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Binding Properties of Guanosine-2′,3′,5′-triisobutyrate

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Abstract□To study the behavior of nucleic acid base in a nonpolar organic solvent, chloroform, we synthesized a derivative of guanosine. This derivative, guanosine-2′,3′,5′-triisobutyrate was obtained by reaction of guanosine with isobutyric anhydride, and identified by TLC, EA, IR and NMR. Hydrogen bonding specificity of this compound was revealed by IR and NMR. The molecules of guanosine-2′,3′,5′-triisobutyrate are self-associated in nonpolar solvent, and hydrogen bonds by imino protons become important as the concentration increases. In the presence of a cytosine derivative, the self-association of the guanosine derivative is destroyed, resulting from interaction with cytosine derivative.

Keywords□guanosine derivative, synthesis, hydrogen bonding specificity

In order to study of specific binding properties of nucleic acids, we synthesized a derivative of guanosine, that is purine base of nucleic acids. This substance is to be used to observe the behavior of nucleic acid derivatives in nonpolar solvent. Binding specificity is demonstrated by self-association property of nucleic acid derivatives and by the intermolecular interaction between drugs and nucleic acid derivatives. Hydrogen bonds play an important role on the binding specificity of nucleic acids. Nonpolar solvents are usually chosen because hydrogen bonding by the solvent is of minor importance. Moreover nonpolar solvent eliminates the vertical stacking of purines and pyrimidines, which is believed to occur in aqueous solution as a result of hydrophobic interaction between the aromatic ring system. Hydrogen bonding specificity of the nucleic acid base, can provide important information about mechanisms of phamocological action and of toxicity of the drug at molecular level. Until now, there are many nucleic acid derivatives, which are soluble in nonpolar organic solvent. However derivatives of guanosine, compounds are rarely known. Rich *et al.*[1] had used 2′,3′-benzylidine-5′-trityl guanosine. In late 1970s, Kyogoku *et al.*[2,3] have used the simpler compound, guanosine-2′,3′,5′-triisobutyrate, but the synthetic method and physicochemical characteristics of that substance have not been reported yet. So we have synthesized it and observed its binding properties.

EXPERIMENTAL METHOD

Materials and Instruments

Guanosine and 2′,3′-benzylidine-5′-triphenyl methyl cytidine (C) were purchased from Lohjin Co., Ltd. and Vega, respectively. Isobutyric anhydride was purchased from Fluka. Chloroform was passed through alumina gel to remove ethanol. All other reagents were analytical grade. IR and NMR spectra were recorded on a Perkin-Elmer 1710 spectrophotometer and Bruker FT-80 MHz spectrometer, respectively. EA was determined on a Perkin-Elmer 240c system.

Synthesis

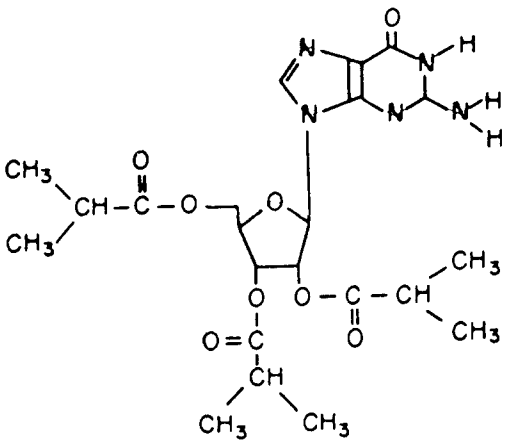
Five grams of guanosine were dispersed in 35 mls of pyridine. Fifteen mls of isobutyric anhydride was added to the guanosine suspension in ice bath. Ice was removed and reaction mixture was stirred for more than 48 hours. End point was confirmed by TLC ($EtOH:CHCl_3 = 5:1$), and remaining isobutyric anhydride was decomposed by ethanol. Pyridine was removed using a vacuum evaporator, then the product (G) was extracted with chloroform. After chloroform was evaporized, G was recrystallized with ethanol.

Spectral measurements

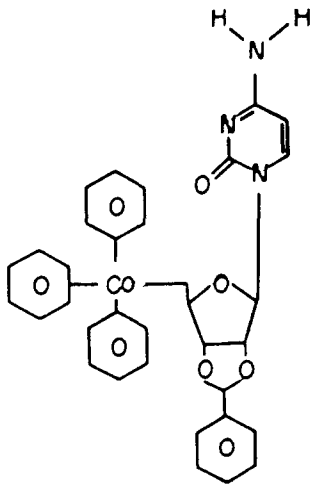
The solvents used for IR and NMR are chloroform and deuterochloroform, respectively. IR and

NMR assignments were supported by spectra in D_2O (data not shown). To examine the self-association, 0.03M and 0.005M solutions were prepared for IR and NMR analysis, respectively, and serial 2-fold dilution were performed for both analysis. In IR analysis molar extinction coefficients at various peaks were calculated to check the self-association and intermolecular interaction. The structure of guanosine 2′,3′,5′-triisobutyrate (G) and 2′,3′,-benaylidine-5′-triphenyl methyl cytidine (C) are as follows.

