Studies on Castor Oil. I. Fatty Acid Composition of Castor Oil[1]

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THE COMPONENT ACIDS of castor oil *(Ricinus communis)* appear to have been first studied in 1848 by Saalmüller (36), who isolated an hydroxy acid and named it ricinoleic acid. The constitution of ricinoleic acid was first established by Goldsobel (9) as 12-hydroxy-9,10-octadecenoic acid and has since been confirmed by a number of investigators (6, 12, 20, 43). Further investigations by Krafft (24), Julliard (17), Meyer (29), Haller (11), Fahrion (8), Eibner and Munzing (7), Myddleton *et al.* (31), Heiduschka and Kirsten (13), and Panjutin and Rapoport (33) established the presence of stearic, dihydroxystearic, oleic, and linoleic acid among the fatty acids of castor oil. Toyama and Ishikawa (39) showed that the two hydroxyl groups in the dihydroxystearic acid are at 9,10 positions. This observation was later confirmed by King (22), who showed that this dihydroxystearic acid is isomeric, with the higher melting (m.p. 132°C.) dihydroxystearic acid obtained by mild oxidation of oleic acid. The saturated acids were usually presumed to be stearic acid (38) although the recent work of Gupta *et al.* (10) has revealed the presence of myristic and palmitic acids.

While there is general agreement regarding the nature and number of component acids, there is no agreement as to their relative proportions. Compositions of castor oil as reported by the various investigators are given in Table I.

Considering the more recent values, those of Kaufmann and Bornhardt (21) were obtained from equations based on theoretical constants of the various acids, in which the experimental values for neutralization value, iodine value, thiocyanogen value, and hydroxyl value of the mixed fatty acids were substituted. They also determined the saturated acids by the Bertram oxidation method and verified the fatty acid composition by a study of the products obtained from the hydrogenation of acetylated castor oil. Riley (35) however contended that the hydroxyl value was not a reliable measure of the amount of hydroxy acids in the mixed acids and recommended the determination of the acetyl value of the oil itself. His procedure for ascertaining the composition had the advantage that linoleic acid was determined spectrophotometrically rather than by calculation from iodine and thiocyanogen values. The estimation of dihydroxystearic acid however was based on its insolubility in ethyl acetate at 0°C. but, because the effect of ricinoleic acid on the solubility of dihydroxystearic acid was not taken into consideration, the values obtained were usually low.

As ricinoleic acid is calculated from the acetyl value after allowing for dihydroxystearic acid, a low value for the latter automatically results in a high value for the former and also a very low value for oleic acid, which is calculated from the iodine value after allowing for linoleic and ricinoleic acids. Since the true thiocyanogen value of ricinoleic acid is not known and, according to Riley (35), the hydroxyl value is not reliable, the composition of castor oil reported by Kaufmann and Bornhardt is unlikely to be accurate. It may be pointed out that both Riley (35) and Gupta *et al.* (10) reported much higher values for linoleic acid determined spectrophotometrically. Since the latter investigators applied Riley's method for calculating the composition, the limitations are the same.

There is therefore a need for a new method for estimating the fatty acid composition of castor oil. Of the various fatty acids present in castor oil only linoleic acid can be determined with any degree of accuracy. The total unsaturated acids may also be estimated accurately if proper precautions are taken in the determination of iodine value. On the other hand, no reliance can be placed on hydroxyl or acetyl values even if they are determined on the glyceride itself because secondary hydroxyl groups cannot be quantitatively estimated by usual acetylation methods (32) and unsaturated groups and mono- or diglycerides interfere. Only the Zerevitinov (46) method is reliable for estimating the hydroxyl groups but is too elaborate for routine analytical work.

