Summary

In the description of this investigation the following points have been presented.

1. Diastatic action of certain vegetable amylases in the absence of inhibitive agencies such as maltose, irreversible adsorption on interfaces due to shaking or on diatomaceous earth or filter paper pulp, is found to be a linear function of time and enzyme concentration throughout large variations in substrate concentration.

2. Adsorptive agents such as extended liquid–vapor surface films caused by continued shaking, diatomaceous earth or filter paper pulp are found to have marked inhibitive effects. The initial presence of small amounts of saponin, gelatin, albumin, casein or peptone, however, prevents this inhibition. Glycine and agar are found to be without effect.

3. Unpurified, ground soy bean diastase does not appear to be subject to adsorption at extended liquid–vapor surface films caused by shaking. Wheat flour and malt sirup enzymes, however, are quite subject to this effect.

4. The Michaelis constant for a flour diastase has been found to be about 0.25 in terms of percentage substrate concentration.

5. A convenient method for the study or determination of diastatic activity is described in which the sugars are fermented out by yeast as rapidly as formed, and the resulting carbon dioxide collected and measured at frequent intervals.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY]

THE CHEMICAL COMPOSITION OF OIL OF RUVETTUS PRETIOSUS, THE "CASTOR OIL FISH"[1,2]

BY WARREN M. COX, JR., AND E. EMMET REID

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Ruvettus pretiosus was described ichthyologically by Cantraine[3] in 1837 and by Lowe[4] in 1841. The latter author reports, "it is affirmed that the bones abound in an oil or marrow, which, when they are sucked incautiously, produces diarrhea." Since this initial observation various in-

[1] The material in this article is extracted from a thesis submitted by Warren M. Cox, Jr., in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the Johns Hopkins University.

[2] Presented to the American Chemical Society at its meeting in Atlanta, Georgia, April, 1930.

[3] F. J. Cantraine, "Memoire sur un Poisson nouveau (*Ruvettus temminckii*) trouvé dans le Canal de Messine, etc." Nouveaux Memoires Academie Royale Sciences et Belles Lettres, Bruxelles **1837**, Vol. 10, 1–2.

[4] Richard T. Lowe, "A Synopsis of the Fishes of Madeira, etc.," *Trans. Zoölogical Society*, London, **2**, 180–181 (1841).

vestigators[5,6,7,8] have extended and confirmed this observation as to the purgative qualities of the oil. The oil from the flesh of the fish is as remarkable in this effect as is the smaller amount obtainable from the bones. Gudger[9] has compiled the available information on this species and on the purgative qualities of its oil. C. B. Nordhoff[10] has recently said, however, that undoubtedly the purgative quality of the oil has been over-emphasized.

It was therefore deemed of interest[11] to undertake a chemical study of the oil with the view of isolating and identifying the purgative principle or principles, if any. Two quarts of the oil were obtained through the kindness of Dr. E. W. Gudger of the American Museum of Natural History and Mr. Donald G. Kennedy, Vaitupu, Ellice Islands, South Seas. The pharmacological evaluation of the "purgativeness" of the oil and its components has been done by Dr. David I. Macht and Dr. Maria J. Barba-Gosé. Their results on the principal constituents of the oil, expressed in arbitrary relative units, are given in Table II. The technique employed by them in determining these values has been published elsewhere.[12]

The chemical analysis of the oil shows it to consist of about equal proportions of saponifiable and unsaponifiable constituents. These are, respectively, fatty acids and normal, straight chain alcohols. The absence of an appreciable amount of glycerol leads to the conclusion that the oil consists primarily of esters of the type, cetyl oleate and oleyl oleate. This conclusion is further warranted by the absence of appreciable amounts of free acid or alcohol, as indicated by the nullity of the acid value, and the fact that the hydroxy acid identified accounts for the acetyl value.[14a] On the basis of 1 kg. of oil, 1.78 mols of fatty acids and 1.28 mols of alcohols have been identified. Since the mols of acid are greater than those of alcohol, a considerable part of the unidentified unsaponifiable matter must have been alcohols of higher molecular weight. The individual constituents which have been identified, with the percentages present, are given in the following table.

[5] Louis Becke, "By Rock and Pool on an Austral Shore," London, 1901, pp. 63 and 148–158.

