

The Production of Protein Cross-linking Agents Derived from Natural Carbohydrates

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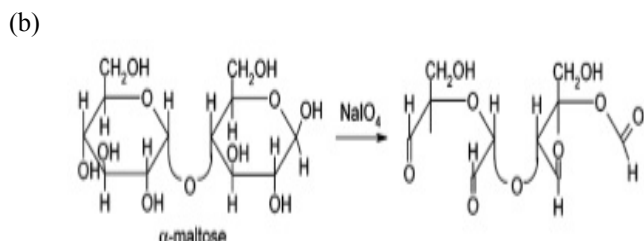
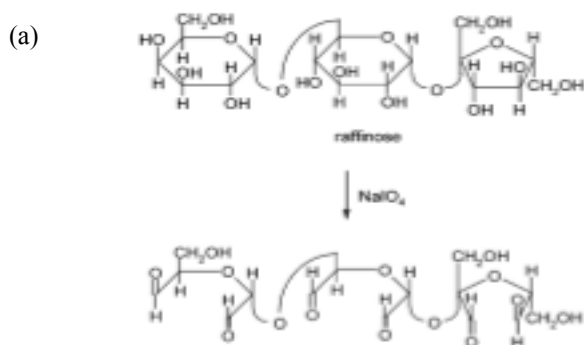
Purpose:

Protein crosslinking agents derived from natural carbohydrates were produced. The polyaldehydes synthesized may be used for cross-linking of proteins and are unique in their poly-functionality and derivation from a natural product.

Introduction:

The premise of this project was to synthesize and characterize novel protein cross-linking agents. Periodate oxidation of 1,2 diols of sugars yields a reagent with aldehyde groups capable of cross-linking proteins.

Dialdehyde cross-linking agents such as glutaraldehyde already exist but here we have synthesized a tetra-aldehyde and a hexa-aldehyde. Carbohydrate-derived cross-linkers offer the potential for relatively low-toxicity as well as an easy method of synthesis. The periodate oxidation of the trisaccharide raffinose and disaccharide maltose was conducted during the course of this project to synthesize a hexa-aldehyde (a) and tetra-aldehyde (b) respectively:



Methodology:

For the synthesis of the hexa-aldehyde approximately 2.5 grams of raffinose was dissolved in 25 ml of water and 4.7g of sodium periodate dissolved in 47 ml of water. The two solutions were mixed at roughly 300 rpm for one hour following which the solution was transferred to a large dish. The solution was allowed to evaporate overnight in the fumehood. Following evaporation, the unpurified product was dissolved for two hours in roughly 15g of methanol per gram of unpurified product (1st extraction) to separate from salt byproducts and impurities. The solution was filtered, transferred to a crystallizing dish, and allowed to evaporate overnight in the fumehood. Occasionally the product wasn't

clearly visible after the evaporation of methanol, the solution was placed in a vacuum oven for approximately an hour.

The protocol used for the production of the 1st extraction of tetra-aldehyde was identical to the one for the hexa-aldehyde except for the following:

- The reaction was conducted with 2.0 g of maltose and 4.9 grams of sodium periodate.
- The 1st extraction was conducted with 35 g of methanol per gram of unpurified product.

In both cases once the 1st extraction product was obtained, a 2nd extraction of the aldehyde product was conducted using a 100g of methanol per gram of 1st extraction product. Upon completion of the 2nd extraction (evaporation of all the methanol), the final polyaldehyde product was stored at 4 °C for future use.

Results:

The two separate oxidation experiments yielded 1 g of hexa-aldehyde and 0.5 g of tetra-aldehyde respectively. The conductivity of both aldehydes was within the 300-600- μ s/cm range, signifying approximately 15% salt contamination. Thin Layer chromatography analysis was conducted using 1% solutions in water of each of the cross-linkers.

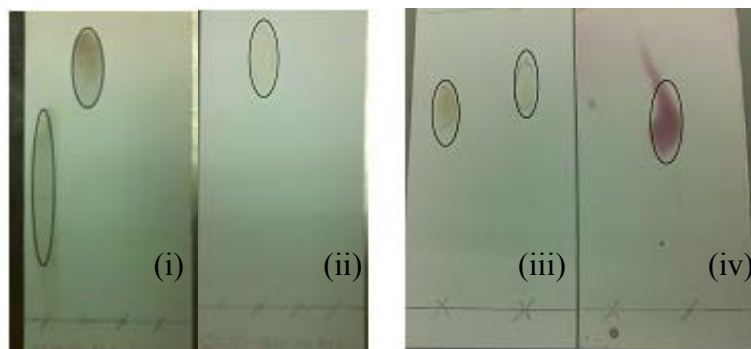


Figure 1: TLC plates initially placed in a chamber filled with 50% methanol and 50 % acetic acid. Plates stained in sulfuric acid (i and iii) or sprayed with Purpald (ii and iv). Pencilled Xs at bottom of each plate indicate where samples were added. Samples, left to right for plates i and ii: Raffinose, Hexa-aldehyde, Sodium iodate, Sodium periodate. Retention factor (Rf) is the rate of travel of sample divided by rate of travel of solvent. Rf hexa-aldehyde=0.9, Rf raffinose=0.51. Samples, left to right for plates iii and iv: maltose, tetra-aldehyde. Rf maltose=0.69, Rf tetra-aldehyde=0.8.

Both the hexa-aldehyde and the tetra-aldehyde were confirmed to be different from the original sugar and the salts (Fig. 1 i, ii). The spraying of Purpald, which detects aldehydes, only labeled the spots representing synthesized products (Fig. 1 ii, iv).

Conclusion:

The methodology explored in this application note is a simple method for producing different carbohydrate-derived cross-linkers. The aldehydes produced during the course of this project will be further explored as protein cross-linkers.