

## Free Acids in Lac Resin

N. PRASAD, S. C. AGARWAL, J. N. CHATTERJEA\* and S. C. SENGUPTA\*\*

Chemistry Division, Indian Lac Research Institute, Namkum, Ranchi-834 010,

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Lac resin has been found to contain 10.70% free fatty and 2.7% free aldehydic terpenic acids. The free fatty acids are composed of (i) saturated and unsaturated straight, chain lengths of  $C_{14}$ ,  $C_{16}$  and  $C_{18}$ , (ii) 6-ketotetradecanoic, (iii) 6-hydroxy-tetradecanoic (butolic), (iv) 16-hydroxyhexadecanoic, (v) 16-hydroxyhexadec-*cis*-9-enoic, (vi) *threo*-9,10-dihydroxy of  $C_{14}$  and  $C_{16}$  chain lengths and (vii) *threo*-9,10,16-trihydroxy hexadecanoic (aleuritic) acids, while the terpenic acids constitute a mixture of jalaric, laccijalaric and a new aldehydic acid ( $C_{14}H_{18}O_2$ ).

**S**TUDIES on the constitution of lac resin<sup>1-8</sup> and its two fractions, the pure<sup>4,10</sup> and the soft<sup>6,9</sup> resins, revealed that at least 18 constituent acids, comprising non-hydroxy and hydroxy fatty acids of  $C_{14}$  to  $C_{18}$  carbon chains and terpenic acids take part in the resin formation. It is believed that these acids are present in the resin mainly through ester<sup>1</sup> and acylal<sup>7-9</sup> linkages. Some workers<sup>10,11</sup>, however, have casually indicated that a few of these acids are free in the resin to some extent. Since the presence of some of the constituent acids in the free state might have some interesting bearing on the resin formation, a systematic study was necessary to arrive at a decisive conclusion. The present paper relates to such an attempt to isolate and identify the free acids present in lac resin.

**Isolation of free acids:** A 30% solution of dewaxed lac in 95% ethanol was exhaustively extracted with *n*-hexane and 12 fractions collected. TLC examination showed the fractions to contain mainly non-, mono-, di- and tri-hydroxy acids. However, butolic acid (6-hydroxytetradecanoic acid) appeared to be the major component of fractions 4 to 12. The fractions were combined and after removal of the neutral fraction and traces of wax, methylated and then acetylated<sup>12</sup>. The acetylated product was fractionated with urea<sup>13</sup> to give adducted (A) and non-adducted (B) esters. TLC examination on silver nitrate impregnated plates<sup>14</sup> revealed that A consisted of saturated and unsaturated non-hydroxy and  $\omega$ -acetoxy esters along with keto ester while B of keto esters along with mono-, di- and triacetoxy esters.

Fractionation of A with increasing proportions of ether in light petroleum on a column of silica gel impregnated with silver nitrate<sup>15</sup> afforded the pure components. Similarly, pure constituents from B were separated over a column of silica gel. The pure constituents were identified by adopting various methods as described in the experimental.

In the above investigation, the presence of free aleuritic acid has only been indicated but its exact

amount, as well as the presence of jalaric and other aldehydic terpenic acids could not be obtained, although their presence in sufficient amounts was reported earlier<sup>10,11</sup>. This may be due to the fact that these acids are practically insoluble in hexane. The following alternate method was, therefore, tried to achieve their isolation.

*Palas* seedlac was dissolved in  $Na_2CO_3$  solution, the sodium salts of lac resin salted out with NaCl solution and the neutral fraction separated from the mother liquor. Half of the liquor was treated for estimation of aleuritic acid and the other half for the terpenic acids. TLC of the mother liquor showed that the aldehydic acids were mainly composed of jalaric acid with small amount of laccijalaric acid and a new aldehydic acid recently isolated by Prasad<sup>16</sup>. Table 1 summarises the percentage of the free acids isolated so far from lac resin in the present study.

TABLE 1—PERCENTAGE OF FREE ACIDS

1. Saturated straight ; chain lengths of $C_{14}$ , $C_{16}$ and $C_{18}$	0.80
2. Unsaturated straight ; chain lengths of $C_{14}$ , $C_{16}$ and $C_{18}$	0.06
3. 6-Ketotetradecanoic	0.45
4. 6-Hydroxytetradecanoic	2.37
5. 16-Hydroxyhexadecanoic	0.57
6. 16-Hydroxyhexadec- <i>cis</i> -9-enoic	0.77
7. <i>Threo</i> -9,10-dihydroxy of chain lengths $C_{14}$ and $C_{16}$	1.37
8. <i>Threo</i> -9,10,16-trihydroxyhexadecanoic (aleuritic)	4.89
9. Aldehydic terpenic	2.68
Total	12.40

The results obtained in the present study confirmed the findings of Sengupta<sup>11</sup> who reported the presence of free acids to the tune of 8-10% in lac resin. Though the presence of  $C_{18}$  straight chain free acids have been indicated, no indication has been observed for the presence of such hydroxy saturated or unsaturated acids in lac resin.

