DITERPENES OF *LARIX* OLEORESINS

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Abstract—Oleoresins from most species of the genus *Larix* have been analysed by GLC for their diterpene composition. Common to all are the abietadiene and isopimaradiene acids usual in Pinaceae with the corresponding aldehydes and alcohols in small amounts. Epimanool also occurs in all, but larixol and its acetate is confined to *L. decidua* and *L. gmelini*, and epitorulosol to the remainder. In the hybrid *L. Xeurolepis* both of these last two compounds are present.

INTRODUCTION

THE GENUS *Larix*, or larches (Pinaceae) comprises[1] some seven Eurasian and three North American species, three interspecies hybrids, and one entity, *L. pendula* (Sol.) Salisb., whose status either as an American species now existing in cultivation, or as a hybrid which arose in cultivation, is still uncertain. Like other genera of the Pinaceae the larches yield oleoresin by tapping though this is effected by boring into the heartwood[2] rather than by the more usual scarification of the sapwood. The chemistry of only two of these resins has been reported on, that of *L. decidua* Miller, formerly available commercially under the name of Venice turpentine, and that of *L. russica* (Endl.) Sabine ex Trautv. (*L. sibirica* Ledeb.) which still finds uses in Russia today.[3,4] Heartwood extracts of three other species, *L. laricina* (Du Roi) Koch,[5] *L. lyallii* Parl.[6] and *L. gmelini* (Rupr.) Kuzeneva[7] have been examined, but diterpenes are only reported from the last. *L. decidua* resin contains large amounts of larixyl acetate (I)[8-11] together with lesser amounts of the free diol larixol (II) and epimanool (III).[10,12] The neutral fraction has also been shown to contain small amounts of aldehydes including dehydroabietal and isopimaral.[12] *L. russica* also contains epimanool,[13] but a compound first thought to be larixol[14] proved to be epitorulosol

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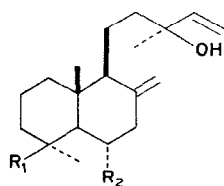[9] HAEUSER, J. (1965) *Bull. Soc. Chim. Fr.* 2645.

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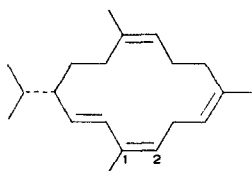
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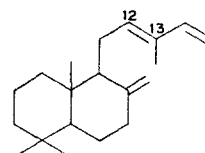
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(I) R₁ = Me ; R₂ = OAc   (VII)   (IX) 12,13 - *trans*
(II) R₁ = Me ; R₂ = OH   (VIII) 1, 2-Dihydro,   (X) 12,13 - *cis*
(III) R₁ = Me ; R₂ = H   1-hydroxy
(IV) R₁ = CH₂OH; R₂ = H
(V) R₁ = CHO; R₂ = H
(VI) R₁ = COOH; R₂ = H

(IV).[13,15] It also contains large amounts of hydrocarbons including the monocyclic thunbergene (cembrene) (VII),[14] biformene (IX or X)[13] and abietadiene[16] as well as abietal and abietol.[13] The acid components of *L. russica* comprise the usual range of abietane and pimarane compounds[17] but in *L. decidua* seem not to have been reported on.

