

**NOVEL DAMMARANE SAPOGENINS,
THEIR USE AS ANTI-CANCER AGENTS,
AND A PROCESS FOR PRODUCING SAME**

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TECHNICAL FIELD

This invention relates to novel dammarane sapogenins, their use in anti-cancer applications, and a process of producing the dammarane sapogenins. More particularly, the invention pertains to a novel group of dammarane sapogenins
10 obtained by chemical cleavage of dammarane saponins extracted from panax ginseng, panax quinquefol, panax notoginseng and other species in the ginseng family, and a novel preparation of anticancer agent containing one or more of these novel sapogenins for the treatment of cancer, particularly multi-drug resistant cancers, as well as a process for producing these novel sapogenins.

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BACKGROUND

Since the beginning of the last decade, anti-cancer research has been increasingly directed to the discovery of novel anti-cancer agents obtained from
20 natural sources, as well as identifying and preparing synthetic compounds found in natural sources.

Ginseng saponins (dammarane saponins, also called "ginsenosides", which are effective ingredients that organically exist in panax ginseng, panax quinquefol,
25 panax notoginseng and other species in the ginseng family) and sapogenins (those that do not naturally exist in the ginseng plant or other species in the ginseng family and can be derived only through chemical structure modification by cleavage and/or semi-synthesis of dammarane saponins), as natural-source root compounds, have been broadly researched for their anti-cancer characteristics. Some of them have
30 been reported to have anti-cancer effects, of which, for example, ginsenoside Rh2 [3-O- β -D-glucopyranosyl-20(s)- protopanaxadiol] has been reported for its anti-cancer activities, including induction of differentiation and apoptosis in cancer cells, inhibition of the growth of human ovarian cancer in nude mice after oral administration, and the ability to inhibit the multiplication of multi-drug resistance
35 (MDR) cancer cells while used with other chemotherapy drugs in vitro.

Ginsenoside Rg3 [3-O-[β -D-glucopyranosyl(1 $\rightarrow$ 2)- β -D-glucopyranosyl]-20(s)- protopanaxadiol] has been reported to inhibit the invasion by various cancer

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cells and suppress the proliferation of human prostate cancer cells in vitro, and to inhibit lung metastasis in mice and peritoneal metastasis in rats.

5 A metabolite of ginseng saponin produced by human intestinal bacteria, Mc [20-O-[α -L-arabinofuranosyl (1 $\rightarrow$ 6)- β -D-glucopyranosyl]-20(s)-protopanaxadiol], has been reported to inhibit the vascularization of tumors and extravasation of cancer cells.

10 While conventional chemotherapy agents directly attack the cancer cells and exhibit severe adverse side effects, some ginseng saponins and sapogenins, as well as their intestinal bacteria metabolites, have been reported to have inhibitory effects on cancers by induction of cancer-cell apoptosis and /or by suppression of vascularization of cancers with few adverse side effects.

15 In the case of treatment of cancers with ginseng saponins, it has been reported that saponins which are metabolized to sapogenins by intestinal bacteria have anti-cancer effects. It has also been reported that ginseng saponins with a hydroxyl at C-20(R), or 20(R) epimers, such as 20(R)-Rh2 and 20(R)-Rg3 have much lower biological activities than those with a hydroxyl at C-20(S), or 20(S) epimers, such as 20(S)-Rh2 and 20(S)-Rg3 respectively. Currently, mixtures of 20(R) and 20(S) epimers are very difficult if not impossible to separate. Thus the mixture has lower efficacy than that of 20(S) epimer. Furthermore, all previously discovered ginsenosides and sapogenins either have sugar moieties at C-3, C-6 or C-20, or have a hydroxyl at C-20, or have both.

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SUMMARY OF INVENTION

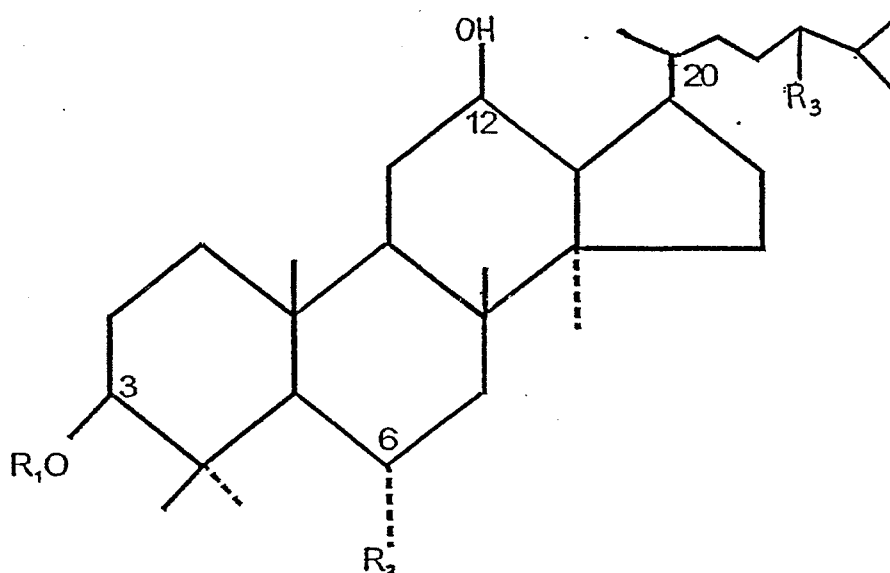
This invention relates to a group of novel sapogenins, their use in anti-cancer applications, and to a process for their production. More particularly, this invention pertains to a novel group of dammarane sapogenins, PAM-120, PBM-110 and PBM-100 (the dammarane sapogenin structure in these three sapogenins is specifically clean of any sugar moieties (glycons) at any position and a hydroxyl at C-20) and PAN-20 and PAN-30 (the dammarane sapogenin structure has sugar moieties (glycons) but is free of hydroxyl at C-20), obtained by chemical cleavage of dammarane saponins. The invention also includes a novel application of the said sapogenins for anti-cancer treatment by using them separately or together, and/or jointly with other drugs, as well as to the process of producing these novel

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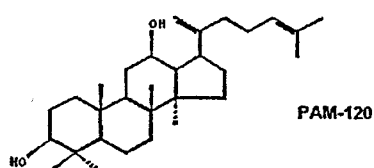
sapogenins. Said novel dammarane sapogenins show surprising anti-cancer effect when applied. In particular, the novel dammarane sapogenins show unexpected and superior activity against multi-drug resistant cancers.

5 The invention is directed to a sapogenin according to the formula:



wherein R1 is H, glc or glc¹⁻² glc, R2 is H or OH, R3 is H or OH; and when R1, R2 and R3 are H, there are double bonds at positions 20(21) and 24(25); and when
 15 R1 is H, R2 is OH and R3 is OH, there are double bonds at positions 20(22) and 25(26); and when R1 is H, R2 is OH and R3 is H, there are double bonds at positions 20(22) and 24(25); and when R1 is glc, R2 is H and R3 is H, there are double bonds at positions 20(21) and 24(25); and when R1 is glc¹⁻²glc, R2 is H and R3 is H, there are double bonds at positions 20(22) and 24(25); and
 20 pharmaceutically acceptable compositions incorporating said sapogenins.

