

Bioactive Glycosides from Solanaceous and Leguminous Plants

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Introduction

Because solanaceous and leguminous natural sources are so widely distributed and are used as foodstuffs and folk medicines, we focussed on these plants and are trying to develop natural medicines after proving the effectiveness of these crude drugs and finding the lead compounds among these natural sources.

A. Solanaceous Plants

1. C₂₇ Steroids (*Solanum* genera)

We have examined the chemical constituents so far of about thirty-five *Solanum* genera (Table 1) and some solanaceous plants including *Solanum dulcamara*¹, *Solanum nigrum*², and *Solanum lyratum*³, well known as anticancer folk medicines since ancient times. Hence, isolated lots of spirostane, furostane, spirosolane and solanidane glycosides, and provided much new information.⁴ From *S. lyratum* the solanidane derivatives showing antitumor activity were obtained (Fig. 1).³

A variety of glycosides were subjected to cytotoxicity tests against various tumor cell lines (PC-6, MCF-7, SW620, NUGC-3, and P-388) and it was shown that the component steroidal glycosides are effective and their activities depend not only upon the type of aglycone but also upon the sort of terminal sugar. The chacotriosyl group (Table 2) in

the sugar residue, and the spirostanol derivative in the aglycone moiety (Table 3) were the most effective.⁵ We propose that the steroidal glycosides enter cells *via* endogenous lectins which are specific receptors for the sugar moiety of the steroidal glycoside. In order to verify this we have to repeat the pharmacological tests. They could be recognized as moderately active anticancer agents which don't show much effectiveness, but they may be developed as natural medicines without side effects. Actually, southern Chinese ingest habitually solanaceous plants as preventives against cancer. Thus,

Table 1. *Solanum* spp. so far examined in our laboratory

<i>Solanum lyratum</i> (4, -, 4)	<i>S. torvum</i> (8, 6, -)
<i>S. nigrum</i> (2, -, 6)	<i>S. gilo</i> (5, -, -)
<i>S. japonense</i> (3, -, -)	<i>S. sisymbriifolium</i> (2, -, -)
<i>S. biflorum</i> (0, 2, -)	<i>S. achrolocucum</i> (3, -, 4)
<i>S. aculeatissium</i> (-, 2, -)	<i>S. integrifolium</i> (1, -, -)
<i>S. dulcamara</i> (6, -, -)	<i>S. surattense</i> (2, -, -)
<i>S. indicum</i> (4, -, 4)	<i>S. ferox</i> (-, 3, -)
<i>S. melonga</i> (-, 1, 2)	<i>S. stramonifolium</i> (3, -, -)
<i>S. toxicarium</i> (3, 5, -)	<i>S. jurpeva</i> (3, -, -)
<i>S. khasianum</i> (-, -, 2)	<i>S. vanhuerchii</i> (-, -, 4)
<i>S. mammosum</i> (-, -, 2)	<i>S. cynathem</i> (-, -, 2)
<i>S. xanthocarpum</i> (-, -, 2)	<i>S. lycocarpum</i> (-, -, 4)
<i>S. paniculatum</i> (7, -, -)	<i>S. tuberosum</i> (9, 4, -)
<i>S. verbascifolium</i> (3, -, -)	<i>S. viarum</i>
<i>S. abutiloides</i>	<i>S. sanitwongsei</i>
<i>S. macrocarpon</i>	<i>S. aethiopicum</i>
<i>S. anguivi</i>	<i>S. demissum</i>
<i>S. chacoense</i>	

Numbers in parenthesis show the number of the compounds obtained from the respective aerial part, underground part, and fruit, and minus shows parts not yet investigated. Plants with no parenthesis are under investigation.

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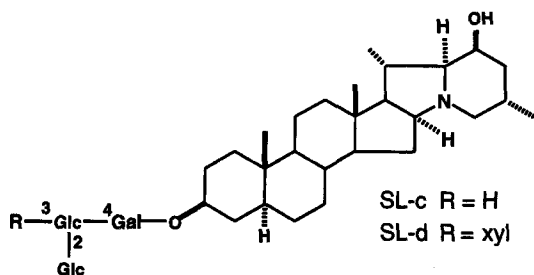


Fig. 1. SL-c and SL-d isolated from *S. lyratum*.

we have substantiated their effectiveness. Furthermore, since *Solanum nigrum* is used for the herpes caused by *Varicella zoster* virus, we applied its 0.5% extract cream to the patients. It brought a good result. We are now measuring the anti-HSV activities of various glycosides using Vero cell to obtain the relationship between the structures and the activities.

