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SYNTHESES AND STUDIES IN POTENTIAL TUBERCULOSTATICS

The author wishes to acknowledge his deep indebtedness to Dr. J.B. Stenlake for suggesting the problem and for his stimulating direction throughout, and also to thank Professor J.P. Todd for his continued interest and encouragement.

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HISTORICAL

INTRODUCTION:

There has been a rapid decline in mortality from respiratory tuberculosis since 1947 in England and Wales. According to Logan and Benjamin[1], in 1954 deaths of persons between 20 and 24 years of age was only one sixteenth that of 1947 and, in 1953, for men and women between the ages of 45 and 54, it was less than one third of the 1947 level.

In Scotland, according to Drolet and Lowell[2], the death rate had dropped from 4,004 in 1947 to 1,341 in 1953. Expressed in relation to population the tuberculosis death rate had decreased from 80 to 26 per 100,000, a fall of 68 per cent. The major contribution to this remarkable decline is due undoubtedly to chemotherapy with streptomycin, p-amino-salicylic acid and isonicotinic acid hydrazide[1,2]. However, despite this encouraging progress there were 342,900 patients suffering from tuberculosis in England and Wales at the end of 1955 and the annual loss to the country's economy was estimated to be in the region of 25 million person-days[1], whilst in America in 1956 an estimated minimum of 25 million pounds was spent on this disease[3].

Archaeological discoveries of skeletons with tuberculous lesions have proved that tuberculosis has plagued mankind since remote antiquity. The disease is characterised by the formation in the tissues of nodular bodies, or tubercles, and in its pulmonary form coughing, lassitude and loss of weight are symptomatic. The causative organism Mycobacterium tuberculosis, or the tubercle bacillus, was first isolated by Koch in 1882. Practically no tissue or organ in the body is immune from its attack, so that extra-

pulmonary forms such as laryngitis, osteomylitis, meningitis and a host of others, including the rapidly fatal form called miliary tuberculosis, or "galloping consumption", also exist. The disease is infectious and it is well known that a high proportion of the population has been infected during childhood.[1] Fortunately, these infections in the vast majority of cases are mild and clinically asymptomatic. However, once the disease becomes established treatment is prolonged and costly. This intractability is one of the worst features of the disease and is due to a unique host-parasite relationship and the emergence of drug-resistant strains of tubercle bacilli.

In diseases of bacterial origin, modern chemotherapeutic agents inhibit the growth of the causative organism and, with the exception of tuberculosis, once the disease has been arrested the host's defensive mechanisms relatively rapidly destroy the parasite. In tuberculosis, however, although tuberculostats arrest the progress of the disease the host's defensive mechanisms act more slowly and with less certainty, so that the micro-organism can remain viable in the tissues for long periods. In certain forms of the disease, such as caseous pulmonary tuberculosis, the organism is isolated within the cavity, thus making penetration of the drug to the site of infection difficult. Tuberculostats are, therefore, used as valuable adjuncts to the traditional treatment of bed rest, collapse therapy and surgery.

Evaluation of potential tuberculostats is carried out either <u>in vitro</u>, in which the minimum concentration necessary to arrest growth of the

tubercle bacillus on some special media (such as that of Dubos[4] or Peizer and Schaefer[5]) is determined, or *in vivo*, in experimental animals such as mice or guinea pigs infected with a virulent strain of *Myco. tuberculosis*. The advantage of a reliable *in vitro* test is twofold, (1) synthesis of the relatively large quantities of substance necessary for *in vivo* testing is avoided and (2) from the results, an indication is obtained as to whether the drug warrants further investigation. The egg-agar media of Peizer and Schaefer is particularly valuable and no substance which has exhibited antituberculous activity in man has failed to arrest growth of the organism in this media. On the other hand, *in vivo* methods have the advantage of comparing the effect of the drug in tuberculous-infected animals with the established untreated disease in a control group of the same animal. The test group receive daily administration of the drug, whilst no treatment is given to the control group. The criterion for the detection of activity is the increase in the time of death of 50 per cent. of the test animals compared with the untreated controls, or a comparison of progress of the disease in the liver, lungs and spleen of sacrificed animals in both groups.

The search for new tuberculostatic agents assumes the following broad pattern:-

(1) screening of new antibiotics;

(2) screening of known chemotherapeutic agents not previously tested against the tubercle bacillus;

(3) synthesis of new compounds to discover other structures with antituberculous activity; and

(4) modification of the structures of known tuberculostats, with the object of determining structure-activity relationships and increasing the chemotherapeutic index.

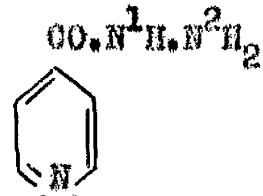
Clinical trial of a drug is only initiated if it shows high *in vivo* and *in vitro* activity, together with a favourable chemotherapeutic index.

While the common antibiotics such as penicillin, the tetracyclines and chloramphemicol as well as a host of sulphonamides, have been spectacularly effective in many diseases of bacterial origin, they are without significant effect in tuberculosis and offer no advance on earlier remedies, such as chaulmoogra oil and gold salts. Chronologically, the sulphones are the first of the modern tuberculostats. The antibacterial activity of 4,4'-diaminodiphenyl sulphone was reported by Buttle, Dewing, Foster, Smith and Stephenson[6] in 1937 and in 1939 Rist, Bloch and Hamon[7] showed that it possessed antituberculous activity. The discovery of the antibiotic streptomycin by Schatz, Bugie and Waksman[8] in 1944 and p-amino-salicylic acid by Lehmann[9] in 1946 heralded a new era in the treatment of the disease. This was followed in the same year by the introduction of the thiosemicarbazones by Domagk[10] and more recently isonicotinic acid hydrazide has been added to the armamentarium of the clinician in the fight against tuberculosis.

ACID HYDRAZIDES IN THE CHEMOTHERAPY OF TUBERCULOSIS:

Although isonicotinic acid hydrazide (isoniazid) (1) had been synthesized by Meyer[11] as long ago as 1912, some forty years were to elapse before its introduction as a therapeutic agent in tuberculosis.

The events leading up to this discovery started in France in 1945,

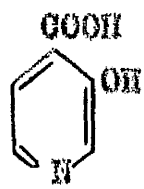


(I)

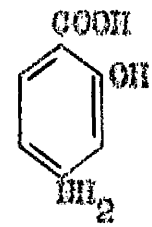
when Chorine[12] observed that the vitamin nicotinamide possessed anti-tuberculous activity, but that nicotinic acid, despite its vitamin activity, was not tuberculostatic. He rightly concluded that the vitamin activity and the antituberculous activity of nicotinamide were not related to each other. The significance of this observation passed unnoticed until 1946 when McKenzie, Malone, Kushner, Olesen and Subbarow[13] rediscovered the antituberculous activity of nicotinamide. They also prepared derivatives of nicotinamide, in which a hydrogen of the amide group was replaced by an isopropyl, pyridyl, or thiazolyl group, and found that these derivatives possessed both types of activity. Introduction of an amino, chloro, or butoxy group in position 6 of the pyridine nucleus, however, gave rise to derivatives which possessed neither antituberculous nor vitamin activity. Contrary to Chorine, they concluded that both the vitamin activity and the antituberculous activity of nicotinamide were, in fact, related to each other.

Whilst preparing 3-hydroxyisonicotinic acid (II), the pyridine analogue of p-aminosalicylic acid (III), Fox[14] discovered that the intermediary 3-aminoisonicotinic acid (IV) and its methyl ester[15] possessed antituberculou activity but no vitamin activity, and concluded that both types of activity were independent of each other, thus confirming the conclusion reached

earlier by Chorine. While both these compounds were about half as active



(II)



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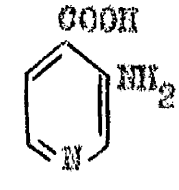
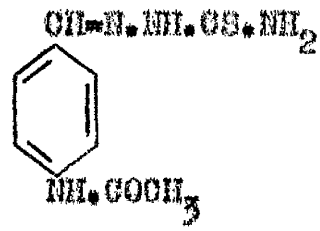


(IV)

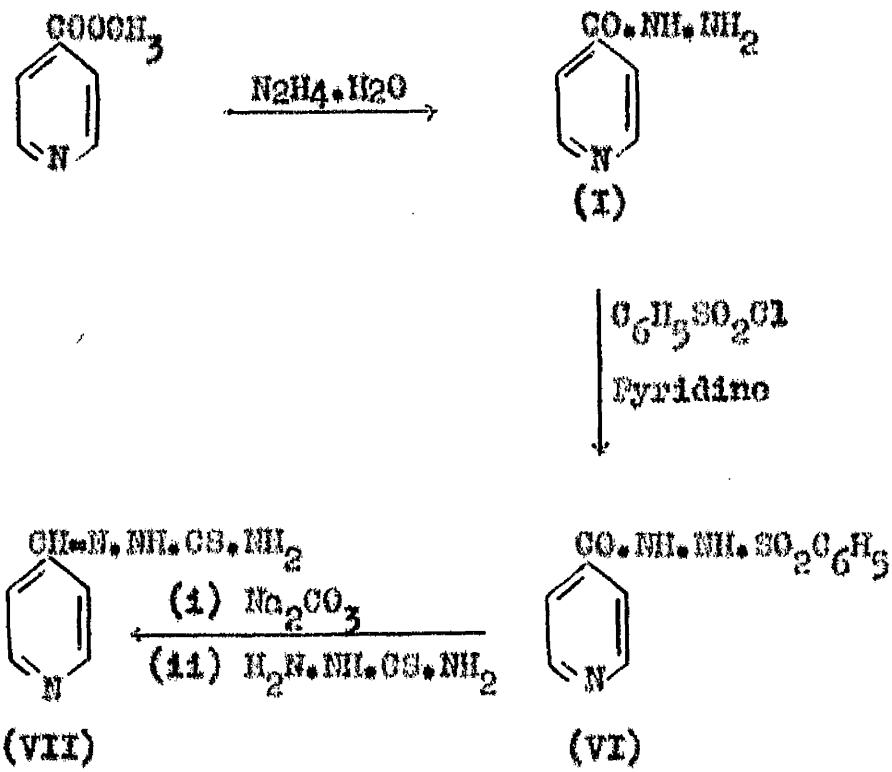
as nicotinamide and therefore of no interest clinically, they served to indicate that antituberculous activity in the pyridine field was not necessarily limited to nicotinamide or to substances with vitamin activity. This meant, in effect, that the field was wide open and theoretically at least tuberculostatic activity might exist in any pyridine structure. In consequence, Fox extended this work to the synthesis and examination of various o-amino and o-hydroxynicotinic acid and isonicotinic acid derivatives[16] and concluded from the inactivity of these compounds that any positional or structural deviation from 3-aminoisonicotinic acid, or its methyl ester, resulted in loss of activity. The high degree of specificity existing between these two structures and their antituberculous activity led to the abandonment of this line of investigation.

With the discovery of p-acetamidobenzaldehyde thiosemicarbazone (Tibione) (V) by Domagk and his associates[10,17] as a new and clinically effective tuberculostat[18,19,20], another sphere of endeavour was opened to workers in the field. In the light of this new lead, Fox[16] was prompted to synthesise isonicotinaldehyde thiosemicarbazone, which was examined by Grunberg and Leivant[21] and found to be more active *in vivo*



(V)

than compound (V). isoNicotinaldehyde thiosemicarbazone (VII) was



synthesized according to the above scheme, the last step being carried out by a modification of the McFadyen-Stevens reaction[22]. Since both isonicotinic acid hydrazide (I) and its benzenesulphonyl derivative (VI) were carboxylic acid derivatives closely related to the compounds under investigation, they also were submitted for testing against mouse tuberculosis. The latter proved to be devoid of activity, but isonicotinic acid hydrazide exceeded that of any other known compound - whether synthetic or antibiotic. Almost simultaneously this important

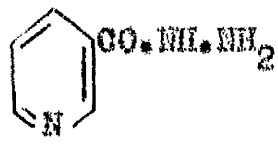
discovery was reported by other workers in the field[23,24].

The first clinical trials of this new and highly active tuberculostat were reported by Robitzek and Selikoff[25]. They selected for treatment patients in an advanced state of caseous-pneumonic tuberculosis, who were considered to be clinically hopeless. The dose administered was 4 mg. per kg. of body weight per day. A remarkable improvement was observed - there was a return of appetite, an increase in body weight, a decrease in cavity size and a general feeling of well-being. No serious toxic symptoms were observed, although twitching of the extremeties, vertigo, constipation, headache and myopia occurred in a few cases. It was not possible in these preliminary studies to observe emergence of resistant strains of tubercle bacilli, but the authors assuming the theory of adaptation made this prediction, which unfortunately has been substantiated by an overwhelming mass of clinical evidence over the past five years.

Immediately following the discovery of the high <u>in vivo</u> antituberculous activity of isoniazid, a considerable number of cognate compounds were prepared and examined in order to explore the structure-activity relationships of this clinically promising agent.

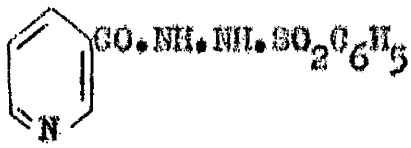
Fox and Gibas[26] examined the isomeric pyridine carboxylic acid hydrazides, nicotinic acid hydrazide (VIII) and picolinic acid hydrazide (IX) (Meyer[11]), and their benzenesulphonyl derivatives[27], (X) and (XI) respectively.

Compounds (VIII), (X) and (XI) were found to be inactive, and compound (IX), although possessing high <u>in vivo</u> activity, was very toxic.



(VIII)

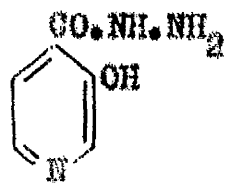
(IX)



(X)

(XI)

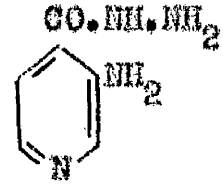
They therefore concluded that, as far as antituberculous activity among pyridine carboxylic acid hydrazides was concerned, the 4-position was the one of choice and that arylsulphonation of N^2 resulted in loss of activity. The possibility that antituberculous activity might reside in other carboxylic acid hydrazides led them to examine benzoic acid and p-nitrobenzoic acid hydrazides[28], but these were devoid of activity. Other aromatic and some aliphatic acid hydrazides were also examined by Bernstein, Lott, Steinberg and Yale[23] but again no activity was found. Fox and Gibas[26] decided to limit structural modifications to the addition of substituents to the pyridine nucleus and accordingly prepared 3-hydroxyisonicotinic acid hydrazide (XII), which, however, was found to be inactive.



(XII)

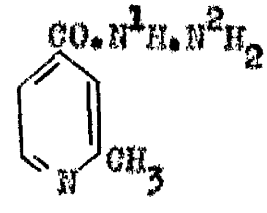
Further studies on the effect of substitution in the pyridine ring were carried out by Bernstein and his associates[29] and, with the exception of

3-aminoisonicotinic acid hydrazide (XIII), the compounds prepared were all devoid of activity.



(XIII)

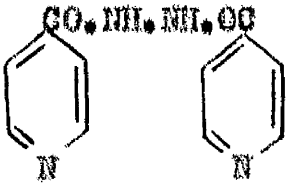
Later work by Isler and his colleagues[30,31,32] on the effect of nuclear substitution revealed that 2-methyl-isonicotinoylhydrazine (XIV) possessed approximately the same antituberculous activity as isoniazid. Lengthening of the side chain on the 2-position of the nucleus, or the introduction of an alkyl group into any other position of the ring, led to abolition of activity. Numerous derivatives of 2-methyl-isonicotinoylhydrazine were also synthesized by condensation with aldehydes, ketones, sugars and carboxylic acid chlorides and, although antituberculous activity was diminished, they were less toxic. Reduction of the Schiff's base link of the hydrazones gave N^2-alkyl derivatives in which activity was still retained.



(XIV)

Further modification of the pyridine ring of isoniazid by reduction to a piperidine ring was carried out by Fox[33] and Bernstein[29] but, again, this led to abolition of activity. Modification of the ring N function was also examined[29]; quaternization resulted in loss of activity, but formation of the N-oxide gave a compound which still retained a high degree

of activity although it was less than isoniazid itself. Although N^2-arylsulphonation of isoniazid resulted in abolition of activity, Fox and Gibas[26], continuing their investigations to determine the structural limitations of the isoniazid molecule, examined 1,2-di-isonicotinoylhydrazine[34] (XV) and found that it was highly tuberculostatic.



(XV)

It therefore appeared that modification of N^2 was not incompatible with antituberculous activity in every instance and, accordingly, 1-isonicotinoyl-2-D-glucosylhydrazine (XVI) and 1-isonicotinoyl-2-isopropylhydrazine (XVII) were synthesized and tested on mice infected with Myco. tuberculosis var. hominis (H37Rv)[25]. Both were relatively atoxic and exhibited a high degree of activity and consequently were submitted for clinical trial[35]. After brief clinical testing, however, the former was withdrawn due to its relative instability.

(XVI)

(XVII)

It now became apparent from the activity of these derivatives that

structural variation involving the terminal nitrogen of the hydrazide moiety could be accomplished without consequential loss of activity. As a result, a very large number of derivatives of isoniazid prepared by condensation with aldehydes, ketones, acid anhydrides and acid chlorides were examined[24,29,36,37]. Almost without exception they were found to be markedly tuberculostatic.

From all these structure-activity studies based on the isoniazid molecule, the following generalizations emerged:-

(1) with few exceptions, introduction of a substituent into the pyridine ring results in loss of *in vitro* and *in vivo* activity;

(2) reduction of the pyridine nucleus results in loss of *in vitro* and *in vivo* activity;

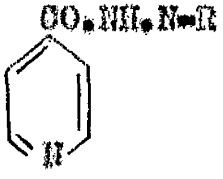
(3) N^2-alkylation or acylation gives rise to derivatives which have low *in vitro* but high *in vivo* activity;

(4) condensation of isoniazid with aldehydes and ketones, including sugars, yield hydrazones which show both *in vitro* and *in vivo* activity;

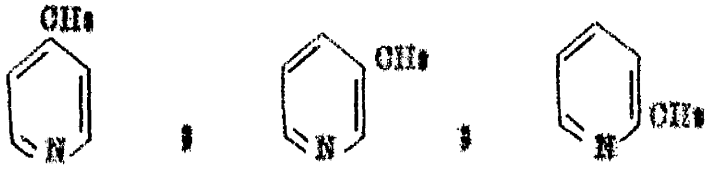
(5) N^2-arylsulphonation abolishes *in vitro* and *in vivo* activity.

An interesting series of nine hydrazones obtained by condensing the three isomeric pyridine aldehydes with the three isomeric pyridine carboxylic acid hydrazides was examined by Kakimoto and Yamamoto[30]. The three derivatives (XVIII) obtained from isoniazid showed *in vitro* activity, which they claimed was superior even to that of isoniazid.

A parallel investigation was carried out by Saikachi, Aramaki and Achi[39],



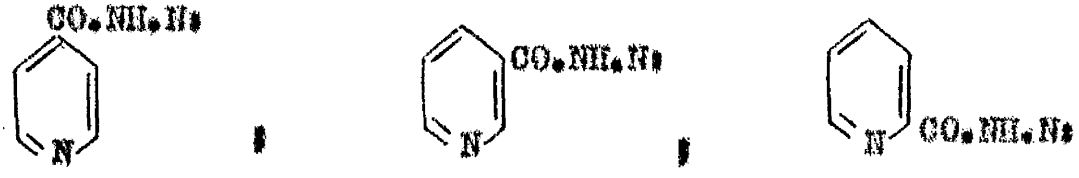
where R =



(XVIII)

who prepared the 2-furfurylidene-(5-nitro) derivatives (XIXa), (XIXb) and (XIXc) of the isomeric pyridine carboxylic acid hydrazides. Again the derivative with isoniazid (XIXa) was found to possess a high degree of activity.

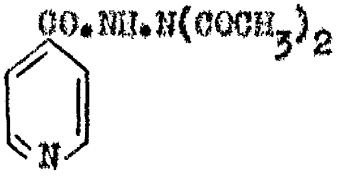
R =



(a) (b) (c)

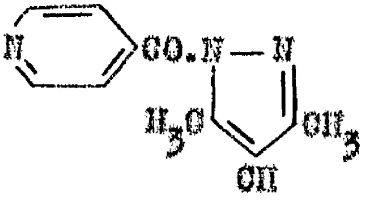
(XIX)

Observing the wide structural variation throughout which antituberculous activity is maintained, Fox and Gibas[40] extended their investigation to dialkyl substituted derivatives of isoniazid. Two types were possible, either N^2-dialkyl or N^1N^2-dialkyl derivatives. It seemed reasonable to expect the former to be active, since most of the alkylidene derivatives[36] and also 1-isonicotinoyl-2-diacetylhydrazine (XX), in which both hydrogen atoms on N^2 are replaced, exhibited activity[41,42]. As anticipated, the N^2-



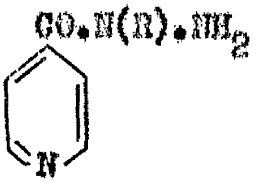
(XX)

dialkyl derivatives were active although their activity was much less than that of the parent substance. It was by no means certain what influence would be exerted by a substituent on N^1 and, in fact, N^1N^2-dialkyl derivatives proved to be either inactive or to possess greatly diminished activity. Loss in activity was ascribed to the presence of a substituent on N^1. One of the derivatives prepared and found inactive during this investigation was 3,5-dimethyl-1-isonicotinoyl pyrazole (XXI), in which all three hydrazine hydrogen atoms have been replaced. $N^1N^2N^2$-trialkyl derivatives were next synthesized[43] and, again, as was anticipated, they exhibited no activity.