G



C



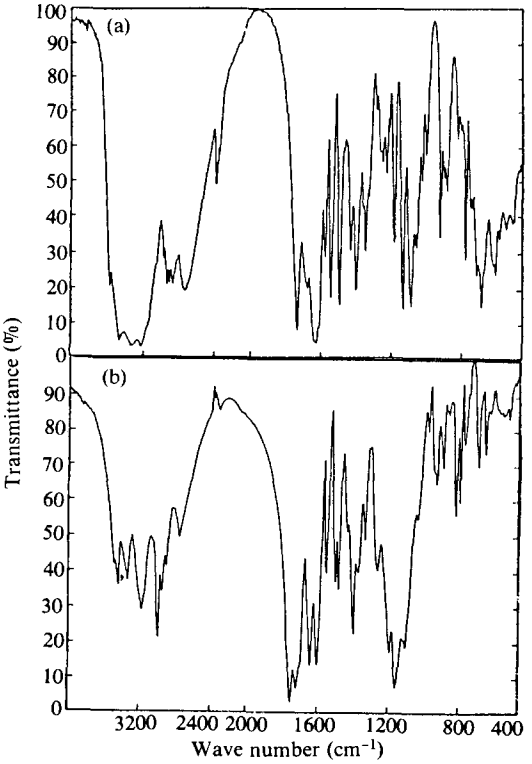


Fig. 1. Infrared spectra of reactant, guanosine (a) and product, G (b).

RESULTS AND DISCUSSION

The final product ($C_{22}H_{31}N_5O_8$) is colorless fine powder. The solubility in chloroform is sufficient for IR and NMR measurement. EA, IR and NMR were utilized to identify the product. EA experiment shows the weight percents of carbon, hydrogen and nitrogen are 52.99, 6.28 and 13.35, respec-

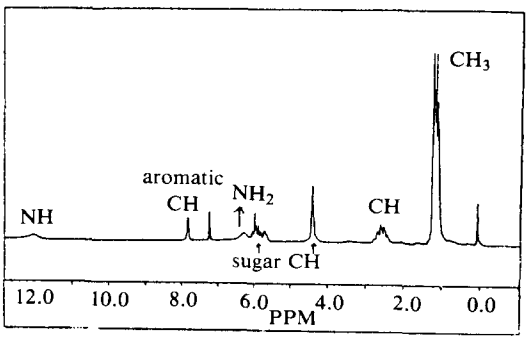


Fig. 2. NMR spectrum of G in deuterochloroform solution.

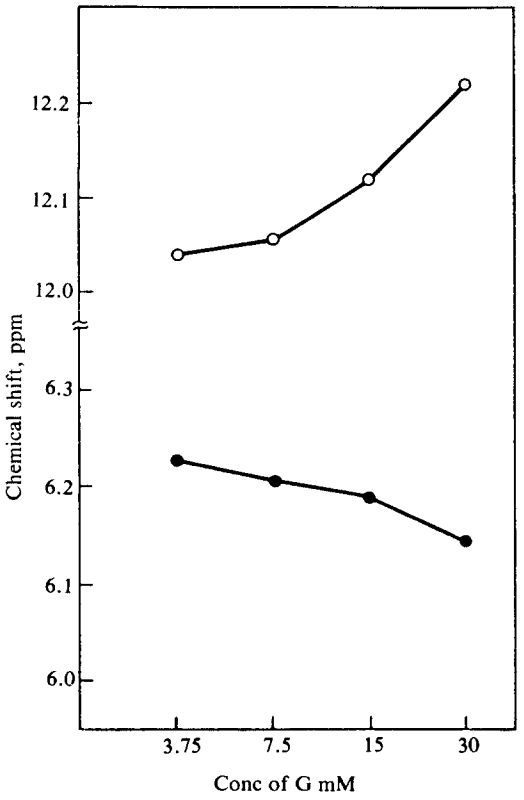


Fig. 3. Effects of the concentration of G on chemical shifts of the amino (●) and imino (○) protons in deuterochloroform solution

tively. IR spectra of the reactant and the product (Fig. 1) are used to check functional groups. In the spectrum of the product, there are no OH stretching peak in 3200-3500 cm^{-1}, which exist in the that of reactant guanosine. Aliphatic CH peaks in 2900-3000 cm^{-1} are strongly enhanced by isobutrylation. NMR spectrum was obtained to identify the complete structure (Fig. 2).

