

### 1111. Shellac. Part II.\* Some Minor Aliphatic Constituents.

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In addition to aleuritic acid and 6-hydroxytetradecanoic acid, hydrolysis of lac resin furnishes the n-C<sub>14</sub>, -C<sub>16</sub>, and -C<sub>18</sub> acids and their mono-olefinic derivatives (0.5%), 6-oxotetradecanoic acid (0.25%), 16-hydroxyhexadecanoic acid (0.5%), 16-hydroxyhexadec-9-enoic acid (1.5%), *threo*-9,10-dihydroxytetradecanoic acid (3%), and *threo*-9,10-dihydroxyhexadecanoic acid (1%). Lac resin is shown to contain some ether-linked hydroxy-acids which remain as polymers after alkaline hydrolysis.

LAC RESIN has long been considered to be a mixture of cross-linked polyesters derived from straight chain or cyclic hydroxy-acids. Evidence concerning the structure of these hydroxy-acids has been presented from time to time but until recently there was agreement only about aleuritic acid and shellolic acid (for detailed references see ref. 1). To these two must be added one aliphatic constituent, butolic acid,<sup>1-3</sup> and a number of cyclic acids,<sup>3,4</sup> and we now report several additional minor aliphatic constituents which together account for about 7% of the lac we used.

During our investigations we realised that lac resin contains ether bonds as well as ester bonds with the result that alkaline hydrolysis gives not only monomeric acids, but also some ether-linked polymeric fragments. Thereafter we separated the monomeric and polymeric hydrolysis products and concentrated our attention on the former. We were led to this conclusion by the isolation of a fraction consisting mainly of ether-linked aleuritic acid units. This fraction, of molecular weight  $625 \pm 15$ , gave only palmitic acid when submitted to iodination-deiodination;<sup>1</sup> it was unaffected by alkaline hydrolysis, and it gave aleuritic acid along with some mono- and di-hydroxypalmitic acids when submitted to acid hydrolysis. Since the substance was unchanged when treated with acetone and anhydrous copper sulphate and gave no recognisable degradation fragments when treated with periodate-permanganate reagent<sup>5</sup> it did not contain any vicinal diol group. The presence of ether links between hydroxy-acids, in addition to the more common ester bonds, will be important in understanding the nature of the polymeric resin.

*Non-hydroxy-acids.*—In several attempts to separate the lac hydrolysate by adsorption chromatography the first eluate always contained a mixture (0.5%) of saturated and unsaturated non-hydroxy-esters. These two types of ester were separated on columns of silicic acid impregnated with silver nitrate<sup>6</sup> and each was subsequently shown, by gas-liquid chromatography, to contain C<sub>14</sub>, C<sub>16</sub>, and C<sub>18</sub> members. The saturated esters behaved in the same way as the n-esters on Apiezon-L and on QF-I columns. The unsaturated esters were separated by preparative gas-liquid chromatography and then submitted to periodate-permanganate oxidation.<sup>5</sup> The C<sub>16</sub> and C<sub>18</sub> acids were  $\Delta^9$  compounds, accompanied by only traces of the  $\Delta^8$  isomers, the C<sub>14</sub> fraction contained the  $\Delta^9$  compound (~50%) along with significant amounts of the  $\Delta^5$  and  $\Delta^6$  isomers and minor amounts of the  $\Delta^7$  and  $\Delta^8$  isomers.

*$\omega$ -Hydroxy-acids.*—After the non-hydroxy-esters,  $\omega$ -hydroxy-esters were eluted from silicic acid columns along with other hydroxy-esters. These were conveniently separated by acetylation and treatment with urea when the  $\omega$ -acetoxy-esters more readily formed an adduct.<sup>7</sup> The adduct contained a mixture of saturated and unsaturated  $\omega$ -hydroxy-C<sub>16</sub>

\* Part I, *J.*, 1963, 5768.