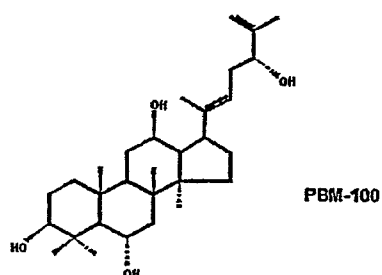
The invention in one embodiment is directed to a sapogenin according to the formula:



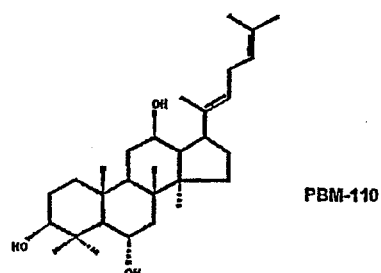
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The invention in a second embodiment is directed to a sapogenin according to the formula:

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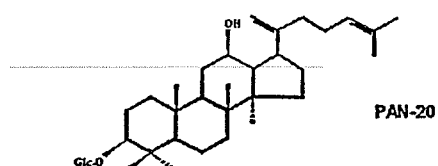


The invention in a third embodiment is directed to a sapogenin according to the formula:



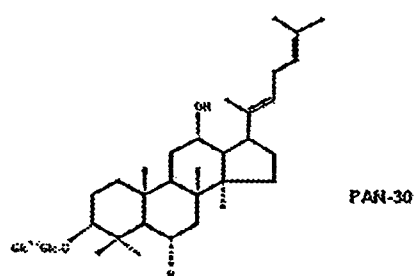
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The invention in a fourth embodiment is directed to a sapogenin according to the formula:



The invention is a fifth embodiment is directed to a sapogenin according to the formula:

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10 The invention also pertains to the use of a sapogenin according to the formula of the invention in treating cancer cells in a human being suffering from cancer, comprising killing cancer cells, inducing apoptosis in cancer cells, or inhibiting multiplication of cancer cells, or any combination thereof. The sapogenins of the invention are particularly useful in treating drug resistant cancer cells (MDR) in a human being suffering from cancer, comprising using the
15 sapogenins either singly, or in combination with one another, or in combination with other chemotherapy agents.

The invention also pertains to a method of treating cancer in human beings or other animals suffering from cancer comprising administering to said human
20 beings or other animals a therapeutically effective amount of a composition comprising one or more of PAM-120, PBM-100, PBM-110, PAN-20 and PAN-30.

The method can comprise a pharmaceutically effective amount of PAM-120, PBM-100, PBM-110, PAN 20 and PAN-30, with or without one or more
25 pharmaceutically acceptable carriers. The active ingredient can be administered in a dosage of between 5 micrograms to 50 grams per 1 kg body weight per day. A preferred range is 50 micrograms to 5 grams per 1 kg body weight per day. The

form of the composition can be selected from the group consisting of an orally administrable form, an injectable form, and a topically applicable form.

5 The orally administrable form can be selected from the group consisting of a tablet, a powder, a suspension, an emulsion, a capsule, a granule, a troche, a pill, a liquid, a spirit, a syrup and a lemonade. The injectable form can be selected from the group consisting of a liquid, a suspension and a solution. The topically applicable form can be selected from the group consisting of a drop, a paste, an ointment, a liquid, a powder, a plaster, a suppository, an aerosol, a liniment, a
10 lotion, an enema and an emulsion. The composition can be administered to human beings or other animals who are receiving one or more other anti-cancer treatments. The composition can be formulated with one or more other anti-cancer agents, for additive treatment effects, or synergistic treatment effects on multi-drug resistance cancers or any other cancer type.

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The invention also includes the incorporation of the sapogenins according to the invention in foods, health foods, nutritional products, natural products and alternative medicine products.

20 The invention also pertains to a process of preparing a sapogenin which comprises producing a ginsenoside extract from plants selected from the group consisting of panax ginseng, panax quinquefol and panax notoginseng, and proceeding according to the following steps: (a) mixing the ginsenoside extract with water; (b) (i) mixing the ginsenoside extract and water with a short-chain (1-5
25 carbon) alkali-metal alcoholate solution or a hydroxide-ethanol solution to produce a mixture; and (ii) placing the resultant mixture in a reaction tank so that the resultant mixture can undergo chemical reactions under required high temperature and high pressure; or (c) (i) alternatively, mixing the ginsenosides extract with ethanol; (ii) mixing the extract and ethanol with alkali-metal alcoholates solution to produce a
30 mixture, and (iii) placing the resultant mixture in a reaction tank so that the resultant mixture can undergo chemical reactions under required high temperature and high pressure; (d) after the reaction is completed, collecting an intermediate product of a mix of ginsenosides and sapogenins from the ethanol mixture; and (e) separating the desired sapogenins from the intermediate saponin-sapogenin mixture
35 by silica-gel-column chromatography.

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The alkali metal can be potassium or sodium. The hydroxide can be sodium hydroxide or potassium hydroxide. The alkali-metal alcoholates solution or the concentration of hydroxide-ethanol solution can be 5~50% (W/V). The alcohol can have 1~5 carbon atoms. The temperature of the reaction tank can be between
5 150~300°C and the reaction pressure can be between 2.5~8.4 MPa.

BRIEF DESCRIPTION OF DRAWINGS

In drawings which illustrate specific embodiments of the invention, but
10 which should not be construed as restricting the spirit or scope of the invention in any way:

Figure 1 illustrates a graph of tumor inhibiting effect of various ginsenosides on B16 cells.
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Figure 2 illustrates a graph of tumor inhibiting effect of various ginsenosides on drug resistant human breast cancer cells MCF7r.

Figure 3 illustrates a plot of the synergistic effect of PAM-120 with cisplatin
20 on drug resistant human breast cancer cells MCF7r.

Figure 4 illustrates a plot of the synergistic effect of PAM-120 with taxol on drug resistant human breast cancer cells MCF7r.

Figure 5 illustrates a graph of the therapeutic effect of PAM-120 on mouse
25 intracranial human malignant glioma (U87) model.

Figure 6 illustrates a graph of the therapeutic effect of PAM-120 on mouse
30 subcutaneous human malignant glioma (U87) model.

Figure 7 illustrates a flow chart of two processes which can be used to obtain the sapogenins according to the invention.

DESCRIPTION

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Throughout the following description, specific details are set forth in order to provide a more thorough understanding of the invention. However, the invention

may be practiced without these particulars. In other instances, well known elements have not been shown or described in detail to avoid unnecessarily obscuring the invention. Accordingly, the specification and drawings are to be regarded in an illustrative, rather than a restrictive, sense.

