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CHROMATOGRAPHIC SEPARATION OF THE ALKALINE HYDROLYSIS PRODUCTS OF SHELLAC

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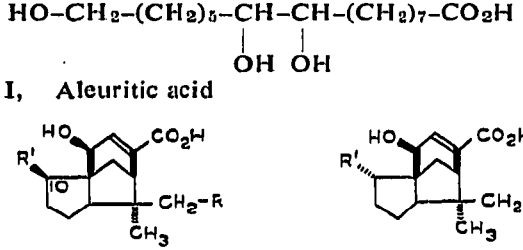
SUMMARY

The methyl ester–trimethylsilyl ethers of the alkaline hydrolysis products of Super Blonde Shellac were analysed by gas–liquid chromatography. The results were compared with the thin-layer chromatographic and column chromatographic patterns of different fractions, analysed as the methyl esters. The main hydroxy fatty acid of shellac, *viz.*, *threo*-aleuritic acid, was completely separated from the terpene acids and a separation of some of the terpene components was achieved on a 3% SE-30 column. The terpene acids were also analysed on a column of 15% EGSS-X and 1% HI-EFF-8 BP with essentially similar results. The hydrolyzate is complex and a number of components are yet to be identified in the pure state, accounting for the numerous gummy fractions obtained in column chromatography.

INTRODUCTION

Shellac[1] is a polymeric resin of commercial value, secreted by the insect *Laccifer lacca* Kerr, commonly found in heavy infestation on certain specific host trees in the forests of India, Burma and Thailand. The resin is mainly a complex mixture of polyesters composed primarily of *threo*-aleuritic acid (I) and a number of closely related sesquiterpene acids of the cedrene skeleton (Fig. 1). The structure of the first known terpene acid, shellolic acid (III) was established by Yates and Field[2]. A large number of minor fatty acids[3] present in shellac have also been characterized. All of the above acids were obtained by the strong alkaline hydrolysis (with *ca.* 20% NaOH for 14 days) of shellac. However, under relatively mild alkaline hydrolysis conditions (with *ca.* 7% NaOH for 5 h), two acids with aldehydic groups, jalaric acid[4] (VII) and laccijalaric acid[5] (VIII), were reported. It is believed[4] that these two primary acids undergo a Cannizzaro reaction to give the corresponding alcoholic and acidic components mentioned in Fig. 1 on treatment with strong alkali. The epimeric centre involved in the above compounds is the C_{10} position.



I, Aleuritic acid

II, R = H, R′ = CO_2H: laccishellolic acid
III, R = OH, R′ = CO_2H: shellolic acid
IV, R = OH, R′ = CO_2H; *epi*-shellolic acid
V, R = H, R′ = CO_2H; *epi*-laccilaksholic acid
VI, R = OH, R′ = CO_2H; *epi*-laksholic acid
VII, R = OH, R′ = CHO; jalaric acid
VIII, R = H, R′ = CHO; laccijalaric acid

Fig. 1. Components of shellac.

EXPERIMENTAL AND RESULTS

Shellac was hydrolyzed as described in an earlier paper[6] and esterified with either diazomethane or methanolic hydrogen chloride. The column chromatography of the methyl esters was carried out on silica gel and the laccishellolate zone was eluted with 2–5% ethyl acetate in benzene, followed by the shellolate zone with 10% ethyl acetate in benzene, and finally the epilaksholate zone with 25–30% ethyl acetate in benzene.

Trimethylsilyl (TMS) derivatives were prepared by the reaction at room temperature of 0.5 ml of Sil-prep (hexamethyldisilazane–trimethylchlorosilane–pyridine, 3:1:9, v/v/v) with the dry hydroxy ester for 1 h in a Reacti-vial (Pierce Chemical Co., Rockford, Ill., U.S.A.) fitted with a Teflon stopper. The solvent was then removed under a slow stream of nitrogen and the residue dissolved in 100–300 μl of hexane.