(XXI)

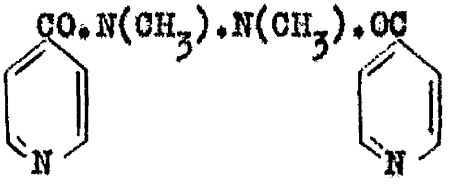
To determine the effect of the presence of a substituent on N^1 alone the three derivatives (XXII), in which R is a methyl, benzyl or isopropyl group, were also prepared.



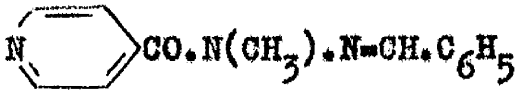
(XXII)

Two of these derivatives were devoid of activity, but surprisingly the third, 1-isonicotinoyl-1-isopropylhydrazine, (R = isopropyl), exhibited high *in vivo* activity.

A similar investigation carried out by Cymerman-Craig and Willis[44] showed that the derivatives (XXIII) and (XXIV), in which all three hydrazino hydrogens are replaced, were also inactive.

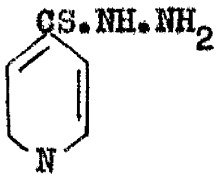


(XXIII)



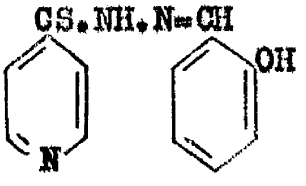
(XXIV)

Further modification of the acid hydrazide moiety was carried out by Konig, Siefkin and Offe[45], by replacing the oxygen function by sulphur to give substances which incorporated some of the structural features of isoniazid and the thiosemicarbazones. Among the compounds examined were thioisonicotinic acid hydrazide (XXV) and its benzylidene and salicylidene derivatives (XXVI) and (XXVII), respectively.



(XXV)

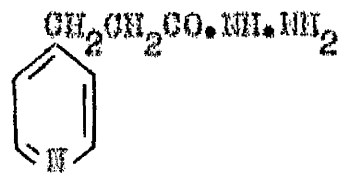
(XXVI)



(XXVII)

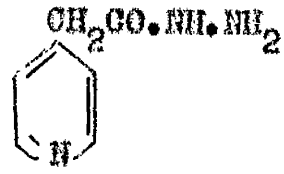
Their tuberculostatic activity *in vitro* was about half that of isoniazid.

The effect of separating the acid hydrazide group from the pyridine nucleus has been studied by Katritzky[46], using β-4-pyridyl-propionic acid hydrazide (XXVIII), β-4-pyridylacrylic acid hydrazide (XXIX) and 4-pyridylacetic acid hydrazide (XXX). No activity was found in these compounds and it appeared that, for tuberculostatic activity, the acid hydrazide group should be directly attached to the pyridine ring. The dihydrochlorides of compounds (XXVIII) and (XXX) have also been examined by Gardner, Smith, Wenis and Lee[47], who found the former inactive but the latter exhibited some activity in mouse tuberculosis.



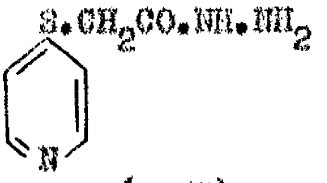
(XXVIII)

(XXIX)

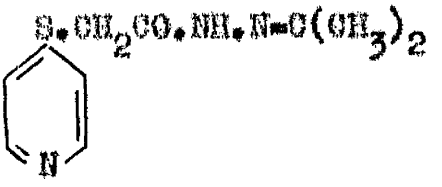


(XXX)

Separation of the acid hydrazide group from the pyridine nucleus by a thiomethylene group was investigated by Takahashi, Shibasaki and Uchibayashi[48], who prepared (4-pyridylthio)-acetic acid hydrazide (XXXI) and its isopropylidene derivative (XXXII).



(XXXI)



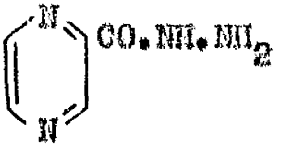
(XXXII)

No activity was shown by the former and the activity of the latter compound was not recorded.

The success of isoniazid and its derivatives as tuberculostatic agents stimulated the search to discover new tuberculostats among other heterocyclic carboxylic acid hydrazides. Yale[49] prepared and examined several different types and found that activity is not confined to the isonicotinic acid series. The most active compounds examined were

(XXXIII)

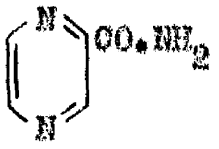
(XXXIV)



(XXXV)

2-furoic acid hydrazide (XXXIII) and thiophene-2-carboxylic acid hydrazide (XXXIV). The acid hydrazides of pyrrole, thiazole, pyrimidine and pyrazine showed only slight *in vivo* activity and were not considered

sufficiently promising to warrant further investigation. Although

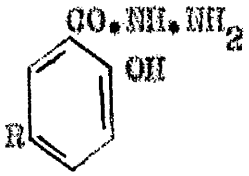


(XXXVI)

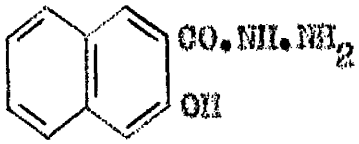
pyrazinoic acid hydrazide (XXXV) showed little or no activity, the amide (pyrazinamide)[50] (XXXVI) is highly tuberculostatic. Like isoniazid, its development as a tuberculostat originated from the discovery of the antituberculous activity of nicotinamide[12], of which it is the pyrazine analogue. Pyrazinamide is used extensively in America, although a recent survey by Jones and his associates[51] draws attention to serious hepato-toxic effects resulting from prolonged therapy with this drug.

Cinchoninic acid hydrazides and some of their hydrazones have also been examined[24,52] for tuberculostatic properties, but none compared favourably with isoniazid when tested *in vitro*.

The simple aliphatic and aromatic carboxylic acid hydrazides examined by Bernstein[29] and Fox[26] were inactive. Buu-Hoi and his co-workers[53] investigating non-pyridinoid acid hydrazides discovered that the 5-substituted salicylic acid hydrazides (XXXVII, R = Cl, Br) and 2-hydroxy-3-naphthoic acid hydrazide (XXXVIII) exhibited activity. To decrease toxicity the NH_2 of the hydrazide group was blocked by hydrazone formation. A very large number of these derivatives was prepared and examined *in vitro* and although many showed activity none was as active as isoniazid.

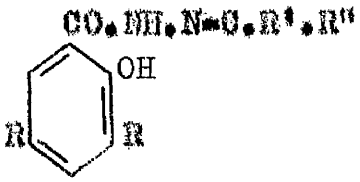


(XXXVII)

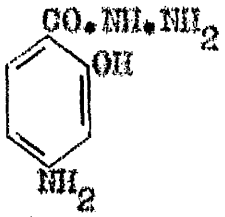


(XXXVIII)

In vitro tuberculostatic activity approaching that of isoniazid



(XXXIX)



(XL)

is claimed by Klosa[54] for the hydrazones (XXXIX, R = H, or Br) prepared by condensing salicylic acid and 3,5-dibromosalicylic acid hydrazides with aldehydes and ketones. As a result of in vitro tests Misaki[55] has found that p-aminosalicylic acid hydrazide (XL) is superior to p-aminosalicylic acid but inferior to isoniazid.

An early investigation carried out by Shavel and his co-workers[56] included some aliphatic acid hydrazides, among which was cyanacetic acid hydrazide (XLI). According to the results of in vitro testing the authors concluded that this substance was inactive. A subsequent examination by Hartl[57] showed that this compound was highly active in vivo, and in preliminary clinical trials Scheu[58] claimed that its activity was of the same order as isoniazid.

$$NC.CH_2CO.NH.NH_2$$

(XLI)

Again, hydrazone formation by condensation with aldehydes and ketones[59]

resulted in compounds which were active but did not exceed in activity either isoniazid or cyanacetic acid hydrazide. The early promise shown by cyanacetic acid hydrazide has not, however, been sustained and, according to Bernon, Aulenier and Tricoire[60], this drug is two to three times more toxic than isoniazid, and although quite well tolerated its effect when administered alone is transient. The authors suggest that its chief value lies in delaying emergence of resistant strains of tubercle bacilli in patients undergoing treatment with isoniazid, streptomycin and p-aminosalicylic acid. The acetamido derivative (XLII) of cyanacetic acid hydrazide has also been reported by Shavel[56] to be inactive.

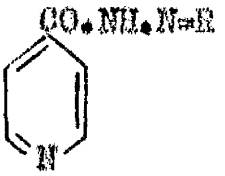
$$CH_3CO.NH.CH(CN)CO.NH.NH_2$$

(XLII)

Whilst isoniazid is the most potent of the acid hydrazides yet discovered, several of its hydrazones have been re-examined in this country, in Australia and in Russia, with a view to discovering the tuberculostat of choice in this series.

Rubbo, Edgar and Vaughan[61] in Australia have compared *in vivo* activities and toxicities of isoniazid and seven of its hydrazones, the results of which are shown in Table I. They concluded that the acute toxicity of isoniazid was appreciably reduced by hydrazone formation and, since the level of activity is of the same order, these compounds possess a more favourable chemotherapeutic index. The veratrylidene derivative (XLIIIa) was potentially the most promising clinical agent and the

TABLE I



(XLIII)

(XLIII)	R	Highest dilution inhibiting growth of H37Rv in serum synthetic medium*	Toxicity compared with isoniazid.
a	Veratrylidene	4096000	0.30
b	Vanillylidene	2048000	> 0.01
c	o-Vanillylidene	1024000	0.20
d	Salicylidene	1024000	0.35
e	3,4-Dihydroxy-benzylidene	1024000	0.03
f	Piperonylidene	2048000	0.22
g	Benzylidene	2048000	0.25
h	p-nitrobenzylidene	1024000	-
Isoniazid (for comparison)		2048000	1

* Reciprocal of the molar concentration.

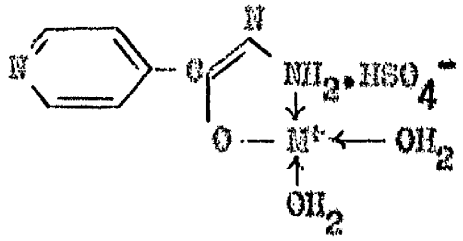
authors extended their investigation to some aspects of the properties and pharmacology of this derivative.

Below pH 2.4 the drug was hydrolysed rapidly to isoniazid and veratraldehyde, although when administered as compressed tablets to human beings 60 per cent. of the drug remained unchanged. The optimal

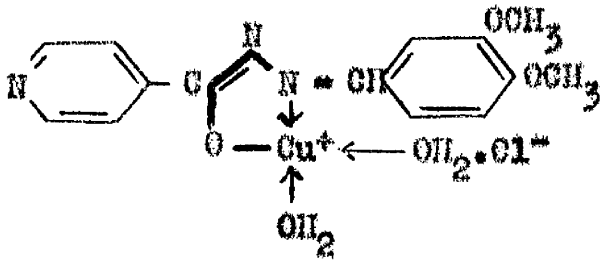
daily dose in man was 20 mg. per kg. of body weight, administered twice daily in 10 mg. doses. The serum concentration on this regimen varied between ten and forty times the minimal concentration necessary to inhibit Myco. tuberculosis. A prolonged acute toxicity trial in humans showed that the dose recommended was safe, and no acute or toxic symptoms were displayed by the patients. The authors regard this compound as being potentially superior to isoniazid and are continuing their clinical examination of the drug.

An earlier report in this country by Bavin, James, Ray, Lazare and Seymour[62] on the antituberculous evaluation of the salicylidene derivative (XLIIId) is in substantial accord with the results expressed by Rubbo and his co-workers, and similar results were obtained in Russia where the vanillylidene derivative (XLIIIb) has shown promise in preliminary clinical trials[63].

Rubbo[61] also investigated some metal chelates (XLIV) of isoniazid with iron, cobalt and zinc sulphates, as well as the copper chelate (XLV) of its veratrylidene derivative. All showed marked antituberculous activity in vitro and in vivo but, due to their high level of local and acute toxicity, they were not considered as potential tuberculostats. Peterson, Bayer, Offe and Domagk[64], however, have also prepared metal complexes of isoniazid with ferrous sulphate, cobalt chloride, zinc chloride and manganese chloride and have claimed that these complexes are tuberculostatic and non-toxic.



(XLIV)



(XLV)

An extension of the work of Fox[16] and Bernstein[29] on sulphonhydrazides has been reported by Domagk[24], who prepared the p-toluenesulphonyl derivative of isoniazid (XLVI) and by Dornow and Wedekind[65], who prepared the p-acetylaminobenzenesulphonyl derivatives of isoniazid (XLVII), nicotinic acid hydrazide and picolinic acid hydrazide.

(XLVI)

(XLVII)

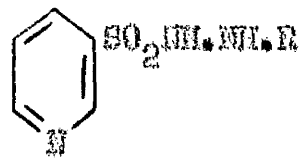
Again, none of these heteroaromatic sulphonhydrazides exhibited activity.

Several aromatic sulphonhydrazides have been subjected to *in vitro* and *in vivo* examination[23,56], but apart from 2-benzenesulphonyl-1-phenylhydrazine (XLVIII), which exhibited slight activity, no significant inhibition was observed.

$$C_6H_5SO_2NH.NH.C_6H_5$$

(XLVIII)

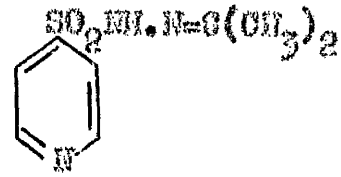
Continuing the search for new antituberculous structures, Domnov[65] synthesized pyridine-3-sulphonhydrazide (XLIX, R = H) and 1,2-di-(pyridine-3'-sulphonyl)-hydrazine (XLIX, R = 3-SO_2-C_5H_4N). Both



(XLIX)

compounds were inactive *in vitro* - a result later independently confirmed by Anderson[66] and Valik and Plasek[67] in the case of the former compound (XLIX, R = H). The latter workers also prepared and examined

(L)



(LI)

pyridine-2-sulphonhydrazide (L) and 1-isopropylidene-2-pyridine-4'-sulphonylhydrazine (LI); compound (LI) was found to be inactive but compound (L) showed some *in vitro* activity.

THE PROBLEM OF DRUG RESISTANCE

Perhaps in no other disease is the development of drug-resistance so vividly illustrated as in tuberculosis. Even with streptomycin, p-aminosalicylic acid and isoniazid, as well as other less effective chemotherapeutic agents, initial success is often followed by failure associated with the emergence of drug-resistant strains of tubercle bacilli. But whilst this undoubtedly presents a serious problem in the treatment of the disease it is not insoluble, and the emergence of drug-resistance and the existence of cross-resistance may yield useful indications of the mode of action of the various substances.

In clinical practice, the problem of drug resistance is satisfactorily overcome by combined drug therapy provided cross-resistance does not occur. Thus Rubbo and his associates[61] found that tubercle bacilli resistant to 1-isonicotinoyl-2-veratrylidenehydrazine (XLIIIa) were also resistant to isoniazid. With these two chemotherapeutic agents complete cross-resistance occurs and their mechanism of action must, therefore, be similar. Cuthbert and Bruce[60] have also found that isoniazid plus p-aminosalicylic acid, or isoniazid-streptomycin, is superior to combined therapy with isoniazid and its salicylidene derivative (XLIIId), since mutual prevention of the development of cross-resistance does not occur with the latter combination.

The soundness of combined therapy has recently been demonstrated experimentally by Middlebrook[69], who has shown that, whereas the rate of production of mutants resistant to high concentrations of isoniazid is 1 in 10^5 and for streptomycin-resistant mutants 1 in 10^6, the

frequency of double mutants is approximately the product of these two.

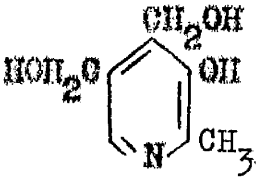
Intermittent multiple-drug regimens of streptomycin-p-amino-salicylic acid, streptomycin-isoniazid and p-aminosalicylic acid-isoniazid are recognized to have a high degree of effectiveness in the treatment of tuberculosis, and, according to Muschenheim[70], the drug of prime importance is isoniazid. A British Medical Research Council investigation[7] has concluded that a combination of p-aminosalicylic acid and isoniazid is very effective, both clinically and bacteriologically. The duration of treatment is important and should be continued over a period of at least one year, and preferably for eighteen months or longer[72]. Joiner, MacLean, Marsh and Carroll[73], using all three pairs in rotation each for two periods of one month, found that short-term therapy led to a high incidence of relapse on cessation of treatment.

According to Weiser, triple-drug therapy, in which all three drugs are administered either simultaneously or in rotation, offers no advantage over dual-drug therapy[74]. There is overwhelming evidence that continuous anti-microbial therapy should be maintained and, if toxic symptoms develop during treatment, medication should not be stopped but alternative chemotherapeutic agents substituted[72].

The salt formed between isoniazid and p-aminosalicylic acid[75] has been investigated by Clegg[76], who found it to be as effective as isoniazid and relatively less toxic. In preliminary clinical trials, no case of complete drug-resistance developed, although in some patients partial resistance was observed. The author considers that this salt merits further clinical investigation.

MODE OF ACTION OF ISONIAZID

Recently, several interesting observations have been made which might ultimately lead to a complete understanding of the mode of action of isoniazid. Popo[77] observed that inhibition of endogenous respiration produced in the tubercle bacillus by isoniazid can be completely neutralised by sodium pyruvate, α-ketoglutarate and especially by pyridoxine (LII). It is suggested that isoniazid, due to its structural similarity to pyridoxine, may act by a type of competitive antagonism to this essential metabolite.



(LII)

Barclay, Koch-Weser and Ebert[78] have reported that the tubercle bacillus can fix ^{14}C-labelled isoniazid and they put forward the theory that the mechanism of action of the drug may well be governed by a mechanism resembling simple physical adsorption. A decrease in the pyridine nucleotide concentration in the blood of tuberculous experimental animals, which returned to normal on administration of isoniazid, has led Patiala[79] to conclude that isoniazid acts by inhibiting enzyme reactions in which pyridine nucleotides are involved. Goldman[80] isolated the isoniazid nucleotide analogue of diphosphopyridine nucleotide (DPN), in which the nicotinamide moiety of the latter has been replaced by isoniazid. DPN is normally reduced by addition of a proton to the 4-position of the pyridine ring, but since this position is substituted in its isoniazid

analogue (D-INAH-N) he concludes that activity of isoniazid may be due to intracellular formation of an inactive analogue with a concomitant reduction in cellular oxidative metabolism.

Knox[91] suggests that high concentrations of isoniazid act by blocking the formation of a specific peroxidase (almost certainly not catalase) essential for the growth of the tubercle bacillus. Mutants deficient in the gene(s) responsible for the synthesis of this peroxidase cannot grow in a medium in which peroxides are formed, but can grow in such a medium provided the peroxides present can be destroyed, e.g. by haemin or catalase. They could also grow in a medium in which peroxides are not formed. Experimental evidence supporting this theory has been forthcoming from Albert, Knox and Reen[92] and Cohn, Oda, Kovits and Middlebrook[93], who found that haemin destroys isoniazid when both are shaken together aerobically at pH 7. Di-isonicotinoylhydrazine and isonicotinic acid are produced by the reaction, the former being an intermediate stage in the formation of the latter. The effect of peroxides has been studied by Darry and his co-workers[94], who found that agents promoting the growth of isoniazid-resistant tubercle bacilli are also capable of reducing the concentration of hydrogen peroxide. Tirunerayanan and Visscher[95] have shown that peroxidase is essential for the activity of isoniazid and tentatively suggest that isoniazid in presence of peroxidase forms dihydroxyisonicotinic acid, which then breaks down to products such as hydrogen peroxide and formaldehyde which are toxic to the tubercle bacillus.

A direct quantitative relationship between antituberculous activity

and ability to form metal chelates with the cupric ion has been advanced by Corl and Marquardt[86] for p-aminosalicylic acid, isoniazid and certain benzalthiosemicarbazones. Erlenmeyer, Sorkin and Roth[87] found that the *in vitro* activity of isoniazid is increased about tenfold in presence of cupric ions, and Cymerman-Craig and his co-workers[88] and others[40] have shown that N^1-alkyl derivatives of isoniazid, which are incapable of forming copper complexes, are devoid of activity. The only recorded example which does not fit the theory is 1-(isonicotinoyl)-1-isopropylhydrazine (XXII, R=CH$(CH_3)_2$), which shows high *in vivo* activity yet does not chelate with the cupric ion[40].

Maher and his colleagues[89] have attempted to co-ordinate the experimental observations of isoniazid resistance and mode of action, and suggest that isoniazid probably forms a metal chelate which then competes with hydrogen peroxide for possession of sites on the catalase molecule. This would result in an accumulation of hydrogen peroxide which would be fatal to the tubercle bacillus.

Kruger-Thiemar[90] has advanced the theory that isoniazid permeates the bacterial cell membrane, so that its concentration inside and outside is the same. In presence of peroxidase, isoniazid is converted to isonicotinic acid, which exists as an anion above pH 6 and cannot diffuse out of the bacterial cell, where it therefore accumulates and disturbs the activity of diphosphopyridine nucleotide. This results in the tubercle bacillus being unable to oxidise substrate hydrogen to water. A compensatory enhanced oxidation of substrate hydrogen takes place whereby more hydrogen peroxide than usual is produced and this is

the real cause of inhibition, presumably by enhancement of catabolic processes.

Holmes and Rubbo have recently rejected[91] the peroxide accumulation hypothesis of isoniazid action on the grounds that, if inhibition of growth of Myco. tuberculosis were due to intracellular accumulation of hydrogen peroxide, then the inhibitory action of isoniazid should be antagonized by a substance which (i) could penetrate the cell wall, (ii) could not affect the growth of the organism, (iii) did not react with isoniazid, and (iv) could reduce hydrogen peroxide. Sodium nitrite fulfilled all these requirements, but the authors were unable to detect any diminution of tuberculostatic activity of isoniazid in presence of this substance.

