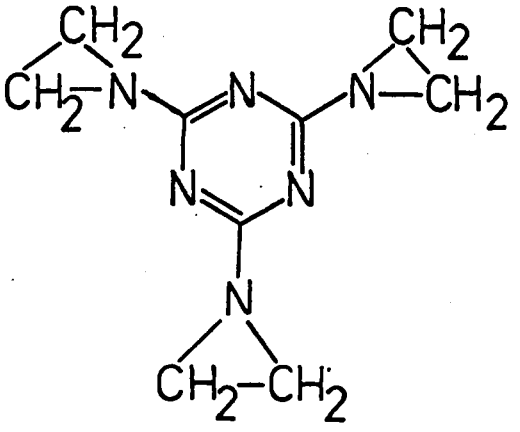
(i) bis-2-chloroethylmethylamine (HN2, nitrogen mustard, embichin); (ii) nitromine (HN2 oxide); (iii) noembichin; (iv) chlorambucil; (v) N,N-di-(2'-chloroethyl)-2-naphthylamine (R48); (vi) N,N-di-(2'-chloroethyl)-4-aminophenylbutyric acid (CB1348); (vii) melphalan; (viii) cyclophosphamide; (ix) degranol; (x) dopan; (xi) asaline; (xii) myleran (G.T. 41); (xiii) mannitol myleran; (xiv) nonane (1,9-dimethanesulphonoxynonane); (xv) 2,4,6-triethyleneimino-s-triazine (TEM); (xvi) N,N',N''-triethylenephosphoramide (TEPA); (xvii) N,N',N''-triethylenethiophosphoramide (THIOTEPA); (xviii) 1,2:3,4-diepoxybutane and (xix) 1,1'-bis(2,3-epoxypropyl)-4,4'-dipiperidine. These are used for the treatment of Hodgkin's disease, chronic lymphatic, lymphocytic and myeloid leukaemia, neuroblastoma, rhabdomyosarcoma, lymphosarcoma, malignant melanoma, and many related disorders.[12,13,14] Most of these compounds lack selectivity in their action and while destroying leukaemic tissue also cause damage to haemopoietic and other growing tissues. None of these compounds acts as a cure of any neoplastic disease; however, their tumour-inhibiting effect lends hope for the development of truly effective drugs.

PLATE 3

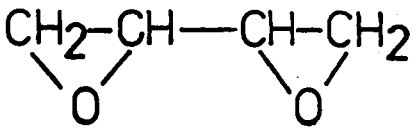
BIOLOGICAL ALKYLATING AGENTS



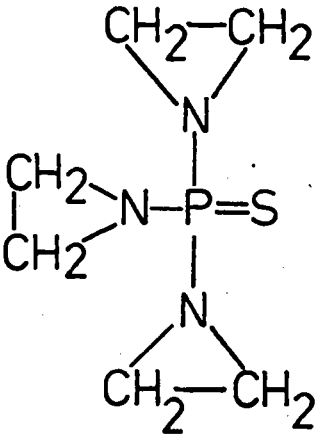
Mannitol Myleran



TEM, 2,4,6-triethyleneimino-s-triazine



1,2,3,4-Diepoxy-butane



Thiotepa

ANTIMETABOLITES

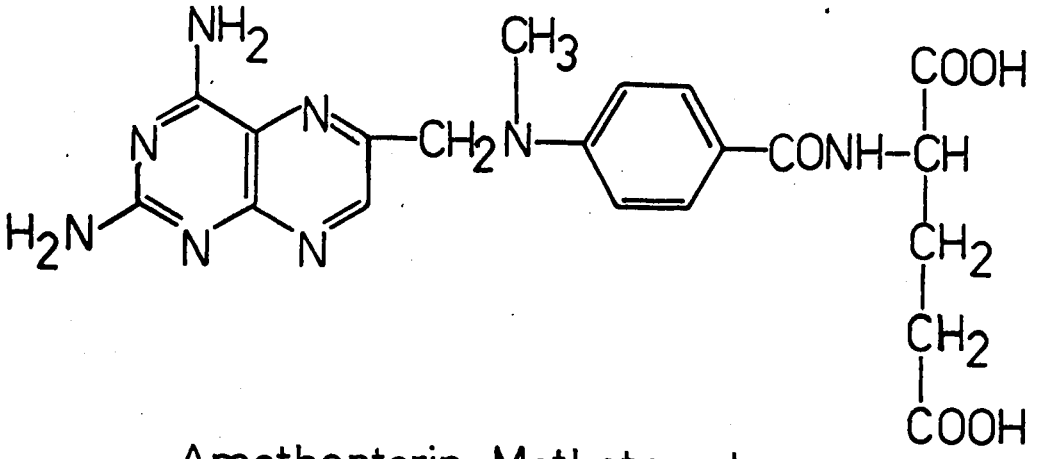
Alongside the alkylating agents came the development of metabolite antagonists or enzyme inhibitors which have proved their value in carcino-chemotherapy. The biological activity of an antimetabolite depends on interference with the synthesis or utilization of a normal metabolite in the body. The most important classes of antimetabolites of value in carcino-chemotherapy are the folic acid antagonists, and the structural analogues of purines and pyrimidines. Folic acid and its precursors play a vitamin-like part in the production and maturation of red blood cells, and this prompted their use in the treatment of some forms of anaemia. In 1948, Farber and his co-workers[15] noted that the administration of folic acid to leukaemic children failed to improve their anaemia and may possibly have accelerated the disease. This observation resulted in the application of anti-folic acid compounds in the treatment of acute leukaemia. They reported temporary remissions in acute leukaemia in children produced by folic acid antagonist, aminopterin. This finding was of great importance as it gave a tremendous impetus to the study of folic acid antagonists as well as to cancer chemotherapy as a whole. Of many analogues of folic acid prepared since then, amethopterin[16] (methotrexate) is the most successful, and it is now a standard drug. Amethopterin is the only known drug that will produce actual "cures" in one form of human neoplastic disease, the choriocarcinoma of the pregnancy state.[33]

In combination with 6-mercaptopurine and cortisone, it eradicates all symptoms and laboratory evidence of acute leukaemia in some children. Unfortunately this cure is not a permanent one and the disease not only returns but becomes resistant to antifolic acid drugs. Some tumours which have proved resistant to methotrexate have responded well to vincaleukoblastine, an alkaloid derived from periwinkle. The activity of these drugs depends on interference with the role of folic acid in the biosynthesis of purines and pyrimidines, resulting in a deficiency of these bases and hence impairment of the subsequent nucleic acid synthesis of purine-containing enzymes with slowing down effects on cell division and growth. The drugs resemble the vitamin chemically, but are sufficiently different to be unable completely to substitute for it in the cell.

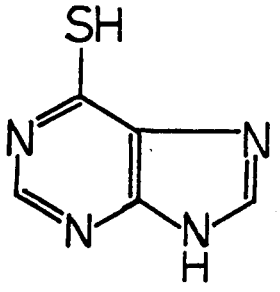
Mammals are able to synthesise their nucleic acids from simple chemical precursors, purines and pyrimidines. Cell division depends upon the synthesis of deoxyribonucleic acid (DNA) in the nucleus, which is required for the duplication of the hereditary material - the chromosomes - during interphase (the period between successive cell divisions). The rationale of the exogenously supplied antipurines and antipyrimidines in carcino-chemotherapy is of interference with nucleic acid biosynthesis by their metabolic incorporation into a "fraudulent" DNA (or RNA) thereby providing the cell with the wrong precursors. In certain cases they upset

PLATE 4

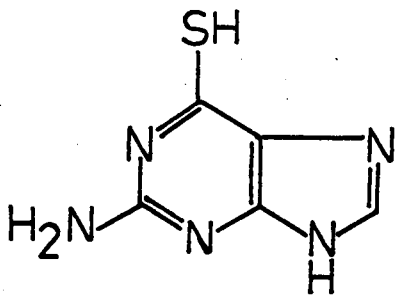
ANTIMETABOLITES



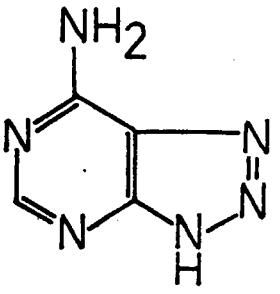
Amethopterin, Methotrexate



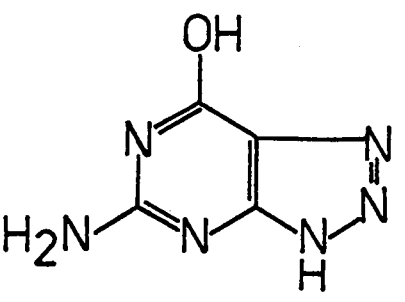
6-Mercaptopurine



Thioguanine



8-Azaadenine

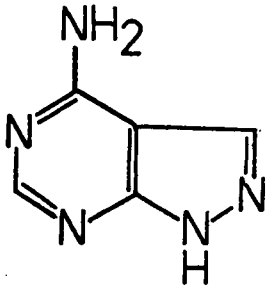


8-Azaguanine

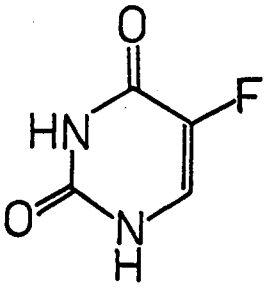
the protein synthesis or interfere with the enzyme systems, which are themselves not involved in nucleic acid synthesis but are normally concerned in the biosynthesis of nucleic acid from the normal purines and pyrimidines. Thus it has recently been suggested that 6-mercaptopurine acts as a tumour-inhibitor by inhibiting the synthesis of nicotinamide-adenine-dinucleotide.[17] Hitchings and his co-workers[18] first prepared 6-mercaptopurine from hypoxanthine; and Burchenal et al.[19] found the drug effective in inducing remissions in acute leukaemia and chronic myelocytic leukaemia. It has been suggested[20] that 6-mercaptopurine is the drug of choice for the treatment of acute leukaemia in adults. Its riboside has been found to have a better therapeutic index in experimental animals than the parent drug. After a time acute leukaemia becomes resistant to the drug, and further treatment with other drugs of the same group becomes futile. However, there is no cross-resistance between the purine antagonists and the antifolic acid drugs. Many other purine analogues have been prepared and found effective as carcino-chemotherapeutic drugs. Thus thioguanine; 2,6-diaminopurine; 6-chloropurine; 8-azaguanine; and the ribosides of most of them have been synthesised and tested.[1,14] Most have been used clinically. Other interesting compounds[21] in this group are many derivatives of 6-mercaptopurine; thioguanosine; 9-β-D-xylofuranosyladenine; psicofuranine; 8-azaadenine; 2,6-diamino-8-azapurine and 8-azahypoxanthine. Robins[22] prepared some

PLATE 5

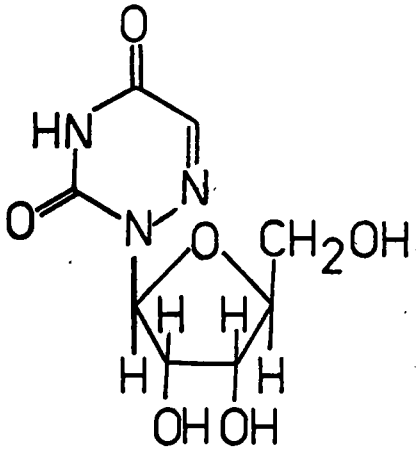
ANTIMETABOLITES



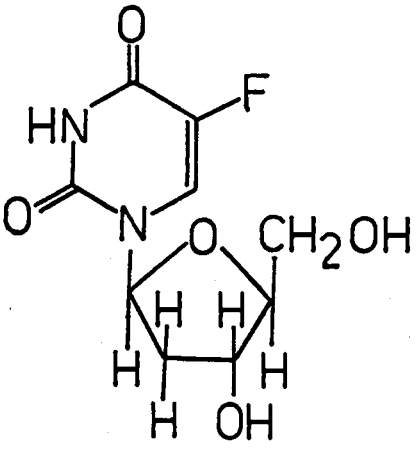
4-Aminopyrazolo-[3,4-d]pyrimidine



5-Fluorouracil



6-Azauridine



5-Fluoro-2'-deoxyuridine

4-aminopyrazolo[3,4-d]pyrimidines, which were tested by Skipper et al.[23] and found to be effective against resistant strains of adenocarcinoma 755 and leukaemia L1210. Unluckily, these compounds led to severe liver toxicity and their application as clinical agents remains unrealised.

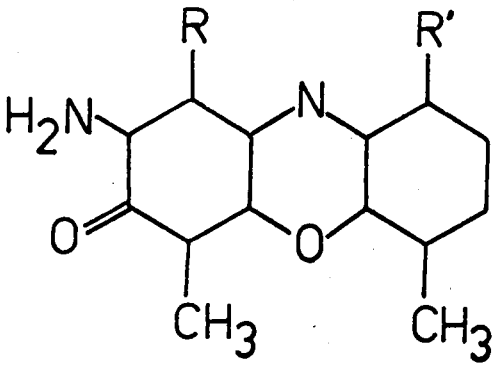
A new series of antagonists of pyrimidines has been recently developed: 5-fluorouracil[24] and its riboside and deoxyriboside.[25] These have shown promise in large scale clinical trials. Another promising antipyrimidine is 6-azauridine which is surprisingly free from any side-effects and lacks cross-resistance with 6-mercaptopurine and amethopterin.

ANTIBIOTICS

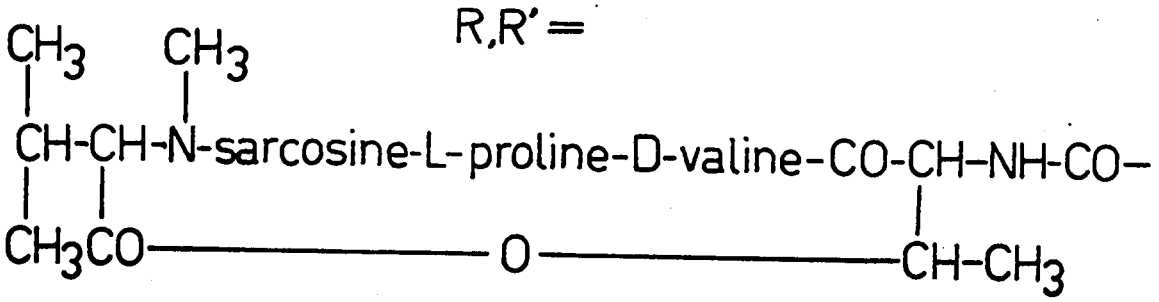
In view of the success of penicillin, streptomycin and other antibiotics in the control of bacterial infection it is logical to search for anti-neoplastic activity in products from microbial systems. Actinomycin D was isolated by Waksman and his colleagues and its anti-tumour activities studied by Farber et al.[14] Mitomycin C has been isolated and used clinically by Japanese workers.[26a] Both actinomycin C and mitomycin C are of clinical value. Other antibiotics possessing anti-tumour activity are azaserine and 6-diazo-5-oxo-L-norleucine (DON).[26b]

PLATE 6

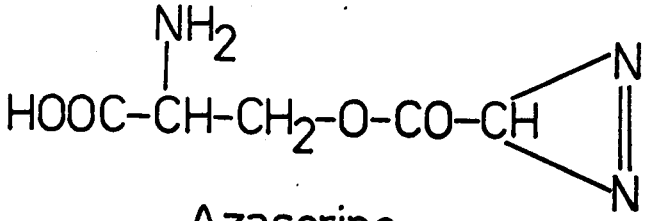
ANTIBIOTICS



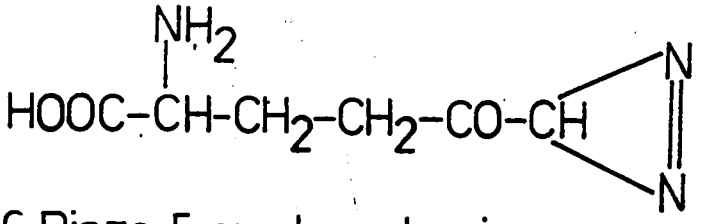
R,R' =



Actinomycin D



Azaserine



6-Diazo-5-oxo-L-norleucine

PLANT PRODUCTS

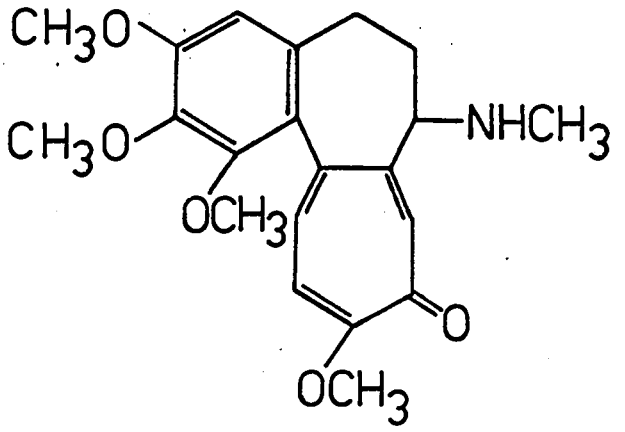
A number of plant extracts have been tested for their anti-tumour activity. Colchicine isolated from Colchicum autumnale L. has the ability to arrest mitosis of plant and animal cells. It has been extensively studied in cancer research. Its synthetic derivative, demecolcine (colchamine), is effective against chronic myeloid leukaemia.[27] Colchamine ointment has been found to cure skin cancer in the early stages.[28] Podophyllotoxin is another antimitotic agent found in podophyllin resin, but clinical trials with this have been disappointing.[29] Vincaleukoblastine (VLB; vinblastine) and vincristine (VC; leurocristine)[30,33] derived from periwinkle possess anti-tumour properties. Both VLB and VC have the ability completely to suppress Hodgkin's disease; and are capable of producing profound remissions in lymphomas. Moreover, VC is useful in acute lymphocytic leukaemia. Some other alkaloids, e.g. emetine and narcotine, exhibit antimitotic properties.

