PATENT SPECIFICATION

NO DRAWINGS

Inventor: LUIS ERNESTO MIRAMONTES

842,683

Date of Application and filing Complete Specification: June 16, 1958.

No. 19184/58.

Complete Specification Published: [illegible]

Index

Inter

ERRATA

SPECIFICATION No. 842,683

Page 2, line 18, *for* "R1" *read* "R^1"
Page 3, line 44, *after* "infra-red" *insert* "absorption"
Page 3, line 68, *for* "recrystallisation" *read* "crystallisation"
Page 3, line 95, *for* "sagogenin" *read* "sapogenin"
Page 4, line 18, *for* "ethyl-hexane" *read* "ethyl acetate-hexane"

THE PATENT OFFICE
12th December, 1960

[illegible] derivatives produced, thereby.

More particularly, the present invention is directed to the separation of hecogenin from a complex mixture of sapogenins. This mixture typically comprises hecogenin, mixed with non-oxosapogenins such as tigogenin, sarsasapogenin and diosgenin.

Such complex mixtures of sapogenins which contain oxo compounds, primarily hecogenin, in the past have been resolved by the application of various solvents to effect a selective solution of the desired compounds and fractional recrystallisation from the crude mixture. Such prior processes were most laborious and required numerous extractions and recrystallisations to effect the separation of the products. These processes were not efficient and the compounds produced were of questionable purity.

The process of this invention has as a first or primary step the treament of the sapogenin mixture with a hydrazine to convert the oxo-compounds to the hydrazone derivatives. These hydrazones have a marked solubility in polar solvents, as compared to their relative insolubility in the useful solvents for the non-oxo compounds of less polar character.

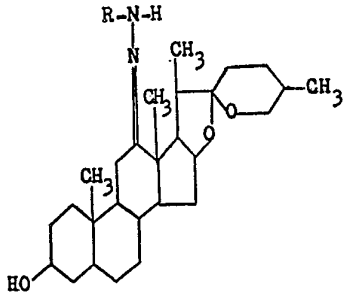
It has been found that formation of the novel oily hydrazone derivatives of hecogenin in the sapogenin mixture can be produced by

[*Price 3s. 6d.*]

[illegible] Price 3s. 6d.

[illegible] are oily liquids and trituration with the solvent is more efficient, sharper separations and purer products are obtained.

The oily hydrazone intermediates of this invention are compounds of the general structural formula:



wherein R is hydrogen, an alkyl radical containing from 1 to 6 carbon atoms or a —CS—NH_2 radical. Where R is hydrogen, the compounds can be converted to the azine derivative by reaction with the required ketone or aldehyde, such as acetone, formaldehyde or butanone.

The hydrazone derivatives may be formed by refluxing the sapogenin mixture with an alkaline solution of the hydrazine in a polar solvent for a period of about 5 to 48 hours, preferably 18 to 36 hours. Excess of the

[illegible] Price 3s.

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Inventor: LUIS ERNESTO MIRAMONTES

842,683

Date of Application and filing Complete Specification: June 16, 1958.

No. 19184/58.

Complete Specification Published: July 27, 1960.

Index at acceptance:—Class 2(3), U(3:4A1:4D:4X:5).

International Classification:—C07c.

COMPLETE SPECIFICATION

New Sapogenins and improvements in or relating to the Treatment of Mixtures containing Sapogenins

We, G. D. SEARLE & Co., a Corporation organized and existing under the laws of the State of Delaware, United States of America, of P.O. Box 5110, Chicago 80, Illinois, United States of America, 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:—

This invention relates to the resolution of a mixture of sapogenins and to the novel hydrazone and azine derivatives produced thereby.

More particularly, the present invention is directed to the separation of hecogenin from a complex mixture of sapogenins. This mixture typically comprises hecogenin, mixed with non-oxosapogenins such as tigogenin, sarsasapogenin and diosgenin.

Such complex mixtures of sapogenins which contain oxo compounds, primarily hecogenin, in the past have been resolved by the application of various solvents to effect a selective solution of the desired compounds and fractional recrystallisation from the crude mixture. Such prior processes were most laborious and required numerous extractions and recrystallisations to effect the separation of the products. These processes were not efficient and the compounds produced were of questionable purity.

