Similar wave length shifts have been observed with the lactic dehydrogenase-DPNH-carboxylic acid ternary complexes. The lower amidines, through 

\[ \text{ADH-DPNH+ACETAMIDE} \]

excitation 328nm, ADH 102 

\[ \text{DPNH 0.85 

\text{PHosphate}} \]

ionic strength:01, 

\[ \text{pH 2.0} \]

2.3C

490 470 450 430 410 390 370 m 350

Fig. 1.

structural analogs, there is strong suggestion that the ADH—DPNH-amide and ADH—DPN-fatty acid complexes, in which no net reaction can occur, represent abortive complexes formed between ADH, DPNH, aldehyde and ADH, DPN, alcohol.

The complete data will be given in a forthcoming paper in this journal.


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Isolation of Palustric Acid from the Oleoresins of North-European Pine and Spruce by Partition Chromatography

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Partition-chromatographic analyses of the resins of North-European (Scotch) pine, Pinus sylvestris, and (Norway) spruce, Picea abies, have shown that these contain palustric acid. Palustric acid was discovered in 1954 by Loeblich, Baldwin and Lawrence in the gum and wood rosin of two American pine species, Pinus palustris and Pinus caribaea. The acid has subsequently been isolated from the resin of a further American pine, Pinus elliottii, and from gum rosin, wood resin and tall oil resin isolated from this species. The acid has also been found as a component in tall oil rosin from North-European pine (Bruun and Gåsland), but has not yet been isolated from the natural rosin of North-European tree species or other non-Ameri-
can species. Similarly as other abietic acids, palustic acid may be transformed by isomerization into other resin acids and may also be formed by similar molecular rearrangements from other resin acids, for instance, from levopimamic acid by heat treatment.\(^1\)

**Experimental.** The analyzed samples consisted of resin that had recently oozed from the trunks of 60-year-old living trees of pine and spruce from which the bark had been removed to increase the oleoresinous exudation. The acid value of the pine resin was 125 mg and that of the spruce resin 110 mg of potassium hydroxide per g, which figures indicate that the samples contained 67 and 59 % resin acids (based on an assumed mean equivalent weight of 302). The respective saponification values were 137 and 124 mg of potassium hydroxide per g.

In order to obtain correct analytical data on the resin acid contents of the natural, unchaged resins, the samples were chromatographed without prior separation of the resin acids from other components, primarily terpenes, present in the samples. These latter components were not found to cause any difficulties in the chromatographic analyses.

The chromatographic analyses were performed according to Ref.\(^1\) with aminopyridine and furfuryl alcohol as the immobile phase on silicic acid and isooctane as the eluant. For details, see Ref.\(^3\).

**Results.** The chromatograms of the pine and spruce resins are reproduced in Fig. 1. Each chromatogram exhibits four peaks. The peak volumes and peak areas are given in Table 1.

**Table 1.** Chromatographic data for the resin acids in oleoresins from North-European pine (**Pinus silvestris**) and spruce (**Picea abies**).

<table>
<thead>
<tr>
<th>Peak No. in Fig. 1</th>
<th>Peak volume ml</th>
<th>Peak area % of total area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>190</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>ca. 340</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>ca. 470</td>
<td>5</td>
</tr>
</tbody>
</table>

**Pinus silvestris**

<table>
<thead>
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<th>Peak No. in Fig. 1</th>
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<td>5</td>
</tr>
</tbody>
</table>

**Picea abies**

According to our earlier studies\(^3\) peak 1 consists of palustic acid, peak 2 may include pimaric acids, l-abiatic acid and levopimamic acid, peak 3 contains neoabiatic acid and peak 4 dehydrogenated resin acids.

In order to confirm the identity of the acid in peak 1, the respective eluant fractions were combined, the isooctane removed by reduced pressure, and the resulting acid dissolved in acetone and reprecipitated with cyclohexylamine.\(^4\) A sample of palustic acid was also precipitated as its cyclohexylamine salt under the same conditions. The cyclohexylamine salts, which were isolated in a yield of 85—90 %, were dissolved in optically pure absolute ethanol and the ultraviolet absorption spectra of the solutions recorded. The recorded ultraviolet spectra (Fig. 2) showed that the cyclohexylamine salts of the acids in peak 1 of pine and spruce were identical with the spectrum for the cyclohexylamine salt of the sample of palustic acid. The specific extinction coefficient (\(c\)) for the latter salt at 285—296 mg/l is 25.6, while the value...
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Fig. 2. Ultraviolet absorption spectra of palustric acid (in form of its cyclohexylamine salt) isolated by partition chromatography from oleoresins of North-European pine (a) and spruce (b) and the spectrum of the cyclohexylamine salt of a palustric acid sample (c). Curves d and e gives the absorption spectra of the cyclohexylamine salts of the acids in peak 2 (in Fig. 1) from pine and spruce respectively. The specific extinction coefficients \( \alpha \) were calculated to refer to the acids rather than to the salts.

of very pure palustric acid reported by Loeblich, Baldwin and Lawrence is 30.1. This implies that the palustric acid sample we have employed as reference compound contained 83 % palustric acid. (The ultraviolet spectrum of the latter acid in absolute ethanol gave the same value for the specific extinction coefficient.) The palustric acid content of the pine resin was thus 15 % and that of the spruce resin 21 % of the total resin acids in the sample. Palustric acid hence represents a major component in the resin of North-European pine and spruce. The palustric acid content is higher in the spruce resin than in any pine species, where its content has been found to vary between 7 and 12 % (see Ref.5).

We have also isolated by cyclohexylamine precipitation the acids in peak 2 and determined the ultraviolet absorption of the precipitate in absolute ethanol. The recorded spectrum for the acids in peak 2 for pine resin (Fig. 2d) exhibits a maximum absorption, \( \alpha = 16.6 \), at 241 \( \mu \text{m} \) and another, \( \alpha = 12.0 \), at 272 \( \mu \text{m} \). These maxima are characteristic for \( \lambda \)-abietic and levopimaric acids, respectively; the absorption values indicate that the \( \lambda \)-abietic acid content in peak 2 for the pine is about 18 % or 11 % of the total resin acid fraction and the levopimaric acid content in the same peak is 63 % or 37 % of the total resin acid fraction (in these calculations the contribution of levopimaric acid to the \( \alpha \)-value of \( \lambda \)-abietic acid has been taken into account). The corresponding values of \( \alpha \) for spruce (Fig. 2 e) are 9.0 and 12.4, which imply that the abietic acid content is 7.5 %, or 4.5 % of the total resin acids, and the levopimaric acid content 65 %, or 39 % of the total resin acids. In American pine species the abietic acid content is near 10 % and the levopimaric acid content between 24 and 31 %. The distribution of resin acids in the oleoresin of the North-European spruce thus differs essentially, particularly with respect to palustric acid, but also with respect to abietic acid, from that in the pine species.

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