paper. The conversion of *cis*-caffeic acid to esculetin could proceed through a hydroxylated intermediate or through a quinone. The hydroxylation reaction, step 2, is similar to the hydroxylation of phenols by an ascorbic acid–iron system studied by Undenfriend[2]. The lactone formation, step 3, is analogous to the spontaneous cyclization of *cis*-*o*-coumarin acid to coumarin[1]. An alternative mechanism involves oxidation to the quinone, step 4, with lactone formation by a Michael addition β to the carbonyl followed by aromatization, step 5. This is less likely, however, because Michael additions do not generally occur under mild acid conditions.

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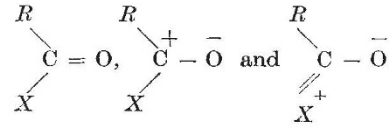
A Proposed Interpretation of Infra-red Spectral Changes occurring upon the Interaction of Polynucleotides

It has been found that the sodium salts of polyadenylic and polyuridylic acids interact[1–4] with formation of a two-stranded helix[1] with a structure very similar to deoxyribonucleic acid. More recently it was observed that the infra-red spectrum[5] of the mixture of these two acids showed the following marked changes from the summation of the curves of the components : a 39 per cent reduction in intensity of the 1,627 cm.$^{-1}$ band and a 7 cm.$^{-1}$ shift to higher frequency ; a decrease in peak height (because of strong overlap the band was not integrated) and an 11 cm.$^{-1}$ shift to higher frequency of the 1,661 cm.$^{-1}$ band. The interaction of the sodium salts of polycytidylic and polyinosinic acids[6] has now been observed in the infra-red[7] with the following result : a marked decrease in intensity of the 1,651 cm.$^{-1}$ peak of the sodium salt of polycytidylic acid (the intensities have not yet been put on a quantitative basis) and a 19 cm.$^{-1}$ shift to higher frequency of the 1,678 cm.$^{-1}$ peak of the sodium salt of polyinosinic acid.

It is possible that an understanding of these changes may be reached by taking into account the change in environment of the vibrating groups after interaction has occurred. A consideration of the helical structure of deoxyribonucleic acid[8,9] and of the polynucleotide pairs[1,6] reveals that the heterocyclic rings are covered on both sides by other such rings at a distance of about 3·4° A. This is no more than a van der Waals separation, and is insufficient to admit water molecules between the planes of the two rings. One result of this helical configuration is, therefore, that the dielectric constant of the immediate environment of the vibrating groups must be considerably diminished, even though water remains in the area which is not between the base pairs.

The purine and pyrimidine bases are hydrogen bonded to water before interaction and to other bases after interaction, but the hydrogen bonds existing after interaction should be stronger than those that existed before[10]. The observed spectral changes, thus, are probably not caused (directly) by the new hydrogen bonds since the usual effect of hydrogen bonding is to decrease frequency and increase intensity rather than the reverse.

The following qualitative considerations, on the other hand, suggest that the observed changes are consistent with a decrease in dielectric constant. The principal resonance forms of the carbonyl group[11] are :



Hydrogen bonding favours the dipolar forms with a resulting shift to lower frequency. A marked decrease in dielectric constant of the medium, however, should diminish the contribution of the forms involving a charge separation and result in a shift to higher frequency. A second result of a decrease in dielectric constant is that there should be a smaller change in dipole moment with the vibration of the bond responsible for the absorption and a consequent reduction in intensity. Experimental results in support of these expectations are cited below.

In studies of frequency and intensity changes in approximately a dozen solvents[12,13] (see also ref. 11), it has been shown that the carbonyl stretching bands show a general trend of increase in frequency and decrease in intensity as the dielectric constant of the medium is decreased. Thus, for example, the frequencies of several ketones increase 11–14 cm.$^{-1}$ on going from chloroform to hexane and 8–9 cm.$^{-1}$ on going from benzene to hexane. There are also 18–36 per cent and 15–20 per cent reductions in intensity for the same solvent changes. *NN*-Diethylacetamide[13] showed a frequency increase of 38 cm.$^{-1}$ for the first solvent change and 15 cm.$^{-1}$ for the second, with intensity reductions of 24 and 20 per cent, respectively. *NN*-Diphenylacetamide showed a similar trend for those solvents in which it was soluble. The directions and approximate magnitudes of these spectral changes are thus consistent with those observed with the polynucleotides. While there are undoubtedly other factors contributing to the observed spectral changes, it is suggested that the above-mentioned decrease in dielectric constant may very well be the most important cause.

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