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New group of oligosaccharides excreted in honeydew from scale insects *Stigmacoccus* sp. And *coccus hesperidium* L.

Novo grupo de oligossacarídeos excretado no "honeydew" das cochonilhas *Stigmacoccus* sp. E *coccus hesperidum* L.

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ABSTRACT

*Analysis of the honeydew from scale insects **Stigmacoccus** sp. and **Coccus hesperidium** (L.) were carried out by paper chromatography, FAB-MS and GC-MS. The results showed three components (tri-, tetra- and penta-saccharides) which were composed by 1,4 glucose(s) linked to the glucose of sucrose. The findings therefore imply significant and novel metabolic transformations of sugars by the insect and/or microbial symbionts. From these results, structures and common names as "Stigmatriose, Stigmatetraose and Stigmapentose" were proposed for these oligosaccharides because they were found initially in **Stigmacoccus** sp. honeydew.*

Key words: *Homoptera, oligosaccharides, FAB-MS, CG-MS, structures.*

RESUMO

*Análise da secreção doce ou açucarada produzida pelas cochonilhas **Stigmacoccus** sp. e **Coccus hesperidium** (L.) foi realizada pelas técnicas de cromatografia de papel, bombardeamento de átomo rápido-espectrometria de massas (FAB-MS) e cromatografia gasosa-espectrometria de massas (GC-MS). Os resultados mostraram a presença de três oligossacarídeos (tri-, tetra- e pentassacarídeos) os quais são compostos por uma ou mais moléculas de 1,4-glucopiranose ligadas à posição 1-glucopiranose do dissacarídeo sacarose. A constatação da presença destes oligossacarídeos na secreção doce ou açucarada das espécies citadas sugere um significante e novo metabolismo de transformação de açucares pelo inseto e/ou por simbiontes microbianos. A partir destes resultados, e por não existirem referências sobre o assunto, sugerem-se as fórmulas estruturais e os nomes comuns de "Estigmatriose, Estigmatetraose e Estigmapentaose" pelo fato de terem sido inicialmente encotrados no honeydew de **Stigmacoccus** sp.*

Palavras-chave: *Homoptera, oligosacarideos, FAB-MS, CG-MS, estruturas.*

INTRODUCTION

Scale insects feeding on plant phloem sugars excrete a syrup termed honeydew, as in ergot disease, which contains several oligosaccharides. The synthesis of these oligosaccharides from sucrose by transglucosylation reactions has been proposed as an osmoregulatory mechanism in these insects (FISHER et al., 1984).

Little is known about the composition of the honeydew secreted by Coccoidea, and particularly nothing appears to have been published, based on modern analytical techniques, on the honeydew produced by insects of the genus ***Stigmacoccus*** and also even of the common ***Coccus hesperidum*** L. Concerning the latter, WHITE & MAHER (1953) described a maltosyl-fructofuranoside arising by glucosyl transfer to sucrose. GRAY & FRAENKEL (1953) implied that this "fructomaltose" was widely associated with scale insects, aphids and bees.

Most of the studies on scale insect honeydew oligosaccharides have concentrated on

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Homoptera: Aleyrodidae such as ***Bemisia*** sp. (whiteflies), in which the unusual disaccharide trehalulose was first identified in ***B. tabaci*** (BYRNE & MILLER, 1990). Later, HENDRIX & WEI (1994) identified bemisiose as another ***B. tabaci*** unusual trisaccharide. ISAACS et al. (1998), studying the composition of cucurbit phloem sap and ***B. tabaci*** honeydew, suggested that glucose made up 60% of the honeydew sugars. The cucurbit plant sap contained low levels of sucrose and raffinose. However, no sucrose or melezitose were detected in any honeydew samples. DAVIDSON et al. (1994) suggested that the unusual disaccharide trehalulose, an important constituent in honeydew of ***B. argentifolii***, is produced by obligate intracellular microorganisms residing in this insect's mycetomes. Some larger oligosaccharides in this honeydew may be produced by certain ***Bacillus*** spp. residing in or on the insects, and may contribute with artefacts to the composition of honeydew collected from rather heterogeneous surfaces. Such bacteria are not involved in an obligate relationship with the insect (DAVIDSON et al., 1994). Consequently, the observation of the uncontaminated excretion of honeydew on long (ca. 5 cm) hyaline anal extensions of an yet undescribed species of genus ***Stigmacoccus***, though near ***S. asper*** Hempel (BOGO et al., 1999), prompted the application of modern linkage analysis methodology to define the composition of the oligosaccharides which arise entirely from enteric transformation of the plant sugars, sucrose and glucose, ingested by the scale insect in phloem sap.

