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Pectic polysaccharides from Chinese herbs: structure and biological activity

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Bioactive pectic polysaccharides have been isolated from Chinese herbs, and their structure, activity and modes of action have been studied. Complement-activating pectin from *Angelica acutiloba* contained a variety of neutral galactosyl chains which attached to the rhamnogalacturonan core (ramified region), and these neutral galactosyl chains were essential for the expression of complement activating activity, but the polygalacturonan moiety modulated the mode of activation by the ramified region. Another pectin-like polysaccharide, bupleuran 2IIb from *Bupleurum falcatum* showed a potent immune complex clearance-enhancing activity. Bupleuran 2IIb may enhance Fc receptor expression on the macrophage surface via the ramified region as an active site. An anti-ulcer pectin, bupleuran 2IIc from *B. falcatum* consists mainly of partially branched polygalacturonan in addition to the ramified region and the rhamnogalacturonan II-like region, and the polygalacturonan region may contribute somewhat to the expression of activity. Collectively considered results have indicated that the pharmacological activity of each of the pectic polysaccharides may depend on their fine chemical structure.

INTRODUCTION

Although the incidence of many infections that were once common on a global scale has declined, endogenously caused chronic disorders have been on the increase during the second half of the 20th century. Because of this, traditional medicine has begun to attract worldwide attention.

Chinese herbal medicine, so-called Kampo medicine, is now very attractive in Japan because it is possible to treat diseases which are still difficult to cure using modern medicine, and at present more than 80% of medical doctors are using Kampo medicine in Japan. Therefore, scientific evaluation of the efficacy of these medicines is very important. Recently, several pharmacological activities have been reported in polysaccharides isolated from plants containing Chinese herbs (Franz, 1989; Yamada & Kiyohara, 1989; Wagner, 1990) in addition to the frontier review of Whistler *et al.* (1976) as shown in Table 1. Most of these activities have been observed in pectic polysaccharides. Therefore, it is possible to use pectic polysaccharides as medicines.

The general structure of acidic pectic polysaccharides is known to comprise an $\alpha(1 \rightarrow 4)$ linked polygalacturonan region, and a ramified region which consists of rhamnogalacturonan core substituted neutral sugar chains—such as arabinogalactan and arabinan—as the side chains (Darvill *et al.*, 1980; Aspinall, 1980). Pectin and pectic arabinogalactan are classified as major pectic polysaccharides. Pectin consists of a large quantity of polygalacturonan region with a small quantity of ramified region, whereas pectic arabinogalactan consists mainly of ramified region.

In order to understand the biological activity of pectic polysaccharides on a molecular level, the following two problems should be clarified: (i) what is the minimum essential carbohydrate structure for the expression of each biological activity; and (ii) how do these polysaccharides express biological activity. However, very few studies on the fine chemical structure of active polysaccharides and the structural requirement for these activities have been carried out. The present authors have studied the structure and pharmacological activity of several bioactive pectic polysaccharides from Chinese herbs in order to resolve these problems (Yamada & Kiyohara, 1989).

In this paper, three recent studies on bioactive polysaccharides from Chinese herbs are presented.

Complement activating (anti-complementary) polysaccharides from the roots of *Angelica acutiloba*

The complement system consists of over 20 serum proteins including 9 complement components (C1–C9)

Table 1. Pharmacological activity of polysaccharides isolated from plants

Immune system	Complement activating activity Anti-tumor activity Mitogenic activity Stimulation of macrophage phagocytosis Stimulation of tumor-cytotoxicity by macrophage Induction of cytokines (IL-1, LAF, TNF-α, IFN-b2) and oxygen radical Enhancing activity of NK cell cytotoxicity Anti-inflammatory activity
Coagulation system	Anti-coagulant activity
Digestive system	Anti-emetic activity Anti-ulcer activity
Others	Hypoglycemic activity Anti-glaucoma activity

