acids were distilled helped to make the separation possible. Repeated fractionation of the fractions with high iodine number and high rotation finally gave an ethyl ester which had a constant boiling point (214° at 10 mm.) and the theoretical iodine number. The specific optical rotation of this fraction was +55.4°. This same rotation was obtained from several fractions made by the above method. Boiling points were recorded by a calibrated millivoltmeter attached to a thermocouple at the top of the column. Reflux ratios of over six to one were maintained. The pressure was kept within less than 0.3 mm. by an automatic pressure regulator.

Gorlic Acid.—After the constants of ethyl gorlate were obtained, the ester was saponified and the free acid liberated. After washing and drying, the acid was distilled at 10 mm. in the Podbielniak apparatus. It gave a horizontal distillation curve upon the first fractionation. About 20% decomposed, remaining in the distilling flask as a thick, dark brown liquid. The constants of pure gorlic acid were determined and are shown in Table III. Mol. weight, sample 0.1609 g.; 0.1 *N* potassium hydroxide 5.79 cc.; mol. wt., 277.8; calcd., 278.2.

Methyl Gorlate.—Gorlic acid was esterified with absolute methyl alcohol in the same manner as described above for the preparation of ethyl gorlate. A single distillation in the Podbielniak apparatus gave several fractions with identical constants and a horizontal distillation curve. The constants of methyl gorlate are given in Table III.

Anal. Calcd. for $C_{18}H_{32}O_2$: C, 78.01; H, 11.03. Found: C, 78.00; H, 11.07.

CALCULATIONS FOR SPECIFIC OPTICAL ROTATION

Compound	Grams	$CHCl_3$, cc.	Ang. rotn. 100-mm. tube	$[\alpha]^{25}D$
Gorlic acid	1.7382	25	+4.22°	+60.7°
Ethyl gorlate	2.1302	25	+4.72°	+55.4°
Methyl gorlate	1.6555	25	+3.82°	+57.7°

In making the iodine number determination we observed the fact, mentioned by others, that more than the usual amount of Hanus solution must be added or the results will be low. We used three times the usual amount.

Summary

A liquid fatty acid possessing a high optical activity has been isolated from two chaulmoogra oils, *Carpotroche brasiliensis* and *Oncoba echinata*.

The constants of the pure acid and those of its methyl and ethyl esters have been determined.

RIO DE JANEIRO, BRAZIL RECEIVED JANUARY 4, 1938

[CONTRIBUTION FROM THE INTERNATIONAL LEPROSY CENTER, RIO DE JANEIRO]

Analysis of Chaulmoogra Oils. I. *Carpotroche brasiliensis* (Sapucainha) Oil

BY HOWARD IRVING COLE AND HUMBERTO T. CARDOSO

Although chaulmoogra oils have been used intensively in the treatment of leprosy for the past fifteen years, no accurate quantitative analyses of these oils have been made. They are today bought and used on the simple criteria of free fatty acid content and a certain minimal optical activity. The former indicates roughly the quality and age of the oil and the latter, supposedly, the approximate percentage of the therapeutically active hydnocarpic and chaulmoogric glycerides. Actually hydnocarpic and chaulmoogric acids differ in optical rotation by 15% and, furthermore, as we shall show, there may be present in considerable amounts a third optically active acid, gorlic acid. All attempts at quantitative analysis have failed because of the difficulty of completely separating hydnocarpic from chaulmoogric acid and of separating these two from the other acids present. None of the ordinary methods of oil analysis yield satisfactory results. We believe that the analysis of oils so extensively used medicinally is fundamental to improvement in the character of the leprosy drugs to be made from them. If a satisfactory general method of analysis could be found the analysis of all the important chaulmoogra oils should be a comparatively easy matter. We have worked out such a method and have analyzed one of the more difficult of these oils, *Carpotroche brasiliensis*, by this method. The analyses of *Oncoba echinata*, *Hydnocarpus wightiana*, *Hydnocarpus anthelmintica* and *Taraktogenos kurzii* will follow shortly.