The unsaturated acids indicated at Sl. Nos 2 and 6 appear to be the precursors of various mono-, di- and tri-hydroxy acids. These free acids as such

\* Chemistry Department, Patna University, Patna-800 005.  
\*\* Indian Mica and Micanite, Kodarma, Bihar.

or in loose combination may be considered as the primary building blocks of lac resin. This contention has been further supported by Singh *et al.*<sup>25</sup>, who have succeeded (i) in isolating 4 essentially pure acid esters (jalaric ester—I and II and laccijalaric ester—I and II) constituting the bulk of soft resin, (ii) in synthesising the possible key compounds (jalaric ester—I and laccijalaric ester—I) of lac resin. They have reported that 16-hydroxy hexadec-9-enoic acid seems to play a key role in the elaboration of these compounds by the insect.

### Experimental

All m.p.s and b.p.s are uncorrected. Light petroleum refers to the fraction b.p. 40-60. Silica gel for column chromatography was 100-200 mesh. TLC was carried out on silica gel layers (0.3 mm) containing 15% gypsum; visualization agent—iodine vapours or heating at 150°. Mixed solvent composition is by volume. Perkin-Elmer Infracord 700A and AIMIL, Gas-liquid chromatography equipment were used for spectral and constituent analyses, respectively.

Dewaxed shellac (100 g), prepared from *Palas* seedlac by the method of Khurana *et al.*<sup>4</sup>, was dissolved in 95% methanol (300 ml) and exhaustively extracted with 1 litre lots of *n*-hexane in succession. Altogether 12 fractions, totalling 7.06 g, which were 2.98, 1.25, 0.97, 0.63, 0.42, 0.29, 0.27, 0.09, 0.06, 0.05, 0.04 and 0.014 g, respectively, were isolated. The tlc examination of the fractions (solvent system, 2% acetic acid in ethyl acetate) along with authentic samples revealed that all the fractions were mixtures of mainly non-, mono-, di-, tri-hydroxy and keto acids. However, butolic acid was found as the major component in fractions 4 to 12. These fractions were, therefore, mixed together and repeatedly extracted with warm light petroleum. The warm extract yielded a crystalline mass (0.65 g) on cooling which, after recrystallisation from aqueous methanol, melted at 56-57°. The acid was identified as butolic (6-hydroxytetradecanoic) acid on the basis of its tlc ( $R_f$  0.73), mixed m.p. (55-56°), oxidative degradation and ir spectrum 3225  $\text{cm}^{-1}$  (OH), 1710  $\text{cm}^{-1}$  (COOH).

As the nature of the first 3 fractions and the combined one, left after separation of butolic acid, was similar, they were combined, taken in ether and extracted with 10%  $\text{Na}_2\text{CO}_3$  solution (100 ml  $\times$  4) leaving the wax and neutral portions in the ether. The acidic mass (6.04 g) recovered on acidification of  $\text{Na}_2\text{CO}_3$  extract, was converted into methyl esters by refluxing with 1% methanolic  $\text{H}_2\text{SO}_4$  for 4 hr. The methyl esters were then acetylated<sup>18</sup> with acetic anhydride (35 ml) at 100° for 4 hr to yield 6.6 g acetylated esters.

**Urea-fractionation<sup>18</sup>**: The acetoxy esters (6.5 g) were dissolved in methanol (70 ml), urea (12 g) added and warmed to get a clear solution. The solution was allowed to stand at room temperature for 48 hr. The adduct was separated, washed with methanol saturated with urea, decomposed with

warm water and extracted with ether yielding adducted acetoxy ester (A; 2.12 g). Similarly, non-adducted acetoxy (B; 4.41 g) was also recovered. TLC of the fractions on silver nitrate impregnated plates<sup>14</sup> indicated A to contain saturated and unsaturated non-hydroxy and  $\omega$ -acetoxy esters along with keto esters [solvent system, light petroleum: ether (80:20)] while B to contain keto esters along with mono-, di- and tri-acetoxy esters.