TABLE 1. DITERPENOID
(After

RR$_t$	Footnote No.	Thunbergol 0.56	(*epi*-)Manool 0.58	Pimarate 0.91	Sandaraco-pimarate 1.0	Laevopimarate/palustrate 1.14	Isopimarate 1.17	Dehydro-abietate 1.42	Abietate 1.58
L. decidua	(1)	0.0	11.4 5.1 4.3–21.8	0.2 0.15 0.0–0.5	1.15 0.3 0.8–1.8	7.9 2.9 2.6–14.6	16.4 3.2 12.3–22.0	4.1 4.0 0.85–14.6	10.95 5.8 3.0–23.0
L. decidua bulk sample	(2)	0.0	7.5	0.5	1.0	12.5	18.5	1.8	7.6
L. gmelini	(3)	0.0	13.0 2.8 9.5–16.0	0.0	1.85 0.45 1.4–2.3	5.95 3.1 2.3–8.8	16.4 1.3 15.0–18.0	5.1 0.8 3.9–5.6	6.55 2.0 4.3–8.3
L. russica	(4)	0.75	12.75	Tr	1.5	2.0	7.5	0.5	2.7
L. kaempferi	(5)	6.7 3.0 3.6–10.8	18.6 19.5 2.3–38.5	1.7 1.6 0.3–4.0	0.85 0.3 0.6–1.25	6.7 4.3 1.1–10.8	21.0 6.65 16.0–30.0	5.9 2.95 3.1–9.1	8.25 7.6 2.6–19.4
L. occidentalis	(6)	11.0 3.2 9.2–16.0	4.4 2.65 0.8–7.5	1.55 1.0 0.7–3.1	1.35 0.15 1.2–1.45	11.95 3.15 10.0–18.3	22.8 1.6 21.7–25.8	7.65 5.3 2.0–16.0	12.7 3.3 8.3–16.6
L. laricina	(7)	Tr?	2.8 3.2 0.3–8.8	0.95 0.75 0.2–2.0	3.35 1.1 2.05–5.1	11.4 4.0 7.5–18.3	26.7 6.85 18.4–35.4	7.2 5.05 1.5–12.9	10.2 2.2 9.7–13.8
L. lyallii	(8)	0.0	20.6 5.25 16.0–26.4	Tr?	0.95 0.1 0.8–1.05	7.8 1.55 6.3–9.4	12.4 1.45 10.8–13.6	3.0 1.3 2.0–4.5	3.55 0.7 2.8–4.2
L. potanini/griffithiana	(9)	1.85 2.2 0.0–5.7	5.6 5.5 0.5–14.0	12.05 8.35 3.2–24.1	1.45 0.5 1.0–2.1	12.5 3.25 8.2–16.7	22.6 4.5 19.0–31.3	8.4 5.8 1.8–17.2	12.6 3.65 7.8–14.5
L. X eurolepis	(10)	2.5 1.1 2.1–4.0	15.0 4.6 10.0–21.2	2.95 2.8 0.4–6.0	1.25 0.4 0.8–1.7	8.4 1.4 5.3–9.5	20.3 4.0 17.7–26.2	6.2 3.8 3.3–11.8	6.2 1.6 4.5–8.1
L. pendula	(11)	0.0	10.7 2.15 8.6–13.5	0.35 0.35 Tr–0.8	1.6 0.55 1.0–2.2	7.3 1.8 5.3–9.5	18.6 2.85 16.0–22.3	8.55 2.35 5.8–11.5	5.3 0.8 4.2–6.1

The figures under each head are the means (in bold type), standard deviation, and range encountered, based on total diterpenoids = 100%. Other components, relatively invariant throughout the genus, are as follows: thunbergene (RR$_t$ 0.28) mean ($\bar{X}$) 0.3, range (of species means) 0–0.9, excepting *L. russica*, $\bar{X}$ 6.5; trans-biformene (RR$_t$ 0.35) $\bar{X}$ 0.6, range 0–1.6; *epi*-manoyl oxide (RR$_t$ 0.39) $\bar{X}$ 0.4, range 0–0.8; abietatriene and abietadiene (RR$_t$s 0.43 and 0.46) $\bar{X}$ 0.45 together, range tr.–1.0, excepting *L. russica*, $\bar{X}$ 7.5 and 24.5 respectively; laevopimaral/palustral (RR$_t$ 1.07) $\bar{X}$ 1.4, range 0–2.2; isopimaral (RR$_t$ 1.13) $\bar{X}$ 1.4, range 0.5–2.75; dehydroabietal (RR$_t$ 1.34) $\bar{X}$ 0.7, range 0.1–1.4; abietal (RR$_t$ 1.42) $\bar{X}$ 0.9, range 0.6–1.7, excepting *L. russica*, $\bar{X}$ 3.3; laevopimarol/palustrol (RR$_t$ 1.59) $\bar{X}$ 1.0, range 0.5–1.8, excepting *L. russica* $\bar{X}$ 0.0; neo-abietal (RR$_t$ 1.81) $\bar{X}$ 0.4, range 0–0.75, excepting *L. lyallii* $\bar{X}$ 2.1; neoabietol (RR$_t$ 2.71) $\bar{X}$ 0.25, range 0–0.8, excepting *L. lyallii* $\bar{X}$ 2.4.