The best approach is to separate the hydroxy acids from the nonhydroxy acids and determine the composition of the nonhydroxy acids by standard methods. After the total unsaturation and the amount of oleic and linoleic acids have been determined, the ricinoleic acid content can be calculated and dihydroxystearic acid obtained by difference. Such a procedure would have the advantage that the calculations would not be based on acetyl or hydroxyl values, the determination of the saturated acids by the Bertram oxidation method, or the estimation of dihydroxystearic acid by its insolubility. The urea-adduct method described by Saletore and Achaya (37) for separating hydroxy acids from mixtures of fatty acids is

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TABLE I

Fatty Acid Composition of Castor Oil

Investigators	Saturated Acids, %	Oleic Acid, %	Linoleic Acid, %	Ricinoleic Acid, %	Dihydroxystearic Acid, %
Eibner and Munzing (7)	3.0	9.0	3.0	80.0	....
Myddleton *et al.* (31)	8.0	negligible	7.0	84.0	1.0
Heiduschka and Kirsten (13)	3.4	6.8	1.4	82.0	1.3
Panjutin and Rapoport (33)	0.3	7.2	3.6	87.8	1.1
Kaufmann and Bornhardt (21)	2.4	7.4	3.1	87.0	0.6
Riley (35)	0.9	nil	5.4	92.6	0.6
Gupta *et al.* (10)	1.0–2.0	negligible	4.5–5.0	91.4–94.9	0.7–1.0
Present Study— I	3.0[a]	5.4	3.4	85.5	2.4
II	3.5[a]	5.1	3.5	86.0	1.9
III	3.4[a]	5.8	3.5	85.7	1.6

[a] Saturated nonhydroxy acids.

unlikely to result in quantitative separation. In fact, many workers do not realize that unaltered fatty acids of castor oil cannot be obtained since the very method of preparation results in the formation of estolides (2, 15, 16, 27, 30, 47).

Therefore in the present investigation the method of separation was based on methyl esters which were prepared in such a manner as to be truly representative. The esters of hydroxy acids were then separated by the usual procedure employed in preparative organic chemistry, *viz.*, reaction with a cyclic anhydride of a dibasic acid and extraction of the resultant half ester with aqueous alkali. The composition was also confirmed by the estimation of saturated acids of nonhydroxy esters in one case and estimation of dihydroxystearic acid by a newly developed method. The experimental work consisted of the following steps: a) preparation of methyl esters of castor oil; b) separation of the nonhydroxy esters; c) estimation of unsaturation in the total methyl esters and the nonhydroxy esters fraction; d) estimation of polyethenoid acid; and e) estimation of dihydroxystearic acid.

Experimental

Raw Materials. Three lots of castor oil from a local oil mill were used in the course of the present investigation. There were no significant differences in their physical and chemical constants. Average values were: specific gravity 31/31°C. 0.958; $n_{40°C.}$ 1.4712; $[\alpha]_D^{31°C.}$ 4.75; acid value 2.3; sap. value 182.7; iodine value (Wijs 30 min.) 30–33°C. 84.3, 15–20°C. 82.6; hydroxyl value 163.0; $E_{1cm.}^{1\%}$ at 234 mμ before isomerization 5.8, after isomerization 33.1; unsaponifiable matter 0.5%. For certain control experiments, peanut oil (I.V. 89.4, S.V. 191.6), hydrogenated groundnut oil (I.V. 0.5, S.V. 188.2), methyl stearate (I.V. 0.2, S.V. 188.4), and methyl oleate (prepared from U.S.P. grade oleic acid) (I.V. 82.6, S.V. 193.7) were also employed. All the solvents were redistilled. Succinic and phthalic anhydrides were prepared by reacting the corresponding acids with acetic anhydride according to the procedure described by Vogel (41). In general, the A.O.C.S. (1) analytical methods were followed. Hydroxyl values were determined by the pyridine-acetic anhydride method of Burton and Robertshaw (5). Linoleic acid was estimated by the spectrophotometric method of Hilditch *et al.* (14).