MISCELLANEOUS COMPOUNDS

In addition to the above main groups of carcino-chemotherapeutic substances, many other compounds have been tested on an empirical basis, and a few have been found to be active. Urethane is the most important of such compounds. It has been used in the treatment of chronic myeloid leukaemia.[21] Recently it has been found that 1-methyl-1-nitrosourea[33] passes the so-called blood brain barrier and destroys

PLATE 7

PLANT PRODUCTS

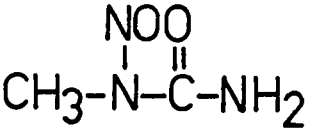


Demecolcine, Colchamine

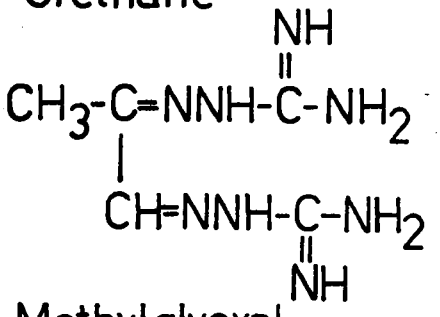
MISCELLANEOUS COMPOUNDS

$H_2NCOOC_2H_5$

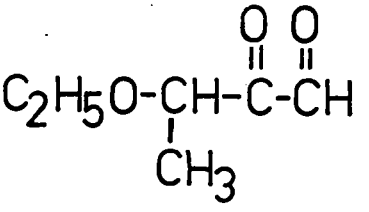
Urethane



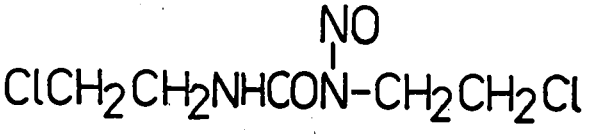
1-Methyl-1-nitrosourea



Methylglyoxal bisguanylhydrazone



3-Ethoxy-2-keto-butyraldehyde



1,3-Bis(2-chloroethyl)-1-nitrosourea

leukaemic cells in the brain tissues. Its chief drawbacks are that it is extremely difficult to handle and decomposes at body temperature ($37^{\circ}C$). Another urea derivative, 1,3-bis(2-chloroethyl)-1-nitrosourea, is far more stable and still shows a high degree of efficacy in the intracerebral situation. This is under active development at the present. Glyoxal bisguanylhydrazone,[31] and the corresponding methylglyoxal bisguanylhydrazone, have shown tumour-inhibiting activity. The latter compound also known as Methyl GAG is most effective[33] in acute myelogenous leukaemia both in the child and the adult. Preliminary clinical trials in acute myelogenous leukaemia have demonstrated that Methyl GAG effects a higher response rate than does 6-mercaptopurine, the previous drug of choice. Unfortunately it has its own drawbacks, (i) it must be administered intravenously and (ii) it is toxic. 3-Ethoxy-2-ketobutyraldehyde[32] and related compounds also possess carcinostatic action.

THE SYNTHESIS OF NEW PURINE ANALOGUES

Several analogues of the naturally occurring purines and pyrimidines are antagonists of many biological systems.[34] Significant tumour inhibition has been demonstrated with 8-azaguanine, with 6-mercaptopurine, with 4-aminopyrazolo[3,4-d]pyrimidine, with 5-fluorouracil, and with other substances which are related to the bases which occur in natural nucleotides.[1,35,36,37] One of the most promising approaches to cancer chemotherapy, therefore, would seem to be by the use of purine and pyrimidine antimetabolites. The *modus operandi* of synthetic organic compounds in living systems is complex, because the science of the relation of chemical structure to biological activity has not advanced sufficiently to allow one to synthesise selectively a biologically active compound without resorting to trial and error. Thus many of the above mentioned compounds were first tested as it was thought that they might act as antagonists in the biosynthesis of nucleic acids; but it has recently been suggested that 6-mercaptopurine acts as a tumour-inhibitor by inhibiting the synthesis of nicotinamide-adenine-dinucleotide.[17] To devise a successful research scheme, one must rely on intuition, experience and a fair degree of luck. With these thoughts in mind it was decided to synthesise some new heterocyclic compounds as possible anti-tumour agents and to explore some interesting chemistry along the way.

Although many purine analogues, obtained by simple structural alteration of naturally occurring purines, have been tested, the structural requirements are still far from clear. It seems probable, however, that a purine antimetabolite should closely resemble the purines found in the naturally-occurring nucleotides, and in nucleic acids.[38] In fact, after an examination of the structures of a few hundred purine derivatives tested for their possible anti-cancer activity; it has been shown[cf.34] that (a) the active analogues of naturally occurring purines are those in which the new group or atom introduced is not greatly different in size from the one replaced, (b) the more active compounds seem to result from an alteration at a single site of the structure of adenine, hypoxanthine or guanine, and (c) active analogues have resulted from replacement in adenine, hypoxanthine or guanine of C_2 or C_8 by nitrogen or by substitution at C_2 or C_6, but not at any other position.

Deoxyribonucleic acid is made up from two polynucleotide chains wound spirally around the same axis, and held together by hydrogen bonds to form a double helix. The phosphate groups and sugar residues are disposed on the outside of the helix, and the purine and pyrimidine bases lie inside the helix, and are hydrogen-bonded in specific pairs. The nitrogen atom at the 1-position in the purine ring (Fig. 1.1) is always involved in the hydrogen bonding, and the nitrogen atom at the 9-position is bound

to a sugar residue. In addition, the 6-position has a substituent capable of forming a hydrogen bond with the pyrimidine. As a working hypotheses it is suggested[cf,38] that these features may be "essential" in an antimetabolite, and that nitrogen atoms at other positions in the ring may be relatively unimportant and serve only to control the electron-densities at the important positions. It may be noted that 4-aminopyrazolo[3,4-d]pyrimidines (Fig. 1.2), which are tumour-inhibiting, have these suggested "essential" features, but that the related 7-aminopyrazolo[4,3-d]pyrimidines (Fig. 1.3) which are not tumour-inhibitory, do not.

It therefore seemed of interest to attempt the synthesis of derivatives of 5-azaindole (Fig. 1.4) and of diazaindoles (Fig. 1.5) having one nitrogen atom at a position equivalent to the 1-position in purine (Fig. 1.1) and another at a position equivalent to the 9-position in purine. This then forms the basis of the work towards attempted synthesis of 5-azaindole and the successful synthesis of many pyrazolo[4,3-c]pyridines.

The observation of Markees and Kidder,[39] and Gorton and Shive[40] that 5-amino-7-hydroxyimidazo[b]pyridine (1-deazaguanine, Fig. 1.6) is a potential antagonist for guanine has prompted the synthesis of pyrazolo[3,4-b]pyridines. In these compounds (Fig. 1.7) the nitrogen atom at the 1-position of the purine ring has been eliminated, the nitrogen atom at 3-position survives and the nitrogen atom at 7-position has been interchanged with the

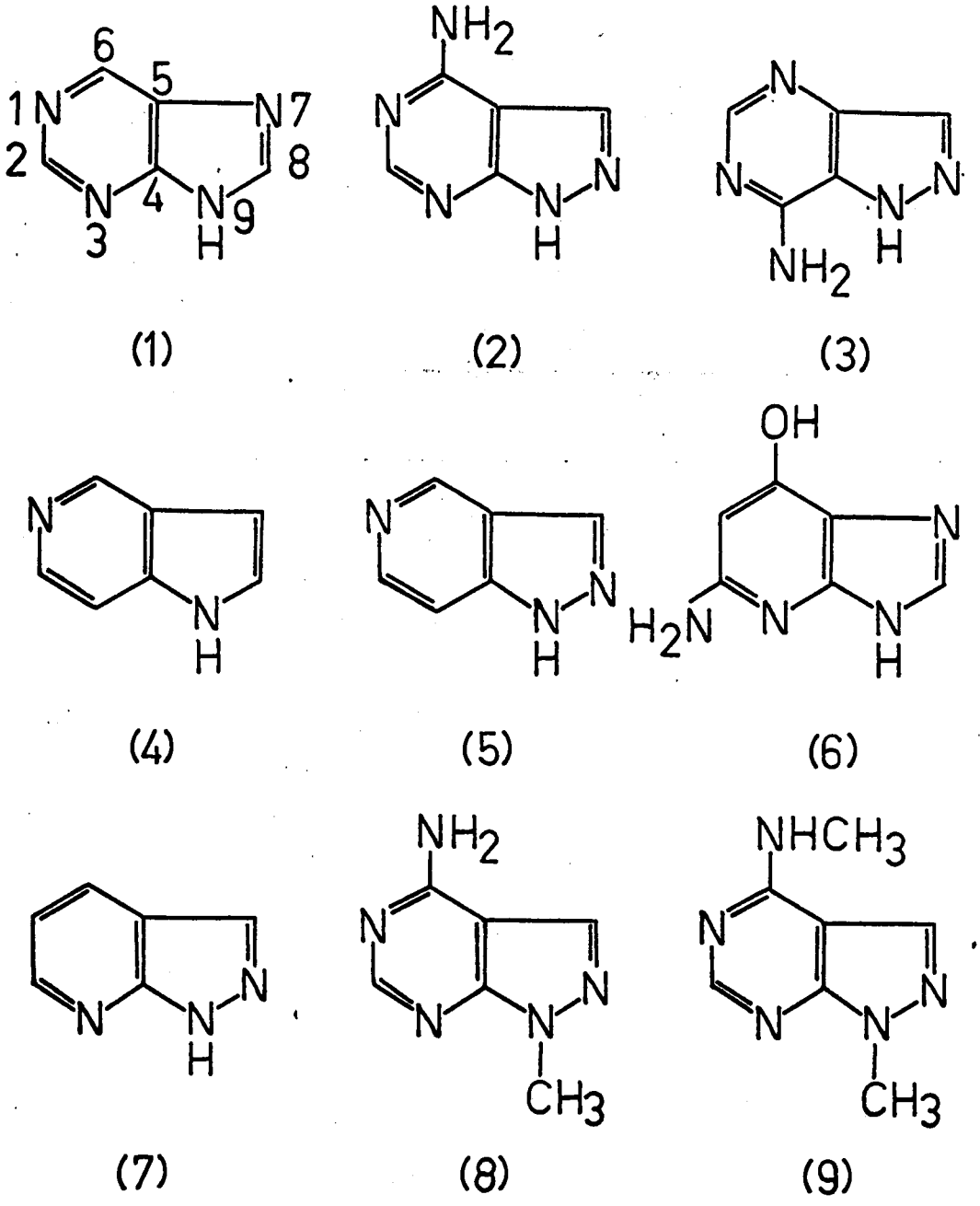

6
5
1N
N7
2
8
N
4
N
9
3
H
NH2
N
N
N
N
H
N
N
N
N
NH2
H
(1)
(2)
(3)
N
N
H
N
N
H
OH
N
H2N
N
N
H
(4)
(5)
(6)
N
N
N
H
NH2
NHCH3
N
N
N
N
N
N
CH3
CH3
(7)
(8)
(9)

FIGURE 1

carbon atom at 8-position. This formed the second line of approach. Because of the close similarity in structures of pyrazolo[3,4-b]pyridines and pyrazolo[4,3-c]pyridines, the above speculation was also coupled with an investigation of their chemical and physical properties.

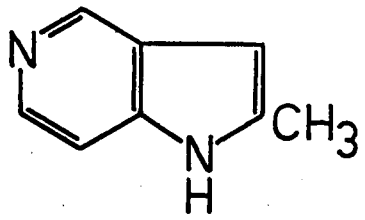
The third and final line of approach towards possible anti-cancer compounds originated from a study of the findings of Robins[22,47] and Robins and co-workers.[41,42,43] Skipper, Robins and Thomson[44] found that 4-aminopyrazolo[3,4-d]pyrimidine (Fig. 1.2) and 1-methyl-4-aminopyrazolo[3,4-d]pyrimidine (Fig. 1.8) inhibit the growth of Adenocarcinoma 755 and Leukaemia 5178 in mice. Hsu, Robins and Cheng[45] observed the inhibition of cellular growth by 4-aminopyrazolo[3,4-d]pyrimidine in certain tissue culture studies. 1-Methyl-4-methylaminopyrazolo[3,4-d]pyrimidine (Fig. 1.9)[44] has been found to exhibit a similar activity against Adenocarcinoma 755 and Leukaemia 5178. As an extension of the earlier work on pyrazolo[4,3-c]pyridines[46] and pyrazolo[3,4-b]pyridines, it was decided to synthesise some compounds possessing pyrazolo[3,4-d]-pyrimidine ring system and fulfilling the requirements of potentially active anti-cancer compounds (see sections (a), (b), and (c) p. 22).

RESULTS AND DISCUSSION

This discussion is divided into four sections. First we will consider the attempted synthesis of 5-azaindoles, secondly the successful synthesis of many pyrazolo[4,3-c]pyridines, thirdly the preparation of some pyrazolo[3,4-b]pyridines, and finally the synthesis of some pyrazolo[3,4-d]pyrimidines.

(a) 5-AZAINDOLE.-

A few syntheses of 5-azaindoles have been described in the literature. In 1948 Clemo and Swan[48] prepared 2-methyl-5-azaindole (Fig. 2.10) in 1% yield. Herz and Tocker[49] have described the synthesis of some substituted 5-azaindoles (in milligram quantities) through the Bischler-Napieralski reaction of acyl derivatives of 2-(2-pyrrole)ethylamine; and 5-azaindole itself has been prepared by Möller and Süs[50] following a long synthetic route with low overall yield. A recent claim[51] to a new synthesis has been questioned.[52] None of these methods were very convenient, therefore two approaches were made to find a more convenient synthesis for the 5-azaindole system (which, however, were unsuccessful). The first was patterned after the Reissert indole synthesis.[53] In the Reissert indole synthesis, o-nitrotoluene is condensed with diethyl oxalate and the resulting ester hydrolysed to o-nitrophenylpyruvic acid (Fig. 2.11). Reductive cyclization then gives indole-2-carboxylic acid (Fig. 2.12). This synthesis[53,54] has found wide application



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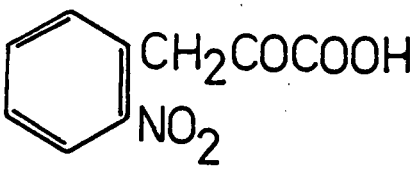

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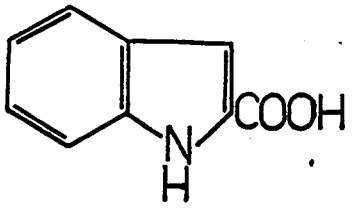

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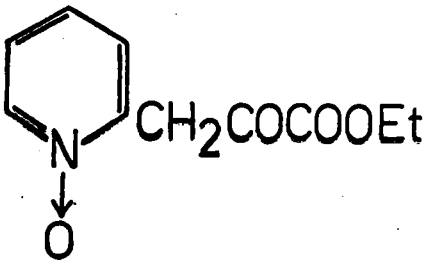

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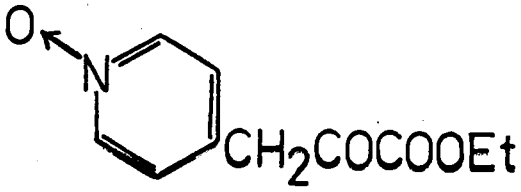

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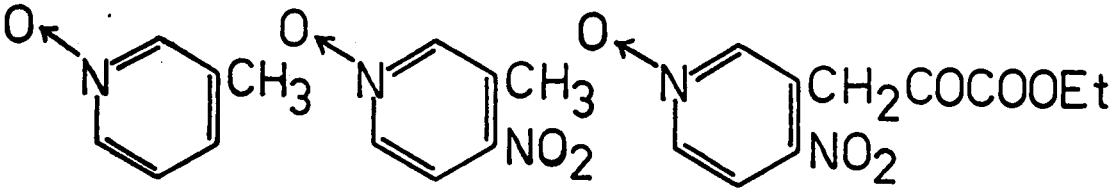
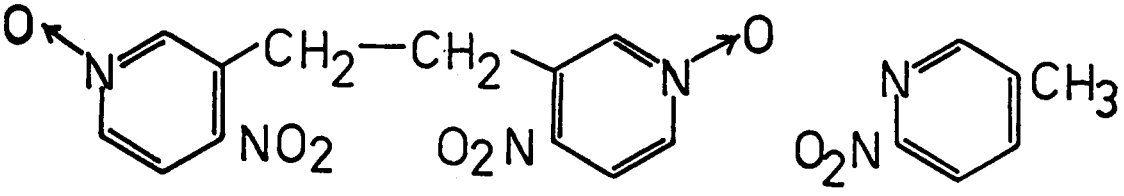


(15) (16) (17)



(18)

(19)

FIGURE 2

for the preparation of substituted indoles, and it was thought worthwhile to attempt to adapt it for the preparation of 5-azaindoles. It may be noted that ethyl 2-pyridylpyruvate 1-oxide (Fig. 2.13) and ethyl 4-pyridylpyruvate 1-oxide (Fig. 2.14) have already been prepared[55] from 2-(and 4-)picoline 1-oxide, and this suggested that the early stages in the proposed synthesis might be successful.

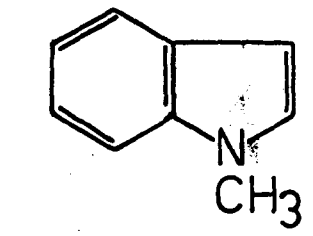
3-Picoline 1-oxide (Fig. 2.15) was accordingly nitrated and the resulting 4-nitro-3-picoline 1-oxide (Fig. 2.16) treated with diethyl oxalate in presence of potassium and sodium ethoxides under a variety of conditions. However, the desired ethyl 3-(4-nitropyridyl)-pyruvate 1-oxide (Fig. 2.17) could not be obtained; only 4,4'-dinitro-1,1'-dioxy-3,3'-dipicolyl (Fig. 2.18) was isolated. An analogous compound was obtained by Lesiak[56] following attempts to condense diethyl oxalate with o-ethyltoluene.

