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Major O-glycans from the nest of *Vespula germanica* contain phospho-ethanolamine

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Abstract—We describe here the structural deciphering of four wasp O-glycans. Following purification of a mixture of glycoproteins from nests of the common wasp *Vespula germanica* L. (Hymenoptera), their substituting O-glycans were liberated by reducing β-elimination and characterised using a combination of high resolution NMR and mass spectrometry analyses. Besides ubiquitously found in the insect cells GalNAc-ol and Gal(β1–3)GalNAc-ol compounds, two novel O-glycans carrying a 2-aminoethyl phosphate group were described for the first time here. We suggest that they present the following structures: **Etn-*P*-(*O*→6)-GalNAc-ol** and **Etn-*P*-(*O*→6)-[Gal(β1–3)]GalNAc-ol**. In conjunction with previous studies, these results suggest that a 2-aminoethyl phosphate group may act as an alternative to sialic acid for conferring charges to glycoproteins.

Keywords: Insect; O-Glycan; Phosphoethanolamine (*P*Etn); NMR

1. Introduction

Although mammal O-glycans have been extensively studied, little information is available on the structure of these compounds in other phyla. Punctual studies in parasites,[1,2] birds[3] or invertebrates[4] demonstrated a wide variety of organisations that generate a staggering structural diversity within this class of compounds. So far, large scale systematic studies on the structure of O-glycans outside phylum of mammals have only been undertaken in amphibians [glycobase.univ-lille1.fr] and in fishes,[5] which permitted to identify several hundreds different compounds. This heterogeneity is essentially localised in the non-reducing end of O-glycans, whereas their core structures are remarkably conserved throughout evolution. On top of a marked inter-species variability, it is noteworthy, that a single species or even a single tissue may synthesise a large number of different O-glycans. For example, in humans, it is estimated to several hundreds in the tracheo-bronchial mucus isolated from a single individual,[6] whereas up to 58 different O-glycans have been characterised from oviducal mucus of the amphibian species *Triturus alpestris* [D. Florea et al., unpublished data].

Compared with the wide heterogeneity observed in most animals, insects seem to synthesise a surprisingly low number of very simple O-glycans. So far, studies conducted on several leptidoptera cell lines suggested that the O-glycosylation in insects was restricted to GalNAc-α-Ser/Thr and Galβ1–3GalNAc-α-Ser/Thr.[7–9] Furthermore, no charged residues have ever been observed on insect O-glycans, whereas these compounds are usually abundantly substituted by either sialic residues or by sulfate groups. Charged glycoconjugates have

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previously been observed in insect, but to our knowledge, no sialic acid has ever been observed associated to any glycoconjugate, glycolipid or glycoprotein. In particular, a family acidic and zwitterionic glycolipids substituted by glucuronyl and phospho-ethanolamine has been described in a species of synanthropic fly (*Calliphora viscina*).[10–11] Similarly, charged phosphono-ethanolamine substituted N-glycans were observed in the locust *Locusta migratoria*.[12] These results suggested that the presence of phosphated substituents may be a common feature in insect glycans and a possible alternative to sialic acid to confer a charge to glycoconjugates. In order to investigate the possible occurrence of charged O-glycans in insects, as starting material we used common wasp's nest (*Vespula germanica*), which is mainly made from wasp saliva [www.inra.fr/Internet/Produits/HYPPZ/RAVAGEUR/6vesger.htm] and thus highly enriched in insect glycoproteins. Such strategy has already been successfully applied to swallow's nests, which were shown to be associated with salivary mucin-type glycoproteins substituted by unusual O-glycans.[3,13,14] Along ubiquitous GalNAc-α-Ser/Thr and Galβ1–3GalNAc-α-Ser/Thr compounds, the present work establishes for the first time the existence of charged insect O-glycans substituted by a phospho-ethanolamine group.

2. Material and methods

2.1. Material

Wasp's nests were collected by the fire brigade of Villeneuve d'Ascq. The nest material and wasps were manually sorted out and dried at 100 °C for 48 h. The wasp's nests were mixed until obtaining a powder and finally suspended (48 h at 4 °C) in phosphate buffered saline (50 mM KH_2PO_4, 150 mM KCl at pH 7) containing 0.8% β-mercaptoethanol. The extract was centrifuged at 6000 rpm and the supernatant repetitively dialysed against large volumes of water during 72 h at 4 °C.

