THE PREPARATION AND ANTIBACTERIAL ACTIVITY OF 2-PHENACYLPYRIDINE AND RELATED KETONES

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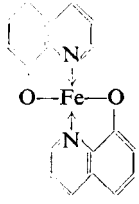
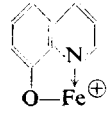
IT has been shown[1] that, of the 7 mono-hydroxyquinoline isomers, only 8-hydroxyquinoline (oxine) exhibits antibacterial properties, and this alone of the isomers is capable of binding metals by chelation. Oxine is non-toxic to *Staph. aureus* unless traces of iron (or copper) are present in the medium[2]. Evidence has been presented which indicates that oxine probably exerts its antibacterial effects by penetrating into the cell as a lipoid soluble complex in which 1 atom of metal is bound by 2 molecules of oxine (1:2 complex) (I), whereas the toxic effects inside the cell are due to the unsaturated 1:1 complex (II)[3]. The introduction of *N* atoms



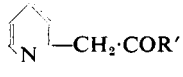
I



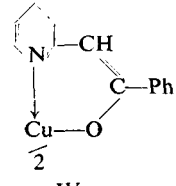
II

into the aromatic rings of the oxine molecule to give aza-oxines results in the reduction of the lipoid solubility of the system and a decrease in the antibacterial properties, whereas both these effects are reversed by increasing the lipoid solubility by further substitution with alkyl groups. The success which has attended the investigations of the co-ordinating properties of the oxine type of molecule, and their implications in the antibacterial activities of these compounds, has stimulated the search for useful antibacterial substances amongst potential complexing agents[1,4,5,6,7,8].

This present study was envisaged as an attempt to provide further information concerning co-ordination phenomena and antibacterial properties. The 2-picolylketone system (III) was chosen because



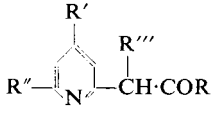
III



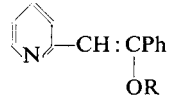
IV

Goldberg *et al.*[9] had already shown that the parent ketone, 2-phenacylpyridine (III, R = H, R′ = Ph) could co-ordinate with cupric ions. Co-ordination presumably occurs via the enolic form of this substance to yield a copper complex (IV), which may be regarded as analogous to the oxine-copper complex. Furthermore, the 2-picolylketone system offers many possibilities for suitable modifications. These include the alteration of the electron density at the nitrogen atom by substitution of the pyridine ring, and the variation of the percentage of the enolic form and the acidity of the enol function by substitution of the methylene and phenyl groups. Such changes could be used to vary the stability and the lipoid solubility of the metal complexes[10,11] and so makes possible an examination of co-ordination phenomena and antibacterial activity.

Preparation of the compounds. The *C*-alkyl derivatives of 2-phenacylpyridine (V, R = R″ = H, R = Ph, R‴ = alkyl groups) were prepared



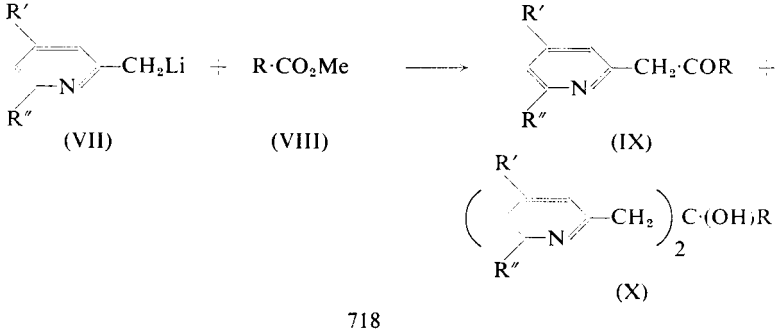
V



VI

by the reaction of the sodio-derivative of 2-phenacylpyridine with the appropriate alkyl halide. Methyl, ethyl, propyl, allyl and benzyl halides gave the *C*-derivatives exclusively (Compounds 2 to 6), but 2-dimethylaminoethyl chloride gave the *O*-derivative (VI; R = $CH_2 \cdot CH_2 \cdot NMe_2$) (Compound 11). The *C*-aroyl compounds (V; R′ = R″ = H, R = Ph, R‴ = CO Aryl) (Compounds 8 to 10) were prepared by the reaction of the appropriate anhydride or acid chloride with the sodio-derivative of 2-phenacylpyridine. Acetic anhydride, on the other hand, gave the *O*-acyl compound (VI; R = Ac) (Compound 12). The details of the methods of preparation and the proof of the structures of the above compounds have been given elsewhere[12].

Those substances possessing substituents in the phenyl ring of 2-phenacylpyridine (Compounds 17 and 19 to 25), or those derived by the replacement of the phenyl by other groups (Compounds 13 to 16), were prepared by the condensation of 2-pyridylmethyl-lithium with the methyl ester of the appropriate acid using a 2 : 1 molar ratio of lithium derivative to ester. As outlined in the following scheme, there is a possibility of the reaction proceeding to give the tertiary alcohols (X) as well as the desired



(VII) (VIII) (IX) (X)

ketones (IX). When the above-mentioned molar ratios were used, however, acylation of 2-pyridylmethyl-lithium with the methyl esters of furoic, naphthoic, benzoic and halogen, methoxy, and methyl substituted benzoic acids yielded the desired ketones in 60 to 80 per cent. yields and tertiary alcohols could not be isolated from the products of the reaction. Likewise, the ketone was the major product when methyl *p*-aminobenzoate was employed as the acylating agent and a 3 molar proportion of 2-pyridylmethyl lithium was used (cf. Scholfield[13] and Schofield and Nunn[14], who used those molar proportions for the condensation of *o*-aminobenzophenone with the lithium derivatives of pyridyl bases). From the reactions in which methyl esters of aliphatic acids were employed, both tertiary alcohols (X; R′ = R″ = H, R = Me or Et) and the desired ketones (IX; R′ = R″ = H, R = Me or Et) could be isolated, e.g., the tertiary alcohol and the ketone were isolated in yields of 30 per cent. and 25 per cent. respectively from the reaction product of methyl acetate and 2-pyridylmethyl-lithium (cf. Levine *et al.*[9]).

