Oligosaccharides components in traditional Chinese medicines and the liposome carrying these oligosaccharides

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Traditional Chinese medicines often contain oligosaccharides which are considered to have important roles in their healing ability. Julibroside I (**2**) isolated from *Albizzia julibrissin* has an ester glycosidic bond, which can be selectively cleaved with lithium iodide in the presence of lutidine and allyl alcohol. As the oligosaccharide is essential for the biological activity of **2**, the product allyl α-L-arabinofuranosyl-(1,4)-[β-D-glucopyranosyl-(1,3)]-α-L-rhamnopyranosyl-(1,2)-β-D-glucopyranoside (**6**) obtained was transformed to the neoglycolipid *N*-decanoyl-1-decylaminoethyl-2-O-α-L-arabinofuranosyl-(1,4)-[β-D-glucopyranosyl-(1,3)]-α-L-rhamnopyranosyl-(1,2)-β-D-glucopyranoside, which will be tested for its anti-tumor activity in the KB cell.

Recent research on glycobiology reveals the important roles of glycoconjugates in immune response, viral and bacterial infection, differentiation, and inflammation. Triterpenoid glycosides which are often found as major components in traditional Chinese medicines are the ones of these examples.

Among the many triterpenoid glycosides, glycyrrhizin (**1**) (Fig. 1) isolated from *Glycyrrhiza glabra* var. *grandlifera* has been utilized as an anti-inflammatory medicine. "Stronger Neo-Minophagen C", an injection fluid containing 0.2% glycyrrhizin, 0.1% cysteine and 2.0% glycine in saline, for example, is used to cure chronic hepatitis. This plant belongs to the great family of *Leguminosae* (bean family), which often provides many structually similar substances having potent and interesting bioactivities.

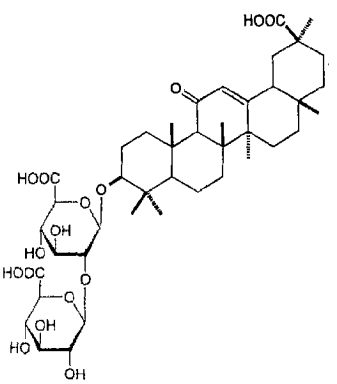

Fig. 1. The Structure of glycyrrhizin (**1**).

Recently one of the authors (T. Ikeda) and his coworkers succeeded in the isolation and structure determination of the most complex triterpenoid glycoside reported so far, Julibroside I ($C_{108}H_{174}O_{53}$, **2**), from the dried bark of *Albizza julibrissin* DURAZZ (silk tree in English, and nemunoki in Japanese) which is also a bean family plant.[1] This plant contains a large amount of Julibroside I (**2**) analogs, each of which has an ester glycoside linked to the quartenary carboxylic acid on C-28 of the oleanane type triterpene. The elimination of this ester glycoside dramatically decreased the anti-tumor activities for the KB cell.[2] This observation suggests that the tetrasaccharide, α-L-arabinofuranosyl-(1,4)-[β-D-glucopyranosyl-(1,3)]-α-L-rhamno-pyranosyl-(1,2)-β-D-glucopyranose, is essensial in the anti-tumor activity of Julibroside I.

Therefore we are interested in the study to test if the tetrasaccharide itself shows the anti-tumor activity or not, and we embarked on the preparation of the neoglycolipids carrying this tetrasaccharide.

Many neoglycolipids, in which either the saccharide part or lipid part is the same as the natural material and the other is completely artificial, have been prepared for testing the biological functions of oligosaccharides.[3]

On the other hand, multivalency in carbohydrate-protein interaction is well-known to cause the enhancement of binding affinity to lectins or receptors, and this phenomenon is referred to as the cluster effect. Liposomes carrying carbohydrate ligands would satisfy this cluster effect, and the carbohydrate ligands displayed on the surface of liposome can be correctly oriented to bind the appropriate lectins or receptors due to the flexibility of liposome surfaces. In order to make stable liposomes, double-chain lipids are generally chosen as the aglycons of neoglycolipids because single-chain glycolipids show detergent-like properties and are not suitable for this purpose. Ceramide which exists in the bilayer liposomal cell membrane as a lipid part of gangliosides actually has two chains composed of sphingosine and fatty acid. Many different types of double-chain glycolipids mimicking the structure of natural glycosyl ceramides such as bissulfone lactosides (**3**), double-tailed amide glycoside (**4**), and dipalmitoylphosphatidylamine glycoside (**5**) (Fig. 2) have been reported.