MATERIALS AND METHODS

Honeydew from scale insect ***Stigmacoccus*** sp. infesting ***Schizolobium excelsum*** (leguminous native from Amazonia) and ***Coccus hesperidum*** (L.) infecting ***Hedera*** sp. (ornamental garden) were collected (10-20 µl) separately by capillarity into glass tubes.

Oligosaccharides isolation

Whatman No. 3 MM paper was used for the isolation of sugars. The solvent system used was propan-1-ol: ethyl acetate: water (7:1:2). For preparative isolation of sugars, viscous honeydew and standards (fructose, glucose, sucrose and raffinose) were diluted by 10 times and then loaded onto the paper as a spot (100μl) and run for 48-55 hours. To elucidate qualitatively the honeydew sugars, the air dried chromatogram was taken through a dip tank, containing aniline hydrogen phthalate reagent (BOGO, 2001). After drying, the paper was heated at 120 °C in an oven for 20min. Oligosaccharides were eluted preparatively in warm water, repurified where necessary, and freeze-dried prior to saccharide analysis and bioassay.

Linkage analysis. Oligosaccharide composition was determined by a combination of FAB-MS analysis of permethylated derivatives (DELL et al., 1994) and GC-MS analysis after the standard procedure of hydrolysis, reduction and peracetylation had been applied to the permethylated saccharides (CARPITA & SHEA, 1989). GC-MS was performed in a 30-m x 0.2-mm DB-5 capillary column in a temperature gradient 90-190°C (20°C min^{-1}), 190-210°C (1°C min^{-1}), 210-300°C (25°C min^{-1}) in A a Fisons 8000 system in electron impact mode, and B a VG autospek Q system in chemical ionisation mode to show molecular ions of derivatised monosaccharides. Spectra were obtained at an ionising potential of 70 eV and a source temperature of 250°C. The GC-MS injector temperature was set at 350°C.

RESULTS AND DISCUSSION

Paper chromatography of honeydew revealed sugars with chromatographic mobilities corresponding to tri-, tetra- and penta-saccharides. The molecular masses of permethylated tri-, tetra- and penta-saccharides confirmed that they were all hexose polymers with molecular-ions in FAB-MS of m/z 658, 862 and 1067 (M+Na= 681, 885 and 1090), respectively (Figure 1).

The tri-, tetra- and penta-saccharide in ***Stigmacoccus*** sp. honeydew appeared identical to the analogous components of ***C. hesperidum*** honeydew.

From the ion-current intensities of components of tri-, tetra-, and penta-saccharides were revealed a systematic increase in the proportion of 1,4-glucopyranose to 2-fructofuranose and 1-glucopyranose with each unitary increase in the number of monosaccharides (Figure 2).

Linkage analysis by GC-MS fragment (Figure 3) showed for both ***Stigmacoccus*** sp. and ***C. hesperidum*** oligosaccharides, fructofuranose linked in the 2 position, glucopyranose linked in both 1 and 4 positions and glucopyranose linked in the 1 position, as it is in sucrose, indicating the presence of an "Stigma" series of compounds.

Since these oligosaccharides also appear to be novel sugars (LIPTAK et al., 1991), structures were proposed for these oligosaccharides (Figure 4). The common names "Stigmatriose, Stigmatetraose and Stigmapentaose" are suggested for these sugars

