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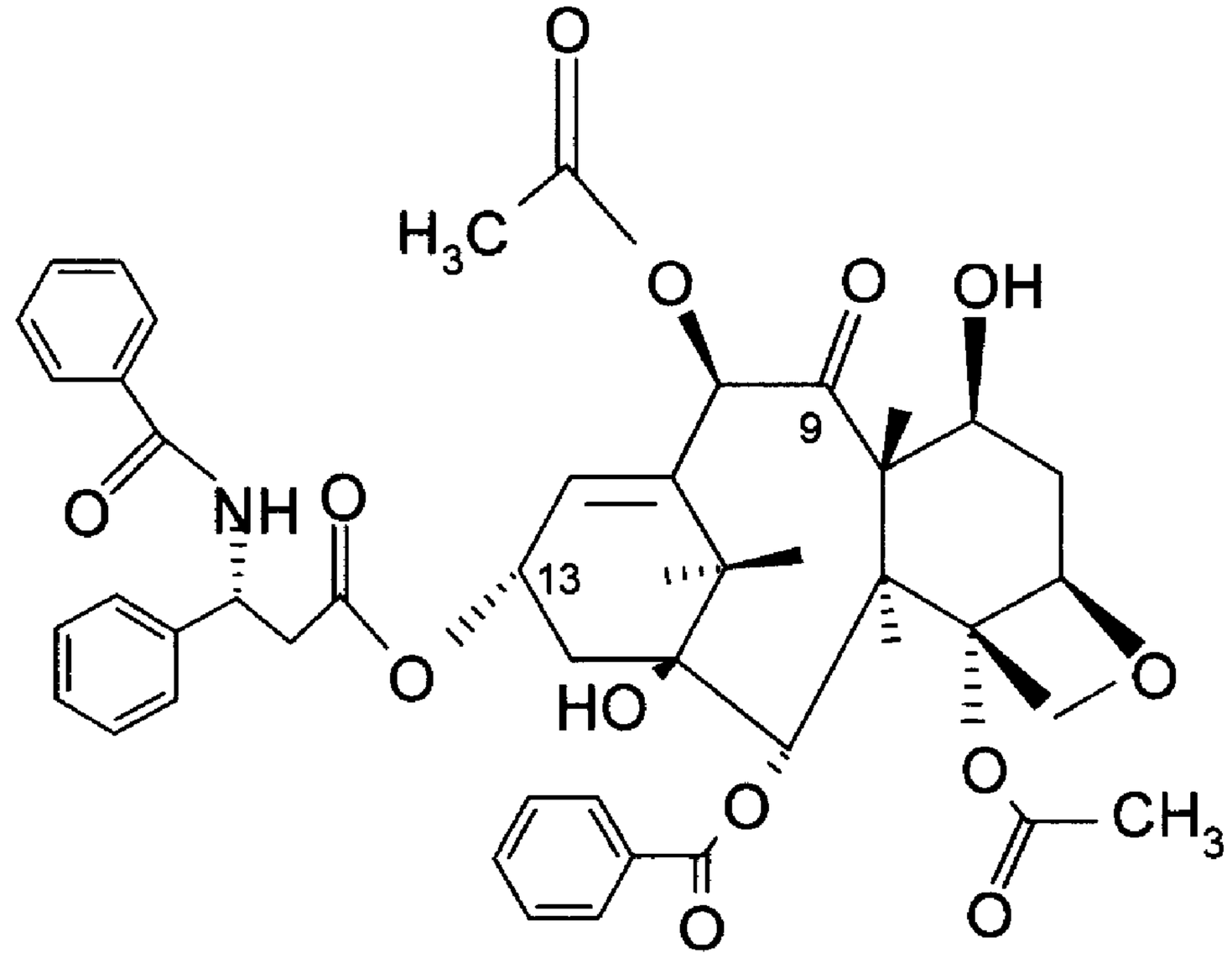
(54) Titre : PROCEDE D'ISOLEMENT DU PACLITAXEL ET DE LA 9-DIHYDRO-13-ACETYLBACCATINE III
(54) Title: PROCESS FOR THE ISOLATION OF PACLITAXEL AND 9-DIHYDRO-13-ACETYLBACCATIN III



(57) Abrégé/Abstract:
An improved method for isolating taxanes, particularly paclitaxel and 9-dihydro-13-acetylbaccatin III from crude extract of naturally occurring Taxus species including treating the extract by dry column chromatography. The advantage is better yield with less effort and at reduced expense.

PROCESS FOR THE ISOLATION OF PACLITAXEL AND 9-DIHYDRO-13-ACETYLBACCATIN III

ABSTRACT

An improved method for isolating taxanes, particularly paclitaxel and 9-dihydro-13-acetylbaccatin III from crude extract of naturally occurring *Taxus* species including treating the extract by dry column chromatography. The advantage is better yield with less effort and at reduced expense.

