Effect of Toluene and Similar Compounds on the Carbohydrate-Anthrone Reaction


Toluene interferes with the anthrone reaction for hexoses causing a decrease in the absorption maximum at 625 m\(\mu\) and producing a second absorption band with a maximum at 475 m\(\mu\). Xylene, benzene, and cyclohexene also interfere, whereas cyclohexane, o-chlorobenzene, and benzoic acid do not.

It was noted that, when the anthrone test was applied to the nondialyzable constituents of human urine, an absorption band always appeared with a maximum at about 475 m\(\mu\); this band was absent when the test was applied to the dialyzable fraction and was usually absent with sugar standards. This experience with the sugars led us to believe there was some variable in our procedure.

Methods

To Lumetron tubes containing 2 ml. of carbohydrate solution 5\(\frac{1}{2}\) ml. of anthrone reagent (0.2 gm. of anthrone in 10 ml. of H\(_2\)O and 100 ml. of H\(_2\)SO\(_4\)) was rapidly added, while mixing. The tubes were heated in a boiling-water bath for 3\(\frac{1}{2}\) min., after which they were wiped dry and allowed to cool. The percentage transmittance was measured in 1-cm. cells with a DK-2 Beckman recording spectrophotometer\(^*\) using a blank containing H\(_2\)O in place of the carbohydrate solution as a reference. Some of the carbohydrate samples contained reagent-grade toluene or another possible interfering substance.

\(^*\)Beckman Instruments Co., Spinco Division, Palo Alto, Calif.
Results and Discussion

Figure 1 shows the effect of toluene on the anthrone reaction with galactose. The usual absorption band at 625 m\(\mu\) appears, but it is decreased (by nearly half when saturated with toluene) when toluene is present. In addition, an absorption maximum appears (greater than at 625 m\(\mu\) when saturated with toluene) at 475 m\(\mu\). The effect increases with an increase in toluene concentration and is similar for all hexoses tried (glucose, galactose, mannose, and fructose). In fact, the absorbance at 475 m\(\mu\) is proportional to the amount of toluene present. The results were so consistent that the solubility of toluene in a standard galactose solution was estimated to be 38 mg./100 ml. of solution.

The anthrone test for 6-deoxyhexoses (rhamnose and fucose) is only slightly affected by toluene and the second absorption band does not appear. The toluene is very probably incorporated into the green chromogen resulting (1) from the reaction between anthrone and hydroxymethyl furfural, producing a new chromogen with an absorption maximum at 475 m\(\mu\); this type of complex probably does not occur with methyl furfural. The toluene effect is eliminated by passing nitrogen gas through the carbohydrate solution for 10 to 15 min.

Anthrone was reconstituted according to the method of Morris (2);
the crystalline and the filtrate fractions (after evaporation of the solvent) gave the same results, which proves that impurities in the anthrone are not the cause of the change in the absorption spectra.

A 90-ml. sample of the toluene was fractionally distilled into 9 separate 10-ml. fractions. Each of the fractions, including the residual fraction, gave the same effect, which strongly indicates that the change in the absorption is not due to an impurity in the toluene.

Xylene, producing a second absorption band at 485 m\(\mu\), interferes with the anthrone reaction to a lesser degree than toluene. Benzene produces a second maximum at 455 m\(\mu\) and interferes even less than xylene. Cyclohexene destroys the color, while cyclohexane, o-chlorobenzene, 2,2,4-trimethyl pentane, and benzoic acid (0.2% w/v) have no effect.

It has been known for some time that tryptophane (3) interferes with the anthrone reaction, and this reaction is not highly recommended for determining the starch content of plants (4) because all interfering substances cannot be removed. When the anthrone reaction is used for the estimation of carbohydrates in the presence of other substances, an absorption curve should be run to check for possible interferences.

**References**