UNIVERSITI PUTRA MALAYSIA

PREPARATION OF BETULINIC ACID DERIVATIVES

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By

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PREPARATIONS OF BETULINIC ACID DERIVATIVES

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February 2004

Chairman : Associate Professor Faujan Bin Hj Ahmad, Ph.D.

Faculty : Science and Environmental Studies

Betulinic acid, 3β-hydroxy-lup-20(29)-ene-28-oic acid is a pentacyclic triterpene. Esterification reactions of betulinic acid were investigated. Betulinic acid esters such as 3-*O*-succinyl-betulinic acid, 3-*O*-(2′,2′-dimethylsuccinyl)-betulinic acid, 3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid and 3-*O*-glutaryl-betulinic acid were prepared. Their structures were determined by spectroscopic methods.

Optimization studies on the esterification reactions were carried out for betulinic acid with succinic anhydride and with 2′,2′-dimethylsuccinic acid only. The effects of varying parameters such as the mole ratio, temperature, time course and mole of catalyst were investigated.

Generally, the optimal conditions for preparing 3-*O*-succinyl-betulinic acid was obtained by mixing betulinic acid with succinic anhydride in a 1:8 ratio and refluxing in 0.124 mole of pyridine for 24 hours at a temperature of 115°C ; the optimal conditions for the preparation of 3-*O*-(2′,2′-dimethylsuccinyl)-betulinic

acid was obtained by refluxing betulinic acid and 2′,2′-dimethylsuccinic acid in a 1:6 ratio with the presence of 0.124 mole of pyridine for 24 hours at a temperature of 85°C. The percentage conversions as detected by gas chromatography analysis were 53.5 and 56.2%, respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai Memenuhi keperluan untuk ijazah Master Sains

PENYEDIAAN TERBITAN ASID BETULINIK

Oleh

MOHD TAJUDIN BIN MOHD ALI

Februari 2004

Chairman : Professor Madya Faujan Bin Hj Ahmad, Ph.D.

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Asid betulinik, asid 3β-hydroksi-lup-20(29)-ene-28-oik adalah terpena daripada kumpulan pentasiklik triterpena. Dalam kajian ini, tindakbalas pengesteran asid betulinik telah dikaji. Ester bagi asid betulinik seperti 3-*O*-suksinil-asid betulinik, 3-*O*-(2′,2′-dimetilsuksinil)-asid betulinik, 3-*O*-(3′,3′-dimetilsuksinil)-asid betulinik dan 3-*O*-glutaril-asid betulinik telah disediakan melalui tindakbalas pengesteran. Struktur bagi ester tersebut ditentukan melalui kaedah spektroskopi.

Kajian pengoptimuman bagi tindakbalas pengesteran telah dijalankan hanya bagi tindakbalas antara asid betulinik dengan suksinik anhidrida dan asid 2′,2′-dimetilsuksinik sahaja. Beberapa parameter seperti seperti nisbah bahan tindakbalas, suhu, masa, dan jumlah mol pemangkin telah dikaji.

Secara umumnya, penyediaan 3-*O*-suksinil-asid betulinik diperolehi melalui kaedah refluk bagi campuran asid betulinik dan suksinik anhidrida dengan nisbah 1:8 di dalam piridina sebanyak 0.124 mol, selama 24 jam dan pada suhu 115°C, manakala

115°C, manakala ester 3-*O*-(2′,2′-dimetilsuksinil)-asid betulinik disediakan melalui kaedah yang sama dengan nisbah bahan tindakbalas antara asid betulinik dan asid 2′,2′-dimetilsuksinik adalah 1:6 di dalam piridina sebanyak 0.124 mol, selama 24 jam dan pada suhu 85°C. Peratus penghasilan ester masing-masing melalui analisis kromatografi gas adalah 53.5% and 56.2%.