2. C₂₈ Steroids

Ergostane derivatives were also obtained from the genus of *Datura*⁶, *Tubocapsicum*⁷, *Physalis*⁸, *Nicandra*⁹, and *Petunia*¹⁰ in parallel with the studies on *Solanum* genera. Chemically interesting structures are shown

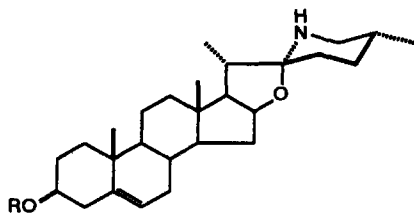


Table 2. Cytotoxicities of Solamargine, SPHA, SPHB, SPHC, and Solasodine (GI₅₀ μg/ml)

R	PC-6	MCF-7	NUGC-3	P388	
5-FU	0.31	1.84	2.21	0.06	
CDDP	0.19	2.95	0.08	0.01	
solamargine	-glc ⁴ -rha 2 rha	2.66	2.16	1.95	1.54
	-glc ² -rha	16.76	7.64	10.09	11.93
	-glc ⁴ -rha	35.91	25.96	34.42	Over
-glc	8.04	9.59	10.76	Over	
solasodine	-H	4.25	1.62	1.47	2.18

in Fig. 2.

3. 26-Aminocholesterol Derivative

From *Solanum abutiloides*, three 26-aminocholesterol derivatives (an example shown in Fig. 3) were isolated.¹¹ These new

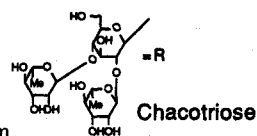
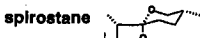
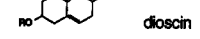

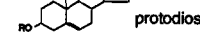
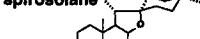
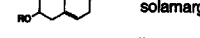
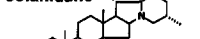
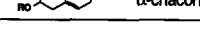


Table 3. Cytotoxicities of dioscin, protodioscin, solamargine, and α-chaconine (GI₅₀ μg/m...

	PC-6	MCF-7	NUGC-3	P388	SW620
5-FU	0.31	1.84	2.21	0.06	1.43
CDDP	0.19	2.95	0.08	0.01	0.63
 spirostane  dioscin	1.12	0.64	0.59	0.40	0.64
 furostane  protodioscin	1.53	1.86	1.69	1.67	1.83
 spirostane  solamargine	2.66	2.16	1.95	1.54	1.62
 solanidane  α-chaconine	1.83	1.54	1.43	1.58	1.46

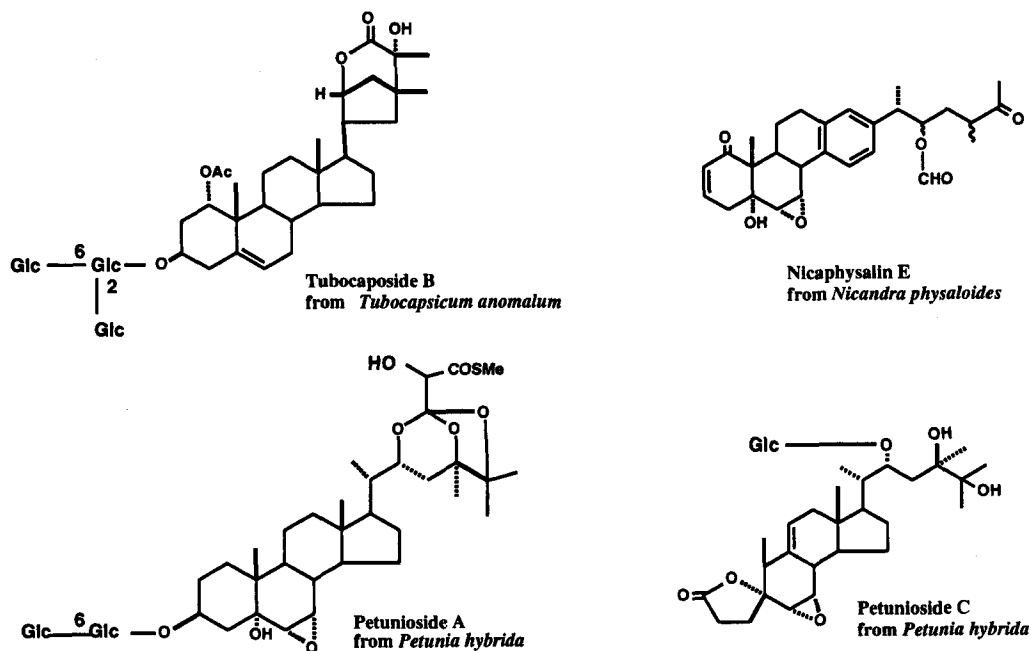


Fig. 2. C₂₅ steroidal saponins from some solanaceous plants.

natural models are regarded as important key intermediates to the spirosolane and solanidane derivatives on the biogenetic pathway, and could be agents for inhibiting the biosynthesis of cholesterol.

