carbenes than meets the eye at first glance.

The year 1981 may be regarded as a watershed in the development of the chemistry of multiple-bonded heavier main-group elements. In that year, stable compounds containing a silicon–carbon, silicon–silicon and phosphorus–phosphorus double bonds were isolated and their structures established by single-crystal X-ray diffraction[8]. The isolation of such compounds was made possible by the incorporation of sterically bulky substituents such as adamantyl or 2,4,6-tris(tart-butyl) phenyl groups. It is indeed surprising that it took nearly another decade for the isolation of a carbene such as IV, considering the fact that nucleophilic carbene complexes of the type VIII were investigated extensively by Lappert and coworkers[9] in the University of Sussex, UK in the early seventies. The carbene complexes of type VIII were prepared from the electron-rich olefin precursor VII, which contains an imidazolidine skeleton. Such are the vagaries and vicissitudes of scientific discoveries: often a small step leading to significant pro-

R = CH₃ or C₆H₅
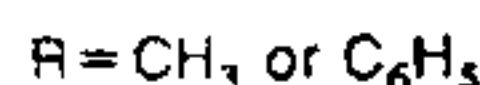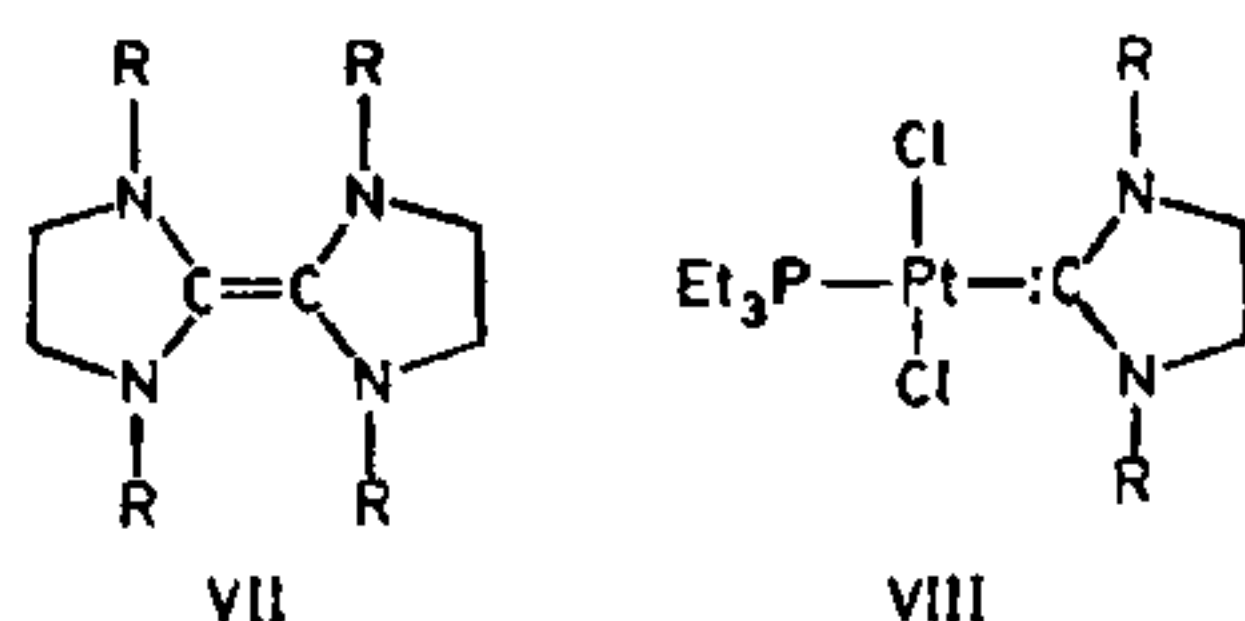
gress may take several years for no apparent reasons.