During the preparation of 4-nitro-3-picoline 1-oxide (Fig. 2.16), some 5-methyl-2-nitropyridine (Fig. 2.19) was also obtained. It seems that nitration of 3-picoline 1-oxide gives a mixture of 4-nitro-3-picoline-1-oxide and 5-methyl-2-nitropyridine 1-oxide, and the latter undergoes deoxygenation. Similar deoxygenations have already been reported.[57,58]

THE HARLEY-MASON INDOLE SYNTHESIS

In 1955 Harley-Mason[59] reported that he had obtained N-methylindole (Fig. 3.20) by condensing N-methylanthranilic acid (Fig. 3.21) with glycollic aldehyde (Fig. 3.22) in hot aqueous solution, and it was suggested that this reaction may be of biogenetic significance. This reaction may be regarded as an extension of the Bischler[60] and Japp-Murray[61] indole syntheses. The Harley-Mason synthesis does not seem to have found further application, and full details have not been published; nevertheless it was decided to attempt the condensation of glycollic aldehyde with 4-anilinonicotinic acid 1-oxide (Fig. 3.23) and 4-methylaminonicotinic acid 1-oxide (Fig. 3.24). Despite many attempts under different reaction conditions the desired condensation could not be effected.

The preparation of the two nicotinic acid derivatives (Fig. 3.23 and 24) required an adequate supply of 4-nitronicotinic acid 1-oxide (Fig. 3.25). This was obtained by a modified method which eliminates the dangers of uncontrolled reactions, and which gave a higher yield than those in the literature.[52,62] This compound (Fig. 3.25) was converted into 4-anilinonicotinic acid 1-oxide (Fig. 3.23) by nucleophilic displacement of the nitro group at C_4 by aniline (cf.52). A modified method has been developed to get the 4-anilino acid (Fig. 3.23) in purer form than that described in the literature.[52] 4-Chloronicotinic acid 1-oxide (Fig. 3.26) was prepared from 4-nitronicotinic acid 1-oxide (Fig. 3.25), and also



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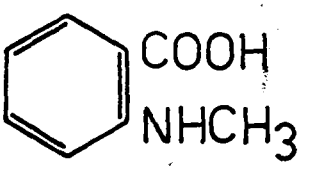

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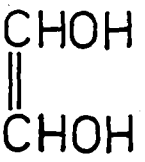

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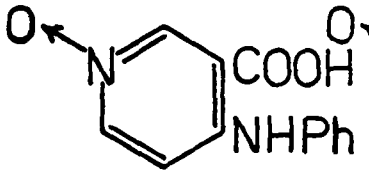

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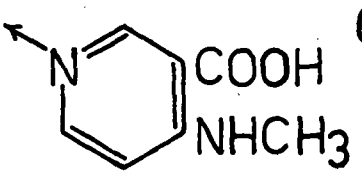

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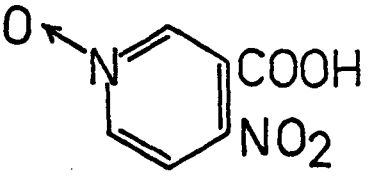

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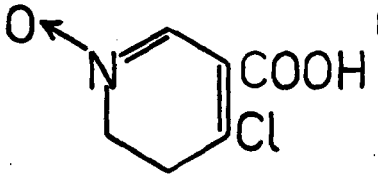

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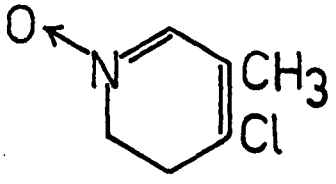

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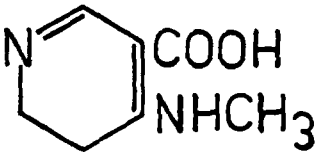

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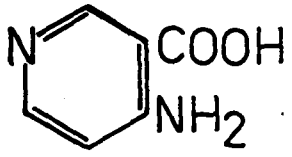

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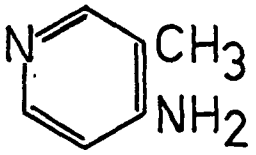

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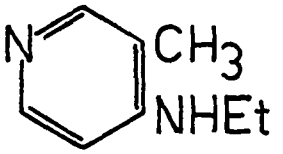

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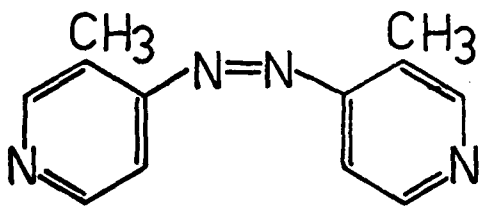

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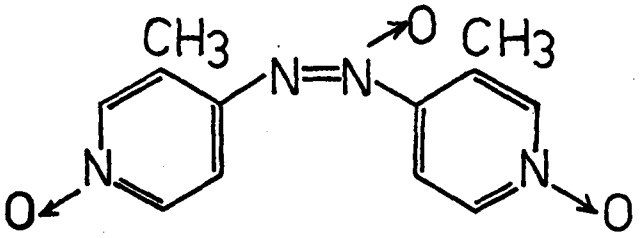


(33)

FIGURE 3

from 4-chloro-3-picoline 1-oxide (Fig. 3.27) by oxidation with chromic acid, and converted into the desired 4-methylaminonicotinic acid 1-oxide (Fig. 3.24) by treatment with aqueous methylamine at 140° under pressure. This was reduced to 4-methylaminonicotinic acid (Fig. 3.28) with hydrogen in presence of palladium-on-carbon.

REDUCTION OF 4-NITRO-3-PICOLINE 1-OXIDE

Prior to the previously mentioned successful preparation of 4-methylaminonicotinic acid and its 1-oxide (Fig. 3.28 and 24) from 4-chloronicotinic acid 1-oxide (Fig. 3.26), it was planned to prepare these compounds (Fig. 3.28 and 24) and the corresponding ethyl derivatives from 4-nitro-3-picoline 1-oxide (Fig. 2.16) by a route based on the preparation of 4-aminonicotinic acid (Fig. 3.29) from 4-amino-3-picoline (Fig. 3.30) as described by Taylor and Crovetti.[63] In this scheme 4-ethylamino-3-picoline (Fig. 3.31) was to be used in place of 4-amino-3-picoline as used by the above workers.

In 1955 Kao, Tilak and Venkataraman[64] extended the original work of Adkins and Cramer,[65] and described the alkylation of amines by refluxing with the requisite alcohol in the presence of Raney nickel. This suggested that 4-nitro-3-picoline 1-oxide (Fig. 2.16) might be converted into 4-ethylamino-3-picoline (Fig. 3.31) by similar treatment with ethanol and Raney nickel.

When 4-nitro-3-picoline 1-oxide (Fig. 2.16) was refluxed with ethanol in the presence of W-2 Raney nickel[66] for 5 hr, however, the main product was found to be 4-amino-3-picoline (Fig. 3.30), together with a small amount of 3,3'-dimethyl-4,4'-azopyridine[67] (Fig. 3.32). The desired 4-ethylamino-3-picoline (Fig. 3.31) was not obtained, even after more prolonged refluxing, but it is of some interest that a very short reaction time resulted in the isolation of 4-amino-3-picoline 1-oxide, together with 3,3'-dimethyl-4,4'-azoxypyridine 1,1'-dioxide (Fig. 3.33).

It was then decided to prepare 4-amino-3-picoline (Fig. 3.30), to methylate it and to use the 4-methylamino-3-picoline in the proposed reaction scheme in place of 4-ethylamino-3-picoline. For this purpose a number of reduction conditions were tried (cf.67) as the literature methods were not satisfactory, and it was found that the catalytic hydrogenation of 4-nitro-3-picoline 1-oxide in methanol in the presence of Raney nickel proceeded smoothly and gave 4-amino-3-picoline (Fig. 3.30) in high yield; but the same product was obtained in quantitative yield following hydrogenation in glacial acetic acid over palladium-on-charcoal. The reduction of 4-nitro-3-picoline 1-oxide using Raney nickel and hydrazine hydrate has also been studied. With excess hydrazine hydrate, 4-amino-3-picoline (Fig. 3.30) was obtained in satisfactory yield.

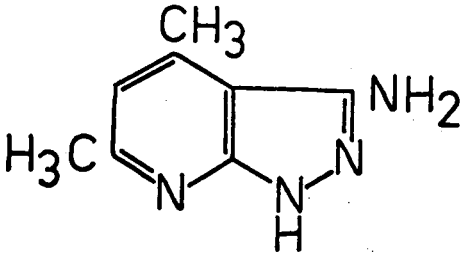
Further work on this line was abandoned in view of the facile preparation of 4-methylaminonicotinic acid, and its 1-oxide, and of 4-anilinonicotinic acid 1-oxide as described earlier.

(b) PYRAZOLO[4,3-c]PYRIDINES.-

A few derivatives of pyrazolopyridines[e.g. 68,69,70] have been described in the literature, but most are hydrogenated derivatives or are derivatives of the alternative diazaindole, pyrazolo[3,4-b]pyridine (Fig. 1.7). 3-Amino-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridine[71] (Fig. 4.34), for example, has been prepared in good yield from 2,4-dimethyl-5-cyanopyridine-6-sulphonic acid (Fig. 4.35). 3-Hydroxy-6-hydrazino-4-methyl-1H-pyrazolo[3,4-b]pyridine dihydrochloride[72] (Fig. 4.36) and 3-hydroxy-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridine[73] (Fig. 4.37) have also been prepared. The only known examples of 1H-pyrazolo[4,3-c]pyridines seem to be 3-amino-4,6-dihydroxy-1H-pyrazolo[4,3-c]pyridine (Fig. 4.38) and 3-amino-7-carboxy-4-hydroxy-1H-pyrazolo[4,3-c]pyridine (Fig. 4.39) and their 2-methyl and 2-phenyl derivatives prepared by Taylor and Hartke[74] from 5-aminopyrazole derivatives.

Benzopyrazoles, on the other hand, are well-known compounds.[75,76] 3-Indazolinone (Fig. 4.40), for example, is readily prepared by refluxing o-hydrazinobenzoic acid (Fig. 4.41) in hydrochloric acid, and it seemed likely that this reaction could be extended to the preparation of pyrazolo[4,3-c]pyridines.

4-Hydrazinonicotinic acid 1-oxide (Fig. 5.42) has already been described in the literature,[77] having been obtained in poor yield from 4-nitronicotinic acid 1-oxide and hydrazine hydrate. It has now been obtained in excellent yield from 4-chloronicotinic acid 1-oxide (Fig. 3.26) and hydrazine hydrate. Refluxing with



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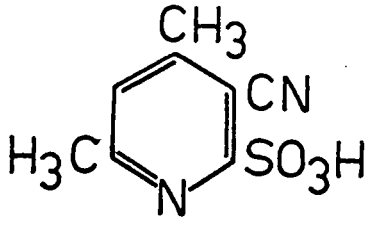

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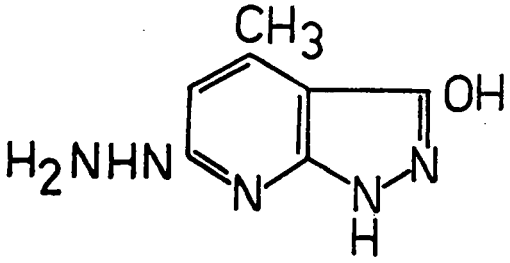

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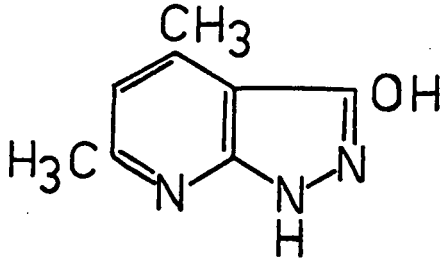

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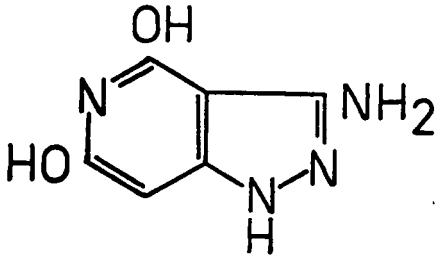

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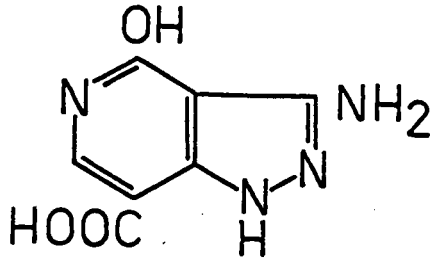

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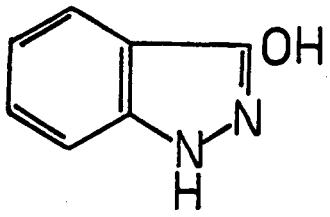

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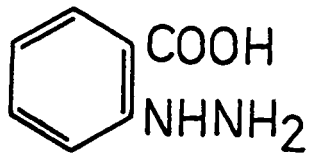


(41)

FIGURE 4

hydrochloric acid then gave the desired 3-hydroxy-1*H*-pyrazolo[4,3-*c*]-pyridine 5-oxide (Fig. 5.43). This was readily reduced to 3-hydroxy-1*H*-pyrazolo[4,3-*c*]pyridine (Fig. 5.44); but attempts to remove the 3-hydroxy group in this compound, or its *N*-oxide, were all unsuccessful. 3-Hydroxy-1*H*-pyrazolo[4,3-*c*]pyridine (Fig. 5.44) was also prepared by the acid-catalysed cyclisation of 4-hydrazinonicotinic acid (Fig. 5.45), itself obtained from 4-chloronicotinic acid[52] (Fig. 5.46) with hydrazine. 4-Chloronicotinic acid in turn was obtained by potassium permanganate oxidation of 4-chloro-3-picoline (Fig. 5.47), itself obtained in good yield[67] by hydrogenation over Raney nickel of 4-chloro-3-picoline 1-oxide (Fig. 3.27).

The facile preparation of 4-hydrazinonicotinic acid 1-oxide (Fig. 5.42) from 4-chloronicotinic acid 1-oxide (Fig. 3.26) with hydrazine suggested that substituted hydrazino compounds could be similarly prepared from substituted hydrazines (such as phenylhydrazine and methylhydrazine). With mono-substituted hydrazines, however, two alternative products could be formed. In one of these, the substituent would be attached to the nitrogen atom adjacent to the pyridine ring; in the other, it would be attached to the nitrogen further from the ring. Somewhat surprisingly, both with phenylhydrazine and with methylhydrazine, the products were found to be those having the substituent on the nitrogen atom adjacent to the ring.

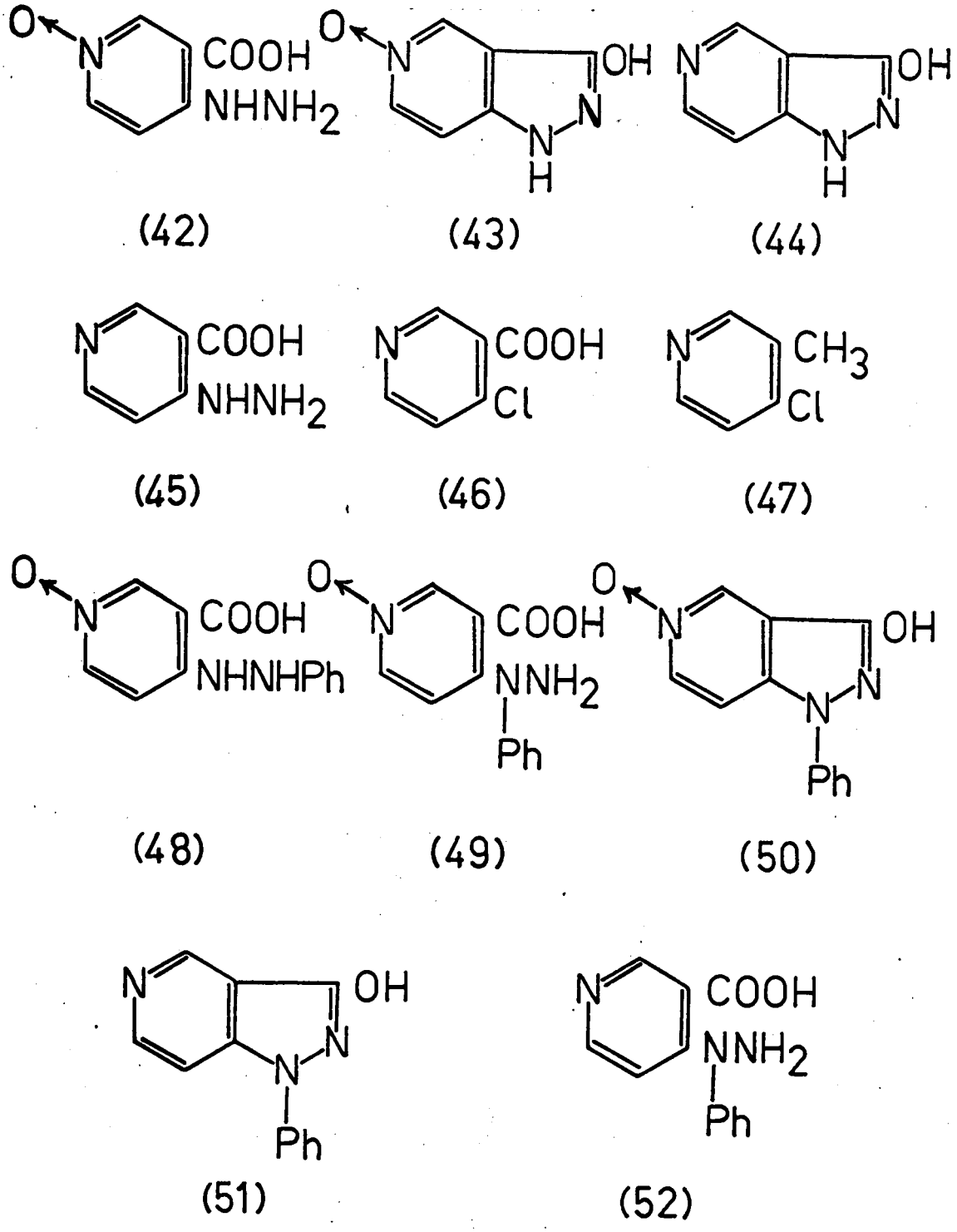
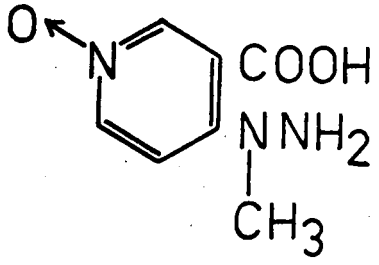

O
N
COOH
NHNH2
OH
N
H
(42)
(43)
(44)
Cl
CH3
(45)
(46)
(47)
NHNHPh
NNH2
Ph
(48)
(49)
(50)
(51)
(52)

FIGURE 5

The reaction of 4-chloronicotinic acid 1-oxide (Fig. 3.26) with phenylhydrazine might be expected to give either the hydrazo-acid (Fig. 5.48), or the diarylamino-acid (Fig. 5.49). Experimentally this reaction gave a mixture of the diarylamino-acid (Fig. 5.49) and the cyclised product, 3-hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine 5-oxide (Fig. 5.50). The acid (Fig. 5.49) was not oxidised by treatment with mercuric oxide or with nitric acid; it gave a positive Liebermann reaction (which hydrazobenzene did not); and the infrared spectrum showed two bands at 3210 cm^{-1} and 3320 cm^{-1} characteristic of compounds with a primary amino group. This acid (Fig. 5.49) was also cyclised to 3-hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine 5-oxide (Fig. 5.50) under quite mild acidic conditions, and the structure of this product follows from the data discussed below. This oxide (Fig. 5.50) was reduced to 3-hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine (Fig. 5.51); and the same product was obtained from 4-(α-phenylhydrazino)nicotinic acid (Fig. 5.52), itself being obtained from 4-chloronicotinic acid (Fig. 5.46) and phenylhydrazine.