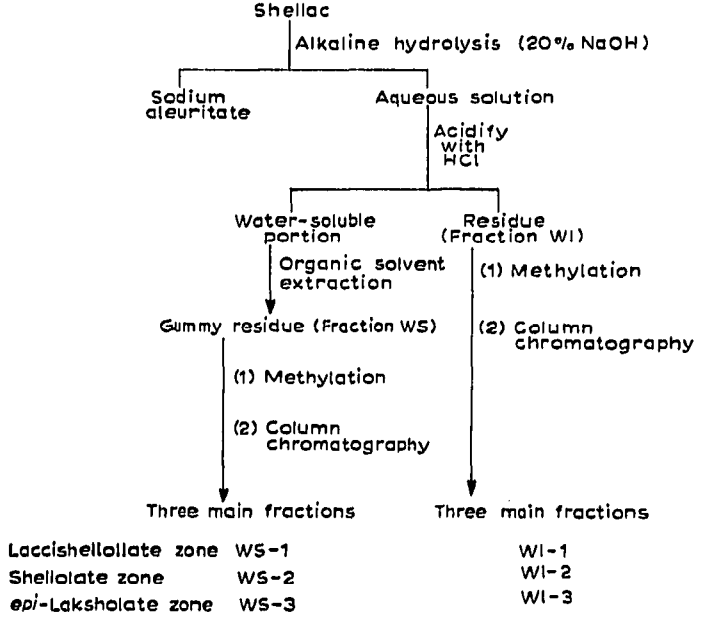
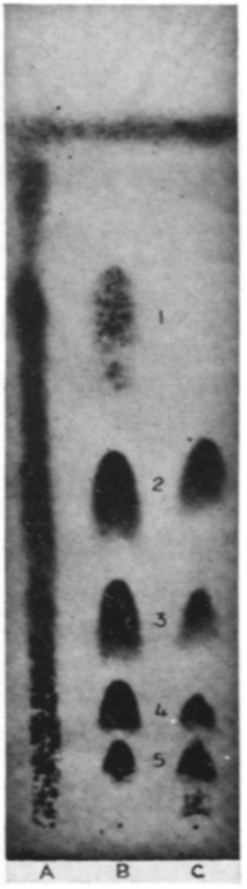


Fig. 2. Scheme for the alkaline hydrolysis and analysis of shellac.

The solution was then stoppered and vortexed vigorously. The solution was centrifuged and the supernatant liquid was injected in 1–4-μl aliquots into the gas chromatograph.

The gas–liquid chromatographic (GLC) analyses were carried out using a Packard 805 gas chromatograph with a U-shaped glass column (6 ft.$\times$$^1/_4$ in. I.D.) and equipped with a flame ionization detector. The stationary phases were (*a*) 1% HI-EFF-8BP on Gas-Chrom Q (100–120 mesh), nitrogen pressure 40 p.s.i. with a column temperature of 230° and detector and injection port temperatures of 260°; (*b*) 15% of EGSS-X on Gas-Chrom P (100–120 mesh), nitrogen pressure 40 p.s.i. with a column temperature of 220° and detector and injection port temperatures



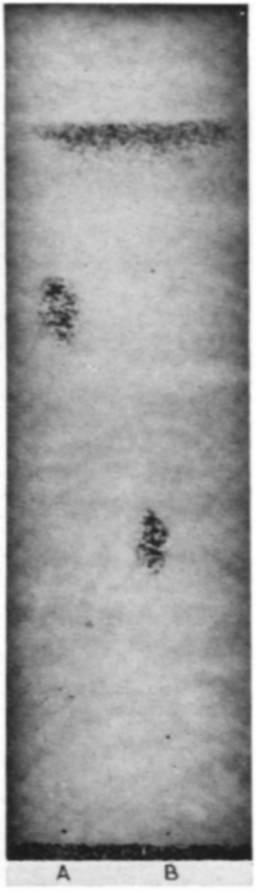


Fig. 3. Thin-layer chromatography of alkaline hydrolysis products of shellac as methyl esters. Adsorbent: Silica Gel G. Solvent system: chloroform–methanol (97:3). The spots were rendered visible by spraying with sulphuric acid and charring after heating. A = extractives of gummy residue (Fraction WI). B = standard mixture of (from top to bottom) 1, laccishellolic acid; 2, shellolic acid; 3, *epi*-shellolic acid; 4, *epi*-laccilaksholic acid; 5, *epi*-laksholic acid. C = extractives of aqueous portion (Fraction WS).

Fig. 4. Thin-layer chromatography of methyl *threo*- and *erythro*-aleuritates on Silica Gel G impregnated with boric acid. Solvent system: diisopropyl ether–methanol (94:6). A = methyl *threo*-aleuritate; B = methyl *erythro*-aleuritate.

of 235°; (*c*) 3% GC-grade SE-30 on Chromosorb WHP (80–100 mesh), with a column temperature of 195° and detector and injection port temperatures of 235°.