<sup>1</sup> W. W. Christie, F. D. Gunstone, and H. G. Prentice, *J.*, 1963, 5768.

<sup>2</sup> S. C. Sen Gupta and P. K. Bose, *J. Sci. Ind. Res. (India)*, 1952, **11**, B, 458.

<sup>3</sup> R. G. Khurana, M. S. Wadia, V. V. Mhaskar, and Sukh Dev, *Tetrahedron Letters*, 1964, 1537.

<sup>4</sup> M. S. Wadia, V. V. Mhaskar, and Sukh Dev, *Tetrahedron Letters*, 1963, 513.

<sup>5</sup> E. von Rudloff, *J. Amer. Oil Chemists' Soc.*, 1956, **33**, 126.

<sup>6</sup> B. de Vries, *Chem. and Ind.*, 1962, 1049; *J. Amer. Oil Chemists' Soc.*, 1963, **40**, 184.

<sup>7</sup> A. P. Tulloch, J. F. T. Spencer, and P. A. J. Gorin, *Canad. J. Chem.*, 1962, **40**, 1326.

esters which were again separated on silicic acid impregnated with silver nitrate. Since the saturated component (0.5%) was reduced to palmitic acid and oxidised to hexadecanedioic acid it must be 16-hydroxyhexadecanoic acid. The unsaturated component (1.5%) was oxidised, as its *O*-methyl derivative, to 7-methoxyheptanoic acid and azelaic acid and must therefore be 16-hydroxyhexadec-9-enoic acid. The absence of any pronounced peak in its infrared spectrum at 10.3  $\mu$  showed it to be the *cis*-isomer. There was also chromatographic evidence for a very small quantity of 14-hydroxytetradecanoic acid. Early reports of saturated<sup>8</sup> and unsaturated<sup>9</sup> monohydroxypalmitic acids in shellac contain no structural details.

**6-Oxotetradecanoic Acid.**—Lac resin is known to furnish 6-hydroxytetradecanoic acid<sup>1-3</sup> and we now find this acid to be accompanied by small amounts (0.25%) of 6-oxotetradecanoic acid. The esters which did not form an adduct in the urea fractionation already described, when eluted again from a silicic acid column, gave this keto-ester. The presence of the keto-group was shown by its infrared spectrum, and its chromatographic behaviour on columns of Apiezon-L and QF-1 was identical with that of synthetic methyl 6-oxotetradecanoate.<sup>1</sup> Its structure was confirmed by the following observations: it was unaffected by methylation with methyl iodide and silver oxide; it was reduced to methyl hydroxytetradecanoate with sodium borohydride and to methyl tetradecanoate by iodination-deiodination; and it was degraded to nonanoic acid and adipic acid by hydrolysis of the amides formed by Beckmann rearrangement of its oximes.

**Dihydroxy-acids.**—After elution of non-hydroxy- and monohydroxy-esters there is obtained a large fraction (~35%) containing aleuritic acid along with some minor components. Repeated chromatography of this eventually gave two pure fractions which were shown to be *threo*-9,10-dihydroxytetradecanoic acid (3%) and *threo*-9,10-dihydroxyhexadecanoic acid (1%). These structures follow from the products of iodination-deiodination and of periodate-permanganate oxidation<sup>5</sup> and from the formation of isopropylidene derivatives. The behaviour of these esters on thin layers of silica impregnated with boric acid<sup>10</sup> showed them to be the *threo*-isomers. With the quantities available neither of these acids shows measurable optical rotation.

From our results we consider the lac resin we examined to contain the acids in Table 1:

TABLE 1.

