

thermal neutrons before being etched, we must subtract the reactor-induced fission tracks, inferred from tracks in the adjacent Lexan sheet to be  $(6.0 \pm 0.8) \times 10^6/\text{cm}^2$ . From this density and the neutron dose we calculate (13, 16) a U concentration in the whitlockite of  $19.4 \pm 1.5$  ppm.

The cosmic-ray neutron flux is a function of the exposure history of the whitlockite. The breccia 14321 as a whole has most probably been irradiated on or near the lunar surface for the past  $25 \times 10^6$  years. Lugmair and Marti (17) find an upper limit on the integrated thermal neutron dose,  $5.6 \times 10^{14}$  neutron/cm<sup>2</sup>, consistent with such an exposure history. If we assume that the whitlockite has been irradiated only in situ, the neutron-induced fission track density would be  $< 3.5 \times 10^6/\text{cm}^2$  and the total spontaneous fission contribution (Table 1) is  $(8.54 \pm 0.73) \times 10^7/\text{cm}^2$ .

According to an alternative explanation, the whitlockite may have been irradiated  $\sim 3.95 \times 10^9$  years ago prior to its incorporation into the breccia. The neutron flux would be unknown, and any number of excess tracks could have been produced. However, our observations of crushed feldspar crystals from rock 14321 with a high-energy electron microscope (650 keV) have provided no evidence for the retention of etchable tracks from an irradiation prior to breccia formation. Dran *et al.* (18) have observed etched sections with a scanning electron microscope and likewise have concluded that existing tracks were erased during breccia formation. We therefore believe that it is extremely unlikely that the excess tracks resulted from neutron-induced fission, either recently or  $3.95 \times 10^9$  years ago.

One further possibility is that, since rock 14321 is heterogeneous, the neutron exposure limits might apply only to those sections of the breccia that have been less irradiated than the whitlockite. This possibility, though unlikely, cannot be ruled out because it postulates a special history for one crystal, which is too small to permit its neutron exposure to be measured by the technique of Lugmair and Marti (17).

Because  $^{244}\text{Pu}$  and  $^{238}\text{U}$  fission tracks are indistinguishable in appearance, we cannot determine solely from fission tracks both the track-retention age  $T$  of the whitlockite and the enrichment factor  $f$ . The simplest and to us the

likeliest possibility is that the whitlockite is contemporaneous with other minerals in the breccia, and we therefore equate  $T$  with the age of an igneous clast in rock 14321, determined by the Rb-Sr method to be  $3.95 \pm 0.04 \times 10^9$  years old (19). The spontaneous fission contribution of 19.4 ppm of U in  $3.95 \times 10^9$  years is  $(5.66 \pm 0.42) \times 10^7/\text{cm}^2$ , and we ascribe the remaining tracks,  $(2.88 \pm 0.82) \times 10^7/\text{cm}^2$ , to  $^{244}\text{Pu}$ . The ratio  $\rho_{\text{Pu}}/\rho_{\text{U}}$  is then  $0.51 \pm 0.15$  (horizontal shaded band in Fig. 1). From Fig. 1 we see that the enrichment factor would then be  $f \approx 1.28 \pm 0.4$  if the age is  $3.95 \pm 0.04 \times 10^9$  years. The relative contributions of  $^{244}\text{Pu}$  and  $^{238}\text{U}$  spontaneous fission are thus consistent with the value for  $(\text{Pu}/\text{U})_0$  of 0.013 at  $T_0 = 4.58 \times 10^9$  years, an age of  $3.95 \times 10^9$  years for the component minerals within the breccia, and the geochemically similar behavior of Pu and U in the whitlockite.

