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CHEMISTRY OF LAC RESIN—III*

LAC ACIDS—3: AN INTEGRATED PROCEDURE FOR THEIR ISOLATION FROM HARD RESIN; CHROMATOGRAPHY CHARACTERISTICS AND QUANTITATIVE DETERMINATION†

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Abstract—A modified procedure for the isolation of "hard resin" from seed-lac is described. An integrated procedure for the isolation of various lac acids from the "hard resin" hydrolysate has been developed. Paper and thin-layer chromatography characteristics of various lac acids and their methyl esters are reported. Quantitative estimation of aleuritic acid and aldehydic acids in "hard" and "soft resins" of various origins has been carried out.

LITERATURE methods[1] for the isolation of aleuritic acid[2-4] (I) and shellolic acid[2,4-6] (III) are, at best, cumbersome and cannot be adopted for the isolation of the new lac acids recently described by us.[7,8] As a step towards the structure elucidation of lac resin it was considered essential to develop, for lac acids, an isolation procedure which would furnish not only the various lac acids known at present,[7,8] but will also be amenable to systematic screening for any more new lac acids.‡ We now describe such a procedure. Furthermore, to serve as reference data, we have collected paper and thin-layer chromatography (TLC) characteristics of the various lac acids and their methyl esters.

Hard resin and its hydrolysis

Lac resin is a complex mixture of polyesters of varying molecular sizes and has been separated[10] into "soft" and "hard" resin (so-called "pure resin" of Tschirch and Ludy[3]) fractions by solvent extractions. Since, "hard resin" which accounts for ~70% of total lac resin (cf. Fig 1), appeared to be the right raw material for further purification with the ultimate objective of isolating a homogeneous lac resin fraction for structural investigation, it was decided to base the integrated procedure for the isolation of various lac acids on "hard resin" rather than on the total lac resin or seed-lac.

The method finally worked out for the isolation of "hard resin" is depicted in Fig 1 and is an adaptation of the method of Tschirch and Ludy[3] with the important modifications of using 78% aqueous ethanol, rather than absolute ethanol,§ for dewaxing

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‡ Weinberger and Gardner[9] describe the isolation of two acids, named lacolic lactone and kerrolic acid, from the hydrolysates of shellac fractions, obtained by solvent segregation of shellac. By the procedure described in the present paper, we have been unable to detect these.

§ In a separate experiment it was found that lac wax has a solubility of ~0·6% (at 28°) in absolute ethanol.

and, the precipitation of "hard resin" from ethanolic solution by ether, rather than by water.

Next, the saponification of "hard resin" was investigated. In view of the known[7] susceptibility of jalaric acid (VII), a primary lac acid,[7] to undergo Cannizzaro reaction on extended exposure to aqueous alkali, the rate of hydrolysis of "hard resin" with KOHaq. (1N) was studied. The saponification was found to be essentially complete after 5 hr reaction at 30 ± 1°. Therefore, in order to minimise side reactions, the initial hydrolysis of "hard resin" was restricted to a 5 hr period at room temp. After studying several unsuccessful procedures (e.g. urea adductation, fractional distillation of derived Me esters, column chromatography) for the isolation of lac acids from such a hydrolysate, a reasonably successful method, which is outlined in Fig 2, could be worked out. Since, on acidification of alkaline solution, the sparingly soluble acids precipitate as a gum, which "takes up" considerable quantities of jalaric and related acids, a second treatment of this gum with alkali is essential. Finally, an extended (24 hr) treatment with alkali is given to convert all the residual jalaric acid (VII) into its Cannizzaro products, before esterification with diazomethane, since the reaction of jalaric acid with diazomethane is quite complex.[7] That aleuritic acid forms a water-sparingly soluble sodium salt was first observed by Gidvani.[4] By following the procedure outlines in Fig 2, it is now possible to isolate all the primary lac acids in a pure state.*

Paper and thin-layer chromatography

For monitoring the separation of lac acids, paper chromatography and TLC proved invaluable; GLC proved to be of limited help, as under a variety of conditions, dimethyl shellolate and dimethyl epishellolate underwent decomposition on GLC columns. Paper chromatography of butolic, aleuritic and shellolic acids has been studied earlier by Sen Gupta.[11] The solvent systems for paper chromatography and TLC, found most suitable during the present work are given in Tables 1–2 along with the R_f values of various lac acids and their methyl esters to serve as reference data; the use of solvent system containing ammonium carbonate buffer, for paper chromatography of lac acids, is based on the work of Hartley and Lawson[12] on the paper chromatography of organic acids.

