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THE STRUCTURE OF NEOANTIMYCIN

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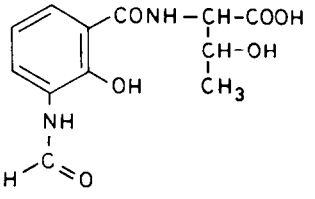
Abstract—Neoantimycin is an antibiotic obtained from *Streptoverticillium orinoci*. The antibiotic is related to antimycins and the structure is reported.

THE isolation of neoaureothin, neoantimycin and ochramycin from *Streptoverticillium orinoci* was reported[1] recently. The present paper concerns the elucidation of the structure of neoantimycin (I).

Neoantimycin is a white crystalline compound, m.p. (dec.) 121–122°. The analytical values and mol wt (mass spec. 698) indicate the formula I ($C_{36}H_{46}N_2O_{12}$). It gives a strong positive reaction (violet) with $FeCl_3$, and with CH_2N_2, a monomethyl-derivative (II) is obtained ($FeCl_3$ test negative) m.p. 108–110°. mol. weight (mass. spec.) 712. calc. for $C_{37}H_{48}N_2O_{12}$. 712. Therefore it can be assumed that II is derived from I by etherification of a phenolic OH.

This is proved by comparison of the NMR spectra of I and II. The IR and UV data of neoantimycin show the relationship to antimycin A_1 (IIIa) and A_3 (IIIb).[2–5] The UV spectra of neoantimycin (I), IIIa, IIIb and blastmycic acid (IV)[2,3,5,7–9] are almost identical.



IV

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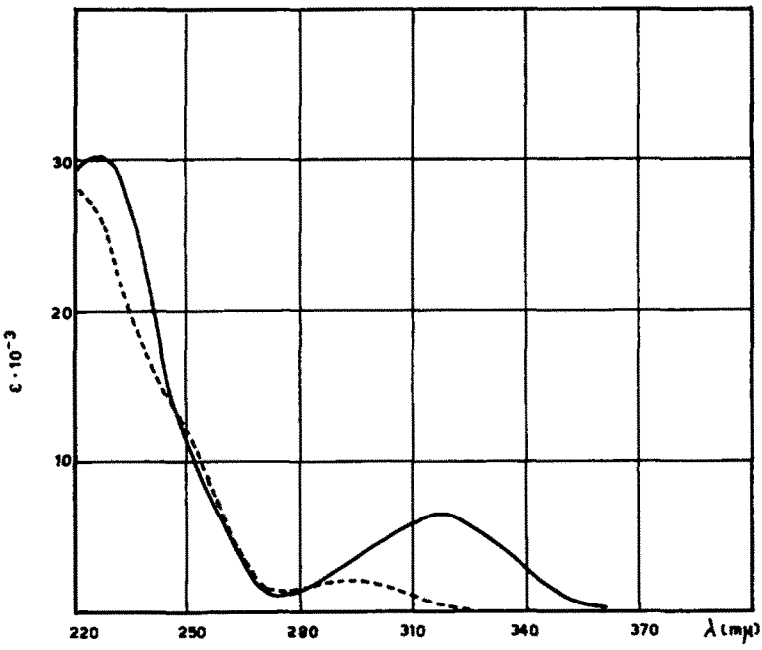
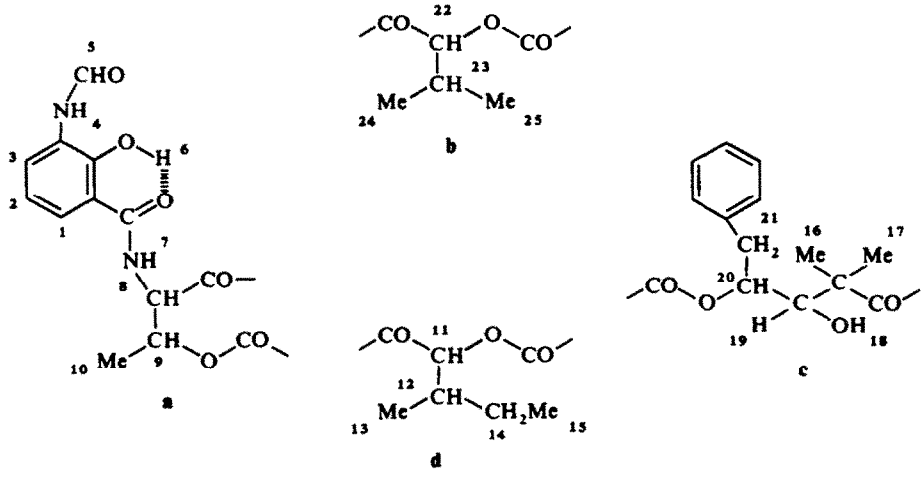


FIG. 1 UV spectra of neoantimycin (———) and neoantimycin, methyl ether (– – – –).

The NMR spectrum of I indicates the presence of the following groups in the neoantimycin molecule:

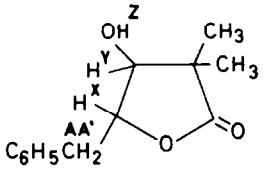


Structure determination of the hydrolysis products of neoantimycin (I). Neoantimycin (I). in common with other antimycins. is readily hydrolysed by mild alkali: the acidified hydrolysate yields a neutral and an acidic fraction:

(i) The neutral fraction furnished a crystalline compound (V). $C_{13}H_{16}O_3$. m.p. 115–116°. whose IR spectrum reveals OH stretching bands at 3623–2425 cm^{-1}

and a C=O stretching frequency of γ-lactone at 1778 cm^{-1}. The NMR spectrum (acetone-d_6) shows one Ph group at 7·30 δ. two equivalent Me's at 1·25 δ (6H). and 5 other protons in the region of 3–5 δ. giving rise to an AA'XYZ type spectrum. The AA' part is a doublet of 6·8 Hz with $\delta_A = \delta_{A'} = 3{\cdot}10$ (—CH_2Ph). the X part is a 6 line pattern centred at 4·78 δ (—CHOAc) with splitting of 6·8 and 3·5 Hz; the signal gives 4 lines at 4·00 δ (CH—OH)* with separations of 3·5 and 5·5 Hz. that collapses to a doublet (3·5 Hz) by addition of trace of D_2O; the Z signal is a doublet of 5·5 Hz at 4·65 δ. which disappears with D_2O. It follows that: H_Z is an OH proton coupled to H_Y alone. H_Y interacts with H_X with $J_{X,Y} = 3{\cdot}5$ Hz. and $\frac{1}{2}|J_{AX} + J_{A'X}| = 6{\cdot}8$ Hz.

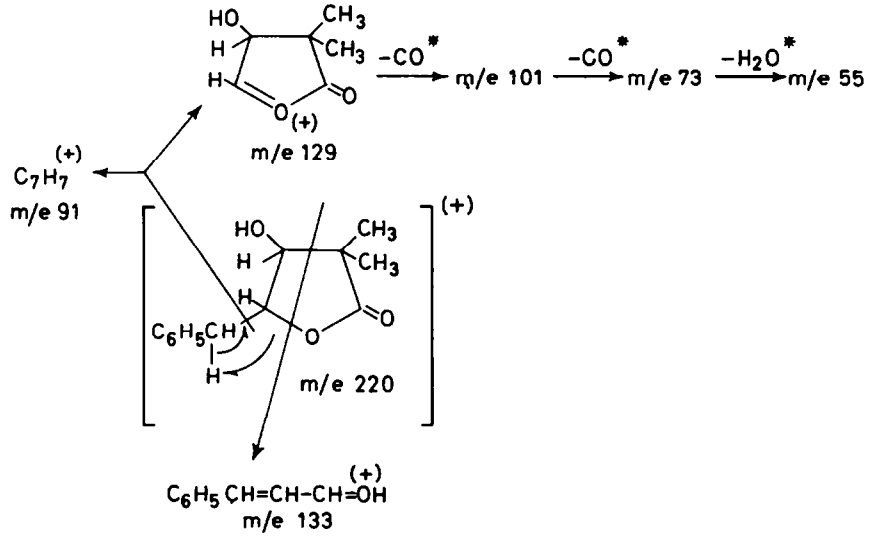
From these data the compound was considered to be the γ-lactone (V) derived from 2,2-dimethyl-3,4-dihydroxy-5-phenyl-valeric acid (VI).



V

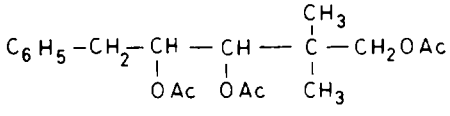
The mass spectrum of V confirms the assigned structure; a molecular ion peak at *m/e* 220 is present. from which are derived the important fragments reported in Scheme 1.

SCHEME 1

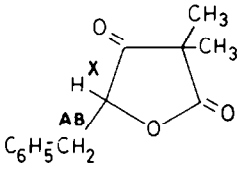


* H_Y goes down-field (5·11 δ) on acetylation (Experimental).

Structure V is also supported by chemical data. Acetylation of V furnishes a monoacetate (Va). m.p. 82–83°. $C_{15}H_{18}O_4$. whose IR spectrum shows no OH bands and γ-lactone and acetate absorptions. Reduction of V with LAH furnishes a triol (VII) readily acetylated to a triacetate $C_{19}H_{26}O_6$ (VIII). The $NaIO_4$ oxidation of the triol furnishes phenylacetaldehyde and 3-hydroxy-2,2-dimethylpropionaldehyde which readily dimerizes to 4-hydroxy-5,5-dimethyl-2-[2-hydroxy-1,1-dimethyl-ethyl]1,3-dioxane.[10] These data indicate that triol VII is 2,2-dimethyl-5-phenyl-pentan-1,3,4-triol. Mild oxidation of V with chromic acid furnishes a keto-lactone (IX). m.p. 47–48°. $C_{13}H_{14}O_3$. which shows ketone (1757 cm^{-1}) and γ-lactone (1802 cm^{-1}) IR bands. and the loss. in the NMR spectrum. of the $—CH_YOH_Z$ group. The spectroscopic properties of IX support the structure of 2.2-dimethyl-3-keto-4-hydroxy-6-phenyl-valeric acid. γ-lactone.



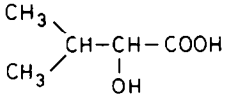
VIII



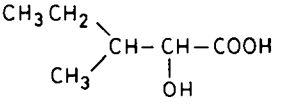
IX

As the IR spectra of I and II do not show C=O bands of a γ-lactone. it must be assumed that the lactone (V) is formed when the alkaline solution of hydrolysed neoantimycin is acidified.

(ii) The acid material. formed during alkaline hydrolysis of neoantimycin. was esterified with diazomethane. The product was separated into a volatile fraction and a residue by vacuum distillation. Gas chromatographic analysis indicated that the distillate contained two compounds in the ratio 1:1. These have been isolated by preparative gas chromatography and. on the grounds of their analytical. chemical and spectroscopic properties. identified as the Me esters of (*S*)(+)-α-hydroxy-isovaleric acid[11] (X) and 2*S*,3*S*(+)-α-hydroxy-β-methyl-valeric acid[12] (XI).



X



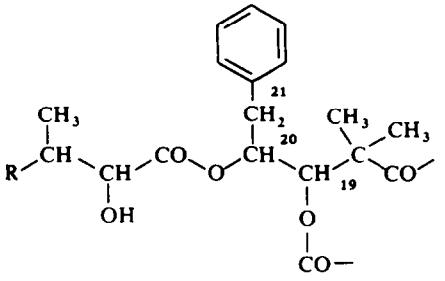
XI

(iii) Purification of the residue after distillation by preparative TLC gives an amorphous solid. whose IR and UV data are close to those of the Me ether. Me ester of blastmycic acid (IV). However, the NMR spectrum reveals that the sample obtained was a mixture of Me ether. Me ester of blastmicic acid (IV) and Me ether. Me ester of N-(3-formylamino-salicyloyl)α-amino-crotonic acid (dehydro-blastmicic acid), (XV) methyl ester, in the ratio 3:2 (Experimental).

As expected. the alkaline hydrolysis of this compound. followed by acidic treatment with HCl. furnished formic acid. 3-amino-salicylic acid and threonine.* On the basis of these results. the fourth unit was assigned the structure of Me ether. Me ester of blastmicic acid (IV).

Determination of the sequence in neoantimycin. The four units isolated after hydrolysis of neoantimycin (I) account for all the C atoms of the antibiotic. The presence of one free OH (apart from the phenol) in neoantimycin has been observed.

The free alcoholic OH in neoantimycin must be placed in the unit V. i.e. on fragment (c). follows from spectroscopic and chemical evidence.



