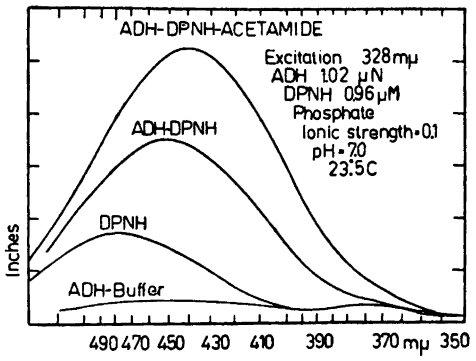

Fig. 1.

Similar wave length shifts have been observed with the lactic dehydrogenase-DPNH-carboxylic acid ternary complexes [3]. The lower amides, through *iso*butyramide, fluoresce more intensely than the ADH—DPNH complex, but higher molecular weight amides, though actually quenching, to varying extents, the fluorescence of ADH—DPNH, still exhibit the wave length shift. By analogy with kinetic experiments with formate [1], it is presumed that the very strong ternary complex formation observed with the longer chain amides has its effect on the "off" velocity of the ternary complex. The dissociation constants of the ternary complex, ADH—DPNH-amide, $K_{ER,I}$, and the ADH-amide complex, $K_{E,I}$, vary from 5 000 μM and 50 000 μM, respectively, for acetamide to 11 μM and 160 μM, respectively, for *n*-hexamide. $K_{EI,R}$ varies from 0.05 μM for acetamide to 0.02 μM for *n*-hexamide. From emission spectral data at 350 mμ the amides show a further quenching of the ADH—DPNH fluorescence when the ternary complex is formed. No effect, however, is seen with the ADH—DPN complex. As indicated by initial velocity measurements, the amides compete for the aldehyde binding site.

Ternary complex formation with the longer chain amides and acids is a logical consequence of the substrate specificity studies on liver ADH with different aldehydes and alcohols recently reported by one of us (A.D.W.) [6]. If it is considered that aldehydes and amides, and alcohols and acids, in this system, function as 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.

1. Theorell, H., Nygaard, A. P. and Bonnichsen, R. *Acta Chem. Scand.* **9** (1955) 1148.
2. Theorell, H. and Winer, A. D. *Arch. Biochem. Biophys. In press.*
3. Winer, A. D. and Schwert, G. W. *J. Biol. Chem.* **234** (1959) 1155.
4. Winer, A. D. and Schwert, G. W. *Science* **128** (1958) 660.
5. Theorell, H. *Advances in Enzymol.* **20** (1958) 31.
6. Winer, A. D. *Acta Chem. Scand.* **12** (1958) 1695.

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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 silvestris*, 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 rosins of two American pine species, *Pinus palustris* and *Pinus caribaea* [1]. The acid has subsequently been isolated from the rosin of a further American pine, *Pinus elliotti*, and from gum rosin, wood rosin and tall oil rosin isolated from this species [2]. The acid has also been found as a component in tall oil rosin from North-European pine (Bruun and Gåsland [3,4]), but has not yet been isolated from the natural rosins of North-European tree species or other non-Ameri-

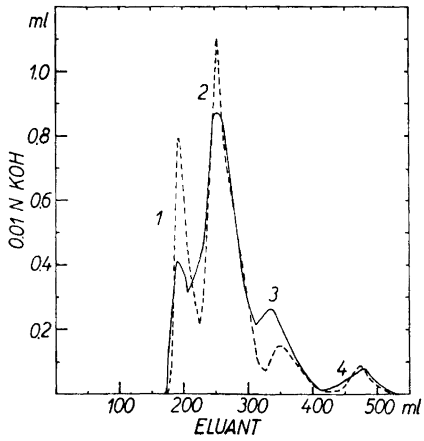

Fig. 1. Chromatogram of the rosin acids in the oleoresins of North-European pine (———) and spruce (— — —). Ordinate gives consumption of 0.010 N alcoholic potassium hydroxide in the titration of 5-ml eluent volumes employing phenolphthalein as indicator. Peak 1 contains palustric acid. For the compositions of the other peaks, see text.

can species. Similarly as other abietic acids, palustric acid may be transformed by isomerization into other rosin acids and may also be formed by similar molecular rearrangements from other rosin acids, for instance, from levopimaric acid by heat treatment [1,5].

Experimental. The analyzed samples consisted of rosin 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 rosin was 125 mg and that of the spruce rosin 110 mg of potassium hydroxide per g, which figures indicate that the samples contained 67 and 59 % rosin 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 rosin acid contents of the natural, unchanged rosins, the samples were chromatographed without prior separation of the rosin 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 *iso*octane as the eluent. 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 rosin-acids in oleoresins from North-European pine (*Pinus silvestris*) and spruce (*Picea abies*).

