ETHYL ESTERS OF HYDNOCARPUS OIL STABILISED WITH CREOSOTE

By G. E. Foster, E. L. Kendrick, E. Walton and W. D. Williams

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The use of chaulmoogra and hydnocarpus oils in the treatment of leprosy has been long established and the oils are still extensively used, in spite of the introduction of modern drugs of the sulphone type. These oils, which consist largely of the glyceryl esters of chaulmoogric and hydnocarpic acids,[1] containing characteristic unsaturated 5-membered rings in their molecules, undergo oxidation and become acid on storage. On this account the oils usually cause irritation on injection and much work has been done in attempting to remedy this defect.

Hofmann,[2] after attempting to isolate the active principle of chaulmoogra oil, approached the problem by isolating the fatty acids obtained by saponification of the oil and esterifying them with ethanol. The resulting mixture of ethyl esters consisted of a low-boiling oil, easily purified by distillation under reduced pressure, and it quickly found a place under the name "Antileprol" in the treatment of leprosy. Subsequently, it was found that the ethyl esters prepared similarly from hydnocarpus oil were equally effective and they have since been included in the British Pharmacopœia.

The ethyl esters, during the past 25 years, have not entirely fulfilled their early promise, for on storage in the presence of air they develop peroxides[3] and become acid. Such esters cause much pain and irritation when injected. Purification by redistillation or by treatment with reducing agents, such as sodium bisulphite solution, is of little value unless the product is used immediately. Iodination of the esters[4] was successful in reducing the incidence of irritation but the iodised esters gave rise to discolouration at the site of injection and never enjoyed great popularity. A more fruitful approach consisted in the addition of anti-oxidants to inhibit oxidation and, in our experience, the best stabiliser of this type is creosote. The use of creosote in Brazil, where a combination of esters of chaulmoogra oil with 4 per cent. of creosote was being used, came to our knowledge in 1941. During the past decade the ethyl esters of hydnocarpus oil stabilised with creosote have enjoyed wide use by leprologists and it is the purpose of this communication to give some account of the stability and standardisation of the preparation.

Stability Tests

For our study of the stability of the ethyl esters of hydnocarpus oil, with and without the addition of creosote, it was decided to use three grades of esters which had been prepared by (1) using no special precautions, (2) refining by chemical treatment and (3) purifying by redistillation. Deterioration was assessed by observing the increase in acid value of the preparations and the development of peroxide. For

the latter determination a test based upon the B.P. method for determining the ascaridole content of chenopodium oil was found convenient and was first brought to our notice by Mr. Y. T. Kruithof, of the Medical Service, Netherlands Guiana, to whom we are indebted. Details of the test are as follows. Into a glass stoppered tube, of about 25 ml. capacity, place 10 ml. of 30 per cent. w/v solution of potassium iodide and 3 ml. of 4N hydrochloric acid. Add 10 ml. of ethyl esters of hydnocarpus oil, shake for 10 minutes and titrate the liberated iodine with 0·1N sodium thiosulphate, using mucilage of starch as indicator.

The preparations, stored in completely filled stoppered bottles and in open half-filled bottles, were kept at 40° C. for 3 months after which they were re-examined. The results are summarised in Table I.

TABLE I

KEEPING TESTS ON SAMPLES OF ETHYL ESTERS OF HYDNOCARPUS OIL

	Initial examination		Examination after 3 months at 40° C.					
			Filled and stoppered			Open and half filled		
Sample	Peroxide Test 0·1N sodium thiosulphate ml.	Acid Value	Reference Letter	Peroxide Test 0·1N sodium thiosulphate ml.	Acid value	Reference Letter	Peroxide Test 0·1N sodium thiosulphate ml.	Acid value
Esters	13·0	0·59	A.	12·2	0·89	B.	25·7	2·46
Esters + 4 per cent. v/v of Creosote ..	12·6	1·02	C.	7·7	1·64	D.	8·8	1·64
Refined esters ..	1·1	0·58	E.	0·6	1·42	F.	22·9	3·18
Refined esters + 4 per cent. v/v of creosote ..	1·0	1·04	G.	0·4	3·12	H.	0·7	3·16
Redistilled esters	0·35	0·61	I.	0·1	0·61	J.	19·2	1·47
Redistilled esters + 4 per cent. v/v of creosote	0·43	1·14	K.	0·4	2·01	L.	1·5	2·62

From these experiments it is evident that samples kept in completely filled stoppered bottles exhibit good preservation, even when no creosote is added. Refined esters became coloured during the test and were less satisfactory than the redistilled product. When air is present, the stabilising effect of added creosote is marked.

