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(54) Title: PRODUCTION OF HYDROXY ACIDS

(57) Abstract

A method of producing a hydroxycarboxylic acid comprises hydrolysing a complex mixture of esters, such as wool wax, to a predetermined degree of hydrolysis less than 90 %. This partial hydrolysis makes it possible to optimise the recovery of hydroxycarboxylic acid.

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PRODUCTION OF HYDROXY ACIDS

This invention relates to the production of hydroxy acids, particularly but not exclusively alpha-hydroxy carboxylic acids. Wool wax (otherwise known as wool grease or lanolin) contains high molecular weight esters. It is known to react wool wax with a small stoichiometric excess of a strong base, such as caustic soda, to totally hydrolyse (saponify) the esters to yield alcoholic and acidic components. The alcoholic and acidic products can be refined as desired to provide various commercial grades of products, the products being used principally in the manufacture of pharmaceuticals, cosmetics and toiletries.

Total saponification of wool wax has been practised for many years, with the primary objective of isolating the alcoholic fraction which is rich in cholesterol and is used as an emulsifier. The fatty acid fraction has been less important commercially. It consists of a mixture of a large number and variety of acids and is sometimes used as such, or it is refined.

The esters of wool wax have a variety of acid components. These acids include certain hydroxy acids, and these acids have become commercially important in recent times. For example, the use of low molecular weight (C_2 - C_{10}) alphahydroxy carboxylic acids (AHAs) in skin care creams has grown considerably.

The skin care industry today employs a range of AHAs which occur in a variety of natural sources such as fruits, milk and honey. However, many of those actually used will be manufactured synthetically. Typical examples are lactic, citric, tartaric, glycolic and malic acids. These acids are incorporated into face creams etc. and are demonstrably effective in reducing wrinkles and some of the effects of ageing. The skin rejuvenating effect of AHAs is believed to be a consequence of their relatively high acidity compared to non-hydroxylated carboxylic acids of similar molecular weight. The low pH environment created by AHAs promotes a more rapid exfoliation/desquamation of the skin. The body's natural reaction to this is the renewal of the cells in the epidermis, which improves its appearance.

Patents and patent applications exist covering the formulation of AHAs into skin creams and lotions and incorporate a wide range of acids in their claims. For example, European patent application no. 0413528A1 describes the use of AHAs containing aliphatic carbon chains C_1-C_{19}.

Some of the fatty acids claimed to be effective in skin creams and lotions can be found in wool wax. For example, alpha-hydroxy hexadecanoic acid (C_{16}) is observed at significant levels in the fatty acid fraction. Wool wax fatty acids as such do not appear to have attracted much attention from the skin care industry. However, the value of wool wax acids in skin care products is now beginning to be appreciated. For example, Beiersdorf AG, European patent application no. 0555776A1, covers the fractionation of wool wax acids using solvents of differing polarity, to obtain acid mixtures rich in the AHAs of interest. This is not a new principle, for articles describing this approach appeared in the scientific press over thirty years ago (eg. Noble, Eisner and Scanlan, JAOCS, Vol 37, January 1960, pages 14-16). A similar principle was also described by Downing (Aust. J. Appl. Scie., Vol 14, No

1, pages 50-56).

Additionally the ability of AHA's to form copper chelates was employed by Milburn and Truter to isolate them from wool wax acids (J. Appl. Chem., Vol 12, April 1962, pages 156-160). We have found Truter's method is very effective, but the Beiersdorf methods are tedious to operate and not very efficient.

We have now found a way of obtaining hydroxy acids, particularly AHAs from complex esters such as wool wax, whereby the product is enriched in those acids most desired.

According to the present invention there is provided a method of producing at least one hydroxycarboxylic acid from a mixture containing at least one hydroxyester and at least one ester that is not a hydroxyester, comprising hydrolysing said mixture, and ceasing said hydrolysis when a degree of hydrolysis less than 90% has been reached.

We have found that, by using partial instead of complete saponification, the composition of the acid mixture produced is different from that produced by complete saponification. By routine trial and experiment, the degree of saponification can be found which optimises the acid mixture produced for the particular end requirements.