The reaction between 4-chloronicotinic acid 1-oxide (Fig. 3.26) and methylhydrazine gave a resinous product from which the expected 4-(α-methylhydrazino)nicotinic acid 1-oxide (Fig. 6.53) could not be obtained. However the crude material was readily converted by treatment with hydrochloric acid into 3-hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine 5-oxide (Fig. 6.54). The yield of 3-hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine 5-oxide was



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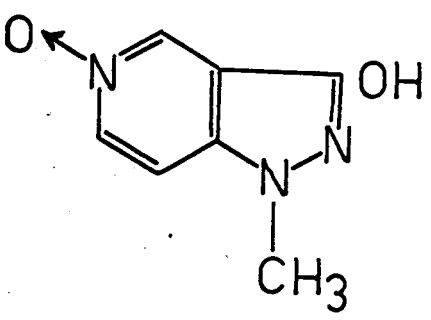

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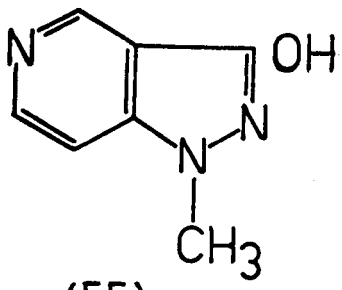

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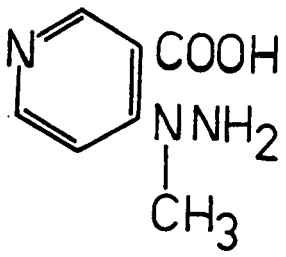

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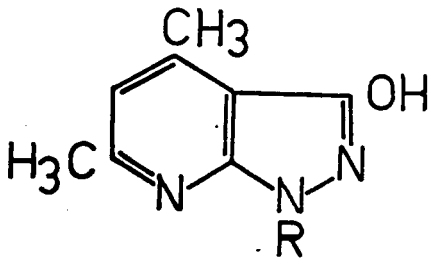

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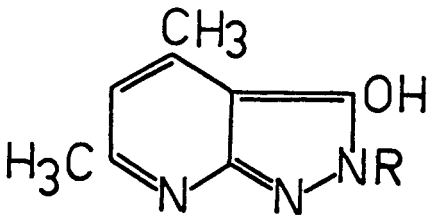

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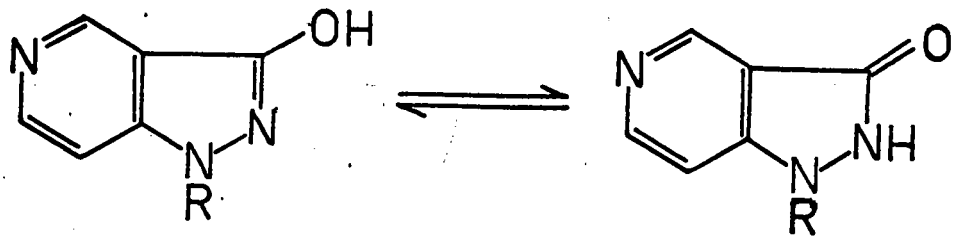


(59) (60)

FIGURE 6

considerably increased when the reaction between 4-chloronicotinic acid 1-oxide (Fig. 3.26) and methylhydrazine was carried out in an atmosphere free from carbon dioxide.

The reaction between 4-chloronicotinic acid (Fig. 5.46) and methylhydrazine also gave a gummy product from which the expected 4-(α-methylhydrazino)nicotinic acid (Fig. 6.56) could not be isolated. However, the gum gave 3-hydroxy-1-methyl-1H-pyrazolo-[4,3-c]pyridine (Fig. 6.55) in good yield on acid-catalysed cyclisation. This compound was also obtained by catalytic hydrogenation of 3-hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine 5-oxide (Fig. 6.54).

Taylor and Barton[78] have shown that 1-substituted pyrazolo[3,4-b]pyridines (e.g. Fig. 6.57) are colourless and stable, but that 2-substituted derivatives (e.g. Fig. 6.58) are deep red and thermally unstable. This is of interest in connection with the structures of the present compounds. The three N-oxides (Fig. 5.43, 50; Fig. 6.54) and the three deoxy-compounds (Fig. 5.44, 51; Fig. 6.55) were all found to be colourless to yellow, and all were stable. Moreover the three N-oxides in dilute alkali gave similar absorption spectra (Fig. 7); and the three deoxy-compounds in dilute alkali also gave similar absorption spectra (Fig. 8).

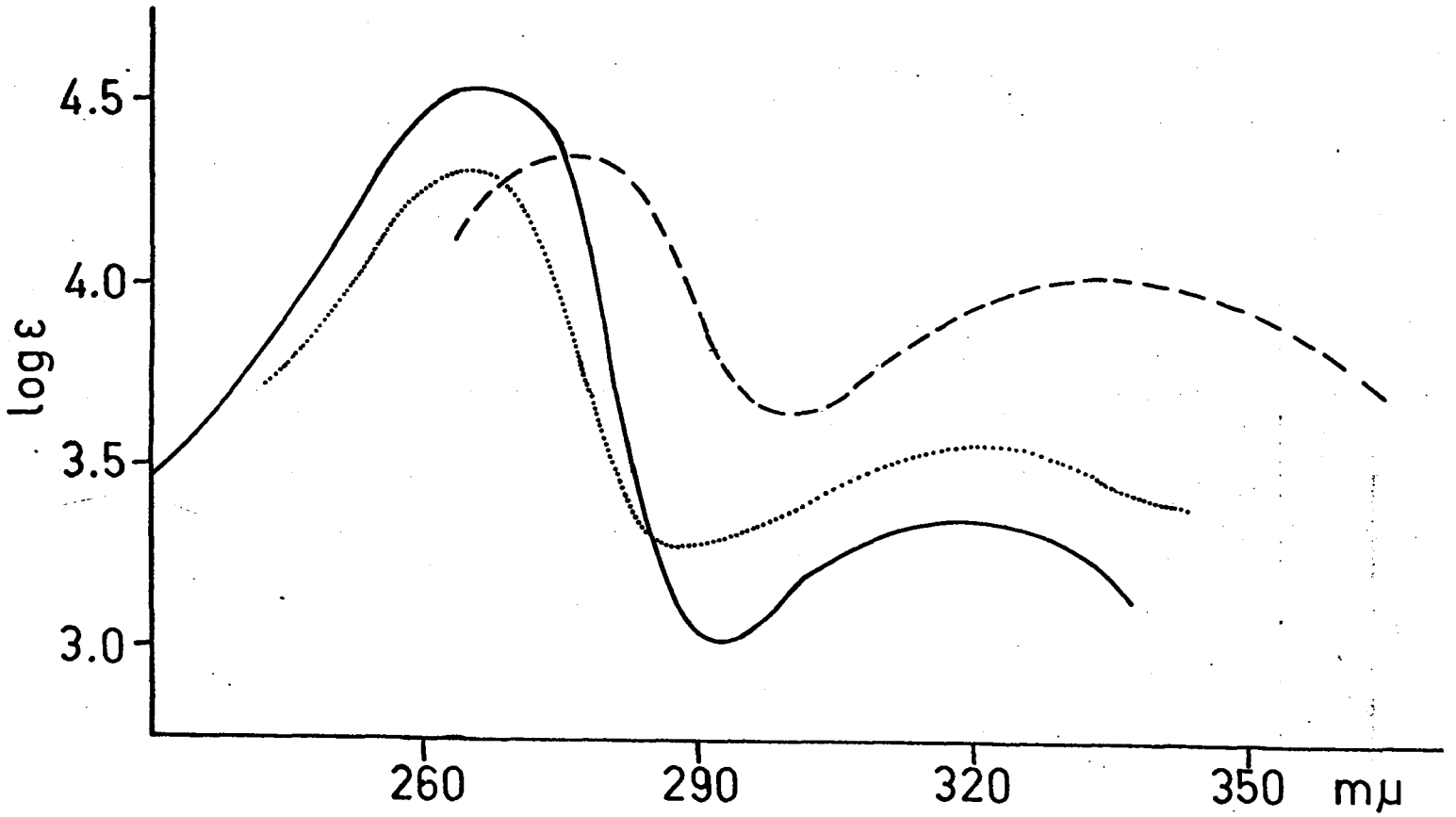


FIGURE 7. Absorption spectra of 3-hydroxy-1H-pyrazolo[4,3-c]pyridine 5-oxide (Fig. 5.43, ———), 3-hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine 5-oxide (Fig. 5.50, – – – –), and of 3-hydroxy-1-methyl-1H-pyrazolo-[4,3-c]pyridine 5-oxide (Fig. 6.54, ·····) in N-sodium hydroxide.

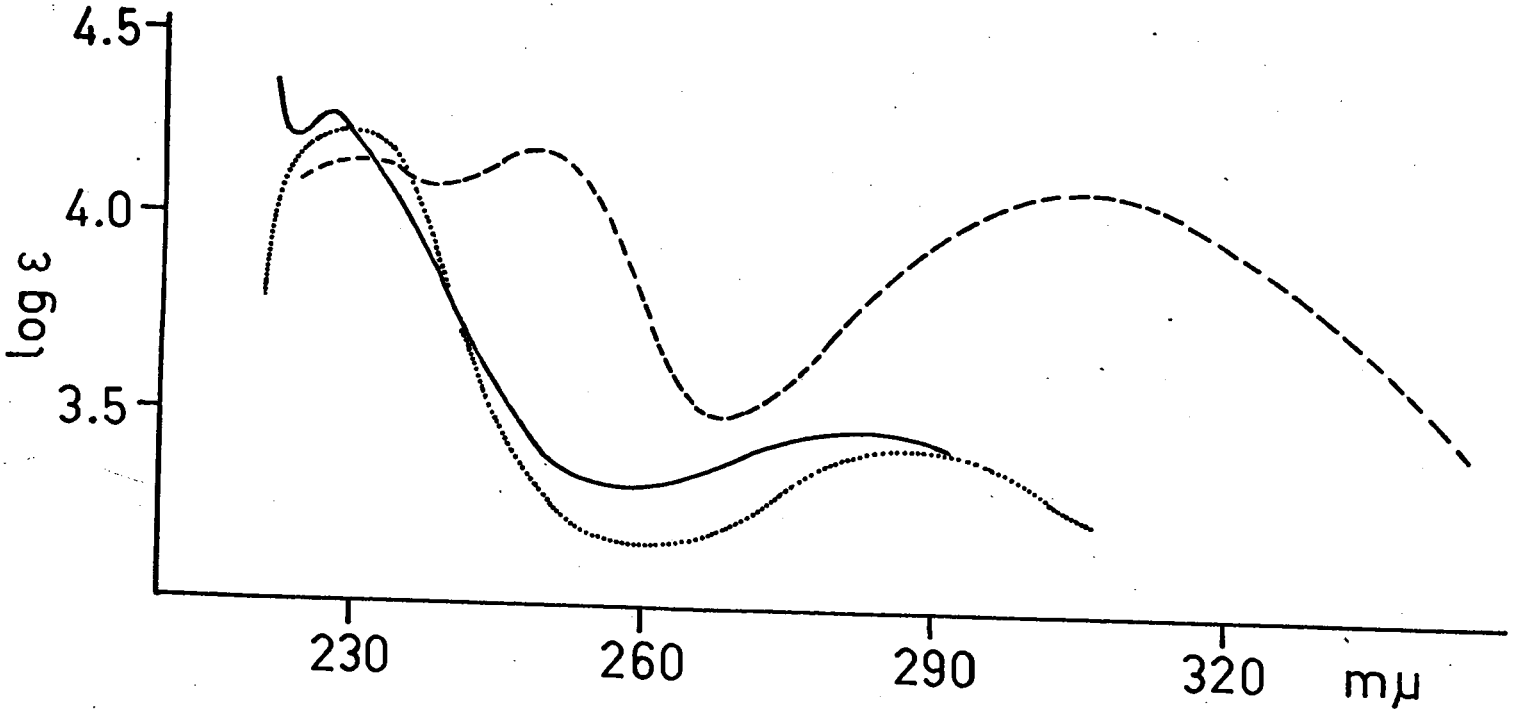


FIGURE 8. Absorption spectra of 3-hydroxy-1H-pyrazolo[4,3-c]pyridine (Fig. 5.44, ——), 3-hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine (Fig. 5.51, - - - -), and of 3-hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine (Fig. 6.55, ·····) in N-sodium hydroxide.

With all these compounds, lactam-lactim tautomerism (59 ⇌ 60) is possible. Indeed, the deep yellow colour of the 3-hydroxy-1H-pyrazolo[4,3-c]pyridines (Fig. 6.59, R=H, Ph, CH_3) suggests that these compounds exist predominantly as the lactams (Fig. 6.60, R=H, Ph, CH_3); and this is supported by the fact that the infrared spectrum of (Fig. 6.59, R=H) showed a very strong carbonyl peak (1630 cm^{-1}). On the other hand, the 5-oxide showed only a very weak carbonyl peak, suggesting that this derivative exists predominantly in the lactim form; and conversion into the hydrochloride gave a salt which again gave strong carbonyl absorption. The other compounds of the series behaved in an analogous way: the 5-oxides showed very weak carbonyl peaks, but the 5-oxide hydrochlorides, and the deoxy-bases, showed strong carbonyl absorption. All the 5-oxides and deoxy compounds gave violet colours with ferric chloride solution.

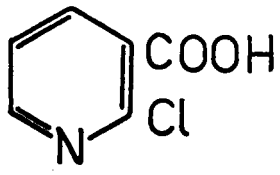
(c) PYRAZOLO[3,4-b]PYRIDINES.-

Several derivatives of pyrazolo[3,4-b]pyridine have already been described in the literature;[69,70] most have been prepared from the corresponding pyrazole derivatives. On two previous occasions, however, derivatives of this ring system have been prepared by treating substituted ethyl 2-chloronicotinate with hydrazine hydrate. These are 3-hydroxy-6-hydrazino-4-methyl-1H-pyrazolo[3,4-b]pyridine dihydrochloride (Fig. 4.36)[72] and 3-hydroxy-4,6-dimethyl-1H-

pyrazolo[3,4-b]pyridine (Fig. 4.37).[73] Moreover, the facile preparation of pyrazolo[4,3-c]pyridines[46] from 4-hydrazinonicotinic acids (preceding section) suggested that the isomeric pyrazolo[3,4-b]pyridines could be similarly prepared from 2-hydrazinonicotinic acids.

The preparation of 2-chloronicotinic acid (Fig. 9.61) from nicotinic acid 1-oxide (Fig. 9.62) has already been described in the literature,[63] but in our hands the method gave low yields. With some modifications better results were obtained. In addition, the method of purification of nicotinic acid 1-oxide has also been modified to give much purer product.

The reaction between 2-chloronicotinic acid (Fig. 9.61) and hydrazine hydrate gave a resin from which the expected 2-hydrazinonicotinic acid (Fig. 9.63) could not be isolated. However, treatment of the crude material with hydrochloric acid gave the desired 3-hydroxy-1H-pyrazolo[3,4-b]pyridine (Fig. 9.64). The reaction of 2-chloronicotinic acid (Fig. 9.61) with methylhydrazine similarly gave 3-hydroxy-1-methyl-1H-pyrazolo[3,4-b]pyridine (Fig. 9.65); again the anticipated intermediate i.e. 2-(α-methylhydrazino)-nicotinic acid (Fig. 9.66) could not be isolated. 3-Hydroxy-1-phenyl-1H-pyrazolo[3,4-b]pyridine hydrochloride was similarly prepared using phenylhydrazine instead of hydrazine or methylhydrazine. Attempts to obtain the free base resulted in decomposition of the starting material.



