Newer Synthetic Structures of Interest as Tuberculostatic Drugs[1,2]

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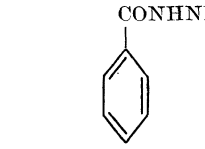
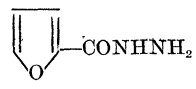
THE *in vitro* growth of the tubercle bacillus is inhibited by an enormous range of organic compounds, few of which exhibit *in vivo* activity. To merit classification as a tuberculostat of interest therefore, a compound should possess marked activity *in vivo*. This criterion has been used in the selection of compounds for discussion in this paper. However, even within the narrow range of compounds possessing *in vivo* activity, assessment of tuberculostats is difficult because tests, techniques, and interpretations differ from laboratory to laboratory. It is not uncommon to have the same compound simultaneously classified active and inactive by two different sources.

Illustrative of this difficulty is the report presented by H. J. White at the Gordon Research Conferences in August 1952. Using a mouse survival test with a bovine strain D_4 of tubercle bacillus, White found benzoylhydrazine I, 2-furoylhydrazine II, 2-thenoylhydrazine III, and 2-thiazolylhydrazine IV all active. When the tuberculostatic activity of pyrazinamide V was discovered, White put the compound through his test and failed to detect activity even in enormous doses. It was obvious therefore that the bovine strain D_4 was not suitable as a screening organism and a switch was made to human strain H37 Rv. With the latter strain, compounds I, II, and III were found to be inactive, compound IV was found still active, and pyrazinamide was, of course, also active.



D_4 (bovine), active
H37 Rv (human), inactive

I

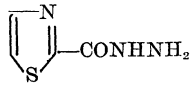


D_4, active
H37 Rv inactive

II

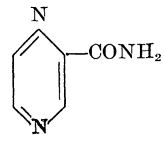
D_4, active
H37 Rv, inactive

III



D_4, active
H37 Rv, active

IV



D_4, inactive
H37 Rv, active

V

[1] Based on a talk given before the Division of Medicinal Chemistry at the 123rd ACS National Meeting in Los Angeles, March 15–19, 1953.

[2] Contribution No. 333 from the Research Laboratories, Hoffmann-La Roche Inc.

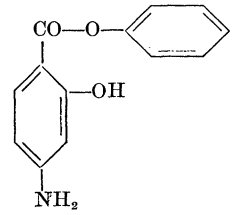
The importance of selecting the proper test organism is further pointed up by the fact that Jouin and Buu Hoi (*1*) screened a large series of chemical compounds, using an *in vitro* test with an avian tuberculosis organism, and missed thereby the antituberculosis activity of nicotinamide.

Similarly, differences in test animals, and even in strains of the same test animal, produce important quantitative differences in the results obtained. In the light of these difficulties, the newer synthetic tuberculostats selected for discussion here are presented uncritically, i.e., without weighting the values assigned to them by the original investigator.

Aliphatic and Isocyclic Compounds

The first notable successes in the search for agents active against tuberculosis were achieved with compounds of the benzenoid series. There have been several recent advances in the field which are claimed to merit attention. Two of these concern modifications of *p*-aminosalicylic acid (PAS).

One of the principal drawbacks to PAS is the rapidity with which it is eliminated, making large and frequent doses necessary to maintain adequate blood levels. Various attempts have been made to overcome this deficiency, with only questionable success. In 1951, Freire, Rist, and Grumbach (*2*) announced the discovery of phenyl *p*-aminosalicylate VI, also known as Fr 7, as their solution to this difficulty.

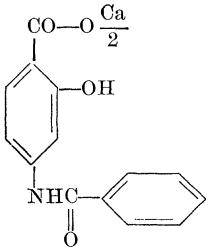


VI (Fr 7)

When administered parenterally in oil solution or aqueous suspension, the compound is supposedly ten times more active than PAS and approximately equal to streptomycin. Orally, it is rated no better than the parent PAS. The difference in parenteral vs oral activity is ascribed to the supposition that the insolubility of the compound via the parenteral route retards elimination and permits the development of higher blood levels for prolonged periods, whereas, orally, it is hydrolyzed to PAS and hence is no better than the latter. The validity of the claim is questioned

by Bavin *et al.* (*3*), who found the compound to be no better than PAS.

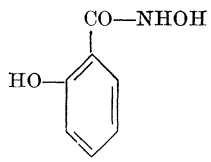
Early this year, another modification of PAS has been proposed as a treatment for genitourinary tuberculosis (*4*). The compound, calcium *p*-benzamidosalicylate VII, is better than PAS in two respects; it produces a sustained and more uniform level of PAS in the blood and it is completely free of the side reactions that accompany the use of the latter drug. The compound has been given to tuberculosis patients



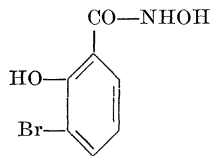
VII

in doses of 15 g/day at intervals of 8 hr and it has been intimated that the dose should probably be higher. Its *in vivo* activity is, therefore, apparently no better than that of PAS and, strangely enough, it is reported to be completely inactive *in vitro*.