2.2. Purification of glycoprotein material

The dialysed material was fractionated on a Sepharose 6B column (100 × 4 cm) equilibrated and eluted with 100 mM Tris–HCl buffer (pH 8.0). Fractions were tested for carbohydrates by orcinol reagent and positive fractions were pooled, dialysed against water and lyophilised, yielding 25 g of material was obtained starting from 200 g of wasp's nest powder.

2.3. Amino acids and sugar analysis

The amino acid composition analysis was performed as previously described[15] and the amino acids were analysed by HPLC (Spectra physics 8100) in reverse phase with water PicoTag® (3.9 × 150 mm) column. Elution was performed using a binary gradient of sodium acetate, 0.1 M of triethylamine and acetonitrile, the flow rate was 1 mL/min during 17 min at 45 °C.

The monosaccharide composition analysis was performed according to Zanetta et al.,[16] after acid-catalysed methanolysis and analysis of the liberated O-methyl-glycosides as heptafluorobutyrate derivatives. In order to achieve the complete cleavage of the GalNAc-6-phosphate bond, the double methanolysis/acylation procedure was applied.[17] The volatile derivatives were separated by gas chromatography on a Carlo Erba GC 8000 chromatograph equipped with a 25 m × 0.25 mm CP-Sil5 CB Low bleed/MS capillary column, 0.25 μm film phase (Chrompack France, Les Ullis, France) coupled to a Finigan Automass II mass spectrometer.

2.4. Isolation of oligosaccharide–alditols

The purified material (about 1 g) was submitted to reductive β-elimination for 72 h at 37 °C in 100 mM NaOH containing 1 M $NaBH_4$. The reaction was stopped by the addition of DOWEX 50 × 8 (25–50 mesh, H^+ form) at 4 °C until pH 6.5. After filtration on glass wool and evaporation to dryness, boric acid was eliminated by repetitive distillation as its methyl ester in the presence of methanol. The material was submitted to a cationic exchange chromatography on DOWEX 50 × 2 (200–400 mesh, H^+ form) to remove residual peptides. The fractions containing sugars were purified on Bio-Gel P2 column (Bio-Rad), (150 × 2.5 cm). Finally,

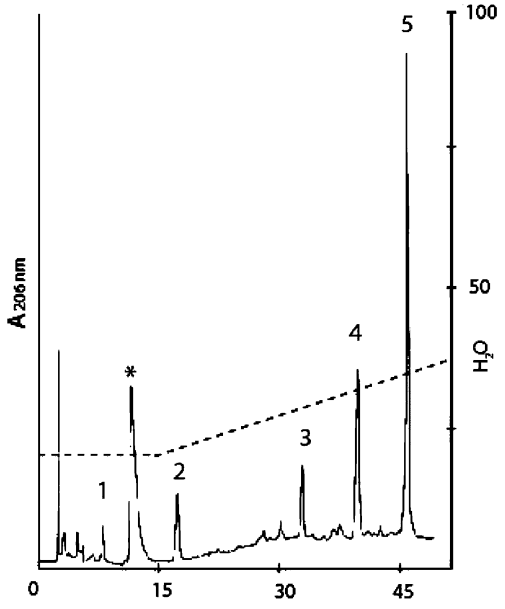

Figure 1. HPLC separation of released oligosaccharide–alditols on a primary amine-bounded silica column. *, non-carbohydrate material.

oligosaccharide–alditols were fractionated on DOWEX 1×2 column (mesh 200–400, $HCOO^-$ form). Oligosaccharide–alditols were desorbed with water and pH was adjusted to 7 with sodium hydroxide.

2.5. Separation of oligosaccharide–alditols

Compounds eluted with water were purified by HPLC (Fig. 1) on a primary amine-bounded silica column (Supelcosyl™, LC-NH_2, 4.6 mm × 250 mm, Supelco Inc., Bellefonte, PA, USA) using a gradient of MeCN–water and flow rate of 1 mL/min. Oligosaccharide–alditols were detected by UV spectroscopy at 206 nm.

