their pheromone. The (*E,Z*)-isomer apparently had less effect on the response of *S. exitiosa* males to their pheromone but it seemed to reduce the attractiveness of the (*Z,Z*)-isomer when the two were mixed in a 1 : 1 ratio.

The final test in this series, a trapping experiment, was conducted in October 1973, in a peach orchard near Byron. Thirty-six traps (Zoecon Pherocon 1C) were arranged in a complete randomized block. A glass petri dish containing 10 μg of (*E,Z*)-3,13-octadecadien-1-ol acetate was placed in the bottom of each of 12 traps; three virgin female *S. pictipes* were placed in each of 12 traps; and 12 traps were empty. Between 10:30 a.m. and 3:00 p.m. (E.D.T.) of the first day, the traps that contained the synthetic pheromone caught 1335 male *S. pictipes*; the traps containing females caught 114 males; and the empty traps caught no males. During the next 4 days, the pheromone traps caught an additional 263 males.

In view of the high percentage of recovery of released *S. pictipes* males in previous experiments, and the marked attractiveness of the synthetic pheromones to the males of both species, we anticipate that the synthesized materials will be useful in efforts to control these pests either by trapping or by disruption. It is possible that one compound or a mixture of the two may be useful in controlling both species via the communication disruption technique.

J. H. TUMLINSON
Insect Attractants, Behavior and Basic Biology Research Laboratory, Agricultural Research Service, Gainesville, Florida 32604

C. E. YONCE
Southeastern Fruit and Tree Nut Research Station Laboratory, Byron, Georgia 31008

R. E. DOOLITTLE
R. R. HEATH
Insect Attractants, Behavior and Basic Biology Research Laboratory, Agricultural Research Service, Gainesville, Florida 32604

C. R. GENTRY
Southeastern Fruit and Tree Nut Research Station Laboratory, Byron, Georgia 31008

E. R. MITCHELL
Insect Attractants, Behavior and Basic Biology Research Laboratory, Agricultural Research Service, Gainesville, Florida 32604

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9. Methylene chloride and pentane are suitable solvents for *S. pictipes* and *S. exitiosa* crude extracts, respectively [M. L. Cleveland and L. L. Murdock, *J. Econ. Entomol.* **57**, 762 (1964); C. R. Gentry, A. A. Sekul, C. E. Yonce, *J. Georgia Entomol. Soc.* **7**, 247 (1972)].
10. A glass column, 0.6 cm (inside diameter), packed with 28 g of Bio-Sil HA (Bio-Rad Laboratories), was eluted sequentially with 100 ml of pentane, 100 ml of a mixture of pentane and ether (95 : 5), 200 ml of a mixture of pentane and ether (90 : 10), and 200 ml of ether. The flow rate was maintained at 120 ml/hour, and 10-ml fractions were collected.
11. The following stainless steel gas chromatographic columns were used with the conditions noted; for SE-30, 5 percent on 80/100 mesh Chromosorb G-HP, 4 m by 4 mm (inside diameter), column temperature at 225°C, He flow at 80 cm³/min; for OV-101, 5 percent on 80/100 Chromosorb G-HP, 2 m by 2 mm (inside diameter), column temperature at 170°C, He flow at 20 ml/min; for Carbowax 20M, 5 percent on 80/100 Chromosorb G-HP, 1 m by 2 mm (inside diameter), column temperature at 180°C, He flow at 20 ml/min; for SP 2300, 3 percent on 100/120 Supelcoport, 8 m by 2.3 mm (inside diameter), column temperature at 230°C, He flow at 20 ml/min; for Dexsil, 60 m by 0.08 cm capillary (inside diameter), column temperature at 235°C, He flow at 22.5 cm/sec; and for DEGS, support-coated open tubular (Perkin-Elmer Corp.), 45 m by 0.05 cm, column temperature at 190°C, He flow at 23 cm/sec.
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14. A 25 cm by 0.62 cm (outside diameter) stainless steel column was packed with dry Adsorbosil-2-ADN (20 percent) (Applied Science Laboratories). This is silica treated with silver nitrate and is intended for use in thin-layer chromatography. The particle size ranges from about 1 to 11 μm. The column was eluted with benzene at a flow rate of 1 ml/min and a head pressure of 1000 pounds per square inch. The column effluent was monitored with a refractive index detector.