Preparation of Methyl Esters. As estolide formation occurs during the isolation of free fatty acids from castor oil (15), the desired methyl esters could not be prepared from the acids by conventional procedures. Attempts to prepare methyl esters by reaction of metallic soaps (Ca, Pb, Ag) with methyl iodide or dimethyl sulphate resulted in low yields or incomplete methylation. Direct methanolysis of castor oil either with alkaline (26, 44) or acidic (4) catalysts occurred to the extent of 90% as determined by the estimation of the separated glycerol, thus the product contained about 10% of unconverted glycerides. Similarly the methyl esters were contaminated with unconverted glycerides when the procedure of Young and Craig (45) was tried. Therefore in order to get the pure methyl esters it was necessary to achieve complete saponification of the glycerides and methylation under such conditions that estolide formation was prevented.

Preliminary experiments, by saponifying castor oil with methanolic potassium hydroxide, splitting the acids *in situ* with an excess of methanolic mineral acid, and refluxing for esterification to occur, showed that products having hydroxyl values of 162–163 could be easily obtained. Since these values were very close to that of the oil itself (163.0), it was obvious that no side reactions, such as estolide formation, involving the hydroxyl group had occurred. A systematic investigation with hydrochloric acid as catalyst resulted in the adoption of the following procedure: 100 g. of castor oil were saponified by refluxing for 3 hrs. with a solution of 40 g. of potassium hydroxide in 400 ml. of methanol and then cooled to room temperature. The soap solution thus obtained was acidified by adding 240 ml. of methanolic hydrochloric acid (122.9 g. HCl/liter) portionwise with continuous shaking. Esterification was achieved by allowing the mixture to stand overnight at room temperature or by refluxing for 3 hrs., after which about 80% of the alcohol was distilled off. The residue was cooled to room temperature, diluted with 250 ml. of water, and extracted with 500 ml. of ether. The extract was washed with water until free from hydrochloric acid, then with four 200-ml. portions of 2% aqueous potassium carbonate, and finally with water. After the extract had been dried with anhydrous sodium sulphate, ether was distilled off. The last traces of ether were removed by heating the methyl esters under vacuum (2–3 mm. Hg) on a water bath. The yield was 94–95%.

Fatty acids were recovered in the usual manner from the carbonate and subsequent water washes and were esterified in the same way as the oil. An additional yield of methyl esters of about 4% was thereby obtained, bringing the combined yield to 99.3%.

The methyl esters of the three samples of castor oil were prepared by this procedure, and the average physical and chemical constants were as follows: Sp. Gr. 33/33°C. 0.921; $n_{40°C.}$ 1.4549; $[\alpha]_{33°C.}^{D}$ 4.45; A.V. 0.5; S.V. 182.9; I.V. (Wijs 30 min.) 15–20°C. 80.3; OH.V. 162.0; $E_{1cm.}^{1\%}$ 234 mμ before isomerization 7.0, after isomerization 29.9; unsaponifiable matter 0.3%.

Resolution of Methyl Esters. The separation of hydroxy esters from nonhydroxy esters of castor oil fatty acids by treatment with dibasic acid anhydrides was studied by Kurtz and Schaffer (25), who found that the reaction was not quantitative in amyl acetate or dioxane, while in pyridine tarry products were formed. A nonreacting hydrocarbon, toluene, was therefore chosen as solvent in the present work. A number of experiments were made with phthalic anhydride and succinic anhydride, either alone or in the presence of catalysts, and the results are summarized in Table II.

The nonhydroxy esters obtained with 900% excess succinic anhydride and 48 hrs. of refluxing (expt. 10) still showed an hydroxyl value of about 5.0. In order to determine whether this value was due to unreacted hydroxy esters or an inherent limitation in the analytical method, hydroxyl and acetyl values were determined for a number of materials known to contain no hydroxy acids. Methyl stearate, methyl oleate, peanut oil, hydrogenated peanut oil, and methyl esters of hydrogenated peanut oil were found to have hydroxyl values of 0, 4.5, 8.2, 3.0, and 0 and acetyl values of 2.1, 4.8, 5.7, 2.8, and 2.0, respectively. Since these hydroxyl values are of the same order of magnitude as those obtained for the nonhydroxy ester fraction from castor oil, it is reasonable to conclude

TABLE II

Reaction Between Methyl Esters and Dibasic Acid Anhydrides [a]

