CHAPTER 1

Ginsenosides: Chemistry, Biosynthesis, Analysis, and Potential Health Effects

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Contents

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Abstract

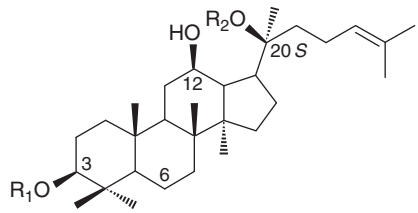
Ginsenosides are a special group of triterpenoid saponins that can be classified into two groups by the skeleton of their aglycones, namely dammarane- and oleanane-type. Ginsenosides are found nearly exclusively in *Panax* species (ginseng) and up to now more than 150 naturally occurring ginsenosides have been isolated from roots, leaves/stems, fruits, and/or flower heads of ginseng. Ginsenosides have been the target of a lot of research as they are believed to be the main active principles behind the claims of ginsengs efficacy. The potential health effects of ginsenosides that are discussed in this chapter include anticarcinogenic, immunomodulatory, anti-inflammatory, antiallergic, antiatherosclerotic, antihypertensive, and antidiabetic effects as well as antistress activity and effects on the central nervous system. Ginsensoides can be metabolized in the stomach (acid hydrolysis) and in the gastrointestinal tract (bacterial hydrolysis) or transformed to other ginsenosides by drying and steaming of ginseng to more bioavailable and bioactive ginsenosides. The metabolization and transformation of intact ginsenosides, which seems to play an important role for their potential health effects, are discussed. Qualitative and quantitative analytical techniques for the analysis of ginsenosides are important in relation to quality control of ginseng products and plant material and for the determination of the effects of processing of plant material as well as for the determination of the metabolism and bioavailability of ginsenosides. Analytical techniques for the analysis of ginsenosides that are described in this chapter are thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) combined with various detectors, gas chromatography (GC), colorimetry, enzyme immunoassays (EIA), capillary electrophoresis (CE), nuclear magnetic resonance (NMR) spectroscopy, and spectrophotometric methods.

I. INTRODUCTION

Ginsenosides are triterpenoid saponins found nearly exclusively in ginseng and they have been the target of a lot of research as they are believed to be the main active principles behind the claims of ginsengs efficacy. Ginseng refers to species within the genus *Panax* (Araliaceae

family) that comprise approximately 14 species of slow-growing perennial plants with fleshy roots (Choi and Wen, 2000). They grow naturally in the Northern Hemisphere in eastern Asia and North America and are cultivated in minor scale in the Northern part of Europe. The most widely used *Panax* species is *P. ginseng* (Korean or Asian ginseng). It was first cultivated around 11 BC and has a medical history (as wild herb) stretching back more than 5000 years (Yun, 2001a,b), and is considered as one of the most valuable medicinal herbs in traditional Asian medicine. Another member of the genus is *P. quinquefolium* (American ginseng), which was valued by the American Indians long before the arrival of Europeans in the New World and since the eighteenth century American ginseng has been cultivated in North America for medicinal purposes. Other commonly used *Panax* species in herbal medicine are *P. japonicus* (Japanese ginseng), *P. notoginseng* (Sanchi ginseng), and *P. vietnamensis* (Vietnamese ginseng). Ginseng is also one of the most commonly used herbal medicinal remedies by American consumers. In Europe, ginseng is also counted among the top retail products, but unlike the United States, where ginseng preparations are classified as dietary supplements, ginseng products in Europe and particularly in Germany are treated as drugs. To day, most of the research has been focused on *P. ginseng* that has been used in traditional Asian medicine as a tonic and a panacea that can promote longevity. Nowadays, ginseng is used mainly to increase resistance to physical, chemical, and biological stress and boost general vitality. This activity of ginseng has been described as ''adaptogenic'' in most of the alternative medicine literature. However, immune system modulation, antistress activities, and antihyperglycemic activities are among the most notable features of ginseng in *in vitro* studies and in clinical trials. Extensive recent *in vitro*, *in vivo*, and epidemiological research also suggests that ginseng may have a cancer preventive effect. The roots of ginseng is the plant part of ginseng that has mainly been used for medicinal purposes and consequently most investigations have been performed on ginseng roots, although medicinal effects of other plant parts of ginseng has been demonstrated. Up to now more than 150 naturally occurring ginsenosides have been isolated from roots, leaves/stems, fruits, and/or flower heads of *Panax* species, of which most of them can be classified into two groups by the skeleton of their aglycones, namely dammarane-type and oleanane-type (Fig. 1.1; Table 1.1). Furthermore, studies of changes in ginsenoside composition due to different traditional processing of ginseng roots such as white and red ginseng have been undertaken, and this has led to the identification of a series of ginsenosides in processed ginseng products with interesting bioactivities of which many are considered as being degradation products of naturally occurring ginsenosides.

FIGURE 1.1 Protopanaxadiol-type ginsenosides

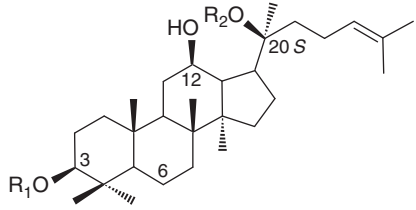


20(*S*)-protopanaxadiol (20(*S*)-PPD, $R_1 = R_2 = H$)

No.	Ginsenosides	R_1	R_2
1	Ginsenoside F_2	–Glc	–Glc
2	Ginsenoside Ra_1	–Glc^2–^{1}Glc	–Glc^6–^{1}Ara(p)^4–^{1}Xyl
3	Ginsenoside Ra_2	–Glc^2–^{1}Glc	–Glc^6–^{1}Ara(f)^2–^{1}Xyl
4	Ginsenoside Ra_3	–Glc^2–^{1}Glc	–Glc^6–^{1}Glc^3–^{1}Xyl
5	Ginsenoside Rb_1	–Glc^2–^{1}Glc	–Glc^6–^{1}Glc
6	Ginsenoside malonyl-Rb_1	–Glc^2–^{1}Glc^6– Malonoyl	–Glc^6–^{1}Glc
7	Ginsenoside Rb_2	–Glc^2–^{1}Glc	–Glc^6–^{1}Ara(p)
8	Ginsenoside malonyl-Rb_2	–Glc^2–^{1}Glc^6– Malonoyl	–Glc^6–^{1}Ara(p)
9	Ginsenoside Rb_3	–Glc^2–^{1}Glc	–Glc^6–^{1}Xyl
10	Ginsenoside Rc	–Glc^2–^{1}Glc	–Glc^6–^{1}Ara(f)
11	Ginsenoside malonyl-Rc	–Glc^2–^{1}Glc^6– Malonoyl	–Glc^6–^{1}Ara(f)
12	Ginsenoside Rd	–Glc^2–^{1}Glc	–Glc
13	Ginsenoside malonyl-Rd	–Glc^2–^{1}Glc^6– Malonoyl	–Glc
14	Ginsenoside 20(*S*)–Rg_3	–Glc^2–^{1}Glc	–H
15	Ginsenoside Rh_2	–Glc	–H
16	Ginsenoside Rs_1	–Glc^2–^{1}Glc^6– Ac	–Glc^6–^{1}Ara(p)
17	Ginsenoside Rs_2	–Glc^2–^{1}Glc^6– Ac	–Glc^6–^{1}Ara(f)
18	Ginsenoside Rs_3	–Glc^2–^{1}Glc^6– Ac	–H
19	Chikusetsusaponin la	–Glc^6–^{1}Xyl	–H

(*continued*)

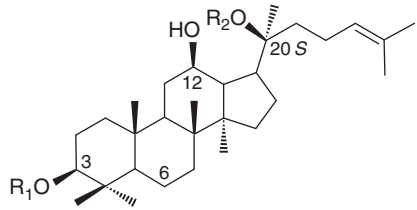
FIGURE 1.1 Protopanaxadiol-type ginsenosides (*Continued*)



20(*S*)-protopanaxadiol (20(*S*)-PPD, R_1=R_2=H)

No.	Ginsenosides	R_1	R_2
20	Chikusetsusaponin III	–Glc^{2}–^{1}Glc (6 | ^{1}Xyl)	–H
21	Chikusesusaponin VI	–Glc^{2}–^{1}Glc (6 | ^{1}Xyl)	–Glc^{6}–^{1}Glc
22	Gypenoside IX (= notoginsenoside Fd)	–Glc	–Glc^{6}–^{1}Xyl
23	Gypenoside XV	–Xyl^{2}–^{1}Glc	–Glc^{6}–^{1}Xyl
24	Gypenoside XVII	–Glc	–Glc^{6}–^{1}Glc
25	Notoginsenoside D	–Glc^{2}–$^{1}Glc^{2}$–^{1}Xyl	–Glc^{6}–$^{1}Glc^{6}$–^{1}Xyl
26	Notoginsenoside Fa	–Glc^{2}–$^{1}Glc^{2}$–^{1}Xyl	–Glc^{6}–^{1}Glc
27	Notoginsenoside Fc	–Glc^{2}–$^{1}Glc^{2}$–^{1}Xyl	–Glc^{6}–^{1}Xyl
28	Notoginsenoside Fe	–Glc	–Glc^{6}–$^{1}Ara(f)$
29	Notoginsenoside K	–Glc^{6}–^{1}Glc	–Glc
30	Notoginsenoside L*	–Glc	–Glc^{2}–$^{1}Ara(p)$
31	Notoginsenoside L*	–Glc^{2}–^{1}Glc	–Glc^{6}–^{1}Glc
32	Notoninsenoside R_4	–Glc^{2}–^{1}Glc	–Glc^{6}–$^{1}Glc^{6}$–^{1}Xyl
33	Pseudo-dinsenoside F_8	–Glc^{2}–^{1}Glc (6 | Ac)	–Glc^{6}–$^{1}Ara(p)$
34	Pseudo-ginsenoside RC_1	–Glc^{2}–$^{1}Glc^{6}$–Ac	–Glc
35	Quinquenoside R_1	–Glc^{2}–$^{1}Glc^{6}$–Ac or –Glc^{2}–^{1}Glc	–Glc^{2}–$^{1}Glc^{6}$–Ac or –Glc^{2}–^{1}Glc

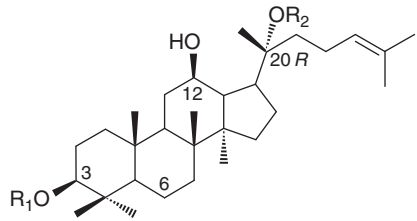
FIGURE 1.1 Protopanaxadiol-type ginsenosides (*Continued*)



20(*S*)-protopanaxadiol (20(*S*)-PPD, $R_1 = R_2 = H$)

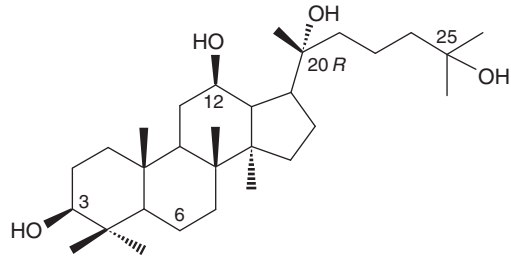
No.	Ginsenosides	R_1	R_2
36	Quinquenoside I	–Glc^{2}–$^{1}Glc^{6}$–(*E*)-2-Butenoyl	–Glc
37	Quinquenoside II	–Glc^{2}–$^{1}Glc^{6}$–(*E*)-2-Octenoyl	–Glc^{6}–^{1}Glc
38	Quinquenoside III	–Glc^{2}–^{1}Glc (6-Ac)	–Glc
39	Quinquenoside V	–Glc^{2}–^{1}Glc	–Glc^{6}–$^{1}Glc^{4}$–$^{1}\alpha$Glc
40	Yesanchinoside J	–Glc^{2}–^{1}Glc (6-Ac)	–Glc^{6}–$^{1}Glc^{6}$–^{1}Xyl

*Ginsenoside **30** and **31** has been given the name notoginsenoside L by different authors (Yoshikawa *et al.*, 2001; Ma *et al.*, 1999).

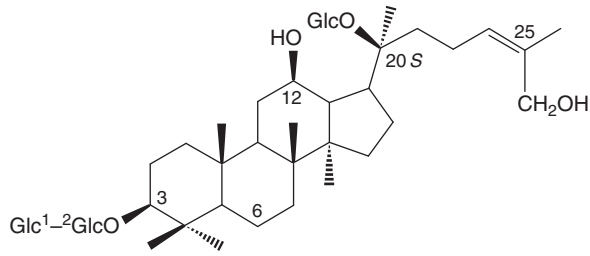


No.	Ginsenosides	R_1	R_2
41	Ginsenoside 20(*R*)–Rd	–Glc^{2}–^{1}Glc	–Glc
42	Ginsenoside 20(*R*)–Rg_3	–Glc^{2}–^{1}Glc	–H
43	Ginsenoside 20(*R*)–Rs_3	–Glc^{2}–$^{1}Glc^{6}$–Ac	–H

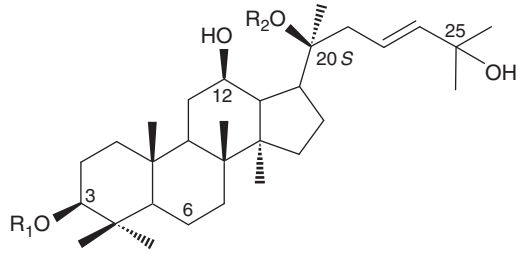
FIGURE 1.1 Protopanaxadiol-type ginsenosides (*Continued*)



20(*R*)-dammarane-3 β, 12 β, 20, 25-tetrol (25-OH PPD, **44**)

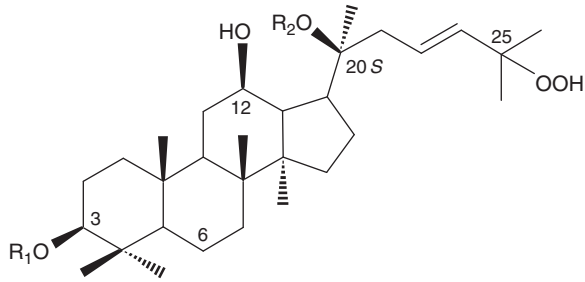


Quinquenoside L_2(**45**)



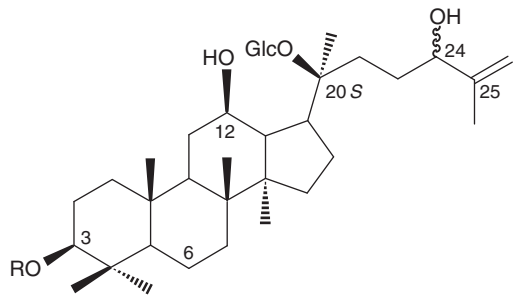
No.	Ginsenosides	R_1	R_2
46	Majoroside F_4	–Glc	–Glc
47	Notoginsenoside A	–Glc^2–^{1}Glc	–Glc^6–^{1}Glc
48	Quinquenoside L_3	–Glc	–Glc^6–^{1}Glc
49	Vina-ginsenoside R_8	–Glc^2–^{1}Glc	–Glc
50	Yesanchinoside H	–Glc^2–^{1}Glc	–Glc^6–^{1}Xyl

FIGURE 1.1 Protopanaxadiol-type ginsenosides (*Continued*)



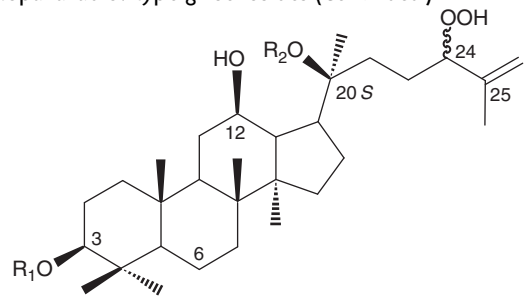
No.	Ginsenosides	R_1	R_2
51	Floralginsenoside E	$-Glc^{2}-{}^{1}Glc$	–H
52	Floralginsenoside F	–Glc	–Glc
53	Floralginsenoside G	$-Glc^{2}-{}^{1}Glc^{6}$–Ac	–Glc
54	Floralginsenoside O	$-Glc^{2}-{}^{1}Glc$	$-Glc^{6}-{}^{1}Ara(f)$
55	Notoginsenoside E	$-Glc^{2}-{}^{1}Glc$	–Glc
56	Notoginsenoside K*	$-Glc^{2}-{}^{1}Glc$	$-Glc^{6}-{}^{1}Glc$

*The name notoginsenoside K has been used for ginsenoside **56** (Yoshikawa *et al.*, 1997a, 1998, 2001) and for ginsenoside **29** (Ma *et al.*, 1999; Sun *et al.*, 2005b).

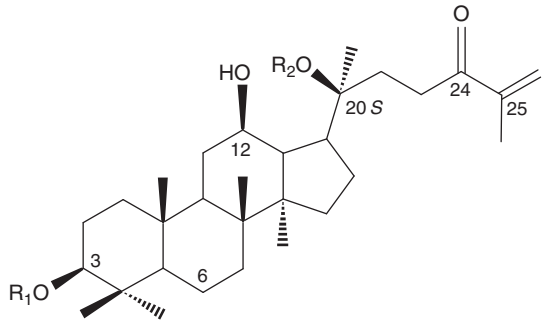


No.	Ginsenosides	R
57	Ginsenoside 24(*R*)–Rg_7	–Glc
58	Ginsenoside 24(*S*)–Rg_7 (= Majoroside F_2)	–Glc
59	24(*S*)–Vina-ginsenoside R_9	$-Glc^{2}-{}^{1}Glc$
60	24(*R*)-Vina-ginsenoside R_9 (= Majoroside F_1)	$-Glc^{2}-{}^{1}Glc$

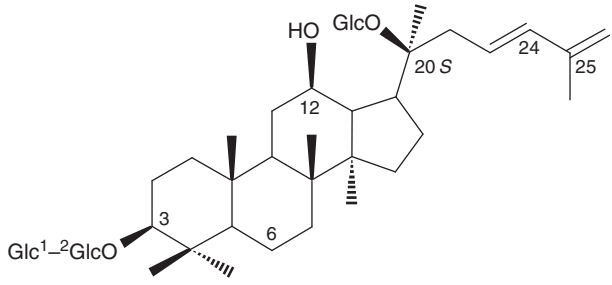
FIGURE 1.1 Protopanaxadiol-type ginsenosides (*Continued*)



No.	Ginsenosides	R_1	R_2
61	Ginsenoside I (epimer of **60**)	–Glc^2–^1Glc	–Glc
62	Ginsenoside II (epimer of **59**)	–Glc^2–^1Glc	–Glc
63	Floralginsenoside H	–Glc^2–$^1Glc^6$–Ac	–Glc
64	Floralquinquenoside D	–Glc	–Glc
65	Notoginsenoside C	–Glc^2–^1Glc	–Glc^6–^1Glc

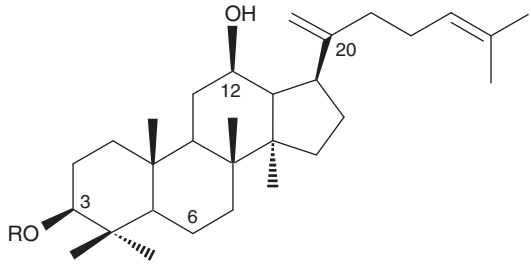


No.	Ginsenosides	R_1	R_2
66	Ginsenoside III	–Glc^2–^1Glc	–Glc
67	Notoginsenoside B	–Glc^2–^1Glc	–Glc^6–^1Glc

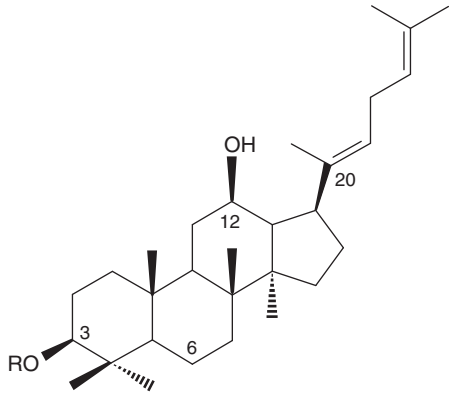


Quinquenoside L_1 (**68**)

FIGURE 1.1 Protopanaxadiol-type ginsenosides (*Continued*)

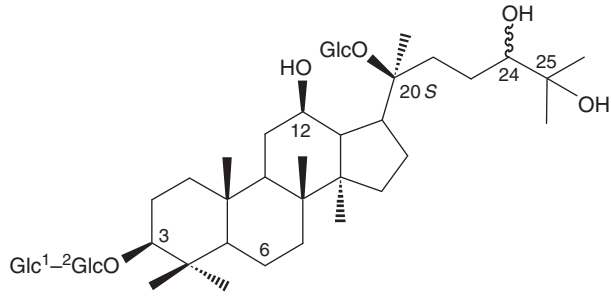


No.	Ginsenosides	R
69	Ginsenoside Rk_1	$-Glc^{2}-^{1}Glc$
70	Ginsenoside Rk_2	–Glc
71	Ginsenoside Rs_5	$-Glc^{2}-^{1}Glc^{6}-Ac$

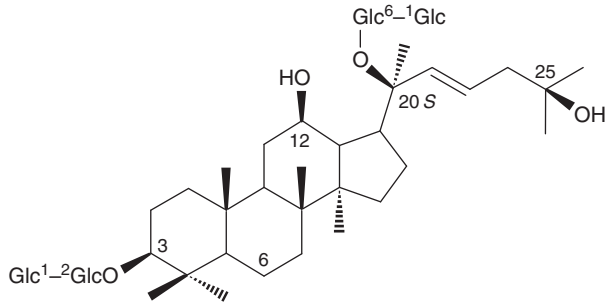


No.	Ginsenosides	R
72	Ginsenoside Rg_5	$-Glc^{2}-^{1}Glc$
73	Ginsenoside Rh_3	–Glc
74	Ginsenoside Rs_4	$-Glc^{2}-^{1}Glc^{6}-Ac$

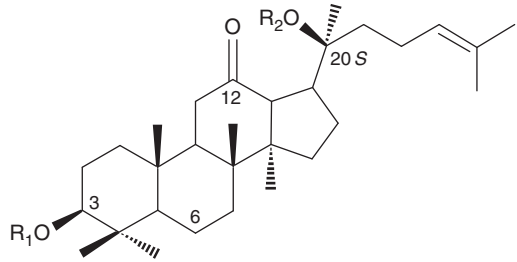
FIGURE 1.1 Protopanaxadiol-type ginsenosides (*Continued*)



Vina-ginsenoside R_{13} (**75**)

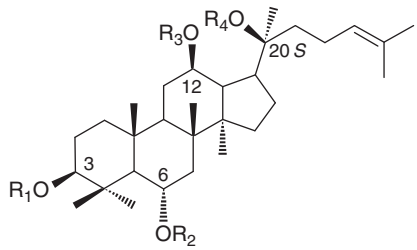


Koryoginsenoside R_2 (**76**)



No.	Ginsenosides	R_1	R_2
77	Chikusetsusaponin LN_4	$-Glc^{6}-^{1}Xyl$	$-Glc^{6}-^{1}Ara(p)$
78	Chikusetsusaponin LT_5	–Glc	$-Glc^{6}-^{1}Glc$
79	Chikusetsusaponin LT_8	–Glc	–Glc

FIGURE 1.1 Protopanaxatriol-type ginsenosides (*Continued*)



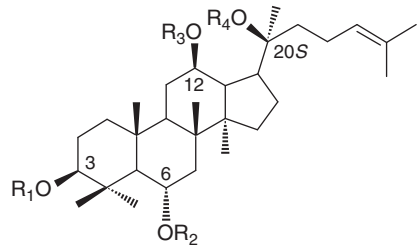
20(*S*)-protopanaxatriol (20(*S*)-PPT, $R_1 = R_2 = R_3 = R_4 = H$)

No.	Ginsenosides	R_1	R_2	R_3	R_4
80	Ginsenoside F_1	–H	–H	–H	–Glc
81	Ginsenoside F_3	–H	–H	–H	$-Glc^{6}-{}^{1}Ara(p)$
82	Ginsenoside F_5	–H	–H	–H	$-Glc^{6}-{}^{1}Ara(f)$
83	Ginsenoside la	–Glc	–H	–H	–Glc
84	Ginsenoside Re	–H	$-Glc^{2}-{}^{1}Rha$	–H	–Glc
85	Ginsenoside malonyl-Re	–H	$-Glc^{2}-{}^{1}Rha$-Malonoyl	–H	–Glc
86	Ginsenoside Rf	–H	$-Glc^{2}-{}^{1}Glc$	–H	–H
87	Ginsenoside 20-gluco-Rf	–H	$-Glc^{2}-{}^{1}Glc$	–H	–Glc
88	Ginsenoside Rg_1	–H	–Glc	–H	–Glc
89	Ginsenoside acetyl-Rg_1	–H	$-Glc^{6}$–Ac	–H	–Glc
90	Ginsenoside malonyl-Rg_1	–H	$-Glc^{6}$–Malonoyl	–H	–Glc
91	Ginsenoside 20(*S*)–Rg_2 (= Chikusetsusaponin I)	–H	$-Glc^{2}-{}^{1}Rha$	–H	–H
92	Ginsenoside 20(*S*)–Rh_1	–H	–Glc	–H	–H
93	Ginsenoside Rh_5*	–H	–Glc	–H	$-CH_3$
94	Chikusetsusaponin L_5	–H	–H	–H	$-Glc^{6}-{}^{1}Ara(p)^{4}-{}^{1}Xyl$
95	Chikusetsusaponin L_{10}	–H	–H	–Glc	–H
96	Floralginsenoside M	–H	$-Glc^{2}-{}^{1}Rha$	–H	$-Glc^{6}-{}^{1}Ara(f)$

(*continued*)

FIGURE 1.1 Protopanaxatriol-type ginsenosides (*Continued*)

No.	Ginsenosides	R_1	R_2	R_3	R_4
97	Floralginsenoside N	–H	–Glc^{2}–^{1}Rha	–H	–Glc^{6}–^{1}Ara(p)
98	Floralginsenoside P	–Glc^{2}–^{1}Glc	–H	–H	–Glc^{6}–^{1}Ara(p)



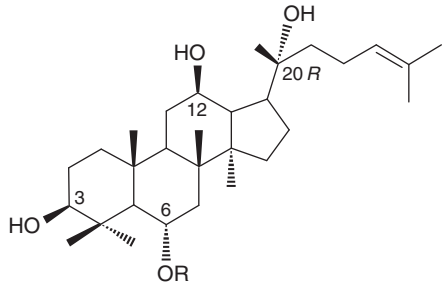
20(*S*)-protopanaxatriol (20(*S*)-PPT, $R_1 = R_2 = R_3 = R_4 = H$)

No.	Ginsenosides	R_1	R_2	R_3	R_4
99	Floralquinquenoside E	–H	–Glc^{2}–^{1}Rha	–H	–Glc^{6}–^{1}Xyl
100	Notoginsenoside M **	–H	–Glc^{6}–^{1}Glc	–H	–Glc
101	Notoginsenoside N	–H	–Glc^{4}–^{1}Glc	–H	–Glc
102	Notoginsenoside R_1	–H	–Glc^{2}–^{1}Xyl	–H	–Glc
103	Notoginsenoside R_2	–H	–Glc^{2}–^{1}Xyl	–H	–H
104	Notoginsenoside R_3	–H	–Glc	–H	–Glc^{6}–^{1}Glc
105	Notoginsenoside R_6	–H	–Glc	–H	–Glc^{6}–^{1}αGlc
106	Notoginsenoside U	–H	–H	–H	–Glc^{6}–^{1}Glc
107	Koryoginsenoside R_1	–H	–Glc^{6}–(*E*)-2-Butenoyl	–H	–Glc
108	Pseudo-ginsenoside RS_1	–H	–Glc^{2}(6-Ac)–^{1}Rha	–H	–Glc
109	Pseudo-ginsenoside RT_3	–H	–Xyl	–H	–H
110	Vina-ginsenoside R_4	–Glc^{2}–^{1}Glc	–H	–H	–Glc

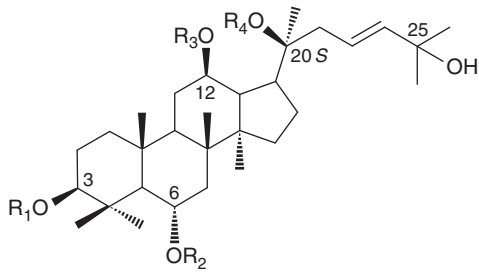
*Ginsenoside **93** isolated from the roots of *Panax vietnamensis* and named ginsenoside Rh_5 by Tran *et al.* (2001) should not be confused with ginsenoside Rh_5 (**141**) isolated from the leaves of *P. ginseng* (De-Quiang *et al.*, 2001).

