TABLE I

TRIAZOLINE PYROLYSES

Compd	Tube packing	Temp, °C	Yields, % 4 or 7	12 + 13	C_2F_4
3	Glass	325	74	Very little[a]	Not investigated
3	Ni	250	2	77	45
6	Glass	300	80	None	None
6	Ni	325	67	Very little[b]	None

[a] 22% of triazoline was unreacted. [b] 14% of triazoline was unreacted.

(triplet, CH_2) and ϕ 73.0 (CF_3) and 163.6 (CF). The *trans* isomer showed bands at τ 2.76 (phenyl) and 5.95 (CH_2) and ϕ 75.1 (CF_3) and 177.4 (CF).

Anal. Calcd for $C_{10}H_7F_6N$: C, 47.07; H, 2.77; F, 44.67; N, 5.50. Found: C, 46.89; H, 2.96; F, 44.43; N, 5.75.

Anal. Calcd for $C_{11}H_7F_8N$: C, 43.29; H,2.31; F, 49.80; N, 4.59. Found: C, 43.29; H, 2.47; F, 49.72; N, 4.49.

The pyrolysis of **3** on nickel balls furnished the highest yield of **12**, **13**, and tetrafluoroethylene and, therefore, was used to obtain these materials. Compound **12** shows nmr spectral bands at τ 2.81 (phenyl) and 5.49 (CH_2) and ϕ 73.4 (CF_3) and 51.1 (CF). The C=N bond absorbs at 5.9 μ. Unfortunately, **12** was too reactive with atmospheric moisture to obtain a good analysis. However, it was converted into the imidoyl ester and tetrazole. See below. Tetrafluoroethylene was easily identified in the liquid nitrogen trapped material by mass spectroscopy. N-Benzyltrifluoroacetamide[11] (**13**) was identified by a comparison of its physical and spectral properties with those of an authentic sample prepared by slowly adding an excess of trifluoroacetic anhydride to benzylamine in ether. The volatiles were removed and the residue was sublimed to give white crystals, mp 70–71°. Its nmr spectra show bands at τ 2.35 (NH), 2.87 (phenyl), and 5.78 (CH_2) and ϕ 76.2 (CF_3).

1-Benzyl-5-trifluoromethyltetrazole (**15**).—A mixture of 2.0 g (0.0098 mole) of N-benzyltrifluoroacetimidoyl fluoride (**12**), 0.7 g (0.011 mole) of sodium azide, and 25 ml of acetonitrile was stirred in a stoppered flask overnight at room temperature. The mixture was filtered and the filtrate was evaporated to 2.1 g of yellow oil. This was purified by preparative gas chromatography on column D at 190°, retention time 18.5 min. Its nmr spectrum shows one band at ϕ 61.3 (CF_3).

(11) N. P. Garnbaryan, L. A. Simonyan, and I. L. Knunyants, *Proc. Acad. Sci. USSR*, **155**, 305 (1964).

Anal. Calcd for $C_9H_7F_3N_4$: C, 47.37; H, 3.09; F, 24.8; N, 24.56. Found: C, 47.44; H, 3.02; F, 24.70; N, 24.76.

Methyl N-Benzyltrifluoroacetimidoate (**14**).—A mixture of 1.0 g of N-benzyltrifluoroacetimidoyl fluoride, 25 ml of methanol, and 2.0 g of sodium bicarbonate was stirred in a stoppered flask overnight at room temperature. The solvent was evaporated before filtration. The resulting pale yellow oil weighed 1.0 g and was nearly pure. It was further purified by gas chromatography on column D at 183°, retention time 8.7 min. It shows a C=N bond at 5.9 μ.

Anal. Calcd for $C_{10}H_{10}F_3NO$: C, 55.30; H, 4.64; F, 26.24; N, 6.45. Found: C, 55.30; H, 4.85; F, 26.01; N, 6.72.

