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Chemistry of antiprotozoal nitroimidazoles*

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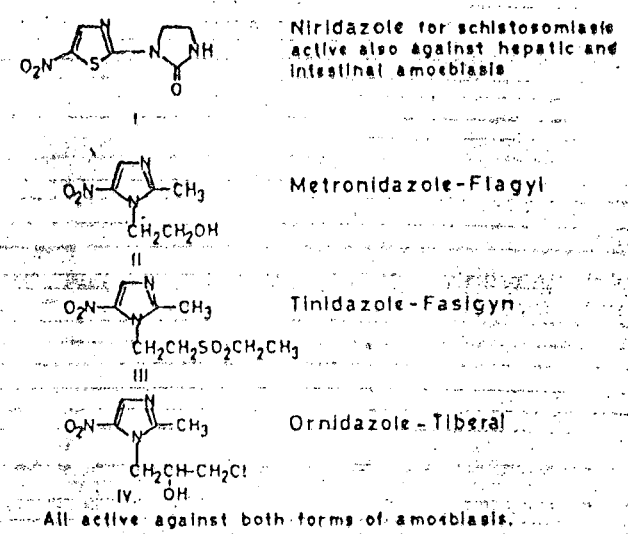
I wish to thank Prof. G. S. R Subba Rao for his introduction and Dr. S. P. Bhatnagar and the other authorities of Reckitt & Colman of India Limited for extending me the opportunity of participating in this symposium and presenting the results of some of our researches. The subject I am going to talk about today is on medicinal chemistry, the chemistry of antiprotozoal nitroimidazoles, in keeping with the tradition that has already been established by my predecessors. Now, medicinal chemistry as many of you may be aware reminds us of an Indian movie. There is a little bit of everything for everybody. There is a little bit of love for the romantic-minded a little bit of fight for the bloody minded, a little bit of clowning for the fun-lovers and a little bit of crime for the mystary-oriented people. In a way, medicinal chemistry resembles this because it has something for every one — thus there is chemistry for those who are interested in organic synthesis. For the biologically minded people, there is quite a bit of biological activity; for those who are attracted to reaction mechanisms, there is some scope as the talks of my eminent predecessors very amply illustrated and there is some fun for spectroscopy-loving people also. Although much of medicinal chemistry is routine, for the perceptive organic chemist, it does offer quite a few challenges and provides some amusement and I hope that my talk which follows now would illustrate these points.

I would begin my talk by introducing to you Niridazole (I) (Slide 1) which perhaps may be claimed to have caused much interest in the chemistry of nitro heterocycles; may be nitrofurans preceded this a little bit. Now Niridazole is a drug of Ciba-Geigy and was introduced for schistosomiasis. It is quite active, one of the best schistosomicidal drugs. But what was interesting was the fact that it was also active against hepatic and intestinal amoebiasis. Around the time this discovery was made, the second molecule which you see on slide 1 which is a 2-methylnitroimidazole with a hydroxyethyl chain, known as Metronidazole (II) was undergoing trials for antitrichomonal activity and taking a cue from the experience with niridazole, it was tested for antiamoebic activity. In fact, it was found to be a very pronounced amoebicide and came to be introduced in the clinic. Now of course metronidazole is available in this country in a big way. More recently, another nitroimidazole called Tinidazole (III) has been introduced in this country and many other nitroimidazoles are known for antiprotozoal activity; for example I would like to mention Ornidazole (IV). Tinidazole is a discovery of Pfizer and ornidazole, of Roche. All these compounds are active against both forms of amoebiasis. They are also active against trichomonas, giardia and most importantly — and this is a very recent development — metronidazole has been shown to have pronounced activity against anaerobic infections.

Slide 1

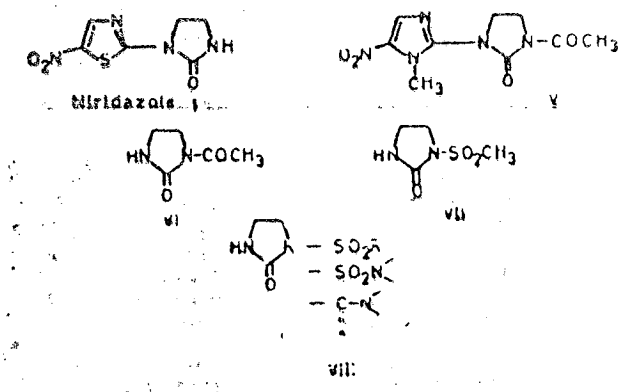


To come back to what we have done, I would again start with niridazole in the next slide (2). This is a nitrothiazolyl imidazolidinone and around the year 1972 when we took upon developing antiamoebic drugs as a major effort at Ciba Geigy Research Centre, Goregaon, there was already an internal lead available in nitroimidazole chemistry and this was inspired by the niridazole molecule. In other words, the imidazolidinone moiety that was present in niridazole was now hooked on to the methylnitroimidazole with an added acetyl group and this com-

*Contribution No. 684

Slide 2

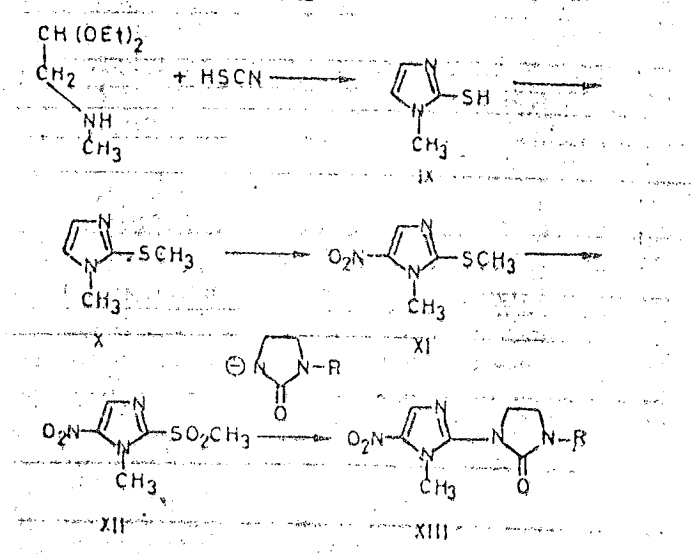
Nitroimidazoles - work at CIBA-GEIGY Research Centre



pound (V) was found to be a very active amoebicide, and therefore there was very understandable interest in developing this compound further. But there was a problem in its synthesis. I shall be discussing the actual synthetic route subsequently I could anticipate this and tell you that the problem arose in the coupling step of the two halves of the molecule. The conditions used were such that they would lead to disproportionation of the acetylimidazolidinone (VI). This caused a few side reactions and therefore the route was found to be rather unmanageable for large scale synthetic work. We, more or less, inherited this lead when we chose to work on amoebiasis as a major priority. We thought that we could perhaps beat this problem by introducing some other groups (VIII) which may be expected to be just stable for the coupling reaction but not so stable that they would not come off later and then we wished to introduce the acetyl group as a last step in our synthetic sequence. It so turned out that we chose, at a very early stage of the game, methane sulphonyl imidazolidinone (VII) as a partner in this reaction and we did come out with the product. Unfortunately, the methylsulphonyl group tended to be so stable that we could not take it off and therefore the original idea of using this as a route to compound V did not succeed. But fortunately for us this was not a great disappointment because the compound (XVII) we could get by using methanesulphonyl imidazolidinone (VII) in fact proved to be a little bit more active than V and a very acceptable one from medicinal chemistry point of view. In the course of this exercise, of course, we tried to employ various other protected imidazolidinones like the one carrying sulphonyl group or a carbonyl or a thiocarbonyl group. Many of these proved to be unstable to a smaller or larger degree under the usual conditions of the coupling reaction; but they also provided interesting chemistry which I will be subsequently discussing.