The name notoginsenoside M has been used for both ginsenoside **100 (Yoshikawa *et al.*, 2001) and for ginsenoside **116** (Ma *et al.*, 1999).

FIGURE 1.1 Protopanaxatriol-type ginsenosides (*Continued*)

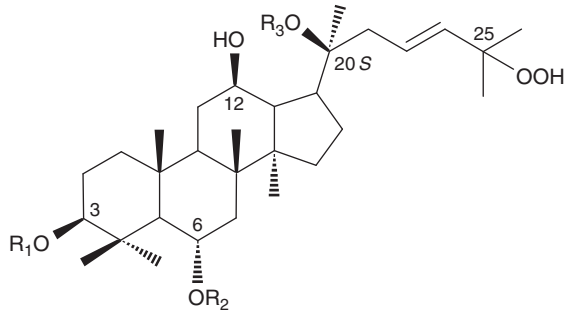


No.	Ginsenosides	R
111	Ginsenoside 20(*R*)–Rg_2	–Glc^{2}–^{1}Rha
112	Ginsenoside 20(*R*)–Rh_1	–Glc

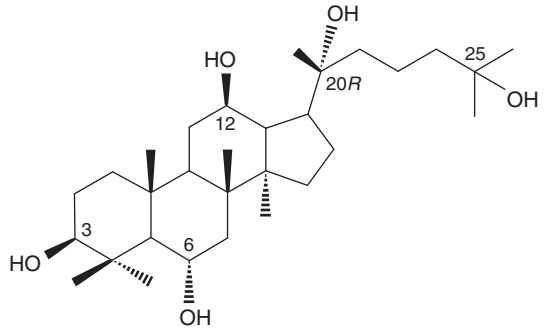


No.	Ginsenosides	R_1	R_2	R_3	R_4
113	Chikusetsusaponin L_{9a}	–H	–H	–Glc	–H
114	Majoroside F_6	–Glc	–Glc^{2}–^{1}Rha	–H	–Glc
115	Notoginsenoside H	–H	–Glc^{2}–^{1}Xyl	–H	–Glc
116	Notoginsenoside M	–H	–Glc	–H	–Glc

FIGURE 1.1 Protopanaxatriol-type ginsenosides (*Continued*)

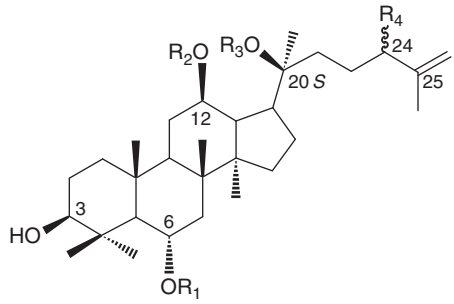


No.	Ginsenosides	R_1	R_2	R_3
117	Ginsenoside Rh_6	–H	–H	–Glc
118	Floralginsenoside B	–H	–Glc	–Glc
119	Floralginsenoside D	–Glc	–H	$-Glc^{6}-{}^{1}Ara(f)$
120	Floralginsenoside I	–H	$-Glc^{2}-{}^{1}Rha$	–Glc
121	Floralginsenoside K	$-Glc^{2}-{}^{1}Glc$	–H	–Glc
122	Floralquinquenoside A	–H	–Glc	–H
123	Floralquinquenoside C	–H	$-Glc^{2}-{}^{1}Rha$	–H

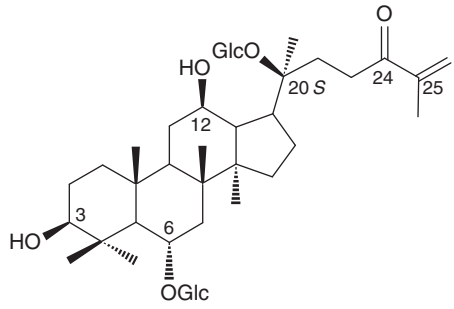


20(*R*)-dammarane-3β, 6α,12β, 20, 25-pentol (25-OH PPT, **124**)

FIGURE 1.1 Protopanaxatriol-type ginsenosides (*Continued*)

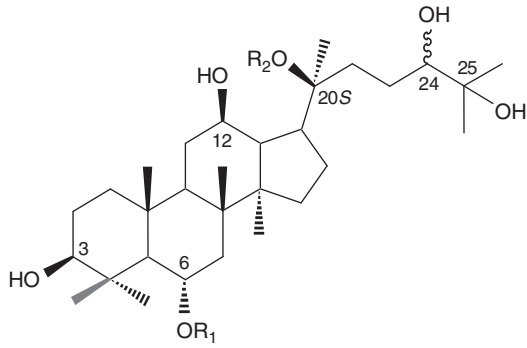


No.	Ginsenosides	R_1	R_2	R_3	R_4
125	Chikusetsusaponin L_{9bc}	–H	–Glc	–H	–OH
126	Floralginsenoside A	–Glc	–H	–Glc	–OOH
127	Floralginsenoside C	–H	–H	–Glc ^{6}–^{1}Ara(p)	–OOH
128	Floralginsenoside J	–Glc^{2}–^{1}Rha	–H	–Glc	–OOH
129	Floralginsenoside La	–Glc^{2}–^{1}Rha	–H	–Glc	–α-OH or –β-OH
130	Floralginsenoside Lb	–Glc^{2}–^{1}Rha	–H	–Glc	–α-OH or –β-OH
131	Floralquinquenoside B	–Glc^{2}–^{1}Rha	–H	–H	–OOH
132	Ginsenoside M_{7cd}	–H	–H	–Glc	–OH
133	Notopanaxoside A	–Glc	–H	–H	–OH

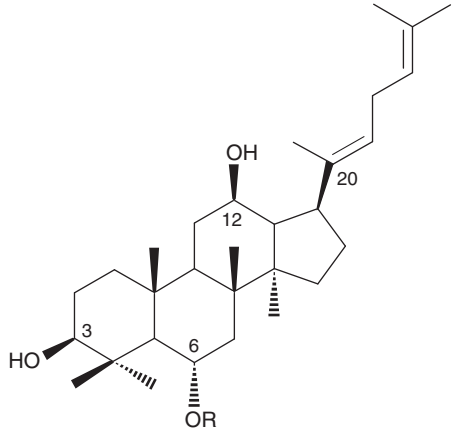


Vina-ginsenoside R_{25} (**134**)

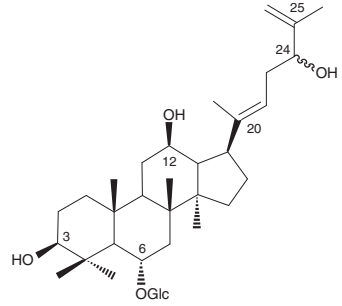
FIGURE 1.1 Protopanaxatriol-type ginsenosides (*Continued*)



No.	Ginsenosides	R
135	Ginsenoside Rg_6	$-Glc^{2}-{}^{1}Rha$
136	Ginsenoside Rk_3	–Glc
137	Ginsenoside Rs_7	$-Glc^{6}-Ac$

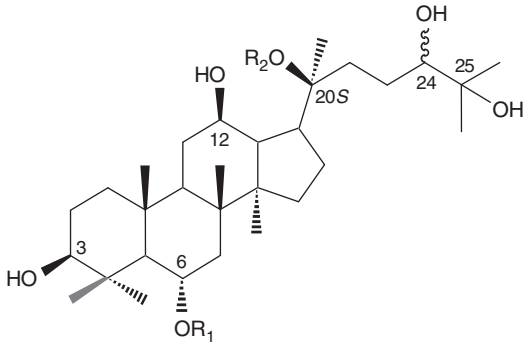


No.	Ginsenosides	R
138	Ginsenoside Rh_4	–Glc
139	Ginsenoside Rs_6	$-Glc^{6}-Ac$
140	Ginsenoside F_4	$-Glc^{2}-{}^{1}Rha$

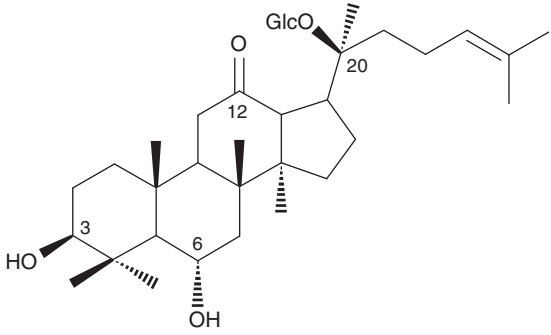


Ginsenoside Rh_5 (**141**)

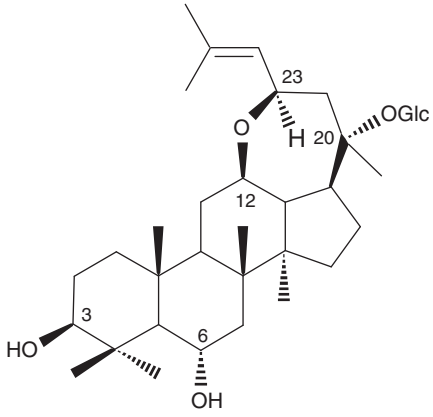
FIGURE 1.1 Protopanaxatriol-type ginsenosides (*Continued*)



No.	Ginsenosides	R_1	R_2
142	Notoginsenoside J	–Glc	–Glc
143	Quinquenoside L_9	–Glc^2–^{1}Rha	–H
144	Vina-ginsenoside R_{12}	–Glc	–H

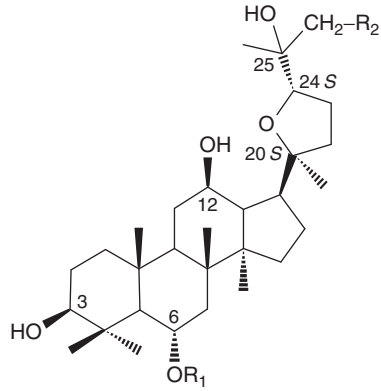


Ginsenoside Rh_8 (**145**)



Ginsenoside Rh_9 (**146**)

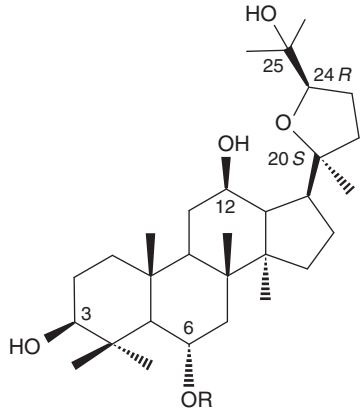
FIGURE 1.1 Ocotillol-type ginsenosides



24(*S*)-Ocotillol ($R_1 = R_2 = H$)

No.	Ginsenosides	R_1	R_2
147	24(*S*)–Majonoside R_1	$-Glc^{2}-^{1}Glc$	–H
148	24(*S*)–Majonoside R_2	$-Glc^{2}-^{1}Xyl$	–H
149	Protopanaxatriol oxide II	–H	–H
150	24(*S*)–Pseudo-ginsenoside F_{11}	$-Glc^{2}-^{1}Rha$	–H
151	24(*S*)–Pseudo-ginsenoside RT_4	–Glc	–H
152	Vina-ginsenoside R_1	$-Glc^{2}-^{1}Rha$ (6 | Ac)	–H
153	Vina-ginsenoside R_2	$-Glc^{2}-^{1}Xyl$ (6 | Ac)	–H
154	Vina-ginsenoside R_5	$-Glc^{2}-^{1}Xyl^{4}-\alpha Glc$	–H
155	Vina-ginsenoside R_6	$-Glc^{2}-^{1}Xyl$ ($^{6}_{1}$| αGlc)	–H
156	Vina-ginsenoside R_{14}	$-Glc^{2}-^{1}Xyl$	–OH

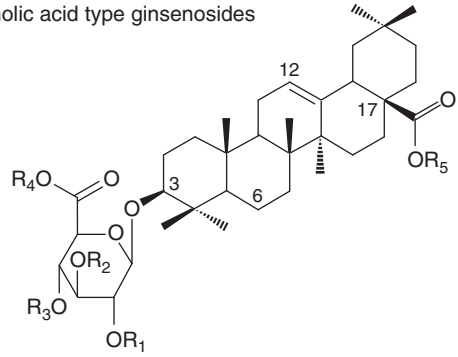
FIGURE 1.1 Ocotillol-type ginsenosides (*Continued*)



24(*R*)-Ocotillol (R = H)

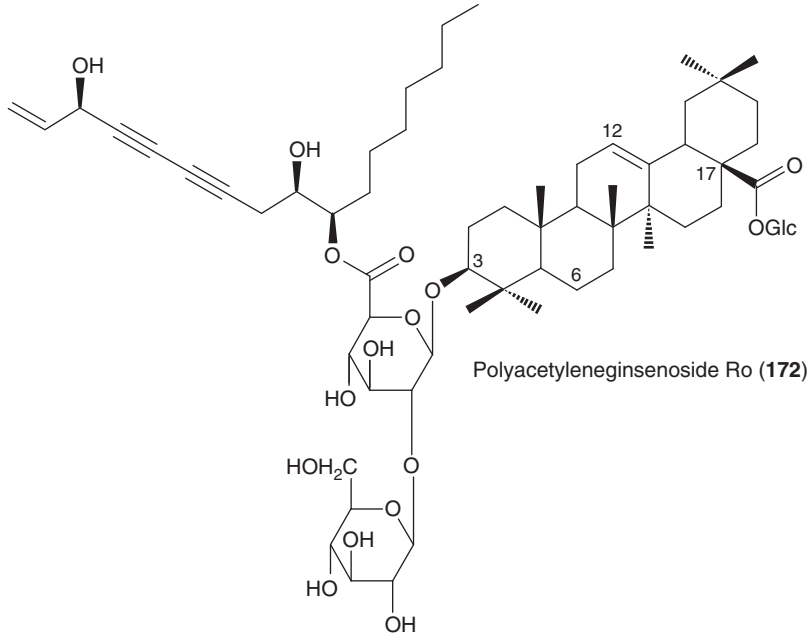
No.	Ginsenosides	R
157	24(*R*)–Majonoside R_1	–Glc^{2}–^{1}Glc
158	24(*R*)–Majonoside R_2 (= 24(*R*)–Pseudo-ginsenoside RT_2)	–Glc^{2}–^{1}Xyl
159	24(*R*)–Pseudo-ginsenoside F_{11}	–Glc^{2}–^{1}Rha
160	24(*R*)–Pseudo-ginsenoside RT_5	–Glc
161	24(*R*)–Vinaginsenoside R_1	–Glc^{2}–^{1}Rha (6-Ac)

Oleanolic acid type ginsenosides



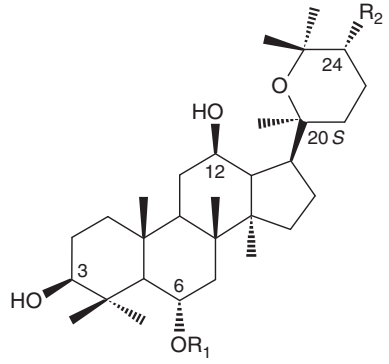
Oleanolic acid 3-*O*-β-glucuronide ($R_1 = R_2 = R_3 = R_4 = R_5 = H$)

No.	Ginsenosides	R_1	R_2	R_3	R_4	R_5
162	Ginsenoside Ro (= Chikusetsusaponin V)	–Glc	–H	–H	–H	–Glc
163	Ginsenoside Ro methyl ester	–Glc	–H	–H	$-CH_3$	–Glc
164	Ginsenoside R_{OA}	–Glc	–H	–H	–H	$-Glc^{6}-{}^{1}Glc$
165	Chikusetsusaponin lb	–H	–H	–Ara(f)	–Glc	–H
166	Chikusetsusaponin IV	–H	–H	–Ara(f)	–H	–Glc
167	Chikusetsusaponin IVa	–H	–H	–H	–H	–Glc
168	Hemsloside-Ma_3	–Glc	–Ara(p)	–H	–H	–Glc
169	Pseudo-ginsenoside RP_1	–Xyl	–H	–H	–H	–H
170	Pseudo-ginsenoside RT_1	–Xyl	–H	–H	–H	–Glc
171	Zingibroside R_1	–Glc	–H	–H	–H	–H



Polyacetyleneginsenoside Ro (**172**)

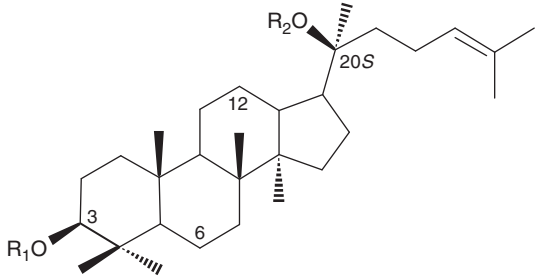
Panaxatriol-type ginsenosides



20(*S*)-panaxatriol (20(*S*)-PT, $R_1 = R_2 = H$)

No.	Ginsenosides	R_1	R_2
173	Vina-ginsenoside R_{10}	–Glc	–OH
174	Vina-ginsenoside R_{11}	–Glc^2–^{1}Xyl	–OH

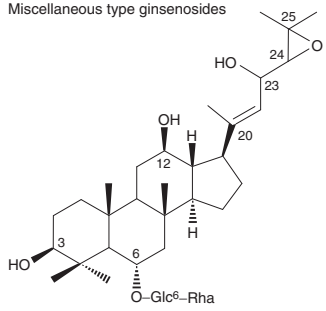
Dammarenediol-type ginsenosides



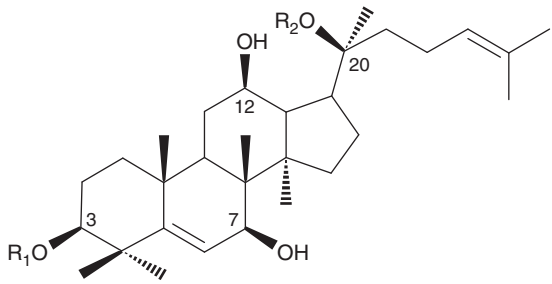
20(*S*)-dammarenediol ($R_1 = R_2 = H$)

No.	Ginsenosides	R_1	R_2
175	Notoginsenoside I	–Glc^2–^{1}Glc	–Glc^6–^{1}Glc
176	Vina-ginsenoside R_3	–Glc	–Glc^2–^{1}Glc
177	Yesanchinoside I	–Glc^2–^{1}Glc	–Glc^6–^{1}Glc^6–^{1}Xyl

Miscellaneous type ginsenosides



Ginsenoside Rg_8 (**178**)



No.	Ginsenosides	R_1	R_2
179	Ginsenoside Rh_7	–H	–Glc
180	Notoginsenoside G	$-Glc^2-^1Glc$	–Glc
181	Quinquenoside IV	$-Glc^2-^1Glc$	$-Glc^6-^1Glc$
182	Yesanchinoside G	$-Glc^2-^1Glc$	$-Glc^6-^1Xyl$

FIGURE 1.1 Ginsenosides isolated from fresh and/or processed ginseng material (roots, leaves, fruits, flower buds), including medicinal preparations based on plant material from ginseng species. Ara(*p*) = α-L-arabinopyranosyl; Ara(*f*) = α-L-arabinofuranosyl; Glc = β-D-glucopyranosyl; Rha = α-L-rhamnopyranosyl; Xyl = β-D-xylopyranosyl; Ac = acetyl.

The fact that ginseng is a very popular phytomedicine used all around the world, a huge quantity of work has been carried out during the last 30–40 years in order to develop analytical methods for the identification, quantification, and quality control of ginsenosides in raw materials, extracts, and marketed products as well as for the determination of their metabolization in humans and their pharmacokinetics. Among all the classical techniques usually employed for phytochemical analysis high-performance liquid chromatography (HPLC) combined with ultraviolet (UV) and/or mass spectrometry (MS) detection has been the method of choice for the analysis of ginsenosides in the last 20 years.

The aim of this chapter is to highlight the present state of knowledge on the chemistry, biosynthesis, analysis, and pharmacological effects of ginsenosides. The latter clearly demonstrate the potential health effects of this interesting group of compounds.

II. CHEMISTRY

A. Chemical structure of ginsenosides and distribution in plants

Ginsenosides with a few exceptions share a similar basic structure, consisting of a saturated 1,2-cyclopentanoperhydrophenanthrene (sterane or gonane) steroid nucleus. They are classified into two groups by the skeleton of aglycones, namely dammarane-type and oleanane-type. Ginsenosides

TABLE 1.1 Distribution of ginsenosides in different plant parts (fresh, dried, and/or processed) of *Panax* species (Araliaceae) and *Gynostemma pentaphyllum* (Cucurbitaceae)

Family/species	Common name	Ginsenosides			References
		Roots/rhizomes	Leaves/stems	Fruits/flower buds	
Araliaceae					
Panax bipinnatifidus Seem. (= *P. japonicus* C. A. Meyer var. *bipinnatifidus* (Seem.) C. Y. Wu et Feng; *P. pseudoginseng* Wall. var. *bipinnatifidus* (Seem.) H. L. Li)	Feather-leaved bamboo ginseng	**5**, **12**, **84**, **101**, **158**, **162**, **166**, **167**	—	—	Zhu *et al.*, 2004
P. ginseng C. A. Meyer	Korean ginseng, Chinese ginseng, Asian ginseng	**2–14**, **15**[a]**–18**[a], **32**, **34**, **35**, **42**[a], **43**[a], **69**[a]**– 72**[a], **74**[a], **76**, **84**, **86–88**, **91**, **92**, **102**, **103**, **107**, **111**, **112**, **135**[a]**–140**[a], **159**[b], **162–164**, **172**	**1**, **5**, **7**, **10**, **12**, **57**, **58**, **80**, **81**, **84**, **88**, **92**, **112**, **117**, **132**, **141**, **145**, **146**, **179**, 20(*S*)-PPT, 20(*S*)-PT	**5**, **7**, **10**, **12**, **15**, **24**, **34**, **44**, **51–55**, **61–63**, **66**, **73**, **80–82**, **84**, **88**, **91**, **92**, **96–98**, **108**, **110**, **112**, **114**, **118–121**, **124–130**, 20(*S*)-PPD	Besso *et al.*, 1982; Dou *et al.*, 2001; Fuzzati *et al.*, 1999, 2000; Haijiang *et al.*, 2003; Kim *et al.*, 1995; 1996; Kitagawa *et al.*, 1983; Kite *et al.*, 2003; Lui and Staba, 1980; Liu *et al.*, 2005; Ma *et al.*, 2005; Morita *et al.*, 1983; Nakamura *et al.*, 2007a; Park *et al.*, 2002a,b,c; Qui *et al.*, 1998, 2001;

					Ryu *et al.*, 1997; Samukawa *et al.*, 1995; Sanada *et al.*, 1974a,b; Sanada and Shoji, 1978; Shibata, 2001; Uvarova *et al.*, 2000; Wan *et al.*, 2007; Wang *et al.*, 2004, 2007; Yahara *et al.*, 1976a,b, 1979; Yoshikawa *et al.*, 2007a,b; Yu *et al.*, 2005; Zhang *et al.*, 2002; Zhu *et al.*, 2004
P. japonicus C. A. Meyer (= *P. pseudoginseng* Wall. subsp. *japonicus* (C. A. Meyer) H. Hara)	Japanese ginseng	**5**, **9**, **10**, **12**, **19**, **20**, **26–28**, **32**, **40**, **50**, **84**, **88**, **91**, **103**, **159**, **162**, **163**, **165–167**, **177**, **179**, **182**	**78–81**, **95**, **96**, **114**, **125**	—	Kondo *et al.*, 1971; Lee *et al.*, 1977; Lin *et al.*, 1976; Lui and Staba, 1980; Morita *et al.*, 1983; Tanaka *et al.*, 1985; Yahara *et al.*, 1977, 1978; Zhu *et al.*, 2004; Zou *et al.*, 2002

(*continued*)

TABLE 1.1 (*continued*)