Experiment No.	Time of Reflux, Hours	Excess Anhydride,[b] %	Unreacted Esters		Remarks
			%	Hydroxyl value	
1	15	100	72.7	133.3	Phthalic anhydride
2	15	100	63.9	122.9	Succinic anhydride
3	16	100	15.9	5.3	Succinic anhydride + 4% H_2SO_4. Mixture charred
4	16	100	16.0	4.7	Succinic anhydride + 2% H_2SO_4. Mixture charred
5	16	100	16.1	5.7	Succinic anhydride + 5% naphthalene-2-sulphonic acid. Mixture charred
6	16	100	16.4	6.1	Succinic anhydride + 2% naphthalene-2-sulphonic acid. Mixture charred
7	18	900	17.1	5.4	Succinic anhydride
8	24	900	13.3	11.1	
9	36	900	12.5	8.3	
10	48	900	11.8	5.2	
11	48	900	14.3	20.1	Phthalic anhydride
12	48	....	99.1	.....	5 g. methyl esters of peanut oil treated with 14.5 g. succinic anhydride

[a] About 5 g. of methyl esters of castor oil dissolved in 100 ml. of toluene were used in each experiment.
[b] Hydroxyl value basis.

that quantitative separations have been achieved under the conditions of experiment number 10. In a control experiment (No. 12) methyl esters of peanut oil were almost quantitatively recovered as unreacted esters which had constants comparable with those of original esters (before, S.V. 190.4, I.V. 89.3; after, S.V. 190.8; I.V. 87.6), thus showing that there was no reaction between unsaturated materials, *e.g.*, methyl oleate or methyl linoleate and succinic anhydride.

The methyl esters of castor oil were accordingly resolved into hydroxy and nonhydroxy ester fractions by the following procedure: 10 g. of the methyl esters were refluxed with 29 g. of succinic anhydride (10 times the required quantity on OH.V. basis) and 100 ml. of toluene for 48 hrs. The toluene was distilled off under partial vacuum (15 in. Hg pressure). After cooling to room temperature, the residue was extracted with six 50-ml. portions of ether. Unreacted anhydride was removed by washing the extracts with 100-ml. portions of water. Succinyl esters were separated by extracting the ethereal solution with six 100-ml. portions of 2% aqueous potassium carbonate. Emulsions were broken by the addition of salt and settling for 3 hrs. Entrained nonhydroxy esters were removed from the carbonate extracts by re-extracting each of them with 100 ml. of ether. Nonhydroxy esters were obtained by washing the ether solutions with water until free from alkali, drying over anhydrous sodium sulfate, distilling off ether, and finally heating under vacuum (2–3 mm. Hg) on a water bath.

The combined carbonate extracts and washings were concentrated to 500 ml. The succinyl derivative was then hydrolyzed by boiling the concentrate for 3 hrs. after addition of 25 g. of solid potassium hydroxide. The hydrolyzate was cooled, acidified with dilute sulphuric or hydrochloric acid, and then extracted with 200 ml. of ether. Hydroxy acids were recovered from the extract in the usual manner. The corresponding weight of hydroxy esters was obtained by multiplying the weight of hydroxy acids by the factor 1.047. For the determination of chemical constants these acids were converted into methyl esters by the procedure outlined above.

The reproducibility of this method was checked by a series of trials with the methyl esters of all three lots of castor oil. Results obtained in one case are listed in Table III.

In these experiments the losses varied between 1.1 and 2.6%. The average values for the compositions of nonhydroxy and hydroxy portions of the methyl esters, after correcting for these losses, are recorded in Table IV together with their chemical constants.

Estimation of Unsaturation. In view of the recorded observation of Toyama and Ishikama (40) that 12-hydroxystearic acid reacts with the Wijs or Rosenmund and Kuhnhenn reagents, depending upon the temperature and time of contact, a comparative study of the standard Wijs method at two temperatures and the bromometric method described by Kamath (18) was undertaken. Preliminary experiments with castor oil by the bromometric method showed that, with 100% excess of 0.2 N bromine in carbontetrachloride at temperatures between 31–33°C., complete addition took place within 60 min. The results obtained with castor oil, methyl esters of castor oil, acetylated castor oil, peanut oil, and methyl esters of peanut oil are summarized in Table V.

