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Nuclear Magnetic Resonance Spectra of Alkaloids. I. The Complete Structure of Lunacrine and Lunine

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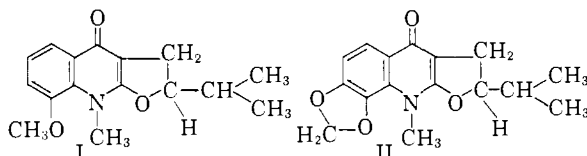
Lunacrine and lunine have been shown to have the structures I and II, respectively, by nuclear magnetic resonance spectroscopy.

The structure I was proposed for lunacrine, the principal leaf alkaloid of *Lunasia amara* Blanco, on the basis of chemical, ultraviolet and nuclear magnetic resonance studies.[3] The nuclear magnetic resonance studies are presented in this communication. The analysis of the nuclear magnetic resonance spectrum of lunacrine has provided unequivocal evidence that the methoxyl group is at position 8 and that the side chain has the isopropyl structure, and, in addition, all other features of the structure have been confirmed. The complete proof of structure by means of nuclear magnetic resonance spectroscopy of the companion alkaloid lunine is presented here also.



Experimental

All compounds were studied as approximately 10% solutions in $CDCl_3$ at an applied magnetic field strength of 14,096 gauss and a hydrogen precession frequency of 60 mc. The zero of reference was taken as benzene in an external annular cell, the shifts of certain of the peaks being measured by the audio frequncy side band method[4] in cycles per second (c.p.s.). In general, the frequencies of only a few lines were measured directly; the other frequencies were obtained by interpolation. The accuracy of the measured frequencies is ±1 c.p.s. In multiplets where the lines are not too far apart, the frequency differences are good to 0.1 c.p.s.; the additional significant figure being necessary in the analysis of multiplets arising from spin–spin interactions. The sign of the shift is chosen to be positive when the resonance falls at a higher applied field than the reference. The shift of any peak in dimensionless units can be determined by dividing the frequency obtained by linear interpolation between measured peaks by 60. This corresponds to the definition $\delta = 10^6(H - H_{ref})/H_{ref}$.

The spectrometer employed for these measurements was a Varian Associates V-4300-C high resolution nmr spectrometer with associated V-4102-SM magnet system equipped with a VK-3506 flux stabilizer. Samples were placed in 5-mm. thin-walled (0.015″) glass tubes and rotated at several hundred r.p.m. by a small air turbine during the recording of the spectra. A special precision annular cell (Wilmad Glass Co., Landisville, N. J.) was employed for the shift measurements. Audiofrequency side bands were generated with a Hewlett–Packard 200-CD audio oscillator and measured with a Hewlett–Packard 521C electronic counter. The spectra were recorded using two recorders (Varian model G-10) run simultaneously at the appropriate different gain settings necessary for the various line intensities.

(1) National Heart Institute, Bethesda, Md.

(2) Varian Associates, Palo Alto, Calif.

(3) S. Goodwin and E. C. Horning, THIS JOURNAL, **81**, 1908 (1959).

(4) J. T. Arnold and M. E. Packard, *J. Chem. Phys.*, **19**, 1608 (1951).

Interpretation

The spectrum of lunacrine shown in Fig. 1 consists of seven well-defined groups of lines; these groups are labeled with the letters a through g going from low field to high field. The observed frequencies for the multiplets are given in Table I. It may be concluded on the basis of chemical shifts alone that the following assignments can be made: multiplets a and b to the hydrogen nuclei of the aromatic system, multiplet c to the α-hydrogen atom of the dihydrofurano ring, the pair of strong lines d to the N-methyl- and methoxyl-hydrogen nuclei, the multiplet e to the β-hydrogen atoms of the dihydrofurano ring, and the multiplets f and g to the side-chain hydrogen atoms.[5] Moreover, the total integrated intensities of the groups are completely consistent with this assignment. The ratios of the measured areas are as follows: for the low gain spectrum, a:d:g, 1:6:6 and for the high gain spectrum, a:b:c:e:f, 1:2:1:2:1.

