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SEPARATION OF RESIN ACIDS FROM FATTY ACIDS IN RELATION TO ENVIRONMENTAL STUDIES

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SUMMARY

Kraft mills pulping coniferous wood species discharge to their receiving waters effluents containing mixed resin and fatty acids. To study the fish-toxic properties of these fractions it is desirable to be able to separate them without causing double bond isomerizations in the acid-labile resin acid fraction. A method is described using toluenesulphonic acid as catalyst under very mild conditions and four examples of its application are given. It was not possible to separate tall oil samples without a low degree of resin acid isomerization.

INTRODUCTION

The common resin acids are tricyclic diterpenes which possess abietane or pimarane skeletons. These co-occur with fatty acids in parenchyma cells of commercially important coniferous wood species. Resin and fatty acids are the main components of tall oil, which is a by-product of the kraft pulping of pinewood. Wood resin is also comprised of these substances and it may be recovered either by tapping living pine trees or by solvent extraction of weathered pine stumps[1,2]. Resin and fatty acids are separated from each other and from various neutral or unsaponifiable substances by fractional distillation *in vacuo* at tall oil or naval stores processing plants. Both acid fractions are valuable feedstocks to the chemical industry.

It has long been known that resin acids are toxic to fish[3]. Canadian research in recent years has established that two major contributors to the toxicity of whole kraft effluent are resin acids and polyunsaturated fatty acids[4-6]. In such studies it obviously is desirable to be able to isolate these fractions with minimum chemical change in composition of the reactive resin acids. This is not easily achieved because of the presence of the conjugated diene function in members of the abietane family. The standard method in wood chemistry relies on partial esterification of the mixed acids with methanol–sulphuric acid as catalyst[7]. This depends on steric hindrance around the tertiary carboxyl group thereby blocking attack by the esterifying agent. Such a method is satisfactory for the quantitative determination of total resin acids and also for the recovery of the fatty acid fraction. However, the use of strong mineral

acid causes double bond isomerization in the abietane-type resin acids and can give rise to misleading results in subsequent fish toxicity bioassays.

Two previous analytical procedures, based on gel permeation chromatography, successfully avoided the isomerization of double bonds. Zinkel and Zank[8] used diethyl ether as solvent and a polystyrene matrix, whereas Chang[9] preferred to use tetrahydrofuran with a different grade of polystyrene. In both methods differential refractometers were utilised and very small amounts only of mixed acids were applied to the columns. For fish toxicity bioassay work these methods have disadvantages including the use of low boiling solvents, inability to process more than one sample at a time, and difficulty in scaling up the sample size for preparative use.

For the past several years in this laboratory the separation of resin acids from fatty acids has been accomplished through an adaptation of the selective esterification procedure in which toluenesulphonic acid (TSA) replaces mineral acid as catalyst. A brief description of this technique is the purpose of this paper and some examples of its practical application are given. Previous investigators have used TSA for the titrimetric estimation of resin acids in tall oil fractions[10,11] but this work was performed without physical separation of the resin acids from the fatty acids. While the method was in use in this laboratory, a patent was granted for use of TSA in the commercial separation of tall oil resin and fatty acids[12].

EXPERIMENTAL

Preparation of various samples of mixed acids

Whole kraft effluent samples from a British Columbia Interior mill were processed through Amberlite XAD-7 resin beds as described elsewhere[6]. The ether-soluble portions of the methanol eluates from the resin beds comprised substantially the fish-toxic fraction and included the mixed fatty and resin acids.

Coarse woodmeal (passing a 3-mm screen) was prepared by passing air-dried Douglas fir wood [*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco] through a Wiley mill. The woodmeal was extracted with petroleum ether (b.p. 65–110°) in an all-glass Soxhlet extractor for 24 h and the extract recovered.

Ten sockeye salmon (*Oncorhynchus nerka*) fingerlings, which had died during a static bioassay test with dehydroabietic acid as toxicant, were freeze-dried, ground in a tissue homogenizer with chloroform and the slurry extracted three times with chloroform in a separatory funnel. The combined chloroform extract was washed with water and dried.

A sample of tall oil from a Western Canadian supplier was used to study optimization of the separation. A fresh tall oil sample was obtained from Longview Fibre Company at Longview, Washington, and used in one of the examples.

Separation of resin acids from fatty acids

Optimum conditions for separation of mixed acids from tall oil or wood extractives were as follows. A sample of tall oil or wood extractives (1–2 g) was dissolved in methanol (50 ml) containing 1 ml of a 10% methanol solution of TSA. The flask was flushed with nitrogen and stoppered, then stored in a refrigerator for 16 h at 36°F. The reaction product was transferred to a separatory funnel with the addition of isooctane (100 ml) and was extracted three times with 5% aqueous KOH

solution. The isooctane layer was washed successively with 5% KOH and water and dried. The combined alkaline extract was washed with isooctane, acidified with acetic acid, and the resin acid fraction recovered by extraction with benzene. The fatty acid methyl ester fraction was recovered from the isooctane phase.