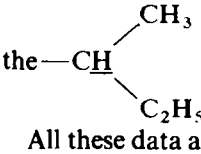
e

Structures such as (e) are not supported by the NMR spectrum of neoantimycin (see Discussion of NMR spectrum and Fig. 5). especially:

(i) As H-20 does not show other couplings except those with H-21B and H-21A. $J_{19,20}$ must be zero.

(ii) Then H-19 in structure (e) should be a singlet lying in the 4·5–5·5 δ region of the spectrum. but no singlet is present between 1·33–8·50 δ.

(iii) The CH—OH group is an AB quartet (3·66. 3·31 δ) with coupling across oxygen of 12·0 Hz. which excludes further interaction with vicinal protons. Actually in structure (e) there is a proton vicinal to this group which should be coupled, because it is very difficult to explain a dihedral angle of 90° in such a freely rotating side-chain.

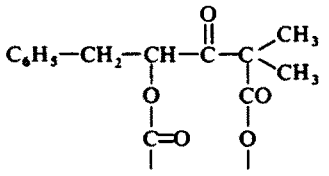
(iv) Moreover. decoupling experiments have shown that the proton of the isopropyl group (H-23) is coupled to the doublet at 5·43 δ ($J_{23,22} = 3{\cdot}5$) and the proton of the  group (H-12) is coupled to the doublet at 4·72 δ ($J_{12,11} = 8{\cdot}2$).

All these data are not compatible with structure (e). Consequently we can conclude that. not only the alcoholic proton must be placed on fragment (c) but. as coupling between H-19 and H-20 is zero. and $v_{19,20} \cong 90°$. these two hydrogens must be part of a large ring. These conclusions are also supplied by chemical evidence:

Mild chromic acid oxidation of neoantimycin. methyl ether (II) furnishes neooxoantimycin, methyl ether (XII), m.p. 100–102°. $C_{37}H_{46}N_2O_{12}$, m.w. 710 (mass

* The configuration of the threonine was not elucidated.

spec.) calc. 710, which does not have absorption in the OH region of the IR spectrum. Moreover. the NMR spectrum shows that oxidation of fragment (c) had occurred yielding (c') (see Discussion of the NMR spectrum and Fig. 6) whereas fragments

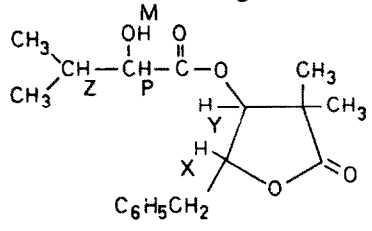


c'

(a). (b) and (d) are unchanged. Mild reduction of XII with $NaBH_4$ gives II in 50–60% yield confirming that no undesired rearrangements occurred during the oxidation of II to III. Mild alkaline hydrolysis. under N_2. of XII furnishes three of the four units isolated by analogous treatment of II: the Me esters of X and XI in 1:1 ratio and the Me ether Me ester of IV. Lactone V was not isolated in this case. but a substantial yield of keto-lactone (IX) was obtained. In this way. it has been confirmed that in neoantimycin only one free OH is present (apart from the phenol). and the OH is located on fragment (c).

At this stage. we can assume that neoantimycin is a cyclic polyester. resulting from the condensation of the four units (IV. VI. X and XI). and 6 different structures must be considered.

Among the products obtained from alkaline hydrolysis of I and II a crystalline product (XIII). $C_{18}H_{14}O_4$. was repeatedly isolated in low yield. The UV spectrum of XIII shows low absorption between 250–260 mμ and the IR indicates the presence of OH. γ-lactone and ester group. Mild alkaline hydrolysis gives the lactone V and α-hydroxy-isovaleric acid: hence the following structure (XIII) was assigned. This



XIII

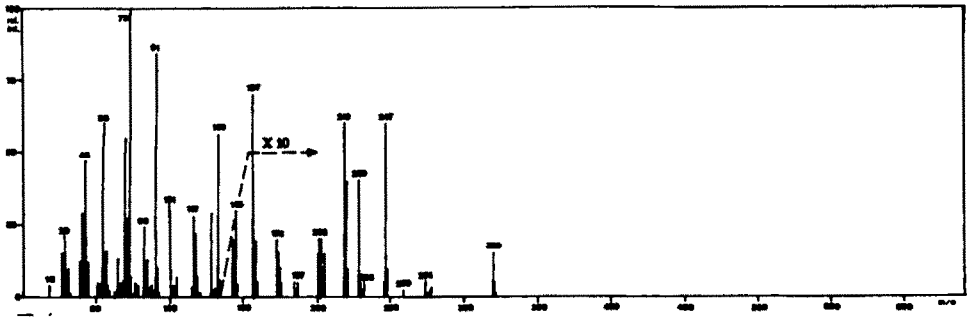
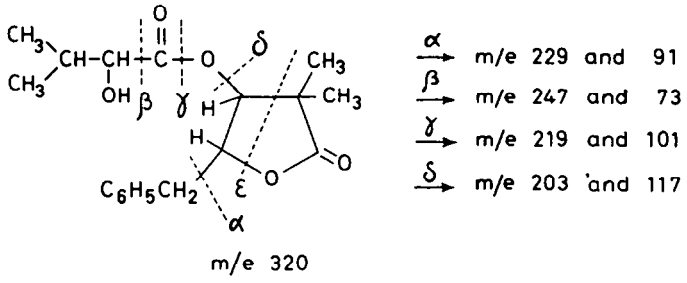


FIG. 2 Mass spectrum of XIII.

is in good agreement with the NMR and mass spectroscopic data (Fig. 2). In Scheme 2 are represented the simple cleavages that give rise to important peaks in the mass spectrogram.

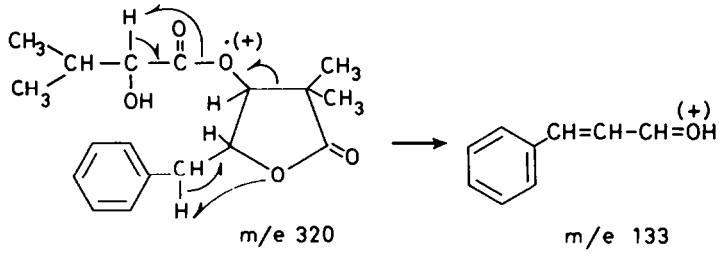
SCHEME 2



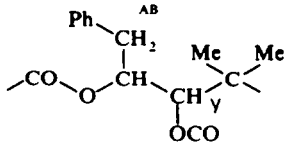
Moreover. the fragment of mass 233 is produced by the breakdown at ε accompanied by migration of an H atom.

The ions of mass 220 and 118 are due to the cleavages at γ and δ both with transfer of an H atom. The intense peak at *m/e* 133 may correspond to the fragment formed by the cleavages γ and ε by the mechanism shown in Scheme 3.

SCHEME 3

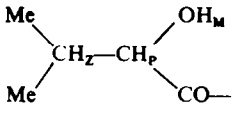


The NMR spectrum (acetone-d_6) shows: one phenyl in a single peak at 7·30 δ. two Me singlets at 1·14 and 1·33 δ. two Me doublets at 0·93 and 1·04 δ with J = 6·8 and J = 7·0 Hz. one proton in a complex absorption between 2–2·5 δ partially overlapped by acetone-d_5 signal.* and 6 other hydrogens in the field region 3–5·5 δ. Four protons of the latter are arranged in an ABXY spectrum: of the AB part only 3 lines are visible. the X signal at 5·03 δ gives 8 lines (splitting of 7·5. 5·8 and 3·8 Hz). the Y signal is a doublet of 3·8 Hz at 5·61 δ. It was deduced that $\delta_A \cong \delta_B$ = 3·0. J_{XY} = 3·8. and $|J_{AX} + J_{BX}|$ = 13·3 Hz. From chemical shift values. the AB protons must be bonded to the phenyl and each X and Y proton to an —C—CO group. Hence the following sequence was proved:

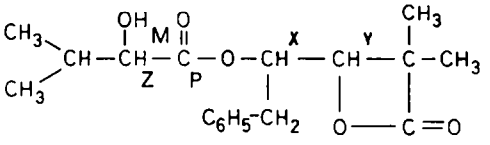


* In $CDCl_3$ this proton is a broad signal of ~40 Hz centered at 2·10 δ.

The two remaining protons shows a doublet at 4·36 δ with $J_{obs} = 6{\cdot}5$ which disappears with a trace of D_2O (OH_M). and a four line pattern of 6·5 and 4·0 Hz (H_P). that collapses to a doublet of 4·0 Hz with D_2O. It must be concluded that H_P ($\delta_P = 4{\cdot}14$: J_{MP} (obs) = 6·5) is also coupled to the proton near 2 δ (H_Z) with $J_{PZ} = 4{\cdot}0$, and H_Z is responsible for the interaction of the two Me's at 0·93 and 1·04 δ. Therefore the following sequence must be present:



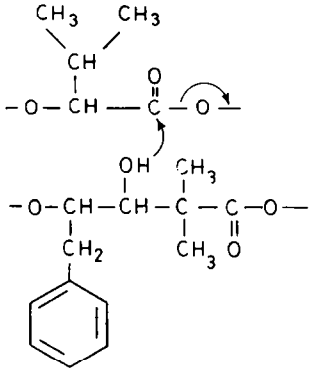
These fragments may be arranged in structures XIII or XIIIa:



XIII a

Structure XIII is favoured as the value of $J_{XY} = 3{\cdot}8$ Hz is the same as that found in the 5-membered ring of V (3·5 Hz). and its acetyl derivative (3·8 Hz).

In XIII the OH group which in neoantimycin is free. is esterified by α-hydroxy-isovaleric acid. This suggests that hydrolysis of neoantimycin involves a rearrangement of the type indicated:



The occurrence of a similar rearrangement is observed also during the thermal treatment of neoantimycin and of its methyl ether.

The mass spectra of neoantimycin (I) and its methyl ether (II) (see discussion of mass spec. and Fig. 8 and 9) show:

(a) important fragments of weight M-132 due to the loss of XI from the molecular ions of I and II.

(b) very abundant ions due to the fragmentation of XI.

It seems that the hydroxy acid (XI) is in some way "different" from the other components as regards the mass spectra of the antibiotic. As during the recording of the mass spectra the compounds are volatilized at 200°. it cannot be excluded that a rearrangement with expulsion of XI occurs during this thermal treatment.

In order to verify this hypothesis. neoantimycin methyl ether (II) was heated *in vacuo* at 150–160° for 3 hr. Two compounds were obtained. the acid XI and a solid compound (XIV) $C_{31}H_{36}N_2O_9$. which account for all the C atoms of the intact antibiotic. The UV spectrum of XIV is reported in Fig. 3. The IR spectrum

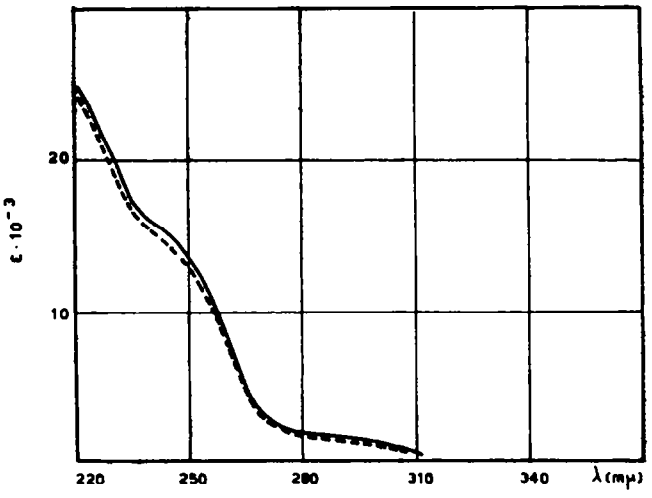
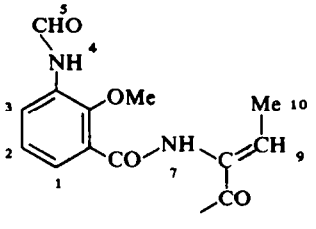
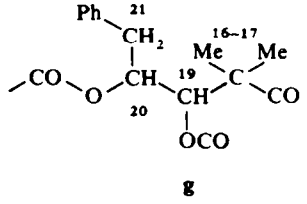


FIG. 3 UV spectra of XIV (———) and XV, methyl ester (– – – –).

