The Transesterification and Chromatographic Treatment of Fish Liver Oils as a Means of Concentrating Vitamin A

M. L. KARNOVSKY, W. S. RAPSON, and N. J. VAN RENSBURG, Department of Chemistry, University of Capetown, South Africa

THE fact that vitamin A alcohol is adsorbed from suitable solvents onto alumina or similar adsorbents, whereas its esters are not retained, has been used as a method for the separation and concentration of the alcohol (1, 2, 3, 4). Although it is usual to apply chromatography as a sequel to the saponification of the oil and isolation of the unsaponifiable fraction, Swain (1) has reported the successful chromatographic concentration of vitamin A after an unspecified selective hydrolysis of its esters. This author has found also that selective saponification of vitamin A esters in the presence of glycerides is impracticable (1b), and it was thought by the present authors that transesterification methods might give some interesting results.

Patents have been filed for the use of transesterification techniques in concentrating the vitamin A of fish liver oils (5) and the carotenoids of palm oil (6). In these methods a large proportion of the ethyl or methyl esters of the fatty acids, formed by catalytic transesterification of the glycerides, is removed by distillation under high vacuum and the active material thus concentrated in the residue. A series of experiments was conducted in this laboratory to investigate the possibility of preferential transesterification of vitamin A esters in glyceride fats, and the removal of the free vitamin A alcohol from the esters of the fatty acids by chromatographic methods. Accordingly, a number of soupfin shark† and hake* liver oils were transesterified catalytically in ethyl alcohol as described in the experimental section, and the extent of liberation of vitamin A alcohol determined by a very simple chromatographic method.

Tables 1 and 2 record some preliminary results. In Table 1 an analogy is drawn between the behavior of vitamin A alcohol contained in the unsaponifiable fraction of a hake liver oil and the vitamin A of a transesterified hake liver oil on chromatographic examination. In Table 2 the results obtained on chromatographing original and transesterified fish liver oils are compared. Only very little of the vitamin A of untreated liver oils is seen to be adsorbed from benzene, and these are therefore regarded as containing but little vitamin A alcohol, most of the vitamin A present being esterified (c.f. 4), and thus passing directly into the filtrate. Intermediate results, exemplified by columns 2 and 3 of Table 1, in which some of the vitamin A present in the material examined is in the alcohol form and is adsorbed and some is esterified and passes through the column in the benzene, were encountered in later experiments where the conditions of the transesterification reaction were such that the process did not go to completion.

Following the promising results of the preliminary experiments, the effects of varying the conditions of the transesterification reaction were examined, i.e. ratio of oil to alcohol, time of reaction, and temperature of reaction, with the object of establishing the optimum conditions and determining whether preferential attack on the vitamin A esters could be achieved. Tables 3, 4, and 5 and Fig. 1 represent the results obtained. It is clear from Table 3 and Fig. 1 that for satisfactory reactions at room temperature considerable excess of ethyl alcohol is necessary—dilute solutions of the oil in alcohol giving the best results. From Tables 4 and 5 it is clear that, at room temperature, at least one or two hours should be allowed in order to achieve efficient conversion of vitamin A esters to vitamin A alcohol whereas on refluxing the mixture almost complete conversion is obtained in a short time. The final conditions found to be satisfactory were a dilution of oil in alcohol of 1 to 5 or 10, by weight, refluxed for 20 minutes. The concentration of the sodium ethoxide catalyst was about 0.3% throughout, obtained by adding 0.1% of sodium to the alcohol.

TABLE 1

Behavior of Vitamin A Alcohol and Ester on Chromatographic Examination

Weight of oil chromatographed (gm.)		A. 1.0370	B. 0.9986	C. 1.0741
Fraction I (50 ml. benzene)	Weight (gm.)	0.8679	0.8850	1.0507
	Percentage total weight	83.7	88.6	97.8
	$E^{1\%}_{1\,cm.}$ 328 mμ value	0.12	9.3	4.08
	Percentage of total recovered vitamin A	2.40	58.52	52.18
Fraction II (50 ml. benzene with 0.5% ethyl alcohol)	Weight (gm.)	0.1369	0.0600	0.0439
	Percentage total weight	13.2	6.0	4.1
	$E^{1\%}_{1\,cm.}$ 328 mμ value	29.4	1.5	85.6
	Percentage of total recovered vitamin A	93.01	0.65	45.72
Fraction III (50 ml. ethyl alcohol)	Weight (gm.)	0.0769	0.0829	0.0140
	Percentage total weight	7.4	8.3	1.3
	$E^{1\%}_{1\,cm.}$ 328 mμ value	2.6	68.8	12.3
	Percentage of total recovered vitamin A	4.58	40.68	2.09

A. Unsaponifiable matter of hake liver oil in arachis oil.
B. Unsaponifiable matter of hake liver oil in hake liver oil.*
C. Transesterified hake liver oil in hake liver oil.*

NOTE: Transesterifications carried out with alcohol : oil ratios = 10:1 by weight, at room temperature, for 2 hours.

