PURIFICATION AND ESTERIFICATION OF CHAULMOOGRA OILS

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INTRODUCTION

Although many new drugs and modifications of old drugs are constantly being tested for their therapeutic effect in cases of leprosy, most leprologists still rely on the ethyl esters of chaulmoogra oils or on the oils themselves for routine treatment of the disease. It is now generally recognized that for injection purposes purified chaulmoogra oils are superior to the best crude oils. The method of purification given by Perkins (5) is quite simple and satisfactory. For the sake of those not familiar with it, it is included in this article.

On the other hand, the methods of esterification given in the literature are often empirical, and usually no attempt is made to determine the percentage of esters in the final product. We consider chemical control highly important if a uniform standard drug is to be obtained. Consequently, we include here a detailed description of our most recent method of preparing the ethyl esters, with explanations of the reasons for and means of chemical control, so that anyone wishing to do so may make the standard drugs. The factors controlling the esterification process, and the influence of sulphuric acid as a catalyst on the reaction rate of esterification, are discussed. A description of an improved vacuum still for distilling the esters is given, with a diagram and directions for operation. Methods of preparing iodized and creosoted esters, and purified chaulmoogra oils, are included.

PURIFICATION OF CHAULMOOGRA OIL

The two principal chaulmoogra oils used today are those obtained from the seeds of *Hydnocarpus wightiana* and *H. anthelmintica.* The oil that originally was called "chaulmoogra," obtained from *Taraktogenos kurzii,* is no longer very widely used for several reasons, among which may be mentioned its higher cost, its greater tendency to become rancid, and its poorer quality as marketed. The physical

455

constants and chemical analysis of the oil from the seed of *Carpo-troche brasiliensis*, which occurs abundantly in Brazil, indicate that it should be as effective as the above-mentioned oils, though as yet it has not received sufficient clinical trial to determine its true place in the chemotherapy of leprosy.

Newly prepared, cold-pressed wightiana or anthelmintica oil from fresh, selected seeds is generally nonirritating, and after filtration and sterilization it may be used for injection purposes without further purification. Outside of India, Siam and neighboring countries, however, the oil is generally from six months to one year or more old when it reaches the consumer, and upon injection it is often round to be irritating. While this irritation is not primarily due to the free fatty acid in the oil,[1] this factor affords us a convenient index as to the quality, age and probable irritant properties of the oil. *H. wightiana* oil, of good quality, shipped in well-cleaned, steel oil drums, reach such countries as the Philippine Islands and Brazil with a free fatty acid content of from 2 to 3 percent. When such oils are to be used for injection purposes the authors prefer to purify them, especially as the process is a simple one. The resulting product is quite bland and nonirritant, of a pale yellow color (considerably lighter in color than the original oil), and with only a slight odor (3).

Method of purification.—The free fatty acids are removed by washing the oil with a solution of caustic alkali. The only difficulty encountered is in the separation of the oil from the resulting emulsion. The following method has proven satisfactory.

Seventy-five liters of hot water (90°C.) are run into a 200-liter alkali-proof steam kettle, such as is described under ''apparatus for esterification.'' Four hundred grams of lye are dissolved in the water, 50 liters of oil are added, and the mixture is gently stirred. If the oil contains more than 3 percent of free fatty acid, a correspondingly increased amount of lye must be used. In this case a considerable amount of solid sodium acid chaulmoograte will be formed, and the stirring must be very gentle to avoid the formation of a fine, inseparable emulsion. Heating the mixture with steam for several hours may be necessary to facilitate separation of the oil. The emulsion finally separates into a fairly clear lower layer and a thick creamy upper layer which still contains a considerable proportion of soap.

After two days the slightly opalescent lower layer is drawn off, hot water (90°C.) is added to the 150-liter mark of the tank, and the contents are stirred, covered and allowed to settle over night. The washing with hot water is repeated four times (or more if necessary), twenty-four hours being allowed for separation on each occasion. It is not advisable to heat the creamy mixture with water for

[1] Experiments to prove this have been made and will be published shortly.

too long a time, as free fatty acids may be formed. If the emulsion does not separate, hot dilute alkali may be added and the mixture heated for several hours. Addition of cold, dilute alkali with subsequent heating usually results in much less rapid separation. Addition of salt is not advised, as in that case most of the soap will remain in the oil.