Whilst control of the degree of hydrolysis is important, the other reaction conditions can also have an influence, particularly for example the temperature. The effect of temperature variation on any particular system should advantageously be investigated when determining the optimum reaction conditions for any particular saponification.

Preferably, at least one of the or each hydroxycarboxylic acid is an alpha-hydroxycarboxylic acid.

In the particular case of wool wax, and the production therefrom of an alpha-hydroxy carboxylic acid-rich fraction, we have found that the highest concentrations

of AHAs are obtained when the degree of hydrolysis is quite small. As the extent of hydrolysis is increased, the AHAs become progressively diluted with non-hydroxy acids.

Typically, the hydrolysis is ceased at a degree of hydrolysis in the range of 5% - 50%, preferably 10% to 30%, more preferably 15% to 25%. A degree of hydrolysis of 20% is often particularly suitable.

It is desirable to select the degree of hydrolysis by performing the steps of :

(a) hydrolysing the mixture of esters to a predetermined degree of hydrolysis;

(b) forming the or each hydroxycarboxylic acid from the product of step (a);

(c) determining the % yield of the or each hydroxycarboxylic acid;

(d) repeating steps (a) to (c) until the degree of hydrolysis necessary to produce a required yield has been reached.

Usually these steps would be carried out until the maximum yield has been achieved.

The yield of hydroxycarboxylic acid is temperature dependent, so it is generally preferable also to carry out the steps (a) to (c) at a plurality of different temperatures in order to determine the temperature at which the maximum yield is obtained. A temperature of 80°C has been found to be quite effective, particularly when the mixture of esters is wool wax.

The mixture of esters is generally a complex mixture of esters. The mixture of ester may contain over 100 different esters, or even over 1000 different esters. The mixture typically contains a plurality of hydroxy esters and a plurality of esters that are not hydroxyesters.

Usually, the mixture of esters includes at least one fatty acid ester. The invention works particularly well when the mixture of esters is a wax, and is especially useful when said wax is wool wax.

It is preferred that at least one of the esters that form the mixture of esters include at least one component that, on hydrolysis, can yield a sterol. The esters may also include at least one component that, on hydrolysis, can yield an aliphatic alcohol. It is desirable that at least one ester of the mixture has a molecular weight greater than or equal to 172, more desirably greater than or equal to 186. Over fifty percent of the esters may have a molecular weight greater than or equal to 172, more desirably greater than or equal to 186. It is possible for substantially all the esters to have a molecular weight greater than or equal to 172, more desirably greater than or equal to 186.

It is especially preferred to recover said hydroxycarboxylic acid after ceasing said hydrolysis. One way to do this comprises treating the partially hydrolysed mixture of esters to form a treated mixture including the or each hydroxycarboxylic acid, then separating the or each hydroxycarboxylic acid from the treated mixture to form a product rich in the or each hydroxycarboxylic acid. Another way comprises separating a salt of the or each hydroxycarboxylic acid formed by said partial hydrolysis to form a mixture rich in the or each salt, then treating the or each salt to form a product rich in the or each hydroxycarboxylic acid. The treatment step would usually comprise acidification, for example with sulphuric acid.

The recovery can be carried out so that said product rich in the or each hydroxycarboxylic acid contains at least 50% of the or each hydroxycarboxylic acid. More preferably the product rich in the or each hydrocarboxylic acid would contain at least 90% of the or each hydroxycarboxylic acid. Even more preferably the product rich in the or each hydroxycarboxylic acid would contain substantially 100% of the or each hydroxycarboxylic acid.

The separation is conveniently carried out by liquid-liquid extraction. The extract is preferably the

mixture rich in the or each hydroxycarboxylic acid, or the mixture rich in the salt of the or each hydroxycarboxylic acid; and the raffinate is preferably alkanol, formed during the hydrolysis, and unreacted ester. The alkanol in the raffinate may subsequently be recovered from the unreacted ester. The unreacted ester is preferably recycled.

The hydroxycarboxylic acid preferably comprises alpha-hydroxy-n-palmitic acid, alpha-hydroxyisostearic acid and/or alpha-hydroxymyristic acid.