2.6. NMR analysis

NMR spectra were acquired on a Bruker ASX 400 NB spectrometer equipped with double resonance ($^1H/X$) broadband inverse *z*-gradient probe head (^1H at 400.33 MHz and ^{31}P at 162.06 MHz). Before analysis, samples were treated twice with ^2H_2O (99.97% ^2H atoms, Euriso-top, CEA, Saclay, France) and then dissolved in 250 μL of ^2H_2O in Shigemi® tubes (Shigemi Inc., Alison Park, United States). ^1H chemical shifts (δ) were expressed in parts per million (ppm) related to the methyl signal of acetone as internal standard (δ_H 2.225). ^{31}P chemical shift has been determined with 10% (v/v) phosphoric acid in D_2O as external standard (δ_P 0.0). All delays and pulses were performed for each one- and two-dimensional experiments. 2D experiments were carried out using standard Bruker pulse program.

2.7. Mass spectrometry analysis

Samples were reconstituted in water and analysed by mass spectrometry on a LCQ DK XP+ ion trap (Thermo Finnigan) instrument. Samples were mixed with an equal volume of MeOH–0.1 M aqueous formic acid and infused directly at 50 nL/min using the nanoflow probe option for MS and MS^n analyses.

Table 1. Amino acid and carbohydrate compositions of total material (number of residues per 100)

Amino acids				Monosaccharides	
Arg	4.3	Leu	6.8	Xyl	2.9
Lys	5.6	Met	0.8	Rha	4.1
Ala	8.6	His	2.9	Fuc	4.0
Thr	7.3	Phe	3.8	Glc	18.2
Gly	10.1	Glu	9.4	Gal	37.3
Val	5.2	Asp	9.5	Man	17.4
Ser	10.2	Cys	0.5	GlcNAc	7.7
Pro	9.2	Tyr	1.6	GalNAc	8.4
Ileu	4.2			NeuAc/Gc	0

3. Results and discussion

3.1. Nature of the material

The reduced material was analysed for amino acids and carbohydrates before chromatographic separation, as described in material and methods section. A carbohydrate to protein ratio of 1 to 1 was calculated, which established the presence of large quantities of glycoproteins within this material. As shown in Table 1, simultaneous presence of large quantities of GalNAc, GlcNAc, Gal and Man residues is indicative of the presence of both N- and O-glycans. In conjunction with high content of O-glycans, the prevalence of four amino acids Ser, Thr, Gly and Pro, which accounted for more than 36% of the crude material total amino acids, suggested

Table 2. ^1H NMR chemical shifts of reduced O-glycans

Residues	Protons	Oligosaccharide–alditols				
		GalNAc-ol **1**	Gal(β1–3)GalNAc-ol **2**	*P*Etn(6)Hexen-ol **3**	*P*Etn(6)GalNAc-ol **4**	Gal(β1–3)[*P*Etn(6)]GalNAc-ol **5**
GalNAc-ol/Hexen-ol	H-1/H-1′	3.741/3.678	n.d.	3.619/3.526	3.741/3.681	3.797/3.744
	H-2	4.251	4.395	4.259	4.263	4.403
	H-3	3.849	4.064	5.835	3.870	4.088
	H-4	3.389	3.507	5.835	3.439	3.561
	H-5	3.927	4.195	4.409	4.087	4.356
	H-6/H-6′	3.668/3.647	3.69/3.63	3.920/3.799	3.942	3.94
	NAc	2.055	2.050	—	2.058	2.053
Gal	H-1	—	4.478	—	—	4.480
	H-2	—	n.d.	—	—	3.562
	H-3	—	n.d.	—	—	3.677
	H-4	—	3.901	—	—	3.896
	H-5	—	n.d.	—	—	3.727
*P*Etn	CH_2N	—	—	3.274	3.274	3.274
	CH_2OP	—	—	4.108	4.108	4.108

*P*Etn: Phosphoethanolamine; Hexen-ol: 3-hexene-1,2,5,6-tetrol.

the presence of mucin-type glycoproteins in the material, as previously described for swallow's nests.[14] On the other hand, small amounts of rhamnose and xylose are indicative of the presence of contaminating vegetal material in the mixture, as expected considering its nature. In order to enrich the starting material in glycoproteins, it was further purified by size exclusion chromatography on a Sepharose 6B column. Carbohydrate containing fractions were then collected for glycan analyses. It is noteworthy that no trace of any type of sialic acid was ever detected at any stage of the purification from crude material to purified O-glycans. In the present work, we concentrated in the deciphering of eventual mucin type *O*-linked glycans as suggested by composition analyses.