In the next slide (3) I would like to introduce to you the synthetic sequence employed for our target molecules. The sequence starts with a compound that one designates as 1-methyl-2-mercaptoimidazole also known as methimazole or thiamazole (IX) which itself is an antithyroid drug. This is readily obtained from methylamino acetaldehyde and thiocyanic acid.

Slide 3

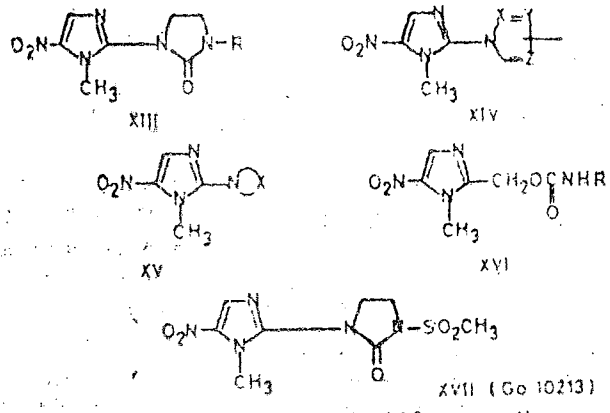


Methimazole can be methylated to the S-methyl derivative (X) which can be nitrated to give 1-methyl-2-methylmercapto-5-nitroimidazole (XI). Unfortunately, the methylmercapto group in this imidazole itself is not amenable to displacement reactions. But it becomes so when it is oxidised with some kind of peracid to the sulphone group. The sulphone XII is now amenable to nucleophilic displacement readily and this is in fact the kind of situation that a medicinal chemist will exult in. In this case, he has a starting material which he could attack with a variety of nucleophiles to produce really a large number of compounds that can satisfy the needs of a medicinal chemistry research programme. The condensation reaction itself as far as the substituted imidazolidinone (VIII) is concerned is carried out by using the sodium salt prepared *in situ* in dimethyl formamide. DMF is convenient for several reasons and it also gives some side reactions which will be seen presently. The sodium salt of the protected imidazolidinone is generated in DMF solution and subjected to the coupling reaction with the sulphone XII, which is often exothermic. Hence it is carried out at room temperature or sometimes even below. I may also point out at this stage that while other leaving groups can presumaby serve just as well, they are not as readily available and this happens to be the most

satisfactory way of synthesising the molecules XIII.

Now to continue with the medicinal chemistry aspect, as I had warned you earlier, XII is the kind of molecule the medicinal chemist revels in using and we did of course treat this with as many nucleophiles as one can imagine. The protected imidazolidinones were only a few of the nucleophilic substrates. We have used triazolidinones which were already available with us because one of my co-workers had oxidised them to triazolinones and used them for Diels Alder reactions. We used oxazolidinones, thiazolidinones and pyrrolidinones. The nucleophiles were varied even more widely. We had azoles like pyrrole, pyrazoles, imidazoles, thiazoles tetrazoles and a few other saturated azaheterocycles like pyrrolidine, piperidine, morpholine and so on. In the next slide (4), I would proceed to show a variety of compounds (XIII-XV) that were synthesised by this method and indicate to you what turned out to be active. The molecules that we derived by using

Slide 4

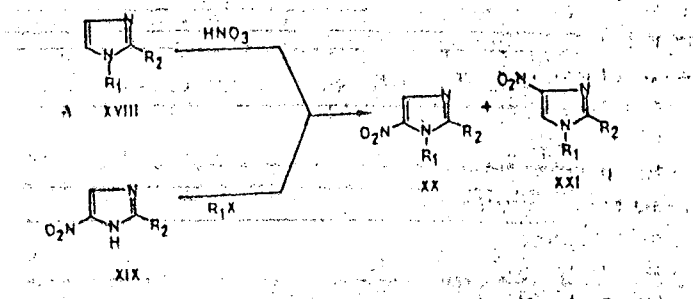


Go 10213 chosen out of >400 preparations.

triazolidinones were not active but we had consistent and very high activity among compounds derived from imidazoles and pyrazoles. We were not very successful in having active compounds with azacycloalkyl moieties, except the one with pyrrolidine which turned out to be moderately active. Very many different types of chemical exercises were carried out to produce other types of compounds. Thus we had 5-nitroimidazole-2-methanol which we could derivatise to compounds, one of which (XVI) turned out to be highly active. This kind of exercise was spread over 3 to 4 years and was carried out by senior colleagues, Drs. Sudarsanam, Arya, George and Nair and by a number of assistants among whom I would like to mention specially Mr. R. K. Shah and Mrs. S. J. Shenoy. The exercise resulted in the elaboration of more than 400 preparations on which biological screening was carried out. Out of these, we chose ultimately a compound designated with the code number Go 10213 (XVII) for clinical development. Go 10213 is more active than other known clinically useful nitroimidazoles in a variety of parameters — intestinal amoebiasis, hepatic amoebiasis, giardiasis and trichomoniasis. It has high *in vitro* antibacterial activity against anaerobes and we hope that this activity would continue to show up in the clinic as well. At the moment Go 10213 (XVII) is under advanced clinical trials. While I am not sure that it would get into a Guinness record, we do think that it is one of the most active amoebicides available in the world to-day.

