Tetrahedron Vol 30, pp. 3689 to 3693. Pergamon Press 1974. Printed in Great Britain

CHEMISTRY OF LAC RESIN—VII[a]

PURE LAC RESIN—3: STRUCTURE[b]

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(*Received in the UK* 1 *May* 1974; *Accepted for publication* 25 *June* 1974)

Abstract—It has been found that gas-liquid-chromatography of methyl esters of lac acids can be carried our after formylation of free OH groups. By this method, it has been found that pure lac resin is based on terpenic acids (essentially jalaric acid) and aleuritic acid in a 1 : 1 ratio and not in a 2 : 1 ratio as suspected earlier. This conclusion is clearly supported by a comparision of 220 MHz PMR spectra of pure lac resin derivatives with suitable reference samples. The PMR spectral data, taken along with the earlier results lead to the most plausible structure **11** for 'pure lac resin'.

In continuation of our earlier investigations[1,2] on pure lac resin we wish to report a reliable procedure for the estimation of constituent lac acids by GLC and the finding that pure lac resin, like jalaric ester-II (**10**), is *essentially* derived from equimolar proportions of jalaric (**5**) and aleuritic acids (**2**). Furthermore, on the basis of PMR spectral data and earlier degradation experiments[2], it has become feasible to propose a most plausible formula for pure lac resin.

GLC determination of individual lac acids. It has been stated earlier[1] that attempted GLC of pure dimethyl shellolate/dimethyl epishellolate (on 0·25% SE 30 or 1% Silicone QF supported on 60–80 mesh Chromosorb W) led to decomposition and hence, estimation of these and other lac acids was carried out in terms of GLC of their CrO_3 oxidation products (after esterification). However, this procedure, which we had referred to as semi-quantitative, has two major drawbacks in that the yields of oxidation products from terpenic lac acids are in the range 60–75% only and secondly, as much as 35% of the total oxidation products from jalaric acid (**5**), the major terpene lac acid, remained unidentified. In view of this, work was continued to develop a more straightforward method of estimation.

Less volatile organic compounds are often subjected to GLC after converting them first into more volatile derivatives.[3] Probing experiments on lac acids (as Me esters) revealed that their formyl

$CH_3 \cdot (CH_2)_7 \cdot CHOH \cdot (CH_2)_4COOH$

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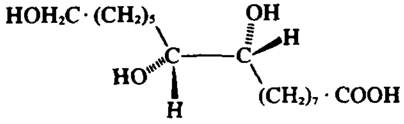

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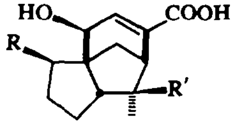
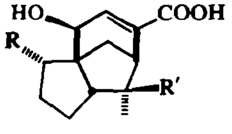


3: R = COOH, R′ = CH_2OH
4: R = R′ = CH_2OH



5: R = CHO, R′ = CH_2OH
6: R = COOH, R′ = CH_2OH
7: R = R′ = CH_2OH
8: R = COOH, R′ = Me
9: R = CHO, R′ = Me

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[a] Part VI: *Tetrahedron* **30**, 867 (1974).

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derivatives* lend themselves eminently to GLC. Table I gives the relative retention times of the various compounds investigated. For quantitative analysis[6], ethyl triacetyl aleuritate was found to be a suitable internal standard. All compounds (Table 1) responded linearly to increments and, could be estimated within ±3% in mixtures of different ratios. Table 1 also gives the weight ratio/area ratio factor for some relevant lac acids.