[6] Augustin Krämer, "Der Purgierfisch der Gilbertinseln," Globus, 1901, Vol. 79, pp. 181–183.

[7] Max Weber, "Siboga Expeditie, Monographie I. Introduction et Description de l'Expedition," Leiden, 1902, pp. 96–97.

[8] Frau A. Weber-van Bosse, "Ein Yahr am Bord I. M. S. Siboga," Leipzig, 1905, pp. 243–244.

[9] E. W. Gudger, "A New Purgative, the Oil of the 'Castor Oil Fish,' Ruvettus," *Boston Med. Surg. J.*, 192, 107–111 (1925).

[10] C. B. Nordhoff, *Natural History*, 28, 40–45 (1928).

[11] We wish to acknowledge our indebtedness to Dr. E. W. Gudger and Dr. D. I. Macht for bringing this problem to our attention.

[12] D. I. Macht and Barba-Gosé, *J. Proc. Soc. Exptl. Biol. Med.*, 28, 772–774 (1931).

TABLE I

THE COMPOSITION OF RUVETTUS OIL

Ruvettus							
Fatty Acids (54.4%)				Unsaponifiable (48.5%)			
Unsaturated	52%	Saturated	1.5%	Unsaturated	28.5	Saturated	18.5
Palmitoleic	?	Stearic	1.5%	Oleyl alc.	14.3	Tetradecyl alc.	trace
Oleic	40%						
Gadoleic	1.3			Cholesterol	0.15	Cetyl alc.	16.2
Erucic	0.3			Squalene	trace	Octadecyl alc.	2.1
$C_{24}H_{46}O_2$	trace						
$C_{26}H_{50}O_2$	trace						
Hydroxyoleic	7% (approx.)					Glycerol	trace

The absence of any appreciable quantity of saturated acid, and especially the absence, in a fish oil, of more than traces of acids more highly unsaturated than oleic, together with the noteworthy completeness of the oleic acid series from C_{16} to C_{26}, makes the oil of more than passing interest. The hydroxy oleic acid seems to be an isomer of ricinoleic acid, but possesses none of the chemical or pharmacological characteristics of this acid. The pharmacological evaluations of the principal components are given in the following table.

TABLE II

RELATIVE PURGATIVE ABILITY OF OILS AND FRACTIONS

Sample	No. of Rats	"Purgativeness"
Emulsion carbon black	...	0
Olive oil	4	10
Castor oil	3	25
Ruvettus oil	7	23
Ethyl oleate	9	18
Ethyl hydroxyoleate	4	14
Oleyl acetate	7	29
Cetyl acetate	6	32

These results seem to indicate that the oil is in the castor oil class, the relatively large doses required for purgation indicating a mild laxative. It is also evident that no one constituent is responsible for this action, and that the effect of the combined constituents accounts for the action of the whole oil. Squalene had been reported[13] as having purgative action but it is present here only in traces. It is also to be noted that cetyl acetate has recently been fed in rather large amount in the course of a metabolism experiment[14] and no observation is reported as to an ensuing diarrhea. One of us (W. M. C.) ingested an ounce of the whole Ruvettus oil *per os* with no noticeable effects.

Experimental

The two quarts of oil were of different color, one a very pale straw-yellow (I) and the other verging toward a light reddish-yellow (II). The first

[13] H. J. Channon, *Biochem. J.*, **20**, 400–408 (1926).

[14] R. Mancke, *Z. physiol. Chem.* (Hoppe-Seyler), **162**, 244 (1926–1927).

was obtained by boiling the flesh and skimming off the oil, and the second by roasting. Both were very pleasant smelling liquids with no suggestion of the fishy odor characteristic of highly unsaturated fish oils, and were wax-like solids at zero degrees with no definite solidification point. The physical characteristics are presented in Table III.

TABLE III

PHYSICAL CHARACTERISTICS OF RUVETTUS OIL[14a]

	I	II
Density 25°/4°	0.8697	0.8700
Iodine no. (Wijs)	83.9	74.4
Saponification no.	115.4	124.0
Refractive index 25°/D	1.4633	1.4628
Acid value	0	0
Reichert-Meissl no.	0	0

Separation of the Saponifiable and Unsaponifiable Constituents.—Exploratory work was done with oil I, while II was reserved for the final complete analysis. It was found in these preliminary experiments that the large amount of unsaponifiable substances present served to make their extraction extremely difficult, unless the correct ratio of water, alcohol and ether was used. The following procedure was adopted.