1,1-Difluorotetramethylcyclopropane (**16**).—A solution of 10 g (0.035 mole) of the triazoline **3** and 10 g (0.119 mole) of tetramethylethylene was pyrolyzed on nickel balls at 240–260° according to the procedure given above in the section on pyrolyses. The addition required 30 min. The material in the liquid nitrogen trap was redistilled at room temperature at 3 mm into another such trap cooled in liquid nitrogen. The distillate weighed 10.9 g, from which a yield of 18% was calculated by using integrated peak areas in the gas chromatographic analysis on column E at 70°, retention time 12 min. The pure material, which has a camphorlike odor, was isolated with column D at 70°, retention time 16.1 min. Its nmr spectrum showed a single triplet at τ 8.90 with a coupling constant of 2 cps owing to the interaction of two fluorine atoms. The nmr spectrum of 1,1-difluoro-2,2-dimethylcyclopropane shows a triplet for the methyl group absorption at τ 8.81 with J = 2 cps.[12] The mass spectrographic analysis showed a parent peak at 134 mass units. The infrared spectrum was identical with that of a sample prepared by a standard technique.[13] A mixture of 18.5 g of sodium chlorodifluoroacetate, 15 ml of tetramethylethylene, and 100 ml of diethylene glycol dimethyl ether was refluxed for 32 hr. The oil (10.4 g) which separated on the addition of ice-water (500 ml) was dried over $MgSO_4$ and then analyzed and purified by gas chromatography on column D at 143°, retention time 3.0 min, 11% yield based on the salt used.

Anal. Calcd for $C_7H_{12}F_2$: C, 62.66; H, 9.02; F, 28.32. Found: C, 62.72; H, 8.99; F, 28.30.

Acknowledgment.—The authors express their gratitude to Dr. R. A. Henry for his suggestions and encouragement and to Mr. Joe Johnson for obtaining the mass spectral data.

(12) R. A. Mitsch, *J. Am. Chem. Soc.*, **87**, 758 (1965).

(13) J. M. Birchall, G. W. Cross, and R. N. Hazeldine, *Proc. Chem. Soc.*, 81 (1960).

The Mass Spectra of Tetracyclines

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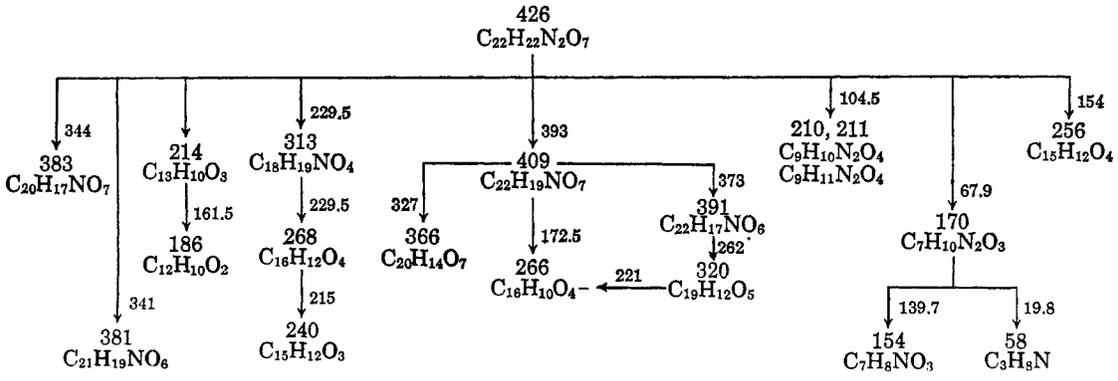
The mass spectra of nine tetracyclines were obtained. Eight of them exhibited strong molecular ion peaks. It is shown that the mass spectra of tetracyclines can be used to determine the nature and location of various functional groups in the molecule by examination of the changes that they cause in the fragmentation pattern and by application of the shift technique. The major fragmentations of the ring system are systematic and can be followed by the observation of metastable ions in 16-ev spectra.

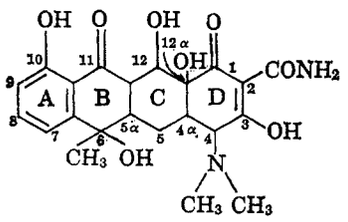
The tetracyclines are one of the most important classes of broad spectrum antibiotics. The first tetracyclines were isolated from *Streptomyces* cultures, but it was soon discovered that the 5, 6, and 7 substituents could be greatly modified without decreasing the antibiotic activity of the compound. This finding has led to the syntheses, both chemical and biological, of a large number of modified tetracyclines in the search for new and more effective antibiotics. Because of the large number of functional groups present in the molecule, conventional methods of structure determination are laborious. However, in mass spectrometry one can take advantage of these functional groups because of the directing influences of the heteroatoms on the molecule's fragmentation processes. From the study of a combination of accurate mass measurements and fragmentation patterns it should be possible to determine both the nature and the location of the various functional groups in tetracyclines.