3.2. Isolation and NMR studies of oligosaccharide–alditols

Purified material was submitted to alkaline borohydride degradation and the released reduced oligosaccharides fractionated by HPLC on an amino-bonded column eluted with a gradient of water/acetonitrine. As shown in Figure 1, five peaks labelled **1**–**5** were individually collected and systematically analysed by 400-MHz ^{1}H NMR spectroscopy. Chemical shifts of the protons of individual constituents obtained from sequential ^{1}H and ^{31}P NMR experiments have been compiled in Table 2. Complete NMR analyses revealed that compounds **1** and **2** are simple O-glycans ubiquitously found in insects while compounds **4** and **5** are novel *2-aminoethyl-phosphate* group substituted O-glycans. Then, compound **3** was assigned as a degradation product of both compounds **4** and **5**.

^{1}H NMR signals of compound **1** were attributed owing to ^{1}H–^{1}H COSY and TOCSY experiments. Their chemical shifts perfectly matched with those of **GalNAc-ol** unit, as previously described in the literature.[18] In particular, it was unambiguously characterised by its H-2, H-5 and NAc signals at 4.251, 3.927 and 2.055 ppm, respectively. Peak **2** was an extension of previous compound with a 3-*O*-linked β-Gal unit, as shown by the strong downfield shift of the H-2 and H-5 signal of GalNAc-ol and the observation of the β-anomeric proton at δ 4.478 ppm ($^{3}J_{1,2}$ = 7.4 Hz).[18,19] Therefore, compound **2** corresponded to the **Gal(β1–3)GalNAc-ol** disaccharide. These data are in agreement with previous studies on the structure of insect *O*-linked glycans that showed the existence of these two components in leptidoptera cells.[8]

As for two previous compounds, 2D-relayed COSY spectrum of compounds **4** (Fig. 2A) showed a spin system attributed to a terminal GalNAc-ol unit. However, H-5 and H-6,6′ signals of this unit showed significant downfield shifts ($\Delta\delta$ +0.16 and +0.28 ppm, respectively) compared with compound **1** (Table 2), which were

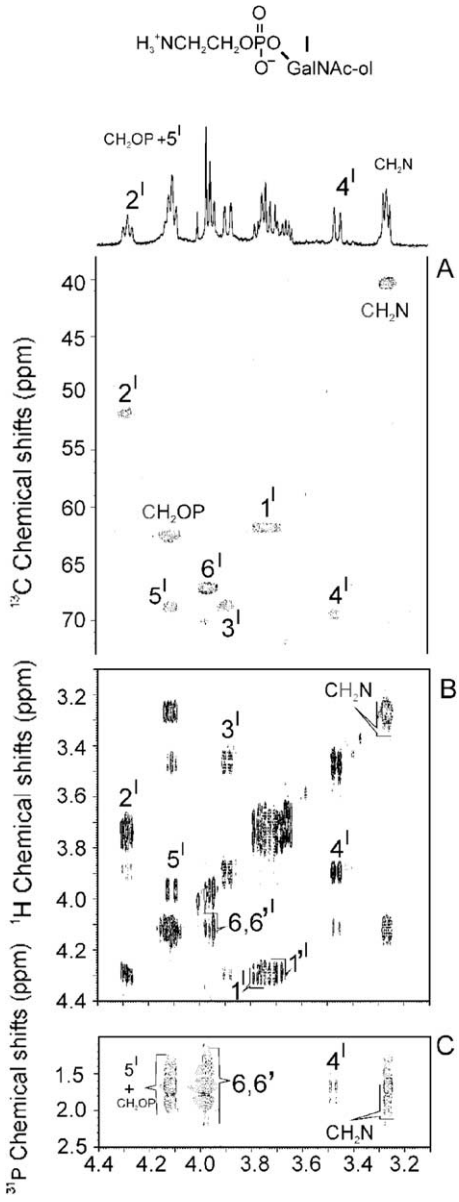

Figure 2. NMR spectra of oligosaccharide–alditol **4**. (A) ^{1}H–^{13}C HMQC spectrum; (B) ^{1}H–^{1}H COSY spectrum; (C) ^{1}H–^{31}P HMQC–TOCSY spectrum with 120 ms as mixing time.

