each other. The catalysts were: platinum-alumina containing 7% of platinum,[22] and chromia-alumina promoted with cerium and potassium.[23]

(22) H. Pines, R. C. Olberg and V. N. Ipatieff, THIS JOURNAL, **70**, 533 (1948).

(23) R. C. Archibald and B. S. Greensfelder, *Ind. Eng. Chem.*, **37**, 356 (1945).

Acknowledgment.—The authors express their thanks to Dr. D. F. Mason of the Chemical Engineering Department, for the mass spectrographic gas analysis and to Miss H. Beck for the elementary analysis.

EVANSTON, ILLINOIS

[CONTRIBUTION FROM THE NAVAL STORES STATION, U. S. DEPARTMENT OF AGRICULTURE[1]]

The Isolation of a New Resin Acid from Gum Rosin—Palustric Acid

BY VIRGINIA M. LOEBLICH, DORIS E. BALDWIN AND RAY V. LAWRENCE

RECEIVED SEPTEMBER 20, 1954

A new primary acid of the abietic type, named palustric acid, has been isolated from gum rosin by means of partition chromatography. The presence of this acid in the oleoresin of *Pinus palustris* and *Pinus caribaea* has been established. The physical constants and ultraviolet absorption spectrum have been obtained. Palustric acid has been isolated as an intermediate product in the acid and heat isomerization of levopimaric acid to abietic acid.

While a large number of acids have been reported as being present in pine oleoresin and rosin, on further investigation several of these acids have been shown to be mixtures. Only about seven resin acids from these sources have been characterized fully.[2-6] The most successful method for isolation of pure resin acids has been the recrystallization of certain amine salts of the resin acids from suitable solvents.[7,8] Since similar separation problems in fatty acid chemistry had been solved by the application of partition chromatography using an amine as the immobile phase, the method developed by Ramsey and Patterson[9] for the saturated straight-chain fatty acids C_{11} to C_{19} was applied to mixtures of the known resin acids. This technique divided the previously characterized resin acids into four groups (Fig. 1, curve 1). The acids eluted at fraction number 22 were a mixture of di- and tetrahydroresin acids. The second group (peak effluent volume 380–390) contained dextropimaric, isodextropimaric, levopimaric and *l*-abietic acids. Neoabietic acid was the only acid eluted in fractions 47–59 and dehydroabietic acid was the only acid eluted in fractions 61–77.

When rosin and oleoresin were chromatographed by this method, five peaks were obtained which did not correspond to the peaks of previously isolated resin acids (Fig. 1, curve 2). The peak effluent volumes of these unknown acids are 70, 310, 720, 790 and 890. Of these, the acid with a peak effluent volume of 310 has been isolated. On the basis of the ultraviolet spectrum this peak contained between 55 and 70% of an unknown acid which had a characteristic ultraviolet absorption maximum at 265–266 mμ. The other acidic material in this peak had no characteristic ultraviolet absorption and was probably a mixture of dextro- and isodextropimaric acids. The new acid was obtained pure by repeated recrystallization of the acid in fractions 25 through 33 from methanol.

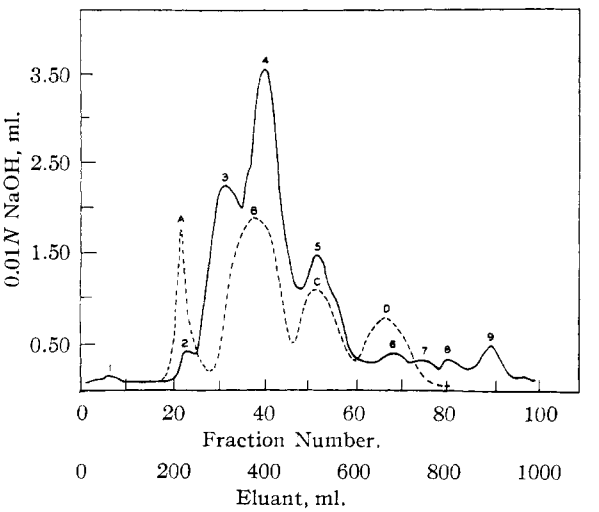

Fig. 1.—Curve 1, ---; chromatogram of pure resin acids: peak A, di- and tetrahydroresin acids; peak B, dextropimaric acid, isodextropimaric acid, levopimaric acid, *l*-abietic acid; peak C, neoabietic acid; peak D, dehydroabietic acid. Curve 2, ——; chromatogram of WW gum rosin: peak 3, palustric acid.