I would like to go on to a different type of synthesis of Go 10213 which we attempted. I would like you to note that this is a 5-nitroimidazole derivative. The location of the nitro group at position 5 is critical because if one were to move this nitro group and place it at position 4, the resultant molecule is not as active any more. There is no 4-nitroimidazole known to be useful as an amoebicide. It becomes quite important to know where the nitro group sits in these molecules, because the methods of synthesis, the two commonly employed ones which I will show in the next slide (5), usually lead to a mixture of the two

Slide 5

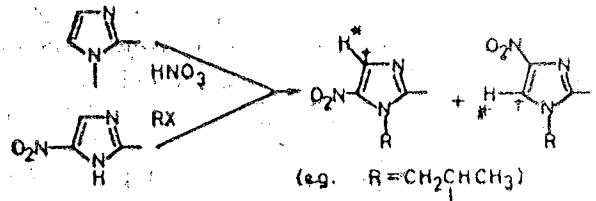


isomeric nitro compounds. For example, if a 1, 2-disubstituted imidazole XVIII is nitrated, a mixture of 4-(XXI) and 5-nitro (XX) derivatives is obtained; or a 2-substituted-4-nitroimidazole (XIX) can be used (positions 4 and 5 in these molecules are equivalent because of prototropy). These upon alkylation give a mixture of XX and XXI and of course one can manipulate the reaction conditions to produce one or the other as a major product in the mixture. If one is lucky, one may even succeed in getting an exclusive product but more often than not one ends up in getting a mixture of these compounds. The mixture has to be resolved and the components identified. All possible spectroscopic techniques have been utilised for this purpose like IR, UV, proton NMR Mass

spectrometry and even dissociation constants. But when we were indulging in these exercises, we noted that all these had some limitations and in the next slide (6), I would like to illustrate this with a particular example. I have summarised the two synthetic routes on the slide — please note again that one

Slide 6

Distinction between 4- and 5-nitro 1-substituted imidazoles



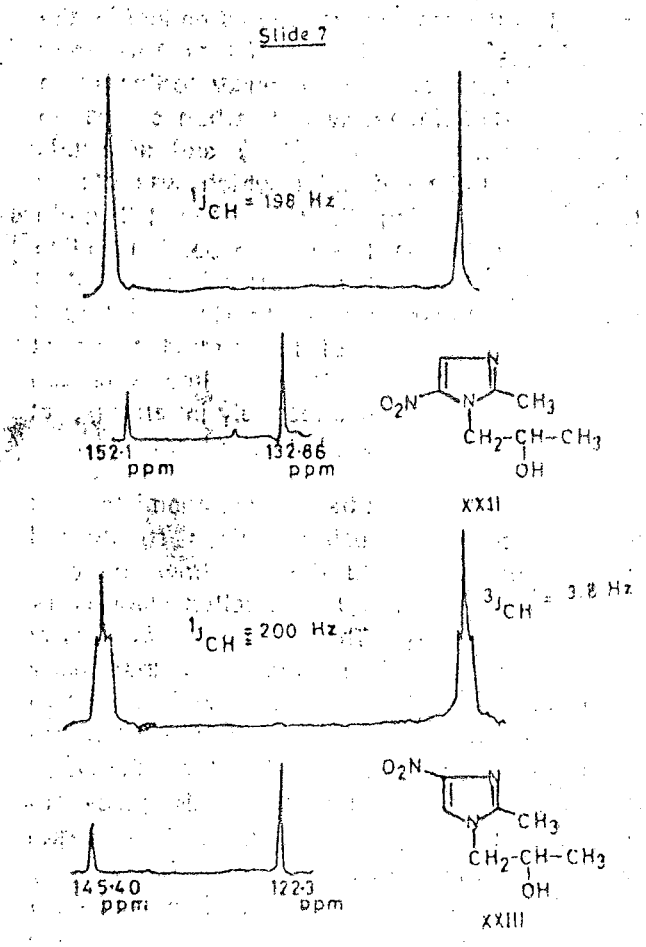
	XXII	XXIII
UV λmax(95% EtOH)	313 nm	302 nm
^{1}H NMR.		
δ H $CDCl_3$	7·82	7·72
δ H DMSO-d	8·05	8·21
Δ·δ	0·23	0·49
^{13}C NMR		
δ C†	132·9(d)	122·3 (d x t)
$^1J_{CH}$	199 Hz	200 Hz
$^{3(4)}J_{CH}$	0	3.8 Hz

nitrates the appropriately substituted imidazole or alkylates N-unsubstituted nitroimidazole. The reaction can constitute a terminal step or one can use this as a precursor for target compounds. No matter what one does, one has to know what one starts with and this is where one has to be able to differentiate between these two compounds. The problem looks to be apparently trivial but I assure you that it is not so easy. UV absorption spectra of these classes of compounds for example have been used and I can illustrate the limitation with the example (XXII) where R is an isopropanol side chain. This happens to be an active antiamoebic agent called secnidazole, although not very widely used. The UV absorption spectra of the two isomers (5- and 4-nitro), differ by only 11 nanometers. In the proton NMR spectrum in $CDCl_3$ the two imidazole protons are only slightly differentiated with hardly a margin of 0.10 ppm in chemical shift. Of course, when the nitroimidazoles carry at position 1, a proton-bearing carbon atom as part of an alkyl side chain, there is a slightly larger chemical shift difference (0 3-0.4 ppm) of these protons. Those of the 5-nitroisomers are more deshielded as one would expect. In DMSO-d_6 as a solvent, as is known in other cases, the situation improves. There is a greater demarcation or differentiation between these two isomers with respect to the proton at C-5 or C-4 as the case may be. $\Delta\delta$ between $CDCl_3$ and DMSO-d_6 solvents is larger for the C-5 proton in the 4-nitro isomer. This allowed us to say whether we are handling a 5- or 4-nitroimidazole. But we soon realised that all these techniques depending upon rather small and subtle differences had limitations when analysed. Unless both isomers were in hand, it would be quite risky to deduce structures by using any of these criteria mentioned. We felt that we could make some contribution in this area by looking at the ^{13}C NMR spectra of these pairs of compounds. Our arguments were as follows :

Proton bearing carbon atoms in these two isomers can be easily located and their coupling pattern analysed. From the presence of a 3 bond CH coupling or its absence one can allocate a given compound either to the 4-nitro series or 5-nitro series since only the former can exhibit the coupling. We further thought that there could be a sizeable difference in the chemical shifts of these carbon atoms which are noted by a dagger. In the case of the 5-nitro isomer, C-4 is attached to a double-bonded nitrogen (N-3) whereas in the other case, C-5 is attached to a more or less formally saturated N atom (N-1) and these speculations turned out to be true as I would show here (slide 6). In the 5-nitro series, C-4 i.e. the carbon atom marked by a dagger has a chemical shift of 132.9 ppm and is a simple doublet with a large one bond coupling whereas in the 4-nitro series (XXIII) C-5 is seen at 122.3 ppm as a large doublet with a further fine structure which is a triplet with a three bond CH coupling of 3.8 Hz. Please note that these carbon chemical shifts are around 133 ppm and 122 ppm while the chemical shift of C-2 in a few nitro imidazoles we have studied is about 140 ppm when it is proton bearing. This has some relevance to what I would be discussing later.

In slide 7, I have a pictorial representation of the arguments based on ^{13}C NMR spectroscopy in terms of the spectra of secnidazole XXII and its isomer XXIII. In the case of XXII, C-5 is seen in the broad band decoupled spectrum as a singlet at 132.9 ppm. When we look at the coupling pattern in the gated spectrum, we see that it is a plain doublet with a splitting of about 200 Hz. In the isomeric 4-nitro imidazole XXIII, C-5 is seen at 122.3 ppm. The coupling information shows it to be a large doublet with a one bond coupling of 200 Hz, with substructure due to a 3 bond coupling of 3.8 Hz. It is to be specially noted that C-4 in secnidazole (XXII) has a chemical shift of approximately 133 ppm while in its

Slide 7



4-nitroisomer, C-5 is seen at 122 ppm and in both cases, the one bond coupling is of the order of 200Hz.