tentatively attributed as the consequence of a substitution in C-6 position. The ^{1}H NMR spectrum showed two unknown additional signals at δ 3.274 and 4.108 ppm that, respectively, correlated with carbon signals at δ 40.61 and 62.64 ppm on a heteronuclear ^{1}H–^{13}C HMQC spectrum (Fig. 2A). On ^{1}H–^{1}H COSY spectrum (Fig. 2B), both signals showed a clear ^{3}J correlation cross-peak that established the vicinal position of the two corresponding carbons. These NMR parameters matched with those previously observed for CH_2 groups of phospho-ethanolamine (*P*Etn) present in various polysaccharides[20] and suggested the presence of such substituent in C-6 position of GalNAc-ol residue. On these bases, signals at δ 3.247 and 4.108 ppm were

assigned as CH_2N and CH_2OP protons, respectively. In agreement with CH_2OP attribution, the multiplet at δ 4.108 showed a supernumerary coupling (about 7 Hz) resulting from the vicinal coupling constant $^3J_{H,P}$. ^1H–^{31}P HMQC experiment confirmed the presence of a phosphorus atom owing to the observation of an intense signal at δ 1.709 ppm (data not shown). It also permitted to directly correlate phosphorus atom through $^3J_{H,P}$ correlation to H-6,H-6′ of GalNAc-ol at δ 3.942 ppm on one side and to CH_2OP proton signals δ 4.108 ppm on the other side. Furthermore, a very low intensity signal was observed at δ 3.274 ppm and attributed to a $^4J_{H,P}$ correlation with CH_2N protons. In agreement, ^1H–^{31}P HMQC-TOCSY experiment extended $^3J_{H,P}$ correlations to CH_2N proton signals at δ 3.274 ppm and to H-5 and H-4 of GalNAc-ol residue (Fig. 2C). Taken together, NMR data clearly established the structure of compounds **4** as **[$H_2NCH_2CH_2O(HPO_3)$](6)GalNAc-ol**. Then, MALDI-TOF analysis in positive mode of compound **4** showed three major peaks at *m/z* 368.9, 384.9 and 390.9 (data not shown), which were assigned to $[M+Na]^+$, $[M+K]^+$ and $[M+2Na-H]^+$ adduct ions of **[H_2NCH_2-$CH_2O(HPO_3)$](6)GalNAc-ol**, in agreement with previous NMR analyses.

As observed on 2D-relayed COSY spectrum (Fig. 3), compounds **5** showed two complete spin systems assigned to a β-Gal residue and to a GalNAc-ol unit. Their proton chemical shifts were very similar to those observed for compound **2** and are in agreement with the presence of **Gal(β1–3)GalNAc-ol** disaccharide, except for the H-5 and H-6,6′ downfield shifts of GalNAc-ol unit at δ 4.356 and 3.94 ppm. As for compound **4**, the presence of a *P*Etn group was established by the two previously assigned correlated CH_2N signal at δ 3.274 ppm and CH_2OP signal at δ 4.108 ppm H-5 and H-6,6′ downfield shifts of GalNAc-ol unit permitted to position the *P*Etn group on the C-6 of GalNAc-ol unit. These data established the structure of compound **5** as **Gal(β1–3)[$H_2NCH_2CH_2O(HPO_3)$]-6-GalNAc-ol**.