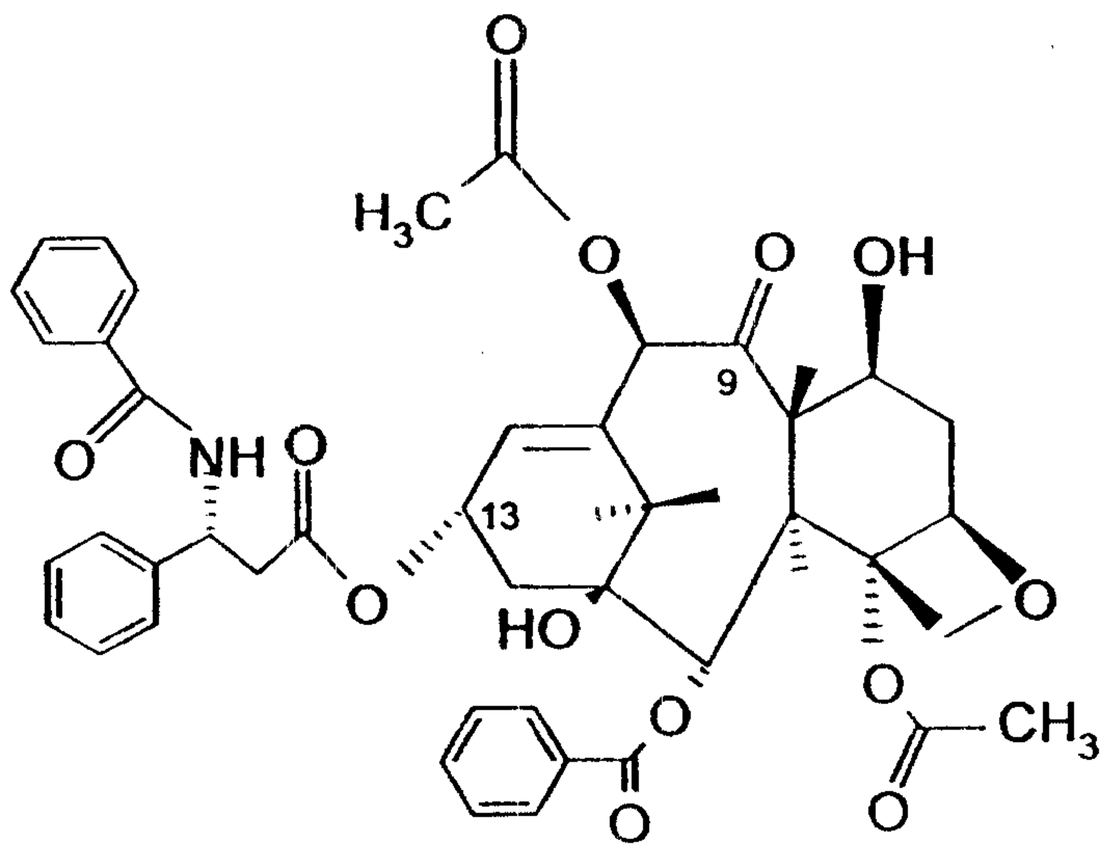
PROCESS FOR THE ISOLATION OF PACLITAXEL AND 9-DIHYDRO-13-ACETYLBACCATIN III

FIELD OF THE INVENTION

The present invention relates to a process for the isolation of taxanes and is particularly concerned with a process for the isolation of paclitaxel and 9-dihydro-13-acetylbaccatin III, from a *Taxus* species.

BACKGROUND OF THE INVENTION

Paclitaxel (Taxol™), represented by the following structural formula:



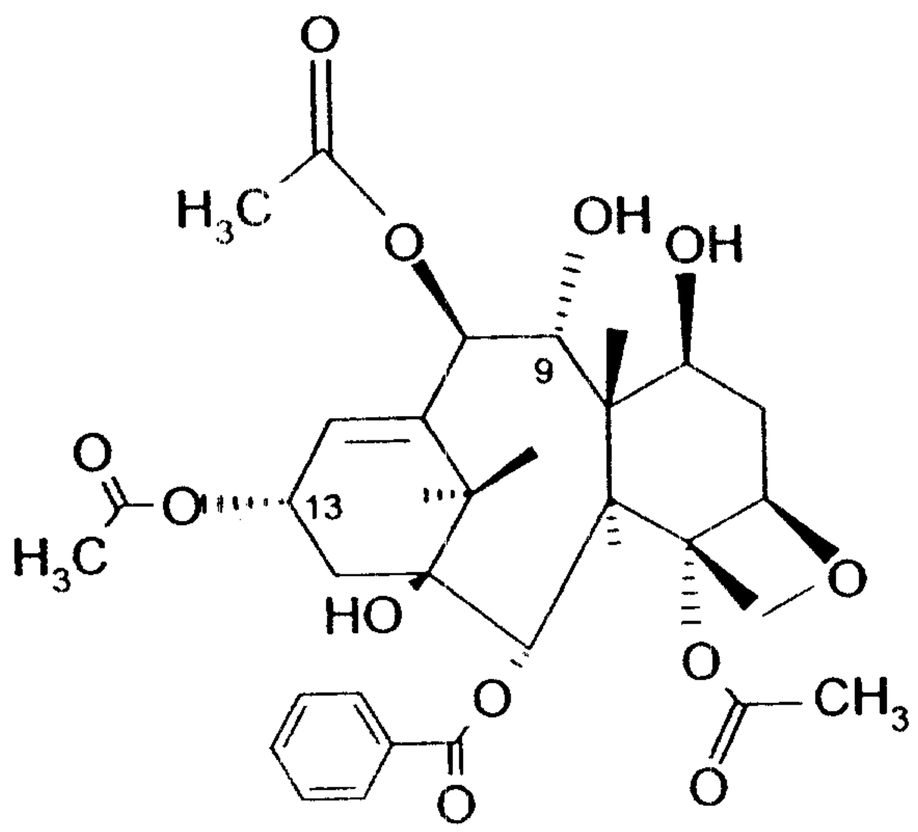
is a potent antitumor compound. Paclitaxel exhibits a unique mechanism for preventing the growth of cancer cells by affecting the microtubules, which play an important role in cell division and other cell functions. At the beginning of cell division, a large number of microtubules are produced, and as the division reaches an end, the microtubules are normally broken down.

However, paclitaxel prevents microtubules from breaking down, which has the effect of clogging up cancer cells to an extent that the cells cease to grow and divide.

Taxol™ is clinically effective for the treatment of refractory human ovarian and breast cancer, and has exhibit promising activity against a number of other types of cancers such as liver, peritoneal, cervical, prostate, colon, and esophageal cancers.

Taxol™ was primarily extracted from the bark of the Pacific yew *Taxus brevifolia*. Unfortunately, the yew grows very slow, approximately eight inches per year, and therefore the tree is a limited source of Taxol™. This has lead researchers to seek alternative means for producing Taxol™ and analogs thereof which may display superior antitumor activity, for example derivatives of Taxol™ that have enhanced solubility. 9-dihydro-13-acetylbaccatin III, a taxane diterpene, has been found to be a useful precursor in the preparation of such analogs (see U.S. Patent No. 5,440,056 issued to Klein et al. on August 8, 1995).

9-dihydro-13-acetylbaccatin III, represented by the following structural formula:



can be obtained from, for example *Taxus canadensis* which is more widely distributed than *Taxus brevifolia*. Hence, 9-dihydro-13-acetylbaccatin III is available more abundantly than Taxol™.

Conventional methods for the isolation of taxanes, including Taxol™ and 9-dihydro-13-acetylbaccatin III, generally comprise the steps of extracting taxanes from plant material with an alcoholic solvent; defatting the extract with a defatting agent; and separating and purifying the individual taxane by chromatography.

Prior art methods disclose the use of various types of chromatographic techniques to separate Taxol™ and derivatives thereof. For example, U.S. Patent No. 5,380,916 issued to Rao on January 10, 1995, describes a process using reverse phase liquid chromatography. Although reverse phase chromatography can be successful in separating the taxanes, such methods require a large amount of solvent, and hence are expensive. Moreover, reverse phase chromatography is time consuming, since it can take up to a few days to accomplish the solvent development for the column.