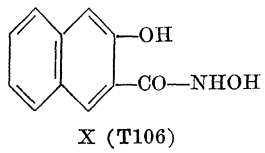
A report by Urbanski (*5*) in 1950 revealed that salicyloyl hydroxamic acid VIII was actively tuberculostatic in mice infected with strain H37 Rv. Issue was taken with this finding by Gardner, Wenis, and Smith (*6*), who prepared the compound and found it to be completely inactive. Apparently undaunted by this adverse judgment, Urbanski and his co-workers (*7*) recently reported on two new hydroxamic acid derivatives, namely, 3-bromosalicyloyl hydroxamic acid IX (T40) and 3-hydroxy-2-naphthoyl hydroxamic acid X (T106).



VIII



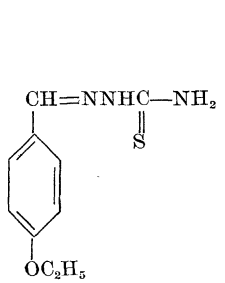
IX (T40)



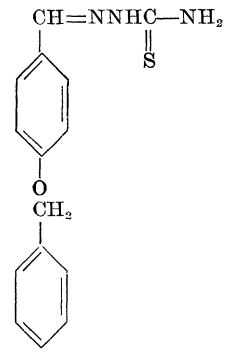
X (T106)

In guinea pig survival experiments using tubercle bacilli of human strain H37 Rv, T40 is said to be almost on a par with streptomycin. Clinically, T40 is claimed to be effective in tuberculous meningitis, miliary tuberculosis, and pulmonary tuberculosis, and gives evidence of radiological improvement in cases of the last category.

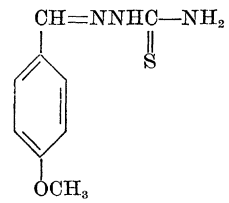
Many changes have been rung on the Tibione structure and most of these changes have been concerned with the subordinate grouping para to the thiosemicarbazone moiety. None of the compounds so derived has as yet been shown to be conclusively better than Tibione itself, though protagonists for one structure or another have appeared from time to time. Two new structures advanced within the past year are *p*-ethoxybenzaldehyde thiosemicarbazone XI (*8*) and *p*-benzyloxybenzaldehyde thiosemicarbazone XII (*9*). The *p*-ethoxy derivative XI is the next higher homologue to Tb II, one of the preferred German formulations, and is not likely to be much different. The benzyloxy derivative XII is reported as being very active in guinea pigs infected with human strain H37 Rv but —what is particularly stressed—is its low order of toxicity.



XI



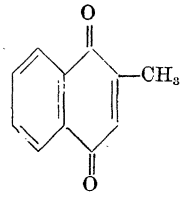
XII



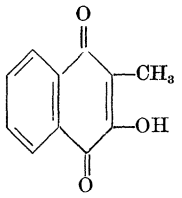
Tb II

It has been known for some time that naphthoquinones in general, and the synthetic vitamin K, 3-methyl-1,4-naphthoquinone (Menadione) XIII, in particular, possess *in vitro* antibiotic properties against a variety of organisms (*10–12*) including the tubercle bacillus (*13, 14*). It has also been known that phthiocol XIV, a compound structurally related to the synthetic vitamin Ks, is a normal component of the tubercle bacillus (*15*). This combination of facts has led Panisset and his co-workers (*16*) to investigate the *in vivo* activity of the synthetic vitamin Ks, Menadione XIII, and Synkayvite[3] (tetrasodium 2-methyl-1,4-naphthohydroquinone diphosporic acid ester) XV, in mice infected with M. tuberculosis var. hominis-H37 Rv and with the bovine type (Ravenel strain), respectively. Their findings show that Menadione exerts a marked tuberculostatic effect against the human type

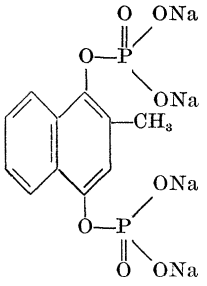
[3] Trade name of Hoffmann-La Roche Inc.



XIII Menadione



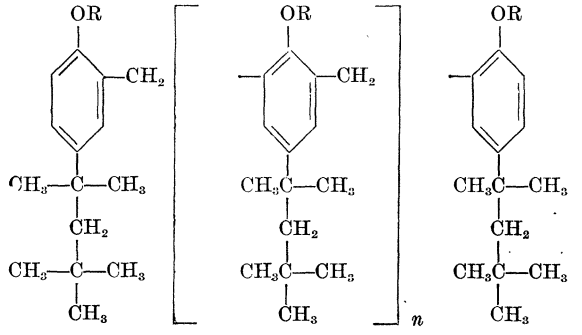
XIV Phthiocol



XV Synkayvite

of organism and that Synkayvite is active against the bovine type. In neither case did the activity appear of a high order, but the compounds are of interest because they may stimulate further work on the tuberculostatic possibilities of the quinoidal type of structure.

