between the Human-H37-TPT and the *M. leprae* no. 1 TPT and that the crossing between timothy and *M. leprae* no. 1 TPT's was very marked, facts which also correspond with the titration curves. These results indicate that further work along this line may be fruitful. Discussion as to the significance of different parts of the curves in relation to the nature of the products will be reserved for another paper.

I wish to express my appreciation to Dr. H. S. Simons for his advice in calculation of the base-binding capacities.

Summary

A comparative study of the proteins isolated from five different strains of human type tubercle bacillus culture filtrates, grown under identical conditions, yielded the following results:

The yields of protein were very much alike, *viz.*, about 0.2 g. per liter of original medium.

The nitrogen content of four of them was 14% and of the fifth 15.2%.

The tuberculin potency, determined by means of the skin test in tuberculous guinea pigs, showed some variation, but this was not marked. All five proteins were potent tuberculins.

All five proteins proved to be identical serologically, when compared by the precipitin reaction. They were type specific, in that they could be distinguished easily by this method from proteins made similarly from other acid-fast bacilli, as had been shown in earlier work. The protein from the bovine type tubercle bacillus, while distinctive, was related to some extent to the human type proteins. Polysaccharide was not responsible for the immunological reactions, since it gave no cross reactions with any of the antisera.

Acid–base-combining capacity curves throughout the range of *p*H 2 to 11, as determined by electrometric titration, using the hydrogen electrode, showed a much closer correspondence between the five proteins from human type tubercle bacilli than between them and proteins made similarly from other types of acid-fast bacilli.

PHILADELPHIA, PENNA. RECEIVED FEBRUARY 3, 1937

[CONTRIBUTION FROM INTERNATIONAL LEPROSY CENTER, RIO DE JANEIRO]

Hydnocarpic and Chaulmoogric Acids and Ethyl Esters

BY HOWARD IRVING COLE AND HUMBERTO CARDOSO

In making quantitative analyses of various chaulmoogra oils, it was necessary for us to know accurately the optical rotation and the boiling or melting points of pure hydnocarpic and chaulmoogric acids and their ethyl esters. The published data were not only incomplete but varied so widely that we found it necessary to prepare these substances in the pure state and redetermine their constants before proceeding with our analyses. Undoubtedly most of the errors found in the literature were due to impurities, since hydnocarpic acid is completely separated from chaulmoogric acid and from palmitic acid only with extreme difficulty. Hydnocarpic and chaulmoogric acids are optically active. They contain a double bond in the pentene ring and are classed theoretically as unsaturated acids as they absorb iodine or bromine in the theoretical amounts. On the other hand, being solid acids they behave physically more like the solid saturated acids, hence standard methods of separating unsaturated from saturated acids cannot be used for their isolation.

Methods of Separation

Pure chaulmoogric acid can best be obtained from the oil of *Oncoba echinata* or *Hydnocarpus alcalae* as these oils do not contain hydnocarpic acid. Analyses of chaulmoogra oils to date show no oil which contains hydnocarpic but not chaulmoogric acid, hence to obtain the former it must be separated from chaulmoogric acid. This was first accomplished qualitatively in 1905 by Power and his co-workers by fractional precipitation of their barium salts.[1] Other workers have used fractional crystallization of the free fatty acids or their salts, fractional distillation of the acids or methyl or ethyl esters, or some combination of these methods. Even so, complete purification is extremely difficult. For the preparation of pure hydnocarpic acid, *H. Wightiana* oil is recommended, as it does not contain palmitic acid whose physical properties are so close to those of hydnocarpic acid as to make complete separation of the latter next to impossible.

We have been able to separate completely hydnocarpic from chaulmoogric acids by employing a combination of

(1) F. B. Power and M. Barrowcliff, *J. Chem. Soc.*, **87**, 888, 896 (1905).