* Containing approximately equal amounts of vitamin A in each component of mixture.

From the fact that the increase in weight of the fatty acid esters in the reaction (glycerides → esters of ethyl alcohol) was considerable in all cases where the degree of conversion of vitamin A ester to alcohol was high, and the failure to achieve any good result by diminishing the amount of alcohol or the time of reaction, it was concluded that preferential attack on the vitamin A esters was not feasible.

Experimental

1. *Isolation of Unsaponifiable Matter.* Where required this was carried out by the S.P.A. method (7) using 4 extractions with ether (8).

2. *Transesterification.* The transesterification reactions were carried out by dissolving the oil (about 3

† *Galeorhinus canis* (Rond.). See Molteno *et al.* (11). A full report on the characteristics of the oil of this species will appear as Part 29, "South African Fish Products," J.S.C.I.

* *Merluccius capensis* (13).

TABLE II

Comparison of State of Vitamin A in Original and Transesterified Fish Liver Oils

		Soupfin I Liver oil		Soupfin II Liver oil		Hake Liver oil	
		O.	T.	O.	T.	O.	T.
Weight of oil chromatographed (gm.)		1.1608	1.1510	1.4714	1.1022	1.3246	1.0751
$E^{1\%}_{1cm.}$ 328 mμ of oil chromatographed		9.13	7.72	1.86	1.63	9.00	7.17
Fraction I (50 ml. benzene)	Weight (gm.)	1.1282	0.9810	1.3993	1.0500	1.2735	1.0070
	Percent. total weight chromatographed	97.12	85.23	95.13	95.28	96.16	93.63
	$E^{1\%}_{1cm.}$ 328 mμ value	9.01	0.67	1.92	0.21	9.09	0.33
	Percent. of total recovered vitamin A	99.02	8.34	95.88	15.05	97.72	4.49
Fraction II (50 ml. benzene plus 0.5% ethyl alcohol)	Weight (gm.)	0.0262	0.1812	0.0182	0.0465	0.0773	0.0231
	Percent. total weight chromatographed	2.26	5.74	5.31	4.22	5.83	2.15
	$E^{1\%}_{1cm.}$ 328 mμ value	0.89	14.89	0.46	26.5	2.77	293.4
	Percent. of total recovered vitamin A	0.22	34.28	1.29	83.75	1.81	91.62
Fraction III (50 ml. benzene) plus 0.5% ethyl alcohol)	Weight (gm.)	0.0030	0.0129	0.0415	0.0042	0.0024	0.0014
	Percent. total weight chromatographed	0.26	1.12	2.82	0.38	0.18	0.13
	$E^{1\%}_{1cm.}$ 328 mμ value	18.07	332.1	1.90	1.86	0.24	69.2
	Percent. of total recovered vitamin A	0.53	54.39	2.82	0.54	0.10	1.35
Fraction IV (50 ml. ethyl alcohol)	Weight (gm.)	0.0034	0.0254	.....	0.0078	0.0069	0.0171
	Percent. total weight chromatographed	0.29	2.26	.....	0.71	0.52	1.59
	$E^{1\%}_{1cm.}$ 328 mμ value	7.36	9.30	.....	12.26	4.13	11.08
	Percent. of total recovered vitamin A	0.24	2.98	.....	0.68	0.24	2.56
Total oil recovered (%)		100.0	104.2	103.2	100.6	102.7	105.9
Total vitamin A recovered (%)*		96.8	88.6	102.4	81.9	99.2	96.0

O = original fish liver oil. T = transesterified fish liver oil. All transesterifications as in Table I.

* Somewhat erratic recoveries, as no elaborate precautions to avoid oxidation were taken in preliminary experiments. Losses highest in chromatograms where adsorption highest.

gm.) in the required weight of pure, absolute alcohol, to which sufficient Na had been added to give a concentration of 0.1%, or 0.3% as NaOEt. The mixture was then thoroughly shaken, and where the ratio of alcohol:oil was greater than 2:1 became homogeneous, and was allowed to stand, with occasional shaking. Where separation occurred, continuous shaking was necessary. The mixture was held at room temperature (about 20°C.), or as was finally decided, was refluxed, for the period required, and then poured into cold water (twice the volume of alcohol used). At least three extractions with peroxide-free ether followed—each volume of ether used being equal to that of the water present. The ethereal solution was washed once or twice with distilled water, distilled into a small flask in a waterbath, and the product dried under vacuum in a waterbath. A little acetone was added and drawn off as above to facilitate elimination of last traces of water. The product was weighed.