Two hundred gram batches of the oil were refluxed with 1000 cc. of 2 *N* alcoholic potash for thirty minutes. About 700 cc. of alcohol was distilled off and the soap poured into 2 liters of hot water in a 6-liter Florence flask. After cooling, 2 liters of ether were added, throughly shaken and the layers separated. Only about 1 liter of ether is separable here. This extraction was repeated three times with 1-liter portions of ether, the extracts combined, washed with water and the ether evaporated. The alkaline liquors were acidified and likewise extracted three times with 500-cc. portions of ether. On evaporation of these extracts the free fatty acids were obtained. Both fractions, the

TABLE IV

SEPARATION OF SAPONIFIABLE AND UNSAPONIFIABLE CONSTITUENTS

The iodine numbers were determined by the Wijs method.

Oil	Weight, g.	Unsaponifiable g.	Unsaponifiable %	Unsaponifiable I no.	Saponifiable g.	Saponifiable %	Saponifiable I no.	Saponifiable Neut. no.	Saponifiable Av. mol. wt.
I	200	98.3	49.3	78.8	107.5	53.8	93.3	189.5	295.7
	150	75.5	50.3	79.0	82.3	54.8	92.3	188.0	298.4
II	200	(81)		57.5	107.0	53.8	93.4	190.2	294.4
	200	94.0	47.0	58.5	110.0	55.0	92.6	187.6	299.1
	200	95.0	47.4	58.0	110.0	55.0	92.1	187.6	298.1
Average			48.5			54.4	92.7	188.6	297.1

[14a] At the end of the analysis it was thought necessary, in the light of the hydroxy acid and alcohols that had been identified, to determine the acetyl value. Only a small sample of oil (I) was available and this had become somewhat rancid, as an acid value of 6.3 would indicate. Its acetyl value was 11.4. Considered in conjunction with the increase in acid value, and the possible acetylation of small amounts of free alcohol and cholesterol, this does not exclude the possibility that some of the hydroxy acid was present as an inner anhydride.

saponifiable and unsaponifiable, were heated to 90–100° in a stream of carbon dioxide until all water had been removed. The data are given in Table IV.

The sum of these two fractions gives a percentage of 102.9, against a theoretical for oleyl oleate of 103.3. The iodine number and the neutralization number of the fatty acids are 92.7 and 188.6, respectively. This compares suggestively with the theoretical values of 90 and 198.9 for pure oleic acid.

Analysis of the Saponifiable Fraction.—The standard method for the separation of saturated and unsaturated fatty acids, the lead soap–ether method,[15] gave no saturated insoluble lead soap. By means of the Twitchell lead soap–alcohol procedure[16] no better result was obtained. Accordingly the free fatty acids were esterified and then fractionated at 5 mm.

Three hundred and twenty-one grams was dissolved in 320 cc. of ethyl alcohol containing 3.5% HCl by volume, 84 g. of calcium chloride (anhydrous) added, and refluxed for two days. After thorough washing there was but 0.2% free acid present. Fractionation was carried out in a small column.

TABLE V

FRACTIONATION OF ETHYL ESTERS OF THE SAPONIFIABLE FRACTION

Fractions	to 180°	180–190°	190–195°	Residue
Weights	11.3	125.8	140.4	65.2
Percentage	3.3	36.7	40.8	19.2
Iodine no.	77.2	81.3	82.2	88.3
Saponification no.	190.7	191.4	186.6	159.7
Molecular wt.	294	293	300.7	351.3

By applying the Twitchell lead soap–alcohol procedure to the lower-boiling fraction, 0.05 g. of an acid was obtained which melted at 74.5° and had a molecular weight of 293.3. The remainder was reësterified and redistilled. It boiled at 205–213 (15 mm.), had an iodine number of 82.3, d_4^{25} 0.8665 and n_D^{25} 1.4511. The calculated iodine number of ethyl oleate is 81.2 and the density is recorded[17] as d_{25} 0.8671.

Fractions 180–190° and 190–195°.—These two fractions were combined and redistilled at 15 mm.