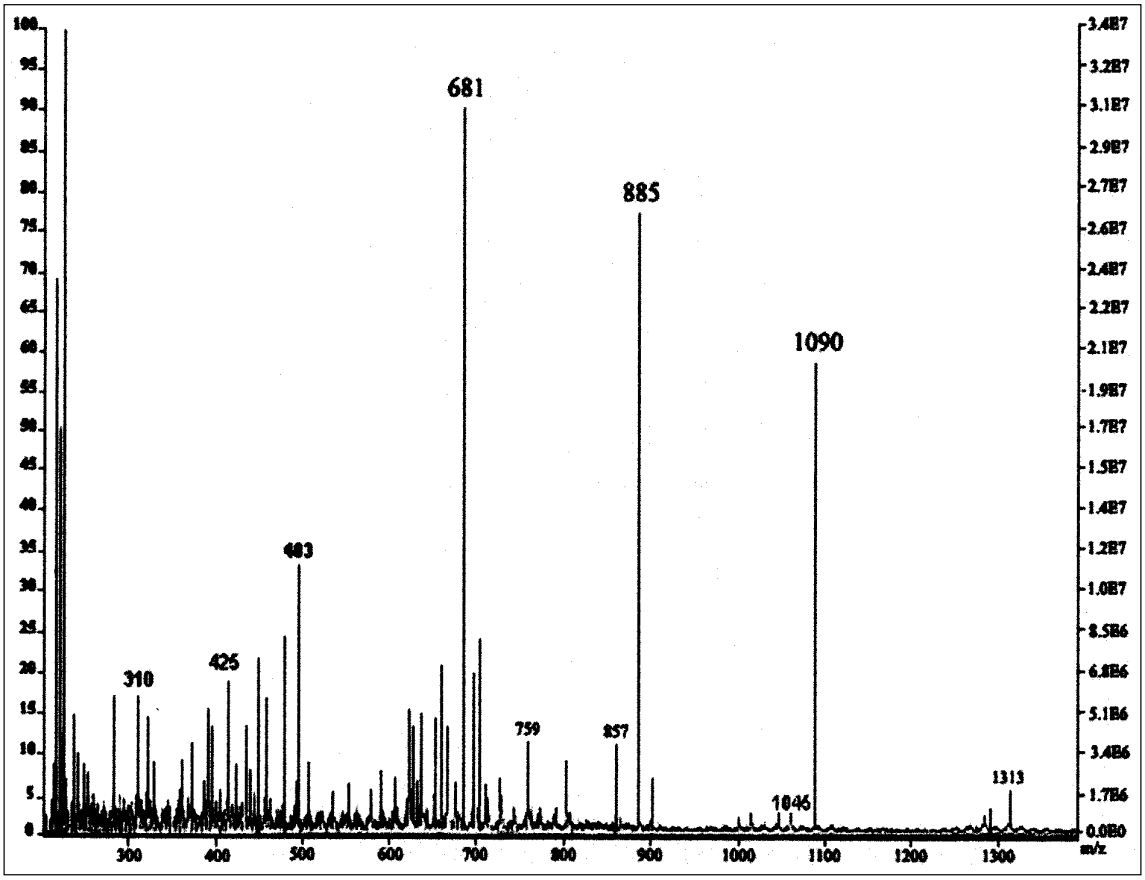

Figure 1 – FAB-MS of permethylated oligosaccharides from ***Stigmacoccus*** sp. The ions m/z 681, 885 and 1090 belong to tri-, tetra- and penta-saccharides, respectively.

because they were found initially in ***Stigmacoccus*** sp. honeydew.

Analysis of the phloem sap from the host plant of the ***Stigmacoccus*** sp. and ***C. hesperidum***, obtained by excising the scale insect *in situ* to leave embedded mouth parts, showed that sucrose and glucose were the only sugars. There was no evidence of any of the oligosaccharides which has been recognised in the excreted honeydew.

Trehalose and trehalulose have already been described as components of ***Myzus persicae*** (FISHER et al., 1984) and ***B. tabaci's*** honeydew (BATES et al., 1990). However, the occurrence of maltose, trehalose, trehalulose and a hexose-hexitol appear to be unique findings for scale insects, extending the range of natural occurrence of these sugars.

The significant difference between the composition of excreted honeydew and the plant sap is attributed to metabolism within the scale insect. At present it is not possible to differentiate between activity by the insect's enzymes and those of any microbial symbionts such as were described for other Coccoidae (DAVIDSON et al., 1994; BATES et al., 1990). However, the present application of modern analytical techniques to even very small amounts of natural material demonstrates the potential for recognising and discovering minor components in very small volumes of scale insect excreta. It also emphasises the complex biotransformations within scale insects, which in the present example of ***Stigmacoccus*** sp. form an integral part of a food web involving a wide range of flying insects. Such insects are seen to feed on the clear colourless honeydew droplets at the end of the long wax anal filaments of insects attached to the bark of large forest trees in Brazil.