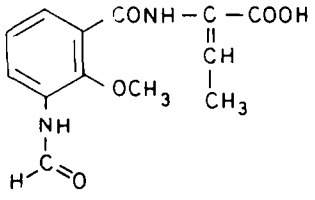
displays carbonyl absorption and does not show an OH band. The band at 1779 cm^{-1} has been attributed to a γ-lactone function as it was also found in V. Va and in XIII. The NMR spectrum (Fig. 7) indicates the presence of fragments **b**. **g** and **f**:





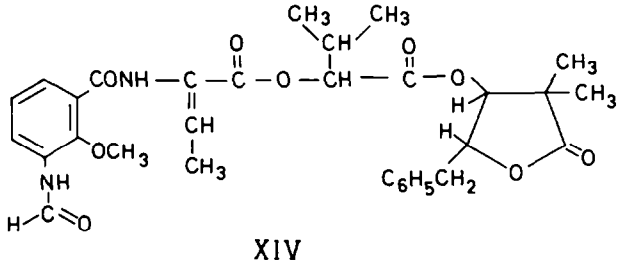
Mild alkaline hydrolysis of XIV followed by treatment with CH_2N_2. furnishes V, VIII, the Me ester of X and an amorphous solid XV. The UV spectra of XV, Me ester and XIV are similar (Fig. 3). The IR shows in the region 5–7 μ the same absorptions of XIV except the γ-lactone band at 1779 cm^{-1}. The NMR spectrum indicates the

presence in XV, Me ester of the fragment **f**. On the basis of the data the structure of N-(3-formylamino-salicyloyl-α-amino-crotonic acid. Me ether was assigned to XV.



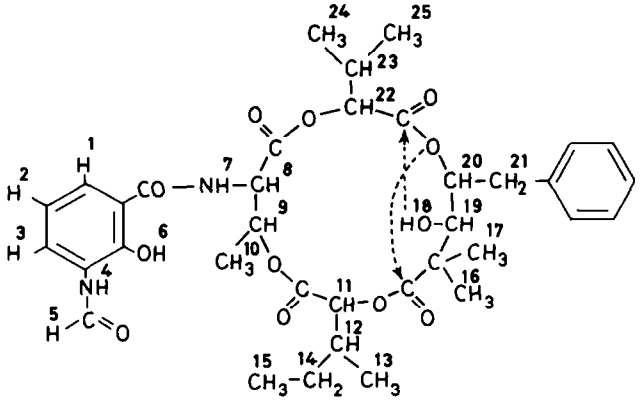
XV

The above data. the spectroscopic characteristics of XIV and examination of the mass spectrum (Fig. 10) indicates that XIV has the following structure:

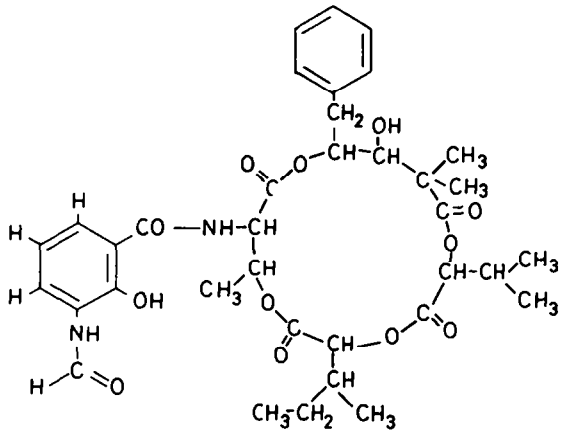


XIV

The formation of the double bond in XIV may be ascribed to a β-elimination process. As the group eliminated is the acid (XI). we can assume that in the antibiotic the fragments **a** and **d** are joined together. This fact reduces the number of theoretically possible structures from 6 to 2. i.e. I and XVI:



I



XVI

The formation of XIV from the neoantimycin. Me ether (II) can be explained by assuming two successive intramolecular transacylations. as indicated by the dotted arrows in I. In both cases. 1.5 intramolecular interactions are involved. The elimination of the acid (XI) can be related to analogous reactions on synthetic blastmycic acid.[13,14] On the contrary. the formation of XIV cannot be easily explained by chemically plausible rearrangements. On this basis. structure I seems more likely for neoantimycin.

This conclusion may be confirmed by independent spectroscopic considerations.

(i) Structure I better explains some features of the NMR spectra of neoantimycin and neooxoantimycin. methyl ether: the two methyl groups of the isovaleric unit are not equivalent; their strong shift difference (0·34 δ) and especially the very high-field position of one of them (0·44 δ) suggests this unit is near a strong anisotropic group. like phenyl.* in such a way that one isopropyl Me is in front of the ring. i.e. ca. perpendicular to the aromatic plane (shielding conical region).

Among the high number of conformations that this molecule may adopt. it is not unlikely to suppose that one would be preferred. always keeping in mind the possibility of the hydroxyl (OH-18) H-bonding† to one of the carbonyls of the large ring (e.g. —CH^{22}—CO—O—). As we found a coupling constant of zero between two vicinal protons (H-20 and H-19). we must conclude that. with a dihedral angle of $\cong 90°$. the large ring of the molecule should indeed exist in a preferred conformation, at least in that half of the ring containing C_{19}—C_{20}.

Then, with the aid of Dreiding models, we can see that all the conformations that satisfy the condition of $\vartheta = 90°$. lead to the isopropyl group in front of the phenyl. with one Me very near to it (0·44 δ), and the other a little further away (0·78 δ): but this holds only if the sequence is as in I. In XVI the isovaleric unit is too far away from the phenyl.

* The substituted benzene ring is anyway too far away.

† From the chemical shift of H-18 in acetone solution this H-bond seems very weak.

(ii) Moreover the choice in favor of I over XVI was substantiated by the mass spectral data of neooxoantimycin. Me ether (XII; Fig. 4) which does not undergo rearrangement with elimination of α-hydroxy-β-methyl-valeric acid. as it does in neoantimycin.

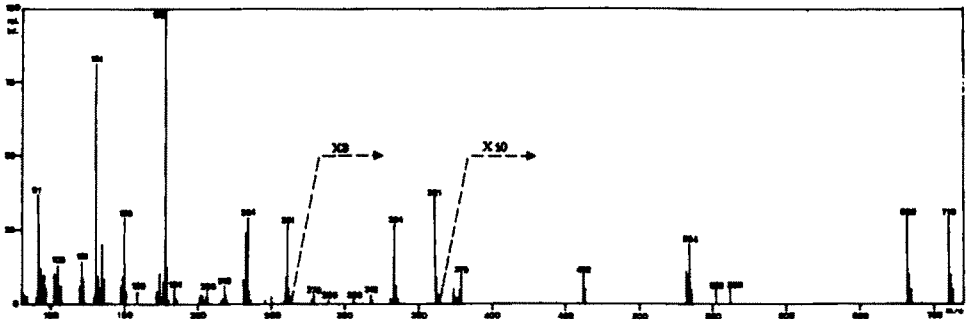
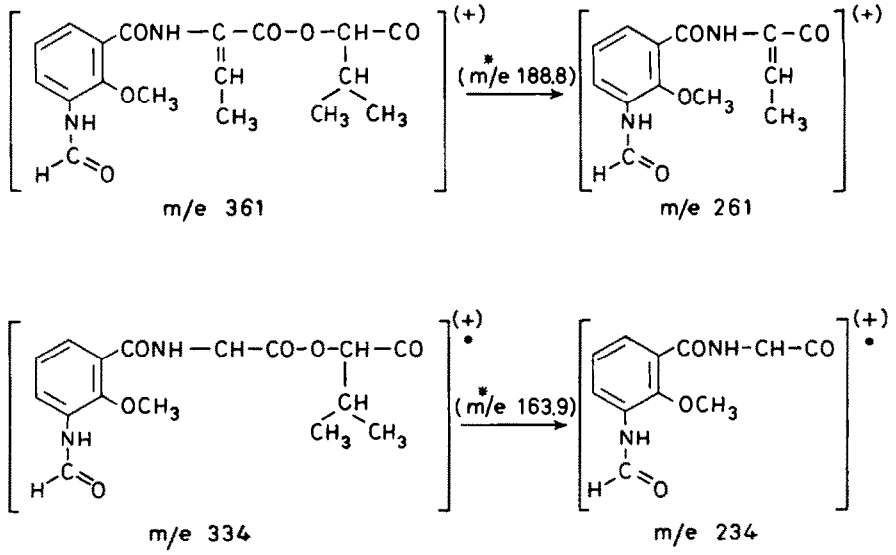


FIG. 4 Mass spectrum of OMe-neooxoantimycin XII.

The two transitions 361 → 261 and 334 → 234 with the appropriate metastable ions (Scheme 4) confirms the partial sequence IV–X* for XII and consequently for I and II.†

By treatment of XII with D_2O in acetone. the two protons of the two N-atoms are exchanged with deuterium. The mass spectrum of the exchanged product shows that the weight of the two couples of ions reported in Scheme 4 are increased by two mass units according to their structures.

SCHEME 4

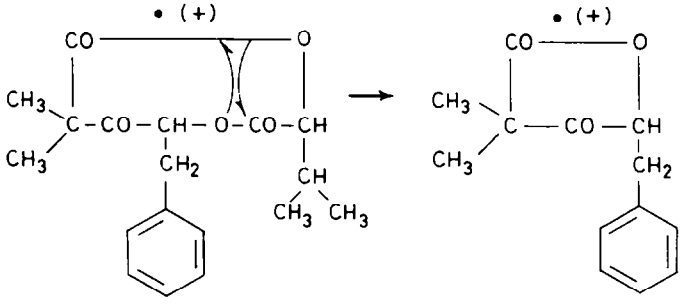


* Conventionally we assume that in a partial sequence each oxyacid is linked to the following one by its carboxyl group.

† It was proved that no rearrangement occurs during the oxidation of II to XII. (Introduction).

Moreover. in the spectrum of XII. two peaks at *m/e* 318 and 218 are present and. unaffected by treatment with D_2O. for which we propose the structure reported in Scheme 5:

SCHEME 5

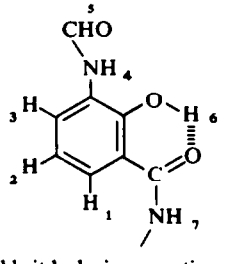


The fragment of mass 318 requires the presence of the partial sequence X–VI or VI–X in the structure of neoantimycin.

All the chemical and spectroscopic evidence supports the formula I for neoantimycin.

DISCUSSION OF NMR SPECTRA

Neoantimycin. (Fig. 5). The low field part of the spectrum shows: one chelated* OH at 12·87 δ which exchanges immediately with vapour TFA, two NH protons of amidic character (they do not exchange with TFA) at 9·06 δ (N<u>H</u>CHO) and 8·30 δ (d, $J_{NH,CH} = J_{7,8} = 9{\cdot}0$ Hz); one formyl hydrogen at 8·54 δ (slightly broad s. N—CHO); and 8 aromatic protons: 5 almost equivalent hydrogens in a single absorption at 7·30 δ. must be attributed to a Ph group bonded to a CH_2: the other 3 protons give an AMX pattern with a triplet at 6·92 δ (H-2), a double doublet at 8·51 δ (H-1) and another double doublet at 7·73 δ (H-3), $J_{1,2} = J_{2,3} = 8{\cdot}0$, $J_{1,3} = 1{\cdot}3$ Hz). The presence of one *meta* and two *ortho* couplings indicates the presence of three vicinal protons. The substitution on the aromatic ring follows from the very low shift value of H-1.† which must be *meta* to the OH and in the *ortho* position to the CO and from the presence of the strongly chelated OH. that requires the same CO in *ortho* position to the OH:



* Even in $CDCl_3$ this signal is at low field: it lacks in neoantimycin. Me ether.

† The benzamide shows the protons *ortho* to the —$CONH_2$ group at 7·92 δ (acetone). The further downfield shift found here may be explained by the perfect coplanarity of the benzene ring and the —CONH— group because of intramolecular H-bonding. As a consequence H-1 experiences the deshielding effect of the CO, and the *peri* effect of the amidic nitrogen, on which some positive charge is localized.