TABLE VI

REDISTILLATION OF ETHYL ESTERS

	to 200°	200–205°	205–210°	210–212°	212°	Residue
Weights	13.7	12.1	60.4	128.3	16	9.3
Iodine nos.	77.2	80.2	82.1	81.6	83.6	84.8
Sapon. nos.	186.2	183.5	183.4	184.2	179.5	171.6

The lows were combined with the first fraction of the previous distillation and treated as described there.

Oleic acid has been best purified by crystallization of its lithium salt. Moore,[18] Armstrong and Hilditch,[19] and Tsujimoto and Kimura[20] have proposed a certain tech-

[15] G. S. Jamieson, *J. Assoc. Off. Agri. Chem.*, **11**, 301 (1928); **12**, 44 (1929).

[16] F. J. Twitchell, *J. Ind. Eng. Chem.*, **13**, 806 (1921).

[17] E. Kröber, *Z. physik. Chem.*, **93**, 648 (1919).

[18] C. W. Moore, *J. Soc. Chem. Ind.*, **38**, 322T (1919).

[19] E. F. Armstrong and T. P. Hilditch. *ibid.*, **44**, 43T (1925).

[20] M. Tsujimoto and K. Kimura, *J. Chem. Ind.* (Japan), **26**, 891 (1923); **23**, 1007 (1920).

nique. It was found convenient to dissolve the free acid in 95% alcohol and neutralize it with aqueous lithium hydroxide solution of such a strength as to make the alcohol about 80%. The lithium salt crystallizes beautifully.

Fraction 200–205°.—The lithium salt–alcohol method gave two crops of crystals, the fatty acids from which had iodine numbers of 76.4 and 77.5, and molecular equivalents of 286.7 and 285.3, indicating the majority to be oleic acid, with some saturated acid present. The lithium soap soluble in alcohol was converted to the free acid and had a molecular equivalent of 275.3—suggesting palmitoleic acid.

Fraction 205–210°.—This fraction was treated as above; the constants of the fatty acids from the lithium salts agreed fairly well with those for oleic acid. When they were treated by the lead soap–alcohol method, 0.1 g. of insoluble lead salt was obtained. The free fatty acid had an iodine number of 39 and a molecular equivalent of 282, indicating a very impure sample of stearic acid.

Twenty-five per cent. of the ester used in the above procedure was not accounted for in the crystalline lithium salts. When the volume was readjusted for the weight of acid remaining in solution, the lithium salt still would not crystallize. The calcium salt of the acid was soluble in alcohol, and the barium salt could not be made to crystallize. After acetylation the saponification and neutralization numbers showed conclusively that an hydroxy acid was present. Purification of the remaining 3.2 g. by precipitation of the lead salt from alcohol and extraction with two 50-cc. portions of boiling petroleum ether, followed by acetylation, confirmed the presence of a C_{18} mono-ethylenic hydroxy acid.

TABLE VII

THE HYDROXYOLEIC ACID OF RUVETTUS OIL

	Saponification		Neutralization		Iodine	
	Number	Mol. wt.	Number	Mol. wt.	Number	Mol. wt.
Soluble Li salts after acetylation	295.7	378	168	334	70.6	359
Insol. Pb salt after acetylation	349	321	169.6	330	..	...
Calcd. for hydroxyoleic acid	330	340	165	340	77.6	340

In connection with this acid it is to be noted that C. W. Moore[21] has isolated a dihydroxyoleic acid from the oil of a South Sea whale.

Fraction 210–212°.—The fatty acids of this fraction were treated by the procedures above; the acid from the lithium salt gave iodine numbers agreeing well with that for pure oleic acid. There was also present, though in smaller amount, soluble lithium salts. After acetylating the free acid from these the neutralization, saponification and iodine numbers were, respectively, 163.5, 298.2 and 74.2. These values are further confirmatory for the hydroxyoleic acid.

Residue from Original Fractionation.—The residue, 65 g., from the first fractionation of the esters, Table V, was refractionated at 5 mm.

TABLE VIII

REFRACTIONATION OF RESIDUE

	to 200°	200–205°	205–215°	Residue
Weight, g.	19.1	10.2	10.7	26.2
Iodine no.	86.5	90.0	90.9	81.3
Sapon. no.	211.5	216.0	206.6	..