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This invention relates to a physically obtained group of novel compounds as follows:

- Dammara-20(21)-diene-3,12-diol (named as PAM-120);
- Dammara-20(22E)-diene-3,12,24-triol (named as PBM-100);
- 10 - Dammara-20(22E)-diene-3,6,12-triol (named as PBM-110);
- 3-O- β -D-glucopyranosyl-dammara-20(21)-diene-3,12-diol (named as PAN-20); and
- 3-O-[β -D-glucopyranosyl(1 $\rightarrow$ 2)- β -D-glucopyranosyl]-dammara-20(22E)-diene-3,12-diol (named as PAN-30).

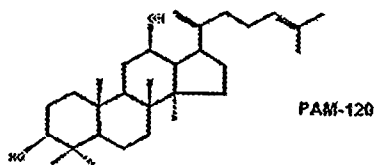
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The chemical formulas, structures and spectrum characteristics of the above listed novel compounds are shown on the following pages:

Sapogenin PAM-120

20 **Dammara-20(21)-diene-3,12-diol (named as PAM-120)**

(1) Structural formula:



- (2) Molecular formula: $C_{30}H_{50}O_2$
- (3) Molecular weight: 442.723
- 25 (4) The 1H -NMR spectrum (300 MHz, C_5D_5N) has shown signals at δ 5.28 (1H, br.t), δ 5.14 (1H, s), δ 4.90 (1H, s), δ 1.67 (3H, s), δ 1.60 (3H, s), δ 1.23 (3H, s), δ 1.06 (3H, s), δ 1.03 (3H, s), δ 0.95 (3H, s) and δ 0.90 (3H, s).
- (5) The ^{13}C -NMR spectrum (75.4 MHz, C_5D_5N) has shown signals at δ 39.57 (C-1), δ 28.31 (C-2), δ 78.02 (C-3), δ 40.30 (C-4), δ 56.46 (C-5), δ 18.84 (C-6), δ 35.46 (C-7), δ 37.53 (C-8), δ 51.03 (C-9), δ 39.61 (C-10), δ 32.76 (C-

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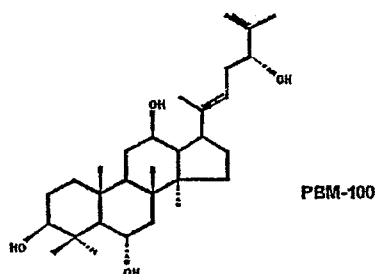
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δ 72.51 (C-12), δ 48.29 (C-13), δ 51.27 (C-14), δ 32.68 (C-15), δ 27.12 (C-16), δ 52.51 (C-17), δ 15.91 (C-18), δ 16.61 (C-19), δ 155.57 (C-20), δ 108.18 (C-21), δ 33.91 (C-22), δ 30.82 (C-23), δ 125.38 (C-24), δ 131.24 (C-25), δ 25.81 (C-26), δ 17.81 (C-27), δ 28.73 (C-28), δ 16.34 (C-29) and δ 17.06 (C-30).

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Sapogenin PBM-100**Dammara-20(22E)-diene-3,12,24-triol (named as PBM-100)**

(1) Structural formula:



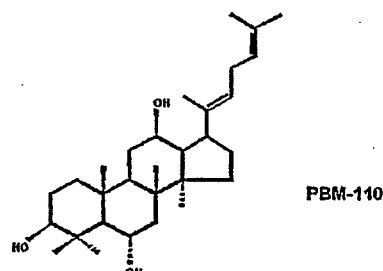
- 10 (2) Molecular formula: $C_{30}H_{50}O_4$
 (3) Molecular weight: 474.721
 (4) The 1H -NMR spectrum (300 MHz, C_5D_5N) has shown signals at δ 5.31 (1H, br.t), δ 5.22 (1H, s), δ 4.82 (1H, s), δ 1.95 (3H, s), δ 1.81 (3H, s), δ 1.66 (3H, s), δ 1.64 (3H, s), δ 1.47 (3H, s), δ 1.19 (3H, s), δ 1.06 (3H, s) and δ 1.03 (3H, s).
 15 (5) The ^{13}C -NMR spectrum (75.4 MHz, C_5D_5N) has shown signals at δ 39.48 (C-1), δ 27.52 (C-2), δ 78.48 (C-3), δ 40.42 (C-4), δ 61.86 (C-5), δ 67.77 (C-6), δ 47.69 (C-7), δ 41.48 (C-8), δ 50.55 (C-9), δ 39.48 (C-10), δ 32.02 (C-11), δ 72.63 (C-12), δ 50.47 (C-13), δ 50.73 (C-14), δ 32.69 (C-15), δ 27.52 (C-16),
 20 δ 50.92 (C-17), δ 17.80 (C-18), δ 17.70 (C-19), δ 140.11 (C-20), δ 13.23 (C-21), δ 124.63 (C-22), δ 30.04 (C-23), δ 78.00 (C-24), δ 149.90 (C-25), δ 110.54 (C-26), δ 17.80 (C-27), δ 28.94 (C-28), δ 16.56 (C-29) and δ 17.14 (C-30).

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Sapogenin PBM-110**Dammara-20(22E)-diene-3,6,12-triol (so named as PBM-110)**

(1) Structural formula:

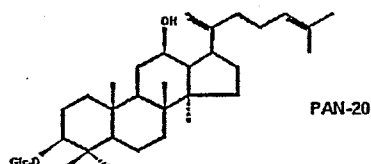


- 5 (2) Molecular formula: $C_{30}H_{50}O_3$
- (3) Molecular weight: 458.722
- (4) The 1H -NMR spectrum (300 MHz, C_5D_5N) has shown signals at δ 5.31 (1H, br.t), δ 5.51 (1H, t, $J=7.2$ Hz), δ 2.01 (3H, s), δ 1.85 (3H, s), δ 1.65 (3H, s), δ 1.64 (3H, s), δ 1.47 (3H, s), δ 1.19 (3H, s), δ 1.03 (3H, s) and δ 1.01 (3H, s).
- 10 (5) The ^{13}C -NMR spectrum (75.4 MHz, C_5D_5N) has shown signals at δ 39.48 (C-1), δ 27.52 (C-2), δ 78.48 (C-3), δ 40.42 (C-4), δ 61.86 (C-5), δ 67.77 (C-6), δ 47.69 (C-7), δ 41.48 (C-8), δ 50.55 (C-9), δ 39.48 (C-10), δ 32.02 (C-11), δ 72.63 (C-12), δ 50.47 (C-13), δ 50.73 (C-14), δ 32.69 (C-15), δ 27.52 (C-16), δ 50.92 (C-17), δ 17.80 (C-18), δ 17.70 (C-19), δ 140.11 (C-20), δ 13.23 (C-21), δ 124.63 (C-22), δ 30.04 (C-23), δ 124.63 (C-24), δ 131.33 (C-25), δ 25.76 (C-26), δ 17.50 (C-27), δ 28.94 (C-28), δ 16.56 (C-29) and δ 17.14 (C-30).
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Sapogenin PAN-20**3-O- β -D-glucopyranosyl-dammara-20(21)-diene-3,12-diol (named as PAN-20)**