Demonstration by linkage analysis should therefore justify full recognition of the "Stigma" series

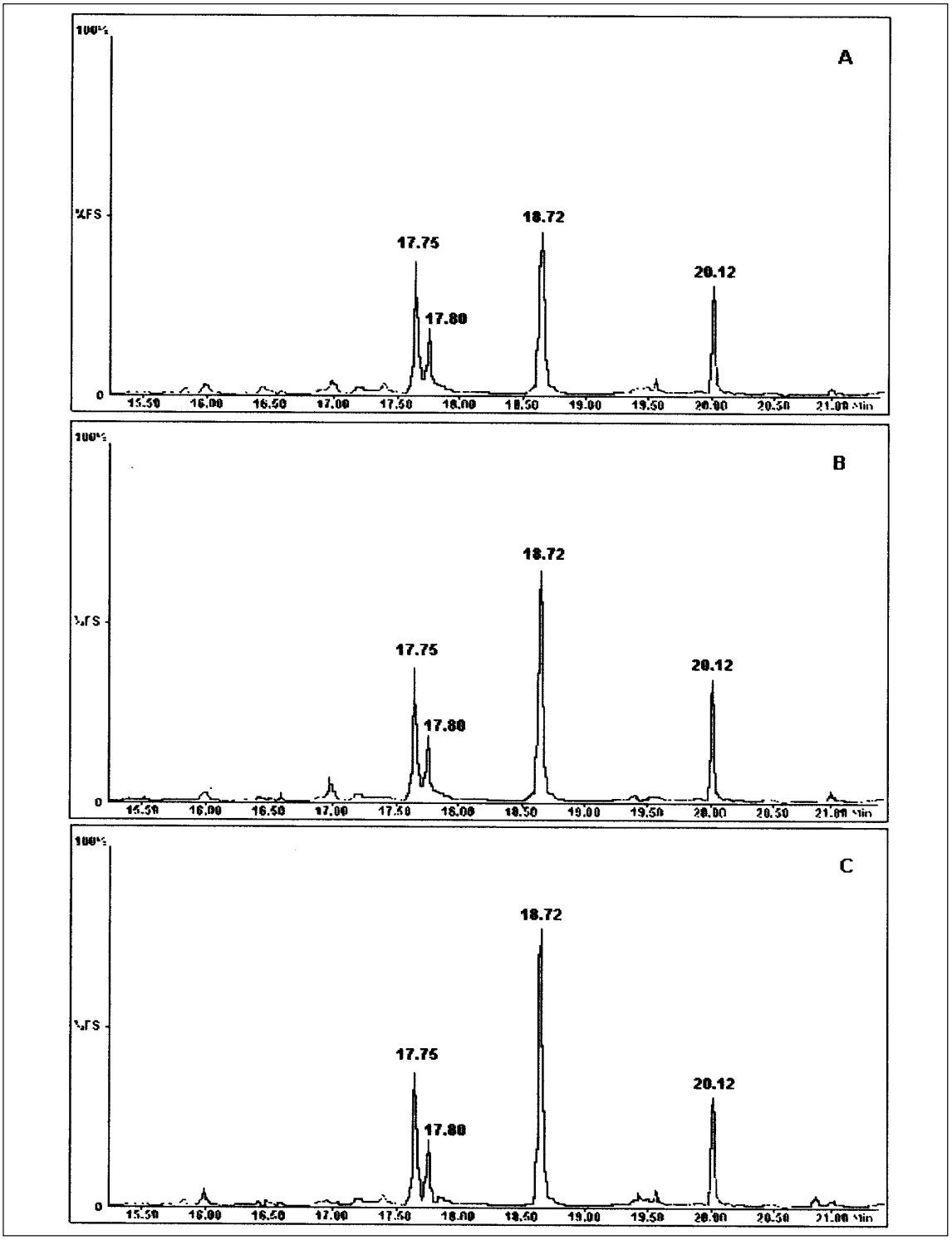

Figure 2 – Gas-chromatography retention times of monosaccharides from Stigmatriose (A), Stigmatetraose (B) and Stigmapentaose (C), showing total ion chromatogram of 2-fructofuranose (17.75 min), 1-glucopyranose (18.72 min) and 1,4-glucopyranose (20.12 min).