The multiplets c and e (Table I) belong to the sub-spectrum which would arise from an ABX system of nuclei in the notation of Bernstein, Pople and Schneider[6] where A and B are spin-coupled nuclei having δ_{AB} and J_{AB} of comparable magnitude which are also spin-coupled with another nucleus, X, having a large chemical shift relative to A and B. In this case A and B refer to the β-hydrogen atoms of the dihydrofurano ring and X to the α-hydrogen atom. The fact that one of the methylene-hydrogen atoms is *cis* to the side-chain while the other is *cis* to a hydrogen atom causes them to be non-equivalent. The analysis of this multiplet can be carried out according to either the notation of the Canadian group or to that of Anderson.[7] In the notation of Anderson the four lines involving one spin orientation of nucleus X may be taken to be the first, second, third and fifth lines of multiplet e, while the other four lines are from the other orientation of X. Field dependence studies support this assignment. All of the line positions and relative intensities of multiplet e can be accounted for with the following set of parameters: δ_{AB}, 16.7 (at 60 mc.); J_{AB}, 15.3; J_{AX}, 9.9; and J_{BX}, 8.7 c.p.s. The set of frequencies

(5) Several tables of carefully measured chemical shifts are becoming available. After taking account of the differences in the methods of referencing, the assignments given above could be shown to be consistent with the data of these tables. However, the spectra reported here have been assigned on the basis of unpublished exploratory work on related model compounds at Varian Associates.

(6) H. J. Bernstein, J. A. Pople and W. G. Schneider, *Can. J. Chem.* **35**, 65 (1957).

(7) W. A. Anderson, *Phys. Rev.*, **102**, 151 (1956); C. F. Callis, J. R. Van Wazer, J. N. Shoolery and W. A. Anderson, THIS JOURNAL, **79**, 2719 (1957).

