extracted with ethyl acetate. The aqueous phase was then filtered through a column of Dowex 50 (H^+) and concentrated to a syrup (2.4 g). This syrup was dissolved in liquid ammonia (100 ml) and sodium (1.3 g) was added in small pieces over a period of 3 h with stirring. Dowex 50 (NH_4^+) (24 g) was added and stirring was continued for 1 h, after which time the ammonia was allowed to evaporate. The last traces were removed over sulphuric acid in a vacuum. The product was treated with water (150 ml) and filtered. The filtrate and washings were concentrated to 25 ml and the thiocresol present was extracted with ethyl ether (3 × 25 ml). Paper chromatography (ethyl acetate-acetic acid-water, 3:1:1) of the aqueous phase revealed the presence of several components, two of which appeared in the region where xylosylserine could be expected. These spots were developed with ninhydrin and with silver nitrate—ethanolic sodium hydroxide.

The substance giving the stronger and slightly faster spot was isolated chromatographically pure after repeated fractionations on thick filter paper, using the same solvent system. It crystallised from aqueous ethanol and the substance (25 mg) melted at 230—240° (decomp.) and had $[\alpha]_{578}^{20} - 65°$ (*c* 0.1, water). [Found: N 5.9. $C_8H_{15}O_7N$ requires: N 5.9].

The substance (1 mg) was dissolved in M hydrochloric acid (0.1 ml) and kept at 100° overnight. The hydrolysate was treated with Dowex 3 (free base) and concentrated. A paper chromatographic examination revealed the presence of xylose and serine.

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Studies on Components in Wood

3. Gas-Chromatographic Separation of Resin Acids of the Pimaric Acid Type

HENRIK H. BRUUN and GÖRAN PENSAR

The Institute of Wood Chemistry, Åbo Akademi, Åbo, Finland

In a previous article [1] we have presented some studies of the gas-chromatographic separation of mixtures containing those resin acids which constitute the main components of resin acid material both of native and of industrial origin. In the present communication we wish to report briefly on some gas-chromatographic separation experiments with a number of pimaric acids, partly with different degree of hydrogenation.

The pimaric acids constitute one of the two main groups of resin acids from softwood trees such as pine (*Pinus*), spruce (*Picea*), douglas-fir (*Pseudotsuga*) and larch (*Larix*) as well as from industrial products made from these species. The pimaric acids are characterized by the presence of a vinyl group which is absent in the acids belonging to the second main group, the abietic acids. The pimaric acids are in several cases more stable toward thermal isomerization than the abietic acids and they show certain individual characteristics which deserve attention with regard to their utilization in the pulp industry.

Pimaric acid and isopimaric acid are contained in many resin products [2] and recent gas-chromatographic analyses indicate that this may be the case with sandaracopimaric acid also.[1] Whether hydrogenated pimaric acids are present in such materials has not been established but it seems probable that technical products contain these acids at least in small amounts. Analytical methods suitable for their determination have not yet been developed.*

* Regarding analytical methods for the two ordinary pimaric acids, *i.e.* pimaric and isopimaric acid, see Ref. 2; for separation of these acids in the presence of sandaracopimaric acid, see Refs. 3, 4, and for gas-chromatographic separation of these three acids from abietic acids, see Ref. 1.

Table 1. Relative retention values (r_P) and separation factors (α) for methyl esters of resin acids.[a]

No.	Methyl ester of	r_P	α
1.	Dihydro-Δ13(14)-pimaric acid	0.85	—
2.	Δ13(14)-Isopimaric acid	0.87	1.03
3.	Dihydropimaric acid	0.91	1.04
4.	Dihydrosandaracopimaric acid	0.93	1.02
5.	Tetrahydroabietic acid[b]	0.94	1.01
6.	Pimaric acid[c]	1.00	1.05
7.	Sandaracopimaric acid	1.08[d]	1.08
8.	Tetrahydroabietic acid[b]	1.11	1.03
9.	Dihydroabietic acid	1.11[d]	1.00
10.	Dihydroisopimaric acid[e]	1.25	1.14
11.	Levopimaric acid	1.28[d]	1.03
12.	Palustric acid	1.28	1.00
13.	Isopimaric acid	1.40[d]	1.09

[a] The retention value for the methyl ester of pimaric acid has been taken as 1.00 and the retention values for the other acids are thus given relative to that of pimaric acid. The separation factor is the ratio between the retention value for the acid in question and that of the preceding acid in the table.

[b] The methyl ester of tetrahydroabietic acid is eluted in two fractions.[1]

[c] This substance contained about 10 % of an unidentified acid, probably sandaracopimaric acid (peak 7 in Fig. 1a).

[d] Previously determined value.[1]

[e] This substance contained about 7 % of unidentified acids (peak 14 in Fig. 1a).