8 February 1974; revised 1 April 1974

Raman Spectroscopic Investigation of Gramicidin A′ Conformations

Abstract. *Gramicidin A′ is believed to form transmembrane channels in lipid bilayers and biological membranes. The first Raman spectroscopic study of gramicidin A′ is presented. Evidence is found for two types of conformation. One type is found in the powder and has a Raman spectrum similar to that of model polypeptides with β hydrogen bonding. The second type is found when gramicidin A′ is dissolved in dimethyl sulfoxide.*

The antibiotic gramicidin A (GA) is a linear pentadecapeptide (15 residues) with the sequence HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NH-CH_2CH_2OH (*1*). There is good evidence that dimers of GA can form transmembrane channels in lipid bilayers (*2–4*). This evidence includes the highly specific conductance (10^7 ohm^{-1} cm^{-2} $mole^{-1}$) of GA in lipid bilayers compared to carriers such as valinomycin (10^3 ohm^{-1} cm^{-2} $mole^{-1}$) (*2*), the second-order dependence of the ionic conductivity of these membranes on GA concentration (*3*), and the persistence of this conductivity when the lipid bilayer "freezes" (*4*).

Several possible structures for GA and its dimer have been proposed. On the basis of unpublished infrared spectra (*5*), Sarges and Witkop (*1*) have suggested that GA forms "side-by-side" dimers by intermolecular parallel β hydrogen bonding, similar to that found in β-keratin (*6*). This model, however, does not explain the membrane activity of GA.

More recently, on the basis of conformational energy calculations, Urry and co-workers have proposed a left-handed helical structure which they call a $\pi_{(L,D)}$ helix (*7, 8*). This structure is characterized by having C=O groups positioned alternately parallel and antiparallel to the helical axis, unlike the well-known α helix found inside most globular proteins, which has C=O groups only parallel (*6*). The $\pi_{(L,D)}$ helix is made possible by the existence of alternating L and D residues in the peptide chain. Urry (*7*) predicts that this type of helix can form "head-to-head" dimers by an *intermolecular* hydrogen bond pattern similar to the antiparallel β-pleated sheet. In contrast, the *intramolecular* hydrogen bonding of GA is postulated

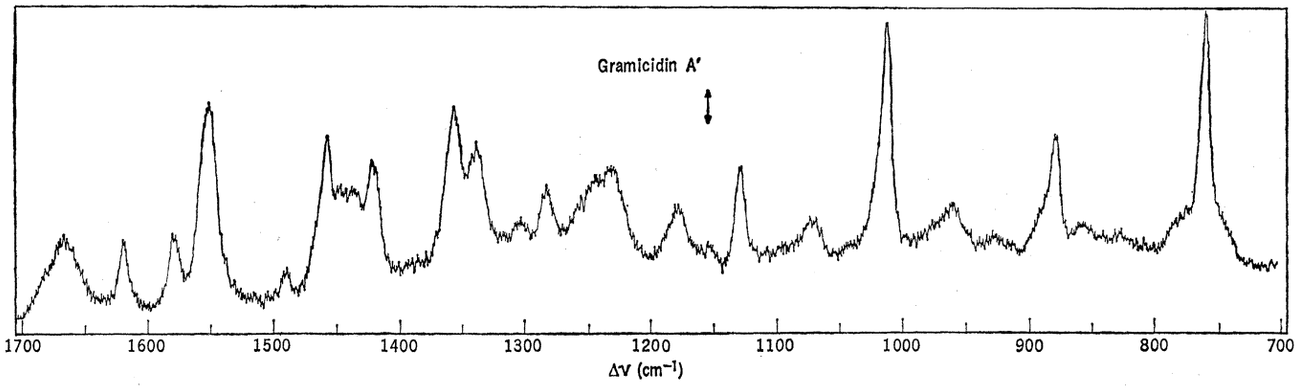

Fig. 1. Raman spectrum of gramicidin A′ powder from 700 to 1700 cm^{-1}. The spectrum was recorded with incident power 20 mw, excitation at 4880 Å, scanning speed 0.2 cm^{-1}/sec, and spectral resolution 3 cm^{-1}. The arrow indicates 300 counts per second. The incident radiation was polarized perpendicular to the scattering plane and no analyzer was used.

to be in a parallel β-pleated sheet pattern. The whole structure forms a cylindrical channel which could act as a pore for ions. It is possible to have several types of $\pi_{(L,D)}$ helices, differing in the number, n, of residues per turn in the helix; these have been denoted $\pi^{n}_{(L,D)}$. Urry predicts that the $\pi^{6}_{(L,D)}$ dimer can span a lipid bilayer and have the observed selectivity of GA ($NH_4^+ > K^+ > Na^+$).

Nuclear magnetic resonance has been used to study GA′ dissolved in dimethyl sulfoxide (Me_2SO); GA′ is a mixture of GA, GB, and GC, where GB and GC differ from GA in position 11, with tyrosine and phenylalanine replacing tryptophan. Although the measured NH-CH coupling constants of GA′ have values consistent with the dihedral angles calculated for a $\pi^{6}_{(L,D)}$ helix, there is no compelling evidence that GA′ has the proposed $\pi^{6}_{(L,D)}$ helix conformation in solution.