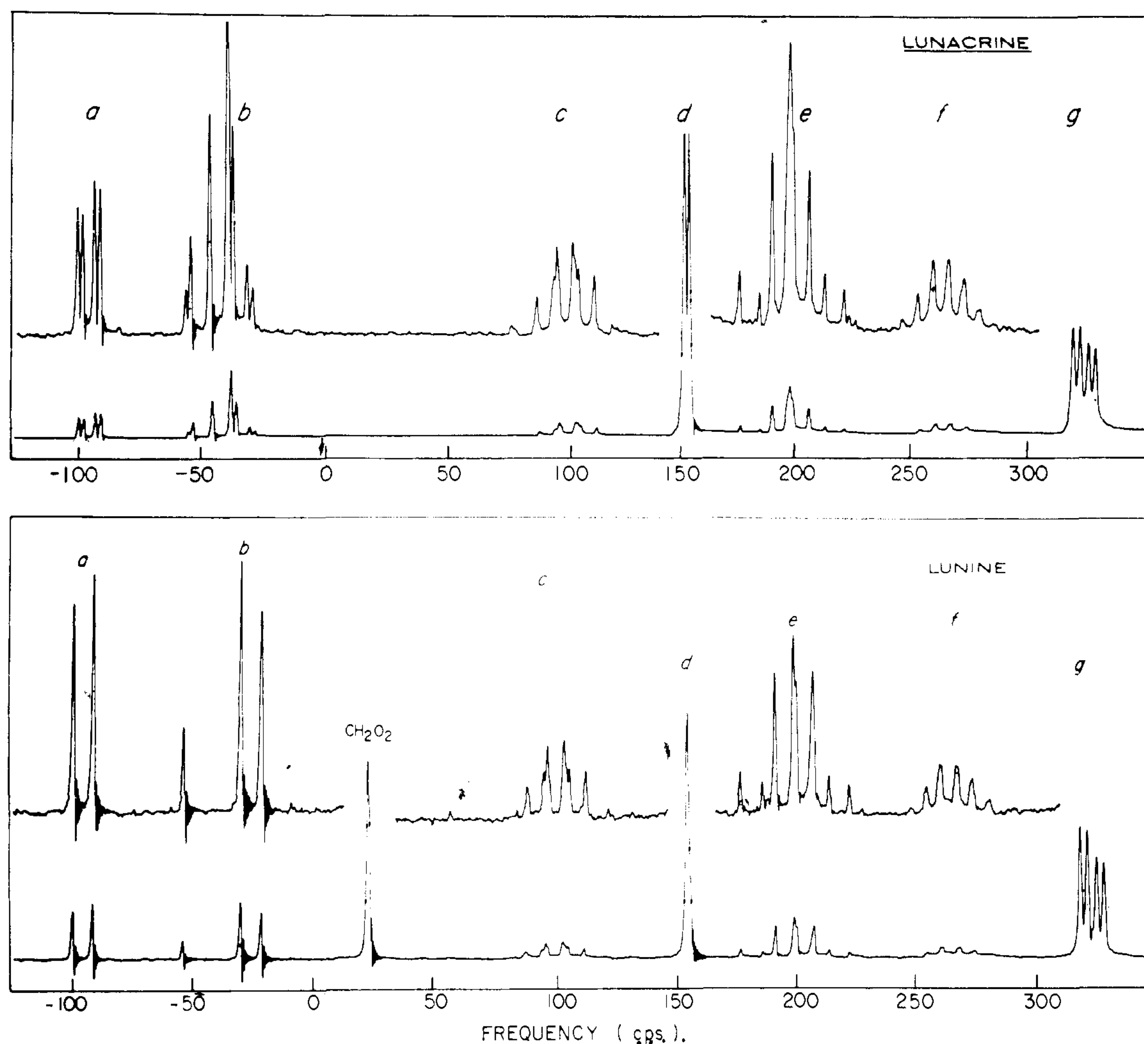


Fig. 1.—Nuclear magnetic resonance spectra of lunacrine (upper) and lunine (lower) in deutero-chloroform solution at 60 mc. The frequency is relative to benzene. The spectra shown are composites of simultaneous low and high gain recordings.

calculated for a system having these parameters is in excellent agreement with the observed frequencies (see Table I). Multiplet c has the slight additional complication arising from spin coupling between the X proton and one additional nucleus, Y, of spin $^1/_2$ which doubles all of the lines of the X pattern with a spacing corresponding to J_{XY} = 7 c.p.s.; two combination lines result from the close similarity of the coupling constants.

The fact that only one hydrogen atom, Y, is needed on the side-chain carbon atom attached to the α-position of the dihydrofurano system to account for the complexity of multiplet c suggests that the arrangement of the atoms in the side chain corresponds to an isopropyl group. In that case the multiplets f and g should display, respectively, a fourteen line pattern and a doublet structure due to spin-coupling between the tertiary hydrogen atom and the methyl-hydrogen atoms of the $-CH(CH_3)_2$ system. However, if this coupling should happen to be the same as the coupling to the ring hydrogen atom (–OCH) which has been found to be 7 c.p.s., then an eight-line pattern with intensities 1:7:21:35:35:21:7:1 would be expected in theory, although in practice the weaker lines would probably be lost in the noise. The six lines of multiplet f, in which the central lines are of equal intensity, are in good agreement with this prediction.

Multiplet g displays the predicted doublet structure with a 7 c.p.s. spacing, but in addition each line of the doublet is further split into two lines 3 c.p.s. apart. This can be explained by considering the rotational conformations of the isopropyl group about the bond joining it to the ring. Figure 2 illustrates one of the three possible potential minima. Due to the lack of symmetry it is clear[8,9] that the residence times in the three conformations can differ slightly and that as a result the magnetic environments of the two methyl groups will not be identical. This amounts to only 3 c.p.s. out of 60 × 10^6 c.p.s. but is clearly observable. A spectrum was obtained at 40 mc. for a check and as

(8) P. M. Nair and J. D. Roberts, THIS JOURNAL, **79**, 4565 (1957).

(9) J. N. Shoolery and Bryce Crawford, Jr., *J. Mol. Spec.*, **1**, 279 (1957).

TABLE I

RESONANCE FREQUENCIES (60 Mc.)