Quantitative estimation of aleuritic acid and aldehydic acids

From the work already described (cf. Fig 2) it is clear that the fatty acid, aleuritic acid and the cedrene derivative, jalaric acid constitute important building blocks of "hard resin". It was thought worthwhile to obtain a quantitative picture of the role of these acids in the constitution of lac resin.

Since, as already stated GLC could not be utilised for present purpose, chemical methods for the estimation of these acids were sought. The estimation of aleuritic acid has been described by a number of workers,[13–15] who have utilised the cleavage of α-glycol function in aleuritic acid either by lead tetraacetate[13] or by periodic acid.[14] During the present work the periodic method[14] was found to be quite satisfactory and was adopted for the estimation of aleuritic acid (after alkali hydrolysis) in "hard" and "soft resin" (Table 3).

* The artefacts, laksholic and *epi*-laksholic acids are best prepared by alkali treatment of jalaric acid. Butolic acid,[7] as will be shown in a subsequent publication, is not a constituent acid of hard resin.

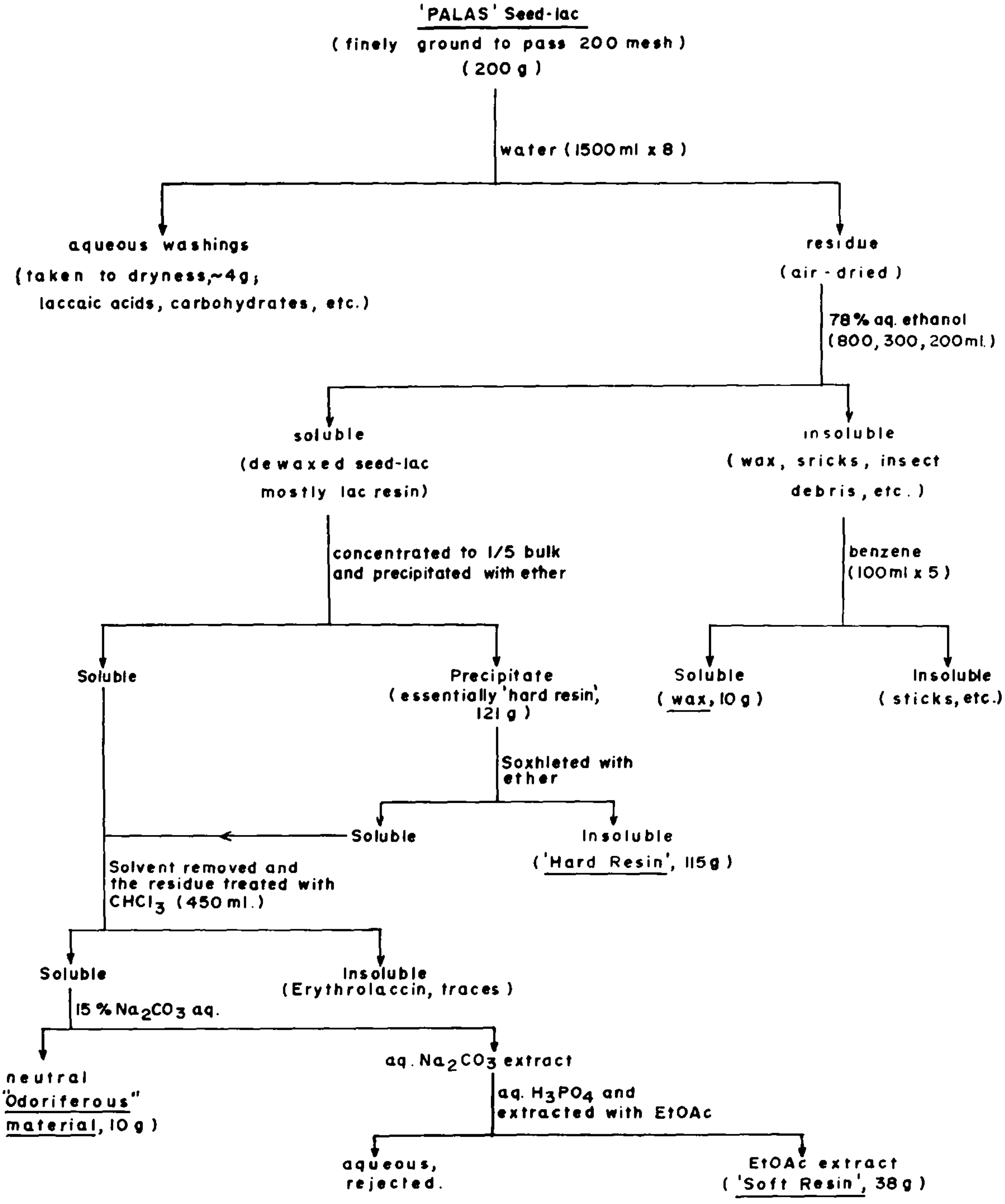
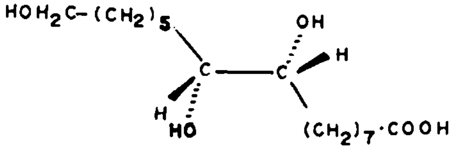