Notes: (1) 14 samples, mostly from Bedgebury National Pinetum forest plots and including five different strains or varieties. (2) Material obtained by tapping and kindly supplied by Bruder Unterweger, Thal

[15] SHMIDT, E. N., REZVUKHIN, A. I. and PENTEGOVA, V. A. (1967) *Khim. Prirod. Soed.* **3** (1), 61.
[16] LISINA, A. I. and PENTEGOVA, V. A. (1965) *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk* (2), 96.
[17] SHMIDT, E. N., KASHTANOVA, N. K., VOL'SKII, L. N., CHIRKOVA, M. A. and PENTEGOVA, V. A. (1970) *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk* (5), 118.

The published results do not give a clear picture of quantitative compositions or allow comparisons to be made between species. The object of this work was, as far as possible, to survey the genus as a whole to obtain a comparative picture, and to see if this suggested relationships or provided chemical keys to identification.

RESULTS

Since it was not practicable to collect all the samples in the wild, recourse was made to cultivated trees. All the species have been grown in England except for the Chinese *L. mastersiana* Rehd. et Wils., which seems never to have been introduced here or elsewhere. It has had to be omitted. Sources of samples are given in the notes to the Table. Most of the cultivated trees are among those listed by Mitchell.[18] It was not feasible to tap the trees for resin but small quantities could usually be found exuding from the trunks from small wounds, etc. Only fairly fresh, sticky material was collected but inevitably some alteration of some susceptible components may have occurred. However, comparison of the average values for fourteen *L. decidua* samples collected in this way with those for a commercial sample obtained by tapping showed a certain quantitative, but no qualitative, disparity.

An objection to relying on cultivated trees is that they may be descended from restricted

COMPOSITION OF *Larix* RESINS
methylation of acids)

Isopim-arol 1·66	(epi-)-Torulosate 1·73	Neoa-bietate 1·81	(epi-)-Torulosal 2·04	Larixol 2·07	Dehydro-abietol 2·16	Abietol 2·25	Larixyl acetate 2·41	? 2·59	(epi-)-Torulo-sol + acetate 3·08	Other peaks footnote No.
0·9 0·55	0·0	3·7 1·75	Tr?	12·8 5·25	0·25 0·25	0·7 0·75	22·1 8·1	0·55 0·65	Tr?	(12)
0·3–2·6		1·5–6·9	0·0–Tr?	4·8–24·0	0·0–0·8	0·0–2·6	12·0–37·0	0·0–1·5		
1·0	0·0	5·6	0·0	3·1	Tr	1·1	33·0		0·0	(13)
0·2 0·15	0·0	2·15 1·35	0·0	23·7 9·9	0·3 0·3	0·3 0·3	16·6 3·85	Tr?	Tr?	(14)
Tr–0·4		0·7–3·9		13·7–37·5	Tr–0·7	Tr–0·65	15·0–20·4			
0·0	Tr?	Tr	0·0	0·0	3·0	11·2	0·0	0·0	1·0	(15)
1·25 0·6	0·4 0·35	3·55 3·7	0·65 0·45	0·0	1·7 1·7	1·1 0·45	0·0	1·2 0·45	8·55 3·4	
0·5–1·9	Tr–0·9	0·4–8·8	Tr–1·2		0·25–4·1	0·5–1·4		0·5–1·75	4·0–12·2	
1·5 0·5	0·6 0·5	5·1 3·0	Tr	0·0	0·8 0·4	2·15 0·9	0·0	1·7 0·95	3·55 3·0	
0·8–2·2	0·0–1·2	1·7–8·8			0·3–1·4	1·2–3·5		0·3–2·7	0·5–8·6	
2·95 1·2	1·1 0·8	7·05 3·05	0·9 0·85	0·0	1·55 1·25	1·95 1·5	0·0	3·1 1·35	3·35 3·45	(16)
1·8–4·9	0·0–2·2	4·0–13·3	Tr–2·1		0·3–3·2	0·6–4·4		1·7–4·7	0·3–9·5	
1·95 0·3	0·4 0·35	4·5 0·85	1·65 0·45	0·0	1·35 0·15	2·45 1·0	0·0	1·25 0·2	19·2 2·55	(17)
1·7–2·3	0·0–0·6	3·6–5·3	1·3–2·2		1·2–1·5	1·4–3·4		1·0–1·4	16·8–21·8	
1·0 0·55	0·0	5·4 2·85	Tr?	0·0	1·85 1·45		0·0	2·3 1·1	0·95 0·8	(18)
0·5–1·8		2·9–9·9			0·4–4·5			1·0–4·0	0·0–1·8	
1·15 0·4	2·45 0·55	2·5 1·0	0·4 0·1	2·75 2·1	0·95 0·45	1·05 0·75	12·7 2·5	Tr?	6·95 1·7	
0·8–1·7	1·7–3·0	1·6–3·9	0·3–0·5	0·4–5·2	0·6–1·6	0·6–2·2	9·0–14·6		4·4–8·1	
1·1 0·15	5·45 2·2	1·55 0·65	0·95 0·8	3·5 2·35	0·9 0·2	0·75 0·35	12·2 1·5	Tr?	11·4 3·3	
1·0–1·3	3·6–7·9	0·8–2·2	0·5–2·2	1·1–6·4	0·7–1·1	0·3–1·1	10·5–14·1		7·9–14·9	