At the completion of the keeping test the samples were sent to Dr. A. C. White, of the Wellcome Research Laboratories, Beckenham, who carried out irritation tests by intracutaneous injection of 0·1 ml. of a 25 per cent. dilution of each preparation in liquid paraffin into guinea-pigs. His results are summarised in Table II, in which the numbers beside the letters in the two columns are based upon the intensity of raction in 2 series of experiments involving 12 guinea-pigs.

TABLE II

IRRITATION TESTS

(Letters refer to samples in Table I)

B (26)	>	A (10)
D (10)	>	C (7·5)
F (27)	>	E (10·5)
G (7·8)	>	H (6·5)
J (31·5)	>	I (6·5)
K (7·5)	>	L (6·2)

The preparations fell roughly into two groups and B, F and J were clearly the most irritant samples tested.

It seemed of interest to complete our stability studies by examination of samples of esters which had been kept at room temperature for periods up to 5 years. As was to be expected from our experiments at 40° C., it was found that the esters, with and without creosote, kept satisfactorily in completely filled and well closed containers, very little increase in peroxide and only slight rises in acid values occurring. As an example of what can happen, however, a sample of esters, stored for 5 years at room temperature, was found to give a reading in the peroxide test of 140 ml. of 0·1N sodium thiosulphate. A sample of the corresponding product, containing 4 per cent. v/v of creosote, prepared from the same esters and stored under identical conditions for the same period gave a reading of only 0·15 ml. of 0·1N sodium thiosulphate when tested.

STANDARDISATION

The analytical control of ethyl esters of hydnocarpus oil stabilised with creosote is surrounded by difficulties. Both constituents are mixtures of indefinite composition and no specific methods for their estimation are available. Application of the usual analytical methods for oils and fats, however, has provided a useful control for purposes of manufacture. Table III summarises data on 10 freshly prepared batches of esters, selected at random from many, and Table IV gives data for the corresponding preparations containing creosote. The reference numbers of the samples are correlated; for example, sample No. 1 in Table IV was prepared with sample No. 1 of esters in Table III.

TABLE III

ANALYTICAL DATA ON SAMPLES OF ETHYL ESTERS OF HYDNOCARPUS OIL

Sample	Specific Gravity at 15·5° C.	Saponification value	Acid value	Iodine value	Optical rotation	Refractive Index at 20° C.	Peroxide test 0·1 N sodium thiosulphate ml.
1	0·9105	193·3	0·42	92·2	+45·64°	1·4580	1·6
2	0·9099	193·2	0·22	92·0	+46·37°	1·4600	0·6
3	0·9100	193·0	0·20	91·1	+46·33°	1·4610	0·2
4	0·9100	193·6	0·29	90·5	+46·41°	1·4605	1·0
5	0·9100	192·6	0·21	92·9	+46·51°	1·4605	0·75
6	0·9101	192·1	0·22	92·6	+46·45°	1·4605	0·9
7	0·9102	192·0	0·20	93·0	+46·25°	1·4595	1·6
8	0·9106	190·6	0·11	91·0	+46·8°	1·4580	0·5
9	0·9099	193·3	0·21	90·1	+46·96°	1·4605	0·5
10	0·9111	192·3	0·23	91·3	+47·4°	1·4601	0·4

ESTIMATION OF CREOSOTE

We have spent much time in search of a reliable method for the estimation of creosote in the stabilised esters but we have discovered no entirely satisfactory assay process. It is possible to remove creosote from a solution of the esters in petroleum ether by shaking with N sodium hydroxide solution, the creosote being recovered from the alkaline extract by acidifying and extracting with ether. It is difficult, however, to remove the solvent from the final extract without loss of creosote. In some experiments, recoveries of creosote amounting to 100 per cent.

TABLE IV

ANALYTICAL DATA ON SAMPLES OF ETHYL ESTERS OF HYDNOCARPUS OIL WITH 4 PER CENT V/V OF CREOSOTE

Sample	Specific gravity at 15·5° C.	Acid value	Optical rotation at 20° C.	Peroxide test 0·1N sodium thiosulphate ml.
1	0·9177	1·2	+43·89°	1·0
2	0·9173	0·72	+44·53°	0·65
3	0·9173	0·73	+44·45°	0·25
4	0·9170	0·85	+44·55°	0·65
5	0·9171	0·79	+44·6°	0·9
6	0·9173	0·69	+44·1°	0·2
7	0·9170	0·75	+44·44°	0·15
8	0·9177	0·34	+44·62°	0·45
9	0·9168	0·6	+45·04°	0·4
10	0·9180	0·74	+45·4°	0·3

were obtained, but in others the results were less satisfactory. The separated esters may also be recovered and weighed by removal of the solvent from the initial petroleum ether extract; the residue being dried at 100° C. for 15 minutes. Results correct to within 1 per cent. of theory were obtained for the ester content so determined.

