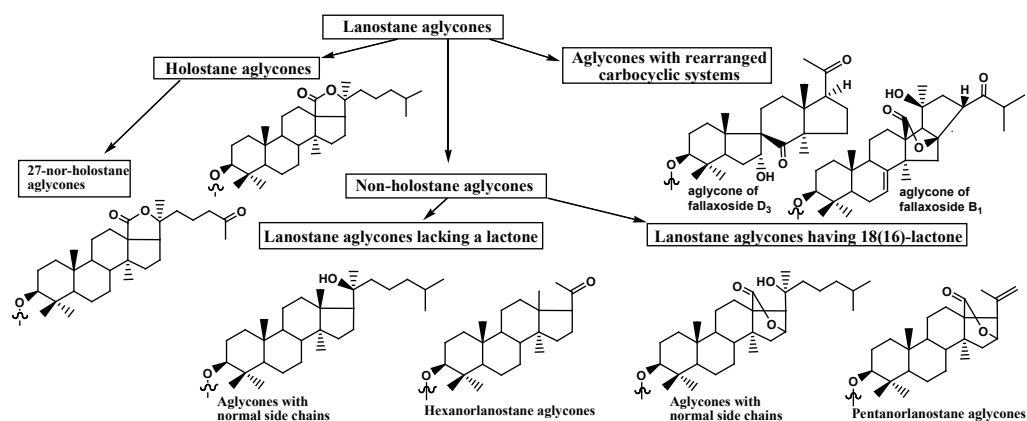


A.S.SILCHENKO, V.I. KALININ, S.A. AVILOV

## Structural diversity and some biosynthetic peculiarities of triterpene glycosides from the sea cucumbers

*Key words: sea cucumbers, triterpene glycosides, biosynthesis.*

Sea cucumbers (class Holothuroidea) are the marine invertebrates belonging to the phylum Echinodermata biosynthesizing unusual for other animals metabolites – triterpene glycosides. The lanostane aglycones and carbohydrate chains comprise the molecules of triterpene glycosides. There are some structural types of the aglycones: holostane type (lanostane derivatives with 18(20)-lactone) and non-holostane type, which is subdivided into groups having 18(16)-lactone or lacking a lactone. There are also the aglycones with shortened or normal side chains within each group of the aglycones. Carbohydrate chains of these compounds differ from each other by the diverse monosaccharide residues composing them, by the number and sequence of the residues and therefore by the architecture. Some of the sugar chains contain from one to three sulphate groups.



The applying of modern techniques of isolation and structural elucidation allowed the discovering of some minor glycosides possessing very interesting structural features. Some glycosides are characterized by unusual aglycones [1 and references herein]. Among them are: synaptoside A<sub>1</sub> from *Synapta maculata* having 7-keto-8(9)-en fragment in holostane aglycone,

\* SILCHENKO Alexandra Sergeevna – PhD, Senior Researcher, KALININ Vladimir Ivanovich – DSc, Leading Researcher, AVILOV Sergey Alexandrovich – DSc, Leading Researcher (G.B. Elyakov Pacific Institute of Bioorganic Chemistry, FEB RAS, Vladivostok, Russia). \*E-mail: alex@piboc.dvo.ru

cucumariosides A<sub>8</sub> and A<sub>9</sub> from *Eupentacta fraudatrix* having unique 18,20-dihydroxy fragment in the aglycones without lactone ring, cucumarioside A<sub>12</sub> from the same sea cucumber and 27-nor-25-oxo-holotoxin A<sub>1</sub> from *Psolus fabricii* the representatives of very rare 27-nor-triterpenoids; cucumarioside H<sub>8</sub> from *E. fraudatrix* and magnumoside A<sub>1</sub> from *Massinium magnum* having unique 16(22)- and 20(24)-epoxy-groups in the aglycones, correspondingly; unprecedented octanorlanostane aglycone of cladoloside C<sub>4</sub> from *Cladolabes schmeltzii*. Finally, the series of glycosides (fallaxosides) with uncommon aglycones was found in the sea cucumber *Cucumaria fallax*. Majority of them contained non-holostane aglycones without lactone having 8(9)-double bond and oxygen-containing functionalities at C-7 and C-11. Two compounds – fallaxosides B<sub>1</sub> and D<sub>3</sub> characterized by unprecedented rearranged lanostane carbocyclic systems in their aglycones. The first of the above has an 18(16)-lactone and additional 16,23-five-membered cycle; the latter has no a double bond, a lactone ring and a side chain and contain two cyclohexane and two cyclopentane rings and a spiro carbon.

