The polymer fraction of albumin was eluted with the void volume. When the dextran content in the buffer solution was increased, the void volume increased indicating that the gel had shrunk owing to the increased osmotic pressure in the buffer-dextran solution. The dimer and monomer fraction of albumin were also eluted later, the higher the concentration of dextran in the buffer. On the contrary the fourth peak emerged with the same volume independent of the dextran concentration.

The different values of the void volume and the elution volume were used for calculations. The mean value of the elution volume of the fourth fraction in seven runs was used as total volume.

As demonstrated in Fig. 1 the presence of dextran in the buffer has a great influence on the elution volume of albumin. When no dextran is present in the buffer the partition coefficient, K_{av},[4] for the monomer fraction of albumin is 0.42, which is in good agreement with the values found by others.[4] With 1 and 2 % dextran in the buffer the corresponding K_{av} values are 0.52 and 0.58. In a separate experiment using another column packed with a different batch of Sephadex G-200 the corresponding K_{av} value was 0.72, when 4 % Dextran 150 was added to the buffer.

The results suggest that gel filtration can be used to determine the exclusion properties of the polymer solutions used as eluents. It is also possible that this modification of gel filtration can be used for increased resolution in separation work, for instance by using dextran gradients. This investigation will be described in detail in a later publication.

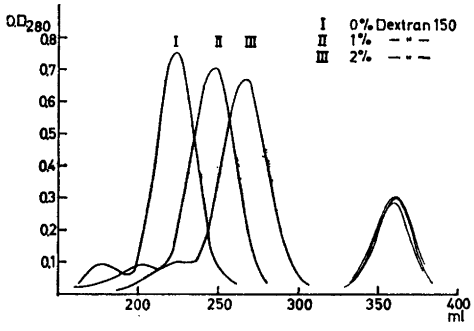


Fig. 1. 55 mg of human serum albumin chromatographed on a column of Sephadex G-200 with various concentrations of Dextran 150 in the eluent. The first eluted peak ("albumin polymers") is not shown in the diagram.

The work was supported by *Svenska Sällskapet för Medicinsk Forskning, Konung Gustaf V:s 80-årsfond,* and the *Medical Faculty, University of Uppsala.*

1. Laurent, T.C. *Biochem. J.* **89** (1963) 253.
2. Laurent, T.C. *Acta Chem. Scand.* **17** (1963) 2664.
3. Porath, J. and Flodin, P. *Nature* **183** (1959) 1657.
4. Laurent, T.C. and Killander, J. *J. Chromatog.* **14** (1964) 317.
5. Granath, K.A. *J. Colloid. Sci.* **13** (1958) 308.
6. Pedersen, K.O. *Arch. Biochem. Biophys. Suppl.* **1** (1962) 157.

Received September 18, 1965.

The Constituents of Conifer Needles

III.* Isolation of β-D-Glucosides of Guaiacyl Glycerol from *Pinus silvestris* L.

OLOF THEANDER

Träkemiska avdelningen, Svenska Träforskningsinstitutet, Stockholm Ö, Sweden

Further studies on the low-molecular weight components of the water-soluble fraction of an ethanol or acetone extract of *Pinus silvestris* L. needles [1] (collected in the autumn) have resulted in the isolation of shikimic acid, sequoitol, L-rhamnose, D-mannitol and two new glucosides.

Carbon column chromatography using gradient elution with aqueous ethanol separated the two glucosides. Higher oligosaccharides and other compounds present in the glucoside-containing fractions were removed by chromatography on cellulose columns. Final purification was accomplished by paper chromatography.

One glucoside (I), isolated in about 0.1 % yield of the water-soluble compo-

* Part II. *Acta Chem. Scand.* **16** (1962) 607.