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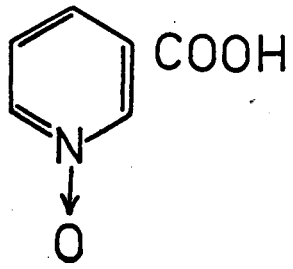

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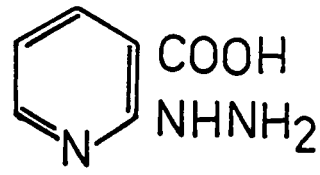

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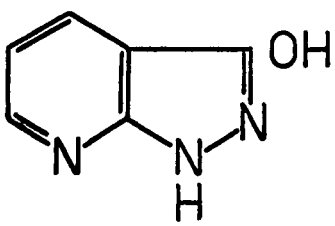

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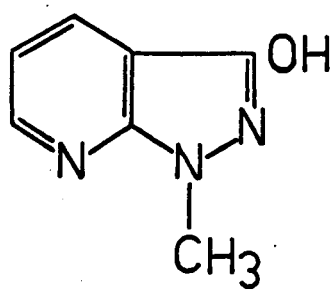

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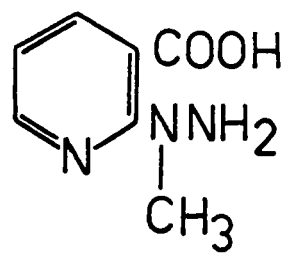

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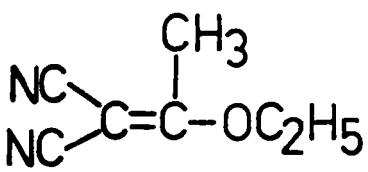

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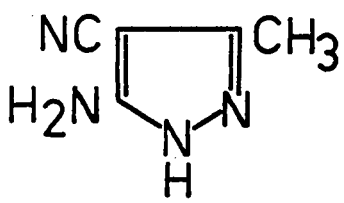
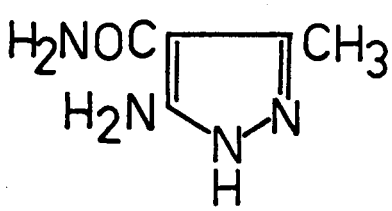


(68)



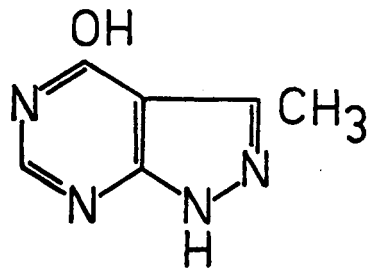
(69)

FIGURE 9

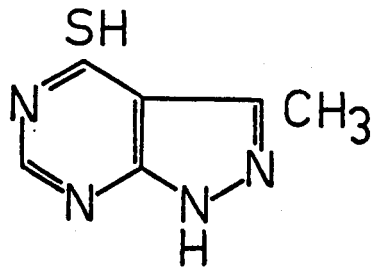
(d) PYRAZOLO[3,4-d]PYRIMIDINES.-

Robins[22] first developed a general method of preparing pyrazolo[3,4-d]pyrimidines from a pyrazole intermediate and Cheng and Robins[41] later utilised the method for synthesising a variety of substituted pyrazolo[3,4-d]pyrimidines. Methylethoxymethylenemalononitrile (Fig. 9.67) was prepared from triethyl orthoacetate, malononitrile and acetic anhydride following the method of Cheng and Robins.[41] In our hands, the method gave a purer product, possibly because of the slight modification in the procedure of isolating methylethoxymethylenemalononitrile from the reaction mixture. With hydrazine hydrate, this compound gave 5-amino-4-cyano-3-methylpyrazole (Fig. 9.68), which on partial hydrolysis gave 5-amino-4-carboxamido-3-methylpyrazole (Fig. 9.69). Reaction with formamide gave 4-hydroxy-3-methylpyrazolo[3,4-d]-pyrimidine (Fig. 10.70); and on thiation this was converted into 4-mercapto-3-methylpyrazolo[3,4-d]pyrimidine (Fig. 10.71).

Similarly, reaction of 5-amino-4-cyano-3-methylpyrazole (Fig. 9.68) with urea and with thiourea gave 4-amino-6-hydroxy-3-methylpyrazolo[3,4-d]pyrimidine (Fig. 10.72) and 4-amino-6-mercapto-3-methylpyrazolo[3,4-d]pyrimidine (Fig. 10.73) respectively. Finally, reaction of urea and of thiourea with 5-amino-4-carboxamido-3-methylpyrazole (Fig. 9.69) gave 4,6-dihydroxy-3-methylpyrazolo-[3,4-d]pyrimidine (Fig. 10.74) and 4-hydroxy-6-mercapto-3-methylpyrazolo-[3,4-d]pyrimidine (Fig. 10.75) respectively in good yields.



(70)



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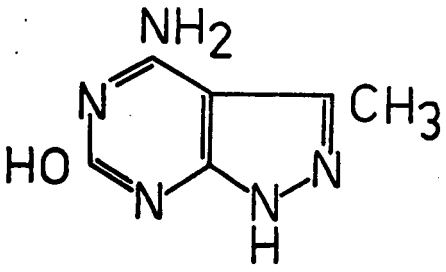

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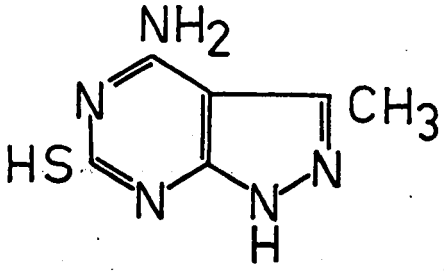

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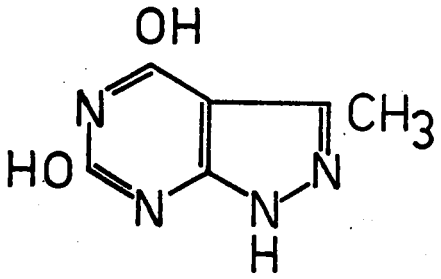

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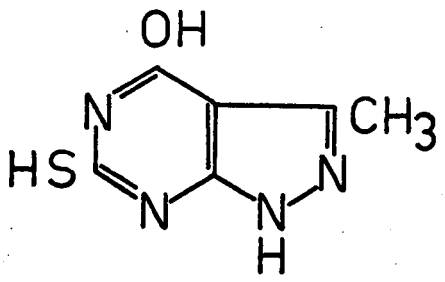

(75)

FIGURE 10

EXPERIMENTAL

(a) 5-AZAINDOLE.-

3-Picoline 1-oxide.- 3-Picoline (46.6 g) was oxidised[79] with hydrogen peroxide (30%, 85 ml) in glacial acetic acid (300 ml) to give 3-picoline 1-oxide as a colourless hygroscopic oil, b.p. 117.5-118.5°/4 mm, 100-102°/0.7 mm (35.8 g; 64%).

4-Nitro-3-picoline 1-oxide.- (i) Nitration of 3-picoline-1-oxide (12.7 g) using the method of Taylor and Crovetti[63] gave 4-nitro-3-picoline 1-oxide, m.p. 136-137° (lit., m.p. 136-137°), (12.1 g, 69%).

(ii) The following method was adapted from that used by den Hertog and Combe[80] for the preparation of 4-nitropyridine 1-oxide. In a number of experiments it gave yields ranging from 87% to 94%.

A mixture of 3-picoline (37.6 g), hydrogen peroxide (30%; 150 ml) and glacial acetic acid (150 ml) was heated at 50° for 48 hr. The resulting light yellow solution was carefully evaporated under reduced pressure, while the temperature was raised gradually. The distillation was stopped when no further distillate was obtained at 100°. Concentrated sulphuric acid (80 ml) was added to the residue, with cooling, and the resulting solution added carefully to a mixture of fuming nitric acid (120 ml) and concentrated sulphuric acid (80 ml) kept in an ice-bath. When the addition had been completed the mixture was placed in a water-bath, the temperature

was slowly raised to 90°, and maintained at this temperature for 2 hr. The temperature was raised to 100° for a further hour, and after cooling to room temperature the mixture was poured onto ice. 4N Sodium hydroxide was added with cooling and vigorous stirring until the solution was only slightly acidic. Almost pure 4-nitro-3-picoline 1-oxide (33.78 g) precipitated; after recrystallisation from acetone it had m.p. 136-137°. Concentration of the mother liquor gave a further quantity (8 g). The total yield was 41.78 g (87%).

In another experiment, 4-nitro-3-picoline 1-oxide (66.65 g) was obtained in 94% yield from 3-picoline (50 g).

(iii) In a further experiment the reaction mixture (from 75.2 g 3-picoline) was heated at 100° for $2\frac{1}{2}$ hr. Working up in the usual way gave 4-nitro-3-picoline 1-oxide (80 g), and the liquors gave an additional product (3 g, m.p. 91-93°). Recrystallisation from ethanol and sublimation at 92°/18 mm gave 5-methyl-2-nitropyridine as colourless lustrous needles, m.p. 94-95° (Found: C, 52.4; H, 4.6; N, 20.4. Calc. for $C_6H_6N_2O_2$: C, 52.2; H, 4.35; N, 20.3%). The m.p. was not depressed by admixture with a specimen prepared from 2-amino-5-methylpyridine by the method of Wiley and Hartmann[81] (1 g of 5-methyl-2-nitropyridine was obtained from 3 g of the amine). The infrared spectra of the two specimens were also identical.

Attempted condensation of 4-nitro-3-picoline 1-oxide with ethyl oxalate.- Herz and Murty[52] reported the failure of their attempt to condense ethyl oxalate with 4-nitro-3-picoline 1-oxide. However, no details were given and a reinvestigation of this problem was undertaken. The following experiments were based upon similar methods reported in literature for the corresponding benzene compounds.

(i) Freshly distilled ethyl oxalate (7 g) was added to a solution (prepared under nitrogen and cooled to room temperature) of potassium (2.8 g) in absolute ethanol (42 ml), and the mixture stirred for 15 min.[cf.55] A suspension of 4-nitro-3-picoline 1-oxide (7.3 g) in absolute ethanol (60 ml) was added and the mixture stirred for 2 hr in an atmosphere of nitrogen and then kept at room temperature for 24 hr. It was concentrated to one-third its volume by distillation under reduced pressure on a steam-bath, and anhydrous benzene (40 ml) added. The resulting precipitate was collected, washed several times with benzene and then ethanol, and treated with 10% hydrochloric acid until the solution was distinctly acid. The residue was collected, washed several times with hot water, recrystallised from aqueous acetic acid and further purified by dissolution in concentrated hydrochloric acid followed by precipitation with water, to give 4,4'-dinitro-1,1'-dioxy-3,3'-dipicolyl as a pale yellow solid, m.p. 242-244° (Found: C, 46.6; H, 3.2; N, 18.3. Calc. for $C_{12}H_{10}N_4O_6$: C, 47.1; H, 3.3; N, 18.3%). The m.p. was not depressed by admixture with an authentic specimen prepared by

the method of Taylor *et al.*[82] The infrared spectra of the two specimens were also identical. In the present work 0.9 mole of n-butyl nitrite and 0.9 mole of sodium were used in place of 0.83 mole of n-butyl nitrite and 0.78 mole of sodium used by the above authors; but the yield was the same (53%) as reported earlier. This shows that using excess of n-butyl nitrite and sodium has no effect on the yield of 4,4'-dinitro-1,1'-dioxy-3,3'-dipicolyl.

The filtrate obtained after separation of 4,4'-dinitro-1,1'-dioxy-3,3'-dipicolyl was extracted five times with chloroform. Evaporation of the extract under reduced pressure gave an orange oil. This solidified after 24 hr in a refrigerator. On recrystallisation from acetone a colourless solid (2 g) was obtained, m.p. 98.5°, which was identified as oxalic acid. The orange substance could not be identified because of the extremely small amount available.

The aqueous acidic solution remaining after these operations was dark red in colour. It was left overnight when it deposited a solid (2.1 g). This was insoluble in acetone, chloroform, ethanol and benzene. On recrystallisation from water-alcohol a compound, m.p. 280° (decomp.) was obtained; this was found to be a mixture of potassium hydrogen oxalate and its monohydrate.

(ii) The method used was essentially similar to that used by Kermack, Perkin and Robinson[83] for the synthesis of indole derivatives.

A solution of sodium (1.84 g) in absolute alcohol (20 ml) was cooled, and gradually mixed with ethyl oxalate (5.84 g). 4-Nitro-3-picoline 1-oxide (3.08 g) was then added and the sides of the flask were washed with a further 20 ml of alcohol. The mixture was well shaken and then kept in a thermostat at 35-40° for four days. The reddish-brown reaction mixture was added to ice and acidified by the addition of a mixture of ice and hydrochloric acid. After evaporation of the alcohol, a yellow solid separated, which on purification as usual gave 4,4'-dinitro-1,1'-dioxy-3,3'-dipicolyl (0.16 g), m.p. and mixed m.p., 242-244°.

(iii) This method was based upon that used by Piers et al.[84] for the synthesis of potassium enolate of ethyl 3-benzylthio-2-nitrophenylpyruvate.

A mixture of potassium (1.18 g), ethyl oxalate (4.38 g), super dry alcohol (50 ml) and 4-nitro-3-picoline 1-oxide (3.85 g) was kept at room temperature for six days, then concentrated to one-third its volume, and dry benzene added until no further precipitation occurred. From the crude precipitate (4.3 g), 4,4'-dinitro-1,1'-dioxy-3,3'-dipicolyl (1.5 g) was obtained.

(iv) The above experiment was repeated using an atmosphere of nitrogen. From 4-nitro-3-picoline 1-oxide (15.4 g), 4,4'-dinitro-1,1'-dioxy-3,3'-dipicolyl (3.8 g) was obtained.

The filtrate and washings obtained were concentrated to give a stioky dark reddish-brown very hygroscopic material. It

was treated with hot pure dry acetone to remove unreacted material, but no 4-nitro-3-picoline 1-oxide could be isolated.

(v) In another experiment the reaction was carried out in benzene medium. Potassium (2.35 g) was dissolved in absolute ethanol (25 ml) and to this was added ethyl oxalate (8.75 g) followed by 4-nitro-3-picoline 1-oxide (7.7 g) and pure dry benzene (250 ml). The mixture was shaken well and left for twentyfive days at room temperature. The precipitated material was purified as usual to give 4,4'-dinitro-1,1'-dioxy-3,3'-dipicolyl (0.95 g).

4-Nitronicotinic acid 1-oxide.- This compound (12 g) was prepared by oxidation of 4-nitro-3-picoline 1-oxide (25 g) with sodium dichromate (81 g) in concentrated sulphuric acid at 45-55°. cf.52,62 After two recrystallisations from acetone it melted at 170° with spontaneous decomposition.

A modified method of preparing 4-nitronicotinic acid 1-oxide.- The method used earlier[52,62] for the preparation of 4-nitronicotinic acid 1-oxide was modified. The new method was found to give better yields, and it altogether eliminated any danger of destruction of the starting material by uncontrollably violent reactions, which were faced many times in preparing this acid by the literature methods.[52,62]

4-Nitro-3-picoline 1-oxide (10 g) was added slowly with stirring to cooled concentrated sulphuric acid (35 ml). In

another flask sodium dichromate (24 g) was dissolved in concentrated sulphuric acid (35 ml) and cooled to 0° in an ice-bath. The cooled solution of 4-nitro-3-picoline 1-oxide as prepared above was then added dropwise. The mixture was maintained at 20-30° with an ice-bath. After the addition was complete (1.5 hr), the 1-oxide container was washed with concentrated sulphuric acid (5 ml) and the washings poured into the reaction vessel. It was kept at room temperature for $\frac{1}{2}$ hr and then warmed to 45-55° in a water-bath. After 20 mins. the inside temperature rose to 60°. The flask was taken out of the water-bath and cooled in an ice-bath until the internal temperature fell to 20°. It was again warmed to 45-55° for 5 hr, care being taken to keep the internal temperature below 55°. The reaction mixture was then cooled slowly to room temperature, and poured with stirring over crushed ice (250 g). The mixture was stirred well and left in the cold-room overnight, when a precipitate separated. It was filtered, washed with ice-cold water, and a very pale green solid was obtained. This was dried overnight <u>in vacuo</u> over concentrated sulphuric acid and sodium hydroxide pellets. Yield, 8.95 g (75%); m.p. 172° (decomposition).

For purification the compound was dissolved in the minimum of dilute ammonium hydroxide, filtered, and reprecipitated by addition of dilute hydrochloric acid. 4-Nitronicotinic acid 1-oxide was then isolated as a colourless powder, m.p. 172° (decomposition). Yield, 8.25 g (69%); the earlier literature[52,62] yield was 56%.

4-Anilinonicotinic acid 1-oxide.- This compound was obtained in 50% yield by the nucleophilic displacement of the nitro group of 4-nitronicotinic acid 1-oxide by aniline following essentially the method of Herz and Murty.[52]

The product could not be readily recrystallised from dimethylformamide and water as reported by the above authors. Hence the following method of purification was followed. The compound was dissolved in just sufficient dilute sodium hydroxide and filtered to remove any undissolved impurity. By careful addition of dilute hydrochloric acid the pH of the solution was brought to 7, the solution being kept in an ice-bath during the addition. After 4 hours at 0°, a small amount of an orange-yellow material separated. It was filtered off and discarded. The clear light yellow solution was kept in an ice-bath, and dilute acetic acid was added to it drop by drop with thorough stirring. The pH was adjusted at 3 to 4, and the mixture left in the ice-bath for two hours, when a deep yellow precipitate of 4-anilinonicotinic acid 1-oxide, m.p. 245-246° (decomposition) separated. This was further purified by recrystallisation from aqueous acetic acid to yield 4-anilinonicotinic acid 1-oxide, m.p. 252-254° (decomp.) (Found: C, 62.5; H, 4.45; N, 12.0. Calc. for $C_{12}H_{10}N_2O_3$: C, 62.6; H, 4.4; N, 12.2%) (lit. m.p. 244-245°).