The isolation of stable, 'bottleable' carbenes such as IV and VI has opened up a vast area of study of the electronic structures and chemical reactivity of this exciting class of compounds.

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First 3D structure of a DNA virus

M. R. N. Murthy

The three-dimensional structure of the single-stranded-DNA (ss-DNA) canine parvovirus reported[1] by Rossmann's group at Purdue University, USA, is the first report of a DNA-virus structure determined at near-atomic resolution by X-ray crystallographic procedures.

Parvoviruses cause a number of diseases in animals, including man. Young or unborn animals are specially susceptible to autonomous parvoviruses. Canine parvovirus is a remarkable example of the emergence of a new pathogen. It spread widely within a few months of its first discovery in 1978 and is now endemic in all populations of canids.

Canine parvovirus particles are spherical in shape, approximately 255 Å in diameter, and have a relative mass of 5.5 to 6.2 million daltons. The viral protein coat encapsulates an ss-DNA genome of about 5000 nucleotides. The number and type of distinct structural proteins in different parvovirus capsids are variable. Canine parvovirus protein coat consists of 60 copies of a combination of three proteins, VP2, VP3 and VP1. In contrast, empty virus particles—devoid of their DNA—contain predominantly VP2. The viral protein subunits are organized in icosahedral symmetry. Despite the complexity of organization of the protein subunits and nucleic acid, the virus is found to form exceptionally well-ordered crystals under favourable conditions. The three-dimensional structure of this virus has now been determined by X-ray diffraction techniques. Rossmann and coworkers have been able to determine this large structure essentially by exploiting the phase information hidden in the molecular symmetry of the virus particle. Rossmann himself has been responsible for the development of much of the theoretical background required for structure determination or refinement of biological macromolecules by this method.