U.S. Patent No. 5,530,020 issued to Gunawardana et al. on June 25, 1996, discloses a process for isolating 9-dihydro-13-acetylbaccatin III from *Taxus canadensis* which employs planet coil countercurrent chromatography (PCCC). Some of the disadvantages associated with this procedure are that it is complex, it can be used to purify only a small amount (milligrams) of chemical, and it entails high cost.

SUMMARY OF THE INVENTION

An object of the present invention is to provide an improved method for isolating taxanes, particularly Taxol™ and 9-dihydro-13-acetylbaccatin III, from a *Taxus* species.

According to one aspect of the present invention, there is provided a method for isolating taxane from a crude extract of a *Taxus* species comprising the step of subjecting the crude extract to dry column chromatography.

The method of the present invention may be practiced on a small laboratory scale as well as a large industrial scale.

Conveniently, the taxane is obtained from extraction of the plant material with an alcoholic solvent, such as methanol or ethanol. Preferably, the column to be used with the method of the present invention includes a nylon tube packed with silica gel having a microsphere size of about 70-200 μm. The ratio of crude extract to silica gel ranges from 1:10 to 1:15, and preferably 1:14. The elutant is a solvent system comprising chloroform and methanol (95:5), or chloroform, methanol and acetone (10:1:0.5).

In one embodiment of the invention, the method includes the step of defatting the crude extract with a defatting agent such as hexane, pentane, petroleum ether, isooctane or mixtures thereof. Preferably, hexane is used.

In another embodiment of the invention, Taxol™ may be further purified by normal phase chromatography. Preferably, the eluting solvent for normal phase chromatography is dichloromethane and ethyl acetate (8:2), or a gradient solvent system including hexane and acetone (starting from 80:20 and progressing to 55:45). The normal phase chromatography step may be repeated several times. For small scale production, it is preferred that the normal phase chromatography step be repeated at least once.

In accordance with another aspect of the present invention, there is provided a method for obtaining taxane from a *Taxus* species comprising the steps of extracting a taxane from a ground *Taxus* species using an alcoholic solvent to obtain a first taxane-containing extract; concentrating the first taxane-containing extract to obtain a taxane-containing concentrate; extracting the taxane from the taxane-containing concentrate with chloroform or dichloromethane to form a second taxane-containing extract; concentrating the second taxane-containing extract to obtain a taxane-containing residue; separating the taxane into taxane-containing fractions by subjecting the taxane-containing residue to dry column chromatography in a dry chromatography column; and recovering any individual taxane from the taxane-containing fractions in the column.

In another embodiment to the present invention, Taxol™ may further be purified by the steps of combining all the taxane-containing fractions containing Taxol™ and 9-dihydro-13-acetylbaccatin III to form a taxane-containing solution; crystallizing the 9-dihydro-13-acetylbaccatin III out of the taxane-containing solution with an alcoholic solvent to obtain 9-dihydro-13-acetylbaccatin III crystals and a Taxol™-containing solution; purifying the Taxol™-containing solution by normal phase column chromatography.

The advantages of the present invention are to provide a method for the isolation of taxanes which is quick, easy to perform, cost-effective and efficient, both in small and large scale productions.

Having thus described the invention, reference will no be made to the accompanying drawings illustrating the preferred embodiments and in which:

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a flow diagram of a small scale process (Example 1) for isolating taxanes from *Taxus canadensis* in accordance with one embodiment of the present invention; and

Figure 2 is a flow diagram of a large scale process (Example 2) for isolating taxanes from *Taxus canadensis* in accordance with a second embodiment of the present invention.

DETAILED DESCRIPTION

The starting material for use in this invention is a plant material selected from species of Taxes, particularly *Taxus canadensis*. The taxanes can be obtained from barks, roots, wood, stems, needles, seeds or mixture thereof. Preferably, the twigs or needles or mixtures thereof are used. The plant material used may be fresh or dried.

The plant material is ground and extracted with a polar solvent such as an alcohol, preferably methanol. The extraction continues for 24 hours at room temperature, and is filtered. The extract is concentrated to about 10% of the original volume by evaporation. About an equal amount of water is added to the concentrate. The aqueous solution is extracted several times with a defatting agent such as hexane, pentane, petroleum ether, isooctane or mixtures thereof.

Preferably, the solution is extracted four times with hexane to give an aqueous layer and a non-aqueous layer. The aqueous layer is extracted five times with chloroform or dichloromethane, and the non-aqueous layer is discarded. The chloroform or dichloromethane extract is concentrated to dryness. The residue obtained is dissolved in a suitable solvent or mixtures thereof such as a mixture of chloroform, methanol and acetone (10:1:0.5), and fractionated by dry column chromatography in order to separate the taxanes contained in the residue.

Dry column chromatography (DCC) provides an alternate method to thin layer chromatography (TLC) and preparative column chromatography. The cost is much less compared to that of preparative liquid column chromatography which requires complex equipment. Samples which are separable on silica gel or neutral alumina TLC plates can also be separated by DCC. One of the advantages of DCC is the direct transferability of the conditions from TLC to DCC. All work done to determine the optimum conditions for separation of the sample on DCC can be done preliminarily on TLC plates. Once determined, these conditions can be applied to DCC which will give a degree of separation similar to that obtained on the thin-layer plate. DCC provides the further advantage that the number of fractions to be processed depends on the number of cuts made which does not usually exceed ten, and most often, does not exceed four or five cuts, which is in contrast to the "liquid-flow" columns which can require a large (>50) number of fractions to be collected. Another advantage of DCC is the use of nylon columns and fluorescent absorbents which provides simplified detection and isolation procedures. Therefore, compared to the "liquid-fill" column chromatography, dry column chromatography is often a quicker, easier and less expensive technique.

The procedures for dry column chromatography are generally set out in "Progress in Separation and Purification", Majorie, M., E.S. Perry and C.J. van Oss Ed., John Wiley & Sons, 1970, volume 3, pages 73-95.

Briefly, the general procedures for accomplishing dry column chromatography involve the use of a column including a nylon tube, but other types of tubes such as glass may also be used. The tube is placed in acetone overnight and air dried to remove any solvent. The column is prepared by sealing the tube at one end, and inserting a small pad of cotton or glass wool from the other end. The sealed end of the tube is pierced to provide a vent, and the tube is dry packed with Alumina or silica gel until it is sturdy enough to stand upright in a clamp. The sample may be poured directly onto the tube, or dissolved in a small volume of the solvent to be used for development, and then distributed evenly on the top of the column. Alternatively, the sample may be coated onto a small amount of adsorbent, dried, ground, and then added to the top of the column. The sample is passed down the column with a solvent. A constant liquid head of solvent of about one to two centimeters should be maintained at all times. The column should never run dry.