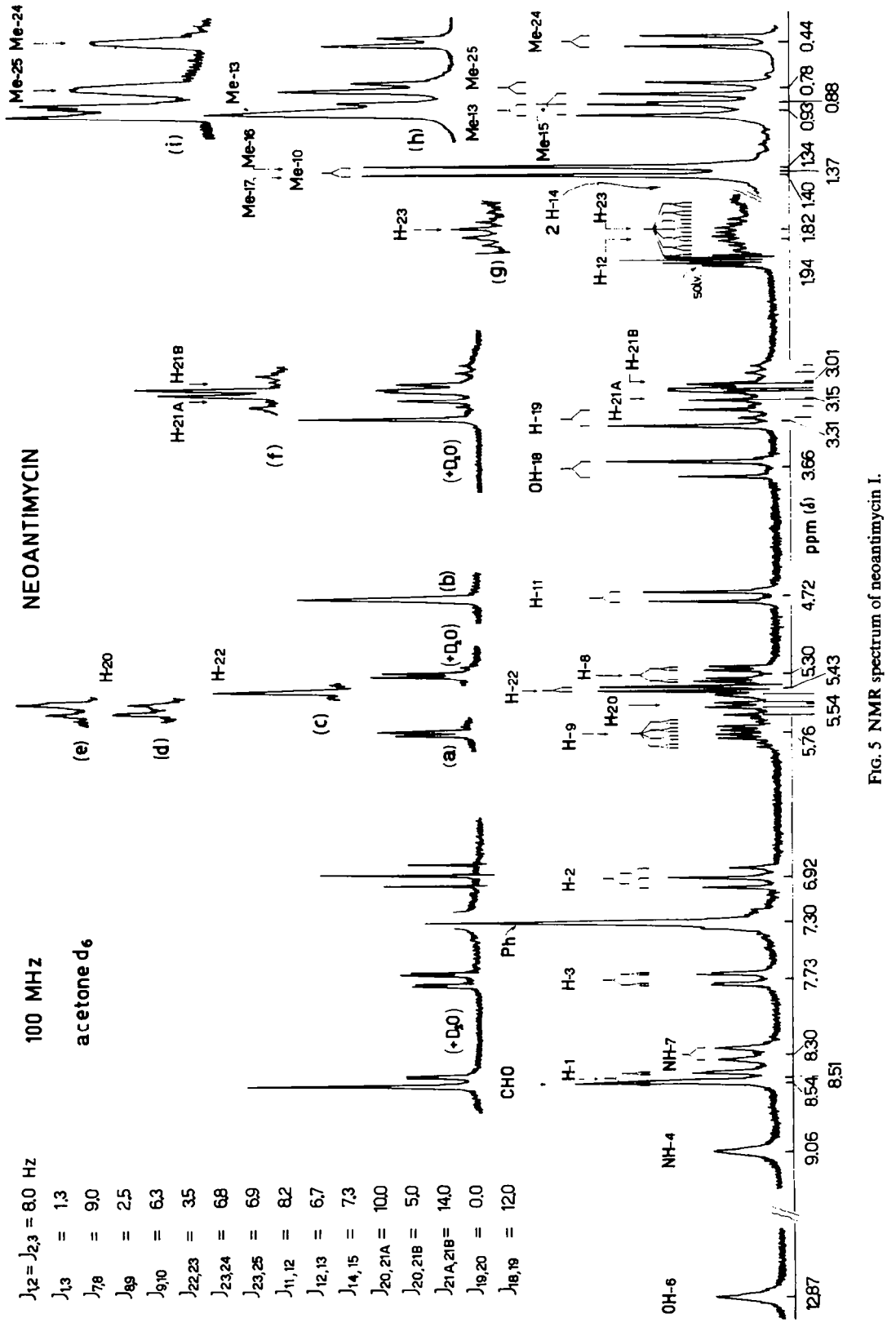
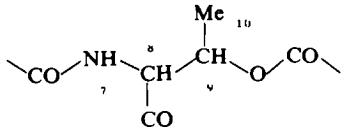


FIG. 5 NMR spectrum of neoantimycin I.

With a trace of D_2O the OH and NH signals disappear.

At higher field. a complex absorption of 4 protons occurs: (1) a double quartet at 5·76 δ (H-9): (2) a four line pattern centred at 5·54 δ (H-20): (3) a small doublet at 5·43 δ (H-22): (4) a double doublet at 5·30 δ (H-8).

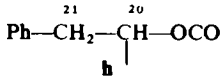
The double quartet at 5·76 δ collapses into a small doublet of 2·4 Hz by decoupling of the Me at 1·37 δ (Fig. 5a); whereas the 2·5 Hz splitting was removed on irradiation at 5·30 δ. The proton at 5·30 δ shows splitting of 2·5 and 9·0 Hz; as its 9·0 coupling vanishes with a drop of D_2O. it must be correlated to the NH doublet of 9·0 Hz at 8·30 δ. The following sequence:



where the substitution at C-8 and C-9 is suggested by the chemical shift values of H-8 and H-9. was thus proved. and fragment **a** can be drawn up.

The 4 line pattern of one proton at 5·54 δ. with splitting of 6·0 and 9·0 Hz. is the X part of an ABX spectrum: the AB part. of 8 lines. lies in the 3 δ region of the field. From the analysis* $J_{AB} = 14{\cdot}0$. $J_{AX} = 10{\cdot}0$. $J_{BX} = 5{\cdot}0$. $\delta_A = 3{\cdot}01$. $\delta_B = 3{\cdot}15$ are obtained.

The value of J_{AB} suggests a geminal coupling. and the chemical shifts of H_A and H_B are in agreement with a CH_2 in the allylic or benzylic position. Taking into account that a Ph group is bonded to a CH_2, and that no other absorption can be attributed to such a methylene. the sequence **h** is complete:



The chemical shift of H-20 as well as that of the other signals in this part of the spectrum (4·5–5·8 δ) indicates at least the presence of the substituent group —OCO— in the vicinity of each proton. provided that no double bond is present in the molecule.

The small doublet of 3·5 Hz at 5·43 is decoupled (Fig. 5c) on irradiation at 1·82 δ: between 1·6 and 2·0 8 lines are visible with splitting of 3·5 Hz at the top of a complex absorption integrating for 2H.† The reverse experiment (irrad. at 5·43 δ) leads to an apparent quartet of 7 Hz (Fig. 5g); which has to be interpreted as the inner part of a septet due to a proton in an isopropyl group —C<u>H</u>Me_2: this proton at 1·82 δ is indeed coupled to the methyls at 0·78 ($J = 6{\cdot}9$) and 0·44 δ ($J = 6{\cdot}8$).‡ Hence the 8 visible lines are part of a septet of doublets. and consequently fragment **b** has been proved.

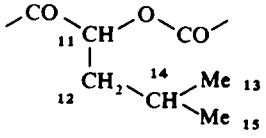
* This analysis was confirmed by decoupling: irrad. of Hx at 5·54 ($\Delta\nu = -243$ Hz) leads to the AB quartet (Fig. 5f): reversal experiment converts the X signal into a doublet of 9 Hz ($\Delta\nu = +226$ Hz) and resp. in a doublet of 6–6·5 Hz ($\Delta\nu = +249$). Decoupling is not perfect. because of the small δ_{AB} (Fig. 5d and 5e).

† The integrals were measured also in $CDCl_3$ where no solvent absorptions are present.

‡ Irrad. at 1·82 δ (Fig. 5i) perturbs all the Me protons between 0·44–0·93 δ because of the presence in this area of the other hydrogens coupled to the methyls. This attribution was possible because the irrad. at 1·97 δ decouples the doublet at 0·93 leaving unchanged the doublets at 0·44 and 0·78 and the triplet at 0·88 δ (see later).

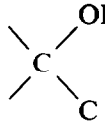
The large difference in chemical shift of the two sets of Me protons in the isopropyl group has already been discussed.

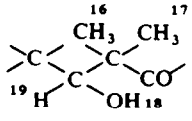
The other proton near 2 δ is completely overlapped by H-23 and acetone-d_5 peak. is responsible for the 8·2 Hz doublet at 4·72 δ; on irradiation at 1·94 δ. J = 8·2 vanishes (Fig. 5b): at the same time. the Me doublet at 0·93 δ is decoupled (Fig. 5h).* Moreover. there is one Me which may be either a triplet at 0·88 δ. or a doublet centred at 0·84 δ, and two protons in a broad absorption between 1·3–1·5 δ. It follows that these 10 protons may be arranged as in **d** or **d'**:



d'

We have discarded fragment **d'** because H-11 is a sharp doublet (4·72 δ). and. as a consequence. it must be near to only one hydrogen (H-12. $J_{11,12}$ = 8·2).

There are still two singlet methyls at 1·34 and 1·40 δ overlapping with the doublet centred at 1·37 δ (Me-10).† The last pattern is an AB quartet at 3·31 and 3·66 δ with J_{obs} = 12·0 Hz which collapses to a singlet of 1 proton (3·31 δ) with a drop of D_2O. while the signal at 3·66 disappears. This pattern must be due to a group of the type  which. together with the above singlet methyls can give rise to a fragment such as **i**. The chemical shift of H-19 has indicated both saturated C atoms near to it. The junction of fragment **i** to fragment **h** to give **c**. is suggested by the NMR



i

spectrum of OMe-neooxoantimycin and definitely proved by the spectrum of the hydrolysis product V.

This could not be deduced from neoantimycin alone. because unfortunately the coupling constant between H-19 and H-20 is zero. It means that the dihedral angle between H^{20}—C—C—H^{19} is $\cong 90°$; an occurrence which is not difficult to explain since such a molecule could indeed be expected to exist in a preferred conformation (Introduction).

* Decoupling is not perfect because the irradiation was made here a little lower (at 1·97 δ) to avoid as much as possible. perturbation of the other methyls.

† The possibility of having three doublets with the same chemical shift was ruled out by the comparison with the spectrum at 60 MHz. where the separation between the two stronger peaks is 3·8 Hz. while the splitting (coupling constant) of the doublet at 1·37 remains 6·3 Hz.

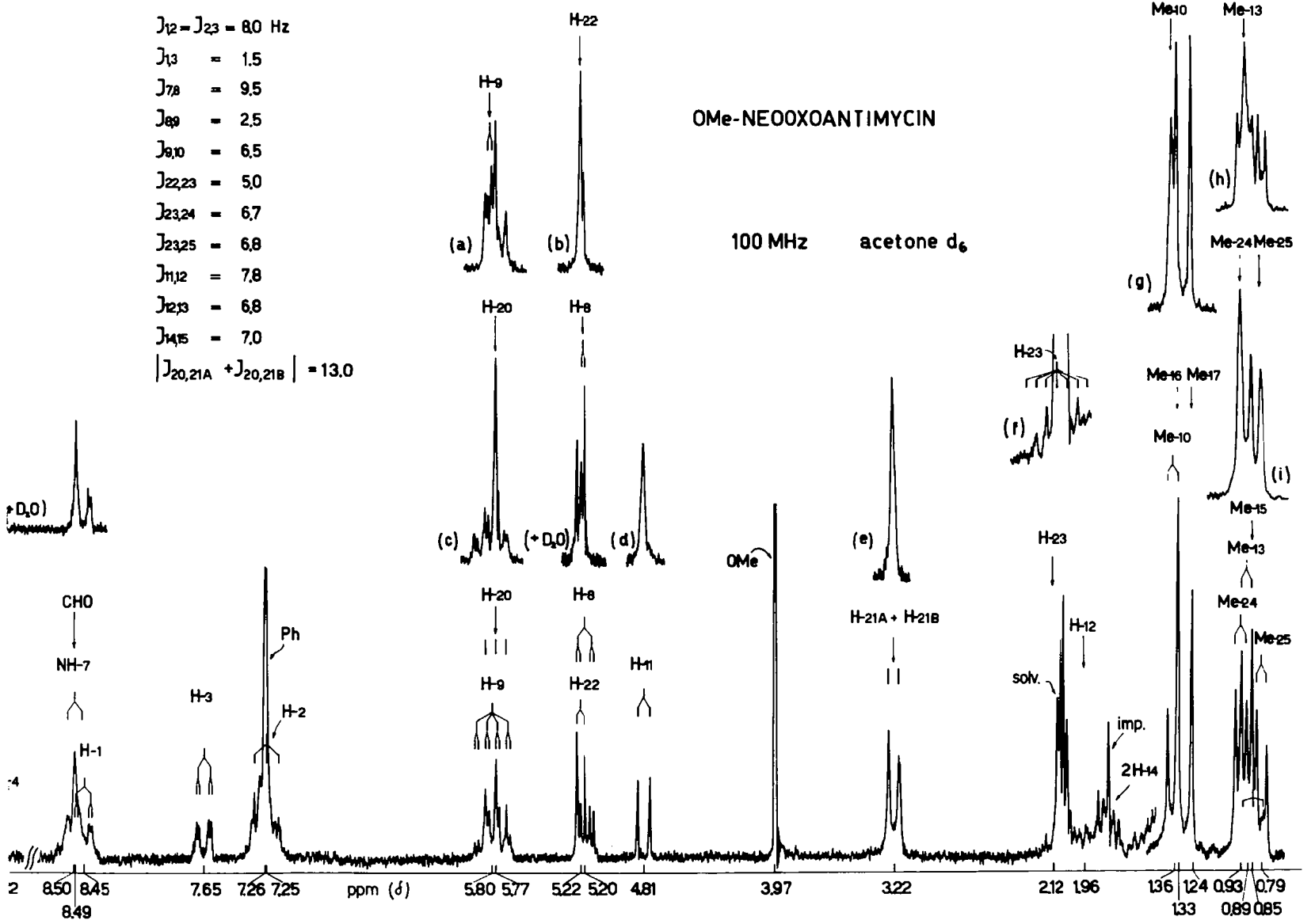
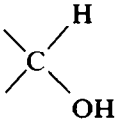
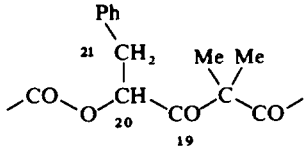


FIG. 6 NMR spectrum of OMe-neooxoantimycin XII.