TABLE III

Resolution of Castor Oil Esters (Lot II) Into Hydroxy and Nonhydroxy Esters

Methyl Esters, g.	Hydroxy[a] Esters, g.	Nonhydroxy Esters, g.	Loss, %	After Distributing Loss	
				% Nonhydroxy Esters	% Hydroxy Esters
9.98	8.63	1.18	1.7	12.0	88.0
10.20	8.82	1.25	1.3	12.4	87.6
10.04	8.72	1.22	1.0	12.2	87.8
10.04	8.74	1.17	1.3	11.8	88.2
10.03	8.73	1.18	1.2	11.9	88.1
10.01	8.68	1.23	1.0	12.4	87.6

[a] Calculated by multiplying the weight of hydroxy acid by 1.047.

TABLE V

Iodine Values by the Bromometric and Wijs Methods

Material	Bromometric 31–33°C., 60 mm.		Wijs, 30 min.	
	Corrected	Uncorrected	31–33°C.	15–20°C.
Castor oil	80.3	84.6	84.5	83.1
Methyl esters of castor oil	80.6	82.3	83.3	80.6
Acetylated castor oil	45.3	79.9	72.8	63.6
Peanut oil	73.8	88.8	89.9	89.4
Methyl esters of peaunt oil	81.4	87.2	88.1	87.3

It is noted that the corrected I.V. of methyl esters of castor oil agrees well with corresponding Wijs 15–20° C. I.V., also with the corrected I.V. of the oil itself. Therefore the standard Wijs method employed at 15–20°C. in an accurate measure of the total unsaturation of the methyl esters of castor oil and can be used in calculating the fatty acid composition.

Estimation of Polyethenoid Acids. The methyl linoleate contents of the methyl esters and the nonhydroxy esters were determined according to the method of Hilditch *et al.* (14). As the nonhydroxy esters

TABLE IV

Constants of Nonhydroxy and Hydroxy Esters from Castor Oil

Castor Oil	Methyl Esters	Average Yield, %	Saponification Value	Iodine Value		Acid Value	Hydroxyl Value	$E^{1\%}_{1cm.}$ 234 mμ	
				Wijs 15–20°C.	Woburn			Before Isomerization	After Isomerization
I	Nonhydroxy	11.8	202.4	....	88.7	0.3	5.2	72.1	243.5
	Hydroxy	88.2	180.0	79.1	....	0.3	176.2	2.0	2.0
II	Nonhydroxy	12.1	201.8	....	85.7	0.4	5.6	56.9	239.2
	Hydroxy[a]	87.9	179.8	79.2	....	0.6	175.4	2.4	2.5
III	Nonhydroxy	12.7	202.9	....	86.9	0.3	5.2	71.0	232.6
	Hydroxy	87.3	180.2	80.0	....	0.2	175.7	1.6	1.7

[a] 0.5% unsaponifiable matter.

showed appreciable absorption before isomerization, the standard Wijs method is unlikely to give accurate results, consequently the Woburn method of Von Mikusch and Frazier (42) was used to determine the total unsaturation.

Procedure for Calculating the Composition. a) All the octadecadienoic acids of the original methyl esters and the nonhydroxy esters were calculated as methyl linoleate from the $E^{1\%}_{1cm.}$ 234 mμ values after isomerization. b) From the I.V. of the nonhydroxy esters the quantity of methyl oleate was calculated after allowing for methyl linoleate. The content of saturated nonhydroxy esters was obtained by difference. c) from the iodine value of the original methyl esters the content of methyl ricinoleate was calculated after allowing for methyl oleate and methyl linoleate. The content of methyl dihydroxystearate was obtained by difference from the total hydroxy esters. The composition of the three samples of castor oil are included in Table I for the purposes of comparison with those of previous studies.