### Chromatography of adducted esters A (2.10 g):

The fraction A was chromatographed in 6 lots on silica gel columns impregnated with silver nitrate<sup>18</sup> (10 g) mixed with hyffosuperpel (5 g) and eluting with 100 ml lots of light petroleum-ether mixtures and collecting in 10 ml portions. The fractionation was monitored by tlc on silver nitrate impregnated plates and the corresponding fractions combined, giving finally 6 fractions (A-1 to A-6; 2.02 g). The fractions A-1 and A-2 were saturated ( $R_f$  0.65) and unsaturated ( $R_f$  0.46) non-hydroxy esters, respectively while the fractions A-3 and A-5 were saturated ( $R_f$  0.32) and unsaturated ( $R_f$  0.25)  $\omega$ -acetoxy esters, respectively. Fraction A-4 appeared to be keto ester ( $R_f$  0.61) and A-6 was a mixture of esters. Fractions A-1 and A-2 were identified by glc as mixtures of saturated and unsaturated  $\text{C}_{14}$ ,  $\text{C}_{16}$  and  $\text{C}_{18}$  ordinary esters respectively on the column of polyethylene glycol adipate (PEGA, 10% w/w) at a column temperature of 200° by running separately with standard esters.

### $\omega$ -Acetoxy esters:

**Saturated**: The hydroxy acid derived from fraction A-3 was shown to be 16-hydroxy hexadecanoic acid on the basis of the following evidences.

The acid, on crystallisation from ether, melted at 92-93° (lit<sup>17</sup> 91-93°). Mixed m.p. 93-94°; ir (methyl ester, m.p. 52-53°): 3278  $\text{cm}^{-1}$  (OH), 1047  $\text{cm}^{-1}$  (primary OH) and 1718  $\text{cm}^{-1}$  (ester carboxyl). Oxidation<sup>18</sup> of the acid gave 1,16-hexadecane dioic acid<sup>19</sup>, m.p. 118-119°. The chain length determination<sup>20</sup> showed that the ester was mainly  $\text{C}_{16}$  in its chromatographic behaviour over glc. (Found: C, 70.67; H, 11.72.  $\text{C}_{16}\text{H}_{32}\text{O}_2$  requires C, 70.59; H, 11.76%). (A.V. = 200.7, H.V. = 194.0 and acid equiv. = 279.0).

**Unsaturated**: The liquid unsaturated hydroxy acid isolated from A-5 was confirmed as 16-hydroxy hexadec-*cis*-9-enoic acid on the basis of its oxidation<sup>21,22</sup> to  $\text{C}_7$  and  $\text{C}_6$  dibasic acids, hydrogenation to saturated  $\omega$ -hydroxy acid and ir spectrum. The methyl ester did not show any peak at 968  $\text{cm}^{-1}$  corresponding to a *trans* double bond indicating its configuration as *cis*. (Found: C, 70.22; H, 10.57.  $\text{C}_{16}\text{H}_{30}\text{O}_2$  requires C, 71.11; H, 11.11%).

**Keto ester (A-4)**: The acid recovered from fraction A-4 melted at 70-71° (mixed m.p. 70-71°) was identified as 6-keto tetradecanoic acid on the basis of tlc (methyl ester,  $R_f$  0.61), ir (two peaks at 1737  $\text{cm}^{-1}$  and 1718  $\text{cm}^{-1}$  corresponding to keto and ester carboxyl groups, respectively), semi carbazone derivative<sup>23</sup> (m.p. 129-130°) and sodium

borohydride reduction to 6-hydroxy tetradecanoate (methyl butolate).

**Chromatography of non-adducted esters B :**

The non-adducted acetoxy esters **B** (4.4 g) were chromatographed over silica gel into 4 fractions (**B-1** to **B-4**; 4.21 g) with increasing proportions of ether in light petroleum. TLC showed **B-1**, **B-2** and **B-3** to be 6-ketotetradecanoate ( $R_f$  0.61), acetoxy butolate ( $R_f$  0.42) and diacetoxy ester ( $R_f$  0.28), respectively. **B-4** was mainly triacetoxy ester ( $R_f$  0.12). The acids recovered from **B-1** and **B-2** were identified in usual manner as described earlier.

**Diacetoxy ester B-3 :** The acid derived from fraction **B-3** formed isopropylidene<sup>24</sup> derivative. The derivative was a liquid which moved as single spot ( $R_f$  0.62). The corresponding  $R_f$  value for the derivative of an authentic 9-10-dihydroxy tetradecanoic acid was 0.61 in the solvent system, light petroleum : ether : methanol (50 : 40 : 10). The dihydroxy acid has  $R_f$  0.30 and its methyl ester  $R_f$  0.25. The hydroxy ester behaved in a similar manner as methyl *threo*-isomer on a tlc plate impregnated with boric acid<sup>24</sup> in the solvent system, light petroleum : ether (60 : 40). Chromic acid as well as potassium permanganate and periodate oxidation<sup>21</sup> yielded azelaic acid as one of the oxidation products. The other fission products may presumably be either pentanal or heptanal if the dihydroxy acid is  $C_{14}$  or  $C_{16}$ . Since either of the two fission products could not be isolated in pure form, it is presumed that the fraction **B-3** is probably a mixture of *threo*-9-10-dihydroxy  $C_{14}$  and  $C_{16}$  acids. The melting point of the acid (m.p. 83-87°) was, however, not sharp.