Results. Retention values and separation factors for the methyl esters of the investigated pimaric acids are given in Table 1. Corresponding values for the methyl esters of some other resin acids are also included for comparison.[1] Fig. 1 shows two chromatograms illustrating the gaschromatographic separation of a number of the acids in question. From the separation factors given in the table it is evident that the eight pimaric acids studied here may be separated with the method utilized in this investigation but that the separation is not complete for all of the acids. The esters of dihydro-Δ13(14)-pimaric acid and Δ13(14)-isopimaric acid are eluted in such a short interval that they give only one peak on the chromatogram. The symmetry of this peak depends on the relative amounts of the two acids. The same is true for the methyl esters of dihydropimaric acid and dihydrosandaracopimaric acid. In addition, there is an overlap between the peaks of the two acid pairs mentioned which makes their identification even more difficult. The methyl ester of pimaric acid gives a third peak which also shows a certain degree of overlap with the previous peak. In contrast, the methyl esters of dihydroisopimaric, sandaracopimaric, and isopimaric acid are completely separated from the rest of the acids. If the sample contains abietic acids, *i.e.* tetrahydroabietic acid, palustric acid, or levopimaric acid, the separation and identification of some of the pimaric acids becomes more difficult. Thus the first eluted isomer of the methyl ester of tetrahydroabietic acid [1] gives a peak on the chromatogram which coincides with the common peak of dihydropimaric acid and dihydrosandaracopimaric acid. If the concentration of tetrahydroabietic acid is high the two Δ13(14)-acids are also overlapped. The methyl esters of the palustric and levopimaric acids are eluted so close to the ester of dihydropimaric acid that an overlap occurs also in this case.

Considering the relation between the number of double bonds and the retention time (for abietic acids, see Ref. 1) it appears natural that the methyl esters of hydrogenated acids (1, 3, and 4 in Table 1) are eluted before those of their nonhydrogenated or dehydrogenated forms. This behavior is to be expected for a column of polar character.

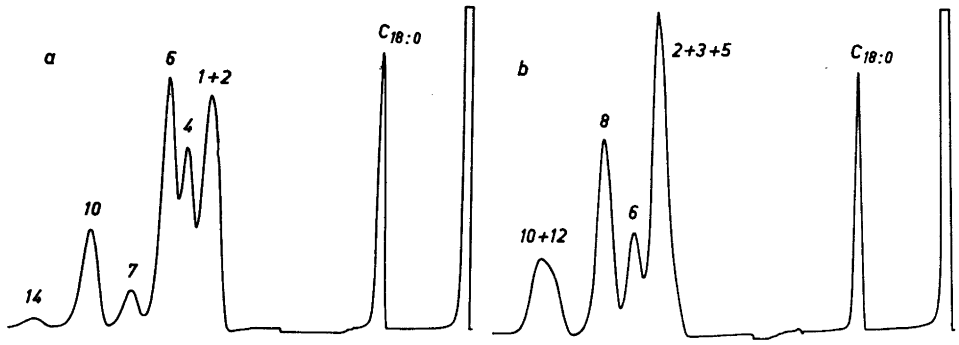

Fig. 1. Gas-chromatograms of (a) an artificial mixture of methyl stearate ($C_{18:0}$) and methyl esters of dihydro-Δ13(14)-pimaric acid (1), Δ13(14)-isopimaric acid (2), dihydrosandaracopimaric acid (4), pimaric acid (6), and dihydroisopimaric acid (10); peak 7 is probably due to sandaracopimaric acid originating from the sample of pimaric acid and peak 14 to an unidentified acid (possibly isopimaric acid) originating from the sample of dihydroisopimaric acid, (b) an artificial mixture of methyl stearate ($C_{18:0}$) and methyl esters of Δ13(14)-isopimaric acid (2), dihydropimaric acid (3), tetrahydroabietic acid (5 and 8), pimaric acid (6), dihydroisopimaric acid (10) and palustric acid.

In summary, it may be stated that the method used in the present study achieves separation of the pimaric acids investigated although the separation is not completely satisfactory for some of the acids. The presence of certain abietic acids interfers with the analysis.

Experimental. A Perkin-Elmer Gas chromatograph Model F 11 equipped with a flame ionization detector was used for the investigation. The recorder was a Leeds & Northrup Speedomax, Model H (2.5 mV, 30″/h). The chromatographic column was made of stainless steel and its dimensions were 10′ × 1/8″. The packing was 5 % ethylene glycol succinate (EGS) on Cromosorb W (80–100 mesh). The investigation was performed at a column temperature of 210°C while the temperature of the injection chamber was about 235°C. Nitrogen was used as carrier gas (Q_{N_2} = 35 ml/min).

For the gas-chromatographic analysis the resin acids were transformed into their methyl esters by treatment with diazomethane in ether solution. The esters were then dissolved in acetone to solutions of about 1 % concentration. The amount of resin acid injected in each analysis was of the order of 5 μg. Methyl stearate (Perkin-Elmer A 007) was used as internal standard in all injections.

With the exception of the ordinary pimaric acid all of the pimaric acids studied were obtained from L. J. Gough, Borough Polytechnic, London, who has provided the following melting point data: Δ13(14)-isopimaric acid 104–106° (sinters at 101.5°), dihydropimaric acid 243–245° (evacuated capillary), dihydro-Δ13(14)-pimaric acid 183.5–187.5° (evacuated capillary), dihydroisopimaric acid 182–183° (evacuated capillary), 173–178° (open capillary), dihydrosandaracopimaric acid 180–183° (evacuated capillary). The pimaric acid (m.p. 215–218°) and the tetrahydroabietic acid were obtained from T. F. Sanderson, Hercules Powder Company, Wilmington, Del., U.S.A. and the palustric acid was furnished by R. V. Lawrence, U.S. Dept. of Agriculture, Olustee, Florida, U.S.A. We wish to express our thanks to these persons for the samples.

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