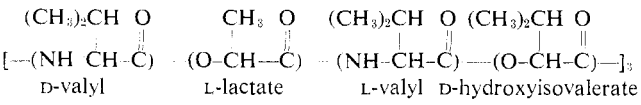
A Small-Angle X-Ray Diffraction Study of Valinomycin and Its Potassium Isothiocyanate Complex in Solution†

W. R. Krigbaum,* F. R. Kuegler,‡ and H. Oelschlaeger

ABSTRACT: Earlier conformational studies of valinomycin, both in solution and in the crystalline state, show that this molecule adopts different conformations in polar and nonpolar solvents, and that a third conformer is involved when a cation is complexed. Diffraction measurements are reported for valinomycin and its K^+ complex in two polar solvents, ethanol and butanol. Despite the experimental difficulties inherent in applying this technique to such a small molecule (*mol* wt 1110), the data clearly demonstrate that a conformational transition occurs when valinomycin in polar solvents binds a cation. The ratio of the experimental radii of gyration of the free and complexed forms, 1.30, is in reasonable agreement with the ratio, 1.26, calculated from models of the proposed structures. Values are also reported for the partial specific volume, $\bar{v}_2$, of valinomycin and its KSCN complex in a number of solvents. For valinomycin, $\bar{v}_2$ decreases by 10% as the polarity of the solvent is increased, while the complex shows a similar (but smaller) variation of $\bar{v}_2$ with solvent polarity.

Valinomycin is a cyclic dodecadepsipeptide having the sequence



This macrocyclic antibiotic has been shown to enhance the cation permeability of mitochondria (Pressman, 1965), erythrocytes (Tosteson *et al.*, 1967; Harris and Pressman, 1967), and lipid bilayers (Andreoli *et al.*, 1967; Mueller and Rudin, 1967) and, or more particular interest, to exhibit a selectivity for Rb^+ or K^+ in preference to Na^+ or other of the alkali metal ions. The ability of valinomycin to act as a mobile carrier to facilitate cation transport across membranes is intimately connected with the fact that it can adopt different molecular conformations, depending upon the polarity of the solvent and whether a cation is bound.

The conformation of valinomycin has been studied in solution by a variety of methods and also in the crystalline state. Ivanov *et al.* (1969) examined the molecule in solution by infrared (ir), optical rotatory dispersion (ORD), and dipole moment measurements, and these workers, as well as Haynes *et al.* (1969), Ohnishi and Urry (1969, 1970), and Urry and Ohnishi (1970), utilized nuclear magnetic resonance (nmr) for conformational studies. In addition, Pinkerton *et al.* (1969) presented a preliminary report of the crystal structure of the K^+ complex, while more recently Duax *et al.* (1972) reported the crystal structure of valinomycin as crystallized from a nonpolar solvent, isooctane. The results of these studies may be summarized as follows.

In the K^+ complex the molecule has the shape of a bracelet, with the six amide NH groups intramolecularly hydrogen bonded to the preceding amide carbonyls to form ten-membered rings. All six ester carbonyls point inward to give six-fold coordination about the unhydrated potassium ion. We will refer to this conformer as form C. The valyl HN-α-CH hydrogens are all gauche. Concerning the isopropyl side chains, the spin coupling constants indicate the α-CH-β-CH protons are trans in the valyl residues and gauche in the hydroxyisovaleryl residues. This molecular conformation is compatible with the result of the partial crystal-structure analysis reported for the potassium aurichloride complex by Pinkerton *et al.* (1969). Furthermore, the structure of the complex appears to be quite rigid, as evidenced by the observation that changes in the solvent polarity from ethanol to 3:1 heptane–ethanol do not affect the shape of the ORD curve (Ivanov *et al.*, 1969), and that the α-proton chemical shifts are nearly independent of temperature (Ohnishi and Urry, 1970). Haynes *et al.* (1969) showed that the nmr spectrum of the carefully dried complex in $CDCl_3$ was unaffected by the addition of 2 μl of H_2O/ml of solution.