The presence of additional methyl-substituents in 2-methylpyridine allows of the possibility of the reaction with lithium yielding a mixture of lithium derivatives which may result in a complex mixture of ketones and tertiary alcohols being formed upon acylation. However, there is evidence that the 4-methyl group is less susceptible to attack by lithium than the 2-methyl group because Erlenmeyer and colleagues[15] were unsuccessful in their attempts to prepare 4-pyridylmethyl-lithium. From the product of the reaction of 2:4-dimethylpyridine (2:4-lutidine) with an equimolecular proportion of phenyllithium followed by a semi-molecular proportion of methyl benzoate, we were able to isolate 4-methyl-2-phenacylpyridine (IX; R″ = H, R′ = Me, R = Ph) in a 65 per cent. yield. Similarly, the use of methyl *m*-chlorobenzoate as the acylating agent resulted in 2-*m*-chlorophenacyl-4-methylpyridine in a 78 per cent. yield. (The fact that the 2-methyl position has been acylated is demonstrated by the green colours given by ethanolic ferric chloride solution with the ketones derived from 2:4-dimethylpyridine, and by the metal co-ordination properties of these compounds.) It is evident that the reaction of equimolecular proportions of phenyllithium and 2:4-dimethylpyridine gives 4-methyl-2-pyridylmethyl-lithium almost exclusively. Under comparable conditions, 2:6-dimethylpyridine gave 6-methyl-2-phenacylpyridine (82 per cent. yield) and 2-*m*-chlorophenacyl-6-methylpyridine (80 per cent. yield) respectively. Carbinols could not be isolated from any of the above reactions involving dimethylpyridines.

The pertinent analytical data and certain properties of those ketones not already reported in an earlier communication, are recorded in Table I and the attached footnotes. Details of the two carbinols (X; R = Me or Et; R′ = R″ = H) isolated are also given in the footnote to this table.

Experimental

Chemical

Microanalyses were made by Mr. G. S. Crouch, School of Pharmacy, University of London. Equivalent weights, except those of picrates, were

TABLE I

KETONES PREPARED FROM 2-METHYLPYRIDINE, 2:4- AND 2:6-DIMETHYLPYRIDINE

	Compound	M.pt. °C.	Physical form	Found			Formula	Required			Yield per cent.	Equivalent	
				C	H	N		C	H	N		Reqd.	Found
13	1:2'-Pyridylpropan-2-one (*a*)	—	yellow oil	—	—	—	C_9H_9NO	—	—	—	25	135	137
14	1:2'-Pyridylbutan-2-one (*b*)	—	,,	—	—	—	$C_{10}H_{11}NO$	—	—	—	35	149	147
15	α-(2'-Furoyl)-2-methylpyridine (*c*)	50–51	yellow prisms (*i*)	—	—	—	$C_{11}H_9NO_2$	—	—	—	48	187	190
16	α-(1'-Naphthoyl)-2-methylpyridine (*d*)	48	,, ,, (*i*)	82·8	5·4	5·8	$C_{17}H_{13}NO$	82·55	5·3	5·7	68	247	246
17	2-*p*-Aminophenacylpyridine	119·5	,, ,, (*j*)	73·2	5·8	13·2	$C_{13}H_{12}N_2O$	73·6	5·7	13·2	50	106	107
18	2-*p*-Hydroxyphenacylpyridine	148	,, ,, (*j*)	74·0	5·1	6·5	$C_{13}H_{11}NO_2$	73·2	5·2	6·6	—	211	213
19	2-*p*-Methoxyphenacylpyridine	84·5	,, ,, (*j*)	74·2	5·7	6·3	$C_{14}H_{13}NO_2$	74·0	5·7	6·2	75	227	227
20	2-*p*-Methylphenacylpyridine	67	,, ,, (*k*)	79·5	6·15	6·6	$C_{14}H_{13}NO$	79·4	6·0	6·6	80	211	211
21	2-*p*-Fluorophenacylpyridine	92·5	,, ,, (*j*)	72·6	4·8	6·4	$C_{13}H_{10}NOF$	72·55	4·65	6·5	76	215	211
22	2-*o*-Chlorophenacylpyridine (*e*)	—	yellow oil ,,	67·9	4·4	—	$C_{13}H_{10}NOCl$	67·4	4·35	—	74	232	230
23	2-*p*-Chlorophenacylpyridine (*f*)	89	yellow prisms (*j*)	—	—	—	$C_{13}H_{10}NOCl$	—	—	—	80	232	235
24	2-*p*-Bromophenacylpyridine	99	,, ,, (*j*)	56·5	3·7	4·9	$C_{13}H_{10}NOBr$	56·5	3·65	5·1	72	276	274
25	2-*m*-Chlorophenacylpyridine	58	,, ,, (*k*)	67·4	4·45	6·3	$C_{13}H_{10}NOCl$	67·4	4·35	6·05	70	232	229
26	6-Methyl-2-phenacylpyridine (*g*)	78·5	,, ,, (*k*)	—	—	—	$C_{14}H_{13}NO$	—	—	—	82	211	212
27	2-(*m*-Chlorophenacyl)-6-methylpyridine	53	,, ,, (*k*)	68·7	5·0	5·85	$C_{14}H_{12}NOCl$	68·4	4·9	5·7	85	246	244
28	4-Methyl-2-phenacylpyridine (*h*)	53	,, ,, (*k*)	79·8	6·3	6·8	$C_{14}H_{13}NO$	79·6	6·2	6·6	65	211	213
29	2-(*m*-Chlorophenacyl)-4-methylpyridine	84	,, ,, (*j*)	68·4	4·7	5·7	$C_{14}H_{12}NOCl$	68·4	4·9	5·7	78	246	243