Whilst the chemotherapy of tuberculosis has produced striking advances in the past decade much still remains to be done - many problems remain to be solved. This much, however, is certain - as new antituberculous structures are discovered, as new aspects of the organism's nutrition are unfolded and as new light is shed on the biophysics and biochemistry of drug action - the answer will be found.

DISCUSSION

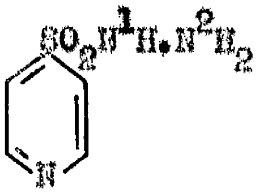
INTRODUCTION:

It was apparent that, although isoniazid and a very large number of its derivatives were potent tuberculostatic agents[16,23,24,29,36,37,61], a high degree of specificity nevertheless existed, in so far as structural modification combined with retention of activity was almost invariably confined to the acid hydrazide moiety. The fact that this group must be directly attached to the pyridine ring[46,49] and, for optimum activity and minimum toxicity to position 4 of that ring, suggested that the investigation of the sulphonic acid analogues of isoniazid and its derivatives would constitute a not unpromising, even if somewhat speculative, approach to the chemotherapy of tuberculosis.

It had been demonstrated that pyridine-3-sulphonhydrazide was devoid of activity[65,66,67], and in this respect it resembled its carboxylic acid analogue, nicotinic acid hydrazide[26]. Search of the literature at the onset of this investigation failed to reveal any reference to pyridine-2-sulphonhydrazide or pyridine-4-sulphonhydrazide but, after its completion, attention was drawn to work carried out by Talik and Plazek[92], who reported activity for pyridine-2-sulphonhydrazide and, while unable to isolate pyridine-4-sulphonhydrazide, they were successful in preparing its isopropylidene derivative (LI), which, however, was found to be inactive. Again, it is of interest to note the activity of pyridine-2-sulphonhydrazide, the analogue of picolinic acid hydrazide, which is active but very toxic[26].

Although N^2-arylsulphonation of isoniazid gave rise to derivatives devoid of activity[16,24,29], it was decided that investigation of analogou

substances, in which the sulphonyl group was attached to the 4 position of the pyridine ring and not to an aromatic ring, might be productive of antituberculous properties. Accordingly, the synthesis of pyridine-4-sulphonhydrazide (LIII) and its derivatives with aldehydes, ketones, sugars, acid anhydrides and acid chlorides, as well as its N^2-alkyl derivatives, was undertaken in order to test this hypothesis.



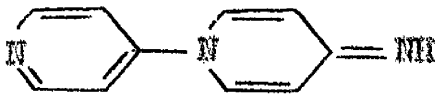
(LIII)

OXIDATION OF 4-THIOPYRIDONE WITH NITRIC ACID AND WITH HYDROGEN PEROXIDE:

4-Thiopyridone (LIV) appeared to be the logical starting point from which to embark on these syntheses, and several routes to the parent compound, pyridine-4-sulphonhydrazide, using for the most part conventional synthetic methods, seemed feasible. These are shown schematically on page 33.

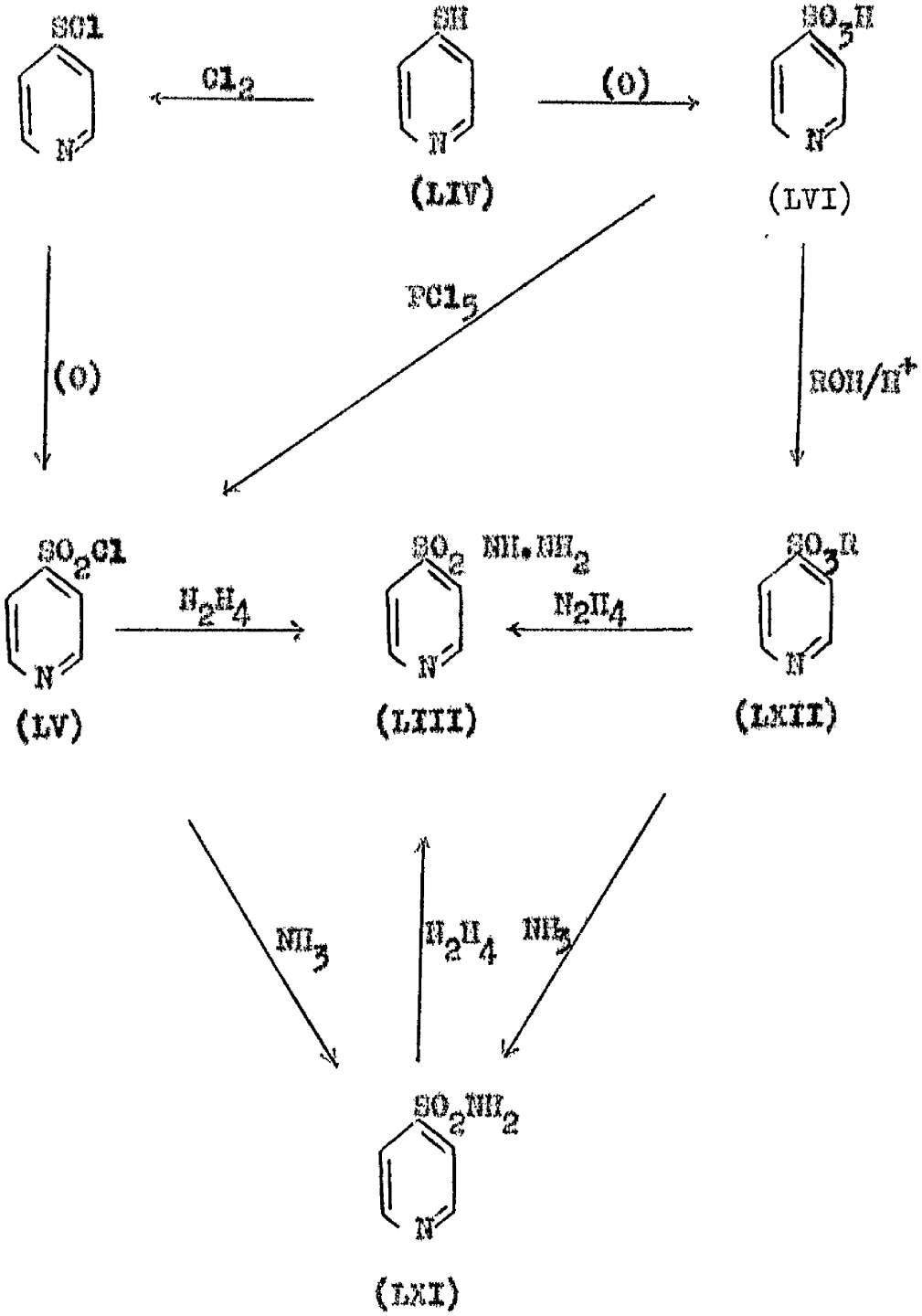
Reference to the literature disclosed an unsuccessful attempt by King and Ware[93] to synthesise pyridine-4-sulphonyl chloride (LV) *via* the sodium salt of pyridine-4-sulphonic acid (LVI), using phosphorus pentachloride. During the reaction, thionyl chloride was produced, which suggested that the SO_2 group was at some stage being detached from the nucleus. Instead of obtaining the expected pyridine-4-sulphonyl chloride they obtained either 1-(4'-pyridyl)-pyridine-4-imine (LVII) or 1-(4'-pyridyl)-4-pyridone (LVIII), depending upon the method of

isolating the product.



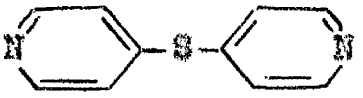
(LVII)

(LVIII)



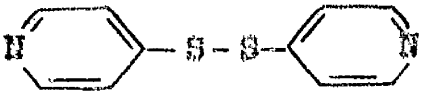
(LXI)

Further attempts to prepare the sulphonyl chloride by chlorinolysis of 4-thiopyridone in acetic acid at room temperature were unsuccessful and, on removing the solvent below 50° and treating the residue with concentrated ammonia, they obtained di-4-pyridyl sulphide (LIX) and also 4-chloropyridine, which again pointed to C - S cleavage. The



(LIX)

action of bromine on 4-thiopyridone in acetic acid, under similar conditions, gave di-4-pyridyl disulphide (LX) instead of the required sulphonyl bromide.



(LX)

A method, however, was found in the patent literature[94] whereby pyridine-4-sulphonamide (LXI) was obtained by the action of dry ammonia gas on ethyl pyridine-4-sulphonate (LXII, R=C_2H_5) and it was decided to prepare these two intermediates and investigate their reaction with hydrazine.

Curtius and his pupil Lorenzen[95] studying methods for the preparation of hydrazides of aromatic sulphonic acids have reported that esters of the latter undergo hydrolysis rather than hydrazinolysis with hydrazine hydrate, according to the following reaction:-

$$C_6H_5SO_3{\cdot}C_2H_5 + H_2N{\cdot}NH_2{\cdot}H_2O \longrightarrow C_6H_5SO_2{\cdot}NH{\cdot}NH_2 + C_2H_5OH + H_2O$$

$$\longrightarrow C_6H_5SO_3H{\cdot}NH_2NH_2 + C_2H_5OH$$

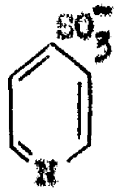
By using anhydrous hydrazine it seemed reasonable to expect the formation of pyridine-4-sulphonhydrazide from ethyl pyridine-4-sulphonate. Again, although Lorenzen[95] had reported no reaction between amides of aromatic sulphonic acids and hydrazine hydrate, it was considered that the interaction of pyridine-4-sulphonamide and anhydrous hydrazine merited investigation as a possible route ~~route~~ to the desired compound.

4-Thiopyridone was prepared by the action of phosphorus penta-sulphide on 4-pyridone, as described by King and Ware[93]. These authors used a temperature between 60 and 70°, but Japanese workers[40] found that it required a temperature of 150°. This reaction was carried out several times during the course of the present work and it was found that the reaction temperature and the yield of 4-thiopyridone depended to a large extent on the quality of the phosphorus pentasulphide used. Almost white samples of phosphorus pentasulphide reacted at about 70° and gave approximately the literature yield, whereas dark grey samples required a considerably higher temperature (ca. 120°) and gave decreased yields of product.

Pyridine-4-sulphonic acid was prepared by nitric acid oxidation of 4-thiopyridone, as described by Koenigs and Kinne[96], the product obtained having the same decomposition melting point of 135° as described by these authors, who identified their product by analysis and by the preparation of its barium and silver salts. It was, therefore, assumed

that it was authentic material. Several attempts to esterify the sulphonic acid by passing dry HCl into a refluxing methanolic or ethanolic solution[94] were unsuccessful, starting material being recovered each time. Potentiometric titration with sodium hydroxide gave an equivalent of 166 (theoretical for pyridine-4-sulphonic acid 159), the curve having one very small inflexion succeeded by a steep inflexion, typical for a strong-acid, strong-base neutralization. In the course of a check on the authenticity of the sulphonic acid it was found that efforts to prepare the barium and silver salts were inconclusive and an alternative method for the preparation of the sulphonic acid was therefore adopted.

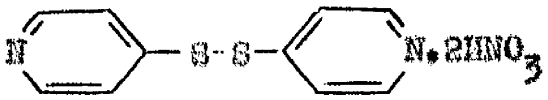
Sodium pyridine-4-sulphonate was obtained by King and Ware[93] by oxidation of 4-thiopyridone with hydrogen peroxide in presence of sodium hydroxide. It was, therefore, decided to carry out the oxidation using hydrogen peroxide in glacial acetic acid. In contrast to the product obtained by nitric acid oxidation, a substance melting with decomposition at 333° was isolated. The same product was obtained from the eluate, when an aqueous solution of sodium pyridine-4-sulphonate was passed through a cation exchange resin column. Potentiometric titration with sodium hydroxide gave an equivalent of 160 (theoretical 159), the curve being indicative of a strong-acid, strong-base neutralization. The ultra-violet absorption spectrum gave the characteristic absorption of the pyridine ring and the product was characterized by analysis and conversion to its ammonium salt[93]. Its high melting point and its failure to form a picrate accord with the expected zwitterionic structure of pyridine-4-sulphonic acid (LXIII).



(LXIII)

The material melting at 135°, obtained by nitric acid oxidation of 4-thiopyridone, was therefore subjected to further examination.

Careful fractional crystallization from water and aqueous alcohol gave pyridine-4-sulphonic acid identical in all respects with an authentic sample and also a larger portion of a more soluble substance, melting at 127°. This substance was readily soluble in water, the solution being strongly acidic, and it titrated as a strong acid (equivalent 174). Sodium nitrate was isolated from the neutralized solution and the nitrate radical identified qualitatively in the original acid by a brown-ring test and quantitatively by the determination of the ammonia produced on reduction with Deverda's alloy in a Conway cell, using the micro-diffusion technique[97]. The substance was identified as di-4-pyridyl disulphide di-nitrate (LXIV) by elementary analysis and by the isolation of the parent base, di-4-pyridyl disulphide (LX), identical with authentic material.



(LXIV)

The identity of the base was confirmed by formation of a picrate having a melting point and crystal form identical with di-4-pyridyl disulphide

dipicrate[93], preparation of the platinichloride[96] and oxidation with hydrogen peroxide in acetic acid to pyridine-4-sulphonic acid. The melting point of the di-4-pyridyl disulphide has been variously reported as 155°[96] and 74°[93]. The material isolated from the dinitrate melted at 75°. The disulphide, as prepared by Koenigs and Kinne[96], also melted at 74° and with dilute nitric acid it gave di-4-pyridyl disulphide dinitrate, identical with the product obtained by nitric acid oxidation of 4-thiopyridone. Attempts to esterify the new authentic pyridine-4-sulphonic acid using ethanolic and methanolic hydrogen chloride, however, were still unsuccessful and it was decided to attempt other methods of esterification.

ATTEMPTED ESTERIFICATION OF PYRIDINE-4-SULPHONIC ACID.

The method of Stacey[98] using ethanol and trifluoracetic anhydride as catalyst failed to effect esterification and so also did the method of Wegscheider and Furcht[99] using dimethyl sulphate, at 100°. Treatment of pyridine-4-sulphonic acid with ethereal diazomethane in presence of a few drops of water to catalyse the reaction[100] gave a neutral substance which had a melting point of 330° (decomp.).

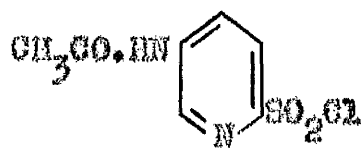
(LXV)

Elementary analysis, its solubility in water and its insolubility in organic solvents showed it to be N-methyl-4-sulphopyridine betaine

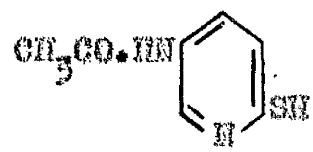
(LXV). This compound was later reported by Larivé, Collet and Bomillauber[101], who obtained it by heating together sodium pyridine-4-sulphonate and dimethyl sulphate at 150°. They, however, quote melting points of 340° and 345° for their product.

THE ACTION OF CHLORINE ON 4-THIOPYRIDONE AND ATTEMPTED ISOLATION OF PYRIDINE-4-SULPHONYL CHLORIDE.

Inability to procure the desired ester compelled the adoption of



(LXVI)



(LXVII)

a new line of approach. Like King and Ware[93], Caldwell and Kornfeld[102] had experienced difficulty in preparing 5-acetamidopyridine-2-sulphonyl chloride (LXVI) from the corresponding sulphonic acid. Chlorosulphonic acid, thionyl chloride, sulphuryl chloride, sulphur chloride and benzotrichloride all failed to yield the desired product. In each case, only unchanged sulphonic acid or dark non-crystalline masses, having none of the properties of a sulphonyl chloride, were obtained. The required compound was, however, obtained in good yield by passing a slow stream of chlorine gas into a cold hydrochloric acid solution of 5-acetamido-pyridine-2-thiol (LXVII).

This method was applied to 4-thiopyridone, chlorine being passed into the hydrochloric acid solution till the temperature showed no further

tendency to rise and excess chlorine was present. No product precipitated, however, and the reaction mixture was allowed to evaporate to dryness at laboratory temperature in vacuo. Treatment of the residue with ammonia and extraction with ether gave a white solid (melting point 74°), which did not depress when mixed with a genuine sample of di-4-pyridyl disulphide. Since the disulphide is known to be formed as an intermediate stage in the preparation of sulphonyl chlorides by this method[103,104], it appeared that the reaction time of ten minutes given was insufficient. The reaction was repeated and chlorine passed in for about two hours followed by evaporation at laboratory temperature, as before, and gave a small yield of pyridine-4-sulphonic acid (m.p. 333°). It was, therefore, apparent that pyridine-4-sulphonyl chloride was being formed but at some stage in its isolation was undergoing hydrolysis to the sulphonic acid. This considered with the observations of King and Ware[93] led to the conclusion that pyridine-4-sulphonyl chloride was not only thermolabile, decomposing to sulphur dioxide and 4-chloropyridine, but also sensitive to hydrolysis by aqueous hydrochloric acid at room temperature. It was, therefore, deemed essential to keep the temperature low and at the same time to remove the water and hydrochloric acid as rapidly as possible. The method of Caldwell and Kornfeld[102] was accordingly modified by carrying out the chlorinolysis at about -10° and attempting to freeze-dry the reaction mixture. This proved to be difficult for, although the reaction mixture readily solidified initially, as the volume decreased during the freeze-drying process it became semi-solid and further evaporation then proceeded only very slowly.

To speed up evaporation under high vacuum the temperature was raised

to 0°, but again the white solid residue obtained proved to be pyridine-4-sulphonic acid. Since hydrolytic decomposition appeared to predominate over desulphonation at 0° it seemed necessary to remove water from the product as rapidly as possible and chlorinolysis was attempted in dilute hydrochloric acid to achieve this end, but the sulphonic acid was still obtained. Isolation of pyridine-4-sulphonyl chloride by low temperature evaporation was, therefore, considered to be impracticable.

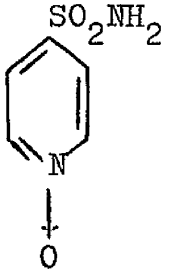
Precipitation by change of solvent was next attempted. Chlorinolysis was carried out at about 15° in glacial acetic acid[104] but instead of removing the solvent at 50°, as had been done by King and Ware[93], the reaction mixture was poured into acetone, dioxan or ether. No product separated out; removal of solvent followed by basifying the residue and extracting with ether gave di-4-pyridyl sulphide (LIX).

In the preparation of isonicotinoyl chloride hydrochloride, Bornow and Wedekind[69] extracted the product from the reaction mixture using ether. Accordingly, it was decided to attempt extraction of pyridine-4-sulphonyl chloride from hydrochloric acid solution using chloroform or ether. Again, no product could be isolated. This method was repeated neutralising the reaction mixture with triethylamine prior to chloroform extraction, but the only identifiable product obtained was triethylamine hydrochloride.

SYNTHESIS OF PYRIDINE-4-SULPHONAMIDE:

Failure to isolate pyridine-4-sulphonyl chloride led to the temporary abandonment of this line of approach to the problem and

attention was focussed on the preparation of pyridine-4-sulphonamide. This was readily achieved by pouring the cold chlorinolysis mixture into an excess of well-cooled concentrated ammonia, which gave the required sulphonamide, m.p. 172-3°, in good yield (58%). Talik and Plazek[105] also prepared this compound by low temperature chlorinolysis of 4-thiopyridone in hydrochloric acid containing 30% hydrogen peroxide, which apparently they found essential. Their product had a melting point of 168-9° and their yield was approximately 20%. They, also, were unable to isolate the sulphonyl chloride. The sulphonamide was characterized by elementary analysis, formation of a picrate, m.p. 186-7° (Talik and Plazek[105] give 178°) and also by its conversion to pyridine-4-sulphonamide-N-oxide (LXVIII) with hydrogen peroxide. The N-oxide has been described in the literature by Ochiai and Naito[106], who prepared it by chlorinolysis of 4-thiopyridone-N-oxide followed by ammonolysis of the N-oxide sulphonyl chloride.



(LXVIII)

ATTEMPTS TO SYNTHESIZE PYRIDINE-4-SULPHONHYDRAZIDE:

At this juncture, two routes to pyridine-4-sulphonhydrazide seemed to be open (i) by direct hydrazinolysis of pyridine-4-sulphonyl chloride, as prepared in solution in hydrochloric acid, and (ii) by hydrazinolysis of pyridine-4-sulphonamide. Both methods were, therefore, investigated

concurrently. The sulphonyl chloride in hydrochloric acid was poured into an excess of well-cooled 50% hydrazine hydrate and left overnight at 0°. No product separated and the mixture was taken down to dryness under reduced pressure. Much frothing occurred as solid material separated out and the only identifiable product obtained was hydrazine hydrochloride. The experiment was repeated but heating was discontinued immediately solid material started to separate. Fractional crystallization of the solid from alcohol to remove hydrazine hydrochloride gave a small amount of 4-pyridylhydrazine hydrochloride. The filtrate was taken down to dryness, during which much frothing again occurred and fractional crystallization from alcohol gave hydrazine hydrochloride, hydrazine sulphate and a small amount of 4-pyridylhydrazine sulphate. Two other crystalline solids, which were not identified, were also isolated. The formation of 4-pyridylhydrazine, in which the pyridine ring is not directly linked to sulphur, and the appearance of sulphur in the form of the sulphate ion again pointed to desulphonation of pyridine-4-sulphonyl chloride. It was not inconceivable, however, that the required pyridine-4-sulphonhydrazide was being formed, but under the alkaline conditions, desulphonation to 4-pyridylhydrazine was taking place. A comparable rearrangement and desulphonation has been reported by Naito and Ochmori[106,107] for nitrobenzene-o- and -p-sulphonamide and pyridine-2- and -4-sulphonamide 1-oxide, under alkaline conditions.

Neutralization of the excess hydrochloric acid with triethylamine followed by treatment with a slight excess of hydrazine was equally unsuccessful.