The situation is more complicated for valinomycin containing no complexed cation. The preferred conformation in nonpolar solvents (which we will designate as form A) resembles the K^+ complex in having all six amide NH groups hydrogen bonded intramolecularly. However, the crystal structure (Duax *et al.*, 1972) indicates a different intramolecular hydrogen bonding scheme, with two of the six forming 13-membered rings. Also, two of the ester carbonyls are found to point toward the center, while four are directed outward (two parallel to the axis of the bracelet and two perpendicular to this axis). These features are not in agreement with the deductions based upon solution studies but, as Duax *et al.* (1972) have pointed out, this conformation is compatible with the observations of solution studies. Coupling constant measurements indicate the HN-α-CH protons are again gauche for the L-valyl residues, but are cis for the D-valyl residues. The isopropyl α-CH-β-CH protons are trans in the valyl residues and gauche in the hydroxyisovaleryl residues, as was the case for the C form.

ORD, ir, nmr, and the temperature dependence of the chemical shifts all indicate that the A form of valinomycin is in dynamic equilibrium with a second conformer, which we will refer to as form B, and that the latter is favored in more polar solvents such as methanol and dimethyl sulfoxide. This

† From the Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706. *Received July 19, 1972.* This work was supported by U. S. Public Health Service Grant HE 12157-0351 GP III from the National Institutes of Health.

‡ Present address: Oesterreichische Stickstoffwerke, Linz, Austria.

form has only the three D-valyl NH groups intramolecularly hydrogen bonded, and all six HN–α-CH protons cis. The isopropyl groups of the valyl residues are more nearly freely rotating in form B. The ORD curves (Ivanov *et al.*, 1968) give clear evidence of the equilibrium between the A and B forms. Ohnishi and Urry (1969) found that although only one type of valyl NH is hydrogen bonded intramolecularly, both exchange with deuterium at nearly the same rate in dimethyl sulfoxide solution. Hence, these authors propose two dish-shaped B conformers having nearly the same energy, and interconverting *via* the A form.

The cation selectivity of valinomycin appears to be related to the cavity size required to accept an unhydrated ion. Mayers and Urry (1972) have performed conformational energy calculations as a function of the cavity radius for a valinomycin analog having methyl side chains. They were able to demonstrate a lower conformational energy for the K^+ and Rb^+ complex, as compared to those of Na^+ and Cs^+, simply in terms of the cavity radius. Prestegard and Chan (1970) have pointed out that the hydration energies for the Na^+, K^+, Rb^+, and Cs^+ ions (97, 77, 70, and 63 kcal/mol, respectively) make it energetically less favorable to strip the hydration shell from a Na^+ in order to form the complex.

Concerning the mechanism of facilitated ion transport, we conclude that valinomycin will exist as the B conformer in the aqueous phase. The same form, or a minor variant of it, should also exist at the lipid–water membrane interface. This conformation is expected to have surface-active properties, since the shape is somewhat disk-like, with all of the hydrocarbon groups covering one face and the polar amide and carbonyl groups on the other face. Complexation of the K^+ leads to a transformation to the C form, which effectively surrounds the cation by a sheath having a hydrophobic external surface. The hydrocarbon region of the lipid bilayer could be easily traversed by this form, and the cation could be expelled at the other interface by the reverse of the previous conformational transformation. This mechanism does not specify the mode of anion transport; however, it does emphasize the point that the ability of valinomycin to adopt different conformations plays a vital role in its facilitation of ion transport across membranes.

This paper reports an investigation of free valinomycin and its K^+ complex in solution in ethanol and butanol by small-angle X-ray diffraction. Since this method provides information concerning the overall molecular size, it provides independent evidence about the existence and equilibrium concentrations of the three conformers described above. In addition, we report values of the partial specific volume for valinomycin and the complex in several solvents.

Experimental Section

Materials and Apparatus. Valinomycin was obtained from Calbiochem. The complex was formed by mixing stoichiometric amounts of valinomycin and KSCN in ethanol solution, evaporating to dryness, and recrystallizing from hexane or toluene.

The partial specific volumes were calculated from density measurements made at 21° with the Anton Paar DMA-02c precision density meter which has been described by Kratky *et al.* (1969). This instrument is capable of measuring densities with a precision of a few parts in the sixth decimal place using less than 1 ml of solution.