B. Leguminous Plants

1. C-28 Me Oleanane

a. Flowers of *Pueraria lobata*. Since the flower of *Pueraria lobata* (Puerariae Flos) is used to counteract drinking, we examined various *in vivo* tests starting from alcohol metabolism and obtained results

shown in Chart 1.¹² From the crude saponin fraction (PF-SP in Chart 1), soyasaponin I, kaikasaponin III, and kakkasaponin I were isolated.

Isoflavonoid fr. (PF-IF) contained kakkalide as major ingredient.¹³

b. Whole Plants of *Abrus cantoniensis*.

This crude drug (Abri Herba) has been used as a folk medicine for infectious hepatitis in China. The clinical efficacy of this drug was recently confirmed in China. On separation two crude saponin fractions were obtained. One is less polar than the other.

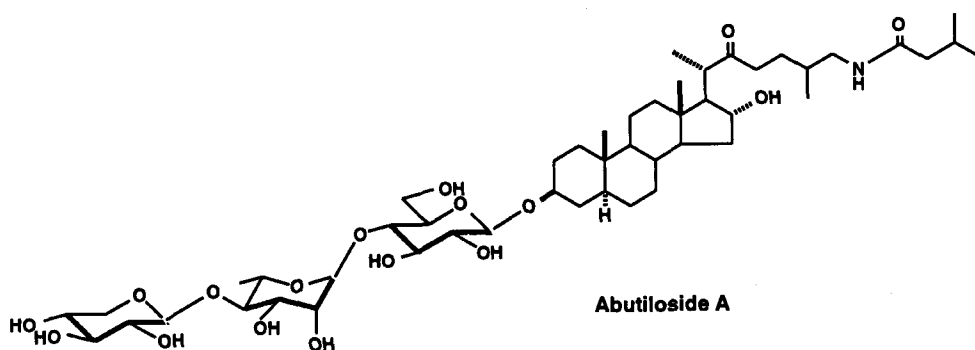


Fig. 3. 26-Aminocholesterol derivative from *S. abutiloides*.

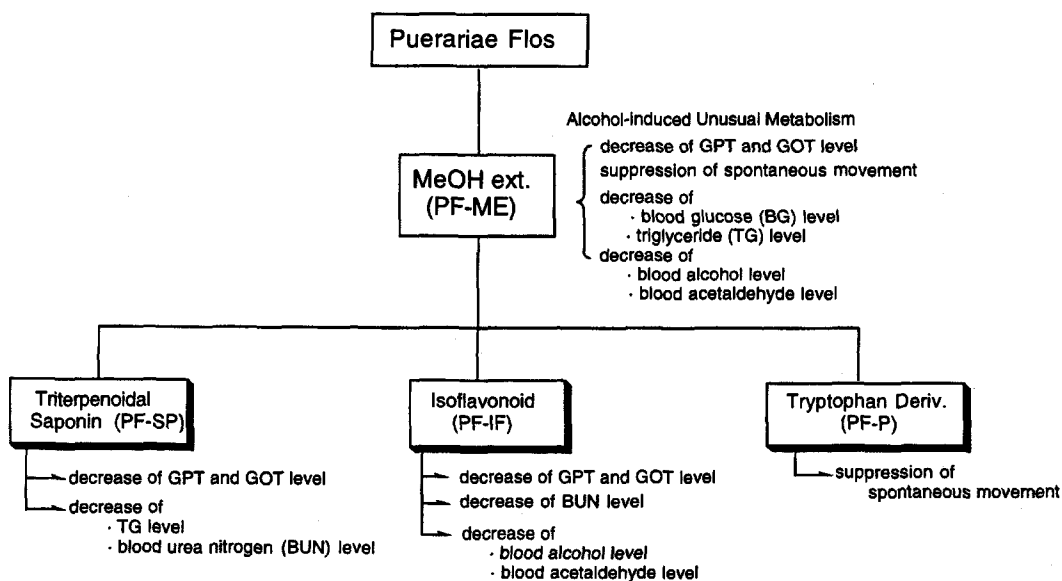


Chart 1. Pharmacological effects of Puerariae Flos and its constituents.

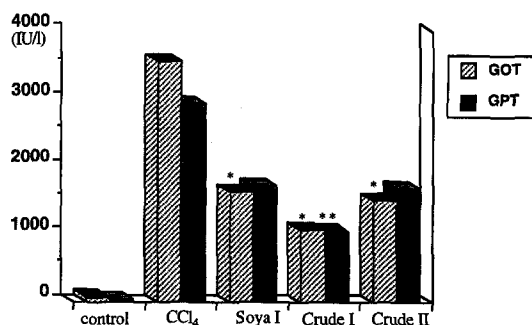


Fig. 4. Effects of Abri Herba extract on mice treated with CCl₄.

