of potassium hydroxide in 5 cc. of water was added to a solution of 1.84 g. of histamine dihydrochloride in 5 cc. of water. The resulting solution was diluted with 100 cc. of ethyl alcohol, and 1.67 g. of pyridoxal was added. The initial bright yellow color gradually faded, and a thick, white precipitate appeared slowly. After a half hour, the mixture was cooled in ice. The white material was collected on a filter and washed with water until free of salt. It was then washed with alcohol and finally with ether; yield, 0.76 g. (29%); m. p. 252–253° (dec.). The analytical sample was dried at 100° (1 mm.) for four hours.

Anal. Calcd. for $C_{13}H_{16}N_4O_2$: C, 59.98; H, 6.20; N, 21.53. Found: C, 60.14; H, 6.10; N, 21.39.

Summary

Pyridoxal has been condensed with several amines to form Schiff bases. Most of these pyridoxylideneamines have been hydrogenated, yielding the pyridoxylamines: pyridoxyltyramine, pyridoxyltryptamine, pyridoxyl-β-phenylethylamine, pyridoxylhistamine, pyridoxylbenzylamine and pyridoxylisobutylamine. These compounds show an activity nearly as great as that of pyridoxine in rats.

RAHWAY, NEW JERSEY RECEIVED JULY 3, 1948

[CONTRIBUTION FROM HERCULES EXPERIMENT STATION, HERCULES POWDER COMPANY]

Resin Acids. V. The Composition of the Gum Oleoresin Acids of Pinus Palustris

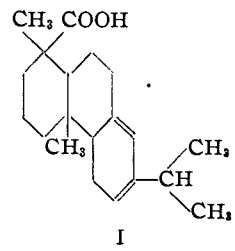
BY GEORGE C. HARRIS

For more than a century an extensive literature has dealt with the acids of gum oleoresin but only a limited number of valid results have been obtained. The reasons for this are that resin acids are unstable substances that easily change and are isomerized on treatment with heat and strong acids, and possess a great tendency to oxidize even with atmospheric oxygen. Especially variable are the primary acids present in unmodified oleoresins.

Because of their capacity for forming mixed crystals, the separation of mixtures of them into pure constituents by simple fractional crystallization often has been very difficult. It is, therefore, not surprising that many investigators working with oleoresins of different sources and using different separation methods obtained acids with different constants and gave them ever different names. Besides fractional crystallization, the only other technique available heretofore was the formation of insoluble sodium salts whereby levopimaric and dextropimaric acids[1] were isolated. A large number of "sapinic" (abietic-type) acids, which do not form crystalline salts have been reported as pure isomers obtained by fractional crystallization. Some of these are α-alepic, α-sapinic, Dupont's pineic,[2] Suzuki's densipimaric,[3] and Vocke's sapinic acid.[4] All of these were eventually shown to be mixtures.[5]

We have been more successful in the isolation of pure resin acids by applying and further developing a new technique,[6] the amine salt method for the separation of resin acids from non-resin acid material and for the isolation of pure resin acids. With the aid of this technique and others reported in the literature, it has been possible to determine fully, for the first time, the composition of the resin acids fraction of the oleoresin from *Pinus palustris*. In the following, the pertinent material reported previously in papers of this series will be brought together with new work in describing the composition of the primary resin acids.

For this investigation, as well as for the isolation of resin acids, reported earlier, a large batch of oleoresin was collected from the "longleaf" pine, *Pinus palustris*, and stored at 0–3° in the dark, out of contact with air. The acidic material was first separated from the turpentine as cyclohexylamine salts.[7] The regeneration of the acids from the amine salts was carried out using a weak acid, boric acid, at temperatures of 50° or below in order to retain the primary resin acids unaltered. The isolation of 15% of levopimaric acid, I, from this mixture of acids using butanolamine has been described.[7] However, it was not concluded to be



I

the total amount of levopimaric acid in the mixture because in the isolation of resin acids with the amine salt technique it was found that the desired separation was obtained but not always in quantitative fashion. To determine this value, a Diels–Alder addition reaction modified by Fleck and Palkin[8] was used whereby the resin acids in dry *n*-

(1) These words have purposely been written as one word since the compounds are not steroisomers as the prefixes *levo-* and *dextro-* would imply.

(2) G. Dupont, *Bull. soc. chim.*, **35**, 1207 (1923).