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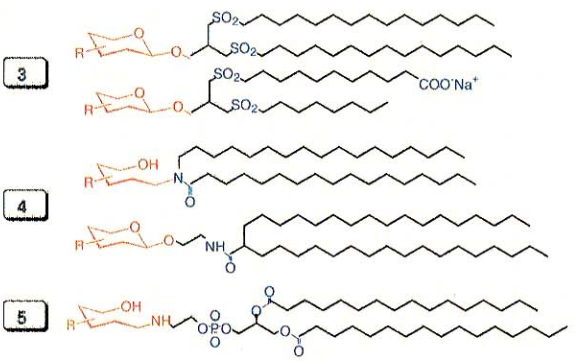

Fig. 2. Neoglycolipid reported so far.

The crude Julibroside I (**2**) and its analogues were easily obtained from the methanol extract of the original plant by passing through Amberlite XAD II and were eluted with 80% methanol (yield is *ca* 2 w/w%). The ester glycosidic bond was selectively cleaved by treatment with allyl alcohol, lithium iodide and lutidine (70%). Alkaline hydrolysis, however, afforded a very poor result.[4)]

The allyl α-L-arabinofuranosyl-(1,4)-[β-D-glucopyranosyl-(1,3)]-α-L-rhamnopyranosyl-(1,2)-β-D-glucopyranoside (**6**) thus obtained was then converted into the double-tailed amide glycoside (Fig. 3). The allyl tetrasaccharide (**6**) was ozonolyzed to afford the aldehyde (**7**), which reacted with decylamine in the presence of sodium cyanoborohydride to give 1-decylaminoethyl-2-O-tetrasaccharide (**8**) (overall 80%). This compound (**8**) was acylated with decanoyl chloride in a biphasic solution of THF / 2N NaOAc[5)] to afford *N*-decanoyl-1-decylaminoethyl-2-O-α-L-arabinofuranosyl-(1,4)-[β-D-glucopyranosyl-(1,3)]-α-L-rhamnopyranosyl-(1,2)-β-D-glucopyranoside (**9**) (85%).

The anti-tumor activity of the neoglycolipid (**9**) for the KB cell is now under investigation.

It appears that the method shown here is generally applicable not only to the triterpene glycosides but also to other traditional Chinese medicines which have an ester glycosidic bond. We are also developing effective techniques to selectively cleave the ether glycosidic bond, such as the glycosidic bond on C-3 of Jublioside I (**2**),

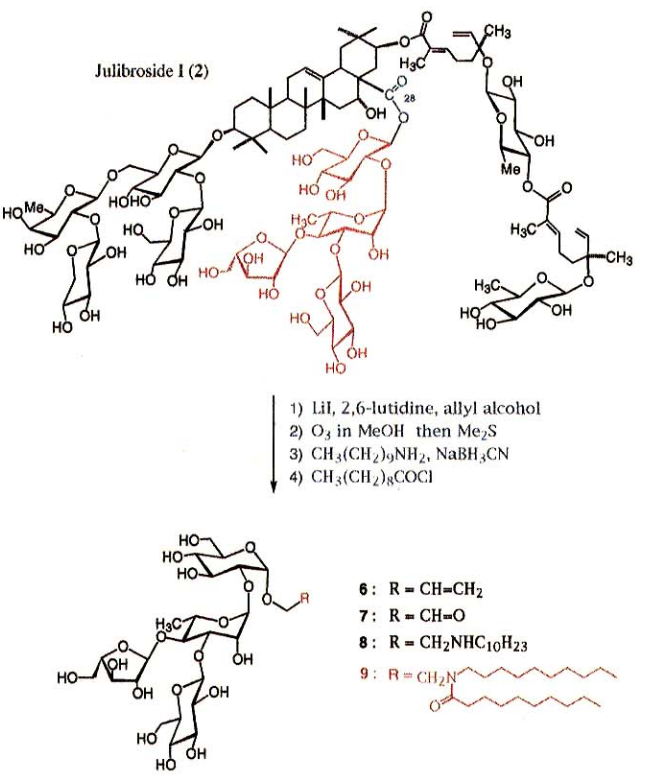

Fig. 3. Synthesis of neoglycolipid (**9**) from Julibroside I (**2**).

without affecting the ester glycosidic bond by using endoglycosidases.

References

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