ELSEVIER

Carbohydrate Research 256 (1994) C1–C4

CARBOHYDRATE RESEARCH

Preliminary communication

Protonic and thermal activation of sucrose and the oligosaccharide composition of caramel

Jaques Defaye * and José Manuel García Fernández *,[1]

CNRS and CEA, Département de Recherche Fondamentale sur la Matière Condensée / SESAM, Centre d'Etudes de Grenoble, F-38054 Grenoble, France

(Received December 20th, 1993; accepted January 19th, 1994)

The controlled thermal treatment of sucrose, preferably with an acidic catalyst such as acetic or citric acid, is a manufacturing process used in the large-scale production of caramel. On a smaller scale, the boiling down of a sucrose syrup containing lemon juice is widely used in the preparation of caramel for home-made products. Other industrial processes for the production of so-called "caramel color", used as an additive in beer or soft drinks, involve the heat treatment of sucrose, D-glucose, or starch in the presence of alkaline catalysts [1].

The products of caramelization include volatile and nonvolatile components, and the volatile fraction is reasonably well characterized [1,2]. However, in spite of much effort [1,3] the composition of the major portion of the nonvolatile fraction of caramel, i.e., ~ 95% of the total product, remains poorly characterized [1]. The GLC–EI and –CIMS analysis of permethylated samples [3] has revealed the presence of 1,6-anhydro-β-D-glucopyranose and certain disaccharides such as cellobiose, maltose, isomaltose, and gentiobiose. The presence of difructose dianhydrides has also been suggested. A highly branched fructoglucan has also been claimed to be a constituent of the product of thermal treatment of sucrose in the presence of citric acid [4]. The oligosaccharide composition of caramel is reminiscent of a protonic activation scheme that has been recently investigated in our laboratory for sucrose using anhydrous hydrogen fluoride as catalyst [5]. With this model in mind, the structural elucidation of a major part of the oligosaccharide composition of caramel has now almost been achieved.

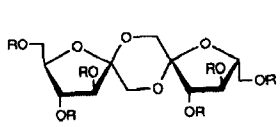
* Corresponding authors.

[1] Present address: Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, E-41071 Sevilla, Spain.

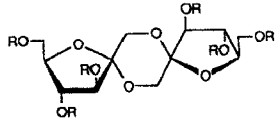
0008-6215/94/$07.00 © 1994 Elsevier Science B.V. All rights reserved
SSDI 0008-6215(94)00047-J

Freeze-dried samples of commercial soft caramel †, from the treatment of sucrose at 160°C in the presence of 0.1% AcOH and containing originally 20–30% water and no more than 0.6% of sucrose, were acetylated [5] in pyridine–Ac_2O. Positive-ion FABMS (*m*-nitrobenzyl alcohol, NaI) of the peracctylated material proved surprisingly simple. There were almost exclusively two well-defined series of cationized quasimolecular ions from (*i*) *m/z* 413 to 2430 and (*ii*) from *m/z* 599 to 2327, respectively, with peaks appearing at intervals of 288 mass units for both series. The first series of peaks evidently relates to acetylated hexose and hexose oligosaccharides up to dp 8, while the second series of quasimolecular ions may relate [5] to acetylated diketohexose dianhydrides and their glycosylated counterparts with 1 to 6 hexosyl substituents.

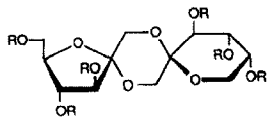
Subsequent fractionation of the crude acetylated caramel by flash chromatography with a gradient of 1:1 → 1:3 light petroleum ether–EtOAc, followed by EtOAc as eluent, resulted in an approximate partition according to molecular weight. The first and second fractions (~ 37%) contained peracetylated D-glucose and D-fructose (^{13}C NMR) as well as residual sucrose. The third fraction (*m/z* 599), ~ 18% of the total mixture, showed the presence of five isomeric acetylated difructose dianhydrides in the respective ratio of ~ 4:12:6:1:2, as determined from the signals [6] in the anomeric region of the ^{13}C NMR spectra. They were found at 103.1 ppm for the di-β-D-fructofuranose 1,2′:2,1′-dianhydride derivative (**1**), at 101.5 and 99.5 ppm for the α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride derivative (**2**), at 101.5 and 95.0 ppm for the α-D-fructofuranose β-D-fructopyranose 1,2′:2,1′-dianhydride derivative (**3**), and at 92.8 and 94.7 ppm for the α-D-fructopyranose β-D-fructopyranose 1,2′:2,1′-dianhydride derivative (**4**). Two further signals, at 102.6 and 98.6 ppm, were assigned to a di-β-D-fructofuranose 1,2′:2,3′-dianhydride hexaacetate (**5**) on the basis of values found for the corresponding 6,6′-diglucosylated derivative [6,7]. The structure of dianhydrides **1**–**5** was fully confirmed following isolation of the pure compounds by subsequent column chromatography using a gradient of 3:1 → 1:1 light petroleum ether–EtOAc and comparison (mp, $[\alpha]_D$, ^{13}C NMR) with authentic samples [8,10].