ACKNOWLEDGEMENTS

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TLC	thin layer chromatography
FT-IR	fourier transform infra red
GC	gas chromatography
NMR	nuclear magnetic resonance
HPLC	high performance liquid chromatography
TI	therapeutic index
R_f	retention time

CHAPTER 1

INTRODUCTION

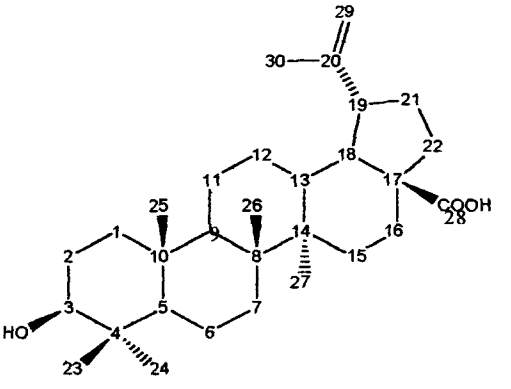
The lupane type pentacyclic triterpene betulinic acid (1), 3-β-hydroxy-lup-20(29)-ene-28-oic, was isolated from higher plants . It has a molecular of formula $C_{30}H_{48}O_3$ with three active sites at C-3, C-20 and C-28 . This natural product can be chemically derived from betulin (2), a substance found in abundance in the white birch (*Betula alba*) (Pisha *et al.*,1995).

Betulinic acid has been reported to possess antitumor activity toward cultured human melanoma cell in vitro and vivo model. (Kim, *et al.*, 1998). Other biological activities reported for betulinic acid include anti-inflammatory activity (Recio *et al.*, 1995) , inhibation of phorbol ester-induced epidermal ornithine decarboxylase accumulation in the mouse-ear with subsequent inhibation of the carcinogenic response in the two- stage mouse –skin model (Yasukawa *et al.*, 1991) , allelopathic activity on monocotyledon species *Hordeum vulgara* and *Triticum aestivum* and dicotyledon species *Lactuca sativa* and *Lepidium sativum* (Macias *et al.*,1994). It also has a potential to block the function of DNA polymerase β selectively, so is able to act as an antitumor agent. (Ma *et al.*,1999).

Betulinic acid derivatives can be prepared by a simple modifications of the betulinic acid structure to produce a number of derivatives. The use of chemical catalyst such as mineral acid, acid chloride, anhydride, acetate,

amine or inorganic catalysts for the preparation of betulinic acid derivatives was reported (Hashimoto, *et al.*,1997 , Fujioka, *et al.*,1994). They use pyridine as the solvent, since betulinic acid is soluble in pyridine. However the reaction temperature is usually maintained at a high temperature (115°C).

The products could be isolated either by washing with water, acidifying, washing with alkali or filtration and further purified through chromatography using silica gel column or semi-preparative scale HPLC to afford the pure product (Hashimoto *et al.*, 1997).



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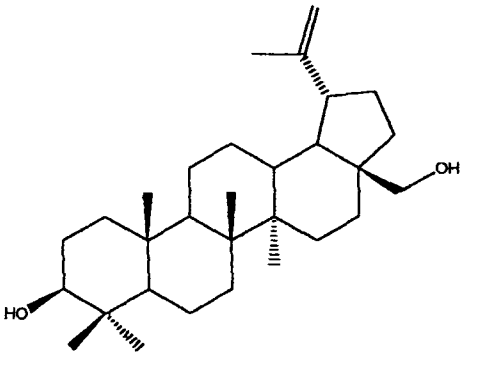


(2)