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## Valinomycin Crystal Structure Determination by Direct Methods

**Abstract.** *The conformation of an uncomplexed form of the antibiotic valinomycin ( $\text{C}_{54}\text{N}_6\text{O}_{18}\text{H}_{90}$ ) has been determined by direct methods including a novel technique for strong enantiomorph discrimination via the calculation and systematic analysis of cosine invariants of a special type. The intramolecular hydrogen bonding scheme and the isopropyl group stereochemistry of uncomplexed valinomycin are compatible with interpretations of spectral measurements for the complexed and uncomplexed molecule in solution but are different from any previously proposed structure. The simple conformational change of a hydrogen bond shift, which could be induced by the process of potassium ion complexing, transforms the uncomplexed into the complexed structure.*

The antibiotic valinomycin has been shown to affect the energy-linked accumulation of alkali ions by mitochondria (1). The structure of uncomplexed valinomycin (Fig. 1a) was found to be more elongated than that of the doughnut-shaped  $\text{K}^+$  complex (Fig. 1b) (2, 3). The conformational difference between the complexed and the uncomplexed forms of valinomycin involves a change in intramolecular hydrogen bonding. Two of the six intramolecular hydrogen bonds in the uncomplexed

molecule are of a type not previously suspected. The hydrogen bonding depicted in Fig. 2a is that observed in the  $\text{K}^+$  complex. The compatibility of this hydrogen bond configuration with the interpretation of spectra of the complexed and uncomplexed forms of valinomycin in nonpolar solutions suggested to various authors (4, 5) that in nonpolar solvents the two forms have at least this hydrogen bonding structure in common. On the basis of their interpretation of spectral data, Ivanov

*et al.* (4) conclude that the conformation shown in Fig. 2a is the only one in which all six amide groupings can participate in mutual hydrogen bonding. There remained the question of wrapping the structure of Fig. 2a about an axis above or below the paper to arrive at conformations with the free carbonyls pointing out of or into the ring, respectively.

In agreement with the interpretation of spectral measurements in nonpolar solvent the uncomplexed structure observed in the crystal has six intramolecular hydrogen bonds (shown schematically in Fig. 2b). Contrary to previous speculation, two of the hydrogen bonds are in 13-membered rings. Two free carbonyls are directed into the cavity (type M), two are directed outward

parallel to the axis of the ring (type P), and two are directed outward perpendicular to the axis of the ring (type Q). An oxygen of each of types M and P is sufficiently exposed at either surface of the cavity to begin coordination with an ion. The ion could then disrupt each of the two hydrogen bonds that are in 13-membered rings. The rounding out of the entire molecule as the ion is drawn in brings the oxygens of type Q into position to form hydrogen bonds with the nitrogen, which is now free, and the observed conformation of the  $K^+$  complex is attained.

The simplicity of this mechanism, which requires no extreme conformational change, seems most compelling, particularly in view of the fact that the formation of the complex may be the

driving force that initiates the conformational change. This sequence of loose complex formation followed by conformational change has been suggested in much less specific terms by Pressman (1). The secondary structure of the uncomplexed form successfully avoids the unfavorable condition of six carbonyl oxygens being directed toward each other, which would occur if the doughnut configuration were retained in the absence of the ion.

A similar correspondence between a complexed and an uncomplexed ionophore was discovered in the x-ray crystal structure determination of the free acid of monensin (6) and the monensin-silver ion complex (7). That correspondence is remarkable in that the free acid could have adopted a straight chain conformation.

Another interesting feature of the uncomplexed valinomycin structure is the conformation of the isopropyl groups. The  $\alpha CH-\beta CH$  conformations are *trans* in the valyl residues and *gauche* in the hydroxyisovaleryl residues. These results agree with the results of proton resonance studies of the complex in solution over the temperature range  $-50^\circ$  to  $30^\circ C$  (3). It is worth noting that if the hydroxyisovaleryl  $\alpha CH-\beta CH$  conformations were *trans* the oxygens of types M and P at one of the cavity surfaces would be greatly shielded from ionic approach.