(*a*) B.pt. 50–60° C./0·5–1 mm., picrate, m.pt. 141–142° C. In addition 2-methyl-1:3-di-(2'-pyridyl)propan-2-ol (X; R = Me; R′ = R″ = H), b.pt. 130–140° C./0·5–1 mm., dipicrate, m.pt. 214° C. (decomp.), was also isolated in 30 per cent. yield, cf. ref. 9.
(*b*) B.pt. 80–90° C./2 mm., picrate m.pt. 146–147° C. In addition 2-propyl-1:3-di-(2'-pyridyl)propan-2-ol (X; R = Et; R′ = R = H), b.pt. 160–170° C./0·5–1·5 mm. dipicrate, m.pt. 196–198° C. (decomp.), was also isolated in 20 per cent. yield, cf. ref. 9.
(*c*) Picrate, m.pt. 149–150° C., see ref. 16.
(*d*) Picrate, m.pt. 201–202° C. (decomp.); found: N, 11·6 per cent.; equiv., 476. $C_{23}H_{18}N_4O_8$ requires; N, 11·8 per cent.; equiv., 476.
(*e*) n_D^{20} 1·5990. Picrate, m.pt. 173° C.; found: N, 12·4 per cent.; equiv., 462. $C_{19}H_{13}N_4O_8Cl$ requires; N, 12·15 per cent.; equiv., 461.
(*f*) See ref. 17.
(*g*) See ref. 18.
(*h*) Picrate, m.pt. 153° C.; found: N, 12·95 per cent.; equiv., 441. $C_{20}H_{16}N_4O_8$ requires; N, 12·7 per cent.; equiv., 440.
(*i*) Solvent, ether.
(*j*) Solvent, 95 per cent. ethanol.
(*k*) Solvent, light petroleum. B.pt. 40–60.

determined by titration with 0·2N-perchloric acid in acetic acid; those of the picrates were determined by titration with 0·02N-sodium hydroxide in 1:1 ethanol-acetone with ethyl bis-2:4-dinitrophenylacetate as indicator.

Preparation of the compounds. The preparation of compounds 2 to 12 has been described in an earlier communication (Beckett and Kerridge[12]).

The general procedure adopted for the preparation of compounds 13 to 17 and 19 to 29 is described below.

Preparation of the lithium derivatives of the methylpyridine bases. The appropriate pyridine base (0·05 mole), freshly distilled over barium oxide, was added with vigorous stirring to a solution of phenyllithium (0·05 mole; from 0·69 g. of lithium and 7·9 g. of bromobenzene) in ether (60 ml.). Addition was carried out under nitrogen and at such a rate that the ether did not reflux. The dark red-brown solution of the lithium derivative was stirred at room temperature for a further period of 15 minutes.

General procedure for the preparation of compounds nos. 13 *to* 16, 19 *to* 29. Since all the compounds were prepared under essentially the same conditions, only the procedure for 2-phenacylpyridine is described.

Methyl benzoate (3·66 g., 0·025 mole), in ether (15 ml.), was added over a period of 10 minutes to a rapidly stirred solution of 2-pyridylmethyl-lithium (0·05 mole) in a nitrogen atmosphere. After the addition of the ester, the mixture was poured onto a slurry of ammonium chloride (4 g.) and crushed ice (30 g.). The ethereal layer was separated and the aqueous phase extracted with ether (3 × 20 ml.) or until the extracts no longer gave a colour with alcoholic ferric chloride solution. The total organic extract was dried ($MgSO_4$), the ether distilled under reduced pressure, and the residue fractionally distilled in vacuum. In this way 2-phenacylpyridine (4·0 g., 75 per cent.), b.pt. 140 to 150° C./2 mm., yellow needles (from ethanol), m.pt. 56° C., was obtained.

Isolation of 2-pyridylmethyl ketones. Picrates, of those compounds which were oils or did not crystallise, were formed by mixing equivalent quantities of picric acid and pyridyl base in ethanol. The pyridyl base was regenerated by dissolving the picrate in a mixture of equal parts of water, ethanol, and acetone and warming with an equivalent quantity of lithium hydroxide. The solution was diluted with water, the free pyridyl base extracted with ether, and the residue obtained by evaporating the ethereal extract chromatographed on alumina. Compounds which had failed to crystallise on standing or after chromatography, usually crystallised on being purified via the picrate.