and their regulators. The complement proteins are activated by a cascade mechanism of classical or alternative pathways. The classical pathway is activated by immune complexes containing IgM and IgG antibodies, and C1 binds to the Fc region of antibody. The complex then activates the following complement system. On the other hand, the alternative pathway is directly activated from C3 by microorganisms, protozoa and some activators such as lipopolysaccharide, and is followed by further activations. Thus, in general, the alternative pathway constitutes the natural defence mechanism of the non-immune host. Complement activation appears to be intrinsically associated with several immune reactions, such as the activation of macrophages and lymphocytes, cellular cooperation, immunopotentiation and regulation of cyclical antibody production (Egivang & Befus, 1984). Several complement-activating (anti-complementary) polysaccharides have been discovered in the hot water extract of Chinese herbs, such as the roots of *Angelica acutiloba* Kitagawa, the leaves of *Artemisia princeps* PAMP, the roots of *Bupleurum falcatum* L., the roots and the leaves of *Panax ginseng* C. A. Meyer, the seed of *Coix lacryma-jobi* var. mayuen and the roots of *Glycyrrhiza uralensis Fisch* et D C., etc. (Yamada & Kiyohara, 1989; Zhao *et al.*, 1991).

Six kinds of complement activating polysaccharides, AGIIa, AGIIb-1, AR-2IIa, 2IIb, 2IIc and IId have been purified from the hot water extract of the roots of *A. acutiloba* (Yamada *et al.*, 1984, 1987; Kiyohara *et al.*, 1988) (Table 2).

AGIIa and AGIIb-1 were classified as pectic arabinogalactans, whereas AR-2IIa, 2IIb, 2IIc and IId were classified as pectins, and contained over 90% galacturonic acid with traces of neutral sugars such as arabinose, galactose and rhamnose. AGIIa and AR-2IId showed the most potent complement-activating potency. AGIIb-1 showed moderate activity and others were weak. Both arabinogalactans were characterized to contain arabino-β-3,6-galactans by structural analysis (Yamada *et al.*, 1984, 1987; Kiyohara & Yamada, 1989*a*), and these arabinogalactans and pectins were shown to be typical complement-activating polysaccharides characteristic of Chinese herbs (Yamada & Kiyohara, 1989). Of these pectins, AR-2IIa, 2IIb, 2IIc and 2IId activate complement via the classical pathway, but only AR-2IId also activates complement via the alternative pathway. These four pectins commonly consist of α(1 → 4) linked polygalacturonan and ramified region. The ramified region contains the rhamnogalacturonan core in which 2-linked and/or 2,4-

Table 2. Characterization of complement activating polysaccharides

	AGIIa	AGIIb-1	AR-2IIa	AR-2IIb	AR-2IIc	AR-2IId
Class	Arabinogalactan		Pectin			
Uronic acid content (%)	2.8	8.7–12.2	91.7	98.6	97.2	97.0
Uronic acid component						
Galacturonic acid (GalA)	+	0.2–0.4	+	+	+	+
Glucuronic acid (GlcA)	—	0.1	—	—	—	—
Neutral sugar component (molar ratio)						
Arabinose (Ara)	1.2	1.8–2.2	0.5	0.9	1.5	1.6
Galactose (Gal)	1.0	1.0	1.0	1.0	1.0	1.0
Rhamnose (Rha)		0.2–0.3	0.4	0.9	1.6	0.7
Xylose (Xyl)			0.06	0.06	0.1	0.09
Mannose	trace			trace		trace
Glucose (Glc)	trace			trace		0.3
Complement activating potency	+++	++	+	+	+	+++