Family/species	Common name	Ginsenosides			References
		Roots/rhizomes	Leaves/stems	Fruits/flower buds	
P. japonicus C. A. Meyer var. *angustifolius* (Burk.) Cheng et Chu	Narrow-leaved Japanese ginseng	**5**, **12**, **88**, **162**, **166**, **167**	—	—	Zhu *et al.*, 2004
P. japonicus C. A. Meyer var. *major* C. Y. Wu et K. M. Feng	Pear ginseng	**5**, **12**, **87**, **91**, **103**, **147**, **148**, **156**, **158**, **162**, **166**, **167**	—	—	Morita *et al.*, 1982, 1983; Tanaka *et al.*, 1985; Tohda *et al.*, 2002; Zhu *et al.*, 2004
P. notoginseng (Burk.) F. H. Chen (= *P. pseudoginseng* Wall. var. *notoginseng* (Burk.) G. Hoo and C. J. Tseng)	Sanchi ginseng,	**1–7**, **9**, **10**, **12**, **14**, **22**, **24–32**, **35–39**, **42**, **47**, **55**, **56**, **65**, **67**, **69**, **72**, **80**, **84**, **87**, **88**, **91**, **92**, **100–106**, **115**,	—	—	Kite *et al.*, 2003; Komakine *et al.*, 2006; Lai *et al.*, 2006; Li *et al.*, 2005; Lui and Staba, 1980; Ma *et al.*, 1999; Matsuura *et al.*, 1983; Sun *et al.*, 2005b, 2006; Wan *et al.*, 2007;

		116, 132, 135, 136, 138, 140, 142, 159[b], **162**[b], **175, 180**			Yoshikawa *et al.*, 1997a,b, 2001; Zhang and Cheng, 2006; Zhou *et al.*, 1981; Zhu *et al.*, 2004
P. pseudoginseng Wall. var. *angustatus* Hara		**20, 21, 86, 103, 162, 166, 167**	—	—	Kohda *et al.*, 1991
P. pseudoginseng Wall. subsp. *himalaicus* Hara	Himalayan ginseng	**1, 5, 9, 12, 24, 84, 88, 91, 102, 103, 109, 148, 150, 151, 158–160, 162, 166, 167, 169, 170**	**9, 12, 33, 84, 159**	—	Morita *et al.*, 1983; Tanaka *et al.*, 1985, 2000; Tanaka and Yahara, 1978
P. quinquefolium L.	American ginseng	**1, 5–15, 24, 34–39, 47, 56, 65, 80, 84, 88–92, 102, 103, 112, 140, 159,**	**5, 7, 9, 10, 12, 14, 45, 48, 68, 84, 91, 92, 143, 159**[b], **162**[b], **176**	**5, 7, 9, 10, 12, 14, 16, 24, 33, 34, 38, 55, 61, 62, 64, 81, 83, 84, 88, 91, 92, 99, 111, 112, 122,**	Christensen *et al.*, 2006; Corbit *et al.*, 2005; Dou *et al.*, 2006; Kite *et al.*, 2003; Ligor *et al.*, 2005; Lui and Staba, 1980; Ma *et al.*, 2006; Nakamura

(*continued*)

TABLE 1.1 (*continued*)

Family/species	Common name	Ginsenosides			References
		Roots/rhizomes	Leaves/stems	Fruits/flower buds	
		162, **167**, **178**, **181**		**123**, **131**, **143**, **150**, **159–161**	*et al.*, 2007b; Popovich and Kitts, 2004a; Wan *et al.*, 2007; Wang *et al.*, 1998, 2001b,c, 2006a; Wills and Stuart, 2001; Wood *et al.*, 2006; Yoshikawa *et al.*, 1998, 2001; Zhu *et al.*, 2004
P. stipuleanatus H. T. Tsai et Feng	Pingpien ginseng	**166**, **167**	—	—	Zhu *et al.*, 2004
P. trifolius L.	Dwarf ginseng	**5**, **9**, **10**, **12**, **28**, **162**	**5**, **9**, **10**, **12**, **28**, **162**	—	Lee and Marderosian, 1981, 1988; Lui and Staba, 1980
P. vietnamensis Ha et Grushv.	Vietnamese ginseng	**5**, **7**, **9**, **10**, **12**, **24**, **26**, **32**, **35**, **40**, **49**, **59**, **60**, **75**, **84**, **87**, **88**,	—	—	Duc *et al.*, 1993, 1994a, b; Tohda *et al.*, 2002; Tran *et al.*, 2001; Zhu *et al.*, 2004

		92, **93**, **102**, **105**, **108**, **110**, **112**, **134**, **138**, **144**, **147–156**, **162**, **167**, **168**, **173**, **174**, **176**			
P. vietnamensis Ha et Grushv. var. *fuscidiscus* K. Komatsu, S. Zhu et S. Q. Cai	Vietnamese ginseng	**5**, **10**, **12**, **84**, **88**, **103**, **158**, **162**	—	—	Zhu *et al.*, 2004
P. zingiberensis C. Y. Wu et K. M. Feng	Ginger ginseng	**5**, **12**, **88**, **92**, **103**, **162**, **166**, **167**, **171**	—	—	Tanaka *et al.*, 1985; Tran *et al.*, 2003; Zhu *et al.*, 2004
Cucurbitaceae					
Gynostemma pentaphyllum Makino	Jiaogulan, five-leaf ginseng	—	**1**, **5**, **6**, **9**, **10**, **13**, **14**, **22–24**	—	Cui *et al.*, 1999; Razovski-Naumovski *et al.*, 2005

[a]This ginsenoside is known to be unique or characteristic for processed ginseng (red ginseng).
[b]This ginsenoside has only been detected in trace or minute amounts by TLC from this ginseng plant part and hence the presence is questionable (Lui and Staba, 1980).

within the dammarane-type consist mainly of three types classified according to their genuine aglycone moieties: protopanaxadiol (PPD), protopanaxatriol (PPT), and ocotillol, whereas ginsenosides of the oleanane-type are classified according to their aglycone oleanolic acid (Fig. 1.1). Other types of ginsenosides isolated from ginseng species include panaxatriol-type and dammarenediol-type ginsenosides (Fig. 1.1). The major ginsenosides in the roots of ginseng include the 20(*S*)-PPDs Rb_1 (**5**), Rb_2 (**7**), Rc (**10**), and Rd (**12**) and the 20(*S*)-PPTs Re (**84**) and Rg_1 (**88**) as well as the malonyl derivatives of the ginsenosides Rb_1, Rb_2, Rc, and Rd (**6**, **8**, **11**, and **13**; Fig. 1.1), which normally account for over 90% of the total ginsenoside content in ginseng roots (Christensen *et al.*, 2006). Consequently, these ginsenosides are used as markers of ginseng quality. The four malonyl derivatives of the ginsenosides Rb_1, Rb_2, Rc, and Rd together with ginsenoside Ro (**162**) and similar esterified ginsenosides are termed ''acidic ginsenosides,'' while the others are termed ''neutral'' ginsenosides.

The most used ginseng roots in herbal medicine and dietary supplements are those from *P. quinquefolium* (American ginseng) and *P. ginseng* (Asian or Korean ginseng). The ginsenoside profiles of these species can be distinguished by ginsenoside Rf (**86**), which is detectable in *P. ginseng* but not in *P. quinquefolium* and several other ginseng species (Table 1.1). Ginsenoside ratios are also indicative of the different types of ginseng even though they may differ among species. The ocotillol-type triterpene 24(*R*)-pseudo-ginsenoside F_{11} (**159**) is present in *P. quinquefolium* whereas it only seems to be present in very minute amounts in *P. ginseng*, and hence a high ginsenoside Rf/24(*R*)-pseudoginsenoside F_{11} ratio (>700) clearly distinguishes Asian ginseng from the American species and may be used to determine whether *P. ginseng* is contaminated with *P. quinquefolium* and vice versa (Chan *et al.*, 2000; Li *et al.*, 2000). In addition, a high Rb_1/Rg_1 ratio with values around 10 or greater is usually indicative of *P. quinquefolium*, while low Rb_1/Rg_1 values usually between 1 and 3 are characteristic for *P. ginseng* (Fuzzati, 2004; Li and Fitzloff, 2002b). The PPD group of ginsenosides dominates quantitatively in *P. quinquefolium* in contrast to *P. ginseng* where the PPT group ginsenosides occur in highest amounts (Fuzzati, 2004; Wan *et al.*, 2007; Wang *et al.*, 1999). Finally, it appears that the profiles of malonyl-ginsenosides can be used to distinguish the species *P. ginseng*, *P. quinquefolium*, and *P. notoginseng*. In *P. quinquefolium*, the levels of malonyl-Rc and malonyl-Rb_2 relative to malonyl-Rb_1 are lower than in *P. ginseng*. In *P. notoginseng*, the most abundant malonyl-ginsenosides is malonyl-Rb_1 and some nonidentified isomeric forms of this ginsenoside (Kite *et al.*, 2003).

P. notogingseng (Sanchi ginseng) is an example of another ginseng species used frequently in herbal medicine, although mostly in Chinese medicine (Lai *et al.*, 2006; Sun *et al.*, 2006). The roots of *P. notoginseng* and/or products based on this species have been intensively investigated for ginsenosides (Lai *et al.*, 2006; Li *et al.*, 2005; Sun *et al.*, 2006; Zhang and Cheng, 2006; Table 1.1).

This species seems to be rich both quantitatively and qualitatively in ginsenosides of the PPD- and PPT-type (Sun *et al.*, 2006; Wan *et al.*, 2007), and the amounts of dammarane-type ginsenosides are clearly higher in *P. notoginseng* compared to the morphologically related species *P. ginseng* and *P. quinquefolium* (Wan *et al.*, 2007). However, ginsenosides of the ocotillol-type and oleanolic acid type seem to be absent in *P. notoginseng* (Table 1.1), although the presence of minute amounts of ginsenoside Ro (**162**) (oleanolic acid type) and 24(*R*)-pseudo-ginsenoside F_{11} (**159**) (ocotillol-type) has been indicated in a single investigation of dried roots of this species (Lui and Staba, 1980). The absence or presence of very minute amounts of ginsenosides of the ocotillol-type and oleanolic acid type in *P. notoginseng* clearly distinguishes this species from other ginseng species investigated (Table 1.1).

Oleanolic acid type ginsenosides (**162**–**172**) seem to be typical constituents of ginseng species and are in particular characteristic for *P. ginseng*, *P. japonicus* (Japanese ginseng), *P. pseudoginseng* subsp. *himalaicus* (Himamayan ginseng), *P. vietnamensis* (Vietnamese ginseng), and *P. zingiberensis* (ginger ginseng) (Table 1.1). Ocotillol-type ginsenosides (**147**–**161**) also seem to be typical constituents of ginseng species, although they only seem to be characteristic for *P. japonicus* var. *major* (pear ginseng), *P. pseudoginseng* subsp. *himalaicus*, *P. vietnamensis*, and *P. quinquefolium* (Table 1.1).

The root/rhizome is the plant part most used in herbal remedies of ginseng and consequently most investigation on ginsenosides has been performed on this plant part. However, ginsenosides are also present in the aerial parts of ginseng species as demonstrated for *P. ginseng*, *P. japonicus*, *P. pseudoginseng* subsp. *himalaicus*, *P. quinquefolium*, and *P. trifolius* (Table 1.1). In particular, the aerial parts of *P. ginseng* and *P. quinquefolium* have been intensively investigated for ginsenosides that have in addition to typical root ginsenosides resulted in the isolation of several new and characteristic ginsenosides such as the floralginsenosides A–P (**51**–**54**, **63**, **96**–**98**, **118**–**121**, and **126**–**128**), La (**129**), and Lb (**130**) from flower buds of *P. ginseng* (Nakamura *et al.*, 2007a; Yoshikawa *et al.*, 2007a,b) and the floralquinquenosides A–E (**64**, **99**, **122**, **123**, and **131**) from the flower buds of *P. quinquefolium* (Nakamura *et al.*, 2007b).

The species *Gynostemma pentaphyllum* (Jiaogulan or five-leaf ginseng) of the Cucurbitaceae is especially rich in gypenosides (gynosaponins), which mainly exists as dammarane-type triterpene glycosides closely related to the ginsenosides (Razmovski-Naumovski *et al.*, 2005). Indeed, a few of the over 90 gypenosides isolated from *G. pentaphyllum* have been shown to be identical with ginsenosides Rb_1 (gypenoside III), Rc, Rb_3 (gypenoside IV), Rd (gypenoside VIII), F_2, Rg_3, malonyl-Rb_1, malonyl-Rd, Rf, gypenoisde XVII, gypenoside IX, and gypenoside XV (Fig. 1.1) (Cui *et al.*, 1999; Kuwahara *et al.*, 1989; Razmovski-Naumovski *et al.*, 2005). These ginsenosides make up to 25% of the total gynosaponin content in the plant and is the first example of ginsenosides found outside of the

Araliaceae family (Liu *et al.*, 2004). Because of its phytochemical similarity with the more expensive ginseng root, *G. pentaphyllum* has attracted much interest as a potential new plant drug. Pharmacological studies of *G. pentaphyllum* have shown that the plant exhibits similar biological activities as the ginseng root probably because of its relatively high ginsenoside content (Razmovski-Naumovski *et al.*, 2005).

The content of total and individual ginsenosides does not only vary between plant organs and species. In particular, the content of ginsenosides in ginseng roots also depends on growing conditions and age of the roots, and internal root size (root hairs, lateral roots, and main roots) (Christensen *et al.*, 2006; Court *et al.*, 1996b; Soldati and Tanaka, 1984; Wills and Stuart, 2001).

B. Ginsenosides produced during steaming and drying of ginseng

The roots/rhizomes of *P. ginseng* are normally prepared before they are used as herbal medicine by drying the fresh roots/rhizomes after peeling off (white ginseng) or prepared by steaming the whole roots/rhizomes followed by drying (red ginseng). Ginsenosides Rb_1 (**5**), Rb_2 (**7**), Rc (**10**), Rd (**12**), Re (**84**), Rg_1 (**88**), and Rg_2 (**91**) are major constituents of white and red ginsengs, while ginsenosides such as quinquenoside R_1 (**35**), Rh_1 (**91**), Rh_2 (**15**), 20(*S*)-Rg_3 (**14**), 20(*R*)-Rg_3 (**42**), Rg_5 (**72**), Rg_6 (**135**), Rs_1–Rs_7 (**16**–**18**, **71**, **74**, **137**, **139**), and Rk_1–Rk_3 (**69**, **70**, **136**) seem to be characteristics for red ginseng as well as the *R*-epimers of Rg_2 (**111**) and Rh_1 (**112**) (Kim *et al.*, 1996; Kitagawa *et al.*, 1983; Park *et al.*, 2002a,b; Ryu *et al.*, 1997; Shibata *et al.*, 1985). Ginsenoside Rg_3 is one of the main artifact components of red ginseng and heat-processed ginseng (Kitagawa *et al.*, 1983; Kwon *et al.*, 2001; Park *et al.*, 2002a,b,c). In addition, white ginseng also contains the malonyl ester of ginsenosides Rb_1, Rb_2, Rc, and Rd (**6**, **8**, **11**, and **13**; Fig. 1.1). In red ginseng, the malonyl group, which is originally attached at the 6″-position of the glucosyl moiety of the above-mentioned ginsenosides, is released and the glycosyl moiety at C_{20}-OH is partly loss of to yield, for example, the deglycosylated ginsenosides Rh_1, Rh_2, and Rg_3 as the artifacts. The ginsenosides Rk_1–Rk_3, Rs_4–Rs_7, Rg_5, and Rg_6 are examples of dehydrated ginsenosides generated by the loss of water from the corresponding ginsenosides with a free hydroxyl group at C-20. For example, ginsenoside Rg_5 and Rg_6 are dehydrated ginsenosides of Rg_3 and Rg_2, respectively (Fig. 1.1). The acetyl group remains at the 6″-position of the glycosyl moiety of some ginsenosides in red ginseng, such as quinquenoside R_1 and ginsenosides Rs_1–Rs_7, because steaming seems to inactivate the deacetylating enzymes (Shibata, 2001).

Finally, it has been shown that storage of dried ginseng roots and its powder at low temperatures (~5 °C) can last for at least 3 months without

any significant loss of ginsenosides (Davidson *et al.*, 2004; Wills and Stuart, 2001). However, it is recommended that such herbal products be stored in packing protecting the products from light and atmospheric moisture (<10%) in order to reduce oxidative and enzymatic degradation of the ginsenosides (Wills and Stuart, 2001).

C. Pharmacokinetics and metabolism of ginsenosides

Medicinal herb products or dietary supplements are normally taken orally, including those based on ginseng. When most medicinal herbs/dietary supplements are taken orally, their constituents are brought into contact with gastric fluids (stomach acids) and by the microflora and enzymes in the large intestine before being absorbed from the gastrointestinal tract. Consequently, a part of the compounds originally present in the medicinal herb products/dietary supplements are metabolized to other components that may be more or less active than the undecomposed (intact) metabolites. The metabolic fate of the components of herbal medicines/dietary supplements therefore could be the key to a better understanding of their biological activity and the pharmacological actions of individual components. Furthermore, it has been suggested that the microfloral metabolic activity is affected by diet change and physiological factors, rather than by variations in the bacteria flora, and could be important in relation to the pharmacological actions and effects of bioactive constituents (Kim *et al.*, 2006). The metabolism and absorption of ginsenosides have been studied intensively in recent years in order to explain the pharmacological actions of ginsenosides and their contribution to the clinical efficacy of ginseng products. From the many investigations that have been undertaken to elucidate the fate of ginsenosides through the gastrointestinal tract using acids, enzymes, and human intestinal bacteria, it is clear that a large part of the intact ginsenosides is metabolized/transformed to ginsenosides with more enhancing biological effects compared with the intact ginsenosides, and that the degradation products of ginsenosides identified in plasma and urine most likely are a result from the metabolism of ginsenosides in the gastrointestinal tract.

For ginsenosides of the PPD-type, such as Rb_1 (**5**), Rb_2 (**7**), and Rc (**10**), it has been demonstrated in both *in vitro* and *in vivo* studies that intestinal human bacteria transform PPD ginsenosides to 20-*O*-β-glucopyranosyl-20(*S*)-PPD (compound K, which is also sometimes named M1 or IH-901) and then finally to 20(*S*)-PPD via a stepwise cleavage of the sugar moieties as illustrated in Fig. 1.2 (Akao *et al.*, 1997, 1998; Hasegawa *et al.*, 1996, 1997, 2000; Shibata, 2001; Tawab *et al.*, 2003; Wakabayashi *et al.*, 1997). The observed intestinal degradation product compound K after oral administration of PPD ginsenosides clearly suggests the presence of bacterial β-glucosidase enzymes that are capable of hydrolyzing the glycosidic linkage. Human intestinal bacteria having β-glucosidase activity that

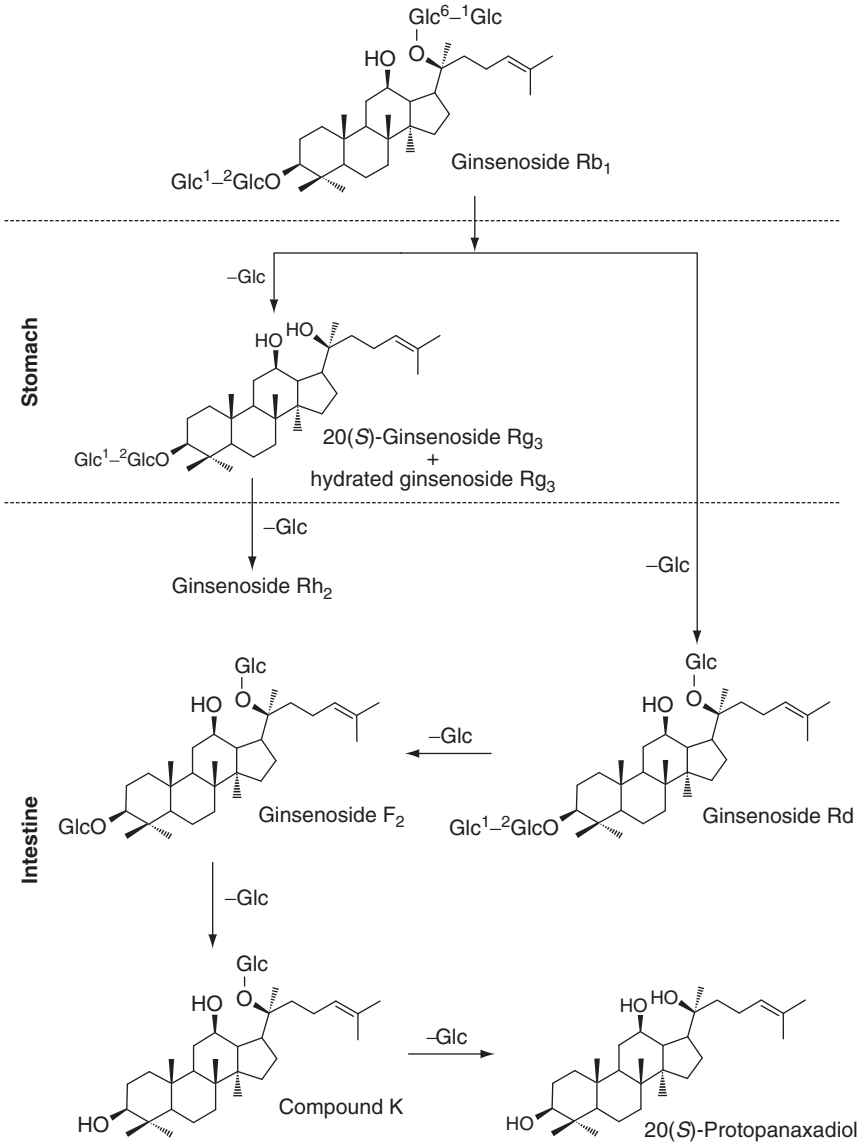


FIGURE 1.2 Possible metabolism (degradation pathway) of protopanaxadiol-type ginsenosides such as ginsenoside Rb_1 by stomach acidity and by human intestinal bacteria.

metabolizes PPDs to compound K includes *Eubacterium* sp. (Akao *et al.*, 1997; Bae *et al.*, 2000, 2004), *Prevotella oris* (Hasegawa *et al.*, 1997), *Streptococcus* sp., and *Bifidobacterium* sp. (Bae *et al.*, 2000, 2004). However, the transformation of the ginsenosides and production of compound K have shown to be very different between human intestinal bacteria (Bae *et al.*, 2000, 2004; Tawab

et al., 2003), and hence the efficiency of conversion and the transformation pathways of ginsenosides may differ greatly due to the diversity of the resident microflora between individuals (Lee *et al.*, 2002a). Compound K has been shown to induce an antimetastatic or anticarcinogenic effect by blocking tumor invasion or preventing chromosomal aberration and tumorgenesis and hence could be one of the key bioactive metabolites after oral intake of ginseng products explaining their potential anticancer activity as described in Section V.A (Lee *et al.*, 1999; Wakabayashi *et al.*, 1997). On the other hand, it has also been shown that the ginsenosides Rb_1, Rb_2, and Rc can be decomposed to ginsenoside Rg_3 (**14**) by mild acid treatment, such as stomach acids (Bae *et al.*, 2004; Han *et al.*, 1982), although a few investigations have indicated that PPD ginsenosides are hardly decomposed in the stomach but reach the intestine in intact forms (Tawab *et al.*, 2003). However, if ginsenoside Rg_3 is produced in the stomach, it can be metabolized to ginsenoside Rh_2 (**15**) and/or 20(*S*)-PPD by human intestinal bacteria as demonstrated in some *in vitro* studies (Bae *et al.*, 2002, 2004). This is also in accordance with pharmacokinetic studies in rats that after intragastric administration of 10 mg/kg ginsenoside Rg_3 resulted in plasma concentrations of approximately 40 and 100 ng/ml of ginsenoside Rh_2 and 20(*S*)-PPD, respectively, 4 h after administration (Xie *et al.*, 2005b). Ginsenoside Rh_2 and 20(*S*)-PPD also seem to be much more physiological active than the intact ginsenosides Rb_1, Rb_2 and Rc. Ginsenoside Rh_2 and 20(*S*)-PPD have, for example, been shown to be much more cytotoxic against tumor cell lines compared with the intact ginsenosides (Bae *et al.*, 2004).