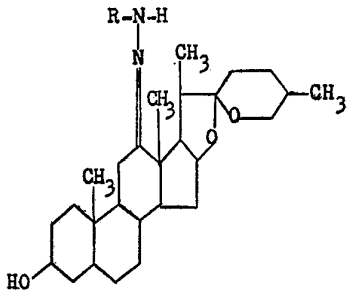
The process of this invention has as a first or primary step the treament of the sapogenin mixture with a hydrazine to convert the oxo-compounds to the hydrazone derivatives. These hydrazones have a marked solubility in polar solvents, as compared to their relative insolubility in the useful solvents for the non-oxo compounds of less polar character.

It has been found that formation of the novel oily hydrazone derivatives of hecogenin in the sapogenin mixture can be produced by heating with a hydrazine of the general formula:

$$R—NH—NH_2$$

wherein R is hydrogen, an alkyl radical containing from 1 to 6 carbon atoms or a $—CS—NH_2$ radical. These hydrazone compounds are obtained as oily liquids which are more soluble in polar solvents, such as the alkanols containing from 1 to 6 carbon atoms in the molecule, than the non-oxo sapogenins. Since they are oily liquids and trituration with the solvent is more efficient, sharper separations and purer products are obtained.

The oily hydrazone intermediates of this invention are compounds of the general structural formula:



wherein R is hydrogen, an alkyl radical containing from 1 to 6 carbon atoms or a $—CS—NH_2$ radical. Where R is hydrogen, the compounds can be converted to the azine derivative by reaction with the required ketone or aldehyde, such as acetone, formaldehyde or butanone.

The hydrazone derivatives may be formed by refluxing the sapogenin mixture with an alkaline solution of the hydrazine in a polar solvent for a period of about 5 to 48 hours, preferably 18 to 36 hours. Excess of the

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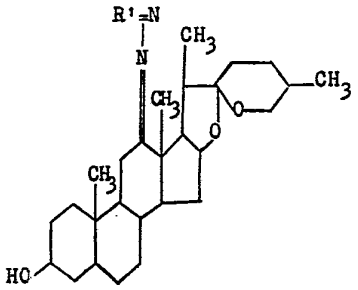
hydrazine usually provides the desired alkalinity. Hydrazines, however, such as phenylhydrazine or phenylsemicarbazine, directly form in the solution solid phenylhydrazones and phenylsemicarbazones. However, when a hydrazine of the general formula R—NH—NH_2 is used, the compound formed is oily.

The solid azines of the general structural formula:



wherein R^1 is an alkylidine radical containing from 1 to 6 carbon atoms or a 12-desoxohecogenin radical are readily isolated and may be purified by washing with solvents. It is desirable, therefore, to convert the hydrazone to the azine. This may be effected in the case of a hydrazone of the above general formula wherein R_1 is other than hydrogen by the following processes:

(a) The hydrazone can be refluxed in an organic acid, to which a small amount of ethanol or a solution of hydrogen chloride in ethanol can be added, for about 3 minutes to an hour, preferably 10 to 30 minutes, and then dried by evaporation of the acid to obtain the corresponding azine.

(b) The hydrazone solution can be vacuum distilled and then heated for a period of 3 minutes to one hour at a temperature of about 180—220° C. preferably about 210° C. for 20 to 40 minutes.

(c) The dry oily hydrazone can be refluxed in a lower alkanol with a small amount of pyridine hydrochloride for a period of about 10 minutes to one hour, preferably 20 to 45 minutes. Thereafter, the solution is rendered alkaline and heated until crystallisation occurs. The solid compound formed is isolated in a conventional mannner.

(d) The hydrazone may be refluxed in high boiling solvents such as tetrahydronaphthalene or the monoethyl ether of ethylene glycol for a period of 10 minutes to one hour.

The lower alkanol solvent containing from 1 to 6 carbon atoms in the molecule in which the hydrazone compound is formed may be methanol, ethanol, 2-propanol or tertiary butanol among which ethanol is preferred.

The pure hecogenin may be recovered from the pure hydrazone or azine by hydrolysis with acid, or refluxing a solution thereof in an alkanol containing from 1 to 6 carbon atoms in the molecule alone or admixed with a less polar solvent such as chloroform for 15 minutes to one hour at a pH of about 1 to 2.

Following initial formation of the hydrazones the solution can be concentrated e.g. by removal of from one-third to two-thirds of the alcoholic solvent by evaporation, whereupon the unreacted non-oxosapongenins precipitate. The desired compounds can be separated by filtration.