At this stage, the difficulties were primarily the separation of a relatively small amount of product from a large amount of hydrazine hydrochloride, complicated possibly by the instability of the compound itself under the experimental conditions of isolation. Attention was turned to an alternative approach to the synthesis of the required hydrazide.

THE ACTION OF HYDRAZINE ON PYRIDINE-4-SULPHONAMIDE:

The next step was the investigation of the reaction between pyridine-4-sulphonamide and hydrazine. Although by no means certain[95], it was expected that the equilibrium which is set up between carboxylic acid amides and hydrazine to give acid hydrazides would also obtain with pyridine-4-sulphonamide and, in this case, give the required product, pyridine-4-sulphonhydrazide.

Pyridine-4-sulphonamide was heated on a water bath with either anhydrous hydrazine or hydrazine hydrate and, after evolution of gas had ceased, excess hydrazine was removed *in vacuo*. The residue crystallized from a large volume of methanol or aqueous ethanol in colourless needles (m.p. 161°) which were soluble in water and insoluble in other organic solvents. On treating the product with hydrochloric acid sulphur dioxide was evolved, sulphur was slowly deposited and from the solution 4-pyridylhydrazine hydrochloride was isolated, identified by its melting point by reduction of ammoniacal silver nitrate in the cold and by formation of a red colour with sodium hydroxide[108]. A structural formula could not be unequivocally assigned to the product for, whilst its analysis and some of its chemical properties indicated a

thiosulphate of 4-pyridylhydrazine and ammonia, no ammonia could be detected on treatment with sodium hydroxide. This product also formed a picrate but, again, the analysis did not permit assignment of a structural formula. The mother liquors from which the reaction product was crystallised were concentrated and gave a small yield of a higher melting product. This gave a satisfactory analysis for 4-pyridylhydrazinium pyridine-4'-sulphonate (LXIX) and, when passed through an anion exchange resin column and the eluate acidified with hydrochloric acid, gave 4-pyridylhydrazine hydrochloride.

(LXIX)

At this stage of the work, a critical appraisal of the methods used and the results obtained led to the following conclusions:-

(1) the difficulty of esterifying pyridine-4-sulphonic acid and the ease with which the N-alkyl-sulphobetaine is formed precluded success in obtaining ethyl pyridine-4-sulphonate;

(2) the reaction between pyridine-4-sulphonamide and hydrazine resulted in cleavage of the C - S link and hence did not warrant further investigation as far as the present work was concerned;

(3) isolation of the sulphonhydrazide from large amounts of other materials, such as hydrazine hydrochloride or triethylamine hydrochloride, was impracticable,

(4) the formation of 4-pyridylhydrazine in certain of the reactions, coupled with the evidence of comparable decomposition of analogous compounds[106,107] under similar conditions indicated

the possibility that the required product itself was possibly unstable.

ISOLATION OF PYRIDINE-4-SULPHONYL CHLORIDE AND THE SYNTHESIS OF PYRIDINE-4-SULPHONHYDRAZIDE.

In the light of these conclusions, the most promising approach for further investigation was considered to be some modification of the extraction techniques already examined which would permit isolation of sulphonyl chloride. If the excess hydrochloric acid could be neutralized without bringing about decomposition of the sulphonyl chloride and if the salt formed during the neutralization were insoluble in the extracting solvent, then it was possible that pyridine-4-sulphonyl chloride might be isolated. Neutralisation with sodium bicarbonate was, therefore, carried out keeping the temperature below 0°. During this process almost colourless, oily globules were observed which dissolved on shaking with chloroform. Removal of the chloroform at about 40° under reduced pressure left a reddish-brown, semi-solid material with an acrid smell, due in part at least to sulphur dioxide. No crystalline product could be isolated on treatment with concentrated ammonia. Using calcium carbonate instead of sodium bicarbonate to facilitate control of the neutralization and at the same time minimise any tendency for the temperature to rise during this process, a cold chloroform solution was obtained which was used to demonstrate the presence of pyridine-4-sulphonyl chloride. The solution when treated with dry ammonia gas gave a white solid which proved to be a mixture of ammonium chloride and pyridine-4-sulphonamide, from which the latter was readily separated by washing

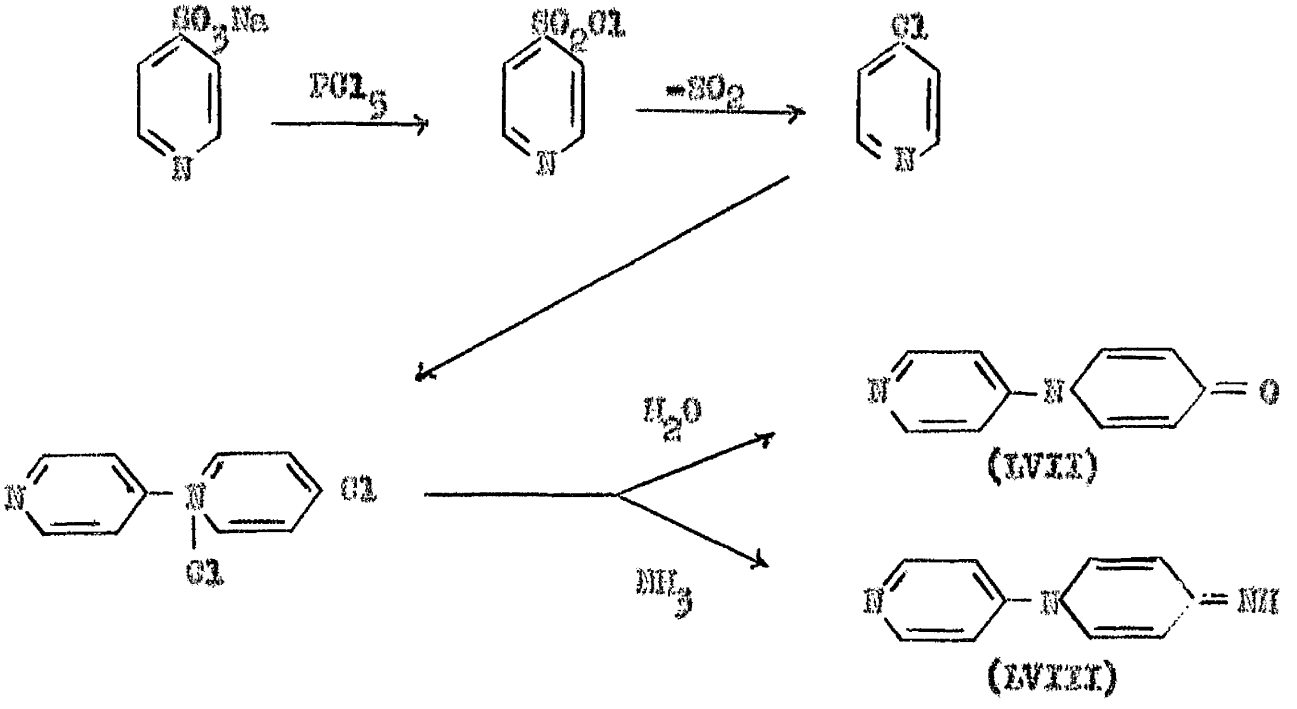
with cold water and crystallizing from water or ethanol to give a product which was identical with authentic material (melting point, mixed melting point and picrate).

The method was forthwith applied to the synthesis of pyridine-4-sulphonhydrazide. The cold chloroform solution containing the pyridine-4-sulphonyl chloride was poured into anhydrous hydrazine (2 moles). Heat was evolved and a white crystalline solid separated from the solution which proved to be a mixture of the required compound and hydrazine hydrochloride. Attempted separation by crystallization of the crude mixture from either methanol or ethanol led to extensive decomposition and the only crystalline product isolated was the by-product, hydrazine hydrochloride. The crude reaction mixture was quite soluble in cold water but, on cooling in ice, a colourless crystalline solid (m.p. 95°), which proved to be pyridine-4-sulphonhydrazide, separated. The weight of crude mixture obtained directly from the reaction indicated an overall yield in the region of 90%, but the recovery of pyridine-4-sulphonhydrazide by the above method of purification was less than 20%. A somewhat less pure product (m.p. 92°) could, however, be obtained in about 60% yield by carefully suspending the product in, and washing with, ice-cold water. This was sufficiently pure for most of the subsequent syntheses undertaken. The pure product reduced ammoniacal silver nitrate in the cold and was sparingly soluble in water, methanol and ethanol, from which it could be recrystallized by cautiously warming to effect solution. It is insoluble in ether, chloroform and benzene and is rapidly decomposed by water, even at 0°. The solution turns yellow and there is a slow evolution of gas.

When stored in air it is gradually transformed into a yellow semi-solid material smelling like pyridine. Formation of a mono-sodic derivative and salts with picric and hydrochloric acids showed its amphoteric character.

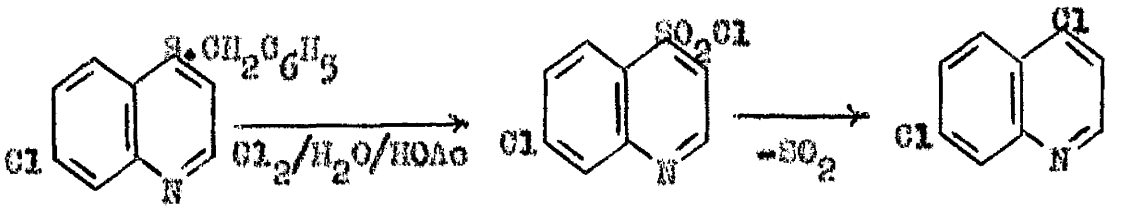
DECOMPOSITION OF PYRIDINE-4-SULPHONYLCHLORIDE:

The chloroform solution remaining after removal of crude sulphonhydroxide yielded 4-chloropyridine identified by conversion to its picrate[93] and also to 4-pyridylhydrazine hydrochloride[108]. The formation of compounds (LVII) and (LVIII) by the action of phosphorus pentachloride on sodium pyridine-4-sulphonate[95] probably proceeds:-

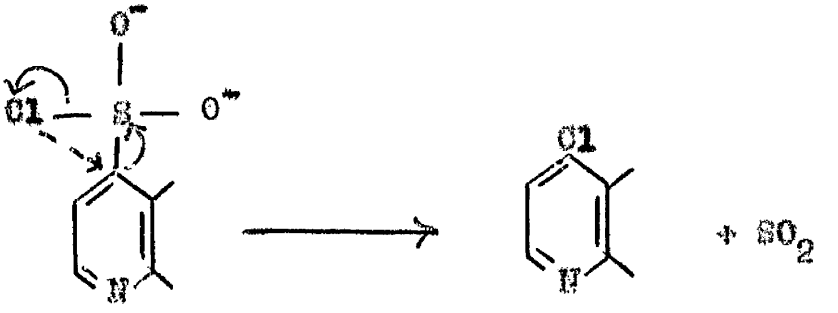


Substantially the same explanation has been advanced by Vibaut and Brockman[93a].

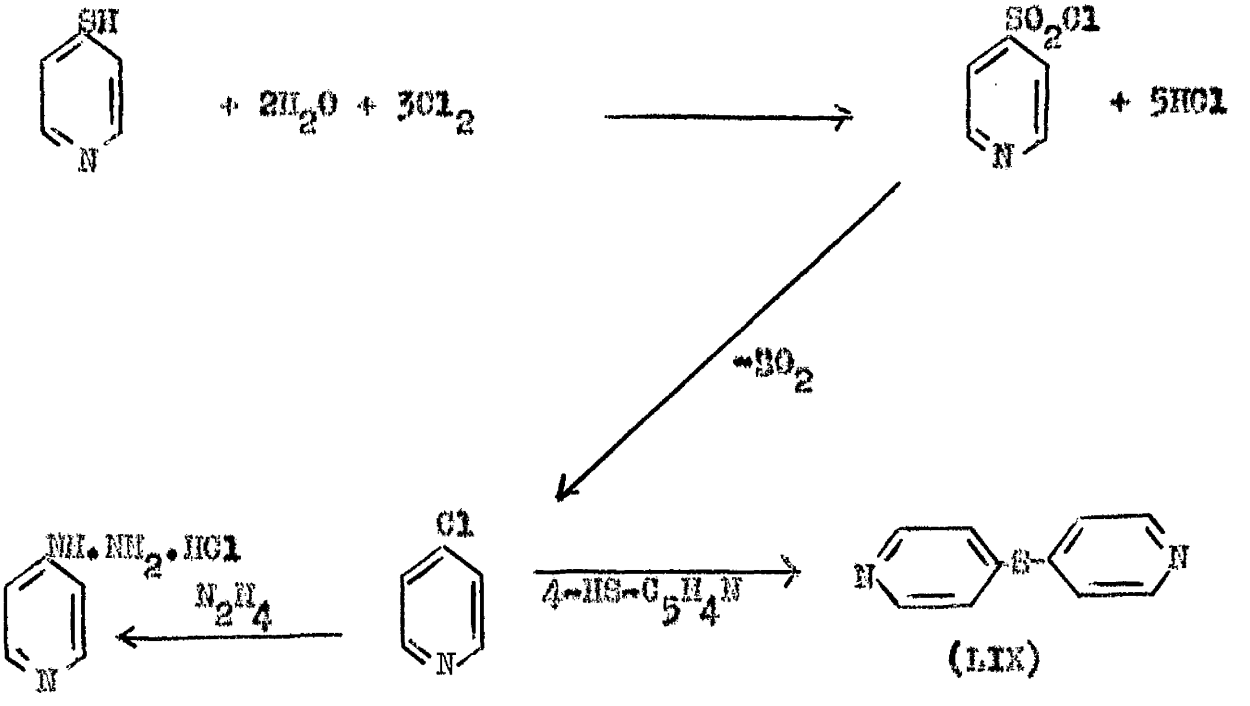
An analogous desulphonation has recently been reported by Kwart and Miller[93b]:-



The cleavage of the C — S bond is depicted:-

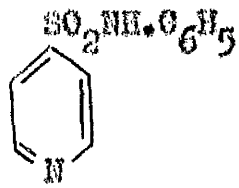


The isolation of di-4-pyridyl sulphide by chlorinolysis of 4-thiopyridone[93] and also the formation of 4-pyridylhydrazine hydrochlori in certain of the earlier attempts to obtain pyridine-4-sulphonhydrazide may also take place by a similar process:-



PYRIDINE-4-SULPHONANILIDE:

Pyridine-4-sulphonyl chloride also condensed with aniline to give the sulphonanilide (LXX). It was necessary to remove all traces of chlorine from the chloroform solution, otherwise a very dark product was obtained which was difficult to purify.

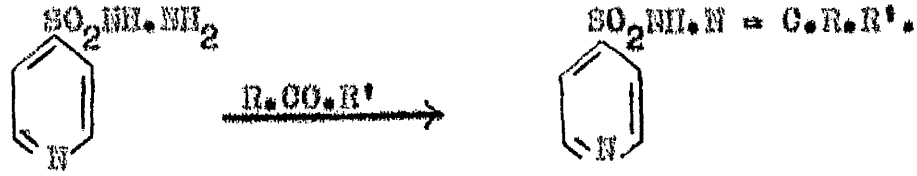


(LXX)

Contrary to the condensation with ammonia, hydrazine and phenyl-hydrazine (p. 57) the anilide remained in solution and was isolated as an oil by filtering off the aniline hydrochloride and evaporating the chloroform. Recrystallisation from aqueous methanol gave the product as a white crystalline solid.

THE REACTION BETWEEN PYRIDINE-4-SULPHONHYDRAZIDE AND ALDEHYDES AND KETONES:

Pyridine-4-sulphonhydrazide reacted readily under the mildest conditions with aliphatic and aromatic aldehydes and ketones, as well with glucose and heterocyclic carbonyl compounds. The reaction can be represented:-



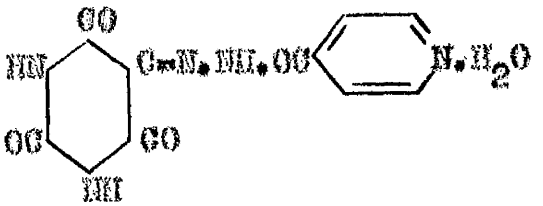
(LXXI)

The general method used involved suspension of the pyridine-4-sulphonhydrazide in methanol (heating to effect solution was not necessary and was not used since it produced decomposition) and adding one molecular proportion of the appropriate carbonyl compound dissolved in methanol. On shaking the reaction mixture, the sulphonhydrazide dissolved and, on leaving at laboratory temperature or, in the case of the more soluble derivatives, at 0° the product eventually precipitated. Analytically pure samples were obtained by recrystallization from the appropriate solvent to constant decomposition point and removing adherent solvent in vacuo. The yields varied from good to excellent. The reaction with acetaldehyde at laboratory temperature was extremely vigorous and gave only semi-solid resinous material. The ethylidene derivative (LXXI, R = H, R' = CH_3) was obtained if the acetaldehyde was added slowly and the reaction mixture cooled externally with ice.

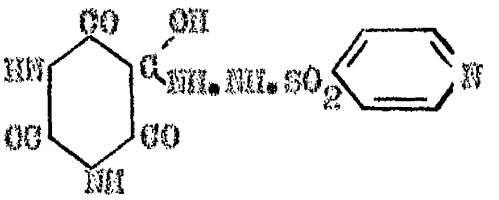
Contrary to the observations of Talik and Plazek[67] the isopropylidene derivative (LXXI, R = R' = CH_3) was found to be stable under normal storage conditions. They also were unable to obtain a picrate. It was found that the picrate readily separated in orange prisms by mixing methanolic solutions of picric acid and 1-isopropylidene-2-pyridine-4'-sulphonylhydrazine. Recrystallization from methanol yielded a sample which gave a satisfactory analysis.

Certain points of similarity are to be observed between the alkylidene and aralkylidene derivatives of pyridine-4-sulphonhydrazide and isoniazid. For example, Fox and Gibas[36] noticed that, in several of the derivatives they prepared, it was often difficult to free the product of

the odour of the parent carbonyl compound, which they ascribed to scission of the Schiff's base linkage, even in the solid state. Some of the derivatives of pyridine-4-sulphonhydrazide, notably the cinnamylidene (LXXI, R = H, R' = $C_6H_5CH{=}CH$) and vanillylidene derivative (LXXI, R = H, R' = 3,4-$(CH_3O)(OH)(C_6H_3)$ also bore a faint odour of the parent aldehyde on storage. The latter compound was not particularly stable and a yellow colour developed after several months. Both isoniazid and pyridine-4-sulphonhydrazide form derivatives with alloxan which have very high melting points and are extremely insoluble in water and most organic solvents. They do dissolve, however, in mineral acid and strong alkali. The almost quantitative yields obtained are probably due to their insolubility. Fox and Gibas[36] have formulated the isoniazid derivative of alloxan as structure (LXXII). From the physical evidence for the structure of alloxan[109] the pyridine-4-sulphonhydrazide derivative is probably best represented by structure (LXXIII). Similarly the chloral derivative of isoniazid is formulated by Vale[110] as the hydrazide (LXXIV) rather than the hydrazone monohydrate (LXXV).



(LXXII)

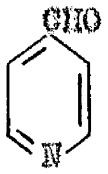


(LXXIII)

(LXXIV)

(LXXV)

Of the many aldehydes and ketones which were condensed with pyridine-4-sulphonhydrazide only two failed to give a pure product. Although condensation occurred readily and in good yield when pure pyridine 4-sulphonhydrazide (m.p. 95°) and redistilled furfural reacted together, the product failed to give a satisfactory analysis. This was probably due to entrainment of solvent by the long fine needles in which the derivative crystallized. Attempted removal of solvent under vacuum even below 100° resulted in discolouration occurring. In this case, however, it was possible to characterize the product by the formation of a picrate. Attempted condensation of isonicotinaldehyde (LXXVI) with pyridine-4-sulphonhydrazide resulted, however, in extensive decomposition with the vigorous evolution of nitrogen even at 0°, and no identifiable product could be recovered.

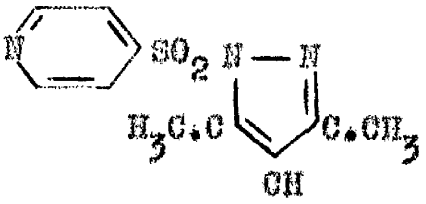


(LXXVI)

Condensation of pyridine-4-sulphonhydrazide with acetylacetone gave 2:4-di(pyridine-4'-sulphonamidoimino) pentane (LXXVII) instead of the

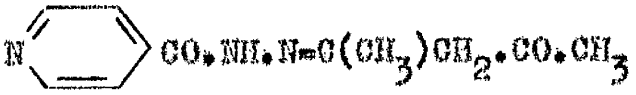
expected 3,5-dimethyl-1-(pyridine-4'-sulphonyl)-pyrazole (LXXVIII).

(LXXVII)

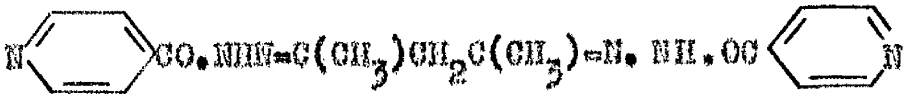


(LXXVIII)

The corresponding reaction between isoniazid and acetylacetone has been reported by Fox and Gibas[40] to give 3,5-dimethyl-1-isonicotinoyl pyrazole (XXI). Yale and his co-workers[110] report that this reaction takes place in two stages with the formation of the mono- and dihydrazones (LXXIX) and (LXXX) respectively.