This new acid was first detected in the oleoresin of *Pinus palustris* and for this reason it was named palustric acid. Since it is present in the pine oleoresin, it is a primary resin acid. Palustric acid has an $[\alpha]_D$ +71.8° (2% EtOH), m.p. 162–167°, and an ultraviolet absorption maximum at 265–266 mμ with a specific absorption coefficient, α, of 30.1 (Fig. 2, curve 1). Palustric acid is an isomerization product of levopimaric acid and on treatment with mineral acid it is isomerized to *l*-abietic acid. It does not react with maleic anhydride at room temperature, but at elevated temperatures it reacts to form an addition product that is identical with the prod-

(1) One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) J. L. Simonsen and D. H. R. Barton, "The Terpenes," Vol. III, Cambridge University Press, 1952, pp. 374–458.

(3) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 1949, pp. 40–68.

(4) G. C. Harris, THIS JOURNAL, **70**, 3671 (1948).

(5) G. C. Harris and J. Sparks, *ibid.*, **70**, 3674 (1948).

(6) L. F. Fieser and W. P. Campbell, *ibid.*, **60**, 159 (1938).

(7) S. Palkin and T. H. Harris, *ibid.*, **56**, 1935 (1934).

(8) G. C. Harris and T. F. Sanderson, *ibid.*, **70**, 334 (1948).

(9) L. L. Ramsey and W. I. Patterson, *J. Assoc. Offic. Agr. Chemists*, **31** 441 (1948).

uct obtained by the reaction of levopimaric acid and maleic anhydride.

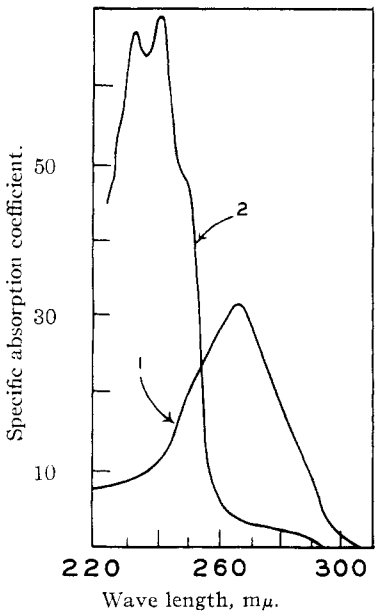

Fig. 2.—Ultraviolet absorption spectra: curve 1, palustric acid; curve 2, HCl isomerized palustric acid.

The six possible positions for two conjugated double bonds in ring B are listed in Table I along with the expected wave length at which the ultraviolet absorption maximum should occur.[10,11] It has been established that the double bonds in levopimaric acid are in the 6–7; 8–14 positions.[12–14] The observed ultraviolet maximum of 265 mμ for palustric acid is in best agreement with the formula having the double bonds in the 5–6; 7–8 positions.

TABLE I

POSSIBLE POSITIONS FOR THE CONJUGATED DOUBLE BONDS IN RING B OF THE RESIN ACID NUCLEUS

Positions of double bonds	Expected ultraviolet absorption maximum, mμ	Positions of double bonds	Expected ultraviolet absorption maximum, mμ
6–7; 8–14	278	6–7; 5–13	278
5–6; 7–8	268	5–13; 8–14	283
7–8; 13–14	278	5–6; 13–14	273

However, Ritchie and McBurney[15] have indicated that the acid isomerization of levopimaric acid proceeds through the formation of a carbonium ion at carbon atom 14 having a double bond in the 7–8 position. If this carbonium ion expells a proton from carbon atom 9, abietic acid is formed; if, instead of position 9, the proton is expelled from carbon atom 13, an acid with the double bonds in the 7–8; 13–14 positions would be formed.

(10) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 1949, pp. 187–188.

(11) H. Booker, L. K. Evans and A. E. Gillam, *J. Chem. Soc.*, 1453 (1940).

(12) L. Ruzicka and S. Kaufmann, *Helv. Chim. Acta*, **23**, 1346 (1940).