In view of the simplicity of this postulated mechanism for the transition from uncomplexed to complexed valinomycin as the  $K^+$  ion enters, the question of whether or not a similarly simple mechanism operates as the ion is released should be raised. The nuclear magnetic resonance spectra of valinomycin in polar solvent indicate the presence of only three intramolecular hydrogen bonds (4), which suggests a much more uncoiled structure. Complexed valinomycin may migrate through the membrane to a more polar region in which the intramolecular hydrogen bonds are competed for by the surroundings strongly enough to open the structure up and effect the release of the ion. On the other hand, the minor conformational change involved in this proposed complexing and the conformational differences in the openings at either side of the valinomycin cavity (with six isopropyl groups at one end and three at the other) suggest that the molecule could be lodged in a membrane pore and act as a pump.

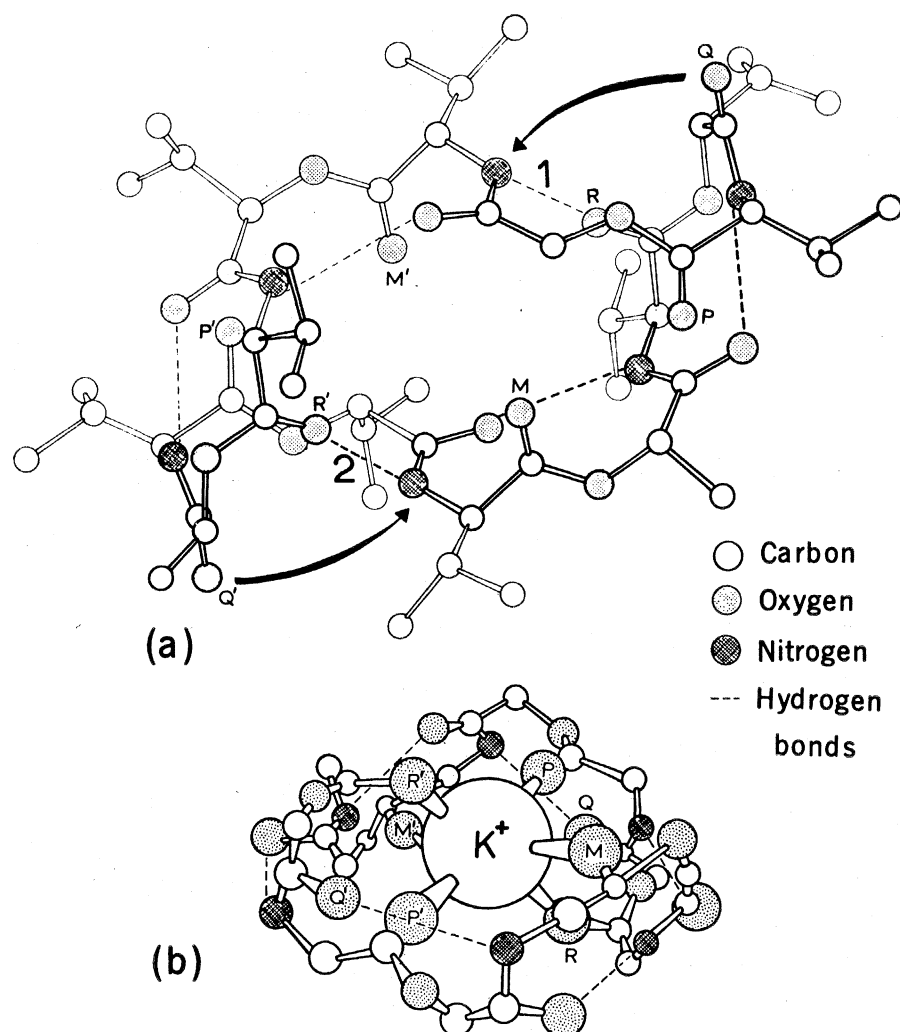


Fig. 1. The uncomplexed (a) and  $K^+$ -complexed (b) conformations of valinomycin. The carbonyl oxygen atoms P, P', M, and M' are those in the most exposed positions and they could begin complexing with  $K^+$  ions. The hydrogen bonds marked 1 and 2 could be disrupted, allowing atoms R and R' to provide the remaining sixfold coordinate for the  $K^+$  ion. The rounding out of the molecule as the ion enters is accomplished by minor conformational changes which bring atoms Q and Q' into position to complete the intramolecular hydrogen bonding of the  $K^+$  complexed ion.