The discovery of the antituberculous effect of the surface-active agents known as the Tritons[4] is an outgrowth of an attempt to study the factors that influence the susceptibility of mice to experimental tuberculosis (*17*). To effect an alteration in the body lipids, Triton A20 was injected intravenously in mice with a tuberculous infection. Unexpectedly, it was observed that the infection was markedly suppressed but further study shows Triton A20 to be too toxic. To circumvent this difficulty, a series of compounds of similar structure was synthesized and tested. Though none of these new compounds is of greater efficacy than Triton A20, some of them appear to be less toxic. According to the investigators, those compounds of



R=CH_2CH_2O—$(CH_2CH_2O)_{15-18}$—CH_2CH_2OH

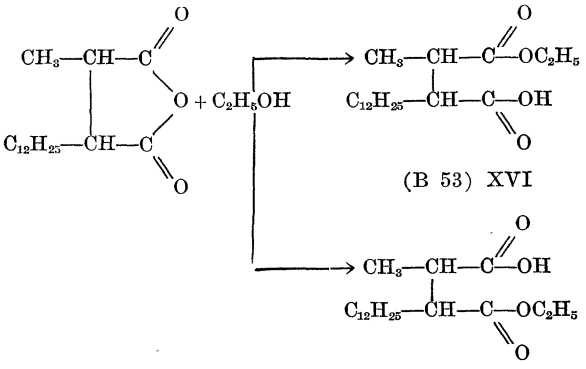
XVI

low molecular weight where *n* equals 0 are very toxic and those in which *n* equals 1 are inactive. For tuberculostatic activity, therefore, *n* must be greater than 1.

[4] Trade name of Rohm and Haas Co.

The *in vivo* tuberculostatic activity of Triton A20 has also been independently reported by Solotorovsky and Gregory (*18*), who find it to be about one-sixth as active as dihydrostreptomycin by parenteral route in mouse infections. More important is the observation that Triton A20 and dihydrostreptomycin act synergistically so that, for example, 2.5 mg of Triton A20 combined with 0.25 mg of dihydrostreptomycin are more effective than either 5 mg of Triton A20 or 1 mg dihydrostreptomycin alone.

Another surface-active substance which has been studied for antituberculous activity is the monoethyl ester of α-methyl α-*n*-dodecyl succinic acid (B 53) XVI. This compound and its isomeric monoester XVII are prepared by treating the corresponding anhydride with ethanol. According to Barry *et al.* (*19*), the compound (B 53) XVI preponderates in the mixture,



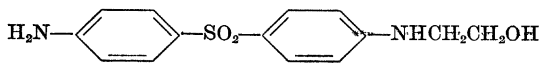
(B 53) XVI

XVII

which is used as such, the presence of the other isomer being ignored. Given orally, in the form of the sodium salt, to guinea pigs infected with human strain H37 Rv at a dose level of 100 mg/kg daily for several months, B 53 is claimed to produce a 20% increase in survival time. This would seem to be a rather modest activity in a compound that is admittedly toxic and which produces a strong hemolytic effect. Nonetheless, it has been clinically tested by local application to tuberculous sinuses and ulcers, where it has been shown to produce rapid healing. Instillation of a buffered 0.5 per cent solution into a tuberculous urinary bladder is claimed to result in the quick elimination of long-standing lesions. Since B 53 activity is antagonized by serum, the compound is unsuited to systemic administration. Barry and his co-workers have therefore extended their studies to dialkyl propane, butane, and pentane diols, and to hydroxyamines with similar carbon skeletons. Although these compounds are held to be promising, no *in vivo* activity data have as yet been presented for them.

Although the sulfones were the first of the modern synthetic tuberculostats, variations of the parent 4,4′-diaminodiphenyl sulfone structure are still under active investigation, as is evidenced by the work of Payne and his collaborators (*47*), who recently reported on the clinical efficacy of 4-amino-4′-(β-hy-

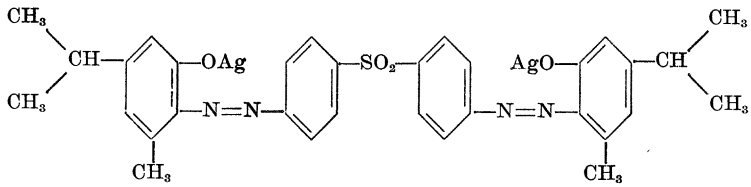
droxyethylamino) diphenyl sulfone. They claim that the compound is a valuable adjunct to streptomycin therapy and that it is well tolerated orally and pro-



4-Amino-4′-(β-hydroxyethylamino) diphenyl sulfone

duces no gastrointestinal irritation in daily doses of 1.5 to 3.0 g. They do not however, recommend that it be used alone and unsupported by other drugs.