(13) *Ibid.*, **24**, 939 (1941).

(14) B. A. Arbusov, *Compt. rend. acad. sci. U.R.S.S.*, **30**, 723 (1941).

(15) P. F. Ritchie and L. F. McBurney, THIS JOURNAL, **72**, 1197 (1950).

Since palustric acid and abietic acid appear to be formed in almost equal amounts in the early stages of the heat isomerization of the levopimaric acid followed by further isomerization of the palustric acid to abietic acid, there must be a rather close relationship between the two acids. Such a relationship would be explained more readily by a structure having the double bonds in the 7–8; 13–14 positions rather than the 5–6; 7–8 positions.

Experimental

Chromatographic Separation of the Known Resin Acids.—The procedure for the chromatographic separation of the fatty acids described by Ramsey and Patterson[9] was used. The only change in the procedure was the use of isoöctane rather than hexane as the eluting solvent. The peak effluent volumes of previously isolated resin acids were determined and are given: tetrahydroabietic acid, 220; dihydroabietic acid, 220; dihydroneoabietic acid, 220; dihydrodextropimaric acid, 220; dihydrolevopimaric acid, 220; dextropimaric acid, 340; isodextropimaric acid, 340; levopimaric acid, 360; *l*-abietic acid, 360; neoabietic acid, 510; and dehydroabietic acid, 670. Two synthetic mixtures were prepared—one containing tetrahydroabietic acid, dihydro dextropimaric acid, dextropimaric acid, levopimaric acid, neoabietic acid and dehydroabietic acid and the other containing dihydroneoabietic acid, dihydrolevopimaric acid, isodextropimaric acid, *l*-abietic acid, neoabietic acid and dehydroabietic acid. When each mixture was chromatographed identical curves were obtained (Fig. 1, curve 1). Although the peak effluent volumes of dextropimaric acid and isodextropimaric acid were 20 ml. prior to the peak effluent volumes of levopimaric acid and abietic acid when the acids were chromatographed separately, only one peak was obtained when a mixture of the acids was chromatographed.

Isolation of Palustric Acid.—A few minor modifications were made in adapting the chromatographic technique to rosin: namely, isoöctane was used in place of hexane as the eluting solvent for the first eighty fractions. The remaining thirty fractions were eluted with 25% benzene–75% isooctane.

The correct amount of 2-aminopyridine–furfuryl alcohol mixture was critical and varied with each control lot of silicic acid. As the silicic acid adsorbed water from the atmosphere, the separation of the three peaks between fractions 25 and 58 (Fig. 1, curve 2) became less distinct. The silicic acid was stored in sealed bottles in a desiccator over a suitable drying agent prior to its use to prevent adsorption of water from the atmosphere.

For the isolation of the various fractions four large columns 31 mm. i.d. × 120 cm. in length were used. Each column contained 200 g. of silicic acid.[16] Four solutions containing 3.5 g. of rosin in 25 ml. of isoöctane were prepared. Twenty ml. of each solution was placed on each column and the remaining 5 ml. was titrated to determine the meq. of acid being chromatographed. The effluent was collected in 100-ml. fractions and the meq. of acid present in each fraction was determined by titrating a 2-ml. aliquot. The columns were run until 100% (±2%) recovery of the acids was obtained. The chromatographic curve of a typical separation was plotted using peak effluent volume as the abscissa and volume of alkali consumed for each 2-ml. aliquot as the ordinate (Fig. 1, curve 2).

To obtain the acid or group of acids in each of the nine peaks, those fractions forming each peak were combined and washed to remove the 2-aminopyridine–furfuryl alcohol mixture as follows: once with water, 3 times with saturated aqueous boric acid solution, once with 2% aqueous citric acid solution, twice with warm water (40°), and once with water at room temperature. The solution was then dried over sodium sulfate and evaporated to a volume of 20–30 ml. under a stream of nitrogen at atmospheric pressure. The extent of separation of the acidic portion of rosin is exemplified by the physical constants of the nine peaks from the chromatogram.

