

## DOCTORAL THESIS

### The molecular mechanism of 20(S)-Protopanaxdiol, a metabolite of ginseng, induced hepatocellular carcinoma HepG2 cell apoptosis and new ginsenosides from the root of panax ginseng C. A. Meyer

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**The Molecular Mechanism of 20(S)-Protopanaxdiol, A  
Metabolite of Ginseng, Induced Hepatocellular Carcinoma  
HepG2 Cell Apoptosis and New Ginsenosides from the Root of  
*Panax ginseng* C. A. Meyer**

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for the degree of Doctor of Philosophy**

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## ABSTRACT

Ginseng, clinically used for thousands of years in China, is now one of the most popular phytomedicines worldwide. Ginsenosides are widely accepted as pharmacologically active components of ginseng from which more than 80 protopanaxadiol (PPD) and protopanaxatriol (PPT) type ginsenosides have been identified and characterized. Upon oral consumption, PPD and PPT type ginsenosides were partly transformed into their respective end metabolites, PPD and PPT. It has been reported that PPD has cytotoxicity on various cancer cell lines, but the molecular mechanism of the action is largely undefined. In this study, we showed the chemical diversity and complexity of ginsenosides in the root of *P. ginseng* and the molecular mechanism of PPD-induced HepG2 cell apoptosis.

Chapter 3 demonstrates the phytochemical studies on the n-butanol fraction of ethanol extracts of root of *Panax ginseng* which led to the isolation of 41 compounds. Six new PPT-type ginsenosides (ginsenosides Re<sub>1-6</sub>, **1-6**), six new PPD-type ginsenosides (ginsenosides Ra<sub>4-9</sub>, **7-12**) and a new phenolic glycoside [4-(1-*E*-propenyl)-2-methoxyphenyl 1-*O*- $\beta$ -D-apiofuranosyl (1 $\rightarrow$ 6)- $\beta$ -D-glucopyranoside, **13**] were determined together with twenty eight known compounds including ten PPT-type ginsenosides [ginsenosides Re (**14**), Rf (**15**), Rg<sub>1</sub> (**16**), Rg<sub>2</sub> (**17**), 20-gluco-ginsenoside Rf (**18**), koryoginsenoside R<sub>1</sub> (**19**), notoginsenoside R<sub>1</sub> (**20**), R<sub>2</sub> (**21**), N (**22**), and yesanchinoside D (**23**)], fourteen PPD-type ginsenosides [ginsenoside Ra<sub>1</sub> (**24**), Ra<sub>2</sub> (**25**), Rb<sub>1</sub> (**26**), Rb<sub>2</sub> (**27**), Rb<sub>3</sub> (**28**), Rc (**29**), Rd (**30**), Rs<sub>1</sub> (**31**), Rs<sub>2</sub> (**32**), malonyl-ginsenoside Rb<sub>1</sub> (**33**), gypenoside XVII (**34**), pseudoginsenoside RC1 (**35**), quinquenoside R<sub>1</sub> (**36**), and vinaginsenoside R<sub>16</sub> (**37**)], an oleanane-type triterpenoid [ginsenoside Ro (**38**)], two phenolic glycosides [4-allyl-2-methoxyphenyl 6-*O*- $\beta$ -D-apiosyl(1  $\rightarrow$  6)- $\beta$ -D-glucoside

(**39**) and eugenol 4-*O*-rhamnosyl (1 → 2) glucoside (**40**)] and a purin glycoside [adenosine (**41**)]. Their structures were elucidated by using spectroscopic methods including 1D and 2D-NMR, high resolution MS and chemical transformation. The unusual  $\alpha$ -D-glucopyranosyl-(1→3)- $\beta$ -D-glucopyranosyl side chain (as found in compounds **1** and **2**) and the eugenol derivatives (compounds **13**, **39** and **40**) were reported in the genus *Panax* for the first time.