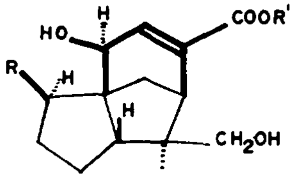
Fig 1. Gross separation of 'Palas' seed-lac.



I

$CH_3(CH_2)_7CHOH{\cdot}(CH_2)_4COOH$

II

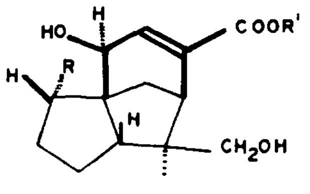


III : R = COOH , R' = H

IV : R = COOMe, R' = Me

V : R = CH_2OH , R' = H

VI : R = CH_2OH, R' = Me



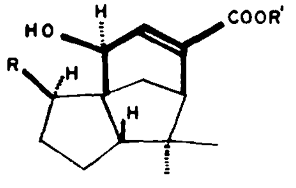
VII : R = CHO , R' = H

VIII : R = COOH, R' = H

IX : R = COOMe; R' = Me

X : R = CH_2OH, R' = H

XI : R = CH_2OH, R' = Me

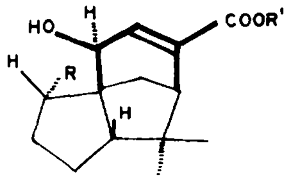


XII : R = COOH, R' = H

XIII : R = COOMe, R' = Me

XIV : R = CH_2OH, R' = H

XV : R = CH_2OH, R' = Me



XVI : R = CHO, R' = H

XVII : R = COOH, R' = H

XVIII : R = COOMe, R' = Me

XIX : R = CH_2OH, R' = H

XX : R = CH_2OH, R' = Me

Of the several chemical methods[16] available for the quantitative estimation of aldehyde (and keto) groups, the silver oxide method of Siggia and Segel[17] and the 2,4-dinitrophenylhydrazine method of Lappin and Clark[18] were selected for assessing their applicability to the estimation of jalaric (and laccijalaric) acid. The results from these two methods, one requiring alkaline and the other acidic medium, were also expected to reveal the presence of any masked aldehyde functions in the lac resin. Though the silver oxide method could be readily standardised using pure jalaric acid,