CHAPTER II

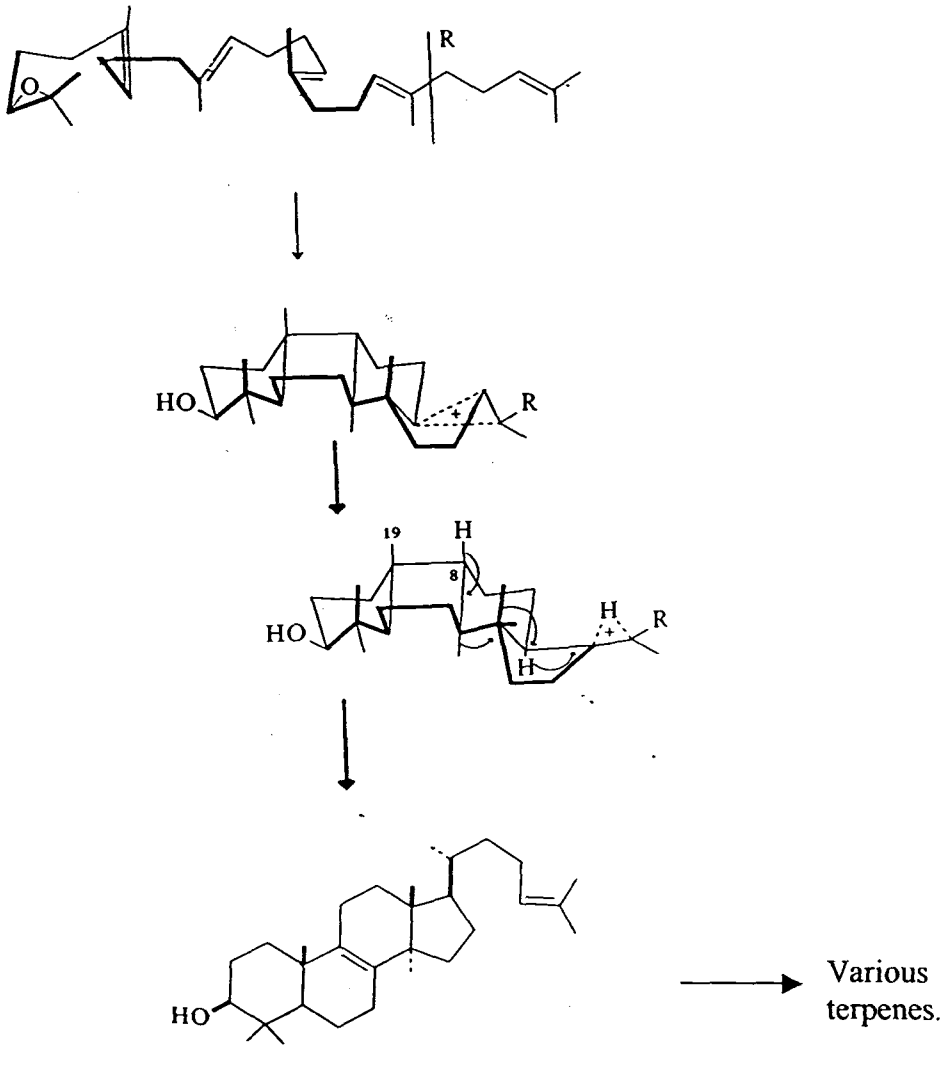
LITERATURE REVIEW

New Potential Natural Product

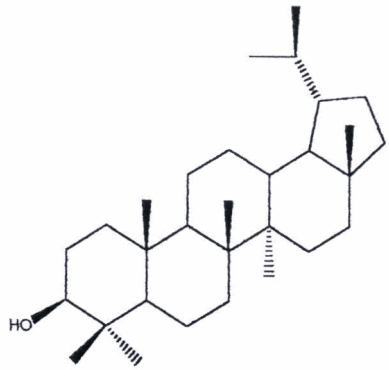
In the recent years, a considerable number of studies has been conducted to the discovery of clinically useful drugs. One of the natural product sources that has become the focus of interest in these studies is pentacyclic triterpenes (Sun *et al.*,1998).

Pentacyclic triterpenes are produced by rearrangement of squalene epoxide (Scheme 1) (Newman .,1972). These compounds are common and are found in most plants. There are at least 4000 known triterpenes. Many triterpenes occur freely but others in special combined forms. Penatcyclic triterpenes have a wide spectrum of biological activities and many of them are very useful in medicine. Several examples of compound which have been categorized in pentacyclic triterpenes groups are betulinic acid (1), betulin (2), lupeol (3), lupane (4), hopane (5), ursane (6) and platanic acid (7) (Patocka 2003).