(1) Structural formula:



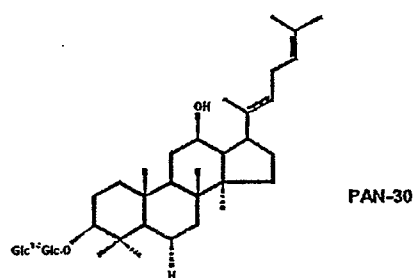
- 5 (2) Molecular formula: $C_{36}H_{60}O_7$
- (3) Molecular weight: 604.863
- (4) The 1H -NMR spectrum (300 MHz, C_5D_5N) has shown signals at δ 4.92 (1H, d, $J=7.5$ Hz), δ 5.29 (1H, br.t), δ 5.14 (1H, s), δ 4.90 (1H, s), δ 1.66 (3H, s), δ 1.60 (3H, s), δ 1.30 (3H, s), δ 1.02 (3H, s), δ 0.98 (3H, s), δ 0.98 (3H, s)
- 10 and δ 0.81 (3H, s).
- (5) The ^{13}C -NMR spectrum (75.4 MHz, C_5D_5N) for aglycon moiety has shown signals at δ 39.34 (C-1), δ 27.13 (C-2), δ 88.82 (C-3), δ 40.26 (C-4), δ 56.47 (C-5), δ 18.52 (C-6), δ 35.40 (C-7), δ 37.12 (C-8), δ 50.91 (C-9), δ 39.74 (C-10), δ 32.73 (C-11), δ 72.47 (C-12), δ 48.30 (C-13), δ 51.26 (C-14), δ 32.74 (C-15), δ 26.78 (C-16), δ 52.52 (C-17), δ 15.86 (C-18), δ 16.52 (C-19), δ 155.58 (C-20), δ 108.19 (C-21), δ 33.91 (C-22), δ 30.82 (C-23), δ 125.39 (C-24), δ 131.25 (C-25), δ 25.81 (C-26), δ 17.81 (C-27), δ 28.73 (C-28), δ 16.83 (C-29) and δ 17.05 (C-30). The ^{13}C -NMR spectrum (75.4 MHz, C_5D_5N) for 3-glucopyranosyl has shown signals at δ 107.00 (C-1"), δ 75.82 (C-2"), δ 78.79 (C-3"), δ 71.94 (C-4"), δ 78.39 (C-5") and δ 63.14 (C-6").
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Sapogenin PAN-30**3-O-[[β -D-glucopyranosyl(1 $\rightarrow$ 2)- β -D-glucopyranosyl]-dammar-20(22E)-diene-3,12-diol (named as PAN-30)**

(1) Structural formula:

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(2) Molecular formula: $C_{42}H_{70}O_{12}$

(3) Molecular weight: 766.587

(4) The ^{13}C -NMR spectrum (75.4 MHz, C_5D_5N) has shown signals at δ 39.17 (C-1), δ 28.00 (C-2), δ 88.82 (C-3), δ 40.14 (C-4), δ 56.29 (C-5), δ 18.33 (C-6), δ 35.24 (C-7), δ 39.60 (C-8), δ 50.66 (C-9), δ 36.91 (C-10), δ 32.10 (C-11), δ 72.49 (C-12), δ 50.33 (C-13), δ 50.91 (C-14), δ 32.54 (C-15), δ 26.64 (C-16), δ 50.80 (C-17), δ 16.35 (C-18), δ 16.49 (C-19), δ 140.06 (C-20), δ 13.07 (C-21), δ 123.21 (C-22), δ 27.35 (C-23), δ 123.54 (C-24), δ 131.16 (C-25), δ 25.60 (C-26), δ 17.66 (C-27), δ 28.73 (C-28), δ 15.72 (C-29) and δ 16.92 (C-30).

The inventors herein have discovered that the dammarane sapogenin structure that is modified to be specifically clean of any sugar moieties (glycons) at any position and free of hydroxyl at C-20 has surprisingly improved effectiveness in treating cancers, particularly in treating multi-drug resistant cancers, compared to sapogenins that have sugar moieties on the structure or a hydroxyl at C-20. The inventors have unexpectedly found that PAM-120, PBM-110 and PBM-100, which all fall into this chemical category, have greater anti-cancer effect than other known saponins and sapogenins. In particular, these three sapogenins, and especially PAM-120, show surprisingly effective activity in the treatment of multi-drug resistant cancers.

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The inventors have also surprisingly and unexpectedly found that a dammarane sapogenin structure which is free of a hydroxyl at C-20, even though there may be a sugar moiety on the structure, demonstrates effective anti-cancer activity, particularly in the treatment of multi-drug resistant cancers. PAN-20 and
5 PAN-30, according to this invention, fall into this latter category.

While the inventors do not wish to be bound by any adverse theories if proved to be unfounded, the inventors offer the following as an aid in understanding the invention. It seems that sapogenins that have no hydroxyl at C-20 compared to
10 sapogenins that have a hydroxyl at C-20 are surprisingly effective in cancer treatment. It also seems that a sapogenin that does not have a sugar moiety (glycon) on the sapogenin structure, is more effective than sapogenins that include a sugar moiety. It also seems that the diol is more effective than the triol. None of this could be predicted, or forecast without testing the sapogenins of the invention.

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According to this invention and varying with the severity of symptoms experienced by the patient, the active daily dose of sapogenin PAM-120 is 0.1 mg – 10 g per kg of body weight, or preferably, 1mg – 1 g per kg of body weight. The active daily dose of sapogenin PBM-110 is 0.1 mg – 10 g per kg of body
20 weight, or preferably, 1mg – 1 g per kg of body weight. The active daily dose of sapogenin PBM-100 is 0.1 mg – 10 g per kg of body weight, or preferably, 1mg – 1 g per kg of body weight. The active daily dose of sapogenin PAN-20 is 0.1 mg – 10 g per kg of body weight, or preferably, 1mg – 1 g per kg of body weight. The active daily dose of sapogenin PAN-30 is 0.1 mg – 10 g per kg of
25 body weight, or preferably, 1mg – 1 g per kg of body weight.

The anti-cancer agent according to this invention contains one or more of the said novel sapogenins PAM-120, PBM-100, PBM-110, PAN-20 and PAN-30, with or without other anti-cancer agent, used with or without one or more
30 pharmaceutically acceptable carriers such as solid and liquid excipients.

The administration forms of the said anti-cancer agents according to the invention are listed as follows:

- Injection forms, including but not limited to intramuscular (IM)
35 injection, intravenous (IV) injection, subcutaneous injection and targeted-tissue injection in aqueous solutions, oil solutions, emulsion, or any forms;

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- Oral forms, including but not limited to tablets, capsules, granules, pills, suspensions, powders, sprits, emulsifiers, and syrups; and
- Topical form, including but not limited to drops, lotions, enemas, ointments, suspensions, paps, pastes, suppositories, aerosols, cataplasmas, emulsifiers, liniments, and plasters.