More promising results were obtained by use of the reaction between nitrous acid and phenols employed by the B.P. for the estimation of morphine in some opium preparations. Details of this process are as follows.

Reagents.

1 per cent. w/v Solution of Sodium Nitrite
Dilute Phosphoric Acid B.P.
Dilute Solution of Ammonia B.P.

Calibration Curve. Prepare a 0·1 per cent. w/v solution of creosote B.P. in water containing a few drops of sodium hydroxide solution. Place 0, 1, 2, 3, 4 and 5 ml. in 50-ml. stoppered cylinders and dilute each to 20 ml. Add 8 ml. of sodium nitrite solution and 6 ml. of dilute phosphoric acid to each cylinder, mix and allow to stand for 15 minutes. Render alkaline by the addition of 12 ml. of dilute ammonia solution, adjust the volume to 50 ml., mix well and allow to stand for 15 to 20 minutes. Measure the light absorption of the reaction mixtures with a Spekker Absorptiometer using 1 cm. cells and a combination of Ilford 603 and heat resisting H503 filters. Construct a calibration curve from the readings obtained.

Assay of Ethyl Esters of Hydnocarpus Oil with Creosote. Transfer a quantity of esters containing 3 to 4 mg. of creosote to a separating funnel and dilute with 20 ml. of ether. Add 15 ml. of water, 8 ml. of sodium nitrite solution and 6 ml. of dilute phosphoric acid. Shake for 15 minutes, add 12 ml. of dilute ammonia solution, mix, allow to separate and run the lower layer into a stoppered cylinder. Wash the ether extract with two quantities, each of 2 ml., of water, adding the washings to the cylinder. Adjust the volume to 50 ml., mix well and allow to

stand for 15 to 20 minutes. Measure the light absorption of the reaction mixture, as above, and estimate the creosote content of the esters from the calibration curve. Use the appropriate specific gravities to calculate the creosote content as per cent. v/v.

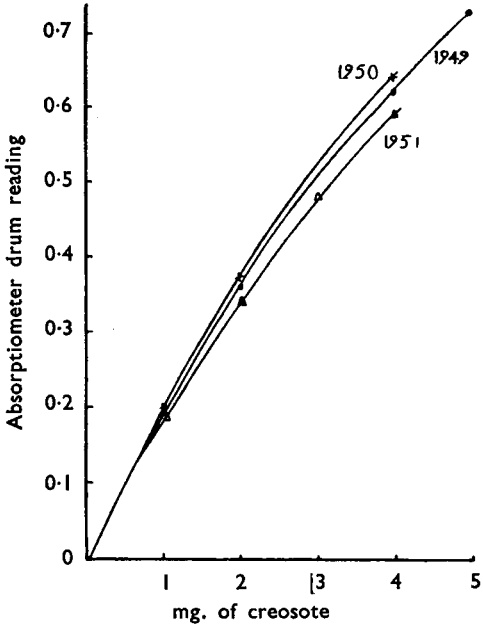

FIG. 1. Calibration curves for batches of creosote; with Ilford 603 and heat resisting H503 filters.

This colorimetric assay process yields good results providing the calibration curve is constructed using a sample of the same creosote as is used for stabilising the esters examined. Unfortunately, the curves for batches of creosote vary, as will be seen by reference to Figure 1. Using an average curve we have obtained figures of 4 to 4·5 per cent. for preparations containing 4·0 per cent. of creosote.

DISCUSSION

The work described in the present paper emerged from the irritation repeatedly observed when hydnocarpus oil and its derivatives were injected during clinical practice. There is little doubt[5] that the principal cause of excessive irritation is the presence of oxidation products of the unsaturated acids of which the oils are esters. The stability experiments performed have afforded conclusive evidence that the ethyl esters of hydnocarpus oil keep well at room temperature when stored in completely filled and well closed containers but, on exposure to air, oxidation readily takes place with the initial formation of organic peroxides. This deterioration can be very largely eliminated by the addition of 4 per cent. of creosote to the esters; at the same time the development of irritant properties is retarded.