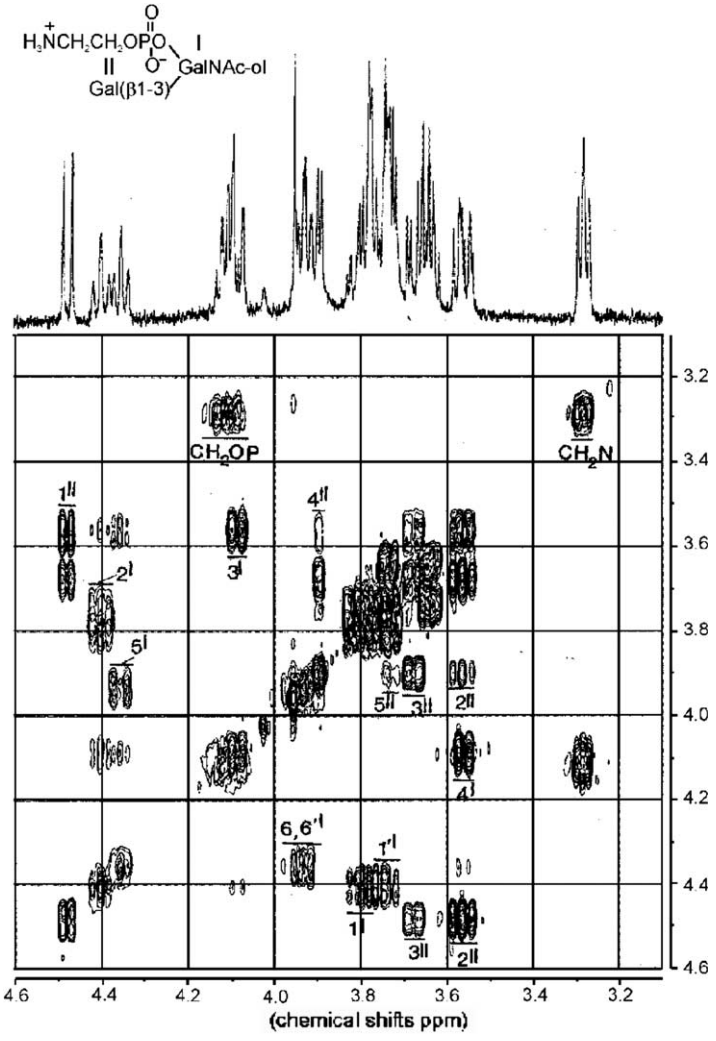

Figure 3. Detail of one-relayed ^1H–^1H COSY spectrum of compound **5** (F1: 4.6–3.1, F2: 4.6–3.1 ppm).

Attribution of this compound was confirmed by multiple CID-MS/MS experiments. ESI-MS analysis in positive mode showed an ion at *m/z* 531.1 that was assigned to the $[M+Na]^+$ adduct of compound **5**. Its fragmentation pattern confirmed the presence of a Gal-GalNAc-ol motif, owing to the presence of a fragment ion at *m/z* 408.2 (Fig. 4A). Loss of a Gal residue from pseudo-molecular ion generated a B- and C-type pair of fragment ions at *m/z* 369.3 and 350.9, respectively, which established that Gal residue is in terminal non-reducing position of the molecule and that phospho-ethanolamine group directly substitutes GalNAc-ol residue. This last point was confirmed by the observation of secondary fragment ions at *m/z* 326.1 and 308.3 attributed to HPO_3-GalNAc-ol motif that resulted from simultaneous cleavages of phosphatidyl bond between H_2N-$(CH_2)_2$ and phosphate group and Gal-GalNAc-ol glycosydic bond. MS^3 analysis from the primary fragment at *m/z* 488 that resulted from loss of H_2N-$(CH_2)_2$ group also generated the HPO_3-GalNAc-ol motif at *m/z* 307.8 and 326.1 (Fig. 4B). Similar analysis realised in negative mode permitted to fragment $[M-H]^-$ adduct of this compound. In accordance with previous data, fragmentation spectrum showed both selective losses of terminal Gal or ethanolamine substituents at *m/z* 327.2/345.2 and 446.2, respectively, and their simultaneous losses to generate phosphorylated GalNAc-ol motif at *m/z* 284.1 (Fig. 4C). Taken together, these data unambiguously confirmed the sequence of compound **5** as previously deduced by NMR analysis.

MALDI-TOF analysis of compound **3** showed three major peaks at *m/z* 293.9, 319.8 and 315.9 (data not shown), tentatively assigned to a 2-aminoethyl phosphate group linked to a $C_6O_3H_{11}$ aglycone. Complete assignment of protons through a COSY experiment permitted to identified the aglycon to a 3-hexene-1,2,5,6-tetrol in accordance with previously published parameters.[21] The presence of CH_2N and CH_2OP proton signals at δ 3.274 and 4.108 ppm, respectively, confirmed the presence of a 2-aminoethyl phosphate group, as in compounds **4** and **5**. Then, deshielding of H-6,6′ of hexane-tetrol residue at δ 3.920/3.799 ppm compared to H-1,1′ parameters at δ 3.619/3.526 ppm established that 3-hexene-1,2,5,6-tetrol is substituted in C-6 position by the 2-aminoethyl phosphate group. Hexene-tetrol containing components are commonly found in reductive elimination products and are thought to originate from a peeling reaction of *O*-linked glycans, releasing C-6