4-Chloro-3-picoline 1-oxide.- The following method was adapted from that used by Ochiai[57] for the preparation of 4-chloropyridine 1-oxide, and was found to be superior to that in the literature.[62]

4-Nitro-3-picoline 1-oxide (10 g) was added in small portions over 45 min. to acetyl chloride (50 ml) at 0°. The reaction mixture was protected with a calcium chloride tube, and warmed at 40° for $\frac{1}{2}$ hr and then at 50° for $\frac{1}{2}$ hr. It was then cooled and added in portions to ice-water (100 ml) with stirring. The solution was neutralised with solid sodium carbonate, and extracted with chloroform (500 ml). Evaporation of the dried extract (potassium carbonate) gave 4-chloro-3-picoline 1-oxide, m.p. 120-122° (7.3 g, 80%).

4-Chloronicotinic acid 1-oxide.- (i) This was prepared in 88.6% yield by treatment of 4-nitronicotinic acid 1-oxide with acetyl chloride according to the method of Taylor and Driscoll.[77] It had m.p. 170° (decomp.), but slowly changed on keeping (166° after one month, 156° after two months).

(ii) 4-Chloro-3-picoline 1-oxide (3.1 g) was slowly added, with shaking, to a mixture of sodium dichromate dihydrate (8 g) in concentrated sulphuric acid (24 ml) in an ice-bath. The temperature was kept at 30-35° during the addition ($1\frac{1}{4}$ hr), and the mixture then warmed (and cooled as necessary) to 50-55° for 6 hr. It was cooled

to room temperature then poured, with stirring, onto crushed ice (100 g), and set aside in the cold-room overnight. The precipitate was collected, washed with ice-cold water, and dried in vacuo to give 4-chloronicotinic acid 1-oxide, m.p. and mixed m.p. 170° (decomp.) (Yield 2.4 g, 65%). The infrared spectrum was identical with that of a sample prepared by the first method.

4-Methylaminonicotinic acid 1-oxide.- Attempts to prepare this compound from 4-nitronicotinic acid 1-oxide by treatment with methylamine under a variety of different reaction conditions were unsuccessful. A mixture of 4-chloronicotinic acid 1-oxide (1 g) and aqueous methylamine (25-30%, 8 ml) was heated at 140° in a steel bomb (10 ml capacity) for 18 hr. The bomb was cooled (6 hr) and the contents filtered. The filtrate was cooled in an ice-bath and the pH adjusted to 3-4 by the dropwise addition of a mixture of concentrated hydrochloric acid and water (1:1). The mixture was then stirred and cooled (ice-bath) for 2 hr, and the solid collected, washed with ice-cold water, and dried (yield 0.7 g, 70.8%), m.p. 240-242° (decomp.). Repeated recrystallisations from water using decolourising carbon gave 4-methylaminonicotinic acid 1-oxide as colourless needles, m.p. 250-252° (decomp.) (Found: C, 50.0; H, 4.75; N, 16.4; O, 28.7. $C_7H_8N_2O_3$ requires C, 50.0; H, 4.8; N, 16.7; O, 28.55%). When heated at 100-110° it became yellow, and reverted to the colourless form on cooling; thermochromism was also observed in aqueous solution.

(ii) In another experiment, the effect of increasing the proportion of methylamine solution, and the time of heating, was investigated. For this purpose 4-chloronicotinic acid 1-oxide (0.5 g) was reacted with methylamine (8 ml) in a steel-bomb at 140° for 24 hr. 4-Methylaminonicotinic acid 1-oxide (0.33 g, yield 66%) was isolated as a colourless solid, m.p. 225-226° (decomp.) after shrinking at 216°. Its infrared spectrum was identical with that of the previous specimen. On recrystallisation from water (decolourising carbon) clusters of needle-shaped crystals were obtained (0.3 g, yield 60%), m.p. 240-242° (decomp.). A mixed m.p. determination of both the samples showed no depression. It follows therefore that increase in proportion of methylamine solution, and time of heating, have no beneficial effect on increasing the yield.

4-Methylaminonicotinic acid.- A mixture of 4-methylaminonicotinic acid 1-oxide (1 g), palladium-on-carbon (0.3 g) and water (70 ml) was hydrogenated at room temperature and atmospheric pressure until the required quantity of hydrogen (131 ml; 7 hr) was absorbed. The reaction mixture was warmed in a water-bath, the catalyst separated and extracted with hot water. The combined filtrates and extracts were evaporated under reduced pressure to 60 ml, and cooled in an ice-bath when unreduced 4-methylaminonicotinic acid 1-oxide (0.27 g) separated, m.p. 242-244° (decomp.). The mother liquor was further concentrated and cooled to give 4-methylaminonicotinic acid (0.6 g, 66%), m.p. 268-270° (decomp.).

Repeated recrystallisations from methanol gave colourless needles of the deoxy acid, m.p. 274-276° (decomp.) (Found: C, 55.5; H, 4.9; N, 18.4. $C_7H_8N_2O_2$ requires C, 55.25; H, 5.3; N, 18.4%).

Dihydroxymaleic acid.- This acid was obtained essentially by the method of Nef[85] and Fenton.[86] From 250 g of tartaric acid, 44 g of dihydroxymaleic acid was obtained.

Glycollic aldehyde.- Following the procedure of Fenton[86] and Fischer and Taube,[87] glycollic aldehyde (5.1 g, 89%) was obtained by heating dihydroxymaleic acid (14.5 g) with dry pyridine (37 ml). After distilling off the pyridine, the syrupy liquid was distilled at 16.5 mm keeping the temperature of the oil-bath at 150°. The inner thermometer stood at 65-75°. The material solidified and had m.p. 96-97°.

Reaction between 4-anilinonicotinic acid 1-oxide and glycollic aldehyde.- (i) A mixture of 4-anilinonicotinic acid 1-oxide (0.77 g), glycollic aldehyde (0.4 g) and pyridine (30 ml) was heated at 130° for 7 hr in an oil-bath. Pyridine was distilled off under reduced pressure, and a solid (0.55 g) separated. After several recrystallisations from acetic acid and water, unchanged 4-anilinonicotinic acid 1-oxide, m.p. 252-254° (decomp.) was obtained. Its infrared spectrum was identical with that of an authentic specimen and mixed m.p. determination showed no depression.

(ii) A mixture of 4-anilinonicotinic acid 1-oxide (0.29 g), glycollic aldehyde (0.15 g) and glacial acetic acid (15 ml) was refluxed for 10 hr. After working up the reaction mixture as usual, unreacted 4-anilinonicotinic acid 1-oxide (0.22 g) was obtained.

(iii) A mixture of 4-anilinonicotinic acid 1-oxide (0.2 g), glycollic aldehyde (0.1 g) and 88-93% orthophosphoric acid (6 ml) was warmed on a steam-bath for 3 hr. After cooling it was poured into ice-cold water (40 ml) and basified with 30% sodium hydroxide; but no solid separated. It was diluted to 220 ml, but again no solid separated. The solution was then cooled in an ice-bath and the pH adjusted to 2-3 by the dropwise addition of concentrated hydrochloric acid, to give unreacted 4-anilinonicotinic acid 1-oxide (0.1 g), m.p. and mixed m.p. 247-249° (decomp.).

Reaction between 4-methylaminonicotinic acid 1-oxide and glycollic aldehyde.- (i) A mixture of 4-methylaminonicotinic acid 1-oxide (0.6 g), glycollic aldehyde (0.25 g) and dry pyridine (15 ml) was heated under reflux (magnetic stirring) in the absence of atmospheric moisture in an oil-bath (120-130°). The pyridine was distilled off under reduced pressure (water-bath/water-pump) and the residue dissolved in water (50 ml), filtered and extracted with ether (300 ml). The ether extract was dried ($MgSO_4$) overnight, and the ether evaporated to give an oil (0.2 g). Its picrate had m.p. 166° (reported m.p. of pyridine picrate is 167°). The aqueous

solution was concentrated to half its original volume, and the pH adjusted to 3-3.2 with hydrochloric acid, when the starting material (0.58 g), m.p. 229-233° precipitated. Its infrared spectrum was identical to an authentic specimen of 4-methylaminonicotinic acid 1-oxide.

(ii) A mixture of 4-methylaminonicotinic acid 1-oxide (0.2 g), glycollic aldehyde (0.08 g) and water (10 ml) was refluxed on a boiling water-bath for 12 hr. The hot reaction mixture was filtered, the filtrate cooled in ice-bath, the pH adjusted to 2.8-3.0 and the precipitate (0.16 g) collected. It had m.p. 238-240° (decomp.) after shrinking at 220°. The filtrate and washings were concentrated to one-third the original volume, then cooled in the refrigerator overnight, when another fraction (0.03 g) was obtained. It had m.p. 226-230° (decomp.) after shrinking at 216°. The recovered materials were shown to be the unreacted starting material.

(iii) The above reaction was repeated in a steel-bomb (except that in this case only 7 ml of water were used) and the contents heated at 140° for 12 hr. The bomb was cooled, the contents washed into a beaker, heated to boiling and filtered, when a dark brown solution was obtained. The pH was adjusted to 3.1-3.4 with hydrochloric acid and the mixture cooled at 0°; but no precipitate formed. The brown solution was then extracted with benzene for 60 hr. The benzene extract was dried (K_2CO_3), filtered, and the benzene evaporated. No product could be obtained from this extract.

The mother liquors were then extracted with ether (24 hr), the ether extract dried (K_2CO_3) and the ether evaporated; but no product was obtained.

The mother liquors were collected and evaporated to dryness on a sand-bath until very little of the solvent remained. It was then diluted with acetone, whereupon a brown substance separated; this was filtered off and dried (0.17 g). The product was washed with alcohol, dissolved in the minimum amount of water, and filtered. To the filtered solution was added excess of acetone, when a precipitate was obtained. Further purification was effected by two repititions of the above procedure (yield 61 mg, m.p. 273-276° (decomp.) after shrinking at 260-265°). When placed in a bath preheated to 170°, it melted at 273-276° (decomp.) after shrinking at 265°. The infrared spectrum differed from that of the starting material. Analysis showed the compound to be 4-methylaminonicotinic acid hydrochloride (Found: C, 44.2; H, 5.05; N, 14.7. $C_7H_9N_2O_2Cl$ requires C, 44.6; H, 4.8; N, 14.85%).

Raney Nickel, W-2.- Raney nickel catalyst was prepared by the method of Mozingo,[66] and the final washings were carried out with ethanol or methanol as required.

Reduction of 4-nitro-3-picoline 1-oxide with Raney nickel in ethanol.- (i) A mixture of 4-nitro-3-picoline 1-oxide (5 g), Raney nickel (25 g) and absolute ethanol (100 ml) was refluxed on a

water-bath for 5 hr. The Raney nickel was filtered off, washed with ethanol, and the alcohol distilled from the filtrate and washings. The residue was distilled at 132-134°/5.5 mm, to give a colourless solid, m.p. 100°, yield 0.4 g (11%). Recrystallisation several times from benzene gave 4-amino-3-picoline, m.p. 108-109°. The material remaining in the distillation flask weighed 2.4 g. Separation of amine mixtures was attempted by the method described in Vogel[88] (p.651, method 2). From this was obtained 3-methyl-4-tosylaminopyridine (1.3 g), which after several recrystallisations from ethanol formed colourless needles, m.p. 212-213° (Found: C, 59.6; H, 5.4; N, 10.4. $C_{13}H_{14}N_2O_2S$ requires C, 59.5; H, 5.4; N, 10.7%). This compound (0.25 g) was heated with 80% sulphuric acid (2.5 ml) on a steam-bath for 3 hr. The resulting solution was diluted with water, basified with ammonium hydroxide and then extracted with ether. The dried ether extract ($MgSO_4$) was evaporated, when 4-amino-3-picoline (0.06 g, 60%), m.p. 107-108° was obtained. On recrystallisation from dry benzene, the m.p. was raised to 108-109° (mixed m.p. with an authentic specimen, 108-109°). The infrared spectra of the two specimens were also identical.

When processed for the isolation of the toluenesulphonamide of the corresponding secondary amine, a light red material was obtained, which after recrystallisation from ethanol, had m.p. 150°. It was shown to be 3,3'-dimethyl-4,4'-azopyridine by mixed m.p. with a specimen obtained in the following experiment.

(ii) A mixture of 4-nitro-3-picoline 1-oxide (5 g), Raney nickel (25 g) and absolute ethanol (400 ml) was stirred and refluxed on a steam-bath for 24 hr. The amine mixture was worked up as above, when 3-methyl-4-tosylaminopyridine (0.85 g, yield 10%) was obtained. It had m.p. and mixed m.p. 212-213°.

After the separation of the above tosyl derivative an impure material (1.2 g), m.p. 95-100° (decomp.) was obtained, and this could not be recrystallised. It was dissolved in dilute hydrochloric acid, filtered, the filtrate cooled, and basified with 10% sodium hydroxide. The material which separated was recrystallised from acetone (the insoluble fraction being discarded) and then from benzene to yield fine red-brown needles (64 mg), m.p. 148-150°. It was sublimed at 100-110°/0.3 mm and resublimed at 85-95°/0.2 mm to give 3,3'-dimethyl-4,4'-azopyridine as orange-red needles (30 mg), m.p. 150° (Found: C, 68.0; H, 5.9; N, 26.3. $C_{12}H_{12}N_4$ requires C, 67.9; H, 5.7; N, 26.4%). Its ultraviolet and visible spectrum (95% ethanol) showed λ_{max} at 296 and 476 mμ.

(iii) A mixture of 4-nitro-3-picoline 1-oxide (2.5 g), W-2 Raney nickel (2.5 g) and ethanol (50 ml) was refluxed for 10 min. Working up gave 4-amino-3-picoline 1-oxide (0.15 g) m.p. 135-136° (lit. m.p. 138°). No 4-amino-3-picoline was obtained. The Raney nickel was extracted with boiling acetone. Evaporation of the acetone and recrystallisation of the residue from acetone gave unreacted 4-nitro-3-picoline 1-oxide (0.24 g), m.p. and mixed m.p.

136-137°, and a less soluble product. Recrystallisation of the latter from dilute acetic acid gave 3,3'-dimethyl-4,4'-azoxypyridine-1,1'-dioxide as dark red needles (0.1 g), m.p. 236-238° (Found: C, 55.1; H, 5.0; N, 21.85. $C_{12}H_{12}N_4O_3$ requires C, 55.4; H, 4.65; N, 21.5%). Its ultraviolet and visible spectrum (95% ethanol) showed λ_{max} at 257 and 402 mμ.

Catalytic hydrogenation of 4-nitro-3-picoline 1-oxide over Raney nickel.- (i) A mixture of 4-nitro-3-picoline 1-oxide (7.3 g), methanol (100 ml), glacial acetic acid (8 ml) and W-2 Raney nickel (8 g) was shaken with hydrogen at ordinary temperature and atmospheric pressure until hydrogen uptake ceased (almost quantitative absorption). The catalyst was collected, washed with hot methanol, and the filtrate basified with 10% sodium hydroxide. The precipitated nickelous hydroxide was collected and extracted with methanol for 48 hr. The combined filtrates and extracts were evaporated in vacuo, and the residue recrystallised from ethanol. The resulting material (4.5 g) was refluxed with tosyl chloride (10 g) in dry pyridine (10 ml) for 2 hr. The pyridine was evaporated in vacuo and the resulting solid treated with 5% sodium hydroxide (30 ml). From the alkaline solution 4-tosylamino-3-picoline (1.3 g) was obtained as a colourless solid, m.p. and mixed m.p. 212-213°. The alkali-insoluble material was washed with water, dried and recrystallised from acetone to give 4-ditosylamino-3-picoline (1.2 g) as colourless needles, m.p. 180° (Found: C, 57.7; H, 4.95; N, 6.4. $C_{20}H_{20}N_2O_4S_2$ requires

C, 57.7; H, 4.9; N, 6.7%). The above 4-ditosylamino-3-picoline (1.1 g) was dissolved in ethanol (10 ml) and refluxed for 1.5 hr with sodium ethoxide (from sodium, 1 g, and ethanol, 15 ml). The mixture was cooled, water (30 ml) was added, and the alcohol evaporated. Dilute hydrochloric acid was added to give pH 7, and the resulting solid collected, washed with water, and then recrystallised from ethanol to give 4-tosylamino-3-picoline as a colourless solid (0.6 g), m.p. and mixed m.p. 212-213°.

The filtrate from the initial separation of 4-tosylamino-3-picoline was evaporated. The residue was dissolved in acetic acid, and the solution neutralised with solid sodium carbonate. This solution was extracted with ether, and the ether evaporated to yield 4-amino-3-picoline , m.p. 107-108° (0.12 g). The total yield of 4-amino-3-picoline was thus 20%.

(ii) A mixture of 4-nitro-3-picoline 1-oxide (1.0 g), methanol (40 ml), glacial acetic acid (2 ml) and Raney nickel (from 1.0 g alloy) was shaken with hydrogen until hydrogen absorption ceased. The catalyst was collected, extracted with methanol and the filtrate and extracts were evaporated. The syrupy product was basified with aqueous sodium hydroxide and the solution continuously extracted with ether for 36 hr. Evaporation gave 4-amino-3-picoline (0.62 g, 89%), m.p. 105-107°.