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This invention also relates to a production process that can be used to commercially produce the above mentioned novel dammarane saponin for anti-cancer applications through chemical cleavage and semi-synthesis of dammarane saponins.

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Figure 7 illustrates a flow sheet of two alternative processes which can be utilized to produce the saponins according to the invention. The production process according to the invention uses general ginsenosides (also called dammarane saponins including Ra, Rc, Rd, Re, etc.) extracted from plants selected from the ginseng family such as panax ginseng, panax quinquefol and panax notoginseng as raw materials. In the process, according to this invention, general ginsenosides are first mixed with water and then with short-chain (1-5 carbon) alkali-metal alcoholate solution or hydroxide-ethanol solution. The mixture is then put into a reaction tank to undergo chemical reactions under required high temperature and high pressure. Alternatively, general ginsenosides are first mixed with ethanol, and then with alkali-metal alcoholates solution. The mixture is thereafter put into a reaction tank to undergo chemical reactions under required high temperature and high pressure. After the required period of time for the reaction to be complete, the intermediate product of a mix of ginsenosides and saponins are collected from the ethanol solution. The next step is to separate the desired dammarane saponins from the intermediate saponin-sapogenin mix by using silica-gel-column chromatography. According to this invention, the said alkali metal can be potassium or sodium, the hydroxide can be sodium hydroxide or potassium hydroxide, the concentration of alkali-metal alcoholates solution or the concentration of hydroxide-ethanol solution can be 5~50% (W/V), and the short chain alcohol can be one with 1~5 carbon atoms. In this invention, during the production process, the reaction tank's temperature can be between 150~300°C and the reaction pressure is between 2.5~8.4 MPa.

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Cancers susceptible to treatment with the compounds of the invention alone or in combination with a chemotherapeutic in accordance with various aspects of the

invention may include both primary and metastatic tumors and hyperplasias, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder and urothelium), female genital tract
5 (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease)), male genital tract (including prostate, seminal vesicles, testes and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma),
10 and tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas). In some aspects of the invention, the compounds of the invention in combination with a chemotherapeutic may also be useful in treating hematopoietic cancers such as leukemias (i.e. chloromas, plasmacytomas and the
15 plaques and tumors of mycosis fungoides and cutaneous T-cell lymphoma/leukemia) and lymphomas (both Hodgkin's and non-Hodgkin's lymphomas).

The compounds of the invention and the chemotherapeutic may be administered in combination separately or as one single combined pharmaceutical
20 composition. The amount of each component administered may be determined by an attending clinician, taking into consideration a variety of factors such as the etiology and severity of the disease, the patient's condition and age and the potency of each component. the components may be administered in accordance with the standard methodologies as, for example, disclosed in the Physician's Desk
25 Reference (PDR) published by Medical Economics Co. Inc. of Oradell, N.J.

One or more pharmaceutically acceptable carriers or excipients may be used to formulate pharmaceutical compositions of the invention, including solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and
30 absorption delaying agents, and the like that are physiologically compatible. In alternative embodiments, the carrier may be suitable for parenteral, intravenous, intraperitoneal, intramuscular, sublingual or oral administration. Pharmaceutically acceptable carriers may include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or
35 dispersion. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be

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incorporated into the pharmaceutical compositions.

Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition may be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the pharmaceutical compositions may be administered in a time release formulation, for example, in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

Sterile injectable solutions can be prepared by incorporating an active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Pharmaceutical compositions may be formulated with one or more compounds that enhance the solubility of the active compounds.

EXAMPLES

Examples of Preparation Processes:

5 **Example 1: Preparation process of producing PAM-120, PBM=100, and PAN-20**

- 10 [1] Ginseng crude extract 10 g was dissolved in 40 mL of 95 % ethanol
- [2] Add 40 mL of 5 N NaOH
- [3] Pour into the reaction tank, and set temperature to 240°C, and
- [4] Reduce temperature to room temperature, and take the products out
- [5] Add HCl to neutralize pH to about 7, and expend the volume to
- 15 [6] Extract 3 times with acetic ester, 100 mL each time
- [7] Combine all the extracts, and reduce the pressure to dry. Thus,
- [8] Grind and dissolved the extract in 20 mL of methanol, and mix the
- [9] Dry up the mixture, and then grind to fine powder
- 20 [10] Load the Silica gel column
- [11] Wash the column with 60 mL of ether:petroleum benzin (1:3), and
- [12] Wash the column with 90 mL of chloroform:methanol (95:5), and
- 25 thus 50 mg of PAN-20 was obtained.

Example 2: Another example of preparation process producing PAM-120, PBM-100, and PAN-20

- 30 [1] 10 g of Ginseng crude extract was added into reaction tank
- [2] Add to the reaction tank 100 mL of 5 N NaOH
- [3] Set temperature to 270°C and pressure to 4.5 Mpa for 1 hour
- [4] Reduce temperature to room temperature, then take out the
- [5] Neutralize the pH to 7 using HCl
- 35 [6] Filter and keep the solids
- [7] Dissolve the solids in 10 mL of 95 % Ethanol
- [8] Add water to make ethanol content less than 5 %

- [9] Sit still overnight
- [10] Filter and keep the solids
- [11] Dry the solids
- [12] Dissolved the solids in 10 mL of methanol
- 5 [13] Filter and keep the solution
- [14] Dry the solution to obtain 3.6 g of products
- [15] Mix the products with 11 g of silica gel
- [16] Grind and then load the silica gel column
- 10 [17] Wash the column with 100 mL of ether:petroleum benzin (1:3),
and thus, 60 mg of PAM-120, and 65 mg of PBM-100 were
obtained
- [18] Wash the column with chloroform:methanol (95:5), and thus 60
mg of PAN-20 was obtained.

15 **Example 3: Comparison of Cancer Cell Inhibition Effects *In Vitro* Between
Ginsenoside 20(S)-Rh2 and Novel Dammarane Sapogenins
PAM-120, PBM-100, PAN-30 and their Composition**

A. Method

Composition: 20(S)-Rh2 was provided by Shenyang Pegasus
20 Pharmaceutical R&D Co., China, with a purity of over 98%. The molecular
weight for Rh2 was 622.3. Sapogenins PAM-120, PBM-100 and PAN-30 were
derived from the process stated in Example 1. The molecular weights of PAM-
120, PBM-100 and PAN-30 were respectively 442.7, 474.7 and 766.6, and the
purity for each of the three agents was higher than 99%. Rh2, PAM-120, PBM-
25 100 and PAN-30 were dissolved 1 gram each separately in 100 mL absolute
ethanol and stored at 4°C. the agents were diluted with RPMI-1640 medium to
the desired concentrations as shown in Table 1.

Cells: Human non-small-cell lung carcinoma H460 cells were incubated
30 in RPMI-1640 medium added with 10% fetal calf serum, 100 units penicillin/ml,
and 100 μ g streptomycin/ml in 5% CO₂ at 37°C.