Verification of Composition. As sufficient quantities of the nonhydroxy esters of Sample II were available, these were converted into fatty acids. By the Twitchell lead salt alcohol method[1] these were found to contain 25.1% of saturated acids. This value corresponded to about 3% of the total acids in the oil and was in excellent agreement with the calculated one of 3.5%.

Estimation of Dihydroxystearic Acid (19)

As estolides may be formed during the isolation of the mixed fatty acids in castor oil, analysis of these (10, 35) for dihydroxy-stearic acid may not be accurate. Therefore analysis of the methyl esters is preferred. Oxidation of dihydroxystearic acid with potassium periodate at pH 8 has been studied by King (23), who reported yields of only 82–95%.

In the present investigation oxidation of dihydroxystearic acid with periodic acid in glacial acetic acid was studied. Preliminary experiments with a sample of dihydroxystearic acid prepared by the mild oxidation of oleic acid (28) (N.V. 177.2, OH.V. 351.6, m.p. 132°C.) showed that the reaction was quantitative with an 80–100% excess of reagent in 10 min. at 15–30°C. Owing to the limited stability of periodic acid solutions a temperature of 15–20°C. is preferred. Values were however affected by unsaturated acids such as oleic, ricinoleic, peanut oil, and linseed oil fatty acids, but stearic acid did not interfere. Therefore, if the methyl dihydroxystearate content of the methyl esters is to be determined, the unsaturation should be of a low order. Either the methyl esters may be hydrogenated, or the original oil may be hydrogenated and the methyl esters prepared from it. The hydrogenation must be done under such conditions that little or no hydrogenolysis occurs and only the double bonds react. A typical procedure for preparing hydrogenated castor oil of low I.V. is as follows: 10 g. of castor oil, 25 ml. of ethanol, and 0.3 g. of Raney nickel are charged into a Parr hydrogenation bottle and shaken at an initial H_2 pressure of 40 p.s.i. at room temperature (30–33°C.) for 5 hrs. Three per cent more of the catalyst are then added, and the hydrogenation is continued for a further 5 hrs. at the same pressure. The product is warmed on a water bath to dissolve the hydrogenated oil. The catalyst is filtered off and washed with hot alcohol. Alcohol is distilled from the filtrate. The residue is taken up with ether, washed free of alcohol with distilled water, and recovered in the usual manner. This hydrogenated castor oil is converted to methyl esters by the above procedure except that 5 hrs. of refluxing was allowed for saponification.

Results with the three samples of castor oil are recorded in Table VI.

Since the hydroxyl values of these materials agree with the average hydroxyl values reported earlier for the oil and methyl esters, no secondary reactions involving the hydroxyl group occurred under these conditions of hydrogenation.

Estimation of Methyl Dihydroxystearate with Periodic Acid. Reagents—M/25 Periodic Acid. About 1.29 g. of reagent grade periodic acid are dissolved

TABLE VI

Properties of Hydrogenated Castor Oils and Their Methyl Esters and the Content of Methyl Dihydroxystearate

Castor Oil	Hydrogenated Oil			Methyl Esters			Methyl Dihydroxystearate, %	
	I.V. Wijs 15–20°C.	Hydroxyl Value	m.p. °C.	Yield, %	I.V. Wijs 15–20°C.	Hydroxyl Value	Periodic Acid Method	Calculated[a]
I	8.4	163.1	82.5	99.2	7.9	162.4	2.7[b]	2.7
II	6.6	161.2	85.0	99.0	6.4	161.2	2.2[b]	1.9
III	6.4	160.9	85.0	98.8	6.1	160.5	1.8[b]	1.6

[a] Same as in Table I.
[b] Average of triplicate determinations.

in 100 ml. of glacial acetic acid. The insoluble matter is allowed to settle down, and the supernatant liquor is decanted. Five ml. of this solution is equivalent to 4 ml. of 0.1 N ferrous ammonium sulfate when potassium ferricyanide is the indicator. Iodic acid does not react with the ferrous salt; a 0.1 M potassium iodate solution acidified with acetic acid gave the end-point, following the addition of one drop of ferrous ammonium sulfate. It reduces periodic acid to iodic acid only. Incidentally this provides a ready method of estimating periodic acid in admixture with iodic acid.

