Specific Cleavage of *O*-Glycosidic Bonds to L-Serine and L-Threonine by Trifluoroacetolysis

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We recently demonstrated that transamidation is effected by treating amides with a mixture of trifluoroacetic acid (TFA) and trifluoroacetic anhydride (TFAA),[1] under conditions when most glycosidic linkages are stable.[2] As the *N*- and *O*-trifluoroacetyl groups introduced during this trifluoroacetolysis are readily hydrolysed off, this should permit the isolation of those oligosaccharide chains in glycoproteins which are *N*-glycosidically linked to L-asparagine. This was also realized when desialylated fetuin was subjected to trifluoroacetolysis.[3] Simultaneously, however, the oligosaccharide chains which are *O*-glycosidically linked to L-serine and L-threonine were also released. In both reactions, part of the 2-amino-2-deoxyhexose residues released during the trifluoroacetolysis was degraded and split off.

Cleavage of *O*-glycosidic linkages to L-serine and L-threonine in glycoproteins and proteoglycans is generally performed by treatment with strong base in the presence of borohydride,[4] a base-catalysed β-elimination followed by reduction of the released glycose residues. The cleavage of the same linkages during trifluoroacetolysis is obviously also a β-elimination, but acid-catalysed.

In order to study this reaction *O*-β-D-xylopyranosyl-L-serine (*1*),[5] *O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-L-serine (*2*)[6] and *O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-fucopyranosyl-L-threonine (*3*)[7,8] were subjected to trifluoroacetolysis under conditions given in Table 1. The products were deacetylated by treatment with 50 % aqueous acetic acid, reduced with sodium borodeuteride, acetylated and analysed by GLC. D-Mannose and maltose, which are not degraded during trifluoroacetolysis,[9] were added as internal standards. The deacetylated and reduced products were identified by GLC-MS, the monomers as their acetates, and the dimers as their fully methylated products. The methylated disaccharide alditol derived from *2* gave an MS indistinguishable from that of an authentic sample, and all spectra were in agreement with the postulated structures.[10]

Table 1. Trifluoroacetolysis of *O*-glycosides of L-serine and L-threonine and of 4-*O*-β-D-galactopyranosyl-D-xylose (*4*). Reactions were performed at 100 °C for 48 h.

Substance	TFA-TFAA	Product	Yield, %
1	1:1	D-Xyl	92
1	1:50	D-Xyl	84
2	1:1	D-Gal	95
2	1:50	β-D-Gal*p*-(1→4)-D-Xyl	92
3	1:1	β-D-Glc*p*-(1→3)-L-Fuc	97
3	1:50	β-D-Glc*p*-(1→3)-L-Fuc	98
4	1:1	D-Gal	94
4	1:50	β-D-Gal*p*-(1→4)-D-Xyl	96

As is evident from the results given in Table 1, the cleavage of the glycosidic linkage to L-serine or L-threonine on trifluoroacetolysis is quantitative. Under the milder conditions (TFA-TFAA, 1:50), the recovery of the sugar moiety is also quantitative. Under the more severe conditions (TFA-TFAA, 1:1), the sugar moieties from *1* and *3* were also recovered unchanged, but the xylose residue in 4-*O*-β-D-galactopyranosyl-D-xylose (*4*) was degraded and split off. The same results were obtained when disaccharide *4* was subjected to trifluoroacetolysis. The reason for the lability of this disaccharide is not clear, but may be due to the absence of an electron-withdrawing effect from a 4-*O*-trifluoroacetyl group or to the impossibility of forming a D-xylofuranose group with a stabilizing trifluoroacetyl group on *O*-5.

The present results threrefore demonstrate that trifluoroacetolysis is a useful reaction for the release and isolation of oligosaccharide or polysaccharide chains *O*-glycosidically linked to L-serine or L-threonine. When a 2-amino-2-deoxyhexose residue is released, it is partially degraded and split off,[3,9] and other glycose residues may also be degraded under severe reaction conditions.

Experimental. Concentrations were performed under reduced pressure at bath temperatures not exceeding 40 °C. GLC was performed on a Perkin-Elmer 3920 instrument fitted with a flame ionisation detector, using a glass capillary column (25 m × 0.25 mm), wall-coated with SE-30 (LKB-Products, Stockholm, Sweden) at 200–320 °C. For GLC–MS, the same column and a Varian MAT 311 A instrument were used. The glass capillary column was connected directly into the ion source of the mass spectrometer.

Trifluoroacetolysis. The glycoside (1 mg), the internal standards (D-mannose, 1 mg and maltose, 1 mg) and TFA-TFAA (1:1 or 1:50, 2 ml) were heated at 100 °C for 48 h in a sealed glass tube. (CAUTION: The reaction mixture is under pressure and highly corrosive, and ade-

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0302-4369/79/030230-02$02.50

quate precautionary measures should be taken.) After cooling to 20 °C, the tube was opened, the solution concentrated to dryness and dissolved in 50 % aqueous acetic acid (5 ml). After 1 h at room temperature, the solution was concentrated to dryness, the product reduced with aqueous sodium borodeuteride and worked up as usual. Part of the product was acetylated and investigated by GLC-MS, another part (from *2*, *3* and *4*) was permethylated [11] and analysed by GLC-MS.

Acknowledgements. We are indebted to Mrs. Lisa Palm-Svensson for her expert technical assistance. This work was supported by grants from the Swedish Medical Research Council (03X-4956), the Swedish Natural Science Research Council and the Medical Faculty, University of Lund.

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Received August 28, 1978.*

* *Editor's note.* Due to circumstances beyond our control, this manuscript has regrettably been delayed.

Dependence of Equilibrium Geometry and Rearrangement Modes on Electron Count in One Class of Trinuclear Complexes of Acetylene

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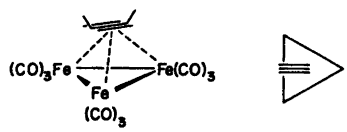
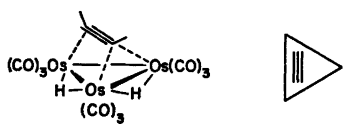
The reaction of ethylene with $M_3(CO)_{12}$, M = Ru, Os leads to two types of complexes in both of which two hydrogens have been stripped from the ethylene.[1] One has a vinylidene ligand, while the other is acetylenic. Another acetylene complex is observed when $Fe_2(CO)_9$ is treated with diphenylacetylene.[2] In both compounds the acetylene is sitting above a triangular base of metals and their associated carbonyls. However, the orientation of the organic π system differs in the two clusters. In $Fe_3(CO)_9C_2Ph_2$ the acetylene is perpendicular to a metal–metal bond, *1*, whereas $H_2Os_3(CO)_9C_2H_2$ has the acetylene parallel to a metal–metal bond, *2*, as do several isoelectronic molecules.[3] We show that this is a consequence of the different electron counts in the two systems (the Os complex has two more electrons than the Fe, if we consider the H's as protonic.), and we calculate a potential energy surface for the intricate relative motion of the acetylene and the metal frame.



1



2

The preference for the perpendicular conformation in the iron system is a consequence of the symmetry properties of the acetylene π orbitals and the cluster fragment levels.[4] In conformation *2* of the neutral iron system there is a low-lying empty orbital which is approximately 90 kJ/mol lower than its counterpart *1*. Occupation of that level causes the reversal of the conformational preference, with

0302-4369/79/030231-02$02.50