Carpotroche brasiliensis, Endl. (Sapucainha) is indigenous to the mountainous forests of the states of Rio de Janeiro, Minas Geraes, Espirito Santo, Bahia, São Paulo and Piauhy. Peckolt, in 1869, was the first to suggest that the oil from the seeds of this tree might be used in place of chaulmoogra oil in the treatment of leprosy.[1] Machado[2] claimed to have found two new acids in this oil which he named carpotrochic and carpotrochinic acids. Da Silva[3] showed that these acids were evidently only mixtures and that the

(1) Souza Araujo, *Intern. J. Leprosy*, **3**, 50 (1935).
(2) Machado, *Ann. soc. med. cir. Rio de Janeiro*, **40**, 189 (1926).
(3) Da Silva, *Rev. brasil. med. pharm.*, **2**, 627 (1926).

main constituents were chaulmoogric and hydnocarpic acids. Paget[4] in 1937 was the first to report the presence of an optically active liquid acid and although he did not obtain it pure he

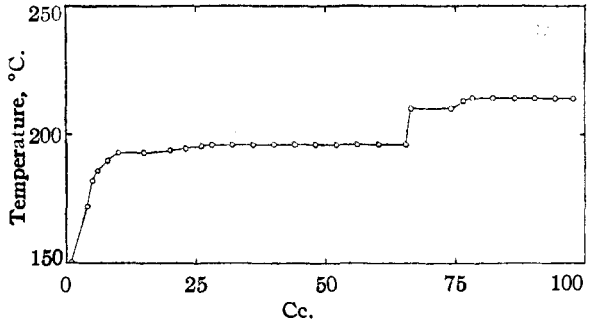

Fig. 1.—Distillation curve of ethyl esters from solid acids (10 mm.).

was able to determine its structure. At the same time we also had obtained this liquid acid and were endeavoring to isolate it in the pure state, a task made very difficult because oleic acid was mixed with it. Since our method of analysis was partly based upon knowing the optical activity and iodine numbers and boiling points of the pure ethyl esters, it was essential that we isolate this acid and determine its constants and those of its ethyl ester. This was finally accomplished.[5] The acid, which we named gorlic, has the same structure as chaulmoogric except for the addition of another double bond between the fifth and sixth carbon atoms of the side chain. It has a high specific rotation (+60.7°) and a high iodine number (182.5). The extremely high rotation of this acid and of hydnocarpic and chaulmoogric acids offer a ready and accurate means of determining the amounts present in a mixture of ordinary fatty acids providing that hydnocarpic can be separated from chaulmoogric and gorlic acids. The amounts of the latter two in a mixture can be computed easily, because of their almost identical optical rotation values and their very different iodine numbers. Hydnocarpic acid has never been separated quantitatively from chaulmoogric acid. Attempts to accomplish this by distillation of the ethyl esters in a Podbielniak high temperature fractionation apparatus[6] were unsuccessful due to the presence of liquid acids (oleic and gorlic). It was necessary first to separate the liquid acids from the solid acids by several crystallizations from 80% ethyl alcohol. The esters made from the solid acid fraction then gave, upon distillation in the Podbielniak still, a sharp separation of the palmitate and hydnocarpate from the chaulmoograte, although the first two were not separated completely from each other (Fig. 1 and Table II). Distillation of the esters from the liquid acid fraction in the above-mentioned still did not give such a sharp separation (Fig. 2 and Table III) but we have been able to compute the amounts of the various constituents present by means of their boiling points, iodine numbers and optical rotations or absence of one or both of the two latter constants.

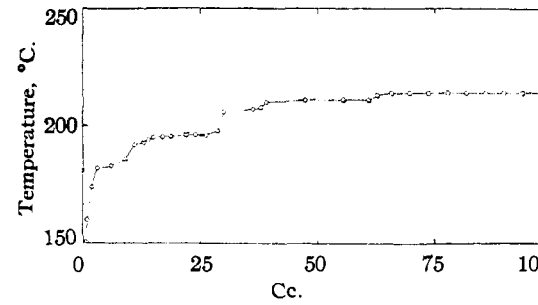

Fig. 2.—Distillation curve of ethyl esters from liquid acids (10 mm.).

