bon site was observed.

The presence of the weakly enriched peaks resulting from the single labeled acetates was especially interesting. They could be interpreted as the consequence of an indirect incorporation of the precursor through propionate (C-7, C-8, C-19)[3] and butyrate (C-6, C-18 and C-5, C-17). In order to test the hypothesis of the butyrate origin of the indicated four carbons, the simultaneous addition of cold butyrate and [2-^{13}C]acetate was investigated. The resulting ^{13}C NMR spectrum of leucomycin A_3 obtained in this experiment showed an important decrease of the relative intensity of the two signals due to C-6 and C-18 (see Table II) reflecting the partial inhibition of the indirect incorporation of butyrate.

Finally [1-^{13}C]butyrate was added to the culture. The resulting ^{13}C NMR spectrum of Ib indicated strong enrichment, as expected, only for C-5 while weak incorporation was observed under these conditions for all the six carbons originating from the carboxyl carbon of acetate. In analogy with the biosynthetic incorporation of butyrate into the antibiotic X-537 A[4] this result is interpreted as the degradation of butyrate into acetate by β-oxidation.

Our results are clearly in contrast to the conclusions of previous studies[1] in which an acetate origin was assigned to C-3 and C-4. However, the biogenetic origin of these two carbons was not elucidated in the present study since no incorporation was evident at these sites by the precursors used.

Based on the results presented above in Tables I and II we propose the biosynthetic scheme shown in Figure 1 (Ib) for the formation of the aglycone of leucomycin A_3.

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Silver Atom–Ethylene Molecular Complex. Matrix Isolation Electron Spin Resonance Study

Sir:

Numerous examples of molecular complexes between olefin molecules and univalent copper or silver cations are known.[1] The formation of a complex between a neutral atom of these elements and an olefin molecule has not been detected, however. We report, in this communication, the electron spin resonance (ESR) spectra of Ag atom–ethylene complex generated within a rare-gas matrix at near liquid helium temperature.

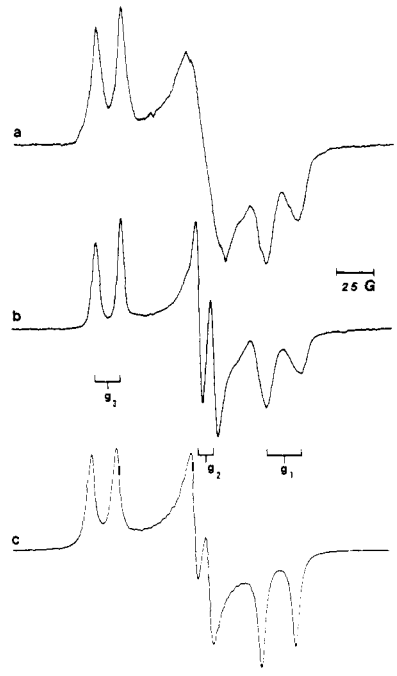


Figure 1. ESR spectra of (a) Ag atom–ethylene (C_2H_4) molecular complexes, (b) Ag atom–ethylene (C_2D_4) molecular complexes, and (c) computer-simulated based upon the parameters given in the text.

The design of the liquid helium cryostat-ESR spectrometer assembly that would permit trapping of high temperature vapor phase species in a rare-gas matrix and observation by ESR of the resulting matrix has been described previously.[2] In the present series of experiments Ag atoms were vaporized from a resistively heated tantalum cell and trapped in a neon matrix together with ethylene molecules introduced through a separate gaseous sample inlet. The composition of the matrix is estimated to be roughly 1000:10:1 for neon atoms, ethylene molecules, and Ag atoms, respectively. The frequency of the spectrometer locked to the sample cavity was 9.410 GHz.

The ESR spectrum of Ag atoms ($4d^{10}5s^1$) isolated in a neon matrix is known.[3] It consists of two sets of sharp, isotropic doublets with the spacings of ~620 and 720 G attributed to ^{107}Ag (natural abundance = 51%, $I = \frac{1}{2}$, $\mu = -0.1130\ \beta_N$), and ^{109}Ag (natural abundance = 49%, $I = \frac{1}{2}$, $\mu = -0.1299\ \beta_N$), respectively. The matrix containing Ag atoms alone appeared white. When trapped together with ethylene, the matrix became red, the ESR signals due to Ag atoms were weak, and a new signal with the overall spread of ~150 G appeared centered about the position corresponding to $g = 2.00$. The new spectrum is assigned to Ag atom–ethylene molecular complexes randomly oriented within the matrix.

The new spectra obtained when normal ethylene (C_2H_4) and perdeuterioethylene (C_2D_4) were used, respectively, are compared in Figures 1a and 1b. The deuteration clearly improves the resolution of the spectrum but does not alter the overall spectral pattern. The latter must hence be attributed to the anisotropic g tensor of the complex and its hyperfine coupling tensor to the Ag nucleus. The following parameters were assessed from the observed spectra.

$$g_1 = 1.972 \pm 0.001$$

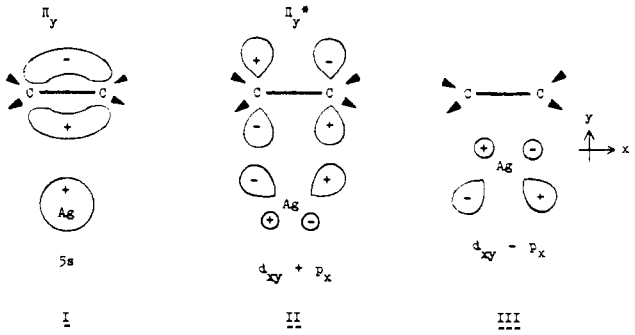
$$g_2 = 2.002 \pm 0.001$$

$$g_3 = 2.042 \pm 0.001$$

$$|A_1|_{Ag} = 22.5 \pm 0.5 \text{ G}$$
$$|A_2|_{Ag} = 10.0 \pm 0.5 \text{ G}$$
$$|A_3|_{Ag} = 16.5 \pm 0.5 \text{ G}$$
$$|A|_H \leq 1.0 \text{ G}$$