Betulin is a valuable component of cosmetic powders and hair conditioners, additive in shampoo and lacquers; first-rate protective coatings from water, chemical and biological resistance, plasticizer for poly vinyl chloride (PVC) and production of polyurethane compounds (Patocka, 2003).



Scheme 1 : An Arrangement of Squalene Epoxide of Pentcyclic Triterpene



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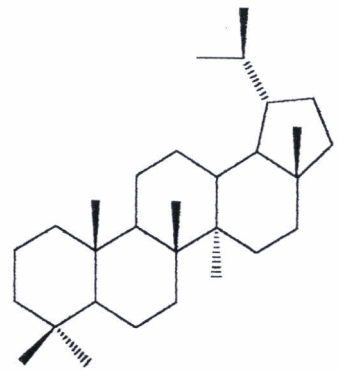

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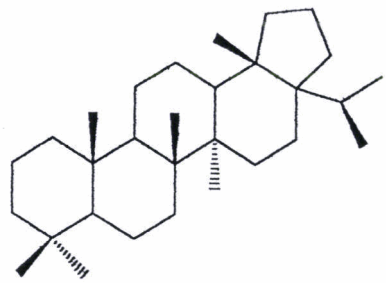

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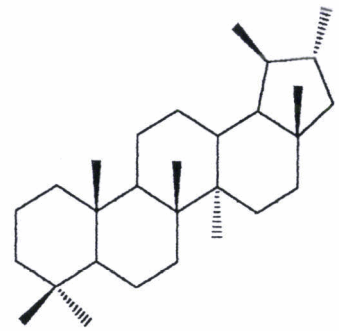

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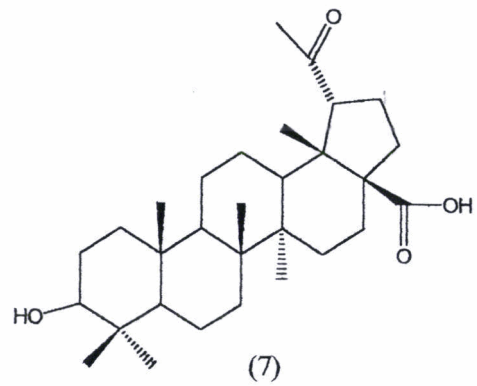


(7)

The antiphlogistic properties of betulin was studied by Recio *et al.*, (1995). Their study revealed a marked inhibition of the carrageenin and serotonin-induced rat paw edema, which was comparable to that of the standard anti-inflammatory agents phenylbutazone and dexamethazone.

Lupeol administered orally or intraperitoneally in a dose of 25-200 mg/kg showed anti-inflammatory activity in rats and mice (Geetha *et al.*, 1998). Lupeol significantly reduced the exudates volume and total leukocyte count in mice (Subhadhirasakul *et al.*, 2000). This compound is also a competitive inhibitor of both trypsin and chymotrypsin (Rajic *et al.*, 2000) and interestingly lupeol does not inhibit cyclooxygenase activity and the synthesis of prostaglandins (Kweifio-okai *et al.*, 1993).

Betulinic Acid : An Introduction

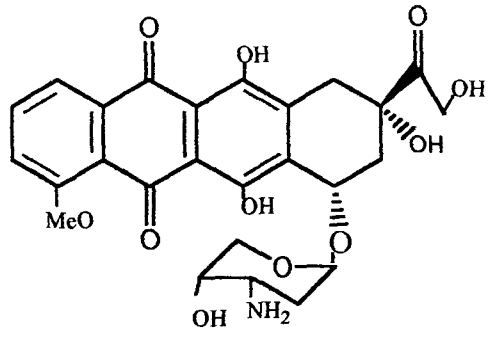
Natural products herbs are alternative pharmacologically active agents apart from modern medicinal drug products. Betulinic acid is one of the natural compound, found in *Ziziphus* species (O'Connell *et al.*, 1988). Methanol extract from the seed of *M .cajipriti* (Ahmad *et al.*,1997 and Malaysian *Callistemon speciosus* D.E (Ahmad *et al.*, 1999) were also reported to contain betulinic acid and ursolic acid .