Diffraction measurements were performed using a medium resolution Kratky camera with Cu Kα radiation and a proportional counter. The distance from the sample to the registration plane was 20.5 cm and the widths of the entrance and detector slits, 250 and 300 μ, respectively, gave a resolution of ~300 Å. Valinomycin, having a molecular weight of only 1110, is significantly smaller than most of the molecules which have been studied by low-angle methods. Since the excess intensity is proportional to the molecular weight, special care was necessary in measuring the solution and solvent curves. During one angular scan 10^4 counts were accumulated at each of 50 angles. Each solution was scanned at least twice and the solvents were scanned several times, and these data were averaged and smoothed before subtraction to obtain the smeared difference scattering curves. Slit desmearing was performed using a modification of the computer program developed by Heine and Roppert (1962). The intensities were placed on an absolute scale using a Lupolen standard calibrated by Kratky and coworkers, as described by Kratky *et al.* (1966) and Pilz and Kratky (1967).

TABLE I: Partial Specific Volumes.

Solvent	$\bar{v}_2$ (cm³/g) Val	$\bar{v}_2$ (cm³/g) K·Val⁺SCN⁻
C_6H_{12}	0.93	
CS_2	0.92	0.89
CCl_4	0.92	0.88
C_4H_9OH	0.88	0.84
$(CH_3)_2SO$		0.84
$CH_3(CO)CH_3$	0.85	
C_2H_5OH	0.85 ± 0.02	0.84 ± 0.02
CH_3OH	0.84	

Partial Specific Volume. The partial specific volume, $\bar{v}_2$, of the solute is given by

$$\bar{v}_2 = \frac{1}{\rho_1}\left(1 - \frac{\rho - \rho_1}{c}\right) \quad (1)$$

where ρ_1 and ρ are the densities (grams/milliliter) of solvent and solution, respectively, and c is the concentration of the solution in grams/milliliter. As a general rule, the densities must be measured to the sixth decimal place in order to determine $\bar{v}_2$ to about 0.1%. Although the instrument is thermostated, slight variation of the sample temperature during measurement is one source of error, and will be more troublesome with solvents having larger coefficients of cubical expansion. The partial specific volume values are also affected by errors in determining the solution concentration. We encountered a lack of reproducibility for the more polar solvents, methanol and ethanol, which we believe is due to the uptake of small (but variable) amounts of water from the air during preparation of the solution and sample injection.

Values obtained for the partial specific volume of valinomycin and its KSCN complex in several solvents are given in Table I. In most cases in which duplicate determinations were made, the $\bar{v}_2$ values agreed to within ±0.01 cm³/g. Ethanol is an exception, and the error limits for this solvent are given in Table I. The partial molar volumes for valinomycin range from 1030 to 930 cm³/mol, corresponding to a reduction of approximately 10% in the partial molar volume on going from the A form in hexane to the B form in methanol.

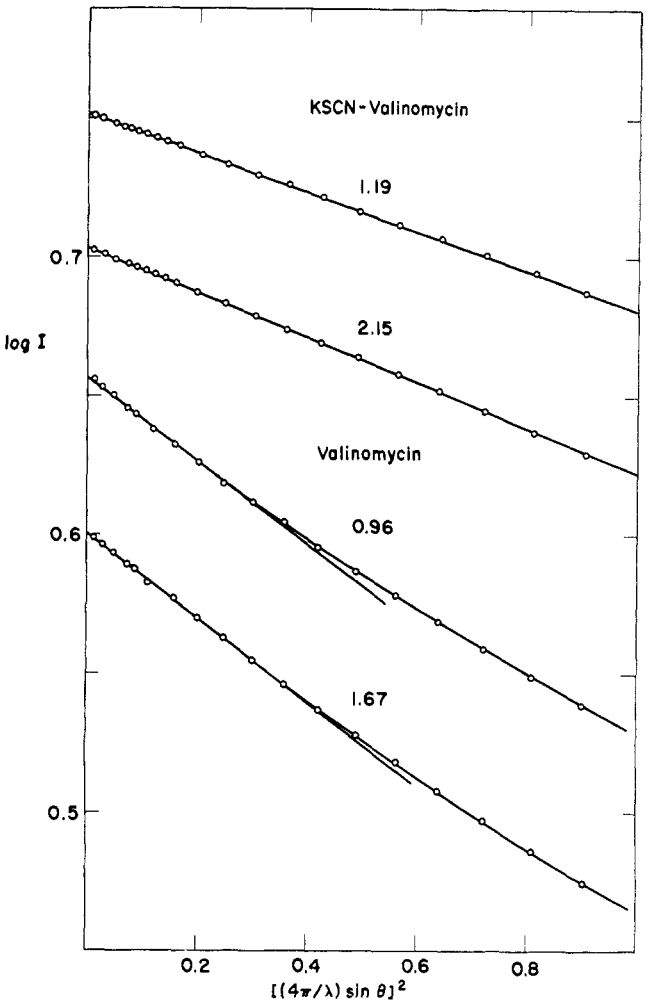


FIGURE 1. Guinier plot of valinomycin data at smaller angles (lower two curves) and its KSCN complex (upper two curves) in butanol.