(3) S. Suzuki, *Chem. Zentr.*, **96**, I, 2383 (1925); **106**, II, 234 (1935).

(4) F. Vocke, *Ann.*, **508**, 11 (1933).

(5) G. Dupont, *Bull. soc. chim.*, **29**, 718 (1921). L. Ruzicka, Fr. Balas and Fr. Vilim, *Helv. Chim. Acta*, **7**, 458 (1924). K. Kraft, *Ann.*, **520**, 133 (1935). K. Kraft, *ibid.*, **524**, 1 (1936).

(6) Fr. Balas, *Časopis Československen. Lékárnictva*, **7**, 320 (1927).

(7) G. C. Harris and T. F. Sanderson, "Resin Acids. I," THIS JOURNAL, **70**, 334 (1948).

(8) E. E. Fleck and S. Palkin, *Ind. Eng. Chem., Anal. Ed.*, **14**, 146 (1942).

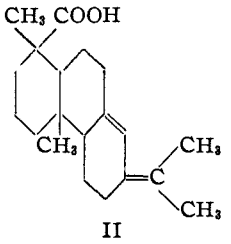
pentane were treated with dry, acid-free maleic anhydride in dry acetone for two hours at room temperature. The levopimaric acid–maleic anhydride adduct and unreacted acids were separated by the precipitation of the latter from an alkaline solution of the mixture by the addition of solid boric acid at a *p*H of 6.2. The amount of levopimaric acid was determined according to the Biot formula

$$x = (C - B)/(A - B)$$

Where A, B and C = the specific rotations of the levopimaric, unreacted and starting acids, respectively.

to obtain values of 30–35% in complete agreement with those of Fleck and Palkin[8] and Wienhaus and Sandermann.[9] In this way, not only agreement with results of other workers, but also assurance of an unaltered starting material was obtained.

The isolation of 5% of neoabietic acid, II, with diethylamine following the isolation of levopimaric acid with butanolamine was also described.[7] Again this was not assumed to be the total amount



II

of this acid in the oleoresin. To determine this value, use was made of the ultraviolet absorption spectrum technique employing a Beckmann spectrophotometer. Since interpretation of results could be simplified by having as few absorbing acids as possible in the mixture, the work was done with the acids from which levopimaric acid was removed quantitatively with acid-free maleic anhydride. An ultraviolet absorption curve,[10] Fig. 1,

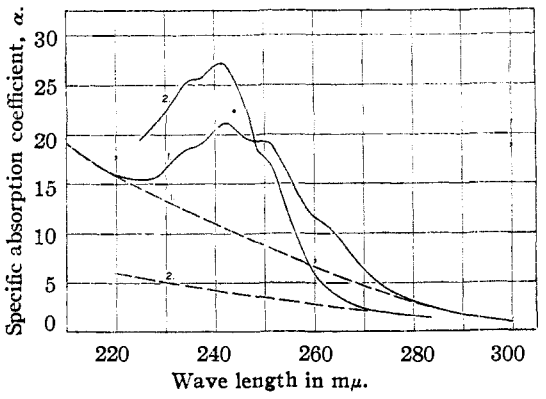
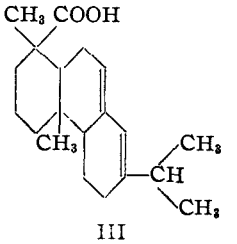


Fig. 1.—Ultraviolet absorption spectra: 1, levopimaric acid-free oleoresin acids; 2, acid-isomerized levopimaric acid-free oleoresin acids.

(9) W. Wienhaus and W. Sandermann, *Ber.*, **71**, 2005 (1938).

(10) The ultraviolet absorption data were determined by Dr. Evelyn V. Cook of this Laboratory.

