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PATENT SPECIFICATION

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The inventors of this invention in the sense of being the devisers thereof within the meaning of Section 16 of the Patents Act 1949 are:—JANY RENZ, ALBERT VON WARTBURG, EMIL ANGLIKER and HANS EMMENEGGER, of Kirschblutenweg 12, Basle, Switzerland, Hebelstrasse 47, Basle, Switzerland, Passwangstrasse 14, Basle, Switzerland, Stockertweg 6, Muttenz, Switzerland, respectively, all Swiss citizens.

COMPLETE SPECIFICATION

Improvements in or relating to Podophyllum Glucosides

We, SANDOZ PATENTS LIMITED of 590 Jarvis Street, Toronto 5, Ontario, Canada, a Canadian Body Corporate, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a product having a cytostatic effect, especially a mitosis inhibiting effect, which product is derived from podophyllum rhizomes.

Podophyllotoxin and certain of its derivatives, e.g. 4'-demethyl-podophyllotoxin, α-peltatin and β-peltatin, which are present in water insoluble resin fractions from podophyllum rhizomes, have a cytostatic effect, but their high toxicity prevents their therapeutic use; although glucosides of these compounds have a lower toxicity and higher water solubility, while having a similar cytostatic effect, these glucosides are very readily split to form glucose and the corresponding toxic aglucone. These glucosides have no advantage over the aglucones for use as oral therapeutic agents, since the aglucones are liberated from these by the digestive ferments. Though fermentative break-down of these glucosides in the animal body can be prevented and their toxicity kept low without substantial loss of cytostatic effect by blocking with a carbonyl compound free hydroxyl groups of the sugar residue, there remains the disadvantage that pure podophyllum glucosides can only be obtained after difficult and expensive purification and separation operations; hence the condensation products of these glucosides with carbonyl compounds are available only in small quantities and at a very high price.

It has now been discovered that the reaction product of an extract of podophyllum rhizomes containing a mixture of at least two cytostatically active principles, with a carbonyl compound has an appreciably more pronounced cytostatic effect than that of a pure podophyllum glucoside with that carbonyl compound. Furthermore, the toxicity of the former product is about the same as that of the latter product, but the former possesses a much wider therapeutic index in comparison and is thus better suited for the treatment of malignant tumours.

The present invention therefore provides a reaction product resulting from the extraction of dried podophyllum rhizomes with a water miscible aliphatic alcohol and directly condensing with benzaldehyde the glucoside mixture contained in said extract after removing non-glucosidal components. Two preferred examples of the podophyllum rhizomes are the rhizomes of *podophyllum peltatum L* and of *podophyllum emodi*. Preferably the removal of non-glucosidal components is effected by diluting the extract with water and shaking out with a chlorinated hydrocarbon, especially dichloroethane or another low molecular weight chlorinated hydrocarbon. Preferably the extract, prior to its being shaken out with the chlorinated hydrocarbon, is treated with a tannin precipitating agent, e.g. lead acetate. Furthermore, it is preferred to proceed in such a way that the glucoside mixture is additionally extracted from the extract diluted with water with a mixture of a chlorinated hydrocarbon, which should have a low molecular weight, containing 10 to 30% by volume of an alcohol of 1 to 5 carbon atoms. The chlorinated hydrocarbon in the chlorinated hydrocarbon/alcohol mixture is suitably chloroform; an especially

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suitable chlorinated hydrocarbon-alcohol mixture is a chloroform/butanol mixture. It is to be noted that the same low molecular weight chlorinated hydrocarbon can be used both for the extraction with that material only and for the extraction with that material in admixture with the said alcohol, and it is only for technical reasons that dichloroethane is preferred for the first mentioned extraction while chloroform is preferred for the second one. The extract of the podophyllum rhizomes used as starting material for the product of the invention may be obtained in any desired manner, e.g. as described in the Examples given hereinafter.

It is stressed that the extract from which the reaction product of the present invention is made is not especially purified, although, of course, most of the cystostatically inactive material is removed by extraction when proceeding as indicated in the Examples. The chlorinated hydrocarbon extraction removes undesired accompanying matter, e.g. aglucones, resins and fatty substances.

It should be noted that podophyllum rhizomes of any type may be used but, as indicated above, rhizomes of *podophyllum peltatum L* and *podophyllum emodi* are preferable for the purposes of the present invention.

The present invention also provides a pharmaceutical composition containing, in addition to a physiologically acceptable carrier, the reaction product of the invention.

In the following Tables the cytostatic effect, toxicity and inhibition of growth of Ehrlich's ascites tumour of the end product of Example 3 (termed herein "SPG 827") was compared with that of the pure podophyllotoxin-benzylidene-β-D-glucoside.

