

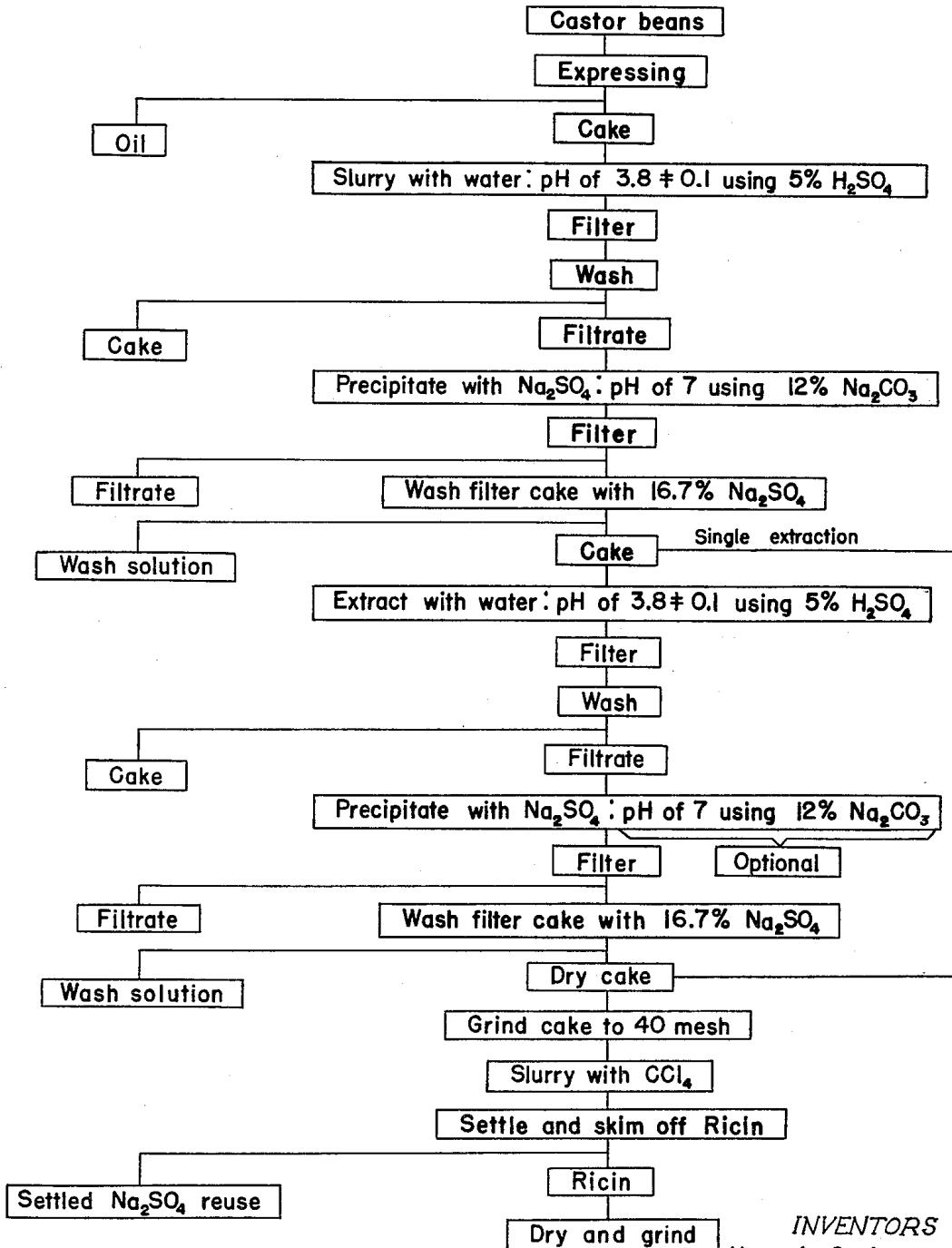
Oct. 23, 1962

H. L. CRAIG ET AL

3,060,165

PREPARATION OF TOXIC RICIN

Filed July 3, 1952



INVENTORS

Harry L. Craig  
O.H. Alderks  
Alsoph H. Corwin  
Sally H. Dieke  
Charlotte L. Karel

BY *George R. Kuehn* ATTORNEY

1

3,060,165

## PREPARATION OF TOXIC RICIN

Harry L. Craig, Cincinnati, and Otto H. Alderks, Wyoming, Ohio, and Alsoph H. Corwin and Sally H. Dieke, Baltimore, and Charlotte L. Karel, Silver Spring, Md., assignors to the United States of America as represented by the Secretary of the Army

Filed July 3, 1952, Ser. No. 297,142

2 Claims. (Cl. 260—123.5)

This invention relates to the method of preparing toxic ricin.