Method of isolation

The procedure for the alkaline hydrolysis and further analysis of the products is described below and is summarized in Fig. 2. During the course of the hydrolysis of shellac with *ca.* 20% NaOH, at room temperature, a major part of the *threo*-aleuritic acid separated out as the sodium salt. Acidification of the alkaline solution then gave a large gummy residue. The aqueous mother liquor carried the maximum

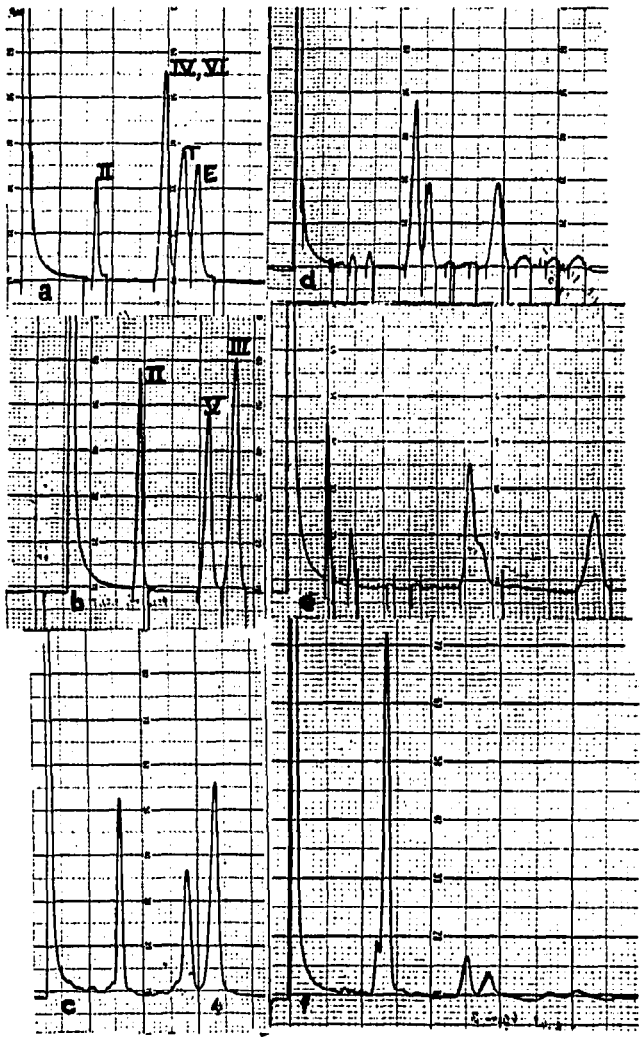


Fig. 5. Gas–liquid chromatogram of shellac hydrolyzate (as methyl esters–trimethylsilyl ethers). Condition: 1% HI-EFF-8BP on Gas-Chrom Q, N_2 pressure 40 p.s.i., column temperature 230°. Chart speed, 2 in./min. (a) *threo*-Aleuritic acid (T), *erythro*-aleuritic acid (E), II, IV and VI (see Fig. 1). (b) Mixture of II, III and V. (c) Total gum from aqueous portion (WS). (d) Fraction WS-1. (e) Fraction WS-2. (f) Fraction WS-3.

amount of the soluble terpene acids. The methyl esters on column chromatography over silica gel gave the individual crystalline components[6] mentioned in Fig. 1. It should be pointed out that all of the crystalline compounds so far reported in the literature are derived from the aqueous portions (Fraction WS, Fig. 2). The major gummy residue (WI) has not been subjected to a close scrutiny as far as the terpene components are concerned, because of the complex and incomplete analytical separations involved. A typical thin-layer chromatographic (TLC) run on the crude methyl esters and an artificial mixture of the crystalline esters isolated is shown in Fig. 3. As the gummy fractions WS and WI appear to be a complex mixture, in order to obtain further information on the components present it would be appropriate to analyse the gums by GLC. Khurana *et al.*[7] have reported the analysis of lac hydrolyzate by GLC of methyl esters and indicate that they are unstable under the conditions used. The methyl esters alone would be expected to have long retention times. In this paper, the GLC analysis of the TMS derivatives of methyl esters is described. The pattern of column chromatographic analysis was similar to that described earlier[6].