The molecular structure of valinomycin was determined by application of direct methods characterized by a novel technique for decisive enantiomorph discrimination. This technique for fixing the enantiomorph (8) in space group  $P2_1$ , based upon calculated cosine invariants (9), had been used successfully in the determination of the crystal structure of aldosterone (10) and applied to the more complicated problem of valinomycin [ $C_{54}N_6O_{18}H_{90}$ , space group  $P2_1$ ,  $a = 23.14(1)$  Å,  $b = 10.356(1)$  Å,  $c = 14.526(6)$  Å,  $\beta = 99.57(5)^\circ$ ] in order further to test its usefulness.

The procedure described here, in contrast to other direct techniques, such as symbolic addition (11) and multiple solution (12), places great stress on calculated values of the cosine invariants, and the identification of those few cosine invariants which are calculated to be negative is of crucial importance (13). In addition, the concept of interacting classes of phases, in order to achieve decisive enantiomorph discrimination, plays an important supporting role.

Approximately 3000 structure invariants,  $\cos(\phi_1 + \phi_2 + \phi_3)$ , were evaluated by using a modification of the triple product formula (9). One hundred of these invariants, most of which on the basis of the calculated values were considered to be  $+1$ , were used in the phasing procedure. However, four of these invariants were calculated to be small or negative and were crucial in the phase determination. In addition to the origin-fixing reflections, two reflections of the type  $h0l$  were introduced, resulting in fourfold ambiguity of phasing (14). The origin was solidly established by using 51 two- and three-dimensional reflections, which were determined via  $\Sigma_2$  relationships (15) to have phases either equal to or approximately equal to 0 or  $\pi$ . Three invariants of the type  $(h4l, \bar{h}4l, 080)$ , in which the  $h4l$  and  $080$  reflections were very intense, had calculated (9) cosines less than 0. The nature of the interactions (8) of these reflections with the others showed that the cosines of these invariants were approximately  $-1$  in value and that these  $\phi_{h4l}$ 's could be used to fix the enantiomorph decisively. The values of ten phases, corresponding to very intense reflections, were then determined to be  $\pm\pi/2$ , approximately. These 61 reflections were used as a basis set in tangent (16) and modified