Ricin is a protoplasmic poison prepared from castor beans after the extraction of castor oil therefrom. It is most effective as a poison when injected intravenously or inhaled, the latter requiring extreme comminution and small particle size to be effective. It is believed that the toxic action is catalytic rather than stoichiometric which probably accounts for the high toxicity of the agent.

Because of its relative instability, ricin must be handled with extreme care. In neutral aqueous solution it is stable only up to 60°–75° C., and in solid form up to 100°–110° C., although for short exposures, temperatures up to 130° C. may be tolerated. It is sensitive to acids, alkalis and halogen and may also be inactivated by mechanical working such as grinding or pulverizing. These factors are of great importance in developing a satisfactory method for preparing the material.

Although ricin has been prepared in crystalline condition in the laboratory in small quantities, it becomes necessary, for purposes of toxicological warfare, to prepare relatively large quantities in a high state of purity. This necessitates that as much as possible of the non-toxic material present be removed in the process.

In preparing the protein material, the castor beans are first ground and pressed to remove most of the oil. The pressed cake still retains about 15% oil and this may be removed by means of solvents which will extract an additional 150 pounds of oil per ton of beans and reduce the oil retained in the cake to a little over 1%. In the event that the expressing step is supplemented by solvent extraction, it is important to prevent detoxification of the protein during the solvent removal step. If residual solvent is removed from the ground beans by blowing with steam, considerable detoxification results. Blowing with nitrogen effectively prevents detoxification but is expensive when carried out on a large scale.

After the oil has been removed, the pressed cake or pomace is extracted by agitating with water at a pH of  $3.8 \pm 0.1$  at 25° C. which removes substantially all of the toxic protein. The extraction process is operative within a pH range of about 3 to 4.5 although the preferred range is about 3.5 to 4. The optimum operating point is a pH of  $3.8 \pm .1$ , as indicated above. A careful pH control is essential in order that as much non-toxic protein as possible may be eliminated and also that the filtration rate may be held at a satisfactory value. Either HCl or H<sub>2</sub>SO<sub>4</sub> may be used to get the desired pH for the extraction water, but H<sub>2</sub>SO<sub>4</sub> is preferred due to its lower corrosion rate and ease of handling in concentrated form. The acid should be used in reasonably dilute form to prevent undue local concentrations during its addition. A 5% concentration is satisfactory.

Following the extraction, the slurry is filtered using either a conventional recessed plate filter or a continuous string discharge vacuum filter. With the latter about 7% of filter aid, based on meal weight, was found necessary for satisfactory filtration.

The filtrate from the water extraction step, which contains the ricin, was treated with a 16.7% solution of Na<sub>2</sub>SO<sub>4</sub> to precipitate the protein. This solution is com-

2

posed of 20 pounds of salt in 100 pounds of water and the amount used was such that the salt content equalled 20% of the filtrate weight. This amount and concentration of salt solution was about optimum considering the factors of cost and toxin recovery. Somewhat higher concentrations and larger amounts of solution can be used, however.

The precipitation process is not limited to the use of Na<sub>2</sub>SO<sub>4</sub>, since a saturated solution of NaCl can be used successfully, but Na<sub>2</sub>SO<sub>4</sub> solution gives better nitrogen fractionation, more rapid precipitation, and can be operated under wider pH limits. It is desirable to raise the pH to about 7–8 before precipitation as this gives better recovery and greater non-toxic nitrogen removal. The pH was raised to this value by using NaOH or Na<sub>2</sub>CO<sub>3</sub>, the latter being preferred. The base used was quite dilute in order to prevent detoxification due to high local concentrations in the solution. A 5% solution of NaOH was used, whereas with Na<sub>2</sub>CO<sub>3</sub> a 12% solution was preferred. In general, this higher pH during precipitation gave a greater non-toxic nitrogen fractionation and at the same time maintained the toxin loss at less than 2%.

After precipitation, the slurry was filtered using from 1 to 4% filter aid, based on slurry weight, for satisfactory filtration; the amount of filter aid needed being dependent on the type of press used. Washing the filter cake with Na<sub>2</sub>SO<sub>4</sub> solution removed additional non-toxic nitrogen which is desirable. In this washing step a 16.7% solution of Na<sub>2</sub>SO<sub>4</sub> was again used. This washing step removed an additional 15% of non-toxic nitrogen from the cake.