It is not yet clear whether, in the method of the invention, it is the AHA esters per se which are most easily hydrolysed, or whether they are selectively esterified with the alcohols which facilitate the largest hydrolysis rate. By analysing the alcohols which are released alongside the fatty acids, it appears that AHAs are predominantly combined with the aliphatic, non-steroidal alcohols. These do have a faster hydrolysis rate than the sterol esters. In any event, the invention provides a method by which high concentrations of AHAs can be produced from wool wax without having to refine the standard, commercial fatty acid fraction.

In the method of the invention, the desired acids (for example alpha-hydroxy carboxylic acids) are formed in a mixture with alcohols and unsaponified esters. Normally, the acids will be recovered from the mixture. It may be necessary to treat the mixture carefully to minimise decomposition or deactivation of the desired acids. For example, alpha-hydroxyacids are relatively more reactive than their non-hydroxy counterparts and have a tendency to form intermolecular condensation products such as lactides. Accordingly, the workup of the soap extract into free fatty acids must be conducted with due regard to the reactive nature of AHAs. Thus, we prefer to liberate the fatty acids using conditions which minimise thermal stress on the sensitive AHA components. The acidification is preferably conducted without an excessive rise in temperature. The

temperature used will depend upon the solvent employed during the saponification step. It is generally preferable to maintain the soap phase as a homogeneous solution prior to acidification, whilst at the same time keeping the temperature as low as possible.

The hydrolysis can be carried out with an alkali, for example a metal hydroxide solution, such as potassium or sodium hydroxide. However, enzymes which will selectively hydrolyse the appropriate esters can be used to achieve a similar or more efficient result. Further, the invention is not limited to the treatment of wool wax, but can equally be applied to other waxes, and the like, containing complex esters.

In the method of the invention, wool wax (or other complex ester-containing materials) are subjected to partial saponification to release an acid product. By "partial saponification", we mean a degree of saponification of less than 90%, and most often considerably lower than this. It will be appreciated that the reaction product will contain not only the desired acids but also alcohols and unreacted esters. In accordance with a further feature of the invention, the unreacted esters (optionally together with the alcohols) can then be fully saponified to release all the alcohols which can then be used as desired, for example for conventional purposes. For example, in the case of producing alpha-hydroxy carboxylic acids, the SV (saponification value) of the alcohols/ester will usually exceed 25mg KOH/g, and the esters can if desired be thereafter saponified.

In order that the invention may be more fully understood, the following examples are given by way of illustration only.

Examples

To exemplify the process, portions of Lanolin BP were saponified under a variety of conditions and the fatty acids thus liberated were analysed. The general procedure for the saponification and separation of the hydrolysis products was as follows.

The Saponification Value (SV) of the lanolin is determined according to the method described in the British Pharmacopoeia. The SV is defined as the number of milligrams of potassium hydroxide (KOH) required to completely saponify 1 gram of the substance. Saponification involves the instantaneous neutralisation of any free acids followed by the slower hydrolysis of esters.

Total or partial saponification is achieved using a calculated quantity of alkali based upon the weight and the SV of the lanolin sample. For reasons of economy, it is usual to employ sodium hydroxide (NaOH) instead of KOH. Accordingly, the quantity of alkali needed for a particular case is calculated as follows.

Total saponification requires: SV milligrams KOH/gram of lanolin

or: $SV \times \frac{40}{56.1}$ milligrams NaOH/gram of lanolin

As will be understood, 40 and 56.1 are the equivalent weights of NaOH and KOH, respectively.

A reduced degree of saponification, for example 50%, requires 50% of the alkali needed for total saponification.

i.e. $\frac{50}{100} \times SV \times \frac{40}{56.1}$ milligrams NaOH/gram of lanolin

In the examples which follow, the calculated weight of caustic soda was dissolved in aqueous ethanol. In the case of a lower temperature saponification, the use of aqueous butanol was found better to dissolve the lanolin.

To conduct the saponification, the mixture of lanolin, aqueous ethanol and sodium hydroxide was placed in a glass flask fitted with a reflux condenser. The flask was seated in an electric mantle and heated until the contents were gently boiling. The boiling point in these cases was about 80°C. For the lower temperature example, the contents were maintained at the desired point using a thermostatic heater. The saponification period was typically three to six hours.

