[Reprinted from the *Philippine Journal of Science*, 46, 1931, 377.]

CAUSES OF IRRITATION UPON INJECTION OF IODIZED ETHYL ESTERS OF HYDNOCARPUS-GROUP OILS[1]

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At the recent conference of leprologists held under the auspices of the Leonard Wood Memorial for the Eradication of Leprosy, the majority of the delegates agreed that the ethyl esters of hydnocarpus-group oils are among the most active drugs at present available in the treatment of leprosy. Several leprologists stated that, by their methods of preparation, a non-irritating oil was essential for the production of esters of low-irritant quality. It is not always possible, however, for institutions situated many thousands of miles from the sources of supply to obtain oil that is fresh and nonirritating. It, therefore, becomes of prime importance that a method be devised by which even intensely irritating oils may be utilized for making relatively nonirritating ethyl esters. In order to accomplish this, it is necessary to take into consideration all the factors that may cause irritation and vary the method of preparation of the esters accordingly.

It has previously been found[2] that irritant properties of ethyl esters may be reduced by (1) elimination of free fatty acids; (2) elimination of decomposition products due to heating or chemical treatment; (3) elimination of volatile and nonvolatile impurities; and (4) addition of 0.5 per cent iodine.

Free fatty acids can be reduced to a minimum (less than 0.2 per cent) by careful neutralization with sodium hydroxide and very thorough washing. Free fatty acids are undoubtedly one of the main causes of irritation. To be certain that the amount present is less than 0.2 per cent, titration with tenth normal alkali should be made a part of the routine procedure for the preparation of the ethyl esters.

[1] Published with the approval of the Director of Health.
[2] Cole, Philip. Jour. Sci. 40 (1929) 503.

Decomposition products, formed upon heating or strong chemical treatment, and other volatile impurities can be largely blown out by steam.

If the esters are distilled, nonvolatile impurities and decomposition products remain in the still as residue. The distilled esters are decidedly more limpid than the undistilled, due probably to the fact that the latter contain some unchanged oil.

Addition of 0.5 per cent iodine markedly reduces the irritant effect of the ethyl esters providing that the method of iodization described below is strictly followed, in which case, the iodine is in the combined form. The presence of free iodine causes irritation.

The inherent irritant quality due to the configuration of the molecule of the compound is, of course, not removable without changing the compound itself. It has been shown that a synthetic compound similar to ethyl hydnocarpate, pure dinormal heptyl ethyl acetate,[3] is even more irritating than ethyl hydnocarpate, while the glyceryl ester of this synthetic compound (corresponding to a natural oil) is bland. This would indicate that part of the irritant effect of the ethyl esters is associated with the ethyl radicle.

A method of preparing hydnocarpus ethyl esters of a standard low-irritant quality, no matter how irritating the original oil may be, has already been described.[4] Since the publication of this process, however, continued experimentation has thrown further light upon the causes of irritation and their prevention.

RELATION BETWEEN IRRITATION AND TYPE AND SHAPE OF CONTAINER

Our standard method[5] for iodizing ethyl esters is as follows:

Fifteen liters of the purified esters are heated in a 20-liter enameled or stainless steel kettle to 140° C. The esters must be thoroughly dried before iodine is added since, if water is present, it effects by catalysis the hydrolysis of several per cent of the esters. If the filtered esters are clear, the heating to 140° C. before adding the iodine will drive off all dissolved water. Seventy-five grams of chemically pure resublimed iodine are added with stirring. The temperature immediately rises to 150° C., at which point it is maintained for exactly thirty minutes, the liquid being stirred occasionally. After cooling, the iodized esters are filtered into bottles (250 cubic centimeters capacity) and

[3] Private communication from C. B. Lara; drug prepared by Roger Adams.
[4] Cole, Philip. Journ. Sci. 40 (1929) 503.
[5] Loc. cit.

sterilized for one hour in an oven at 150° C. The temperature of the contents of the bottles reaches in this time 110° C.

Since this method of iodization was adopted, more than 3,000 liters of ethyl esters (200 lots of 15 liters each) have been iodized and used with practically no complaints of excessive irritation. Smaller institutions, however, might desire to make smaller lots of esters. It was found, in certain recent experimental work, that when the drug was iodized in 1-liter lots instead of the standard 15-liter batches, it was more irritating than usual, although the standard method was carefully followed, except for the fact that these lots were heated in tall, 3-liter glass beakers instead of low, stainless steel or enameled kettles. Experiments were then made using enameled beakers instead of glass beakers, but the drug so prepared was no less irritating. The form of the container was changed from a tall (beaker) type to a shallow (pan) type.[6] The preparations were equally irritating provided that the time of heating to 150° C. and time of cooling to room temperature of these two types were the same. In ordinary practice, however, with the low form (pan type), the drug heats more quickly and cools more rapidly than with the tall form. The longer time necessary to heat and cool the contents of the tall type of container corresponds to overheating of the drug, and we already know that overheating results in an irritant product. This rapid heating and cooling is evidently preferable, for the product in this case was less irritant and, in fact, entirely comparable with that produced by the standard method for 15-liter lots.

