On the Composition of the Fatty Acids of Pine Wood Extract and Tall Oil. A spectrophotometric investigation

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Rather little attention has been called to the composition of the fatty acids contained in the wood of *Pinus silvestris*. Bergström and Trobeck [1] have published data obtained by chemical investigation on the fatty acids obtained by alcohol extraction of pine wood. Amongst saturated acids they found oleic, linoleic and linolenic acids.

The component fatty acids of tall oil have been more closely investigated, the same unsaturated acids having been stated to be present, among others by Sandström and Sandström [2].

The introduction of ultra-violet absorption spectrophotometry has given a more accurate and sensitive tool to substitute the chemical examination. The polyunsaturated fatty acids can be isomerized by alkali yielding more or less conjugated double bond systems which can be detected and even quantitatively calculated from the ultra-violet absorption readings at certain wavelengths. The characteristic peaks of alkali treated linoleic, linolenic and arachidonic acids appear at 2320, 2680–2800 and 3020–3160 Å respectively. Thus Brice *et al.*[3] have found small amounts of linolenic acid in American tall oil whereas Anderson and Wheeler [4] consider linolenic acid to be absent. They stated a slightly higher absorption at 3160 Å than at 2680 Å and assumed this being due to impurities.

The purpose of this investigation has been to analyze the polyunsaturated fatty acids of Finnish pine wood and tall oil and to state if the sulphate cooking process has any effect upon them.

Four specimens of pine were extracted with alcohol and ether successively and the extracts obtained were treated along four tall oil samples as follows.

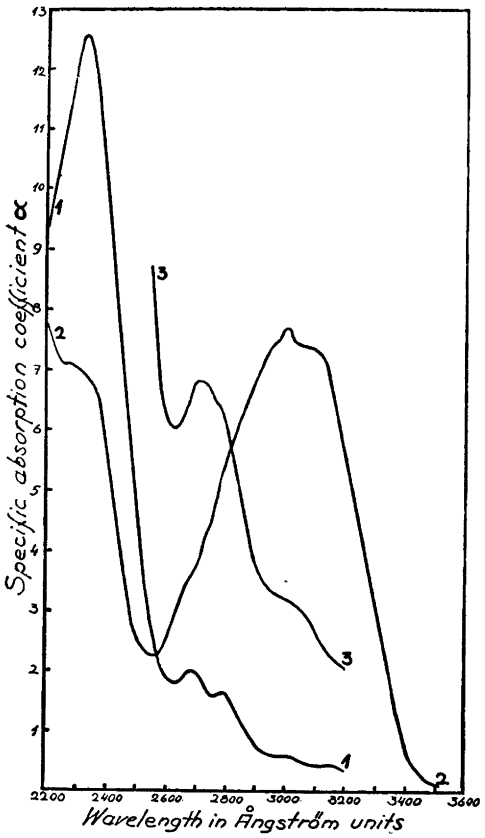
The dark and more polaric constituents were eliminated by dissolving the samples in petroleum ether. The soluble part was then saponified with alcoholic potassium hydroxide and extracted with ether until no more unsaponifiable material could be isolated. The rosin acids were thereafter separated from the fatty acids by esterifying with absolute methanol catalyzed by sulphuric acid and extracting the fatty acid methyl esters by ether from the mixture which had been made alkaninic previous to the extraction. Particular care was taken to obtain the component fractions as free from other components as possible because all of them contain systems of two or more conjugated double bonds.

As storing of the ready samples showed soon to alter the spectral characteristics, the measurements were carried out within 48 hours from the completion of the separation. The separation itself was performed with minimum delay. A Model DU Beckman spectrophotometer was used and the isomerization method of Brice *et al.*[3] employed. Throughout the investigation alcohol was used as the solvent.

The fatty acids and the rosin acids as well as the unsaponifiable materials were examined both in their original condition and after the treatment with the isomerization reagent.

The wood extract fatty acids were thereafter collected and distilled under a pressure of 3 mm and re-investigated spectrophotometrically.

The results show that the absorption curves of the rosin acids, closely resembling those published by Ritchie and McBurney [5] on abietic acid, are not materially affected by the alkali treatment. From 2420 Å



Curve 1: Tall oil fatty acids, untreated
» 2: Pine wood » » »
» 3: » » » » isomerized

the absorption rapidly decreases remaining minimal from 2 600 Å towards the longer wave lengths. The unsaponifiable materials show absorption at about 2 300 Å where a distinct peak of double conjugation appears after the alkali treatment. Another broad absorption band centered at about 3 000 Å seems to become slightly intensified by alkali.