The sample of oil was obtained by cold-pressing authentic fresh *C. brasiliensis* seeds kindly furnished by the Escola Superior de Agricultura of Viçosa, Minas Geraes, from their plantation. Its constants, determined immediately after pressing and after standing for one year are shown in Table I, which also shows constants obtained by other authors. We have found that, although the oil keeps fairly well as indicated in Table I, the seeds deteriorate rapidly. Leprosy drugs made from oil from old seeds are extremely irritating, while those made from oil from fresh seeds, even if the oil itself has stood for over one year, are no more irritating than our standard drugs from *H. wightiana* oil. Paget stated that this irritation was associated with the 9% of tarry acids which he found present in his sample. The presence of these tarry acids indicates an oil made from old seeds. The fact that we found no tarry acids in our oil (following his method of separation) and that drugs made from our oil were not irritating corroborates his statement. A comparison of his approximate analysis with our analysis shows that his tarry acids were formed largely by the decomposition of the gorlic acid,

(4) Paget, *J. Chem. Soc.*, 955 (1937).
(5) Cole and Cardoso, THIS JOURNAL, **60**, 612 (1938).
(6) Podbielniak, *Ind. Eng. Chem., Anal. Ed.*, **3**, 181 (1931); **5**, 119 (1933).

as the percentage of the latter obtained by him is very low. This is what one would expect as gorlic acid is more unstable than either hydnocarpic or chaulmoogric acids.

TABLE I

CONSTANTS OF *carpotroche brasiliensis* OIL

Author		S. gr. at 25°	Refr. in. at 25°	Spec. rot. $[\alpha]^{25}D$	Sap. no.	F. F. A. as % Oleic	Iodine no.
André[7]		0.95	1.475	+53.4	183.7	..	106.1
Da Silva[2]		.95	1.472	+54	185	2.6	108
Rothe[8]		.9486	....	+52	204.4	..	101.6
Paget[1]		.9563	....	+54	199.7	10.8	101.3
Viçosa sample	Fresh	.95	1.4793	+52.4	201.8	1.3	103
	1 yr. old	.955	1.4790	+53.8	201.8	3.6	108

Experimental

Method of Analysis.—The oil (one year old, with constants shown in Table I) was saponified and the free fatty acids liberated and washed in the usual manner. Two hundred grams of the total acids was dissolved in 800 cc. of 80% ethyl alcohol and allowed to stand overnight in an electric referigerator at 0°. The solid acids were separated in a Büchner funnel, redissolved, recrystallized and separated again in the same manner. The two mother liquors were combined and most of the alcohol evaporated. To change any ethyl esters, formed in the above operations, into acids, the mixture was resaponified and the fatty acids liberated and well washed with hot water. They were dissolved in two volumes of 80% alcohol and kept in an electric refrigerator at −10° for two or three days to crystallize out of solution as much as possible of the remaining solid acids. This solid fraction was recrystallized again at −10°, separated, washed and added to the first fraction of solid acids. The filtrate was added to the main liquid acid fraction and the liquid acids were separated by dilution with hot water, then washed and dried. The two fractions, solid and liquid, were then treated separately as follows: they were esterified by boiling for three hours with two volumes of absolute ethyl alcohol and 3% of concentrated sulfuric acid. The esters were separated by dilution and washed. Two volumes of ethyl ether was added and the free fatty acids removed by a 10% solution of sodium carbonate. The ethereal solution was washed several times. The fatty acids were liberated from the carbonate solution and the wash waters esterified and added to the ethereal solution. The ether was evaporated and the esters dried. The two samples of ethyl esters, so prepared, were fractionally distilled at 10 mm. pressure in the Podbielniak Model B high temperature fractionating apparatus. Free fatty acids must be absent in order to obtain sharp distillation curves. Any liquid acid in the solid acid fraction interferes with a sharp separation of the ethyl esters made from the solid acids.

Qualitative Analysis

The free fatty acids of *C. brasiliensis* oil contain palmitic, hydnocarpic, chaulmoogric, gorlic and oleic acids. They were isolated and identified as follows.

Palmitic Acid.—Difficulty was experienced in trying to free this acid from hydnocarpic acid. The easiest method was to cool a fraction of ethyl esters rich in palmitate (Table II, fraction 1) to 0° in an electric refrigerator overnight. The ethyl palmitate which crystallized out was separated, dissolved in 95% ethyl alcohol and cooled to −10° in the ice compartment of the refrigerator. The crystallization was repeated until the m. p. remained constant at 24°. This was changed to palmitic acid; found, m. p. 62.5°, neut. equiv., 256.2.