Effects of Soyasaponin I (Soya I), Crude saponin I (Crude I) and Crude saponin II (Crude II) on CCl₄ induced liver injury by oral administration; Dose was each 500 mg/kg; Each column represents mean of 10 mice. *P<0.05, **P<0.01.

The efficacy of these fractions and the methanolic extract was confirmed towards liver injury by CCl₄. As shown in Fig. 4 these saponins clearly blocked liver injury compared to the untreated CCl₄ control. Especially, the crude saponin fraction showed 70% inhibition.¹⁴ Since the saponin fraction possessed considerable activity, we tried to examine the chemical ingredients. At first, we investigated their sapogenols to get many new sapogenols, named abrisapo-

genols (Fig. 5), along with known ones.¹⁵

The common features of these sapogenols are that they possess a methyl group at C-17 and some oxygen functions on the E-ring. Furthermore, we have isolated several saponins (Fig. 6).¹⁶ New saponins were named abrisaponin. The major saponins were soyasaponin I and abrisaponin I. These saponins have glucuronic acid in the *endo*-sugar chain.

c. Roots of *Pueraria lobata*. This crude drug (*Puerariae Radix*) is widely used as antispasmodic, antipyretic, and antiperspirant medicine in Chinese orthodox medicines, Kampo. We have first found the presence of triterpenoidal saponins in this crude drug (Fig. 7).¹⁷ Among the above actions of this crude drug, antispasmodic action is explained by daidzein. However, the substances responsible for the other actions have never been isolated from the crude drug. Now, we can guess that the triterpenoid saponin might be responsible for antipyretic and antiperspirant actions.

d. The Others. We have obtained saponins from thirteen plants as listed in Table 4.¹⁸ Of these saponins, seventy two saponins