In contrast to the conventional dry column methods which take 15-30 minutes for the solvent to migrate from the top to the bottom of the column, it has been found that the process of the present invention requires from 1 to 3 hours to complete the separation of the desired compounds. Although the separation time is slower than the usual 15 to 30 minutes, DCC is still a faster technique than "liquid-filled" chromatography which can take three to four days to complete. The duration of the chromatographic runs is a function of the length of the column and the viscosity of the solvent. Separation is achieved as the mixture runs down the column. When the mixture reaches the base of the column, the development is stopped and the ends of the column are sealed. The various components of the extract can be identified by the separate bands which can be detected by, for example a UV or visible method. The column is then cut into the desired sections, and the pure compounds are eluted with polar solvents.

The present invention can be best practiced using nylon columns. Alumina or silica gel are suitable adsorbents, silica gel being preferred. Preferably the silica gel has a microsphere size of about 70-200 μm. The dimensions of the column are determined by the amount of silica gel needed to perform the separation. The amount of silica gel required is, in turn, determined by the sample size. When practicing the dry column chromatographic separation of the present invention, it has been found that the ratio of sample size to the amount of silica gel required to separate the sample ranges from 1:10 to 1:15, preferably 1:14. Hence, the amount to silica gel needed is approximately ten to fifteen times, and preferably fourteen times the amount of sample. The above ratios have been found to be optimal with respect to the cost and degree of separation.

Augmenting the amount of silica gel used will increase the cost which can be substantial in large scale production. On the other hand, reducing the amount of silica gel will result in a poor separation.

Having described the dry column chromatographic techniques and conditions suitable for the present invention, a preferred embodiment of the method for isolating taxanes in accordance with the present invention follows. The crude chloroform extract is separated into individual taxanes by charging the chloroform extract onto the top of the column, and adding a solvent mixture of chloroform, methanol and acetone (10:1:0.5) thereto. Once the solvent reaches the bottom of the column, the tube is closed, and the column is sliced into various sections. The compounds from the sliced sections are eluted with a polar solvent such as an alcohol, preferably methanol. To identify the individual taxanes, TLC is performed.

Taxol™ is separated from 9-dihydro-l3-acetylbaccatin III by purification as follows. The fractions are analyzed by TLC. Taxol™ shows a purple green color, and 9-dihydro-13-acetylbacccatin III shows a dark green color on the TLC plate. The fractions containing Taxol™ and 9-dihydro-13-acetylbaccatin III are combined, and extracted with a polar solvent such as an alcohol, in this case methanol. The methanol extract is concentrated to dryness, and the residue is dissolved with methanol to crystallize out the

9-dihydro-13-acetylbaccatin III. The crystallization normally continues overnight at room temperature.

Following crystallization, the 9-dihydro-13-acetylbaccatin III crystals are filtered and the mother liquor is evaporated. The resulting residue is dissolved in a chloroform and methanol solvent system at a ratio of 95:5. The chloroform/methanol solution is purified by normal phase column chromatography. The column is packed with silica gel having microsphere size of 70-200 μm. Useful elutants for this invention can be selected from the standard texts of chromatography. A gradient solvent system of hexane and acetone, with a ratio of hexane to acetone of about 80:20 to 55:45 is found to be particularly useful for the purpose of the present invention. Taxane fractions are collected from the column, and the ones containing Taxol™ are combined and subjected to normal phase chromatography again. The second chromatographic step uses a different developing solvent system, namely a mixture of dichloromethane and ethyl acetate (8:2). The fractions containing Taxol™ are collected and evaporated to dryness. Taxol™ is crystallized by standard techniques known in the art. In the present case, Taxol™ is crystallized from aqueous alcohol solution. For recrystallization of Taxol™, a variety of organic solvents may be used, in this case a solvent mixture of hexane and acetone (3:1) is used. Taxol™ is obtained as white needle-like crystals.

The method of the present invention can also be used for large scale production of taxanes. Generally, the procedures are similar to those mentioned above; however, they may differ in the solvents systems used. The specific conditions and solvent systems used in applying the method of the present invention on a large scale are as follows.

The plant material is ground and extracted with a polar solvent such as an alcohol, in this case ethanol. The extraction is performed at 50°C in an industrial extract unit for about 12 hours. The extract is filtered. The ethanol is evaporated to about 10% of its original volume, and the resulting concentrate is doubled in volume with water. The aqueous solution is extracted four times with 10 L of hexane. The aqueous layer is recovered, and the non-aqueous layer is discarded. The aqueous layer is extracted five times with a non-polar solvent, in this case dichloromethane. The dichloromethane is

evaporated to dryness, and the resulting residue is dissolved in a non-polar solvent or a mixture of solvents, in this case a mixture of chloroform and methanol (9:1). The chloroform and methanol solution is coated onto 1.5 kg of silica gel. The silica gel is dried to evaporate the solvent. The dried silica gel is fractionated by dry column chromatography. The column is made of a nylon, and the absorbent is silica gel. Suitable developing solvents are non-polar elutants or mixtures thereof, for example a mixture of chloroform and methanol (95:5).

The column is developed until a dark-green color fraction reaches the base of the column, at which time, the nylon column is closed by sealing off the ends and cut into portions. The portions containing Taxol™ and 9-dihydro-13-acetylbaccatin III are transferred into a second empty column, shorter in length, and is washed with a polar solvent, in this case methanol. The use of the second column is for practical purpose. The short column is used simply as a mean to hold the silica gel so that it can be washed. The sliced portions could easily have been washed by hand individually. The methanol solution is concentrated to dryness and the residue is washed with methanol, and kept at room temperature overnight. 9-dihydro-13-acetylbaccatin III is obtained as crystals. The crystals are filtered, and the mother liquor is concentrated to dryness. The residue is dissolved in chloroform and purified by normal phase chromatography.

For normal phase chromatography, the preferred adsorbent is also silica gel having a microsphere size of 70-200 μm. The eluting solvent is selected from a variety of solvents known in the art, and is preferably a mixture of dichloromethane and ethyl acetate (8:2). The fractions containing Taxol™ are combined and concentrated to dryness. The residue is dissolved in methanol. The methanol solution is kept in a refrigerator overnight. Taxol™ is obtained as white solids, which are crystallized by standard crystallization techniques. The Taxol™ is further recrystallized by dissolving the solids in small volumes of acetone. The acetone solution is diluted three times with hexane, and the mixture is allowed to sit at room temperature for 6 hours to yield purified Taxol™ as white needles-like crystals.