Reduction of 4-nitro-3-picoline 1-oxide with hydrazine and Raney nickel.- A mixture of 4-nitro-3-picoline 1-oxide (2.5 g), ethanol (50 ml), hydrazine hydrate (99/100 W/W; 4 ml) and W-2 Raney nickel (about 0.1 g) was warmed on the steam bath for 0.5 hr. A further quantity (2 g) of catalyst was then added and a vigorous reaction ensued. The mixture was heated for a further 0.5 hr, the catalyst removed, the filtrate carbon treated, and again filtered. This filtrate was concentrated to small volume, and diluted with a little acetone, and again filtered. The filtrate was then evaporated to dryness. Sublimation of the residue at 95-98°/3.5 mm gave 4-amino-3-picoline (0.45 g, 25.6%) m.p. and mixed m.p. 105-107°. In another experiment, a mixture of 4-nitro-3-picoline 1-oxide (5 g), methanol (50 ml) and hydrazine hydrate (15 ml), was treated with half the Raney nickel prepared from 5 g of Ni-Al alloy, and refluxed for 1 hr. The remaining portion of the catalyst was then added and refluxing continued for a further hour. Working up in the usual way gave 4-amino-3-picoline (2.55 g; 73%), m.p. and mixed m.p. 105-107°. From the mother liquors a small amount (80 mg; 2.3%) of 3,3'-dimethyl-4,4'-azopyridine, m.p. and mixed m.p. 148-150°, was isolated.

Catalytic hydrogenation of 4-nitro-3-picoline 1-oxide over palladium.- A mixture of 4-nitro-3-picoline 1-oxide (10 g), 5% palladium-on-charcoal (4.5 g) and glacial acetic acid (150 ml) was

hydrogenated at 3 atmospheres pressure until hydrogen absorption ceased (36 hr). The mixture was then heated to 70°, the catalyst separated and extracted with hot acetic acid. The combined filtrates and extracts were evaporated under reduced pressure on the steam bath. The residue was distilled at 138-142°/4.5-5 mm to give a syrupy liquid which solidified (7 g, 100%). Recrystallisation from benzene gave 4-amino-3-picoline as colourless needles, m.p. and mixed m.p. 105-107°.

(b) PYRAZOLO[4,3-c]PYRIDINES.-

4-Hydrazinonicotinic acid 1-oxide.- A mixture of 4-chloronicotinic acid 1-oxide (11.3 g), hydrazine hydrate (16 ml) and absolute ethanol (60 ml) was refluxed for 6 hr.[cf.89] The yellow product which separated was collected, dissolved in the minimum quantity of dilute ammonium hydroxide, carbon treated, filtered, cooled in an ice-bath and adjusted to pH 4-5. The resulting yellow precipitate was collected, washed with ice-cold water, dried (10 g, 89%) and recrystallised from water. 4-Hydrazinonicotinic acid 1-oxide was obtained as yellow needles, m.p. 230-231° (decomp.) (Found: C, 42.85; H, 4.2; N, 24.4. Calc. for $C_6H_7N_3O_3$: C, 42.6; H, 4.2; N, 24.85%). This compound has previously been obtained[77] in 22% yield from 4-nitronicotinic acid 1-oxide, and m.p. 231° (decomp.) was reported.

3-Hydroxy-1H-pyrazolo[4,3-c]pyridine 5-oxide.- (i) A mixture of 4-hydrazinonicotinic acid 1-oxide (1.18 g), concentrated hydrochloric acid (3 ml) and water (35 ml) was refluxed for 2 hr, then concentrated to about 10 ml, basified with sodium carbonate, again concentrated to 10 ml, and left overnight at room temperature. The resulting deep yellow precipitate of the sodium salt was collected (0.8 g) and recrystallised from aqueous alcohol to give the sodium salt of 3-hydroxy-1H-pyrazolo[4,3-c]pyridine 5-oxide, m.p. 291-292° (decomp.) (Found: N, 18.3. $C_6H_4N_3O_2Na.3H_2O$ requires N, 18.4%). The sodium salt was recrystallised from acetic acid and then from water to give

3-hydroxy-1H-pyrazolo[4,3-c]pyridine 5-oxide as a very pale yellow amorphous solid, m.p. 252-254° (decomp.) (Found: C, 47.6; H, 3.4; N, 27.7. $C_6H_5N_3O_2$ requires C, 47.7; H, 3.3, N, 27.8%). The ultraviolet absorption spectrum (water) showed λ_{max}, 255 mμ (log ε 4.33) and 295 mμ (log ε 3.6); in 10 N sulphuric acid the spectrum showed maxima at 232 mμ (log ε 4.26) and 292 mμ (log ε 3.6); and in N sodium hydroxide, maxima at 265 mμ (log ε 4.53) and 320 mμ (log ε 3.35) were observed. The infrared spectrum (Nujol) showed a very weak band in the carbonyl region (1625 cm^{-1}).

The hydrochloride was recrystallised from dilute hydrochloric acid and formed fine colourless needles, m.p. 235-237° (decomp.) (Found: C, 38.4; H, 3.3; N, 22.3. $C_6H_5N_3O_2.HCl$ requires C, 38.4; H, 3.2; N, 22.4%). The infrared spectrum (Nujol) showed a strong carbonyl peak at 1625 cm^{-1}.

(ii) The following method was found to be more convenient than that described above. A mixture of 4-hydrazinonicotinic acid 1-oxide (15 g), water (150 ml) and concentrated hydrochloric acid (35 ml) was refluxed for $2\frac{1}{2}$ hr. The clear solution was concentrated to one-third its volume, and cooling gave fine colourless needles of 3-hydroxy-1H-pyrazolo[4,3-c]pyridine 5-oxide hydrochloride (15.7 g, 94%), m.p. 235-237° (decomp.). It was dissolved in the minimum amount of water, filtered, cooled in an ice-bath, and the pH adjusted to 3.4 by the dropwise addition of dilute ammonium hydroxide. The very pale yellow precipitate was collected, washed with water, and then with alcohol,

to give 3-hydroxy-1H-pyrazolo[4,3-c]pyridine 5-oxide (12.6 g, 96% yield) as a very pale yellow amorphous solid, m.p. 252-254° (decomp.).

Attempts to methylate[90] 3-hydroxy-1H-pyrazolo[4,3-c]pyridine 5-oxide with methyl sulphate, or with diazomethane, were unsuccessful; unchanged starting material was the only substance which could be isolated from these experiments.

Attempted thiation[91] of 3-hydroxy-1H-pyrazolo[4,3-c]pyridine 5-oxide was likewise unsuccessful.

Several attempts, based on reaction procedures[22,76,92] for analogous compounds, were made to chlorinate 3-hydroxy-1H-pyrazolo-[4,3-c]pyridine 5-oxide with phosphorus oxychloride (with or without the addition of pyridine or of dimethylaniline), but all were unsuccessful.

4-Chloro-3-picoline.- 4-Chloro-3-picoline 1-oxide (10.1 g) was hydrogenated in methanol (35 ml) over Raney nickel (from 5 g alloy) at room temperature and atmospheric pressure. The catalyst was filtered off and washed with methanol and the filtrate and washings evaporated to give 4-chloro-3-picoline as a colourless oil (6.3 g, 70.3%). It was characterised as the picrate, m.p. 152-153° (lit. 151-152°[52] 152-153° [93]). This substance was unstable at room temperature, hence oxidation was carried on immediately.

4-Chloronicotinic acid.- This acid (4.2 g, 55%) was obtained by potassium permanganate oxidation[52] of 4-chloro-3-picoline (6.2 g).

4-Hydrazinonicotinic acid.- (i) A mixture of 4-chloronicotinic acid (1.8 g), hydrazine hydrate (2.5 ml) and absolute ethanol (15 ml) was heated in an oil-bath at 105-110° for 9 hr. The solvent was then evaporated under reduced pressure and the residue dissolved in the minimum amount of dilute ammonium hydroxide. The filtered solution was cooled, and the pH adjusted to 3-4 with acetic acid. The precipitate was collected, washed with ice-cold water, and dried (yield, 0.7 g). A portion was recrystallised from water. 4-Hydrazinonicotinic acid was obtained as fine colourless needles, m.p. 244-246° (decomp.) (Found: C, 47.1; H, 4.85; N, 27.2. $C_6H_7N_3O_2$ requires C, 47.05; H, 4.6; N, 27.4%). The mother liquors were boiled with concentrated hydrochloric acid and gave the cyclised product (0.84 g) as described below.

(ii) In another experiment, a mixture of 4-chloronicotinic acid (4 g), hydrazine hydrate (5 ml) and absolute ethanol (20 ml) was refluxed for 9 hr under the similar conditions described in the above experiment. Working up gave 4-hydrazinonicotinic acid sesquihydrate (3.2 g, 83%), m.p. 222-224° (decomp.). It was recrystallised from water to give colourless woolly needles, m.p. 222-224° (decomp.) (Found: N, 23.5, 23.2. Calc. for $C_6H_7N_3O_2 \cdot 1.5H_2O$: N, 23.3%). Repeated attempts to dehydrate the sesquihydrate were unsuccessful; but like the anhydrous acid, it readily cyclised to 3-hydroxy-1H-pyrazolo[4,3-c]pyridine, m.p. and mixed m.p. 289-291° (see below).

3-Hydroxy-1H-pyrazolo[4,3-c]pyridine.- (i) A mixture of 4-hydrazinonicotinic acid (1.0 g), concentrated hydrochloric acid

(3 ml) and water (30 ml) was refluxed for $3\frac{1}{2}$ hr, then concentrated to about 10 ml, and set aside in the refrigerator. The crystals which separated were collected (0.53 g), and concentration of the mother liquors gave a further quantity (0.47 g; yield 89%). A portion was recrystallised from a 1:1 mixture of water and concentrated hydrochloric acid to give 3-hydroxy-1H-pyrazolo[4,3-c]pyridine hydrochloride as colourless needles, m.p. 298-300° (Found: C, 42.0; H, 3.6; N, 24.6. $C_6H_5N_3O.HCl$ requires C, 42.0; H, 3.5; N, 24.5%). This hydrochloride (0.5 g) in the minimum amount of water, was adjusted to pH 3 and the precipitate collected. Recrystallisation from methanol gave 3-hydroxy-1H-pyrazolo[4,3-c]pyridine as deep yellow needles (0.24 g; 62%), m.p. 292-294° (decomp., after sintering) (Found: C, 47.3; H, 4.6; N, 28.1. $C_6H_5N_3O.H_2O$ requires C, 47.05; H, 4.6; N, 27.4%). The picrate separated from methanol in deep yellow needles, m.p. 260-262° (decomp.) (Found: C, 39.4; H, 2.5; N, 22.8. $C_{12}H_8N_6O_8$ requires C, 39.6; H, 2.2; N, 23.1%). The ultraviolet spectrum of the base in water showed maxima at 226 mμ (log ε 4.29) and 289 mμ (log ε 3.51); in 10 N sulphuric acid the spectrum showed maxima at 220 mμ (log ε 4.2) and 283 mμ (log ε 3.55); and in N sodium hydroxide, maxima at 227 mμ (log ε 4.25) and 283 mμ (log ε 3.43) were observed. The infrared spectrum (Nujol) showed a very intense peak in the carbonyl region at 1630 cm^{-1}.

(ii) 3-Hydroxy-1H-pyrazolo[4,3-c]pyridine 5-oxide (1.0 g) was hydrogenated in methanol (25 ml) over Raney nickel (from 1.5 g alloy) until the required quantity of hydrogen had been absorbed. Working up

gave 3-hydroxy-1H-pyrazolo[4,3-c]pyridine, m.p. 289-291° (decomp.) (0.44 g; 55%) not depressed by admixture with a specimen prepared as above. The infrared spectra given by the two samples were also identical.

Attempts to methylate,[90] chlorinate[22,76,92] and reduce (lithium aluminium hydride)[92(a)] 3-hydroxy-1H-pyrazolo[4,3-c]pyridine were all unsuccessful.

3-Hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine 5-oxide.- Phenylhydrazine (6 ml) was added with shaking to a suspension of 4-chloronicotinic acid 1-oxide (6.0 g) in absolute ethanol (50 ml), and the mixture refluxed for $4\frac{1}{2}$ hr, then cooled and filtered. The solid was dissolved in dilute ammonium hydroxide, filtered, and reprecipitated with acid (yield 2.25 g). Crystallisation from concentrated hydrochloric acid:water (1:1) gave 3-hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine 5-oxide hydrochloride as colourless needles, m.p. 230-232° (after shrinking) (Found: C, 54.8; H, 3.8; N, 16.2. $C_{12}H_9N_3O_2.HCl$ requires C, 54.7; H, 3.8; N, 15.9%). The infrared spectrum (Nujol) showed an intense band in the carbonyl region, at 1630 cm^{-1}. This hydrochloride (1.5 g) was dissolved in water, and the base reprecipitated by the addition of ammonium hydroxide. Further dissolution and reprecipitation gave 3-hydroxy-1-phenyl-1H-pyrazolo-[4,3-c]pyridine 5-oxide as fine colourless needles (1.24 g), m.p. 291-293° (decomp.) (Found: C, 63.3; H, 4.2; N, 18.3; O, 14.6. $C_{12}H_9N_3O_2$ requires C, 63.4; H, 4.0; N, 18.5; O, 14.1%). The

ultraviolet spectrum in N-sodium hydroxide showed maxima at 277 mμ (log ε 4.35) and 335 mμ (log ε 4.02). The infrared spectrum (Nujol) showed a very weak band at 1625 cm^{-1}.

The original liquors were set aside for several days, and the solid (1.6 g) which separated was collected, dissolved in dilute ammonium hydroxide, filtered, cooled in an ice bath and pH adjusted to 3.4 by the dropwise addition of acetic acid. 4-(α-Phenylhydrazino)-nicotinic acid 1-oxide was obtained as an amorphous cream solid (1.4 g), m.p. 193-195° (decomp.) (Found: C, 59.0; H, 4.8; N, 17.5. $C_{12}H_{11}N_3O_3$ requires C, 58.8; H, 4.5; N, 17.1%). The infrared spectrum (Nujol) showed peaks at 3210 cm^{-1} and 3320 cm^{-1} characteristic of compounds containing a primary amino group. The acid was not oxidised by treatment with mercuric oxide[94] or with nitric acid[95] and gave a positive Liebermann reaction (which hydrazobenzene did not).

4-(α-Phenylhydrazino)nicotinic acid.- A mixture of 4-chloro-nicotinic acid (1 g), phenylhydrazine (1 ml) and absolute ethanol (8 ml) was refluxed. After $\frac{1}{2}$ hr a deep yellow solution resulted. Refluxing was continued for 8 hr and the reaction mixture cooled. The yellow product which separated was collected, dissolved in dilute ammonium hydroxide, the solution filtered and reprecipitated by the dropwise addition of dilute hydrochloric acid to pH 5.5-5.8 (yield, 1.1 g, 76%, m.p. 191-193°, decomp.). Repitition of the above reprecipitation, and recrystallisation from methanol, gave 4-(α-phenylhydrazino)nicotinic acid as stout lemon needles, m.p. 194-197° (decomp.); but despite

repeated attempts satisfactory analyses could not be obtained owing to its ready cyclisation during purification (Found: N, 18.8. $C_{12}H_{11}N_3O_2$ requires N, 18.3%). The infrared spectrum (Nujol) showed bands at 3220 and 3360 cm^{-1}. Like its N-oxide, this was not oxidised by treatment with mercuric oxide[94] or nitric acid,[95] and gave a positive Liebermann reaction.

3-Hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine.- (i) A mixture of the above phenylhydrazinonicotinic acid (0.5 g), water (15 ml) and concentrated hydrochloric acid (1.5 ml) was refluxed for $4\frac{1}{2}$ hr, then filtered and evaporated to one-third its volume, and cooled. The solid which separated (0.32 g, 59%); was recrystallised from concentrated hydrochloric acid:water (1:2). 3-Hydroxy-1-phenyl-1H-pyrazolo[4,3-c]-pyridine hydrochloride was obtained as fine almost colourless needles, m.p. 252-254° (after shrinking) (Found: C, 57.8; H, 4.2; N, 16.9. $C_{12}H_9N_3O.HCl$ requires C, 58.2; H, 4.1; N, 17.0%). The above hydrochloride (0.15 g) was dissolved in water, filtered and the pH adjusted to 4.0-4.5 by dropwise addition of dilute ammonium hydroxide, when fine yellow needles separated. After filtration and washing with a little water, 3-hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine was obtained (100 mg, 78%), m.p. 214-216° (after shrinking). Repeated recrystallisations from methanol gave the analytical sample, m.p. 219-221° (after shrinking) (Found: C, 68.4; H, 4.5; N, 19.6. $C_{12}H_9N_3O$ requires C, 68.2; H, 4.3; N, 19.9%). The ultraviolet spectrum in

N-sodium hydroxide showed maxima at 231 mμ (log ε 4.12), 250 mμ (log ε 4.17) and 306 mμ (4.08). The infrared spectrum (Nujol) showed a sharp band at 1645 cm^{-1}.

(ii) 3-Hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine 5-oxide (0.25 g) in N sodium hydroxide (10 ml) was hydrogenated over Raney nickel (from 1 g alloy) for $3\frac{1}{2}$ hr at atmospheric pressure and room temperature. The reaction mixture was warmed in a water-bath, filtered, the catalyst extracted thrice with hot water and the filtrate and washings were concentrated to half the original volume. The solution was cooled in an ice-bath and the pH brought to 4.5 by the dropwise addition of dilute hydrochloric acid, when yellow needles of 3-hydroxy-1-phenyl-1H-pyrazolo[4,3-c]pyridine separated, which after washing with water and drying had m.p. 203-205° (0.15 g, 65% yield). Two recrystallisations from methanol gave pure product, m.p. and mixed m.p. with the material obtained in previous experiment, 214-216° (after shrinking). The infrared spectra of the two specimens were identical.