If smaller quantities of oil than that mentioned are to be purified, some provision must be made to keep the oil hot during the separation of the emulsion, otherwise the mixture cools too rapidly to allow separation to take place.

Volatile impurities are removed from the oil by passing steam through it for from one to two hours, sufficient steam being used to give an aqueous distillate equal to about one-fifth the volume of the oil. The amount of volatile impurities is very small, but the distillate has a strong odor. After steaming, the oil is allowed to stand until most of the water has settled out. It is then filtered through folded filter paper several times until perfectly clear and free from water.

The purified oil is pale yellow and clear, with a free fatty acid content of not over 0.2 percent. The yield should be from 90 to 95 percent of the original oil. The oil should be bottled, sterilized, tightly sealed and kept in a dark, cool place. It is practically nonirritating on injection.

ETHYL ESTERS OF CHAULMOOGRA OILS

Esterification.—Early methods of esterification involved the preliminary preparation of the free fatty acids, but later the process was shortened considerably by simply treating the oil directly with alcohol and a small amount of acid to serve as catalyst. The simplest method is that suggested by Muir [6] which involves the interaction of the oil, alcohol and catalyst in the presence of sunlight, and subsequent washing with sodium hydroxide and water. The data of two examples of such esterification at ordinary temperatures in sunlight are given in Table 1.

TABLE 1.—*Products of cold esterification of chaulmoogra oils.*

Oil used [a]	Time required for esters to rise, days.	Total time, days	Refractive index at 25°C.	Percent esters formed [b]	Percent free fatty acid
C. brasiliensis [c]	20	20	1.4668	60.0	—
C. brasiliensis	—	41	1.4593	96.1	—
C. brasiliensis	—	63	1.4587	99.0	3.5
H. wightiana [d]	19	41	1.4600	92.4	—
H. wightiana	—	67	1.4588	98.1	3.4

[a] Mixture used: Oil 3 liters, 95 percent alcohol 3 liters, H_2SO_4 192 cc.
[b] Computed from the formula given elsewhere, using refractive index of pure mixed esters of *C. brasiliensis* as 1.4585, and of *H. wightiana* as 1.4584.
[c] *C. brasiliensis*, nD/25° 1.4793; F.F.A. 9.23 per cent.
[d] *H. wightiana*, nD/25° 1.4796; F.F.A. 2.8 per cent.

It can be seen from this table that an excessive amount of time is involved in the cold process when large amounts of esters are to be made. The hot process is therefore generally employed. In any case, refractive index readings should be taken to determine when the reaction is complete. Table 1 shows that if the directions: "Allow to stand for a further period equal to the time required for the rising of the lower layer" (3) are followed the oil will not be completely esterified.

Determination of refraction index.—This is a very simple determination to make with an Abbe refractometer. The temperature must be taken at the same time, as the refractive index varies considerably with the temperature. Perkins (4) has shown that in a mixture of oil and esters the refractive index decreases as the percentage of esters increases. The following formula may therefore be used to determine the percentages of oil and esters in a mixture of the two:

$$\text{Percent esters} = \frac{n \text{ (of oil)} - n \text{ (of sample)} \times 100}{n \text{ (of oil)} - n \text{ (purified esters)}}$$

Where n equals refractive index at 25°C.

The reading is usually taken at room temperature and corrected to 25°C. by adding to the reading 0.0004 for each degree above that temperature or subtracting the same amount for each degree below it. In order to remove acid, glycerol and alcohol, the sample is washed three or four times and then dried before the determination is made.

The influence of free fatty acid upon the refractive index readings in this determination is shown in Table 2. The tests recorded in this table were made by adding weighed amounts of mixed fatty acids to weighed amounts of distilled esters (0.14 percent free fatty acid).

TABLE 2.—*Variation in refractive index and optical rotation of mixed ethyl esters of* H. wightiana *oil with percentage of free fatty acid.*

F.F.A. in esters percent	Optical rotation aD/30°	Refractive index nD/25°
0.14	47.10	1.4584
5.00	47.50	1.4590
10.00	47.86	1.4596
20.00	48.44	1.4604

Rate of esterification.—This varies not only with the method used, but also from start to finish of the reaction, as shown in Text-fig. 1. The velocity falls rapidly after about 70 percent of the oil is converted into esters.