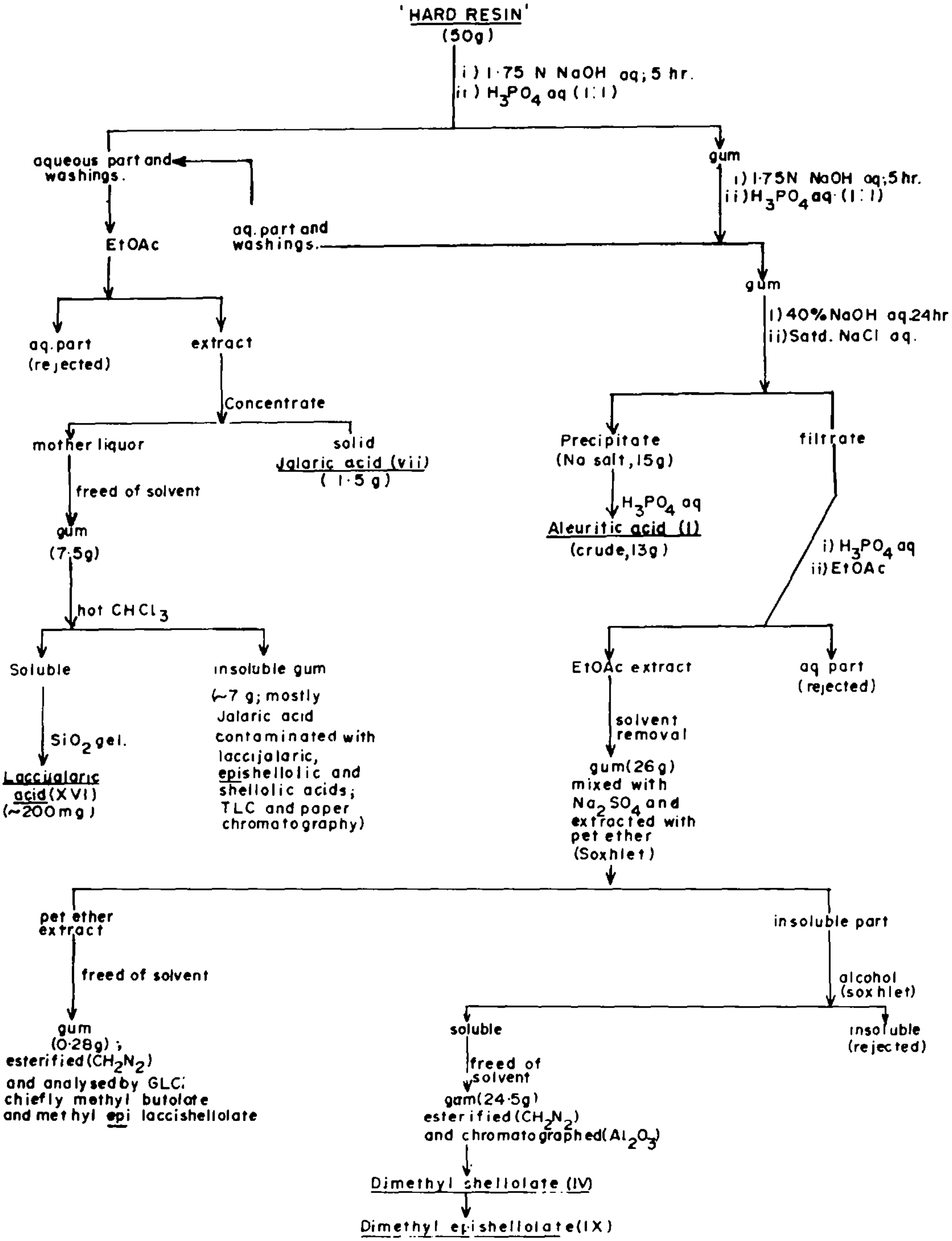


FIG 2. Separation of lac acids from "hard resin" hydrolysate.

it was surprisingly found that in presence of aleuritic acid (synthetic mixtures of jalaric and aleuritic acids) consistently high results were obtained[*] and hence the method was abandoned. The 2,4-dinitrophenylhydrazone method,[18] which depends on the estimation of absorption at 480 mμ of an alkaline soln of the 2,4-dinitrophenylhydrazone, could be easily standardised in terms of jalaric acid[†] and hence was adopted for the estimation of aldehydic acid units in lac resin (Table 3).

The methods discussed above have been applied to the estimation of aleuritic and aldehydic acid residues in "hard" and "soft resin" derived from seedlacs of various origins (Table 3). The significance of these results will be discussed in a later publication. Aleuritic acid residues have been estimated (periodic acid method) by earlier workers[14] in several varieties of seedlacs and shellacs (e.g. 14·34 % for *kusmi* seedlac). Similarly, carbonyl number of a variety of seedlacs and shellacs has been determined[19] by the sodium sulphite method (e.g. carbonyl number = 24·5 mg KOH g for kusmi seedlac, i.e. 12% aldehydic acid content in terms of jalaric acid).