NOTE: Alcohol purified by distillation over Al and KOH (9), and then distilled over calcium was found suitable. The reaction mixture was kept, wherever possible, in an inert atmosphere away from light.

Loss of Vitamin A. This was usually 3-5% in this part of the process.

3. *Chromatographic Separation of Vitamin A Alcohol and Ester* (cf. 3, 4). A column of alumina,* 20 cm. high by 0.8 cm. diameter was used, the receiver being a tared 100-ml. distilling flask. The material under examination (1 gm.) was dissolved in pure, dry benzene (about 50 ml.) and passed through the column over about 1 hour. The column was washed until 65 ml. of benzene had been passed into it, and the receiver (100-ml. distilling flask) was changed. A mixture of 5% EtOH in benzene was then passed down the column (50 ml.) into the new tared receiver.

The solvent was removed from each fraction on a waterbath under vacuum, until constant weight was attained. The vitamin A content of the material in each flask was then determined.

In the preliminary experiments, where more than 2 fractions were collected, the solvents were as indicated in the tables and the procedure as above.

*B.D.H. "alumina for chromatographic analysis," without further activation. Column prepared from dry alumina and washed with benzene before use.

TABLE III

Variation of Degree of Transesterification With Variation of Alcohol/Oil Ratio

[Reaction time 1 hour throughout, at room temperature]

Ratio of oil to alcohol in transesterification reaction (by weight)		1:1	1:2	1:3	1:5	1:10
$E^{1\%}_{1cm.}$ 328 mμ of original oil		9.20	9.20	9.20	9.20	8.53
$E^{1\%}_{1cm.}$ 328 mμ of transesterified product		8.85	8.62	8.91	8.35	7.82
Weight of material chromatographed (gm.)		1.1138	1.1631	0.9873	1.0004	1.1719
Fraction I (Pure benzene 65 ml.)	Weight (gm.)	0.9210	1.0995	0.9472	0.9728	1.1407
	Percentage of total weight	82.7	94.6	95.9	97.3	97.3
	$E^{1\%}_{1cm.}$ 328 mμ	10.14	6.21	4.21	2.84	2.08
	% of total recovered vitamin A	95.4	72.2	51.3	34.4	28.3
Fraction II [Ethyl alcohol (5%) in benzene (50ml.)]	Weight (gm.)	0.1932	0.0609	0.0417	0.0371	0.0325
	Percentage of total weight	17.4	5.2	4.2	3.7	2.8
	$E^{1\%}_{1cm.}$ 328 mμ	2.33	43.15	90.9	141.7	185.4
	% of total recovered vitamin A	4.60	27.8	48.7	65.5	71.7
Recovery of vitamin A (%)	On transesterification	98.7	98.6	101.6	93.6	96.2
	On whole process	98.0	93.0	89.8	89.9	88.9

TABLE IV

Variation of Degree of Transesterification With Time of Reaction, at Room Temperature
[Ratio of oil/alcohol throughout = 1/5 by weight]

Time of reaction (mins.)		10 min.	60 min.	120 min.
$E^{\%}_{1\,cm.}$ 328 mμ of original oil = 9.20				
Weight of oil chromatographed (gm.)		1.1218	1.0004	1.2485
$E^{1\%}_{1\,cm.}$ 328 mμ		8.71	8.35	8.14
Fraction I (65 ml. pure benzene)	Weight (gm.)	1.0163	0.9728	1.2100
	Percent. of total weight	90.5	97.3	96.9
	$E^{1\%}_{1\,cm.}$ 328 mμ	7.15	2.84	1.66
	% of total recovered vitamin A	80.0	34.4	20.3
Fraction II [50 ml. ethyl alcohol (5%) in benzene]	Weight (gm.)	0.1018	0.0371	0.0412
	Percent. of total weight	9.1	3.7	3.3
	$E^{1\%}_{1\,cm.}$ 328 mμ	17.88	141.7	191.0
	% of total recovered vitamin A	20.0	65.5	79.7
Recovery of vitamin A (%)	On transesterification	99.0	93.6	98.2
	On whole process	92.1	89.9	95.5

NOTE: As far as possible, light was excluded, and vessels filled with N_2 or CO_2. Occasionally somewhat more material was recovered than was used, due to incomplete removal of solvents.

Recovery of Vitamin A. Loss of vitamin A, using the 2-fraction separation above, was usually of the order of 1-5%.