The time necessary for complete esterification is decreased by:
(*a*) Increasing the amount of alcohol above that theoretically neces-
sary; (*b*) decreasing the amount of water present;[2] (*c*) increasing,
up to a certain point, the amount of sulphuric acid used as catalyst;
(*d*) increasing the temperature, from room temperature to the boil-
ing point of the mixture; (*e*) mixing the two liquid layers more
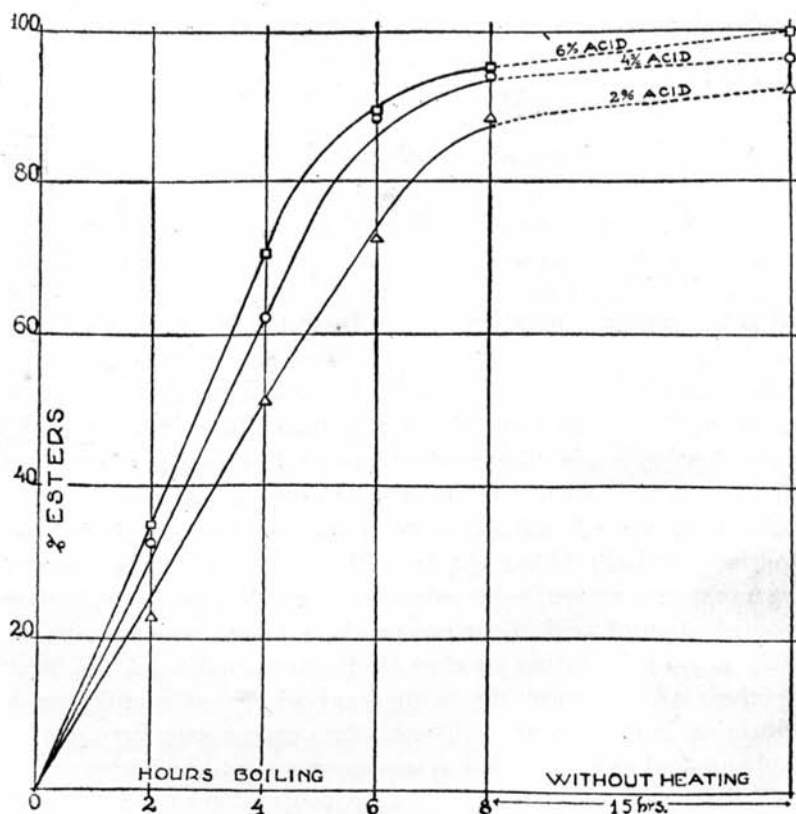
TEXT-FIG. 1. Curves showing variation of rate of esterification of chaul-
moogra oil with different concentrations of sulphuric acid.

intimately, which is accomplished by boiling vigorously. It has been
shown (4) that sulphuric acid produces a more favorable distribution
of the alcohol and glycerol in the two liquid layers than does hydro-
chloric acid, and, as a result, a higher final value of ethyl esters.

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[2] The amount of water actually increases as the reaction proceeds, because of
the formation of water as one of the products of reaction between the alcohol and
any free fatty acid that may be present. This excess water is at least partly
taken up by the sulphuric acid. It is obvious that the alcohol and acid should be
as concentrated as possible at the start of the reaction.

From the above resumé it can be seen that, in any given mixture that is esterified by boiling vigorously, further decrease in the time of esterification must be obtained by either increasing the concentration or the amount of alcohol and acid in proportion to the amount of oil present.   In routine production it is not economical to use alcohol of higher concentration than 96 percent, or sulphuric acid of more than 66° Baumé.   Therefore one can increase either the amount of alcohol, or that of acid, or both.   Increase in the amount of alcohol beyond one volume to one of oil unduly decreases the effective capacity of the apparatus.

The best proportions to use will depend upon many factors.   For instance, at the Culion Leper Colony, where steam is purchased at a flat monthly rate and is available day and night, it is cheaper to esterify for a longer time than to use increased amounts of alcohol and acid.   Boiling for forty-eight hours with one-half as much alcohol as oil is the standard practice there.   In India, Muir ([6]) used more alcohol and acid (approximately the mixture given in Table 1), and esterified for twenty hours.   In Brazil, where alcohol is cheap and where we must make our own steam, it is more convenient to complete the esterification in eight hours than to have three shifts of assistants to tend the boiler over night.   In order to complete the esterification in eight hours it was found necessary to increase the amount of alcohol to two times, and of acid to eight times, that used in the Culion process. There was some question in our minds as to whether this large increase in concentration of acid might not result in more decomposition and possibly a more irritating product, but this happily did not prove to be the case.   In most places the convenience of completing an esterification in eight hours will more than compensate for the extra alcohol and acid used.   If alcohol is very expensive, the excess may be recovered from the first wash water by neutralizing the latter with lime.