OMe–neooxoantimycin. The spectrum of OMe–neooxoantimycin (Fig. 6), compared to that of neoantimycin and OMe-neoantimycin, had clearly shown the loss of the AB pattern at 3·66 (H-18) and 3·31 δ (H-19). i.e. of the group  while H-20 goes down-field by 0·23 δ. Moreover. the 2 protons of —CH_2—Ph group (H-21A and H-21B) are slightly deshielded by 0·16 and 0·09 δ; here. the AB part is a doublet of 6·5 Hz with $\delta_A \cong \delta_B = 3{\cdot}22$. which collapses to a singlet (Fig. 6e) on irradiation of H_X (H-20) at 5·77 δ (triplet of 6·5 Hz $= \frac{1}{2}|J_{AX} + J_{BX}|$, overlapped by the H-19 absorption). The reverse procedure (irradiation at 3·22 δ) confirms this assignment (Fig. 6c). Then. one or both Me singlets move up-field (1·33 and 1·24 δ).

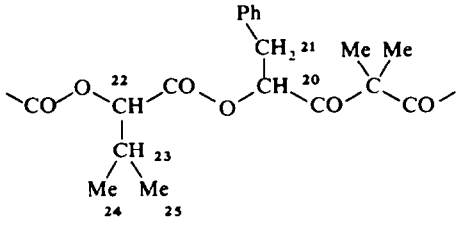
The data proves that oxidation of OMe-neoantimycin occurs at the fragment **i** and suggests the sequence **c'**:



c'

The other parts of the whole spectrum are substantially unchanged in respect to neoantimycin and OMe-neoantimycin. the only exception being the isovaleric acid unit. Here H-22 goes up-field by 0·21 δ (d of 5·0 Hz at 5·22 δ). H-23 moves down-field by 0·30 δ (2·12 δ. hidden by acetone $-d_5$ absorption): $J_{22,23}$ also changes from 3·5 to 5·0 Hz. The two Me doublets are either both shifted down-field from 0·44 (Me-24) and 0·78 δ (Me-25) to 0·79 (Me-24) and 0·93 δ (Me-25). or one remains unchanged (0·79 δ. Me-25). and the other moves down-field by 0·49 δ (0·93 δ. Me-24). The second hypothesis seems more probable.

The data may be interpreted by a change of conformation of the molecule due mainly to the lack of the OH-18. and with long-range effects of the strongest anisotropic group (i.e. Ph) on the proton of the isovaleric acid residue. At this point it is significant to observe that all protons of fragments **a** and **b** are strictly unchanged (chemical shifts and coupling constants): as a consequence the isovaleric acid unit must be the nearest to the Ph group. i.e. in the sequence:



The above assignments were made by decoupling experiments. The H-22 doublet of 5·0 Hz at 5·22 δ collapses on irradiation at 2·12 δ (H-23) (Fig. 6b): the reverse (irrad. of H-22 at 5·22 δ) leads to a partially visible quintet of 6·8 Hz centred at 2·12 δ

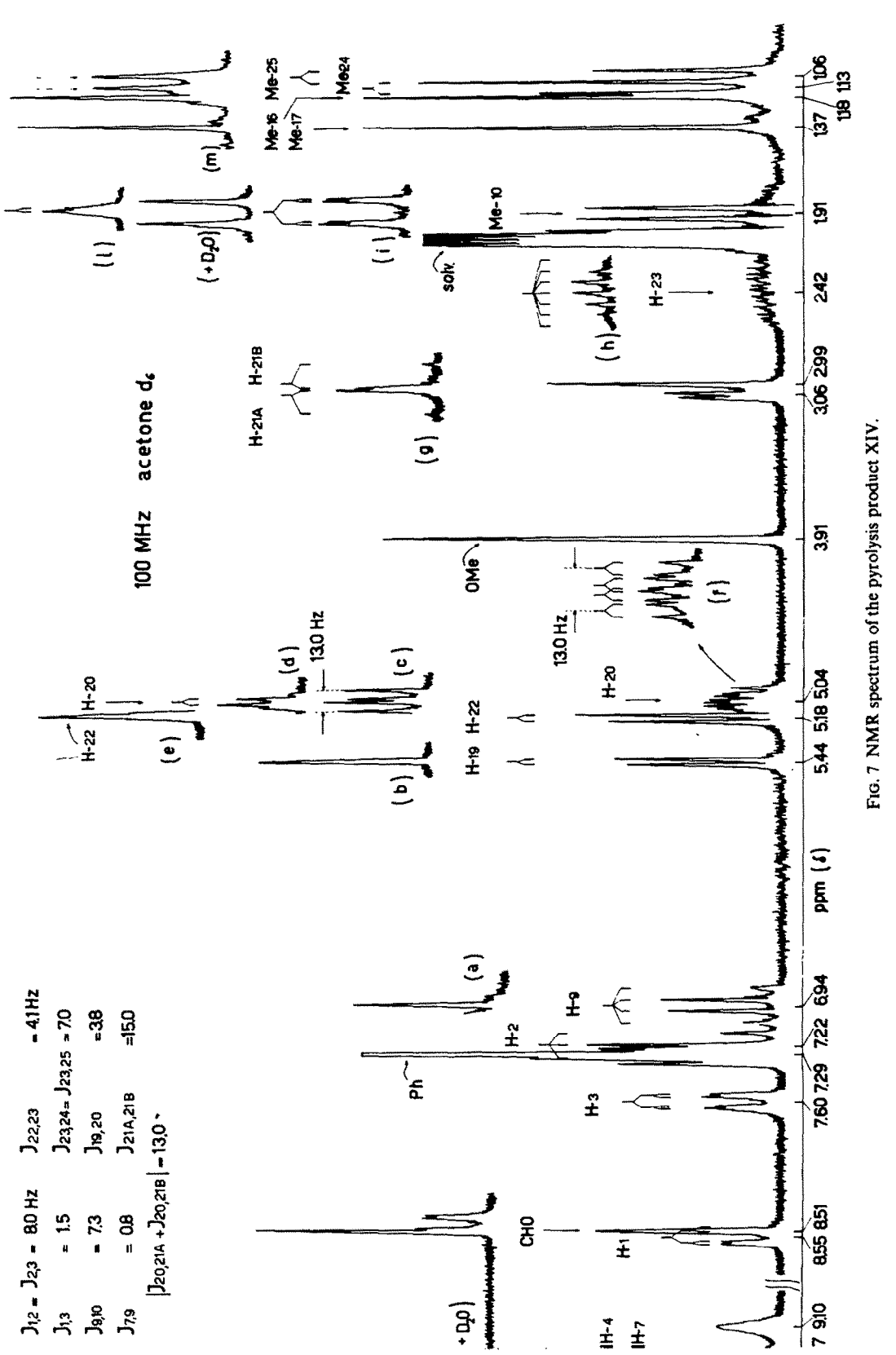
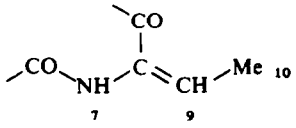


FIG. 7 NMR spectrum of the pyrolysis product XIV.

(Fig. 6f) ($CH(CH_3)_2$ septet). On irradiation at 2·12 δ. the Me absorptions are simplified: the triplet at 0·85 δ (Me-15) and the doublet at 0·89 (Me-13) are unchanged. whereas the Me doublets at 0·93 and 0·79 δ (Me-24 and Me-25) become singlets (Fig. 6i). H-12 was discovered at 1·96 δ. by the collapse of H-11 doublet at 4·81 δ on irradiation with $\Delta\nu = +285$ Hz (Fig. 6d). Decoupling H-12 (Fig. 6h) the Me-13 doublet at 0·89 becomes a singlet. The Me coupled to H-9 lies at 1·36 δ as shown by irradiation of H-9 (Fig. 6g). and by the inverse experiment i.e. irradiation at 1·36 δ. the double quartet of H-9 at 5·80 becomes a small doublet of 2·5 Hz ($J_{8,9}$) (Fig. 6a).

H-8 gives a double doublet at 5·20 δ of 2·5 and 9·0 Hz; the strong coupling of 9·0 Hz ($J_{7,8}$) vanishes with D_2O: and hence this signal must be due to the vicinal amidic hydrogen.

The pyrolysis product (XIV) (Fig. 7). The most interesting feature of the NMR spectrum of XIV is a quartet of one proton at 6·94 δ ($J = 7·3$ Hz), which led to the determination of the cleavage point of the large ring of neoantimycin. This proton is coupled to the Me at 1·91 δ (dd of 7·3 and 0·8 Hz Fig. 7i): their interaction is shown by decoupling experiments (Fig. 7a and 7b). As the small splitting (0·8) of the Me disappears with D_2O, this Me must be long-range coupled to an amidic NH group. The following sequence:



provides that the shift of the quartet (6·94 δ) indicates a proton on a conjugated double bond. and the value of 1·91 for the Me is in agreement with its allylic position.

The low-field part of the spectrum is very similar to the corresponding one of neoantimycin. with the exception that the chelated phenolic OH lacks. and the NH doublet of 9·0 Hz (H-7) is here a broad singlet. The two NH protons lie at 9·27 and 9·10 δ; the formyl signal at 8·51 δ; the three aromatic protons. in an AMX pattern with *ortho* and meta couplings. have the same chemical shift as in neoantimycin methyl ether. Fragment **f** was thus proved. The remaining 5 hydrogens of the Ph group are almost equivalent: they must be correlated to the CH_2 at ~ 3 δ; the CH_2 protons form the AB part of an ABX spectrum. where the X signal is an eight line pattern at 5·04 δ (Fig. 7f); irradiation of H_X (Fig. 7g) leads to the AB quartet with a geminal coupling (J_{AB}) of 15·0 Hz. $\delta_A = 2·99$ and $\delta_B = 3·06$. The X proton gives $|J_{AX} + J_{BX}| = 13$ Hz and another coupling of 3·8 Hz; this is evidenced by strong irradiation at ~ 3 δ: H-20 becomes a doublet of 3·8 Hz (Fig. 7d).* while the 3·8 splitting vanishes on irradiation at 5·44 δ (H-19) (Fig. 7c). The reverse experiment (decoupling H-20) collapses the H-19 doublet (Fig. 7b). Chemical shift values of both H-19 and H-20 indicate they are both bonded to a —OCO— group. Taking into account that H-19 shows no further splitting. we have drawn up the sequence **g**.

In the high-field part of the spectrum. two Me doublets of 7·0 Hz appear at 1·06 and 1·13 δ and are both decoupled on irradiation at 2·42 δ (Fig. 7m): at the same time the doublet of 1 H at 5·18 δ (H-22) collapses (Fig. 7e). The reverse experiment (irrad.

* The broad doublet obtained is a consequence of the non-identical chemical shift of the methylene protons.

at 5·18 δ) leads to a quintet of 7·0 Hz centred at 2·42 δ (H-23) which must be the visible part of a septet (Fig. 7h). These eight protons are thus easily arranged in the isovaleric unit of fragment **b**.

DISCUSSION OF MASS SPECTRA

The mass spectra of neoantimycin (I; Fig. 8) and its methyl ether (II; Fig. 9) are. as expected. similar and show a peculiar behaviour.