For ginsenosides of the PPT-type, such as Rg_1 (**88**) and Re (**84**), several studies have demonstrated that these compounds are metabolized to ginsenoside Rh_1 (**92**) and ginsenoside F_1 (**80**) and then finally to 20(*S*)-PPT under different conditions via a stepwise cleavage of the sugar moieties as illustrated in Fig. 1.3 (Hasegawa *et al.*, 1996, 1997; Tawab *et al.*, 2003). After oral administration of ginsenoside Rg_1, the compound may be transformed in the stomach to ginsenoside Rh_1 and hydrated derivatives of Rh_1 (Han *et al.*, 1982; Sun *et al.*, 2005a; Tawab *et al.*, 2003). However, not all ginsenoside Rg_1 is hydrolyzed in the stomach and intact ginsenoside Rg_1 may reach the large intestine, where it is metabolized by intestinal bacteria to ginsenoside F_1 and 20(*S*)-PPT (Hasegawa *et al.*, 1996; Tawab *et al.*, 2003). Ginsenoside Re, on the other hand, may be hydrolyzed by gastric fluids to ginsenoside Rg_2 (**91**) that is then converted in the intestine to ginsenoside Rh_1 by the elimination of rhamnose through intestinal bacteria (Fig. 1.3). Intact ginsenoside Re may also reach the large intestine where it can be metabolized by intestinal bacteria to ginsenoside F_1 and 20(*S*)-PPT via ginsenoside Rg_1. Besides intestinal bacteria, several food microorganisms have shown to be able to produce specific forms of ginsenosides, including those produced by intestinal human bacteria (Chi *et al.*, 2005; Kim *et al.*, 2006). This clearly shows that it

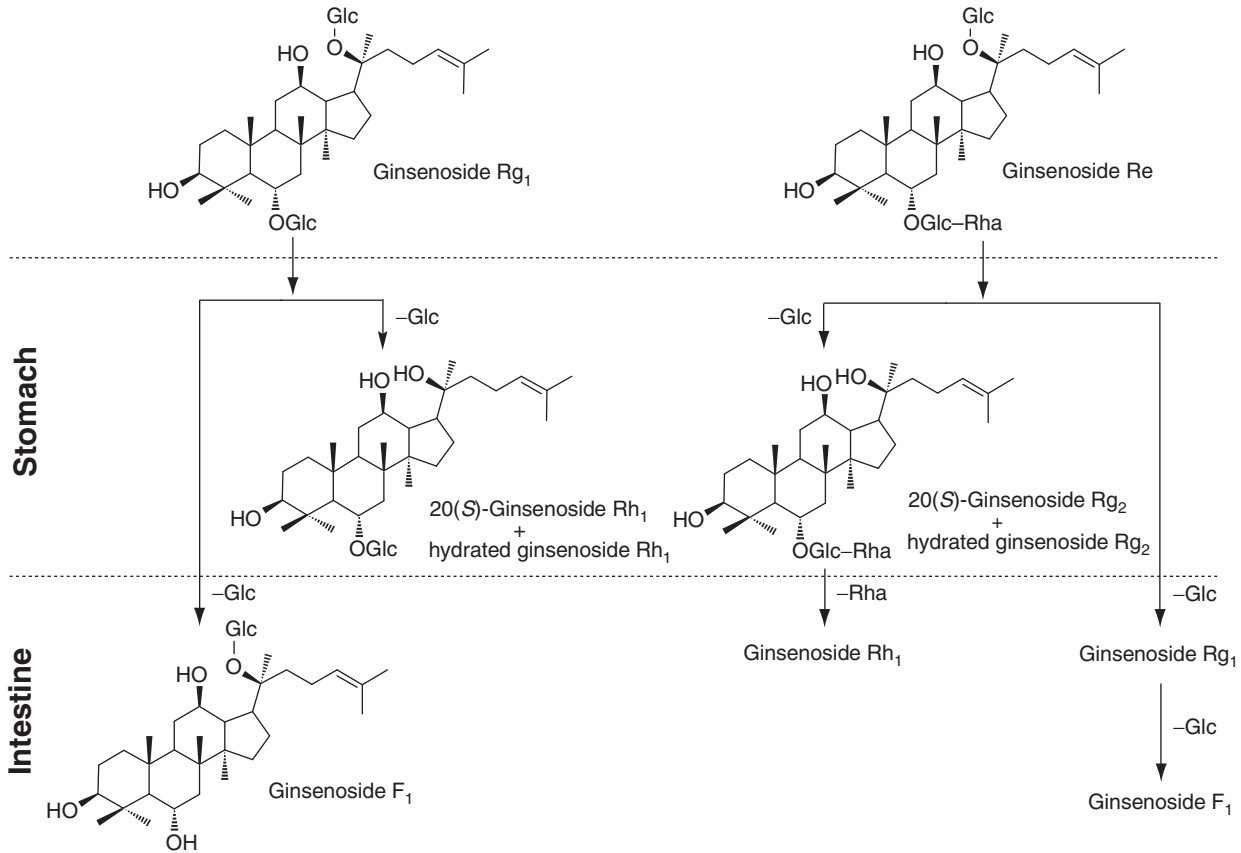


FIGURE 1.3 Possible metabolism (degradation pathway) of protopanaxatriol-type ginsenosides such as ginsenoside Rg_1 and Re by stomach acidity and by human intestinal bacteria.

would be feasible to develop a specific bioconversion process to obtain specifically designed functional products by the appropriate combination of ginsenoside substrate and specific microbial enzymes from food microorganisms (Chi *et al.*, 2005).

Pharmacokinetic studies in rats and/or humans have demonstrated that ginsenosides when taken orally can be detected in plasma and urine samples as intact ginsenosides or deglycosylated degradation products (Hasegawa *et al.*, 1996, 1997, 2000; Li *et al.*, 2004a,b, 2007a,b). The main degradation products detected in urine and plasma samples after oral intake of PPD and PPT ginsenosides are the monoglycosylated ginsenosides compound K, ginsenoside Rh_1 and ginsenoside F_1 and hydrated products of these, which clearly indicates that ginsenosides are transformed in the gastrointestinal tract as illustrated in Figs. 1.2 and 1.3, and further that the metabolized products are absorbed from the human gastrointestinal tract. Deglycosylated ginsenosides are usually more readily absorbed into the bloodstream acting as active compounds than the corresponding undecomposed ginsenosides (Tawab *et al.*, 2003). The concentrations of the deglycosylated ginsenosides in plasma after oral administration of ginseng products have been shown to be in the range where a significant physiological effect can be expected, whereas the bioavailability of intact ginsenosides is very poor compared with the deglycosylated ginsenosides. Consequently, the direct physiological effect of intact ginsenosides *in vivo* can therefore be discussed and clearly need further investigations. In humans, compound K and ginsenoside F_1 are usually detected in plasma from 7 h after the intake of ginseng and in urine from 12 h after the intake, whereas ginsenoside Rh_1 is detectable from 1 h in plasma and 3 h in urine after oral intake (Tawab *et al.*, 2003). Investigations on the pharmacodynamics of compound K after intravenous administration to mice have shown that compound K is mostly excreted as bile; however, some compound K are esterified with fatty acids at C-3 of the aglycone moiety or C′-6 of the glucose moiety in the liver (Hasegawa *et al.*, 2000). Consequently, the esterified forms of compound K are accumulated longer in the liver than compound K itself. Further, it has been shown that esterified compound K inhibits tumor growth more than compound K *in vivo* (Hasegawa *et al.*, 2000). These results suggest that liver enzymes also play a role in the metabolism of ginsenosides and in the formation of active principles of ginsenosides in the body.

III. BIOSYNTHESIS

Ginsenosides are biosynthesized via the isoprenoid pathway in the cytosol with mevalonic acid as the precursor for isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are the two C_5 starting units in the biosynthesis of ginsenosides and other terpenoids

(Dewick, 2004; Haralampidis *et al.*, 2001; Linsefors *et al.*, 1989). By using expressed sequence tag analysis, it has been possible to identify several candidate genes encoding for the enzymes geranyl diphosphate synthase (GPS), farnesyl diphosphate synthase (FPS), and squalene synthase (SS), involved in the various biosynthetic steps from IPP and DMAPP to squalene (Fig. 1.4A) (Choi *et al.*, 2005; Jung *et al.*, 2003; Lee *et al.*, 2004a; Tansakul *et al.*, 2006). Further candidate genes that appear to play a critical and important role in the biosynthesis of ginsenosides have been identified. However, further elucidation of the structure and physiological function of these genes is necessary before their role in the ginsenoside biosynthesis can be determined (Choi *et al.*, 2005; Luo *et al.*, 2005). As illustrated in Fig. 1.4A, triterpenes are not formed by an extension of adding an IPP C_5 unit to a growing chain of isoprenoid units such as farnesyl diphosphate. Instead, two molecules of farnesyl diphosphate (C_{15}) are joined tail to tail to yield the hydrocarbon presqualene diphosphate (Fig. 1.4A). Loss of the diphosphate group from presqualene diphosphate results in the formation of an unfavorable primary cation, which, however, via a Wagner-Meerwein 1,3-alkyl shift will lead to the formation of a more favorable tertiary carbocation and achieve the required C-1–C-1′ bond. Breaking the original C-1–C-2′ bond will then give an allylic cation that via supply of hydride from an NADPH cofactor will result in the formation of squalene (Blagg *et al.*, 2002; Dewick, 2004; Haralampidis *et al.*, 2001; Torssell, 1983). The next step in the biosynthesis of ginsenosides is the formation of squalene-2,3-oxide (squalene oxide or oxidosqualene), a reaction that is catalyzed by squalene epoxidase requiring O_2 and NADPH cofactors. If squalene oxide is suitablly positioned and folded on the enzyme surface, the resulting polycyclic triterpene structures formed can be rationalized in terms of a series of cyclizations, followed by a sequence of concerted Wagner-Meerwein migrations of methyls and hydrides. Thus, if squalene oxide is folded on the cyclized enzyme, cycloartenol synthase, it will approximate a *chair-boat-chair-boat* conformation, and the transient tetracyclic protosteryl cation will then be produced with these conformational characteristics (Dewick, 2004). This cation may then undergo a series of Wagner-Meerwein 1,2 shifts, thus, for example, creating cycloartenol (Fig. 1.4B), the precursor of a series of plant sterols such as β-sitosterol (Lee *et al.*, 2004a; Tansakul *et al.*, 2006). Should squalene oxide be folded on to another type of cyclase enzyme, in a *chair-chair-chair-boat* conformation, then the transient dammarenyl cation will be formed with different stereochemical features to the prostosteryl cation (Dewick, 2004). Direct quenching of the dammarenyl cation with water leads to epimeric C-20 dammarenediols (Fig. 1.4B), which are precursors of the dammarane-type triterpene saponins (Dewick, 2004; Haralampidis *et al.*, 2001; Tansakul *et al.*, 2006; Torssell, 1983). Most ginsenosides have *S* configuration at C-20 and it has been demonstrated that water addition

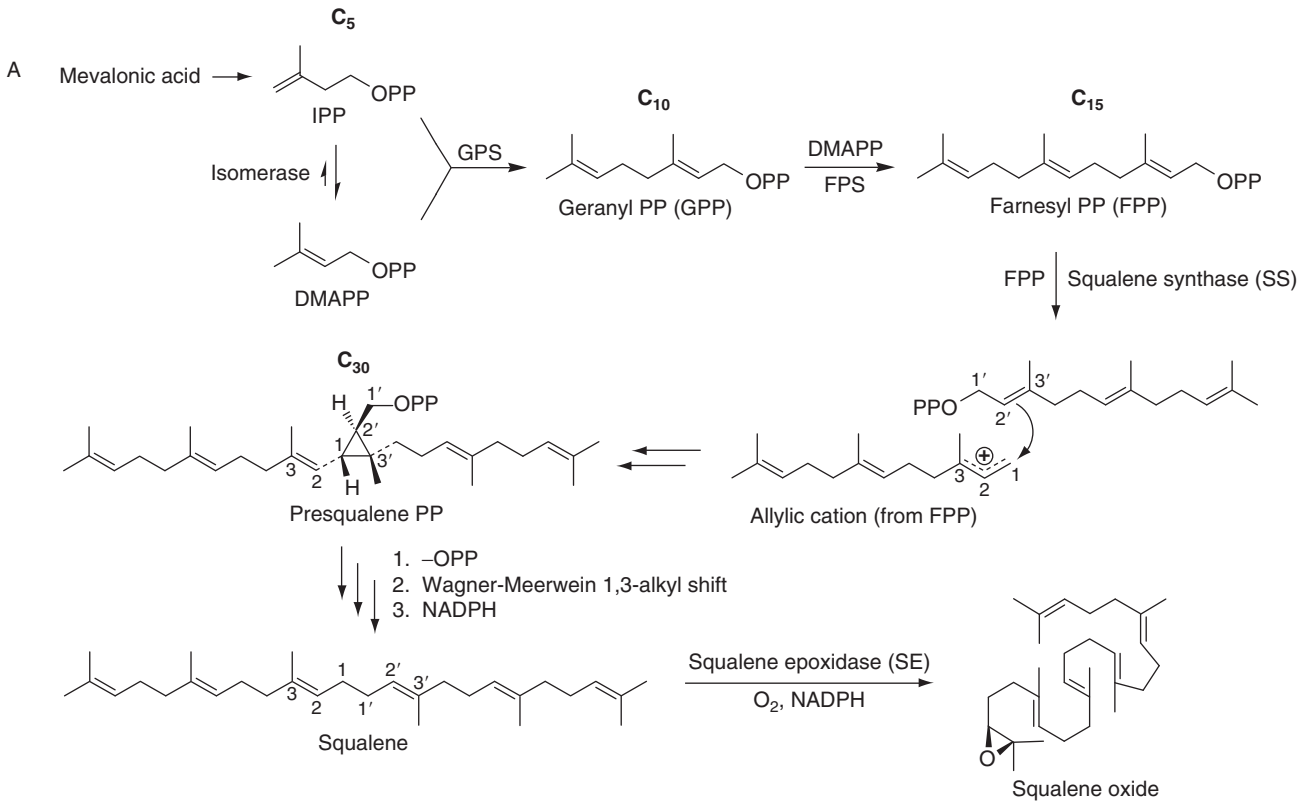


FIGURE 1.4 (*Continued*)

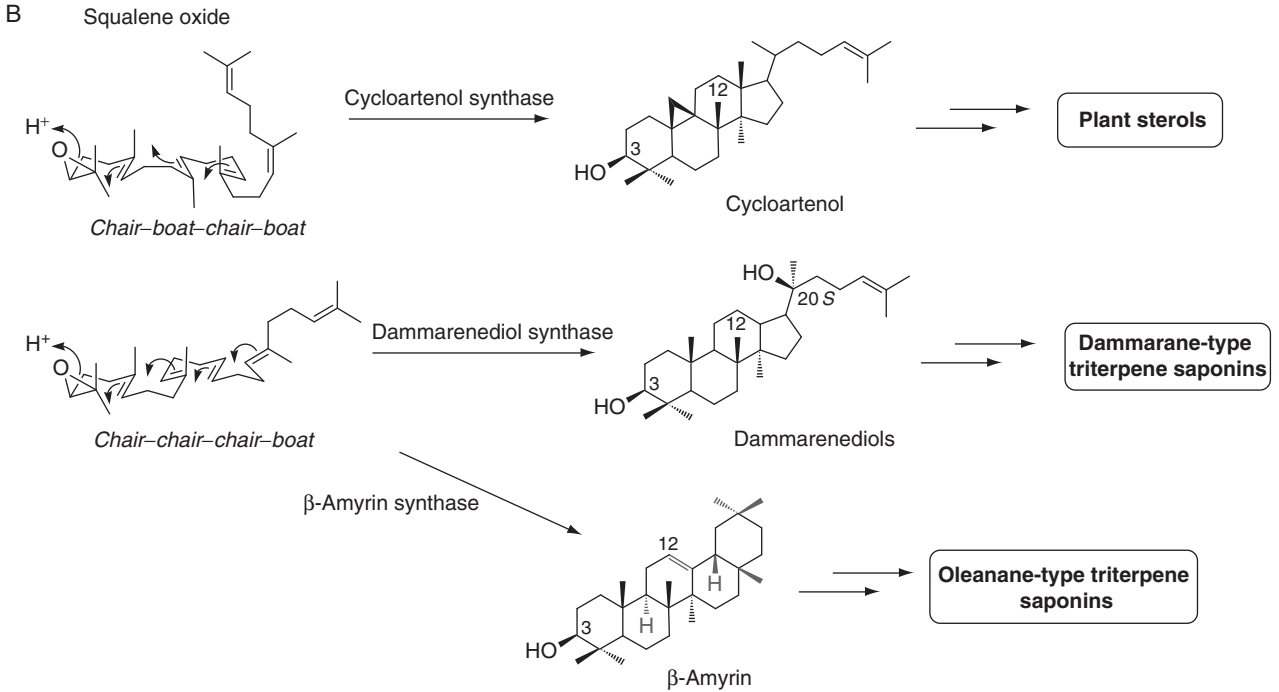


FIGURE 1.4 Proposed biosynthetic route for the biosynthesis of (A) squalene oxide (squalene-2,3-oxide) via the isoprenoid pathway and (B) triterpene saponins of the dammarane-type and oleanane-type from squalene oxide. PP, diphosphate group; GPS, geranyl phosphate synthase; FPS, farnesyl phosphate synthase; NADPH, nicotinamide adenine dinucleotide phosphate.

to the dammarenyl cation is stereospecific and that quenching of the carbocation by water addition, which terminates the cyclization reaction, is strictly enzymatic controlled (Kushiro *et al.*, 1997; Tansakul *et al.*, 2006). A few ginsenosides of the dammarenediol type have been isolated from the roots of ginseng species, including *P. notoginseng* (**175**), *P. vietnamensis* (**176**), and *P. japonicus* (**177**) (Table 1.1, Fig. 1.1), and appear to be directly biosynthesized from dammarenediol followed by glycosylation (Fig. 1.5). Hydroxylation of dammarenediol at C-12 leads to PPD and further hydroxylation at C-6 then to PPT, oxidation processes that most likely proceed via cytochrome P450 monoxygenase enzymes (Choi *et al.*, 2005; Haralampidis *et al.*, 2001; Jung *et al.*, 2003; Tansakul *et al.*, 2006). Biosynthesis of ginsenosides from PPD and PPT triterpene aglycones involves glycosylation primarily at the C-3 and/or C-20 positions on the skeleton for PPD-type ginsenosides (**1–79**) and at C-6 and C-20 positions for PPT-type ginsenosides (**80–82**, **84–97**, **99–109**, **111**, **112**, **115–120**, **122–146**), although a few PPT-type ginsenosides such as ginsenoside Ia (**83**); chikusetsusaponin L_{10} (**95**) and L_{9a} (**113**); floralginsenoside P (**98**), D (**119**), and K (**121**); vina-ginsenoside R_4 (**110**); and majoroside F_6 (**114**) have different glycosylation patterns with glycosylation at C-3 and/or C-12 positions. The glycosylation of triterpene aglycones proceeds via triterpene-glucosyltransferase enzymes, of which several candidates have been identified (Wang *et al.*, 2005, 2006b; Yue and Zhong, 2005).

The biosynthesis of ocotillol-type ginsenosides has to the best of my knowledge not been investigated. However, they may be biosynthesized via epoxidation of the double bond at C-24–C-25 followed by an intramolecular nucleophilic attack of the hydroxyl group at C-20 as illustrated in Fig. 1.6 (route a). On the other hand, a nucleophilic attack of the hydroxyl group in position C-20 at C-25 of the epoxide group will then lead to the rare panaxatriol-type ginsenosides, such as vina-ginsenoside R_{11} (**174**) (Fig. 1.6, route b) isolated from the roots of *P. vietnamensis* (Table 1.1). Addition of water to the epoxide group will finally lead to hydrated PPD-type ginsenosides (Fig. 1.6, route c), such as notoginsenoside J (**142**).

Alternatively the dammarenyl cation may undergo a Wagner-Meerwein 1,2-alkyl shift to give the baccharenyl cation. A pentacyclic ring system can now be formed by cyclization on the double bond, giving a new five-membered ring and the tertiary lupenyl cation (Fig. 1.7). Ring expansion in the lupenyl cation by bond migration gives the oleanyl system that by Wagner-Meerwein 1,2-hydride shifts leads to β-amyrin, which is the precursor for the oleanane-type triterpene saponins including those found in ginseng (Dewick, 2004; Haralampidis *et al.*, 2001; Tansakul *et al.*, 2006; Torssell, 1983), as illustrated in Fig. 1.7.

As described above, the cyclization of squalene oxide is a biosynthetic branching point not only for phytosterols and triterpenes but also for dammarane- and oleanane-type ginsenosides. In ginseng, the enzyme

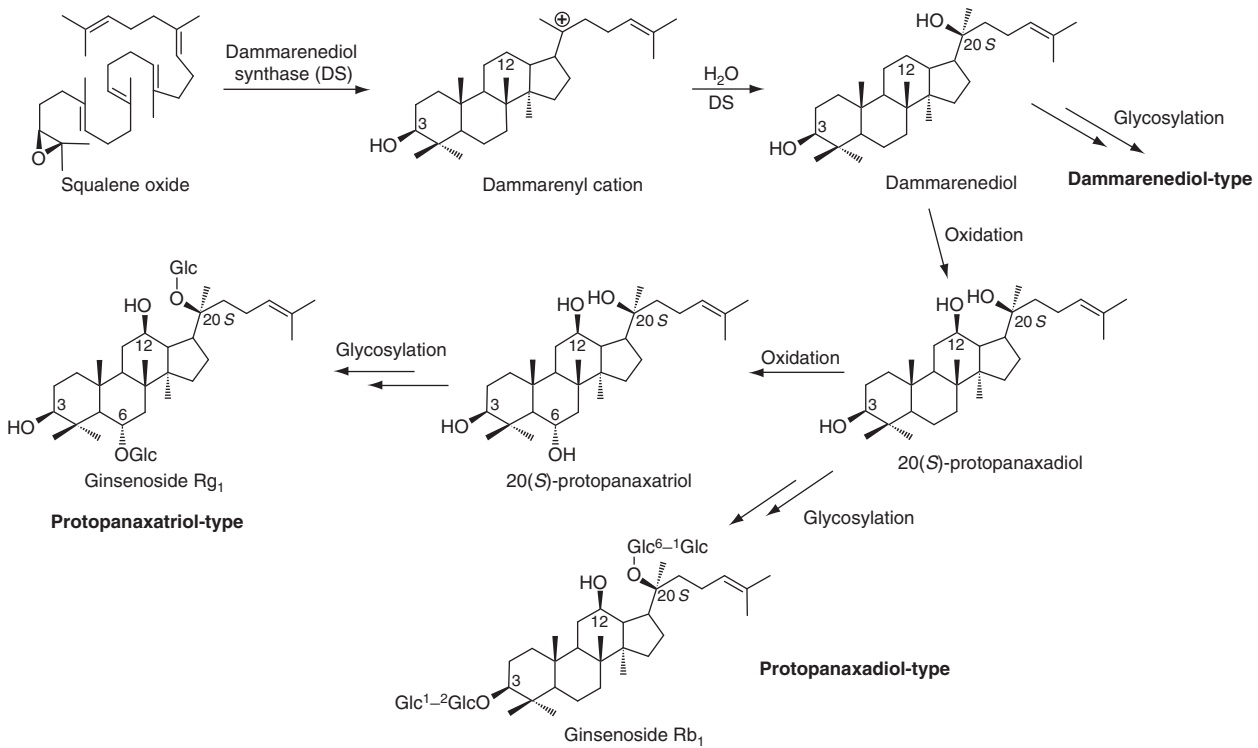


FIGURE 1.5 Possible biosynthetic route for ginsenosides of the dammarenediol-, protopanaxadiol-, and protopanaxatriol-type via dammarenediol.

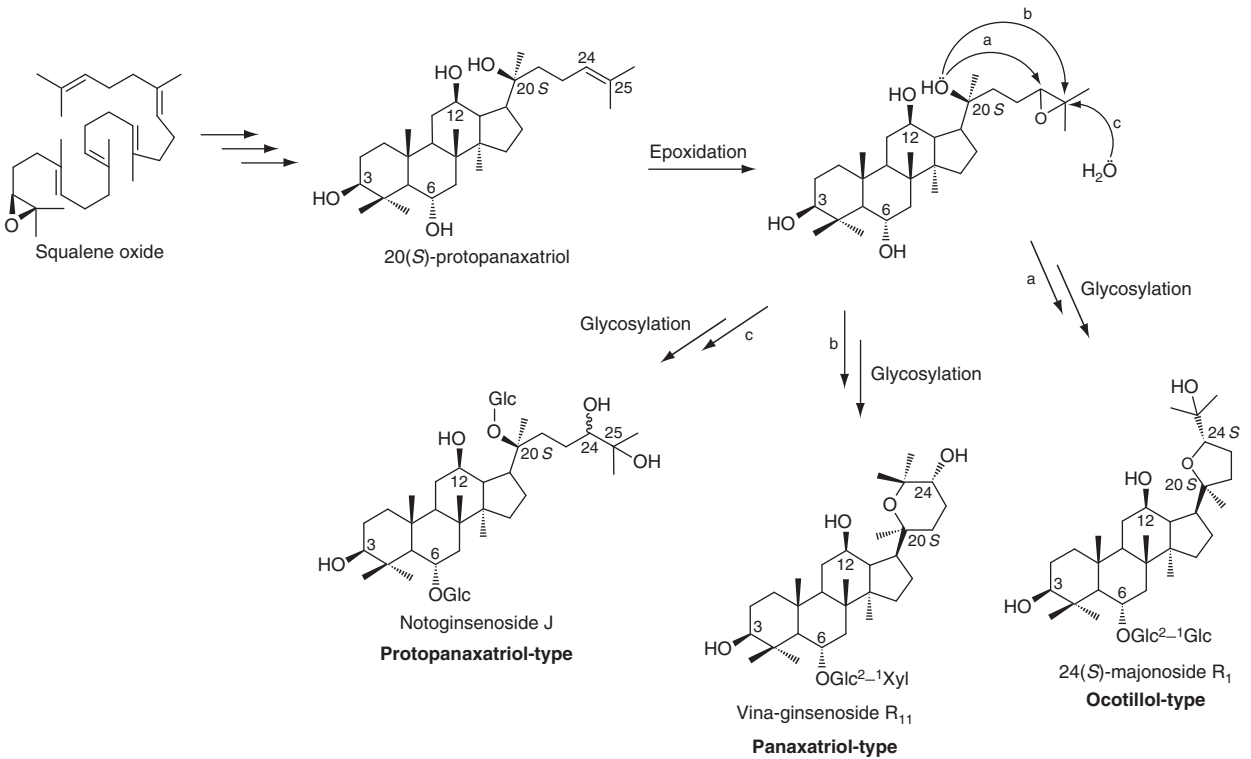


FIGURE 1.6 Possible biosynthetic route for ginsenosides of the ocotillol-, panaxatriol-, and protopanaxatriol-type via 20(*S*)-protopanaxatriol.