TABLE 1. PAPER CHROMATOGRAPHY[a] CHARACTERISTICS OF LAC ACIDS

No.	Acid	R_f (Solvent system)[b]	
		A	B
1	Butolic (II)	1	0·85
2	Aleuritic (I)	0·82	0·78
3	Jalaric (VII)	0·71	0·67
4	Shellolic (III)	0·54	0·52
5	*Epi*shellolic (VIII)	0·43	0·40
6	Laksholic (V)	0·70	0·67
7	*Epi*laksholic (X)	0·70	0·64
8	Laccijalaric (XVI)	0·76	0·71
9	Laccishellolic (XII)	0·57	—
10	*Epi*laccishellolic (XVII)	0·52	—
11	*Epi*laccilaksholic (XIX)	0·74	—

[a] Ascending chromatography; Whatman No. 1 paper; solvent front, 14 cm; temp 29 ± 1°. Visualization agents: (i) 50 mg bromophenol blue and 200 mg citric acid in 100 ml water,[12] (ii) for detection of ketonic or aldehydic acids: 400 mg 2,4-dinitrophenylhydrazine in 100 ml 2N HCl aq.

[b] Solvent system A: ammonia buffer[12] (30 parts: 7·2 g of ammonium carbonate in 95 ml water and 7·5 ml NH_4OH aq sp. gr. = 0·88), n-BuOH (35 parts) and EtOH (35 parts). Solvent system B: ammonia buffer, n-BuOH and EtOH (30:52:35).

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[*] Apparently, the α-glycol linkage of aleuritic acid is involved in some abnormal reaction, because similar high results were also obtained in presence of ethylene glycol; that no direct oxidation of α-glycol linkage by Ag_2O is involved is clear from the fact that aleuritic acid alone fails to consume any Ag_2O.

[†] The addition of alkali to a 2,4-dinitrophenylhydrazone soln produces an intense wine-red colour, the absorption maximum (λ_{max} 480 mμ) and specific intensity (ε_{480}, 2·72 × 10^4) of which have been demonstrated[18] to be *nearly* independent of the structure of the carbonyl compound. However, it was experimentally established that an alkaline soln of jalaric acid 2,4-dinitrophenylhydrazone[7] has λ_{max} 480 mμ, ε 1·21 × 10^4 and these values were employed for estimating jalaric acid (and laccijalaric acid) residues in lac resin.

Table 2. Thin layer chromatography[a] characteristics of lac acids and their methyl esters

No.	Compound	R_f (solvent system)[b]		
		Acids		Methyl esters
		A	B	C
1	Butolic (II)	0·72	0·52	0·53
2	Aleuritic (I)	0·28	0·10	0·20
3	Jalaric (VII)	0·42	0·20	—
4	Shellolic (III)	0·43	0·22	0·42
5	*Epi*shellolic (VIII)	0·38	0·13	0·35
6	Laksholic (V)	0·34	0·15	0·26
7	*Epi*laksholic (X)	0·23	0·08	0·13
8	Laccijalaric (XVI)	0·68	0·55	—
9	Laccishellolic (XII)	0·67	0·50	0·77
10	*Epi*laccishellolic (XVII)	0·67	0·47	0·71
11	Laccilaksholic (XIV)	—	0·37	0·56
12	*Epi*laccilaksholic (XIX)	0·43	0·20	0·35

[a] Silica gel layers (0·3 mm), from silica gel containing 15% plaster of Paris; solvent front, 10 cm; temp 29 ± 1°. Visualization agent: conc H_2SO_4 or a soln of chlorosulphonic acid in AcOH (1:2).

[b] Solvent systems:

A: 2% AcOH in EtOAc
B: EtOAc, benzene and AcOH (70:30:0·5)
C: Toluene, EtOAc and acetone (7:4:4).

Table 3. Estimation of aleuritic and aldehydic acid residues in lac resins of various origins

Seedlac		Aleuritic acid (%)[a]		Aldehydic acids (%)[b]	
Name	Host tree	"Hard resin"	"Soft resin"	"Hard resin"	"Soft resin"
Palas	*Butea monosperma* Lamk.	38·5	16·9	31·1	36·1
Kusmi	*Schleichera oleosa* Lour.	45·1	26·5	37·2	34·6
Jalari	*Shorea talura* Roxb.	38·9	28·6	37·2	34·1
Ber	*Zizyphus mauritiana* Lamk.	38·8	23·4	41·0	36·8

[a] Values obtained after hydrolysis: Values obtained before hydrolysis (i.e. for available α-glycol linkages in lac resin, in terms of aleuritic acid) for "hard" and "soft" resins of various origins were respectively: Palas (11·2; 9·8), Kusmi (11·5; 12·5), Jalari (12·2; 11·7) and Ber (11·6; 17·1).