The final electron-density map computed at 3.25-Å resolution allowed confident tracing of the 584 residues of VP2, except for the amino-terminal 38 residues. The viral coat protein folds into a structure, a part of which resembles the now familiar eight-stranded β-barrel motif found in several ss-RNA plant and animal viruses (Figure 1). The barrel structure not only conforms to the standard topology, but also includes some of the helical intrusions between β-strands found in the structure
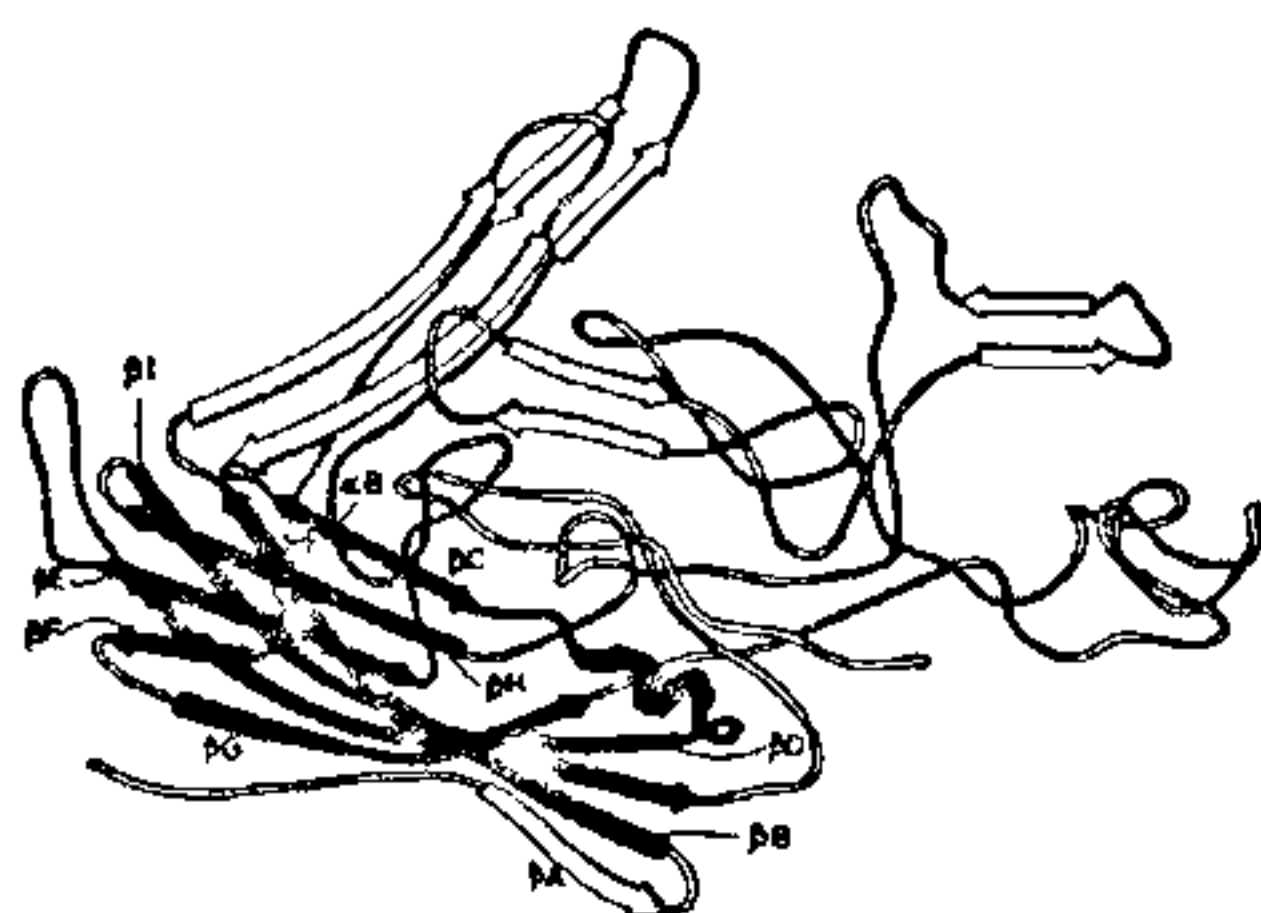
Figure 1. Ribbon drawing of the three-dimensional fold of canine parvovirus VP2. The secondary-structure elements have been identified by correspondence to those of the plant virus, southern bean mosaic virus. The shaded region corresponds to the eight-stranded β-barrel motif found in the coat protein structures of most of the plant and animal icosahedral viruses. [Redrawn from the original with permission from Prof. M G. Rossmann, © American Association for the Advancement of Science.]

of other spherical viruses. However, this motif is only a third of the VP2 protein and the remaining part of the polypeptide folds into a mostly irregular structure. Similar large-scale unordered structures are not usually observed in the folded structure of globular proteins. Surprisingly, this structure appears as an insertion between the β-strands β-G and β-H and consists of 219 residues. This region also corresponds to the 'FMDV loop' of animal picornaviruses. These similarities in the polypeptide folds of different viruses are meaningful in terms of plant and animal virus evolution.