The mass spectra of nine different tetracyclines were obtained: tetracycline hydrochloride, chlortetracycline hydrochloride (aureomycin hydrochloride), oxytetra-

(1) Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, Calif.

SCHEME I
METASTABLE DIAGRAM FOR 5α,6-ANHYDROTETRACYCLINE





tetracycline

cycline (terramycin), 5α,6-anhydrotetracycline, 12α-deoxytetracycline, dedimethylaminotetracycline, 6-demethyl-6-deoxytetracycline, 6-demethyltetracycline, and tetracyclinenitrile. All were reasonably volatile without extensive thermal decomposition except oxytetracycline, which charred somewhat in addition to evaporating. The spectra correlated quite well for almost all of the compounds. Because of this correlation, it is possible to discuss most of the major fragmentation processes for the entire series in terms of those for two compounds, 5α,6-anhydrotetracycline and dedimethylaminotetracycline, and to use the various other compounds primarily for labeling studies on various parts of the molecule;[2] for example, the chlorine atom labels the aromatic ring in aureomycin, and in 6-demethyltetracycline all of the fragments containing C-6 should be shifted 14.016 mass units. This technique requires extreme care in its application, since not only may the change in substitution have some direct effect on the fragmentations, but it also may change the number and availability of hydrogen atoms, causing shifts differing from the expected ones by 1.008 or 2.016 mass units. In this series as in other polycyclic systems certain types of functional groups may dominate the fragmentation pattern. The strongest influence in directing ring fragmentations in the tetracyclines is the dimethylamino group, as it is in the steroids.[3a] In dedimethylaminotetracycline the carbonyl groups are the strongest directing influence.

All of the compounds except the nitrile gave a strong molecular ion peak.[3b] The first peak observed in the nitrile spectrum is for the loss of water. The behavior of all of the other compounds in the high mass region is similar to that of 5α,6-anhydrotetracycline (see Figure 1). There is a peak at *m/e* 409.112 for the loss of NH_3, then one at 391.106 for the loss of H_2O from 409, one at 366.072 for the loss of CH_3NCH_2 from 409, and also peaks at *m/e* 383.101 for the loss of CH_3NCH_2 from the molecular ion and at 381.120 for the loss of $CONH_3$. There is also a peak at *m/e* 364.058 for the loss of the dimethylamino group as $(CH_3)_2NH$ from the 409 fragment and a peak at 336.063 for the loss of CO from the 364 fragment. These transitions were all established by the observation of metastable ions (see Scheme I). The only other peak in the spectrum with the ring system still intact is at *m/e* 320.067 for loss of CH_3NCH_2 and CO from the 391 fragment. In 12α-deoxytetracycline there are peaks at *m/e* 322.083 and 393.122 for the corresponding fragments.

The linear arrangement of the four rings in the tetracycline system does not facilitate cleavages involving more than one ring like those found in many steroid systems.[4] As in many steroid systems the fragmentations are quite complex and the structures drawn for the various fragments are not necessarily the best or the only possibilities. In general they have been chosen because they relate closely to the structure of the molecule. Almost all cleavages in the tetracyclines occur in rings C and D because of the aromatic ring A and the ease of aromatizing ring B. The cleavages of ring D lead to quite intense peaks in the spectra of compounds with a 4-dimethylamino group. In steroid systems the dimethylamino group completely dominates the fragmentation pattern.[5,6] Its importance in this system can be seen from the observation that the largest fragment peaks in the spectrum of 5α,6-anhydrotetracycline are 58.068 (Ia), 55.042 (Ib), 98.061 (Ic), 71.074 (Id), and 84.081 (Ie). (See Chart I). In the 12α-deoxy compound there is a strong peak at *m/e* 100.076 (If), rather than at *m/e* 98, since there is now hydrogen available at the ring junction. A more interesting manifestation of the importance of the dimethylamino group is the large peak at *m/e* 313.132 (II) and the observation of the corresponding peak in the spectrum of each of the other compounds which have dimethylamino groups. The metastable ion at 229.5 shows that this peak arises directly from the molecular ion. The peak at 268.073 (III), is formed by the loss of $(CH_3)_2NH$ from the 313 fragment by a Mc-

(2) K. Biemann, *Tetrahedron Letters*, **No. 15**, 9 (1960).