Example 1

Referring to Figure 1, 2 kg of twigs and needles of *Taxus canadensis* were ground. The ground material was placed in a 10 L flask, and extracted with 4 L of methanol. The extraction was continued at room temperature for 24 hours, following which, the solution was filtered. 3 L of fresh methanol was added to the flask to extract any remaining taxanes. Again, the extraction proceeded for 24 hours at room temperature, and the solution was filtered. The filtrates were combined and concentrated to approximately 800 mL. 800 mL of water was added to the concentrate. The aqueous solution was extracted four times with 3 L of hexane. The aqueous layer was recovered and the non-aqueous layer was discarded. The aqueous layer was extracted five times with 600 mL of chloroform. The chloroform solution was concentrated to dryness and the residue was dissolved in 150 mL of a solvent mixture of chloroform, methanol and acetone (10:1:0.5), and fractionated by dry column chromatography (DCC). A nylon column having dimensions of 2 inches x 30 inches was used. The column was packed with silica gel having microsphere size of about 70-200 μm. The elutant was a mixture of chloroform, methanol and acetone (10:1:0.5).

The chloroform, methanol and acetone solvent mixture containing the taxanes was charged into the column. The elutant was added, being careful to keep a constant one to two centimeters of liquid head at all times. Once the elutant reached the bottom of the column, further column development was prevented. The time required for chromatographic run was approximately 2 hours. The column was cut into 10 portions. Taxol™ and 9-dihydro-13-acetylbaccatin III were determined to be in portions 5 and 6 by TLC analysis (samples were sprayed with 10% H_2SO_4 in ethanol followed by heating at 100-105 ° C for a couple of minutes). The two portions were combined and extracted with methanol. The methanol solution was concentrated to dryness. The residue was dissolved in 50 mL methanol and kept at room temperature overnight. Some colorless crystals were formed. The crystals were filtered and recrystallized from methanol. 2 g of white needle-like crystals were obtained and identified by NMR as 9-dihydro-13-acetylbaccatin III, purity ≥98%, melting point 202-204 °C, molecular weight 630.28, chemical formula $C_{33}H_{42}O_{12}$.

^{1}H-NMR (400 MHz, $CDCl_3$): 8.07 (d, 2H, 2', 6'-Ar-H), 7.58 (dd, 1H, 4'-Ar-H), 7.45 (dd, 2H, 3', 5'-Ar-H), 6.17 (d, 1H, H-10), 6.14 (dd, 1H, H-13), 5.73 (d, 1H, H-2), 4.93 (d, 1H, H-5), 4.41 (dd, 2H, H-7, H-9), 4.28 (d, 1H, H-20a), 4.14 (d, 1H, H-20b), 3.02 (d, 1H, H-3), 2.51 (ddd, 1H, H-6a), 2.26 (s, 3H, 10-OAc), 2.18 (m, 2H, H-14), 2.17 (s, 3H, 4-OAc), 2.12 (s, 3H, 13-OAc), 1.91(dd, 1H, H-6b), 1.91 (s, 3H, 18-CH_3), 1.79 (s, 3H, 19- CH_3), 1.66 (s, 3H, 16- CH_3), 1.23 (s, 3H, 17-CH_3) ppm.

^{13}C-NMR (100 MHz, $CDC1_3$): 170.55, 170.48, 169.38, 167.00, 139.58, 134.88, 133.69, 130.05, 129.18, 128.62, 84.03, 82.05, 78.74, 76.77, 76.55, 73.91, 73.49, 73.18, 69.74, 47.07, 44.84, 43.00, 37.92, 35.29, 28.27, 22.84, 22.56, 21.34, 21.24, 14.86, 12.48 ppm.

The mother liquor from the crystallization of 9-dihydro-13-acetylbaccatin III was concentrated to dryness and dissolved in chloroform and methanol (95:5), and purified by normal phase chromatography. A 1.5 cm x 50 cm column was used. The column was packed with silica gel. The eluting solvent was a decreasing gradient system of hexane and acetone (starting from 80:20 and progressing to 55:45). Fractions of 40 mL were collected. The fractions containing Taxol™ were combined and purified again with normal phase chromatography. The eluting solvent for the second normal phase chromatography purification step was dichloromethane and ethyl acetate (8:2). The fractions which contained Taxol™ were combined and concentrated to dryness, and dissolved in aqueous methanol and kept in a refrigerator overnight to yield solid crystals, which were filtered and recrystallized from hexane and acetone (3:1) to further yield white needle-like crystals identified by NMR as Taxol™, yield 300 mg (0.015%), melting point 200-205 °C, molecular weight 853.9, chemical formula $C_{47}H_{51}NO_{14}$.

^{1}H-NMR (400 MHz, $CDC1_3$): 8.12 (d, 2H-Ar-H), 7.73 (d, 2H-Ar-H), 7.61 (dd, 1H-Ar-H), 7.51 (m, 5H-Ar-H), 7.41 (m, 4H-Ar-H), 7.38 (dd, 1H-Ar-H), 6.98 (d, 1H, N-H), 6.27 (s, 1H, 10-H), 6.23 (dd, 1H, 13-H), 5.78 (dd, 1H, H3'), 5.67 (d, 1H, 2-H), 4.94 (d, 1H, 5-H), 4.79 (dd, H-2'), 4.40 (dd, 1H, 7-H), 4.30 (d, 1H, 20-Ha), 4.19 (d, 1H, 20-Hb), 3.79 (d, 3-H), 3.54 (d, 1H, -OH), 2.51 (ddd, 1H, 6-Ha), 2.46 (d, 1H, -OH), 2.38 (s, 3H, 4-

OAc), 2.33 (m, 2H, 14-H), 2.24 (s, 3H, 10-OAc), 1.89 (ddd, 1H, 6-Hb),1.79 (s, 3H, 18-CH_3), 1.68 (s, 3H, 19- CH_3), 1.24 (s, 3H, 16-CH_3), 1.14 (s, 3H, 17- CH_3) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$): 203.60, 172.69, 171.25, 170.33, 166.97, 141.94, 137.91, 133.70, 133.56, 133.14, 131.97, 130.18, 129.09, 129.02, 128.68, 128.36, 127.00, 84.37, 81.13, 79.00, 77.18, 76.48, 76.26, 75.53, 74.89, 73.14, 72.38, 72.17, 58.59, 54.98, 45.56, 43.14, 35.64, 35.57, 26.84, 22.61, 21.79, 20.83, 14.83, 9.53 ppm.