The low iodine values indicate that there is only a trace of unsaturation greater than mono-ethylenic. In confirmation of this, no ether-insoluble bromide could be isolated from the most highly unsaturated fraction.

[21] Moore, *J. Soc. Chem. Ind.*, **38**, 322T (1919).

The high saponification numbers were paralleled by high free acid and this was shown to be caprylic acid, due undoubtedly to decomposition. Because of this the two lower fractions were combined and redistilled at 9 mm., the main fraction boiling at 221–223° at this pressure. The saponification number (178) indicated gadoleic acid (calculated 181); iodine number, 76.4; calculated, 81.8. Complete identification of this acid was difficult; when hydrogenated with Adams' catalyst[22] the ester absorbed the theoretical amount of hydrogen, but the free acid melted at 65°—indicating a mixture; when oxidized according to the procedure of Lapworth and Mottram,[23] the dihydroxy acids obtained melted low, 118–121°, again indicating a mixture. Purification of the gadoleic acid from the contaminant oleic acid was accomplished by crystallizing a dilute alcoholic solution of their lithium salts. After freeing the acid from the first crystals it had a neutralization number of 181.8 (calculated 181), corresponding to a molecular weight of 305.4 (gadoleic acid, 310). The iodine number was 77.4 (gadoleic acid, 81.8).

Fraction 205–215°.—After saponification and distillation of the caprylic acid the free acids were treated by the lithium salt method. The acids from the first crop of crystals melted at 30° and had a molecular weight by titration of 343, and an iodine number of 64.2. Theoretical values for erucic acid, $C_{22}H_{42}O_2$, are 338 and 75. The acid obtained from the second crystals was liquid at room temperature but had a molecular weight of 338.1 and an iodine number of 71.5. This fraction is chiefly erucic acid.

Residue.—A small amount of lithium salts was isolated from the thick red oil left in the still. After repeated recrystallizations from various solvents, the neutralization number and iodine number of the free acid indicated a mixture of $C_{24}H_{46}O_2$ and $C_{26}H_{50}O_2$.

Glycerol.—The merest trace of glycerol could be detected, as the tribenzoate, in the aqueous residue from 200 g. of the oil, after saponification and separation of the saponifiable and unsaponifiable constituents.

Unsaponifiable Fraction.—No satisfactory method has been devised for the separation of higher saturated and unsaturated alcohols.[24] The best methods have seemed to be crystallization from acetone[25] and fractional distillation. Neither of these methods was satisfactory in our hands.

Edeleanu[26] reported the interesting fact that aromatic and cyclic unsaturated hydrocarbons are soluble in liquid sulfur dioxide, while paraffins and naphthenes are almost completely insoluble. Moore, Morrel and Egloff[27] and Zerner, Weiss and Opalski,[28] confirmed and extended this observation. When the total unsaponifiable fraction was treated with this solvent it was found that only the unsaturated constituents were dissolved, and that excellent separation could be obtained.

The extraction was performed in the usual type Soxhlet apparatus save that the condenser jacket was open at the top for the introduction of pow-

[22] R. Adams and R. L. Shriner, THIS JOURNAL, **45**, 2171 (1923).

[23] A. Lapworth and E. N. Mottram, *J. Chem. Soc.*, **127**, 1628 (1925).

[24] T. P. Hilditch and J. A. Lovern, *J. Soc. Chem. Ind.*, **48**, 359–365, 365–368 (1929).

[25] Y. Toyama, *Chem. Umschau*, **31**, 61–67, 153–155 (1924).

[26] L. Edeleanu, *Petroleum*, **9**, 862–864; *Bull. Am. Inst. Mining Eng.* 2313–2332 (1914).

[27] R. J. Moore, J. C. Morrel and G. Egloff, *Met. Chem. Eng.*, **18**, 396–402 (1918).

[28] Zerner, Weiss and Opalski, *Z. angew. Chem.*, **35**, 253–256 (1922).

dered carbon dioxide. When the extraction is complete, the condenser tube is attached to a rubber connection leading to a Dewar flask. The next morning the solvent is found in this flask and a new extraction begun.

A comparison of the separation afforded by the three methods is given in Table IX.