Saponification under any set of conditions is finished when an aliquot of the reaction mixture is found to contain only a minimal amount of residual caustic soda. This is determined by titration with standard hydrochloric acid, using phenolphthalein indicator. For practical purposes, the residual amount should not exceed 1% of the original weight of NaOH used in the reaction.

Immediately after the saponification reaction, the mixture consists essentially of a solution or suspension of:

* Sodium salts (soaps) of liberated fatty acids
* Lanolin alcohols liberated by hydrolysis of esters
* Unhydrolysed lanolin esters.

To separate the desired fatty acid components from the alcohols and esters, partitioning of the mixture between the polar, aqueous solvent and a similar volume of immiscible, nonpolar solvent was used. The nonpolar solvent extracts the alcohols and esters, whilst the fatty acid soaps remain with the aqueous phase. Accordingly, the aqueous solvent was provided by adding more water or aqueous ethanol to the saponification mixture. The nonpolar solvent used was hexane.

The reaction mixture, together with the solvents,

was placed in a separating funnel fitted with a stopper. The warm mixture was then carefully mixed by swirling, allowing vapour pressure to escape via the stopcock. The funnel was then allowed to stand until the two solutions separated. The lower aqueous soap phase was drained off into a beaker. The upper, nonpolar extract was then drained off and kept separate. The aqueous phase was poured back into the separating funnel and the extraction process repeated with fresh aliquot of warm hexane.

At least three extractions were performed. More extractions will improve yields and purity, but three was considered sufficient for the current purpose. The combined hexane extracts were backwashed with warm aqueous ethanol, to recover traces of soap which may have been absorbed. The backwashes were combined with the soaps phase.

The soaps phase was now treated to recover the fatty acids. This was achieved by careful addition of sulphuric acid until the pH fell to 2.5-3, as determined by pH papers. At this point, the fatty acids separate from the aqueous solution and float off. They could be simply decanted off, but in order to maximise yields, the acids were extracted with ethyl acetate in a separating funnel. Three extractions were performed and combined. The ethyl acetate solution was then backwashed with brine to remove traces of absorbed water. Finally, the ethyl acetate was removed by distillation under vacuum in a rotary evaporator, operating at 60°C. The weight of fatty acids obtained was recorded to enable yields to be calculated.

Analysis of the fatty acid fractions was conducted using a Perkin Elmer model 8600 gas chromatograph. This was fitted with a 30 metre capillary column, having an internal diameter of 0.54 mm and coated internally with a 1.5 micron film of stationary phase DB1, a dimethylpolysiloxane. The carrier gas was helium, at an inlet pressure of 60 KPa. Detection was achieved by means of a flame ionisation detector operating at 350°C. The 0.5 microlitre sample was

injected by microsyringe, through a split/splitless injector module held at 325°C.

The chromatography was conducted with the following temperature profile. Initial column temperature was 150°C, which was held for a period of 2.5 minutes. After this period, a ramp of 5°C/minute was applied until the temperature reached 325°C. The column was then held at 325°C for 7 minutes.

Prior to injection, the fatty acids were converted into trimethylsilyl derivatives. This improves resolution by the chromotagraphic column, provides more uniform peaks on the chromatogram and more accurate quantitations. Trimethylsilylation involves the replacement of hydrogen atoms in hydroxyl and carboxyl groups with the trimethylsilyl radical [$-Si(CH_3)_3$] and is a particularly valuable technique in the analysis of hydroxyacids. It is preferred to the more conventional methylation technique, which can lead to thermal degradation and less accurate quantitations due to peak tailing.

The trimethylsilyl derivatives were prepared by reacting the fatty acid mixture with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane. This reagent mixture is available commercially. The preparative details are as follows

To about 0.1g of fatty acid mixture in a sample tube add 1ml of the BSTFA reagent. Heat the mixture to 60°C for 1 minute. Add 5ml heptane to dilute the mixture and transfer it to a small separating funnel. Add 5ml of water to quench the excess BSTFA reagent. Stopper the funnel and shake vigorously for about fifteen seconds. Allow the phases to split, then drain off the aqueous phase into another funnel. Retain the heptane extract. Add a further 5ml aliquot of heptane to the aqueous phase and repeat the shaking and separation. Conduct a third extraction with 5ml heptane. Transfer the combined heptane extracts into a 20ml volumetric flask and dilute to the mark with heptane.