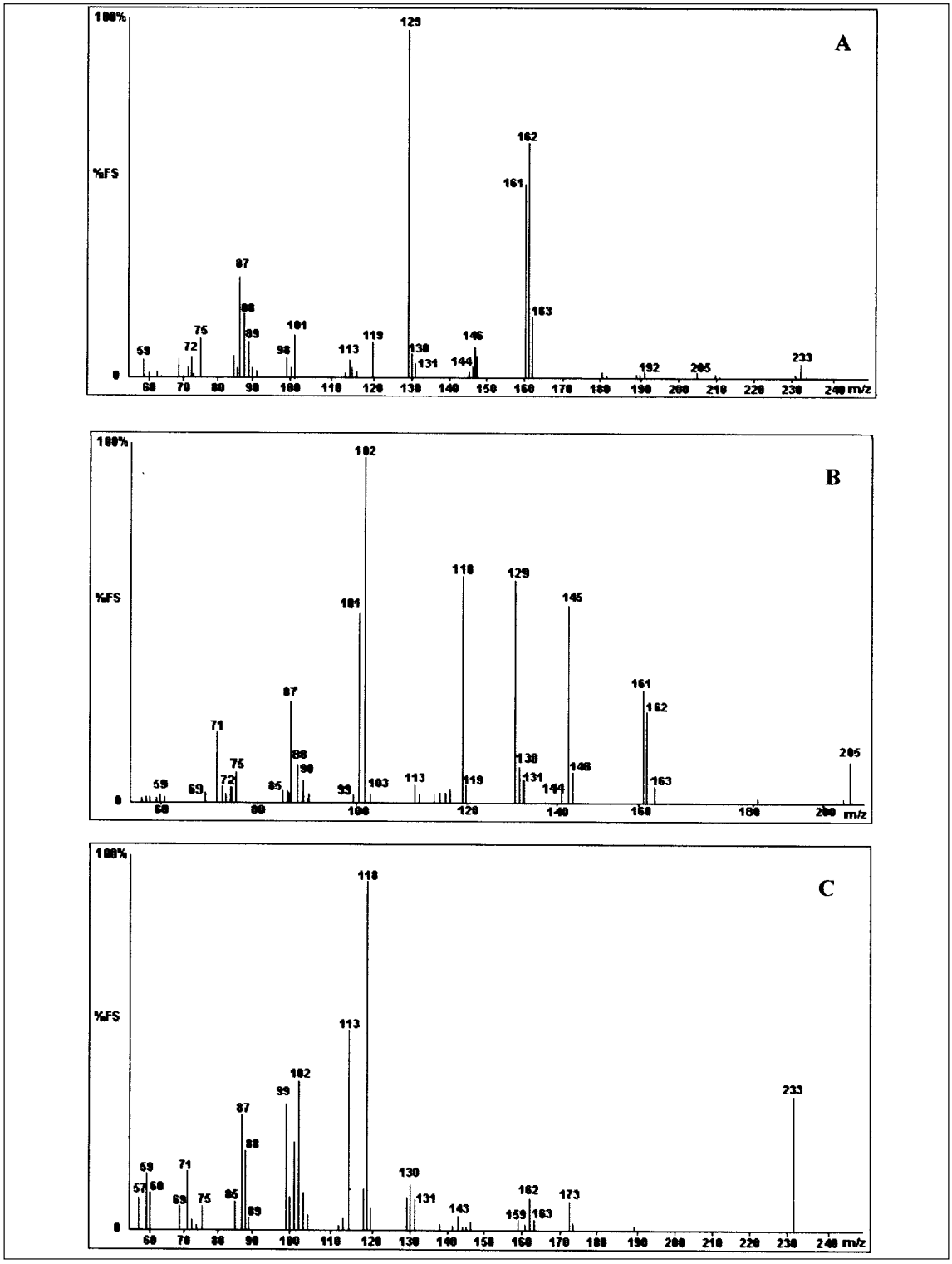

Figure 3 - Mass spectra of 2-linked fructofuranose with retention time (Rt) at 17.75 min (A), 1-linked glucopyranose with Rt at 18.72 min (B) and 1,4-linked glucopyranose with Rt at 20.12 min (C).