Studies in Chapter 4 showed that PPD inhibited the cell growth and induced apoptosis in human hepatocarcinoma HepG2 cells. PPD induced the intrinsic as well as the extrinsic apoptotic pathways. PPD treated cells showed a massive cytoplasmic vacuolization and a dramatic change of Endoplasmic reticulum (ER) morphology. The occurrence of ER stress was supported by the observations that PPD treatment affected the ER stress-associated genes and proteins expression. PPD induced the phosphorylation of PERK and eIF2 $\alpha$ , the splicing of XBP1 mRNA, and the cleavage of AFT6, suggested that PPD activated the three previously described arms of unfolded protein response (UPR). These observations suggest that PPD-induced apoptosis involves an ER stress response. Knockdown of one of the three UPR limbs by specific siRNA did not affect the PPD-induced apoptosis but transfection of cells with CHOP siRNA significantly reduced the PPD-mediated apoptosis. Western blotting analysis showed that PPD-stimulated downregulation of Bcl-2 protein, increase of DR5 protein, activation of caspase-8 and cleavage of PARP were significantly inhibited in CHOP knockdown cells. Taken together, these observations support the hypothesis that PPD induces HepG2 cell apoptosis through ER stress pathway which may provide a new target pathway for cancer chemotherapy using ginsenosides.

In conclusion, this study provides a new insight to the role of ER stress in PPD- induced HepG2 cell apoptosis and the exploitation of these findings may facilitate the establishment a new molecular target of PPD and PPD-type ginsenosides and lead to the development of new PPD analogs for cancer chemotherapy. As for phytochemical study of ginseng, 13 new compounds have been isolated and characterized. The existence of these new components illustrates the chemical diversity and complexity of saponins in *P. ginseng*.

## TABLE OF CONTENTS

DECLARATION.....	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vi
LIST OF FIGURES.....	xi
LIST OF TABLES.....	xix
LIST OF ABBREVIATIONS.....	xx
<b>CHAPTER ONE: GENERAL INTRODUCTION.....</b>	<b>1</b>
1.1. <i>Panax ginseng</i> .....	1
1.1.1. Phytochemical Studies on Ginseng.....	2
1.1.1.1. Saponins from the Root of <i>P. ginseng</i> .....	2
1.1.1.2. Saponins from the Red Ginseng, the Leaves, Flower Buds, and Fruits of <i>P. ginseng</i> .....	4
1.1.1.3. Other Constituents of <i>P. ginseng</i> .....	5
1.1.1.4. Metabolism Properties of Ginsenosides .....	6
1.1.2. Pharmacological Action of Ginseng and Ginsenosides.....	8
1.1.2.1. Anti-tumor Effects.....	8
1.1.2.1.1. Inhibition of proliferation.....	9
1.1.2.1.2. Induction of Programmed Cell Death.....	10
1.1.2.1.3. Prevention of Metastasis and Invasion.....	11
1.1.2.1.4. Effects on Multi-drug Resistance.....	12
1.1.2.1.5. Effects on angiogenesis.....	12
1.1.2.2. Cardiovascular Protective Effects.....	14

1.1.2.3. Immunomodulatory Effects.....	15
1.1.2.4. Effects on the Central Nervous System (CNS) .....	16
1.1.2.5. Anti-diabetic Actions.....	18
1.2. Endoplasmic Reticulum and Endoplasmic Reticulum Stress.....	19
1.2.1. Endoplasmic Reticulum.....	19
1.2.1.1. Structure of ER.....	20
1.2.1.2. Protein Folding and Quality Control in ER.....	21
1.2.1.3. ER-Associated Degradation (ERAD) .....	23
1.2.2. Endoplasmic Reticulum Stress .....	24
1.2.2.1. The Unfolded Protein Response.....	25
1.2.2.1.1. A common role for BiP.....	26
1.2.2.1.2. The PERK Pathway.....	27
1.2.2.1.3. The IRE1 Pathway.....	28
1.2.2.1.4. The ATF6 Pathway.....	29
1.2.2.2. ER Stress-associated Apoptosis.....	29
1.2.2.2.1. CHOP.....	31
1.2.2.2.2. Death Receptors .....	32
1.2.2.2.3. IRE1 $\alpha$ /TRAF2/ASK1/JNK.....	32
1.2.2.2.4. The Bcl-2 Family Proteins.....	33
1.2.2.2.5. Caspases 12/4 .....	35
1.3. Aims of Study.....	35
<b>CHAPTER TWO: MATERIALS AND METHODS.....</b>	<b>37</b>
2.1. General .....	37
2.2. Plant Materials .....	38
2.3. Extraction and Isolation.....	38

