DETECTION OF NON-REDUCING CARBOHYDRATE COMPOUNDS WITH COMPLEX CUPRATES(III)

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INTRODUCTION

Owing to the anomalous valency state of the central copper atom the complexes of trivalent copper act as powerful oxidizing agents, especially in an alkaline medium. This property of the complex bound copper(III) was used for the first time by BECK[1] as a semi-quantitative analytical method for the oxidative degradation of proteins. By far the most useful application of these complex copper(III) salts is to the oxidation of polyhydroxy compounds. This reaction can be followed electrometrically or spectrophotometrically and has been used *e.g.* in the quantitative estimation of tartrates[2] or for the detection of sugars and their derivatives on paper chromatograms[3]. The potassium periodatocuprate(III) reagent used by BONNER[3] for this purpose has in the complex anion the ligand of another strong oxidizing agent, namely periodate. It seemed interesting to investigate the detection of carbohydrates and other organic compounds by means of the telluratocuprate(III) anion $[Cu(TeO_6)]^{9-}$, where the only oxidizing agent is the trivalent copper. The different oxidizing properties associated with the two complex ions were considered as a possible means of distinguishing between the structures of various compounds. This means of identification proved unsuccessful and in fact on comparing our results with those of BONNER[3], no qualitative difference could be found in the action of periodatocuprates(III) and telluratocuprates(III). The new reagent, however, is worth investigating for its high sensitivity to a wide range of organic compounds, *e.g.* the various non-reducing carbohydrates dealt with in this paper.

EXPERIMENTAL

Reagent

The 0.05 *M* telluratocuprate(III) solution was prepared as described by JENŠOVSKÝ[4-6] and BONNER[3], using the equivalent amount of potassium tellurate instead of potassium periodate.

Sample solutions

The samples were dissolved in ethyl alcohol in concentrations of 0.1 % w/v.

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Paper chromatography

The descending technique was employed, using Whatman No. 1 or No. 3 filter paper in a *n*-butanol–acetic acid–water (10:1:3) system, except in the case of the higher oligosaccharides, where the system *n*-propanol–ammonia–water (6:3:1) was used.

Detection of the spots

The paper chromatograms were thoroughly dried and, when free from all acid residues, were sprayed with the reagent. The sugar compounds give white spots on a distinctive deep yellow-brown background. The background disappears after a few minutes but a permanent record of the spots can be made either by marking with pencil or by spraying the chromatogram, immediately after the maximum contrast of the spots has been reached, with a solution of rosaniline as described by BONNER[3]. Red spots on a greenish yellow background were then obtained. A second method for stabilizing the chromatograms, especially useful where Whatman No. 3 paper was used, consisted in spraying with TTC* solution (2 % w/v sol. of TTC in water mixed with the same volume of 1 *N* NaOH) at 50–60° in the dark[7,8]. At the positions occupied by the oxidized compounds deep rosy spots appeared on a pink background. For the location of water insoluble carbohydrate derivatives, the standard dipping technique was found more suitable than spraying.

TABLE I

R_F VALUES OF NON-REDUCING FREE OLIGOSACCHARIDES, GLYCITOLS, GLYCOSIDES, GLYCOSANS AND GLYCONIC ACID LACTONE LOCATED BY MEANS OF TELLURATOCUPRATE(III) REAGENT WITH A SENSITIVITY OF 1 μg

Compound	*Solvent system*	R_F
Saccharose	*n*-BuOH–AcOH–H_2O (10:1:3)	0.08
Trehalose		0.55
Raffinose	*n*-PropOH–NH_4OH–H_2O (6:3:1)	0.48
Stachyose		0.41
D-Glucitol (Sorbitol)	*n*-BuOH–AcOH–H_2O (10:1:3)	0.05
1,5-Anhydro-D-glucitol		0.25
D-Mannitol		0.05
myo-Inositol		0.05
Methyl β-D-glucopyranoside	*n*-BuOH–AcOH–H_2O (10:1:3)	0.22
n-Propyl β-D-glucopyranoside		0.61
Isopropyl β-D-glucopyranoside		0.55
Isobutyl β-D-glucopyranoside		0.73
tert.-Butyl β-D-glucopyranoside		0.64
Cyclohexyl β-D-glucopyranoside		0.72
Phenyl β-D-glucopyranoside		0.55
m-Hydroxyphenyl β-D-glucopyranoside[a]		0.52
o-Hydroxymethylphenyl β-D-glucopyranoside (Salicin)		0.52
Phenyl β-cellobioside		0.35
1,6-Anhydro-β-D-glucopyranose	*n*-BuOH–AcOH–H_2O (10:1:3)	0.45
D-Galactonic-γ-lactone	*n*-BuOH–AcOH–H_2O (10:1:3)	0.21

[a] *m*-Hydroxyphenyl β-D-glucopyranoside gives, as all free phenols, a stable red-brown coloration without a further stabilizing spray.

