(80 mg) was recrystallised from hexane giving colourless plates of ketone III, m.p. 79.5–80.5°. (Found: C 77.82; H 7.73. Calc. for $C_{14}H_{16}O_2$: C 77.75; H 7.46).

The yellow molecular compound with perchloric acid crystallised out from a solution of ketone III in the acylation mixture after a few minutes. The same result was obtained when 2,4,6,7-tetramethylbenzofuran (II) was used as starting material. In the presence of water the molecular compound gave the ketone III in 98 % yield.

2-Benzofuranyl methyl ketone. A solution of benzofuran (1.18 g) in the acylation reagent (15 ml) was kept for 2 h at room temperature and then washed with water and sodium bicarbonate solution. The ethyl acetate phase was evaporated and the residue was recrystallised from hexane. The product (1.05 g, 66 %) was sublimed (bath temp. 80°, 1 mm Hg) and the sublimate was identified as 2-benzofuranyl methyl ketone by m.p. 72–73°,⁴ dinitrophenylhydrazone, m.p. 265° (decomp.) and reduction to 2-benzofuranylmethylcarbinol, m.p. 40–41°.¹⁰ The reduction was done by stirring the ketone with an aqueous solution of potassium borohydride.

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Received October 31, 1963.

The Effects of Protonation, Alkylation and Cooling on the Fluorescence of Purine Nucleosides in Liquid Solution

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In a recent publication¹ we elaborated on the previously known fluorescence of adenosine and guanosine in acid solutions. A reasonable hypothesis derived from this work is that alkylations of these compounds should produce fluorescent derivatives to the extent that the alkyl groups stabilize the same electron pairs as those involved in the protonations. In accordance with this expectation we have found that adenosine and guanosine yield fluorescent derivatives when methylated with dimethyl sulfate. Guanosine also reacts with nitrogen mustard (2,2'-dichloro-N-methyldiethylamine) and diazomethane with a high yield of fluorescent substances.

Dimethyl sulfate and nitrogen mustard are supposed to alkylate the purine nucleosides mainly in the positions which are protonated in acid solutions, *i.e.* N-1 in adenosine and N-7 in guanosine.^{2,3} These data are confirmed by our observation that neither the absorption spectra nor the fluorescence activation spectra of the fluorescent derivatives of the purine nucleosides depend on whether the fluorescence has been elicited by protonation or by alkylation, except when diazomethane is used as alkylating agent. The latter reagent is known, however, not to alkylate guanosine at N-7.⁴

When solutions of the fluorescent guanosine derivatives were cooled down to 193°K, the fluorescence intensity increased 10- to 50-fold. A mixture of water (1 volume) and methanol (3 volumes) was used as solvent to avoid freezing (Fig. 1).

The fluorescence of the adenosine derivatives either increased slightly (1-methyl adenosine), or did not change significantly (adenosine at pH 2.5), or even almost disappeared (adenosine triphosphate at pH 3.5) when their solutions were cooled. The absorption spectra of the solutions did not change appreciably on cooling. This

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Fig. 1. Guanosine methylated with dimethyl sulfate. Fluorescence intensity at 390 nm as a function of temperature. pH = 6.6. Solvent: 1 volume water, 3 volumes methanol. Exciting light: 274 nm. Absorbance at 274 nm = 0.07 (1 cm light path).

indicates that the absence of any substantial increase of the fluorescence at low temperatures was not due to insolubility of the fluorescent compound. We tentatively conclude that thermal activation energy is needed not only in collisional quenching processes, but also in some step preceding

the singlet-singlet emission of the adenosine derivatives. This step may be a thermally activated internal conversion from a non fluorescent n, π^* singlet state to a fluorescent π, π^* singlet state only slightly above the n, π^* state. The relative magnitudes of the activation energies of such an internal conversion and of the quenching process conceivably determine the shape of the curve relating fluorescence intensity to temperature. The fluorescence was found to be proportional to the first power of the intensity of exciting light. Hence, reaction between two excited molecules is not a prerequisite for fluorescence in our systems.

The possible role of oxygen quenching and a detailed analysis of the relationship between the temperature and the fluorescence as well as further separation and identification of the fluorescent and non fluorescent derivatives of the purine nucleosides are the subjects of current investigations.

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Received October 16, 1963.