Optical Properties of Sugar Flavazoles

Abstract. The absorption of glucose flavazole was measured in the ultraviolet and visible regions of the spectrum. Three maxima were observed: 267, 335, and 410 mμ. The absorption at 410 mμ follows Beer's law with 70 percent propanol or water as solvent. The molar absorptivities for glucose, maltose, and lactose flavazoles were the same and equal to 3.7×10^3.

Reducing sugars react with *o*-phenylenediamine and excess phenylhydrazine to produce characteristic derivatives termed flavazoles. The experimental conditions of the reaction have been reviewed, and a number of derivatives have been tabulated (*1*). The reaction is adaptable to oligosaccharides as well as to monosaccharides, although lower yields have been reported (*2*). This is not a serious handicap when paper chromatography is used for purification. A series of starch dextrins from four to eight glucose units were readily separated and purified by this method, thus permitting their preparation on a semimicro scale.

The flavazoles are bright yellow and can be estimated by colorimety. The spectrum through the ultraviolet and visible ranges was measured for glucose flavazole. The intensity of the absorption taken at 410 mμ is proportional to concentration within the limits of experimental error. Furthermore, the chromophoric group does not appear to be influenced by substitution at positions 4 or 6 with other hexose units. The molar absorptivity at 410 mμ for glucose, maltose, and lactose flavazoles was found to be the same within experimental error (2 to 3 percent).

Glucose, maltose, and lactose flavazoles were prepared by the conventional procedure (*2*). Four crystallizations were usually adequate to remove all traces of impurities as revealed by paper chromatography. Paper chromatography is a much more sensitive method for detecting impurities than is the melting point. This is particularly true of the oligosaccharides. The solvent system, six parts 1-butanol, four parts pyridine, and three parts water, used frequently for carbohydrates was used. The derivatives after crystallization were dried in a vacuum oven for 6 hr at 50°C and stored over calcium chloride. The absorbancies were read in 70 percent propanol (Eastman-redistilled) against a solvent blank in each case.

A Beckman model DU spectrophotometer was used for the absorption studies, and 1-cm quartz cells were used in the ultraviolet as well as in the visible spectrum.

Two absorption peaks, 267 and 335 mμ, were found in the ultraviolet, and one, 410 mμ, was found in the visible spectrum (Fig. 1). The latter was selected for quantitative studies because it is in the visible range and therefore can be used with a simple colorimeter. It should be noted, however, that the absorption in the ultraviolet is much more intense than it is in the visible. Molar absorptivities were as follows: at 267 mμ, 3.98×10^4; at 335 mμ, 1.01×10^4; at 410 mμ, 3.7×10^3.

A slight solvent effect could be observed. When distilled water was used as solvent in place of 70 percent propanol, the peak in the visible was shifted to 405 mμ. The height of the peak, however, was not significantly changed.

The absorption obeyed Beer's law very well over the workable range of the Beckman instrument. An exact correspondence between glucose, maltose, and lactose flavazoles could be observed when the absorbancy readings were plotted against the molar concentration (the molecular weight of glucose flavazole is 336 and of maltose and lactose flavazole, 498). An equally good check was obtained when water was used as solvent, and the absorbance was measured at 405 mμ instead of at 410 mμ. Since mono- and disaccharide flavazoles are only sparingly soluble in water but appreciably soluble in 70 percent propanol, the latter solvent is preferable.

It has been pointed out in a previous communication (*3*) that paper chromatography of flavazoles could be used to advantage in determining the structure of oligosaccharides. Acidic or enzymic hydrolysis of an oligosaccharide flavazole yielded a number of fragments consisting of reducing sugars and flavazoles of a lower degree of polymerization. These could be separated by paper chromatography and subsequently identified, permitting a formulation of the original oligosaccharide. It would be of advantage if such qualitative observations could be combined with quantitative estimations. A number of methods are currently available for estimating carbohydrates separated by chromatography (*4*). Glucose and maltose flavazole fragments can be easily eluted from paper chromatograms and determined