Betulinic acid was potentiated to inhibit the growth of tumor cell. It was observed that concentration of betulinic acid in tumor cell was nearly

twice the level observed in liver, compared to the distribution of betulinic acid in mouse blood, liver, lung and kidney. The HPLC-electro spray MS analysis of betulinic acid is useful for preclinical or clinical evaluations of this potential antitumor agent (Shin *et al* .,1999). In contrast, various drugs derived from natural product sources, such as adriamycin (8), etoposide (9), and vincristine (10), and their derivatives have been tested for efficiency against melanoma. However, these compounds exhibited low response rates, transient complete responses and high toxicity (Thompson *et al.*, 1992).

Several betulinic acid derivatives were prepared by Kim *et al*,. (1998) and tested against human melanoma cell line (MeL-2) to determine the structural requirements of betulinic acid for the biological effect. They reported that the free carboxylic acid group at position C-28 has an important role to increase the melanoma selective cytotoxicity activities or toxic effect. They also make efforts to modify betulinic acid as an initial lead compound especially at the C-3 hydroxyl group in order to develop therapeutic agents to arrest the replication of the human immunodeficiency virus (HIV).

Betulinic acid was reported to inhibit DNA polymerase β with IC_{50} value of 14. DNA polymerase β, is the most accurate enzyme that create an exact copy of DNA .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OH
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OH
OH
MeO
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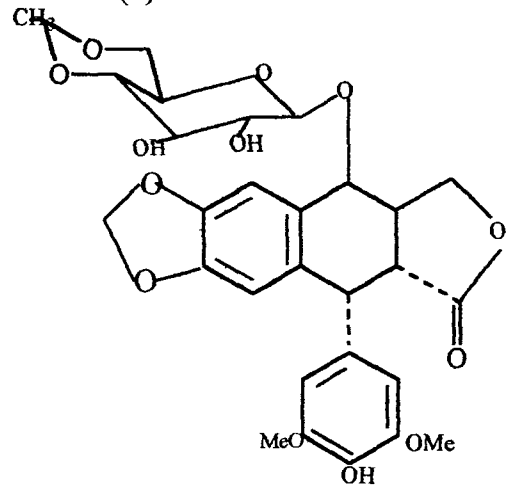
O
OH
NH2

(8)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O
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OH
OH
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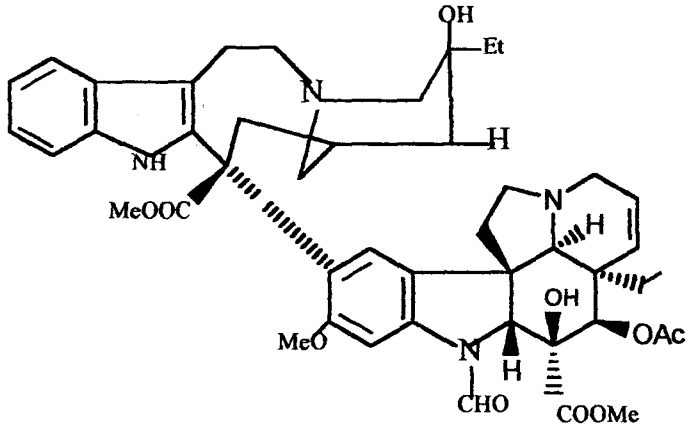
MeO
OMe
OH

(9)


OH
Et
N
H
NH
MeOOC
N
H
OH
OAc
MeO
N
H
CHO
COOMe

(10)