Peak No. in Fig. 1	Peak volume ml	Peak area % of total area
	Pinus silvestris	
1	190	18
2	250	59
3	ca. 340	18
4	ca. 470	5
	Picea abies	
1	190	25
2	250	60
3	ca. 340	10
4	ca. 470	5

According to our earlier studies [3] peak 1 consists of palustric acid, peak 2 may include pimaric acids, *l*-abietic acid and levopimaric acid, peak 3 contains *neo*abietic acid and peak 4 dehydrogenated rosin acids.

In order to confirm the identity of the acid in peak 1, the respective eluant fractions were combined, the *iso*octane removed by reduced pressure, and the resulting acid dissolved in acetone and reprecipitated with *cyclo*hexylamine [6]. A sample of palustric acid was also precipitated as its *cyclo*hexylamine salt under the same conditions. The *cyclo*hexylamine salts, which were isolated in a yield of 85—90 %, were dissolved in optically pure alsolute ethanol and the ultraviolet absorption spectra of the solutions recorded. The recorded ultraviolet spectra (Fig. 2) showed that the *cyclo*hexylamine salts of the acids in peak 1 of pine and spruce were identical with the spectrum for the *cyclo*hexylamine salt of the sample of palustric acid. The specific extinction coefficient (a) for the latter salt at 265—266 mμ is 25.0, while the value

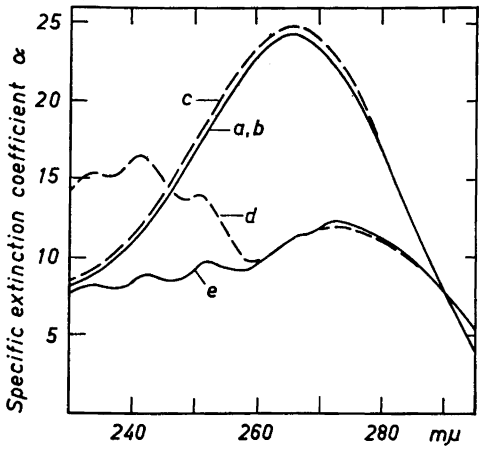

Fig. 2. Ultraviolet absorption spectra of palustric acid (in form of its *cyclo*hexylamine salt) isolated by partition chromatography from oleoresins of North-European pine (*a*) and spruce (*b*) and the spectrum of the *cyclo*hexylamine salt of a palustric acid sample (*c*). Curves *d* and *e* gives the absorption spectra of the *cyclo*hexylamine salts of the acids in peak 2 (in Fig. 1) from pine and spruce respectively. The specific extinction coefficients α were calculated to refer to the acids rather than to the salts.

of very pure palustric acid reported by Loeblich, Baldwin and Lawrence [1] 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 rosin 21 % of the total rosin acids in the sample. Palustric acid hence represents a major component in the rosin of North-European pine and spruce. The palustric acid content is higher in the spruce rosin than in any pine species, where its content has been found to vary between 7 and 12 % (see Ref.[2]).

We have also isolated by *cyclo*hexylamine 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 rosin (Fig. 2d) exhibits a maximum absorption, $\alpha = 16.6$, at 241 mμ and another, $\alpha = 12.0$, at 272 mμ. These maxima are characteristic for *l*-abietic and levopimaric acids, respectively; the absorption values indicate that the *l*-abietic acid content in peak 2 for the pine is about 18 % or 11 % of the total rosin acid fraction and the levopimaric acid content in the same peak is 63 % or 37 % of the total rosin acid fraction (in these calculations the contribution of levopimaric acid to the α-value of *l*-abietic acid has been taken into account). The corresponding values of α 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 rosin acids, and the levopimaric acid content 65 %, or 39 % of the total rosin acids. In American pine species [2] the abietic acid content is near 10 % and the levopimaric acid content between 24 and 31 %. The distribution of rosin 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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1. Loeblich, V. M., Baldwin, D.E. and Lawrence, R.V. *J. Am. Chem. Soc.* **77** (1955) 2823.
2. Baldwin, D. E., Loeblich, V. M. and Lawrence, R. V. *Ind. Eng. Chem Data Series* **3** (1958) 342.
3. Bruun, H. H. and Gåsland, S. *Acta Chem. Scand.* **13** (1959). 598.
4. Bruun, H. H. *Paper and Timber (Finland)* **41** (1959) 73.
5. Loeblich, V. M., Baldwin, D. E., O'Connor, R. T. and Lawrence, R. V. *J. Am. Chem. Soc.* **77** (1955) 6311.
6. Harris, G. C. and Sanderson, T. F. *J. Am. Chem. Soc.* **70** (1948) 334.

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