A Versatile Copper Reagent for Sugar Chemistry Demonstrations

By F. W. PRICE

Laboratory experiments and demonstrations in high school and college courses in biology and biochemistry usually include experiments on the identification and composition of nutritionally important sugars. Those usually dealt with are the monosaccharides glucose, fructose, and galactose and the disaccharides maltose, sucrose, and lactose. One experiment is the well-known test with Benedict's reagent, which is an alkaline solution of cupric ions in which the copper is complexed with citrate. Sugars possessing an available aldehyde group reduce alkaline copper solutions when heated with them, and a characteristic orange or brick-red precipitate of cuprous oxide is formed. All of the previously mentioned sugars except sucrose give this reaction; hence they are called reducing sugars.

Limitations of Benedict's Solution

Alkaline copper solutions such as Benedict's are sensitive but nonspecific; that is, they detect reducing properties but do not help to identify different individual sugars. A common laboratory experiment shows how the nonreducing disaccharide sucrose is converted by acid hydrolysis to a reducing substance by testing with Benedict's reagent before and after carrying out the hydrolysis reaction. The implication is that the sucrose has been converted to glucose and fructose; however, one cannot justifiably deduce this from these results alone. The hydrolytic action of dilute acid or amylases on starch solution is similarly demonstrated by the subsequent reduction of Benedict's reagent; but again one cannot, strictly speaking, conclude that glucose or any other sugar is the end product of hydrolysis. Maltose is hydrolyzed to glucose by maltase. Benedict's reagent would be of no use in demonstrating this because both maltose and glucose are reducing sugars.

Classroom demonstrations of sugar chemistry would be more interesting and logical if a copper reagent were used that would (i) give a qualitatively distinctive reaction with disaccharides, irrespective of reducing properties, which is quite different from that given by any of the common disacharides; and (ii) enable individual disaccharides to be characterized by detection of the nonglucose component in the acid hydrolysate by reactions that are qualitatively characteristic of these monosaccharides. The following tells how to prepare and use a copper reagent that fulfills these criteria.

The Cuprammonium Reagent

Preparation

To a 5% w/v [weight per volume] aqueous solution of cupric sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$,

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add strong (S.G. 0.880) from a buret with continual swirling. Just enough ammonia should be added to redissolve the initial precipitate of cupric hydroxide, and the pH should now be exactly 9.7. The resulting deep-blue solution of cuprammonium sulfate seems to be stable for many months at room temperature.

Reaction of Disaccharides

When cuprammonium reagent, with or without added water, is heated in a test-tube in a boiling water bath for five minutes, a dark-brown film, presumably of cupric oxide, appears on the walls of the test-tube below the liquid surface. Under specified conditions (described below) monosaccharides prevent this reaction but disaccharides do not. This "brown film" reaction may therefore be used to distinguish between the two classes of sugars. To 5 ml of cuprammonium reagent in a test-tube add 0.5 ml of a 5% w/v solution of disaccharide (lactose, glucose, or maltose) and place in a boiling water bath for exactly five minutes. Remove and examine. The presence of a dark-brown, adherent film on the sides of the test-tube indicates the presence of disaccharide. Monosaccharides do not give the test under these conditions.

Reaction of Monosaccharides

Into a test-tube place 5 ml of cuprammonium reagent, 5 ml of 5% w/v solution of monosaccharide (glucose, galactose, or fructose) and mix. (At room temperature, glucose gives a pale-blue precipitate after standing a few minutes. This reaction is not given by any other monosaccharide so far tested; therefore it appears to be truly specific for glucose within the carbohydrate class.) Place the tube in a boiling water bath and leave for exactly five minutes without agitating or disturbing it. Remove and examine. The three monosaccharides each give distinctive reactions (table 1).

Characterization of the Disaccharides

Because the three monosaccharides that enter into the composition of the above-mentioned disaccharides give distinctive color reactions with cuprammonium reagent, the composition of the disaccharides may be demonstrated by acid hydrolysis followed by testing with cuprammonium reagent. This should reveal the nature of the monosaccharide constituents. Maltose yields glucose only on hydrolysis, and lactose and sucrose give galactose and fructose, respectively, in addition to glucose. Fortunately, glucose does not interfere with the characteristic reactions given by galactose and fructose with cuprammonium reagent. Hence their presence in the hydrolysates of the respective disaccharides may be demonstrated and the identity of the parent disaccharide thereby proven.

Table 1. Reactions of 5% w/v monosaccharide solutions (5 ml) with cuprammonium reagent (5 ml).

Monosaccharide	*Supernatant*	*Precipitate*
Glucose	blue	light blue,* copious
Fructose	deep yellow	dark brown, granular
Galactose	pale yellow	dark green, flocculent

* Precipitate appears at room temperature shortly after mixing the cuprammonium reagent with the glucose solution.

Table 2. Reactions of 5% w/v disaccharide solutions (5 ml) with cuprammonium reagent (5 ml) before and after acid hydrolysis.