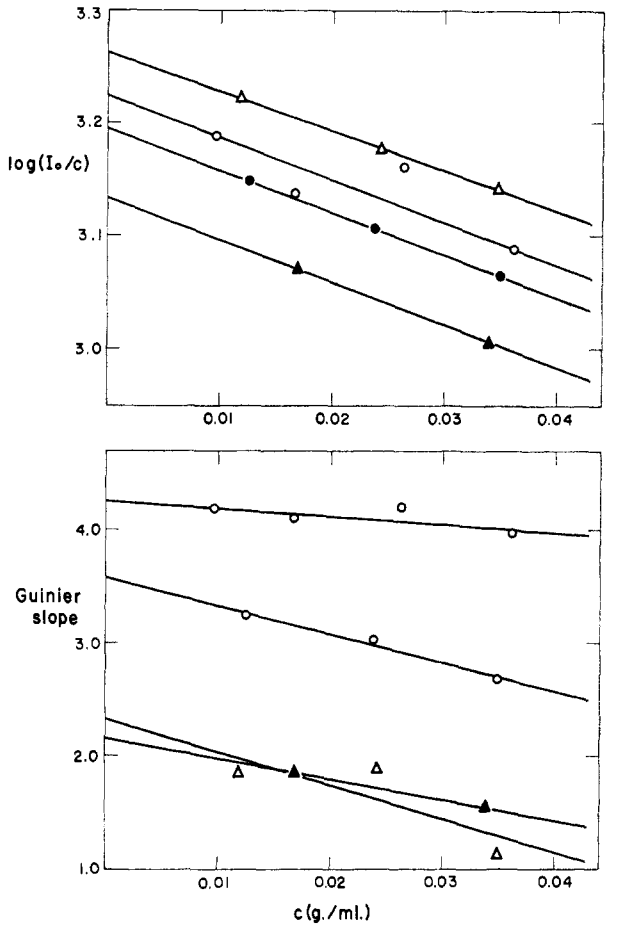


FIGURE 2. Extrapolation of the concentration dependence of the intercepts (above) and slopes (below) of the Guinier plots for valinomycin (circles) and its KSCN complex (triangles) in ethanol (●,▲) and butanol (○,△).

Radius of Gyration and Molecular Weight. Following the treatment of Guinier (1939), the intensity scattered at sufficiently small angles by a collection of N identical particles of arbitrary shape may be adequately represented by the relation

$$I(h) = Nn^2 i_e \exp(-R^2h^2/3) \qquad (2)$$

where n is the number of electrons per particle, i_e is the Thomson scattering factor for one electron at zero angle and R is the electronic radius of gyration of the particle. The reciprocal lattice vector **h** has the magnitude $(4\pi/\lambda) \sin \theta$, where λ is the wavelength of the radiation and θ is the Bragg angle. According to 2, the radius of gyration, R, can be evaluated from the initial slope of a plot of ln $I(h)$ *vs.* h^2, which is referred to as a Guinier plot. The molecular weight, M, can be determined from I_0, the antilogarithm of the intercept at zero scattering angle, using the following relation

$$M = \frac{1}{i_e N} \frac{I_0}{P_0} \frac{a^2}{Dc(\Delta z)^2} \qquad (3)$$

where i_e is the Thomson scattering factor for one electron, N is the Avogadro number, P_0 is the intensity of the primary beam per centimeter of length in the plane of registration, D is the sample thickness in centimeters, and c is the solution concentration in grams per milliliter. The difference, Δz, in moles of electrons per gram, is given by $(z_2 - \bar{v}_2 d_1)$, where z_2 is the number of moles of electrons per gram of solute and d_1 is the electron density of the solvent in moles per milliliter.