Gas–liquid chromatography of methyl threo- and erythro-aleuritates as the trimethylsilyl derivatives

Threo–erythro pairs of hydroxy fatty acids and their derivatives normally show substantial differences in solubility and they are also readily identified by TLC on silica gel impregnated with boric acid[8]. A typical TLC run on methyl *threo–erythro*-aleuritates is shown in Fig. 4. Only the *threo*-aleuritic acid is present in shellac. It

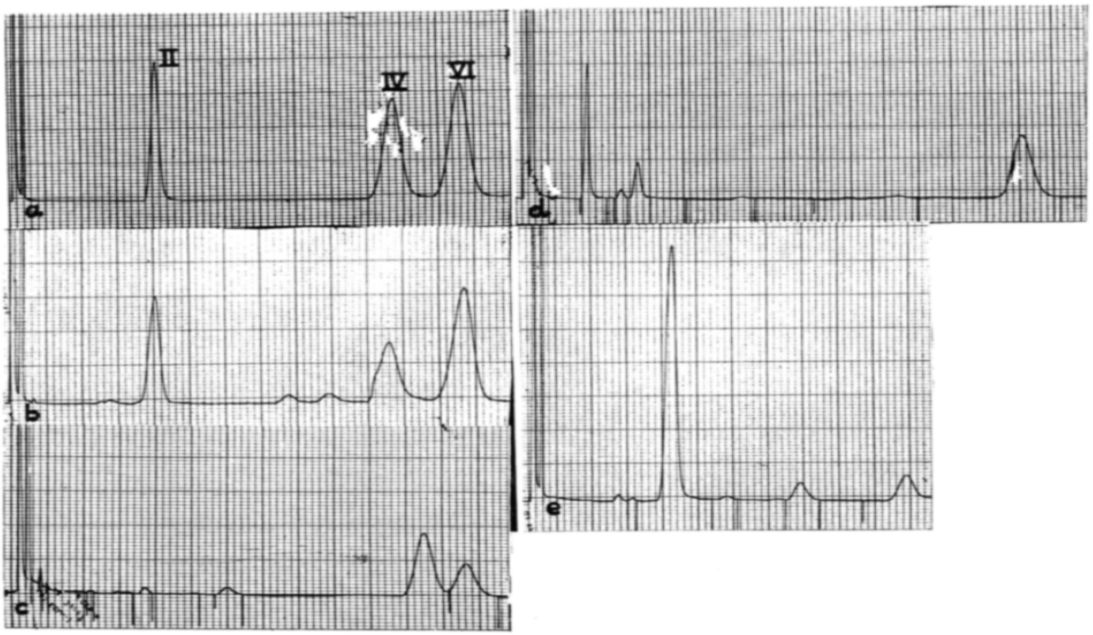


Fig. 6. GLC analysis of shellac hydrolyzate (as methyl ester–trimethylsilyl ether). Conditions: 15% of EGGS-X on Gas-Chrom P, N_2 pressure 40 p.s.i., column temperature 220°. Chart speed, 2.5 in./min. (a) Mixture of II, IV and VI. (b) Fraction WS. (c) Fraction WS-1. (d) Fraction WS-2. (e) Fraction WS-3.

has been mentioned earlier that some part of this acid appears in the gummy fractions after the alkaline hydrolysis of shellac. Earlier, Eglinton and Hunneman[9] noted that *threo–erythro* pairs of 9,10-dihydroxy- as well as 9,10,18-trihydroxyoctadecanoates are well separated by the GLC of the TMS derivatives on 3% SE-30 on Gas-Chrom Q, the *erythro*-isomer preceding the *threo*-isomer, which is the reverse of the usual pattern in TLC analysis. On 3% SE-30, 1% HI-EFF-8BP and 15% EGSS-X,