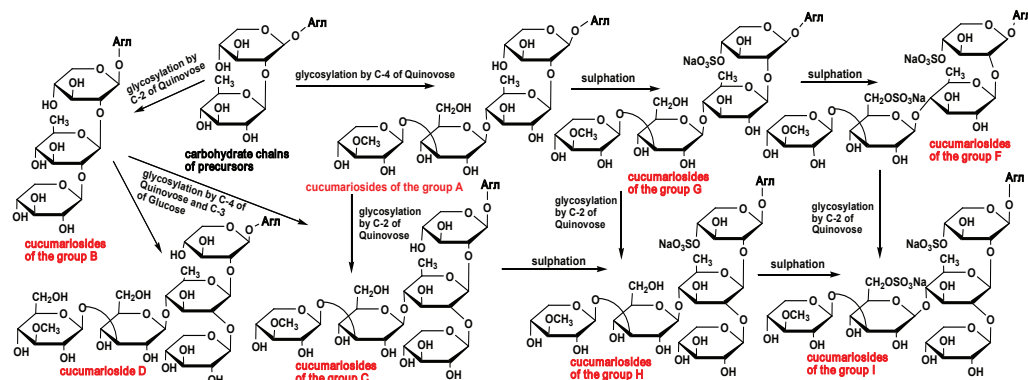
The pathways of biosynthetic transformations of these unique aglycones were suggested. Thus fallaxoside B<sub>1</sub> was formed as a result of an enzymatic intramolecular aldolic condensation of 1,6-diketo precursor leading to the formation of additional C-23–C-16 covalent bond. The formation of fallaxoside D<sub>3</sub> was explained by the proton induced Meinwald or pinacol-pinacolone-like rearrangement of 8,9-epoxy or 8,9-dihydroxy precursor, correspondingly, that leads to shifting of 7(8)-covalent bond to 7(9)-position with a size decreasing of cycle B to five-member one and formation of spiro-quaternary center between the rings B and C and a keto group at C-8 position [3].

Some uncommon structural features were also found in carbohydrate chains of the glycosides. The glycosides of *Synapta maculata* were characterized by pentasaccharide sulphated carbohydrate moiety, containing 3-O-methylglucuronic acid, which was found first in the glycosides of sea cucumbers. Moreover it does not occur in the glycosides from plants or sponges but comprises the capsule polysaccharides of bacteria. The sugar chains of the glycosides from *Staurocucumis turquetii* and *S. liouvillei* contain rare 3-O-methylquinovose residue that is considered as chemotaxonomic character of the genus *Staurocucumis*. Cucumariosides of the group B from *E. fraudatrix* and colochiroside E from *Colochirus robustus*, correspondingly, contain linear and branched trisaccharide chains. Cladoloside N from *Cladolabes schmeltzii* has three xylose residues (as first, second and third units) and cladolosides of the groups F and H have two quinovose residues (as second and fifth units) in the carbohydrate chains. High-polar glycosides with sulphated hexasaccharide sugar chains were first discovered in the same sea cucumber. Some of cladolosides were characterized by the presence of non-methylated sugars as terminal units in hexasaccharide chains. Colochiroside E from *C. robustus*, cladoloside J<sub>1</sub> from *C. schmeltzii*, psolusoside B from *Psolus fabricii* and kurilosides A and C from *Thyonidium kurilensis* were characterized by the unusual architecture of sugar chains.

So, having the data array concerning the structural diversity of triterpene glycosides from the sea cucumbers, we can analyze their biogenetic relationships and biosynthetic pathways. The species *Eupentacta fraudatrix* and *Cladolabes schmeltzii* are convenient objects for the analysis of carbohydrate chains biosynthesis since the high diversity of these parts of their glycosides. The synthesis and elongation of glycosides sugar moieties occur through the consequent attachment of monosaccharides to the certain positions of the forming chain. The sulphation is frequently considered as a final stage of sugar chain biosynthesis.