TABLE 1

Cytostatic Action in vitro

Material	DE_{50}
SPG 827	0.5 µg/ml
Podophyllotoxin-benzylidene-β-D-glucoside	3.5 µg/ml

(De_{50} = that dosage which inhibits by 50% the increase of P-815 mastocytoma cells in vitro)

Furthermore, compared with SPG 827, approximately seven times the amount of podophyllotoxin - benzylidene - β - D - glucoside were required to obtain total inhibition of mitosis in a culture of embryonic fibroblasts of fowls.

TABLE 2

Toxicity (mouse, acute)

Material	DL_{50}
SPG 827	220 mg/kg i.v. 680 mg/kg p.o.
Podophyllotoxin-benzylidene-β-D-glucoside	250 mg/kg i.v. 760 mg/kg p.o.

In Table 3 the intraperitoneal treatment was effected twice daily with a total of 16 injections. Subsequently determination of the total number of tumour cells in the abdominal cavity and comparison with untreated control mice was effected.

TABLE 3

Inhibition of growth of Ehrlich's ascites tumour

Dosage (mg/kg)	% inhibition of tumour growth	
	SPG 827	Podophyllotoxin-benzylidene-β-D-glucoside
2	92	0
5	100	0
10	100	0
20	100	7
40	100	47

The product of the invention is intended for the treatment of malignant tumours in animals, e.g. carcinomas, sarcomas and condyloma acuminatum; the products of Examples 3 and 4 are especially suitable for treating humans. It may be used externally or orally, either alone or in the form of a pharmaceutical composition for enteral or external administration. In order to produce pharmaceutical compositions the product of the invention may be processed with one or more inorganic or organic pharmaceutically tolerable adjuvants as exemplified by the following:

for tablets and dragees: lactose, starch, talc or stearic acid,

for suppositories: natural or hardened oils or waxes.

Furthermore, suitable preserving agents, stabilizing agents, wetting agents, solubilizers, sweetening and colouring materials, or flavourings may be incorporated.

It has been found useful to use SPG 827 for oral therapy in capsules of 10—50 mg, the daily dosage being 150—500 mg of SPG 827. For patients who are unable to swallow capsules, or for local application to tumours which are externally accessible, a solution containing 100 mg of SPG 827 per ml of solution can be used with advantage.

The following Examples illustrate the invention without, however, limiting it.

EXAMPLE 1:

Dried rhizomes of *podophyllum emodi* containing about two per cent of glucosides are finely ground and exhaustively extracted with methanol at room temperature. The united extracts are concentrated to approximately $\frac{1}{3}$ of their volume, and then shaken out with dichloroethane, after addition of half the volume of water. This dichloroethane solution, which is preferably washed once more with water, contains the aglucones, resins, fatty materials and accompanying matter and is practically free of podophyllum glucosides. The aqueous-methanolic solution is united with the wash-water from the dichloroethane fraction and this in turn shaken out with a 4:1 chloroform-butanol mixture.

The united chloroform-butanol extracts are evaporated, the residue dissolved in three parts of benzaldehyde and the solution stirred for two hours at room temperature with two parts of zinc chloride. It is then diluted with an equal amount of water and the condensation product shaken out with chloroform. By squirting the concentrated chloroform solution into benzine and washing out the resulting precipitate with benzine the condensation product is obtained free of benzaldehyde and benzoic acid. This precipitation operation may be repeated a number of times in order to obtain a particularly low aglucone content.

A product is obtained constituted by weight an aglucone and resin fraction (about 4%), podophyllotoxin - benzylidene - β - D - glucoside (about 57%), 4'-demethyl-podophillotoxin-benzylidene-β-D-glucoside (about 2%), unreacted glucosides (about 3%) and unidentified accompanying matter (about 34%).

EXAMPLE 2:

In manner analogous to that described in Example 1 a product is obtained from dried rhizomes of *podophyllum peltatum L* containing about half a per cent of glucosides, which product is constituted by weight an aglucone and resin fraction (about 7%), podophyllotoxin - benzylidene - β - D - glucoside (about 18%), β - peltatin - benzylidene - β - D-glucoside (about 13%), 4' - demethylpodophyllotoxin - benzylidene - β - D - glucoside (about 10%), α-peltatin benzylidene - β - D-

glucoside (about 8%), unreacted glucosides (about 5%) and unidentified accompanying matter (about 39%).