The other reagents, *viz.*, potassium iodide (10%), sodium thiosulfate (0.1 N), starch solution (1.0%), and chloroform are the same as for I.V. determinations.

Procedure. About 1 g. of the methyl esters of hydrogenated castor oil is weighed into a 250-ml. iodine flask and is dissolved in 10 ml. of glacial acetic acid. This solution is cooled in a water bath at 15–20°C. Five ml. of 0.04 M periodic acid, previously cooled to the same temperature, are then added. The flask is shaken well, stoppered tightly, and kept in the dark at 15–20°C. for 10 min. Twenty-five ml. of 10% KI solution and 10 ml. of chloroform are then added and after vigorous shaking the liberated iodine is titrated against 0.1 N thiosulfate with starch as indicator. A blank with 5 ml. of the reagent is conducted under identical conditions.

$$\text{Methyl dihydroxystearate} = \frac{(B - S) \times N \times 16.525}{W}$$

where B = ml. of 0.1 N thiosulfate for blank
S = ml. of 0.1 N thiosulfate for sample
N = normality of thiosulfate
W = weight of sample in grams.

Methyl dihydroxystearate contents of the three samples of castor oil are recorded in Table VI. The excellent agreement between these and those obtained by calculation bears out the accuracy of this method of estimation.

Discussion

The usual method of preparing methyl esters involves isolation of the fatty acids and their subsequent esterification. In the present method the saponified fat was converted directly into methyl esters in quantitative yields and without undesirable by-products.

Reaction with succinic anhydride in toluene resulted in quantitative separation of hydroxy from nonhydroxy esters in one operation, in contrast to the two or more steps required with other dibasic acid anhydrides such as phthalic anhydride. The method was reproducible, and losses were only 1–3%. The fact that methyl esters of peanut oil were recovered unchanged after treatment with succinic anhydride demonstrated that this reagent does not react with unsaturated groups or cause undesirable isomerizations.

The recovered hydroxyesters had consistently lower hydroxyl values than would be expected of a mixture containing 97 parts of methyl ricinoleate and 3 parts of methyl dihydroxystearate. In view of the observed effects of unsaturated acids on acetyl and hydroxyl values and the probability of incomplete acetylation (32) these low hydroxyl values may not be significant. In any case only the yields of the hydroxy and nonhydroxy esters were considered in determining the composition, and no reliance has been placed on the constants of the hydroxy esters.

The nonhydroxy esters however show certain peculiarities in that the amount of conjugated acids has perceptibly increased (Table IV). The unsaturation must therefore be determined by the Woburn method. The comparative study of iodine value by Wijs and bromometric methods has brought out the importance of temperature (Table V). This effect may be more pronounced with hydroxy oils than with others. In the case of the methyl esters the total consumption of the halogen in the bromometric method (I.V. uncorrected) is comparable with that of Wijs at the same temperature (I.V. Wijs 30–33°C.) while that corrected for substitution is comparable with Wijs at 15–20°C. Because during the summer months the average temperatures of this country (India) are well over 30°C. and the Wijs method is accurate only at temperatures below 20°C., the bromometric method is often used. Since differences between the corrected and uncorrected iodine values are greater with the oils than their methyl esters, more substitution apparently occurred with the glycerides.

The three lots of castor oil, although purchased at different times (1947, 1949, 1950), had almost identical fatty acid compositions. Previously Gupta *et al.* (10) found a similar agreement in the compositions of castor oils from various sources. However the composition deduced from the present study differs in many respects from those of previous investigations. The differences are probably not due to the origin and the history of the oils but may be attributed to the use of different analytical methods and different equations for calculating the composition.