Various experiments were conducted to explore the effect of changes in reaction time, temperature, TSA concentration and the substitution of naphthalene sulphonic acid for TSA or mineral acid for acetic acid in acidification of the resin acid soaps.

Gas chromatography

Control samples of mixed acids were methylated with an ethereal solution of diazomethane. Separated resin acid fractions were methylated similarly. Fatty acid methyl ester fractions also were methylated to ensure the absence of traces of free resin acids.

The gas chromatograph used in most of this work was a Hewlett-Packard Model 5711 A fitted with dual hydrogen flame ionization detectors and coupled to a Hewlett-Packard Model 3370 B digital electronic integrater. Choice of column and operating conditions are listed under Fig. 2. Earlier work on the separation of resin and fatty acid fractions from the fish-toxic portions of kraft effluent samples was performed using a Hewlett-Packard Model 7620 A gas chromatograph equipped with dual hydrogen flame ionization detectors. Operating conditions are listed under Fig. 1.

RESULTS AND DISCUSSION

In some preliminary experiments, a synthetic mixture was prepared using the resin acid fraction of a sample of Douglas fir wood and various C_{18} fatty acids which commonly are present in wood extracts. A clean separation of fatty acids from resin acids with quantitative recovery resulted when a methanolic solution of this mixture containing 1.7% TSA was shaken at room temperature for 2 h or for 16 h. No resin acid isomerization occurred and no emulsification problems were encountered.

An application of the method to the separation of a fish-toxic kraft effluent sample is shown in Fig. 1. The unsaponifiable fraction of this extract was first removed via column chromatography on DEAE-Sephadex A-25 according to the procedure of Zinkel and Rowe[13]. The separation was very clean and it can be seen that no significant degree of isomerization occurred. It was subsequently shown by fish bioassay tests that both the fatty acid and resin acid fractions of this sample were toxic.

Extension of the method to the separation of tall oil samples was not straight-forward. Considerable problems were encountered with emulsions. These were alleviated by using isooctane as the preferred solvent for the fatty acid fraction, by using 5% KOH solutions rather than 5% NaOH solutions for removal of the non-esterified resin acid fraction, and by employing benzene for the final recovery of the resin acids. Additions of small amounts of saturated brine or methanol were also beneficial in overcoming the emulsion problem.

Difficulty was also encountered in preventing double bond isomerization of the resin acid fraction when separating tall oil. If reaction times were too short or the temperature was too low, incomplete separation without isomerization was observed. On the other hand, if reaction times were prolonged, or the temperature
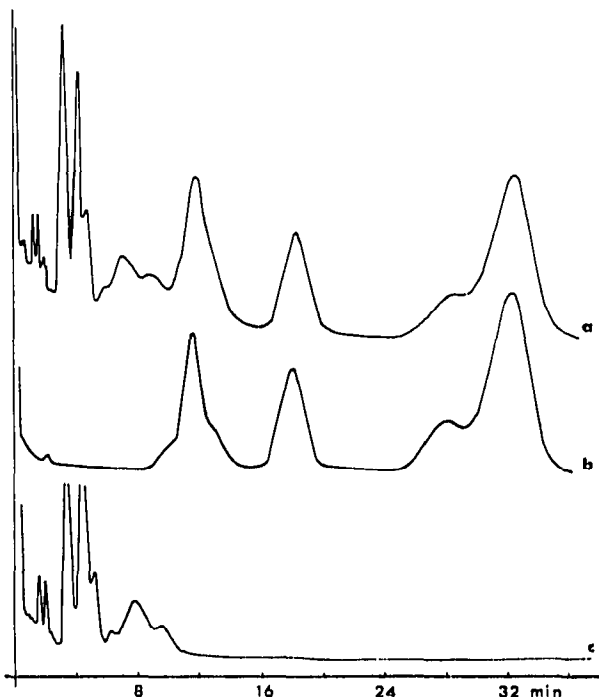
Fig. 1. (a) Methyl esters of a mixture of fatty and resin acids recovered from whole kraft effluent. (b) Methyl esters of resin acids after separation. (c) Methyl esters of fatty acids after separation. Column, 6 ft. × 1/8 in. O.D. stainless steel packed with 10% EGSS-X on 100–120 mesh Gas-Chrom P. Hewlett-Packard Model 7620 A gas chromatograph; detector, flame ionization; range, × 10³; attenuation, 100; column temperature, 175° isothermal; injector temperature, 200°; detector temperature, 250°; carrier gas, nitrogen at a flow-rate of 70 ml/min; solvent, diethyl ether.

elevated, complete separation was achieved but always at the expense of some isomerization. This situation is illustrated in Fig. 2, which was conducted on a fresh sample of tall oil. Some isomerization has taken place, as is seen by comparing the relative heights and widths of peak 2 (abietate) with peaks 1 and 3 (palustrate and unresolved dehydroabietate with neoabietate) in the original sample with the corresponding peaks in the recovered resin acid fraction.