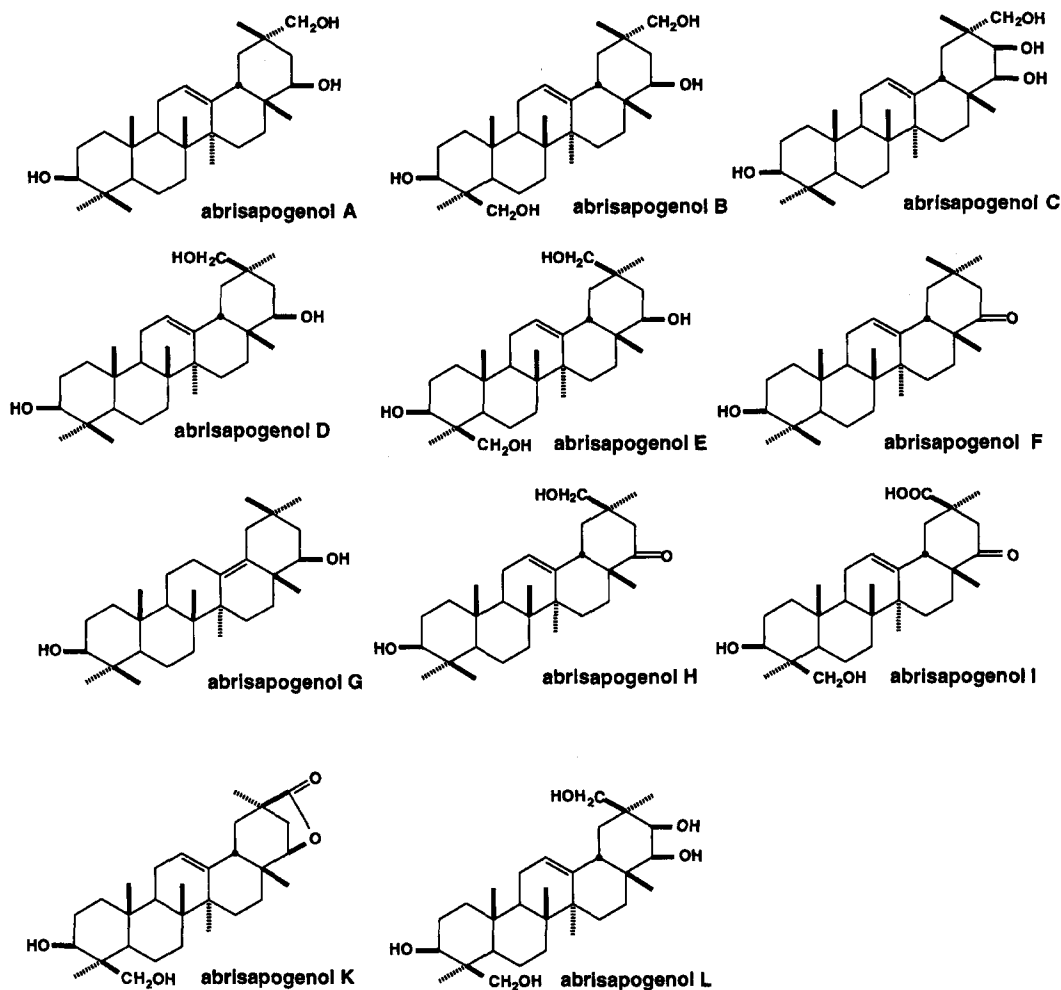


Fig. 5. New sapogenols from Abri Herba.

were new ones including novel twenty one sapogenols.

The known oleanane saponins mostly belong to the type with a carboxylic group at C-28, some of which are used as anticough medicines. In contrast, we deal with the oleanane triterpene saponins replaced by a methyl group and it might be predicted that these type saponins are effective for liver injury. These kinds of saponins are not very abundant except in the legume family. We would expect that the oleanane glycosides in the leguminous plants are able to explain the effectiveness of the respective crude drugs.

e. Structure-Hepatoprotective Relationship

On the way of our hepatoprotective study, from the reason that the antipyretic and perspiration of *Puerariae Radix* might be related to the inflammatory reaction, we have investigated the conditions for *in vitro* assay method using immunological liver injury of primary cultured rat hepatocytes. This method is regarded as one of the highly correlated to human hepatitis. This slide shows preparations of antiserum against the rat liver plasma membranes and primary cultured rat hepatites. The hepatoprotective activities were quantified by measuring the

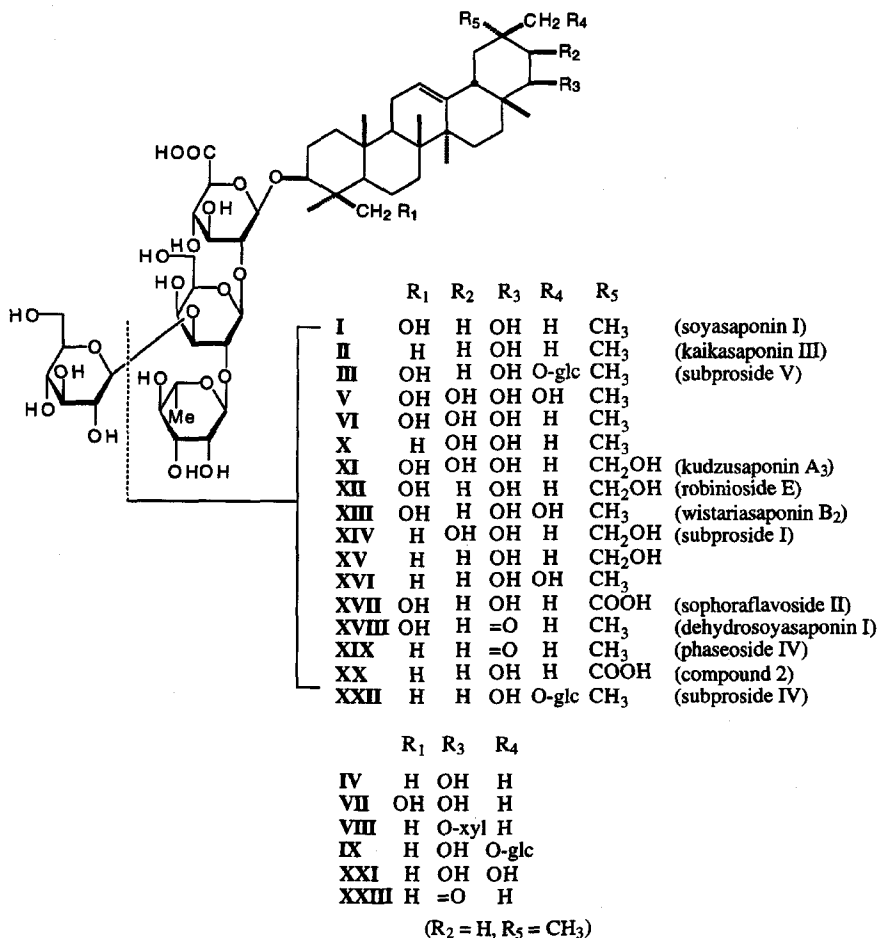


Fig. 6. Abrisaponins from Abri Herba.