TABLE IX

SEPARATION OF SATURATED FROM UNSATURATED ALCOHOLS. COMPARISON OF METHODS

1	Acetone	Melting points, °C.			33–34		36		38
		I nos. of fractions distd. from last prod.							12, 16 and 52
2	Distillation	Fractions	1	2	3	4	5	6	7	Residue
		I nos.	19	18	23	43	59	69	76	146

				Iodine numbers		
3	SO_2 extraction	Time of extn.	Extd., %	Original	Extract	Residue
	A	5 hrs.	47.0	58	106	13
	B	5 hrs.	13.9	13	65	2.2

The efficiency of extraction is dependent on the fineness of the material, and it is advantageous, after the first extraction, again to shave the material. Some saturated alcohol is dissolved with the large amount of unsaturated material present, but fortunately the solubility of pure cetyl alcohol in liquid sulfur dioxide is very small.[29] The procedure may also be successfully applied to mixtures of fatty acids, but in this case a larger amount of saturated acid is dissolved.

Fractionation of the Sulfur Dioxide Insoluble Fraction.—One hundred and seven grams of the material insoluble in sulfur dioxide was acetylated and distilled at 15 mm.

TABLE X

DISTILLATION OF SATURATED ALCOHOL ACETATES

	to 199°	199–202°	202–203°	Residue
Weights	9.3	81.2	17.0	11.8
Iodine nos.	1.1	1.2	1.7	8.5
Sapon. nos.	196.1	194.2	186.3	182.9

To 199°.—This fraction, on redistillation, yielded 1.1 g. boiling below 190°; saponification number, 200.3. On crystallization of the alcohol from 90% ethyl alcohol, the crystals melted at 45–47°. The solid remaining after evaporation of the mother liquors melted at 38–40°—indicating the presence of a small amount of tetradecyl alcohol (m. p. 37.5°).

Fraction 199–202°.—The boiling point of this substance agrees well with the accepted value for cetyl acetate.[30] The saponification number of cetyl acetate is 197.5, the density (I. C. T.) d_4^{20} 0.859; density found, d_4^{25} 0.856. The alcohol obtained on saponification melted at 49.5°. This fraction is thus cetyl acetate.

Fraction 202–203°.—This was redistilled and the main portion identified as cetyl acetate. One gram, as a higher boiling fraction, had a saponification number of 189.5,

[29] W. F. Seyer and R. W. Ball, *Trans. Roy. Soc., Canada*, [3] 19, 149–151 (1926).

[30] J. Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," The Macmillan Co., 6th ed., p. 244.

and the recrystallized alcohol melted at 57–58°, indicating the presence of octadecyl alcohol (m. p. 59°).

Residue.—The majority of this fraction, after saponification, was identified as octadecyl alcohol by its melting point, 58.5°.

Fractionation of the Sulfur Dioxide Soluble Fraction.—The fraction was distilled directly at 15 mm.

TABLE XI

DISTILLATION OF THE SULFUR DIOXIDE-SOLUBLE FRACTION

	to 185°	185–200°	200–206°	Residue
Weights	6.5	27.4	30.0	48.9
Iodine nos.	62.9	79.5	92.8	121.0

Fraction 200–206°.—The constants of this fraction agree with those given for oleyl alcohol: b. p. (15 mm.) 200–206°; I, no. 92.8; n_D^{25} 1.4611; d_4^{25} 0.860. Toyama[31] gives b. p. (15 mm.) 205–210°, n_D^{20} 1.4607, d_4^{20} 9.8489. Bouveault and Blanc[32] give b. p. (13 mm.) 200°, d_4^0 9.862. The theoretical iodine number for oleyl alcohol is 94.7. This agreement makes the identity of the fraction certain.

Fractions 185–200° and "to 185°."—Cetyl alcohol boils at 189° at 15 mm., so that that dissolved by sulfur dioxide would appear in these fractions. This was the case, and no satisfactory method has been found to separate the cetyl and oleyl alcohols.

Residue.—The large residue is not surprising when it is remembered that no attempt was made to free the original unsaponifiable material from sterols and possible hydrocarbons. Part of the material was distilled at 1.5 mm., and the fraction 210–260° gave a small amount of squalene.