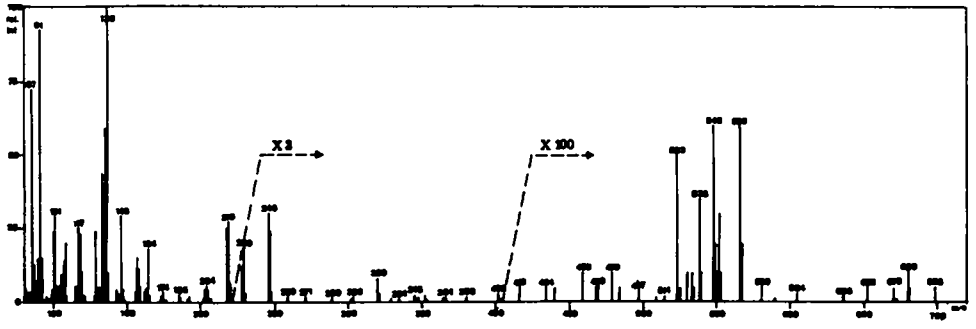


FIG. 8 Mass spectra of: neoantimycin I

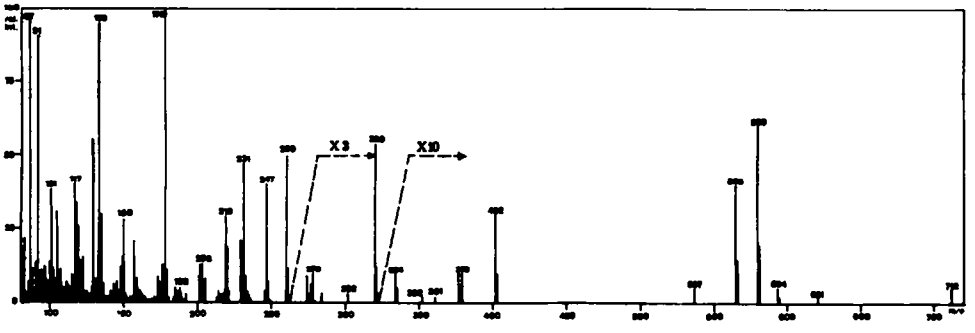


FIG. 9 OMe-neoantimycin II

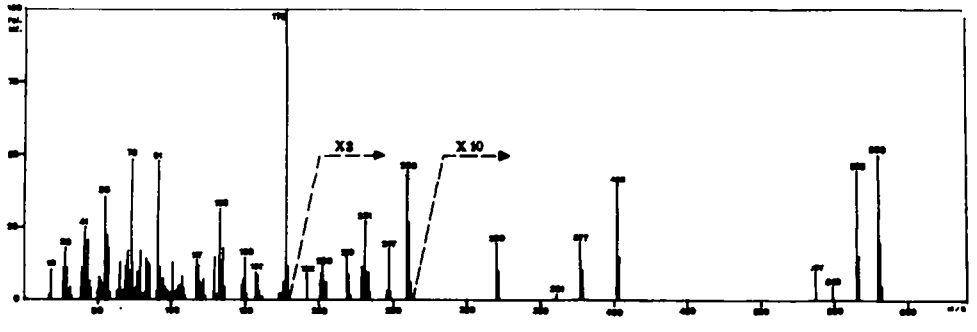
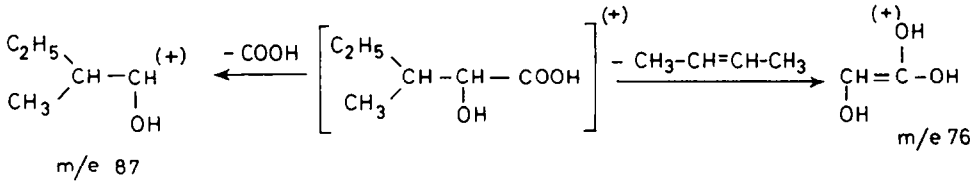


FIG. 10 Pyrolysis product XIV

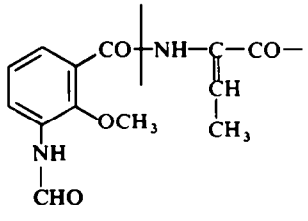
Both easily eliminate α-hydroxy-β-methyl-valeric acid (XI) yielding the corresponding M-132 fragments. In the low mass regions of these spectra we find very strong peaks at *m/e* 87 and 76 due to the XI fragmentation as shown in Scheme 6. By thermal

SCHEME 6



reaction of II the product XIV with a molecular weight of 580 (132 less than (II)) was obtained. showing a fragmentation pattern (Fig. 10) very like to that of I and II. The spectrum of XIV does not contain the peaks at *m/e* 87 and 76. confirming that this product is formed from II by elimination of XI. Such behaviour* must be attributed to the free alcoholic OH group of II. In fact. the elimination of XI from XII. in which the free OH group of II has been oxidized to a ketone. cannot be observed either by electron impact or by thermal reaction. The elucidation of the structure and fragmentation pattern of XIV is the key for the interpretation of the spectra of I and II. The presence of the **l** moiety in the molecule of XIV. already proved by intense peaks at *m/e* 261 (**1**), 260 (**1**-H), 231 (260-CHO),† 178 (**m**) and 152 (**m**-CO)† suggests four different arrangements as reported in Fig. 11.

All these peaks and that at *m/e* 361 (weak) contain the (**m**) moiety. In fact they are shifted 14 mass lower in the spectrum of I.



I

On the other hand the two couples of peaks at *m/e* 319–320 and 219–220 correspond to fragments without the **l** moiety. so that they are also present in the spectrum of I. and are complementary to the couples of peaks at *m/e* 261–260 and 361–360 respectively.

The ion of mass 402 results from the loss of the fragment (**m**) from the molecular ion. and the couple of complementary mass peaks at *m/e* 203 and 377 are derived from the breakdown at ξ (Fig. 11. formulas **1** and **2**). This evidence is in favour of

* The temperature required for the volatilization of (I) and (II) in the ion source is about 220–230°. We can not exclude a partial thermal rearrangement accompanied by elimination of (XI). but the presence of the metastable ion for the transition $M^+ \rightarrow (M\text{-}132)^+$ indicates that this fragmentation is. at least in part. an electron impact effect.

† The marked transition are supported by the presence of the appropriated metastable ions.

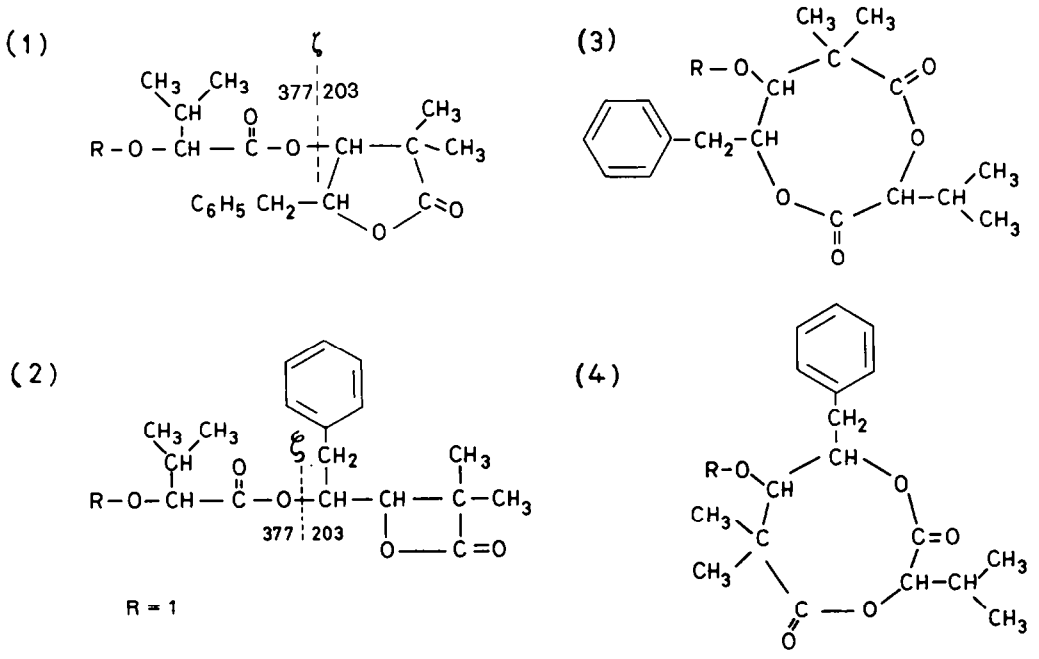


FIG. 11

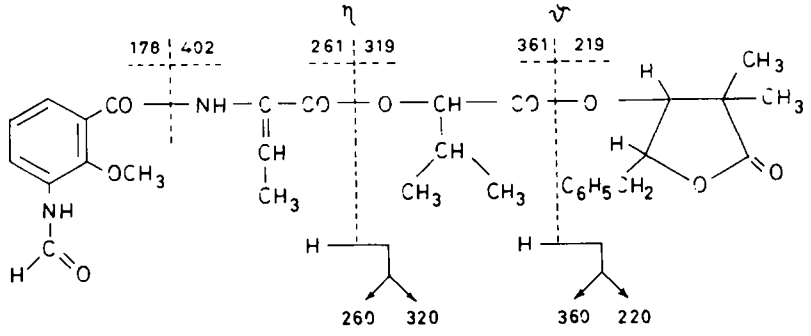
the structures **1** and **2**. but the conclusive confirmation of the structure of XIV has been obtained from the spectrum of XIII (Introduction). a partial hydrolysis product of I.

Compound XIII shows a fragmentation pattern identical to that of XIV. except of course the fragments containing the **1** group (which is not present in XIII).

This fact proves that **1** is the correct structure. The cleavage at η in XIV (Scheme 7). yielding the two abundant complementary couples of ions of mass 320–319 and 260–261. must be easy. and accounts at a time for the weakness of the peaks at *m/e* 361. compared with its complementary at *m/e* 219.

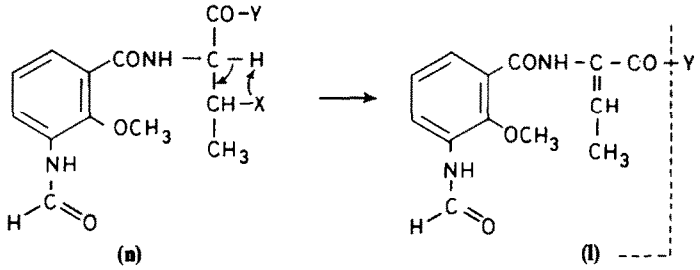
In fact the 361 fragment can be formed only by a competitive fragmentation (at ϑ) with the preferred 320–319 couple. whilst 219–220 can be derived also from 320 by cleavage at ϑ (Scheme 7).

SCHEME 7



The product II. from which XIV is derived by elimination of XI. does not contain the **l** moiety of XIV but contains the partial structure **n**. shown in Scheme 8.

SCHEME 8



It follows that the double bond of **l** must be formed as a result of the leaving of the hydroxy acid (XI). whose original position must be as indicated in **n** (Scheme 8).

EXPERIMENTAL

All m.ps were determined on a Kofler type hot-stage m.p. block and are uncorrected. IR spectra were determined on a Perkin–Elmer model 21 double beam recording spectrophotometer. All UV spectra were run in EtOH on a Beckman recording spectrophotometer model DK-2A. The NMR spectra were recorded with Varian Hr-100, HA-100 and A-60 spectrometers: decoupling experiments were performed in "field sweep" or "frequency sweep": the integrals were measured with a 405CR Hewlett–Packard digital voltmeter. Chemical shifts are measured in ppm (δ) from TMS as int. stand.; coupling constants are in Hz. The mass spectra were obtained on a Hitachi RMU6D (single focus) instrument with ionizing potential of 70 V. Samples were directly introduced into the ion source heated at 200–250. TLC were performed on Merck Silica Gel HF_{254}.

Neoantimycin (I). Supplies of neoantimycin were obtained as previously described.[1] The material used was crystallized from EtOAc-hexane: m.p. 121–122°. $[\alpha]_D^{25}$ in Chf (*c.* 1) +58·3: UV (Fig. 1) λ_{max} (mμ): EtOH 227. 319. log ε 4·48. 3·80: NaOH 0·01N in EtOH 222. 342. $E_{1cm}^{1\%}$ 600. 140: HCl 0·01N in EtOH 227. 311. $E_{1cm}^{1\%}$ 343. 75: IR (Chf). cm^{-1} 3571 (w). 3448 (w). 1761 (s). 1706 (s). 1647 (m). 1613 (w). 1597 (w). 1531 (s). 1188 (s). The NMR spectrum is reported in Fig. 5: Mol. wt. 698 (MS and vap. press. osm. in benzene or dioxane). The MS is shown in Fig. 8. (Found: C, 61·78; H, 6·59; N, 3·92; O, 27·61. $C_{36}H_{46}N_2O_{12}$ requires: C. 61·88: H. 6·63: N. 4·00: O. 27·48%).