* Triphenyltetrazolium chloride.

TABLE II

R_F VALUES OF VARIOUS SUBSTITUTED CARBOHYDRATE COMPOUNDS DETECTED BY MEANS OF TELLURATOCUPRATE(III) REAGENT WITH A SENSITIVITY OF 5 TO 10 μg
System *n*-butanol–acetic acid–water (10:1:3).

Compound	R_F
1,2,3,6,2',3',4',6'-Octa-O-acetyl-β-maltose	1.0
3,4,6-Tri-O-acetyl-D-glucal	1.0
1,2-O-Isopropylidene-α-D-glucofuranose	0.70
1,2,5,6-Di-O-isopropylidene-α-D-glucofuranose[a]	0.98
1,2-O-Isopropylidene-5,6-anhydro-α-D-glucofuranose	0.95
1,6-Anhydro-2-O-*p*-tolyl-β-D-altropyranose[b]	0.85

[a] Does not react with the telluratocuprate(III) but develops a deep rosy coloration with the TTC reagent without any previous treatment. This remarkable reaction is difficult to explain as the compound is perfectly stable in alkaline medium and has one single hydroxyl group in the C-3 position.

[b] Sensitivity = 50 μg.

RESULTS

The miscellaneous carbohydrate compounds tested for the applicability of the telluratocuprate(III) reagent can be divided into three groups according to the sensitivity of the reagent.

(1) The unsubstituted sugars, glycosides, glycosans and anhydrosugars are detectable in amounts of 1 μg.

(2) In substituted sugars, *e.g.* in fully acylated sugars easily hydrolysable in the strong alkaline medium, or in various alkylidene derivatives with at least one pair of vicinal hydroxyl groups, the sensitivity varies between 5 and 10 μg. In some cases, *e.g.* 1,6-anhydro-2-O-*p*-tolyl-β-D-altropyranose, a compound with a single hydroxyl pair adjacent to the large *p*-tolyl group, the limit of sensitivity was shifted to 50 μg.

(3) The non-reactive substances include especially the alkali-stable polytopic alkylidene and arylidene derivatives such as the various di- and tri-benzylidene or isopropylidene derivatives of monoses and glycitols, *e.g.* 1,2,5,6-di-O-isopropylidene-α-D-glucofuranose.

The telluratocuprate(III) reagent has been used with success for the detection of sugar compounds in plant extracts and in mixtures of enzymic transglycosylation products. R_F values of carbohydrate compounds located with the reagent are summarized in Tables I and II.

DISCUSSION

The results summarized in this paper as compared with those of BONNER[3] indicate the interesting fact that in both periodatocuprates(III) and telluratocuprates(III) applied as locating reagents in paper chromatography the only oxidizing agent is the trivalent copper. The sensitivity of telluratocuprate(III) is in most cases even slightly higher than that of periodatocuprate(III) as, after spraying, a picture is developed in which white spots show up very vividly against a deep yellow-brown background.

SUMMARY

The use of potassium telluratocuprate(III) is described for the detection of non-reducing carbohydrate compounds on paper chromatograms. The sensitivity of the reagent is 1 μg to unsubstituted sugars and glycosides and 5 to 10 μg to a number of carboxylic acid esters and alkylidene carbohydrate derivatives tested.

REFERENCES

1 G. BECK, *Mikrochemie ver. Mikrochim. Acta*, 35 (1951) 1, 152.
2 L. JENŠOVSKÝ, *Collection Czech. Chem. Commun.*, 22 (1957) 313.
3 T. G. BONNER, *Chem. & Ind. (London)*, 1960, 345.
4 L. JENŠOVSKÝ, *Chem. Listy*, 50 (1956) 1956; *C. A.*, 50 (1956) 14438.
5 L. JENŠOVSKÝ, *Z. Anorg. Allgem. Chem.*, 307 (1961) 208.
6 L. JENŠOVSKÝ, *Z. Anorg. Allgem. Chem.*, 307 (1961) 219.
7 K. WALLENFELS, *Naturwissenschaften*, 37 (1950) 491.
8 W. E. TREVELYAN, D. P. PROCTER AND J. S. HARRISON, *Nature*, 166 (1950) 444.