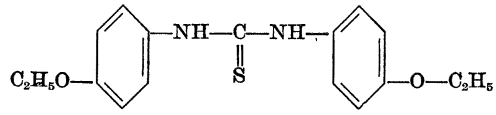
Another new sulfone has also been reported by Weiller and Rymer (*48*). This compound, known as J-51, is said to be the silver salt of 4,4′-bis-(azo-para-isopropyl-meta-cresol) diphenyl sulfone and probably has the following structure:



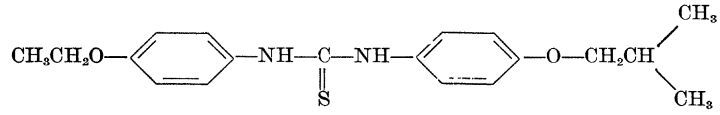
J-51

The compound has been tested clinically in 50 patients and is said to be definitely beneficial both clinically and radiologically. Moreover, only one toxic reaction with the drug was obtained in this series. This reaction took the form of a severe diahorrea which necessitated withdrawal of the drug.

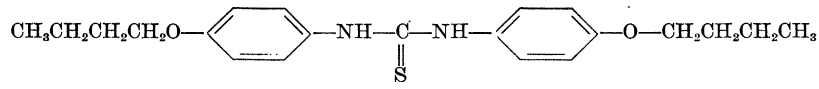
Most recently, an entirely new class of antituberculous compounds was announced by Heubner *et al.* (*49*) and by Mayer, Eisman, and Konopka (*50*). These workers discovered that 4,4′-diethoxythiocarbanilide possessed high antituberculosis activity in mice infected with the H37 Rv strain. In a subsequent investigation of over 300 thiocarbanilides and related structures, they found that the structure-activity relationship in this series is quite specific. For example, the following variations in structure resulted in loss of activity: (1) shortening of the alkoxy group to methoxy or lengthening it to octyloxy, (2) replacement of the alkoxy groups with an alkyl group branched at the carbon adjoining the ring, (3) replacement of both alkoxy groups with halogens or dialkylamino groups, (4) removal of one of the alkoxy groups, (5) shifting the alkoxy groups to the 2 or 3 position, (6) placement of a second substituent (methyl, halogen, or amino) in the ring, (7) substitution of a methyl group on one of the ureido nitrogens, (8) replacement of the thiocarbanilide moiety with the corresponding carbanilide, guanidine, guanylthiourea or dithiobiuret.



According to these investigators, the results of delayed and limited therapy experiments in mice and guinea pigs, together with the high therapeutic index shown by some of the thiocarbanilides, warrant their consideration as therapeutic agents in tuberculosis. Moreover, they claim there is no cross resistance with either streptomycin or isoniazid and that, on the basis of their preliminary studies, no resistant forms arise in animal experiments. The activities of certain of their compounds, notably 4-ethoxy-4′-isobutoxy thiocarbanilide (Su 1795) and 4,4′-di-*n*-butoxythiocarbanilide (Su 1906), are said to exceed those of PAS or streptomycin and to approach that of isoniazid in guinea-pig experiments.

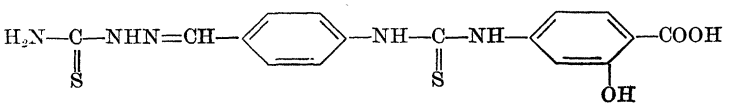


(Su 1795)



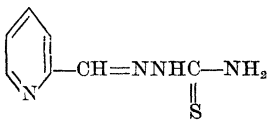
(Su 1906)

Quite independently, Harrington, D'Arcy Hart, and Rees in England (*51*), intrigued by the observation that certain compounds containing the thiocarbamido grouping manifested *in vitro* anti-tuberculous activity, conceived the idea of combining in one molecule PAS, *p*-aminobenzaldehyde thiosemicarbazone, and the thiocarbamido group. The result was a thiocarbanilide, a compound said to be more active than PAS and comparable to the thiosemicarbazones in mouse experiments. The structure of this thiocarbanilide is as follows:

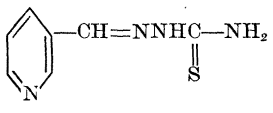


Pyridine Derivatives

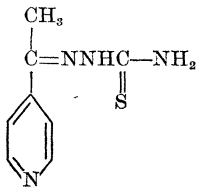
The most significant advances in the search for synthetic tuberculostats have been made in the field of the heterocycles, most notably the nitrogen heterocycles of the pyridine series. Chronologically, the first pyridine compounds to merit consideration as tuberculostats of interest—other than nicotinamide and its immediate derivatives—are the three isomeric pyridyl aldehydes and one pyridyl ketone thiosemicarbazone. Actually, the α-isomer, picolinaldehyde thiosemicarbazone XVIII (*20*) is too toxic for practical use (*21*), and the methyl 4-pyridyl ketone thiosemicarbazone XIX is principally interesting because it is the only active pyridyl ketone derivative known. The β-isomer, nicotinaldehyde thiosemicarbazone XX, was prepared independently in Switzerland (*22*), France (*23*), and the United States (*24*), and is regarded by the French workers as much superior to Tb I on the basis of animal studies (*23, 25, 26*). At least partial confirmation of this view has been obtained here (*21*).