2-*p*-*Aminophenacylpyridine* (*Cpd. no.* 17). Methyl *p*-aminobenzoate (3·8 g.; 0·025 mole), in ether (15 ml.) was added, under an atmosphere of nitrogen, to a solution of 2-pyridylmethyl-lithium (0·075 mole; from 1·03 g. of lithium in 90 ml. of ether). The mixture was stirred at room temperature for 4 hours and then decomposed and worked up as described under "General procedure."

2-*p*-*Hydroxyphenacylpyridine* (*Cpd. no.* 18). 2-*p*-Methoxyphenacylpyridine (2 g.) was heated with hydriodic acid (15 ml.), sp.gr. 1·7, and red phosphorus (0·2 g.) for 1 hr. at 130 to 140° C. The cooled solution was neutralised with ammonia, and the precipitated base extracted with ether (3 × 15 ml.). After distilling off the ether, under reduced pressure, the residue was recrystallised from 70 per cent. aqueous ethanol to give 2-*p*-hydroxyphenacylpyridine (1·3 g., 70 per cent., m.pt. 148° C.)

The pertinent analytical details for the above compounds and others are given in Table I.

Metal sensitivity tests. The sensitivities of the compounds towards biologically important metal ions were determined by the method of Butler, Irving and Ring[19] as modified by Hollingshead[20]. Sensitivities are expressed in Table III in terms of pL, which is defined as $-\log_{10}$ limiting concentration of metal in gram equivalents per litre which gives a colour or precipitate under conditions of the test.

The molar ratio of glycine necessary to prevent precipitation at *p*H 7·3 of selected compounds was determined using the same concentration of the reagent (0·0032M) as in the above tests and a 0·0016M concentration of cupric ions. The results are summarised in Table IV.

Bacteriology

Determinations of minimum inhibitory concentration. Nine species of bacteria (National Collection of Type Cultures) were revived on recovery media (Table II), and then transferred to maintenance media. After incubating for 24 hours on recovery media, a washed suspension in glass-distilled water was prepared containing 10,000 organisms per ml.

TABLE II

Strain of bacteria	Culture media		Test
	Recovery	Maintenance	
Staph. aureus 6571 ..	Nutrient broth (Oxoid C.M.1)	Nutrient agar (Oxoid C.M. 55)	Peptone water (Oxoid C.M.9 + 0·5 per cent. dextrose)
B. subtilis 3610	,,	,,	,,
Corynebact. hofmannii 231	,,	,,	,,
Bact. coli 86	,,	,,	,,
Proteus vulgaris 4175 ..	,,	,,	,,
Sh. sonnei 8220	,,	,,	,,
Salm. typhimurium 5170 ..	,,	Dorset egg (Oxoid P.M.5)	,,
Myco. phlei 8151	Glycerol broth (Oxoid C.M.1 + 10 per cent. glycerol)	,,	,,
Str. pyogenes 8198.. ..	Serum broth (Oxoid C.M.1 + 10 per cent. normal horse serum [B.W. and Co.])	Blood agar (Oxoid C.M.55 + 10 per cent. rat blood [Heparinised])	Serum broth

The compounds to be tested were prepared in M/40 concentration in 70 per cent. ethanol and then serially diluted with glass distilled water so that each dilution was twice as great as the preceding one in the series. To 1·8 ml. of the test media (see Table II) was added 0·1 ml. of these dilutions and 0·1 ml. of bacterial suspension, determinations being carried out in quadruplicate. The dilutions of the substances under test covered the range M/1600 to M/800,000. Incubation was at 37° C. for 48 hours except for tests involving *Mycobacterium phlei* in which the period was extended to 5 days. The minimum inhibitory concentration was taken as the greatest dilution showing no growth at the end of the specified period. Oxine was used as a control substance in all the tests. Concentrations of M/800 and M/400 were employed for some of the compounds tested.

The results are summarised in Tables V and VI.

Tests showing the role of metal ions. For each compound being studied, 5 test systems were prepared in sterile glass distilled water. One

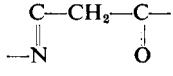
contained only the compound in minimum inhibitory concentration. A second and third contained the compound in minimum inhibitory concentration with equivalent amounts of ferrous sulphate and cupric sulphate respectively. The remaining 2 tubes contained the equivalent amount of these two metal salts alone. All test systems were inoculated and the final volume (2 ml.) contained 2000 washed organisms. They were incubated at 37° C. and subcultures of 0·1 ml. made at fixed times after inoculation. The subculture tubes were incubated for 24 hours and the presence or absence of visible growth noted.

The organisms used were *Proteus vulgaris* and *Bacillus subtilis*. The results are summarised in Tables VII and VIII.

Results and discussion

Metal Co-ordination Properties

It has been shown[21] that substitution by alkyl substituents of the methylene group of aceto-acetic ester results in a reduction in the percentage of the enol form; the effectiveness of the substituents in accomplishing this effect is ethyl>methyl>*n*-propyl. Likewise, the order of the groups in reducing the percentage of the enol form in acetyl acetone is ethyl>methyl>benzyl[21]. Since 2-methylpyridine is regarded as an ammono ketone ether[22], the tautomeric system of 2-phenacylpyridine (XI) may be



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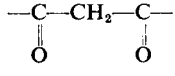
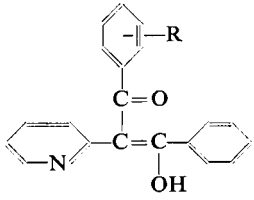


(XII)

considered as analogous to a β-diketone (XII). Substitution of the active methylene group in 2-phenacylpyridine with methyl, ethyl, *n*-propyl, allyl, and benzyl groups led to a complete absence of the enol form. This was demonstrated by failure of the compounds to give colours with ethanolic ferric chloride solution (contrast with the parent compound) and the presence of only one absorption peak at 247 mμ in the ultra-violet absorption spectra of ethanolic solutions of the compounds (see Beckett and Kerridge[12]), the absorption peak at 337 mμ characteristic of the enol form of 2-phenacylpyridine being absent. Furthermore, unlike the ultra-violet absorption spectra of the parent molecule, the spectra of these alkyl substituted derivatives were unaffected by the presence of cupric ions.