« Assay Method for Hepatoprotective Drugs Using Primary Cultured Rat Hepatocytes Injured with Antiserum against Rat Liver Plasma Membranes »

Arao T. et al., *Biol. Pharm. Bull.*, 20 (9), 988-991 (1997).

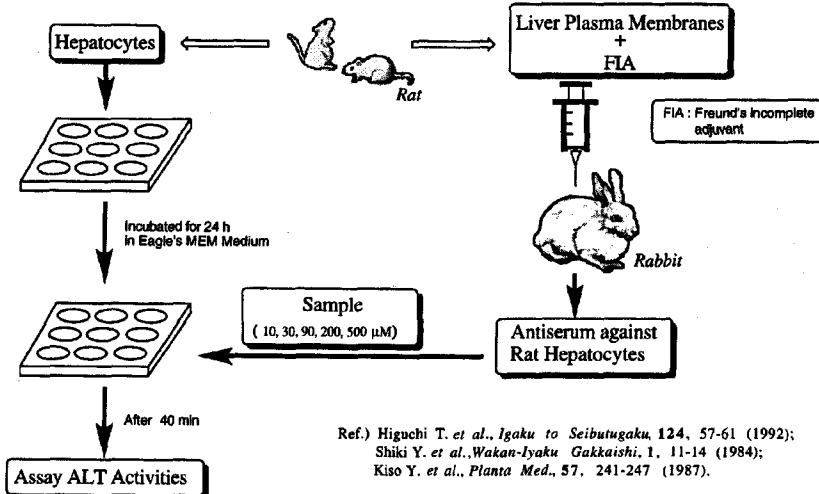


Table 4. Continued

Plants	Parts	Total OGs	New Aglycones	New OGs
<i>Pueraria thomsonii</i>	root	5	0	2
<i>Pueraria thomsonii</i>	flower	3	0	0
<i>Robinia pseudo-acacia</i>	bark	13	0	10
<i>Sophora subprostrata</i>	root	11	4	7
<i>Sophora flavescens</i>	root	4	0	3
<i>Vicia faba</i>	seed	1	0	0
<i>Vigna angularis</i> cv. Dainagon	seed	2	0	0
<i>Vigna unguiculata</i> cv. Chuguro	seed	2	0	0
<i>Wisteria brachybotrys</i>	bark	9	0	5
Total 33	41	172	21	72

« Structure Hepatoprotective Relationships of Oleanene Glucuronides »

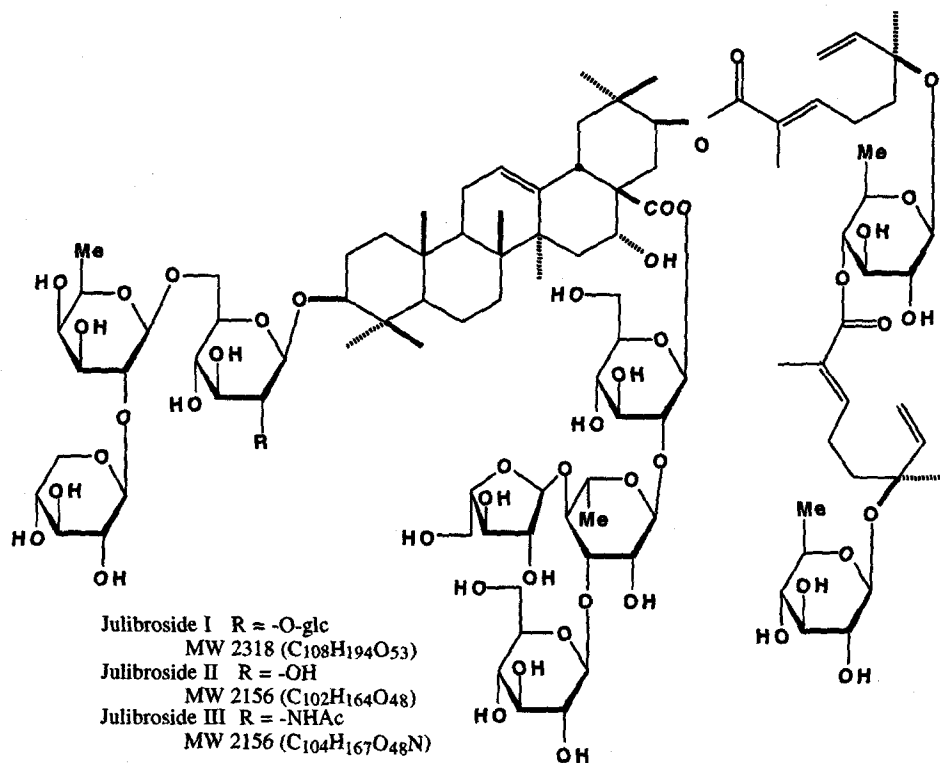
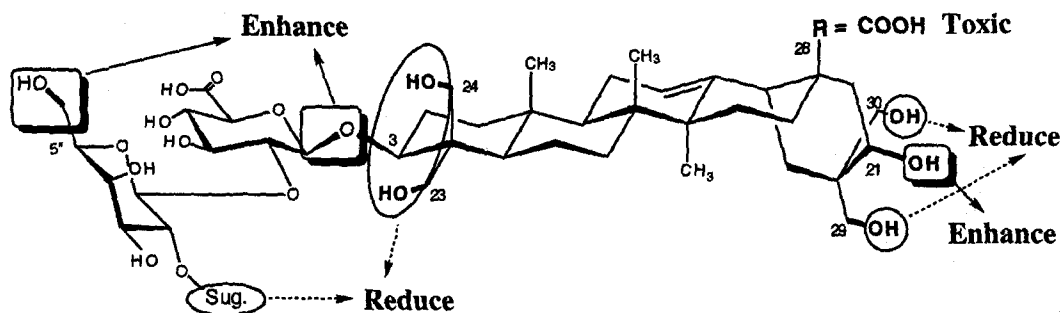