Tetradecanoic (20%)	}	0.5%	16-Hydroxyhexadec-9-enoic	1.5%
Hexadecanoic (50%)			<i>threo</i> -9,10-Dihydroxytetradecanoic	3%
Octadecanoic (5%)			<i>threo</i> -9,10-Dihydroxyhexadecanoic	1%
Tetradecenoic (5%)			Aleuritic	22%
Hexadecenoic (15%)			Unidentified aliphatic acids	8%
Octadecenoic (5%)			Unidentified polyhydroxy-compound	2%
6-Oxotetradecanoic		0.25%	Non-aliphatic acids	24%
6-Hydroxytetradecanoic		5%	Polymeric acids	32%
16-Hydroxyhexadecanoic		0.5%		

## EXPERIMENTAL

Light petroleum had b. p. 40–60°. Gas-liquid chromatography was carried out on methyl esters with a Pye Argon chromatograph and 4-ft. columns of Celite coated with Apiezon L (2.5%, 5%, or 10%), the silicone elastomer SE-30 (10%), or the fluorinated silicone QF-1 (10%). Relative retention times are reported as "carbon numbers,"<sup>11</sup> and the abbreviation g.l.c. after the name of a compound indicates that its "carbon number" is listed in Table 2. Separations by column chromatography using neutral alumina or silicic acid were monitored by thin-layer chromatography with silica gel G (Merck) and with various mixtures of ether, light petroleum, and methanol as developing solvent; the separated components were detected by exposure of the plate to iodine vapour. Silica (20 g.) mixed with silver nitrate (10 g.) was used where appropriate and the iodination-deiodination reaction for removal of hydroxyl groups was carried out as already described.<sup>1</sup>

<sup>8</sup> A. Tschirch and F. Lüdy, *Helv. Chim. Acta*, 1923, **6**, 994.

<sup>9</sup> D. N. Gupta, *J. Indian Inst. Sci.*, 1924, **70**, 142.

<sup>10</sup> L. J. Morris, *J. Chromatog.*, 1963, **12**, 321.

<sup>11</sup> F. P. Woodford and C. M. van Gent, *J. Lipid Res.*, 1960, **1**, 188.

TABLE 2.  
Carbon numbers of methyl esters.

Methyl ester	SE-30*	QF-1*	Ap. L*
Tetradecanoate	—	14·0	13·8
Hexadecanoate	—	16·0	15·8
Octadecanoate	—	18·0	17·8
Hexanedioate	—	11·5	9·0
Nonanedioate	—	14·6	11·8
Hexadecanedioate	—	21·7	18·8
7-Methoxyheptanoate	—	10·5	9·1
6-Oxotetradecanoate	—	18·9	15·4
6-Hydroxytetradecanoate	—	18·0	15·8
6-Methoxytetradecanoate	—	15·8	15·1
6-Acetoxytetradecanoate	16·4	19·0	15·9
16-Hydroxyhexadecanoate	—	20·8	18·4
16-Methoxyhexadecanoate	—	19·0	17·7
16-Acetoxyhexadecanoate	19·8	23·0	19·2 (5%)
16-Hydroxyhexadecanoate	—	20·8	18·8
16-Methoxyhexadecanoate	—	19·0	17·9
16-Acetoxyhexadecanoate	19·8	23·0	19·5 (5%)
<i>threo</i> -9,10-Dihydroxytetradecanoate	—	20·8	—
Isopropylidene derivative	16·5	—	—
<i>threo</i> -9,10-Diacetoxytetradecanoate	18·6	—	—
<i>threo</i> -9,10-Dihydroxyhexadecanoate	—	—	19·4 (2·5%)
Isopropylidene derivative	18·5	18·4	18·2
<i>threo</i> -9,10-Dimethoxyhexadecanoate	—	—	17·7
<i>threo</i> -9,10-Diacetoxyhexadecanoate	20·3	—	—
9,10,16-Trihydroxyhexadecanoate	—	—	—
Isopropylidene derivative	—	—	20·3 (2·5%)
9,10,16-Trimethoxyhexadecanoate	—	—	19·7 (2·5%)
9,10,16-Triacetoxyhexadecanoate	23·8	—	—
Di- <i>O</i> -methoxyshellolate	—	—	19·1 (2·5%)
Di- <i>O</i> -acetylshellolate	21·6	—	—

\* 10% unless otherwise indicated in parentheses.