Effect of catalysts on time of esterification.—Keeping the proportions of oil and alcohol constant, experiments were made in our routine apparatus on the effect of varying the amount of sulphuric acid.   The results are shown in Table 3.   Refractive index readings were made at room temperature and corrected to 25°C.

METHOD FOR EIGHT-HOUR ESTERIFICATION

Esterification.—Table 3 shows that when equal volumes of 95 percent alcohol and chaulmoogra oil are boiled vigorously for eight hours with concentrated sulphuric acid, at least six percent of acid

is necessary to obtain a 95 percent yield of esters. However, if the mixture is allowed to stand over night after boiling, the yield is 100 percent, even with 5 percent of acid. The table also shows that complete reliance cannot be placed upon time of esterification for completion of the reaction. Five percent acid gives a lower yield in eight hours than does 4 percent in the run tabulated. It is necessary, therefore, to check the percentage yield by means of refractive index readings.

Our present method of esterification consists in boiling vigorously for approximately eight hours (longer if the refractive index reading indicates the necessity), under a reflux condenser, a mixture of 7 liters of 95 percent ethyl alcohol, 7 liters of *H. wightiana* oil, and 700 cc. of concentrated sulphuric acid (66° Baumé). This mixture is allowed to cool over night, during which time the esterification is completed, as shown in Table 3. The cooling also facilitates handling.

TABLE 3.—*Effect of concentration of sulphuric acid on rate of esterification.*

Percent acid [a]	Refractive index at 25°C. after boiling				Percent esters 8 hrs.[b]	After standing over night [c]	
	2 hrs.	4 hrs.	6 hrs.	8 hrs.		Refractive index	Percent esters
2	1.4746	1.4684	1.4637	1.4603	88.5	1.4595	92.2
4	1.4724	1.4660	1.4603	1.4591	94.0	1.4586	96.3
5	1.4720	1.4547	1.4608	1.4592	93.6	1.4578	100.0
6	1.4720	1.4642	1.4601	1.4588	95.4	1.4578	100.0

[a] The percentage of sulphuric acid in the mixture is figured on the basis of the sum of the oil and alcohol; i.e., 2 percent (200 cc.) of acid was added to 5,000 cc. of oil plus 5,000 cc. of 95 percent alcohol.
[b] Figure from refractive index of oil = 1.4796 and pure esters = 1.4578.
[c] These readings were taken approximately fifteen hours after the heat was turned off.

Washing crude esters.—To remove excess alcohol, acid and glycerol, the crude esters are washed with two volumes of hot water to one of esters about four times, or until the wash water is neutral to litmus. If the esters are to be used without neutralizing or distilling, they are dried in a steam kettle or on a hot water bath and filtered when cold. If they are to be distilled they need not be filtered at this point. If they are to be used undistilled but neutralized, the washed esters are neutralized as described under neutralization.

Distillation.—The advisability of using undistilled instead of distilled ethyl esters has been the subject of much discussion. In bygone years, when we often could obtain only very old oils, distillation of the esters seemed advisable. Today, with good grades of oil (not over 2.5 to 3.0 percent free fatty acid) easily obtainable, it is

questionable whether there remains any good reason, except improvement of color, for distilling esters that have been properly prepared. If, however, esterification is not controlled by refractive index readings, the crude esters may contain as high as 10 to 20 percent of oil. In this case distillation, by removing the unchanged oil, decreases the viscosity of the esters and yields a product of more constant composition and color. On the other hand, distillation undoubtedly increases the irritant decomposition products because of the comparatively high heat necessary to distill at 15 to 20 mm. of mercury pressure, but these compounds can be removed by neutralizing and blowing out with steam.