Pattern	Compound	Frequencies relative to benzene
a	Lunacrine	−100, −98, −93, −91
	III	−82.0, −79.2
	IV	−98, −95.8, −90.6, −88.4
	V	−105, −103, −98, −96
	Lunine	−100, −91.8
b	Lunacrine	−58.2, −54.4,[a] −47.6, −41, −39.0, −33.9, −31.9
	III	−60.1, −54.5,[a] 50.7, −48.1, −45.4, −38.9, −36.1
	IV	−56.5, −52,[a] −48.7, −41.5, −39.8, −34.2, −32.0
	V	−58.5, −55.0,[a] −50.9, −43.4, −41.2, −35.2, −33.2
	Lunine	−30.5, −21.8
c	Lunacrine	86.4, 93.1, 94.7, 101.8, 104.3, 111.4
	Lunine	86.1, 93.1, 94.7, 101.8, 103.9, 110.9
	V	92, 101, 110
d	Lunacrine	151, 153
	Lunine	156
	V	151
e	Lunacrine	
	Obsd.	174.9, 184.6, 190.2, 198.0, 199.8,[b] 206.7, 214.0, 223.0
	Calcd.	174.9, 184.7, 190.2, 198.0, 200.0, 206.9, 213.4, 222.0
	Lunine	176.0, 185.4, 190.9, 198.8, 199.9, 207.0, 213.7, 221.9
	V	180, 189, 198
f	Lunacrine	246.8, 253.5, 260.2, 267.0, 273.6, 280.2
	Lunine	247.9, 254.4, 260.4, 267.4, 273.7, 280.7
g	Lunacrine	318.9, 322.0, 325.9, 329.0
	Lunine	318.2, 321.2, 325.0, 328.0

[a] Chloroform. [b] In the spectrum selected for publication (Fig. 1) this line is not well resolved; however, in other spectra this side peak was clearly present.

expected the doubling of the lines in multiplet g diminished to 2 c.p.s.

The simple rotational conformation picture of Fig. 2 applies strictly only to substituted ethane-like molecules. In lunacrine, the rest of the molecule must also be taken into consideration. The barrier to rotation is probably more complex than the simple threefold barrier of substituted ethanes. The bond angles H–C–O, O–C–CH_2 and H–C–CH_2 are probably not all equal, due to the five-membered ring. Nevertheless, the simple picture provides a way of readily understanding how the observed chemical shift can arise. More detailed considerations indicate that even if all rotational conformations had equal residence times a chemical shift would be possible.[10]

If the side chain were the alternative *n*-propyl group, a completely different spin coupling scheme would apply. The excellent fit of the observed spectrum with that predicted for an isopropyl side chain constitutes unequivocal proof of the presence of this structural element.

The lines resulting from the magnetic resonance of the three aromatic hydrogen nuclei appeared as two multiplets, a and b, of relative intensity 1:2. These three nuclei may be regarded as an ABX system of nuclei in which multiplet a is assigned to the X-nucleus and multiplet b to the other two nuclei. The second line in multiplet b (−54.4 c.p.s.) may be ascribed on the basis of observations from many spectra to the small amount

(10) H. S. Gutowsky, "Technique of Organic Chemistry," A. Weissberger, Ed., Interscience Publishers, Inc., New York, N. Y., 3rd ed., 1958, Chapter on Nuclear Magnetic Resonance.

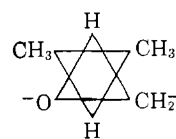
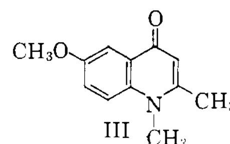


Fig. 2.

of chloroform in the solvent, deuterio-chloroform. Analysis of multiplets a and b as an ABX system leads to excellent agreement of line intensities and positions with experiment when $J_{AX} = 7$, $J_{AB} = 7$, $J_{BX} = 2$; and $\delta_{AB} = 9$ c.p.s. at 60 mc. The multiplet due to A is a triplet since $J_{AX} = J_{AB}$, while that of B is a quartet. It is observed that the high field line of the triplet and the low field line of the quartet form a combination line (−41 c.p.s.), the only line arising from non-equivalent nuclei which is unresolved in the spectrum.

A study of a large number of substituted benzene derivatives revealed that *ortho* hydrogen atoms are characteristically coupled to the extent of 6–9 c.p.s.; *meta* 2—3 c.p.s.; while *para* oriented hydrogen atoms are coupled to an unobservably small extent except in a few unusual cases. Thus, the ABX analysis of the multiplets shows that all three of the hydrogen atoms are adjacent, since A is *ortho* to both B and X. This immediately rules out the positions 6 and 7 of the quinolone system for the methoxyl group.