The idea is further expanded in the next slide (8) where we have the data on a large number of isomeric nitroimidazoles. Concentrating on the chemical shifts as well as coupling pattern, we find that as we had speculated and shown to be true in

Slide 8

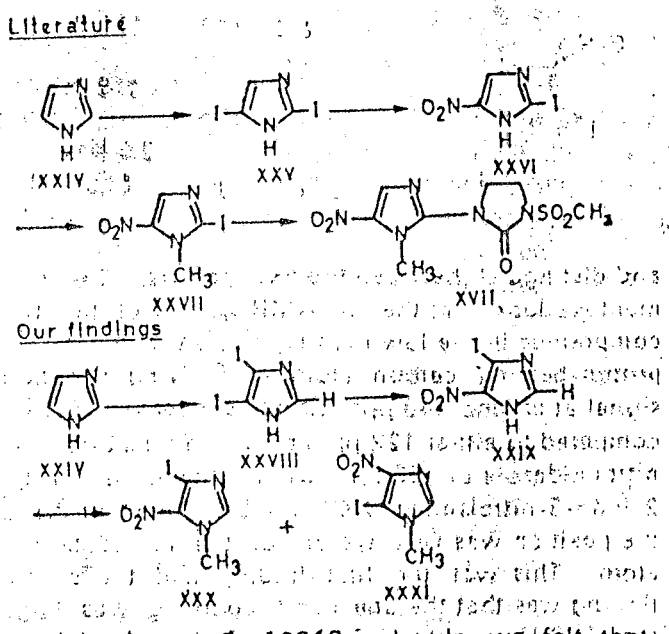
^{13}C NMR spectra of 4-and 5-nitro 1-substituted imidazoles

R	R_1	(δ C-multiplicity)		(δ C-multiplicity)	
CH_3	H	132.1	d x d	120.1	d x quintet
CH_3	CH_3	131.7	d	122.3	d x q
CH_2CH_2OH	CH_3	132.9	d	121.8	d x t
$CH_2CH(OH)CH_3$	CH_3	132.9	d	122.3	d x t
$CH_2CH(OH)CH_2Cl$	CH_3	132.3	d	122.4	d t
$CH_2CH_2SO_2CH_2CH_3$	CH_3	133.1	d	122.1	d x t
p-NO_2-C_6H_4	CH_3	132.4	d	121.5	d
CH_3	SCH_3	133.2	d	123.6	d x q
CH_3	SO_2CH_3	130.4	d	126.5	d x q
CH_3		132.1	d	?	?
CH_3		131.0	d	121.7	d x q

one or two cases, in a bunch of 5-nitroimidazoles C-4 chemical shifts are uniformly around 132 ppm and in 4-nitroimidazoles, C-5 shifts, 122 ppm, with very few exceptions. Again, where R is a proton bearing carbon atom at position 1 of isomeric nitroimidazoles, one finds an appropriate fine structure and multiplicity for C-5 in the 4-nitroimidazole series. This is absent in the 5-nitro series. Even in the absence of such fine coupling as seen in derivatives where R is an aryl group, we do find that the chemical shift difference persists. For example, we can look at a p-nitrophenyl derivative. In the 5-nitro series, C-4 is resonating at 132.4 ppm while in the isomeric 4-nitroimidazole, the signal of C-5 is seen at 121.5 ppm. Thus we can very safely say that even by the use of carbon shifts alone, it should be quite easy to differentiate between these two series.

Now let me take the case where we have to look at carbon 2 also and we happened to get into this problem for a particular reason. This is illustrated in the next slide (9). Thinking about various synthetic

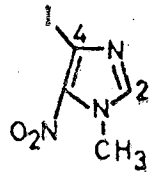
Slide 9



procedures for our Go 10213 molecule, we felt that perhaps we could try a much shorter route using as material which is recorded in the literature. Imidazole (XXIV) is reported to iodinate to 2, 4-diiodoimidazole (XXV). Reaction with HNO_3 is claimed to displace the iodine at C-4 by a NO_2 group to afford 2-iodo-4-nitroimidazole (XXVI). It is further stated in the literature that XXVI can be methylated to 1-methyl-2-iodo-5-nitroimidazole (XXVII) which has been used by two groups of workers. We felt that perhaps we could synthesise this molecule readily and use it conveniently for coupling with methanesulphonyl

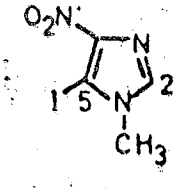
imidazolidinone to afford Go 10213 (XVII). When we started working with this compound, we found that the structure had to be revised. This had been also realized in yet another laboratory. What had in fact transpired was that diiodination of imidazole gave 4, 5-diiodo-imidazole (XXVIII) which goes over to 4-iodo-5-nitro imidazole (XXIX) upon nitration. Methylation under the conditions that had been reported in the literature yielded isomeric methylated derivatives (XXX) and (XXXI).

Now in the next slide (10) I shall show how we have used 13_C NMR spectroscopy to assign structures



XXX

$\delta C(2)$ - 143·7
$^1J_{CH}$ - 217 Hz
$^3J_{CH}$ - 4 Hz
$\delta C(4)$ - 90·8
$^3J_{CH}$ - 12 Hz

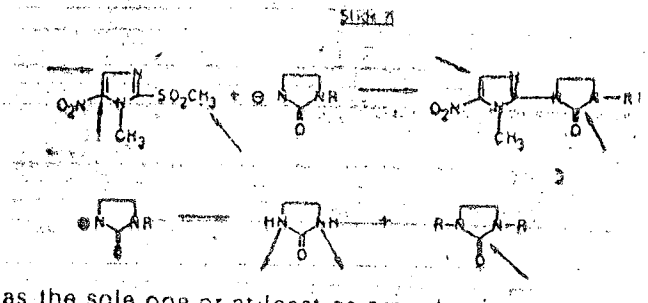


XXXI

δC (2) 139·8
$^1J_{CH}$ 219 Hz
$^3J_{CH}$ 3.5 Hz
$\delta C(5)$ 81.5 (m)

and distinguish between the two isomers. The moment we looked at the 13_C NMR spectra of the two compounds in the low field region, we noted that a proton-bearing carbon atom was giving rise to a signal at around 140 ppm which was way down field compared to either 122 ppm expected for a 2-iodo-4-nitroimidazole or 133 ppm which should be for the 2-iodo-5-nitroisomer (XXVII). On the other hand, the position was very typical of the imidazole C-2 atom. This was the first finding and the second finding was that the one bond coupling was larger than 200 Hz — around 217 to 219 Hz which is again a typical 1 bond coupling of C-2 in imidazole. Therefore, it was quite obvious that in these two compounds, position 2 was free from either iodo or nitro group. Subsequent distinction between the two isomers was possible by looking at the signal of the iodine bearing carbon atom. Even when it is quaternary, an iodine-bearing carbon atom gives rise to a good signal and also has a very peculiar feature which is well recognised in the literature, viz. that the signal appears considerably upfield from the corresponding non-iodinated carbon atom. If it is recalled that in the case where we had no iodine, the carbon signals were picked up at 120 or 130 ppm, the upfield shift produced by the bulky iodine atom can be appreciated since now that carbon shows up around 90 ppm in one isomer (XXX) and then this signal was seen to be a doublet which was clearly due to a 3 bond coupling with the proton at C-2. In the other isomer (XXXI) the carbon-bearing iodine was seen to be a multiplet, obviously because of 3 bond coupling with proton on C-2 and further 3 bond coupling with the protons of the methyl group at position 1. This is an illustration of how one can use 13_C NMR spectroscopy effectively in arriving at structures and distinguishing between isomers.