Neutralization.—Recent experiments (1) have shown that free fatty acids up to 3 percent in the esters do not cause irritation, and many institutions are using unneutralized esters. For instance, the Departamento de Prophylaxia da Lepra at São Paulo uses *H. wightiana* esters, distilled and washed but not neutralized. Although the free fatty acid for their esters as distributed averages 2.4 percent, their record of several years of satisfactory use on a large scale (1,000 liters in 1935) proves that their product is not too irritating for routine use. In their practice distillation is used to maintain a fairly constant product, as a sample of their crude esters analysed 6.27 percent free fatty acid and 92.2 percent of esters. Distillation in this instance actually lowered the free fatty acid content by almost 4 percent, although one cannot rely upon distillation alone for the reduction of free fatty acid. Though unneutralized esters may thus be used, neutralization is recommended in order to ensure a product of more constant composition and lighter color.

Neutralization is usually accomplished by adding the esters to a hot, weak solution of sodium hydroxide or lye. The lye (about 100 grams per 10 liters of esters for every 2.5 per cent free fatty acid) is dissolved in the hot water and the esters are mixed in, twice as much water as esters being used. Care should be taken not to stir too vigorously, as this will form a strong emulsion. Crude esters show a greater tendency to form a strong emulsion when they are neutralized than do distilled esters. In the former case it may be necessary to add salt to break the emulsion. After the mixture has stood 24 hours, the clear lower layer is drawn off and two volumes of hot water are again thoroughly mixed in, after which the mixture is again allowed to stand 24 hours. Washing in this way is repeated four times. The yield is about 96 per cent, and the free fatty acid usually less than 0.15 per cent.

Neutralization may also be accomplished by adding powdered unslaked lime to the esters, mixing thoroughly and allowing the solids to settle completely.

The supernatant liquid is then filtered. In this case the exact amount of lime required for neutralization is added. The esters treated by this process contain about twice as much fatty acid (0.35 percent as those neutralized by the lye process, and there is less improvement in color.

Steaming.—Volatile impurities may be removed by passing steam through the esters until the distillate no longer has an acrid odor. Approximately two hours is usually necessary. Volatile impurities exist in greater quantities in distilled esters than in esters not so highly heated. They are of a decidedly irritating character, hence their removal is advised. Washing thoroughly with boiling water would probably give almost as good results, as may be assumed from the fact that at São Paulo, where that method is used, the resulting product is a satisfactory one.

APPARATUS

The essentials of a plant for the production of chaulmoogra ethyl esters include steam- or waterbath-heated, acid-proof containers with reflux condensers for the esterification; a steam-jacketed, acid- and alkali-proof kettle for washing and neutralizing, and, if distillation is to be included, a high vacuum pump and a glass or iron still.

Apparatus for esterification.—For capacities up to five liters per day, ordinary pyrex glass apparatus is quite satisfactory for the esterification and distillation. For work on a larger scale we have found nothing superior to acid-proof stone-ware pots (20 to 30 liters capacity), heated with steam at atmospheric pressure by placing them in covered tubs of concrete, wood or glazed pottery. Recently we have found it preferable to have removable galvanized iron covers for the steam tubs, so that, after a run, the pot may be lifted out of the tub for cleaning and drying. For a 26-liter jar a reflux condenser of 13 mm. brass pipe 1.5 meters long, with a water jacket of 38 mm. galvanized iron pipe, has sufficient capacity to permit vigorous boiling of the mixture. The jars should not be filled much over half full. They are emptied after a run by siphoning or pressure, then washed with alcohol and dried.

Apparatus for washing.—For washing or neutralizing the oil or esters, a glass-lined or stainless steel kettle is most convenient. A steam-jacketed kettle in which, if necessary, the emulsion may be heated to facilitate separation is almost a necessity. With such a kettle the breaking of the emulsion with salt need not be resorted to. For efficient separation the height of the kettle should be about twice its diameter. It should be provided with a valve at the bottom

for drawing off the wash water and, finally, the esters. Most of the latter may be conveniently separated from any remaining wash water by providing a valve on the side, close to the bottom of the kettle, through which they may be drawn off.

Apparatus for distillation.—Large amounts of esters cannot be distilled conveniently or economically in glass apparatus. For such distillation the iron still shown in Text-fig. 2 is much preferable. This still has an operating capacity of from four to five liters an hour at a pressure of 10 to 15 mm. of mercury. It has several improvements over stills previously described. The column is much shorter, and is filled with quarter-inch wire mesh to prevent frothing over and to increase contact surface of the refluxing liquid. For insulation, a galvanized iron sheet covered with eighth-inch asbestos is substituted for asbestocel, as the latter material is difficult to obtain outside of the United States. Improvements have also been made in methods of feeding and draining. The still may be heated by a gas or oil burner at the bottom, the sides and still-head being heated by the hot air rising in the space between the still and the iron screen. If electric heating is used, besides a 500 watt heater on the bottom it will be necessary to apply heat to the sides of the still and column by wrapping them with chromel wire over asbestos paper.