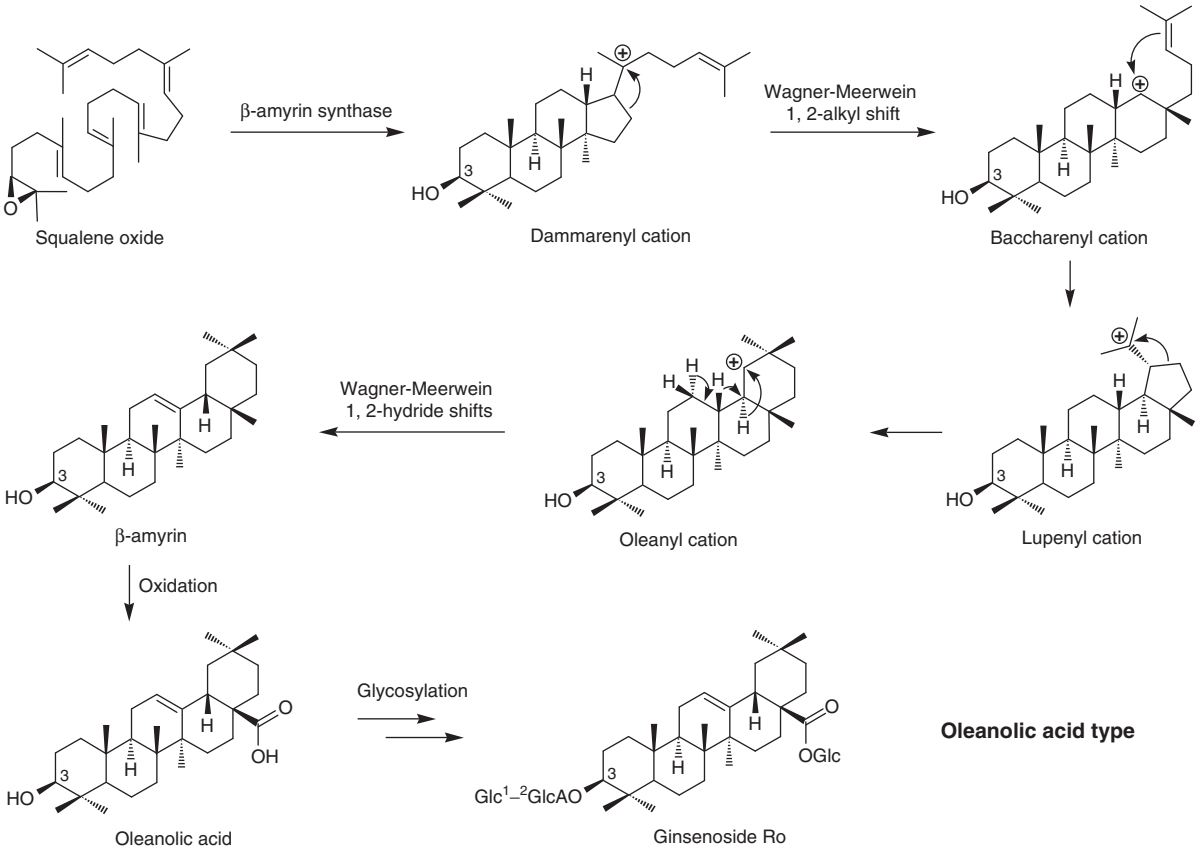


FIGURE 1.7 Possible biosynthetic route for ginsenosides of the oleanolic acid type from squalene oxide.

cycloartenol synthase catalyzes the production of cycloartenol, and β-amyrin synthase is involved in the biosynthesis of oleanane-type triterpenes (Fig. 1.2). The cyclization of squalene oxide into the dammarane skeleton/dammarenediol is catalyzed by squalene oxide (oxidosqualene) cyclase (Abe *et al.*, 1993; Haralampidis *et al.*, 2001; Kushiro *et al.*, 1997), which has recently been identified as dammarenediol-II synthase (Tansakul *et al.*, 2006). The formation of the various types of ginsenosides from dammarenediol and β-amyrin proceeds via various hydroxylation, oxidation, and glycosylation reactions that are catalyzed by various enzymes, of which only a few have been characterized (Choi *et al.*, 2005; Haralampidis *et al.*, 2001; Jung *et al.*, 2003; Yue and Zhong, 2005).

A special type of polyacetyleneginsenoside named polyacetyleneginsenoside Ro (**172**) (Fig. 1.1) and isolated from the roots of *P. ginseng* (Zhang *et al.*, 2002) is clearly formed from the oleanane-type ginsenoside Ro (**162**) and the polyacetylene panaxytriol by a simple esterification reaction. This ginsenoside is an example of a compound biosynthesized from two independently biosynthetic pathways, the mevalonate pathway and the acetate pathway. Polyacetylenes are biosynthesized from oleic acid by β-oxidation and various dehydrogenation and oxidation steps (Christensen and Brandt, 2006), and hence originate from the acetate pathway.

IV. ANALYSIS

Several qualitative and quantitative analytical techniques have been developed for the analysis of ginsenosides for the evaluation of quality of ginseng products and to determine the effect of processing of ginseng roots on the content of ginsensoides as well as for the determination of the metabolism and bioavailability of ginsenosides *in vitro* and *in vivo*. These analytical techniques include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) combined with various detectors, gas chromatography (GC), colorimetry, enzyme immunoassays (EIA), capillary electrophoresis (CE), nuclear magnetic resonance (NMR) spectroscopy, and spectrophotometric methods.

A. Sample extraction

Many types of extraction procedures have been employed for the extraction of ginsenosides from fresh or dry ginseng plant material as well as from ginseng preparations. Characteristic for most of the extraction methods is the use of methanol or ethanol or different aqueous mixtures of these two solvents, which also clearly enhance the extraction performances of these compounds compared with pure methanol or ethanol at room temperature (Anderson and Burney, 1998; Christensen *et al.*, 2006; Fuzzati, 2004; Lou *et al.*, 2006a). In order to enhance the recovery of

ginsenosides, various extraction experiments have been conducted at room temperature or using heat (Du *et al.*, 2004; Christensen *et al.*, 2006; Chuang and Sheu, 1994; Corbit *et al.*, 2005; Fuzzati, 2004; Lou *et al.*, 2006a; Popovich and Kitts, 2004a) or sonification (Corbit *et al.*, 2005; Court *et al.*, 1996a; Fuzzati, 2004; Li and Fitzloff, 2002a,b; Lou *et al.*, 2005) as well as supercritical fluid extraction (Wang *et al.*, 2001a; Wood *et al.*, 2006), microwave-assisted extraction (Chen *et al.*, 2007; Kwon *et al.*, 2003; Shu *et al.*, 2003), and enzyme-assisted extraction (Chen *et al.*, 2007). The use of heat for the extraction of ginsenosides may improve the total yield of ginsenosides but has also proved to degrade the thermally unstable malonyl-ginsenosides into the corresponding neutral ginsenosides. For example, Court *et al.* (1996a) showed that ginsenosides were partial degraded (~50%) after 5 h of soxhlet extraction with pure methanol and after minimum 20 h, total conversion of the malonyl-ginsenosides to their corresponding neutral ginsenosides was achieved (Court *et al.*, 1996a). Based on the huge amount of literature on extraction of ginsenosides from fresh or dried plant material, it appears that simple extraction with 80% of methanol, at room temperature, under stirring or sonification is optimal for the extraction of both neutral and acidic ginsenosides (Christensen *et al.*, 2006; Lou *et al.*, 2006a), whereas extraction with 100% methanol by refluxing at 60–65 °C for approximately 1 h seems to be an optimal extraction procedure of neutral ginsenosides (Corbit *et al.*, 2005).

B. Thin-layer chromatography

In the 1960 and 1970s when the first ginsenosides were isolated from ginseng, the ginsenosides were named Rx according to their R_f values on TLC (x = o, a_1, a_2, b_1, b_2, b_3, c, d, e, f, g_1, g_2, h_1, etc, Fig. 1.1) where x corresponds to the sequence of R_f value of the spots on the TLC plate from the bottom to the top (Shibata *et al.*, 1966). As the ginsenosides often separate chromatographically in groups on TLC containing the same genuine aglycone, this nomenclature seemed to be reasonable at the time with relative few known ginsenosides. Today, this nomenclature system is not practiced anymore. TLC is still commonly used in the analysis of plant material/extracts for ginsenosides due to its easiness of use, low cost, and versatility (Corthout *et al.*, 1999; Dallenbach-Toelke *et al.*, 1987; Fuzzati, 2004; Glensk *et al.*, 2001; Lee and Marderosian, 1981; Ludwiczuk *et al.*, 2002; Lui and Staba, 1980; Schulten and Soldati, 1981; Tani *et al.*, 1981; Vanhaelen-Fastré *et al.*, 2000). Detection of ginsenosides by TLC is usually achieved by using sulfuric acid alone or its mixtures with aromatic aldehydes like vanillin or anisaldehyde. TLC has been shown to be useful to discriminate ginseng species and products based on their content of ginsenosides (Corthout *et al.*, 1999; Ludwiczuk *et al.*, 2002), and in the United States Pharmacopoeia and the European

Pharmacopoeia, TLC is still employed for the identification test of root plant material. *P. quinquefolium* and *P. ginseng* can, for example, be discriminated for their ginsenoside composition by two-dimensional (2D) TLC using a mixture of chloroform, methanol, and water (13:7:2) as the first developing system and a mixture of water, *n*-butanol, and ethyl acetate (5:4:1) as the second solvent developing system (Fig. 1.8). Using this 2D-TLC system, it is possible to separate the ginsenosides Rb_1 (**5**), Rb_2 (**7**), Rc (**10**), Rd (**12**), Re (**84**), Rf (**86**), Rg_1 (**88**), Rg_2 (**91**), and Ro (**162**) (Fig. 1.8; Lui and Staba, 1980), where in particular, ginsenoside Rf is characteristic for *P. ginseng* and therefore commonly used to discriminate this ginseng species from *P. quinquefolium* as described in Section II.A. The

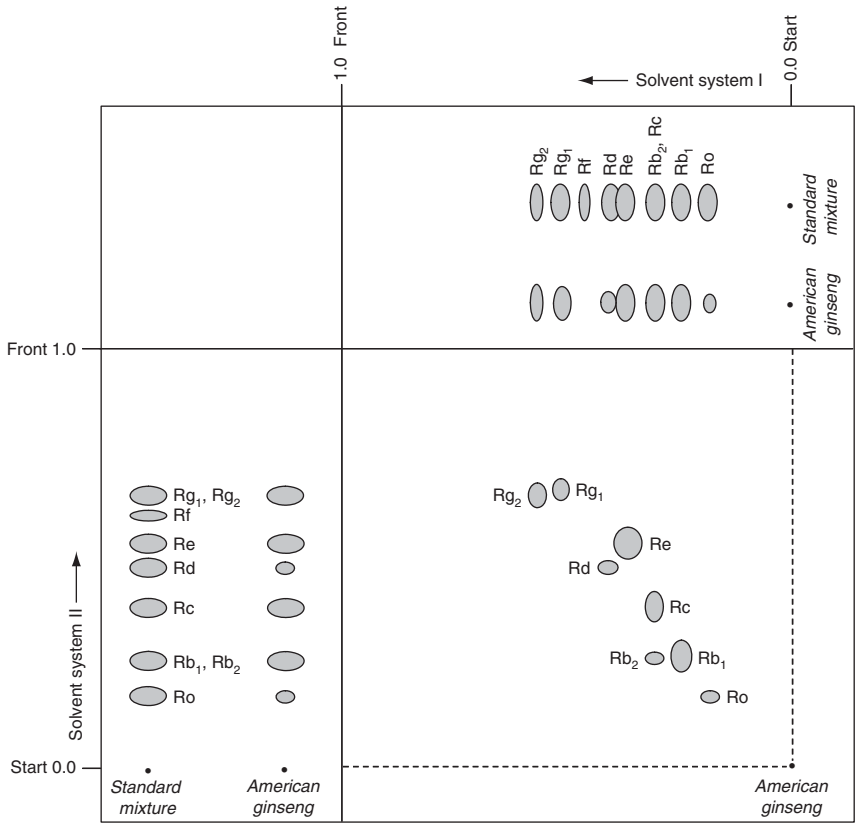


FIGURE 1.8 One-dimensional TLC separation of a standard mixture containing the ginsenosides Ro, Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, and Rg_2 and one- and two-dimensional TLC separation of ginsenosides of *Panax quinquefolium* (American ginseng) root extract. Solvent system I = first developing system containing chloroform–methanol–water (13:7:2); Solvent system II = second developing system containing water–*n*-butanol–ethyl acetate (5:4:1).

reproducibility and accuracy of this form for TLC is, however, limited because of the unstable coloration of the developing reagents, and is therefore not suitable for quantification. In contrast, densiometric determination of ginsenosides by high-performance TLC (HPTLC) offers the advantage of being reproducible, accurate, and selective and having relatively low detection limits (Corthout *et al.*, 1999; Dallenbach-Toelke *et al.*, 1987; Vanhaelen-Fastré *et al.*, 2000). Ginsenosides of *P. ginseng* roots and preparations have been quantified using HPTLC on silica gel F_{254} using chloroform, ethyl acetate, methanol, and water in the ratio 15:40:22:9 as developing system. The ginsenosides were revealed by anisaldehyde reagent and quantification of the ginsenosides Ra_1 (**2**), Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, and Rg_2 were performed by scanning the plates at 535 nm in emission mode. The method was successfully validated with regard to linearity, precision, and accuracy and with a detection limit for the ginsenosides of approximately 10 ng/spot (Corthout *et al.*, 1999). Vanhaelen-Fastré *et al.* (2000) have also successfully used HPTLC for the densitometric determination of major ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, and Rg_1) in *P. ginseng* by employing vapors thionyl chloride for detection, allowing the quantification of ginsenosides both in absorbance mode ($\lambda =$ 275 nm) and in fluorescence mode ($\lambda_{excitation} = 366$ nm, $\lambda_{emission} = 400$ nm) with a practical lower quantification limit of 100 ng.

The relative low detection limits of ginsenosides on TLC have also made it possible to study the pharmacokinetics of these compounds *in vivo*. Odani and colleagues (Odani *et al.*, 1983a,b; Takino *et al.*, 1982) developed a TLC-dual-wavelength method that could be used to investigate the absorption, distribution, metabolism, and excretion of ginsenoside Rb_1 and Rg_1 in rats. They showed that degradation and metabolism of intact ginsenosides occurs in the stomach and large intestine of rats in accordance with other pharmacokinetic studies of ginsenosides in animal and humans (see Section II.C). The developed TLC-densiometry method was also found to be useful for quantiative analysis of ginsenoside Rb_1 and Rg_1 in the different tissue and biofluid samples (Odani *et al.*, 1983a,b).

C. Gas chromatography

In the beginning of 1980 when the analysis of ginsenosides by GC was developed, the analysis of ginsenosides by GC was usually performed directly on the trimethylsillyl derivatives of ginsenosides. However, efficiency of the separation and the number of ginsenosides detected and quantified at that time was only 7 with ginsenoside Rf (**86**) and Rg_1 (**88**) being superimposed in the GC analysis (Bombardelli *et al.*, 1980). Today GC analysis is performed on highly efficient capillary GC columns and the strategy today for GC analysis of ginsenosides is first to hydrolyze the ginsenosides followed by trimethylsillyl-derivatization of their genuine

aglycones in order to simplify and improve the sensitivity of the GC analysis (Cui, 1995; Cui *et al.*, 1993, 1996, 1997, 1998; Fuzzati, 2004). Oxidative alkaline cleavage of the glycosidic chains is preferable compared with acid hydrolysis as the former method does not cause epimerization, hydroxylation, and cyclization (Cui *et al.*, 1993, 1998). The GC-methodology, however, does not allow evaluating complex pattern of the different types of ginsenosides in ginseng and ginseng products. Consequently, the GC methodology is rarely used for the analysis of ginsenosides. However, GC-MS has shown to be sensitive enough to allow the detection and quantification of the genuine aglycones of ginsenosides 20(*S*)-PPD and 20(*S*)-PPT in human urine samples after oral administration of ginseng preparations using panaxatriol as internal standard. Among 65 samples collected from athletes stating the consumption of *P. ginseng* preparations, Cui *et al.* (1996) were able to detect and quantify 20 (*S*)-PPT and 20(*S*)-PPD in 60 samples with a concentration between 2 and 35 ng/ml urine for 20(*S*)-PPT and a concentration of lower than 7 ng/ml urine for 20(*S*)-PPD in all samples. In a similar study on human urinary excretion of ginsenosides after ingestion of ginseng preparations, the same levels of ginsenosides in urine samples was found (Cui *et al.*, 1997). 20(*S*)-PPT occurred in at least sixfold higher concentration than 20(*S*)-PPD even though the daily intake of 20(*S*)-PPD-type ginsenosides was at least twofold higher than the intake of 20(*S*)-PPT-type ginsenosides. These findings clearly suggest that the uptake, metabolism, and excretion of 20(*S*)-PPD-type ginsenosides differ from those of 20(*S*)-PPT-type ginsenosides (Cui *et al.*, 1997).

D. High-performance liquid chromatography

HPLC has been the method of choice for the analysis of ginsenosides in most studies on ginseng plant material and preparations (Asafu-Adjaye and Wong, 2003; Chuang and Sheu, 1994; Li *et al.*, 2001b; Ma *et al.*, 1995; Pietta *et al.*, 1986a; Samukawa *et al.*, 1995; Soldati and Sticher, 1980; van Breemen *et al.*, 1995; Wang *et al.*, 1999, 2006a), gastric fluids (Pietta *et al.*, 1986b), plasma/serum (Jeoung *et al.*, 2005; Li *et al.*, 2004a; Qian *et al.*, 2006; Sun *et al.*, 2005a), urine samples (Li *et al.*, 2004b; Qian *et al.*, 2006), feces (Qian *et al.*, 2006), and tissues (Li *et al.*, 2006), because of its speed, sensitivity, and ability to analyze polar compounds. Different techniques have been used for the detection of ginsenosides such as UV, evaporate light scattering detection (ELSD), fluorescence, and MS.

1. UV detection

Among the different techniques for detection of ginsenosides, UV is the most employed technique because it is by far the most widespread detector type either as a simple UV-detector or in the form of the more

advanced diode array detectors. However, as ginsenosides in general are poor chromophores, their detection is limited to short wavelengths at 200–205 nm. The level of sensitivity for HPLC-UV detection of ginsenosides is not ideal, and because many compounds absorb light at short detection wavelength, many compounds may interfere with the analysis (Fuzzati, 2004; Yap *et al.*, 2005). This feature also limits the choice of solvents and mobile phase modifiers to improve separation. Separation of ginsenosides is commonly achieved by using a reversed-phase C_{18} column with water or phosphate or ammonium acetate buffers and acetonitrile mixtures as mobile phases in isocratic or in gradient elution mode (Fuzzati, 2004; Lou *et al.*, 2006b). The use of phosphate buffer and its concentration has been shown to be very important in order to obtain an optimal resolution of ginsenosides, in particular malonylated and other esterified ginsenosides (Christensen *et al.*, 2006; Fuzzati, 2004). Effective separations of neutral and acidic ginsenosides have been obtained using 10 mM KH_2PO_4 buffer (pH∼5.8–5.9) in combination with aqueous acetonitrile as mobile phases in gradient elution. Such a mobile phase system has, for example, been used in a comparative study on 37 commercial samples of ginseng radix (Chuang *et al.*, 1995). Content of the major ginsenosides Rb_1 (**5**), Rb_2 (**7**), Rc (**10**), Rd (**12**), Re (**84**), Rf (**86**), Rg_1 (**88**), Rg_2 (**91**), and Ro (**162**) and the malonylated derivatives of Rb_1 (**6**), Rb_2 (**8**), and R_c (**11**) (Fig. 1.1) was determined in white and red *P. ginseng* (Korean ginseng) as well as in *P. quinquefolium* (American ginseng) and *P. notoginseng* (Sanchi ginseng) roots. According to this study, *P. notoginseng* possesses the highest total content of ginsenosides followed by *P. quinquefolium* and white *P. ginseng*. Red ginseng was characterized by the absence of malonyl-ginsenosides due to the heating procedure connected with the production of this type of ginseng as described in Section II.B.

The use of phosphate buffer has also been used to determine the distribution of acidic and neutral ginsenosides in fresh or processed roots of *Panax* species and the ratio between acidic and neutral ginsenosides in the roots (Christensen *et al.*, 2006; Chuang *et al.*, 1995; Wills and Stuart, 2001). For example, in a recent study by Christensen *et al.* (2006), it was found that the total ginsenoside content and most individual ginsenosides except for ginsenoside Rg_1 varied significantly between different root sections of different diameters of *P. quinquefolium*, that is root hairs, lateral roots, and main roots of ginseng (Table 1.2; Figs. 1.9 and 1.10). Further, it was shown that the concentration of malonyl-ginsenosides constitutes approximately one-third of the total ginsenoside content in American ginseng roots, independent of size of root sections (Table 1.2). These results are in accordance with similar investigations on *P. quinquefolium* and other ginseng species (Chuang *et al.*, 1995; Wills and Stuart, 2001) and clearly show that malonyl-ginsenosides contribute significantly to the total content of ginsenosides in ginseng roots. The fact that ginseng

TABLE 1.2 The content of ginsenosides (mg/kg fresh weight) in different root sections [diameter 0.5–2.5 mm (root hairs); 5.0–10.0 mm (lateral); 15.0–20.0 and >20.0–38.0 mm (main roots)] of fresh roots from 6-year-old *Panax quinquefolium* (American ginseng) plants grown in Denmark (Christensen *et al.*, 2006)

	Ginsenosides (mg/kg fresh weight)											
Root size class according to root diameter	Rg_1	Re	Rb_1	Rc	Rb_2	Rd	Ro	Malonyl-Rb_1	Malonyl-Rc	Malonyl-Rd	Total ginsenosides	Total malonyl-ginsenosides
0.5–2.5 mm	800a	7420a	8070a	3170a	910a	2650a	310c	4850a	520a	2290a	31000a	7660a
5.0–10.0 mm	500a	4450b	6260b	900b	280b	1250b	470bc	4420ab	260b	1280b	20100b	5960b
15.0–20.0 mm	510a	4290b	5910b	530c	180c	740c	540b	3820b	170b	920c	17600b	4910bc
>20.0–38.0 mm	650a	4390b	6230b	380c	160c	550c	780a	3780b	200b	790c	17900b	4770c

Means within a column followed by different letters are significantly different ($p \leq 0.05$).

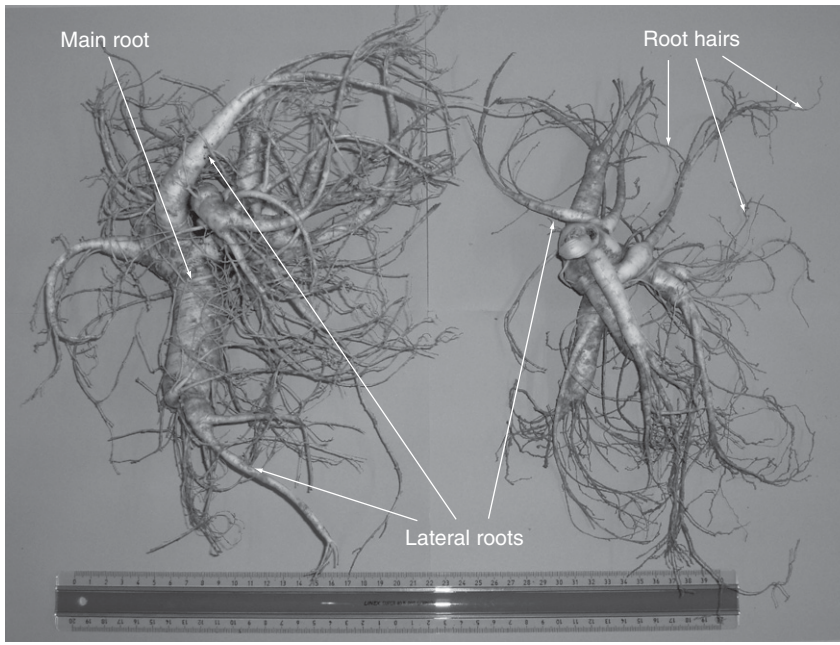


FIGURE 1.9 Ginseng roots from 6-year-old American ginseng plants (*Panax quinquefolium*) grown in Denmark with root hairs, lateral roots, and main roots. Ginseng roots within the same species may not only differ in content of ginsenosides but also in root size.

roots vary in ginsenoside content between root types/diameters may be used to make differentiated ginseng herbal remedies and/or to select and improve the genotypes of ginseng with respect to a higher yield and content of ginsenosides (Christensen *et al.*, 2006).

However, if phosphate buffers are not used in the mobile phases, the resolution of acidic ginsenosides such as malonyl-ginsenosides are very poor and in most cases, these ginsenosides will not be visible in the HPLC chromatograms using UV-detection as illustrated in Figs. 1.10 and 1.11. The importance of the malonyl-ginsenosides (**6**, **8**, **11**, and **13**) must be recognized because of their potential to undergo demalonylation by heat and hydrolysis. This clearly affects the concentration of total ginsenosides because malonyl-ginsenosides are often present in significant amounts in ginseng roots as mentioned previously. Hence, it is important that malonyl-ginsenosides are quantified when evaluating the quality of ginseng preparations and products. The use of phosphate or other types of buffers such as ammonium acetate is therefore recommended in the HPLC analysis of ginsenosides. Malonyl-ginsenosides may also be determined indirectly even though they cannot be observed in the HPLC chromatograms (Court *et al.*, 1996a; Du *et al.*, 2004; Wills and

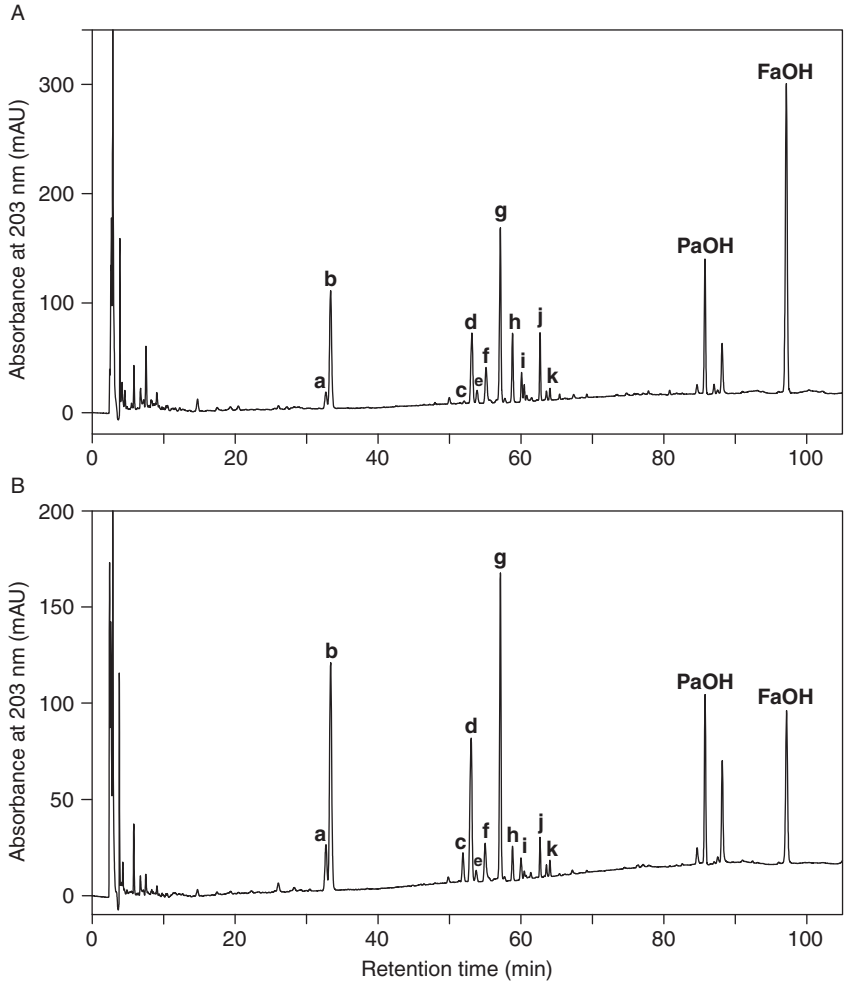


FIGURE 1.10 HPLC chromatograms of 80% aqueous methanolic ginseng extracts. (A) Root hairs (root diameter: 0.5–2.5 mm) and (B) main roots (root diameter: 15.0–20.0 mm) from a fresh root of a 6-year-old American ginseng (*Panax quinquefolium*) plant. Ginsenosides: **a** = Rg_1 (**88**), **b** = Re (**84**), **c** = Ro (**162**), **d** = malonyl-Rb_1 (**6**), **e** = malonyl-Rc (**11**), **f** = malonyl-Rd (**13**), **g** = Rb_1 (**5**), **h** = Rc (**10**), **i** = Rb_2 (**7**), **j** = Rd (**12**), **k** = gypenoside XVII (**24**) (Figure. 1.1). Polyacetylenes: **PaOH** = panaxydol; **FaOH** = falcarinol. Separations performed on a Purospher® STAR reversed-phase (RP)-18 end-capped column (5 μm; 250×4 mm id) with mobile phases consisting of solvent A [10% MeCN–90% 10 mM KH_2PO_4, pH = 5.82 (v/v)] and solvent B [75% MeCN–25% H_2O (v/v)]. Solvent gradient: 0 min 0% B, 5–15 min 15% B, 26 min 20% B, 36 min 22% B, 45 min 33% B, 50 min 35% B, 55 min 40% B, 75 min 80% B, 90–105 min 100% B, and 115 min 0% B.