2.4. Determination of the Absolute Configuration of Sugars.....	43
2.5. Alkaline Hydrolysis of Compounds 6–12 .....	44
2.6. Preparation of 20( <i>S</i> )-Protopanaxadiol and 20( <i>S</i> )-Protopanaxatriol.....	45
2.7. Chemicals and antibodies for cell biological experiments.....	46
2.8. Cell Line and Cell Culture.....	48
2.9. Cell Growth Assay .....	48
2.10. Apoptosis assay .....	48
2.10.1. Propidium Iodide Method.....	48
2.10.2. DAPI Staining.....	49
2.11. Mitochondrial Transmembrane Potential ( $\Delta\psi_m$ ) .....	49
2.12. Western Blotting.....	50
2.13. Immunofluorescence Staining.....	50
2.14. Live-cell ER and Golgi apparatus labeling .....	51
2.15. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) .....	51
2.16. Transient Transfection of siRNA.....	52
2.17. Intracellular Ca <sup>2+</sup> Measurements.....	54
2.18. Proteomic analysis.....	54
2.18.1. Sample Preparation.....	54
2.18.2. Two-Dimensional Gel Electrophoresis.....	55
2.18.3. Silver staining.....	55
2.18.4. Protein Pattern Differential Analysis.....	56
2.18.5. In-Gel Digestion .....	56
2.18.6. Protein Identification by MS.....	57
2.19. Data analysis.....	57



### **CHAPTER THREE: NEW GINSENOSES FROM THE ROOT OF *PANAX***

<b><i>GINSENG</i> C. A. MEYER</b> .....	58
3.1. Introduction.....	58
3.2. Results and Discussion.....	60
3.2.1. Structure Elucidation of New Compounds from the Root of <i>Panax Ginseng</i>	
C.A. Meyer.....	60
3.2.1.1. New Protopanaxatriol-type Ginsenosides .....	60
3.2.1.2. New Protopanaxadiol-type Ginsenosides.....	76
3.2.1.3. New Phenolic Glycoside.....	93
3.2.2. Identification of Known Compounds from the Root of <i>Panax Ginseng</i>	
C.A. Meyer.....	95
3.2.1.1. Protopanaxatriol Type Ginsenosides .....	95
3.2.1.2. Protopanaxadiol Type Ginsenosides.....	101
3.2.1.3. Oleanane Type Ginsenoside.....	109
3.2.1.4. Phenolic Glycosides.....	110
3.2.1.5. Purin Glycoside.....	112

### **CHAPTER FOUR: 20(S)-PROTOPANAXADIOL, A METABOLITE OF GINSENG, INDUCED HUMAN HEPATOCELLULAR CARCINOMA HEPG2 CELL APOPTOSIS THROUGH ENDOPLASMIC RETICULUM STRESS**.....

4.1. Introduction .....	113
4.2. Results .....	117
4.2.1. PPD Inhibited Cell Growth and Induced Apoptosis in HepG2 Cells.....	117
4.2.2. Results of Proteomic Analysis .....	120
4.2.3. PPD Induced Cytoplasmic Vacuolization and Endoplasmic Reticulum	

Patches.....	124
4.2.4. PPD Induced ER stress and UPR.....	130
4.2.5. PPD-induced Mitochondria-mediated Intrinsic Apoptotic Pathway.....	134
4.2.6. PPD Up-regulated Death Receptor DR5 Expression and Activated the Extrinsic Apoptotic Pathway.....	139
4.2.7. PPD Activated ERK and p38 MAPK Pathway but not JNK Pathway.....	141
4.2.8. The Role of three UPR Limbs in PPD-induced Apoptosis.....	144
4.2.9. CHOP was Essential for PPD-induced Apoptosis.....	146
4.2.10. PPD Raises Cytosol Ca <sup>2+</sup> Level.....	149
4.2.11. PPD Induces Downregulation of Calnexin and Calreticulin.....	151
4.3. Discussion.....	153
<b>CHAPTER FIVE: GENERAL DISCUSSION AND CONCLUSION.....</b>	<b>158</b>
5.1 Summary and Conclusion of the Studies.....	158
5.2 New directions from research.....	166
Appendix: NMR, HRMS, UV, and IR spectra of new compounds .....	167
References .....	223
PUBLICATIONS.....	264
CURRICULUM VITAE.....	266