disubstituted rhamnosyl residues alternate with 4-linked galacturonic acid in sequences, and neutral carbohydrate side chains such as arabinan, galactan and arabinogalactan which are assumed to be linked to position 4 of rhamnose (Kiyohara *et al.*, 1988). Digestion of each pectin with endo α(1 → 4) polygalacturonase after de-esterification gave an enzyme-resistant fraction (PG-1a, PG-1b, PG-1c and PG-1d from AR-2IIa, 2IIb, 2IIc, and 2IId, respectively) and several α(1 → 4) linked oligogalacturonides (Kiyohara *et al.*, 1988). Methylation analysis indicated that PG-1a, PG-1b, PG-1c, and PG-1d each consisted of rhamnogalacturonan core with neutral carbohydrate side chains. Therefore these were suggested to be 'ramified' regions in the pectins. The ramified region (PG-1) from each pectin had a more potent complement-activating activity than the corresponding original pectin, but the oligogalacturonides had weak or negligible activities. These results suggest that the complement-activating potency of these pectins is expressed mainly by their ramified regions, and that the activities may be regulated by the polygalacturonan region (Kiyohara *et al.*, 1988). Lithium-mediated degradation of the ramified region from four pectins gave several neutral oligosaccharides which attached to the rhamnogalacturonan core. Because this oligosaccharide mixture reduced activity, and rhamnogalacturonan did not show activity, these results suggest that complement activation by the ramified region may be due to a combination of the rhamnogalacturonan core and the neutral sugar chains (Kiyohara *et al.*, 1989). Digestion of the ramified region with *exo*-α-L-arabinofuranosidase, or *exo*-β-D-galactosidase, or both, did not change the activity (Kiyohara *et al.*, 1989). Because lithium degradation products still showed a significant activity, neutral carbohydrate chains were fractionated from the product by gel filtration on Biogel P-2 and HPLC in order to discover the complement-activating activity of neutral carbohydrate chains attached to the rhamnogalacturonan core. Of these carbohydrate fractions, the high molecular weight fraction and the oligosaccharide, which appeared in the same elution volume with glucopentaose, showed potent activity. This result indicated that neutral carbohydrate chains of oligosaccharide size can express activity. An alternative new β-elimination method for the release of neutral carbohydrate chains was also developed, which attached to 4-linked galacturonic acid residues, in the presence of NaBD4, in order to clarify the details of the linkage region between neutral carbohydrate side chains and rhamnogalacturonan core (Kiyohara & Yamada, 1989*b*). This method was applied for the structural analysis of *exo*-β-galactosidase treated ramified regions in these pectins (Kiyohara *et al.*, 1989). The resulting neutral oligosaccharide-alditols were fully methylated and analyzed by GC-MS, and galactitol-*l-d* or rhamnitol-*l-d* was identified as the reducing terminal in these oligosaccharides. These pectins contained β(1 → 6) linked galactosyl di- to tetra-saccharides, attached to position 4 of the 2,4-disubstituted rhamnosyl residue in the rhamnogalacturonan cores, either directly or through 4-linked galacturonic acid as the common structure. AR-2IIa also contained a large proportion of (1 → 3,6)-linked long β-galacto-oligosaccharide chains, whereas AR-2IIb, -2IIc and -2IId contained only a trace

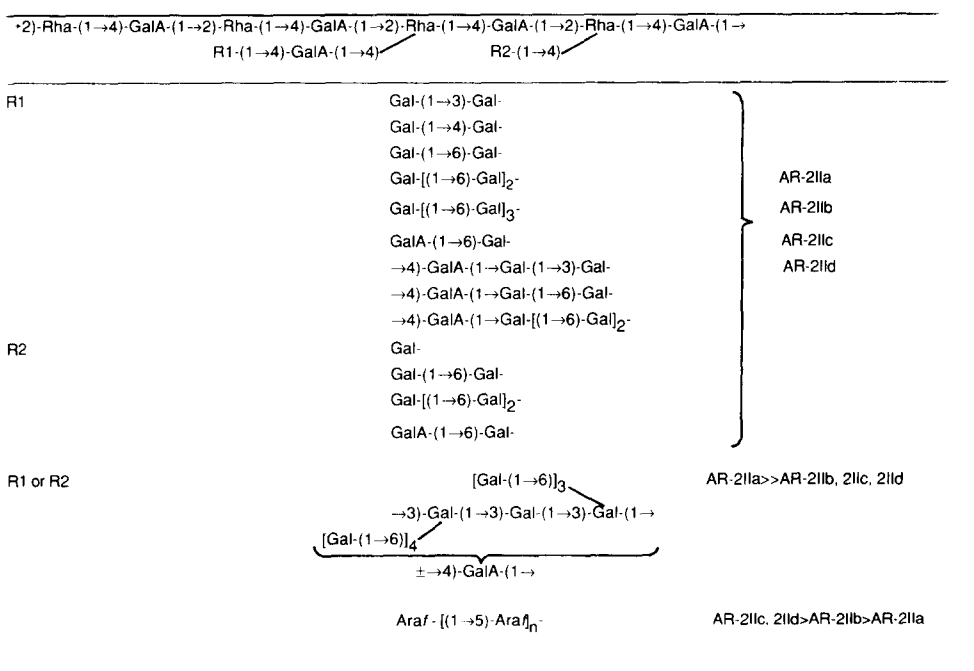

Fig. 1. Structure of enzyme-treated 'ramified' region of complement-activating pectin from *Angelica acutiloba* Kitagawa.

of such a moiety (Fig. 1). With these results it would be expected that original pectins would have more long galactosyl side chains and β-3,6-galactan chains. Of those eliminated neutral oligosaccharides, the (1 → 3,6)-linked long β-galactosyl chain showed potent activity, but the mixture of β(1 → 6)-linked galactotriose, galactotetraose and β(1 → 6)-linked galactooligosaccharides having a rhamnosyl residue as a reducing terminal also showed significant activity (Fig. 2).