Fractions 6–8, peak 1, was a semi-crystalline material

(16) Silicic acid, Mallinckrodt Chemical Works, 100 mesh, suitable for chromatographic analysis by the method of Ramsey and Patterson was used.

having an $[\alpha]_D$ −6° in isoöctane and no characteristic ultraviolet absorption. Fractions 21–24, peak 2, contained, in addition to any di- and tetrahydroresin acids that may be present, several other unidentified products. Fractions 25–33 comprised the third peak and contained the palustric acid. These acids had an $[\alpha]_D$ +66.7°,[17] m.p. 140–145°, and a maximum specific absorption coefficient of 24.4 at 265–6 mμ. Fractions 35–47, peak 4, had an $[\alpha]_D$ −26.7°, m.p. 135–148°, and ultraviolet absorption characteristic of *l*-abietic acid but with a specific absorption coefficient of 33.7. Peak 5, fraction 48–58, contained neoabietic acid having an $[\alpha]_D$ +159.8° and a m.p. 167–169°. Peak 6, fractions 64–70, contained dehydroabietic acid and minor quantities of impurities. Peak 7, fractions 72–77, was a semi-crystalline material with no specific rotation. Ultraviolet absorption analysis gave maxima at 262–264 mμ and 273 mμ and a shoulder at 284–285 mμ with specific absorption coefficients at 32.1, 32.3 and 23.8, respectively. Peak 8, fractions 79–82, and peak 9, fractions 87–93, were semi-crystalline materials with no characteristic ultraviolet absorption. Peak no. 8 had no specific rotation and peak no. 9 had an $[\alpha]_D$ +9.7°. The physical constants for the acids obtained from peaks 6, 7 and 8 do not correspond to the physical constants for any of the previously reported acids found in rosin or oleoresin.

Purification of Palustric Acid.—The acid in fractions 25 through 33 from the four chromatograms was combined and recrystallized from methanol until the specific rotation and melting point of the recrystallized acid and the acid in the mother liquor were identical. This required six recrystallizations. The pure palustric acid had an $[\alpha]_D$ +71.6°, m.p. 162–167°, and its maximum absorption in the ultraviolet region at 265–266 mμ, α 30.1 (Fig. 1, curve 1).

Anal. Calcd. for $C_{20}H_{30}O_2$: C, 79.39; H, 10.00. Found: C, 79.42, 79.35; H, 10.11, 10.08.

An indication of the purity and homogeneity of the palustric acid was obtained in the following manner. The isobutanol amine salt of the pure acid was prepared, recrystallized three times from acetone, and the acid regenerated in isoöctane with saturated aqueous boric acid solution. The melting point and optical rotation of the regenerated acid were identical with the starting material.

Presence of Palustric Acid in Wood Rosin, *Pinus palustris* Oleoresin and *Pinus caribaea* Oleoresin.—From 0.5 to 0.6 meq. of *Pinus palustris* oleoresin, *Pinus caribaea* oleoresin and wood rosin were chromatographed on columns containing 20 g. of silicic acid. The effluent was collected in 10-ml. fractions and 5 ml. of each fraction was titrated to determine the amount of acid present. The chromatographic curve was plotted and the fractions containing palustric acid determined. The remaining 5-ml. aliquots of these fractions were combined, washed in the same manner as described for the large chromatograms, and evaporated to a volume of 10 ml. A 5-ml. aliquot was titrated with standard 0.01 *N* NaOH to determine the concentration, and the remaining 5 ml. was used for ultraviolet absorption analysis. In each case a unipeaked curve was obtained with maximum absorption at 265–266 mμ and a specific absorption coefficient range from 21 to 24. The percentages of palustric acid in the oleoresins and rosins investigated were estimated from the ultraviolet absorption of fractions 25–33 and are listed in Table II.

Mineral Acid Isomerization of Palustric Acid.—A solution of 0.1063 g. of palustric acid in 15 ml. of 0.58 *N* alcoholic HCl was refluxed for 1.5 hr. The solution was cooled and diluted to a volume of 25 ml. with 0.58 *N* alcoholic HCl. The $[\alpha]_D$ of this solution was −83.4°. The ultraviolet absorption spectrum of the acid isomerized palustric acid (Fig. 2, curve 2) indicated approximately 90% conversion to abietic acid. This conversion to abietic acid was of approximately the same extent as that obtained with levopimaric acid. An isomerization carried out under identical conditions using levopimaric acid, having $[\alpha]_D$ −267°, gave a slightly less pure abietic acid having $[\alpha]_D$ −78°.