In view of the extent to which the stabilised esters have been used in leprosy it is desirable that suitable analytical methods should be available to control manufacture. This raises difficulties owing to the constituents of the preparation being mixtures of varying composition. From the analytical data presented in Tables III and IV it is evident that application of the usual methods for the analysis of oils and fats can afford useful information. In particular these data show that both the specific gravity and the optical rotation of the esters are significantly affected by the addition of creosote. An average difference of 1·95° between the optical rotations of the esters, before and after stabilisation, is observed

and this corresponds to approximately 4 per cent. of added creosote. Similarly, taking the specific gravity at 15·5° C. of creosote as 1·086, the average increase in specific gravity roughly agrees with the amount of added creosote. The presence of organic peroxides should be controlled and it would be reasonable to impose a limit of 2 ml of 0·1N sodium thiosulphate for the titration obtained by the peroxide test described. Addition of creosote tends to increase the acid value but there is no reason for the acid value of the stabilised product to be greater than that allowed (1·0) for ethyl esters of hydnocarpus oil B.P. Application of the nitrous acid reaction, employed for morphine estimations, has afforded a useful colorimetric method for the determination of creosote in the stabilised esters but its application is limited owing to the need to prepare a calibration curve using a sample of the actual creosote present in the esters if accurate results are to be obtained.

The success which attended the addition of creosote to the ethyl esters directed attention to stabilising hydnocarpus oil by similar means. This problem is complicated by the fact that the oil, unlike the esters, cannot be commercially purified by re-distillation. The only practical way of overcoming the difficulty is to add creosote to the oil immediately after expression from the seeds of *Hydnocarpus Wightiana* and before oxidation has commenced. This, in fact, has been done and many tons of hydnocarpus oil containing creosote have been used. The problems of analytical control of this preparation are similar to those of the stabilised ethyl esters and have been largely solved by application of the methods described for the esters, with appropriate modification.

Summary

1. The stability of the ethyl esters of hydnocarpus oil B.P. with and without added creosote, has been investigated by keeping samples at 40° C. for 3 months and at room temperature for 5 years.

2. The esters when kept in completely filled well-closed containers undergo little deterioration on storage.

3. When exposed to air the esters rapidly develop peroxides and become acid in reaction. Such esters cause excessive irritation on injection.

4. Addition of 4 per cent. v/v of creosote to the esters very largely inhibits the oxidation which occurs when the esters are kept in partly filled containers.

5. Analytical data are presented for the standardisation of the esters stabilised with 4 per cent. v/v of creosote.

6. A colorimetric method for the determination of creosote in the esters is described.

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DISCUSSION

The paper was presented by DR. G. E. FOSTER.

The CHAIRMAN asked whether guaiacol had been considered as a stabiliser, as it had a local anæsthetic effect and was therefore less painful on injection, and whether other phenolic substances had been investigated in that respect.

MR. A. F. CALDWELL (Singapore) stated that, in Malaya, creosote had been added to hydnocarpus oil. Work had not been done with the official oil because supplies from India were not free from peroxide, but oil from *Hydnocarpus anthelmintica* was found to be satisfactory and of a better quality than the hydnocarpus oil from India. In the treatment of leprosy most patients had some nerve involvements and the clinical results might therefore be different from tests on experimental animals. He referred to the difficulty of trying to assess clinical pain and described his own experience of issuing on one occasion a large number of bottles of hydnocarpus oil known to be free from peroxides. The oil had come back with the complaint that it was very painful. It had then been divided into two portions and reissued. They were subsequently informed that both batches were perfectly satisfactory. It had been found from experience that hydnocarpus oil, sterilised and packed in 1 oz. bottles completely filled, remained free from peroxide for a period of up to 10 years.

DR. F. HARTLEY (London) suggested that it was unexpected to find that creosote was the chosen antioxidant, and asked what criteria the authors had in mind when they referred to it as the "best stabiliser." He was surprised by the degree of precision indicated in the results of the irritation tests.

DR. R. E. STUCKEY (London) asked for information about the method used in selecting creosote as the antioxidant. Recent work had shown that there was a synergistic effect between one class of antioxidants, phenolic bodies, and some acidic substances such as phosphoric or citric acid. It had been shown that citric acid removed the metallic ions which catalysed the oxidation so increasing the efficiency of the phenolic antioxidant. Had the authors tried any such combinations in their experiments? Was there any evidence of the synergistic effect of an acid, such as citric acid, and creosote in stabilising the ester?

DR. G. E. FOSTER, in reply, said that guaiacol had not been tried instead of creosote. The anæsthetic effect mentioned by the Chairman was interesting, because in 1927 a great deal of pain was reported when the ethyl esters were being used. The addition of creosote was tried as

something which would minimise pain owing to its very weak anæsthetic properties, but it was given up at that time because it seemed to be of no particular value. It was not until 1940 that creosote was used in Brazil with beneficial results in reducing the incidence of irritation and with antioxidant effect. He had established that 1 per cent. of creosote was no less irritant than 4 per cent. when added to the preparation, and clinical trials showed the same.

It was gratifying to learn that some of the results obtained by Mr. Caldwell coincided with their own.

Dr. Hartley and Dr. Stuckey had mentioned the use of other anti-oxidants and he would give them fullest information after he had had an opportunity of consulting his colleagues at Dartford.