Curve 1, surprisingly enough showed the absorption characteristics of abietic acid, III, with an inflection (usually a maximum for the pure acid) at 235 mμ and a maximum at 241–242 mμ wave length. This, indeed, was the first evidence of the



III

presence of abietic acid, *per se*, in gum oleoresin. Therefore, it is to be classified henceforth not only as the transformation product of resin acids but also as a primary acid. The quantity was determined as a ratio of the differences of specific absorption coefficients, $\Delta\alpha$, at 241–242 mμ between an arbitrarily defined general absorption (the broken line, Fig. 1, Curve 1) and the maximum at that wave length of the curve for this mixture and that for the pure acid[7] (α = 76.6). It was found to be between 15–20% of the total acids. The presence of neoabietic acid was shown by the fact that at 250 mμ, in place of the usual inflection point at an α lower than the maximum at 235 mμ for pure abietic acid, a decided maximum appeared at an α slightly higher than that of the inflection point at 235 mμ accompanied by the slight bulge in the curve at 264 mμ that is characteristic of the curve for neoabietic acid.[7] The amount of neoabietic acid was calculated in the same manner described for abietic acid using the absorption maximum and general absorption at 250 mμ wave length and found to be between 15–20% of the total acids. These values were checked by isomerizing the neoabietic acid with mineral acid and determining the amount of abietic acid produced, Fig. 1, Curve 2.

At one time it was believed that a fourth two-double-bond, abietic-type acid might be present whose ultraviolet absorption spectrum might be masked by those of levopimaric, neoabietic, and abietic acids. To determine whether this was the case, the butanolamine salts of the levopimaric acid-free resin acids were prepared and fractionated very carefully using the triangular scheme of fractional crystallization. From the specific rotations and ultraviolet absorption curves of the fractions of salts no new acid was in evidence and only the separation of isodextropimaric acid as the more insoluble and neoabietic acid as the more soluble salt was effected. From the foregoing it was concluded that the maleic anhydride-reactive acids fraction of gum oleoresin is composed of levopimaric, neoabietic, and abietic acids (Table I).

One of the characteristics of two-double-bond abietic-type acids is that under acid conditions they are isomerized to abietic acid which under

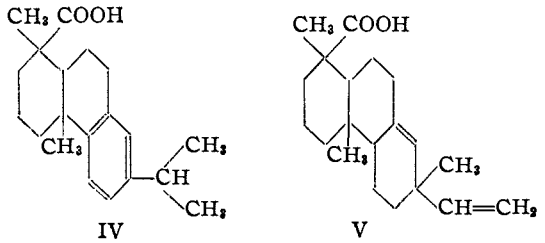
TABLE I

COMPOSITION OF GUM OLEORESIN ACIDS

Acid	Percentages: Harris	Other investigators
Levopimaric	30–35	30–35
Neoabietic	15–20	...
Abietic	15–20	...
Isodextropimaric	8	...
Dextropimaric	8	1–3
Dihydroabietic	4	...
A dihydroabietic	4	4

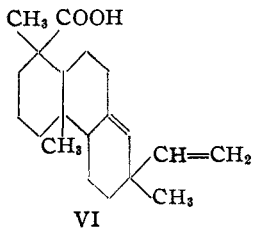
the same conditions is in equilibrium with a small amount of levopimaric acid. The latter reacts with maleic anhydride to give the adduct which can be separated from the unreactive acids. It was in this manner that the maleic anhydride reactive acids, which compose about 75% of the oleoresin, were removed for purposes of studying the remaining 25% of acids, the maleic anhydride unreactive.[11] The total gum acids were treated with maleic anhydride in dry, boiling benzene saturated with dry hydrochloric acid and the unreacted acids separated at a *p*H of 6.2. These acids were found to remain unchanged under these conditions especially dextropimaric acid which was treated under identical conditions and was isolated unaltered.

The composition of this fraction was determined in the following manner. An ultraviolet absorption curve (Fig. 2, Curve 1) showed unequivocally the presence of dehydroabietic acid,[12] IV (Fig. 2, Curve 2), to the extent of 18% of this fraction or 4% of the total oleoresin acids. The butanolamine salts were prepared and fractionally crystallized to obtain the pure salt of isodextropimaric acid,[11] V, in 4% yield of the total acids. In 1939, Fleck and Palkin[13] showed that the oleoresin from *Pinus palustris* contains about 4% of a dihydroabietic acid which was isolated by means of

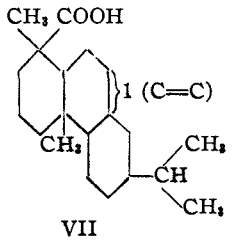


IV

V



VI



VII

(11) G. C. Harris and T. F. Sanderson, "Resin Acids. III," THIS JOURNAL, **70**, 2079 (1938).

(12) L. F. Fieser and W. P. Campbell, THIS JOURNAL, **60**, 2631 (1938).

(13) E. E. Fleck and S. Palkin, *ibid.*, **61**, 1230 (1939).