It has been suggested that, for AR-2IIa, 2IIb, 2IIc and 2IId, the neutral carbohydrate side chains attached to the rhamnogalacturonan core ('ramified region') might be essential for the expression of activity, since each PG-1 from four pectins showed similar potent activity (Kiyohara *et al.*, 1988). Although only AR-2IId activated complement through the classical pathway not the alternative pathway, each PG-1 activated complement through both pathways. It has been suggested that the activity of the ramified region in the pectins might be regulated by the polygalacturonan region. AR-2IId was resistant to *endo*-α-polygalacturonase digestion. When AR-2IId was digested with *endo*-α-polygalacturonase without de-esterification, and pyridylaminated derivatives of the resulting galacturonooligosaccharides were applied to HPLC, polymerized oligosaccharides higher than heptasaccharide were eluted. However, the enzymic digest of de-esterified AR-2IId mainly contained tri- and tetra-galacturono-oligosaccharides, similar to that of AR-2IIc, which has almost the same molecular weight as AR-2IId (Kiyohara & Yamada, 1994). These results indicate that AR-2IId may have a different methyl-ester distribution in the polygalacturonan region to the other three pectins. Collectively considered, these results suggest that this polygalacturonan moiety may modulate the activation of an alternative pathway by the ramified region, and

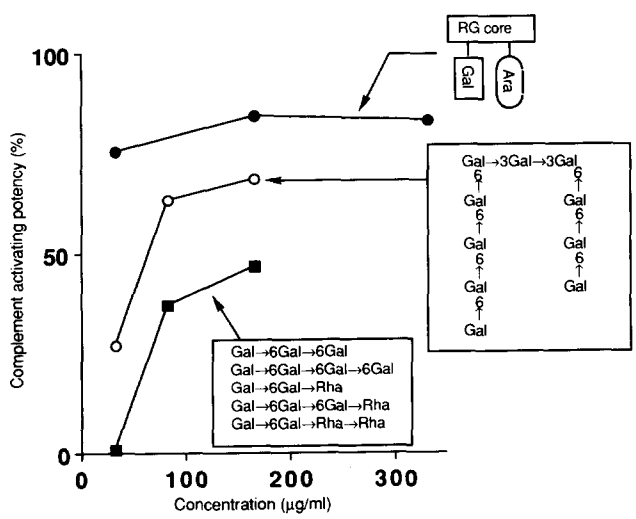

Fig. 2. Complement-activating potency of the ramified region and its neutral oligosaccharides from the pectins of *A. acutiloba*.

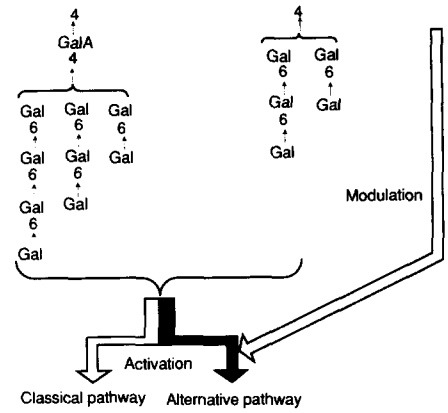

Fig. 3. Structural requirement for complement activation by AR-2IIa, IIb, IIc and IId.

this modulation may be controlled by the distribution of the methyl-ester on the polygalacturonan moiety (Fig. 3). Several complement-activating pectic polysaccharides have been isolated from various herbs (Wagner, 1990). These results have indicated that the neutral carbohydrate chains consisting of β-(1 → 6)-linked galactose attached to the rhamnogalacturonan core may be the minimum essential structure for the expression of complement-activating activity.

