the pattern of the intensities of the X-ray reflexions is very similar in the three crystals, but some changes are observable both on drying and on halogen substitution. Analysis shows (see Appendix 1) that there is incomplete substitution of the Cl by I in the *N*-iodoacetyl derivative.

Iodoacetylgramicidin S crystallized in the presence of excess of iodine

An orthorhombic form of iodoacetylgramicidin S was obtained by Synge in 1952, by crystallizing it from aqueous ethanol in the presence of excess of iodine. Beautiful little brownish-red needle-shaped crystals were formed, elongated along [010] with well-developed {100} and other unidentified domal faces. These showed marked pleochroism, from which it could be deduced that the iodine molecules lay perpendicular to the needle axis. Unlike most of the derivatives examined, the needle axis is not the one which is a multiple of (18·8–19·8 Å), but the optics seem to bear the same relationship to the cell dimensions as in other crystals. $\gamma \parallel b$, and probably $\alpha \parallel a$ and $\beta \parallel c$.

Gramicidin S chloroaurate

The chloroaurate was prepared by Synge in 1952, and grows in the form of colourless, flat needles, elongated along [001], with well developed {100} or {010}.

Of several crystals selected, one was found to have crystallized in a different space group from the others. All of them had the same unit cell dimensions, and most seem to be body-centred, probably having the space group I222; the one exception had the space group $P22_12$.

The crystals were weakly birefringent, with $\alpha \parallel a$, $\beta \parallel c$ and $\gamma \parallel b$.

SUMMARY

1. Single crystals of a series of derivatives of gramicidin S have been prepared and examined by X-ray methods. Their morphology, unit cells and space groups have been determined, and a comparison between crystals in the wet and dry states has been made.

2. The weights of the crystal asymmetric units of the hydrochloride and *N*-acetyl, *N*-chloroacetyl and *N*-iodoacetyl derivatives have been determined by X-ray methods and the results compared with the molecular weights determined by chemical analysis.

3. It is concluded that the gramicidin S molecule is most probably a decapeptide in which two halves of the molecule are related by a twofold axis of symmetry. Some conclusions about the size and shape of the molecules are drawn.

4. Appendix 1 describes the methods of preparation and chemical analyses of the crystals examined.

5. In Appendix 2 possible molecular models for gramicidin S are discussed in relation to current theories of protein structure.

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APPENDIX 1

Preparation of some Derivatives of Gramicidin S

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N (Kjeldahl) determinations were done by the incineration procedure of Campbell & Hanna (1937). Other elementary analyses, unless otherwise stated, were by Weiler and Strauss, Oxford. Evaporations, unless otherwise stated, were done *in vacuo* below 40°.

Starting material: gramicidin S hydrochloride. Specimens I and III (Synge, 1945; Consden, Gordon, Martin & Synge, 1947) were first sent for crystallographic study and used for preparing derivatives. Later preparations have all been crystallized from the same batch of crude material as specimen III. One such preparation was studied by countercurrent distribution by Dr Lyman C. Craig and colleagues

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and gave 90–93% of the preparation in the main peak (see Craig, Gregory & Barry, 1950; Fig. 5). Dr Craig kindly returned the main-peak fraction (tubes 19–31), and this highly purified product is here described as specimen IV.

Gramicidin S sulphate. The hydrochloride (60 mg.) was dissolved in 70% (v/v) aqueous ethanol, and Ag_2SO_4, equivalent to the chloride present, was added in the same solvent. The resulting precipitate was centrifuged off. The combined supernatant and washings, on slow evaporation at atmospheric pressure, yielded the crystals which were sent for study.

Gramicidin S hydriodide. The sulphate was prepared as above but was not crystallized. The solution was treated with BaI_2 (also dissolved in 70% v/v aqueous ethanol) equivalent to the sulphate present. The resulting precipitate was centrifuged off, and the supernatant on slow evaporation at atmospheric pressure yielded large crystals which were sent for study. A more recent *preparation* (analysed air-dry by Pascher, Bonn) gave: C, 49·9; H, 7·1; N (Dumas), 10·5; I, 15·3%. $C_{60}H_{92}O_{10}N_{12},2HI,4H_2O,3C_2H_5.OH$ requires C, 49·4; H, 7·5; N, 10·5; I, 15·8%.

Gramicidin S flavianate. Gramicidin S hydrochloride (30 mg.) was dissolved in 1 ml. of 70% (v/v) aqueous ethanol and treated with 45 mg. of flavianic acid (2:4-dinitro-1-naphthol-7-sulphonic acid) dissolved in 1 ml. of the same solvent. A crystalline precipitate formed immediately, and was filtered off, washed with the same solvent and recrystallized from warm aqueous ethanol. Yield, 35–45 mg. Different preparations have given very variable elementary analyses in the range 1–2 molecules of flavianic acid/decapeptide unit.