Assling, Tyrol, Austria. (3) Four samples from Bedgebury and Kew and including one each of *var. japonica* (Regel) Pilger, and *var. principis-rupprechtii* (Mayr) Pilger. (4) Two samples only from trees (from Siberian seed) growing in the Bergianska Trädgarden, Stockholm. (5) Four samples from Bedgebury, Kew and Inverary Castle. (6) Six samples from Bedgebury, Kew and Wakehurst Place. (7) Six samples, four from Bedgebury and Kew, and two wild from Candle Lake, Saskatchewan. (8) Three wild samples from above Lake Louise, Banff National Park, Alberta. (9) Seven samples, The three given definitely to *L. griffithiana* (Gore's Wood, Borde Hill, Sussex; Strete Ralegh Pinetum, Devon; Coldrennick, Cornwall) contained no thunbergol. Of the remaining four (Borde Hill and Wakehurst Place) one (Borde Hill, Tolls) is given to *L. potanini* and the rest are uncertain. (10) Four samples from Kew. (11) Four samples, two each from Kew and Bedgebury. (12) RR_t 0·53, average ($\bar{X}$) 0·5%. (13) *ditto* 0·6%. (14) *ditto* $\bar{X}$ 0·2%. (15) RR_t 0·5 (neutral) $\bar{X}$ 3·1%; 0·73 (ester) $\bar{X}$ 2·0% and 0·85 (ester) $\bar{X}$1·6%. (16) RR_t 0·82 (Δ^8-isopimarate) in three samples, $\bar{X}$ 0·6%. (17) ditto $\bar{X}$ 0·4%. RR_t 0·53 $\bar{X}$ 1·0%. (18) RR_t 0·82 in four samples, $\bar{X}$ 0·6%.

18 MITCHELL, A. F. (1972) in *Conifers in the British Isles*, Proceedings of the Third Conifer Conference, The Royal Horticultural Society, London.

parentage and so will not reflect the range of variability to be found in the wild. This may be so for the species of which only few samples have been analysed but the fourteen *L. decidua* samples were from several different strains and included var. *polonica* and the forms *silesiaca* and *carpathica*. No greater differences were noticed between these than within the species as a whole.