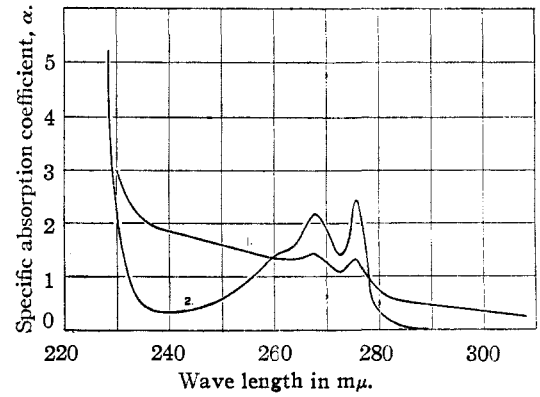


Fig. 2.—Ultraviolet absorption spectra: 1, maleic anhydride–unreactive acids; 2, dehydroabietic acid.

its crystallizable, insoluble lactone.[14] Since the other acids of this fraction do not form this difficultly soluble lactone, a dihydroabietic acid, VII, must be assumed to be a primary product. From these results it appears that some disproportionation may take place even in the tree to result in the formation of equal quantities of dehydroabietic and dihydroabietic acids. By difference, then, dextropimaric acid, VI, is present to the extent of about 8% of which only 4–5% can be separated pure from the early cuts of a distillation of rosin or by crystallization from the mixture after isodextropimaric acid is removed as an amine salt. Therefore, the maleic anhydride-unreactive acids fraction of gum oleoresin is composed of isodextropimaric, dextropimaric, dehydroabietic, and a dihydroabietic acid as in Table I.

Experimental

Determination of Levopimaric Acid Content.—A solution of 50.0 g. of total oleoresin acids, $[\alpha]^{24}D$ −48°,[15] in 50 g. of *n*-pentane was treated with 8.1 g. of triply distilled maleic anhydride in 6 g. of dry acetone. Upon the addition of the maleic anhydride a dark color developed with the simultaneous production of heat. At the end of two hours of agitation, the solution was poured slowly into a 3% solution of aqueous alkali containing a sufficient amount of alkali (10.0 g.) to neutralize the acids. The solution was diluted to a volume of 3 liters and solid boric acid added during rapid agitation to a *p*H of 6.2.

Sodium sulfate, 60 g., was added to coagulate the precipitated resin acids that were filtered and washed well with water to obtain 30 g. of acids with rotation $[\alpha]^{24}D$ +60°. Using the Biot law

$$X = (C - B)/(A - B)$$
$$X(A) + (1 - x)B = C$$
$$X(-276) + (1 - x)\,60 = -48$$

the per cent. of levopimaric acid was calculated as 32%. After several experiments, it was concluded that there is between 30 and 35% of levopimaric acid in the oleoresin.

Investigation of the Unreactive Acids.—The 30 g. of maleic anhydride-unreactive acids with acid number 185 was dissolved in 60 g. of acetone and treated with 9.0 g. of butanolamine (2-amino-2-methyl-1-propanol; Commercial Solvents, Inc.) in 10 cc. of acetone. The salts were filtered and fractionated with acetone as solvent using the triangular scheme of fractional recrystallization. The ro-

(14) R. F. Cox, *ibid.*, **66**, 865 (1944).

(15) All rotations are of 1% solutions in absolute ethanol.

tations of the last few fractions were 0°, +3°, +12°, +29°, +44°, +57°, +88° and +102°. From these rotations and the ultraviolet absorption curves of these fractions it was concluded that the first fraction was the salt of isodextropimaric acid, the last that of neoabietic acid, and the middle fractions were mixtures of salts of neoabietic and abietic acids and acids that do not demonstrate absorption in the ultraviolet region.

Summary

The composition of the acid fraction of American gum oleoresin from *Pinus palustris* has been determined fully for the first time using such techniques as the amine salt method, ultraviolet absorption spectrum technique, and the Diels–Alder addition of maleic anhydride. In addition to the already known levopimaric and dextropimaric acids and a dihydroabietic acid, the following acids were shown to be present as primary acids in oleoresin: neoabietic, abietic, isodextropimaric and dehydroabietic acids.

WILMINGTON, DELAWARE RECEIVED[16] MARCH 10, 1948

(16) Original manuscript received August 9, 1946.