(3) (a) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day Inc., San Francisco, Calif., 1964, pp 43–49. (b) In ref 3a, p 250, there is a mass spectrum of 12α-deoxytetracycline which is missing the 428 (M) group. The 410 peak is mislabeled as M.

(4) Reference 3a, pp 26–120.

(5) W. Vetter, P. Longevialle, F. Khoung-Hou-Laine, Q. Khoung-Hou, and R. Goutarel, *Bull. Soc. Chim. France*, 1324 (1963).

(6) L. Dolejš, V. Hanuš, V. Černý, and F. Šorm, *Collection Czech. Chem. Commun.*, **28**, 1584 (1963).

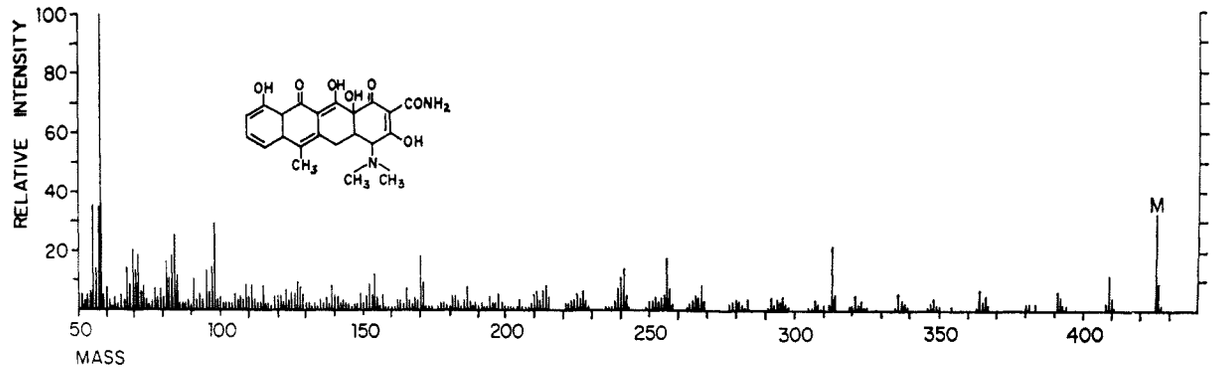


Figure 1.—Mass spectrum of 5α,6-anhydrotetracycline.