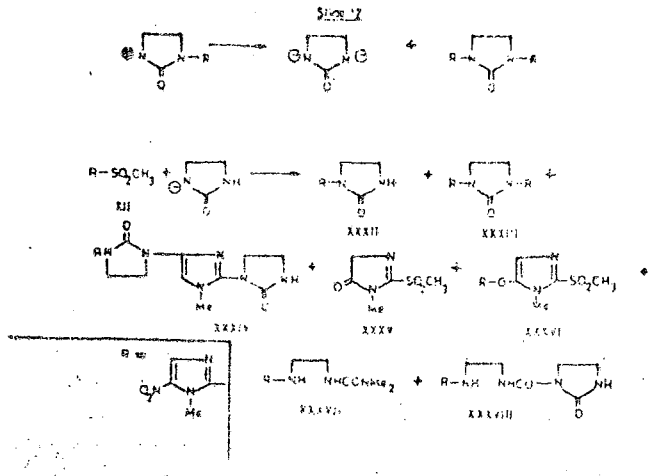
Now I would like to get back to the chemistry that we encountered while using various substituted imidazolidinones. I would like to show the next slide (11) and comment on the so called stability or instability of molecules. This is perhaps in a way parallel to human behaviour. We know that many people are very stable but this is only with respect to normal situations. However, when the latter are sufficiently provocative, you find that 'even the worm turns'. The so-called stability yields place to agitation, sometimes aggression. Let us look at the step where we had united the two moieties, namely 1-methyl-2-methanesulphonyl-5-nitro imidazole (XII) and a N-substituted imidazolidinone to produce Go 10213 and analogues. What we expected and what we got in most cases was the required product either



as the sole one or at least as a predominant one. But very soon we found that there were several vulnerable points in both the partners of this synthetic manoeuvre and these are noted on them by various arrows (slide 11). The arrows indicate sites of nucleophilic attack and these can happen at various places — position 4 of the nitroimidazole, the methyl group of the sulphone, the CO group of the imidazolidinone etc. Among various R substituents on the imidazolidinone N atom, methylsulphonyl was stable but not methylthiocarbamoyl, acetyl or methylcarbamoyl. We found that when one used a base such as sodium hydride to generate the sodium salt of the substituted imidazolidinone, the molecule underwent disproportio-

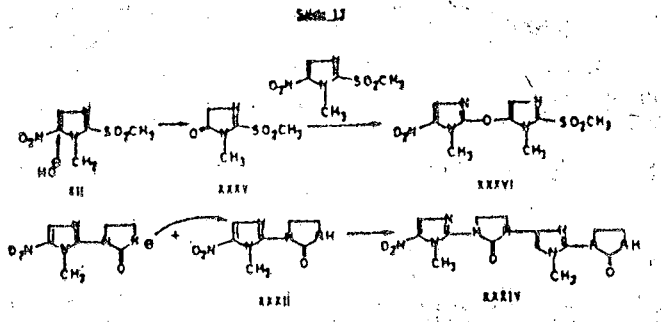
nation to give imidazolidinone (or ethylene urea as it is more popularly known), and a 1, 3-disubstituted imidazolidinone. Ethylene urea has 2 nucleophilic centres which obviously would be sought by electrophiles.

In the next slide (12), I would like to illustrate this by recounting our experience with this sulphone and,



for the sake of convenience, I would use the term R to symbolise the 1-methyl-5-nitro-2-imidazolyl residue. We thought that we would study in some depth the reaction of the sulphone (XII) with the sodium salt of ethyleneurea itself. This turned out to be a veritable mess — of course an attractive one from the chemist's point of view because of the formation of interesting products. One of the major products is the expected and desired one, the nitroimidazolylimidazolidinone XXXII. This is subsequently attacked by sulphone to produce the 1, 3-disubstituted imidazolidinone XXXIII. This is again only to be expected. Then there was a host of unexpected compounds which are listed as XXXIV — XXXVIII. Let us take a look at these and see how they could arise. Compound XXXVII, one can immediately recognise as arising from XXXII due to attack by dimethylamideion. Please recall that the solvent we used for the sake of convenient solubility was DMF; in the presence of sodium hydride apparently there is some dimethylamideion formed and it attacks the carbonyl group of the imidazolidinone ring and cleaves it. There are two possible ways of cleavage which produce the anion of either XXXVII or of an isomer, the former being more stabilised. There is a very easy way of distinguishing between the two, which utilises the alkali-induced shift to long wavelength of the absorption maximum of a 2-amino-5-nitroimidazole with a free NH. The product XXXVII in fact had a large bathochromic shift in the UV absorption spectrum when its alcohol solution was treated with alkali. Product XXXVIII obviously arises by cleavage of the imidazolidinone ring in XXXII, this time by attack by an imidazolidinone anion, the ring opening in the same direction as in the previous case. Now let us see how products XXXIV-XXXVI would have arisen.

In the next slide (13) I would like to speculate on this. Let us look at sulphone XII which is one of our starting materials. If we assume that there is a little moisture around and that hydroxylions are present, we can visualise an addition at position 5; in this case, the addition would be triggered by the presence of the electron-withdrawing sulphonyl group at position 2; subsequent elimination of nitrous acid would give rise to the imidazolidinone XXXV. The enolate of this displaces the methane sulphonyl group in a second molecule of XII to give the product XXXVI. Now let us see where 13_C NMR spectra helped us to assign structures to these compounds. In the 13_C NMR spectrum of the compound XXXVI, we could locate both the imidazole protons and neither of the two showed any fine splitting which clearly indicated to us that both imidazoles were substituted at position 5 — in one half, with a NO_2 group and in the other half, by oxygen. I would now like to turn to the last product XXXIV, which we call 'the train' because we have four imidazole molecules connected in a row. It probably arises as follows — the anion of the primary product XXXII of the reaction attacks another like molecule at position 4, unlike in

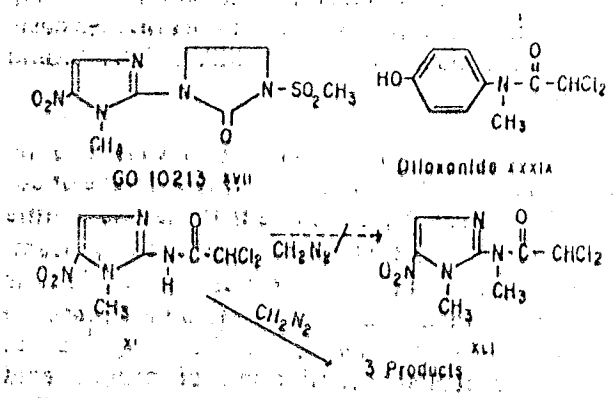


the case of the sulphone XII where the electron withdrawing methylsulphonyl group is available to direct the entry of hydroxyl at position 5. Subsequent loss of HNO_2 would account for the formation of XXXIV. 13_C NMR spectrum showed unambiguously a proton-bearing carbon at position 5 of an imidazole, having 3 bond coupling with a CH_3 group.