To enable quantitation of individual fatty acids within the mixture, the internal standard method is recommended. Because lanolin fatty acids contain many different acids, care must be taken to select an internal standard which does not co-elute with any natural component. After the evaluation of several candidates, the use of n-heptadecanoic acid was selected. In order to accurately quantitate hydroxyacids, it is necessary to determine any difference in the instrument's detector response to the analytes and to the internal standard. This is conveniently determined by measuring the chromotographic peak areas of known mixtures of pure analyte and internal standard. This allows the relative response factor for the system to be calculated.

For convenience, the response factor for alpha-hydroxypalmitic acid, relative to n-heptadecanoic acid, was determined and applied for the quantitation of the other hydroxy acids of interest. The relative response factor across a wide range of compositions was found to be 1.04 (linear regression correlation 0.994).

For quantitative analysis of lanolin fatty acid mixtures, about 0.025g of n-heptadecanoic acid was added quantitatively to the weighed sample (about 0.1g) before addition of the BSTFA reagent.

Lanolin fatty acids contain a complex mixture of structures and researchers have yet to provide a complete identification. However, the desirable alpha-hydroxy acids have been fully resolved and the literature provides a solid basis on which to work. For example, Motiuk (JAOCS, v56, p91-97, 1979) reviewed the efforts of several researchers and provided the following composition. It will be appreciated that a natural material like wool wax or lanolin is subject to a considerable variability in composition. Accordingly, the figures quoted are approximations.

Type	Carbon Chain Length	% approx
alpha-Hydroxyacids/Normal	C_{10}-C_{32}	22
alpha-Hydroxyacids/Iso	C_{12}-C_{34}	5
alpha-Hydroxyacids/Anteiso	C_{11}-C_{33}	1
	Total	28 approx

The most abundant hydroxy acid is the C_{16} member of the normal series, alpha-hydroxy-n-palmitic acid (a), which we have observed to account for 10-15% of the total fatty acid mixture, depending upon the source wool wax. The next most abundant hydroxy acid is the C_{18} member of the iso series, alpha-hydroxyisostearic acid (b) and accounts for 2.5-5% of the total. Third most abundant hydroxy acid is the C_{14} member of the normal series, alpha-hydroxymyristic acid (c). This accounts for 1-2% of the total fatty acids. Fatty acids (a), (b) and (c) together account for the greater proportion of the total hydroxy acids and are convenient markers for assessing AHA activity.

The structure of the most abundant alpha-hydroxy acids is:

(a) CH_3-$(CH_2)_{13}$-CH(OH)-COOH

(b) $(CH_3)_2$-$(CH_2)_{14}$-CH(OH)-COOH

(c) CH_3-$(CH_2)_{11}$-CH(OH)-COOH

The following table shows the experimental results using ethanol as a solvent at a temperature of 80°C.

Degree of Saponification	Yield of Fatty Acids (*)	AHA Content (a+b+c)
100%	50.0%	15.5%
75%	43.4%	17.0%
50%	24.2%	28.4%
30%	16.0%	47.2%
10%	3.3%	33.1%

(*) Based upon weight of lanolin used.

The process is not limited to lanolin, which was used here for illustration. Accordingly, unrefined wool wax or woolgrease may be used, providing the levels of free fatty acids usually present therein are taken into account when calculating the input of alkali. In cases where a significant level of acids pre-exists, the stoichiometric calculation is more appropriately based upon the Ester Value (EV) of the material.

The EV is defined as the number of milligrams of KOH required to completely hydrolyse the esters contained in 1 gram of substance.

Any free acidity is measured by the Acid Value (AV).

The AV is defined as the number of milligrams of KOH required to completely neutralise the free acids contained in 1 gram of substance.

Accordingly, EV = SV - AV

In the case of lanolin, the AV is considered sufficiently small (typically below 1 mg KOH/g) to be neglected in the calculation. The British Pharmacopoeia

specifies the SV of lanolin is 90 to 105 and the AV is 1 max.

The separation of the hydrolysis products may be conducted using a variety of immiscible solvents and may employ a variety of different physical techniques. For example, a continuously operating, countercurrent, liquid/liquid extraction column, as employed for commercial manufacture of wool wax alcohols and acids, may be used.