After filtration the filter cake, which contains the ricin in combination with the Na<sub>2</sub>SO<sub>4</sub>, may be dried and slurried with CCl<sub>4</sub> to separate the ricin by flotation. Separation of the ricin after a single precipitation and washing step is possible, but it is preferred to carry the process through an additional extraction and precipitation step. This is accomplished by slurrying the filter cake in three times its weight of water and the pH of the slurry is again brought to  $3.8 \pm .1$  by means of 5% H<sub>2</sub>SO<sub>4</sub>. The slurry is filtered and a second precipitation is brought about by adding Na<sub>2</sub>SO<sub>4</sub> solution. Although pH control here is not wholly essential it is advantageous to bring the pH to approximate neutrality by adding 12% Na<sub>2</sub>CO<sub>3</sub>. A precipitation time of 45 minutes was necessary to obtain complete removal of the toxin. In filtering out the precipitate, no filter aid was used and the filter cake was washed with Na<sub>2</sub>SO<sub>4</sub> solution on the filter whereby an additional amount of non-toxic nitrogen was removed from the cake. This washing was effective only the first time and repeated washings had little effect in removing further non-toxic nitrogen.

The ricin-Na<sub>2</sub>SO<sub>4</sub> precipitate was dried at about 50° to 60° C. on a hot air tray dryer. The dried product was ground to pass a 40 mesh screen and agitated with 5 times its weight of CCl<sub>4</sub>, which served to separate the ricin from the Na<sub>2</sub>SO<sub>4</sub> by flotation. After settling, the ricin was skimmed off the top. This reduced the Na<sub>2</sub>SO<sub>4</sub> content of the mixture from a previous 40 to 50% down to 15 to 18%. About 1 to 2% of nitrogen remained in the Na<sub>2</sub>SO<sub>4</sub> salt which could then be used for subsequent precipitations.

The final precipitation produced a particle size of 1–2  $\mu$ . On drying the wet cake, however, the ricin cemented together forming larger particles. These could not be broken down to their original size by ordinary grinding methods and since a very fine particle size was necessary in order that the product might be used as a toxic weapon, it was thought desirable to seek some method to prevent the agglomeration or cementing process that took place on drying.

To attempt to affect this result, physical conditions prevailing under the precipitation process were changed.

3

This included changing the temperature of precipitation and the rate of agitation. Other changes included precipitation with only partial saturation of  $\text{Na}_2\text{SO}_4$  and the use of wetting and seeding agents. None of these expedients produced any significant improvement in particle size.

Ordinary dry ball and hammer milling of the dried ricin produced considerable detoxification perhaps due to the generation of excess heat. The use of  $\text{CCl}_4$  slurry plus the use of low temperature and low moisture content of the ricin reduced detoxification during ball milling.

Spray drying proved to be an even better method of securing a reasonably small particle size. Best results were achieved by using a solution having about 20% solids, an inlet temperature of  $150^\circ\text{C}$ . and an atomizing air pressure of 150 to 180 p.s.i. The particle size secured was 6 to 8  $\mu$ .

The best means of securing a small particle size was by air grinding. This was carried out in an apparatus having a chamber with conical top and bottom. The material to be ground has been fed into this chamber and is withdrawn from the bottom and forced back into the center of the chamber tangentially through a venturi. Compressed air of about 100 p.s.i. was fed to the venturi to provide the grinding force. The fines are drawn off the top and the large particles settle to the bottom to be recirculated and reground. This process produced particles having a mass median diameter of 2.5 to 3.5  $\mu$ .

4

Numerous variations are possible in the several steps of the process commencing with the water extraction and precipitation which may be a single or multiple step. Although a single extraction step can be used, as indicated before, some process modifications are necessary for its successful operation on a plant scale. Double extraction proved to be quite efficient but additional steps beyond the second extraction step were not found necessary.

The drawing is self-descriptive and shows the various steps of the process described.

We claim:

1. In a method of preparing toxic ricin from castor beans comprising slurring an expressed castor bean cake with water to remove the water soluble ricin and precipitating the ricin from the filtrate, the further steps which include slurring the precipitate with  $\text{CCl}_4$  and separating the ricin by flotation.

2. A process in accordance with claim 1 in which the precipitate is dried prior to slurring.

#### References Cited in the file of this patent

Kabat et al.: *J. Biol. Chem.*, vol. 168, 1947, pages 629-39.

Kunitz et al.: *J. Gen. Physiol.*, vol. 32 (1948), pages 25-31.