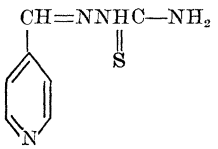
α XVIII



β XX



XIX



γ XXI

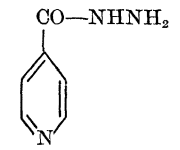
The γ-isomer, isonicotinaldehyde thiosemicarbazone XXI (*20*), is comparable to the β-isomer in activity and is somewhat less toxic. Both the β and γ isomers in comparison with Tb I reveal one noteworthy point of distinction. When the three compounds are tested in mice infected intravenously with human strain H37 Rv, they show activities of approximately the same magnitude. In mice infected intranasally with the same organism, the two pyridine derivatives appear much more active than Tb I. The significance of this difference in activity with variation in route of infection has not yet been elucidated, but it may conceivably have important ramifications in the mechanism of drug action. The pertinent data are presented in Table 1.

The discovery in the Roche Laboratories of isonicotinyl hydrazine and its derivatives as synthetic tuberculostats followed closely upon the preparation of the pyridylaldehyde thiosemicarbazones (*20, 27, 28*). A large number of reports on the activity of isonicotinyl hydrazine in both experimental and clinical tuberculosis have already appeared in the literature, so that a detailed discussion of the subject at this point is unnecessary. However, this much can be said with assurance: isonicotinyl hydrazine XXII is the most potent tuberculostat clinically tested to date. It is also the parent substance to a host of derivatives with marked tuberculostatic properties. These derivatives may be conveniently divided into 4 classes, i.e., the alkylidene derivatives XXIII, the sugar derivatives, the alkyl and aralkyl derivatives XXIV, and the acyl derivatives XXV.

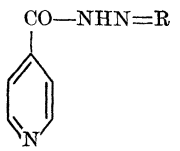
TABLE 1

Activity of Thiosemicarbazones in Mice Infected with Human Strain H37 Rv (Medicated Diet)

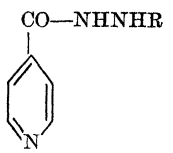
Compound	Intravenous route		Intranasal route	
	Approx. dose, mg/kg	Per cent protection	Approx. dose, mg/kg	Per cent protection
Tb I	125	50	500	30
Nicotinaldehyde thiosemicarbazone	125	83.3	100	55.5
Isonicotinaldehyde thiosemicarbazone	125	80	100	60



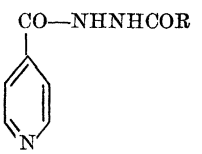
XXII



XXIII

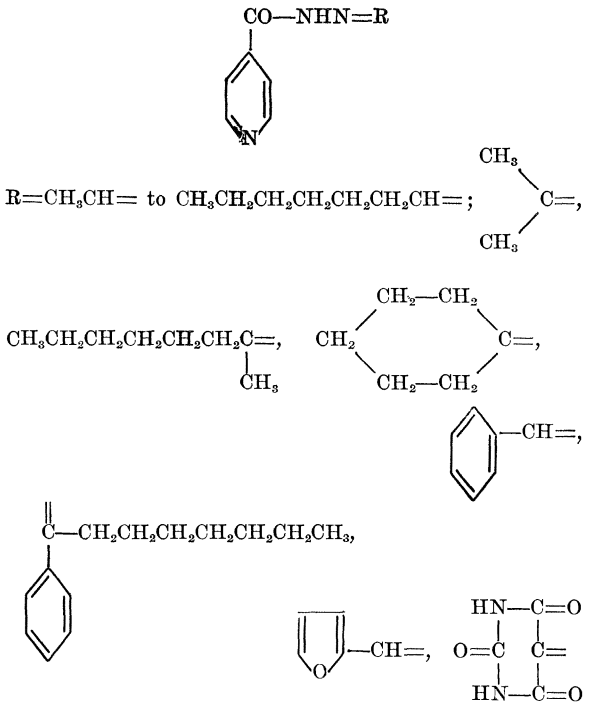


XXIV

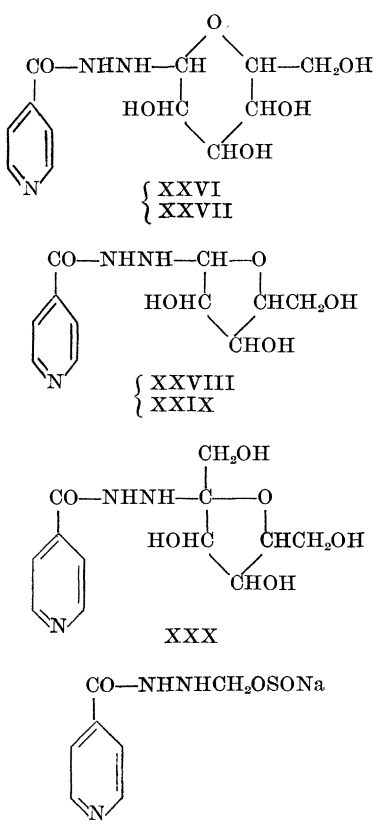


XXV

The alkylidene derivatives prepared by Fox and Gibas (*29*) cover the range of straight-chain aliphatic substituents from ethylidene to heptylidene and of branched-chain aliphatic substituents from isopropylidine through α-methyl heptylidene to cyclohexylidene. A variety of aralkylidenes such as benzylidene and α-heptylbenzylidene and heterocyclic substituents such as furfurylidene and 2,4,5-trioxohexahydropyrimidylidene were also prepared.