In order to extend this method to analysis of lac resin, synthetic mixtures of jalaric acid and aleuritic acid (ratios, 1:1, 2:1) were exposed to alkali [5% KOH aq, 48 h; conditions necessary for hydrolysis of lac resin and Cannizzaro reaction of jalaric acid to *epi*-shellolic (**6**) and epi-laksholic acids (**7**)[7]], the total product isolated, esterified (CH_2N_2), formylated, internal standard added and subjected to GLC (programming). It was soon realised that this sequence failed to give results of quantitative significance: the material eluted on GLC to the extent of 50–60% (as compared to the internal standard) and the peaks no longer corresponded to the original ratios. However, when after a short alkali exposure, silver oxide was added (to oxidise jalaric acid) and the product further subjected to the rest of the sequence as before, peak area ratios acceptably close to the required values, were obtained (though the product still showed incomplete elution 70–75%). Thus, for example, an artificial mixture of jalaric acid (2 parts) and aleuritic acid (1 part, by weight) gave a value of 1·9:1 by this procedure.

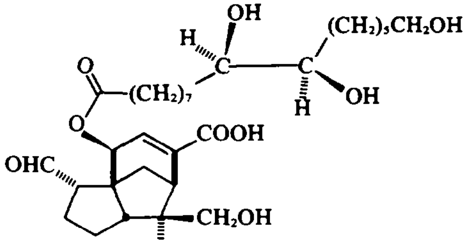

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Table 1. GLC data of formates of LAC acids (as methyl esters)[a]

No.	Lac acid	RRT	Weight ratio/ Area ratio[b]
1.	Butolic acid (**1**)	0·42	0·85
2.	*Epi*-laccishellolic acid (**8**)	1·0	1·25
3.	Shellolic acid (**3**)	2·0	—
4.	*Epi*-shellolic acid (**6**)	2·0	1·28
5.	Laksholic acid (**4**)	2·3	—
6.	*Epi*-laksholic acid (**7**)	2·3	—
7.	Aleuritic acid (**2**)	3·2	1·12

[a] Column: 180 cm × 6 mm, 10% silicone SE30 on 60–80 mesh chromosorb W; temp: 260°; 85 ml H_2/min.

[b] These values are with respect to the internal standard (ethyl triacetylaleuritate).

Jalaric ester-II, a component of soft resin, was clearly shown by the above procedure to be derived from equimolar amounts of jalaric and aleuritic acids, in full accord with the structure **10** established earlier[8] for this ester by unequivocal methods.

Pure lac resin. When the above method was applied to the component analysis of pur lac resin[1], it was consistently found that this material is based on equimolar proportions of jalaric acid (together with some small percentage† of laccijalaric acid, **9**) and aleuritic acid in contradiction of our earlier results (terpenic acids: aleuritic acid, 2:1) based on CrO_3 oxidation procedure[1].

Confirmation of the above conclusion could be obtained, unexpectedly, by a more direct method.

While studying the chemistry of jalaric ester-II (**10**),[8] we methylated (CH_2N_2) this compound, formylated (acetic-formic anhydride) the product and examined its PMR spectrum. The spectrum (60 MHz, $CDCl_3$) showed the olefinic protons (6·66 ppm), the allylic proton (5·90 ppm), both part of the terpene moiety, and the two α-glycol formate protons (5·10 ppm; from the aleuritic acid part) distinctly and in the expected integral ratios of 1:1:2, thus providing a convenient and straightforward method for determing the terpene acid (all terpene lac acids have $-\underset{|}{C}=CH-\underset{|}{C}HOH$ grouping): aleuritic acid ratio. To obtain maximum advantage from this method, we next examined the PMR spectra of jalaric ester-II, pure lac resin and their derivatives at 220 MHz[9] and the data are summarised in Table 2. It is, at once, clear on examining the entries 2 and 5 (Table 2) that in pure lac resin the terpene acid: aleuritic acid ratio is 1:1, just like the ratio established for jalaric ester-II. As a matter of fact, the corresponding spectra in the two series (Table 2) are *essentially* superimposable!