The problems with tall oil may relate to its past history. Tall oil is recovered from the evaporators in a kraft mill in the form of the sodium soap. This is subsequently acidulated with sulphuric acid to free the resin and fatty acids and no doubt some isomerization takes place during this operation. It appears that some of this mineral acid is trapped in the commercial tall oil and this may have a bearing on the experimental difficulty in the resin acid separations. It is apparent that palustrate and neoabietate, which are very sensitive to mineral acids and are difficult to distinguish from isopimarate and dehydroabietate, respectively, by gas chromatography, are being converted into abietate. Another reason why tall oil acids are difficult to separate without double bond isomerization is that the total content of resin acids of the abietane type is very high compared to that seen in Douglas fir wood extracts or whole kraft effluent samples from Western Canadian mills. In the latter case the
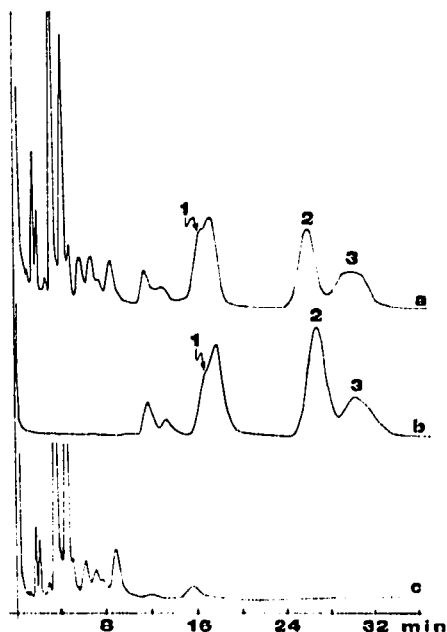
Fig. 2. (a) Mixed tall oil fatty and resin acid methyl esters. (b) Separated resin acid methyl esters. (c) Separated fatty acid methyl esters. Column, as in Fig. 1. Hewlett-Packard Model 5711 A gas chromatograph; detector, flame ionization, range × 10; attenuation, 100; column temperature, 185°; injector temperature, 200°; detector temperature, 250°; carrier gas, nitrogen at a flow-rate of 51 ml/min; solvent, diethyl ether. 1 = Palustrate; 2 = abietate; 3 = dehydroabietate and neoabietate.

main components are the chemically stable isopimarate and dehydroabietate. This reflects the pulping of spruce, Douglas fir and other coniferous species besides pine.

In a third example the technique has been used as part of the isolation scheme for the recovery of insect juvenile hormone analogs of the juvabione type from Douglas fir wood extracts. In this case the biologically active compounds I and II are readily esterified and remain in the fatty acid ester fraction from which they may in turn be removed by extraction with Girard T reagent[14]. In this example, partial esterification with conventional mineral acid catalysts is equally satisfactory since the resin acid fraction is of no subsequent interest.
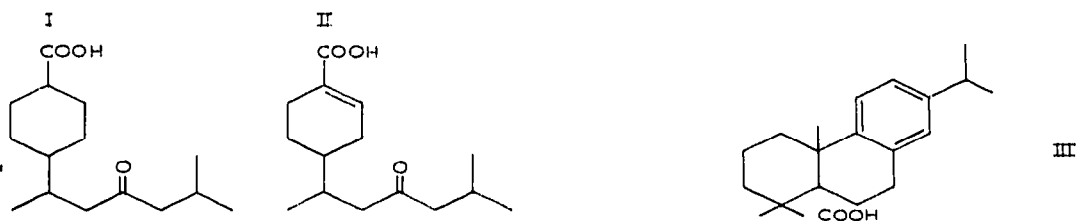
A fourth and final example concerns the post mortem examination of fish which have died as a result of bioassay exposure to dilute solutions of resin acids. Analysis of internal organs for the presence of the resin acids or their metabolites

may yield valuable information as to the cause of death of the experimental animals. The problem here is that fish contain large amounts of lipids, including polyunsaturated fatty acids. The methyl esters of some of these acids unfortunately have retention times similar to the corresponding esters of the resin acids and thereby obscure the presence of the latter. The mixed acid fraction was recovered via solvent extraction of the homogenized total body tissues of dead sockeye salmon fingerlings. After saponification to destroy glyceride esters, the extract was partially esterified with TSA in methanol followed by methylation of the resin acid fraction with diazomethane. In this case the fish had been exposed to dehydroabietic acid (III) and the results of analysis of the resin acid fraction unequivocally showed the presence of this acid in the bodies of the fish. In this simple example the resin acid does not contain acid-labile double bonds. However, the method may equally well be applied to other resin acids which do possess this structural feature. For work on *post mortem* analysis of internal organs it would be preferable to use radioactively labelled acids because of the small amounts of tissue involved and the high dilution factor. Nevertheless, the method does have value in the investigation of fish kills in lakes and rivers which constitute the receiving waters for kraft pulping effluents.

In summary, the method is useful in the preparative-scale separation into fatty and resin acid fractions of the fish-toxic lipid fraction of kraft mill effluent or of tall oil. Small changes in composition of tall oil resin acids cannot be avoided but the procedure is very much less drastic than mineral acid catalysed methods in current use. In special cases the method may also be used to advantage to determine the reason for fish kills where contact with kraft pulping effluents is suspected.

ACKNOWLEDGEMENT

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