To examine the binding specificity of this nucleic

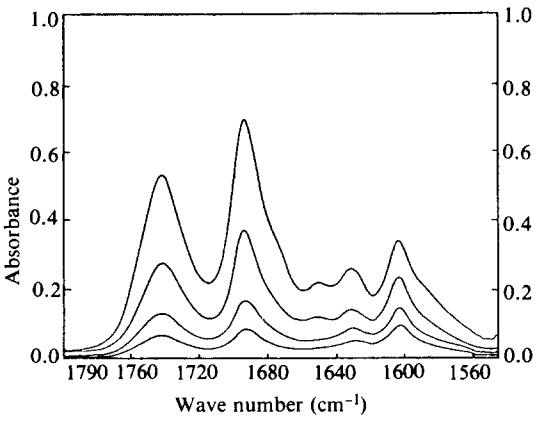


Fig. 4. Effects of the concentration of G in IR 6μregion. (Uppermost peak is obtained from 0.005M solution, and lower peaks are plotted after serial 2-fold dilution of the solution).

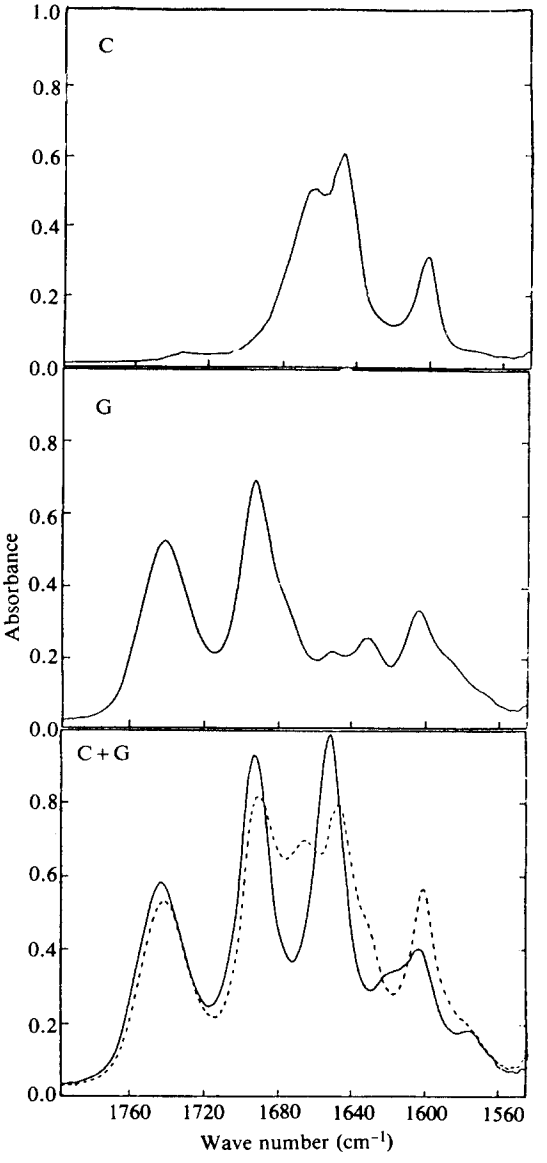


Fig. 5. Infrared spectra of C and G and 1:1 mixture solution in 6u region. They are each present in the chloroform solution at concentration of 0.005M (1 mm KBr cell). The dashed line is the calculated sum of the two spectra, the solid line is the observed sum.

acid derivative with other nucleic acid derivatives or drugs, it is necessary to check its self-association property first. Changes in chemical shift are plotted against concentration (Fig. 3). The changes of the imino and amino proton peaks are indicative of self-association. Imino proton signal shifted downfield, indicates that these protons take part in hydrogen bondings between themselves as concentration increases. In contrast to imino protons, amino proton signal shifted upfield slightly. These amino protons participate in hydrogen bond in a dilute solution, but the hydrogen bonds are probably broken in a concentrated solution. In a concentrated solution, imino hydrogens may play more important role on their self-association phenomena. In regard to the enthalpies calculated nonempirically by Hobza *et al.*[4] among the 4 possible GG pairs, less strongly bonded GG pairs are destroyed and the strongest GG pair (GG I) becomes predominant as concentration increases. To observe the behavior of hydrogen acceptors in hydrogen bonds, IR spectra of G in various concentration are examined (Fig. 4). The values of absorptivity, or molar extinction coefficient at 1694, 1630 and 1600 cm^{-1} are slightly changed, while absorptivity at 1741 cm^{-1} is unchanged against concentration. This reaffirms that G is self-associated. New band appeared at 1650 cm^{-1} in more concentrated solution may be a self-association band. This also indicates that the carbonyl group in sugar moiety is not involved in self-association.

In the presence of C, aliphatic carbonyl group becomes to be involved in interaction. Association band for G-G (1650 cm^{-1}) and C-C (1665 cm^{-1}) are disappeared, moreover aromatic carbonyl peaks for C and G become sharp and intense. New band is shown in the region of 1615 cm^{-1}, which is thought to be G-C association band (Fig. 5). This implies that self-associated form of G and C are disappeared, and nonassociated form and G-C dimer are made in the equimolecular mixture of G and C.

LITERATURE CITED

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