It is our purpose to study how GA facilitates selective ion transport in lipid bilayers. Laser Raman spectroscopy is a sensitive probe of hydrogen bonding patterns in proteins and other biological molecules (*9–11*); we have utilized this technique to study different conformations of the cation carrier molecule valinomycin (*9*). Here we report the first Raman spectroscopic investigation of GA′. Evidence is presented for the possible existence of two different conformations of GA′, with one conformation (possibly the proposed $\pi_{(L,D)}$ helix) predominating in the solid state and the other conformation predominating in Me_2SO.

Gramicidin A′ was obtained commercially (Nutritional Biochemical Co., Cleveland, Ohio) and used without purification. Raman spectra of GA′ powder and $CHCl_3$, dioxane, and Me_2SO solutions were obtained by using a 4-watt Ar^+ laser and a Spex Ramalog IV system. Spectra of the powder contained in a small capillary were obtained with 20 mw of power to avoid burning. Power for solution spectra ranged between 100 and 200 mw. Exciting radiation included laser lines at 5140, 4880, and 4579 Å. The concentration of GA′ in Me_2SO solution was approximately 10 percent (weight to volume), while the GA′ solutions of CH_3Cl and dioxane were near saturation.

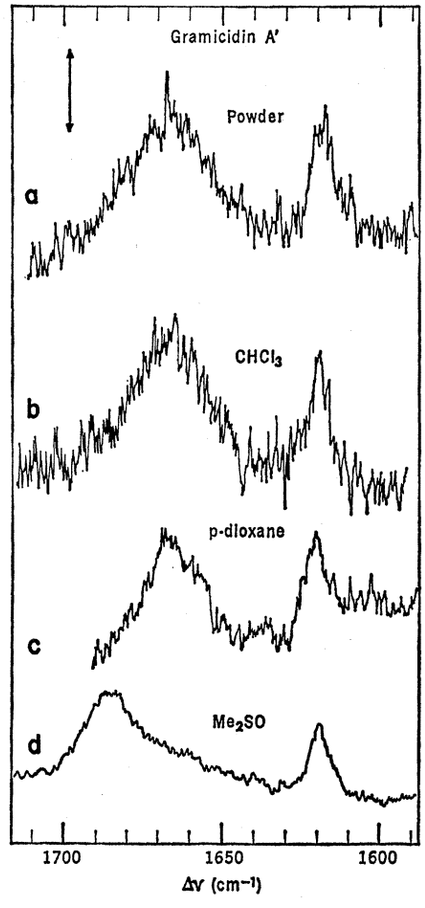

Fig. 2. Raman spectra from 1600 to 1700 cm^{-1} in the amide I region of (a) gramicidin A′ powder, (b) gramicidin A′ in $CHCl_3$, (c) gramicidin A′ in *p*-dioxane, and (d) gramicidin A′ in Me_2SO. The spectra in a, b, c, and d were recorded with incident power 20, 100, 150, and 100 mw, respectively. Other conditions were the same as for Fig. 1.

Figure 1 shows the Raman spectrum of GA′ powder in the range of 700 to 1700 cm^{-1}. By comparing this spectrum with the spectra of the component amino acids, with the spectra of homopolymers of some of these amino acids, and with previous assignments (*9, 10*), we were able to assign many lines to specific residue vibrations. In particular the Trp peaks appear in several places, including the very strong lines at 758, 1012, and 1360 cm^{-1}. The NH vibration of the Trp indole ring appears to be split (1420 and 1432 cm^{-1}); this may be due to interaction of the NH group with other parts of the molecule. The peaks at 835 and 858 cm^{-1} are assigned to Tyr and hence are a rough measure of the amount of GC present in GA′. Vibrations of the Val and Leu residues (which make up 33 percent of the residues) appear at 878, 1125, 1150, and 1340 cm^{-1}. In addition, the peak at 1070 cm^{-1} may be due to the OH group at the end of the polypeptide chain.

We tentatively assign the broad left asymmetric peak at 1665 cm^{-1} to the amide I carbonyl stretch vibration and the multiple peaks at 1231, 1245, and 1285 cm^{-1} to the amide III NH bending vibration. Polypeptides with α-helical, random, and β conformations all have characteristic amide I and III frequencies (*11*). Hence, these regions

are extremely sensitive to hydrogen bonding and reflect the skeletal conformations of the molecule. The peaks seen in GA′ in these regions—the singlet at 1665 cm^{-1} (see Fig. 2a) and the activity at 1231, 1245, and 1285 cm^{-1}—are similar to the Raman spectra (*11, 12*) of model polypeptides with antiparallel β hydrogen bonding (no Raman studies have been reported on model compounds with parallel β hydrogen bonding). The activity on the high-frequency sides of the 1665- and 1231-cm^{-1} peaks may also indicate the presence of other types of carbonyl groups (for example, nonhydrogen bonding carbonyl groups).

