Carbohydrate Research, 28 (1973) 387–389

Note

Periodate oxidation of 2-acetamido-2-deoxy-β-D-glucopyranosyl residues*†

JAMES ELTING, CHENG-CHUN HUANG, AND REX MONTGOMERY‡
Department of Biochemistry, The University of Iowa, Iowa City, Iowa 52242 (U. S. A.)
(Received January 12th, 1973; accepted March 1st, 1973)

The consumption of periodate by carbohydrates has been measured by titrimetric[1] and spectrophotometric[2,3] procedures. The former method determines the amount of periodate remaining in the oxidation reaction by reagents that are specific for that ion, leading therefore to minimal ambiguity. The spectrophotometric methods depend upon the assumption that only the periodate ion absorbs significantly at 222.5 nm[2], 260 nm[3], or 290 nm[4], these wavelengths being chosen to reduce the interference from the iodate ion.

Structural studies of the carbohydrate in ovalbumin have included the Smith degradation of L-β-aspartamido–carbohydrate fragments[5]. The consumption of periodate by two of these fragments, identified[6] as AC-A and AC-C, was monitored spectrophometrically at 260 nm and found to be less than the theoretical amounts by 0.8 and 1.1 moles IO_4^- per mole of glycopeptide, respectively. As two residues of hexosamine were oxidized[5] in each mole of AC-A and AC-C, an explanation for the discrepancy was sought by studying the reaction of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**) with periodate. The results obtained by titrimetry agreed with those reported earlier[7]. The spectrophotometric assays were significantly lower (Table I), and suggested that the oxidation product (**2**) of **1** absorbed at 222.5 nm and 260 nm. Isolation of **2** as an amorphous solid showed λ_{max} 272 nm, ε_{max} 220, ε_{260} 180, and at wavelengths below 240 nm the absorbance increased sharply, with the extinction coefficient at 222.5 nm being of the same order as that at 260 nm and 290 nm. The addition of iodate or periodate to aqueous solutions of **2** gave no indication by spectrophotometry of the formation of complex species. The absorbance of **2** at 260 and 290 nm disappeared after reduction with borohydride, although some absorption at 222.5 nm persisted due to the acetamido chromophore.

From the spectral data it can be calculated that the interference of the IO_3^- and **2** in the oxidation solution accounted for approximately 70–80% of the error found in the spectrophotometric procedure. By extrapolation of the rate of oxidation to zero time, the spectrophotometric procedure indicated 0.70 moles of periodate consumed

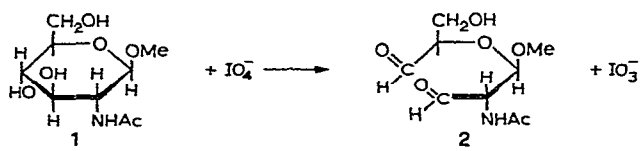
*Dedicated to Dr. Louis Long, Jr., in honor of his 70th birthday.
†This investigation was supported by research grant GM-14013 from the National Institutes of Health.
‡To whom requests for reprints should be made.

TABLE I

OXIDATION OF METHYL 2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSIDE WITH SODIUM METAPERIODATE

Time (h)	*Moles IO_4^- consumed per mole of glycoside*		
	Titrimetric	*Spectrophotometric*	
		260 nm	*222.5 nm*
5	0.51	0.25	0.30
24	1.10	0.62	0.66
51	1.11	0.73	0.75
96	1.13	0.74	0.77
Extrapolation to 0	0.96	0.70	0.70

per mole of **1**, that is, approximately 0.3 moles less than theory. The oxidation of fragments AC-A and AC-C by the same spectrophotometric method as used for **1** gave values of 0.4 and 0.5 moles, respectively, less than theory for periodate consumed per hexosamine residue oxidized. It appears, therefore, that a significant part of the discrepancy may be explained by the absorbance of the oxidized *N*-acetylhexosamine residues in AC-A and AC-C at the wavelength of that assay (260 nm). Furthermore, the error is not likely to be reduced by conducting the assay at 222.5 nm or 290 nm.



1 2

TABLE II

SPECTRAL PROPERTIES OF THE OXIDIZED PRODUCT 2

Property	*Compound* **1**	*Periodate*	*Compound* **2**	*Iodate*
λ_{max} (nm)	<220	222.5	272	<220
ε_{max}		1.07×10^4	220	
ε_{260}	0	1.01×10^3	180	66
$\varepsilon_{222.5}$	92			
I.r. (μm)	3.0, 6.0, 6.4, 9.5		3.0, 6.0, 6.5, 9.7	
P.m.r.	$-OCH_3$ 2.1		2.1	
(p.p.m.)	$-NCOCH_3$ 3.6		3.5	

EXPERIMENTAL

Periodate oxidation of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**). — Aqueous solutions of **1** (0.01M) were oxidized with sodium metaperiodate (0.050M) at 5° in the dark. The reaction was monitored for 96 h by titrimetry and u.v. spectros-

copy at 222.5 nm and 260 nm, exactly as described previously[1-3]. The results are summarized in Table I.

Isolation of oxidation product **2**. — The oxidation of **1** with periodic acid (0.025M) at 5° in the dark was stopped after 48 h by neutralization with a saturated solution of barium hydroxide. After 12 h at 5° the precipitated barium salts were filtered off and the filtrate deionized with Rexyn-300 (H^+, OH^-) resin. The resin had been extensively washed with water before use and shown to yield no u.v.-absorbing material. The u.v. spectrum of the resulting aqueous solution was determined before freeze-drying the solution to an amorphous, colorless residue (**2**) that was dried to constant weight *in vacuo* at room temperature (26°). The u.v. spectrum of **2** in distilled water was the same as that found before dehydration (Table II).

The homogeneity of **2** was indicated by reduction with sodium borohydride to the corresponding trihydric alcohol, the *O*-trimethylsilyl derivative of which was chromatographed on a column (6 ft × 1/8 in.) of 3% SE-52 on Chromosorb W (80 mesh), the temperature being programmed from 150° to 210° at 8° per min. The derivative of **2** emerged as a single peak, having a retention time of 0.40 compared with the *O*-trimethylsilyl derivative of **1**.

The i.r. spectrum of **2** in KBr was similar to **1** in its main absorbances and showed no strong absorbance for aldehyde C=O. Also, the p.m.r. spectra of **1** and **2** in D_2O showed similar chemical shifts from internal tetramethylsilane for the $-OCH_3$ and $-NCOCH_3$ protons.

REFERENCES

1 F. Smith and R. Montgomery, *Methods Biochem. Anal.*, 3 (1956) 153.
2 J. S. Dixon and D. Lipkin, *Anal. Chem.*, 26 (1954) 1092.
3 C. Grado and C. E. Ballou, *J. Biol. Chem.*, 236 (1961) 54.
4 T. Ikenaka, *J. Biochem. (Tokyo)*, 54 (1963) 328.
5 C.-C. Huang and R. Montgomery, *Federation Proc.*, 31 (1972) 1415.
6 C.-C. Huang, H. E. Mayer, and R. Montgomery, *Carbohyd. Res.*, 13 (1970) 127.
7 R. W. Jeanloz and E. Forchielli, *J. Biol. Chem.*, 188 (1951) 361.