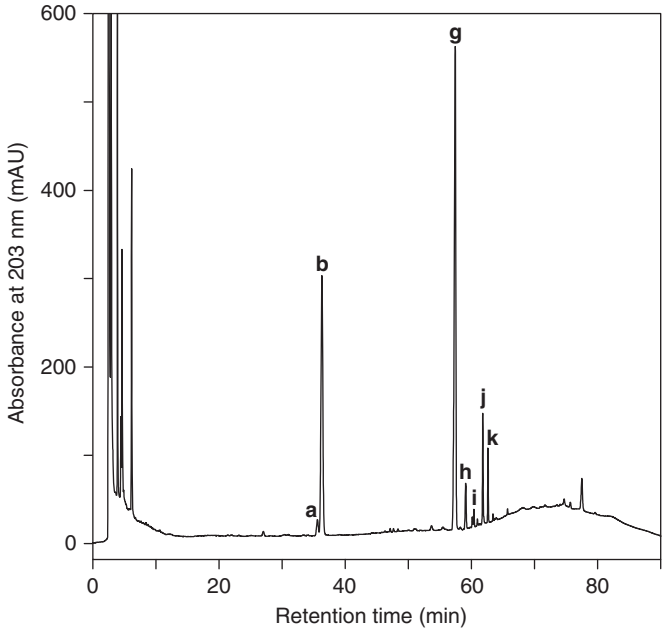


FIGURE 1.11 HPLC chromatogram of 80% aqueous methanolic extract from a fresh root of a 6-year-old American ginseng (*Panax quinquefolium*) plant. Polyacetylenes removed by extracting the roots with ethyl acetate before extracting with aqueous methanol. Ginsenosides: **a** = Rg_1 (**88**), **b** = Re (**84**), **g** = Rb_1 (**5**), **h** = Rc (**10**), **i** = Rb_2 (**7**), **j** = Rd (**12**), and **k** = gypenoside XVII (**24**) (Figure 1.1). Separations performed on a Purospher® STAR reversed-phase (RP)-18 end-capped column (5 μm; 250×4 mm id) with mobile phases consisting of solvent A [10% MeCN–90% H_2O (v/v)] and solvent B [75% MeCN–25% H_2O (v/v)]. Separations performed by the following solvent gradient: 0 min 0% B, 5–15 min 15% B, 26–36 min 25% B, 45 min 35% B, 50 min 40% B, 60–74 min 100% B, and 84–89 min 0% B.

Stuart, 2001). In a study by Wills and colleagues, malonyl-ginsenosides were determined by an indirect HPLC method in which samples of ginseng were analyzed twice. In the first analysis, the amount of neutral ginsenosides was quantified in the extract, after which the extract was hydrolyzed and then analyzed second time for ginsenosides (Du *et al.*, 2004; Wills and Stuart, 2001). The purpose of the hydrolysis process was to convert the malonyl-ginsenosides to their respective neutral ginsenosides. The HPLC chromatograms of both the original and hydrolyzed extracts were compared, and the concentration of malonyl-ginsenosides in the original extract was calculated. The indirect method may also be useful to identify esterified ginsenosides (Court *et al.*, 1996a) as commercial standards are not available for these compounds.

HPLC-UV has also been shown to be useful for the detection and quantification of ginsenosides in biological fluid and tissues. The pharmacokinentics of ginsenoside Rb_1, Rd, Rg_1, and notoginsenoside R_1 (**102**), which are the four main ginsenosides in *P. notoginseng*, have been determined by HPLC-UV in rat serum and various tissues after oral and intravenous administration of ginsenoside extract from *P. notoginseng* (Li *et al.*, 2004a, 2006). The serum and tissue samples were pretreated with solid phase microextraction prior to analysis by HPLC-UV in order to remove excessive interferences and to improve selectivity and sensitivity of the four ginsenosides.

2. Evaporate light scattering detection

The main problems encountered in performing HPLC-UV analyses of ginseng for ginsenosides are the high level of baseline noise and relatively poor sensitivity due the weak UV absorption and the limit choice of solvents and mobile-phase modifiers for improved separation (see Section IV.D.1 and Fuzzati, 2004). ELSD is a fast and relatively cheap and straightforward analytical method based on mass detection in which the chromatographic elute is nebulized by a gas stream (nitrogen) and the vapor enters a heated tunnel, where the solvent evaporates. The resulting analyte particles pass through a narrow light beam, and the scattered light is collected by a photomultiplier. The ELSD signal depends on the number of particles and size of analytes. Because ELSD only responds to nonvolatile analytes, it generates a stable baseline even by gradient elution and hence the use of volatile modifiers in the eluents, in order to obtain better selectivity, can be used by ELSD (Fuzzati, 2004). Consequently, HPLC-ELSD is normally more sensitive than, for example, HPLC-UV, although Li and Fitzloff (2002a) concluded in a direct comparison of these two method when used to quantify the ginsenosides Rb_1 (**5**), Rb_2 (**7**), Rc (**10**), Rd (**12**), Re (**84**), and Rg_1 (**88**) in *P. ginseng* and *P. quiquefolium* that HPLC-ELSD and HPLC-UV are comparable with regard to sensitivity and reproducibility. However, in another comparative study by Li and Fitzloff (2001), a fast HPLC-ELSD method was developed for the determination of 24(*R*)-pseudo-ginsensoide F_{11} (**159**), a minor ocotillol-type ginsenoside in *P. quinquefolium* that has been reported to improve memory performance (see Section V.F). 24(*R*)-pseudo-ginsensoide F_{11} was separated with a Sperisorb ODS-2 C_{18} column using a gradient of acetonitrile and water. Comparison between UV and ELSD detection showed very poor UV absorption due to no double bond in the structure of this ginsenoside. The detection limit of ELSD for 24(*R*)-pseudo-ginsenoside F_{11} was approximately 50 ng whereas the detection limit by UV detection was approximately 1050 ng (Li and Fitzloff, 2001).

HPLC-ELSD have been used to isolate, detect, and quantify all types of ginsenosides from various types of fresh and processed plant material of

ginseng species and preparations (Cao *et al.*, 2003; Fuzzati, 2004; Fuzzati *et al.*, 2000; Kim *et al.*, 2000; Park *et al.*, 1996c; Wan *et al.*, 2006a,b, 2007). White and red ginseng have been analyzed by HPLC-ELSD for their content of the ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf (**86**), Rg_1, Rg_2 (**91**), Rg_3 (**14**), and Rh_1 (**92**) (Park *et al.*, 1996c). Complete separation of these ginsenosides was achieved within 35 min with a LiChrosorb NH_2 column using an acetonitrile-water-2-propanol gradient system. The detection limits ($S/N = 3$) of the ginsenosides ranged from 35 to 150 ng, clearly illustrating its higher sensitivity. This method, for example, has been used to show that steaming of *P. ginseng* roots at high temperatures enhances the yield of ginsenosides such as Rg_5 (**72**) and 20(*S*)-Rg_3 (**14**) and 20(*R*)-Rg_3 (**42**) in red ginseng, which appear to have great impact on the health effects of ginseng (see Section V). Ginsenosides Rg_3 and Rg_5, which were absent in raw ginseng, were detected after steaming, whereas ginsenoside Rg_3 and Rg_5 were the most abundant in the material steamed at 120 °C, accounting for 39% and 19% of total content of ginsenosides, respectively (Kim *et al.*, 2000). Processing of ginseng roots normally also produces a wide variety of other less polar ginsenosides as described in Section II.B, and a HPLC-ELSD method that simultaneously separates and detects both polar ginsenosides and the less polar ginsenosides such as ginsenoside F_4 (**140**), Rg_3, Rg_5, Rg_6 (**135**), Rk_1 (**69**), Rk_3 (**136**), Rs_3 (**18**), Rs_4 (**74**), Rs_5 (**71**) together with the 20(*R*) epimers of Rg_2 (**111**), Rh_1 (**112**), Rg_3, and Rs_3 (**43**) has been developed (Kwon *et al.*, 2001). Separations were achieved within 45 min with an RP-C_{18} column using an acetonitrile-water-acetic acid gradient system. Also acidic ginsenosides such as malonyl-Rb_1 (**6**), malonyl Rb_2 (**8**), malonyl-Rc (**11**), and malonyl-Rd (**12**) have been successfully determined by HPLC-ELSD together with 13 common neutral ginsenosides in *P. ginseng* roots. The compounds were separated on a Hypersil BDS C_{18} column with 8 mM ammonium acetate (pH 7 with ammonium hydroxide) and acetonitrile as mobile phase in a linear programmed gradient system (Fuzzati *et al.*, 2000).

Furthermore, HPLC-ELSD methods for the simultaneous determination of ginsenosides obtained by pressurized liquid extraction of different parts of *P. notoginseng* have recently been described (Wan *et al.*, 2006a,b). Eleven major ginsenosides namely notoginsenoside R_1 (**102**), ginsensoides Rb_1, Rb_2, Rb_3 (**9**), Rc, Rd, Re, Rf, Rg_1, 20(*S*)-Rg_2, and 20(*S*)-Rg_3 were determined by HPLC-ELSD with detection limits between 18 and 98 ng. Separations were achieved in 60 min using a Zorbax ODS C_{18} column eluting with a gradient consisting of acetonitrile and water.

3. Fluorescence detection

Fluorescence is one of the most sensitive detection methods in HPLC analyses. However, as ginsenosides do not contain a suitable fluorescence chromophore they have to be derivatized before detection. Shangguan

et al. (2001) described a novel precolumn derivatization method for the quantitative determination of ginsenosides by HPLC with fluorescence detection. The double bond at the C_{24}–C_{25} position was converted into an aldehyde group by means of ozonolysis. Reaction of the aldehyde group with 9-fluorenylmethoxycarbonyl (FMOC) by hydrazine formed the ginsenosides FMOC-hydrazone following separation by RP-HPLC with gradient elution using methanol-water-0.1% TFA as eluent. Detection was performed with fluorescence (excitation 270 nm and emission at 310 nm) and the detection limits for ginsenosides such as Rb_1 (**5**) and Rg_1 (**88**) were approximately 1 and 2 ng, respectively. However, the limitation of this method is the requirement for a double bond at C_{24}–C_{25} and hence it cannot be used for the detection of all types of ginsenosides (Fig. 1.1). Still as the most common ginsenosides possesses a double bond at C_{24}–C_{25}, this method can still be considered useful for the detection of ginsenosides. Another HPLC method using photoreduction fluorescence detection has been described for the analysis of the ginsenosides Rb_1, Rb_2 (**7**), Rd (**12**), Re (**84**), and Rg_1 (Park *et al.*, 1995). Ginsenosides were separated on a LiChrosorb NH_2 column using acetonitrile and aqueous 2-*tert*-butylanthraquinone (*t*-BAQ) solution. The column effluent was passed through a 45 cm PTFE capillary tube coiled around a 10 W UV lamp to reduce *t*-BAQ in combination with the analyte (ginsenosides) to a highly fluorescent dihydroxy anthracene derivative that was detected by fluorescence detection (excitation 400 nm and emission 525 nm). The method showed reasonable detection limits between 100 and 1000 ng for ginsenosides, which is comparable with those obtained by UV detection (Park *et al.*, 1995).

4. Mass spectrometry

With the development of sophisticated ionization techniques including electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), HPLC-MS techniques have been successfully applied to the online analysis of ginsenosides in extracts and biological fluids (Fuzzati, 2004). In terms of sensitivity and specificity, an MS detector is better than UV or ELSD. Among the various MS methods, the HPLC-MS-MS (or just LC-MS-MS) technique is to date the most sensitive method for detection and quantification of ginsenosides.

LC-APCI-MS has been shown to very useful for the characterization of both neutral ginsenosides as well as thermolabile malonyl-ginsenosides in ginseng extracts (Ma *et al.*, 2005). However, LC-MS with ESI interface is a highly sensitive and soft ionization technique for the LC-MS analysis of thermolabile compounds and is considered to be the best method for the analysis of ginsenosides as it can overcome most problems associated with the thermolabile malonyl-ginsenosides and low molecular ion abundance levels. LC-ESI-MS is characterized by abundant adduct formation

and combined with collision-induced dissociation (CID) methodology, the LC-ESI-MS technique has been shown to be very useful for structure characterization of ginsenosides (Ackloo *et al.*, 2000; Cai *et al.*, 2002; Chan *et al.*, 2000; Cui *et al.*, 2000, 2001; Fuzzati *et al.*, 1999; Kite *et al.*, 2003; Li *et al.*, 2000; Song *et al.*, 2005; van Breemen *et al.*, 1995; Wang *et al.*, 1999). Both positive and negative ionizations of ginsenosides have been studied. The glycosidic linkages, the aglycone, and the attached sugar(s) can be determined by CID MS-MS analyses of $[M+H]^+$ and $[M-H]^-$ ions. Moreover, some alkali and transition metal cations may form strongly bonded attachment ions with the ginsenosides, and positive mode quasi-molecular $[M+H]^+$ ions are therefore often observed together with $[M+Li]^+$, $[M+Na]^+$, and/or $[M+K]^+$. As a result, their CID spectra of the metal attachment ions show a variety of structurally characteristic fragmentation patterns that can give important information about the structure of ginsenosides. Ackloo *et al.* (2000) conducted CID experiments on metal-attachment ions for the characterization of ginsenosides. Positive ESI-MS experiments with alkali metal ions such as Li^+ and Na^+ and transition metal cations such as Co^{2+}, Ni^{2+}, and Zn^{2+} were found to be useful in determining the molecular masses of the ginsenosides, and their CID spectra showed a variety of structure-related fragmentation patterns that could be used to determine the identity of the aglycone, the type of attachment positions of sugars to the aglycone, and the nature of the *O*-glycosidic linkages in the appended disaccharides.

LC-ESI-MS and LC-ESI-MS-MS analysis have been used to detect 25 ginsenosides in *P. ginseng* roots (Fuzzati *et al.*, 1999). The ginsenosides were separated on a reversed-phase Hypersil BDS C_{18} column using a binary eluent (aqueous 8 mM NH_4OAc, buffered to pH 7 with NH_4OH and acetonitrile) under gradient conditions. The investigation revealed the presence of several minor ginsenosides not described previously, including two isomers of ginsenoside a_1 (**2**) and a_2 (**3**) and several malonyl-ginsenosides (Fuzzati *et al.*, 1999). In this study, the neutral ginsenosides exhibited the quasi-molecular ion $[M-H]^-$, together with adduct ions $[M+OAc]^-$ and $[M-CH_2O+AcO]^-$ and doubled-charged adduct species such as $[M-H+OAc]^{2-}$ and $[M+2\ OAc]^{2-}$. Malonyl-ginsenosides exhibited the quasi-molecular ion $[M-H]^-$ together with the quasi-molecular ion $[M-CO_2-H]^-$ and adduct ions such as $[M-CO_2+OAc]^-$, $[M-CO_2+2\ OAc]^{2-}$, and $[M-CO_2-H+OAc]^{2-}$. The presence of double-charged adduct species is due to the thermal instability of malonyl-ginsenosides. The MS-MS spectra of the ginsenosides exhibited fragmentation pattern corresponding to the successive loss of the glycosidic units including the [Aglycone –H]$^-$ ions. However, the technique did not allow a complete structural identification of the isomers and some of the malonyl-ginsenosides (Fuzzati *et al.*, 1999). LC-MS-MS in negative ionization mode has also been applied to investigate the

in vivo metabolism of ginsenoside Rb_1 (**5**) in rats (Qian *et al.*, 2006). Oxygenation and deglycosylation were found to be the major metabolic pathways of Rb_1 in rat. A total of nine metabolites were detected in urine and feces samples collected after intravenous and oral administration of Rb_1. Deglycosylated metabolism of Rb_1 generated other ginsenosides as major metabolites such as ginsenoside Rd (**12**), Rg_3 (**14**), F_2 (**1**), Rh_2 (**15**), or compound K (Fig. 1.2), which clearly indicates that ginsenoside Rb_1 may have many pharmacological activities and may be used as a prodrug (Qian *et al.*, 2006). A prodrug is a pharmacological substance that is administered in an inactive or significantly less active form. Once administered, the prodrug is metabolized *in vivo* into the active compound and therefore intact ginsenosides, such as ginsenoside Rb_1, may therefore be used as prodrugs toward diseases where they are less effective compared to their *in vivo* degradation products. LC-ESI-MS in negative ion mode using selected ions monitoring has been used to develop a method for rapid quantification of ginsenoside Rg_1 (**88**) and its secondary glycoside Rh_2 and the aglycone 20(*S*)-PPT in rat plasma in order to study the pharmacokinetics of ginsenoside Rg_1 (Sun *et al.*, 2005a). The mass spectra of ginsenoside Rg_1, Rh_2, and 20(*S*)-PPT revealed beside quasi-molecular ion $[M-H]^-$ also negative adduct ions $[M+Cl]^-$ at *m/z* 835.50, 673.75, and 511.35, respectively. Sensitivity of the method was further improved by addition of NH_4Cl to the mobile phase. The detection limits for ginsenoside Rg_1 in deprotonated ion mode $[M-H]^-$ was 100 pg and 12.5 pg in adduct ion mode $[M+Cl]^-$. Consequently, the latter method was used for pharmacokinetic studies of ginsenoside Rg_1 in rat plasma. A similar LC-ESI-MS method was used to detect and quantify ginsenoside Rg_3 (**14**) and its metabolites in rat plasma (Xie *et al.*, 2005b) for the study of the pharmacokinetics of this pharmacological active ginsenoside (see Section V). Finally, LC-ESI-MS-MS in negative ionization mode has also been employed for the quantification of ginsenosides Rb_1, Rb_2 (**7**), Rc (**10**), Rd, Re (**84**), Rf (**86**), and Rg_1 in commercial samples of *P. ginseng* and *P. quiquefolium*. Separations were performed on a narrow bore Zorbax C_{18} column with water and acetonitrile as mobile phases. Although ginsenoside Rg_1 and Re coeluted under these conditions, they could be quantified separately using differences in molecular ions and product ions (Ji *et al.*, 2001). Concentrations of the other ginsenosides were determined by peak area of the most abundant product ions. LC-ESI-MS-MS in positive ion mode has been employed for the determination of ginsenosides such as Rb_1, Rb_2, Rc, Rd, Re, Rf, and Rg_1 in root extracts of *P. ginseng* and *P. quinquefolium* (Chan *et al.*, 2000; Wang *et al.*, 1999). In the study of Wang *et al.* (1999), the quantification was performed by selected reaction monitoring choosing $[M+H]^+$ as the precursor ion and monitoring the most abundant fragment ion that was a disaccharide ion for ginsenoside Rb1, Rb2, Rc, Rd, and Re and the $[aglycone+H-3H_2O]^+$ ion for Rf and Rg1.

Detection limits were 2 pg on column. Another LC-ESI-MS-MS method by Li *et al.* (2000) illustrates the use of a triple-quadrupole mass spectrometer for the analysis of ginseng extracts and for the differentiation of isobaric ginsenosides. Li *et al.* (2000) investigated the presence and the concentration ratio of ginsenoside Rf and 24(*R*)-pseudo-ginsenoside F_{11} (**159**) in *P. ginseng* and *P. quinquefolium* based on their baseline chromatographic separation and unambiguous identification using MS-MS. The two ginsenosides were separated with a narrow-bore Waters Spherisorb C_{18} column eluting with 0.1% acetic acid and 5 mM sodium acetate and acetonitrile containing 0.1% acetic acid under gradient conditions and monitored using the multiple reaction monitoring precursor/product ion pairs m/z 823 $[M+Na]^+ \rightarrow 365$ and 801 $[M+H]^+ \rightarrow 143$ during LC-MS-MS analysis with detection limits of 120 pg on-column. Li *et al.* (2000) found that 24(*R*)-pseudo-ginsenoside F_{11} was abundantly present in *P. quinquefolium* and only present in minor amounts in *P. ginseng* and that ginsenoside Rf appears to be absent or under the detection limit in *P. quinquefolium*, clearly showing that these ginsenosides can be used to distinguish *P. ginseng* and *P. quinquefolium*, as described in Section II.A.

While LC coupled with quadrupole MS has been extensively used for especially the quantification of ginsenosides, ion trap MS and quadrupole-time of flight (Q-TOF) MS provide several advantages in the structural analysis of ginsenosides. Ion trap MS with positive and negative ionization modes with its ability to perform ESI combined with multistage MS (MS^n) have been used to analyze ginsenosides rapidly in plant extracts and to provide their structural information (Cui *et al.*, 2000, 2001). In particular, the fragmentation pathways of the quasi-molecular $[M-H]^-$ ion resulted in several significant signals corresponding to the cleavage of the glycosidic bonds and sugars, allowing a relatively straightforward interpretation of the MS^n spectra for structure elucidation of ginsenosides (Cui *et al.*, 2000). The effect of metal cationization (Li^+, Na^+, K^+, Ag^+) on CID of ginsenosides has been investigated by ESI-MS^n (Cui *et al.*, 2001). Metal-cationized ginsenosides were found to have characteristic fragmentation patterns that were found to be useful for convenient screening and identification of ginsenosides in mixtures.

Although a lot of structural information can be obtained through CID in the course of ESI-MS-MS or ESI-MS^n analysis, high-resolution mass spectrometry (HRMS) analysis provides more detailed and more accurate structural information that can be used to identify, especially unknown degradation products or metabolites of ginsenosides. HRMS analysis of ginsenosides has been performed by Q-TOF MS in combination with MS-MS and have shown to be useful not only for confirming the molecular composition but also for studying the structures of various isomers of ginsenosides by applying CID technique (Cai *et al.*, 2002; Song *et al.*, 2005).

Accurate mass measurement for both parent and fragment ions from the HRMS analysis provides information of elemental composition of the analytes. Fragmentation pathways obtained from the interpretation of MS-MS spectrum and assignment of the accurate mass of each fragment ion can not only be used to verify the fragmentation pattern but also be used for differentiating ginsenoside isomers (Cai *et al.*, 2002; Song *et al.*, 2005). HRMS analysis by Q-TOF MS seems to be a very useful technique especially for pharmacokinetic and metabolic studies of ginsenosides due to its sensitivity and selectivity. Recently, Q-TOF MS in combination with ultra-performance liquid chromatography (UPLC) had also been demonstrated to be a powerful tool for herbal metabolomics to discriminate differentially processed herbs such as raw and steamed *P. notoginseng* (Chan *et al.*, 2007). As demonstrated, the UPLC-TOF-MS-based metabolomics approach is promising for the quality control of ginseng and the holistic standardization of ginseng herbal extracts for clinical studies. Finally, UPLC-TOF-MS may also find use for metabonomic studies of endogenous metabolites as well as metabolized ginsenosides in biofluids and tissue samples in order to provide further information on the potential health effects of ginsenosides.

E. Nuclear magnetic resonance spectroscopy

Herbal remedies contain different constituents in which characteristic metabolomic NMR fingerprints can be assigned. Therefore, NMR spectroscopy has been shown to be useful in the quality control of different herbal products such as ginseng (Qin and Zhao, 1999; Yang *et al.*, 2006), St. Johns wort (Bilia *et al.*, 2001), arnica (Bilia *et al.*, 2002), and many other herbs. Yang *et al.* (2006) recently demonstrated the application of 2D NMR spectroscopy for quality control of ginseng products. By combining 2D *J*-resolved NMR spectroscopic methods with principal component analysis, they were able to distinguish different ginseng preparations as well as white and red ginseng roots from each other based on their metabolic profiling. The most important constituents of the metabolic profiling of ginseng products and roots were ginsenosides, polysaccharides, mono- and disaccharides, amino acids, fumaric acid, and inositol. Another application of NMR spectroscopy was demonstrated by Kang *et al.* (2005) who demonstrated by using a combination of NMR spectroscopy and molecular dynamics simulations that ginsenoside 20(*S*)-Rg_3 inhibited Na^+ channel activity but not 20(*R*)-Rg_3. The different effect on Na^+ channel activity observed for 20(*S*)- and 20(*R*)-Rg_3 may explain the different effects observed for these enantiomers in relation to tumor cell invasion and metastasis (Azuma and Mochizuki, 1994; Section V.A).