TABLE II

FRACTIONAL DISTILLATION OF ETHYL ESTERS FROM SOLID FATTY ACIDS OF *C. brasiliensis* OIL (74.8% OF TOTAL FATTY ACIDS)

Fr.	B. p., °C. (10 mm.)	Cc.	Sp. rot. $[\alpha]^{25}D$	Palmitate, %	Hydnocarpate, %	Chaulmoograte, %	Oleate, %
1	153–195	20.0	50.85	3.6	16.4	..	...
2	195–197	20.3	54.16	2.6	17.7	..	...
3	195–197	20.0	56.81	1.7	18.3	..	...
4	195–197	5.1	53.06	0.7	4.4	..	...
5	211–212	9.3	50.88	...	..	8.6	0.7
6	212–214	23.0	53.54	...	..	22.2	0.8
Residue		2.3	...	...	..	..	...
% in solid fraction				8.6	56.8	30.8	1.5
% in total F. F. A.				6.4	42.5	23.1	1.1
% residue in total F. F. A.				1.7			

Hydnocarpic Acid.—Fractions rich in ethyl hydnocarpate, such as Nos. 2, 3, 4, Table II, were refractionated until the specific optical rotation indicated a practically pure product. This was changed into acid and crystallized from 95% alcohol until the m. p. remained constant at 60.5°. Found for hydnocarpic acid: neut. equiv., 251.8; iodine no. 100; spec. rotation +69.3°.

Chaulmoogric Acid.—Pure chaulmoogric acid was obtained easily by twice crystallizing the acid made from the ethyl chaulmoograte of fraction 6, Table II. Found: m. p., 68.5°; neut. equiv., 281; iodine no., 90; spec. rotation, +60.3°.

Gorlic Acid.—Many methods of purifying the gorlic acid were tried but the only one which proved successful was by the repeated fractionation of the higher fractions of the esters from the liquid acids, such as nos. 5, 6 and 7, Table III. Ethyl gorlate; iodine no., calcd. 165.7; found 166.2. The ethyl gorlate was changed to acid; neut. equiv., calcd. 278.2; found, 277.8; iodine no., calcd., 182.5; found, 179.7; spec. rotation, +60.7°.

TABLE III

FRACTIONAL DISTILLATION OF ETHYL ESTERS FROM LIQUID FATTY ACIDS OF *C. brasiliensis* OIL (25.2% OF TOTAL FATTY ACIDS)

Fr.	B. p., °C. (10 mm.)	Cc.	Sp. rot. $[\alpha]^{25}D$	Iodine no.	Palmitate, %	Hydnocarpate, %	Chaulmoograte, %	Oleate, %	Gorlate, %
1	151–161	1.0	34.00	...	0.4	0.6	...	..	..
2	161–189	8.0	32.33	83.6	.2	4.2	...	3.6	..
3	189–197	17.3	38.04	112.8	.2	5.3	...	5.9	5.9
4	197–210	11.0	38.47	141.0	...	..	...	3.3	7.7
5	210–212	23.5	44.03	142.7	...	..	1.9	4.8	16.8
6	212–214	25.0	50.05	152.0	...	..	2.0	2.4	20.6
7	214	11.8	52.58	155.0	...	..	1.1	0.6	10.1
Residue		2.4							
% in liquid fraction					0.8	10.1	5.0	20.6	61.1
% in total fatty acids					.2	2.5	1.3	5.2	15.4
% residue in total F. F. A.				0.6					

(7) André, *Compt. rend. Acad. Sci.*, **181**, 1089 (1925).
(8) Rothe, *Rev. soc. bras. chim.*, **2**, 358 (1931).

Oleic Acid.—Although we were able to free ethyl gorlate from ethyl oleate by fractional distillation we were not able to free the ethyl oleate from the ethyl gorlate. The elaidic acid test is not satisfactory in the presence of gorlic acid as the latter gives a similar reaction. However, oxidation by permanganate yielded dihydroxystearic acid. The presence of oleic acid was confirmed further by the boiling point of the acid and of ethyl oleate and by the correct iodine numbers for mixtures with gorlic acid or ester. The oxidation products of hydnocarpic, chaulmoogric and gorlic acids (hydroxy and keto acids) were present only in very small amounts and were not isolated.