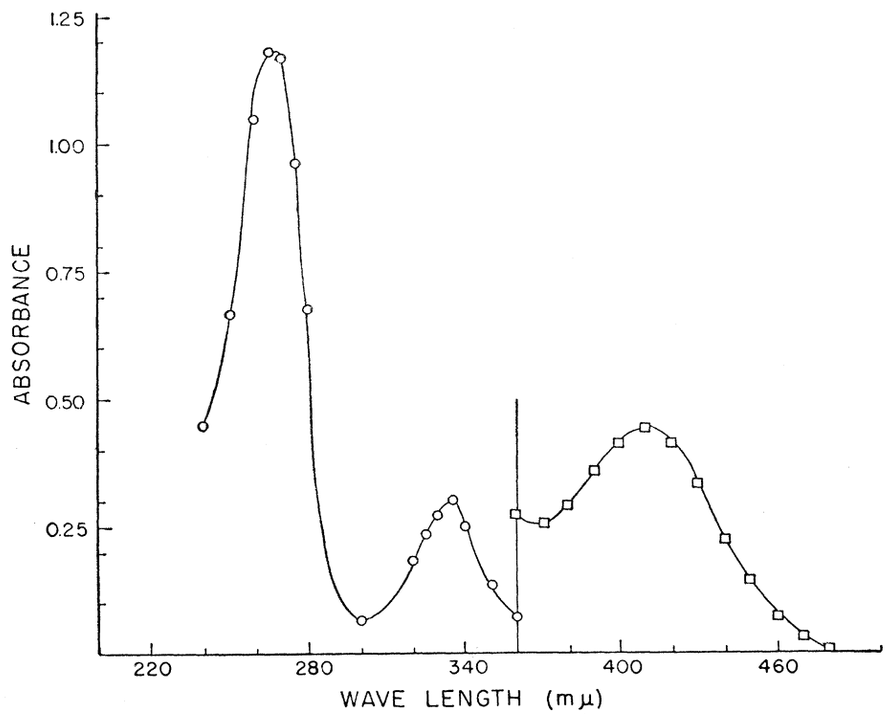

Fig. 1. Absorption spectrum of glucose flavazole in 70 percent 1-propanol. Circles indicate the ultraviolet (0.001 percent) and squares the visible (0.004 percent).

quantitatively by the method described above. For a usual Beckman cell an amount readily obtained from a chromatographic strip (0.25 to 0.50 mg) is adequate for a reliable determination (*5*).

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Deamination of Adenine by Ionizing Radiation

Abstract. A small amount of hypoxanthine is formed when a solution of adenine is irradiated. This has been detected by using C^{14}-labeled adenine and the techniques of paper chromatography and liquid-scintillation counting. The biological significance of this conversion is suggested.

Deamination takes place quite readily when an aminopurine or aminopyrimidine is hydrolyzed with acid (*1*). Ten-percent hydrochloric acid converts cytosine into uracil (*2*). Nitrous acid deaminates adenosine to inosine (*3*) and guanosine to xanthosine (*4*). A similar conversion takes place enzymatically. Intestinal deaminases, for example, convert adenine to hypoxanthine, adenosine to inosine, and adenylic acid to inosinic acid (*5*). The ultraviolet irradiation of adenine solutions has been reported to give trace quantities of hypoxanthine (*6*).

Previous workers had concluded that deamination of this type probably did not take place under the influence of ionizing radiation (*7*). In our studies of the radiation decomposition of nucleotides and related compounds, however, we have found that adenine is converted into hypoxanthine. The yield is small, but significant.

About 250 μl of a 0.1 percent solution of adenine-2-C^{14} (specific activity, 1.3 mc/mmole) were sealed in a vacuum (*8*). To expel any dissolved oxygen, a steady stream of N_2 was bubbled through the solution for about 10 min before the sample was sealed.

A 1.5 kc cobalt-60 source was used for the irradiation. The dosage was calculated by means of the Fricke ferrous sulfate dosimeter. The radiation intensity was 5×10^6 rad/hr.

The irradiation products were analyzed by paper chromatography, with Whatman No. 4 paper and propanol–ammonia–water and butanol–propionic acid–water as solvents (*9*). Twenty-five microliters of the irradiated solutions of adenine were spotted on paper together with 10 μl of 0.1 percent inactive hypoxanthine as carrier. The ultraviolet-absorbing areas were carefully marked out. Autoradiography with x-ray films showed darkening of the film corresponding to the hypoxanthine spot. The radioactivity could have come only from the adenine, showing the conversion of adenine to hypoxanthine. The chromatography of the control was done at the same time as that of the irradiated samples. Thus, bacterial deamination could have been, at most, a minor effect.

In one experiment, the hypoxanthine spot was eluted with 0.1 percent formic acid and rechromatographed with fresh carrier hypoxanthine, with butanol–water (*10*) and isobutyric acid–ammonia–ethylene diaminetetraacetic acid (*11*) as solvents. The radioactivity was concentrated on the hypoxanthine spot, which confirmed the formation of radioactive hypoxanthine.

The hypoxanthine spots were eluted with 0.1 percent formic acid and counted with a liquid scintillator having an internal standard of C^{14}-labeled toluene. The same technique was used for the estimation of residual adenine. The results of the experiments are shown in Table 1.

The biological importance of a deamination of this type may be very great. If it would be legitimate to extrapolate from a high dose level to a low dose level, we may have a possible clue to the origin of a radiation mutation. When the ribonucleic acid of tobacco mosaic virus was heated with HNO_2, the amino bases were converted to the hydroxy bases without splitting of the nucleotide chain. Deamination of a large number of nucleotides resulted in the inactivation of the molecule (*12*). More gentle treatment by controlled action of HNO_2 gave a maximum number of mutations when an average of 1 deamination resulted per 6000 nucleotides (*13*).

Table 1. Formation of hypoxanthine from adenine by ionizing radiation. In all experiments the initial activity was 4.1×10^5 disintegrations per minute (dpm).

Expt.	Dose (10^6 rad)	Residual adenine (%)	Hypoxanthine	
			Act. (dpm)	Amt. (%)
Control	0	100	330	0.08
1	1	87.7	3602	0.88
2	2	75.4	3807	0.98
3	5	60.1	7840	1.9
4	10	44.4	3839	0.9

Because hypoxanthine, xanthine, and uracil normally exist in the keto form (*14*), the deamination of adenine to hypoxanthine will result in the disruption of the hydrogen bonding between adenine and thymine, suggested by the Watson and Crick structure of deoxyribonucleic acid (*15*). From theoretical considerations Lavalle (*16*) has pointed out that deamination of adenine would result in the replacement of an adenine-thymine base pair by a guanine-cytosine pair, altering the molecular code of heredity (*17*).

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