Example 2

Referring to Figure 2, about 45 Kg of fresh needles and twigs of *Taxus canadensis* were crushed into small pieces. The crushed plant material was extracted with 200 L of ethanol at 50 °C in an industrial extract or unit for 12 hours, and filtered. The ethanol extract was concentrated to 20 L, and 20 L of water was added. The aqueous solution was extracted four times with 10 L of hexane. The aqueous layer was recovered and the non-aqueous layer was discarded. The aqueous layer was further extracted five times with 10 L of dichloromethane. The dichloromethane solution was concentrated to dryness. The residue was dissolved in 800 mL of chloroform and methanol (9:1) solution. The solution was coated onto 1.5 kg of silica gel. The silica gel was dried to remove any solvents, and ground to 70-200 μm. The powdered silica gel was fractionated by dry column chromatography. Eight columns were used. The columns were nylon tubes having dimensions of 5 cm x 90 cm packed with silica gel. It should be noted that the exact dimensions of the columns are not crucial as long as the columns are big enough to hold the amount of silica gel required for separation. The developing solvent utilized was chloroform and methanol (95:5).

The powdered silica gel was charged into the top of the column, and the developing solvent was added. After the solvent moved to the base of the column, the column development continued until the first colored fraction moved to the base of the column, at which time the column development was stopped and the column was cut into 10 sections. The time required for the chromatographic run was about 3 hours.

The portions which contained Taxol™ and 9-dihydro-13-acetylbaccatin III were put into a short column, and washed with methanol to elute the compounds. The fractions could also be washed by hand. The methanol solution was concentrated to dryness. A small volume of fresh methanol was added to the residue. The methanol solution was kept at room temperature overnight to yield needle-like crystals, which were filtered and recrystallized from methanol to further yield white needles-like crystals identified as 9-dihydro-13-acetylbaccatin III, yield 10.3g (0.023% based on fresh material).

The mother liquor from the 9-dihydro-13-acetylbaccatin III crystallization was concentrated to dryness, and the residue was dissolved in 150 mL of chloroform and purified by normal phase chromatography (4 cm x 90 cm column of silica gel). The eluting solvent was dichloromethane and ethyl acetate (8:2). 50 fractions 200 mL each were collected. The fractions which contained Taxol™ were combined and concentrated to dryness. The residue was dissolved in aqueous methanol, and the methanol solution was kept in a refrigerator overnight. Taxol™ was obtained as a white solid. The crude Taxol™ was dissolved in 50 mL of acetone, and 150 mL of hexane was added. The mixture was kept at room temperature for 6 hours to give some white needles-like crystals, which were filtered and dried at 60 °C for 4 hours, yield 3.0 g (0.007%), purity ≥99%.

While the foregoing embodiments of the present invention have been described and shown, it is understood that all alternatives and modifications that may be made thereto and fall within the scope of the invention.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method for isolating a taxane from a crude extract of a *Taxus* species comprising the step of subjecting the crude extract to dry column chromatography.

2. The method according to claim 1, wherein said dry column chromatography is performed in a nylon column packed with silica gel.

3. The method according to claim 1 or 2, wherein said ratio of crude extract to silica gel is 1:14.

4. The method according to claim 1 or 2, wherein said column is loaded with a chloroform and methanol solvent system having a ratio of chloroform to methanol of about 95:5.

5. The method according to any one of claims 1 through 3, wherein said column is loaded with a chloroform, methanol and acetone solvent system having a ratio of chloroform to methanol to acetone of about 10:1:0.5.

6. The method according to claim 1, wherein said taxane is selected from the group consisting of Taxol™ and 9-dihydro-13-acetylbaccatin III.

7. The method according to claim 1, wherein said *Taxus* species is *Taxus canadensis*.

8. The method according to claim 1, wherein said crude extract is obtained from the group consisting of the needles and twigs of the *Taxus* species.

9. The method according to any one of claims 1 through 8, wherein said crude extract is obtained by extraction with an alcoholic solvent.

10. The method according to claim 1, further including the step of defatting said crude extract with a defatting agent selected from the group consisting of hexane, pentane, petroleum ether, isooctane and mixtures thereof.

11. The method according to claim 6, further including the step of purifying Taxol™ by normal phase chromatography.

12. The method according to claim 11, wherein said normal phase chromatography is performed using a dichloromethane and ethyl acetate solvent system having a ratio of dichloromethane to ethyl acetate of about 8:2.

13. The method according to claim 11, wherein said normal phase chromatography is performed using a hexane and acetone gradient solvent system having a ratio of hexane to acetone of between about 80:20 and about 55:45.

14. A method for obtaining a taxane from a *Taxus* species comprising the steps of:

(a) extracting a taxane from a ground *Taxus* species using an alcoholic solvent to obtain a first taxane-containing extract;

(b) concentrating said first taxane-containing extract to obtain a taxane-containing concentrate;

(c) extracting said taxane from said taxane-containing concentrate with a solvent system selected from the group consisting of chloroform and dichloromethane to form a second taxane-containing extract;

(d) concentrating said second taxane-containing extract to obtain a taxane-containing residue;

(e) separating said taxane into taxane-containing fractions by subjecting said taxane-containing residue to dry column chromatography, said column containing silica gel microspheres in a size range of between about 70 and about 200 microns;

(f) maintaining a ration of residue to silica gel in a range of between about 1:10 and 1:15; and

(g) recovering any individual taxane from the taxane-containing fractions.

15. The method according to claim 14, further including the step of defatting said taxane-containing concentrate with a defatting agent selected from the group consisting of hexane, pentane, petroleum ether, isooctane and mixtures thereof.

16. The method according to claim 14, wherein said dry column chromatography is a column having a nylon tube packed with silica gel.

17. The method according to claim 14, wherein the ratio of said taxane-containing residue to silica gel is 1:14.

18. The method according to any one of claims 14 through 17, wherein said column is loaded with a chloroform and methanol solvent system having a ratio of chloroform to methanol of about 95:5.

19. The method according to any one of claims 14 through 17, wherein said column is loaded with a chloroform, methanol and acetone solvent system having a ratio of chloroform to methanol to acetone of about 10:1:0.5.

20. The method according to any one of claims 14 through 19, wherein said taxane is selected from the group consisting of Taxol™ and 9-dihydro-13-acetylbaccatin III.

21. The method according to claim 20, wherein said Taxol™ is purified by further purification comprising the steps of:

(i) combining all the taxane-containing fractions containing Taxol™ and 9-dihydro-13-acetylbaccatin III to form a taxane-containing solution;

(ii) crystallizing the 9-dihydro-l3-acetylbaccatin III out of the taxane-containing solution with an alcoholic solvent to obtain 9-dihydro-l3-acetylbaccatin III crystals and a Taxol™ containing solution; and

(iii) purifying said Taxol™ containing solution by normal phase column chromatography.

22. The method according to claim 21, wherein purifying said Taxol™ containing solution by normal phase column chromatography is repeated.