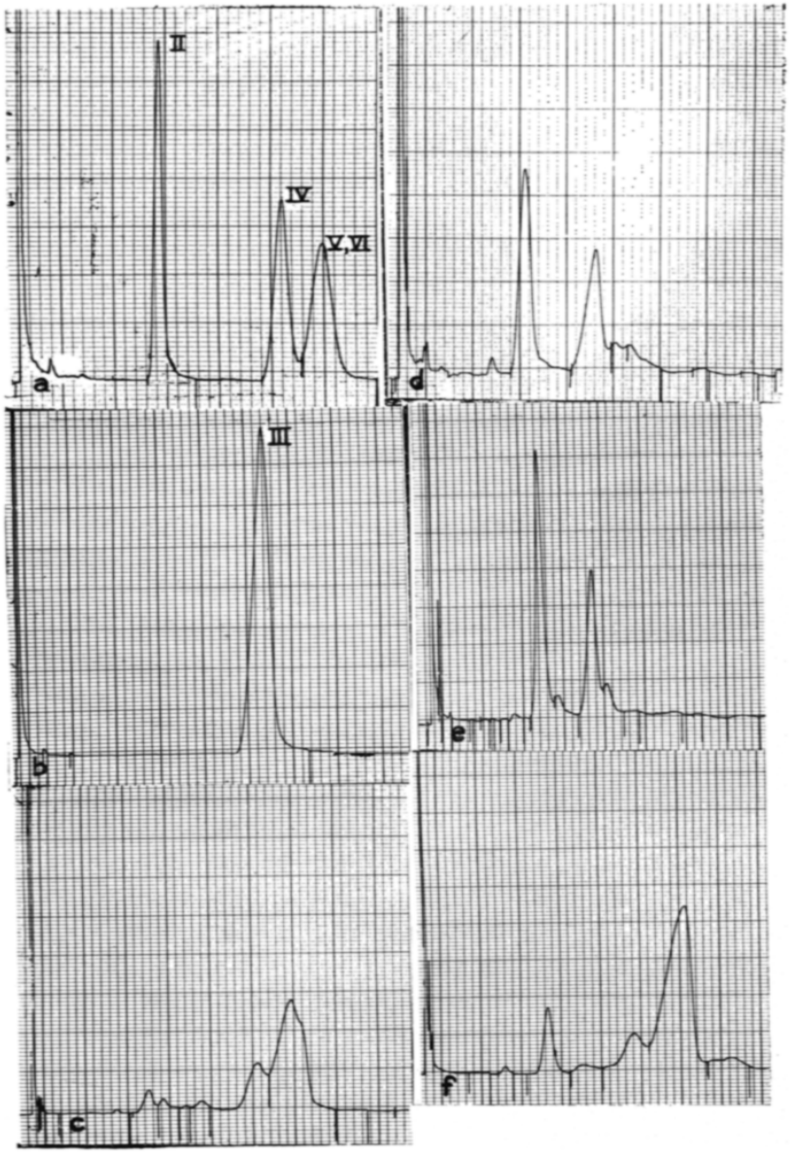


Fig. 7. GLC analysis of shellac hydrolyzate (as methyl ester–trimethylsilyl ether). Conditions: 3% SE-30 on Chromosorb WHP, N_2 pressure 40 p.s.i., column temperature 195°. Chart speed, 2.5 in./min. (a) Mixture II, IV, V and VI. (b) Dimethyl shellolate. (c) Fraction WS. (d) Fraction WS-1. (e) Fraction WS-2. (f) Fraction WS-3.

the *threo*-isomer precedes the *erythro*-isomer in the case of the aleuritates. A typical separation of these isomers on a HI-EFF-8BP column is shown in Fig. 5a.

Analysis of the terpene esters

Prior to the analysis of the alkaline hydrolysis products of shellac, an attempt was made to separate the TMS derivatives of the compounds listed in Fig. 1 on columns of 3% SE-30, 3% QF-1, 1% HI-EFF-8BP and 15% EGSS-X. While the separation of some of the terpene acids was achieved on both the HI-EFF-8BP and EGSS-X columns, the resolution of these acids on a 3% QF-1 column was poor. The best resolution of these compounds, however, was obtained on the SE-30 column. It may be pointed out that TMS derivatives were stable at room temperature for more than 48 h and under the chromatographic conditions used. On the SE-30 column, the TMS derivative of *threo*-aleuritate was clearly separated from the terpene acids as it had a much longer retention time compared with those of the terpene acids. Typical separations of terpene acids are shown in Figs. 5a and b (HI-EFF-8BP), 6a (SE-30) and 7a and b (EGSS-X). A clear separation of laccishellolate, *epi*-shellolate and shellolate can be achieved while *epi*-laccilaksholate and *epi*-laksholate are not separated. It may be recalled from the TLC analysis (Fig. 3) that these two compounds follow each other closely, and a similar pattern was also noticed in the column chromatography. The efficient identification and separation of these two compounds would have to include both TLC and column chromatography.