[b] In terms of jalaric acid.

EXPERIMENTAL

All m.p. and b.p. are uncorrected; m.p. taken on Kofler hot stage. Pet. ether refers to the fraction b.p. 40–60°. UV and visible spectral measurements were made on a Perkin–Elmer spectrophotometer, model 350. Alumina used for chromatography was made neutral by the HNO_3 method[20] and graded according to Brockmann[21]; silica gel was graded according to Hernandez[22]; GLC was carried out on "Varian Aerograph" model 700 using 150 cm × 5 cm column packed with 20% silicone SE 30 on Chromosorb W (60–80 mesh), using H_2 as the carrier gas.

Seedlac of various origins was made available through the courtesy of Indian Lac Research Institute, Ranchi.

"Hard" and "soft" resin. Palas seedlac (200 g; −200 mesh) was stirred with distilled water (1·5 l.) for 15 min, the aqueous part decanted and the process of washing repeated (another 6 times). The washed lac

powder was air-dried (4 days) at room temp and the resulting material (196 g) thoroughly dispersed in 78 % EtOH (800 ml) with mechanical stirring (30 min). The alcohol insoluble material was again treated with two further portions of 78 % EtOH (300, 200 ml) as above. The insoluble material on extraction with hot benzene (100 ml × 5) and usual work up gave light orange shellac wax (10 g).

The total EtOH extract was concentrated under reduced press (water bath) to $\frac{1}{4}$ its volume. The concentrate was cooled and with vigorous stirring slowly diluted with ether (2·5 l.). The ppt was collected by straining through muslin cloth and the product was titurated with ether (400 ml × 3) to furnish an insoluble material (121 g), which was soxhleted (12 hr) with dry ether to finally yield the insoluble portion (115 g) as the "hard resin".

The various ether extracts and washings, mentioned above, were combined and the solvent flashed off. The residue was taken up in $CHCl_3$ (450 ml), filtered and the $CHCl_3$ soln extracted with Na_2CO_3aq (15 %; 250 ml × 6). The alkaline extract was acidified (H_3PO_4aq 1 : 1) and the product taken up in EtOAc (500 ml × 5). The combined EtOAc extracts were washed with brine and worked up to give "soft resin" as a dark red sticky mass (38 g).

The $CHCl_3$ soln, mentioned above, was stripped off the solvent to give the neutral part ("odoriferous material", 10 g).

Exactly the same procedure was employed for the isolation of "hard" and "soft" resins from seedlacs of different origins.

Hydrolysis of "hard" resin and separation of resin acids. The well-powdered hard resin (50 g) was added to NaOH aq (1·75N; 225 ml) and the soln allowed to stand at room temp (28–30°) for 5 hr. The resulting clear soln was acidified with H_3PO_4aq (90 ml; 1 : 1) when a gum separated. The gum was washed with water (100 ml × 3) and the aqueous filtrate and washings combined. The gum was once again treated with NaOH aq and worked up exactly as above to give a gum (*vide infra*) and an aqueous portion. The combined aqueous portions from both the saponifications were extracted with EtOAc (250 ml × 4) and the extracts washed with brine and dried (Na_2SO_4).

Jalaric acid and laccijalaric acid. The EtOAc extract was concentrated (~50 ml) under reduced press and the concentrate on standing for several hr at room temp deposited jalaric acid[7] (1·5 g; m.p. 168–172°).

The above mother liquor was freed of solvent to yield a gum (7·5 g), which was extracted with hot $CHCl_3$ (100 ml). The $CHCl_3$-soluble portion (600 mg) was chromatographed on SiO_2 gel/II (20 cm × 2 cm). The first eluate (8 % EtOAc in C_6H_6; 400 ml) was discarded and the next eluate (12 % EtOAc in C_6H_6; 500 ml) on removal of solvent gave crude laccijalaric acid[8] (200 mg, m.p. 161–163°; after one recrystallization from EtOAc, m.p. 164–165°).