Fig. 8. Albizziae saponins from Albizziae Cortex.

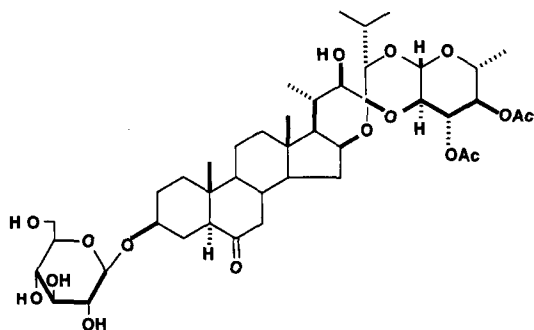


Fig. 9. Growth inhibitor from *Heloniopsis japonica*.

from the above-mentioned ones in respect to the functional group at C-17. Among these saponins, julibrosides II and III possessed strong topoisomerase I activity.

C. Others

1. Cholestane Derivative

During our study on the steroid components among the liliaceous plants, we obtained a novel cholestane derivative, named helojaposide, which is a unique cholestane diglycoside having the ketal carbon at C-23 linked to C-16, and C-2' of the fucosyl moiety (Fig. 9). At 10 ppm this showed a significant inhibitory activity for the root growth of rice.²⁰

2. Cycloartane

Thalictrum spp. is used as a folk medicine in Japan for treating stomach ache. From this herb fourteen cycloartane-type triterpenoidal saponins were isolated (Fig. 10).²¹ Among these the glycosides (thalictoside D, E in Fig. 11, F and G) with the side chain at C-17 possessing a five-membered ring were shown to have strong LTT (lymphocytotransformation test) activity.

D. Transglycosylation

Through our studies on the various glycosides, we have understood that the sugar moiety also plays an important role in the bioactivity, so that we tried to transfer the sugar to another aglycone. Julibrosides possessed anticancer activity, but, when the

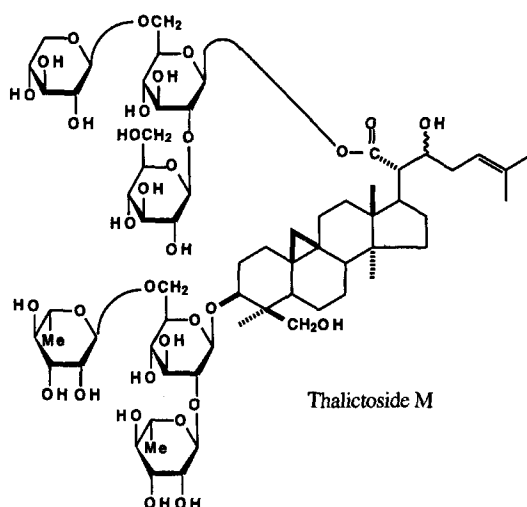


Fig. 10. Thalictoside M from *Thalictri Herba*.