The organization of the protein on the surface of the virus creates 22-Å-long, 70-Å-wide protrusions on the icosahedral threefold axes, and 15 Å depressions on the twofold axes. Cylindrical structures around icosahedral 5-folds are surrounded by 15-Å-deep 'canyons'. In anology with rhinoviruses, the structure of which was also determined by the Rossmann group a few years ago, the authors suggest that the canyon near the icosahedral 5-folds might be the site of receptor attachment. Rhinoviruses are resposible for common cold in humans. According to the 'canyon hypothesis' (Figure 2) proposed by the Rossmann group, the receptor-recognition site, where residues must be conserved to allow virus particles to continue to bind cell-surface
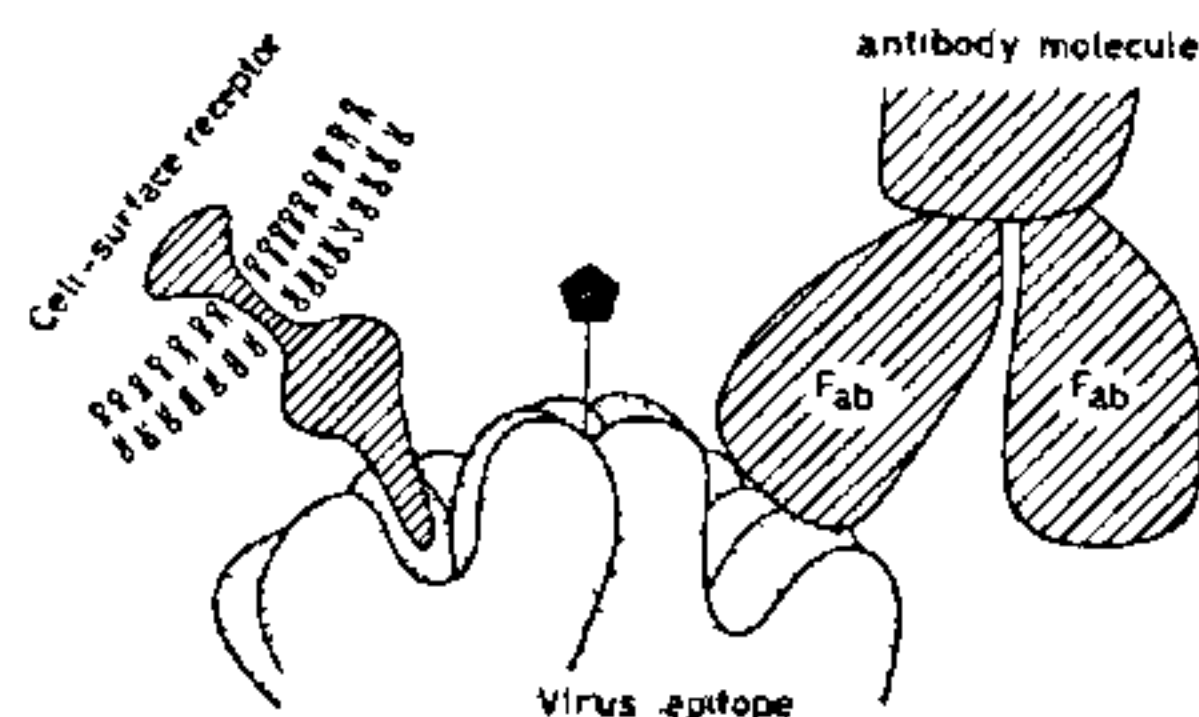
Figure 2. The 'canyon hypothesis' provides a mechanism by which picornaviruses are believed to evade the host immune system. The receptor-binding site is deeply buried on the surface of the virus and hence binding of the large antibody molecules to the site is sterically hindered. New serotypes evolve by mutation of residues outside the canyon. [Reproduced with permission from Prof. M. G. Rossmann, © American Association for the Advancement of Science.]

receptors of susceptible cells, is deeply buried inside the canyon or pit and is inaccessible to antibody molecules. The residues accessible to antibody molecules are on the surface of the virus and do not participate in recognition. Random mutations at these sites can lead to the evolution of new serotypes. These findings on receptor recognition and our understanding of mechanisms of immune surveillance hold promise for design of rational drugs targeted against residues lining the canyon floor. Another interesting feature of this work is the observation of partially ordered nucleic-acid structure. Approximately 13% of the ss-DNA genome is icosahedrally ordered and is visible in the electron-density map. Each protein subunit appears to be in an invariant contact with 11 nucleotides. This is the second clear example of ordered nucleic acid in icosahedral virus particles. However, the functional implications of ordered nucleic-acid structure is not yet clearly understood.

The three-dimensional structure of canine parvovirus will provide a firm foundation for designing future experiments to elucidate the receptor-recognition and antigenic sites of parvoviruses.

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