Aleuritic acid. The gummy acids (*vide supra*) were dissolved in NaOH aq (40 %; 30 ml) and left aside at room temp for 24 hr. To this NaCl aq (satd 35 ml) was added and the mixture left aside for 96 hr. The Na salt which separated was collected, dissolved in water and the soln treated with H_3PO_4aq (1 : 1) and the crude aleuritic acid (13 g; m.p. 94–96°) collected by filtration. The product after *one* crystallization from ethanol gave pure aleuritic acid[4] (m.p. 98–100°).

Dimethyl shellolate and dimethyl epishellolate. The alkaline filtrate, after separating sodium aleuritate was acidified with H_3PO_4aq (1 : 1) and the acids taken up in EtOAc (250 ml × 3). From the EtOAc extract the solvent was flashed off and the resulting gum (26 g) was thoroughly mixed with anhydrous Na_2SO_4 (200 g) and the material extracted in a Soxhlet apparatus, first with pet. ether (16 hr) and finally with ethanol (8 hr).

The light petroleum extract (280 mg) was esterified (CH_2N_2) to give an ester mixture which was found by mixed GLC (temp 225–230°; gas flow, 45 ml/min) with authentic samples, to consist of (increasing retention times) methyl myristate (~15 %), methyl palmitate (~12 %), methyl butolate (~40 %) and methyl *epi*laccishellolate and methyl laccishellolate (same retention time, ~20 %).

The alcohol extract was freed of solvent and the resulting gum (24·5 g) esterified with CH_2N_2; a part (5 g) of the ester mixtures was chromatographed over neutral Al_2O_3/II (28 cm × 3 cm; solvent: C_6H_6 with increasing amounts of MeOH) and the suitable fraction (C_6H_6 and 1 % MeOH in C_6H_6 eluates, 1·23 g) rechromatographed over SiO_2 gel/IIa (22·5 cm × 2 cm). 15 % EtOAc in C_6H_6 eluate (500 ml × 2) gave dimethyl shellolate[7] (211 mg, m.p. 145–148°; recrystallized from C_6H_6, m.p. 152–153°), while 20 % EtOAc in C_6H_6 (500 ml × 6) eluted dimethyl *epi*shellolate (410 mg, m.p. 145–149°; recrystallized from C_6H_6, m.p. 154–155°).

Estimation of aleuritic acid

(a) *In "hard resin".* Palas hard resin (100 mg) was dissolved in gl AcOH (5·0 ml), 20 ml of a soln of periodic acid (5·0 g of para-periodic acid dissolved in 200 ml water and diluted to 1 l. with gl AcOH) added and the mixture kept in the dark at room temp (25–30°). After 0·5 hr, $CHCl_3$ (5·0 ml) and KI aq (10·0 ml; 20%) were added and the liberated iodine titrated against standard $Na_2S_2O_3$ aq (0·9946N) in the usual way.

By this procedure, crystalline, dry aleuritic acid (m.p. 100–101°) showed 99·4% purity.

(b) *In hydrolysed "hard" resin.* Palas hard resin (2·0 g) was dissolved in NaOH aq (40%, 6·0 ml) by slight warming and left aside at room temp (30 ± 2°) for 24 hr. The reaction mixture was acidified (H_3PO_4 aq 1 : 1; 15 ml) and the liberated acids taken up in EtOAc (30 ml × 2), the extract washed with water (10 ml × 4) and dried (Na_2SO_4). The solvent was next flashed off and the aleuritic acid content of the residue estimated, as detailed above, on a small sample (~ 100 mg).

Estimation of aldehydic acids

Palas "hard" resin (~10 mg) was dissolved in aldehyde-free MeOH (25 ml) and 1 ml methanolic 2,4-dinitrophenylhydrazine (twice recrystallized from MeOH and a satd soln prepared in the same solvent) soln and one drop of conc HCl aq added. The mixture was heated at 55–66° for 3 hr, cooled to room temp and KOH aq (5 ml; 10 g KOH dissolved in 20 ml water and diluted to 100 ml with aldehyde-free MeOH) added, when a wine red soln resulted. A blank was also run side by side. The spectrophotometer was adjusted for 100% transmittance at 480 mμ and the optical density of the sample measured at the same wavelength.

Pure, crystallized jalaric acid (m.p. 178–180°) when analysed by the above procedure showed 96–100% purity.

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