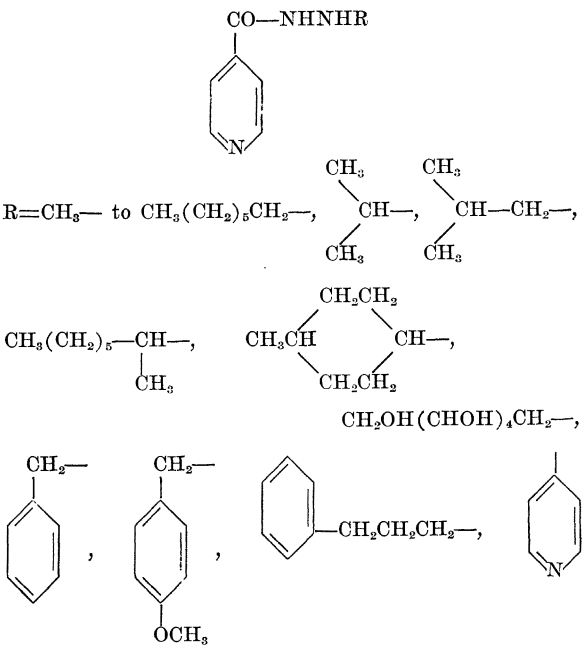


These compounds are for the most part powerfully tuberculostatic in experimental tuberculosis.

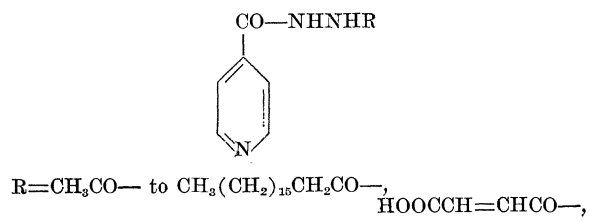


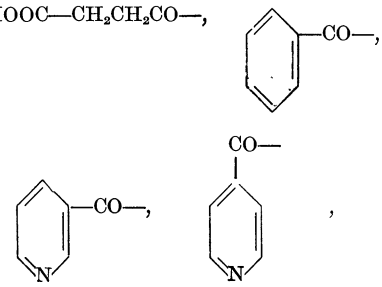
The sugar derivatives of isonicotinyl hydrazine which have been prepared (*30*) include the *d*-glucosyl XXVI, the *d*-galactosyl XXVII, the *d*-xylosyl XXVIII, the *d*-ribosyl XXIX, and the *d*(-)fructosyl XXX. In addition, the sodium formaldehyde sulfoxylate derivative XXXI has been made and studied. These, too, have an activity closely comparable to that of isonicotinyl hydrazine.

The straight-chain alkyl derivatives of isonicotinyl hydrazine prepared by Fox and Gibas (*31*) cover the range from methyl to heptyl and include a sugar alcohol. The branched-chain derivatives include isopropyl, isobutyl, methyl heptyl, and several cyclohexyl substituents. The effect of aralkyl, aryl, and heterocyclic grouping was studied by preparing a series of compounds whose substituents include benzyl, substituted benzyl, γ-phenyl propyl, and γ-pyridyl.



The activity of these compounds is mostly of a high order but no generalized relationship seems to exist between the size or configuration of the substituent group and the tuberculostatic potency of the compound. The effect of acylating isonicotinyl hydrazine has been studied (*32*) with a wide variety of organic acids including the range of fatty acids from acetyl to stearoyl, dicarboxylic acids such as maleic, succinic, and phthalic, and heterocyclic carboxylic acids such as nicotinic, isonicotinic, and furoic.

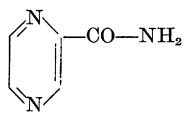


HOOC—CH_2CH_2CO—, 

The acylated derivatives are, in general, very active, and here, too, there is no apparent relationship between size of the substituent grouping and activity.

The Diazines

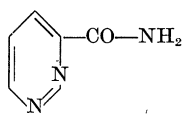
The tuberculostatic activity of pyrazine carboxamide XXXII was first announced by Kushner and his co-workers (*33*), who reported that the compound is several times more active than PAS and approximately seven times as active as nicotinamide. This has been partly confirmed by Rogers *et al.* (*34*), who appear to have prepared the compound independently. The latter group reports an activity of approximately three times that of either PAS or nicotinamide. Clinically, the compound is said to produce an immediate



XXXII

symptomatic improvement which borders on euphoria and seems to be out of proportion to its admittedly moderate activity (*35*). It also produces rapid defervescence and reduction in cough and sputum, even in patients with streptomycin-resistant organisms. Despite these favorable aspects and the mildness of its toxic reactions, pyrazinamide has only limited use because of the rapidity with which drug resistance becomes manifest.