The resins were analysed by GLC (for details, see Experimental) and the results are shown in Table 1. For each component the mean percentage found, together with the standard deviation and the range encountered, are shown. Some minor components have been omitted including many early peaks in the hydrocarbon region where low retention times make identification uncertain. Most of the identified components are ones which have been already reported in *Larix*. Epitorulosal (V) and epitorulosic acid (VI) are new to the genus but might be expected, given the occurrence of the corresponding alcohol. The ester emerging at RR_t 2·59, most abundant in *L. laricina*, has not been identified. It was too minor a component to make isolation practicable and so in an effort to identify it and also to provide further confirmation of the identities of other peaks the neutral and methylated acid fraction of one sample of *L. laricina* resin were submitted to GC–MS. The spectrum of the 2·59 compound showed mass peaks at m/e 317 and 314 (base peak) perhaps corresponding to m-15 and m-18 of a hydroxyabietic acid, though the molecular ion at 322 was not observable.

Epimeric labda-13-ols are not separated by GLC and consequently present results provide no evidence for the configuration of such compounds in these resins. Since, however, all so far reported from *Larix* have proved to be 13-*S* (13-*epi*) this is provisionally assumed to hold true for like compounds in the remaining species.

Thunbergene (VII) and thunbergol (VIII) (cembrene and isocembrol) were identified by comparison with those compounds as occurring in *Pseudotsuga menziessii* (Mirbel) Franco oleoresin.[19,20] Thunbergene has already been reported in *L. russica*[14] and is of common occurrence in the Pinaceae, especially in *Pinus* subgenus Haploxylon.[21] Thunbergol readily dehydrates and partially does so during chromatography; consequently a plateau of dehydration products runs between the thunbergol and thunbergene peaks. The area of this has been counted with the former.

DISCUSSION

The most striking feature of the resin compositions is that 2 species, *L. decidua* and *L. gmelini*, contain large amounts of larixol/larixyl acetate which were not detectable in the remainder. Their presence in *L. gmelini* resin accords with previous findings in the heartwood extract.[7] Of the remaining species *L. russica* is exceptional in having high proportions of neutral abietane compounds as was reported previously,[13,14,16] though the proportion of acids there noted was higher than in this study and is probably more reliable. Epitorulosol, sometimes accompanied by lesser amounts of its monoacetate, is present in all the species from which larixol/larixyl acetate is absent, though the amount present is highly variable even within a species (0·3–9·5% in *L. laricina*). The 3 American species, *L. laricina*, *L. lyallii* and *L. occidentalis* Nutt., together with the Japanese *L. kaempferi* (Lamb.) Carr., are rather similar in composition except that the last 2 contain fairly high amounts of thunbergol

[19] ERDTMANN, H., KIMLAND, B., NORIN, T. and DANIELS, P. J. L. (1968) *Acta Chem. Scand.* **22**, 938.
[20] KIMLAND, B. and NORIN, T. (1968) *Acta Chem. Scand.* **22**, 943.
[21] DAUBEN, W. G., THIESSEN, W. E. and RESNICK, P. R. (1965) *J. Org. Chem.* **30**, 1693.

while the first 2 contain little or none. However one sample of supposed *L. laricina* resin did contain a substantial amount of thunbergol but it has not been included since the identification could not be confirmed. The two closely related Himalayan and W. Chinese species, *L. griffithiana* Carr. and *L. potanini* Batalin, were available only as cultivated trees which present some difficulties of distinction. Since no consistent chemical differences could be detected between samples from trees generally accepted as *L. griffithiana* and those which were believed to be *L. potanini* or were uncertain, the results have been pooled. With two trees the resin found on the bark was wholly anomalous in composition, consisting largely of epimanool (> 50%) and other neutral diterpenes. The reasons for this disturbing finding have not been ascertained and the results have not been included. The average composition of the remaining seven samples was close to the American spp./*L. kaempferi* group but the high pimaric acid content was distinctive.

The hybrid *L. X eurolepis* Henry (*L. decidua* × *L. kaempferi*) shows a resin composition essentially intermediate between those of its parents, containing both larixol/larixyl acetate and torulosol/torulosyl acetate. The resin of the ambiguous *L. pendula* was very close in composition to that of *L. X eurolepis*, also showing these two pairs of compounds. Since larixol is absent from the three certain American species its presence here seems to argue slightly against *L. pendula* belonging in this category, but the composition is in full qualitative accord with a hybrid origin with *L. decidua* × *L. laricina* parentage.