EFFECT OF STIRRING ON IODIZATION

In order to determine whether stirring during the iodization is beneficial or otherwise, two batches of esters were iodized at the same time under identical conditions, except that one lot was gently stirred only during the addition of the iodine, while the other one was vigorously stirred by means of a motor stirrer during the entire heating. The time of heating to 140° C. (15 minutes), the time of heating with iodine at 150° C. (30 minutes), and the time necessary to cool to 40° C. (2 hours) were kept constant for both lots by

[6] It assumed that the container will be more than half filled. The depth of the liquid would then be greater than the diameter in the tall type and should be not much more than half the diameter in the shallow type.

regulating the heat input. No differences in color or irritant properties were observed. Occasional stirring, however, is considered necessary to distribute the heat evenly and to hasten the solution of the iodine. Vigorous stirring is unnecessary.

RELATION BETWEEN IRRITATION AND COLOR

The color of iodized esters varies from a turbid greenish to a clear reddish brown, depending on the temperature and time of heating the iodine with the esters. Low temperatures and short time of heating yield a greenish product. High temperatures and prolonged heating yield a clear reddish-brown product.

Color determinations on over a hundred samples were made in order to determine whether there might not be a definite relationship between color and irritant properties. Direct color measurements were not possible owing to the depth of color; diluted samples were therefore, compared. One cubic centimeter of sample was diluted with 25 cubic centimeters of 95 per cent alcohol. A 5-liter lot of iodized esters was taken as standard and all other lots were compared with this one. Comparisons were made in a Bausch and Lomb colorimeter using 20 millimeters depth for the standard. Table 1 shows typical lots of esters compared in this manner. It is obvious that no definite relationship can be drawn between irritant properties and tint and shade of color. On the other hand, these experiments proved that clear reddish-brown at the overheated end and turbid greenish iodized esters at the underheated end of the range are irritating. The correct range of color and turbidity lies between these extremes, but there can be a considerable variation without noticeable change in irritant properties.

RELATION BETWEEN IRRITATION AND EXPOSURE TO SUNLIGHT, AIR, AND HEAT

Iodized esters were exposed to bright sunlight for fourteen hours in (1) filled, well-stoppered bottles and (2) open beakers. Controls were kept in the dark. The irradiated contents of the stoppered bottles and both the stoppered-bottle and open-beaker controls showed no change, but the preparation exposed to sunlight in the open beakers exhibited a marked change in color. The color became more reddish and paler and the turbidity disappeared.

All lots were then kept in the dark for sixteen days at room temperature (30° C.). Very little change was observed in any except

the open beakers previously exposed to sunlight; in these the color had continued to fade until it was only one-fourth as deep as the control. This indicates that sunlight in the presence of air acts as an accelerator of this reaction, the catalytic effect persisting in the drug even after its removal to the dark. Products affected in this way proved extremely irritating.

In order to determine whether this reaction is accelerated by light or heat rays or both, iodized esters in filled, sealed bottles and in open beakers were heated in the dark for ten days at 50° C. as this was the maximum temperature reached by the drugs in the direct sunlight. At the end of fourteen hours, a slight change of color was observed in the open beakers only. This change increased

TABLE 1.—*Relation of color to irritation in iodized ethyl esters.*[a]

Lot number.	Color in millimeters compared with standard.	Tint.	Number of patients tested.	Irritation.		
				Greater than standard per cent.	Less than standard per cent.	Equal to standard per cent.
38 B (Standard)	20	Green brown	331	.....	.....	.....
V	12	.... do	98	5.1	30.6	64.3
W	17	Turbid green brown	82	7.3	18.3	74.4
X	22	Turbid green brown	73	5.5	32.9	61.6
Z	23	.... do	78	5.1	23.1	71.8

[a] The irritation experiments were performed by the members of the medical staff of the Culion Leper Colony. The standard drug and the samples to be tested were injected usually in the upper arm of the patient. Sufficient esters to produce a wheal one centimeter in diameter was injected by the intradermal method. Comparison were made of the pain upon injection and degree of tissue reaction. Observations were made also after 24, 48, 72 hours and one week.

to a marked degree at the end of ten days, showing that heat in the presence of air can accelerate the same reaction as sunlight and air but, as might be supposed, not nearly so rapidly. The controls show that at 30° C. the change is negligible. Table 2 summarizes the results of these experiments.

SUMMARY

The causes of irritation upon injection of iodized ethyl esters of hydnocarpus-group oils are discussed.

The shape of the container in which iodization is accomplished is important. A low (pan) type of vessel rather than a high (breaker) type should be used.

TABLE 2.—*Effect of light, heat, and air on color and irritation of iodized ethyl esters.*

	Color in millimeters equivalent to 20 millimeters of standard.	
	Filled stoppered bottles.	Open beakers.
Before exposure	22	22
Exposed to direct sunlight, 10 hours	22	* 35
Exposed to direct sunlight, 14 hours	22	* 41
Exposed to direct sunlight, 14 hours plus 16 days in dark	22	* 60
Exposed in dark, 10 days at 30°C	22	24
Exposed in dark, 14 hours at 50°C	22	28
Exposed in dark, 10 days at 50°	22	* 60

* Products marked thus were found to be very irritating upon injection.

Occasional stirring during iodization of the esters is probably beneficial. Continuous vigorous stirring is not necessary.

Experiments show that color comparison cannot be utilized as control in the production of standard relatively nonirritating iodized ethyl esters; time and temperature of heating of the esters with the iodine must be used as the basis of control.

Sunlight or heat in the presence of air soon changes the iodized esters in such a way as to yield an extremely irritating product. This deterioration is accompanied by increased clarity and change in color to reddish brown.

The author wishes to gratefully acknowledge his indebtedness to Dr. C. B. Lara and the medical staff at Culion for performing the irritation tests mentioned in this article.