Now I want to talk about some other similar interesting chemistry that ensued when we wanted to do a very simple manipulation, related to exercises in medicinal chemistry slide 14). We have the molecule Go 10213 (XVII) on the one hand and on the

Slide 14

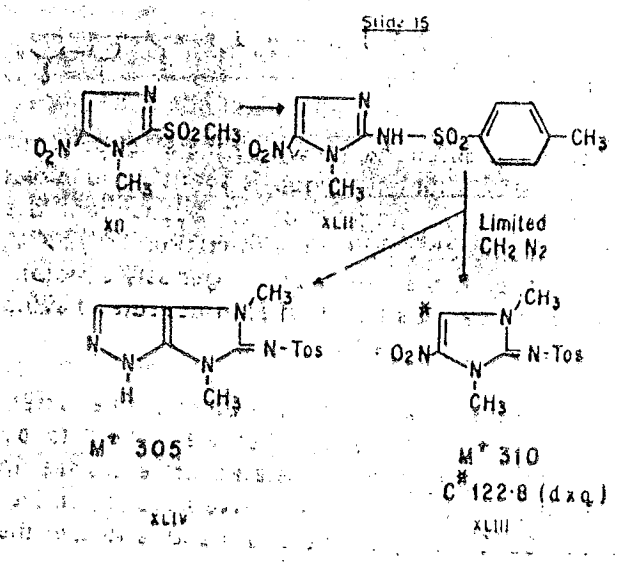
ADDITION OF DIAZOMETHANE TO NITROIMIDAZOLES



other, we have a rather simple N-methyl dichloroacetamide of p-aminophenol which is known as diloxanide (XXXIX) and which is a very good drug against caecal amoebiasis. We also had in our hands as a part of our exercises in this area, 1-methyl-2-dichloroacetylamino-5-nitroimidazole (XL). Since in the case of diloxanide, we knew that the N-methyl group was needed for the activity, we thought we would make the N-methyl derivative (XLI) of XL also. We knew that the NH in XL was acidic and we argued that the simplest and most preferable way of methylation would be to use the old fashioned one, viz to treat XL with diazomethane. Using ethereal diazomethane, obtained from nitrosomethyl urea, we found that the reaction did not go as expected. Instead, we got a mixture of 3 products which happened to be pyrrazoloimidazoles.

Instead of going through what happened in this case, I wish to take up a similar reaction involving the toluene sulphonamide XLII (slide 15) and show you how it became even more complicated. By re-

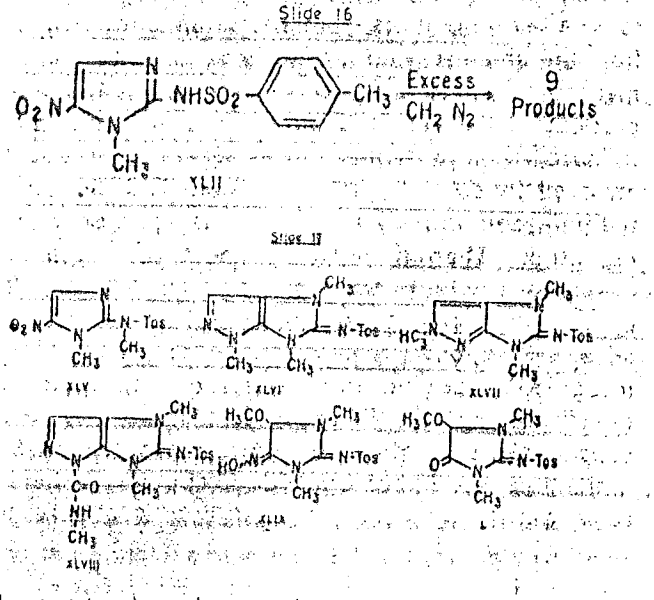
Slide 15



action of the methylsulphonyl nitroimidazole XII with p-toluenesulphonamideion, we got XLII in very high yields. Since the amide NH in XLII was again acidic, we expected methylation to occur readily with diazomethane. When we treated XLII with a limited amount of diazomethane (prepared as usual from nitrosomethylurea with alkali and ether), we did not get the expected derivative XLV. There were two products, one of which was the methyl derivative XLIII wherein the methyl group sits on the nuclear nitrogen. The other product was one wherein diazomethane had seemed to have undergone cycloaddition to give a pyrazolo imidazole XLIV This is what happened with a limited amount of diazomethane.

We thought that use of a larger amount of diazomethane would make the reaction become even more complicated and this happened in fact. In addition to the two products in the earlier slide, we got more products (slide 16). In the next slide (17), the structures of the various products are given. We had both the endo- and exo-N-methyl derivatives XLIII and XLV respectively. We had the unsubstituted pyrazoloimidazole (XLIV), as well as the two methylated products, XLVI and XLVII. Additionally, there were two methylcarbamoyl pyrazoloimidazole derivatives XLVIII and isomer LV. The other two products were XLIX and L.

Slide 16

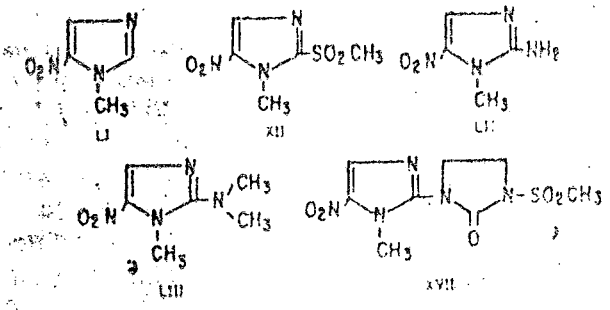


Slide 17

I want to show how and why these were happening in the next slide (18). In trying to unravel the possible mechanisms of these reactions, we noted the fact that many other kinds of nitroimidazoles which we were dabbling with all the time were totally unresponsive to diazomethane — e.g. sulphone (XII), imidazole (LI), amine (LII), dimethylamine (LIII) or even Go 10213 (XVII). This led us finally

Slide 18

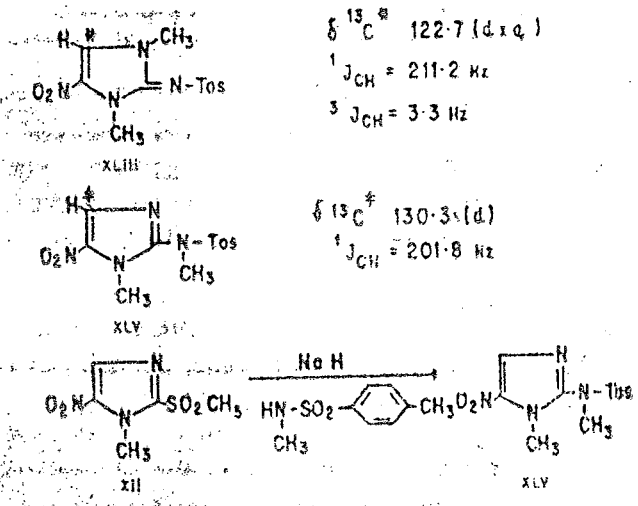
NITROIMIDAZOLES UNREACTIVE TOWARDS DIAZOMETHANE



to get a clue to the structure of the vulnerable intermediate formed in the diazomethane reaction, which gave rise to this plethora of products.