Since the substitution of the active methylene group of a β-diketone system by an electron attracting mesomeric group has been shown to increase the percentage of the enolic component[23], aroyl substitution of 2-phenacylpyridine was expected to have a similar effect. In fact the aroyl derivatives (Bz, $-CO\cdot C_6H_4Cl$-*p*, $-CO\cdot C_6H_4\cdot NO_2$-*p*, $-CO\cdot C_6H_3\cdot(NO_2)_2$–1:3:5) of 2-phenacylpyridine all gave colours with ethanolic ferric chloride solution indicating that the keto-enol tautomerism had not been supressed. All these compounds in ethanolic solution exhibited an ultra-violet absorption peak at 370 mμ (ε values 9000 to 14,000)[12] which indicates the existence of these compounds in the enol form (XIII), rather

than in the alternative form (XIV) which would be expected to absorb at varying wavelengths dependent upon the group R. These compounds, unlike the *C*-alkyl derivatives, precipitated metal ions from aqueous solutions (see Table III).



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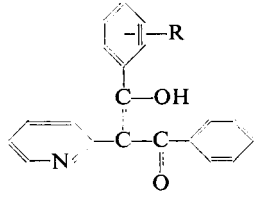
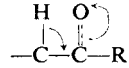


(XIV)



(XV)

It is known that prototropy is facilitated in the system (XV), as shown, if group R is electron attracting[24]. Consequently, the substitution of the phenyl ring of 2-phenacylpyridine by electron attracting groups would be expected to increase the enol/keto ratio and in consequence the apparent acidity of the system. The results of the metal sensitivity tests (Table III) indicate that, of the compounds tested, the *m*-chloro-derivative shows the greatest sensitivity towards metal ions, whereas those compounds in which enolisation is repressed, by the substitution of an electron donating group such as amino or hydroxyl in the phenyl ring, fails to precipitate metal ions under the conditions of the test. Although the sensitivity towards metal ions is dependent upon both the stability and the solubility of the chelate, the observed differences in sensitivity are not due solely to difference in the solubility (e.g., the replacement of a hydrogen atom of the ring by methyl or chlorine would be expected to diminish the solubility of the reagent similarly[11], whereas these derivatives show different sensitivities to metal ions). Moreover, differences in sensitivity were observed in the isomeric mono-chlorophenacylpyridines.

The substitution of a methyl group in the 6-position of 2-phenacylpyridine (Cpd. 26) completely repressed metal precipitation under the conditions of the test. This result is probably due to the steric interference of the group with the co-ordination by the adjacent nitrogen atom, and is comparable with the observed reduction of chelating properties upon the introduction of a methyl group into the 2-position of 8-hydroxyquinoline[25]. On the other hand, 4-methyl-2-phenacylpyridine (Cpd. 28) is more sensitive to metal ions than 2-phenacylpyridine, probably due to the increased electron density at the nitrogen atom in the former compound.

The replacement of the phenyl group of 2-phenacylpyridine by alkyl groups (Cpds. 13 and 14) caused a complete loss of metal-precipitating properties and lowered sensitivity towards metal ions resulted from the replacement of the phenyl by the α-furyl group (Cpd. 15).

It is known that amino-acids and proteins can bind metal ions, e.g., glycine solubilises zinc serum albumin and zinc insulin complexes by successfully competing for the complexed metal ions[26]. Consequently, before a molecule can be considered as a potential complexing agent under

TABLE III

METAL SENSITIVITY TESTS

Metal	Cu^{++} (as $CuSO_4 \cdot 5H_2O$)			Fe^{++} (as ferrous alum)			Fe^{+++} (as ferric alum)			Co^{++} (as $Co(NO_3)_2 6H_2O$)		
*p*H	8·4	7·3	5·3	8·4	7·3	5·3	8·4	7·3	5·3	8·4	7·3	5·3
Cpd. 1†	3·8	3·5	N.P.	2·2*	2·2*	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
" 7†	3·8	3·5	"	2·2*	2·2*	"	"	"	"	"	"	"
" 8†	4·3	3·8	"	2·3	2·2*	"	"	"	"	"	"	"
" 9†	3·8	3·5	"	2·2*	N.P.*	"	"	"	"	"	"	"
" 10†	4·0	3·8	"	N.P.	"	"	"	"	"	"	"	"
" 1	4·5	4·2	"	3·2*	3·2*	"	"	"	"	"	"	"
" 13	N.P.	N.P.	"	N.P.	N.P.	"	"	"	"	"	"	"
" 14	N.P.	N.P.	"	"	"	"	"	"	"	"	"	"
" 15	3·5	3·5	"	"	"	"	"	"	"	"	"	"
" 16	4·5	4·3	3·5	3·8	4·2	"	"	3·8	"	"	"	"
" 17	N.P.	N.P.	N.P.	N.P.	N.P.	"	"	N.P.	"	"	"	"
" 18	"	"	"	"	"	"	"	2·5*	"	"	"	"
" 19	4·2	4·2	3·8	"	3·2	"	"	3·2	"	"	"	"
" 20	4·8	4·5	N.P.	3·5	4·2	2·2	"	N.P.	"	3·5	"	"
" 21	4·5	4·2	3·8	N.P.	4·3	N.P.	"	3·8	"	N.P.	"	"
" 22	3·8	3·8	N.P.	N.P.	N.P.	"	"	N.P.	"	"	"	"
" 23	4·3	4·3	3·8	2·5	4·5	"	"	4·5	"	"	"	"
" 24	4·8	4·2	3·8	2·2	4·2	"	"	3·5	"	"	"	"
" 25	4·8	4·5	4·2	2·8	4·3	"	"	3·8	"	4·2	3·2	"
" 26	N.P.	N.P.	N.P.	N.P.	N.P.	"	"	N.P.	"	N.P.	N.P.	"
" 27	"	"	"	"	"	"	"	"	"	"	"	"
" 28	4·2	4·2	3·5	"	2·2	"	"	2·2	"	"	"	"
" 29	4·8	4·5	3·8	3·2	4·2	"	2·5	4·2	2·5	3·8	2·5	"