The difference between the coupling constants to the two Ag nuclei could not be resolved. The upper limit of the coupling constant to the protons was estimated from the difference in the apparent line widths of the spectra of the deuterated and nondeuterated species. The computer-simulated spectrum (Figure 1c)[4] based upon the g tensor and the Ag coupling tensor given above and a Lorentzian line shape with the line width of 4 G is in excellent agreement with the observed result (Figure 1b).

The interaction leading to the formation of olefin-Ag^+ cation is thought to involve the π orbitals of the double bond and hybrid orbitals of the cation located equidistant from the two unsaturated carbon atoms.[5] Two dative bonds are thought to be formed, a σ-type bond resulting from migration of electrons from the filled bonding π orbital of the olefin molecule into the vacant 5s orbital of the cation (I), and a π-type dative bond resulting from migration into the vacant antibonding π orbital from a filled d-p hybrid orbital of the cation (II).



In the schematic presentation of the orbitals given above, the d-p hybrid orbital involved in the dative π bond (II) should be given as $\Phi = a(4d_{xy}) + b(5p_x)$. We propose that the structural feature of the Ag atom-ethylene observed here is exactly the same as that of olefin-Ag^+ cation and its unpaired electron is located in the essentially nonbonding hybrid orbital (III) given by $\Phi^* = b(4d_{xy}) - a(5p_x)$. The extremely small hyperfine coupling constants to the Ag and the proton nuclei are thus accounted. The positive g shift ($g_3 = 2.042$) must be caused by the d_{xy} part of the orbital when the magnetic field is perpendicular to the xy plane. The negative g shift ($g_1 = 1.972$) could result from the p_x part of the orbital when the field is parallel to the y axis.

We have also succeeded in generating and observing the ESR spectra of Cu atom-ethylene complex. Gold atoms were found not to complex with ethylene. The detailed analyses of the spectra presented here and those of Cu atom-C_2H_4 will be reported soon.

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Iron to Sulfur Bonding in Cytochrome *c* Studied by X-Ray Photoelectron Spectroscopy[1]

Sir:

A classical problem in the field of metalloproteins is the identification of the groups to which the metal is liganded. Direct chemical evidence is often misleading; positive identification usually requires the elucidation of the three-dimensional structure of the protein using x-ray diffraction methods. The development of alternative methods that are simpler and faster to carry out could therefore have wide-ranging implications.

We wish to report here studies which indicate that x-ray photoelectron spectroscopy (XPS) may be a valuable tool in this context. We have chosen horse heart cytochrome *c*,[2] a heme protein of 12386 molecular weight, to study sulfur coordination to iron.

X-ray diffraction analysis indicates that the axial coordination positions of the heme iron are occupied by an imidazol nitrogen of histidine and a thioether sulfur of methionine.[3] There are four sulfur atoms per molecule of cytochrome *c*. All four are effectively thioethers. Two arise from methionines and two arise from cysteines which are covalently bound to the heme across the double bonds of the vinyl groups.[2]

Therefore three sulfurs in the protein have similar chemical environments while the sulfur coordinated to the heme iron would be expected to have a decreased electron density as a result of electron donation to the metal ion. Since core electron binding energies are a function of the chemical environment of the atom, XPS should be useful in differentiating between these two types of sulfurs and thus provide a means of determining the integrity of the Fe-S bond.

The sulfur 2p electron energy region, 160-170 eV, was examined in cytochrome *c*, the cyano derivative of cytochrome *c*, as well as in the lyophilized protein. No other element in these proteins has a core electron binding energy in the sulfur 2p region.

Spectra were obtained from a Hewlett-Packard 5950A ESCA spectrometer. An electron gun was employed in this work in order to eliminate charging effects. The Al Kα x ray line served as the exciting radiation. The large aliphatic C1s peak was used as the reference signal with a binding energy of 284.0 eV.

Oxidized cytochrome *c* in the solid form was obtained from the Sigma Chemical Co. A solution of the protein was prepared at a concentration of 10 mg/ml in 0.01 *M* phosphate buffer (pH 6.9). To ensure complete oxidation, $K_3Fe(CN)_6$ was added to the solution. For the cyano derivative, a sufficient amount of a neutralized KCN solution was added to give a final CN^- concentration of 0.08 *M*. The absorption spectra of these samples were measured before and after XPS analysis on a Cary 14 recording spectrometer.

A few drops of the sample solution were deposited onto gold platens and then mounted onto a cyrogenic probe. The samples were then inserted into the sample preparation chamber of the spectrometer and frozen under a dry nitrogen atmosphere to 173 K. This temperature was previously determined to be appropriate for this experiment since it allowed for the retention of a layer of ice over the protein thus preventing lyophilization. The ice layer was monitored by scanning the C1s and O1s electron region and observing the rate of growth of these signals. At temperatures higher than 175 K the C1s signal increases rapidly in intensity while at 175 K its rate of growth is markedly reduced indicating that the protein surface is covered by a layer of ice. In addition, the O1s peak increases much more rapidly at 175 K than at higher temperatures. Temperatures lower than 170 K re-