Figure 2, b to d, shows the region 1600 to 1700 cm^{-1} for GA′ dissolved in $CHCl_3$, dioxane, and Me_2SO, respectively. The first two solution spectra closely match the powder spectrum of Fig. 2a. GA′ tends to aggregate due to the low solubility of these solvents; however, a similar spectrum appears for nonsaturated solutions of GA′-methanol. The spectrum of GA′ in Me_2SO differs considerably. The amide I peak is shifted from 1665 to 1685 cm^{-1} and a right asymmetry appears. In the amide III region the peak at 1285 cm^{-1} disappears and the peak at 1231 cm^{-1} is reduced in intensity.

An amide I vibration as high as 1685 cm^{-1} is unusual for an ordered conformation. A similar peak is seen in random coil glucagon (*11*), where it is assigned to completely unsolvated amide carbonyl groups. The powder peaks at 1231 and 1285 cm^{-1} become, in Me_2SO, broad regions of activity centered about 1240 and 1265 cm^{-1}. Previous studies in deuterated Me_2SO using other techniques have been interpreted as suggesting that GA′ exists in an ordered conformation that is not α helical (*8*). Whether the conformation of GA′ in Me_2SO is random coil or ordered is not clear, but it is certainly different from any conformation we find in powder, $CHCl_3$, dioxane, or methanol.

Note added in proof: Our powder data are also consistent with a very recently proposed antiparallel double-helical dimer structure (*13*).

KENNETH J. ROTHSCHILD
H. EUGENE STANLEY
Harvard-MIT Program in Health Sciences and Technology and *Department of Physics, Massachusetts Institute of Technology, Cambridge 02139*

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Chromosomes of the Chocolate Gourami: A Cytogenetic Anomaly

Abstract. *The chocolate gourami,* Sphaerichthys osphromonoides, *has the lowest chromosome number reported for fishes, with* 2n = *16 and* n = *8. Other members of the family Belontiidae have somatic numbers of 42 to 48. Chromosome instability is demonstrated by the presence of somatic abnormalities and meiotic multivalents.*

Sphaerichthys osphromonoides is a tropical fish belonging to the largest family of anabantoids, the Belontiidae. This species is native to Malaya and Sumatra and is thought to have originated from a line that came from an anabantid ancestor when an explosive phase of adaptive radiation occurred during the Upper Cretaceous and Lower Tertiary (*1*). This gourami is one of the most specialized belontiids and is classified in the subfamily Trichogasterinae. It is considered by aquarists to be a delicate species that is hypersensitive to temperature change and other external stimuli. The chocolate gourami is not a prolific breeder. Despite intensive studies, its breeding habits are not completely understood (*2*). Until now, *Sphaerichthys* has not been studied cytogenetically.

Specimens used in this study consisted of 21 females and 10 males which were obtained from various tropical fish dealers. Both mitotic and meiotic chromosomes were enumerated ac-

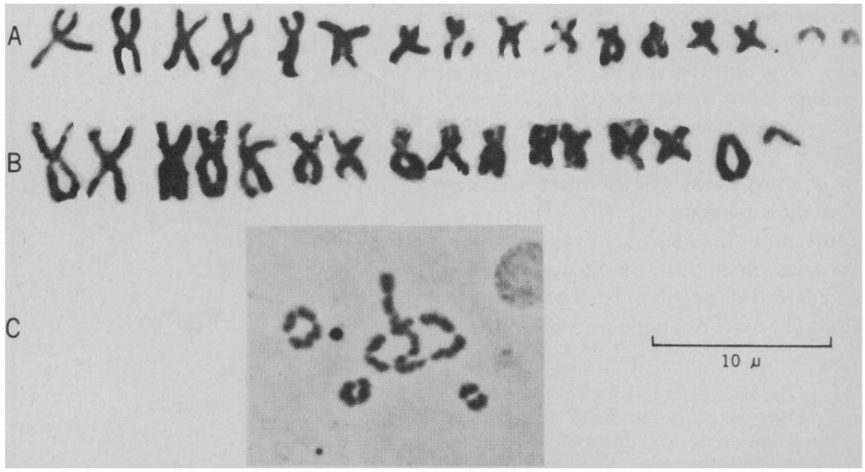

Fig. 1. Mitotic and meiotic chromosomes of the chocolate gourami. (A) Normal somatic complement showing a diploid number of 16. (B) Abnormal somatic complement showing a heteromorphic acrocentric pair. (C) Meiotic spread from testis showing a ring hexavalent, a rod quadrivalent, and three bivalents for a haploid number of 8.