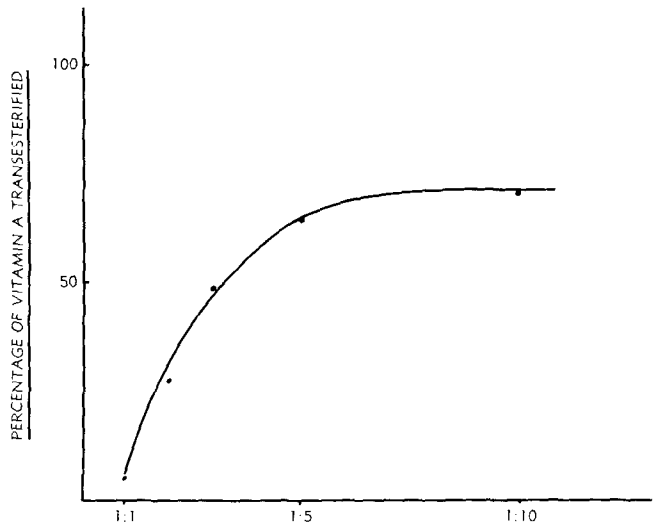

FIG. 1. Variation of degree of transesterification of vitamin A with oil/alcohol ratio. (Conditions as in Table III.)

4. *Determination of Vitamin A.* The method of Dann and Evelyn (10) and an Evelyn colorimeter were used throughout. [As a factor for converting the $E^{1\%}_{1\,cm.}$ 328 mμ values obtained into International units per gram, 1600 has been in common use in this laboratory (12).]

Summary

Fish liver oils containing vitamin A esters have been catalytically transesterified with ethyl alcohol, and the proportions of vitamin A alcohol produced under various conditions have been evaluated chromatographically.

Acknowledgments

The authors acknowledge financial assistance from the National Research Council and Board and from the following industrial concerns: Concentra (Pty.) Ltd.; Irvin and Johnson (S.A.) Ltd.; Marine Products (Pty.) Ltd.; The National Trawling and Fishing Coy.; Ocean Products (Pty.) Ltd.; Union Coast Fisheries (Pty.) Ltd.; and Vitamin Oils (Pty.) Ltd.

TABLE V

Transesterification Under Reflux for Twenty Minutes

Ratio of oil : alcohol		1:10	1:5*
$E^{1\%}_{1\,cm.}$ 328 mμ original oil		8.75	.....
$E^{1\%}_{1\,cm.}$ 328 mμ transesterified product		7.87	6.60
Weight of oil chromatographed (gm.)		1.0155	0.5190
Fraction I (65 ml. benzene)	Weight (gm.)	1.0043	0.2836
	$E^{1\%}_{1\,cm.}$ 328 mμ	0.10	0.19
	% of total recovered vitamin A	1.3	1.8
Fraction II [50 ml. ethyl alcohol (5%) in benzene]	Weight (gm.)	0.0411	0.2354
	$E^{1\%}_{1\,cm.}$ 328 mμ	197.4	12.71
	% of total recovered vitamin A	98.7	98.2
Recovery of vitamin A	On transesterification	93.7	.....
	On whole process	96.2	.....

*An early experiment.

REFERENCES

1. Swain, Prog. Reports Pacific Biol. Station, Fish Res. Bd. Canada (a) No. *49*, 13; (b) No. *50*, 8; (c) 1942, No. *53*, 7.
Swain, J. Fish Res. Bd. Canada 1943, *6*, 113.
2. Morton, Prog. Reports Pacific Biol. Station, Fish Res. Bd. Canada, 1944, No. *58*, 13.
Swain, *ibid.*, 1945, No. *63*, 32.
Swain and McKercher, *ibid.*, 1945, No. *65*, 67.
3. Reed, Wise, and Frundt, Ind. Eng. Chem. (Anal.) 1944, *16*, 509.
Glover, Goodwin, and Morton, Biochem. J., 1947, *41*, 94.
4. Kascher and Baxter, Ind. Eng. Chem. (Anal.), 1945, *17*, 499.
5. Tanaka, C. A., 1941, *35*, 1939[8]—Japanese Pat. 128,235, Jan. 10, 1939.
6. Eckey, C. A., 1947, *41*, 2596[e]—British Pat. 567,682, Feb. 27, 1945.
7. Analyst, 1933, *58*, 203.
8. Karnovsky and Rapson, J. Soc. Chem. Ind., 1947, *66*, 95.
9. A.O.A.C. Methods of Analysis, 6th Ed., 1945, p. 497.
10. Dann and Evelyn, Biochem. J., 1938, *32*, 1008.
11. Molteno, Rapson, Roux, Schwartz, and Van Rensburg, J.S.C.I. 1945, *64*, 172.
12. Vitamins and Hormones, Vol. II. (Edited by Harris and Thiman.) Academic Press, N. Y., 1944, p. 192.
Cf. A.O.A.C. Methods of Analysis, 1945, p. 600.
13. Rapson, Schwartz, and van Rensburg, J.S.C.I., 1944, *63*, 313; *ibid.*, 1945, *64*, 140.
Roux and Molteno, *ibid.*, 1946, *65*, 281.