N.P. = no precipitate. * Colour only. † Reagent concentration 0·0008 molar.

biological conditions, it must be shown that it can compete successfully with amino-acids or proteins for metal ions. The results obtained in the metal sensitivity test are dependent upon the solubility as well as upon the stability of the complexes and although both these factors are important in biological action, a measure of the ability of the reagent to bind metals under biological conditions is not obtained. A simple test of this latter effect has been devised for the present investigation because this preliminary exploration of a series of compounds did not warrant the more detailed study of stability constants of the complexes. The test involved the determination of the sensitivities of the compounds towards cupric ions under standard conditions in the presence of varying amounts of glycine; the molar ratio of glycine necessary to prevent precipitation was observed. It seems reasonable to assume that if a molecule can precipitate copper in the presence of a large molar excess of glycine, then it is likely to form a complex with these ions under biological conditions. The results of the tests upon certain of the compounds of the series are shown in Table IV.

TABLE IV

MOLAR RATIOS OF GLYCINE NECESSARY TO PREVENT PRECIPITATION OF COPPER IONS BY DERIVATIVES OF 2-PHENACYLPYRIDINE

Compound No.:	19	1	23	24	25	28	29
Molar ratio of glycine	4	5	55	45	50	7·5	90

Antibacterial Properties

The bacteriological results (Tables V and VI) indicate that although many of the compounds are capable of co-ordinating with metals such

TABLE V

ANTIBACTERIAL ACTIVITIES OF 2-PHENACYLPYRIDINE AND ITS DERIVATIVES

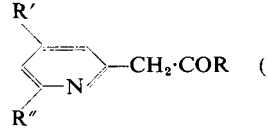
Compound No.	Derivative (I)	M.I.C. in reciprocal molar concentration against*							
		Staph. aureus.	*Str. pyogenes*	*B. subtilis*	*Corynebact. hofmannii*	*Bact. coli*	*Proteus vulgaris*	*Sh. sonnei*	*Myco. phei.*
1	R = H	—	1600	—	—	—	1600	—	—
2	R = Me	400	1600	—	—	—	3200	—	800
3	R = Et	1600	800	—	—	—	1600	—	1600
4	R = Pr^n	6400	1600	1600	1600	—	1600	—	3200
5	R = $CH_2{\cdot}CH:CH_2$	3200	1600	1600	1600	—	1600	—	1600
6	R = CH_2Ph	800	—	—	—	—	1600	—	—
7	R = Bz	800	1600	1600	1600	—	3200	—	1600
8	R = $CO{\cdot}C_6H_4{\cdot}NO_2$-*p*	1600	3200	—	—	1600	1600	—	1600
9	R = $CO{\cdot}C_6H_4Cl$-*p*	1600	1600	—	800	—	800	—	1600
10	R = $CO{\cdot}C_6H_3(NO_2)_2$ -1:3:5 (II)	1600	3200	1600	3200	1600	1600	1600	3200
11	R = $CH_2{\cdot}CH_2{\cdot}NMe_2$	800	—	—	—	—	1600	—	—
12	R = Ac	800	1600	—	—	800	1600	—	—
	Oxine	100,000	100,000	50,000	100,000	1600	800	1600	25,000

— Signifies growth at M/1600.
* All these compounds listed in Table V are inactive at M/1600 concentration against *Salm. typhi.*

as iron or copper (Table III), and many of them exhibit antibacterial effects, the two actions do not appear to be directly related. For example, compounds 2 to 6 which exist in the ketonic form and do not co-ordinate