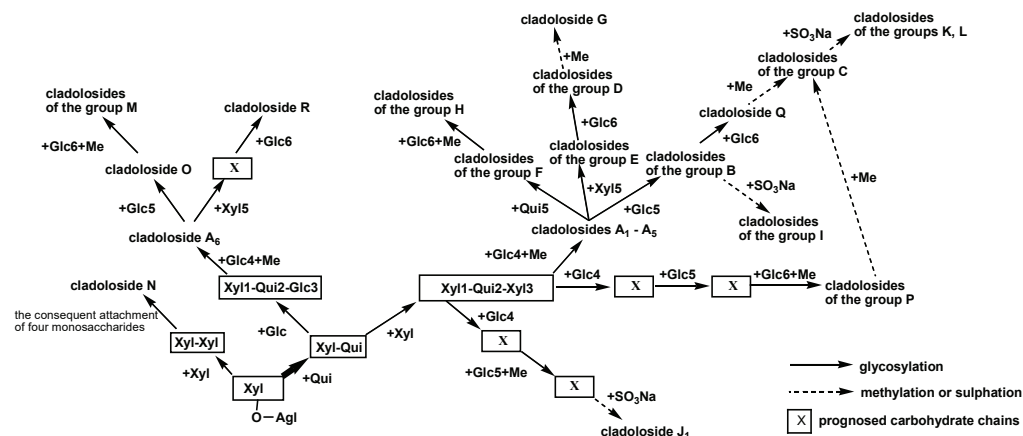
The glycosides of *E. fraudatrix* – cucumariosides of the groups A, B, C, D, F, G, H and I, contain trisaccharide, tetrasaccharide and pentasaccharide chains with or without sulphate groups [2 and references herein, 4]. Cucumariosides of the group A – linear nonsulphated tetraosides could be considered as “basic” group which is actively transformed in the process of biosynthesis. Its sulphation leads to the formation of the chains of cucumariosides belonging to the groups G and F and its subsequent glycosylation by C-2 of quinovose resulted in the formation of cucumariosides of the groups H and I. The formation of cucumariosides of the group C occurred by glycosylation of sugar chain of cucumariosides of the group A without prior

sulphation. So, sulphation and glycosylation are the alternative processes leading to the synthesis of different groups of cucumariosides. The minor triosides – cucumariosides of the group B also could be the biosynthetic precursors of pentaosides belonging to the groups of cucumariosides C, D, H and I when the glycosylation by C-2 of quinovose precedes the glycosylation by C-4 of this residue. Thus the biosynthesis of glycosides carbohydrate chains in *E. fraudatrix* is a mosaic type as its different stages can be shifted in relation to each other in time (heterochrony) and proceed in different consecutions. The result of such mosaicism is the formation of final products (sulphated pentaosides) by different (alternative) pathways.



The glycosides of sea cucumber *C. schmelzii* are characterized by the extreme diversity (19 types) of carbohydrate chains. When analyzing their biogenesis it becomes obvious that each type of chain is biosynthesized by one pathway only as individual consequence of the glycosylation reactions. So this situation is opposite to that observed in *E. fraudatrix*. The first step of diversification of biosynthetic pathways of cladolosides occurs after the formation of xylosides of the aglycones. The mainstream of biosynthesis is the glycosylation of the first xylose residue by the quinovose leading to the formation of all the cladolosides with one exception (the attachment of another xylose unit occurs in the process of cladolide N formation). All consistencies of glycosylation reactions pass through the branching points. At the most branched point cladolosides of the group A with tetrasaccharide chains are located similarly to cucumariosides of the group A in *E. fraudatrix*.

The sulphation of cladolosides always occurs in final biosynthetic stages since sulphate group attaches to terminal methylated monosaccharide residues of penta- and hexasaccharide chains. The methylation of sugar chains is a “termination signal” stopping further elongation of a chain. The presence in some groups of cladolosides of non-methylated terminal residues indicates the attachment of O-methyl directly to synthesizing sugar chain, but not to the monosaccharide that consequently glycosylates forming chain. However in cladolosides 3-O-methylation is not



always a final stage for the whole molecule because after the introduction of this termination group to one half-chain (upper or bottom) elongation of another half-chain could proceed. The time shifting of these stages relative to each other (heterochrony), characteristic for the mosaic type of biosynthesis, lead to the formation of carbohydrate chains with terminal residues with or without 3-O-methyl group in the fourth or sixth positions of the chains.

Thus, comparison of metabolic networks of carbohydrate chains of the glycosides from *E. fraudatrix* and *C. schmeltzii* showed different degree of mosaicism of their biosynthesis. Classical mosaic type of biosynthesis is inherent for the sugar moieties of *E. fraudatrix*. Whereas biosynthesis of carbohydrate parts of glycosides in *C. schmeltzii* has more regulatory (strictly directed) nature but with some character traits of mosaicism.

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