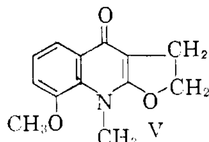
A decision between positions 5 and 8 cannot be made on the basis of spin–spin coupling. The unusual chemical shift of −100 c.p.s. for one of the aromatic hydrogen atoms can, however, be utilized to settle this question. It would be expected that the hydrogen nucleus proximate to the carbonyl group, *i.e.*, at position 5, would be the most perturbed of the aromatic hydrogen atoms of a 2,3-disubstituted-4-quinolone. The spectra of several simple model quinolone derivatives confirm this expectation. In the spectrum of 1-methyl-4-quinolone, the quartet at −100 c.p.s. observed for lunacrine remains essentially unchanged while the multiplet from the remaining hydrogen atoms is much more complex since the hydrogen atoms at positions 2 and 8 have about the same chemical shift as those at 6 and 7. In the spectrum of 1,2-dimethyl-6-methoxy-4-quinolone (III), a doublet is observed at −79.2 and −82.0 c.p.s., well separated from the multiplet resulting



from the other aromatic hydrogen atoms. The 2.8 c.p.s. spacing of this doublet corresponds to the spin-coupling constant of *meta* hydrogen atoms; therefore this low field doublet must be assigned to the 5-hydrogen atom. The expected doublet and quartet are observed for the 7- and 8-hydrogen atoms of III; $J_{78} = 9.4$ and $\delta_{78} = 9.4$ c.p.s. The diamagnetic shift of the resonance frequency of the 5-hydrogen atom of III compared with that of lunacrine is ascribed to the presence of the methoxy group on the adjacent carbon atom. Finally

the simple model compound 1,2-dimethyl-8-methoxy-4-quinolone (IV) was found to have multiplets corresponding to a and b of lunacrine in which the spacings (Table I) and relative line intensities were completely identical with those of lunacrine.

It is of interest to note that in the interpretation of nuclear magnetic resonance spectra, a suitable simple model compound representing a portion of a more complex molecule can be utilized to obtain a "fingerprint" of the portion of the spectrum under consideration. This is analogous to the use of model compounds in interpretations of ultraviolet and infrared spectra. In this instance the model suitable for n.m.r. comparison was much more readily available than the ultraviolet model. In order to obtain evidence for the position of the methoxyl group using ultraviolet spectral comparisons, it is necessary to have the intact dihydrofurano-4-quinolone system. By good fortune, a sample of γ-fagarine was obtained[11]; treatment with methyl iodide followed by hydrogenation afforded dihydro-iso-γ-fagarine (V).[12] The spec-



trum of V confirmed the earlier assignments of the aromatic hydrogen atoms. The spacings in the multiplets a and b (Table I) and the relative intensities of the lines were identical with those in the spectrum of lunacrine. The ultraviolet spectrum of V is, of course, identical with that of lunacrine. All of the other frequencies in the spectrum of dihydro-iso-γ-fagarine agreed well with the lunacrine assignments. The α- and β-dihydrofurano-hydrogen atoms afforded the expected triplets (Table I; multiplets c and e, respectively) having chemical shifts comparable with the corresponding multiplets of lunacrine. It is interesting that the methoxyl- and N-methyl-hydrogen nuclei of V (line d) happen to resonate at the same frequency, whereas in lunacrine they are in slightly different fields.

In summary, the nuclear magnetic resonance spectral assignments for lunacrine are as follows: a, the hydrogen atom at position 5 of the quinolone system; b, the aromatic hydrogen atoms at 6 and 7; c, the α-hydrogen atom of the dihydrofurano ring; d, the methoxyl- and N-methyl-hydrogen atoms; e, the β-hydrogen atoms of the dihydrofurano ring; f, the tertiary hydrogen atom of the isopropyl group; and g, the C-methyl-hydrogen atoms.