Alternatively, the unreacted non-oxasapogenins may be precipitated by addition to the alkanol solution of water to reduce the solubility of the non-oxo compounds.

Still another modification can be used in order to recover the unreacted hydrazine. The entire reaction mixture can be dried *in vacuo* whereby any unreacted hydrazine is removed at the same time. Thereafter the residue is triturated with an alkanol containing from 1 to 6 carbon atoms in the molecule.

The hydrazone derivatives in the alcoholic solution can be hydrolysed directly to produce free hecogenin by acidifying the solution and refluxing it for about 20 to 45 minutes. The pure hecogenin is recovered by crystallisation from the hydrolysis solution, preferably by neutralising the acid hydrolysis product, heating, filtrating, and washing with water.

Frequently it is preferable to purify the hydrazones further. This is done by converting the hydrazones to the corresponding azine derivatives. Thus the pH of the alcoholic solution remaining after removal of the non-oxosapogenin residues is adjusted to 6 by the addition of formic acid, acetic acid or hydrochloric acid to convert the hydrazone derivatives of hecogenin to the azine according to procedure (a) above. It may also be evaporated to dryness and heated according to procedure (b) to convert it to the azine, or the alcoholic hydrazone solution may be refluxed with pyridine hydrochloride to effect the conversion to the azine according to procedure (c), or it may be refluxed in high boiling solvents according to procedure (d). The azine is precipitated from the alcoholic solution by concentrating and cooling. The azine can be further purified by washing it with a lower aliphatic ether or ester, preferably ethyl acetate, amyl acetate, *n*-butyl ether, or dioxane. The azine can be converted to hecogenin by refluxing at a pH of about 1 in a solution of ethanol and chloroform. Neutralisation and concentration results in the crystallisation of hecogenin.

In a further modification, applicable where the hydrazone derivative of hecogenin is hecogenin substituted with hydrazine, the conversion of oily liquid hydrazone derivative of hecogenin to crystallisable solid azine can be effected by condensation with the required

carbonyl compound. The ketone or aldehyde condenses with the NH_2 group to form an azine. Alkanones, such as acetone, methyl ethylketone, diethyl ketone, methyl propyl ketone and ethyl butyl ketone can be used. These azines are solids and form a suitable modified type of solid hydrazone readily purified by crystallisation. The advantage of using a compound of this type in the separation method of the present invention is that in the instance of the unsubstituted oily hydrazone hecogenin, the reaction thereof with a lower alkanone comprises a very easy conversion of the oily hydrazone compound to a solid azine. The alkanone azine derivitave of hecogenin is readily hydrolysed to hecogenin in the same manner as for the hydrazones.

The extractions in the Examples given below were performed upon dry acid hydrolysate of Mexican Agave fourcroyoides. Other plant species from which sapogenin materials comprising a mixture of both oxo and non-oxosapogenins are available by hydrolysis can be used as the starting material mixture; these, depending upon the specific plant material, will vary from one to another to the quantity of hecogenin with respect to other sapogenins, but the procedure for resolving such mixtures as set forth herein can be used.

The following Examples illustrate the invention:

EXAMPLE IA

Two grams of the sapogenin mixture was dissolved in 20 ml of ethyl alcohol, 4 ml of hydrazine hydrate were added and the reaction carried to completion by refluxing for 24 hours. The length of time required for completion will vary in different localities with the boiling point of the alcohol and with the specific mixture, but may be determined conveniently by testing for the disappearance of the carbonyl band from the infra-red curve of a sample. When the reaction was complete 12 ml. of solvent were distilled at atmospheric pressure. The residue was then chilled, preferably at 0° C. for two hours. The crystalline material thus produced was filtered and washed with a small volume of previously chilled alcohol. The tigogenin obtained melted at about 194—198° C.

EXAMPLE IB.

The filtrate containing the hecogenin hydrazine derivative produced according to Example IA was adjusted, preferably with hydrochloric acid, to pH 1.0 and refluxed for about 30 minutes. The mixture was then neutralised to pH 7 with strong aqueous sodium hydroxide and the solvent partially removed by distillation carried out at atmospheric pressure until abundant crystal formation was observed. The mixture was then cooled to room temperature and filtered. The crystals were washed with cold alcohol, followed by copious washing with water. The hecogenin crystals so obtained melted at about 240—245° C. Concentration and recrystallisation of the mother liquors gave a sapogenin mixture which was returned to the process for recovery.