Immune complex clearance-enhancing polysaccharides from the roots of *Bupleurum falcatum*

Association of circulating antigens and antibodies to form immune complexes is a normal phenomenon, and normally this immune complex is eliminated by the reticuloendothelial system. But, if excessive quantities of immune complex are formed, immune complex deposits onto several tissues, in which case the complement system is activated, resulting in anaphylatoxin formation, and inflammation occurs. Therefore, it has been considered that this deposition of immune complex may be one of the causes of auto-immune disease. A primary function of mononuclear phagocytic cells is to bind immune complexes through Fc and complement receptors, followed by subsequent endocytosis and degradation. Therefore, the binding of immune complexes to these cells is an important functional parameter for immune complex clearance, and the enhancing substance has a binding potential role to treat autoimmune diseases. Recently, a new photometric microassay was developed by these authors for immune complex (IC) binding to macrophages in a homologous system using glucose oxidase–antiglucose oxidase complexes (GAG) as a model for IC clearance *in vitro* (Matsumoto *et al.*, 1990). The extracts of Chinese herbs were then tested against immune complex clearance through the Fc receptor of the macrophage by this

assay, and it was found that the acidic pectic polysaccharides, bupleuran 2IIb and 2IIc, from *B. falcatum* L. showed a potent enhancing activity (Matsumoto *et al.*, 1993*a*). Both polysaccharides remarkably enhanced GAG binding activity through Fc receptors of macrophages, and bupleuran 2IIb showed higher activity than bupleuran 2IIc (Fig. 4).

The most active polysaccharide, bupleuran 2IIb, has a molecular weight of 23 000, contains 75.5% uronic acid, and consists of mainly rhamnose, arabinose, galactose and galacturonic acid (Yamada *et al.*, 1989). Bupleuran 2IIb consists of high methyl-esterified and less esterified galacturonan regions, and a rhamnogalacturonan core which is substituted with neutral carbohydrate side chains such as galactan, arabinogalactan and arabinopyranan (Fig. 5) (Yamada *et al.*, 1989). Bupleuran 2IIb also has a potent complement-activating activity (Yamada *et al.*, 1989). The GAG binding enhancing activity of bupleuran 2IIb was reduced by periodate oxidation but not pronase or *endo*-polygalacturonase digestions. This result indicates that neutral carbohydrate chains of bupleuran 2IIb are important for the expression of activity. When bupleuran 2IIb was treated with *endo*-polygalacturonase, the resulting enzyme-resistant ramified region showed a potent activity, but the activities of oligogalacturonides were weak or negligible. This result suggested that the ramified region which consisted of a rhamnogalacturonan core with neutral carbohydrate chains is important for this enhancing activity, similar to the activity of complement-activating polysaccharides.

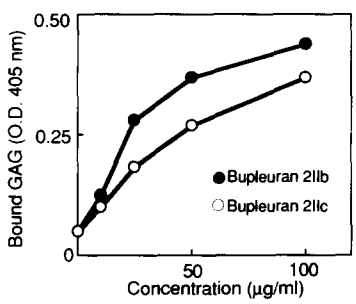

Fig. 4. GAG binding activity through Fc receptor of macrophages by bupleuran 2IIb and 2IIc.

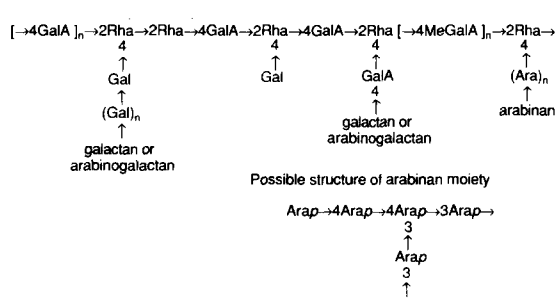

Fig. 5. Possible structural model of bupleuran 2IIb.

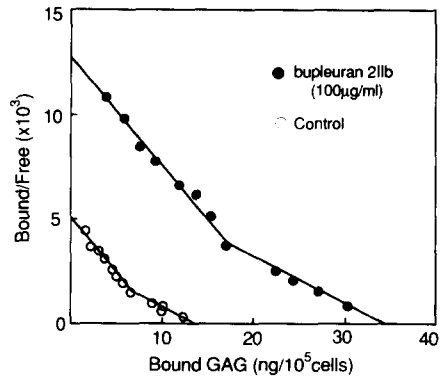

Fig. 6. Scatchard analysis of GAG binding through Fc receptors of macrophages by bupleuran 2IIb.