In vitro treatment: H460 cells were seeded in 96-well flat-bottomed
microtest-plates at 1.2x10³ cells per well, six wells in each group, incubated in
35 humidified 5% CO₂ at 37°C for 24 hours with or without the agents according to
the schedule as shown below.

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Table 1. DOSAGE

Group		Dosage (µg/ml)
Control	No drug	-
5	Rh2	25
	PAM-120	
	PBM-100	
	PAN-30	
10	Rh2	40
	PAM-120	
	PBM-100	
	PAN-30	

15 After the 24 hours of incubation, an equal volume of 10% formalin phosphate-buffered saline containing 0.2% crystal violet was added to each well and left at room temperature for 20 minutes. The plates were then washed twice with distilled water and dried at room temperature. The absorbency of the stained cells at 590nm was then measured using an automatic microtest-plate reader.

20 Average absorbency of the control wells (A_c) without any treatment was calculated, average absorbency of each treatment group (A_{Ti}) was determined, and then the average cell viability of each treatment group (V_i) was derived using the following formula:

25

$$V_i(\%) = \frac{A_{Ti}}{A_c} \times 100\%$$

- 20 -

B. ResultTable 2. CANCER CELL VIABILITY (%)

Group		Absorbency of Stained Cells (M $\pm$ SD)	V(%)	tTest w/ Control	tTest w/ Rh2
5	Control	No drugs	.368 $\pm$.069	100.00	
	Low-dose (25 μ g/ ml)	Rh2	.278 $\pm$.030	78.49	P<0.01
		PAM-120	.220 $\pm$.051	62.08	P<0.01
		PBM-100	.223 $\pm$.040	62.72	P<0.01
		PAN-30	.249 $\pm$.045	70.30	P<0.01
10	High-dose (40 μ g/ ml)	Rh2	.181 $\pm$.049	50.99	P<0.01
		PAM-120	.125 $\pm$.031	35.34	P<0.01
		PBM-100	.130 $\pm$.019	36.51	P<0.01
		PAN-30	.147 $\pm$.032	41.49	P<0.01
15					

20 The results in Table 2 show a significant inhibitory effect on proliferation of H460 cells by each of the novel compounds PAM-120, PBM-100 and PAN-30 (P<0.01 compared with that of the Rh2 control), and a notable increase in inhibitory effect of PAM-120 and PBM-100 on the proliferation of H460 cells (P<0.05 compared with that of Rh2).

25

Example 4: Tumor Weight TestA. Method

30 Forty (40) C57BL/6J mice weighing 18-22 g were randomly divided into four groups: one control group and three treatment groups, each with 10 animals. Mouse sarcoma 180 cells were hyperdermically transplanted into the mice by using a transplantation needle under the right armpit. After the transplantation, all mice formed a tumor. The mix composition of ginsenosides and sapogenins

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including the three novel dammarane sapogenins (PAM-120, PBM-100 and PAN-30), derived as an intermediate product from the process described in Example 2, was prepared into a suspension form. The mice were weighed daily prior to drug administration to determine the actual measurement of drug administered. The drug administration started from 24 hours post tumor transplantation. The mice in the three treatment groups were orally given the mix composition at a daily dose of 0.4 mg/kg, 1.2 mg/kg and 3.6 mg/kg respectively for 8 days using a gastric catheter. The mice in the control group were orally given a normal saline placebo. 24 hours after the last administration of the drug, the mice were sacrificed with an overdose of anesthetics. The weight of the sarcoma in each mouse was measured. The average tumor weight of each treatment group (W_t) and that of the control group (W_c) were calculated, and the tumor inhibition ratio (R_i) of each treatment group was determined with the following formula:

$$R_i(\%) = \frac{W_c - W_t}{W_c} \times 100\%$$

B. Result

Table 3. TUMOR WEIGHT RATIO (%)

GROUP	MICE#	Tumor Weight (g) (M $\pm$ SD)	R(%)	P
Control	10	2.995 $\pm$ 0.621		
Mix 0.4 mg/kg	10	1.269 $\pm$ 0.525	57.63	<0.01
Mix 1.2 mg/kg	10	0.725 $\pm$ 0.270	75.79	<0.01
Mix 3.6 mg/kg	10	0.388 $\pm$ 0.130	87.04	<0.01

The results in Table 3 have demonstrated that oral administration of the subject mix composition, the intermediate product from Example 2, achieves tumor inhibition ratios of 58%, 76% and 87% respectively at the doses of 0.4 mg/kg, 1.2 mg/kg and 3.6 mg/kg, showing a dose related anti-cancer efficacy of the mix of the intermediate product of Example 2 containing the three novel sapogenins PAM-120, PBM-100 and PAN-30 and some other saponins and sapogenins whose structures are known or unknown.

A. Method

$$\text{LPR}(\%) = \frac{\text{ADS}_{(\text{treatment})} - \text{ADS}_{(\text{control})}}{\text{ADS}_{(\text{control})}} \times 100\%$$

B. Result

Table 4. MURINE SARCOMA 180 BEARING MICE
LIFE PROLONGATION RATE

5	Group	%		
		DS ₅₀	ADS(M $\pm$ SD)	LPR(%)
	Control	14	14.7 $\pm$ 5.4	
	Rh2 (10 mg/kg)	22	24.7 $\pm$ 12.6	68.0
10	PAM-120 (10 mg/kg)	38	38.6 $\pm$ 16.4	162.6
	Rh2 (25 mg/kg)	41	44.3 $\pm$ 19.6	201.4
	PAM-120 (25 mg/kg)	77	80.6 $\pm$ 34.4	448.3

15 The anti-cancer effect of the novel sapogenin PAM-120 was indicated by the significant increase in life prolongation of the mice bearing murine sarcoma ($P < 0.01$ compared with the average days of survival in the control). Better anti-cancer effect of novel sapogenin PAM-120 on murine sarcoma than that of Rh2 was demonstrated by the significant increase in life prolongation of the sarcoma-bearing mice ($P < 0.01$, compared with the average days of survival in the relevant Rh2 treatment dose groups). Two mice in the 25 mg/kg composition treatment group survived for the whole 120 days, and were found to have no tumors whatsoever existing in their bodies postmortally.

25 Figure 1 illustrates a graph of tumor inhibiting effect of various ginsenosides on B16 cells. Mouse melanoma tumor B16 cells were cultured with DMEM and 5% serum supplement in 96-well dishes. Cells were then treated with various concentrations of PAN-20, PAN-30, PBM-100, PBM-110, PAM-120 and Rh2, respectively. The number of alive cells were measured using MTT method 24 hours after the treatment and compared with the control samples. All the new compounds showed a significantly higher tumor inhibitory effect than RH2, especially at low concentrations ($p < 0.01$, Student t test).

35 Figure 2 illustrates a graph of tumor inhibiting effect of various ginsenosides on drug resistant human breast cancer cells MCF7r. Human drug resistant breast cancer cells (MCF7r) were cultured with DMEM and 5% serum supplement in 96-well dishes. Cells were then treated with various concentrations of PAN-20, PAN-30, PBM-100, PBM-110, PAM-120, and Rh2, respectively.