CHART I

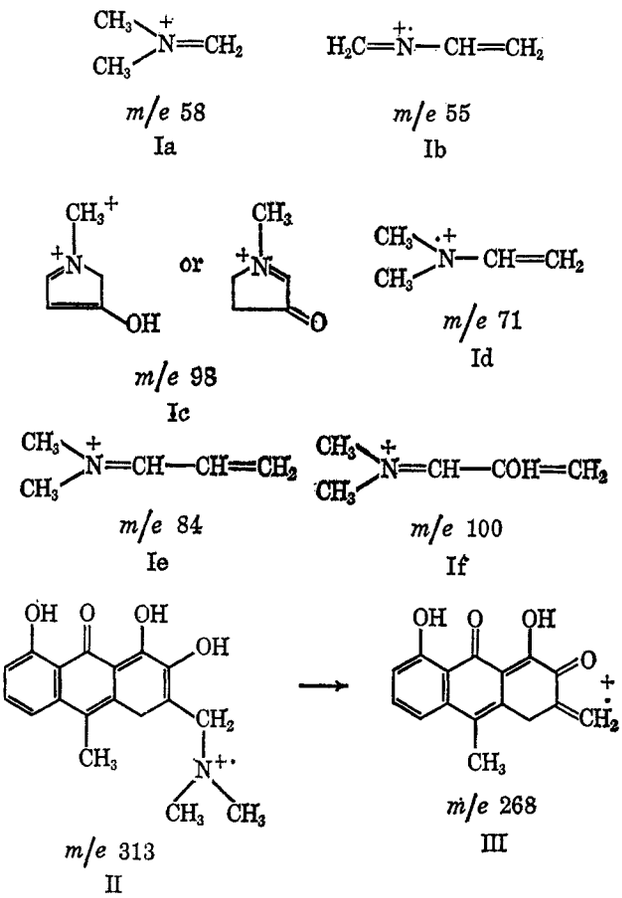


CHART II

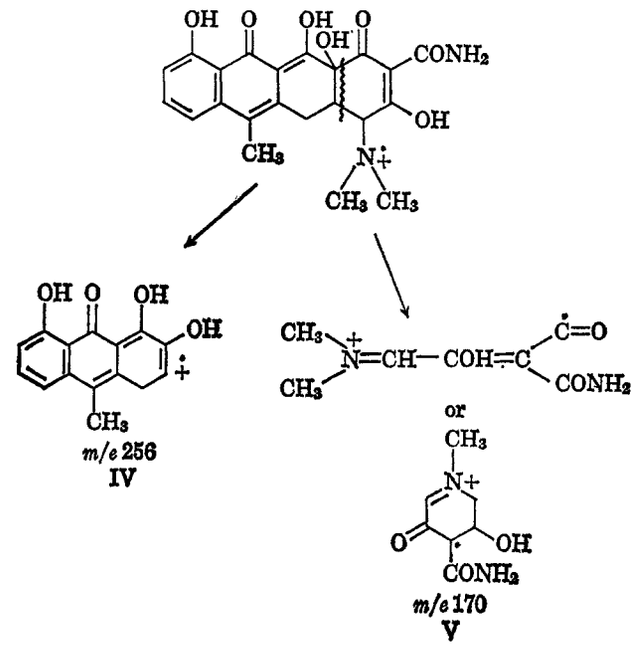
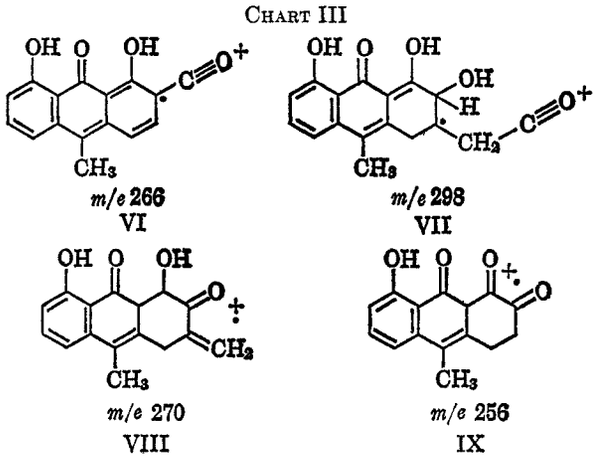


CHART III



Lafferty rearrangement,[7] and the fragment at *m/e* 240.077 is produced by the loss of CO from the 268 fragment. A pair of complementary processes lead to the fragments of *m/e* 256.073 (IV), and 170.069 (V), since the charge and odd electron can be stabilized by either part of the molecule. (See Chart II.) One other cleavage of ring D that occurs to a significant extent is the decomposition of the 409 fragment to a fragment of *m/e* 266.057 (VI). Not all ring D fragmentations, however, are dimethylamino directed. In dedimethylaminotetracycline the fragments 298.083 (VII), 270.088 (VIII), and 256.074 (IX) all come from oxygen-directed reactions. (See Chart III.)

Dedimethylaminotetracycline exhibits some of the same ring C fragmentations as 5α,6-anhydrotetracycline. Two fragments arise directly from the molecular ion in the 5α,6-anhydro compound: 214.064 (X) and 211.076 (XI). The 214 fragment then loses CO to give 186.068. The fragment at 227.070 (XII) also arises through a cleavage of ring C. In the dedimethylamino compound there is also a fragment at *m/e* 201.055 (XIII). (See Chart IV.)

The mass spectrum of oxytetracycline (terramycin) has several anomalous peaks. (See Chart V and Figure 2.) The largest fragments are at *m/e* 72.044 (XI),

(7) F. W. McLafferty and M. C. Hamming, *Chem. Ind.* (London), 1336 (1958).