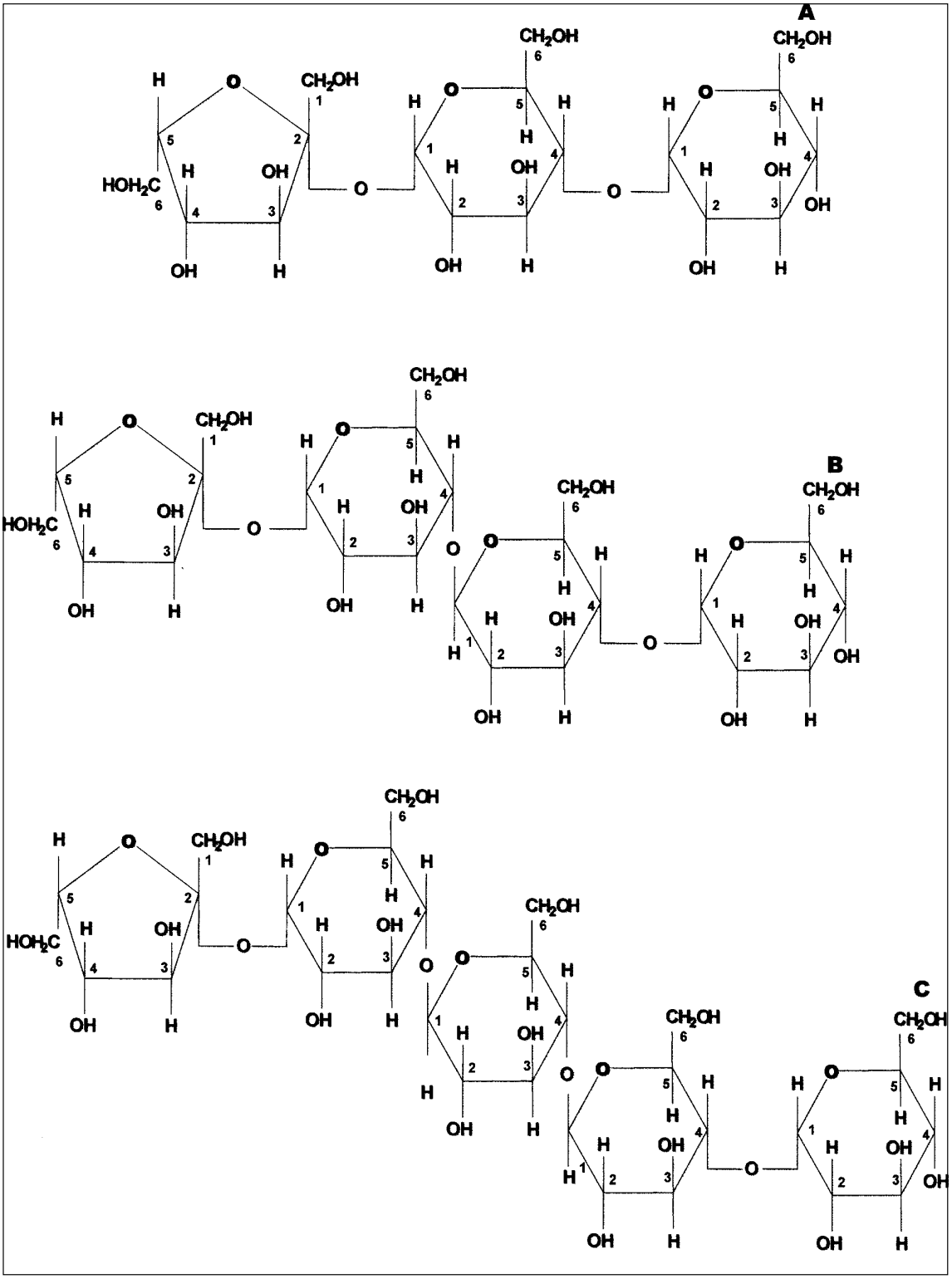

Figure 4 – Proposed structures of Stigmatriose (A), Stigmatetraose (B) and Stigmapentaose (C).

of oligosaccharides and the use of the trivial names. Also between the two quite different scale insects a common pattern of oligosaccharides is seen to exist

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