The number of alive cells were measured using MTT method 24 hours after the treatment and compared with the control samples. All new compounds showed a significantly higher tumor inhibitory effect than Rh2, especially at low concentrations ($p < 0.01$, Student t test).

5

Figure 3 illustrates a plot of the synergistic effect of PAM-120 with Cisplatin on drug resistant human breast cancer cells MCF7r. MCF7r cells were treated with anti-cancer chemotherapy agent Cisplatin at various concentrations in the presence of 10 ug/ml PAM-120. In Figure 3, the first bars in each
10 concentration group represent percentages of alive cells 24 hours after treatment with Cisplatin only. The second bars in each group represent the results of cells treated with Cisplatin and PAM-120.

Figure 4 illustrates a plot of the synergistic effect of PAM-120 with
15 Taxol on drug resistant human breast cancer cells MCF7r. MCF7r cells were treated with anti-cancer chemotherapy agent Taxol at various concentrations in the presence of 10 ug/ml PAM-120 or 20 ug/ml Rh2. In Figure 4, the first bars in each concentration group represent percentages of alive cells 24 hours after treatment with Taxol only. The second bars in each group represent the results of
20 cells treated with Taxol and PAM-120, while the third bars represent the results of cells treated with 20 ug/ml Rh2.

Figure 5 illustrates a graph of the therapeutic effect of PAM-120 on mouse intracranial human malignant glioma (U87) model. Nude mice were
25 intracranially implanted with human malignant glioma cells (U87). On day 10 post tumor implantation, animals were treated with various dosages of PAM-120. Animals treated with 25 mg/kg and 50 mg/kg PAM-120 had significantly longer survival time after tumor implantation ($p < 0.01$, Kaplan Meier analysis).

30 Figure 6 illustrates a graph of the therapeutic effect of PAM-120 on mouse subcutaneous human malignant glioma (U87) model. Nude mice were subcutaneously implanted with human malignant glioma cells (U87). On day 7 post tumor implantation, animals were treated with 25 mg/ml PAM-120 or equal dose of Rh2. Tumor sizes were measured on day 7 (before treatment) and day 24
35 (after the treatment). Both PAM-120 and Rh2 significantly inhibited tumor growth comparing to PBS control animals. Tumor sizes in PAM-120 treated

- 25 -

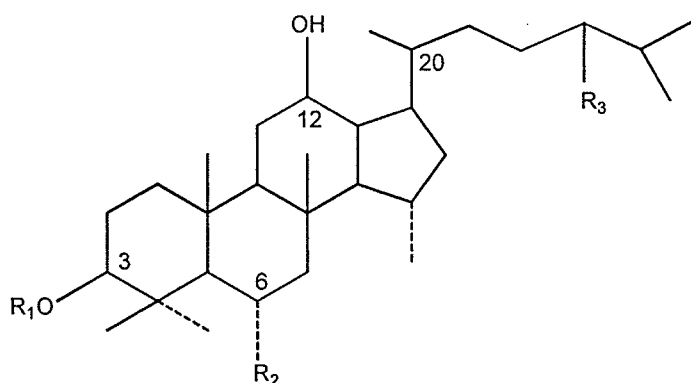
animals were significantly smaller than those in the Rh2 treated animals
($p < 0.05$).

5 Figure 7 illustrates a flow chart of two processes which can be used to
obtain the sapogenins according to the invention.

10 As will be apparent to those skilled in the art in the light of the foregoing
disclosure, many alterations and modifications are possible in the practice of this
invention without departing from the spirit or scope thereof. Accordingly, the
scope of the invention is to be construed in accordance with the substance defined
by the following claims.

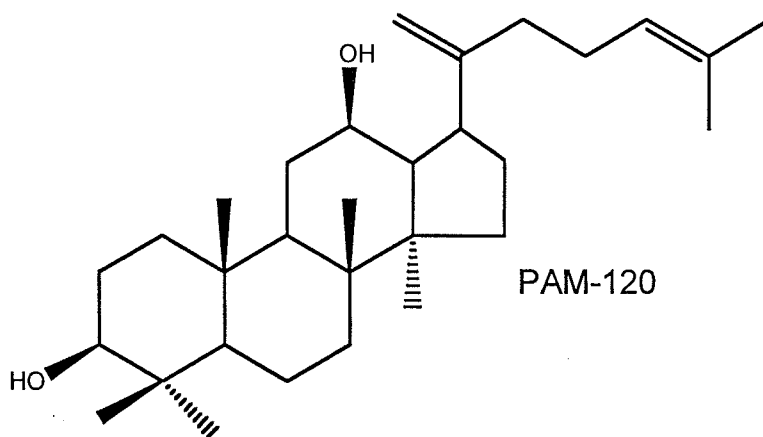
WHAT IS CLAIMED IS:

1. A sapogenin according to the formula:

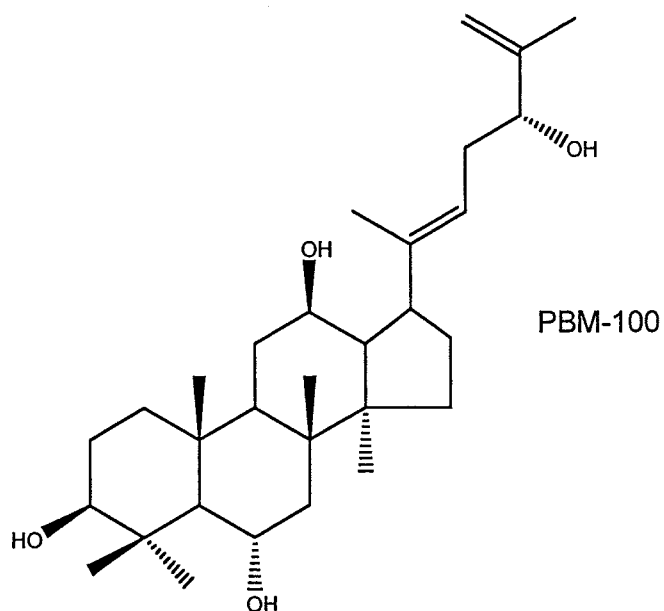


wherein R1 is H, R2 is H or OH, R3 is H or OH; and when R1, R2 and R3 are H, there are double bonds at positions 20(21) and 24(25); and when R1 is H, R2 is OH and R3 is OH, there are double bonds at positions 20(22) and 25(26); and pharmaceutically acceptable compositions incorporating said sapogenins.