fractional vacuum distillation of their ethyl esters in a Podbielniak high temperature fractionating apparatus[2] and fractional crystallization of the free fatty acids made from these esters. The free fatty acids of *Hydnocarpus Wightiana* oil were prepared by saponifying it and liberating the acids with sulfuric acid in the usual manner. They were washed free of mineral acid and glycerol with hot water, solidified and remelted to free them from water. The acids were esterified by dissolving them in five times their weight of 99% ethyl alcohol, then slowly mixing in one part by weight of concentrated sulfuric acid and allowing the mixture to stand overnight. An equal volume of water was then added and the ester extracted with ethyl ether, washed, and free acids removed by a 10% solution of sodium carbonate. The ethereal solution was washed several times with water, then dried with lumps of calcium chloride, filtered and the ether removed. The ethyl esters so prepared were distilled at 10-mm. pressure in a model B Podbielniak high temperature fractionating apparatus. One fractionation separated the ethyl hydnocarpate from the chaulmoograte though not from the other solid and liquid acids present. The fractions were saponified by adding twice their volume of 95% alcohol and 10% excess of potassium hydroxide dissolved in as little water as possible. Complete saponification results after five minutes of boiling. After dilution with 3 or 4 parts of hot water an excess of 15% sulfuric acid was added. The fatty acids rise and solidify on cooling. They were washed four times with hot water and then crystallized to constant melting point and optical rotation from 80% alcohol. Usually two crystallizations were sufficient. It was observed that the melting point was not an accurate criterion of the purity of the compound. The optical rotation, which is very high,

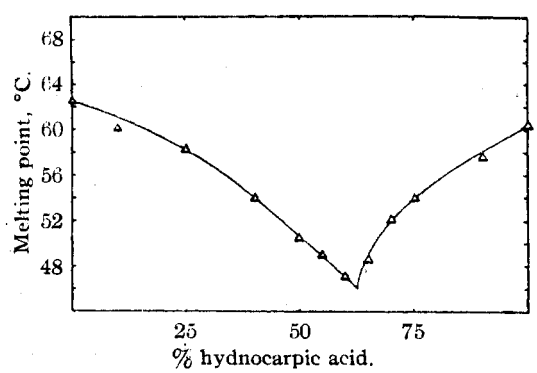

Fig. 1.—Melting point curve of palmitic and hydnocarpic acid mixtures.

was a more sensitive indication of purity as was also the appearance of the crystals. The crystals of pure hydnocarpic and of pure chaulmoogric acid both grow upward in branching forms from the melted acid, as it solidifies. Even a small amount of impurities inhibits this very characteristic growth, yielding instead a flat upper surface. As far as we know this peculiarity has not been reported before.

The pure ethyl esters were prepared from these pure acids as outlined above and these were then redistilled in the Podbielniak apparatus. After taking the constants of these esters they were again changed to acids and crystallized two or more times. These acids showed no change in their constants from those first prepared. Ethyl esters from these acids when distilled gave the same boiling points, refractive indices and optical rotations as the preceding ones. Chaulmoogric acid and ethyl chaulmoograte prepared from *Oncoba echinata* oil gave the same constants as those from *H. Wightiana* oil. The constants of the pure acids and ethyl esters are given in Tables I and II.

TABLE I

CONSTANTS OF HYDNOCARPIC AND CHAULMOOGRIC ACIDS

Acid	Hydnocarpic	Chaulmoogric
Melting point, °C.	60.5	68.5
Specific rotation, $[\alpha]^{25}D$	69.3	60.3
Iodine number (Hanus)	100.7	90.5
Neut. equivalent	251.8	280.9

Remarks: both the acids when pure grow upward in branching crystals from the melted acid as it solidifies.

TABLE II

CONSTANTS OF ETHYL HYDNOCARPATE AND CHAULMOOGRATE

Ester, ethyl		Hydnocarpate	Chaulmoograte
B. p. (10 mm.), °C.		184	206
Sp. rotation, $[\alpha]^{25}D$		61.94	55.42
Specific gravity at	20/4	0.911	0.904
	25/4	.907	.901
	30/4	.904	.898
Refractive index at	20°	1.4597	1.4610
	25°	1.4578	1.4592
	30°	1.4558	1.4573

Melting Point Curves

It was noticed that even a small percentage of palmitic acid lowered the melting point of hydnocarpic acid to a surprising degree. The melting point curve of mixtures of these two acids was determined.

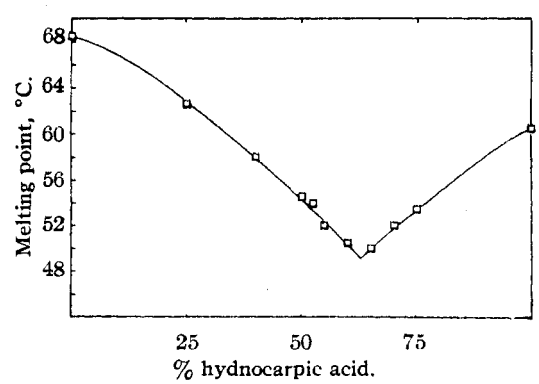

Fig. 2.—Melting point curve of chaulmoogric and hydnocarpic acid mixtures.