Quantitative Analysis

The total fatty acids of the oil, when separated into liquid and solid acids by the method outlined above, consisted of 74.8% solid and 25.2% liquid acids. The separation of course is not complete, but the solid fraction should contain not over 1 or 2% of liquid acids. From the qualitative analysis it is seen that the solid fraction consists essentially of palmitic, hydnocarpic and chaulmoogric acids. The distillation curve (Fig. 1) shows that a sharp separation of chaulmoograte is obtained. If 100 cc. of esters is distilled, the percentage of ethyl chaulmoograte in any fraction (boiling point above 200°) is computed easily from the specific rotation of the fraction, *i. e.*, Fraction No. 6, Table II, (53.54/55.4) × 100 = 96.6%. 96.6 × 23 = 22.2 cc. of ethyl chaulmoograte.

Total % of ethyl chaulmoograte in esters from solid acids = 30.8. 30.8 × 0.748 = 23.1% chaulmoogric acid in solid fraction of F. F. A. The same procedure is used with fractions boiling below 201° to obtain the percentage of hydnocarpic acid, the palmitic being obtained by difference.

The method of computing the percentage composition of the liquid acids in fractions 5, 6 and 7, Table III, is as follows: the percentage of optically inactive acid may be computed by using 55.5° as the specific rotation of both ethyl gorlate and chaulmoograte, *i. e.*, in Fraction 5, (44.03/55.5) × 100 = 79.4%. 100 − 79.4 = 20.6% ethyl oleate. 20.6% of 23.5 cc. = 4.8 cc. oleate. Then if $x = 82$ (iodine no. of both oleate and chaulmoograte) and if $y = 167$ (iodine no. of gorlate), the percentages of gorlate and chaulmoograte can be calculated from the formulas, $x + y = 100$ and $\frac{82x}{100} + \frac{167y}{100}$ = I no. (of fraction). In fraction 4 the specific rotation and iodine number indicated that only gorlate and oleate were present. In fractions 1, 2 and 3, palmitate was obtained by difference after the other esters present had been computed.

TABLE IV

PERCENTAGE COMPOSITION OF THE FATTY ACIDS OF *C. brasiliensis* OIL (FROM TABLES II AND III AND ACCORDING TO PAGET)

Fatty acid	Composition according to Cole, %	Composition according to Paget, %
Hydnocarpic acid	45.0	
Chaulmoogric acid	24.4	65–70
Palmitic acid	6.6	
Gorlic or dehydrochaulmoogric	15.4	9
Oleic acid	6.3	4
Keto acids	..	4
"Tarry acids"	None	9
Residue (decomposition products of distillation)	2.3	..

Summary

A method for the qualitative and quantitative analysis of chaulmoogra oils is described.

The analysis of the fatty acids of *C. brasiliensis* oil as made by this method is given.

RIO DE JANEIRO, BRAZIL RECEIVED JANUARY 8, 1938

[CONTRIBUTION FROM THE INTERNATIONAL LEPROSY CENTER, RIO DE JANEIRO]

Analysis of Chaulmoogra Oils. II. *Oncoba echinata* (Gorli) Oil

BY HOWARD IRVING COLE AND HUMBERTO T. CARDOSO

Oncoba echinata, Oliver, a tall shrub, from the seeds of which oil of gorli or *beurre de gorli* is obtained, is indigenous to West Africa. Gorli oil has been used in the treatment of leprosy. It is solid at ordinary temperatures and resembles that of *Hydnocarpus alcalae* in having a high melting point and in containing chaulmoogric but not hydnocarpic glycerides. Owing to the difficulty of separating these two compounds, the absence of the latter makes this oil a good source of pure chaulmoogric acid. In fact, until recently, the only correct figures for the constants of chaulmoogric acid have been obtained from this oil. The absence of hydnocarpic acid also so simplifies the analysis that oils of this type are the only chaulmoogra oils for which definite quantitative results have been published. Even so, the analyses have been only approximate.

The first analysis of the oil of gorli was made by Goulding and Akers[1] in 1913. They stated that the fatty acids consisted of 85.5% chaulmoogric acid and 12.5% liquid acids. André and Jouatte[2] in 1928 found that the liquid acid fraction contained an optically active liquid fatty acid with a

(1) Goulding and Akers, *Proc. Chem. Soc.* (London), **29**, 197 (1913).

(2) André and Jouatte, *Bull. soc. chim.* **43**, 847 (1928).