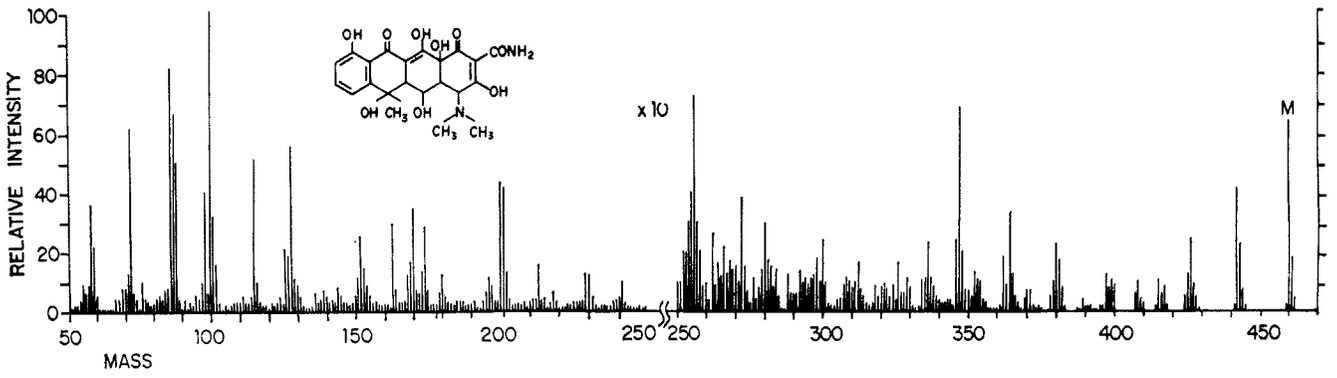


Figure 2.—Mass spectrum of oxytetracycline (terramycin).

CHART IV

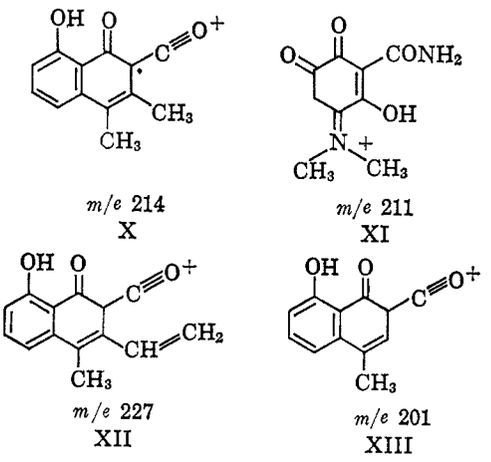
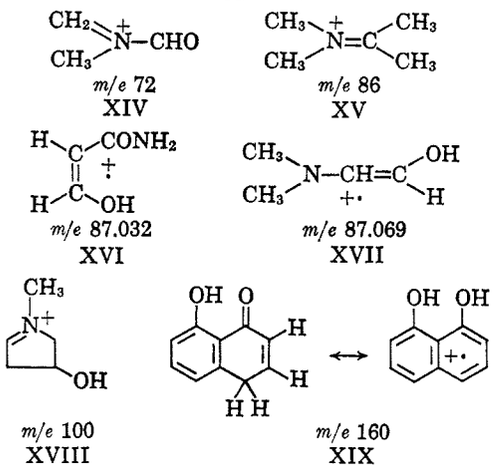


CHART V



86.098 (XV), 87.032 (XVI), 87.069 (XVII), and 100.075 (XVIII), rather than 71, 84, and 98 as in the other dimethylamino compounds. This is probably due to the presence of the 5-oxygen. In the neutral fragments the 5-oxygen is probably oxidized to a carbonyl, making two hydrogens available. However, some of these unusual reactions may be the result of thermal reactions in the solid phase before the sample ever gets into the electron beam, so the structures of these fragments are quite uncertain.

One can also use the mass spectrum of oxytetracycline as an example of how one would locate changes in substitution. The molecular ion is at *m/e* 460.148 corresponding to a composition of $C_{22}H_{24}N_2O_9$. The presence of the dimethylamino group can be inferred from the presence of peaks at *m/e* 58.068 (Ia), 72.044 (XIV), and from the peak at 417.107 for the loss of CH_3NCH_2 from the molecular ion. The presence of an amide function is suggested by the presence of a peak at 426.120 for the loss of H_4NO, NH_3 plus OH. The molecular formula indicates that the ninth oxygen would probably be a hydroxyl group, and it is improbable that it would be on any position but 4, 4α, 5, or 5α. The large peak for the loss of water from the molecular ion suggests that it is probably not on positions 4α or 5α, but it does not completely rule them out. However, peaks are observed at 230.057 for $C_{13}H_{10}O_4$ corresponding to 214 (X), $C_{13}H_{10}O_3$, in 5α,6-anhydrotetracycline and at 201.056 (XIII) for $C_{12}H_9O_3$. These two fragments are consistent only with a 5-hydroxyl group.