Fraction 210–260° (1.5 mm.).—One gram of this fraction was placed in 10 cc. of acetone and dry hydrogen chloride gas passed in until the solution was saturated. On cooling and concentrating, a small amount of white crystals separated which melted, after recrystallization, at 111–130°. Squalene[33] forms a hexahydrochloride which melts indefinitely between 112–126°.[34] This is confirmatory for the presence of this hydrocarbon.

The remaining residues resisted all attempts at further analysis.

Cholesterol.—The digitonin method of Windaus[35] for the separation of sterols was employed on a small sample of the total unsaponifiable fraction which had been previously washed with ice cold methyl alcohol. The flocculent precipitate obtained was washed with alcohol and ether, decomposed with boiling xylene, filtered and the residue from the evaporation of the xylene tested for the presence of cholesterol by the Burchard[36] and Salkowski[37] color reactions. Both were positive.

Summary

1. The oil of *Ruvettus pretiosus* has been analyzed, and consists primarily of cetyl and oleyl esters of oleic and hydroxyoleic acids. The oil is best classified as a liquid wax.

[31] Y. Toyama, *Chem. Umschau*, **31**, 13–17 (1924).

[32] Bouveault and Blanc, *Bull. soc. chim.*, [3] **31**, 1210 (1904).

[33] M. Tsujimoto, *J. Ind. Eng. Chem.*, **8**, 889 (1916); **12**, 63 (1920).

[34] I. M. Heilbron, E. D. Kamm and W. M. Owens, *J. Chem. Soc.*, 1630 (1926).

[35] A. Windaus, *Ber.*, **42**, 238–246 (1909).

[36] R. H. A. Plimmer, "Practical Organic and Biochemistry," Longmans, Green and Co., **1926**, p. 345.

[37] E. Salkowski and W. R. Orndorff, "Laboratory Manual of Physiological and Pathological Chemistry," John Wiley and Sons, Inc., New York, **1904**, pp. 91–92.

2. The constituents present are: acids—stearic, oleic, gadoleic, erucic, $C_{24}H_{46}O_2$, $C_{26}H_{50}O_2$, an hydroxyoleic acid; alcohols—oleyl, tetradecyl. cetyl, octadecyl, cholesterol, glycerol: hydrocarbon—squalene.

3. The purgative properties of the oil have probably been over-emphasized, although there does seem to be a modicum of pharmacological action. This may be dependent on its content of esters of higher alcohols.

4. Extraction with liquid sulfur dioxide has been shown to be a satisfactory method for the separation of the saturated and unsaturated constituents of the unsaponifiable fraction.

BALTIMORE, MARYLAND

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NEBRASKA]

ISOMERIC ALPHA-PARA-NITROPHENYL-BETA-PHENYL-DELTA-KETONIC ACIDS

BY S. AVERY, C. B. BISWELL AND E. E. LISTON

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Attempts to resolve the inactive isomeric α,β-diphenyl-δ-ketonic acids, described in a previous paper,[1] into optically active forms were unsuccessful. It was accordingly decided to synthesize the acids named in the title of this paper in the expectation that these would more readily permit the formation of optical isomers. Although attempts along this line up to the present had not been successful, these inactive nitro acids and their derivatives show certain properties markedly different from all delta-ketonic acids previously studied.

The two isomeric nitriles of α-p-nitrophenyl-β-phenyl-γ-benzoylbutyric acid have been investigated by Allen.[2] He appears, however, not to have interested himself in preparing the acids themselves and no further reference appears in the literature. Further, no work has been found bearing on α-p-nitrophenyl-β-phenyl-γ-trimethylacetylbutyric acid or its derivatives.

In condensing benzalpinacolone with methyl p-nitrophenylacetate in the presence of sodium methoxide, a crystalline substance was formed, easily purified, and without evidence of isomeric admixture. When, however, benzalpinacolone was condensed with ethyl p-nitrophenylacetate in the presence of sodium ethoxide, a crystalline substance was formed which hydrolyzed to an isomer of the one obtained by the methyl condensation route. Though the isomers melted with decomposition at the same temperature, the crystalline forms were distinctly different and their derivatives showed marked variations. An entirely similar result was obtained when benzalacetophenone was condensed, first with methyl p-

[1] Avery and Jorgensen, THIS JOURNAL, **52**, 3628 (1930).

[2] Allen, *ibid.*, **47**, 1733 (1925).