The result of Scatchard analysis of GAG binding to macrophages indicates bupleuran 2IIb enhanced Fc receptor expression on the cell surface (Fig. 6). Fc receptors of macrophages have a variety of biological activities concerned with immune complex clearance, antibody-dependent cell-mediated cytotoxicity, release of arachidonic acid metabolites, release of lysozomal enzymes, release of active oxygen and release of IL-1. Bupleuran 2IIb may regulate these activities. Up-regulation of the Fc receptor by bupleuran 2IIb was also suggested to mediate by *de novo* synthesis of the receptor protein.

Bupleuran 2IIb may bind to the macrophages through the specific polysaccharide receptor for the ramified region, because when the pronase treated macrophages are incubated with bupleuran 2IIb, GAG binding activity is completely inhibited; this stimulation may enhance gene expression, and resulting mRNA may synthesize Fc receptor protein on the macrophage surface (Matsumoto *et al.*, 1993*a*). Excess amounts of immune complex may bind to the newly formed Fc receptors, and then may be degraded by the macrophages.

Anti-ulcer polysaccharides from the roots of *Bupleurum falcatum*

During a study of the polysaccharide components of Chinese herbs, the potent anti-ulcer polysaccharides (bupleuran 2IIb and 2IIc) against HCl–ethanol-induced ulcerogenesis in mice were obtained from the hot water extract of roots of *Bupleurum falcatum* (Yamada *et al.*, 1991*a*). Bupleuran 2IIc showed higher activity than using a clinical anti-ulcer drug, Sucralfate, at the same dose, but the activity of bupleuran 2IIb was a little weaker than bupleuran 2IIc (Fig. 7).

Bupleuran 2IIc has a molecular weight of 63 000 and contains 93.6% galacturonic acid, with small amounts of neutral sugars such as rhamnose, arabinose, galactose, glucose, mannose and xylose. Therefore, bupleuran 2IIc also can be classified as a pectin-like polysaccharide.

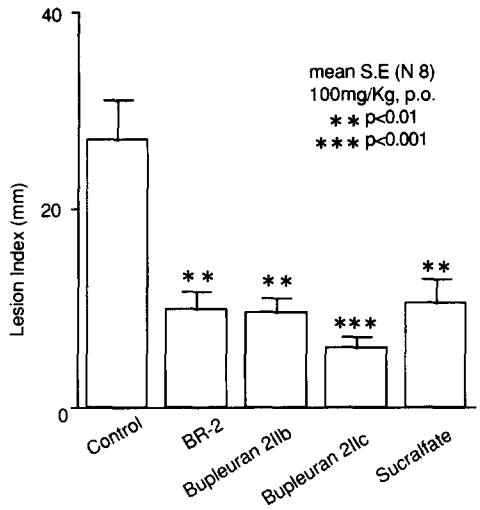

Fig. 7. Effect of bupleuran 2IIb and 2IIc on HCl–ethanol-induced gastric lesions in mice.