In the present study the linoleic acid content of castor oil was calculated from spectrophotometric data. The value is comparable to that reported by Kaufmann and Bornhardt (21) but lower than that found by Riley (35) and Gupta *et al.* (10). These differences parallel differences in the iodine values reported by these investigators.

The oleic acid content was calculated from the iodine value of the nonhydroxy esters after allowing for linoleic acid and on the assumption that the Woburn method measures the true unsaturation. In contrast, Riley and Gupta *et al.* calculated the oleic acid content after making allowance for unsaturation due to both ricinoleic and linoleic acids. Since these workers reported high contents of linoleic and ricinoleic acids, their finding of a negligible value for oleic acid is not surprising. By the present methods of determining acetyl or hydroxyl values even peanut oil, its methyl esters and methyl oleate show hydroxyl values of the order of 5 and significant acetyl values. Therefore calculations based on hydroxyl or acetyl values would probably give high values for ricinoleic acid and correspondingly low values for oleic acid. For this reason ricinoleic acid values were calculated from the iodine values of the methyl esters of the oils after allowing for linoleic and oleic acids. These values are comparable to those reported by Kaufmann (21) but lower than those of Riley (35) and Gupta *et al.* (10). Since the latter investigators obtained low values for dihydroxystearic acid and since every unit of this acid corresponds to nearly two units of ricinoleic acid when calculated from hydroxyl or acetyl values, their high values for ricinoleic acid were not surprising.

The dihydroxystearic acid content was obtained from the total hydroxy acid content after deducting for ricinoleic acid. These values are higher than those

reported in the literature but are likely to be correct because they are not based on acetyl or hydroxyl values or the solubility of the acid in ethyl acetate or ether.

The content of saturated acids, 3–3.5%, was confirmed in one sample by the isolation of the saturated acid from the nonhydroxy ester fraction.

The estimation of α,β-glycolic compounds (3, 34) by oxidation with periodic acid is a standard procedure and does not need any elaboration. However, incidental to the present study, it has been shown that the true periodic acid content of a solution is best determined by titration with ferrous ammonium sulfate because the usual iodometric method determines the total oxidative capacity. The iodometric method is however more convenient for assessing the consumption of periodic acid. The recommended method for dihydroxystearic acid gives results agreeing well with those calculated and thus confirms the composition arrived at in this paper. It should also prove useful in estimating dihydroxy compounds in fats and other materials.

Summary

Methyl esters of castor oil were prepared by saponifying the oil with potassium hydroxide in methanol, splitting the potassium soaps *in situ* with an excess of hydrochloric acid, and esterifying at room temperature. The esters had hydroxyl values comparable with those of the parent oils. The methyl esters were quantitatively resolved into hydroxy and nonhydroxy esters after reacting with succinic anhydride in toluene. The composition of castor oil was calculated from a) amount of nonhydroxy esters, b) methyl linoleate content of methyl esters determined spectrophotometrically, c) iodine value of the methyl esters determined by the Wijs method at 15–20°C., and d) iodine value of the nonhydroxy esters determined by the Woburn method. This composition was confirmed by the estimation of saturated acids in one sample and dihydroxystearic acid in all. Castor oil was readly hydrogenated with Raney nickel in alcohol at room temperature (30–33°C.) without any hydrogenolysis of the hydroxyl groups. Methyl dihydroxystearate content of the methyl esters of this hydrogenated oil was determined by reaction with 80–100% excess periodic acid at 15–20°C.

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A Semi-Micro Procedure for the Separation and Degradation of Long-Chain Fatty Acids[1,2]

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STUDIES of the biochemistry of fatty acids have been greatly facilitated by the use of carbon-14 compounds, and such studies have in turn resulted in the development and refinement of semi-micro procedures for the separation and degradation of long-chain fatty acids. In spite of this, there has not yet appeared in the literature a complete semi-micro procedure for the isolation, separation, and stepwise degradation of radioactive fatty acids from plants or animals. Such a procedure is presented in this paper. For the most part, the procedure can be carried out with equipment usually available in the laboratory, and the few pieces of special apparatus which are required can be assembled with little diffi-

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