EXAMPLE IIA.

10 Grams of the sapogenin mixture was milled to pass a 20 mesh screen and was added to 50 ml. of ethyl alcohol. 10 Ml. of hydrazine hydrate were added and the mixture was refluxed for 24 hours. 15 Ml. of water were then slowly added, with stirring, and the mixture was cooled to 20° C. The tigogenin crystals were then filtered, washed with 5 ml. of diluted ethyl alcohol and with water. The compound melted at about 190—200° C.

EXAMPLE IIB.

The alkaline alcohol filtrate containing 12-hydrazonohecogenin produced according to Example IIA was adjusted to pH 6.0 with hydrochloric acid and refluxed for 15 minutes. The azine derivative of hecogenin so obtained was filtered and washed with hot water. The product may be converted to hecogenin following the procedure set forth in Example VI.

EXAMPLE III.

5 Grams of sagogenin mixture was refluxed for 12 hours with a mixture of 25 ml. of ethylene glycol monoethyl ether and 5 ml. of hydrazine hydrate. 0.05 Gram of activated carbon and 0.5 grams of diatomaceous earth were then added and the mixture was filtered. The solution was chilled to 0° C. and the tigogenin crystals were filtered, washed with a small quantity of cold ethylene glycol monoethyl ether and dried.

EXAMPLE IV.

The filtrate of Example III was distilled to dryness under vacuum giving rise to an oily residue. Chromatography of this material over an adsorbent composed of 85% of silicon dioxide and 15% of magnesium oxide gave a colourless oil, which, when dried, was a resinous material.

Prolonged heating at 210° C. transformed the hydrazone derivative of hecogenin to the corresponding azine as does also the adjustment to pH 6.0 detailed in Example IIB. The third procedure to effect this conversion is illustrated in Example V.

EXAMPLE V.

3 Grams of the resinous material obtained in Example IV were refluxed for 30 minutes with 10 ml. of ethyl alcohol and 0.3 gram of pyridine hydrochloride. A crystalline solid precipitated out of the solution which was filtered from the hot mixture and washed with hot ethyl acetate to eliminate traces of tigogenin and other sapogenins. This azine of hecogenin had a melting point of 329—331° C.

EXAMPLE VI.

To obtain hecogenin from the azine, 2 grams of the azine derivative of hecogenin obtained by the process of Examples II, IV or V, were refluxed for 30 minutes with 10 ml. of ethyl alcohol, 10 ml. of chloroform, 2 ml. of water and 2 ml. of concentrated hydrochloric acid. 0.8 Gram of sodium hydroxide dissolved in 2 ml. of water were then added and distillation was carried out until abundant crystallisation had occurred. The suspension was filtered, the crystals were washed with hot water and dried to obtain hecogenin having a melting point of 245—250° C.

EXAMPLE VII.

Example IA was repeated. The filtrate was dried *in vacuo* and recrystallised three times from ethyl-hexane solution to give crystals having a melting point of 205—207° C. The hydrazono-hecogenin compound when first formed was an oily liquid. It set to a resinous body on drying as in Example IV and may ultimately be purified to solid crystalline form.

EXAMPLE VIII.

10 Grams of the oily hydrazono-hecogenin compound described in Example IV were refluxed with 10 ml. of acetone for 60 minutes. The mixture was cooled to 0—5° C. and the crystals filtered. The 12-isopropyl-azino-hecogenin was recrystallised from a chloroform-acetone solution and melted at 206—208° C.

Following the procedure of this Example, but substituting methyl ethyl ketone, diethyl ketone, and methyl propyl ketone for acetone, there were respectively obtained 12 - methyl-ethyl - azino - hecogenin, 12 - diethyl - azino-hecogenin and 12 - methyl - propyl - azino-hecogenin.

EXAMPLE IX.

Following the procedure of Example VI the 12-isopropylazino-hecogenin was hydrolysed at pH 1 with hydrochloric acid to hecogenin.

EXAMPLE X.