F. Capillary electrophoresis

Very few studies have described the use of CE for the analysis of ginsenosides (Glöckl *et al.*, 2002; Iwagami *et al.*, 1992). Glöckl *et al.* (2002) described a very fast and reliable method for the analysis of ginsenosides Rb_1 (**5**), Rb_2 (**7**), Rc (**10**), Rd (**12**), Re (**84**), Rf (**86**), and Rg_1 (**88**) in ginseng extracts and preparations using micellar electrokinetic chromatography. Capillary zone electrophoresis was not applicable due to the absence of charge in ginsenosides. The analysis was performed using 100 mM borate and 80 mM cholate (pH 10) as mobile phases and a capillary length of 75 cm. Chloramphenicol was used as internal standard. The ginsenosides were separated within 20 min and detected using UV at 200 nm. The analyses of the ginseng extract showed good separation of all ginsenosides with except of ginsenoside Rf, which coeluted with other components in the extract. Validation of the method was performed for the quantification of the major ginsenoside Rb_1 evaluating linearity, precision, and accuracy.

G. Enzyme immunoassay

Enzyme immunoassay (EIA) and enzyme-linked immunosorbant assay (ELISA) are almost synonymous and both methods are based on the principle of immunoassay, that is, it is a biochemical test that measures the concentration of a compound using the reaction of an antibody or antibodies to its antigen. The immunoassay takes advantages of the specific binding of an antibody to its antigen and hence monoclonal antibodies (MAbs) are often used as they usually only bind to one site of a particular molecule. This often provides a more specific, accurate, rapid, and sensitive test for specific compounds, although cross-reactivity occurs. EIA or ELISA techniques has been developed for the qualitative and quantitative determination of ginsenosides in plant extracts and biological fluids using both polyclonal antibodies and MAbs (Fukuda *et al.*, 2000a,b, 2001; Kanaoka *et al.*, 1992; Morinaga *et al.*, 2006; Shoyama *et al.*, 1999; Tanaka *et al.*, 2006; Yoon *et al.*, 1998). The first step in the development of EIA methods is the synthesis of a hapten–carrier protein conjugate. Bovine serum albumin (BSA) in combination with ginsenosides particularly have been used for the preparation of specific MAb in mouse against ginsenoside Rb_1 (**5**), F_1 (**80**), Rf (**86**), Rg_1 (**90**), and Rg_2 (**91**) (Fukuda *et al.*, 2000a; Morinaga *et al.*, 2001; Shoyama *et al.*, 1999; Tanaka *et al.*, 1999) and for the establishment of ELISA assays for the determination of immunoaffinity concentration for ginsenosides (Fukuda *et al.*, 1999, 2000b, 2001). Recently, a simple procedure using periodate oxidation has been developed for coupling ginsenosides with BSA (Morinaga *et al.*, 2006; Yoon *et al.*, 1998). Both the synthetic hapten–carrier protein conjugates and the MAb produced were characterized by matrix-assisted

laser desorption mass spectrometry (MALDI-TOF-MS) (Morinaga *et al.*, 2001, 2006) and have, for example, been used to develop an ELISA assay for the determination of total ginsenoside content in ginseng (Morinaga *et al.*, 2006). The total content of ginsenosides determined by the ELISA was in good agreement with HPLC-UV determinations. The effective measuring range of this ELISA assay was between 20 and 400 ng/ml for total ginsenosides when using ginsenoside Re (**84**) as standard. Furthermore, a highly sensitive ELISA method has been developed for the determination of 20(*S*)-PPT ginsenosides (Jung *et al.*, 2002). Polyclonal antibodies raised against ginsenoside F_1-BSA showed high reactivity to 20(*S*)-PPT ginsenosides and minor reactivities to other ginsenosides. Using ELISA, the detection and quantification range was from 50 pg/ml to 20 ng/ml, and the method was proven to be useful for the determination of 20(*S*)-PPT ginsenosides in biological fluids.

An immunochromatographic assay for detection of ginsenosides Rb_1 and Rg_1 has been developed that uses anti-ginsenoside Rb_1 and anti-ginsenoside Rg_1 MAbs and a detection reagent that contains colloidal gold particles coated with anti-ginsenoside Rb_1 and anti-ginsenoside Rg_1 MAbs (Putalun *et al.*, 2004). This qualitative assay system was found to be useful for a rapid screening method for the detection of ginsenoside Rb_1 and Rg_1 in plants and plant preparations in concentrations down to 2 μg/ml.

Finally, Western and Eastern blotting methodologies have been used to detect and quantify ginsenosides in ginseng in picomole concentrations (Fukuda *et al.*, 1999, 2000b, 2001; Tanaka *et al.*, 2006). The use of this methodology has allowed the direct immunocrystolocalization of ginsenosides and/or individual ginsenosides directly in fresh ginseng roots, thus showing that the highest content of ginsenosides is found primarily in the endodermis cells, followed by the exodermis tissue and the radial vascular bundle (Shoyama *et al.*, 1999; Tanaka *et al.*, 2006).

H. Near infrared spectroscopy

Near infrared spectroscopy (NIRS), a technique based on absorption and reflectance of monochromatographic radiation by samples over a wavelength range of 400–2500 nm, has been successfully applied for food composition analysis, for food quality assessment, and in pharmaceutical production control. NIRS can be used to differentiate various samples via pattern recognitions. The technique is fast and nondestructive method that does not require sample preparation and is very simple to use compared too many other analytical methods such as HPLC. The drawback of NIRS, however, is that the instrument has to be calibrated using a set of samples typically 20–50 with known analyte concentrations obtained by suitable reference methods such as HPLC in order to be used for quantitative analyses. Simultaneous quantification of the

ginsenosides Rb_1 (**5**), Rb_2 (**7**), Rc (**10**), Rd (**12**), Re (**84**), Rg_1 (**88**), Ro (**162**), and the malonylated-ginsenosides **6**, **8**, **11**, and **13** (Fig. 1.1) in *P. quinquefolium* roots has been performed by NIRS (Ren and Chen, 1999). In this study, the NIRS was calibrated by analyzing 26 samples of *P. quinquefolium* roots for their content of ginsenoisdes by HPLC-UV, and for each sample, NIR spectra were collected over 400–2500 nm. The HPLC and spectral data obtained were used to calibrate and cross-validate the NIR instrument for measuring the individual ginsenosides. A similar investigation using NIRS have been used to quantify the ginsenoside Rb_1, Rb_2, Rd, Re, Rf (**86**), and Rg_1 in *P. notoginseng* (Chen and Sorensen, 2000). These investigations showed that the NIRS methods for the quantitative determination of ginsenosides are comparable with those obtained by HPLC-UV.

V. POTENTIAL HEALTH EFFECTS OF GINSENOSIDES

There is extensive literature on the beneficial effects of ginseng. Pharmacological effects of ginseng have been demonstrated in the central nervous system (CNS), the cardiovascular system, and the immune system. Furthermore, extensive preclinical and epidemiological studies have demonstrated that ginseng and ginseng products have potential cancer-preventive effects as well as effects on hyperglycemia (Gillis, 1997; Shibata, 2001; Sticher, 1998; Yun, 2001a, 2003). The active components in ginseng consist mainly of polysaccharides, polyacetylenes, and ginsenosides, of which the ginsenosides are considered to be the major active principles of ginseng (Sticher, 1998). The ginsenosides have demonstrated an ability to target different types of tissues, producing an array of pharmacological responses. Since ginsenosides may produce effects that are different from one another, and single ginsenosides and/or their metabolized products may initiate multiple actions in the same tissue, the overall pharmacology of ginseng and ginseng products is very complex. In the following, some of the most interesting pharmacological effect of ginsenosides, and hence their potential health promoting effects are discussed.

A. Anticarcinogenic effects

Ginsenosides have been shown to exert anticarcinogenic effects *in vitro* and *in vivo* through different mechanisms. Several ginsenosides show direct cytotoxic and growth inhibitory effects against tumor cells, whereas others have been shown to inhibit metastasis and tumor growth. Results from epidemiological and cohort studies with white and red ginseng have clearly demonstrated that they have nonorganic specific preventive effect against cancer and that this effect is likely to be due to their content of

ginsenosides, in particular ginsenoside 20(*S*)- and 20(*R*)-Rg_3 (**14**, **42**), Rg_5 (**72**), and Rh_2 (**15**) (Yun, 2001a, 2003).

1. Cytotoxic and antitumor activity

The cytotoxic and antiproliferative effects of ginsenosides toward human and animal cancer cell lines have been demonstrated in numerous investigations. In a study, Wang *et al.* (2007) tested the cytotoxicity of 10 ginsenosides (20(*S*)-PPD, **5**, **12**, **14**, **15**, **44**, **84**, **88**, **91**, and **124**), isolated from the fruits of *P. ginseng*, toward several human cancer cell lines, including breast cancer cell lines (e.g., MCF-7 cells), lung cancer cell lines (e.g., H838 cells), and prostate cancer cell lines (e.g., LNCaP and PC3 cells). Among the ginsenosides tested, ginsenoside 20(*S*)-PPD, Rh_2 (**15**), and ginsenoside 20(*R*)-25-OH PPD (**44**) showed substantial activity in all cell lines and were clearly the most effective inhibitors of cancer cell growth and proliferation (Wang *et al.*, 2007). For 20(*R*)-25-OH PPD, the IC_{50} values for most cell lines were in the range of 10–60 μM, which was at least twofold lower than for any of the other ginsenosides tested. Both 20(*S*)-PPD and 20(*R*)-25-OH PPD increased programmed cell death (apoptosis) and cell cycle progression in a dose-dependent manner, whereas these effects were less pronounced for ginsenoside Rh_2. It is notably that 20(*R*)-25-OH PPD had stronger effect than ginsenoside 20(*S*)-Rg_3 (**14**) on cell growth inhibition with IC_{50} values being 5- to 15-fold lower than for ginsenoside Rg_3 (Wang *et al.*, 2007), a compound already being marketed for cancer therapy (Liu and Ye, 2004; Shibata, 2001). Furthermore, ginsenoside Rb_1 (**5**), Rd (**12**), and Rg_3 had little or no effect on cell growth and proliferation. The results from the study of Wang *et al.* (2007) clearly suggest that the structural type of dammarane saponin, the number of sugar moieties, and differences in the substituent groups in the side chain of the aglycone affect the anticancer activity of ginsenosides. This is also in accordance with a study of Popovich and Kitts (2002) who found that both ginsenoside 20(*S*)-Rh_1 (**92**) and Rh_2 with a single sugar moiety had antiproliferative effects on human leukemia cells (THP-1), while 20(*S*)-Rg_3 with two sugar moieties did not have a substantial antiproliferative effect on the cells. The effect on cell proliferation of ginsenoside Rh_2 was furthermore found to be of the same magnitude as the aglycones 20(*S*)-PPD and 20(*S*)-PPT, whereas the inhibitory effect of ginsenoside 20(*S*)-Rh_1 was tenfold less. Furthermore, the presence of sugars in PPD and PPT aglycone structures seems to reduce the potency to induce apoptosis as PPD and PPT was found to induce apoptosis to a higher extent than ginsenoside Rh_2, whereas Rh_1 did not induce apoptosis (Wang *et al.*, 2007). This indicates that the position of sugar moieties at C-3 or C-6 also play a role in the anticancer effect of ginsenosides (Odashima *et al.*, 1985; Popovich and Kitts, 2002; Wang *et al.*, 2007). It has been suggested that the antiproliferative effects of ginsenosides and other bioactivities are dependent

on the ability of ginsenosides to interact with cell membrane functions, and hence their hydrophobic character (Attele *et al.*, 1999; Popovich and Kitts, 2002, 2004b; Wang *et al.*, 2007). This is also in accordance with the structure–activity studies on the antiproliferative effects of ginsenosides and the enhanced activity observed for fatty acid conjugate ginsenosides (Hasegawa *et al.*, 2000, 2002).

Ginsenosides of the 20(*S*)-PPD family is the best-studied group of ginsenosides with regard to anticancer effect of which ginsenoside Rh_2 is one of the best studied ginsenosides. Ginsenoside Rh_2 has been shown to suppress proliferation in a number of human cancer cells, including breast, colorectal, prostate, hepatic, intestinal, melanoma, and animal cell lines (Bae *et al.*, 2004; Kikuchi *et al.*, 1991; Kim *et al.*, 1999c; Lee *et al.*, 1996; Odashima *et al.*, 1985; Oh *et al.*, 1999; Ota *et al.*, 1991; Park *et al.*, 1997; Popowich and Kitts, 2004b; Wang *et al.*, 2007). The antiproliferative effect of Rh_2 appears to be linked to its ability to induce apoptosis and/or by arresting cell cycle progression. For example, Rh_2 has been reported to activate caspase-3 protease, a major proenzyme involved in apoptosis, and to arrest cell cycle progression at the G_1 stage of MCF-7 human breast cancer cells, SK-HEP-1 hepatoma cells, and B16-BL6 melanoma cells (Jin *et al.*, 2000; Lee *et al.*, 1996; Oh *et al.*, 1999; Park *et al.*, 1997) and to inhibit tumor growth *in vivo* of nude mice bearing human ovarian cancer cells (Kikuchi *et al.*, 1991; Nagata *et al.*, 1998; Tode *et al.*, 1993). The antiproliferative effects toward cancer cells of other PPD ginsenosides such as Rg_3, Rg_5 (**72**), Rs_3 (**18**), and Rs_4 (**74**) also seem to be due to their ability to induce apoptosis and to perturb normal cell cycle events (Liu *et al.*, 2000; Kim *et al.*, 1999a,b, Min *et al.*, 2006), although the antiproliferative effects of ginsenosides, including PPD and PPT, toward renal proximal tubule cells may be due to a decrease of c-fos and c-jun gene expression (Han *et al.*, 2002).

Compound K, the metabolized ginsenoside of some of the major ginseng PPD ginsenosides, such as ginsenoside Rb_1, Rb_2, Rc, and Rd, as described in Section II.C, has shown to be cytotoxic and to inhibit proliferation of a number of cancer cells such as B16-BL6 mouse melanoma cells and activated rat hepatic stellate cells in a dose-dependent manner, and to induce morphological changes and apoptotic cell death at concentrations between 24 and 40 μM (Lee *et al.*, 2000; Park *et al.*, 2006; Wakabayashi *et al.*, 1998). So despite that some PPD ginsenosides do not show any significant cytotoxic and antiproliferative effects toward cancer cells even in high concentrations, they are to be considered as important prodrugs due to their metabolization into compound K *in vivo* (see Section II.C).

Much less is known about the cytotoxic and antiproliferative effects of the 20(*S*)-PPT family of ginsenosides. Ginsenoside Rh_1 has been reported to inhibit proliferation of the NIH 3T3 mouse fibroblast cell line but did

not influence growth of B16 melanoma cells. Further, it was shown in the study of Wang *et al.* (2007) that ginsenoside Rh_1 and related PPTs such as Re, Rg_1, and Rg_2 had no or only a minor antiproliferative effect when tested against human cancer cell lines. However, one of the main metabolized ginsenosides after oral administration of PPT-type ginsenosides is 20(*S*)-PPT as described in Section II.C. The antiproliferative and antitumor effect of 20(*S*)-PPT has been clearly demonstrated (Hasegawa *et al.*, 2002) and as in the case of PPD-type ginsenosides, many PPT-type ginsenosides may be considered as important prodrugs due to their metabolization into, for example, 20(*S*)-PPT *in vivo* (see Section II.C).

2. Inhibition of tumor cell invasion and metastasis

The prevention of cancer metastasis is important in order to improve the prognosis of cancer patients. The most characteristic step of cancer metastasis is tumor cell invasion of surrounding tissues and vasculature. Kitagawa and colleagues developed an invasion model for estimating tumor cell invasion ability *in vitro* (Kitagawa *et al.*, 1995; Shinkai *et al.*, 1996). In this model, tumor cells are seeded on a primary cultured monolayer of host cells, such as mesothelial or endothelial cells. The tumor cells penetrate the monolayer and grow and form tumor cell colonies underneath the monolayer. The capacity of penetration of tumor cells *in vitro* corresponds well with that of *in vivo* implantation into test animals. Thus, the *in vitro* model allows studying the effects of substances on tumor cell invasion. By using this *in vitro* model, more than 10 ginsenosides have been tested for the inhibition of tumor cell invasion and metastasis (Kitagawa *et al.*, 1995; Shinkai *et al.*, 1996). Ginsenoside 20(*R*)-Rg_3 (**42**) has been found to be a potent inhibitor of invasion of several tumor cells including heaptanoma (MM1), melanoma (B16FE7), human small lung carcinoma (OC10), and human pancreatic adenocarcinoma (PSN-1) cells whereas ginsenoside Rb_2 (**7**), 20(*R*)-Rg_2 (**111**), and 20(*S*)-Rg_3 (**14**) have only shown little inhibitory activity on tumor cell invasion. Neither ginsenoside Rc (**10**), Re (**84**), Rh_1 (**91**), Rh_2 (**15**), nor 20(*R*)-Rh_1 (**112**) was found to have any effect in the model. As demonstrated by Azuma and Mochizuki (1994) and Mochizuki *et al.* (1995), the enantiomers 20(*S*)- and 20(*R*)-Rg_3 appear to have significant inhibitory effect on tumor metastasis growth as demonstrated *in vitro* on two highly metastatic tumor cells, B16-BL6 melanoma and colon 26-M3.1 carcinoma, and *in vivo* by tumor inoculation of B16-BL6 melanoma in mice. However, the effects of 20(*S*)- and 20(*R*)-Rg_3 against pulmonary metastasis *in vitro* and *in vivo* appear to be different, with 20(*S*)-Rg_3 showing the weakest effect *in vivo* and the strongest effect *in vitro* compared with 20(*R*)-Rg_3 (Azuma and Mochizuki, 1994). Furthermore, as ginsenoside Rh_2 with no inhibitory effect on metastasis but with clear antiproliferative effects toward a wide range of cancer cells, as described in Section V.A.1, indicates that

ginsenosides exert stereo- and structure-specific biological actions suggesting that the mechanisms of their actions on cell growth and invasive locomotion are not necessarily the same. The mode of action of ginsenoside Rg_3 on cell invasion and metastasis has been suggested to be related to its ability to inhibit intracellular Ca^{2+} increase without affecting the protein phosphorylation (Shinkai *et al.*, 1996).

3. Inhibition of tumor angiogenesis

Angiogenesis is a physiological process involving the growth of new blood vessels from preexisting vessels and is considered as a normal process in growth and development, as well as in wound healing (Fan *et al.*, 2006; Folkman, 1995). However, this is also a fundamental step in the transition of tumors from a dormant state to a state where the tumor cells grow rapidly (malignant state). Inhibition of angiogenesis therefore prevents tumor growth, proliferation, and secondary metastasis and is essential in the prevention and treatment of cancer (Fan *et al.*, 2006; Folkman, 1995). Only a few studies on the angiosupressive effects of ginsenosides have been performed and mainly concern the ginsenosides Rb_2 (**7**) and 20(*R*)-Rg_3 (**42**). Sato *et al.* (1994) studied the effect of ginsenoside Rb_2 on angiogenesis and metastasis produced by B16-BL6 melanoma cells in syngeneic mice. Intravenous administration of ginsenoside Rb_2 on day 1, 3, or 7 after tumor inoculation resulted in a remarkable reduction in the number of vessels oriented toward the tumor mass, but did not cause a significant inhibition of tumor growth. The angiosuppressive effect was dose-dependent in the range 10–500 μg/mouse. In contrast, intratumoral or oral administration of ginsenoside Rb_2 caused a marked inhibition of both neovascularization and tumor growth. Ginsenoside Rb_2 did not affect the growth of rat lung endothelial cells but inhibited in a dose-dependent fashion the invasion of rat lung endothelial cells into the reconstituted basement membrane (Matrigel), which is considered to be an essential event in tumor neovascularization. Multiple administrations of ginsenoside Rb_2 after the intravenous inoculation of B16-BL6 melanoma cells resulted in a significant inhibition of lung metastasis as compared with the untreated control. The results suggest that the inhibition of tumor-associated angiogenesis by ginsenoside Rb_2 may partly contribute to the inhibition of lung tumor metastasis. Yue *et al.* (2006) examined the ability of ginsenoside 20(*R*)-Rg_3 to interfere with the various steps of tumor angiogenesis. Ginsenoside 20(*R*)-Rg_3 was, for example, found to inhibit the proliferation of human umbilical Vein endothelial cells (HUVEC) with an IC_{50} of 10 nM. Ginsenoside 20(*R*)-Rg_3 also dose dependently suppressed the capillary tube formation of HUVEC on the Matrigel from 1 to 1000 nM in the presence or absence of 20 ng/ml vascular endothelial growth factor (VEGF). The tumor angiosupressive effects and inhibiting effect of metastasis of ginsenoside Rb_2 and 20(*R*)-Rg_3 are probably related to their inhibitive effect on the release of VEGF from tumor cells.

B. Immunomodulatory effects

The immunomodulatory (immunosuppressive and/or imunostimulatory) activities of ginsenosides are closely related to their anticarcinogenic, anti-inflammatory, and antiallergic activities. The immune responses are controlled by T helper (Th) cells and can broadly be categorized into cellular-mediated responses (cell-mediated immunity) mediated by Th1 cells and macrophages and antibody (antibody-mediated immunity) responses directed by Th2 cells. Th cells are involved in activating and directing other immune cells such as cytotoxic T cells and natural killer (NK) cells, and hence are particularly important in the immune system. The development and differentiation of Th cells are strictly regulated by antigen-presenting dendritic cells (DCs). DCs that generate Th1 responses may be used to prevent or treat pathological conditions that are caused by infections and malignant disorders via secretion of type 1 cytokines such as interferon-γ (IFN-γ) and interleukin-2 (IL-2) to facilitate T-cell-mediated cytotoxicity (Takei *et al.*, 2004). In contrast, DCs that generate Th2 responses may be used to prevent or treat conditions in which Th1 responses are disturbed, for example, contact allergy and autoimmune disorders, by secretion of type 2 cytokines, such as IL-4 and IL-10, to help B cells to secrete protective antibodies (Takei *et al.*, 2004). Therefore, any compound capable of modulating especially macrophage activation and/or function, and hence the production of small and large lymphocytes (e.g., NK, T, and B cells), is important in the prevention and treatment of tumors, infectious agents, and chronic inflammatory diseases (e.g., rheumatoid arthritis, asthma, and atherosclerosis) (Rhule *et al.*, 2006). It is well known that various ginseng species have different immunomodulatory activities and that the main active components are ginsenosides. Yu *et al.* (2005) investigated various PPT-type ginsenosides isolated from *P. ginseng* leaves (20(*S*)-PPT, panaxatriol (20(*S*)-PT), F_1 (**80**), Re (**84**), Rg_1 (**88**), Rh_1 (**92**), and 20(*R*)-Rh_1 (**112**)) for their ability to differentially modulate type 1 and type 2 cytokines production from murine splenocytes. Ginsenosides F_1 and Rg_1 were found to influence type 2 cytokines production through regulation of the expression of, for example, IL-4 while ginsenosides Rh_1 and 20(*R*)-Rh_1 influenced type 1 cytokines production by regulation of the production of IL-12 and the expression of IFN-γ and T-bet, the latter being a specific Th1 transcription factor that is thought to initiate development of Th1 while inhibiting Th2 differentiation (Yu *et al.*, 2005). The results clearly showed that PPT-type ginsenosides have different immunomodulatory effects including both immunostimulatory and immunosuppressive effects. This is also in accordance with a study of Cho *et al.* (2002) who found that the ginsenosides Rb_1 (**5**), Rb_2 (**7**), Re (**84**), and Rg_1 (**88**) differently modulated lymphocyte proliferation induced by T lymphocyte mitogens [e.g., concanavalin A (Con A))] and the B lymphocyte mitogen, lipopolysaccharide

(LPS), as well as cytokine IL-2, a potent trigger of lymphocyte proliferation. Ginsenoside Rb_1 and Re significantly enhanced Con A-induced lymphocyte proliferation whereas Rg_1 did not affect the proliferation. On the other hand, Rb_2 strongly blocked the mitogen-induced lymphocyte proliferation with IC_{50} values between 21.8 and 29 μM and moreover Rb_2 inhibited Con A-stimulated IL-2 production with an IC_{50} of 13.3 μM. This clearly shows that ginsenoside Rb_2 is a very potent immunosuppressive agent. Ginsenosides Rb_2 and Rb_1 had no suppressive effects on the proliferation of IL-2-stimulated CD8+ T cells whereas Re and Rg_1 showed strong suppressive effects with IC_{50} values of 57.5 and 64.7 μM, respectively. These results clearly indicate that ginsenosides may modulate lymphocyte proliferation and that the immunosuppressive effects of ginsenosides toward tumor necrosis factor (TNF)-α cytokine production and T cell proliferation are different. Ginsenosides of *P. notoginseng* and *P. ginseng*, such as Rb_1, Rb_2, and Rg_1, have also shown to strongly suppress the production of TNF-α in macrophages treated with LPS (Cho *et al.*, 2001; Rhule *et al.*, 2006). Furthermore, these ginsenosides also seem to suppress the production of other inflammatory cytokines, such as IL-6 and IL-1β (Rhule *et al.*, 2006), and hence demonstrate that widely distributed ginsenosides possesses anti-inflammatory and immunosuppressive properties *in vitro*.

The activation of macrophages and hence the production of various types of lymphocytes has been shown to be important for the prevention and treatment of tumors and infectious diseases. Ginsenoside Rg_1 has been reported to have mainly immunomodulatory effects that increases both humoral and cell-mediated immunities by enhancing activity of Th cells and NK cells responsive to given antigens (Kenarova *et al.*, 1990; Lee *et al.*, 2004b; Lee and Han, 2006). Furthermore, it has been reported that ginsenoside Re activates microphage function to kill tumor cells (Plohmann *et al.*, 1997) and that ginsenosides from red ginseng in combination with other constituents in red ginseng such as melanoidins (Maillard reaction products) have immunomodulatory effects (Lee *et al.*, 2002b). The immunomodulatory effects of red ginseng that may be effective in defending against infections and tumors seem to be closely related to the ability of the constituents of red ginseng to stimulate the production of the multifunctional cytokine TNF-α by macrophages (Lee *et al.*, 2002b).

Finally, it has been shown that maturation of DCs is promoted by metabolized ginsenosides such as compound K. Takei *et al.* (2004) showed that mature DCs differentiated with compound K enhance the differentiation of naive T cells toward the Th1 type depending on IL-12 secretion, which clearly suggests that compound K has immunostimulatoty effects and that this compound is involved in the cancer preventive effects of ginseng and that compound K may be used on DC-based vaccines for cancer immunotherapy (Takei *et al.*, 2004).