EXAMPLE 3:

Extraction of the dried rhizomes of *podophyllum emodi* containing about two per cent of glucosides and condensation with benzaldehyde are effected in manner analogous to that described in Example 1, but the aqueous methanol extract is freed of undesired accompanying materials before washing with dichloroethane by means of a concentrated aqueous lead acetate solution. A condensation product constituted by weight an aglucone (about 0.5%) and resin fraction (about 3.5%), podophyllotoxin - benzylidene - β - D - glucoside (about 75%), 4' - demethyl - podophyllotoxin - benzylidene - β - D - glucoside (about 7%), unreacted glucosides (about 3%) and unidentified substances (about 11%) is thus obtained.

EXAMPLE 4:

A condensation product is obtained from *podophyllum peltatum L* rhizomes containing about half a ~~percent~~ per cent of glucosides in manner analogous to that of Example 3, which product is constituted by weight an aglucone and resin fraction (about 4%), podophyllotoxin - benzylidene - β - D - glucoside (about 50%), ~~B~~ - peltatin - benzylidene - β - D-glucoside (about 15%), 4' - demethyl - podophyllotoxin - benzylidene - β - D - glucoside (about 15%), α - peltatin - benzylidene - β-D-glucoside (about 6%), unreacted glucosides (about 3%) and unidentified accompanying matter (about 7%). It should be noted that the actual composition of the final products obtained depends on the glucoside content of the drug used as starting material. For example, using *podopyllum emodi* as starting material, the following ranges are obtainable:—

aglucone and resin fraction	0 to 10%
podophyllotoxin - benzylidene-β-D-glucoside,	50 ± 40%
4' - demethyl - podophyllotoxin-benzylidene-β-D-glucoside,	15 ± 10%
unreacted glucosides,	0 to 10%
unidentified accompanying matter	0 to 50%

Using *podophyllum peltatum L* as starting material, the following ranges are obtainable:—

aglucone and resin fraction,	0 to 10%
podophyllotoxin-benzylidene-β-D-glucoside,	30 ± 30%
β-peltatin-benzylidene-β-D-glucoside,	30 ± 30%
4'-demethyl-podophyllotoxin-benzylidene-β-D-glucoside,	10 ± 10%
α-peltatin-benzylidene-β-D-glucoside,	20 ± 20%
unreacted glucosides	0 to 10%
unidentified accompanying matter	0 to 20%

WHAT WE CLAIM IS:—

1. A reaction product resulting from the extraction of dried podophyllum rhizomes with a water miscible aliphatic alcohol and the direct condensation with benzaldehyde of the glucoside mixture contained in said extract after removal of non-glucosidal components.

2. A product according to Claim 1, in which the removal of non-glucosidal components is effected by diluting the extract with water and shaking out with a chlorinated hydrocarbon.

3. A product according to Claim 2, in which the chlorinated hydrocarbon is dichloroethane.

4. A product according to either one of Claims 2 and 3, in which the extract is treated with a tannin precipitating agent prior to its being shaken out with a chlorinated hydrocarbon.

5. A product according to Claim 4, in which the tannin precipitating agent is lead acetate.

6. A product according to any one of Claims 2 to 5, in which the glucoside mixture is additionally extracted from the extract diluted with water with a mixture of a chlorinated hydrocarbon containing 10 to 30% by volume of an alcohol of 1 to 5 carbon atoms.

7. A product according to Claim 6, in which the chlorinated hydrocarbon is chloroform.

8. A product according to Claim 6, in which the mixture of chlorinated hydrocarbon and alcohol is a chloroform/butanol mixture.

9. A product according to any one of Claims 1—8, in which the podophyllum rhizomes are *podophyllum peltatum L* rhizomes.

10. A product according to any one of Claims 1—8, in which the podophyllum rhizomes are *podophyllum emodi* rhizomes.

11. A mitosis inhibiting product whenever obtained by the procedure substantially as described in either of Examples 1 and 2.

12. A mitosis inhibiting product whenever obtained by the procedure substantially as described in either of Examples 3 and 4.

13. A product according to Claim 1 having the constitution substantially set forth in Example 1.

14. A product according to Claim 1 having the constitution substantially set forth in Example 2.

15. A product according to Claim 1 having the constitution substantially set forth in Example 3.

16. A product according to Claim 1 having the constitution substantially set forth in Example 4.

17. A pharmaceutical composition containing, in addition to a physiologically acceptable carrier, a composition as claimed in any one of the preceding Claims.

glucoside (about 8%), unreacted glucosides (about 5%) and unidentified accompanying matter (about 39%).