The fatty acids of pine wood have a rather intensive band equally centered at about 3 000 Å. In the isomerization process, however, this band almost disappears and a new one at about 2 700 Å appears. This band does not have the inflexion between the peaks characteristic of conjugated triene acids. However, it becomes visible in the distilled acids thus being indicated to be only obstructed. On the other hand the absorption at 3 000 Å was strongly decreased in the distilled acids and still lessened by alkali. The possible interference of the carotenoid pigments at 3 000 Å is eliminated through the absence of any appreciable absorption at longer wave lengths where most carotenoid pigments according to Karrer and Jucker [6] have characteristic triple peak bands.

The above holds even for the rosin acids and the unsaponifiable materials of tall oil. In the fatty acids, however, there are interesting and different features. The isomerization causes a marked increase in the conjugated diene absorption but there is no change in the triene absorption, where there are distinct conjugated triene peaks already in the spectrum of the untreated fatty acids, thus indicating that there are no more isolated triene systems to be affected by alkali. A very slight absorption at 3 020 and 3 160 Å indicate minute amounts of conjugated tetraene.

Anderson and Wheeler [4] have calculated that the sulphate cooking process corresponds to less than 10 % of the reaction time in the analytical isomerization process. Thus the constancy of the conjugated triene absorption does not agree with the behaviour of linolenic acid.

In Fig. 1 some typical absorption curves are collected.

From these results following conclusions can be derived: The fatty acids of *Pinus silvestris* grown in Finland contain a fatty acid with four conjugated double bonds. During the sulphate cooking this tetraene system is converted into a triene system appearing as "linolenic acid" in the spectrum. Supposedly even conjugated diene systems are formed simultaneously. Fin-

The Enzymic Hydrolysis of Triacetin by Acetylcholine-Esterase and its Inhibition by Various Compounds

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In the course of investigations on the mechanism of action of the inhibition of acetylcholine-esterase (human erythrocytes) by various compounds, triacetin has been used as a substrate. There are proofs for the existence of two active groups, centre I and centre II, of the acetylcholine-esterase molecule. Centre I is a negatively charged group which attracts the positive nitrogen atom of the choline ester. Centre II combines with the acyl group of the ester, acetates being split at a higher rate than other esters. Any acetates, acetylcholine as well as the acetates of a great variety of alcohols [1], are split. Centre II alone is responsible for this hydrolysis [2].

Acetylcholine protects acetylcholine-esterase against the action of tetraethylpyrophosphate (TEPP) [3-5]. Physostigmine also has this protective action against TEPP when acetylcholine is used as substrate. It has now been demonstrated that choline has a similar action.

Choline inhibits the enzymic hydrolysis of triacetin. As expected this inhibition is not competitive. At constant triacetin concentration the degree of inhibition increases with increasing choline concentration to about 70 per cent (corresponding to 0.25 *M* choline). Then the inhibition is constant in the presence of still higher choline concentrations. This inhibition may be due to steric hindrance.

It has been demonstrated recently that, in a mixture of acetylcholine, prostigmine or physostigmine, and acetylcholine-esterase, an equilibrium rate is reached in 10 to 25 minutes, depending upon the inhibitor concentration, after adding the inhibitor or substrate respectively. The inhibition is stronger than during equilibrium when the enzyme has been incubated with the inhibitor, it is less strong when substrate and inhibitor are added simultaneously. This is the result obtained with acetylcholine [3,4,5]. With triacetin the reaction curves are different from those obtained with acetylcholine. Equilibrium is attained immediately after adding triacetin to the incubated enzyme as well as in the case of simultaneous addition of substrate and inhibitor to the enzyme. Therefore, comparing the degrees of inhibition of the hydrolysis of acetylcholine and triacetin this fact must be considered.

Another important result has also been obtained with prostigmine and triacetin. If the concentration of this inhibitor is raised above 10^{-8} *M* keeping the substrate concentration constant, the degree of inhibition is no more increased; on the contrary, it decreases. Even with 10^{-6} *M* prostigmine the inhibition is less than with 10^{-8} *M*.

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nish tall oil does not contain linolenic acid.

This investigation will be published more in detail.

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