In another approach, using the PMR method, pure lac resin, a mixture of 1 part of jalaric acid and 1 part of aleuritic acid and , a mixture of 2 part of jalaric acid and one of aleuritic acid, were each subjected to KOH aq (5%, 48 h), the product methylated (CH_2N_2) and finally formylated and examined by PMR (220 MHz). The results clearly showed 1:1 ratio for pure lac resin. In fact, the

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*Though a variety of hydroxyl derivatives have been exploited for GLC analysis,[3] it is suprising that formates, which can be conveniently prepared even from tertiary alcohols,[4] have not been investigated so far. We find, that triterpene and steroid alcohols, besides polyalcohols based on diterpenes and sesquiterpenes can be conveniently subjected to GLC, after formylation. Formylation is readily achieved by acetic-formic anhydride.[4,5]

†On the average, ratio of jalaric acid: laccijalaric acid was found to be 7:1. It should be noted that this method does not distinguish between the aldehydic acid and the corresponding dicarboxylic acid e.g. jalaric acid and epishellolic acid.

Table 2. PMR spectral characteristics (220 MHz)

No.	Compd.	Derivative‡	Solv.	Signal assignments: ppm (multiplicity,† relative intensity)									
				$C{=}C\underline{H}$	$C{=}C{-}C\underline{H}{-}OCOR$	$C\underline{H}OCOR'$	$C\underline{H}_2OCOR'$	$C\underline{H}OH$	$CH_2O\underline{H}$	$CH_2OCO\underline{H}$	$CHOCO\underline{H}$	$COO\underline{Me}$	$C\underline{H}O$ #
1.	Jalaric ester-II	—	CD_3COOD	6·75(bs, 1)	5·97(bs, 1)	—	—	3·60(m*, 6)		—	—	—	9·75(s, 0·5)
2.	Jalaric ester-II	B	$CDCl_3$	6·63(bs, 1)	5·92(bs, 1)	5·21(bs, 2)	4·22(t,2) 4·00(q, 2)	—	—	8·11(bs, 2)	8·21(s, 1)	3·79(s, 3)	9·81(s, 0·5)
3.	Pure resin	—	CD_3COOD	6·75(bs, 1·3)	5·97(bs, 1·3)	5·04(bs*, 1)	—	3·58(m*, 7·4)		—	—	—	9·75(s, 0·5)
4.	Pure resin	A	CD_3COOD	6·72(d, 1)	6·00(bs, 1)	5·23(bs*, 2)	4·20(bs, 2) 4·03(m, 1·7)	—	—	8·11(s, 1·8)	8·23(s, 1·1)	—	9·75(s, 0·5)
5.	Pure resin	B	$CDCl_3$	6·63(bs, 1)	5·94(d, 1)	5·12(bs*, 2)	4·19(t, 1·8) 3·93(m, 2)	—	—	8·08(bs, 1·9)	8·19(s, 1)	3·81(s, 1·7) 3·72(s, 0·2)	9·76(s, 0·2)

†Except singlets (s), multiplicity of other signals is not very clear and hence indicated multiplicity is apparent: d = doublet, t = triplet, q = quartet, m = multiplet, m* = overlapping multiplets, b = broad, bs* = broad singlet, more like a hump. In all cases position given is that of the centre.

‡A = after formylation; B = after methylation (CH_2N_2) and then formylation.

Invariably during isolation and storage oxidation of the aldehyde function occurs to varying degree. This is responsible for the fractional $C\underline{H}O$ integeration.

spectra of the final products from pure lac resin and 1:1 jalaric acid-aleuritic acid mixture, were virtually indistinguishable.