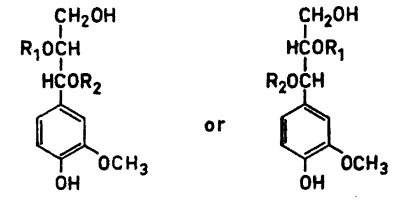
nents was amorphous ($[\alpha]_D^{22}$ −16°) whereas the other (II), obtained in smaller amounts, was crystalline (m.p. 162—166°, $[\alpha]_D^{22}$ + 45°; Found: C 51.0; H 6.77; O 42.3; $C_{16}H_{24}O_{10}$ requires C 51.0; H 6.43; O 42.5). Treatment of either of the glucosides with β-glucosidase yielded D-glucose and a component, indistinguishable from guaiacyl glycerol [1-*C*-(4-hydroxy-3-methoxy-phenyl)-glycerol] by paper chromatography and UV absorption in neutral and alkaline solution. Paper electrophoresis in borate (pH 10) and sulphonated phenylboronic acid (pH 6.5) buffers distinguishes between *threo*- and *erythro*-guaiacyl glycerols.[2] Using this method both aglucones were identified as the *threo*-isomer. Furthermore the aglycone from I was transformed to the tetraacetate, which was identified by m.p., mixed m.p. and thin-layer chromatography with an authentic sample of D,L-*threo*-guaiacyl glycerol tetraacetate.

The intense colour reactions of the two glycosides with phenol reagent "Echtblausalz B" (Merck) and also the UV absorption in neutral and alkaline solutions indicated that the phenolic hydroxyl was free in both substances. Glucoside I gave a strong blue colour with 2,6-dibromo-N-chloroquinonimine (which is characteristic of *p*-hydroxybenzyl alcohols [3]); whereas II gave no colour. This indicated that glucose is linked at the β- or γ-hydroxyl of the glycerol chain in I and at the α-position in II. Neither vanillin nor formaldehyde was formed when I was treated with periodate under conditions by which these products are readily formed from guaiacyl glycerol,[4] showing that glucose is linked in the β-position. On the other hand, formaldehyde (identified as methylene-bis-dimedone) but no vanillin was formed from glucoside II, in agreement with the formula below.

The extract also contains free guaiacyl glycerol, present in larger amount than the two glucosides. The guaiacyl glycerol is optically active ($[\alpha]_D^{22}$ −18°) and has the *threo*-form. The aglucones isolated from the two glucosides also had negative rotations. The absolute configuration of the aglucones is not yet determined.

A preliminary study of the water-soluble fraction of cambium and newly formed needles collected in the spring by paper chromatography and electrophoresis indicated that glucoside I was present.

The isolation of these optically active compounds in pine needles may be of some importance in connection with the biosynthesis of lignin and other wood constituents having phenyl propane carbon structures.



I R_1 = β-D-glucopyranose; R_2 = H

II R_2 = β-D-glucopyranose; R_1 = H

Traces of *threo*-guaiacyl glycerol were recently isolated in the form of its tetraacetate after treatment of *Pinus resinosa* Ait heartwood under acetylation conditions at 50—70° [5] and the related ω-hydroxypropioguaiacone found in small amounts in the bark of *Pinus silvestris* L.[6]

Acknowledgements. The author wishes to thank Professor B. Lindberg for his interest in this work and Professor E. Adler for a sample of D,L-*threo*-guaiacyl glycerol tetraacetate.

1. Assarsson, A. and Theander, O. *Acta Chem. Scand.* **12** (1958) 1319.
2. Gierer, J. and Norén, I. *Acta Chem. Scand.* **16** (1962) 1976.
3. Gierer, J. *Acta Chem. Scand.* **8** (1954) 1319.
4. Adler, E. and Yllner, S. *Acta Chem. Scand.* **7** (1953) 570.
5. v. Rudloff, E. *Chem. Ind. (London)* **1965** 180.
6. Oksanen, H. *Suomen Kemistilehti* **B 38** (1965) 65.

Received September 24, 1965.

Exclusion Chromatography of Barley β-Amylase on Sephadex G-75

MARTTI NUMMI, RAILI VILHUNEN and TOR-MAGNUS ENARI

Laboratory of Brewing, Helsinki, Finland

We have earlier reported the fractionation of barley β-amylases on Sephadex G-100 columns.[1] Both that and the