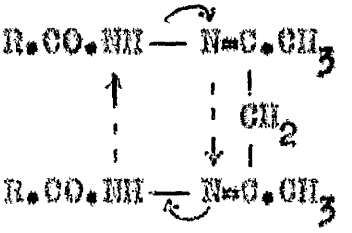
(LXXIX)



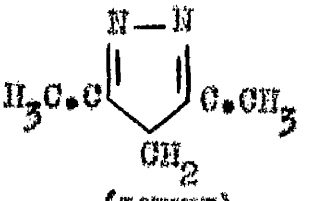
(LXXX)

Cymmerman-Craig and Willis[44], on the other hand, found that the chief product obtained was 1,2-di-isonicotinoyl hydrazine (XV), together with smaller amounts of 3,5-dimethylpyrazole (LXXXI). They suggested

that these two compounds may be formed from compound (LXXX) by the changes illustrated below.

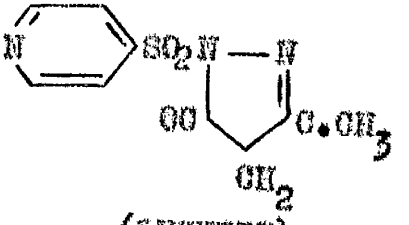


According to Wenner[111] isoniazid gave a non-crystallizable hydrazone (LXXXII) with ethyl acetoacetate, but reaction of the latter with pyridine-4-sulphonhydrazide gave the crystalline derivative 3-methyl-1-(pyridine-4'-sulphonyl)-5-pyrazolone (LXXXIII).



(LXXXI)

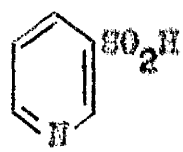
(LXXXII)



(LXXXIII)

The analogue of compound (XVI) was obtained when pyridine-4-sulphonhydrazide was condensed with D-glucose.

The reaction between pyridine-4-sulphonhydrazide and carbonyl compounds is thus seen to run in the main parallel to isoniazid. Some difference, however, is observed with the isomeric pyridine-3-sulphonhydrazide (XLVIII) in that, while pyridine-2-sulphonhydrazide (L) gives the isopropylidene derivative[67] (LI) with acetone, pyridine-3-sulphonhydrazide gives pyridine-3-sulphinic acid[112] instead (LXXXIV).



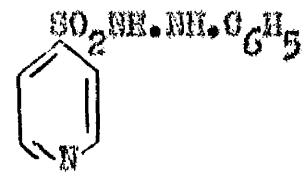
(LXXXIV)

Copper chelation occurs readily with isoniazid and several of its alkylidene derivatives[61,97,98]. On attempting preparation of a copper complex with pyridine-4-sulphonhydrazide in a similar manner[97], extensive decomposition occurred with copious evolution of nitrogen, and the small amount of insoluble coloured product which was obtained could not be purified for the purpose of identification.

ATTEMPTS TO SYNTHESIZE ALKYL DERIVATIVES OF PYRIDINE-4-SULPHONHYDRAZIDE.

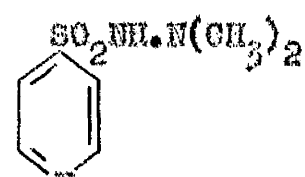
Although hydrazone formation occurred readily with pyridine-4-sulphonhydrazide, the preparation of monoalkyl derivatives proved more difficult. In the isoniazid series, the Schiff's base linkage of the alkylidene derivatives was readily reduced by catalytic hydrogenation under mild conditions[33]. Several attempts at catalytic reduction of the Schiff's base linkage of the corresponding pyridine-4-sulphonhydrazides by varying the solvent, the pressure and the temperature met with no success, the starting material being recovered in each case. Failure

to effect reduction was also experienced using sodium borohydride in methanol, according to the method of Billman and Diesing[113]. The only derivative of this type prepared was 1-phenyl-2-pyridine-4'-sulphonylhydrazine (LXXXV) by condensation of pyridine-4-sulphonyl chloride and



(LXXXV)

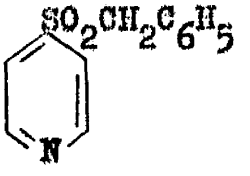
phenylhydrazine in cold chloroform solution. As in the case of pyridine-4-sulphonhydrazide, the yield of crude mixture of phenylhydrazine hydrochloride and product indicated an overall yield in the region of 68%, but attempted recrystallisation from water or alcohol resulted in decomposition of the product and only phenylhydrazine hydrochloride could be isolated. Applying the method of isolation used for pyridine-4-sulphonhydrazide gave a yield of approximately 12% of crude product which was further purified by cautiously warming with ethanol to effect solution and allowing the solution to evaporate spontaneously at laboratory temperature to induce crystallisation. Attempts to condense pyridine-4-sulphonhydrazide with unsymmetrical dimethylhydrazine failed to give 1:1-dimethyl-2-pyridine-4'-sulphonylhydrazine (LXXXVI).



(LXXXVI)

Preparation of alkyl derivatives was also attempted using the

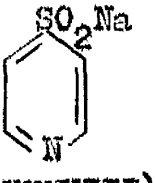
method of Libermann, Grumbach and Rist[114]. Pyridine-4-sulphonhydrazide was cautiously dissolved in warm methanol and addition of a warm solution of sodium ethoxide gave almost instantaneously a white solid which was characterized by analysis as the mono-sodio derivative of pyridine-4-sulphonhydrazide. Efforts to condense the sodio-derivative with alkyl bromides and chlorides gave products which were difficult to free from sodium halide. Benzyl chloride also failed to yield the expected alkyl derivative, but gave instead the corresponding 4-pyridyl sulphone (LXXXVII) which was characterized by elementary analysis and the formation of a picrate.



(LXXXVII)

THE DECOMPOSITION OF PYRIDINE-4-SULPHONHYDRAZIDE:

The condensation of a sulphinate and alkyl halides to give sulphones is well known[112,115,116,117,118] and the intermediate formation of sodium pyridine-4-sulphinate (LXXXVIII) suggested itself as a possible



(LXXXVIII)

explanation of the formation of benzyl 4-pyridyl sulphone (LXXXVII). This was further strengthened by several references in the literature to the decomposition of sulphonhydrazides, according to the following

reaction, by Raschig[119] (X = H), Escales[120] (X = C_6H_5) and McFadyen and Stevens[22] (X = ArCO):-

$$X.NH.NH.SO_2C_6H_5 \longrightarrow XH+N_2+C_6H_5SO_2H$$

More recently Carpino[121] has reported decomposition of 1,1-dibenzyl-2 benzenesulphonhydrazide (LXXXIX) to dibenzyl, nitrogen and benzenesulphinic acid. The same author attempting the synthesis of compound (LXXXIX) by the condensation of benzyl chloride and benzenesulphonhydrazid in presence of triethylamine obtained instead benzyl phenyl sulphone (XC).

$C_6H_5SO_2NH.N(CH_2C_6H_5)_2$ (LXXXIX)

$C_6H_5CH_2SO_2C_6H_5$ (XC)

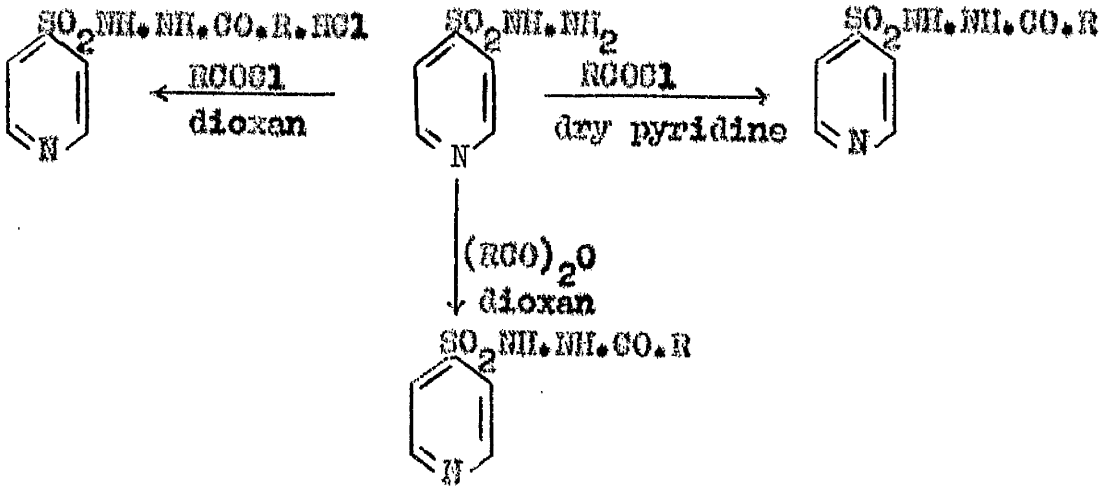
In the light of this and other evidence[122,123,124], it was decided to try to demonstrate that decomposition of pyridine-4-sulphonhydrazide did, in fact, give pyridine-4-sulphinic acid. Pyridine-4-sulphonhydrazid was refluxed for a short time in methanol and the yellow solid separating on the addition of ether was recrystallized from aqueous acetone. The product was acidic, did not form a picrate or reduce cold ammoniacal silver nitrate. Elementary analysis and oxidation with hydrogen peroxide to pyridine-4-sulphonic acid proved its identity to be pyridine-4-sulphinic acid. The same product was obtained by refluxing pyridine-4-sulphonhydrazide with sodium ethoxide and passing an aqueous solution of the product, obtained on precipitation with ether, through a cation exchange resin column.

Condensation of pyridine-4-sulphonhydrazide with alkyl halides

to give a 4-pyridyl sulphone would appear, therefore to proceed through the sulphinic acid. This opens up a new route to alkyl 4-pyridyl sulphones, which might prove interesting from the point of view of the chemotherapy of tuberculosis[7].

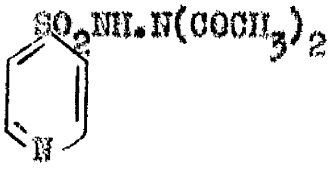
SYNTHESIS OF ACYL DERIVATIVES OF PYRIDINE-4-SULPHONHYDRAZIDE:

Pyridine-4-sulphonhydrazide reacted smoothly with acid chlorides and acid anhydrides, giving well-defined crystalline derivatives in good yield, according to the following scheme:-



Two derivatives were obtained with acetic anhydride, depending on the experimental conditions. With one molecular proportion of the anhydride and using methanol as solvent the monoacetyl derivative (XCI) was obtained, and in presence of excess acetic anhydride without solvent the diacetyl derivative (XCII) was obtained.

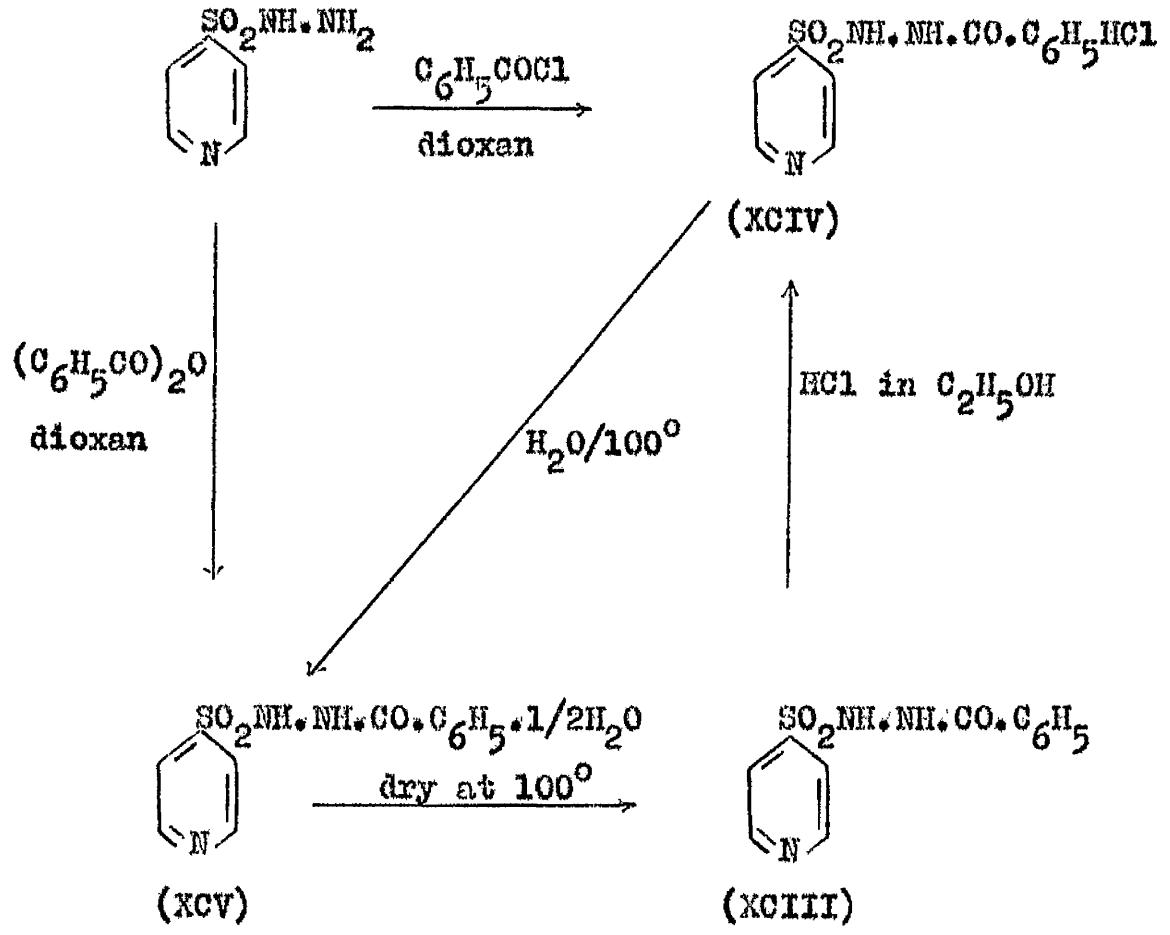
(XCI)



(XCII)

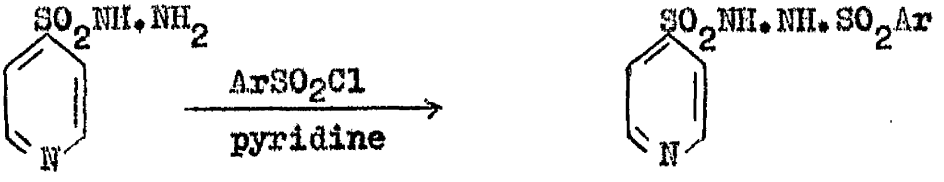
On attempting the condensation of pyridine-4-sulphonhydrazide and benzoyl chloride in dry pyridine as solvent heat was evolved on mixing, but no product separated on standing at 0° for several days. Removal of the solvent *in vacuo* resulted in the evolution of gas and the product turning green. No crystalline derivative could be isolated from the residue. On repeating the experiment and diluting with water without prior removal of solvent a white solid was obtained, which proved to be the benzoyl derivative of pyridine-4-sulphonhydrazide (XCIII). Using dry dioxan as solvent, the benzoyl derivative was obtained as the hydrochloride (XCIV) (m.p. 195° decomp.) on recrystallisation from 90% ethanol. Recrystallisation of the hydrochloride from water in which it was sparingly soluble gave a halogen-free compound (XCV) (m.p. 129° decomp.), which proved to be the hemi-hydrate of the benzoyl derivative; this was also obtained from benzoic anhydride and pyridine-4-sulphonhydrazide using dioxan as solvent. In this case, isolation was achieved by precipitation with ether and crystallising from a mixture of ethanol and ether. The anhydrous base (XCIII) (m.p. 164° decomp.) was obtained by drying the hemi-hydrate at 100°. The hydrochloride was regenerated from the anhydrous base by treatment with ethanolic hydrochloric acid.

The above sequence of reactions is shown schematically on page 62.



ARYLSULPHONYL DERIVATIVES OF PYRIDINE-4-SULPHONHYDRAZIDE:

Pyridine-4-sulphonhydrazide reacted with sulphonyl chlorides to give well-defined crystalline solids in moderate yield:



The sulphonyl chloride, if solid, was dissolved in dry pyridine

(if liquid, no solvent was used) and added to a suspension of pyridine-4-sulphonhydrazide in dry pyridine whilst cooling externally in ice. The pyridine-4-sulphonhydrazide dissolved, the mixture turned orange and bubbles of nitrogen were slowly liberated. The product was isolated by the addition of water and purified by recrystallisation from ethanol. If ethanol was substituted for pyridine as solvent, the sulphonyl chloride reacted preferentially with the alcohol and the liberated hydrochloric acid gave pyridine-4-sulphonhydrazide monohydrochloride. The latter was identified by analysis, equivalent weight and the formation of a picrate identical with pyridine-4-sulphonhydrazide picrate. The same compound was isolated on the addition of pyridine-4-sulphonhydrazide in methanol to a chloroform solution of pyridine-4-sulphonyl chloride in an effort to obtain 1,2-di(pyridine-4'-sulphonyl)hydrazine (XCVI). In another attempt to prepare compound (XCVI) the order of addition of anhydrous

(XCVI)

hydrazine and pyridine-4-sulphonyl chloride was the reverse of that used for the preparation of pyridine-4-sulphonhydrazide. The product obtained again proved to be pyridine-4-sulphonhydrazide (melting point, picrate and salicylidene derivative) and the yield was approximately 60%. Compound (XCVI) was eventually obtained by adding a chloroform solution of pyridine-4-sulphonyl chloride to a suspension of pyridine-4-sulphonhydrazide in dry pyridine. A dark-blue oil was obtained as a

by-product, which was not characterized.

1:2-Di(pyridine-3'-sulphonyl)hydrazine (XLIX) was similarly prepared[65] by addition of pyridine-3-sulphonyl chloride to pyridine-3-sulphonhydrazide (XLVIII). Ethanol was found to be a suitable solvent but, as in the preparation of arylsulphonyl derivatives of pyridine-4-sulphonhydrazide (p. 62), nitrogen was evolved during the reaction despite the precautions taken. The authors do not record any attempt to prepare compound (XLIX) directly from pyridine-3-sulphonyl chloride and hydrazine. The reaction between benzenesulphonyl chloride and hydrazine gives either benzenesulphonhydrazide or 1,2-di(benzenesulphonyl)hydrazine (XCVII), depending upon the order of the addition[95]. In the case of nitrobenzenesulphonyl

$$C_6H_5SO_2NH.NH.SO_2C_6H_5$$

(XCVII)

chlorides, on the other hand, Dann and Davies[122] found that the 1:2-disulphonyl compound could not be obtained directly from the nitrobenzenesulphonyl chloride and hydrazine. This anomalous behaviour they ascribed to the influence of the nitro-group.

EXPERIMENTAL

Melting points are uncorrected.

The author wishes to thank Dr. A.C. Syme, Mr. W. McCorkindale and Miss P. Adams, of the Chemistry Department, for carrying out the micro-analysis.

PREPARATION OF STARTING MATERIALS

Anhydrous Hydrazine:

Hydrazine hydrate (100 g., 2 moles) was heated under reflux on a steam bath with solid sodium hydroxide (90 g.) for about half an hour, access of moisture being prevented by a calcium chloride drying tube attached to the condenser outlet. The mixture separated into two layers and the lower layer, consisting of sodium hydroxide dissolved in the water present in the hydrazine hydrate, was run off. The upper layer consisting of almost pure hydrazine was carefully distilled from potassium hydroxide (15 g.) and the fraction distilling at 114° collected. Yield 45 g.

Chelidonic Acid was prepared by the method described in Organic Syntheses[125].

4-Thiopyridone was prepared from chelidonic acid by the method described by King and Ware[93].

OXIDATION OF 4-THIOPYRIDONE WITH (1) HYDROGEN PEROXIDE AND (2) NITRIC ACID:

(1.) Pyridine-4-sulphonic acid:

4-Thiopyridone (0.5 g.) was dissolved in glacial acetic acid (10 ml.) and 30% hydrogen peroxide (1.6 ml.) added. After heating on a water bath for about half an hour the acetic acid was removed under reduced pressure and the residue, recrystallized from aqueous methanol (charcoal), gave colourless plates (0.25 g., 36%) of pyridine-4-sulphonic acid, melting with decomposition at 333°.

Found: C, 37.5; H, 3.0; N, 0.0%; equivalent (potentiometric titration) 160.

$C_5H_5O_3NS$ requires C, 37.7; H, 3.1; N, 0.0%; equivalent 159.

Method B:

Sodium pyridine-4-sulphonate prepared by alkaline hydrogen peroxide oxidation of 4-thiopyridone, as described by King and Ware[93], was dissolved in water and passed through a cation exchange resin column (Zeo-Karb 225). The eulate was evaporated to dryness and the residue crystallised from ethanol-water gave pyridine-4-sulphonic acid, m.p. 333-4° (decomp.), identical with the product obtained in method A.

Ammonium pyridine-4-sulphonate:
Ammonium pyridine-4-sulphonate:

Pyridine-4-sulphonic acid was treated with a slight excess of dilute ammonia and evaporated to dryness. Crystallisation from aqueous methanol gave colourless crystals of ammonium pyridine-4-sulphonate, m.p. 256° (efferv.). Literature[93] m.p. 256°.

Found:	N, 16.2%
Calc. for $C_5H_6O_3N_2S$	N, 15.9%

Method C:

Di-4-pyridyl disulphide (*vide infra*) (0.5 g.) in glacial acetic acid (10 ml.) and 30% hydrogen peroxide (1.6 ml.) was heated on a water bath for half an hour and the acetic acid removed under reduced pressure. The residue crystallised from aqueous methanol gave pyridine-4-sulphonic acid, m.p. 333° (decomp.),

identical with the product obtained by methods A and B.

(2) Oxidation of 4-Thiopyridone with Nitric Acid:

4-Thiopyridone (2 g.) was heated on a water bath with nitric acid (d. 1.2, 20 ml.) until vigorous reaction accompanied by evolution of oxides of nitrogen commenced[96] (ca. 5 min.). After the reaction had subsided the solution was evaporated to dryness. The residue (3.3 g.) was dissolved by gently warming with water (8 ml.), filtered and, on cooling, pale yellow needles of di-4-pyridyl disulphide dinitrate (1.8 g., 58%) separated. When washed with ice cold water (2 ml.) and dried *in vacuo* the product melted with decomposition at 127°.