A similar technique can be applied to the spectrum of 6-demethyl-6-deoxytetracycline, $C_{21}H_{22}N_2O_7$. The peak at *m/e* 170.068 (V) suggests that the ring D structure of tetracycline is still intact and the peak at *m/e* 380.115, $C_{21}H_{18}NO_6$, indicates the presence of either a 5-, 6-, or 12α-hydroxyl group. The peak at *m/e* 162.052 is $C_{10}H_8O_2$ (XIX), dihydroxynaphthalene, showing that the methyl is missing from the 6-position. No equivalent peak is observed in the mass spectrum of tetracycline, indicating that the oxygen is also missing from the 6-position. In the 5α,6-anhydro compound the double bond hinders the formation of an equivalent fragment. Tetracycline dehydrates easily at this position so in this case also the 174 fragment is not a major peak. The saturated 5α,6 bond does allow this cleavage.

Despite the abundance of functional groups on the ring system the tetracyclines give good, clear, and reproducible mass spectra. The basic tetracycline system cannot be characterized by the presence on any particular peak or any particular pattern which is immediately obvious, but a great deal of information can be gained by the use of the shift technique.[2] For example, there is a large peak at *m/e* 331.142 corresponding to $C_{18}H_{21}NO_5$ in the mass spectrum of tetracycline; the analogous peak in chlortetracycline is 365.103, $C_{18}H_{20}NO_5Cl$, in oxytetracycline 347.137, $C_{18}H_{21}NO_6$, in 5α,6-anhydrotetracycline 313.131, $C_{18}H_{19}NO_4$, in 12α-deoxytetracycline 297.136, $C_{18}H_{19}NO_3$, in 6-demethyltetracycline 299.116, $C_{17}H_{17}NO_4$, in 6-deoxy-6-demethyltetracycline 301.131, $C_{17}H_{19}NO_4$, and in dedimethylaminotetracycline 270.089, $C_{16}H_{14}O_4$. For further examples see Table I. This method is quite useful for the tetracyclines, since modifications of the molecule which are of interest are quite small. Mass