*Preparation and Separation of Lac Esters.*—Lac methyl esters were prepared from "Super Blonde Shellac" by the method previously described<sup>1</sup> or in the following manner. The shellac (22·1 g.) was hydrolysed as before and the acids (23·0 g.) were methylated with diazomethane and then acetylated with acetic anhydride. The acetoxy-esters (30·7 g.), after removal of an ether-insoluble polyhydroxy-compound (0·45 g.), gave a distillate (17·5 g.) at 140° and 10<sup>-3</sup> mm. pressure and a residue (8·7 g.). The distillate, in five portions, was eluted from silicic acid columns with light petroleum containing increasing proportions of ether. Non-acetoxy-esters (0·3%, no ether), monoacetoxy-esters (9·8%, 20% ether), and acetylated aleuritane with other minor components (36·7%, 40% ether) were eluted with the solvents indicated. These fractions, and others obtained from undistilled hydroxy-esters, were examined as described below.

*Evidence for Ether-linked Polymeric Fragments resulting from Alkaline Hydrolysis.*—Lac acetoxy-esters which had been repeatedly submitted to chromatography gave a fraction (370 mg.) which ran as a single spot on thin-layer chromatography both before and after alkaline hydrolysis but was not eluted under the usual conditions of gas-liquid chromatography. This fraction showed the following properties:

(i) The material had molecular weight of  $625 \pm 15$  compared with a value of 618 expected for two methyl aleuritane molecules joined through one ether bond. (The molecular weight was measured with a Mechrolab vapour pressure osmometer with benzene as solvent.)

(ii) After alkaline hydrolysis and treatment with methanolic boron trifluoride, gas-liquid chromatography showed a range of esters including aleuritane. After reacylation triacetoxy- (g.l.c.), diacetoxy- ("carbon number" 20·3, SE-30), and monoacetoxy- ("carbon number" 18·5) palmitates were present. Oxidation of the hydroxy-esters (chromium trioxide-acetic acid) gave mainly heptanedioic and nonanedioic acids (g.l.c.).

(iii) When the original fraction was treated with methanolic boron trifluoride or methanolic hydrogen chloride, without prior alkaline hydrolysis, it behaved in a similar way.

(iv) When hydrolysed and submitted to iodination-deiodination the polymeric product gave methyl palmitate (g.l.c.).

(v) When hydrolysed and oxidised (periodate–permanganate) no short-chain products could be detected.

(vi) The hydroxy-ester resulting from reaction with methanolic sodium methoxide did not form an isopropylidene derivative with acetone and anhydrous copper sulphate.

*Non-hydroxy-acids.*—Gas-liquid chromatographic examination of this fraction showed saturated and unsaturated C<sub>14</sub>, C<sub>16</sub>, and C<sub>18</sub> esters to be present. A portion of this mixture (174 mg.) was eluted, from a column of silicic acid impregnated by silver nitrate, with light petroleum (17 mg.) and with light petroleum containing 1% (78 mg.), 2% (17 mg.), 3% (43 mg.), and 5% ether (1 mg.). The unsaturated material (43 mg.) was subsequently separated into C<sub>14</sub> (4 mg.), C<sub>16</sub> (9 mg.), and C<sub>18</sub> (11 mg.) components by preparative gas-liquid chromatography on a Perkin-Elmer fractometer with 20% Apiezon-L column at 225°. Each fraction was hydrolysed and oxidised.

The C<sub>18</sub> acid (10 mg.), for example, dissolved in water (5 ml.) containing potassium carbonate (180 mg.), was shaken for 24 hr. with a solution of potassium permanganate (1 mg.) and potassium periodate (70 mg.) in water (20 ml.). Excess of oxidant was then destroyed with sulphur dioxide, the solution was neutralised, reduced to about half-volume in a rotary film evaporator, acidified, saturated with sodium chloride, and extracted with ether (5 × 30 ml.). The extract was methylated and shown by gas-liquid chromatography to be a mixture of nonanoic and nonanedioic acids with a trace of decanoic acid.