Preparation of the Maleic Anhydride Addition Product of Palustric Acid.—A 25-ml. isoöctane solution of 0.1 g. of palustric acid and 0.04 g. of maleic anhydride (dissolved in 0.5 ml. of acetone) was allowed to stand at room temperature for 24 hr. The solution was washed with water to remove the maleic anhydride. The ultraviolet absorption spectrum of this solution was identical with the spectrum of the original palustric acid, indicating that the acid did not react with maleic anhydride at room temperature.

A mixture of 0.15 g. of palustric acid and 0.07 g. of maleic anhydride was then heated in an oil-bath at 150° for 2 hours. The product was dissolved in ether, washed neutral with water, dried over sodium sulfate, and evaporated to dryness. The adduct was crystallized from benzene–isoöctane. The melting point, mixed melting point and optical rotation of the maleic anhydride addition product of palustric acid and the maleic anhydride addition product of levopimaric acid were identical; m.p. 223.5–228.5°, $[\alpha]_D$ −27°.

Preparation of Methyl Palustrate.—A 2-g. sample of palustric acid was rechromatographed on a large column. The fractions containing the purified palustric acid were combined and washed as described previously. The cyclohexylamine salt of palustric acid was precipitated by adding 2 ml. of redistilled cyclohexylamine to the isoöctane solution of the acid. The salt was collected, dried under vacuum at room temperature and converted to the acid by shaking an ether suspension of the salt with 3 *N* acetic acid. The ether solution of palustric acid was washed neutral, dried over sodium sulfate and then treated with an excess of an ether solution of diazomethane. After standing 12 hours, the excess diazomethane and ether were removed by distillation. The ester crystallized from methanol; m.p. 25–7°, $[\alpha]_D$ +67.7°. The ultraviolet absorption spectrum of the ester exhibited a maximum at 265–6 mμ with a specific absorption coefficient, α, of 28.0.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.69; H, 10.19. Found: C, 79.14, 79.26; H, 10.08, 10.20.

Palustric Acid from Acid-isomerized Levopimaric Acid.—A 5-g. sample of levopimaric acid, $[\alpha]_D$ −272°, was isomerized at room temperature in 50 ml. of 0.1115 *N* alcoholic HCl solution for 180 minutes. The specific rotation of this solution was −74.3°. The acids were watered out of the alcohol solution and 3.3 g. was chromatographed. The sample contained 8% palustric acid having an $[\alpha]_D$ +62.8°, and an ultraviolet absorption maximum at 265 mμ, α 27.

Palustric Acid from Heat Isomerized Levopimaric Acid.—A 5-g. sample of levopimaric acid, $[\alpha]_D$ −272°, was sealed in a glass tube under vacuum and heated at 155° for five hours. The product had an $[\alpha]_D$ −6°. A 2.8-g. sample of the isomerized product was chromatographed and found to contain 29% palustric acid, having an $[\alpha]_D$ +68.5° and an ultraviolet absorption maximum at 266 mμ, α 28.5.

References to specific products of commercial manufacture are for illustration only and do not constitute endorsement by the U.S. Department of Agriculture.

OLUSTEE, FLORIDA

TABLE II

PERCENTAGE OF PALUSTRIC ACID IN GUM ROSIN, WOOD ROSIN, *Pinus palustris* OLEORESIN AND *Pinus caribaea* OLEORESIN

Oleoresins and rosins investigated	Total acids in peak III,[a] %	Palustric acid in peak III,[b] %	Palustric acid in original material,[c] %
Pinus palustris oleoresin	13	77	10
Pinus caribaea oleoresin	11	75	8
Gum rosin	20	79	17
Wood rosin	18	70	12

[a] The meq. of acid in the palustric acid peak divided by the total meq. placed on the column represents the percentage of the total acids in peak III. [b] The ratio of the specific absorption coefficient of the palustric acid in this peak to the specific absorption coefficient of pure palustric acid represents the percentage of palustric acid in peak III. [c] The percentage of total acids in peak III multiplied by the percentage of palustric acid in peak III represents the percentage palustric acid in the acidic portion of the original material.

(17) The specific rotations are in 95% EtOH unless otherwise specified. On the small samples the specific rotations were determined in the solvent used for ultraviolet analysis.