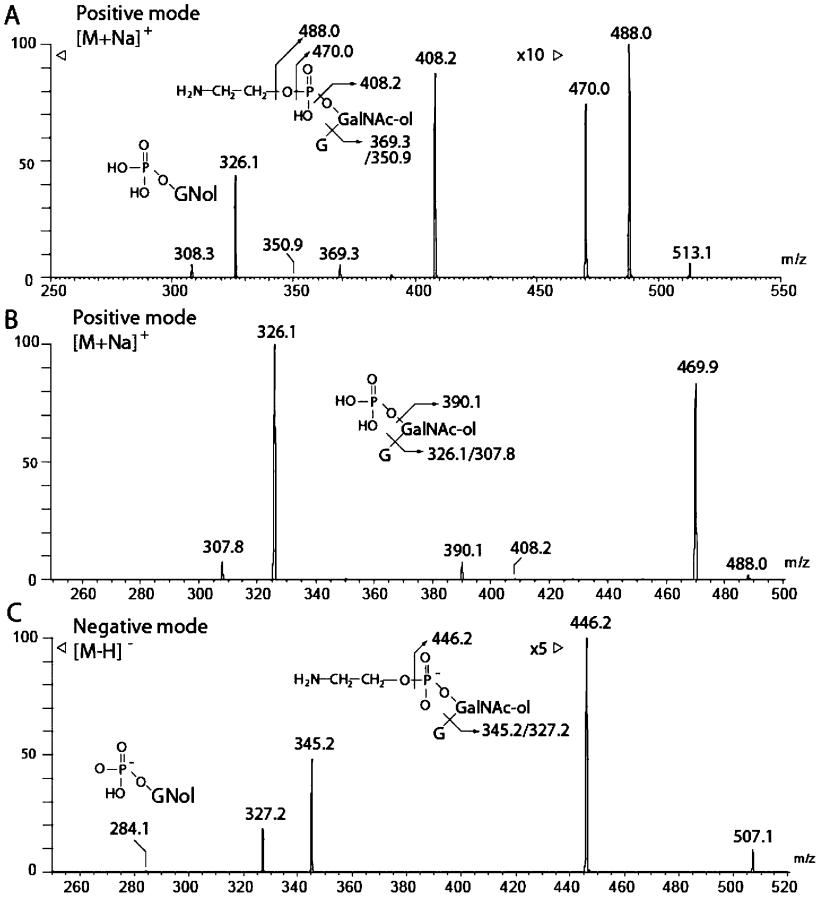

Figure 4. ESI-MS^n fragmentation patterns of compound **5**. (A) MS^2 fragmentation of $[M+Na]^+$ adduct at *m/z* 531.1 in positive mode. (B) MS^3 fragmentation of fragment at *m/z* 488.0 in positive mode and (C) MS^2 fragmentation of $[M-H]^-$ adduct at *m/z* 507.1 in negative mode.

upper branch from C-3 lower branch.[21,22] In this respect, compound **3** appears as a degradation product of both compounds **4** and **5**, which retrospectively confirms the position of 2-aminoethyl phosphate group on C-6 of GalNAc-ol residue.

The four major glycans extracted from wasp nest exhibit typical features of animal mucin-type O-glycans, which strongly suggest that they originate from wasp derived material and not from residues of entrapped vegetal material. Indeed, the hypothesis of a possible vegetal origin for these compounds is most unlikely considering that mucin type O-glycans have, to our knowledge, never been observed in plants, despite much effort done over the last years to uncover such compounds [Dr. P. Lerouge, personal communication]. Observation of GalNAc and Gal(β1–3)GalNAc cores in these components is in total agreement with previous studies realised on insect O-glycosylation. The phospho-ethanolamine containing components described here represent the first known example of insect charged O-glycans and are so far unique in animal kingdom. Although presenting zwitterionic properties, *P*Etn may act as an unexpected substitute for acidic groups to confer charge to O-glycosylated glycoproteins in insects. Considering that phospho-ethanolamine and phosphono-ethanolamine based glycoconjugates have been previously described in two other insect species, whereas neither sialic acid nor sulfate based ones have been, we may envisage the possibility of a distinct structural evolution of insect glycosylation processes. Systematic studies of insect glycoconjugates structures as well as their genetic counterparts are needed to confirm this appealing hypothesis.