Figure 1 illustrates an example of a Guinier plot of some of the data collected at the smaller angles for valinomycin (lower two curves) and its KSCN complex (upper two curves) in butanol. Concentrations are indicated in units of 10^2c (*i.e.*, in the units grams/100 ml). One sees that the innermost portions of the curves are reasonably linear, and that the initial slopes are significantly larger for valinomycin than for the K^+ complex. Both the intercepts (as I_0/c) and the initial slopes of the Guinier plots must be extrapolated to zero concentration in order to eliminate interparticle interference effects. These extrapolations are shown for all samples in Figure 2. Values so obtained for the molecular weight and radius of gyration are collected in Table II.

TABLE II: Molecular Weight and Radius of Gyration.

	Val		K·Val⁺SCN⁻	
Solvent	M	R	M	R
Ethanol	1060	5.0 ± 0.3	920	3.9 ± 0.2
Butanol	1050	5.4 ± 0.3	940	4.4 ± 0.4

The molecular weight values determined from the experimental data for valinomycin in ethanol and butanol, 1060 and 1050, are in quite good agreement with that of the known structure, 1110. If we regard the KSCN complex as two separate ions, the weight-average molecular weight would be 1097. Hence, the molecular weights obtained for the KSCN complex in these same two solvents, 920 and 940, are two low by approximately 15%. These discrepancies must arise from errors in $\bar{v}_2$ and in the absolute value of the intensity I_0. It is clear, however, that neither valinomycin nor its complex with K^+ shows a tendency toward aggregation in these solvents.

Values for the radius of gyration of valinomycin and its KSCN complex are given in columns three and five of Table II. As shown in Figure 2, there is considerable uncertainty in the extrapolation of the slopes to zero concentration for the complex in butanol (open triangles). Despite this uncertainty, the radius of gyration clearly decreases significantly when the complex is formed from the B conformer of valinomycin in polar solvents. We also attempted to obtain data for the A conformer in hexane, but these measurements were terminated due to the low solubility of valinomycin in this solvent.

In order to provide a more quantitative comparison of the experimental radii of gyration with the structures of the proposed conformers, models of the three forms were constructed using Prentice-Hall framework molecular models. The description of Ivanov *et al.* (1968) was followed in constructing the models of conformers B and C, while the structure of Duax *et al.* (1972) formed the basis for model A. The coordinates of all nonhydrogen atoms were measured for each model, and the radius of gyration, R_0 was calculated for each by computer using the relation

$$R_0^2 = \sum_j f_j[(x_j - x_0)^2 + (y_j - y_0)^2 + (z_j - z_0)^2]/\sum_j f_j \quad (4)$$

where f_j is the atomic scattering factor of atom j located at (x_j, y_j, z_j), while (x_0, y_0, z_0) are the coordinates of the electronic center of gravity, *i.e.*, $x_0 = \Sigma_j f_j x_j / \Sigma_j f_j$, etc. The results obtained were R_0 = 5.2 Å for form A (in nonpolar solvents), 6.3 Å for form B (in polar solvents), and 5.0 Å for the K^+ complex in form C (ignoring the SCN^- ion).

Since we expect valinomycin to be predominately in the B form in both ethanol and butanol, there is a significant disparity between the calculated and experimental values. The calculated R_0 for the B form, 6.3 Å, exceeds the average of the experimental R values, 5.2 Å, by 22%. We prefer to give extra weight to the more reliable value obtained for the radius of gyration of the K^+ complex in ethanol, obtaining R = 4.0 Å for the complex. The calculated R_0 value for the C conformer, 5.0 Å, is about 25% larger than the experimental result. Thus, the experimental radii of gyration values for both the A and C forms are smaller than those calculated from the models, and in each case the difference exceeds the anticipated combined errors, about ±10%. It should be pointed out that the experimental R values are obtained directly from the slope of the Guinier plot, whereas the determination of M involves an absolute calibration of intensity and a separate measurement of $\bar{v}_2$. Two possible explanations for these discrepancies can be advanced. The R_0 values calculated according to eq 4 represent the radius of gyration of the molecule *in vacuo*, not in the solvent. Secondly, for molecules as small as valinomycin the solvent can no longer be treated as a continuous medium of uniform electron density.