The anti-ulcer activity of an acidic polysaccharide fraction, BR-2 (mainly containing bupleuran 2IIb and 2IIc) from *B. falcatum* was reduced by periodate oxidation and *endo*-polygalacturonase digestion, but not pronase digestion suggesting the polygalacturonan region may contribute to the expression of the activity (Yamada *et al.*, 1991*a*). The oral administration of BR-2 at doses of 50–200 mg/kg dose prevented the formation of gastric lesions induced by HCl–ethanol (Yamada *et al.*, 1991*a*). The intraperitoneal and subcutaneous administration of BR-2 also reduced this gastric lesion (Sun *et al.*, 1991). Therefore, BR-2 was suggested as working through not only local action but also systemic action in the stomach. BR-2 also inhibited a variety of acute experimental ulcer models such as ethanol-induced ulcer, indomethacin–HCl-induced ulcer, pylorus ligated ulcer, water-immersion stress ulcer and a chronic ulcer model, acetic-acid induced ulcer by oral administration (Sun *et al.*, 1991; Matsumoto *et al.*, 1993*b*). Because the level of prostaglandin E2 in the gastric juice and mucosal layer was not affected by oral administration of BR-2, and the inhibitory effect of BR-2 on HCl–ethanol-induced lesions was suppressed by pretreatment with indomethacin, which is an inhibitor of prostaglandin biosynthesis, it was suggested that endogenous prostaglandins did not mediate the cytoprotective action of BR-2 (Sun *et al.*, 1991). BR-2 at 200 mg/kg caused a significant increase of alcian blue binding to gastric mucosa in rats and mice (Sun *et al.*, 1991). Sucralfate and 16-dmPGE2 also showed a similar increase in rats. However, BR-2 did not change hexosamine and *N*-acethylneuraminic acid contents in gastric juice and gastric mucosa from pylorus-ligated rats. Because alcian blue dye is able to bind to negatively charged materials, these results suggest that BR-2 may bind to the surface of mucosa like a protective coating when administered orally. Oral administration of BR-2 significantly reduced the concentration of acid and pepsin activity in gastric juice from pylorus-ligated rats, but had no significant effect on the volume of gastric juice (Sun *et al.*, 1991). These results suggest that BR-2 can also suppress gastric damage induced by aggressive factors. It has recently been found that oxygen-derived free radicals may be involved in the pathogenesis of mucosal damage induced by HCl–ethanol (Matsumoto *et al.*, 1993*b*). 5–10 mg/ml of bupleuran 2IIc showed the potent scavenging effect of hydroxyl radicals, similar to the effect of the non-enzymatic oxygen radical scavenger, mannitol (Matsumoto *et al.*, 1993*b*). This result indicated that this radical scavenging effect is also involved in the anti-ulcer activity of bupleuran 2IIc. Our results suggested that the major mechanism of mucosal protection by BR-2 may be due to its anti-secretory activity on acid and pepsin, its increased protective coating and its radical scavenging effects, however, it is not involved in the action of endogenous prostaglandins and mucus synthesis (Fig. 8).

Bupleuran 2IIc consists of 85.8% polygalacturonan region and 8.6% ramified region, which contain a rhamnogalacturonan core with neutral sugar chains (Yamada *et al.*, 1991*b*). The polygalacturonan region consists of $\alpha(1 \rightarrow 4)$-linked galacturonic acid with small amounts of 2,4- and 3,4-di-*O*-substituted galacturonic acid (Yamada *et al.*, 1991*b*). The polygalacturonan region has *endo*-polygalacturonase sensitive and resistant blocks, and carboxylmethylated and branched galacturonic acid residues were contained in the enzyme resistant block (Yamada *et al.*, 1991*b*). The ratio of sensitive block to resistant block was 7:3. The ramified region contained several short neutral carbohydrate chains and a $\beta(1 \rightarrow 3,6)$ galactan chain attached to position 4 of either the 2-linked rhamnosyl residue through 4-linked galacturonic acid or 2-linked rhamnose directly in the rhamnogalacturonan core (Yamada *et al.*, 1991*b*). Bupleuran 2IIc also contained another minor part which consisted of 2-methylfucose, 2-methylxylose and apiose in addition to rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose as neutral sugars; and aceric acid, 2-keto-3-deoxy-octurosonic acid (KDO), galacturonic acid

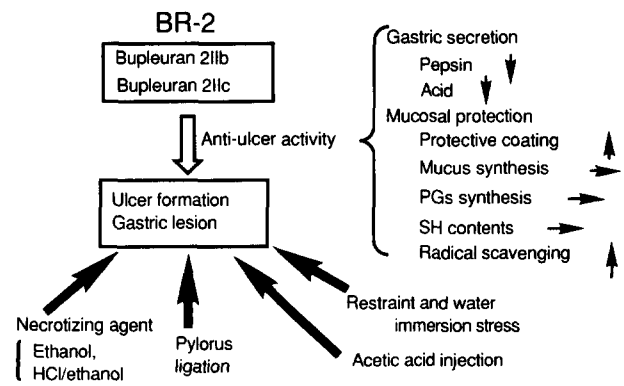

Fig. 8. Possible mechanism of anti-ulcer activity by bupleuran 2IIb and 2IIc.

