Both of the carbon-oxygen bonds have a bond order of $1\frac{1}{2}$. Accordingly, the carbon-oxygen bond distance of 1.26 Å is intermediate between the carbon-oxygen double-bond distance of 1.20 Å in H₂C=O and the carbon-oxygen single-bond distance of 1.43 Å in HO—CH₃.

Carbonate ion, CO₃²⁻, is somewhat more complicated in that three resonance structures are required. The resonance hybrid has three equivalent carbon-oxygen bonds, each having a bond order of $1\frac{1}{2}$. Because the carbon-oxygen bonds in carbonate ion (order $1\frac{1}{2}$) have more single-bond character than those in formate ion (order $1\frac{1}{2}$), they are slightly longer (1.28 Å).

EXERCISE 2.4 One resonance structure for ozone, O₃, is $-\text{O}--\text{O}--\text{O}$. Write two Lewis resonance structures showing all valence electrons and compare the expected oxygen-oxygen bond length with that of hydrogen peroxide (Exercise 2.1).

In each of the foregoing examples, the important resonance structures are equivalent. In some cases, a species is best described by two or more resonance structures that are not energetically equivalent. One such species is protonated formaldehyde, (H₂COH)⁺. Formaldehyde itself may be represented by a Lewis structure in which there are two carbon-hydrogen single bonds and a carbon-oxygen double bond (Exercise 2.3).

In protonated formaldehyde, an additional oxygen-hydrogen single bond is present. Two Lewis structures may be written for (H₂COH)⁺.
application in the pharmaceutical industry was the use of formaldehyde to modify bacterial toxins and viruses; similar procedures are still important commercially. The purpose of this treatment is to kill, inactivate, or so change the virus or toxin as to render it incapable of eliciting its toxic or pathological response, while retaining its ability to elicit an immunogenic response when injected into an animal. The bacterial toxins, when so modified, are known as toxoids.

One of the oldest processes involving protein modification is the treatment of animal hides or hair for human use, as in the tanning of leather. Increased knowledge has led to recent improvements in these ancient procedures. For example, glutaraldehyde, used for cross-linking of proteins, is now also used for tanning leather. It apparently functions similarly by cross-linking collagen in the leather. Similarly, several different modifications are now used to give wool fibers superior performance for clothing. Chlorination or treatment with polyepoxides is being used commercially. The latter primarily react with amino groups. Use has also been made of reagents splitting disulfide bonds for the purpose of obtaining "permanent press" in finished clothing items.

1.3 CURRENT STATUS

The current interest in chemical modifications of proteins is indicated by the many reviews and books on its various aspects. Baker (1967), for example, has published a book dedicated to the organic chemistry of the active site (of enzymes), and Hirs (1967) has edited a detailed compilation of laboratory procedures for proteins. Other reviews, including general discussions of chemical reagents and their reactions, have been published by Cohen (1968), Glazer (1970), Vallee and Riordan (1969), Stark (1970), Shaw (1970), and Spande et al. (1970).

There is now a long series of chemical reagents and reactions used for: (a) synthesizing peptides and proteins (Stewart and Young, 1969), (b) sequential and stepwise degradation of proteins to determine their structures (Stark, 1970), and (c) preparation of derivatives of amino acids to increase their volatilities and detectabilities by vapor phase chromatography (Gehrke et al., 1968). The first type under (a) includes methods which must maintain the integrity of the polypeptide chain and the structures of the side groups of the amino acids, because the objective is to end with a normal polypeptide or protein. Special reagents are used to block or stabilize the partially synthesized protein. In general, however, the methods are milder than (b) and (c) types. This is not the case for the methods used for preparing derivatives for vapor phase chromatography. For this purpose, the only requirement is maintenance of the structure of the individual amino acids. Consequently, the methods used for these derivatives usually involve much harsher conditions than are used for intact proteins.

Many of the methods in use today are sufficiently simple and convenient to be
the well-known formol titration of amino groups (French and Edsall, 1945; Kallen and Jencks, 1966).

\[
\begin{align*}
\text{P-NH}_2 + \text{C}=\text{O} & \rightleftharpoons \\
\text{P-NHCH}_2\text{OH} & \rightleftharpoons \text{P-}\text{NCH}_2\text{OH}
\end{align*}
\]

Formaldehyde is available from commercial sources in 37–40% aqueous solutions, containing small amounts of methanol and known as formalin. In such solutions, it exists primarily as a number of low-molecular-weight polymers of the type \(\text{H}(\text{OCH}_2)_n\text{OH}\). Formaldehyde may also be obtained as a stable solid known as paraformaldehyde, composed of high-molecular-weight polymers of the same type. Heating of paraformaldehyde can be used to generate pure gaseous formaldehyde.

Both sources of polymeric formaldehyde revert to monomer in dilute aqueous solutions. Under such conditions, formaldehyde is in very rapid equilibrium with a hydrated form (Equation 2), and more than 99.9% is in the form of the hydrate.

\[
\begin{align*}
\text{C}=\text{O} + \text{H}_2\text{O} & \rightleftharpoons \text{H}_2\text{O-C-OH} \\
\text{H} & \text{H}
\end{align*}
\]

Compounds I through V (see below) have been shown to result from the reaction of formaldehyde with tyrosine, tryptophan, histidine, asparagine, and cysteine, respectively (French and Edsall, 1945). Presumably, their formation is preceded by the formation of very electrophilic immonium cations (Equation 3),

\[
\begin{align*}
\text{H}_2\text{N-CH}_2\text{OH} + \text{H}_2\text{O} & \rightleftharpoons \text{H}_2\text{N-CH}_2\text{OH} + \text{H}_2\text{O} \\
\end{align*}
\]

which react with the adjacent amino acid side chains. Similar reactions have been postulated to occur in proteins linking the \(\varepsilon\)-amino groups of lysine residues via methylene bridges to neighboring side chains (Fraenkel-Conrat et al., 1947; Fraenkel-Conrat and Olcott, 1948a, 1948b; Fraenkel-Conrat and Mecham, 1949). The postulated reactions are mechanistically similar to the widely studied Mannich reaction (Fernandez and Fowler, 1964; Alexander and Underhill, 1949). One
Organic Chemistry is essentially the chemistry of Carbon. Carbon compounds are classified according to how the Carbon atoms are arranged and what other groups of atoms are attached. Hydrocarbons. The simplest Organic compounds are made up of only Carbon and Hydrogen atoms only.