Discussion

Despite their limitations, the small-angle data do convincingly demonstrate that there is a significant conformational transformation on going from the B conformer of valinomycin in polar solvents to the C form of the K^+ complex. The ratio of the averaged experimental values cited above, R = 5.2 and 4.0 Å, is 1.30. This ratio stands in fair agreement with that of the calculated values for the B and C conformers, which is 1.26. The partial specific volume of valinomycin in ethanol and butanol, 0.85 and 0.88 cm^3/g, would imply that some fraction of the molecules have the A conformation in in the latter solvent. Hence, the somewhat larger R value in butanol is contrary to expectation; however, the difference in the R values in these two solvents is within the combined experimental errors.

The partial specific volumes given for valinomycin in column two of Table I reveal a decrease of about 10% on going from the A form in hexane to the B form in more polar solvents. However, the radius of gyration as calculated from the models is approximately 20% larger for the B form than that of the A form. These apparently divergent trends can be explained if the more compact A conformer contains a cavity which is not occupied by solvent. The same reasoning can be used to explain the decrease in $\bar{v}_2$ on going from the A form of valinomycin in CS_2 or CCl_4 to the complex. The smaller (but still significant) trend of $\bar{v}_2$ for the complex to decrease as the polarity of the solvent is increased may be due to a change in the structure of the solvent in the immediate vicinity of the cation or anion.

References

Audreoli, T. E., Tieffenberg, M., and Tosteson, D. C. (1967), *J. Gen. Physiol. 50*, 2527.

Duax, W. L., Hauptman, H., Weeks, C. M., and Norton, D. A. (1972), *Science 176*, 911.

Guinier, A. (1939), *Ann. Phys. (Leipzig) 12*, 161.

Harris, E. J., and Pressman, B. C. (1967), *Nature (London) 216*, 918.

Haynes, D. H., Kowalsky, A., and Pressman, B. C. (1969), *J. Biol. Chem. 244*, 502.

Heine, S., and Roppert, J. (1962), *Acta Phys. Austr. 15*, 1948.

Ivanov, V. T., Laine, I. A., Abdulaev, N. D., Senyavina, L. B., Popov, E. M., Ovchinnikov, Yu. A., and Shemyakin, M. M. (1968), *Biochem. Biophys. Res. Commun. 34*, 803.

Kratky, O., Leopold, H., and Stabinger, H. (1969), *Z. Angew. Phys. 27*, 273.

Kratky, O., Pilz, I., and Schmitz, P. J. (1966), *J. Colloid Interface Sci. 21*, 24.

Mayers, D. F., and Urry, D. W. (1972), *J. Amer. Chem. Soc. 94*, 77.

Mueller, P., and Rudin, D. O. (1967), *Biochem. Biophys. Res. Commun. 26*, 398.

Ohnishi, M., and Urry, D. W. (1969), *Biochem. Biophys. Res. Commun. 36*, 194.

Ohnishi, M., and Urry, D. W. (1970), *Science 168*, 1091.

Pilz, I., and Kratky, O. (1967), *J. Colloid Interface Sci. 24*, 211.

Pinkerton, M., Steinrauf, L. K., and Dawkins, P. (1969), *Biochem. Biophys. Res. Commun. 35*, 512.

Pressman, B. C. (1965), *Proc. Nat. Acad. Sci. U. S. 53*, 1076.

Prestegard, J. H., and Chan, S. I. (1970), *J. Amer. Chem. Soc. 92*, 14.

Tosteson, D. C., Cook, P. C., Andreoli, T. E., and Tieffenberg, M. (1967), *J. Gen. Physiol. 50*, 2513.
Urry, D. W., and Ohnishi, M. (1970), *in* Spectroscopic Approaches to Biomolecular Conformation, Urry, D. W., Ed., Chicago, Ill., American Medical Association Press, p 263.