Found: C, 35.0; H, 2.5; N, 15.8; Nitrate-N (determined by reduction with Deverda's alloy in a Conway cell and titration of the liberated ammonia)[97] 8.0, 8.2%.

$C_{10}H_{10}O_6N_4S_2$ requires

C, 34.7; H, 2.9; N, 16.2; Nitrate-N, 8.1%.

The combined mother liquors and washings treated with ethanol gave colourless plates of pyridine-4-sulphonic acid (0.53 g.), m.p. 333-4° (decomp.), identical with authentic material. The filtrate was evaporated to dryness and the residue recrystallised from 90% ethanol (22 ml.) gave a further crop of pyridine-4-sulphonic acid (0.41 g.). Total yield 27%.

Di-4-Pyridyl Disulphide:

Di-4-pyridyl disulphide dinitrate (0.4 g.) was basified with dilute ammonia and extracted with ether (10 ml.). The ethereal

solution was washed with a little water, dried (Na_2SO_4) and on evaporating the solvent, an almost colourless oil, remained which set to a crystalline mass of di-4-pyridyl disulphide, m.p. 75-6°. King and Ware[93] give 74-75°. The yield was 0.26 g. (96%). The disulphide prepared by oxidation of 4-thiopyridone with iodine in sodium hydroxide solution, as described by Koenigs and Kinne[96], melted at 74-75°.

Found: C, 54.5; H, 3.4%.

Calc. for $C_{10}H_8N_2S_2$ C, 54.5; H, 3.7%.

The dipicrate (from methanol) decomposed at 230° (literature[93] m.p. 231°).

Found: C, 39.2; H, 1.9; S, 9.6%.

Calc. for $C_{22}H_{14}O_{14}N_8S_2$ C, 38.9; H, 2.1; S, 9.4%.

The platinichloride formed golden yellow plates, which decomposed about 285° (literature[96] reports decomposition without melting at 275°).

Found: C, 18.7; H, 1.6; Pt, 30.96%.

Calc. for $C_{10}H_{10}N_2S_2Cl_6Pt$ C, 19.1; H, 1.6; Pt, 30.95%.

ATTEMPTED ESTERIFICATION OF PYRIDINE-4-SULPHONIC ACID:

Method A[94]:

Pyridine-4-sulphonic acid (1 g.) was suspended in dry ethanol or methanol (10 ml.) and dry hydrochloric acid gas passed in for three hours. After refluxing on a water bath for twenty-five hours the alcohol was removed under reduced pressure. Pyridine-4-

sulphonic acid was quantitatively recovered.

Method B[99]:

Dimethyl sulphate (0.64 g.) and pyridine-4-sulphonic acid (0.8 g.) were heated together on a boiling water bath for four hours. Methanol (2 ml.) was added and the mixture refluxed for a further eight hours. The insoluble material was filtered off, dissolved in water (0.5 ml.), filtered and ethanol (5 ml.) added. Pyridine-4-sulphonic acid crystallized on standing for a short time. The filtrate from the reaction mixture was reduced on a water bath and left a residue of dimethyl sulphate.

Method C[99]:

Pyridine-4-sulphonic acid (0.2 g.) was suspended in methanol (2 ml.) and trifluoroacetic anhydride (0.5 ml.) added. The sulphonic acid did not dissolve on standing at room temperature for one day. It was then heated under reflux on a water bath for half an hour. Pyridine-4-sulphonic acid was quantitatively recovered.

N-Methyl-4-sulphopyridine betaine:

Pyridine-4-sulphonic acid (0.32 g.) suspended in ether (10 ml.) was treated with excess diazomethane. A few drops of water were added to promote the reaction. The reddish-brown solid produced was filtered off, dissolved in boiling water (charcoal), filtered and concentrated to small bulk. Ethanol was added till the solution became faintly opalescent and on standing 0.19 g. (54%) of N-methyl-4-sulphopyridine betaine crystallized as a colourless solid decomposing at 330°. It is soluble in water, neutral to litmus and

insoluble in the common organic solvents.

Found: C, 41.6; H, 3.6; N, 7.6%.

Calc. for $C_6H_7O_3NS$ C, 41.6; H, 4.1; N, 8.1%.

(Larivé[101] records decomposition points of 340° and 345°).

EXPERIMENTS INVOLVING CHLORINOLYSIS OF 4-THIOPYRIDONE:

The method used was essentially that described by Caldwell and Kornfeld[102].

Method I:

Chlorine gas was passed into 4-thiopyridone (1.11 g.), dissolved in a cold mixture of concentrated hydrochloric acid (7.5 ml.) and water (2 ml.) for about ten minutes, keeping the temperature below 5° by external cooling. No product separated and the reaction mixture was then taken down to dryness at laboratory temperature in a vacuum desiccator containing potassium hydroxide (4 days). The pale yellow residue (1.2 g.) was added to concentrated ammonia solution (10 ml.) at 0°. Very little heat was evolved and an oil separated which was extracted with ether and dried (Na_2SO_4). Evaporation of the ether gave an almost colourless oil which crystallised to a white solid (0.6 g., 54%), melting at 74-5°. A mixed melting point with an authentic sample of di-4-pyridyl disulphide was undepressed.

Method II:

The previous experiment was repeated, the chlorine being passed in for two hours. The residue (1.4 g.) obtained on evaporating to dryness

at 1 mm. Hg. below 5° (24 hours) melted between 290 and 300° with decomposition. The residue was divided into three portions - "A", "B" and "C".

"A" was recrystallized to constant melting point and gave pyridine-4-sulphonic acid, m.p. 331-2° (decomp.), from aqueous alcohol.

"B" was basified with ammonia and extracted with ether, as in method I, and gave a very small residue of di-4-pyridyl disulphide melting between 68 and 70°. A mixture melting point with an authentic sample was 71-72°.

"C" was treated with a slight excess of dilute ammonia, evaporated to dryness on a water bath and the residue washed with ether. Recrystallization from aqueous methanol gave ammonium pyridine-4-sulphonate, melting at 256° (efferv.)[93].

Method III:

Method II was repeated using a mixture of concentrated hydrochloric acid (1 ml.) and water (9 ml.) as solvent. A crude residue of pyridine-4-sulphonic acid, m.p. 316-20° (decomp.) was obtained.

Method IV:

Methods II and III were repeated, chlorinolysis being carried out at -5°. Crude pyridine-4-sulphonic acid remained after "freeze drying" the reaction mixture.

ATTEMPTS TO ISOLATE PYRIDINE-4-SULPHONYL CHLORIDE:

Chlorine gas was bubbled through a solution of 4-thiopyridone in glacial acetic acid containing the theoretical quantity of water, as described by Lee and Dougherty[104], the temperature being kept at 15° by external cooling with water. When excess of chlorine was present the reaction mixture was divided into several parts - "A", "B" and "C". "A" was poured into ether, "B" into dioxan and "C" into acetone. In no case did a product precipitate. The solvent was removed *in vacuo* keeping the temperature below 40° and the residue made alkaline with dilute ammonia and extracted with ether. Removal of the ether gave di-4-pyridyl sulphide, m.p. 68-70°, which gave a picrate from methanol, m.p. 228°. The di-4-pyridyl sulphide regenerated from the picrate melted at 70-71° and gave a depressed melting point on admixture with di-4-pyridyl disulphide. (King and Ware[99] give di-4-pyridyl sulphide, m.p. 71°, and dipicrate, m.p. 229°).

PYRIDINE-4-SULPHONAMIDE:

Method A:

4-Thiopyridone (1.11 g.) was dissolved in a cold mixture of concentrated hydrochloric acid (7.5 ml.) and water (2 ml.) in a boiling tube, which was then placed in a mixture of crushed ice and salt contained in a Dewar flask. When the temperature had dropped to -10°, chlorine gas was bubbled through at such a rate that the temperature was maintained at about -5°. When the temperature started to drop the flow

of chlorine through the mixture was increased and, towards the end of the reaction, it was passed through vigorously. The reaction was complete when the solution was yellowish-green in colour and the temperatu had dropped to -10° despite the rapid stream of chlorine passing through the solution. The reaction mixture was then poured on to crushed ice (15 g.) and transferred with stirring to concentrated ammonia (40 ml.) cooled to 0°. The warm reaction mixture was allowed to stand for about three hours and concentrated under reduced pressure on a water bath till some solid material started to separate out. The white solid which separated on leaving overnight in the refrigerator was filtered off, washed with ice-cold water and dried in a hot air oven at 100°. The yield of crude product, m.p. 167-8°, was 0.91 g. (58%). Recrystallisation from ethanol or water gave either large rhombic prisms or white spears, m.p. 172-3°, depending on the rate of crystallisation.

Found: C, 38.29; H, 3.45; N, 17.90%.

$C_5H_6O_2N_2S$ requires C, 37.99; H, 3.83; N, 17.72%.

Pyridine-4-sulphonamide picrate separated on mixing ethanolic solutions of pyridine-4-sulphonamide and picric acid. On washing with a little solvent and crystallising from ethanol the product was obtained as yellow plates, m.p. 186-7° (decomp.).

Found: C, 34.29; H, 1.94; N, 17.07%.

$C_{11}H_9O_9N_5S$ requires C, 34.11; H, 2.34; N, 18.01%.

Pyridine-(4-sulphonamide)-N-oxide:

Pyridine-4-sulphonamide (0.150 g.) was dissolved in a mixture of 30% hydrogen peroxide (0.3 ml.) and glacial acetic acid (0.4 ml.) and

heated between 70-80° on a water bath for five hours. The reaction mixture was concentrated under reduced pressure and on cooling pyridine-(4-sulphonamide)-N-oxide (0.085 g., 49%) was obtained as a brown solid. Recrystallisation from ethanol (5 ml.) (charcoal) gave white prisms which melted with decomposition at 230°. Naito and Dohmori[106] give m.p. 220°.

ATTEMPTED SYNTHESIS OF PYRIDINE-4-SULPHONHYDRAZIDE:

Method A:

Chlorinolysis of 4-thiopyridone (1.11 g.) was carried out as described under the synthesis of pyridine-4-sulphonamide. The yellowish-green aqueous reaction mixture was added to well-cooled 50% w/w hydrazine hydrate (40 g.) and left overnight at 0°. No product was obtained and it was reduced to dryness under reduced pressure, much frothing occurring as solid material separated. The warm molten residue solidified to a hygroscopic solid on cooling. Attempted recrystallisation from ethanol gave hydrazine hydrochloride as the only identifiable product.

Method B:

Method A was repeated but concentration of the reaction mixture was stopped as frothing commenced. After cooling, the solid separating was filtered off, dissolved in boiling ethanol and progressively concentrated to remove hydrazine salts. It gave 4-pyridylhydrazine hydrochloride, m.p. 241° (decomp.).

Found: C, 41.45; H, 4.7; N, 28.76%.

Calculated for $C_5H_8N_3Cl$ C, 41.23; H, 5.54; N, 28.8%.

(Koenigs[108] records 4-pyridylhydrazine hydrochloride, m.p. 238° (decomp.). The concentrated filtrate from the reaction mixture was heated under reduced pressure on a water bath till frothing had practically ceased and the residue boiled with ethanol (ca. 30 ml.). The clear solution was decanted from some molten insoluble material, which consisted chiefly of hydrazine hydrochloride and hydrazine sulphate. On concentrating the ethanol solution and filtering off the hydrazine salts a white crystalline solid (22 mg.) was obtained. Recrystallization from ethanol gave 4-pyridylhydrazine sulphate, m.p. 152° (decomp.).

Found: C, 29.2; H, 4.60%.

$C_5H_9O_4N_3S$ requires C, 28.98; H, 4.38%.

Further concentration of the mother liquors gave small amounts of two products which were not identified, (i) m.p. 232° (decomp.), found: C, 44.7; H, 4.4; N, 21.6%, and (ii) m.p. 236-7° (decomp.), found: C, 35.26; H, 3.5; N, 26.59%.

Method C:

Chlorinolysis of 4-thiopyridone (1.11 g.) was carried out as in the previous experiment and the reaction mixture carefully neutralized with triethylamine, keeping the temperature about 0° by external cooling in an ice-salt mixture. It was then added to an excess of hydrazine hydrate (1 g.). After allowing to stand for three hours, it was evaporated to dryness under reduced pressure. The residue dissolved completely in warm chloroform and, on cooling, gave some hydrazine sulphate (m.p. 254°). Concentrating the chloroform to small bulk gave triethylamine

hydrochloride. No other pure identifiable product could be isolated.

ATTEMPTED SYNTHESIS OF PYRIDINE-4-SULPHONHYDRAZIDE BY HYDRAZINOLYSIS OF PYRIDINE-4-SULPHONAMIDE.

Method A.

Pyridine-4-sulphonamide (0.79 g.) was heated with anhydrous hydrazine (0.9 g.) on a boiling water bath. After a few minutes the reaction mixture separated into two layers but became homogeneous on further heating. Ammonia was evolved and the gentle, intermittent bubbling which accompanied the reaction had almost completely subsided after about half an hour. On evaporation to dryness under reduced pressure a white solid (0.7 g.) was obtained. Recrystallisation from a relatively large volume of methanol, or from aqueous ethanol, gave white needles, m.p. 161° (decomp.).

Found:	C, 24.61;	H, 5.38;	N, 23.98%.
$C_5H_{12}O_3N_4S_2$ requires	C, 25.0;	H, 5.0;	N, 23.3%.

On acidifying with dilute hydrochloric acid sulphur dioxide was liberated and colloidal sulphur produced. Extraction of the latter with carbon disulphide and evaporation of the aqueous solution gave 4-pyridylhydrazine hydrochloride, m.p. 241° (decomp.) from ethanol. (Koenigs[108] gives 4-pyridylhydrazine hydrochloride, m.p. 238°).

An aqueous solution gave a precipitate with lead acetate, which was washed with warm water and dried at 100°.

Found:	Pb, 65.89%.
Calc. for PbS_2O_3	Pb, 64.91%.

With sodium hydroxide no ammonia could be detected, but oily globules, insoluble in the common organic solvents, were produced and on exposure to air a deep pink colour developed. 4-Pyridylhydrazine turns pink on exposure to air[100]. With picric acid it formed a derivative, m.p. 207° (decomp.) from methanol.

Found: C, 39.48; H, 2.42; N, 23.41%.

Method B:

Pyridine-4-sulphonamide (0.79 g.) was dissolved in methanol (10 ml. containing anhydrous hydrazine (0.16 g.) and refluxed on a water bath till no more ammonia was evolved (ca. seven hours). On cooling and standing for three days a product (0.62 g.), identical with the one obtained in method A, separated. Concentration of the mother liquor gave a non-crystalline yellow substance (0.2 g.), m.p. 130-170°. Several recrystallizations from methanol gave a small amount of 4-pyridylhydrazinium pyridine-4'-sulphonate as a white solid, m.p. 226-7 (decomp.).

Found: C, 44.29; H, 4.19; N, 20.68%.

$C_{10}H_{12}O_3N_4S$ requires C, 44.77; H, 4.47; N, 20.89%.

An aqueous solution passed through an anion exchange resin (Deacidite FF) gave, after acidification with dilute hydrochloric acid, 4-pyridylhydrazine hydrochloride[108], m.p. 241° (decomp.) from aqueous ethanol.

PYRIDINE-4-SULPHONAMIDE:

Method B:

4-Thiopyridone (1.11 g.) was dissolved in a mixture of concentrated hydrochloric acid (7.5 ml.) and water (2 ml.) and treated with chlorine, as in method A (p.72). After the reaction was complete the mixture was treated with calcium carbonate (ca. 1 g.) to part neutralise and displace dissolved chlorine, then poured into a flask containing cold chloroform (20 ml.) immersed in an ice-salt freezing mixture. The strongly acid solution was completely neutralized by the portion-wise addition of further small quantities of calcium carbonate and agitating the contents of the flask till effervescence ceased, before adding more calcium carbonate. In all about 8 g. of calcium carbonate was added and throughout the neutralisation the temperature of the mixture remained about -5°. The flask was then stoppered, shaken vigorously and the clear chloroform solution either decanted or filtered from the almost solid white sludge, which was washed with two further portions of cold chloroform (20 ml.). The combined chloroform solution was kept at about 0° over anhydrous sodium sulphate until colourless (ca. 1½ hours). After filtration a steady stream of dry ammonia gas was passed in for 10 minutes. Heat was evolved and the contents of the flask were allowed to cool before filtering off the crude product, which was washed with cold water and dried at 100°, giving 0.93 g. (60%) of pyridine-4-sulphonamide, m.p. 172-3° (from water). A mixed melting point with an authentic sample was undepressed.

Picrate from ethanol m.p. 186-7° (decomp.).

Pyridine-4-sulphonanilide:

4-Thiopyridone (1.11 g., 0.01 mole) was converted to pyridine-4-sulphonyl chloride, as in method B above, and the cold, dry colourless chloroform solution of the latter added to redistilled aniline (1.86 g., 0.02 mole). The mixture was shaken vigorously and left overnight at about 0°. The aniline hydrochloride formed was filtered off and the chloroform removed under reduced pressure. The residual oil was twice recrystallised from 60% methanol to give feathery needles (1.48 g., 65%) of pyridine-4-sulphonanilide, m.p. 135-6°.

Found: N = 12.0%.

$C_{11}H_{10}O_2N_2S$ requires N = 11.96%.

Pyridine-4-sulphonhydrazide:

Chlorinolysis of batches of 4-thiopyridone (5.55 g., 0.05 mole) was carried out, as in method B (p.78) for pyridine-4-sulphonamide. The cold, dry chloroform solution of pyridine-4-sulphonyl chloride was added in 10 to 20 ml. portions to anhydrous hydrazine (3.2 g., 0.1 mole) with vigorous shaking after each addition. Heat was generated and a white crystalline solid separated out. The mixture was left overnight in the refrigerator and the solid filtered and washed with a little ether. The crude mixture of hydrazine hydrochloride and product after drying *in vacuo* was suspended in ice-cold water (15 ml.), quickly filtered, washed with five portions of ice-cold water (ca., 1 ml.

then sucked dry before transferring to a vacuum desiccator containing sulphuric acid and evacuating to 3 mm. Hg. The yield of almost pure product varied between 5.7 and 6.3 g., (66 to 73%), melting with decomposition at 92-3°. This was sufficiently pure for the synthesis of derivatives. An analytically pure sample was prepared by dissolving in a minimum volume of cold water and cooling in ice, or by cautiously dissolving in warm methanol and cooling whereby colourless needles, m.p. 95-6° (decomp.), were obtained on drying in vacuo.

Found: C, 35.01; H, 3.74; N, 24.29; S, 18.2%.

$C_5H_7O_2N_3S$ requires C, 34.71; H, 4.08; N, 24.28; S, 18.5%.

Picrate: The picrate was prepared by mixing ethanolic solutions of pyridine-4-sulphonhydrazide and picric acid. The needles which separated washed with some alcohol and dried in vacuo melted with decomposition at 117-8°.

Found: C, 32.92; H, 2.31; N, 20.73%.

$C_{11}H_{10}O_9N_6S$ requires C, 32.83; H, 2.51; N, 20.89%.

Pyridine-4-sulphonhydrazide is insoluble in benzene, ether and chloroform, sparingly soluble in alcohol, more soluble in methanol and soluble in water. Aqueous solutions slowly turn yellow with the evolution of nitrogen, even at 0°, and heating in methanol produces decomposition, the solution turns yellow, nitrogen is evolved and small amounts of hydrazine sulphate are formed. Prolonged heating (6 hrs.) in methanol and removal of hydrazine sulphate gave a yellow viscous oil which did not crystallize.

Dihydrochloride:

A methanolic solution of pyridine-4-sulphonhydrazide was treated with a saturated methanolic hydrochloric acid solution. A white crystalline solid was obtained which contained a molecule of methanol of crystallisation. When dried *in vacuo* over potassium hydroxide, it melted with decomposition at 122-3°.

Found: C, 25.73; H, 4.78; S, 11.7%.

$C_6H_{13}O_3N_3SCl_2$ requires C, 25.9; H, 4.74; S, 11.5%.

Attempts to remove the methanol by heating at 60° under vacuum resulted in discolouration of the product.

Attempted preparation of a copper complex of pyridine-4-sulphonhydrazide:[8]

Pyridine-4-sulphonhydrazide (0.8 g.) was dissolved in water (30 ml.) and a solution of copper sulphate containing copper sulphate (1 g.) and 2N sulphuric acid (2 ml.) in water (30 ml.) added. Immediate evolution of gas occurred and on standing at 0° for several days a mixture of solids (yellow and blue-green in colour) was deposited. After filtration it was washed liberally with water, ethyl alcohol and ether. The mixture was insoluble in benzene, chloroform, acetone and methanol but soluble in alkali. Attempts to effect a separation were unsuccessful.

PREPARATION OF ALKYLIDENE AND ARALKYLIDENE DERIVATIVES OF PYRIDINE-4-SULPHONHYDRAZIDE:

1-Ethylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.865 g., 0.005 mole) in methanol (5 ml.) was kept cool in ice and cold acetaldehyde (0.5 ml.) added dropwise with shaking. The mixture was reduced to dryness under diminished pressure at laboratory temperature. The residue was dissolved in methanol (30 ml.) by very gently warming and again concentrated under reduced pressure at laboratory temperature till crystallisation commenced. On standing at about 0° colourless needles (0.42 g., 42%) separated, m.p. 120° (decomp.).

Found:	C, 42.52;	H, 4.08%.
$C_7H_9O_2N_3S$ requires	C, 42.21;	H, 4.55%.

Picrate: The picrate, m.p. 113° (decomp.) separated in needles from ethanol.

Found:	C, 36.65;	H, 2.43%.
$C_{13}H_{12}O_9N_6S$ requires	C, 36.44;	H, 2.82%.