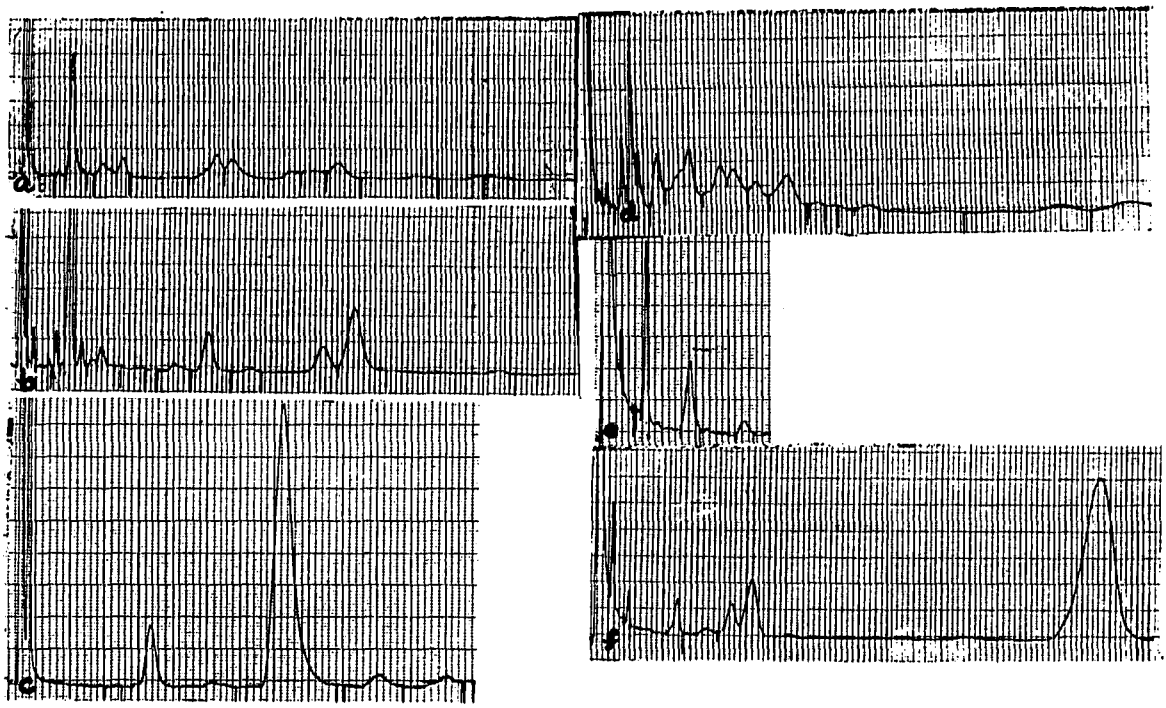


Fig. 8. GLC analysis of shellac hydrolyzate (water-insoluble gum, Fraction WI) as methyl ester–trimethylsilyl ether. Conditions as in Figs. 6 and 7. (a), (b) and (c) Fractions WI, WI-1 and WI-3, respectively, on EGSS-X column. (d), (e) and (f) Fractions WI, WI-1 and WI-3 on SE-30 column. In (f) the peak with the longest retention time is *threo*-aleuritic acid.

Analysis of the gums

The complexity of the lac hydrolyzate is indicated by the GLC of the TMS derivatives of the two parent gums, *viz.*, the methyl esters prepared from the extractives of the aqueous portion (Figs. 5c, 6b and 7c) and the residual gum obtained after alkaline hydrolysis (Figs. 8a and 8d). It is apparent why only a succession of gums were obtained on extensive column chromatography. The column chromatography of the above-mentioned parent gums (WS and WI) can be resolved into three primary zones, the first of laccishellolate, the second of dimethyl shellolate and *epi*-shellolate and the third of *epi*-laccilaksholate and *epi*-laksholate, mentioned in their elution order. Figs. 5d, 5e, 5f, 6c, 6d, 6e, 7d, 7e and 7f represent the above-mentioned individual zones of gummy residues, which precede or follow those fractions in that zone during column chromatography, that are readily crystallisable. The fractions belong to the extractives of the aqueous portion. On the other hand, the extractives of the water-insoluble gum, on a similar chromatographic separation, gave no useful amount of crystalline compounds, except minor amounts of methyl *threo*-aleuritate and *epi*-laksholate, the remainder of the fractions being gums. However, in this case also the three zones could be separated, with the last one as the major fraction. Fig. 8 represents the individual zones from the water-insoluble residue.

It can clearly be seen that the crystalline components are distributed primarily in the aqueous portion and to a smaller extent in the residual portion. Failure to isolate crystalline components from the residual portion is largely due to its being a more complex mixture than the aqueous portion. It is intended to investigate these separations further. Experimental results also indicate that some polymeric fractions may still be present in shellac, even after alkaline hydrolysis[3,10].

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