	Before hydrolysis		*After hydrolysis*	
Disaccharide	*Supernatant*	*Precipitate*	*Supernatant*	*Precipitate*
Lactose	blue	light blue, copious	pale yellow	dark green, flocculent
Maltose	blue	none	blue	light blue,* copious
Sucrose	blue	brown film on walls of tube	deep yellow	dark brown, granular

*Precipitate appears at room temperature shortly after mixing the cuprammonium reagent with the maltose hydrolysate.

The hydrolysis of the disaccharides is carried out as follows:

To 50 ml of 10% w/v lactose solution, contained in a flask or large test-tube, add 5.0 ml of 10N hydrochloric acid. Mix, stand in a water bath, and bring to the boil. Continue boiling for 15 minutes; then cool and neutralize to about pH 8–9 by addition of 10N sodium hydroxide (about 5 ml). Maltose is treated in exactly the same way except that a 5% w/v solution is used. Under these conditions hydrolysis of lactose and maltose is nearly complete. Sucrose is hydrolyzed by taking 50 ml of a 10% w/v solution and adding 0.5 ml of 10N hydrochloric acid. The contents of the tube are brought to 100 C in a boiling water bath and kept at that temperature for two or three minutes, followed by rapid cooling. Under these conditions complete hydrolysis of sucrose occurs. If stronger acid is used or if heating is prolonged, considerable darkening of the sucrose solution will occur. Neutralize the hydrolysate by dropwise addition of 10N sodium hydroxide.

That the disaccharides have been destroyed is shown by performing the "brown film" test, which will now be negative. The presence of glucose in the maltose hydrolysate and of fructose and galactose in the sucrose and lactose hydrolysates, respectively, may be shown by performing the test for the monosaccharides described previously. The reactions given by the hydrolysates are those characteristic of glucose (in the case of the maltose hydrolysate) and of fructose and galactose (sucrose and lactose hydrol-

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Fig. 5. Students in a science methods class try out each other's autoinstructional kits.

pupil for trial use. "Bugs" in the program are identified from results of the pre-test and post-test and from subsequent discussions between the developer and the user. Failing to make objectives and directions clear, omitting critical material, providing too much content for the intended time-span, and making the post-test too difficult are faults commonly uncovered.

Kits are revised on the basis of feedback from the pupil trial and brought to a meeting of the methods class, where they are exchanged among class members for a second trial. Each kit is reviewed and evaluated by a class member and the instructor in terms of criteria previously established for kit development. In this way each student is exposed to the work of his classmates and benefits from the general discussion that follows.

Admittedly it takes an exceptionally able teacher to package his teaching into a shoebox that can be used by his pupils without additional assistance. Most of the kits developed by beginners fall short of the ideal set by the kit-development criteria. One might logically ask: "What, then, do the kit-developers learn?" In general they become sensitive to the need for controlling vocabulary, stating objectives succinctly, clarifying explanations, and relating material to the pupil's abilities, interests, and motives. In addition, they often find that their understanding of basic science concepts increases markedly as they work through the process of developing successful autoinstructional kits to teach these concepts.

One important reason for using the autoinstructional-kit learning procedure in our methods classes is that it introduces a mode of teaching that is consistent with the principles of educational psychology and with the current emphasis on individualizing instruction in the classroom. Inviting methods-class students to participate in the development of autoinstructional programs encourages active rather than passive participation in the learning process while at the same time increasing the students' awareness of the necessity for individualizing classroom instruction. Methods-course students often use their autoinstructional kits in student teaching along with other individualized materials. Thus it is our conviction that the development of autoinstructional kits and other, comparable material in college methods classes causes future teachers to be exceptionally predisposed to the use of individualized materials in their science-teaching.

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ysates); that is, the nonglucose components of the disaccharide in the case of lactose and sucrose. Fortunately, unchanged disaccharide does not interfere with the monosaccharide reactions. Here it should be mentioned that the monosaccharide test, if carried out with the disaccharide solutions (5 ml sugar solution plus 5 ml of reagent), gives reactions that are quite different from the hydrolyzed disaccharides (table 2).

Discussion

Cuprammonium compounds are well known but do not appear to have been previously used as sugar-analysis reagents. The reagent described here is superior to Benedict's reagent in that if carefully used it gives a wide variety of color and precipitation reactions with various common sugars and permits individual sugars to be identified.

There are, of course, many organic reagents that enable different types of sugars to be identified; but some of these may not always be easily or cheaply available and are usually best prepared fresh before use. On the other hand, the cuprammonium reagent is quickly and conveniently prepared from inexpensive and easily available laboratory chemicals and is stable for months.

The color reactions of sugars with the cuprammonium reagent is an empiric finding, and the chemistry involved may be complex. A possible approach to elucidation of the mechanisms involved is suggested by the fact that the cuprammonium ion forms complexes with polyhydroxy compounds including sugars (R. E. Reeves, 1951: "Cuprammonium–Glycoside Complexes," *Advances in Carbohydrate Chemistry* 6: 107-134). It may be that this is involved in the reactions of sugars with cuprammonium reagent to give the observed variety of appearances.