2 Grams of the sapogenin mixture was dissolved in 20 ml. of ethyl alcohol together with 4 ml. of hydrazine hydrate and refluxed for 24 hours. The excess hydrazine was recovered for re-use by concentration of the reaction mixture to dryness. The mixed dry residue obtained in the initial reaction for the hydrazone derivative of hecogenin was refluxed in 10 ml. of ethyl alcohol and filtered from insoluble residue of non-oxosapogenin. The non-oxosapogenin solid filter cake was dissolved in 10 ml. of ethyl acetate and concentrated to a volume of 7 ml., chilled to 0° C. and allowed to stand for 24 hours. The crystals were tigogenin. The hydrazono-hecogenin solution was then adjusted to a pH of 6 and heated under reflux for 15 minutes, concentrated to a volume of 6 ml., chilled to 0° C. and the crystals of the azino derivative of hecogenin were filtered, washed and dried.

WHAT WE CLAIM IS:—

1. A process for the separation of hecogenin (isolated in the form of the 12-hydrazono compound) from a mixture with non-oxosapogenins, which comprises heating the mixtures with a hydrazine of the general formula R—NH—NH_2 where R is hydrogen, an alkyl radical containing from 1 to 6 carbon atoms or a —CS—NH_2 radical, in an alkanol containing from 1 to 6 carbon atoms in the molecule and separating the desired hydrazonohecogenin from the unreacted components of the mixture by differential solubility.

2. A process as claimed in claim 1 wherein the hydrazonohecogenin is hydrolysed in an acid solution to yield hecogenin.

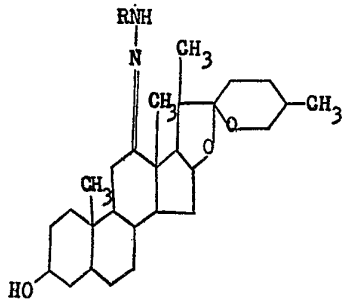
3. A process for the separation of hecogenin (isolated in the form of the 12-hydrazono compound) from a mixture with non-oxosapogenins, which comprises heating the mixture with a hydrazine of the general formula R—NH—NH_2 where R is hydrogen, an alkyl radical containing from 1 to 6 carbon atoms or a —CS—NH_2 radical, in an alkanol containing from 1 to 6 carbon atoms in the molecule, evaporating the mixture to dryness in vacuo, and extracting the hydrazono-hecogenin from the dry mixture with an alkanol containing from 1 to 6 carbon atoms in the molecule.

4. A process for the separation of hecogenin (isolated in the form of the 12-hydrazono compound) from a mixture with non-oxosapogenins, which comprises heating the mixture with a hydrazine of the general formula R—NH—NH_2 where R is hydrogen, an alkyl radical containing from 1 to 6 carbon atoms or a —CS—NH_2 radical, in an alkanol containing from 1 to 6 carbon atoms in the molecule, concentrating the solution until the non-oxosapogenins precipitate, and filtering.

5. A process for the separation of hecogenin (isolated in the form of the 12-hydrazono compound) from a mixture of non-oxosapogenins, which comprises heating the mixture with a hydrazine of the general formula R—NH—NH_2 where R is hydrogen, an alkyl radical containing from 1 to 6 carbon atoms or a —CS—NH_2 radical, in an alkanol containing from 1 to 6 carbon atoms in the molecule, adding a sufficient amount of water to precipitate the non-oxosapogenins, and filtering.

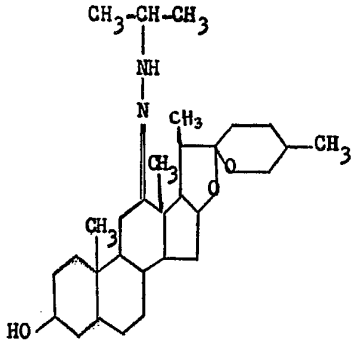
6. A process as claimed in claim 1 substantially as described with reference to any one of Examples 1A, 2A, 3, 4, 7 and 10.

7. Compounds of the general structural formula:

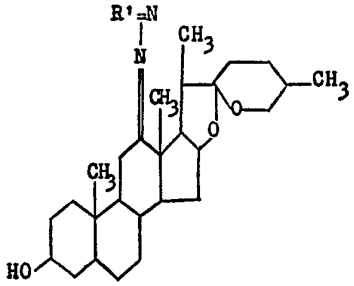


wherein R is hydrogen, an alkyl radical containing from 1 to 6 carbon atoms or a —CS—NH_2 radical.

8. The compound of the structural formula:



9. Compounds of the general structural formula:



where R^1 is an alkylidene radical containing from 1 to 6 carbon atoms or a 12-desoxo-hecogenin radical.

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Agents for the Applicants.

Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press.—1960.
Published by The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.