2. The sapogenin as claimed in claim 1 wherein R1, R2 and R3 are H, and there are double bonds at 20(21) and 24(25).
3. The sapogenin as claimed in claim 1 wherein R1 is H, R2 and R3 are OH, and there are double bonds at 20(22) and 25(26).
4. A sapogenin according to claim 1 having the formula:



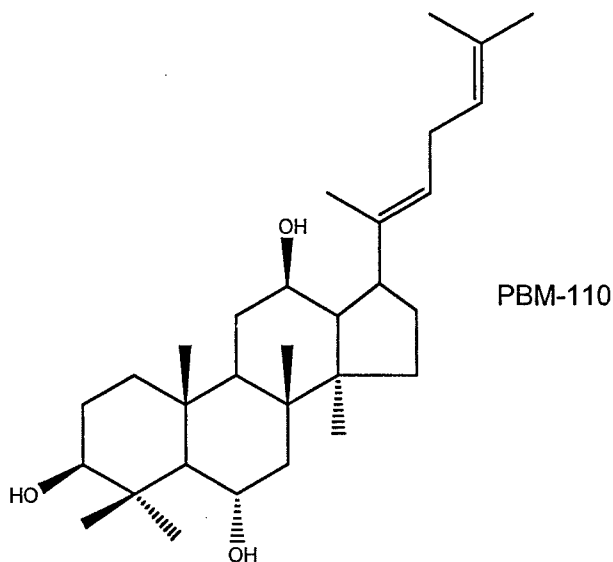
5. A sapogenin according to claim 1 having the formula:



6. A use of a sapogenin according to any one of claims 1 to 5 to treat cancer cells comprising killing cancer cells, inducing apoptosis in cancer cells, or inhibiting multiplication of cancer cells, or any combination thereof.
7. A use of a sapogenin according to any one of claims 1 to 5 to treat multi-drug resistant cancer cells (MDR) comprising using the sapogenin singly, or in combination with one or

more other sapogenins or compositions, or in combination with one or more other chemotherapy agents, or in combination with one or more other anti-cancer treatments.

8. A use of a sapogenin according to any one of the following formulae:



to treat cancer cells comprising killing cancer cells, inducing apoptosis in cancer cells, or inhibiting multiplication of cancer cells, or any combination thereof.

9. A use of a composition comprising one or more of the sapogenins according to claim 8 to treat cancer cells comprising killing cancer cells, inducing apoptosis in cancer cells, or inhibiting multiplication of cancer cells, or any combination thereof.

10. A use according to any one of claims 8 or 9 to treat multi-drug resistant (MDR) cancer cells comprising using the sapogenin or composition singly, or in combination with one or more other sapogenins or compositions, or in combination with one or more other chemotherapy agents, or in combination with one or more other anti-cancer treatments.

11. A composition comprising one or more sapogenins according to any one of claims 1 to

5 to treat cancer.

12. A composition according to claim 11 comprising a pharmaceutically effective amount of one or more of the sapogenins, with or without one or more pharmaceutically acceptable carriers, and one or more chemotherapeutic agents.

13. A composition according to any of claims 11 or 12 wherein the composition is formulated with one or more other anti-cancer agents, for additive treatment effects or synergistic treatment effects, on multi-drug resistant cancers or any other cancer type.

14. A composition according to any one of claims 11 to 13 wherein the composition is administered in a dosage of between 5 micrograms to 50 grams per kg body weight per day.

15. A composition according to any one of claims 11 to 13 wherein the composition is administered in a dosage of between 50 micrograms to 5 grams per kg body weight per day.

16. A composition according to any one of claims 11 to 15, wherein the form of the composition is selected from the group consisting of an orally administrable form, an injectable form, and a topically applicable form.

17. A composition according to claim 16, wherein the orally administrable form is selected from the group consisting of a tablet, a powder, a suspension, an emulsion, a capsule, a granule, a troche, a pill, a liquid, a spirit, a syrup and a lemonade.

18. A composition according to claim 16, wherein the injectable form is selected from the group consisting of a liquid, a suspension and a solution.

19. A composition according to claim 16, wherein the topically applicable form is selected from the group consisting of a drop, a paste, an ointment, a liquid, a powder, a plaster, a suppository, an aerosol, a liniment, a lotion, an enema and an emulsion.

20. A food, health food, nutritional product, natural product, or alternative medicine product, comprising a sapogenin according to any one of claims 1 to 5.

21. A process of preparing a sapogenin according to any one of claims 1 to 5 which comprises producing a ginsenoside extract from plants selected from the group consisting of panax ginseng, panax quinquefol and panax notoginseng, or a sapogenin source from some other plant, and proceeding according to the following steps:

- (a) (i) mixing the ginsenoside extract with water;
- (ii) mixing the ginsenoside extract and water with an alkali-metal alcoholate solution or a hydroxide-ethanol solution to produce a mixture; and
- (iii) placing the resultant mixture in a reaction tank so that the resultant mixture can undergo chemical reactions under high temperature and high pressure effective to produce sapogenins therefrom;

or, alternatively:

- (b) (i) mixing the ginsenoside extract with ethanol;
- (ii) mixing the ginsenoside extract and ethanol with an alkali-metal alcoholate solution to produce a mixture, and
- (iii) placing the resultant mixture in a reaction tank so that the resultant mixture can undergo chemical reactions under high temperature and high pressure effective to produce sapogenins therefrom;
- (c) after the reaction is completed, collecting an intermediate product of a mix of ginsenosides and sapogenins from the mixture; and
- (d) separating the desired sapogenins from the intermediate saponin-sapogenin mixture by silica-gel-column chromatography.

22. A process of according to claim 21 which comprises producing a ginsenoside extract from plants selected from the group consisting of panax ginseng, panax quinquefol and panax

notoginseng, and proceeding according to the following steps:

- (a) (i) mixing the ginsenoside extract with water;
- (ii) mixing the ginsenoside extract and water with an alkali-metal alcoholate solution or a hydroxide-ethanol solution to produce a mixture; and
- (iii) placing the resultant mixture in a reaction tank so that the resultant mixture can undergo chemical reactions at a temperature between 240 - 300EC and at a pressure between 3.5 - 8.4 MPa;

or, alternatively:

- (b) (i) mixing the ginsenoside extract with ethanol;
- (ii) mixing the ginsenoside extract and ethanol with an alkali-metal alcoholate solution to produce a mixture, and
- (iii) placing the resultant mixture in a reaction tank so that the resultant mixture can undergo chemical reactions at a temperature between 240 - 300EC and at a pressure between 3.5 - 8.4 MPa;
- (c) after the reaction is completed, collecting an intermediate product of a mix of ginsenosides and sapogenins from the mixture; and
- (d) separating the desired sapogenins from the intermediate saponin-sapogenin mixture by silica-gel-column chromatography.

23. A process according to any one of claims 21 and 22 wherein the alkali metal can be potassium or sodium.

24. A process according to any one of claims 21 to 23 wherein the hydroxide can be sodium hydroxide or potassium hydroxide.

25. A process according to any one of claims 21 to 24 wherein the alkali-metal alcoholate solution or the concentration of hydroxide-ethanol solution is 5-50% (W/V).

26. A process according to any one of claims 21 to 25 wherein the alkali-metal alcoholate has 1-5 carbon atoms.
27. A process according to claim 21 wherein the temperature of the reaction tank is between 150-300EC and the reaction pressure is between 2.5-8.4 MPa.