It has been shown earlier[1] that pure lac resin, which is essentially homogeneous in terms of mol. wt. species, has a mol. wt. of 2095 ± 110 (Osmometric method). In the light of information disclosed above, it follows that pure lac resin molecule must contain four terpene acid (mostly jalaric acid) and four aleuritic acid units. (Mol. wt. expected for a resin molecule derived from four jalaric acid and four aleuritic acid molecules with loss of seven molecules of water is 2210). In view of the known structure (**10**) of jalaric ester-II, the PMR spectrum of which is closely similar to that of the pure lac resin, and the points of linkage of the constituent acids disclosed earlier[2], structure **11** appears most appropriate for pure lac resin. This structure is in full accord with the various signal intensities in the PMR spectra of the lac resin and its derivatives (Table 2). Keeping in view, the ratio of jalaric and laccijalaric acids (*vide supra*) it follows that out of every two molecules of lac resin, on the average, one of these contains one laccijalaric acid unit. It must be pointed out that it is not considered necessary that the molecular species constituting 'pure lac resin' should be sequence-wise homogeneous or even in terms of jalaric/laccijalaric acid ratio; structure **11** should represent the average situation. Moreover, depending on the age of lac sample and the care taken during its collection, significant oxidation of the aldehyde function, apparently occurs. Thus, it is not significant to establish the aldehyde content, though, in view of the PMR data one could state that out of four terpene acid units, two have aldehyde function.

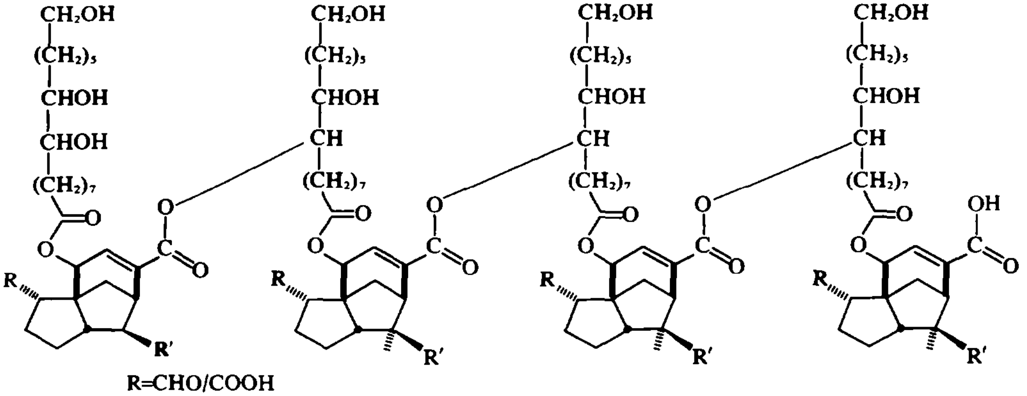
Structure **11** meets the major findings from our earlier chemical degradation studies.[1,2] However, there are some apparent discrepancies and hence a few comments are in order. As has been mentioned earlier, our previous results based on CrO_3 oxidation,[1] as well as UV absorption analysis,[1] has indicated a 2:1 ratio for terpenic acids: aleuritic acid. In the CrO_3 oxidation method, we had ignored the unidentified products and since the elution of the various chromotographic fractions on GLC column was also incomplete, results must have been vitiated. In the case of UV analysis, our reasonable assumption that interesterification of different lac acids should have no effect on absorptivity of various components and the absorptivity of the resulting polyester is purely additive, can now be considered as untenable. A more serious apparent anomaly concerns estimation of aleuritic acid in lac resin[1] by the well-established periodic acid method.[10] Repeatedly, a value of 35 ± 2% has been obtained[1] by the standardised procedure,[10] whereas according to structure **11** (R=CHO, R'=CH_2OH) one would expect a value of ~55%. We have no explanation for this discrepancy, except for the empirical finding that when a mixture of jalaric acid and aleuritic acid (1:1 w/w) is exposed to alkali (to simulate conditions for hydrolysis of lac resin) and the resulting product analysed for aleuritic acid by the periodic acid method, lower values for aleuritic acid are always obtained (Table 3).

EXPERIMENTAL

GLC was carried out on Aerograph model A-350-B using 180 cm × 6 mm aluminium column packed with 10% silicone SE-30 on 60–80 mesh Chromosorb W, using H_2 as the carrier gas. PMR spectra were taken on Varian T-60 spectrometer or HR-220 spectrometer; signals are recorded in (ppm) relative to TMS as zero.