TABLE I

SOME CORRELATIONS IN THE TETRACYCLINE SERIES

5α,6-Anhydro-	Tetra-	Chlor-	12α-Deoxy-	6-Demethyl-	Dedimethylamino-
426.143 (M)	426.143	460.104	428.158 (M)	430.138 (M)	401.111 (M)
$C_{22}H_{22}N_2O_7$	$C_{22}H_{22}N_2O_7$	$C_{22}H_{21}N_2O_7Cl$	$C_{22}H_{24}N_2O_7$	$C_{21}H_{22}N_2O_8$	$C_{20}H_{19}NO_8$
409.116	409.116	443.077	411.131	395.101	366.073
$C_{22}H_{19}NO_7$	$C_{22}H_{19}NO_7$	$C_{22}H_{18}NO_7Cl$	$C_{22}H_{21}NO_7$	$C_{21}H_{17}NO_7$	$C_{20}H_{14}O_7$
391.108	409.116	443.077	393.122	395.101	366.073
$C_{22}H_{17}NO_6$	$C_{22}H_{19}NO_7$	$C_{22}H_{18}NO_7Cl$	$C_{22}H_{19}NO_6$	$C_{21}H_{17}NO_7$	$C_{20}H_{14}O_7$
364.057	364.059	398.032	348.062	350.042	
$C_{20}H_{12}O_7$	$C_{20}H_{12}O_7$	$C_{20}H_{11}O_7Cl$	$C_{20}H_{12}O_6$	$C_{19}H_{10}O_7$	
336.062	336.064	370.024		336.062	
$C_{19}H_{12}O_6$	$C_{19}H_{12}O_6$	$C_{19}H_{11}O_6Cl$		$C_{19}H_{12}O_6$	
313.132	331.141	365.100	297.137	317.125	
$C_{18}H_{19}NO_4$	$C_{18}H_{21}NO_5$	$C_{18}H_{20}NO_5Cl$	$C_{18}H_{19}NO_3$	$C_{17}H_{19}NO_5$	
256.073	256.073	290.035	240.080	242.056	256.073
$C_{15}H_{12}O_4$	$C_{15}H_{12}O_4$	$C_{15}H_{11}O_4Cl$	$C_{15}H_{12}O_3$	$C_{14}H_{10}O_4$	$C_{15}H_{12}O_4$
241.049	241.050	275.010	225.055		241.049
$C_{14}H_9O_4$	$C_{14}H_9O_4$	$C_{14}H_8O_4Cl$	$C_{14}H_9O_3$		$C_{14}H_9O_4$
170.069	170.068	170.069		170.069	
$C_7H_{10}N_2O_3$	$C_7H_{10}N_2O_3$	$C_7H_{10}N_2O_3$		$C_7H_{10}N_2O_3$	
98.061	98.060	98.060	98.061	98.060	
C_5H_8NO	C_5H_8NO	C_5H_8NO	C_5H_8NO	C_5H_8NO	
84.081	84.082	84.081	84.081	84.081	
$C_5H_{10}N$	$C_5H_{10}N$	$C_5H_{10}N$	$C_5H_{10}N$	$C_5H_{10}N$	
71.074	71.073	71.074	71.074	71.074	
C_4H_9N	C_4H_9N	C_4H_9N	C_4H_9N	C_4H_9N	
58.068	58.068	58.068	58.068	58.068	
C_3H_8N	C_3H_8N	C_3H_8N	C_3H_8N	C_3H_8N	

spectral correlations can be used to determine the identity and location of functional groups in the tetracycline molecule.

Experimental Section

The mass spectra were taken on an AEI MS9 double focussing mass spectrometer. The compounds were evaporated directly into the source from a "degussit" tube on a vacuum-lock probe.[8] The source temperature was about 250° above room temperature for tetracycline HCl, chlortetracycline HCl, and oxytetracycline, and was about 300° for the other compounds. The accurate masses of all fragments of significant intensity were determined. Heptacosafluorotributylamine was used as a mass standard for the accurate measurements. The compositions of all fragments not included in Beynon and Williams' tables[9] were calculated on an IBM 7094 computer using a program written by Dr. David Usher, formerly of this department.

The spectra of tetracycline and chlortetracycline were obtained by pyrolysis of the hydrochlorides. The other compounds were all free bases. The tetracycline HCl, chlortetracycline HCl, and terramycin were obtained from of Chas. Pfizer and Sons, Groton, Conn. 12α-Deoxytetracycline, 5α,6-anhydrotetracycline, 6-demethyltetracycline, 6-demethyl-6-deoxytetracycline, dedimethylaminotetracycline, and tetracyclinenitrile were contributed by Dr. J. S. Webb and Dr. J. H. Boothe of the Organic Chemical Research Section, Lederle Laboratories, Pearl River, N. Y.

Spectra of each compound were obtained at ionizing energies of 70 and 16 ev. Metastable peaks are enhanced in the 16-ev spectra. Some 8- and 10-ev spectra were obtained, but these contained all of the larger peaks in the 70-ev spectra. However, some of the smaller peaks totally disappeared, showing that they arise through high-energy processes.[10]

The resonance forms or tautomeric forms chosen for the fragments illustrated, I–XIX, were chosen to illustrate either the origin of the fragment or a point discussed in the text.

Acknowledgment.—The author would like to thank Professor R. B. Woodward for his help and encouragement. This work was supported by the National Institutes of Health.

(8) M. Barber, R. M. Elliott, and T. O. Merren, International Symposium on Mass Spectrometry, Paris, 1964.

(9) J. H. Beynon and A. E. Williams, "Mass and Abundance Tables for Use in Mass Spectrometry," Elsevier Publishing Co., Amsterdam, 1963.

(10) J. H. Beynon, "Mass Spectrometry and Its Applications to Organic Chemistry," Elsevier Publishing Co., Amsterdam, 1960, pp 252–262.