Further enhancements to both AHA activity and physical quality, such as colour and odour, can be obtained by the incorporation of vacuum distillation processes, for example by molecular distillation.

The molecular distillation of the AHA concentrates obtained via the controlled saponification technique provides for paler coloured and lower odour products, which are preferred for many applications.

The use of fractional distillation techniques, such as the so-called topping and tailing method, allows for the desirable AHA components to be further concentrated.

CLAIMS:

1. A method of producing at least one hydroxycarboxylic acid from a mixture containing at least one hydroxyester and at least one ester that is not a hydroxyester, comprising hydrolysing said mixture, and ceasing said hydrolysis when a degree of hydrolysis less than 90% has been reached.

2. A method according to claim 1, wherein, at least one of the or each hydroxycarboxylic acid is an alpha-hydroxycarboxylic acid.

3. A method according to claim 1 or 2, wherein, said hydrolysis is ceased at a degree of hydrolysis in the range of 5 - 50%.

4. A method according to claim 1 or 2, wherein, said hydrolysis is ceased at a degree of hydrolysis in the range of 10 - 30%.

5. A method according to claim 1 or 2, wherein said hydrolysis is ceased at a degree in the range 15 - 25%.

6. A method according to claim 1 or 2, comprising selecting the degree of hydrolysis by performing the steps of :

(a) hydrolysing the mixture of esters to a predetermined degree of hydrolysis;

(b) forming the or each hydroxycarboxylic acid from the product of step (a);

(c) determining the % yield of the or each hydroxycarboxylic acid;

(d) repeating steps (a) to (c) until the degree of hydrolysis necessary to produce a required yield has been reached.

7. A method according to claim 6, wherein the steps (a) to (c) are carried out until the maximum yield has been achieved.

8. A method according to claim 7, further comprising carrying out the steps (a) to (c) at a plurality of different temperatures in order to determine the temperature at which the maximum yield is obtained.

9. A method according to any preceding claim, wherein said mixture of esters includes at least one fatty acid ester.

10. A method according to claim 9, wherein said mixture of esters is a wax.

11. A method according to claim 10, wherein said wax is wool wax.

12. A method according to any preceding claim, including the step of recovering said hydroxycarboxylic acid after ceasing said hydrolysis.

13. A method according to claim 12, wherein said recovery step comprises treating the partially hydrolysed mixture of esters to form a treated mixture including the or each hydroxycarboxylic acid, then separating the or each hydroxycarboxylic acid from the treated mixture to form a product rich in the or each hydroxycarboxylic acid.

14. A method according to claim 12, wherein said recovery step comprises separating a salt of the or each hydroxycarboxylic acid formed by said partial hydrolysis to form a mixture rich in the or each salt, then treating the or each salt to form a product rich in the or each hydroxycarboxylic acid.

15. A method according to claims 13 or 14, wherein said treatment step comprises acidification.

16. A method according to claim 13, 14 or 15, wherein said product rich in the or each hydroxycarboxylic acid contains at least 90% of the or each hydroxycarboxylic acid.

17. A method according to any one of claims 13 to 16, wherein said separation is carried out by liquid-liquid extraction.

18. A method according to claim 17, wherein the extract is the mixture rich in the or each hydroxycarboxylic acid, or the mixture rich in the salt of the or each hydroxycarboxylic acid, and the raffinate is alkanol, formed during the hydrolysis, and unreacted ester.

19. A method according to claim 18, wherein the alkanol in the raffinate is separated from the unreacted ester.

20. A method according to any preceding claim, wherein the hydroxycarboxylic acid comprises alpha-hydroxy-n-palmitic acid, alpha-hydroxyisostearic acid and/or alpha-hydroxymyristic acid.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 95/02848

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C59/01 C07C51/09 C11C1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C C11C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A,3 862 197 (CASTNER) 21 January 1975 see claims 1,2	1
Y	GB,A,428 048 (THE PROCTER & GAMBLE COMPANY) 30 April 1935 see claims 1,2	1
A	GB,A,281 896 (GRIGORI PETROFF) 15 December 1927 see claim 1	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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GB-A-428048		NONE	
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