Operation of the still.—After testing the vacuum, two liters of dried esters are put in through the feed funnel (Text fig. 2, D) and heat is applied with vacuum on. If the first distillate is colored it is returned to the still. Feeding is continuous, the input rate being adjusted to the distillation rate by the stopcock in funnel D. Input and output are recorded, so that the amount of esters in the still is known at all times; the amount is maintained at approximately two liters. To increase the rate of distillation the esters may be preheated to about 100°C. in one-liter pyrex flasks.

The simple method of holding the receiving flasks in place by suction (Text-fig. 2, E) is much preferable to the older method of inserting the stopper into the mouth of the flask. The receiver may be removed for emptying, without stopping the distillation, by closing the stopcock A, and, while holding the receiver, opening stopcock B to the outside air in the direction of the receiver. As the air enters, the receiver drops from the rubber stopper E. In replacing the flask, stopcock C is first closed and the empty receiver is held against the flat rubber stopper E while stopcock B is closed to the outside air. When the air in the receiver is exhausted stopcock C is opened. Thus the pressure in the system is equalized and the distillate, which has meanwhile collected in the pyrex separatory funnel F, may be drained into the receiver by opening stopcock A.

If the still is overheated the distillate is dark. A rate of not more than four liters per hour yields an almost colorless distillate. At five liters per hour,

a slight yellow color is noticeable. If there is water present in the esters, it may cause the boiling over of the liquid in the still. At the end of the day's run the dark liquid residue is drained off through the pipe at the bottom by removing the drain plug. Disassembling for cleaning is seldom necessary. If cleaning does become necessary, it may be accomplished by unscrewing the still-head from the still, heating the parts in a fire until the contents become brittle, and then scraping out the charred material.

In distilling thirty liters a day, the yield of esters should average 90 to 95 percent if the crude esters contain less than 5 percent of unconverted oil.
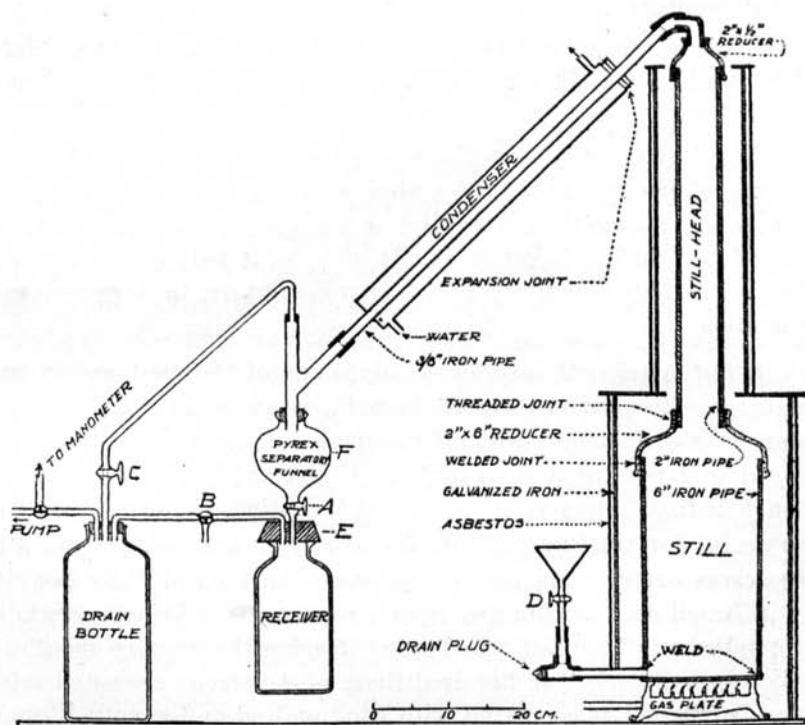
TEXT-FIG. 2. Vacuum still for the distillation of chaulmoogra ethyl esters.