The similarity groupings suggested by diterpene compositions are thus quite different from those arrived at by Stairs[22] from the monoterpene compositions. He found *L. laricina* to be close to *L. occidentalis* (as here) but *L. decidua* was similar to *L. kaempferi* and *L. russica* similar to *L. gmelini*. Monoterpene composition of oleoresins has been increasingly used in systematic studies of conifers in recent years.[23–26] Within a genus qualitative differences in composition seem not to be common but differences in mean quantitative compositions can be significant even for varieties or populations.[23,25,26] Within-species variability can however be so high, even within a population, that percentages of individual components could not be meaningful characters for identification purposes. It may be that synthesis of diterpenes is more specific. Although the majority of compounds found in *Larix* are found in all the species, and indeed other genera of Pinaceae, larixol is confined to 2 species and has not so far been reported from any other genus. Although too few samples of some species have been studied for confidence that the full range of compositional variability has appeared, on present results individuals can be placed in one of the following groups by inspection of their diterpene compositions: American spp. + *L. kaempferi*; *L. decidua* + *L. gmelini*; *L. potanini* + *L. griffithiana*; *L. russica*; *L. X eurolepis* + *L. pendula*. Further subdivision could perhaps be made on the basis of thunbergol presence but this seems a rather variable character.

It has been suggested[23] that comparisons of percentage ratios as well as simple percentages would be advantageous in taxonomic studies but clearly the ideal is a multivariate approach. To see if such an approach was capable of separating the individual samples of the American spp./*L. kaempferi* group into their appropriate species a limited study was made employing

[22] STAIRS, G. R. (1968) *Silvae Genet.* **17**, 182.
[23] ZAVARIN, E. and SNAJBERK, K. (1972) *Phytochemistry* **11**, 1407.
[24] WILKINSON, R. C. and HANOVER, J. W. (1972) *Phytochemistry* **11**, 2007.
[25] ZAVARIN, E. and COBB, F. W. (1970) *Phytochemistry* **9**, 2509.
[26] ZAVARIN, E., SNAJBERK, K., REICHERT, T. and TSIEN, E. (1970) *Phytochemistry* **9**, 377.

the product moment correlation coefficient based on standardized characters.[27] It was found that although the *L. lyallii* samples clustered appropriately, the samples from the other three species were clustering together in a fairly random way.

EXPERIMENTAL

GC columns were 274 × 0·84 cm glass. All the samples were examined on 1 % XE60 on 100–120 mesh diatomite 'CQ' and all quantitative results were from these chromatograms Some early runs were also made on 3 % Versamide 900 on Chromosorb W, and 1 % OV1 on diatomite 'CQ' for checking peak identifications. Relative retention times on XE60 were very dependent on mode of preparation of the column packing, those given in Table 1 are for a packing prepared by evaporation under N_2 with manual agitation of an acetone solution of the XE60. The column temp. was 192°, the FID oven 250° and the injection zone at ~220°. The carrier gas (argon) flow rate was 45 ml/min.

The resin samples were dissolved in ether and filtered from woody matter. Portions were then chromatographed untreated, after acetylation with Ac_2O–pyridine, and after methylation with CH_2N_2 in the presence of a little MeOH. Some samples were also chromatographed after reduction with $NaBH_4$ in MeOH to confirm the identification of aldehyde peaks. The isopimarane and abietane alcohols and aldehydes mostly emerge in the same region as the esters. Their amounts were determined from the chromatograms of the unmethylated material, being normalized to a well-separated peak present in the chromatograms of both the methylated and unmethylated resin, epimanool being usually utilized for this purpose. The amounts so found are of course deducted from the amounts of the esters under which they lie. The earlier XE60 column used (packing prepared in a rotary evaporator, not under N_2) did not separate abietol and dehydroabietol which were therefore determined from the acetates which were separated. This column, however, gave better separations of isopimarate from laevopimarate/palustrate though on neither column were they fully separated. Thus although their total is accurate the individual amounts are only approximate. Peak areas were measured by cutting and weighing of photocopies and no calibration of the detector for different compounds was attempted.

[27] SOKAL, R. R. and SNEATH, P. H. A. (1963) *Principals of Numerical Taxonomy*, p. 290, Freeman, San Francisco.