EXAMPLE 3:

Extraction of the dried rhizomes of *podophyllum emodi* containing about two per cent of glucosides and condensation with benzaldehyde are effected in manner analogous to that described in Example 1, but the aqueous methanol extract is freed of undesired accompanying materials before washing with dichloroethane by means of a concentrated aqueous lead acetate solution. A condensation product constituted by weight an aglucone (about 0.5%) and resin fraction (about 3.5%), podophyllotoxin - benzylidene - β - D - glucoside (about 75%), 4' - demethyl - podophyllotoxin - benzylidene - β - D - glucoside (about 7%), unreacted glucosides (about 3%) and unidentified substances (about 11%) is thus obtained.

EXAMPLE 4:

A condensation product is obtained from *podophyllum peltatum L* rhizomes containing about half a ~~percent~~ per cent of glucosides in manner analogous to that of Example 3, which product is constituted by weight an aglucone and resin fraction (about 4%), podophyllotoxin - benzylidene - β - D - glucoside (about 50%), ~~B~~ - peltatin - benzylidene - β - D-glucoside (about 15%), 4' - demethyl - podophyllotoxin - benzylidene - β - D - glucoside (about 15%), α - peltatin - benzylidene - β-D-glucoside (about 6%), unreacted glucosides (about 3%) and unidentified accompanying matter (about 7%). It should be noted that the actual composition of the final products obtained depends on the glucoside content of the drug used as starting material. For example, using *podopyllum emodi* as starting material, the following ranges are obtainable:—

aglucone and resin fraction	0 to 10%
podophyllotoxin - benzylidene-β-D-glucoside,	50 ± 40%
4' - demethyl - podophyllotoxin-benzylidene-β-D-glucoside,	15 ± 10%
unreacted glucosides,	0 to 10%
unidentified accompanying matter	0 to 50%

Using *podophyllum peltatum L* as starting material, the following ranges are obtainable:—

aglucone and resin fraction,	0 to 10%
podophyllotoxin-benzylidene-β-D-glucoside,	30 ± 30%
β-peltatin-benzylidene-β-D-glucoside,	30 ± 30%
4'-demethyl-podophyllotoxin-benzylidene-β-D-glucoside,	10 ± 10%
α-peltatin-benzylidene-β-D-glucoside,	20 ± 20%
unreacted glucosides	0 to 10%
unidentified accompanying matter	0 to 20%

WHAT WE CLAIM IS:—

1. A reaction product resulting from the extraction of dried podophyllum rhizomes with a water miscible aliphatic alcohol and the direct condensation with benzaldehyde of the glucoside mixture contained in said extract after removal of non-glucosidal components.

2. A product according to Claim 1, in which the removal of non-glucosidal components is effected by diluting the extract with water and shaking out with a chlorinated hydrocarbon.

3. A product according to Claim 2, in which the chlorinated hydrocarbon is dichloroethane.

4. A product according to either one of Claims 2 and 3, in which the extract is treated with a tannin precipitating agent prior to its being shaken out with a chlorinated hydrocarbon.

5. A product according to Claim 4, in which the tannin precipitating agent is lead acetate.

6. A product according to any one of Claims 2 to 5, in which the glucoside mixture is additionally extracted from the extract diluted with water with a mixture of a chlorinated hydrocarbon containing 10 to 30% by volume of an alcohol of 1 to 5 carbon atoms.

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8. A product according to Claim 6, in which the mixture of chlorinated hydrocarbon and alcohol is a chloroform/butanol mixture.

9. A product according to any one of Claims 1—8, in which the podophyllum rhizomes are *podophyllum peltatum L* rhizomes.

10. A product according to any one of Claims 1—8, in which the podophyllum rhizomes are *podophyllum emodi* rhizomes.

11. A mitosis inhibiting product whenever obtained by the procedure substantially as described in either of Examples 1 and 2.

12. A mitosis inhibiting product whenever obtained by the procedure substantially as described in either of Examples 3 and 4.

13. A product according to Claim 1 having the constitution substantially set forth in Example 1.

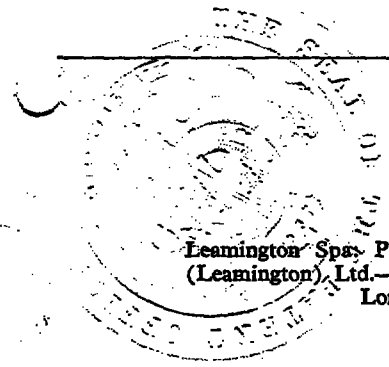
14. A product according to Claim 1 having the constitution substantially set forth in Example 2.

15. A product according to Claim 1 having the constitution substantially set forth in Example 3.

16. A product according to Claim 1 having the constitution substantially set forth in Example 4.

17. A pharmaceutical composition containing, in addition to a physiologically acceptable carrier, a composition as claimed in any one of the preceding Claims.

* C L M S *



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