In the next slide (19), I show very briefly how we distinguished between the two isomeric N-methyl derivatives XLIII and XLV. This was again done by

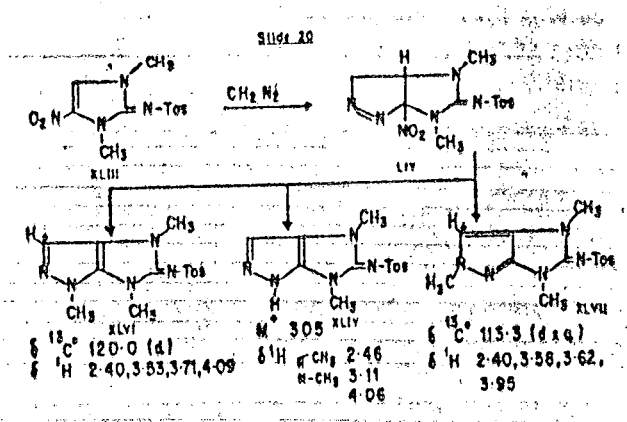
Slide 19



taking recourse to 13_C NMR spectroscopy. The signal of C-4 in XLIII which was easily located, showed in addition to the large one bond coupling, a 3 bond coupling with a methyl group which must be necessarily located on N-3. This was lacking in the isomeric molecule XLV. Now the exo isomer XLV itself was easily identified by comparing with a synthetic specimen that could be obtained by heating sulphone XII with N-methyl-p-toluenesulphonamideion.

In the next slide (20), we show how when the endo-N-methyl derivative XLIII is subjected to reaction with diazomethane, we get most of the products that we had seen earlier, namely two methylatedimidazopyrazoles XLVI and XLVII and the unmethylatedimidazopyrazole XLIV Two methylcarbamoyl imidazopyrazoles were also formed but not shown in this slide. It is now quite obvious as to what was happening. Diazomethane methylates both the endo or nuclear N atom and the exo N in XLII very readily to give rise to the imidazolidinone derivatives XLIII and XLV respectively The endo N-methyl derivative is not so aromatic as the exo-N-methyl isomer and has a relatively active nitro ethylene bond which is vulnerable. This undergoes a cycloaddition reaction with diazomethane to form the intermediate LIV,

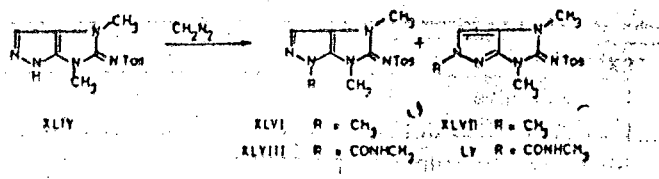
Slide 20



which could lose the elements of nitrous acid to produce the imidazopyrazole XLIV. Since excess diazomethane is around, further methylation is possible. The pyrazole nucleus is quite acidic and undergoes methylation in two possible ways to produce compounds XLVI and XLVII. Also formed are the two methylcarbamoyl derivatives which will be subsequently seen. What is impressive to note is that these two imidazopyrazoles XLVI and XLVII which differ only in the location of the methyl group in the pyrazole half are easily distinguished by resort to 13_C NMR spectroscopy, specifically looking at the signal due to the proton-bearing carbon atom in the aromatic region. This signal is a plain doublet with a large one bond coupling in the case of XLVI, whereas in XLVII, this as one would expect, shows further fine structure due to 3 bond coupling with the methyl protons.

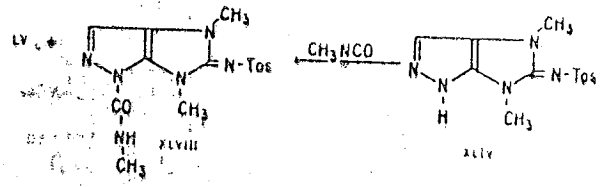
In the next slide (21), I would like to tell you that we did take imidazopyrazole XLIV and again let it react with diazomethane, when besides the methylated derivatives XLVI and XLVII, the methylcarbamoyl derivatives XLVIII and LV were also formed. Now,

Slide 21



we arrived at the structures of these methylcarbamoyl imidazopyrazoles by resorting to conventional analytical and spectroscopic techniques. We could again distinguish between the two isomeric methyl imidazopyrazoles by means of the shifts of the pyrazole carbon atoms which are characteristically different. We supported structure assignments by letting XLIV react with methyl isocyanate when the two methyl carbamoyl derivatives (XLVIII and LV) were formed (slide 22). This led us to wonder as to what the source was for methylisocyanate in this reaction. We were not aware at that time but subsequently learnt it specially from Prof. Pakrashi that when one makes

Slide 22



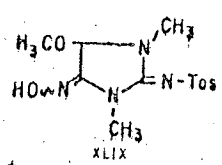
m/e 362 (M^+), 305 ($M^+ - CH_3NCO$)
IR 3400 (NH) 1720 cm^{-1} (CO)
δ^1H 2·42, 3·04 (d, s with D_2O), 3·63, 3·64, 7·86, 4 Ar H

H_3CNCO from $H_3C-N(NO)-CONH_2$

diazomethane from nitrosomethyl urea with aqueous alkali in ether, the reagent so produced has always some methyl isocyanate as a contaminant. There are a few publications in the literature on this phenomenon, but the fact is not too well-known. The percentage of methyl isocyanate formed in this reaction is very critically dependent upon the strength of the alkali and the temperature of the reaction at which one generates the diazomethane. But what is not perhaps adequately known or recognised is the mechanism of formation of methyl isocyanate. This has been investigated using nitrosomethylurea with nitrogen label in two of the three different positions and it appears as if the nitrogen which is involved in methyl isocyanate formation is from the terminal amino group and not either of the other nitrogen atoms, especially the one bearing the methyl group to begin with. For lack of time, I would not like to discuss this any more, but I would like to talk a little bit about the other two compounds from the diazomethane reaction wherein the reagent is not implicated at all.