Having correlated the n.m.r. spectrum of lunacrine with the structure I, it is now possible to employ n.m.r. spectroscopy in the elucidation of the structures of other *Lunasia* alkaloids. Lunine, one of the constituents of the leaf alkaloids present in trace amounts, has been found to have the empirical formula $C_{16}H_{17}O_4N$, to contain one N-methyl group but no methoxyl group, and to be optically active.[13] The immediately obvious fact that lunine differs empirically from lunacrine by the addition of one oxygen atom and the loss of two hydrogen atoms, coupled with the observation that unlike lunacrine, lunine contains no methoxyl group suggested that lunine may be the methylenedioxy analog of lunacrine. Positive color tests (Labat and chromotropic acid) provided evidence for the presence of a methylenedioxy group. The ultraviolet spectrum of lunine, measured in alcohol, was in consonance with the quinolone system (λ_{max} 247, 314 and 325 mμ); however, a bathochromic shift to 333 mμ in acid solution was observed in contradiction to the earlier assumption that, in the ultraviolet, the N-methyl-4-quinolone chromophore characteristically exhibited a hypsochromic shift on acidification.[3] No clear-cut decision could be made from the infrared spectrum; the medium-strong band at 6.09 μ was 0.09 μ lower than the corresponding band in lunacrine and would suggest the 2-quinolone class for lunine.[14] It might be reasonably assumed that a methylenedioxy substituent could influence the infrared and ultraviolet spectra of lunine as compared with lunacrine in the manner described.

The situation was immediately clarified on obtention of the n.m.r. spectrum of lunine (Fig. 1). Striking similarities in the spectra of lunine and lunacrine are immediately obvious. The multiplets of the lunine spectrum are lettered a through g as in the case of the lunacrine spectrum, but the new signal at 23 c.p.s. which has the proper chemical shift, relative intensity and lack of fine structure[15] to qualify for assignment as arising from the methylenedioxy-hydrogen atoms, is indicated on the spectrum by its assignment, CH_2O_2. The essentially complete agreement of the resonance frequencies (Table I) of the multiplets c, e, f and g with the corresponding values obtained for lunacrine as well as the correspondence in relative line intensities and the presence of the N-methyl signal at 156 c.p.s. (d) provide compelling evidence for the α-isopropyl-dihydrofurano-4-quinolone system for lunine similar to lunacrine. The conclusion that these multiplets have the same genesis is supported by field dependence studies.

The two aromatic hydrogen atoms of lunine, multiplets a and b (Fig. 1, the signal at −54.5 c.p.s. is ascribed to chloroform), must be *ortho* to one another because of the magnitude of the spin-coupling constant, *ca.* 8.5 c.p.s. (see Table I). Again as in the case of lunacrine the multiplet of one of the aromatic hydrogen nuceli is at very low field indicating the presence of a hydrogen atom at position 5 in a 4-quinolone system. It is therefore

(11) We are very grateful to Dr. I. J. Pachter, Smith, Kline and French Laboratories, Philadelphia, Pa., and Dr. F. A. Kincl, Syntex, Mexico City, for samples of γ-fagarine.

(12) S. Goodwin, unpublished work.

(13) S. Goodwin, A. F. Smith, A. A. Velasquez and E. C. Horning, in manuscript.

(14) M. F. Grundon, N. J. McCorkindale and M. N. Rodger, *J. Chem. Soc.*, 4284 (1955).

(15) Theoretically it is possible that the hydrogen atoms of the methylenedioxy group of lunine could be chemically non-equivalent since a plane of symmetry through the methylene group is not possible, however such long range effects have not been observed. For an example of non-equivalent methylenedioxy-hydrogen atoms, *cf.* S. Goodwin, J. N. Shoolery and L. F. Johnson, *Proc. Chem. Soc.*, 306 (1958).

required that the methylenedioxy group be at positions 7, 8 and, thus, the structure II may be written for lunine.

Acknowledgments.—We are indebted to Professor H. S. Gutowsky for helpful comments concerning the rotational conformation arguments. We are grateful to Miss A. A. Velasquez for preparing the model compounds III and IV.

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[CONTRIBUTION FROM LEDERLE LABORATORIES, AMERICAN CYANAMID COMPANY]

Biosynthesis of Tetracyclines. I. The Halide Metabolism of *Streptomyces aureofaciens* Mutants. The Preparation and Characterization of Tetracycline, 7-Chloro^{36}-tetracycline and 7-Bromotetracycline