Another diazine with tuberculostatic activity is pyridazine-3-carboxamide XXXIII (*34*), which has been found to be at least as effective as nicotinamide on parenteral administration.

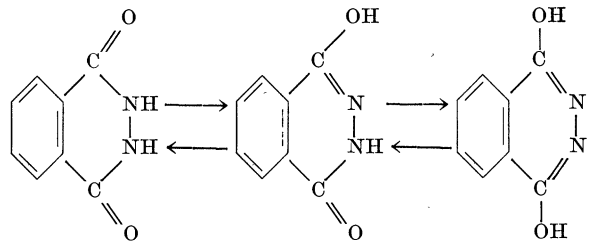


XXXIII

Unlike isonicotinyl hydrazine, both diazines show extreme structure-activity specificity. Any change in structure appears to abolish activity.

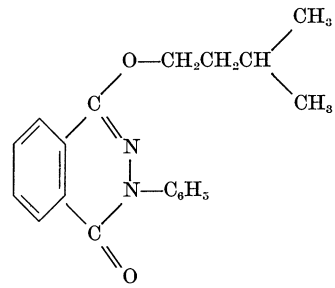
The Phthalylhydrazines

In 1946, Jouin and Buu Hoi (*1*), upon testing a huge series of different chemical structures for *in vitro* activity against an avian type of M. tuberculosis, found N, N′-phthalylhydrazine XXXIV to be markedly active. Three years later, in 1949, Buu Hoi and



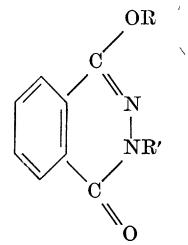
XXXIV

his co-workers (*36*) investigated derivatives of phthalylhydrazine and reported that 1-isoamyloxy-3-phenyl-4-keto-3,4-dihydrophthalazine XXXV is more active in a mouse survival test against human type of tubercle



XXXV

bacilli than is streptomycin. More recently, in a continuation of this work, the French investigators have listed a series of phthalylhydrazine derivatives which are claimed to be very active in mouse tuberculosis, particularly in chronic forms of the disease (*37*). The compounds studied include the following structures:



	R	R′
1)	—$CH_2CH_3CH(CH_3)_2$	—C_6H_5 (compound XXXV)
2)	—$CH_2C_6H_5$	—$CH_2C_6H_5$
3)	—$CH_2(CH_2)_{10}CH_3$	—$CH_2(CH_2)_{10}CH_3$
4)	—$CH_2(CH_2)_{10}CH_3$	—C_6H_5
5)	—	—C_6H_5

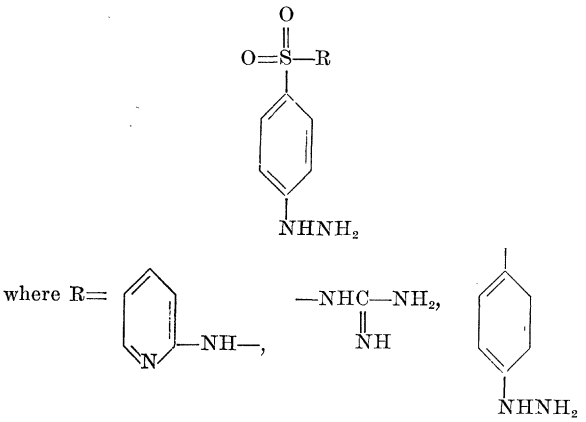
When tested against certain human strains of tubercle bacilli, the compounds proved to be superior to streptomycin plus PAS, but against human strain H37 Rv

their activity is much less spectacular than that of streptomycin. It was noted, however, that the action of the compounds given subcutaneously in oil solution seemed to persist long after cessation of treatment, presumably because of slow adsorption. The investigators are currently modifying the phthalylhydrazine structure in the hope of obtaining less toxic compounds.

Partial confirmation of these results has been obtained by Bavin *et al.* (*3*), who claim that 1,4-diketo-3-phenyltetrahydrophthalazine (compound 5, above) gives promising results in the usual test in mice.

Miscellaneous Hydrazines

Before the advent of the modern chemotherapeutants for tuberculosis, some Japanese investigators (*38–40*) reported that phenylhydrazine inhibits tuberculous changes in guinea pigs. Unfortunately, phenylhydrazine also produces a profound anemia and was therefore deemed unsuited for clinical application. Subsequently, Corper and Cohn (*41*) refuted the tuberculostatic activity of phenylhydrazine while confirming its hemolytic effect. Nonetheless, Takeda and his co-workers, on the basis of the original claim, conceived the idea of modifying phenylhydrazine structure with suitable substituents so as to retain the antituberculous activity and diminish its toxicity. Accordingly, they have prepared and studied compounds of the general type:



These compounds are reported to be active against tubercle bacilli of human type strain H_2 in guinea pigs (*42–44*). The *p,p'*-dihydrazinodiphenyl sulfone, for example, is said to be simultaneously more active and less toxic than the analogous *p,p'*-diaminodiphenyl sulfone (*44*). Moreover, preliminary clinical trials with the compound in nodular type leprosy are reported to be encouraging (*45*).