Power and Barrowcliff in their original work on hydnocarpic acid reported that systematic

(2) W. J. Podbielniak, *Ind. Eng. Chem., Anal. Ed.*, **3**, 181 (1931); **5**, 119 (1933).

fractional crystallization of the free fatty acids of *H. Wightiana*, *H. anthelmintica* or *T. Kurzii* oils always yielded mixtures which melted at 48–50°C.[1] They concluded that they were dealing with a molecular mixture of chaulmoogric acid and its homolog, hydnocarpic acid. By making up mixtures of these two pure acids and plotting the melting point curve, it was found that the lowest melting mixture was not at the point of a molecular mixture. Mixtures of the two acids do show, however, a very sharp drop in melting point and a loss of the characteristic crystalline formation of the pure acids (Fig. 2).

Optical Rotation

Because of the very high specific optical rotations of hydnocarpic and chaulmoogric acids (69.3 and 60.3°, respectively), the determination of this value is a very convenient means of ascertaining the purity of the acid. Especially is this so when one is able to employ, as we have done, an electric sodium lamp in conjunction with a precision polarimeter. Two different observers have been able to check readings with each other within ±0.02°.

The specific optical rotation of chaulmoogric acid often has been reported too high because there has been mixed with it a small amount of the more optically active hydnocarpic acid. Thus Stanley and Adams[3] report +61.9°, Power and Barrowcliff[1], +62.1° and Hinegardner and Johnson,[4] +62.2°. Goulding and Akers[5] report +60.0° while we obtain +60.3°.

In the case of hydnocarpic acid most of the optical rotation values given in the literature are too low due to the presence of palmitic or even chaulmoogric acid. We, however, closely check the value given by Stanley and Adams,[6] +69.4°, although their value for ethyl hydnocarpate, +70.5°, evidently is in error.

CALCULATIONS FOR SPECIFIC OPTICAL ROTATION

Compound	Source, oil of	Grams	$CHCl_3$, cc.	Ang. rotn., 100-mm. tube	$[\alpha]^{25}D$
Chaulmoogric acid	*H. Wightiana*	2.5526	25	+6.16°	+60.3°
Chaulmoogric acid	*O. echinata*	3.2175	25	+7.77°	+60.3°
Ethyl chaulmoograte	*O. echinata*	2.6479	25	+5.87°	+55.42°
Hydnocarpic acid	*H. Wightiana*	4.9422	50	+6.86°	+69.3°
Ethyl hydnocarpate	*H. Wightiana*	1.9978	25	+4.95°	+61.94°

Summary

The published data on the physical constants of hydnocarpic and chaulmoogric acids and ethyl esters are incomplete and inaccurate. Methods of preparation of the pure acids and ethyl esters are described and their physical constants have been determined. The melting point curves for mixtures of hydnocarpic and palmitic and of hydnocarpic and chaulmoogric acids have been determined.

(3) W. M. Stanley and R. J. Adams, THIS JOURNAL, **48**, 2395 (1926).

(4) W. S. Hinegardner and T. B. Johnson, *ibid.*, **51**, 1503 (1929).

(5) E. Goulding and N. C. Akers, *Proc. Chem. Soc.* (London), **29**, 197 (1913).

(6) W. M. Stanley and Roger Adams, THIS JOURNAL, **51**, 1515 (1929).

RIO DE JANEIRO, BRAZIL RECEIVED FEBRUARY 9, 1937

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NOTRE DAME]

Some Reactions of Dihydroxyfluoboric Acid

BY J. W. KROEGER, F. J. SOWA AND J. A. NIEUWLAND[1]

A previous paper[2] from this Laboratory described the preparation and some of the properties of dihydroxyfluoboric acid and advanced a tentative structure for this compound. The present work was undertaken in order to throw some light on the structure of this acid and to present certain peculiar reactions which it undergoes.

The literature on the various acids of boron and fluorine has been reviewed by Meerwein and Pannwitz[3] and by the authors.[2] Meerwein describes a compound which he calls boron fluoride-dihydrate which is striking in its similarity to dihydroxyfluoboric acid. They have similar boiling and melting points, their indices of refraction and densities agree rather closely and the melting points of their dioxane derivatives are practically the same.

Dihydroxyfluoboric acid may be written in any of three ways

(1) The major portion of this work was completed before the death of Dr. Nieuwland in June, 1936.

(2) Sowa, Kroeger and Nieuwland, THIS JOURNAL, **57**, 454 (1935).

(3) Meerwein and Pannwitz, *J. prakt. Chem.*, **141**, 123 (1934).