C. Anti-inflammatory and antiallergic effects

The anti-inflammatory and antiallergic properties of ginsenosides are more or less directly linked to their immunostimulatory and anticarcinogenic effects as well as in diseases where inflammatory conditions play a significant role such as in atherosclerosis and neurodegenerative diseases. Allergic diseases of type 1, such as asthma, allergic rhinitis, atopic dermatitis, and food allergy afflict up to 20% of the human population in many countries (Park *et al.*, 2003). Allergen reactivity in these allergic diseases is based on immunoglobin E (IgE)-mediated pharmacological processes in a variety of cell populations, in particular basophils and mast cells. Degranulation of basophils and mast cells with antigen cross-linked IgE releases histamine, prostaglandins, leukotrienes, and cytokines affecting macrophages, lymphocytes, eosinophils, and neutrophils, causing tissue injuries and inflammatory diseases. Cytokines and/or bacterial LPS induce nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression in, for example, macrophages and hence a production of nitric oxide (NO) and prostaglandins (PGs), respectively. A sustained production of NO and PGs has been implicated in the pathogenesis of inflammatory diseases and cancer (Gillis, 1997).

Several ginsenosides have shown to reduce the expression of iNOS and COX-2 and to inhibit the production of NO and PGs in macrophages as well as the inhibition of nuclear factor (NF)-κB transcription factor, which regulates iNOS and COX-2 gene expression. Ginsenoside Rh_1 (**92**) and Rh_2 (**15**) and ginsenoside 20(*S*)-PPT, a metabolite of, for example, Rh_1 or Rg_1 (**88**), and compound K, a metabolite of, for example, ginsenoside Rb_1 (**5**), have been reported to inhibit the production of NO and PGE_2 and to inhibit the activation of NF-κB, in LPS-stimulated murine macrophages (RAW 264.7 cells) (Oh *et al.*, 2004; Park *et al.*, 1996a, 2003, 2004, 2005). The inhibition of NF-κB and COX-2 expression has also been demonstrated for compound K in mouse ear edema induced by the prototype tumor promoter 12-*O*-tetradecanylphorbol-13-acetate (Lee *et al.*, 2005). The results suggest that these ginsenosides can inhibit NO and PGs production by regulation of the signal transduction related to the activation of NF-κB. The anti-inflammatory effects of ginsenosides have also been demonstrated in microglial cells, which are resident macrophages of the CNS (Bae *et al.*, 2006; Wu *et al.*, 2007). Wu *et al.* (2007) found that the PPDs ginsenoside Rb_2 (**7**) and Rd (**12**) and the PPTs ginsenosides Rg_1 and Re (**84**) were able to inhibit LPS-induced NO formation and TNF-α production due to the inhibition of NF-κB in N9 microglial cells. Correspondingly, Bae *et al.* (2006) demonstrated that ginsenosides Rg_3 (**14**) and Rh_2 were able to inhibit the production of NO and the expression of COX-2, TNF-α, and IL-1β in BV-2 microglial cells induced by LPS and IFN-γ, while they increased the expression of the anti-inflammatory

cytokine IL-10. Thus, these ginsenosides may be used in the prevention or treatment of inflammatory diseases, such as allergic inflammation and neurological diseases (e.g., Alzheimer's and Parkinson's diseases) as well as cancer (Bharti and Aggarwal, 2002; Oh *et al.*, 2004; Wu *et al.*, 2007).

The antiallergic effect of ginsenosides has been studied *in vitro* and *in vivo* on rodent peritoneal mast cells and on IgE-induced passive cutaneous anaphylaxis (PCA), the latter being a model for study of type 1 sensitivity reactions. Ginsenosides Rb_1, Rc (**10**), Rd, F_2 (**1**), and Rh_1 have been shown to inhibit histamine and/or leukotriene release from peritoneal mast cells (Choo *et al.*, 2003; Park *et al.*, 2004; Ro *et al.*, 1998), whereas ginsenoside Rh_1, Rh_2, and compound K have been shown to be potent inhibitors of the PCA reaction in rodents (Choo *et al.*, 2003; Park *et al.*, 2003, 2004). The inhibitory activity of Rh_1, Rh_2, and compound K on the PCA reaction was found to be more potent than the commercial antiallergic drug disodium cromoglycate (Choo *et al.*, 2003; Park *et al.*, 2003, 2004). These ginsenosides furthermore showed a membrane stabilizing effect and it has been suggested that this membrane stabilizing effect, which may prevent membrane perturbations, is the main cause for their antiallergic activity (Choo *et al.*, 2003; Park *et al.*, 2003, 2004).

D. Antiatherosclerotic and antihypertensive effect

Many studies have shown that ginseng has a protective effect on the development of atherosclerosis that may lead to myocardial infarction and other cardiovascular diseases. The preventive effects on cardiovascular diseases of ginseng include its potential antihypertensive and antiatherosclerotic effects. Ginsenosides are likely to be responsible for some of these effects as they have been shown to have inhibitory effects on platelet aggregation and to suppress thrombin formation as well as an effect on blood vessel contraction.

One of the major effects of ginsenosides on the cardiovascular system is due to their ability to reduce sympathetic nerve activity and with increase vascular relaxation resulting in lowered blood pressure. This relaxing effect of ginsenosides on the cardiovascular system is partially due to the release of endothelial NO or a labile nitroso compound that liberates NO. NO relaxes blood vessels, in part, by stimulating the production of cyclic GMP in the smooth muscle (Kim *et al.*, 1994). Ginsenosides have depressant action on cardiomyocete contraction that may be mediated, in part, through increased NO production. This is also in accordance with several animal studies in rats that have demonstrated that ginsenosides such as ginsenoside Rb_1 (**5**), Re (**84**), and Rg_1 (**88**) cause endothelium-dependent vascular relaxation and an increase in the tissue content of cyclic GMP in rat aorta, and hence an increased NO production (Chen, 1996; Kim *et al.*, 1994; Kang *et al.*, 1995a,b; Scott *et al.*, 2001). The

improved effect of red ginseng on the vascular endothelial dysfunction in patients with hypertension is possibly due to an increased production of NO (Sung *et al.*, 2000), although it has been shown that ginsenoside-induced vasorelaxation may also involve Ca^{2+} activated K^{+} channels in vascular smooth muscle cells (Li *et al.*, 2001a).

It has also been shown that ginsenosides enhance cerebral blood flow in rats (Kim *et al.*, 2002b) and reduces plasma cholesterol levels and prevent the formation of atheroma in the aorta of rabbits fed on high cholesterol diet (Kang *et al.*, 1995b). This antiatherosclerotic effect of ginsenosides such as ginsenoside Rg_2 (**91**), 20(*S*)-Rg_3 (**14**), and 20(*R*)-Rg_3 (**42**) may be due to their strong inhibitory activity on platelet aggregation (Kimura *et al.*, 1988; Matsuda *et al.*, 1986), regulation of cyclic GMP and cyclic AMP levels, and their inhibitory effect on the conversion of fibrinogen to fibrin (Matsuda *et al.*, 1986; Park *et al.*, 1996b). Ginsenosides have been furthermore shown to be relatively potent platelet-activating factor antagonists (Jung *et al.*, 1998) as well as potential regulators of total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol (see Section V.G) and anti-inflammatory compounds (see Section V.C), which also may play a role in the prevention of the development of atherosclerosis and other cardiovascular diseases (Liu and Xiao, 1992).

E. Antistress activities

Cortisol (corticosterone) is a vital hormone produced by the adrenal cortex and is often referred to as the ''stress hormone'' as it is involved in the response to stress. IL-6 is a multifunctional cytokine produced by a variety of cells, including immune cells (macrophages, T, and B cells), fibroblasts, neurons, and glial cells, in response to infection, trauma, and stress (Gadient and Otten, 1997). It is well known that stress increases cortisol and plasma IL-6 level, and hence these are good antistress markers. For example, the catecholamines, norepinephrine (noradrenaline), and epinephrine (adrenaline) are involved in the increase of plasma IL-6 level induced by CNS stimuli such as stress, whereas cortisol can suppress plasma IL-6 levels (Reichlin, 1993; Takaki *et al.*, 1994). Kim *et al*. demonstrated that several common ginsenosides [e.g., Rc (**10**) and 20(*S*)-Rg_3 (**14**)] as well as compound K (Fig. 1.2), the major intestinal metabolite of PPD ginsenosides, are able to inhibit stress-induced cortisol levels in mice, clearly indicating the potential antistress activity of ginsenosides (Kim *et al.*, 1998a, 2003a). Furthermore, Kim *et al.* (2003b) investigated the effect of ginseng saponins on plasma IL-6 in nonstressed and immobilization-stressed mice. Ginseng total saponins, ginsenosides Rb_2 (**7**), Rd (**12**), and Rg_1 (**88**) administered intraperitoneally attenuated the stress-induced increase in plasma IL-6 level. Intracerebroventricular injection of each ginsenoside did not affect plasma IL-6 level induced by immobilization stress. Ginsenosides Rb_2 and

Rd were shown to significantly decrease IL-6 level in basal state macrophage cells (RAW 264.7) and to decrease the catecholamine (norepinephrine and epinephrine)-induced IL-6 release as well. Ginsenoside Rg_1 effectively blocked epinephrine- but not norepinephrine-induced IL-6 release. It was suggested that the inhibitory action of ginseng saponins against the immobilization stress-induced increase of IL-6 level is partly periphery, mediated by blocking catecholamine-induced increase of IL-6 level in macrophages rather than in CNS (Kim *et al.*, 2003b). The antistress effect of ginseng total saponin and ginsenoside Rg_3 and Rb_1 toward immobilization stress has also been demonstrated by investigating the brain level of endogenous polyamines (Lee *et al.*, 2006b), which are essential for cellular growth, proliferation, regeneration, and differentiation of the brain, and are also well-known stress stimuli markers. In this study, it was found that ginsenoside Rg_3 and Rb_1 blocked the activity of the enzyme ornithine decarboxylase, involved in the metabolism and catabolism of polyamines, and attenuating the levels of the polyamine putrescine. Thus, ginsenoside Rg_3 and Rb_1 may play a neuroprotective role in the immobilization-stressed brain (Lee *et al.*, 2006b).

F. Effects on the CNS

Various ginseng species have been shown to have both stimulatory and inhibitory effects on the CNS, and may modulate neurotransmission. Ginsenosides, and in particular ginsenoside Rb_1 (**5**), Rg_1 (**84**), and Re (**88**), seem to play a major role in these effects (Attele *et al.*, 1999; Rausch *et al.*, 2006).

1. Memory, learning, and neuroprotection

Central cholinergic systems have been implicated in mediation learning and memory processes (Perry, 1986). Because scopolamine is a cholinergic receptor antagonist, the performance impaired by scopolamine may result in a dysfunction of central cholinergic mechanisms, and hence may result in memory deficits (Yamaguchi *et al.*, 1995, 1997). Results from animal studies have shown that Rb_1, Rg_1, and Re prevent scopolamine-induced memory deficits (Benishin *et al.*, 1991; Yamaguchi *et al.*, 1995, 1996b). Yamaguchi *et al.* (1996a) showed that sugar moieties at C-6 and C-20 as in ginsenoside Rg_1 and Re are important for the ameliorating effect of ginsenosides on the performance impaired by scopolamine in rats. These ameliorative effects of Rg_1 and Re have been shown to be closely related to an increase of choline acetyltransferase activity in the medial septum of young and aged rats (Yamaguchi *et al.*, 1997). Ginsenoside Rb_1 and Rg_1 have also shown to be capable of partially reversing scopolamine-induced amnesia by improving cholinergic activity and having partial neurotrophic and neuroprotective effects (Radad *et al.*, 2004b).

Furthermore, it has been demonstrated that ginsenoside Rb_1 increases the uptake of choline in central cholinergic nerve endings (Benishin, 1992), and facilitates the release of acetylcholine from hippocampal slices (Benishin *et al.*, 1991; Lee *et al.*, 2001). These results clearly suggest that ginsenosides may facilitate learning and improve the basic synaptic transmission as well as nerve growth.

Ginsenosides also seem to have a neuroprotective effect where nerve growth also plays an important role. Ginsenosides have in several *in vitro* studies shown to increase survival of cultured neuronal cells and to enhance the outgrowth of their neuritis. For example, ginsenoside Rb_1 has shown to increase the neurite outgrowth of cultured cerebral cortex neurons (Kim *et al.*, 1998b; Sugaya *et al.*, 1988) and to stimulate neurite outgrowth of PC12 cells in the absence of nerve growth factor (Rudakewich *et al.*, 2001). The ability of ginsenosides to regenerate neuronal networks has also been demonstrated in SK-N-SH cells for PPD-type saponins such as ginsenoside Rb_1 and Rb_3 (**9**) and notoginsenoside R_4 (**32**) and Fa (**26**), while PPT- (**84**, **87**, **88**, **91**, **102**, **103**), ocotillol- (**148**, **150**–**153**, **155**), and oleanolic acid type (**162**, **167**) saponins had no effect on neurite outgrowth (Tohda *et al.*, 2002). This clearly indicates that some ginsenosides are able to extend axons and dendrites in neurons that may compensate for and repair damaged networks in, for example, the dementia brain. Furthermore, it has been shown that ginsenosides Rb_1 and Rg_1 protect neurons from excitotoxicity induced by, for example, glutamate and oxidative stress caused by hydrogen peroxide and promote neurite lengths and neurite numbers of dopaminergic cells after exposure to 1-methyl-4-phenylpyridinium (Radad *et al.*, 2004a,b), which is an active metabolite selectively toxic to dopaminergic neurons *in vitro*. Interestingly, ginsenosides Rb_1 and Rg_1 also seem to be able to reverse the cell death caused by 1-methyl-4-phenylpyridinium (Rudakewich *et al.*, 2001). These beneficial effects of ginsenosides, and in particular ginsenoside Rb_1 and Rg_1, are primarily mediated through scavenging of free radicals and improving energy metabolism (Radad *et al.*, 2004b) as well as their ability to block Ca^{2+} over-influx into neuronal cells and inhibit Na^+ channel activity (Kim *et al.*, 1998b, 2002a, 2005b; Lee *et al.*, 2006d; Radad *et al.*, 2004b). In particular, Ca^{2+} loading exceeding the capacity of Ca^{2+} regulating mechanisms could activate several cell death-related genes and pathways leading to apoptosis and cell death (Radad *et al.*, 2006; Rausch *et al.*, 2006; Said *et al.*, 2000).

Glutamate that is a major neurotransmitter in the mammalian nervous system not only plays a role in the development of the brain and learning but is also a potent neurotoxin when present in excess at synapses (Plaitakis and Shashidharan, 2000; Rausch *et al.*, 2006). Glutamate excitotoxicity has been shown to contribute to neuronal degeneration in acute conditions such as stroke, epilepsy, hypoglycemia, and chronic

neurodegerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases (Radad *et al.*, 2006; Rausch *et al.*, 2006). Although the pathogenesis of glutamate toxicity is not fully understood, it appears that ginsenosides and ginseng plants may have beneficial effects toward the above-mentioned diseases due to their neurotrophic and neuroprotective effects (van Kampen *et al.*, 2003). Also the anti-inflammatory effects of ginsenosides seem to play an important role in neurodegenerative diseases such as Alzheimer's disease (Joo *et al.*, 2005), which is primarily caused by cell death due to chronic inflammation and cell stress.

2. Neurotransmitter modulation

Ginsenosides have in *in vitro* studies shown that they may modulate nerve transmission by decreasing or even increasing the availability of neurotransmitters (Kimura *et al.*, 1994; Tsang *et al.*, 1985; Xue *et al.*, 2006). Xue *et al.* (2006) demonstrated that both ginsenoside Rb_1 (**5**) and Rg_1 (**88**) increased neurotransmitter release in undifferentiated and differentiated PC12 cells. The promoted neurotransmitter release of ginsenoside Rb_1 was found to be due to an increasing phosphorylation of synapsin phosphoproteins through the cyclic AMP-dependent protein kinase pathway, whereas the similar effects observed for ginsenoside Rg_1 were independent of the phosphorylation of the synapsins. On the other hand, Tsang *et al.* (1985) demonstrated that ginseng extracts, and hence also ginsenosides, concentration dependently inhibits the uptake of γ-aminobutyric acid (GABA), glutamate, dopamine, norandrenaline, and serotonin in rat brain synaptosomes. GABA is an inhibitory neurotransmitter in mammalian CNS and it has been shown that ginsenosides compete with agonists for binding to $GABA_A$ and $GABA_B$ receptors and hence modulate neurotransmission (Kimura *et al.*, 1994). The regulation of GABAergic neurotransmission may be important in the action of ginsenosides.

G. Effect on metabolic processes

The effects of ginseng and ginsenosides on metabolic processes are in particular due to their ability to activate peroxiome proliferator-activated receptors (PPARs) that are transcription factors and part of a large family consisting of steroid/thyroid hormone receptors. Ginsenosides have been shown to change the expression of PPAR α and γ. PPAR α is expressed in the kidney, the liver, the muscles, as well as in adipose tissues and activation results in upregulation of genes involved in the triglyceride-lowering effects through transcriptional activation of apolipoprotein (apo) C-III and lipoprotein lipase (Auwerx *et al.*, 1996; Hertz *et al.*, 1995) as well as fatty acid β-oxidation (Desvergne and Wahli, 1999). PPAR α has also been demonstrated to increase the concentration of HDL levels

through induction of apo A-I and -II gene expression in humans (Staels and Auwerx, 1998). Thus, PPAR α is an important regulator of lipid metabolism and hence is an important target for the prevention and treatment of lipid disorders, cardiovascular diseases, obesity, and diabetes. PPAR γ is primarily expressed in adipose tissue and is also involved in lipid metabolism and plays an important role in insulin resistance and hence is an important molecular target for particular obesity and diabetes as discussed in more detail in Section V.H. It has been shown that ginseng and a mixture of ginsenosides obtained by extraction are able to regulate total cholesterol, triglycerides, and HDL cholesterol *in vitro* and *in vivo* and hence are able to regulate lipid metabolism through activation of PPAR α (Yoon *et al.*, 2003). This is also in accordance with another study where ginsenoside Rf (**86**) was identified as one of the major active components that regulate lipoprotein metabolism by interacting with PPAR α acting as a PPAR α antagonist (Lee *et al.*, 2006a). Ginsenoside Rf may therefore have therapeutic applications in relation to the prevention and treatment of various diseases, including lipid disorders, cardiovascular diseases, obesity, and diabetes.

H. Antidiabetic effects

According to the World Health Organization (WHO), more than 180 million people suffer from diabetes and more than 90% of these have type 2 diabetes (T2D) and this number is likely to be doubled by 2030 due to the increasing prevalence of obesity (Wild *et al.*, 2004). T2D is characterized by insulin resistance, low fasting glucose levels (hyperglycemia), and high concentrations of triglycerides in the blood and hence also the risk for especially cardiovascular diseases. In recent years, clinical trials and animal experiments have demonstrated that ginseng and ginsenosides are able to lower blood glucose (Attele *et al.*, 2002; Cho *et al.*, 2006; Chung *et al.*, 2001; Lee *et al.*, 2006c; Sotaniemi *et al.*, 1995; Vuksan *et al.*, 2000; 2001; Xie *et al.*, 2002a,b, 2004, 2005a; Yun *et al.*, 2004), to increase insulin sensitivity (Attele *et al.*, 2002; Han *et al.*, 2006; Shang *et al.*, 2007; Yun *et al.*, 2004), and to regulate lipid metabolism (Han *et al.*, 2006; Yoon *et al.*, 2003; Yun *et al.*, 2004), and even to reduce body weight (Attele *et al.*, 2002; Han *et al.*, 2005; Kim *et al.*, 2005a; Sotaniemi *et al.*, 1995; Xie *et al.*, 2002a,b, 2005a; Yun *et al.*, 2004). Hence, ginsenosides may be used for the prevention and treatment of T2D. However, antidiabetic effects have only been demonstrated for a few specific ginsenosides including Rb_1 (**5**), Re (**84**), Rh_2 (**15**), and the aglycone 20(*S*)-PPT. Antidiabetic effect has been demonstrated for ginsenoside Re in ob/ob diabetic mice (Attele *et al.*, 2002) and ginsenoside Rh_2 has been shown to increase insulin secretion and to lower plasma glucose in Wistar rats (Lee *et al.*, 2006c). Furthermore, it has been shown that the antidiabetic

effects of ginsenoside Rb_1 (Shang *et al.*, 2007) and 20(*S*)-PPT (Han *et al.*, 2006) are probably related to their ability to activate peroxisome PPAR γ. PPAR γ is a member of the nuclear receptor of ligand-activated transcription factors that regulate the expression of key genes involved in lipid and glucose metabolism and adipocyte differentiation (Desvergne and Wahli, 1999; Tobin and Freeman, 2006). PPAR γ is primarily expressed in adipose tissue, and activation of PPAR γ improves the ability of adipocytes to store lipids, thereby reducing lipotoxicity in muscle and liver (Bays *et al.*, 2004). The genes expressed by activation of PPAR γ depend largely on the type of activating ligand present as these recruit a different set of cofactors (Semple *et al.*, 2006). Hence, the transcriptional response of the PPAR γ can result in either cofactors that lead to increased lipid storage and decreased energy expenditure (e.g., transcriptional factor-2 (TIF-2)) or recruitment of cofactors that lead to increased insulin-stimulated glucose uptake and positive regulation of glucose metabolism and energy expenditure (e.g., the steroid receptor coactivator-1) (Burgermeister *et al.*, 2006; Schupp *et al.*, 2005). In general, the activation of PPAR γ causes body-wide lipid repartitioning by increasing the triglyceride content in adipose tissue and lowering free fatty acids triglycerides in circulation, liver, and muscle, thereby improving insulin sensitivity (Han *et al.*, 2006). The thiazolidinediones (TZDs) are full PPAR γ agonist often prescribed in the clinical treatment of T2D, as insulin sensitizing drugs. However, severe side effects such as edema development, weight gain, heart enlargements, and hepatoxicity are seen in relation to the use of TZDs (Larsen *et al.*, 2003; Pan *et al.*, 2006). These undesirable side effects are believed to be caused by the fact that TZDs are full PPAR γ agonists (Barroso *et al.*, 1999). Partial PPAR γ agonists are ligands that activate PPAR γ in a more selective way than full agonists and furthermore appear to promote more beneficial recruitment of cofactors, and hence are not believed to have the same side effects as full agonists. The potential antidiabetic effects of ginsenoside Rb_1 and 20(*S*)-PPT have been shown to be due to promotion of adipocyte differentiation via PPAR γ activation and increased activation of glucose transporter 4 (GLUT4) associated with insulin sensitivity in adipocyte tissue (Han *et al.*, 2006; Shang *et al.*, 2007). Therefore, Rb_1 and 20(*S*)-PPT seem to improve insulin resistance by reducing lipotoxicity in muscle and liver through increasing ability to store lipids in adipocytes and enhancing insulin sensitivity through increase of GLUT4 expression in adipocyte. However, further investigations of the antidiabetic effects of Rb_1 and 20(*S*)-PPT are needed to classify these ginsenosides as potential antidiabetic agents with no side effects.

Some investigations have indicated that the hypoglycemic effect of ginseng products depends both on the ginsenoside content and on the

profile, clearly indicating that the antidiabetic effect of ginseng depends on the concentration of single ginsenosides and that not all ginsenosides possesses antidiabetic effects (Sievenpiper *et al.*, 2003a,b, 2004; Vuksan *et al.*, 2000, 2001).

VI. CONCLUSION

Ginsenosides are a unique group of compounds that have demonstrated a multiple of pharmacological effects including anticarcinogenic, immunostimulatory, antiatherosclerotic, antihypertensive, and antidiabetic effects as well as effects on the CNS and stress. The various pharmacological effects of ginsenosides are probably due to their resemblance in chemical structure/nature with triterpenoid steroid hormones and their amphiphilic nature being able to intercalate into plasma membranes (Attele *et al.*, 1999). Their amphiphilic properties of ginsenosides can lead to changes in the membrane fluidity, and thus affect membrane function, elicitating a cellular response. Moreover, like steroid hormones, they are lipid-soluble signaling molecules that can traverse the plasma membrane and interact with membrane anchored receptors, and ion channels as well as nuclear receptors initiating genomic effects (Attele *et al.*, 1999). Pharmacokinetic studies have also confirmed that ginsenosides are bioavailable and that to some extent they are metabolized in the gastrointestinal tract to deglycosylated ginsenosides whose ability to traverse membranes are even better than intact ginsenosides, which further add to the multifunctional pharmacological activities of this group of compounds. Finally, the diversity in ginsenoside structures, including structural isomerism (Fig. 1.1), furthermore contributes to the multifunctional pharmacological effects of ginsenosides. Certainly, the argument can be raised that evidence for most pharmacological effects of ginsenosides has been obtained from *in vitro* studies, many of which have not been confirmed *in vivo* and certainly not in humans with the exception of ginsenoside 20(*S*)-Rg_3 (**14**) and 20(*R*)-Rg_3 (**42**), which is used in anticancer treatment (Shibata, 2001). Nevertheless, the demonstration of the health effects of ginseng and ginseng preparations in epidemiological and cohort studies and the fact that ginsensoides are bioavailable and can initiate effects at the plasma membrane by interacting with multireceptor systems and that ginsenosides are able to traverse the membrane and produce genomic effects clearly indicates that ginsenosides have potential health effects in humans.

The pharmacological effects of ginseng and ginseng preparations and hence their quality are clearly dependent on their content of ginsenosides.

Consequently, many types of analytical methods have been developed for the identification and quantification of ginsenosides in raw materials and preparations. In particular, analytical HPLC combined with UV, evaporate light scattering, and/or MS detection have shown to be useful for the analysis of nearly all types of ginsenosides. So far, focus have been primarily on the quantification of major ginsenosides in roots from *P. ginseng*, *P. quinquefolium*, and *P. notoginseng* such as ginsenoside Rb_1 (**5**), Rb_2 (**7**), Rc (**10**), Rd (**12**), Re (**84**), Rf (**86**), Rg_1 (**88**), and Rg_2 (**91**) as these compounds are the most common ginsenosides and have shown a multifunctional pharmacological effects. However, the pharmacological effects of many ginsenosides and their potential metabolized products have so far not been investigated that also include many of the new types of ginsenosides isolated in recent years from the aerial parts of several ginseng species (Table 1.1). Therefore, many interesting pharmacological effects of ginsenosides are yet to be discovered and perhaps this may reveal further and new insight in the pharmacological effects of ginsenosides and their potential health effects that in the end may result in new ginseng herbal remedies or medicinal products.

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