3-Hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine 5-oxide.- Methylhydrazine (6 ml) was added to 4-chloronicotinic acid 1-oxide (3 g) and absolute ethanol (20 ml) in a flask fitted with a condenser and sodalime tube. The mixture was refluxed for 6 hr then evaporated to dryness under reduced pressure. The resulting gum was treated with water (35 ml) and concentrated hydrochloric acid (6 ml), the solution

refluxed for $2\frac{1}{2}$ hr, and then concentrated to one-third its volume. The product (3 g, 86%, m.p. 218-220° decomp.) was collected and recrystallised from methanol. 3-Hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine 5-oxide hydrochloride was obtained as fine colourless needles, m.p. 224-226° (decomp.) (Found: C, 41.3; H, 4.1; N, 20.7. $C_7H_7N_3O_2$.HCl requires C, 41.7; H, 4.0; N, 20.8%). The infrared spectrum (Nujol) showed an intense band in the carbonyl region (at 1625 cm^{-1}). This hydrochloride (0.34 g) in dry methanol (50 ml) was treated with Amberlite CG 400 (8 ml) (preparation described below) and the mixture kept overnight with occasional stirring. The resin was filtered off, washed thrice with methanol, and the filtrate and washings evaporated to dryness to give 3-hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine 5-oxide (0.2 g, 72%), m.p. 234-236° (decomp.). The analytical sample was prepared by recrystallisation from methanol and was obtained as clusters of almost colourless fine needles, m.p. 236-238° (decomp.) (Found: C, 50.5; H, 4.3; N, 25.0; O, 19.6. $C_7H_7N_3O_2$ requires C, 50.9; H, 4.3; N, 25.45; O, 19.4%). The ultraviolet spectrum (in N sodium hydroxide) showed maxima at 265 mμ (log ε 4.3) and 321 mμ (log ε 3.55). The infrared spectrum (Nujol) showed a weak band in the carbonyl region (at 1627 cm^{-1}). The picrate was prepared and recrystallised from methanol to give deep yellow needles, m.p. 206-208° (decomp.) (Found: N, 21.2%. $C_{13}H_{10}N_6O_9$ requires N, 21.3%).

Preparation of Ion Exchange Resin.- A mixture of Amberlite CG 400 (8 ml) in 4% sodium hydroxide solution (25 ml) was allowed to stand

for 24 hr with occasional stirring. The resin was collected by filtration and washed with water until the filtrate was free from alkali; it was finally washed three times with dry methanol before use.

(ii) In another experiment, a mixture of 4-chloronicotinic acid 1-oxide (1.5 g), methylhydrazine (2 ml) and absolute ethanol (10 ml) was refluxed for 6 hr without exclusion of atmospheric carbon dioxide. Working up gave much smaller yield of 3-hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine 5-oxide hydrochloride (0.4 g, 2%), m.p. 224-226° (decomp.). Attempts to isolate the intermediate 4-(α-methylhydrazino)-nicotinic acid 1-oxide were unsuccessful.

3-Hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine.- (i) A mixture of 4-chloronicotinic acid (1.5 g), absolute ethanol (10 ml) and methylhydrazine (3 ml) was refluxed for 9 hr in a flask fitted with a condenser and sodalime tube, then evaporated to dryness under reduced pressure. The resulting gum was treated with water (20 ml) and concentrated hydrochloric acid (3 ml), and the solution refluxed for 3 hr, then concentrated to one-third its volume. The product (1.62 g, 91%, m.p. 273-275° after shrinking) was collected and recrystallised from methanol. 3-Hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine hydrochloride was obtained as very fine colourless needles, m.p. 274-276° (after shrinking) (Found: C, 45.2; H, 4.4; N, 22.4. $C_7H_7N_3O.HCl$ requires C, 45.3; H, 4.3; N, 22.6%). This hydrochloride (0.8 g) on treatment

with Amberlite CG 400 (8 ml, regenerated immediately before use with 4% sodium hydroxide for 24 hr) in methanol (50 ml), gave the free base. This was purified by dissolution in methanol and reprecipitation with dry acetone. 3-Hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine was obtained as yellow needles (0.64 g, 98%) m.p. 205-207° (decomp., after shrinking) (Found: C, 47.7; H, 5.1; N, 23.7; $C_7H_7N_3O.1.5H_2O$ requires C, 47.7; H, 5.7; N, 23.85%). The ultraviolet absorption spectrum (in N sodium hydroxide) showed maxima at 230 mμ (log ε 4.22) and 288 mμ (log ε 3.41). The infrared spectrum (Nujol) showed a sharp intense band at 1627 cm^{-1}. The picrate was prepared in methanol and recrystallised from 95% ethanol to give shining golden prisms, m.p. 242-244° (decomp.) (Found: C, 41.7; H, 2.8; N, 22.35. $C_{13}H_{10}N_6O_8$ requires C, 41.3; H, 2.7; N, 22.20%).

(ii) 3-Hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine 5-oxide (92 mg) was hydrogenated in methanol (30 ml) over Raney nickel (from 0.5 g alloy) for $2\frac{1}{2}$ hr. 3-Hydroxy-1-methyl-1H-pyrazolo[4,3-c]pyridine (82 mg, 99%) separated from methanol in yellow needles, m.p. and mixed m.p. 205-206° (decomp.). The infrared spectra given by the two samples were also identical.

Spectra.- Infrared spectra were determined with a Perkin-Elmer 137 Infracord; ultraviolet spectra were determined using a Perkin-Elmer 137 UV Spectrophotometer.

(c) PYRAZOLO[3,4-b]PYRIDINES.-

Nicotinic acid 1-oxide.- The following method was found to be superior to that given in the literature.[63,96]

A mixture of nicotinic acid (20 g), 30% hydrogen peroxide (32 ml) and glacial acetic acid (200 ml) was heated on the steam-bath for 4 hr, then evaporated to dryness under reduced pressure. Water (60 ml) was added and the mixture again evaporated. The residue was dissolved in boiling water (220 ml), the solution filtered and diluted with ethanol (50 ml). On cooling, colourless lustrous needles (15.6 g, 70%) of nicotinic acid 1-oxide, m.p. 259-261° (decomp.) separated. A small portion was recrystallised from water to give an analytical sample, m.p. 260-262° (decomp.) (Found: C, 51.9; H, 3.75; N, 10.1. Calc. for $C_6H_5NO_3$: C, 51.8; H, 3.6; N, 10.1%). The literature records m.p. 249° (decomp.)[96] and 254-255 (decomp.)[63].

2-Chloronicotinic acid.- A mixture of nicotinic acid 1-oxide (2.4 g) and phosphorus oxychloride (25 ml, freshly distilled over phosphorus pentachloride) was refluxed on a sand-bath for 11 hr. Excess phosphorus oxychloride was evaporated under reduced pressure, and the syrupy residue poured, with stirring, over crushed ice (25 g). The mixture was cooled in an ice-bath for 4 hr, then filtered, and the residue washed with water and dried (yield, 1.4 g, m.p. 170° decomp.). Recrystallisation from 95% ethanol gave 2-chloronicotinic acid as light tan prisms. When heated rapidly it melted at 173-175° (decomp.); but

when heated very slowly it sintered and darkened at 170°, and turned black at 192-195° (Found: C, 46.3; H, 2.8; N, 8.85. Calc. for $C_6H_4NO_2Cl$: C, 45.7; H, 2.6; N, 8.9%). Concentration of the original mother liquors to half volume gave 4-chloronicotinic acid (0.16 g), m.p. and mixed m.p. 163-164°.

3-Hydroxy-1H-pyrazolo[3,4-b]pyridine.- A mixture of 2-chloronicotinic acid (0.76 g), 99% hydrazine hydrate (2 ml) and absolute ethanol (10 ml) was refluxed for 9 hr, the apparatus being protected with a soda-lime tube. The mixture was evaporated to dryness under reduced pressure, the residue treated with water (10 ml) and concentrated hydrochloric acid (1.5 ml) and the solution refluxed for $2\frac{1}{2}$ hr, then filtered and evaporated to small volume. Anhydrous acetone (30 ml) was added and the product (0.54 g) collected. This could not be recrystallised. Purification was effected by dissolving the product (0.15 g) in the minimum amount of water. The solution was filtered and the pH adjusted to 5.0 with dilute ammonium hydroxide. Two such treatments gave 3-hydroxy-1H-pyrazolo[3,4-b]pyridine as a pale yellow powder, m.p. 204-205° (decomp.) (Found: C, 46.7; H, 4.5; N, 27.6. $C_6H_5N_3O.H_2O$ requires C, 47.05; H, 4.6; N, 27.4%). The hydrochloride, obtained by recrystallisation of the base from concentrated hydrochloric acid:water (1:1) was obtained as orange-red prisms, m.p. 260-263° (Found: C, 42.3; H, 3.3; N, 24.3. $C_6H_5N_3O.HCl$ requires C, 42.0; H, 3.5; N, 24.5%).

3-Hydroxy-1-methyl-1H-pyrazolo[3,4-b]pyridine.- A mixture of methylhydrazine (2.0 ml), 2-chloronicotinic acid (1.0 g) and absolute ethanol (10 ml) was refluxed for 8 hr, the apparatus being protected by a soda-lime tube, then evaporated to dryness. The resulting gum was treated with water (12 ml) and concentrated hydrochloric acid (2 ml), the solution refluxed for $2\frac{1}{2}$ hr, filtered, and concentrated to one-third its volume. The yellow product (0.9 g, 76%) which separated on cooling was recrystallised from methanol. 3-Hydroxy-1-methyl-1H-pyrazolo[3,4-b]-pyridine hydrochloride was obtained as yellow prisms, m.p. 246-248° after sintering (Found: C, 45.7; H, 4.5; N, 22.4. $C_7H_7N_3O.HCl$ requires C, 45.3; H, 4.3; N, 22.6%).

A portion (0.2 g) of the above hydrochloride was dissolved in water (3 ml), the solution filtered, cooled, and adjusted to pH 5.5 with dilute ammonium hydroxide. Recrystallisation from acetone gave the free base as fine colourless needles, m.p. 166-167° after sintering (Found: C, 56.25; H, 4.65; N, 27.6. $C_7H_7N_3O$ requires C, 56.4; H, 4.7; N, 28.2%).

3-Hydroxy-1-phenyl-1H-pyrazolo[3,4-b]pyridine hydrochloride.- A mixture of 2-chloronicotinic acid (0.5 g), phenylhydrazine (0.4 ml) and absolute ethanol (5 ml) was refluxed for 8 hr, the apparatus being protected with a soda-lime tube. Excess ethanol was evaporated and the residue treated with water (10 ml) and concentrated hydrochloric acid (2.5 ml) and refluxed for 3 hr. The solution was filtered and

evaporated to one-third the original volume, when light tan prisms separated. The product was collected, washed once with little water and dried (0.3 g), m.p. 216-218° (decomp.). The product was twice, recrystallised from 95% ethanol but the m.p. remained unchanged. Despite repeated attempts satisfactory analyses for carbon and hydrogen could not be obtained (Found: N, 16.6, 16.9. $C_{12}H_9N_3O.HCl$ requires N, 17.0%). Many attempts to get the free base were also unsuccessful and invariably resulted in decomposition.

(d) PYRAZOLO[3,4-d]PYRIMIDINES.-

Methylethoxymethylenemalononitrile.- A mixture of malononitrile (42 g), triethyl orthoacetate (104 g) and acetic anhydride (145 g) was refluxed for 3 hr.[41] The reaction mixture was then left overnight at room temperature, when long colourless needles separated. Filtration and washing twice with a little cold ethanol gave methylethoxymethylene-malononitrile (49.4 g), m.p. 93-94°. The mother liquor and washings were evaporated to nearly dryness under reduced pressure, filtered off from the accompanying liquid, washed three times with water:ethanol (2:1) and twice with a little of cold ethanol to give a second fraction (26.2 g), m.p. 89-91°. This portion was of light grey colour. The total yield was thus 75.6 g (87.4%). A small amount of the first fraction was recrystallised from water to give colourless needles, m.p. 93-94° (Found: C, 61.7; H, 6.0; N, 20.1. Calc. for $C_7H_8N_2O$ C, 61.75; H, 5.9; N, 20.6%). This compound has been previously obtained in 83.7% yield and m.p. 88.5-89.5° has been reported.[41]

5-Amino-4-cyano-3-methylpyrazole.- 5-Amino-4-cyano-3-methylpyrazole (36 g) was obtained by heating methylethoxymethylene-malononitrile (49.4 g) with hydrazine hydrate (34 g) and absolute ethanol (25 ml) according to the method of Cheng and Robins.[41] It had m.p. 163°.

5-Amino-4-carboxamido-3-methylpyrazole.- This compound (25 g) was obtained by partial hydrolysis of 5-amino-4-cyano-3-methylpyrazole (27 g) with concentrated sulphuric acid (110 ml) using the method of Taylor and Hartke.[74]

4-Hydroxy-3-methylpyrazolo[3,4-d]pyrimidine.- The method of Taylor and Hartke[74] was modified as follows.

A mixture of 5-amino-4-carbamido-3-methylpyrazole (2.0 g) and formamide (12 ml) was refluxed for 1 hr on a sand bath, then cooled and poured into water (60 ml). The precipitate (1.8 g, 87%) was collected and recrystallised from water to give 4-hydroxy-3-methylpyrazolo-[3,4-d]pyrimidine as fine colourless needles, m.p. 335-336° in agreement with the literature.[74]

4-Mercapto-3-methylpyrazolo[3,4-d]pyrimidine.- A mixture of finely powdered 4-hydroxy-3-methylpyrazolo[3,4-d]pyrimidine (0.66 g), powdered phosphorus pentasulphide (3.6 g) and anhydrous pyridine (15 ml) was refluxed for 3 hr.[cf.42] The pyridine was then evaporated under reduced pressure, on the steam-bath, water (50 ml) was added and the mixture set aside at room temperature for 30 min, then heated on the steam-bath for 3 hr, and filtered. The filtrate was acidified with glacial acetic acid and the crystalline product which separated on standing was collected, washed and dried (yield 0.38 g, m.p. 341-343° decomp.). Recrystallisation could not be effected and purification was carried out by dissolution in dilute sodium hydroxide and reprecipitation with glacial acetic acid. 4-Mercapto-3-methylpyrazolo[3,4-d]pyrimidine was obtained as a light grey powder, m.p. 345-347° (decomp.) after darkening and shrinking (Found: C, 43.5; H, 4.0; N, 33.4. $C_6H_6N_4S$ requires C, 43.4; H, 3.6; N, 33.7%).

4-Amino-6-hydroxy-3-methylpyrazolo[3,4-d]pyrimidine.- A mixture of 5-amino-4-cyano-3-methylpyrazole (1.5 g) and urea (3.0 g) was heated at 165° for 20 min., then at 190° for another 20 min., until the liquid melt solidified. The cooled solid was dissolved in 10% sodium hydroxide, filtered, and acidified while hot with acetic acid. The precipitate was collected, washed with warm water and dried (yield 1.4 g). It could not be recrystallised and the analytical sample was obtained by two repititions of the above procedure. 4-Amino-6-hydroxy-3-methylpyrazolo[3,4-d]pyrimidine was obtained as a pale yellow powder, m.p. $>360^{\circ}$ (Found: C, 39.75; H, 4.8. $C_6H_7N_5O.H_2O$ requires C, 39.3; H, 4.95%).

4-Amino-6-mercapto-3-methylpyrazolo[3,4-d]pyrimidine.- A mixture of 5-amino-4-cyano-3-methylpyrazole (1.5 g) and thiourea (2.7 g) was heated at 180° for 30 min., then at 200° for 10 min., until the liquid melt solidified. The cooled melt was dissolved in hot 10% sodium hydroxide, filtered, and the hot filtrate carefully acidified with acetic acid. The precipitate was collected, washed with warm water and dried (yield 1.1 g). It could not be recrystallised and the analytical sample was obtained by two repititions of the above procedure. 4-Amino-6-mercapto-3-methylpyrazolo[3,4-d]pyrimidine was obtained as a light yellow powder, m.p. $>360^{\circ}$ (Found: C, 39.7; H, 4.3; S, 17.8. $C_6H_7N_5S$ requires C, 39.8; H, 3.9; S, 17.7%).

4,6-Dihydroxy-3-methylpyrazolo[3,4-d]pyrimidine.- A mixture of 5-amino-4-carboxamido-3-methylpyrazole (1.0 g), and urea (2.0 g) was heated at 165° for 20 min., then at 190-195° for 20 min., until the liquid melt solidified. The cooled solid was dissolved in 2N sodium hydroxide, the solution filtered, and acidified while hot with glacial acetic acid. The product was collected, washed, and dried (yield 1.0 g). It could not be recrystallised and the analytical sample was obtained by reprecipitation from hot basic solution. 4,6-Dihydroxy-3-methylpyrazolo-[3,4-d]pyrimidine was obtained as a colourless powder, m.p. $>360^{\circ}$ (Found: C, 41.0; H, 4.2; N, 32.1. $C_6H_6N_4O_2.\frac{1}{2}H_2O$ requires C, 41.1; H, 4.0; N, 32.0%).

4-Hydroxy-6-mercapto-3-methylpyrazolo[3,4-d]pyrimidine.- A mixture of 5-amino-4-carboxamido-3-methylpyrazole (1.0 g) and thiourea (2.0 g) was heated at 180-185° for 30 min., and at 200° for 10 min. Dissolution in hot 2N sodium hydroxide and reprecipitation with glacial acetic acid gave the crude product (yield 1.1 g) which was recrystallised from water. 4-Hydroxy-6-mercapto-3-methylpyrazolo[3,4-d]pyrimidine was obtained as fine colourless needles, m.p. $>360^{\circ}$ (Found: C, 39.3; H, 3.7; N, 30.7. $C_6H_6N_4OS$ requires C, 39.6; H, 3.3; N, 30.8%).

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