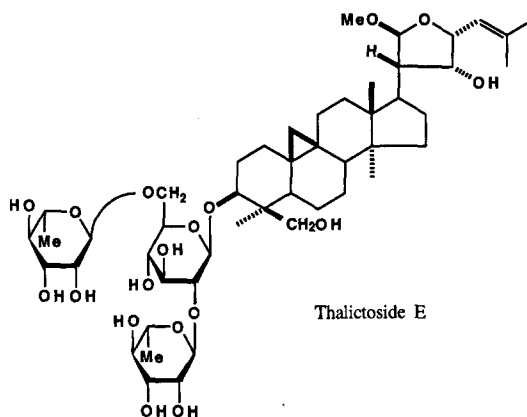


Fig. 11. Cycloartane glycoside having LTT activity.

ester sugar linkage at C-28 was taken off, the product turned out to have no activity. Therefore, it was supposed that this sugar moiety was important for the activity. Hence, we cut off this sugar moiety as an allyl glycoside. Next, we subjected this compound to ozonolysis to give an aldehyde, which was subsequently converted to an amine by reductive amination with decylamine using cyanoborohydride. The resulting amine was reacted with decanoyl chloride to give a *N*-acyl derivative (Chart 2).²² Pharmacological tests for the product are currently in progress. Next, we removed the allyl group at an anomeric center and applied the Schmidt

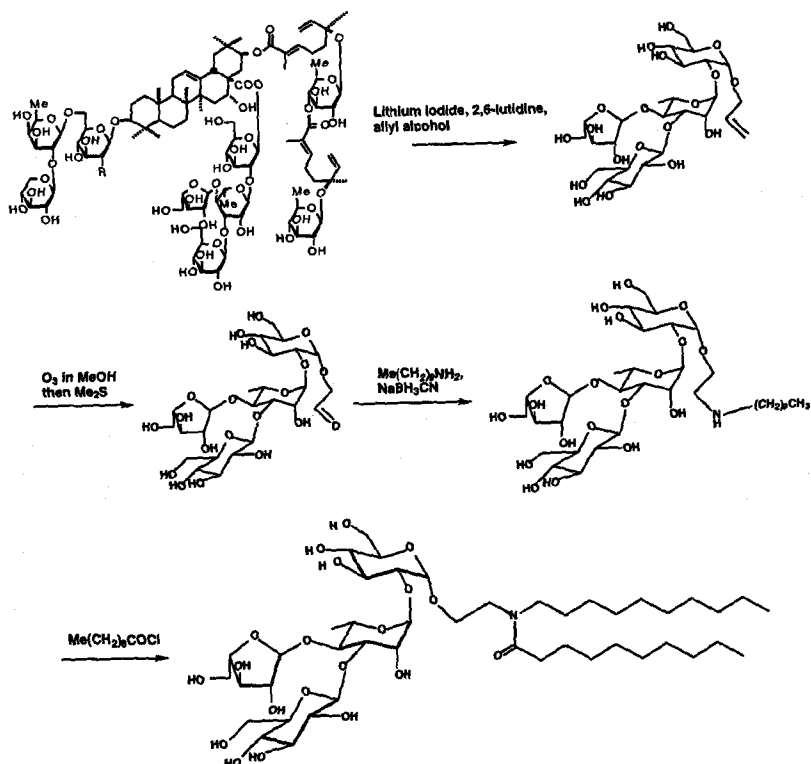


Chart 2. Synthesis of a neoglycolipid.

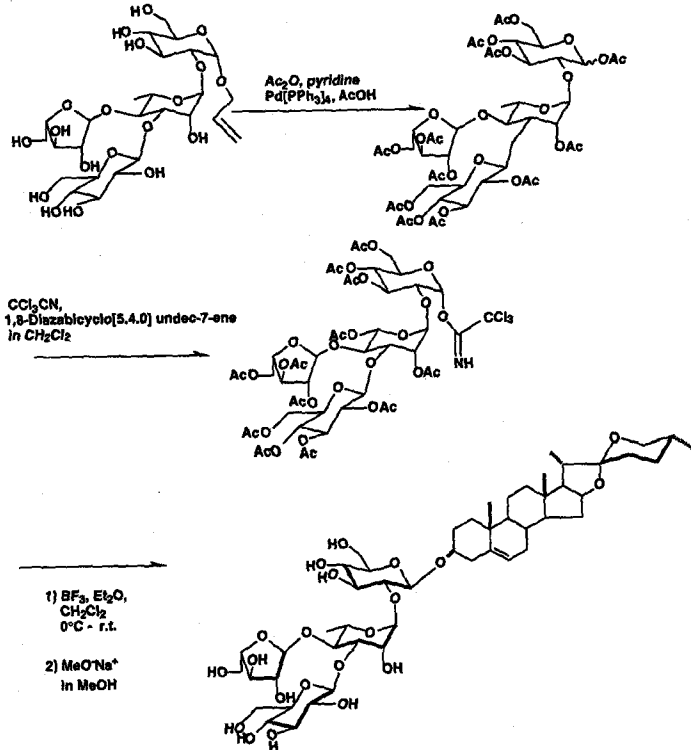


Chart 3. Transglycosylation into the spirostanol.

method. Thus we obtained cholesteryl and diosgenin glycosides in β -form (Chart 3).

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