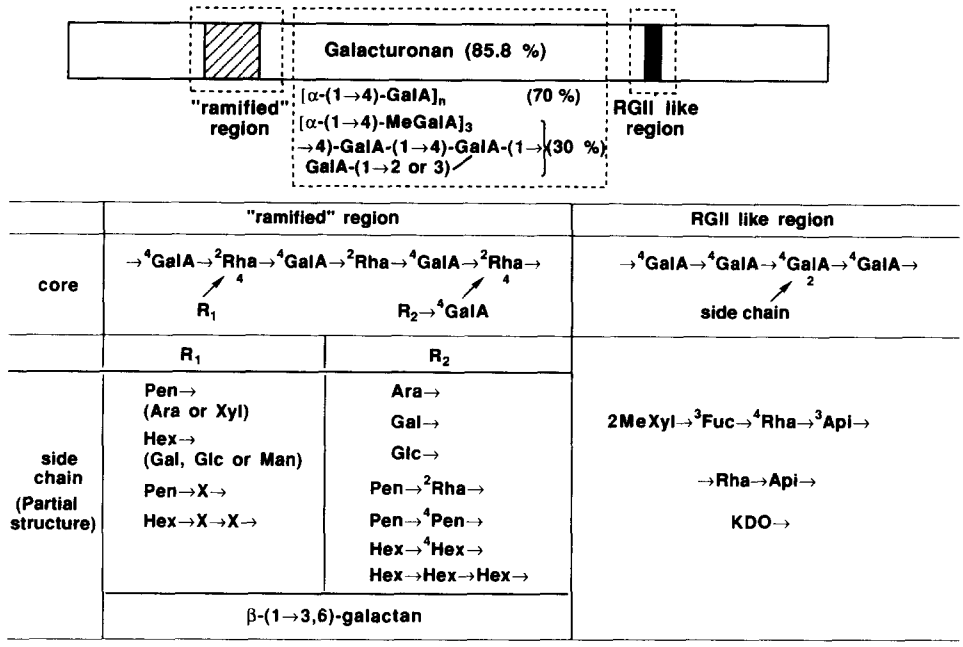

Fig. 9. Possible structural model of bupleuran 2IIc.

and glucuronic acid as acidic sugars (Yamada *et al.*, 1991*b*). Darvill *et al.* (1978) first reported on the isolation and characterization of rhamnogalacturonan II (RG-II), which was obtained from sycamore cell wall. RG-II consisted of more component sugars such as 2-methylfucose, 2-methylxylose, apiose, KDO, 3-deoxy-D-*lyxo*-2-heptulosaric acid (DHA) and aceric acid, in addition to common sugar from plants (Spellman *et al.*, 1983; York *et al.*, 1985; Stevenson *et al.*, 1988). The minor part in bupleuran 2IIc was very similar to RG-II, but no other groups have reported it until now. Puvanesarajah *et al.* (1991) recently reported the partial structure of RGII, which consists of several side chains containing apiose or KDO, and these chains are probably attached to α(1 → 4)-linked galacturonan main chains. When the RG-II-like region from bupleuran 2IIc was treated by lithium degradation, the same neutral oligosaccharides (→ Xyl → 3Fuc → 4Rha → 3Api → and → Rha → Api →), which were obtained from RG-II, were detected by GC-MS. These results concluded that the anti-ulcer polysaccharide, bupleuran 2IIc, contained rhamnogalacturonan II (Fig. 9).

Bupleuran 2IIc might contain the ramified region and RG-II in the same pectin molecule, because bupleuran 2IIc is a homogeneous pectin. This is the first discovery of purified pectin containing RG-II. It has recently been found by the present authors that immunostimulating pectic polysaccharide isolated from *Glycyrrhiza uralensis* contains similar component sugars to RG-II (Zhao *et al.*, 1991). Therefore, it is possible that many pectins contain a ramified region, polygalacturonan region and RG-II-like region in the same polysaccharide molecule.

Although the anti-ulcer activity of BR-2 decreases after *endo*-polygalacturonase digestion, the activity of apple pectin, which contains over 95% galacturonic acid, was not so significant, and polygalacturonic acid from oranges showed significant activity, but this was lower than BR-2 (Yamada *et al.*, 1991*a*). These results indicate that not only the polygalacturonan moiety, but also other minor parts such as the ramified region and rhamnogalacturonan II, and branched galacturonic acid, may be important for the expression of the anti-ulcer activity of bupleuran 2IIb and 2IIc.

CONCLUSIONS

The three active polysaccharides under consideration all belong to the pectic polysaccharides. Results presented herein suggest that pectic polysaccharides may be involved in several pharmacological activities of these crude drugs. However, each pharmacological activity of the pectic polysaccharides may depend on their fine chemical structure. Further studies on the structure–activity relationship of these bioactive polysaccharides should provide useful data for clarification of their modes of action.

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