The C<sub>16</sub> acid was similarly degraded to heptanoic acid, octanoic acid (trace), and nonanedioic acid whilst the C<sub>14</sub> acid gave mainly pentanoic and nonanedioic acids accompanied by lesser amounts of the C<sub>6</sub>–C<sub>9</sub> monobasic acids.

*ω-Hydroxy-acids.*—A chromatographic fraction (22 g.), enriched in ω-hydroxy-esters but also containing other hydroxy-esters, was heated at 100° for 4 hr. with acetic anhydride. After removal of the excess of anhydride at 70° under reduced pressure the acetylated esters (24.9 g.) were dissolved in methanol (200 ml.) along with urea (35 g.). Next day the adduct was decomposed with water to release the adduct-forming esters (2.27 g.). These, treated a second time with urea, gave an adduct-forming fraction (0.71 g.) which contained saturated and unsaturated ω-acetoxy-C<sub>16</sub> esters (g.l.c.) along with two minor components of "carbon number" 17.7 and 15.8 (5% Ap. L.). The major components gave a single compound (g.l.c.) when hydrogenated and furnished the corresponding hydroxy-esters (g.l.c.) when deacetylated.

Saturated and unsaturated acetoxy-esters (688 mg.) were separated on a column of silicic acid impregnated with silver nitrate (30 g.). Most of the saturated ester (154 mg.) was eluted with light petroleum containing 10% ether and most of the unsaturated ester (200 mg.) with light petroleum containing 15% ether.

The saturated acetoxy-ester was deacetylated<sup>12</sup> by methanol-sodium methoxide to the hydroxy-ester (m. p. 50–52°, lit.,<sup>13</sup> 55°). This was reduced by iodination-deiodination to methyl palmitate accompanied by about 2% of methyl myristate, and oxidised by chromium trioxide in aqueous acetone and sulphuric acid<sup>14</sup> to an acidic compound which, after methylation, behaved like methyl hexadecanedioate (g.l.c., m. p. 51–52°, lit.,<sup>15</sup> 52°). Hydrolysis of the acetoxy-ester gave 16-hydroxyhexadecanoic acid, m. p. 90–91° (lit.,<sup>13</sup> 95°) (Found: C, 70.9; H, 11.8. Calc. for C<sub>16</sub>H<sub>32</sub>O<sub>3</sub>: C, 70.6; H, 11.8%).

The unsaturated acetoxy-ester (73 mg.) was deacetylated and the hydroxy-ester (65 mg.) was methylated by refluxing with silver oxide and methyl iodide in tetrahydrothiophen 1,1-dioxide for 8 hr. The reaction mixture was treated with an aqueous solution of potassium cyanide, extracted with ether, and traces of the dioxide were finally removed by percolation through alumina from which the methoxy-ester (71 mg. g.l.c.) was eluted with benzene-ether (3:1). Its acid, oxidised in potassium carbonate solution with potassium permanganate and potassium periodate, gave azelaic acid (g.l.c.) and 7-methoxyheptanoic acid (g.l.c.). *cis*-16-Hydroxyhexadec-9-enoic acid melted at 17–19° and showed no absorption peak at 10.3 μ (Found: C, 70.4; H, 10.8. C<sub>16</sub>H<sub>30</sub>O<sub>3</sub> requires C, 71.1; H, 11.2%).

*6-Oxoletradecanoic Acid.*—When the acetoxy-esters which did not form a urea adduct were re-converted into hydroxy-esters by hydrolysis and methylation, and again separated by chromatography on silicic acid, a keto-ester was eluted with benzene-ether (9:1). This keto-ester (g.l.c.)