[CONTRIBUTION FROM HERCULES EXPERIMENT STATION, HERCULES POWDER COMPANY]

Resin Acids. VI. Kraft's Proabietic Acid,[1] a Mixture of Primary Resin Acids

BY GEORGE C. HARRIS AND JACQUELINE SPARKS

The purity and homogeneity of Kraft's proabietic acid was suspected for the following reasons: (1) its preparation involved only the concentration of the dextrorotatory constituents in the oleoresin after removal of as much levopimaric acid as possible by fractional crystallization, a technique shown to be inoperable in resin acid chemistry, (2) the height of the maximum at 243 mμ, log K_{molar} = 3.86, for the substance was too low for absorption maxima of pure resin acids in that region, 243 mμ, and (3) the irregularities in the ultraviolet absorption curve between 265 and 278 mμ indicated the presence of residual levopimaric acid and some dehydroabietic acid. In the light of the first reason, the isolation of the dextrorotatory neoabietic acid,[2] $[\alpha]^{24}D$ +159°, from oleoresin in 15–20% yield cast further doubt on the homogeneity of proabietic acid.

The acid prepared in this Laboratory was similar in physical constants (melting point 159–162°, and rotation $[\alpha]^{24}D$ + 10°) to that reported by Kraft with exception of the position of the intense band in the ultraviolet absorption spectrum of the substance. Kraft's acid demonstrated its most intense band of absorption at 243 mμ, α = 24.3, and ours at 250 mμ, α = 27.0. A discrepancy in the most intense band of absorption of abietic acid was also described in an earlier paper.[3] Kraft reports it as being at 237.5 mμ and we have found it repeatedly to be at 241 mμ.[2] These differences are not alarming inasmuch as Kraft's work was done on a Hilger-type apparatus whereby transmission was determined by measurements made on a photographic plate and ours was done with a Beckmann spectrophotometer which utilizes a photoelectric cell and gives accurate determinations below 250 mμ, the region in which a Hilger-type apparatus is inaccurate.

In working the oleoresin to obtain the crystalline acids, 80% aqueous acetone was used more effectively than petroleum ether[4] to obtain the same type of acid mixture with rotation $[\alpha]^{24}D$ − 95°, and melting point 140–145°, in better yield. The ammonium salts were prepared as directed[1] in 1% aqueous ammonia solution and filtered. The resulting acids regenerated with carbon dioxide from the soluble ammonium salts in the filtrate, with rotation, $[\alpha]^{24}D$ − 24°, were again treated with 1% ammonia and the unreacted acids regenerated with rotation, $[\alpha]^{24}D$ + 10°. This material was crystallized from aqueous alcohol with the same rotation, melting point 159–162°, and ultraviolet absorption characteristics, Fig. 1, Curve 1.[5] Since the presence of levopimaric acid was suspected from the ultraviolet absorption curve[1] of the mixture, the material was treated under anhydrous conditions with maleic anhydride at room temperature in the absence of acid to obtain an unreacted fraction, with rotation $[\alpha]^{24}D$ +39°, from which can be calculated 9–10% of levopimaric acid in Kraft's acid. An ultraviolet absorption curve, Fig. 1, Curve 2, was determined of the levopimaric acid-free acids to detect the presence and determine more accurately the amounts of neoabietic acid, with intense band at 250 mμ and slight bulge at 265 mμ, and abietic acid with intense band at 241 mμ. The amounts were determined according to a method described in an earlier paper[6] and found to be 25% of neoabietic and 10% of abietic acids.

Further attempts to resolve the mixture to pure constituents consisted of the preparation and fractional crystallization of the butanolamine salts according to the triangle scheme. The salt of isodextropimaric acid was obtained as the insoluble fraction, to show the presence of this acid in the mixture, and that of neoabietic acid as the more soluble fraction, to confirm its presence.

(1) K. Kraft, *Ann.*, **524**, 1 (1936).

(2) G. C. Harris and T. F. Sanderson, "Resin Acids. I," THIS JOURNAL, **70**, 334 (1948).

(3) K. Kraft, *Ann.*, **520**, 133 (1935).

(4) F. Vocke, *Ann.*, **508**, 14 (1933).

(5) The ultraviolet absorption data were obtained by Dr. Evelyn V. Cook, of this Laboratory.

(6) G. C. Harris, "Resin Acids, V," THIS JOURNAL, **70**, 3671 (1948).