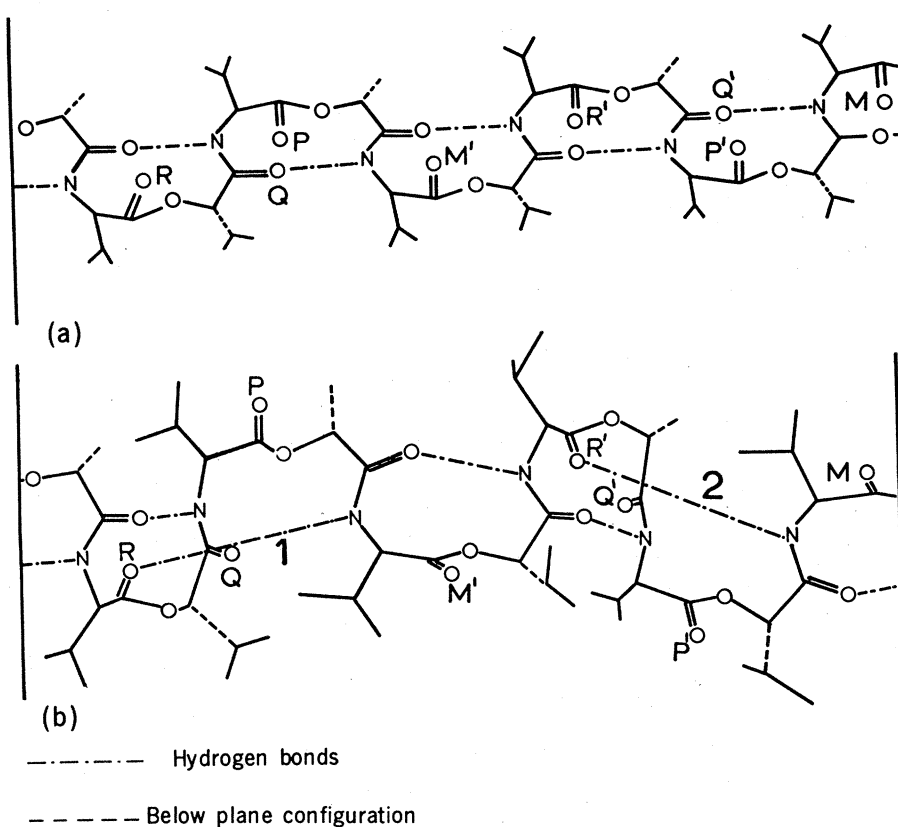


Fig. 2. The intramolecular hydrogen bonding scheme in complexed (a) and uncomplexed (b) valinomycin. The vertical bars at left and right should be brought together by curving the plane of the paper toward the observer. The carbonyl oxygen atoms  $M$ ,  $P$ , and  $R$  comprise the  $K^+$  coordination sphere in the complexed molecule. The source of the conformational difference is that the oxygen atoms involved in hydrogen bonds 1 and 2 are  $R$  and  $R'$  rather than  $Q$  and  $Q'$ .

tangent (17) formulas to determine the values of 452 phases. Modified and simple tangent figures of merit on the four trials required because of the  $h0l$  reflections of ambiguous phase showed no preferred solution. Inspection of the phasing of some reflections of the  $\Sigma_1$  type and the  $020$  and certain  $h1l$  phase values, strongly indicated by the phasing process, suggested the most promising solution. The 51 strongest peaks in the  $E$ -map (the Fourier synthesis in which the coefficients are the normalized structure factors  $E$  rather than the conventional structure factors  $F$ ) formed an oval. Structure factor calculations based upon those peaks gave an  $R$  value (defined as  $\Sigma||F_o| - |F_c||/\Sigma|F_o|$ ) of 36 percent for 5982 reflections. Two subsequent difference maps led to the unambiguous identification of the 78 non-hydrogen atoms of the structure. Three cycles of isotropic refinement on all non-hydrogen atoms reduced the  $R$  factor to 15 percent.

When comparing the true phases, as determined from the structure, with the phases that led to the solution, it was

found that the average magnitude of the error in the 43 three-dimensional phases of the basis set was  $18^\circ$ . This good agreement suggested that an initial  $E$ -map with more than 452 terms might give the full structure. To test this hypothesis, the 61 basis phases were again used as input to the modified tangent procedure in order to calculate 552 phases. In the initial  $E$ -map based on these 552 phases, 57 of the 60 strongest peaks were found to be at an average distance of 0.15 Å from the refined atomic positions. Seventeen of the remaining 21 atoms were also present in the 552-phase  $E$ -map at an average distance of 0.27 Å from refined positions. The four atoms not in the map were terminal carbons on isopropyl groups exhibiting the greatest thermal motion.

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## Revegetation following Forest Cutting: Mechanisms for Return to Steady-State Nutrient Cycling

**Abstract.** Dense stands of a woody, successional species, *Prunus pensylvanica* L., develop rapidly, with early closure of canopy and rapid attainment of high values of net annual production and nutrient accumulation. Such rapid growth following disturbance tends to minimize losses of nutrients from the ecosystem, thus promoting a return to steady-state cycling characteristic of a mature forest.