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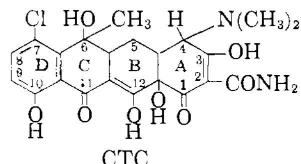
Quantitative measurements have been made of the chloride and bromide metabolism, as regards incorporation into the tetracyclines, of three *Streptomyces aureofaciens* mutants. These three are closely related genetically, but differ from each other in the quantitative features of their halide metabolism. They represent two metabolic classes. The classification is based on the independence or dependence on concentration of chloride of the rate of conversion of chloride to 7-chlorotetracycline.[1] The mutants differ substantially in their susceptibility to inhibition by bromide of the incorporation of chloride into 7-chlorotetracycline. This inhibition has been shown reversible by excess chloride. Halogenation in all three mutants is substantially inhibited by thiocyanate; the degree of reversibility of this inhibition varies among the mutants. The total terminal tetracyclines concentration of these systems is independent of chloride and bromide concentration from values near zero to values ten times the stoichiometric equivalent of the total tetracyclines potential of the system. The total tetracyclines potential is the sum of the millimoles per liter of 7-chlorotetracycline, 7-bromotetracycline and tetracycline present at the end of the fermentation cycle. Withholding halide or inhibiting halide utilization results in biosynthesis of equimolar amounts of the unsubstituted product, tetracycline. The fermentation end products from these mutants do not contain any significant quantities of chlorinated organic materials except 7-chlorotetracycline. Tetracycline, 7-chloro^{36}-tetracycline and 7-bromotetracycline have been prepared biosynthetically through the use of *Streptomyces aureofaciens* Duggar. Some of their properties have been determined. The preparation of 7-chloro^{36}-tetracycline started with HCl^{36} and proceeded in good yield in a manner involving substantially no isotopic dilution.[2]

Introduction and Discussion

The abilities of a variety of biological systems to incorporate the four halides into halogenated organic compounds of many types are well known. Metabolic experiments with *Streptomyces aureofaciens* Duggar grown in media containing chloride^{36} and in media containing no available halogen except chloride ion have shown that chloride can serve the organism efficiently as the sole source of the chlorine in the broad-spectrum antibiotic, 7-chlorotetracycline (CTC), resulting from the mold's metabolic processes.[3] Similar experiments



using media containing no available halogen except bromide ion have shown that bromide can serve the organism efficiently as the sole source of the bromine in the broad spectrum antibiotic, 7-bromotetracycline (BTC).[4] Witholding chloride and bromide[5] or inhibiting halide utilization[6] prevents the formation of CTC and BTC and results in the formation of equivalent amounts of tetracycline (TC). In the mutants described here, bromination is accomplished at a lesser rate than chlorination; for bromide levels not toxic to the organism, this reduction in halogenation rate is accompanied by the formation of more TC. The total terminal tetracyclines concentration of these fermentation systems is independent of the chloride and bromide concentration from values near zero to values ten times the stoichiometric equivalent of the total tetracyclines potential of the particular system. (Total tetracyclines potential is the sum of the millimoles per liter of 7-chlorotetracycline, 7-bromotetracycline and tetracycline present at the end of the fermentation cycle.)

The three mutants described here are coded BC-41, S-1055 and S-580. Mutants S-1055 and S-580 are direct isolates from cultures of BC-41. All are descendants of the original *S. aureofaciens* A-377 soil isolate of Duggar. They resemble each other in the over-all aspects of their halide metabolism but differ in the quantitative features of their halide metabolism, falling into two classes. Class I contains those mutants whose rate of chloride utilization for CTC is independent of chloride ion concentration; Class II includes those whose rate of chloride utilization depends on

(1) The trademarks of the American Cyanamid Company for tetracycline and 7-chlorotetracycline are Achromycin and Aureomycin, respectively.

(2) A summary of the material presented here has previously been published: A. P. Doerschuk, J. R. D. McCormick, J. J. Goodman, S. A. Szumski, J. A. Growich, P. A. Miller, B. A. Bitler, E. R. Jensen, M. A. Petty and A. S. Phelps, THIS JOURNAL, **78**, 1508 (1956).

(3) M. A. Petty and M. Matrishin, Abst. of Papers, 118th Meeting, American Chemical Society, 1950.

(4) P. Sensi, *Il. Farmaco Sci. Ed.*, **10**, 346 (1955).

(5) G. Rolland and P. Sensi, *Farm. sci. e. tec. (Pavia)*, **10**, No. 1, 37 (1955).

(6) A. Gourevitch, M. Misiek and J. Lein, *Antibiotics and Chemotherapy*, *V*, No. 8, 448 (1955).