We could recognize one very readily as being the oxime XLIX (Slide 23), which exists as a mixture of E and Z isomers. This is very much evident from the proton NMR spectrum. The corresponding ketone in this case is the lactam L. The structure can be as-

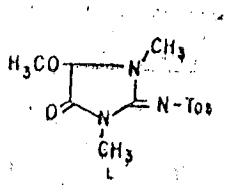
Slide 23



M^+ at m/e 326

^1H n.m.r.

	Major	minor
CH_3	2·42	2·42
2 N-CH_3	3·10	3·41
1 O-CH_3	3·30	3·41
	3·34	3·48
$H_3CO-C-H$	5·68	5·19
N-OH	6·91	5·99
4 Ar H		



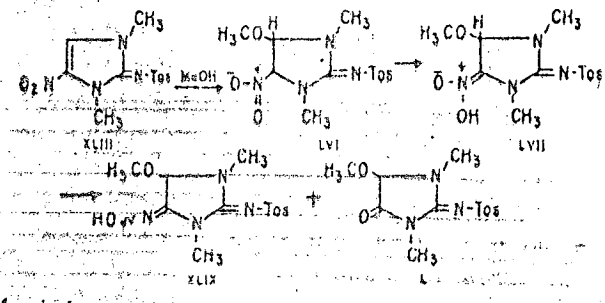
M^+ at m/e 311,
$\nu_{C=O}$ 1760 cm^{-1}

^1H n.m.r.

CH_3	2·46
2 N-CH_3	3·06
1 O-CH_3	3·39
	3·44
$H_3CO-C-H$	5·00
4 Ar H	

signed on the basis of the available data. In the next slide (24), I speculate as to how these could have been formed. Obviously methanol and alkali used in the preparation of diazomethane are implicated.

Slide 24

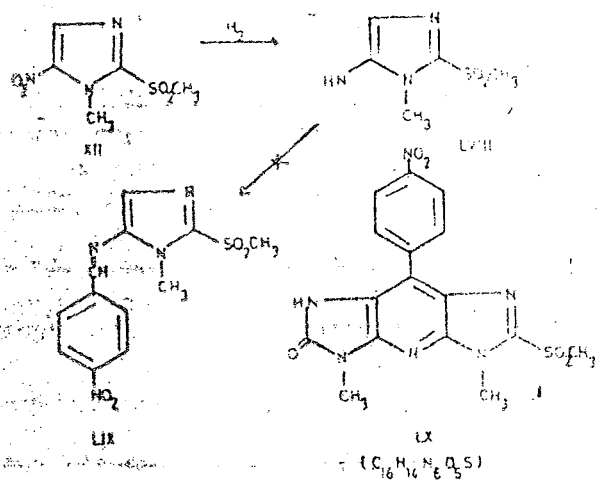


Much interesting chemistry emanated thereby due to what may be considered as sloppy laboratory techniques. What we think must be happening is that 1, 3-dimethyl-2-tosylimino-5-nitroimidazoline (XLIII), which we have already seen has a highly vulnerable and reactive nitroethylene group, undergoes addition of methanol under alkaline catalysis. Species LVI is thus produced which can then go by well-recognised routes either to the oxime XLIX or by reduction with nitrous acid to ketone L (Nef reaction).

Now I would like to go to one last interesting bit of chemistry that we encountered in nitroimidazoles and this arose out of our endeavours to make some reduction products of the active nitroimidazoles. The reason for this is that people have always been speculating that the mechanism of antiprotozoal activity of the nitroimidazoles was somehow connected with the reduction of nitro group to various unidentified species. The end product of reduction must be the aminoimidazoles which are unstable because they are liable to ring opening. We thought that it would be interesting to make some aminoimidazoles from the

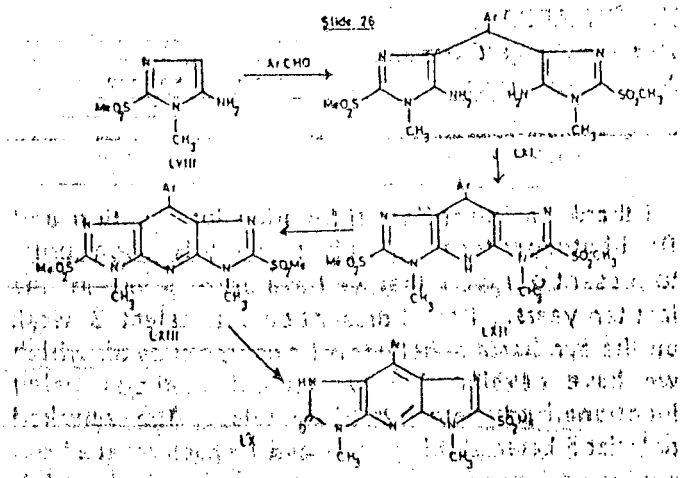
vast array of nitroimidazoles available to us and in most cases, we could get the required compounds by trapping them with various aryl or acyl chlorides, isocyanates and so on and they behaved quite respectably in these manouvres. However, we did come across one interesting phenomenon, accounting which I would like to conclude my lecture and this is shown in the next slide (25). The sulphone XII was a compound that we were handling in kilogram quantities for the synthesis of Go 10213. We thought that it

Slide 25



would be only just if we let it be also reduced catalytically. Incidentally compound XII itself was inactive as an antiamoebic compound. In the event, reduction did produce the expected amino sulphone LVIII and we could characterise it in a variety of ways. When we used p-nitrobenzaldehyde in trifluoroacetic acid to make the Schiff base LIX, to our surprise, we got in rather low yield, quite a complex product for which the molecular formula could be immediately derived as $C_{16}H_{14}N_6O_5S$ by resorting to elemental analysis and mass spectrometry. The proton spectrum revealed some signals due to NH, methyl groups and so on. The protons of the p-nitrophenyl group were also seen; but what was immediately strikingly apparent was the total absence of an azomethine proton (CH=N). Putting together various data that were available to us, we concluded that the compound is best represented as LX, namely an imidazopyridoimidazole with a pendant p-nitrophenyl group. We have requested Prof. Venkatesan to do X-ray crystal structure for this, hoping that it would confirm our speculations. In the last slide (26), I have tried to rationalise these observations.

Slide 26



We look upon the 5-amino-1-methylimidazole sulphone (LIII) as having some enaminic character at C-4. Therefore it should be susceptible to electrophilic attack and two such molecules can be brought in unison by p-nitrobenzaldehyde to produce a benzylidinebis-imidazole LXI. This can undergo cyclisation by losing the elements of ammonia, to produce the dihydropyridine LXII. Dihydropyridines are known to be susceptible to aerial oxidation. Hence one can imagine that LXII can be oxidised to the aromatic compound LXIII and if one would allow the preferential fortuitous loss of *one* methylsulphonyl group in presence of trifluoroacetic acid, one can arrive at the structure of the final product as LX.

I hope that the results that I have presented till now would have shown you that the remarks I made about medicinal chemistry bearing resemblance to Indian movies are fully justified — a little bit of everything for everybody's taste. I thank you for your patience.