Gramicidin S chloroaurate. Gramicidin S hydrochloride (24 mg.) was dissolved in 70% (v/v) aqueous ethanol (2 ml.) and commercial 'brown gold chloride' (75 mg.) dissolved in the same solvent was added. Water was then added drop by drop to the resulting clear solution until turbidity resulted. The solution was then allowed to evaporate slowly, giving long yellow *needles* in bundles, from which the mother liquor was decanted. The needles were washed successively with 30% ethanol and water, and analysed air-dry. [Found: C, 41·9; H, 5·9; N (Kjeldahl), 9·0; Au (combustion residue), 19·3%.

$$C_{60}H_{92}O_{10}N_{12},1{\cdot}8HAuCl_4,0{\cdot}2HCl,2C_2H_5.OH$$

requires C, 41·4; H, 5·7; N, 9·1; Au, 19·2%.]

N-Acetylgramicidin S. Gramicidin S hydrochloride (40 mg.) was dissolved in pyridine (4 ml.) and acetic anhydride (1 ml.). After keeping for 24 hr. at room temp. the mixture was evaporated to dryness, and water was added to the resulting gummy product, in which it was insoluble. After repeated evaporation with additions of fresh portions of water, the residue was dissolved in 0·8 ml. of ethanol, water was added to turbidity, and evaporation was allowed to proceed slowly in air at room temp. Large crystals resulted, which were recrystallized in the same way. The behaviour of the solvent of crystallization is described in the main paper. Yield, after drying *in vacuo* at room temp. over H_2SO_4–soda lime, 40·5 mg. The *product* lost 4% of its wt. on further drying over P_2O_5 *in vacuo* at 100° and, after drying in this manner for analysis, had $[\alpha]_D^{22} - 313°$ in 70% (v/v) aqueous ethanol (*l*, 0·5; *c*, 1·5). [Found: C, 62·2; H, 7·7; N (Kjeldahl), 13·5%. $C_{64}H_{96}O_{12}N_{12},0{\cdot}5H_2O$ requires C, 62·2; H, 7·9; N, 13·6%.] No chloride was detected in the product by testing with $AgNO_3$ in aqueous ethanol, and amino N was absent (Van Slyke nitrous acid procedure, 30 min. reaction time).

N-Chloroacetylgramicidin S. Gramicidin S hydrochloride (100 mg.) was treated with water (20 ml.) and chloroform (B.P., 10 ml.). Na_2CO_3 (700 mg.) was added to the resulting mixture which was kept at 0°. Two portions of chloroacetyl chloride (each 0·13 ml.) were added with shaking at intervals of 5 min. and the mixture was shaken for a further 10 min. The chloroform layer was separated, and the aqueous layer extracted with two further 10 ml. portions of chloroform. On evaporation, the combined chloroform extracts yielded 90 mg. of clear brown gum, which was dissolved in 3 ml. of ethanol. Water was then added until crystallization started. This continued on allowing slow evaporation in air. The resulting large crystals were freed from mother liquor and washed by decantation successively with 30 and 10% (v/v) aqueous ethanol and then with several changes of water. The *product* (35 mg.) was analysed air-dry. [Found: C, 56·8; H, 7·5; N (Kjeldahl), 11·9; Cl (Carius), 5·3%.

$$C_{64}H_{94}O_{12}N_{12}Cl_2,3H_2O,1{\cdot}5C_2H_5.OH$$

requires C, 56·8; H, 7·7; N, 11·9; Cl, 5·0%.] No chloride was detected in the product with $AgNO_3$ in aqueous ethanol.

N-Iodoacetylgramicidin S. Crude *N*-chloroacetylgramicidin S (55 mg.) was dissolved in acetone (2 ml.) and NaI (90 mg.) dissolved in acetone (3 ml.) was added (cf. Conant, Kirner & Hussey, 1925). Immediate precipitation occurred, presumably of NaCl. The mixture was kept overnight. Water was then added and the precipitate dissolved; on addition of excess of water the derivative of gramicidin S was precipitated. The precipitate was washed thoroughly with water and dried *in vacuo* at room temp. over H_2SO_4–soda lime (yield, 56 mg.). The *product*, which was considerably less soluble in aqueous ethanol than *N*-chloroacetylgramicidin S, gave large crystals on slow evaporation in air at room temp. from solution in a mixture of water, ethanol and acetone. These were analysed air-dry. The iodine determination was kindly done by Mrs R. V. Pitt-Rivers of the National Institute for Medical Research. Chlorine was determined by subtraction of the expected weight of AgI from that of silver halide obtained in a Carius determination. (Found: C, 52·0; H, 6·5; N, 10·6; I, 12·8; Cl, 1·8%. $C_{64}H_{94}O_{12}N_{12}I_{1{\cdot}6}Cl_{0{\cdot}4},3H_2O,2C_2H_5.OH$ requires C, 51·6; H, 7·1; N, 10·6; I, 12·8; Cl, 0·9%.)