General procedure for esterification, formylation. The appropriate lac acid/pure fraction (1g) was dissolved in EtOH (20 ml) and a soln of ethereal diazomethane added till an excess persisted. After 3 min at room temp, the product was freed of solvents under reduced pressure and acetoformic anhydride reagent[4] (12 ml) added, warmed on a steam-bath for 5 min and the clear soln left aside at room temp for 25 h. The excess reagent was flashed off under suction from a water-bath to furnish the required product, as a foam/gum.

General procedure for hydrolysis- Ag_2O oxidation, esterification and formylation. Pure lac resin (544 mg)



R=CHO/COOH
R'=CH_2OH/CH_3

11

Table 3. Effect of alkali treatment on the estimation of aleuritic acid in presence of jalaric acid

		% Aleuritic acid (± 1%)		
No.	Treatment	Aleuritic acid + jalaric acid (1 : 1, w/w)	'Pure lac resin'	Aleuritic acid
1.	5% KOH aq., 30–35° 48 h	44	33	97
2.	5% KOH aq., 30–35° 10 days	33	31	—
3.	1·6% KOH aq., 100° 13 h	31	29	102
	(expected value)	(50)	(55)	(100)

was mixed with 5% KOH aq (6 ml) and after keeping at room temp (27°) for 5 h, it was added to a Ag_2O suspension (prepared from 1g $AgNO_3$ and 15 ml 10% KOH aq) and the mixture kept aside at room temp for 24 h. The precipitated Ag was filtered off and thoroughly washed with water. The filtrate and washings were combined, acidified with 1N HCl and extracted with EtOAc. Removal of solvent after washing and drying (Na_2SO_4) yielded a product (0·5g) which was esterified and formylated as above.

In case of jalaric acid and jalaric acid-aleuritic acid mixture, the first alkali treatment was omitted and the material directly submitted to Ag_2O oxidation etc.

Programmed GLC of the final products from pure lac resin or from lac acid mixtures was carried out at 250 to 320° at H_2 gas flow of 50 ml/min.

Ethyl triacetylaleuritate. Aleuritic acid (3g), EtOH (100 ml), conc H_2SO_4 (5 ml) were refluxed (5 h) and then worked up with EtOAc to give ethyl aleuritate (2·6g). This was taken up in Ac_2O (20 ml) and pyridine (2 ml) and the mixture refluxed for 3 h. The mixture was worked up with EtOAc to give a product (2·7g) showing a single spot on TLC (SiO_2-gel layers; solvent: 30% EtOAc in C_6H_6). This product was passed through a column of SiO_2-gel (IIA; 22 cm × 1·5 cm; elution with 15% EtOAc in C_6H_6) to get ethyl triacetylaleuritate as a liquid (2·4 g). IR: no OH absorption; C=O 1735 cm^{-1}. PMR (CCl_4): three $C\underline{H}_3COO$ (3H, s, 1·96 ppm; 6H, s, 2·05 ppm); $C\underline{H}_2OCOCH_3$ and $COOC\underline{H}_2CH_3$ (4H, overlapping multiplets between 3·86 and 4·20 ppm); $C\underline{H}OCOCH_3$ (2H, broad triplet, 4·88 ppm).

Estimation of aleuritic acid. Procedure followed for results shown in Table 3 was as follows: For example, a mixture of jalaric acid (254 mg) and aleuritic acid (251 mg) was dissolved in 5% KOH aq (6 ml) and the soln kept aside for 48 h at room temp. (30–35°). The reaction mixture was acidified (H_3PO_4 aq, 1 : 1) and the liberated product taken up in EtOAc (15 ml × 2), the extract washed with water (5 ml × 4) and dried. The solvent flashed off and the aleuritic acid content of the residue estimated on 100 mg sample by the usual procedure.[10]

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