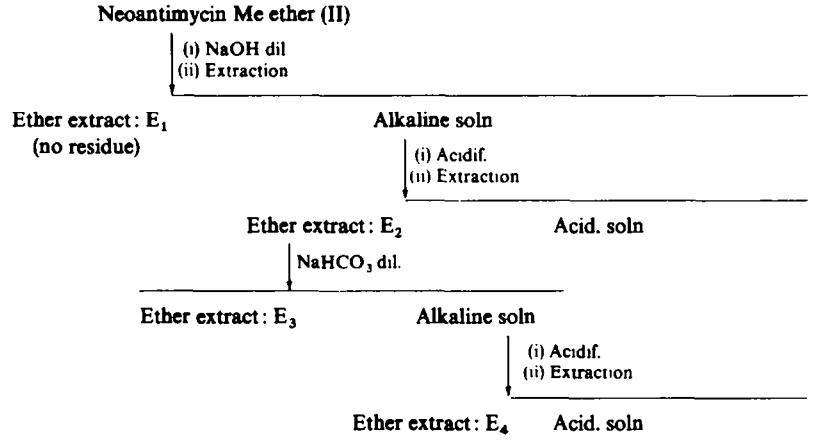
Neoantimycin. Me ether (II). A soln of 1 g neoantimycin in 100 ml ether was cooled in ice–salt mixture and an excess of ethereal CH_2N_2 soln was added. After 5 min. the soln was allowed to remain for 30 min at room temp before the solvent was removed at 50° under a stream of dry air. The residue was crystallized from EtOAC–hexane to yield 900 mg of pale yellow crystals (negative $FeCl_3$ test). After recrystallization m.p. 108–110°. $[\alpha]_D^{25}$ in CCl_4 (*c.* 1) +2·01: UV (Fig. 1) λ_{max}^{EtOH} 293 mμ. log ε 3·32: IR (Chf). cm^{-1} 3571 (w). 3425 (w). 1761 (s). 1706 (s). 1667 (m). 1608 (w). 1587 (m). 1515 (s). 1190 (s): NMR (acetone-d_6):

δ0·45 (d. $J_{23,24}$ = 6·8. Me-24)
0·80 (d. $J_{23,25}$ = 6·8. Me-25)
0·86 (d. $J_{14,15}$ = 7·0. Me-15)
0·91 (d. $J_{12,13}$ = 6·8. Me-13)
1·34 (s. Me-16)
1·37 (d. $J_{9,10}$ = 6·5. Me-10)
1·41 (s. Me-17)
3·0–3·2 (AB part of ABX. H-21A. H-21B)
3·31 (d. $J_{18,19}$ = 12·0. H-19)
3·67 (d. $J_{18,19}$ = 12·0. H-18)
3·97 (s. MeO—)
δ4·66 (d. $J_{11,12}$ = 7·8. H-11)
5·20 (dd. $J_{8,9}$ = 2·5. $J_{7,8}$ = 9·0. H-8)
5·45 (d. $J_{22,23}$ = 3·0. H-22)
5·56 (4 lines. part X of ABX. H-20)
5·75 (dq. $J_{8,9}$ = 2·5. $J_{9,10}$ = 6·5. H-9)
7·25 (t. $J_{1,2}$ = $J_{2,3}$ = 8·0. H-2)
7·28 (s. phenyl)
7·69 (dd. $J_{2,3}$ = 8·0. $J_{1,3}$ = 2·5. H-3)
8·40 (dd. $J_{1,2}$ = 8·0. $J_{1,3}$ = 2·5. H-1)
8·50 (s. broad. H-5)
8·54 (s. $J_{7,8}$ = 9·0. H-7)
9·22 (broad. H-4)

Mol. wt. 712 (MS. Fig. 9). (Found: C, 62·44; H, 6·90; N, 3·77. $C_{37}H_{48}N_2O_{12}$ requires: C, 62·34; H, 6·79; N. 3·93%).

Hydrolysis of neoantimycin (I). The alkaline hydrolysis of I gave compound V, XII, X, XI and IV and was analogous to the hydrolysis of II (see later). Nevertheless, the presence of the free phenolic OH in I caused the formation of undesirable oxidation products.

Alkaline hydrolysis of neoantimycin. Me ether (II). 750 mg of II hydrolysed with 5% NaOH in MeOH aq at room temp. The hydrolysis, controlled by TLC, was complete in ca. 2 hr.



γ-Lactone (V). The ext E_3 gave a solid residue (180 mg). After repeated crystallization from benzene–hexane m.p. 115–116°: $[\alpha]_D^{25}$ in Chf (*c*, 1) +66·0: UV and NMR are reported in the Introduction: IR (Chf) cm^{-1} 3623–3509 (w). 1778 (s). Mol. wt. 220 (MS): MS see Scheme 1. (Found: C. 70·90: H. 7·10. $C_{13}H_{16}O_3$ requires: C. 70·89: H. 7·32%).

Acetylation of γ-lactone (V): (Va). γ-lactone (V) was treated with Ac_2O–pyridine 10:1 (5 ml) at room temp for 20 hr. The mixture was diluted with water and extd with ether. The crude oil, from the ether, was chromatographed on Silica Gel with 15% EtOAc in hexane: after a small amount of a yellow oil. the major product (50 mg) was eluted and crystallized from cyclohexane to yield Va (45 mg). After recrystallization. m.p. 82–83°. $[\alpha]_D^{25}$ in CCl_4 (*c*. 1) +98·7. IR (Chf) cm^{-1} 1779 (s). (1792 in CCl_4). 1748 (s): NMR (CCl_4): 1·08 and 1·23 (2s. $\rangle C(Me)_2$). 2·02 (s. MeCOO—). 2·8–5·2 (ABXY pattern: δ_A = 3·01. δ_B = 2·80 $PhCH_2$—: δ_X = 4·66. —CHOAc: δ_Y = 5·11 —CHOAc: J_{AB} = 14·2. J_{AX} = 7·8. J_{BX} = 5·7. J_{XY} = 3·8). MS *m/e* (1%): 262 (0·13). 234 (0·05). 233 (0·1). 220 (0·2). 219 (1·3). 217 (0·2). 216 (0·7). 202 (0·2). 201 (0·1). 177 (0·1). 176 (0·6). 175 (4). 174 (0·4). 173 (0·2). 172 (0·3). 171 (3·2). 157 (0·1). 145 (0·3). 143 (1·2). 134 (0·6). 133 (7). 131 (0·1). 118 (1·5), 117 (0·1), 116 (0·2), 115 (3·7), 114 (0·5), 105 (1), 104 (0·2), 103 (0·7), 102 (0·2), 101 (1·8), 92 (1), 90 (0·2), 89 (0·4), 85 (1·7), 84 (0·5), 83 (2·8), 79 (0·2), 78 (0·5), 77 (1), 73 (0·3), 72 (4·5), 71 (1), 70 (7), 69 (0·5), 67 (0·6), 65 (2·5), 63 (0·7). 57 (1·7). 56 (0·5). 55 (3). 54 (0·1). 53 (0·2). 52 (0·2). 51 (1). 44 (2·2). 43 (100). 42 (2). 41 (4·5). 40 (0·2). 39 (3). (Found: C. 68·87: H. 6·96. $C_{15}H_{18}O_4$ requires: C. 68·68: H. 6·92%).

Reduction of γ-lactone (V) *by LAH: triol* (VIII). 250 mg of V in ether (20 ml) were added dropwise to an ethereal soln 0·35 M of LAH (15 ml). After 2 hr at room temp. the mixture was refluxed for 3 hr. The excess of reagent was destroyed. and the mixture acidified and extd with ether. The crude triol. obtained as a yellow oil (250 mg) was isolated by acetylation.

Acetylation of the triol (VII): *triacetate* (VIII). 120 mg of VII were treated with Ac_2O–pyridine 10:1 (3 ml) at room temp for 24 hr. The mixture was diluted with water and extd with ether. The oil (130 mg), from the ether, was chromatographed on Silica Gel (30 g) with 20% EtOAc in hexane. The triol triacetate (VIII: 110 mg) was distilled at 100–102°/0·001 mm as a colourless oil $[\alpha]_D^{25}$ in CCl_4 (*c*. 1) +31·52: IR (CCl_4) cm^{-1} 1755 (s). 1740 (s): NMR (CCl_4): 0·87 and 0·88 (2s. $\rangle CMe_2$). 1·87. 1·95. 2·14 (3s. 3 MeCOO—): 2·65 (d, *J* = 7·0, $PhCH_2$—CH), 3·53 and 3·82 (AB q, J_{AB} = 11·5 $-CH_2-OAc$), 4·88 (d, *J* = 10, CH—

CHOAc). 5·25 (td. J = 7·0. $PhCH_2$—CH<CH—, OAc). 7·29 (s. Ph). (Found: C. 65·01: H. 7·47. $C_{19}H_{26}O_6$ requires: C. 65·12: H. 7·48%).

Periodate oxidation of the triol VII. To VII (132 mg) in dioxane (4 ml) and water (3 ml) was added 0·04N $Na3O_4$ (35 ml), a N_2 stream carrying volatile products into 2,4 DNPh reagent. After 1 hr. no ppt was observed. Extn of the aqueous soln with ether afforded an oil (125 mg) showing two spot on TLC (30% EtOAc in hexane). The residue was purified by preparative TLC (15% EtOAc in hexane):

Phenylacetaldehyde. The compound with higher R_f corresponded to phenylacetaldehyde by TLC (5% EtOAc in benzene) and by GLC on a column of DEGS (20% on Chromosorb W. 180°). and gave the 2.4 DNPh (50 mg), m.p. 119–121 identical mix m.p., (UV. IR) with authentic phenylacetaldehyde 2.4 DNPh.

3-Hydroxy-2.2-dimethylpropionaldehyde. This was obtained by prep TLC as an oil which. after two days. solidified as white crystals (45 mg), m.p. 88–90° (benzene–hexane), identical (mix m.p. and IR) with the authentic dimer of 3-hydroxy-2,2-dimethylpropionaldehyde, m.p. 90–91°.* Distillation of both compounds produced identical samples of 3-hydroxy-2.2-dimethylpropionaldehyde (b.p. 90–95/15 mm). In addition the 2.4 DNPh. m.p. 190–192°. was identical (mix m.p. and IR) with an authentic sample.

Oxidation of γ-lactone (V):*γ-oxolactone* (IX). A 110 mg sample of V in acetone (10 ml) was treated at 0–5°, with a soln (0·65 ml) of Na_2CrO_4 (0·5 g) and 0·5 ml H_2SO_4 made up to 2 ml with water. TLC (EtOAc: hexane 1:1 R_f (V) ca. 0·40. R_f (IX) ca. 0·65) showed that the oxidation was complete in 3 hr. The mixture was diluted with water and. after addition of $NaHCO_3$ until pH ca. 6. and $NaHSO_3$. was extracted with ether. The crude product (110 mg). from the ether. was chromatographed on Silica Gel (15 g) with 30% EtOAc in hexane to elute pure γ-oxolactone (IX) as white crystals (105 mg), m.p. 47·5–48° (hexane). $[\alpha]_D^{25}$ in CCl_4 (*c.* 1) +103·8. UV and NMR see Introduction: IR (Chf) cm^{-1}: 1802 (s). 1757 (s). (Found: C. 71·43: H. 5·52. $C_{13}H_{14}O_3$ requires: C. 71·54: H. 6·47%).

γ-Lactone (XIII). After crystn of V a less polar compd was isolated from the mother liquor by prep TLC giving 35 mg of XIII. m.p. 109–111° (hexane): IR (Chf) cm^{-1}: 3571 (w). 1779 (s) (1792 in CCl_4). 1742 (s): NMR (Introduction). Mol. wt. 320 (MS): MS see Fig. 2. (Found: C. 67·71: H. 7·35. $C_{18}H_{25}O_5$ requires: C. 67·48: H. 7·55%).

Acetylation of γ-lactone (XIII). Compd XIII. with Ac_2O–pyridine 10:1. gave a monoacetate. m.p. 113–114° (hexane): IR (CCl_4) cm^{-1} 1792 (s). 1751 (s). (Found: C. 66·40: H. 7·15. $C_{20}H_{26}O_6$ requires: C. 66·28: H. 7·23%).

Hydrolysis of γ-lactone (XIII). Compd XIII (15 mg) was hydrolysed with 5% NaOH in MeOH at room temp in ca. 1 hr. The alkaline soln. diluted with water and acidified. was extd with ether. The ether extract. washed with 2N $NaHCO_3$. was treated with ethereal CH_2N_2 soln. analyzed by GLC on a column of Carbowax 20 M:KOH:Chromosorb W (15:5:80) at 115°. gave a single peak. identical with that of X. Me ester. prepd. later.

γ-Lactone (V). The $NaHCO_3$ washings acidified and extd with ether gave a solid m.p. 115–116° (benzene–hexane). identical (mix m.p.. IR) with V.