TABLE VI

ANTIBACTERIAL ACTIVITIES OF KETONES PREPARED FROM 2-METHYLPYRIDINE, 2:4- AND 2:6-DIMETHYLPYRIDINE



Cpd. No.	Derivative (III)	M.I.C. in molar concentration against*			
		Staph. aureus	*Str. pyogenes*	*B. subtilis*	*Proteus vulgaris*
13	R′ = R″ = H; R = Me	—	—		—
14	R′ = R″ = H; R = Et	—	—		—
15	R′ = R″ = H; R = 2-furyl	—	—		800
16	R′ = R″ = H; R = 1-naphthyl	800	3200		3200
17	R′ = R″ = H; R = $C_6H_4{\cdot}NH_2$ -*p*	—	—	—	1600
18	R′ = R″ = H; R = $C_6H_4{\cdot}OH$ -*p*	—	—	—	1600
19	R′ = R″ = H; R = $C_6H_4{\cdot}OMe$ -*p*	1600	1600	—	1600
20	R′ = R″ = H; R = C_6H_4Me -*p*	—	3200	—	3200
21	R′ = R″ = H; R = C_6H_4F -*p*	—	—	6400	3200
22	R′ = R″ = H; R = C_6H_4Cl -*o*	—	—	3200	3200
23	R′ = R″ = H; R = C_6H_4Cl -*p*	—	1600	3200	1600
24	R′ = R″ = H; R = C_6H_4Br -*p*	—	3200	—	6400
25	R′ = R″ = H; R = C_6H_4Cl -*m*	—	—	—	6400
26	R′ = H; R″ = Me; R = Ph	—	—	1600	3200
27	R′ = H; R″ = Me; R = C_6H_4Cl -*m*	—	—	25,000	3200
28	R′ = Me; R″ = H; R = Ph	—	—	25,000	3200
29	R′ = Me; R″ = H; R = C_6H_4Cl -*m*		—	50,000	3200
	Oxine	100,000	100,000	50,000	3200

— Signifies growth at M/1600.
* Compounds 17 to 29 are inactive at M/1600 concentration against *Corynebact. hofmannii, Bact. coli, Salm. typhi, Sh. sonnei,* and *Myco. phei.*

with metals, exhibit antibacterial activities of a higher order and against more organisms than do the metal co-ordinating compounds 1 and 19 to 25. Furthermore, even the enol ether (Cpd. 11) and enol ester (Cpd. 12) are antibacterials.

Although the correlation (see Compounds 13 to 29) between the antibacterial effect recorded as minimum inhibitory concentration and the chelating power of the molecules as determined by the precipitation tests is slight, it was decided to investigate a few of the metal chelating compounds to see whether metal ions played any part in their possible bactericidal action. Two similar non-chelating compounds were used as controls. Oxine was used as reference compound because it is known that it fails to exert its bactericidal action upon certain organisms in distilled water if certain concentrations of ferrous or cupric ions are absent.

The role of metal ions in the antibacterial action. The results reported in Table VII show the effect of ferrous and cupric ions on the bactericidal action of compounds 2, 24, 25, and 26 and of oxine on *Proteus vulgaris* in glass-distilled water. Compounds 24 and 25 coordinate with metals both in the absence and in the presence of 45 and 50 molar proportions of glycine, respectively, but compounds 2 and 26 fail to precipitate metal ions under the conditions of the test used. All four of these compounds, in contrast to oxine, are bactericidal to *Proteus vulgaris* in the absence of metals, and both ferrous and cupric ions potentiate this activity, although the effect is slight. However, since these metal ions have a similar effect on compounds 2 and 26 as on compounds 24 and 25, this observed potentiation of activity is probably independent of chelation phenomena; it could be attributed to the additive effect of two independent bactericidal actions because the concentrations of metal ions used are themselves bactericidal.

Compounds 28 and 29 are highly active against *B. subtilis* (Table VI), and are bactericidal in the presence of cupric but not ferrous ions (Table VIII). In contrast to these results, oxine is bactericidal in the presence of both cupric and ferrous ions (Table VIII). This difference in effect of cupric and ferrous ions on the bactericidal activity of compounds 28 and 29 may be due to the lower order of metal chelation of the latter ions (Table III).

Although complete success has not attended this attempted design of potential antibacterial agents in which biological activities can be correlated with metal chelating properties, this mechanistic chemotherapeutic approach has, at least, led to compounds exhibiting antibacterial properties. Two interesting facts which may have practical applications also arise from the investigation.

(1) Nearly all the compounds of the series possess activity, albeit of a low order, against *Proteus vulgaris.* This fact, coupled with the difficulty of erradicating *Proteus vulgaris* in certain conditions of infection and the low toxicity of the compounds, e.g., compounds 28 and 29 can be tolerated in mice at doses as high as 620 mg./kg. by subcutaneous injection, makes certain compounds worthy of a more detailed investigation.

TABLE VII

THE EFFECT OF FERROUS AND CUPRIC IONS ON THE ACTIVITY OF SELECTED COMPOUNDS AGAINST *Proteus vulgaris* IN GLASS DISTILLED WATER

Compound No.	Concn.	Metal	Concn.	Time of subculture in hours 0	1	2	3	4	5	6	8
		Fe	M/3200	+	+	−	−	−	−	−	−
		Cu	M/3200	+	+	−	−	−	−	−	−
2	M/3200	—	—	+	+	+	−	−	+	−	−
2	M/3200	Fe	M/3200	+	+	−	−	−	−	−	−
2	M/3200	Cu	M/3200	+	−	−	−	−	−	−	−
26	M/3200	—	—	+	+	+	−	−	+	−	−
26	M/3200	Fe	M/3200	+	−	−	−	−	−	−	−
26	M/3200	Cu	M/3200	+	−	−	−	−	−	−	−
		Fe	M/6400	+	+	−	−	−	+	+	−
		Cu	M/6400	+	+	+	−	+	−	−	−
24	M/6400	—	—	+	+	+	−	−	−	−	−
24	M/6400	Fe	M/6400	+	+	−	−	−	−	−	−
24	M/6400	Cu	M/6400	+	+	+	−	−	−	−	−
25	M/6400	—	—	+	+	+	+	−	+	−	−
25	M/6400	Fe	M/6400	+	+	−	−	−	−	−	−
25	M/6400	Cu	M/6400	+	+	−	−	−	−	−	−
Oxine	M/6400	—	—	+	+	−	−	−	+	+	+
Oxine	M/6400	Fe	M/6400	+	+	−	−	−	−	−	−
Oxine	M/6400	Cu	M/6400	+	+	+	−	−	−	−	−
—	—	—	—	+	+	+	−	−	+	+	+