1-isoPropylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.865 g.) in methanol (5 ml.) was treated with acetone (0.5 ml.) and shaken. On standing colourless prisms separated and were filtered off. The filtrate was concentrated to small bulk, when a further crop was deposited. Recrystallisation from ethanol gave prisms (0.6 g., 59%), melting with decomposition at

147° when dried *in vacuo*.

Found: C, 45.53; H, 5.19%.

$C_8H_{11}O_2N_3S$ requires C, 45.07; H, 5.2%.

1-isoPropylidene-2-pyridine-4'-sulphonylhydrazine picrate was obtained as orange prisms (from methanol), m.p. 135-6° (decomp.).

Found: C, 30.2; H, 3.24; N, 19.2%.

$C_{14}H_{14}O_9N_6S$ requires C, 30.0; H, 3.2; N, 19.0%.

The following compounds were synthesized by mixing a suspension of pyridine-4-sulphonhydrazide (m.p. 92-3°) in methanol, and a methanolic solution containing an equimolecular proportion of the appropriate carbonyl compound. The product separating was crystallized from a suitable solvent and dried *in vacuo*. The synthesis of 1-benzylidene-2-pyridine-4'-sulphonylhydrazine is typical.

1-Benzylidene-2-pyridine-4'-sulphonylhydrazine:-

Pyridine-4-sulphonhydrazide (0.865 g., 0.005 mole) suspended in methanol (5 ml.) was shaken with benzaldehyde (0.53 g., 0.005 mole), dissolved in methanol (5 ml.) until the solution was homogeneous. On standing colourless highly refractive prisms or needles (1.15 g., 88%) separated, m.p. 138-9° (decomp.). Recrystallisation from ethanol, aqueous ethanol or methanol raised the melting point to 141-2° (decomp.).

Found: C, 55.33; H, 4.17%.

$C_{12}H_{11}O_2N_3S$ requires C, 55.17; H, 4.25%.

It is soluble in hot ethanol, methanol but is insoluble in water and in ether.

1-p-Methoxybenzylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) and p-methoxybenzaldehyde (0.68 g., 0.005 mole) were reacted, as described in the general method above. The solid separating (1.25 g., 86%) crystallized from ethanol, or methanol, in colourless needles, m.p. 153-6° (decomp.).

Found: C, 54.05; H, 4.10%.

$C_{13}H_{13}O_3N_3S$ requires C, 53.60; H, 4.50%.

1-Cinnamylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was reacted with cinnamaldehyde (0.61 g., 0.005 mole), as described in the general method. A solid (1.28 g., 69%) separated, which crystallized in lemon-coloured shining flakes, m.p. 140° (decomp.) from ethanol.

Found: C, 58.01; H, 4.47%.

$C_{14}H_{13}O_2N_3S$ requires C, 58.54; H, 4.56%.

1-m-Methylbenzylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) treated with m-methylbenzaldehyde (0.60 g., 0.005 mole), as described in the general method, gave a white crystalline solid (0.34 g.) On addition of water to the

filtrate from this solid a further crop of crystals (0.73 g.) was obtained: the total yield was 77%. Recrystallization by dissolving in ethanol and adding water dropwise till opalescent gave white needles, m.p. 136-7° (decomp.).

Found:	C, 56.91;	H, 4.75%.
$C_{13}H_{13}O_2N_3S$ requires	C, 56.72;	H, 4.76%.

1-Piperonylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was reacted with piperonal (0.75 g., 0.005 mole), as described in the general method, to give a pale yellow solid (1.31 g., 83%). Crystallization from ethanol or methanol (charcoal) did not remove the colour from the product, which melted with decomposition at 163-4°.

Found:	C, 51.48;	H, 3.75%.
$C_{13}H_{11}O_4N_3S$ requires	C, 51.15;	H, 3.64%.

1-Salicylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was reacted with salicylaldehyde (0.61 g., 0.005 mole), as described in the general method. The product (1.24 g., 89%) recrystallised from ethanol and dried *in vacuo* gave colourless prisms, m.p. 166° (decomp.).

Found:	C, 52.11;	H, 4.3%.
$C_{12}H_{11}O_3N_3S$ requires	C, 51.90;	H, 4.0%.

1-p-Hydroxybenzylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was reacted with p-hydroxybenzaldehyde (0.61 g., 0.005 mole), as described in the general method. The solution turned bright red and fine glistening needles were deposited. Washing with a small volume of ethanol and recrystallizing from ethanol (charcoal) gave colourless needles (0.90 g., 71%), m.p. 168-9° (decomp.).

Found: C, 51.9; H, 3.98%.

$C_{12}H_{11}O_3N_3S$ requires C, 51.98; H, 4.0%.

1-p-Dimethylaminobenzylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was treated with p-dimethylaminobenzaldehyde (0.75 g., 0.005 mole), as described in the general method. The solution quickly changed to a deep orange colour and a crystalline solid (1.15 g., 76%) was deposited. Recrystallization from a large volume of ethanol or methanol (charcoal) gave yellow needles, m.p. 190° (decomp.).

Found: C, 55.39; H, 5.4%.

$C_{14}H_{16}O_2N_4S$ requires C, 55.26; H, 5.27%.

1-D-Glucosyl-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was added in small portions to anhydrous D-glucose (0.9 g., 0.005 mole) dissolved in methanol (30 ml.) and gently warmed to effect solution. On standing at 0° for several days, 0.63 g. (37%) of product was obtained. Concentration of the

mother liquor to small bulk at laboratory temperature gave a further 0.22 g. The total yield of pure product was 50%, m.p. 156° (decomp.).

Found: C, 39.39; H, 4.93%.

$C_{11}H_{17}O_7N_3S$ requires C, 39.4; H, 5.1%.

1-Furfurylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was treated with freshly distilled furfuraldehyde (0.40 g., 0.005 mole), as described in the general method. The product (0.81 g., 66%) crystallised in long white needles. Crystallization from aqueous methanol, aqueous ethanol or benzene gave a product, m.p. 128-9° (decomp.), when dried for eight hours at 80° under high vacuum and later for three days under the same conditions. A sample (from benzene) was submitted for analysis.

Found: C, 49.39; H, 4.45%.

$C_{10}H_9O_3N_3S$ requires C, 47.81; H, 3.61%.

With picric acid it gave prisms of 1-furfurylidene-2-pyridine-4'-sulphonylhydrazine picrate, (m.p. 161° decomp.) from ethanol.

Found: C, 40.46; H, 2.24; N, 17.44%.

$C_{16}H_{12}O_{10}N_6S$ requires C, 40.0; H, 2.5; N, 17.5%.

Attempted synthesis of 1-isonicotinylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (m.p. 95-6°) (0.005 mole) was treated with freshly-distilled isonicotinaldehyde (0.54 g., 0.005 mole), as

described in the general method. The solution immediately started to turn yellow and it was transferred to the refrigerator. The colour quickly changed to deep red with copious evolution of nitrogen. After two days a dark amorphous solid had separated. More methanol was added, the solution boiled with charcoal three times and the filtrate concentrated to 3 ml. Ether was added till a faint opalescence persisted. Feathery yellow crystals separated after several hours at 0^{0}, but on filtering off they became semi-solid and could not be handled.

1-Vanillylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) condensed with vanillin (0.76 g., 0.005 mole), as described in the general method, to give very fine needles. The mother liquor after filtration was treated with water till cloudy, when a further crop of crystals was obtained. The total yield was 1.05 g. (65%). Recrystallisation from ethanol gave long fine needles, m.p. 120-1° (decomp.).

Found: C, 50.26; H, 4.69%.

$C_{13}H_{13}O_4N_3S$ requires C, 50.01; H, 4.26%.

The product was soluble in ether, ethanol and methanol and insoluble in water. The solid gradually turned pale yellow on standing in air and the smell of vanillin was apparent.

1-Veratrylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was reacted with veratraldehyde (0.83 g., 0.005 mole), as described in the general method.

The white solid separating was washed with a little methyl alcohol and recrystallised from ethanol to give long white needles (1.05 g., 65%), melting with decomposition at 165° on drying at 80° in vacuo.

Found: C, 52.09; H, 4.2%.

$C_{14}H_{15}O_4N_3S$ requires C, 52.33; H, 4.7%.

1-α-Phenylethylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) reacted with acetophenone (0.60 g., 0.005 mole), as described in the general method, to give a colourless solid (1.05 g., 76%). Recrystallisation from ethanol gave thick prisms, m.p. 148-9° (decomp.).

Found: C, 57.03; H, 4.71%.

$C_{13}H_{13}O_2N_3S$ requires C, 56.72; H, 4.76%.

1-β-Phenylethylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) and freshly-distilled phenylacetaldehyde (0.60 g., 0.005 mole) reacted together, as described in the general method, to give a white crystalline solid (1.2 g., 80%). Recrystallisation from methyl alcohol gave colourless prisms, m.p. 110° (decomp.).

Found: C, 56.09; H, 4.47%.

$C_{13}H_{13}O_2N_3S$ requires C, 56.72; H, 4.76%.

1-cyclohexylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was reacted with freshly-

distilled cyclohexanone (0.49 g., 0.005 mole), as described in the general method. The white solid separating was filtered off and the mother liquor concentrated to give a further small crop of crystals. The total yield was 0.96 g. (75%). Recrystallisation from ethanol gave colourless pyramids, m.p. 142° (decomp.).

Found:	C, 52.4; H, 5.66%.
$C_{11}H_{15}O_2N_3S$ requires	C, 52.17; H, 5.90%.

1-cycloHeptylidene-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was reacted with freshly-distilled cycloheptanone (0.56 g., 0.005 mole), as described in the general method, to give 1.15 g. (86%) of product. Recrystallisation from methanol gave colourless prisms, m.p. 166° (decomp.).

Found:	C, 54.0; H, 6.2%.
$C_{12}H_{17}O_2N_3S$ requires	C, 53.93; H, 6.4%.

1-Hexahydro-2":4":6"-trioxo-5"-pyrimidylidene-2-pyridine-4'-sulphonyl-hydrazine:

Pyridine-4-sulphonhydrazide (0.005 mole) was treated with alloxan (0.71 g., 0.005 mole), as described in the general method. The product separated almost quantitatively as a white, micro-crystalline solid (1.48 g.), which was liberally washed with methyl alcohol and dried at 3 m Hg. It did not melt below 360°.

Found:	C, 34.47; H, 3.10%.
$C_9H_9O_6N_5S$ requires	C, 34.28; H, 2.88%.

Attempted removal of the elements of water at 80° under high vacuum result in the product turning pink in colour. It was very insoluble in the common organic solvents and in water, but dissolved in strong alkali and mineral acid.

Condensation of pyridine-4-sulphonhydrazide with acetylacetone: 2:4-Bi(pyridine-4'-sulphonamidoimino)pentane:

Pyridine-4-sulphonhydrazide (0.365 g.) suspended in dry ethanol (8 ml.) was treated with acetylacetone (1.0 ml.) and shaken till homogeneous. On standing overnight at 0° a white crystalline solid (0.95 g., 92%) separated. Recrystallization from a relatively large volume of methanol gave small glistening needles, melting at 162-3° (decomp.).

Found: N = 20.5%.

$C_{15}H_{18}O_4N_6S_2$ requires N = 20.3%.

It is sparingly soluble in methanol, ethanol and almost insoluble in benzene, ether and chloroform.

Condensation of pyridine-4-sulphonhydrazide with ethyl acetoacetate: 3-Methyl-1-pyridine-4'-sulphonyl-5-pyrazolone:

Pyridine-4-sulphonhydrazide (0.173 g., 0.001 mole) in methanol (1 ml. was shaken with ethyl acetoacetate (0.13 g., 0.001 mole) in methanol (1 ml and left overnight at 0°. A white crystalline solid (0.18 g., 75%) separated. Recrystallization from methanol gave white needles and prisms, melting with decomposition at 124-5°.

Found: N, 17.7%.

$C_9H_9O_3N_3S$ requires N, 17.6%.

ATTEMPTED SYNTHESIS OF ALKYL AND ARALKYL DERIVATIVES OF PYRIDINE-4-SULPHONYLHYDRAZIDE:

A.

In the following experiments 1-isopropylidene-2-pyridine-4'-sulphonylhydrazine and 1-benzylidene-2-pyridine-4'-sulphonylhydrazine (0.0033 mole) were used.

Method I:

The appropriate compound was dissolved in sufficient methanol and hydrogenated at 20° and one atmosphere pressure, using Adams' platinum oxide catalyst (25 mg.). There was no uptake of hydrogen after three hours and on filtering and evaporating the solution to dryness the starting material was recovered in each case.

Method II:

Method I was repeated using methanolic hydrochloric acid as solvent and warming, if necessary, to effect solution. Starting material was again recovered.

Method III:

Methods I and II were repeated using hydrogen at five atmospheres pressure. Starting material was recovered.

Method IV:

Hydrogenation was attempted using glacial acetic acid as solvent and hydrogen at a pressure of one atmosphere. No reduction took

place.

Method V.

Method I was repeated using ethanol as solvent at a temperature of 50° and hydrogen at a pressure of one atmosphere. Starting material was recovered.

B.

1-Benzylidene-2-pyridine-4'-sulphonylhydrazine (0.26 g.) in dry methanol (5 ml.) was treated portion-wise with sodium borohydride[115] (0.075 g.), the mixture being cooled externally with cold water. No product separated on the addition of water (ca. 1.5 ml.). Dilute hydrochloric acid was added dropwise until the solution remained turbid and on standing at 0° white needles separated. 1-Benzylidene-2-pyridine-4'-sulphonylhydrazine, m.p. 141° (decomp.), was recovered on recrystallising from aqueous ethanol.

Reduction of 1-isopropylidene-2-pyridine-4'-sulphonylhydrazine using sodium borohydride was also unsuccessful.

C.

Method I.

Pyridine-4-sulphonhydrazide (0.865 g., 0.005 mole) was added in small amounts to sodium (0.115 g., 0.005 g. atom) dissolved in warm ethanol (10 ml.). The mixture became homogeneous and almost immediately a white precipitate rapidly separated. Benzyl chloride (0.64 g., 0.005 mole) was added and the mixture heated under reflux on a steam bath. Yellowing of the solution occurred after a few minutes and heating was discontinued. Some white suspended material consisting chiefly of

sodium chloride was filtered off and the filtrate concentrated and cooled gave the mono-sodio derivative of pyridine-4-sulphonhydrazide as a white solid. It was washed with a little ethanol and purified by dissolving in ethanol and precipitating several times with ether. The yield was 0.68 g. (70%) and it melted about 285°. It is soluble in water, giving an alkaline solution, and insoluble in organic solvents.

Found: C, 30.3; H, 3.0; Na = 11.6%.

$C_5H_6O_2N_3SNa$ requires C, 30.6; H, 3.1; Na = 11.6%.

Method II.[114]

The previous experiment was repeated using pyridine-4-sulphonhydrazide (0.865 g., 0.005 mole), sodium (0.23 g., 0.01 g. atom) and benzyl chloride (1.28 g., 0.01 g. mole), the latter being added rapidly before the mono-sodio derivative separated and refluxed on a steam bath for four hours. The sodium chloride was removed as before and progressive concentration finally gave a product (0.25 g.) free of chloride. Recrystallisation from aqueous methanol gave shining plates, m.p. 169-70° (decomp.), of benzyl 4-pyridyl sulphone. Yield 10%.

Found: C, 61.16; H, 4.6; N, 6.1%.

$C_{12}H_{11}O_2NS$ requires C, 61.8 ; H, 4.7; N, 6.0%.

On mixing methanolic solutions of benzyl 4-pyridyl sulphone and picric acid benzyl 4-pyridyl sulphone picrate separated in needles, (m.p. 191°), containing one molecule of solvent of crystallisation.

Found: N, 11.41%.

$C_{19}H_{16}O_{10}N_4S$ requires N, 11.33%.

1-Phenyl-2-pyridine-4'-sulphonylhydrazine.

4-Thiopyridone (1.11 g., 0.01 mole) was converted to pyridine-4-sulphonyl chloride, as in method B (p. 78). The cold dry chloroform solution containing the sulphonyl chloride was added to redistilled phenylhydrazine (2.16 g., 0.02 mole) in chloroform (10 ml.) and shaken. The mixture deposited a brown solid (3.0 g.) on standing overnight at 0°. This was filtered off, washed with some chloroform and adherent solvent removed *in vacuo*. It was suspended in ice-cold water, washed till free of chloride ion and again dried *in vacuo*. The crude product (0.3 g., 12%) was carefully dissolved in ethanol (*ca.* 20 ml.) by gentle heating and allowed to evaporate spontaneously at laboratory temperature, when a white micro-crystalline solid separated. It is insoluble in water and ether. Attempted recrystallization from water resulted in the formation of dark-brown semi-solid material.

Found: N = 16.72%.

$C_{11}H_{11}O_2N_3S$ requires N = 16.67%.

Attempted synthesis of 1:1-dimethyl-2-pyridine-4'-sulphonylhydrazine.

Pyridine-4-sulphonyl chloride in chloroform was poured into two molecular proportions of unsymmetrical dimethylhydrazine, as in the previous experiment. Very little solid separated after standing at 0° for several days. The chloroform was removed at about 40° and the brown residue treated with ice-cold water, whereupon bubbles of gas were evolved and most of the residue went into solution. The insoluble material, after filtration and drying *in vacuo*, was dissolved in methanol

and ether added till cloudy. Only oily semi-solid material separated which did not crystallize on long standing at 0° or *in vacuo*. It was then dissolved in ethanol and hydrogen chloride passed in, followed by addition of ether till a faint opalescence persisted. Again, an oily non-crystallizable product was obtained.

THE DECOMPOSITION OF PYRIDINE-4-SULPHONHYDRAZIDE:

Method A:

Pyridine-4-sulphonhydrazide (0.43 g.) was heated under reflux for 15 minutes with methanol (5 ml.) and allowed to cool. A small amount of hydrazine sulphate was removed by filtration and the filtrate treated with ether till faintly opalescent and kept overnight at 0°. The pale yellow solid which had separated was dissolved in water (*ca.* 1 ml.) and acetone added dropwise till the solution remained turbid. On standing pyridine-4-sulphinic acid (75 mg., 21%) separated as lemon-yellow needles, which when washed with acetone and ether and dried *in vacuo* melted with decomposition at 139-40°.

Found:	C, 42.6;	H, 3.69;	N, 9.63%.
$C_5H_5O_2NS$ requires	C, 42.0;	H, 3.52;	N, 9.79%.

Pyridine-4-sulphinic acid is soluble in water (acid to litmus), warm methanol and ethanol and insoluble in acetone and ether. It did not form a picrate in methanol or reduce cold ammoniacal silver nitrate. The yellow colour slowly changed to green on standing in air.

Oxidation of Pyridine-4-Sulphinic Acid:

A small quantity of pyridine-4-sulphinic acid was evaporated to

dryness with a few drops of 30% hydrogen peroxide. The white residue recrystallized from aqueous ethanol gave pyridine-4-sulphonic acid, m.p. 330-1° (decomp.). This oxidation was repeated in presence of a few drops of dilute ammonia and gave ammonium pyridine-4-sulphonate, m.p. 256° (decomp.)[93], from aqueous ethanol.

Method B.

Pyridine-4-sulphonhydrazide (0.43 g.) was dissolved in methanol (ca. 2 ml.) and heated under reflux for several hours with sodium (0.06 g.) in ethanol (ca. 5 ml.). The solution was cooled, filtered and ether added to the filtrate to precipitate the product, which was dissolved in water (ca. 2 ml.) and passed through a column of Zeo-Karb 225. The eluate was reduced to dryness under reduced pressure and the yellow residue recrystallized from aqueous acetone gave pyridine-4-sulphinic acid, m.p. 139-40° (decomp.) as lemon-yellow needles.

Oxidation with hydrogen peroxide gave pyridine-4-sulphonic acid, m.p. 330° (decomp.)

SYNTHESIS OF ACYL DERIVATIVES OF PYRIDINE-4-SULPHONHYDRAZIDE:

1:1-Diacetyl-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.865 g.) was added in small portions to acetic anhydride (5 ml.) and the mixture shaken. Heat was evolved and complete solution was effected by gentle warming on a water bath. On cooling, colourless prisms (0.96 g., 69%) separated out. The product recrystallised from ethanol and dried *in vacuo* melted with

decomposition at 161°.

Found:	C, 42.09;	H, 3.7;	S, 12.30%.
$C_9H_{11}O_4N_3S$ requires	C, 42.03;	H, 4.3;	S, 12.45%.

1-Acetyl-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.432 g., 0.0025 mole) was suspended in methanol (5 ml.) and acetic anhydride (0.51 g., 0.0025 mole) added. On standing, small white needles (0.35 g., 65%) separated out. On recrystallising from ethanol and drying in vacuo the product melted at 181-2° (decomp.).

Found:	C, 39.25, 39.4;	H, 3.65, 4.07%.
$C_7H_9O_3N_3S$ requires	C, 39.07;	H, 4.22%.

1-β-Hydroxycarbonylacryloyl-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.865 g., 0.005 mole) was added portion-wise with shaking to finely powdered maleic anhydride (0.49 g., 0.005 mole) in dry dioxan (6 ml.) and gently warmed on a water bath to give a clear solution. On cooling, a white crystalline solid (1.1 g., 66%) was precipitated. It was crystallised from a relatively large volume of 90% methanol and dried in vacuo, giving white needles, m.p. 166-7° (decomp.).

Found:	C, 40.13;	H, 3.20%.
$C_9H_9O_5N_3S$ requires	C, 39.85;	H, 3.35%.

1-β-Hydroxycarbonylpropionyl-2-pyridino-4'-sulphonylhydrazine:

Pyridino-4-sulphonhydrazide (0.865 g., 0.005 mole) was added to dioxan (8 ml.) containing succinic anhydride (0.5 g., 0.005 mole) and shaken to effect solution. After several days, a white crystalline solid (1.0 g., 73%) was deposited. Recrystallization from methanol gave white, silky needles, m.p. 179-80° (decomp.) when dried *in vacuo*.