Sphingolipids of Mushrooms†

Benjamin Weiss* and Richard L. Stiller

ABSTRACT: Ceramides and cerebrosides isolated from cultivated and wild mushrooms together comprise about 0.3–0.7% of the cell dry weight which is approximately 5% of the wet weight. The long-chain bases obtained from these sphingolipid fractions are (1) 4-hydroxyheptadecasphinganine, (2) 16-methyl-4-hydroxyheptadecasphinganine, (3) 4-hydroxyoctadecasphinganine, (4) 17-methyl-4-hydroxyoctadecasphinganine, (5) 18-methyl-4-hydroxynonadecasphinganine, (6) 19-methyl-4-hydroxyeicosasphinganine, (7) 20-methyl-4-hydroxyheneicosasphinganine, (8) 20-methyl-4-hydroxyheneicosa-X_1-sphingenine, (9) 20-methyl-4-hydroxyheneicosa-X_2-sphingenine, (10) 4-hydroxydocosasphinganine, (11) 4-hydroxydocosa-X_1-sphingenine, and (12) 4-hydroxydocosa-X_2-sphingenine. The occurrence in biological materials of bases 5, 7, 8, 9, 10, 11, and 12 has not been previously reported. The major normal fatty acids are palmitate, stearate, octadecenoate and octadecdienoate, whereas the predominant hydroxy fatty acids are hydroxytetradecanoate, hydroxypentadecanoate, hydroxypalmitate, and hydroxystearate. The hexose from the cerebrosides of the wild mushrooms is glucose.

The order Eumycophyta, true fungi, consists of three classes of increasing morphological complexity, Phycomycetes, Ascomycetes, and Basidiomycetes; included in the last class are mushrooms. Although considerable work has been done on the structural determination of the toxic peptides and central nervous system (CNS) active principles, which act at postganglionic parasympathetic effector sites, of poisonous mushrooms, genus *Amanita*, little is known of their lipid composition. Sphingolipids are considered to be ubiquitous constituents of cell membranes and it was therefore of interest to know of the presence or absence of these compounds in cells which elaborate substances active on the CNS. Since the CNS-active compounds are substituted tetrahydrofurans and isoxazole derivatives (Wieland, 1968; Simons, 1971) which would be soluble in lipids, it was thought that the knowledge obtained might assist in understanding the mechanism of transport of these substance across cell membranes as well as yielding information concerning the nature of receptor sites. It was also hoped that this study might disclose the presence of novel long-chain bases[1] which would serve as models in organic syntheses in future studies for the search of metabolic antagonists in the treatment of diseases of the CNS. Therefore, we investigated the sphingolipid content of several species of *Amanita* along with the cultivated edible mushroom, *Agaricus bisporus*, and attempted to characterize each component of these complex lipids.

Methods and Materials

Infrared spectroscopy on KBr disks and column chromatography (cc) were performed as described previously (Weiss and Stiller, 1970a,b). All reagent grade solvents were distilled and the column packings, silicic acid (Merck or Mallinckrodt), and Florisil (100 mesh) were washed several times by suspension in methanol and centrifugation; after additional washing of the Florisil with water, the adsorbents were dried at 115°. Column fractions were analyzed for long-chain bases, serine, ethanolamine (Meltzer, unpublished results), hexose (Radin *et al.*, 1955), phosphate (Marinetti, 1962), and ester groups (Meltzer, 1958).

Analytical thin-layer chromatography (tlc) plates (20 × 20 cm) containing silica gel G (0.2 mm thick) were prepared (Weiss and Stiller, 1965) and preparative plates (2 mm thick) were purchased from Brinkmann Instrument Co. Ascending chromatography with solvent systems chloroform–methanol–water (100:42:6) (Carter and Hirschberg, 1968) and chloroform–methanol–concentrated NH_4OH (65:35:5) (Rouser *et al.*, 1965) were used to examine the purity of fractions obtained by cc. Preparative tlc with the former solvent system was employed when further purification was necessary. Components were detected after separation by a variety of reagents (Mangold, 1969), preferably iodine vapor, and removed by successive suspension of the adsorbent-containing band in warm cholroform–methanol (2:1) and methanol with centrifugation.

Gas chromatography (gc) was done on a Perkin-Elmer 881 equipped with flame ionization detector. A 6.0 ft × 0.25 in. i.d. glass column packed with 3% SE-30 on gas chrom Q was used for the determination of long-chain bases, long-chain alcohols, fatty acids, and simple sugars. The nitrogen flow rate was 15 ml/sec and the injector and detector temperatures were 270°. Column temperatures of 195 and 240° were employed for determining bases of chain length up to C-18 and

† From the Division of Neuroscience, New York State Psychiatric Institute, and Department of Biochemistry, College of Physicians and Surgeons Columbia University, New York, New York 10032. *Received May 5, 1972.* This study was supported in part by Public Health Service Research Grant NB 06300-05 from the National Institute of Neurological Diseases and Stroke.

[1] The nomenclature used is that recommended by IUPAC-IUB Commission on Biochemical Nomenclature. In all cases, the configurations at carbon atoms 2, 3, and 4 are unknown.