**Triacetoxy ester B-4 :** Fraction **B-4** on hydrolysis afforded a trihydroxy acid (0.29 g) which melted at 99-100°. The acid was identified as *threo*-9,10,16-trihydroxyhexadecanoic acid (aleuritic acid) by the established methods.

**Isolation of free aldehydic terpenic acids :** Powdered *Palas* seedlac (100 g) was dissolved in  $Na_2CO_3$  solution (4%) by warming and diluting the solution to 1 litre with warm water. The cold solution was filtered to remove wax and impurities. The sodium salts of lac resin were then salted out with saturated NaCl solution and allowed to stand overnight. The clear liquor was decanted and the residue washed twice with NaCl solution. The solutions were mixed together and extracted with ether to remove the neutral portion, if any. The liquor portion was then divided into two parts (X) and (Y).

The first part (X) was decomposed with dil.  $H_2SO_4$ , impurities filtered and the solution extracted with ethyl acetate. The resinous mass thus obtained was examined by tlc, [solvent system, ethyl acetate : acetic acid (100 : 2)], plate sprayed with 2,4 DNP solution which revealed it to be a mixture of jalaric ( $R_f$  0.44), *laccijalaric* ( $R_f$  0.65) and an unknown aldehydic acid ( $R_f$  0.56). The resinous mass was then digested with chloroform

and 2 fractions, chloroform-soluble and chloroform-insoluble, were separated.

**Chloroform-insoluble fraction :** It was gummy in nature. TLC showed it to be a mixture of mainly jalaric and *laccijalaric* acid ( $R_f$  0.42 and 0.65). It was chromatographed over silica gel eluting with increasing proportion of ethyl acetate in benzene and collecting altogether three fractions. The first fraction ( $R_f$  0.42) was confirmed as jalaric acid by adopting the established procedures. The second fraction was a mixture of jalaric and *laccijalaric* acids while the third fraction was identified as *laccijalaric* acid ( $R_f$  0.65).

**Chloroform-soluble fraction :** It was evaporated to dryness to get a viscous liquid mass which showed a single spot ( $R_f$  0.56) on tlc plate sprayed with 2,4-DNP reagent. The acid could not be crystallised and hence 2,4-DNP derivative was prepared which melted at 113-115°. IR : 1740  $cm^{-1}$  (-CHO), 1725  $cm^{-1}$  (-COOH) and 1610, 1550  $cm^{-1}$  (-C=C, absence of -OH group). UV :  $\lambda_{max}^{EtOH}$  210 nm and  $\epsilon=7,280$ . (Found : C, 73.18 ; H, 7.30.  $C_{18}H_{18}O_8$  requires C, 73.17 ; H, 7.31%). A.V.=228.8, acid equiv.=246. Mol. formula  $C_{18}H_{18}O_8$ . It appears that the acid does not resemble either jalaric or *laccijalaric* acid but seems to be a new one. The structure is being studied.

In another lot, the resinous mass containing the mixture of aldehydic acids was treated with excess of aqueous 2,4-DNP solution. There was immediate separation of yellow precipitate. The mixture was heated and allowed to stand overnight. The precipitate was filtered and repeatedly washed with dil. HCl. The derivative, on recrystallisation from absolute MeOH, melted at 204-207° with decomposition. The weight of the pure derivative was 4.4 g which amounts to 2.68 g of aldehydic acids on the basis of jalaric acid, the major component.

**Isolation of free aleuritic acid :** The second part (Y) of the mother-liquor was made just acidic with dil.  $H_2SO_4$  and extracted with *n*-butanol. The extract was dried over anhydrous  $Na_2SO_4$ , distilled and finally evaporated to dryness to get a viscous compound (0.47 g from 200 ml mother liquor). TLC showed it to be a mixture of 2 components ( $R_f$  0.22 and 0.79) indicating one of the components as aleuritic acid [ $R_f$  0.21 in the solvent system ethyl acetate-acetic acid (100 : 2)]. The amount of aleuritic acid in this compound was estimated by adopting the method of Sengupta<sup>25</sup> and found to be 4.83%.

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