LEPROSY DRUGS MADE FROM ETHYL ESTERS

Unmodified ethyl esters of chaulmoogra oil are generally too irritant for injection purposes; they are, therefore, usually compounded with other substances. Reduction in irritation may be accomplished by dilution with other oils, such as cod-liver or olive. This dilution of the drug naturally reduces the therapeutic effect of an injection of a given volume. In the case of dilution with purified chaulmoogra oil

(which is nonirritating) there is only a slight reduction in therapeutic effect.

Iodine, creosote, menthol, thymol and other such substances reduce the irritant effects of the esters by means other than dilution. In some cases this result is probably due to the anesthetic properties of the added substances. In others it is probably due to their chemical combination with some very irritating substance present in the esters in small amounts. This we believe to be the explanation of the effect of adding iodine.

The three most widely used mixtures today are esters diluted with chaulmoogra oil, esters with creosote added, and esters heated with iodine.

Ethyl esters with chaulmoogra oil.—Pure, nonirritating chaulmoogra oil can be added to the ethyl esters up to 20 percent without unduly increasing their viscosity or their absorbability. Warming this mixture before injection is advisable, as it reduces the viscosity. Creosote is also sometimes added to this mixture, in 4 percent concentration.

Ethyl esters with creosote.—This is one of the most widely used mixtures. Beechwood creosote, U. S. P., is added to the dried ethyl esters at room temperature. The concentration most generally employed is 4 percent, but as high as 10 percent has been used. Although iodized esters are slightly less irritating, the creosoted esters are useful for treating patients found sensitive to iodine, and some physicians prefer them for routine use. An idea of their popularity in Brazil may be obtained from a report of the Departamento de Prophylaxia da Lepra of São Paulo. During the first ten months of 1935 approximately five hundred liters of 4 percent creosoted esters were distributed as compared with one hundred and twenty liters of 2 percent iodized esters and three hundred liters of various other mixtures.

Ethyl esters with iodine.—Ethyl esters with 0.5 percent iodine are probably more extensively used than any other leprosy drug. Their popularity is justified, as they are among the least irritating and most active drugs at present available. One of their drawbacks is that the pigmentation caused by the iodine when the drug is given intradermally remains visible for some time. However, this coloration has some virtue in that it aids the physician by defining for some time the areas that have been treated.

One of us has already shown ([1,2]) that the method of preparing iodized esters has an important bearing upon the reduction of irritation. The method finally evolved was adopted as standard by the Leonard Wood Memorial Conference ([3]). It has been used for many years at the Culion Leper Colony, and has been found to produce a standard product of constant quality.

The method of preparation is as follows: The ethyl esters are heated rapidly in an enamelled or stainless steel pan to 140°C. The proper amount of iodine is stirred in, and the stirring is continued until it is dissolved. The esters react with the iodine, raising the temperature almost immediately to 150°C. By regulating the heating, this temperature is maintained for exactly thirty minutes, with occasional stirring. The esters are then cooled, filtered and placed in ampules or bottles and sterilized. To be certain that the reaction is complete the iodized esters should be stored for two weeks before use.

Several precautions should be observed in preparing the iodized esters by this method. In order that the esters may heat or cool rapidly, the depth of the liquid in the pan should not be much more than one-half the diameter, otherwise a more irritating product results. The esters must be clear and free from water. Continuous stirring is not necessary, but occasional stirring is probably beneficial. The iodized esters should be kept tightly sealed in a cool, dark place as sunlight or heat in the presence of air soon change them into an extremely irritating product.

SUMMARY

1.   A method for the purification of chaulmoogra oils is given.

2.   It is shown that the time of esterification of chaulmoogra oil is not a reliable criterion for determining the completion of the reaction. Refractive index readings should always be used.

3.   A table showing the influence of free fatty acid on the refractive index and optical activity of mixed ethyl esters of *Hydnocarpus wightiana* oil is given.

4.   The factors affecting the rate of esterification are enumerated and discussed.

5.   Experiments that show the influence of sulphuric acid on the rate of esterification are recorded.

6.   A method for the esterification of chaulmoogra oil in approximately eight hours is described.

7.   Apparatus for the preparation, distillation and purification of the ethyl esters of chaulmoogra oil is described.

8.   The method of vacuum distillation of ethyl esters in an iron still is included, with a diagram of the still and directions for its operation.

9. Methods for the preparation of creosoted and iodized esters are given in detail, including precautions to be observed.

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