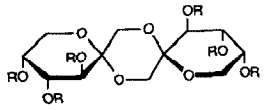
1 R = OAc
6 R = H



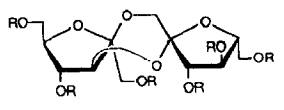
2 R = OAc
7 R = H



3 R = OAc
8 R = H



4 R = OAc
9 R = H



5 R = OAc
10 R = H

† Produced by Nigay, S.A., F-42110 Feurs (France); food ingredient "soft caramel" conforming with AFNOR NF V 00-100, Nigay 1395 SMA6.

The fourth fraction, ~ 17% of the total crude material, showed a net quasi-molecular ion at m/z 701. Signals of acylated anomeric carbon atoms of α- and β-D-glucopyranose moieties were clearly observed at 89.0 and 91.7 ppm, respectively, indicating that this fraction contained the glucobiose components already reported in caramel [3]. Therefore, it was not further characterized.

Two further fractions, accounting for ~ 29% of the total mixture, were also collected by flash chromatography. They exhibited two series of quasimolecular ions in FABMS ranging from (*i*) m/z 887 to 2327 for the first series and (*ii*) from m/z 989 to 2430 for the second series. Intervals of 288 mass units for both series of peaks confirmed that the first was related to glycosylated difructose dianhydrides with 1 to 6 glucosyl substituents. The second series evidently relates to glucooligosaccharides having dp 3–8. No significant difference was found in the ^{13}C NMR spectra of both fractions which, from comparison and correlation of both their FABMS and ^{13}C NMR spectra, differed solely by their dp. Characteristic signals at δ 89.0, 91.7, 95.8, and 101.0–101.5 ppm indicated that both anomeric configurations were present in the glucooligosaccharide series of products. Such behaviour is clearly reminiscent of a glucooligosaccharide reversion product that had been previously isolated by hydrogen fluoride treatment of D-glucose, starch, and cellulose [11].

Signals of the anomeric carbon atoms of substructures of acetylated di-β-D-fructofuranose (103.1 ppm), α-D-fructofuranose β-D-fructofuranose (101.5–101.0 and 99.5 ppm), α-D-fructofuranose β-D-fructopyranose 1,2′ : 2,1′-dianhydride (101.5–101.0 and 95.0 ppm), and di-β-D-fructofuranose 1,2′ : 2,3′-dianhydride (102.6 and 98.6 ppm) were also observed in the ^{13}C NMR spectra of both fractions. According to the FABMS data, such a difructose dianhydride framework must contain from 1–6 glucosyl residues. On the other hand, the absence of signals in the region of 85.0–83.5 ppm in the ^{13}C NMR spectra of these fractions precluded any substitution at O-3 or O-4 of the fructofuranose moieties [5–7]. The glycosylation at O-3, O-4, or O-5 of fructopyranose dianhydride moieties has been found to be much less favoured than that at the primary OH group of fructofuranose subunits, even with much stronger protonating catalysts [5]. Therefore, the glucosylation may occur almost exclusively at primary OH groups in dianhydrides **6–8** and **10**, giving rise to the tri- and tetra-saccharide components. Thus, further glucosylation has to proceed preferentially at the already branched glucosyl residues. This general pattern resembles that previously found for the higher oligosaccharide material produced by activation of sucrose with anhydrous hydrogen fluoride at high sucrose concentration [5]. However, contrary to the latter case in which thermodynamic components predominate, the kinetic difructofuranose dianhydride substructures prevail in caramel.

In conclusion, our preliminary analysis of the oligosaccharide material of "soft caramel" proves the presence of a significant proportion (~ 18%) of difructose dianhydrides **6–10**, and of an almost equivalent amount of glucosylated difructose dianhydride derivatives, besides the previously identified glucobioses [3] and higher oligomers. It must be stressed that large amounts of these spirodioxanyl pseudooligosaccharides may then also be formed in any thermal and proton-catalyzed

treatment of ketose-containing materials. The scope of such conversions on the production of sucrose- and fructooligosaccharide-based food ingredients is currently under investigation in our laboratory.

1. Acknowledgments

The authors thank the Ministerio de Educación y Ciencia of Spain and the CNRS for postdoctoral fellowships to J.M.G.F., and Dr. Henri Nigay (Nigay S.A.) for providing the samples of caramel. The results were obtained in the framework of the "Groupement de Recherche no. 1008" of the CNRS.

2. References

[1] P. Tomasik, M. Palasiński, and S. Wiejak, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 203–278.
[2] L. Cottier, G. Descotes, C. Neyret, and H. Nigay, *Ind. Aliment. Agric.*, 106 (1989) 567–570.
[3] H. Tschiersky and W. Baltes, *Z. Lebensm. Unters. Forsch.*, 189 (1989) 132–137.
[4] M. Manley-Harris and G.N. Richards, *Carbohydr. Res.*, 240 (1993) 183–196.
[5] J. Defaye and J.M. García Fernández, *Carbohydr. Res.*, 251 (1994) 17–31.
[6] J. Defaye and J.M. García Fernández, *Carbohydr. Res.*, 237 (1992) 223–247.
[7] J. Defaye and J.M. García Fernández, *Carbohydr. Res.*, 251 (1994) 1–15.
[8] J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 136 (1985) 53–65.
[9] J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 152 (1986) 89–98.
[10] T. Taniguchi, M. Sawada, T. Tanaka, and T. Uchiyama, *Carbohydr. Res.*, 177 (1988) 13–20.
[11] J. Defaye, A. Gadelle, and C. Pedersen, *Carbohydr. Res.*, 110 (1982) 217–227.