23. The method according to claim 22, wherein said normal phase column chromatography is performed using a dichloromethane and ethyl acetate solvent system having a ratio of dichloromethane to ethyl acetate of about 8:2.

24. The method according to any one of claims 21 or 22, wherein said normal phase chromatography is performed using a hexane and acetone gradient solvent system having a ratio of hexane to acetone of about 80:20 to about 55:45.

FIGURE 1

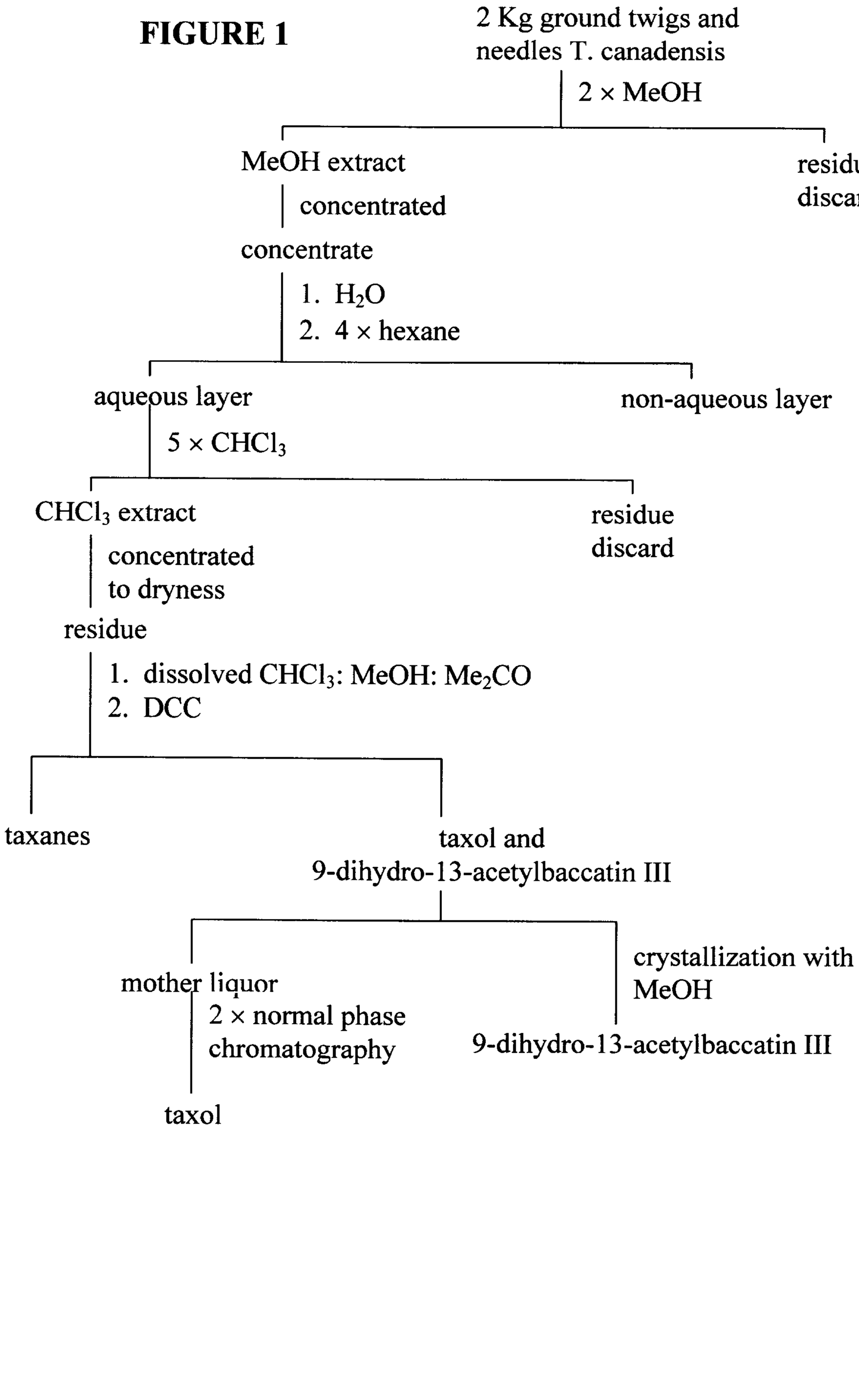
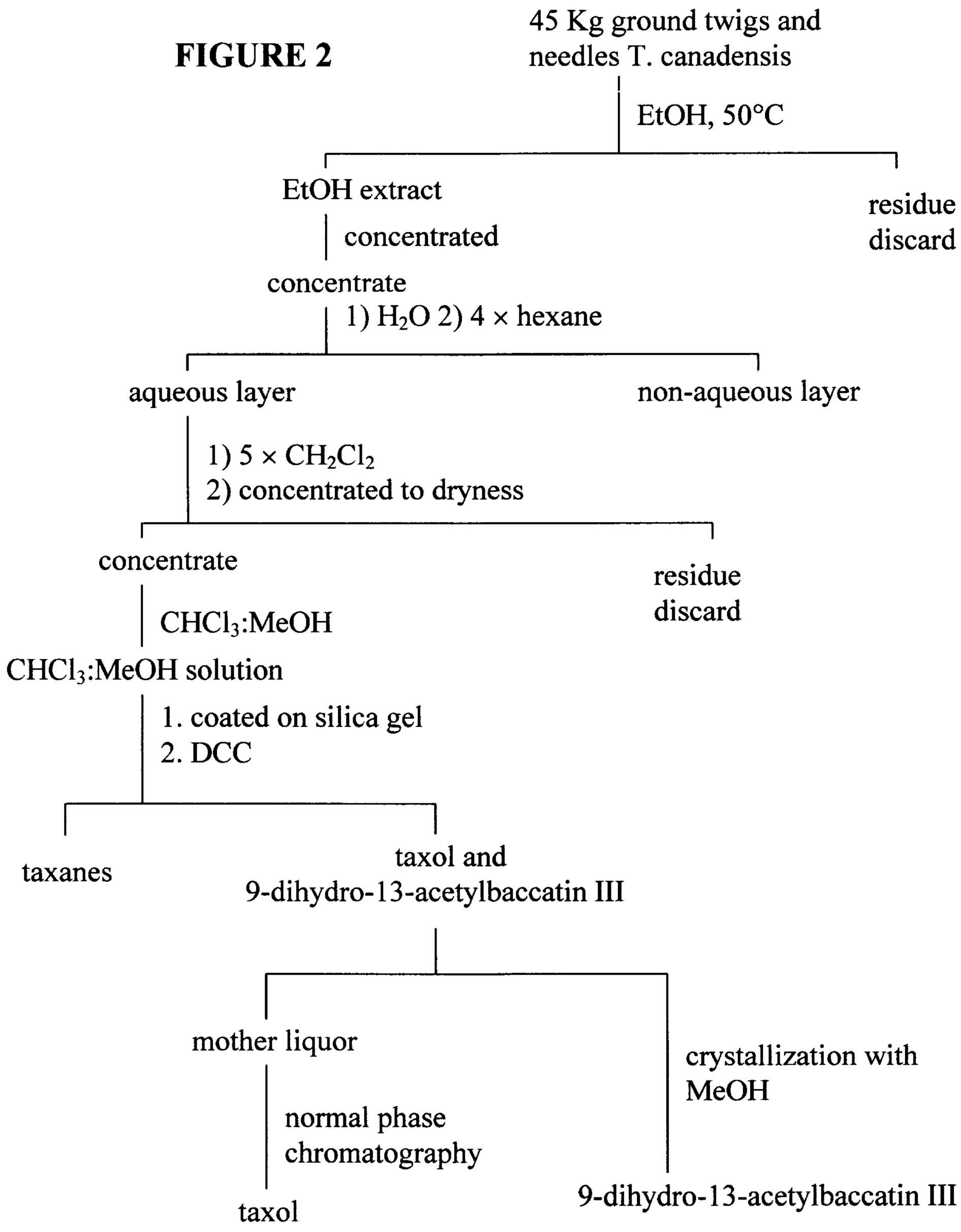
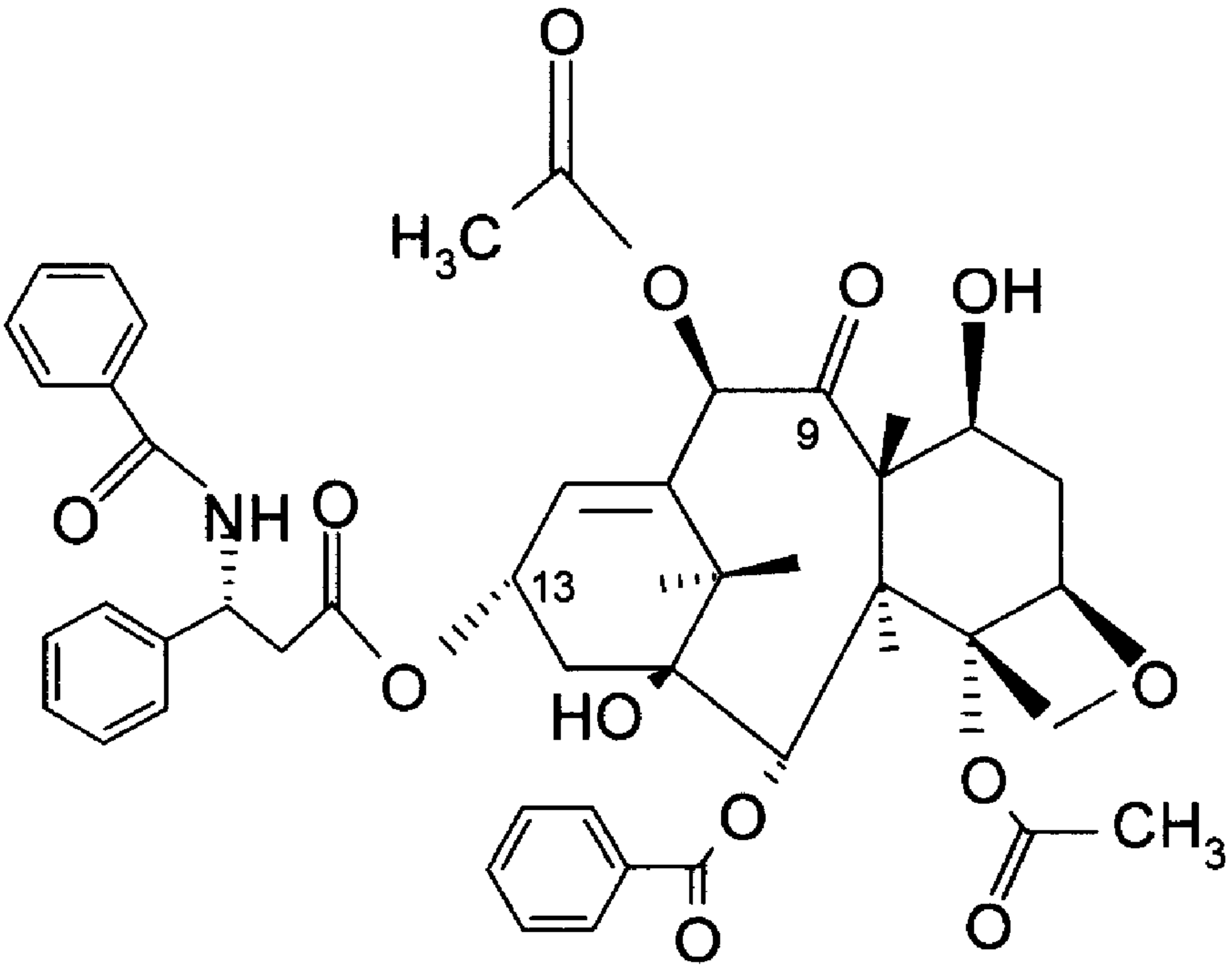