(2) Compounds 27, 28 and 29 are almost specific in their action against *B. subtilis*, e.g., compound 29 is inactive against 7 of the organisms used in the present work at concentrations of M/1600 but is active in concentrations as low as M/50,000 against *B. subtilis*.

TABLE VIII

THE EFFECT OF FERROUS AND CUPRIC IONS ON THE ACTIVITY OF SELECTED COMPOUNDS AGAINST *B. subtilis* IN GLASS DISTILLED WATER

Compound No.	Concn. 1/M	Metal	Concn. 1/M	Time of subculture in hours 0	1	2	3	4	5	6	8
		Fe	25,600	+	+	+	−	−	+	+	+
		Cu	25,600	+	+	−	−	−	+	+	+
28	25,600	—	—	+	+	+	−	−	+	+	+
28	25,600	Fe	25,600	+	+	+	−	−	+	+	+
28	25,600	Cu	25,600	+	+	+	−	−	−	−	−
		Fe	51,200	+	+	+	−	+	+	+	+
		Cu	51,200	+	+	+	−	−	+	+	+
29	51,200	—	—	+	+	+	−	−	+	+	+
29	51,200	Fe	51,200	+	+	+	−	−	+	+	+
29	51,200	Cu	51,200	+	+	+	−	−	−	−	−
Oxine	51,200	—	—	+	+	+	−	+	+	+	+
Oxine	51,200	Fe	51,200	+	−	−	−	−	−	−	−
Oxine	51,200	Cu	51,200	+	−	−	−	−	−	−	−
—	—	—	—	+	+	+	−	−	+	+	+

SUMMARY

1. Substituted acyl and aroyl derivatives of 2-methylpyridine and 2:4- and 2:6-dimethylpyridine have been prepared as potential antibacterial and chelating agents, and their sensitivities towards metal ions determined. The effect of glycine upon the precipitation of cupric ions by certain of the compounds is recorded.

2. The bacteriostatic values of these compounds against Gram-positive and Gram-negative organisms are reported. The effect of metal ions on the bactericidal action of selected compounds has been investigated and compared with their effect upon the action of oxine.

3. Some of the substances possess a specific action against *B. subtilis*.

One of us (K. A. K.) thanks the Pharmaceutical Society for the award of a scholarship.

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DISCUSSION

The paper was presented by DR. A. H. BECKETT.

DR. F. HARTLEY (London) said that although in the particular series chosen it had not been possible to draw the rather interesting conclusions which Albert was able to obtain, nevertheless it was only from the examination of potentially useful series that fundamental correlation between action and structure could be determined. Another potentially useful aspect of the paper was whether it could add anything useful to the detection and evaluation of metals by the reagents which had been studied. From Table III it appeared that there might be one or two interesting possibilities. By analogy with 8-hydroxyquinoline it might well be the case that if other metallic ions were studied some specific colour or precipitation would be obtained.

DR. G. E. FOSTER (Dartford) asked why iron and copper had been chosen for the chelating test. Bacteria were sensitive to other metals. Had the authors any data with regard to the chelating properties of their compounds with calcium and magnesium?

MR. H. D. C. RAPSON (Dorking) said that at least three mechanisms for the bactericidal action of chelating agents are possible, firstly a lipoid soluble form of the compound diffuses into the bacterium and forms a metal complex inside; secondly, a lipoid soluble complex with a metallic ion diffuses into the bacterium and then dissociates with the liberation of metallic ions or unsaturated complexes; and, thirdly, more complicated processes. Hence it is important to investigate more directly and precisely the chelating ability of the compounds.

DR. G. BROWNLEE (London) referred to the statement in the paper "Oxine is non-toxic to *Staph. aureus* unless traces of iron (or copper) are present in the medium" and said that that was not what Albert *et al.* had shown. They showed that a substantial part of the activity of oxine could be reversed under very special conditions of choice of medium and testing. At least two other authors have shown that this simplification of the possible mechanism of action would have to be critically investigated before it could be accepted that chelation played the only part in the mechanism. That copper and iron—and, incidentally, magnesium—played a part seemed to be implicit in the discussion, but in his view one ought, in all humility, to say "Is it true?" Of course nothing of this detracted from the value of the investigation.

DR. A. H. BECKETT, in reply, agreed with Dr. Hartley that some of the compounds might have analytical uses and infra-red studies would be of interest. Iron and copper were chosen for the test since those metals are implicated in the activity of oxine against *Staph. aureus*. Since the work was only a preliminary study, a quickly performed test seemed desirable when the substances were not of a high order of antibacterial activity. On the points raised by Dr. Brownlee, he agreed that the conditions of Albert's tests were rather different from practical conditions, but nevertheless such work was essential to limit the variables. The paper was an attempt to provide further information on compounds which could compare with metals based on the premise that chelation could be involved in certain types of antibacterial activity.