S(+)-α-hydroxy-isovaleric acid (X). *2S,3S(+)-α-hydroxy-β-methylvaleric acid* (XI). *N-(3-formylamino-salicyloyl)threonine* (IV). The extract E_4 gave an acidic oil (430 mg), which was methylated with CH_2N_2. The mixture of Me esters was heated at 50–60°/0·001 mm collecting volatile products in a trap cooled with dry ice. The volatile fraction (130 mg). analyzed by GLC on a column of Carbowax 20 M:KOH:Chromosorb W (15:5:80) at 115°. showed two peaks in 1:1 ratio. The two components were separated by prep GLC on a 1 m column packed with bentone (8·5 g. 20% Celite) plus diisodecylphthalate (7·5 g. 25% Celite at 150°):

S(+)-α-Hydroxy-isovaleric acid (X). *Me ester.* The first eluted was a colourless oil $[\alpha]_D^2$ in CCl_4 (*c.* 1) +17·5. (α_{578} +18·8: α_{546} +22·4: α_{436} +46·1: α_{364} +90·8: α_{313} +186·5): Mol. wt. 132 (MS). The retention time [columns of: (i) Carbowax 20M:KOH:Chromosorb W 15:5:80. 115°: (ii) DEGS. 15% on Chromosorb W. 90°]. IR and NMR showed the product to be identical with the sample of S(+)-α-hydroxy-isovaleric acid. Me ester prepared[11] from S(+)-valine: $[\alpha]_D^{25}$ in CCl_4 (*c.* 1) +17·8 (α_{578} +18·8: α_{546} +22·3: α_{436} +46·5: α_{364} +91·1: α_{313} +188·4).

2S,3S(+)-α-Hydroxy-β-methyl-valeric acid (XI) *Me ester.* The second eluted was a colourless oil $[\alpha]_D^{25}$ in CCl_4 (*c*, 1) +25·3 (α_{578} +27·3; α_{546} +31·6; α_{436} +62·0; α_{364} +115·1; α_{313} + 220·8); Mol. wt. 146 (MS);

* The dimer of 3-hydroxy-2.2-dimethyl-propionaldehyde was reported to have the structure of 4-hydroxy-5.5-dimethyl-2(2-hydroxy-1.1-dimethyl-ethyl)-1.3-dioxane ([10]).

GLC retention time (on the same columns of the former compd X), showed the product to be identical with the sample of 2S,3S(+)-α-hydroxy-β-methyl-valeric acid, Me ester prepared[12] from 2S,3S(+)-isoleucine: $[\alpha]_D^{25}$ in CCl_4 (*c*, 1) +23·8 (α_{578} +25·7: α_{546} +29·4; α_{436} +56·9: α_{364} +105·2: α_{313} +198·4).

N-(3-*Formylamino-saliculoyl*)*threonine* (IV). *Me ether. Me ester.* The residue (after distillation of the volatile fraction), purified by prep TLC (EtOAc–cyclohexane 1:1), gave an amorphous solid (240 mg): UV was nearly identical with that of II (λ_{max}^{EtOH} 293, log ε 3·32). IR (Chf), cm^{-1}: 3390 (m), 1742 (s), 1704 (s), 1661 (s), 1608 (w), 1585 (m), 1511 (s). The NMR spectrum (acetone–d_6) showed the product to be a mixture of IV, Me ether, Me ester (ca. 60%) and N-(3-*formylamino-salicyloyl*)-α-*amino-crotonic acid Me ether* (XV) *Me ester* (ca. 40%):

	(IV). Me ether. Me ester	(XV). Me ester
Me-10	δ 1·23 (d. $J_{9,10}$ = 6·3)	δ 1·82 (dd. $J_{9,10}$ = 7·0: $J_{7,10}$ = 0·8)
OH	2·87 (s)	—
COOMe	3·92 (s)	3·95 (s)
OMe	3·77 (s)	3·77 (s)
H-9	4·15 (qd. $J_{9,10}$ = 6·3: $J_{8,9}$ = 2·5)	6·76 (q. $J_{9,10}$ = 7·0)
H-8	4·77 (dd. $J_{8,9}$ = 2·5: $J_{7,8}$ = 9·3)	—
H-1	7·64 (dd. $J_{1,2} = J_{2,3}$ = 8·0)	7·54 (dd) $J_{1,2} = J_{2,3}$ = 8·0
H-2	7·22 (t. $J_{1,3}$ = 1·8)	7·20 (t) $J_{1,3}$ = 1·8
H-3	8·38 (dd)	8·60 (dd)
CHO	8·48 (broad s)	8·48 (broad s)
2NH	8·13–9·63 (broad)	8·13–9·63

Hydrolysis of N-(3-*formylamino-salicyloyl*)*threonine* (IV). *Me ether, Me ester.* A sample of IV, Me ether, Me ester (220 mg) was hydrolyzed with 5% NaOH (5 ml) at 100° for 20 min. The alkaline soln was adjusted at pH 1·0 with H_2SO_4 and then concentrated to dryness *in vacuo* collecting the volatile acid in a dry ice-cooled trap.

Formic acid. The distillate was neutralized with NaOH and dried *in vacuo*. The Na salt. in 2 ml 0·5N H_2SO_4. was continuously extracted with ether. and the volatile acid. analysed by TLC (EtOH–NH_4OH–H_2O 40:2:8) and paper chromatography (n-BuOH sat. 3N NH_4OH) was shown to be formic acid. The Me ester. obtained with CH_2N_2. had GLC retention time (Ucon LB 550X. 10% Chromosorb W. 18°) identical with that of Me formate.

3-*Amino-salicylic acid.* The residue from the distillate was hydrolysed with 6N HCl (7 ml) in a sealed tube at 120° for 12 hr. The hydrolysate concentrated to dryness. gave a residue which. in H_2O. was adjusted to pH 3. The ether extract of the aqueous soln gave a tan coloured solid (104 mg) which was purified by sublimation at 140°/0·01 mm. to give white crystals m.p. 233–235° (dec). Mix m.p. and UV. IR. NMR showed the product to be identical with an authentic sample of 3-amino-salicylic acid ([5]). (Found: C. 54·77: H. 4·42: N. 9·34. $C_7H_7NO_3$ requires: C. 54·90: H. 4·61: N. 9·15%).

Threonine. The aqueous soln was concentrated to dryness *in vacuo*, dissolved in BuOH–AcOH–H_2O 4:1:1 and passed through a column of cellulose (5 g). The product obtained from the fractions with positive ninhydrin test showed the same R_f value as threonine on bidimensional paper (phenol–H_2O 4:1. BuOH–AcOH–H_2O 4:1:5) and was identified as threonine by an Aminoacid Analyser Beckman Spinco Mod. 120.[1]

Neooxoantimycin Me ether (XII). A 380 mg sample of II in acetone (25 ml) was treated. at room temp. with a soln (2·0 ml) of Na_2CrO_4 (0·5 g), 0·5 ml of H_2SO_4 and water to 2 ml. TLC (EtOAc–hexane 1:1. R_f (II) ca. 0·25. R_f (XII) ca. 0·32) showed that the oxidation was complete in approx. 3 hr. The mixture was diluted with water and. after addition of $NaHCO_3$ until pH ca. 6. and $NaHSO_3$. was extracted with ether. The crude product (375 mg) from the ether. was chromatographed on Silica Gel (30 g) with 30% EtOAc in hexane (350 ml) to elute a red oil showing no absorption at 254 mμ. The major product (140 mg. 37% yield). eluted with 35% EtOAc–hexane (150 ml). was crystd from EtOAc–hexane to yield 130 mg of white crystals. After recrystn. m.p. 100–101°. $[\alpha]_D^{25}$ in Chf (*c*. 1) +10·07: UV λ_{max}^{EtOH} = 293 mμ. log ε 3·36: IR (Chf). cm^{-1}: 3390 (w). 1761 (s). 1704 (s). 1667 (m). 1608 (w). 1585 (m). 1513 (s). 1188 (s). NMR. see Fig. 6. Mol. wt. 710 (MS): MS. see Fig. 4. (Found: C. 62·35: H. 6·50: N. 3·80. $C_{37}H_{46}N_2O_{12}$ requires: C. 62·53: H. 6·52: N. 3·94%).

Reduction of Neooxoantimycin, Me ether (XII) *by* $NaBH_4$: *neoantimycin Me ether* (II). A sample of XII (80 mg) in 1,2-dimethoxy-ethane, was reduced at 0° with $NaBH_4$. TLC (EtOAc cyclohexane 1:1) showed that the reduction was complete in ca. 20 min. The mixture, diluted with acetone and neutralized

with acetic acid, was concentrated to a small volume and extracted with ether. The residue was purified by prep TLC (40% EtOAc in cyclohexane) and crystd from EtOAc–hexane to give crystals (50 mg). m.p. 108–110°, identical (mix m.p., $[\alpha]_D^{25}$, IR and NMR) with the sample of II prepared above.

Hydrolysis of neooxoantimycin Me ether (XII). A sample of XII (70 mg) was hydrolysed in N_2 atm with 3% NaOH in MeOH at room temp. The hydrolysis, followed by TLC. was complete in ca. 30 min. and was worked up as described for the hydrolysis of II.

γ-Lactone (IX). The ethereal extract, containing the neutral fraction, gave an oil (15 mg) which was purified by prep TLC (EtOAc–cyclohexane 1:1) and crystd from hexane: m.p. 47–48°. Mix m.p. and IR showed the product to be identical with IX prepared above.

S(+)-α-Hydroxy-isovaleric acid (X). *2S,3S(+)-α-hydroxy-β-methyl-valeric acid* (XI), *N-(3-formylamino-salicyloyl)threonine* (IV). Compds X and XI were isolated as Me esters by the method described. Analogously, IV Me ether. Me ester was isolated. The latter was contaminated with N-(3-formylamino-salicyloyl)-α-amino-crotonic acid Me ether, (XV), Me ester.

Pyrolysis of neoantimycin, Me ether (II). 115 mg of II was heated at 155–160°/0·01 mm collecting volatile products in a trap cooled with dry ice. The pyrolysis, followed by TLC, was complete in ca. 3 hr.

2S,3S(+)-α-Hydroxy-β-methyl-valeric acid (XI). *Me ester.* The volatile fraction, treated with CH_2N_2 and analysed by GLC (Carbowax 20M: KOH–Chromosorb W 15:5:80 at 115°) gave a single peak. GLC retention time showed the product to be identical with the sample of XI, Me ester prepared above.

Pyrolysis compound XIV. The residue of the pyrolysis was an amorphous solid (85 mg) which was purified by chromatography on Silica Gel (15 g) with EtOAC–hexane 1:1. The first 100 ml eluted gave a small amount of a yellow oil. The major product (62 mg, 65% yield), eluted with 120 ml, was purified by prep TLC (EtOAc–hexane 1:1), $[\alpha]_D^{25}$ in CCl_4 (*c*, 1) +53·22: UV reported in Fig. 3. NMR in Fig. 7: IR (Chf) cm^{-1}: 3413 (w), 1779 (s), (1792 CCl_4), 1764 (s), 1704 (s), 1684 (s), 1661 (sh), 1608 (w), 1587 (m), 1513 (s), 1289 (s), 1250 (s), 1200 (m), 1127 (s). Mol. wt. (580 (MS): see Fig. 10). (Found: C, 64·16: H, 6·40: N, 4·63. $C_{31}H_{36}N_2O_9$ requires: C, 64·12; H, 6·25; N, 4·83%).

Pyrolysis of neoantimycin (I). Pyrolysis of I was carried out under the conditions described for II. The same products, XI Me esters and XIV are obtained after methylation of both the volatile fraction and the residue. The yield of XIV was much lower.

Pyrolysis of neooxoantimycin, Me ether (XII). 20 mg of XII, heated under the same conditions as described for II, gave no volatile acid. Attempt to separate the different components of the pyrolysis residue were ineffective.

Hydrolysis of the pyrolysis products (XIV). 50 mg of XIV were hydrolysed with 5% Na_2CO_3 in MeOH aq at room temp. The hydrolysis was complete in ca. 1 hr.

γ-Lactone (V), *γ-lactone* (XIII). The alkaline soln was extracted with ether. The semisolid residue from the ether. showed on TLC (EtOAc–cyclohexane 1:1) two components (R_f ca. 0·40. 0·53). Separation of the mixture by prep TLC gave crystals of V m.p. 115–116° and XIII m.p. 109–110° (mix m.p. and IR identical with the samples of V and XIII obtained before).

S(+)-α-Hydroxy-isovaleric acid (X), *N-(3-formylamino-salicyloyl)-α-amino-crotonic acid, methyl ether* (XV). The alkaline soln was adjusted at pH 1·0 and then continuously extracted with ether. The solvent was removed at low temp and the residue methylated with CH_2N_2, was shown to be X, Me ester by GLC (Carbowax 20M:KOH:Chromosorb W 15:5:80 at 115°). The residue (after distillation of the volatile fraction), purified by prep TLC (R_f ca. 0·15: EtOAc–cyclohexane 1:1) gave XV. Me ester as an amorphous solid: UV in Fig. 3: IR (Chf) cm^{-1}: 3390 (m), 1704 (s), 1684 (s), 1662 (sh), 1605 (w), 1585 (m), 1511 (s). The NMR spectrum showed the same signals as reported for XV, Me ester present as impurity in the sample of IV, Me ether, Me ester.

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