These analytical results indicate that substitution of chlorine by iodine probably did not exceed 80%. Accordingly in a later preparation, in which the highly purified specimen IV had served as starting material, the treatment with NaI in acetone was done under reflux for 30 min., following Friedman & Rutenburg (1950). However, the I/N ratio in the product was even lower (1·15 atoms of I/12 atoms of N; the I determination was kindly done by Miss B. W. Simpson of the Rowett Research Institute).

Iodine addition compound of N-iodoacetylgramicidin S. On mixing aqueous ethanolic solutions of iodoacetylgramicidin S and iodine an immediate yellow precipitate formed. This could be recrystallized by slow evaporation in air at room temp. from aqueous ethanol or aqueous dioxan to give large red crystals. The product has not been analysed. It was assumed to be a molecular compound with iodine rather than the product of any chemical reaction (cf. Cramer, 1954).

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APPENDIX 2

Possible Molecular Models for Gramicidin S and their Relationship to Present Ideas of Protein Structure

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The knowledge that has accumulated on the precise structure of amino acids and simple peptides makes it possible to build a number of models which may represent the detailed arrangement of the atoms in gramicidin S. These models must be judged in relation to the evidence described that the molecule behaves as a decapeptide in which two pentapeptide halves are related by a twofold axis of symmetry, and that the atoms tend to be concentrated in layers 4·8 Å apart in a direction at right angles to this twofold axis. In addition, the results of the studies made by Abbott & Ambrose (1953) of the infrared spectra of gramicidin S crystals have to be considered.

Abbott & Ambrose (1953) showed that, in the two main series of crystals examined, hexagonal *N*-acetylgramicidin S and orthorhombic gramicidin S hydrochloride, the infrared dichroism of the crystals is strong and is consistent with an orientation of the CO and NH bonds not far from parallel with the *c* axis, i.e. normal to the strongly reflecting planes of spacing 4·8 Å. Further, they favour a folded α-chain configuration on the evidence that the frequency of the C=O stretching mode in gramicidin S is 1646 cm.$^{-1}$, similar to that of α- and not β-keratin, evidence which is supported by observations on the dichroism of the absorption bands in the 4500–5000 cm.$^{-1}$ region. These observations suggest that we should concentrate our model-building on chain configurations which have been put forward for α-folded proteins, particularly those related to the Pauling, Corey & Branson (1951) α-helix and similar helices. But since there is some doubt about the reliability of conclusions based on small shifts in the frequencies of spectral lines (Ehrlich & Sutherland, 1953), we have thought it best to include also models based on extended β-chain configurations, while accepting the evidence of Abbott & Ambrose (1953) on the general orientation of the CO and NH groups in our crystals. In our model-building we have not considered the possibility put forward by Gavrilov and others (Silaev, Trefilova & Ioanisiani, 1951; Ioanisiani, Gavrilov & Plekhan, 1954; Akimova & Gavrilov, 1954; Reznichenko, 1954) that the peptide structure of gramicidin S may be modified chemically by internal condensation between certain carbonyl and imino groups. None of our data at present are sufficiently precise to exclude such a condensation, but it would be incompatible with most of the models we have so far built.

Within the framework of a simple peptide molecule the following models appear to us most worthy of serious consideration.

α_{11} *Ribbon.* Abbott & Ambrose (1953) have put forward a decapeptide model for gramicidin S based on the Huggins (1943) α_{11} ribbon configuration. Their particular version has not a strict twofold axis of symmetry; it is possible, however, to build a model on this configuration which has the required symmetry. It is illustrated in Fig. 1 and is composed of two four-residue α_{11} ribbons joined at the two ends by the remaining two residues. The rupture of the hydrogen-bonding system at the two proline residues caused by the eclipse of the NH groups allows freer rotation at these two points, and the two halves of the ring can be so oriented that they are related exactly by a twofold axis of symmetry passing through the centre of the ring.