Found: C, 38.12; H, 4.2%.

$C_9H_{11}O_5N_3S.\frac{1}{2}H_2O$ requires C, 38.29; H, 4.29%.

The sample was dried under high vacuum at 100°.

Found: C, 39.36; H, 4.29%.

$C_9H_{11}O_5N_3S$ requires C, 39.56; H, 4.06%.

1-β-Hydroxycarbonylbenzoyl-2-pyridino-4'-sulphonylhydrazine:

Pyridino-4-sulphonhydrazide (0.865 g., 0.005 mole) was shaken with phthalic anhydride (0.74 g., 0.005 mole) dissolved in dry dioxan (25 ml.). A white solid (0.97 g., 60%) was quickly precipitated. Recrystallization from ethanol or from water gave a white micro-crystalline solid which when dried *in vacuo* over potassium hydroxide melted with decomposition at 158°.

Found: C, 49.0; H, 3.26%.

$C_{13}H_{11}O_5N_3S$ requires C, 48.6; H, 3.45%.

1-Benzoyl-2-pyridino-4'-sulphonylhydrazine hemi-hydrate:

Pyridino-4-sulphonhydrazide (0.432 g., 0.0025 mole) was added to benzoic anhydride (0.56 g., 0.0025 mole) in dioxan (5 ml.) and shaken

till homogeneous. It was left overnight and the product precipitated by the addition of ether. It crystallised in tufts as the hemi-hydrate from an ethanol-ether mixture and melted at 128-9° (decomp.). The yield was 0.28 g., (40%).

Found:	C, 49.95; H, 4.6%.
$C_{12}H_{11}O_3N_3S.\frac{1}{2}H_2O$ requires	C, 50.33; H, 4.22%.

1-Benzoyl-2-pyridine-4'-sulphonylhydrazine hydrochloride:

Pyridine-4-sulphonhydrazide (0.865 g., 0.005 mole) in dry dioxan was added with shaking to benzoyl chloride (0.70 g., 0.005 mole) in dry dioxan (5 ml.) and left overnight. The white solid (1.3 g., 85%) was filtered off and gave almost colourless plates (from 90% ethanol), melting with decomposition at 194-5°.

Found:	C, 45.92; H, 3.74%.
$C_{12}H_{12}O_3N_3SCl$ requires	C, 45.82; H, 3.86%.

The hydrochloride is sparingly soluble in cold water but soluble in hot water, giving a strongly acid solution.

1-Benzoyl-2-pyridine-4'-sulphonylhydrazine:

1-Benzoyl-2-pyridine-4'-sulphonylhydrazine hydrochloride was dissolved in hot water. On cooling, white needles melting with decomposition at 129° separated. On drying, in vacuo at 100°, the product melted with decomposition at 164°.

Found: C, 51.9; H, 3.95%.

$C_{12}H_{11}O_3N_3S$ requires C, 52.90; H, 4.00%.

The hemi-hydrate, m.p. 128-9° (decomp.) when dried *in vacuo* at 100° also melted at 164° with decomposition.

An ethanolic solution of the base gave 1-benzoyl-2-pyridine-4'-sulphonylhydrazine hydrochloride (m.p. 195° decomp.) with ethanolic hydrochloric acid.

An attempted preparation of 1-benzoyl-2-pyridine-4'-sulphonylhydrazine using benzoyl chloride in pyridine:

Pyridine-4-sulphonhydrazide (0.865 g.) was suspended in dry pyridine (8 ml.) and treated dropwise with benzoyl chloride (0.70 g.) in dry pyridine (4 ml.). Heat was evolved and the mixture became homogeneous on gently warming on a water bath. No product separated after two days at 0°. The pyridine was removed under reduced pressure on a water bath, but decomposition occurred, bubbles of gas being liberated from a dark-green liquid residue which could not be crystallised.

The experiment was repeated without removing the pyridine. Isolation of the product was achieved by the addition of cold water to the reaction mixture. Recrystallisation from aqueous methanol and drying *in vacuo* at 100° gave 1-benzoyl-2-pyridine-4'-sulphonylhydrazine m.p. 163-4° (decomp.).

1-isoNicotinoyl-2-pyridine-4'-sulphonylhydrazine:

isoNicotinoyl chloride hydrochloride was prepared from isonicotinic acid (2 g.), according to the method of Dornow and Wodekind[65]. The isonicotinoyl chloride hydrochloride was added to pyridine-4-sulphonhydrazide (0.865 g.) in dry pyridine (10 ml.) and allowed to stand for a few minutes. The product was isolated by addition of water, filtered, washed and recrystallized from 95% methanol to give colourless prisms, m.p. 120-1° (efferv.). The yield was 0.31 g. (20%) of product containing one molecule of methanol of crystallization.

Found: C, 46.26; H, 4.5; N, 17.7%.

$C_{12}H_{14}O_4N_4S$ requires C, 46.45; H, 4.55; N, 18.06%.

1-Phenylcarbamoyl-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazine (0.865 g., 0.005 mole) suspended in methanol (5 ml.) was treated with phenyl isocyanate (0.6 g., 0.005 mole) dissolved in methanol (5 ml.). On shaking, the mixture became homogeneous and slightly warmer, and almost immediately a white precipitate separated. The reaction mixture was allowed to stand overnight and gave 1.2 g. of product. Yield 82%. Recrystallization from a large volume of methanol (200 ml.) gave colourless refractive prisms, melting at 200° (decomp.). The product was dried at 100° for eight hours for analysis.

Found: N = 19.11%.

$C_{12}H_{12}O_3N_4S$ requires N = 19.17%.

It is insoluble in water, ethanol and ether.

Synthesis of 1-Allylthiocarbamoyl-2-pyridine-4'-sulphonylhydrazine.

Pyridine-4-sulphonhydrazide (0.865 g., 0.005 mole) in methanol (5 ml.) was treated with freshly distilled allylisothiocyanate (0.49 g., 0.005 mole) in methanol (2 ml.). Some solid material separated on standing overnight at 0°. The mother liquor was concentrated under reduced pressure at room temperature, when a further yield of product was obtained. Recrystallization by dissolving in methanol with gentle heating and allowing to cool gave 0.35 g. (25%) of 1-allylthiocarbamoyl-2-pyridine-4'-sulphonylhydrazine as colourless prisms, m.p. 149° (decomp.), with previous sintering and darkening about 140°.

Found:	N, 20.6%.
$C_9H_{12}O_2N_4S_2$ requires	N, 20.6%.

THE SYNTHESIS OF SULPHONYL DERIVATIVES OF PYRIDINE-4-SULPHONHYDRAZIDE:

Attempted synthesis of 1-pyridino-4'-sulphonyl-2-toluene-p-sulphonylhydrazine:

p-Toluenesulphonyl chloride (0.95 g., 0.005 mole) was added in small portions to pyridine-4-sulphonhydrazide (0.863 g., 0.005 mole) in dry methanol (15 ml.), shaking after each addition. The reaction mixture was left for several days at 0° and pyridine-4-sulphonhydrazide monohydrochloride (0.71 g., 67%) separated from the solution in very pale yellow needles. It was recrystallized from a methanol-ether mixture and when dried in vacuo melted with decomposition at 106°. It dissolved in water, was strongly acidic, gave a positive chloride reaction and reduced cold ammoniacal silver nitrate.

Found: C, 29.20; H, 3.7%; equivalent (titration) 207.5

$C_5H_8O_2N_3SCl$ requires C, 28.64; H, 3.85%; equivalent 209.5.

With picric acid in methanol it gave pyridine-4-sulphonhydrazide picrate, (m.p. 117° decomp.).

1-Pyridino-4'-sulphonyl-2-toluene-p-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.432 g., 0.0025 mole) was treated dropwise with p-toluenesulphonyl chloride (0.48 g., 0.0025 mole) in dry pyridine (5 ml.), the reaction mixture being cooled externally with cold water. The mixture turned orange in colour and nitrogen was slowly liberated. The solid remaining undissolved after addition was complete was dissolved by stirring, and water (ca. 15 ml.) slowly added whilst cooling in crushed ice. The pale yellow solid separating was washed with water (ca. 20 ml.) and sucked dry at the pump.

Crystallization from ethanol gave glistening needles (0.32 g., 40%), melting with decomposition at 153-4° with previous darkening about 150°.

Found:	C, 44.03;	H, 3.97%.
$C_{12}H_{13}O_4N_3S_2$ requires	C, 44.03;	H, 4.00%.

1-Benzenesulphonyl-2-pyridine-4'-sulphonylhydrazine:

Pyridine-4-sulphonhydrazide (0.432 g., 0.0025 mole) was suspended in dry pyridine (5 ml.). Benzenesulphonyl chloride (0.44 g., 0.0025 mole) was added dropwise with external cooling in cold water. The reaction mixture turned orange in colour and nitrogen was slowly liberated. The undissolved solid was broken up with a glass rod and stirred till homogeneous. The flask containing the reaction mixture was transferred to crushed ice, water (ca. 15 ml.) was slowly added and a pale brown solid separated. It was filtered off, washed liberally with water and sucked dry at the pump. Recrystallization from ethanol gave glistening needles or plates (0.31 g., 40%), which decomposed at 141-2° with previous darkening about 139°.

Found:	C, 42.33;	H, 3.73%.
$C_{11}H_{11}O_4N_3S_2$ requires	C, 42.17;	H, 3.54%.

Attempted Synthesis of 1:2-Di(pyridine-4'-sulphonyl)hydrazine:

Method I.

4-Thiopyridone (1.11 g., 0.01 mole) was treated with chlorine gas, as in method B (p.78) for the preparation of pyridine-4-sulphonamide. Anhydrous hydrazine (0.40 g., 0.015 mole) in dry methanol (5 ml.) was

added slowly to the stirred solution of pyridine-4-sulphonyl chloride in cold dry chloroform. Hydrazine hydrochloride started to separate out and the mixture was left overnight at 0° before filtering. The filtrate was evaporated below 50° under reduced pressure till its volume was about 5 ml., when heating was discontinued. The remainder of the solvent on evaporation left a yellow solid residue which dissolved in methanol and deposited white needles (m.p. 90°-140°) on concentrating. Attempted recrystallization from methanol resulted in decomposition, giving a semi-solid product.

Method II:

4-Thiopyridone (1.11 g., 0.01 mole) was converted to pyridine-4-sulphonyl chloride, as under method B, for the preparation of pyridine-4-sulphonamide. Pyridine-4-sulphonhydrazide (0.97 g., 0.005 mole) in methanol (20 ml.) was added in portions to the cold dry chloroform solution of pyridine-4-sulphonyl chloride. Heat was evolved and the reaction mixture was shaken and cooled in water after each addition. The mixture was left overnight at 0° and gave pyridine-4-sulphonhydrazide monohydrochloride (0.83 g.), which was crystallised by shaking with methanol, filtering and adding ether to the filtrate until it was faintly opalescent. On standing, pale yellow needles were deposited which melted with decomposition at 106°. The product was soluble in water, strongly acidic, gave a positive chloride reaction and reduced ammoniacal silver nitrate in the cold.

Equivalent (titration), 206; picrate from methanol, m.p. 117° (decomp.).

Method III:

4-Thiopyridone (2.22 g., 0.02 mole) was converted to pyridine-4-sulphonyl chloride, as in the previous experiments. The cold, dry chloroformic solution was treated dropwise, shaking and cooling in ice with slightly more than one and one-half molar proportions of anhydrous hydrazine (0.64 g.). Very little heat was generated and the reaction mixture turned slightly yellow in colour. On standing at 0°, an off-white solid was precipitated. It was filtered off, sucked as dry as possible, washed with ice cold water (ca. 20 ml.) and dried *in vacuo* to give 2.2 g. (63%) of pyridine-4-sulphonhydrazide as white needles, m.p. 94-5° (decomp.) from methanol.

Picrate from methanol 110° (decomp.).

The derivative with salicylaldehyde crystallized from ethanol, melted at 166° with decomposition.

The chloroform filtrate from the product was cautiously concentrated under reduced pressure at about 40° and, when the volume was about 2 ml., the last traces of chloroform were removed at laboratory temperature. The semi-solid residue was extracted with ether leaving brown solid material. The ethereal solution was washed with a little water and dried (Na_2SO_4). The ether was removed *in vacuo* at laboratory temperature to give an almost colourless mobile liquid (ca. 0.35 g.), with an odour not unlike pyridine.

Picrate from alcohol, m.p. 144-5°. King and Ware[93] quote 4-chloro-pyridine picrate, m.p. 146°.

The remainder of the liquid was refluxed for 15 hours with

anhydrous hydrazine (2 ml.) in alcohol (8 ml.) and taken down to dryness under reduced pressure on a water bath. The residue was crystallized from alcohol and melted with decomposition at 240°. Koenigs[103] quotes m.p. 238° (decomp.) for 4-pyridylhydrazine hydrochloride. 4-Chloropyridine was similarly isolated as a by-product in the preparation of pyridine-4-sulphonhydrazide by the method described on page 79.

Synthesis of 1:2-di(pyridine-4'-sulphonyl)hydrazine:

4-Thiopyridone (1.0 g.) was converted to pyridine-4-sulphonyl chloride, as in the previous experiments, and added dropwise with vigorous shaking to a well-cooled solution of pyridine-4-sulphonhydrazide (0.865 g.) in dry pyridine (10 ml.) contained in a flask immersed in crushed ice. Globules of a pale-green oil separated out and floated on the surface of the chloroform. The flask was transferred to the refrigerator, left overnight and the solid product filtered off and washed with ether. It was suspended in cold water (10 ml.), filtered and dried *in vacuo*, giving 0.95 g. (60%) of product, m.p. 173-4° (decomp.). It is very insoluble in ethanol, methanol, acetone, ether, benzene, chloroform, petroleum ether, acetic acid and hot or cold water. It dissolved in 20% sodium hydroxide solution, giving a yellow solution with a pyridine-like smell, and also in concentrated hydrochloric acid. It was obtained as a white micro-crystalline solid by dissolving in hot dimethylformamide, adding an equal volume of alcohol and allowing to stand overnight at about 0°. On filtering off, washing liberally with hot alcohol and finally ether the product still melted at 173-4° with decomposition.

Found:	C, 38.54; H, 3.64%.
$C_{10}H_{10}O_4N_4S_2$ requires	C, 38.22; H, 3.21%.

The chloroform filtrate from the reaction mixture contained some orange globules floating on the surface. On evaporating to dryness under reduced pressure on a water bath, a small amount of a dark-blue oil was obtained. Attempted crystallization and also formation of a picrate were unsuccessful.

BACTERIOLOGICAL RESULTS

BACTERIOLOGICAL TESTING OF DERIVATIVES OF PYRIDINE-4-SULPHONYHRAZIDE.

The *in vitro* evaluation of the antituberculous activity of some of the compounds synthesised in this work was measured against *Myco. tuberculosis* var. *hominis* strain CN3679 in Peizer and Schaefer media, media with 33% blood and Dubos media. The author is indebted to Dr. S.R.M. Bushby of the Wellcome Foundation Ltd. for carrying out these tests, the results of which are recorded in Table 2.

For the tests in Dubos media the drugs were added to the media at a concentration of 1,000 µg. per ml., sterilised by tyndallisation at 60°C on two successive days, and then diluted aseptically in two-fold decrements in volumes of 2 ml. in 5 ml. screw-cap bottles. For the tests in Peizer and Schaefer media, the drugs were added to physiological normal saline at ten times the desired strength, sterilised similarly and then serially diluted in two-fold decrements. Volumes of 0.2 ml. were then transferred to 5 ml. screw-cap bottles and 1.8 ml. of the medium added to each at 56°C to give final concentrations of the same order as that used in the Dubos media. The media was then incubated in a sloped position.

The inoculum for the Dubos media was one drop of a seven days old Dubos culture, centrifuged and concentrated to half of the original volume, while for the Peizer and Schaefer media about one hundredth of this inoculum was used, namely, one drop of a four days old culture without concentration run over the surface of the media.

The tests in Dubos media were read after seven and fourteen days incubation at 37°C, and those in the Peizer and Schaefer media after

fourteen and twenty-one days. For the purpose of comparison, the test was also carried out on isoniazid.

TABLE 2

COMPOUND
1-Benzylidene-2-pyridine-4'-sulphonylhydrazine
1-Cinnamylidene-2-pyridine-4'-sulphonylhydrazine
1-cycloHexylidene-2-pyridine-4'-sulphonylhydrazine
1-p-Dimethylaminobenzylidene-2-pyridine-4'-sulphonyl-hydrazine
1-p-Methoxybenzylidene-2-pyridine-4'-sulphonylhydrazine
1-Ethylidene-2-pyridine-4'-sulphonylhydrazine
1-isoPropylidene-2-pyridine-4'-sulphonylhydrazine
1-α-Phenylethylidene-2-pyridine-4'-sulphonylhydrazine
1-m-Methylbenzylidene-2-pyridine-4'-sulphonylhydrazine
1-l-Glucosyl-2-pyridine-4'-sulphonylhydrazine
1-Piperonylidene-2-pyridine-4'-sulphonylhydrazine
1-Salicylidene-2-pyridine-4'-sulphonylhydrazine
1-Hexahydro-2"-4"-6"-trioxo-5-pyrimidylidene-2-pyridine-4'-sulphonylhydrazine
1-p-Hydroxybenzylidene-2-pyridine-4'-sulphonylhydrazine

TABLE 2

Minimum inhibitory concentration µg./ml. in media							
Feiger and Schoetez		33% blood		Dubos			
Test 1	Test 2	Test 1	Test 2	Test 1		Test 2	
21 day	21 day	7 day	7 day	7 day	14 day	7 day	14 day
1000	1000	125	"	1000 (±1000)	1000	1000	1000
1000	1000	125	"	250	500	500	500
1000	1000	125	"	500	1000	500	1000
1000	1000	125	"	250	1000 (± 500)	250	500
1000	1000 (±1000)	125	"	1000 (±500)	1000 (±500)	1000	1000
125	250	125	"	62	125	62	125
1000 (±1000)	1000	125	"	250 (±31)	500 (±250)	250 (±125)	500
1000	1000	125	"	1000	1000	1000	1000
1000	1000	125	"	1000	1000	500	1000
1000	1000 (±1000)	125	125	250	1000 (±500)	250	500
1000	1000	125	"	500	1000 (±500)	500	500
1000 (±1000)	1000	125	"	1000 (±500)	1000	1000	1000
1000	1000	125	"	1000	1000	1000	1000
500	500	125	"	250	250	125	250

TABLE 2 (contd.)

COMPOUND
1-cycloHeptylideno-2-pyridino-4'-sulphonylhydrazino
1-β-Phenylethlideno-2-pyridino-4'-sulphonylhydrazino
1-Acetyl-2-pyridino-4'-sulphonylhydrazino
1:1-Diacetyl-2-pyridino-4'-sulphonylhydrazino
1-Benzoyl-2-pyridino-4'-sulphonylhydrazino hydrochloride
1-o-Hydroxycarbonylbenzoyl-2-pyridino-4'-sulphonyl-hydrazino
1-β-Hydroxycarbonylpropionyl-2-pyridino-4'-sulphonyl-hydrazino
1-β-Hydroxycarbonylacryloyl-2-pyridino-4'-sulphonyl-hydrazino
1-Benzenosulphonyl-2-pyridino-4'-sulphonylhydrazino
1-Pyridino-4'-sulphonyl-2-toluene-p-sulphonylhydrazino
1:2-Di(pyridino-4'-sulphonyl)hydrazino
isoNicotinoylhydrazino (for comparison)

TABLE 2 (contd.)

Minimum inhibitory concentration µg./ml. in media							
Polgar and Schecter		35% blood		Dubos			
Test 1	Test 2	Test 1	Test 2	Test 1		Test 2	
21 day	21 day	7 day	7 day	7 day	14 day	7 day	14 day
1000	1000	125	-	1000	1000	1000	1000
1000	1000	125	-	250	250	125 (±62)	250
1000	1000	125	125	1000	1000	1000	1000
1000	1000	125	125	1000	1000	1000	1000
1000 (±1000)	1000	125	125	500	500	500	500
1000	1000	125	-	1000	1000	1000	1000
1000	1000 (±1000)	125	125	1000	1000	1000	1000
1000	1000	125	-	500	1000	500	1000
1000 (±1000)	1000	125	125	1000	1000 (±1000)	1000 (±1000)	1000
1000 (±1000)	1000 (±1000)	125	125	1000	1000	1000	1000
1000	1000	125	125	1000	1000	1000	1000
0.06	0.06	0.03	0.03	0.03	0.06	0.03	0.06

(±) SIGNIFIES CONCENTRATIONS AT WHICH THERE WAS PARTIAL INHIBITION OF GROWTH.

CONCLUSION.

From the results recorded in Table 2 none of the compounds examined exhibited significant inhibitory action compared with isoniazid, and it is concluded that replacement of the carbonyl group in the isonicotinic series by a sulphonyl group leads to the abolition of tuberculostatic activity.

BACTERIOLOGICAL SCREENING OF PYRIDINE-4-SULPHONAMIDE.

The structural similarity of pyridine-4-sulphonamide to sulphanilamide prompted an examination of the former for bacteriostatic activity against a variety of micro-organisms. The author wishes to express his thanks to Dr. E.O. Morris of the Microbiology Department of this College for carrying out the tests, the results of which are recorded in Table 3.

TABLE 3

Organism	Concentration in broth*			
	M/800	M/1600	M/3200 ...	to M/819,200
Staph. aureus	+	+	+	+
E. coli	+	+	+	+
Ps. aeruginosa	-	+	+	+
Strept. faecalis	+	+	+	+

* Concentrations shown are those in broth, i.e. M/800 represents M/40 diluted 1.20 in broth.

CONCLUSION:

Pyridino-4-sulphonamide shows little or no inhibitory action against the organisms tested.

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