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References

1. Jones, C.; Todeschini, A. R.; Agrellos, O. A.; Previato, J. O.; Mendonca-Previato, L. *Biochemistry* **2004**, *43*, 11889–11897.
2. Huang, H. H.; Tsai, P. L.; Khoo, K. H. *Glycobiology* **2001**, *11*, 395–406.
3. Strecker, G.; Wieruszeski, J. M.; Cuvillier, O.; Michalski, J. C.; Montreuil, J. *Biochimie* **1992**, *74*, 39–51.
4. Meikle, P.; Richards, G. N.; Yellowlees, D. *J. Biol. Chem.* **1987**, *262*, 16941–16947.
5. Inoue, S.; Inoue, Y. In *Glycoproteins II:* New comprehensive biochemistry; Neuberger, A., van Deenen, L. L. M., Eds. 1997; Elsevier; Vol. 29b, pp 243–402.
6. Lamblin, G.; Degroote, S.; Perini, J. M.; Delmotte, P.; Scharfman, A.; Davril, M.; Lo-Guidice, J. M.; Houdret, N.; Dumur, V.; Klein, A.; Rousse, P. *Glycoconjugate J.* **2001**, *18*, 661–684.
7. Thomsen, D. R.; Post, L. E.; Elhammer, A. P. *J. Cell. Biochem.* **1990**, *43*, 67–79.
8. Lopez, M.; Tetaert, D.; Juliant, S.; Gazon, M.; Cerutti, M.; Verbert, A.; Delannoy, P. *Biochim. Biophys. Acta* **1999**, *1427*, 46–61.
9. Kramerov, A. A.; Arbatsky, N. P.; Rozovsky, Y. M.; Mikhaleva, E. A.; Polesskaya, O. O.; Gvozdev, V. A.; Shibaev, V. N. *FEBS Lett.* **1996**, *378*, 213–218.
10. Weske, B.; Dennis, R. D.; Helling, F.; Keller, M.; Nores, G. A.; Peter-Katalinic, J.; Egge, H.; Dabrowski, U.; Wiegandt, H. *Eur. J. Biochem.* **1990**, *191*, 379–388.
11. Helling, F.; Dennis, R. D.; Weske, B.; Nores, G.; Peter-Katalinic, J.; Dabrowski, U.; Egge, H.; Wiegandt, H. *Eur. J. Biochem.* **1991**, *200*, 409–421.
12. Hård, K.; van Doorn, J. M.; Thomas-Oates, J. E.; Kamerling, J. P.; van der Horst, D. J. *Biochemistry* **1993**, *32*, 766–775.
13. Hanisch, F. G.; Uhlenbruck, G. *Hoppe-Seyler's Z. Physiol. Chem.* **1984**, *365*, 119–128.
14. Wieruszeski, J. M.; Michalski, J. C.; Montreuil, J.; Strecker, G.; Peter-Katalinic, J.; Egge, H.; van Halbeek, H.; Mutsaers, J. H.; Vliegenthart, J. F. *J. Biol. Chem.* **1987**, *262*, 6650–6657.
15. Bidlingmeyer, B. A.; Cohen, S. A.; Travin, T. L. *J. Chromatogr.* **1984**, *336*, 93–104.
16. Zanetta, J. P.; Timmerman, P.; Leroy, Y. *Glycobiology* **1999**, *9*, 255–266.
17. Trinel, P. A.; Maes, E.; Zanetta, J. P.; Delplace, F.; Coddeville, B.; Jouault, T.; Strecker, G.; Poulain, D. *J. Biol. Chem.* **2002**, *277*, 37260–37271.
18. Kamerling, J. P.; Vliegenthart, J. F. G. In *Biological Magnetic Resonance*; Plenum Press: New York, 1992; Vol. 10.
19. Coppin, A.; Maes, E.; Morelle, W.; Strecker, G. *Eur. J. Biochem.* **1999**, *265*, 94–104.
20. Shashkov, A. S.; Kondakova, A. N.; Senchenkova, S. N.; Zych, K.; Toukach, F. V.; Knirel, Y. A.; Sidorczyk, Z. *Eur. J. Biochem.* **2000**, *267*, 601–605.
21. Florea, D.; Maes, E.; Haddad, M.; Strecker, G. *Biochimie* **2002**, *84*, 611–624.
22. Capon, C.; Wieruszeski, J. M.; Lemoine, J.; Byrd, J. C.; Leffler, H.; Kim, Y. S. *J. Biol. Chem.* **1997**, *272*, 31957–31968.