<sup>12</sup> E. J. Gauglitz and L. W. Lehmann, *J. Amer. Oil Chemists' Soc.*, 1963, **40**, 197.

<sup>13</sup> P. Chuit and J. Hausser, *Helv. Chim. Acta*, 1929, **12**, 487.

<sup>14</sup> M. Matic, *Biochem. J.*, 1956, **63**, 168.

<sup>15</sup> P. Chuit, *Helv. Chim. Acta*, 1926, **9**, 274.

was unchanged by methylation but was reduced by sodium borohydride to a hydroxy-ester (g.l.c.) which could be methylated (g.l.c.). The original ester showed carbonyl absorption in its infrared spectrum and hydroxyl absorption in the spectrum of its reduction product. Iodination-deiodination of the hydroxy-ester gave methyl myristate as almost the only product. The oximes (35 mg.) from the keto-ester (33 mg.) were submitted to Beckmann rearrangement;<sup>1</sup> hydrolysis of the resulting amides gave nonanoic and adipic acid (g.l.c.). Both the keto-acid (m. p. 68–69°) and its semicarbazone (m. p. 129–131°) were identical with synthetic specimens.<sup>1</sup>

*threo-9,10-Dihydroxytetradecanoic Acid.*—Further chromatography of the impure acetylated aleuritane, eluted from a silicic acid column with light petroleum-ether (6:4), eventually gave a fraction enriched in the diacetyl derivative of this hydroxy-acid and another containing the C<sub>16</sub> homologue.

The acetoxy-ester (g.l.c.) was deacetylated<sup>12</sup> and the hydroxy-ester converted into methyl myristate by iodination-deiodination. Oxidation with chromium trioxide in aqueous acetone and sulphuric acid<sup>14</sup> gave mainly nonanedioic acid (g.l.c.). The dihydroxy-ester formed an isopropylidene derivative (g.l.c.) with dry acetone and anhydrous copper sulphate. The dihydroxy-acid, m. p. 78.5–79.5° [lit.,<sup>16</sup> 80° for the (±)-acid] (Found: C, 64.5; H, 10.9. Calc. for C<sub>14</sub>H<sub>28</sub>O<sub>4</sub>: C, 64.6; H, 10.8%), was oxidised by periodate-permanganate<sup>5</sup> to nonanedioic acid (g.l.c.). The methyl ester, m. p. 58–60° (Found: C, 65.4; H, 10.9. Calc. for C<sub>15</sub>H<sub>30</sub>O<sub>4</sub>: C, 65.7; H, 11.0%), behaved in the same way as methyl *threo-9,10*-dihydroxystearate on a thin layer of silica impregnated with boric acid.<sup>10</sup>

*threo-9,10-Dihydroxyhexadecanoic Acid.*—When the diacetoxy-C<sub>16</sub> ester was deacetylated and then iodinated-deiodinated it gave methyl palmitate. Periodate-permanganate oxidation<sup>5</sup> of the dihydroxy-acid gave heptanoic acid and nonanedioic acid. Its methyl ester formed an isopropylidene derivative (g.l.c.) and behaved in the same way as methyl *threo-9,10*-dihydroxystearate on thin layers of silica impregnated with boric acid.<sup>10</sup> The dihydroxy-acid melted at 93–94° [lit.,<sup>17</sup> 87° for the (±)-acid] (Found: C, 66.8; H, 11.0. Calc. for C<sub>16</sub>H<sub>32</sub>O<sub>4</sub>: C, 66.6; H, 11.2%), and its methyl ester at 62–66° [lit.,<sup>17</sup> 65° for the (±)-ester] (Found: C, 68.6; H, 11.3. Calc. for C<sub>17</sub>H<sub>34</sub>O<sub>4</sub>: C, 67.5; H, 11.3%).

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<sup>16</sup> B. W. Boughton, R. E. Bowman, and D. E. Ames, *J.*, 1952, 671.

<sup>17</sup> T. P. Hilditch, *J.*, 1926, 1828.