Because terrestrial plant communities have always been subjected to various forms of natural disturbances, such as wind storms, fires, and insect outbreaks, it is only reasonable to consider recovery from disturbance as a normal part of community maintenance and repair. Although the structural basis of recovery from disturbance has long been recognized, the functional basis is only now beginning to be understood, largely as a result of the whole ecosystem studies of nutrient cycling at the Hubbard Brook Experimental Forest in New Hampshire (1-3).

At Hubbard Brook, experimental clear-cutting and subsequent herbicidal spraying of a 15.6-hectare watershed-ecosystem triggered a chain of events which led to pronounced changes in ecosystem function (3, 4). Significant increases in decomposition rates, nitrification, streamwater concentrations of dissolved inorganics (most notably nitrate-nitrogen), total hydrologic runoff, and erosion have been reported (2, 3, 5, 6). The combined effect of deforestation and suppression of vege-

tative regeneration with herbicides caused extreme open or loose cycling of nutrients (nutrient dumping), which were flushed from the forest soils into streams where they caused eutrophication.

The cutting and herbicide experiment raises important and fundamental questions about both the general impact of disturbance on the functioning of ecosystems and the means by which

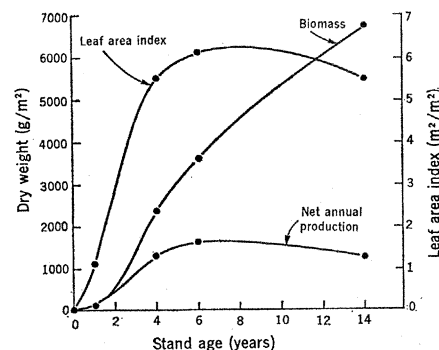


Fig. 1. Relationships of biomass, net annual production, and leaf area index to stand age.

such impact is attenuated by natural recovery processes. Because similar trends are produced by commercial clear-cutting practices in the Northeast (7), it is of considerable interest to understand these recovery processes, especially as they are related to nutrient circulation. We present data to show the importance of a fast-growing, short-lived, successional tree species, pin cherry (*Prunus pensylvanica* L.), in reducing the degradative nutrient losses found at Hubbard Brook, and discuss the implications for ecosystem stability and forestry practice. Disturbance is here defined as destruction of vegetation, or parts of vegetation, whether natural or man-induced.

Erosional and nutrient element losses from a forest ecosystem following disturbance are diminished by any form of vegetative regeneration, there being a roughly inverse relationship between the rate of regeneration and the amounts of erosional and nutrient losses (8). To measure the rate of recovery following cutting, we sampled naturally occurring stands of pin cherry in different stages of recovery (1, 4, 6, and 14 years after cutting). To minimize site differences between stands, we sampled stands which were all in north-central New Hampshire, all on the same geologic formation, all subjected to the same disturbance (clear-cutting), all broadly equivalent in drainage and elevation, and, most importantly, all densely stocked with, and dominated by, the same successional species—pin cherry.

We measured the rate of recovery of the ecosystem in terms of amount of biomass, rate of biomass accumulation (by measuring net annual primary production for each stand), rate of canopy closure as indicated by leaf area index (9), and rate of accumulation of nutrients (nitrogen, calcium, magnesium, potassium, and sodium) in plant tissues (10).

Our approach to biomass and production estimation (14) followed harvest techniques based on the allometric relations of individual sample trees (11, 12). Three to five replicate chemical analyses were made for each major tissue (leaves, current twigs, older branches, dead branches, roots, stem wood, and stem bark) from each of the three older stands (4, 6, and 14 years), and included macro-Kjeldahl nitrogen and cation analyses by atomic absorption spectrophotometry (13). For a particular stand, weights of the different plant parts were multiplied by the ap-