2 Kg ground twigs and needles T. canadensis
2 × MeOH
MeOH extract
residue
discard
concentrated
concentrate
1. H_2O
2. 4 × hexane
aqueous layer
non-aqueous layer
5 × $CHCl_3$
$CHCl_3$ extract
residue
discard
concentrated to dryness
residue
1. dissolved $CHCl_3$: MeOH: Me_2CO
2. DCC
taxanes
taxol and 9-dihydro-13-acetylbaccatin III
mother liquor
crystallization with MeOH
2 × normal phase chromatography
9-dihydro-13-acetylbaccatin III
taxol

FIGURE 2


45 Kg ground twigs and needles T. canadensis
EtOH, 50°C
EtOH extract
residue discard
concentrated
concentrate
1) H_2O 2) 4 × hexane
aqueous layer
non-aqueous layer
1) 5 × CH_2Cl_2
2) concentrated to dryness
concentrate
residue discard
$CHCl_3$:MeOH
$CHCl_3$:MeOH solution
1. coated on silica gel
2. DCC
taxanes
taxol and 9-dihydro-13-acetylbaccatin III
mother liquor
crystallization with MeOH
normal phase chromatography
taxol
9-dihydro-13-acetylbaccatin III


O
H3C
O
O
OH
9
O
NH
O
13
O
HO
O
O
O
CH3
O