Conclusion. It was indicated earlier in this paper that the tuberculostatic activity of the various compounds would be presented uncritically, i.e., without weighing the values assigned to them by the original investigators. Experience has shown, however, that many compounds that are initially reported to show great promise never mature through the normal transitional phases of drug development. Some of them never leave the laboratory for the clinic and others, having reached the clinic, fail to qualify as acceptable drugs. It is to be expected then that many of the compounds discussed here will in time be only of academic, or at most, historic interest. But that is not necessarily the full measure of their importance. To the chemist, at least, these overt failures may provide the stimuli and the points of departure for further and possibly more successful research. For example, the failure of the sulfathiadiazoles as tuberculostats led Behnisch and his co-workers (*46*) to the discovery of Tb I and the benzenoid thiosemicarbazones. Similarly, the distinct but relatively inadequate activity of 3-aminoisonicotinic acid proved to be an important stimulus to the discovery of isonicotinylhydrazine (*28*). Perhaps, in much the same way, several of the newer structures—if not in themselves successful—may prove to be fruitful sources of inspiration for further research.

One thing is certain, further research is still very much in order. Although it is too early to pass any final judgments on the role of isonicotinylhydrazine in the control of tuberculosis, it seems likely that no one drug will provide the complete answer. The chemotherapy of tuberculosis in the future will probably involve a combination of two or more drugs, each of which will attack the tubercle bacillus via a different route, so that clinical arrest (if not sterilization) can be obtained before the emergence of resistant strains.

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Cyanopsin, A New Pigment of Cone Vision

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THE three visual pigments known heretofore are formed by combinations between two retinenes and two visual proteins or opsins. Retinene$_1$ combines with rod opsin (scotopsin) to make rhodopsin, or with cone opsin (photopsin) to make iodopsin. Retinene$_2$ combines with rod opsin to make porphyropsin (*1–3*). Clearly a fourth combination is possible: retinene$_2$ with cone opsin. This has now been prepared. It is a blue, light-sensitive pigment, with an absorption maximum at 620 mμ. We propose to call it *cyanopsin.*

It is synthesized as follows. An extract of dark-adapted rods and cones from the chicken retina contains a mixture of rhodopsin and iodopsin. With deep red light, to which rhodopsin is insensitive, the iodopsin alone is bleached irreversibly to a mixture of all-trans retinene$_1$ and cone opsin. To this is added a small amount of the specific cis isomer of retinene$_2$ which, when mixed with rod opsin, forms porphyropsin (*2, 3*). Added in this instance to cone opsin, it forms cyanopsin.

The synthesis of cyanopsin is completed within 5 minutes in the dark at room temperature. The absorption spectrum of the resulting solution is measured in the dark, and again after bleaching in deep red light. The former spectrum minus the latter is the difference spectrum of cyanopsin. This is shown in Fig. 1.

In red light, cyanopsin bleaches to a straw-colored mixture of all-trans retinene$_2$ and cone opsin. The absorption falls in the red and yellow, simultaneously rising in the blue and violet. At about 502 mμ—the "isosbestic" point—the extinction does not change. Above this wavelength the difference spectrum is positive, with a maximum at 620 mμ; below this wavelength it is negative, with a minimum at about 407 mμ, the absorption maximum of retinene$_2$.

Such a difference spectrum is defined by the equation

(Spectrum of cyanopsin) − (Spectrum of all-trans retinene$_2$ + opsin) = Difference spectrum

Our preparation of cyanopsin was formed in a chicken retinal extract in the presence of a variety of impurities. A difference spectrum, however, is by its nature "pure." It represents only the change in spectrum of the photosensitive material bleached by light—in this case cyanopsin—regardless of whatever else is present. We can measure also the spectrum of a mixture of crystalline all-trans retinene$_2$ and opsin. The sum of these spectra, according to the above equation, should represent the absorption spectrum of pure cyanopsin.

Such an estimate of the spectrum of cyanopsin is shown in Fig. 2. It was obtained by adding together the difference spectrum of Fig. 1 and the spectrum of retinene$_2$ plus opsin shown in Fig. 2. This procedure has two arbitrary aspects. Not having a pure preparation of chicken cone opsin, we have substituted here a good preparation of cattle rod opsin. The opsins possess only the protein absorption band at about 280 mμ, and have very low extinctions at wavelengths longer than 310 mμ. Their own absorption therefore does not affect appreciably the spectra shown in Fig. 2. They do, however, have an effect upon the spectrum of retinene$_2$. The retinenes couple spontaneously in solution with the amino groups of opsins and other proteins to yield loosely bound complexes

[1] We should like to dedicate this paper to Professor Otto Loewi on his eightieth birthday. The chemistry of excitation owes much to him; and in a broader sense excitation and vision are very much his province.

[2] This research has been supported in part by funds from the Rockefeller Foundation and the Office of Naval Research. We are grateful also to the Organic Research Laboratory of Distillation Products Industries of Rochester, N. Y., for a gift of crystalline retinene$_2$.