ANALYSIS OF THE NON-RUBBER CONSTITUENTS OF LATEX FROM *ALSTONIA ANGUSTILOBA*

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MORRIS, M.D. & HALIMAHTON MANSOR. 1992. Analysis of the non-rubber constituents of latex from *Alstonia angustiloba*. Latex samples of four *Alstonia angustiloba* (pulai) trees from two different locations were analysed. The total solids contents of the samples varied from 28 to 36%. On average, the solids portion consisted of 81% resins, 11% rubber and 8% residue. No large variation was observed in the composition of latex between the trees. The pulai latex was also found to be quantitatively similar in composition to that of jelutong. Separation of the acetone solubles of the resin fraction by column chromatography (silica, hexane:ethyl acetate) gave two major fractions. Structural elucidation of these fractions revealed that the resin of pulai latex consisted of four main compounds, *viz* lupeol acetate as a major component (25% dry weight), lupeol (15%), α-amyrin and its acetate (15%) and a trace amount of β-amyrin acetate.

Key words: *Alstonia angustiloba* - latex - non-rubber fraction resins - acetone solubles - lupeol acetate - lupeol - amyrin

Introduction

Alstonia angustiloba is the largest of the *Alstonia* tree species and is native to the Malay Peninsula and other tropical regions of Asia (Burkill 1935). Known as pulai in Malay, it contains a latex which can be easily extracted by tapping. The latex has been used by various native tribes in traditional medicines commonly in the treatment of sores and skin diseases (Browne 1955).

Early reports on the properties and composition of the latex were purely descriptive. Eaton (1910) found it to be similar to that from jelutong (*Dyera costulata*) but inferior in strength, in readiness to coagulate and in tendency to turn yellow. Hooper (1905) stated that the latex does not yield rubber and nearly half of it consists of resins. A preliminary chemical analysis of the latex from *A. angustiloba* has shown that it does contain a small amount (5-8%) of natural rubber along with larger amounts (20 to 28%) of non-rubber material, the remainder being water (Halimahton & Morris 1990). These findings were in accord with a previous

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*Present address: *21 Stapleford Panshanger, Welwyn Garden City, Herts AL7 2PD, England* analysis of latex from one sample of *A. scholaris*, the other pulai species, that indicated 2.43% rubber and 22 to 55% solids (Ullah *et al.* 1980).

In view of the large proportion of non-rubber material in the latex from *A. angustiloba* and its reputed biological activity (Gupta *et al.* 1981), a more detailed analysis of the latex constituents was considered worthwhile. In order to gain some insight into the possible variability in composition of the latex, quantitative analyses on latex from four randomly selected wild trees of different geographical locations were carried out.

Materials and methods

The *A. angustiloba* trees were selected at random from two sites in the Kuala Lumpur area of Peninsular Malaysia, namely Segambut and Kepong. The trees were tapped using a full spiral cut at about 1.3 *m* from the ground. Latex was collected until flow naturally ceased after 1 to 2 *h*.

The water content of the latex was determined by drying samples at 40 to 50°*C* *in vacuo* until constant weight. The dried latex was extracted successively with acetone and petroleum ether (60-80°*C*) by Soxhlet extractor for 48 *h*. Both extracts and the final residue were dried under reduced pressure.

Thin layer chromatography (TLC) was carried out on silica gel plates using hexane:ethyl acetate (17:3) as eluant. Plates were developed by spraying with 29% sulphuric acid followed by heating at 105°*C*.

Column chromatography was also carried out on silica gel, eluting successively with hexane:ethyl acetate (17:3) and hexane:ethyl acetate (7:3).

Nuclear magnetic resonance (NMR) spectroscopy was performed using a Brucker AC-P 300 FT-NMR System at 300 *MHz* (^{1}H) and 175 *MHz* (^{13}C). Samples were dissolved in deuterated chloroform and tetramethylsylane (TMS) was used as an internal standard.

Mass spectrometry (ms) was carried out on a JEOL JMS-D300 instrument with a column heating rate of 10°$C\ min^{-1}$ from 60 to 300°*C* and an ionisation potential of 70 *eV*.

Results and discussion

Fractionation of the latex

The method of fractionation of the latex into water, acetone soluble resins, hydrocarbon soluble rubber and insoluble residue was based on that of Tezuka *et al.* (1980). The overall compositions of the *Alstonia* latex samples in this study are given in Table 1 while the mean composition of the solids portions is given in Table 2. The total solids contents of the latex samples fall within the range of 28 to 36% described earlier (Halimahton & Morris 1990). These solids have been found to consist on average of 81.1% resins, 11.0% rubber and 7.7% residue. In overall quantitative terms, the pulai latex is similar in composition to the more commonly exploited jelutong latex (Table 2). There is also some similarity to the natural latices from sorva (*Couma macrocarpa*) and sorvinha (*Couma utilis*) in terms of fractional composition, but latices from chicle (*Manilkara chicle*) and chiquibul (*Manilkara zapotilla*) have significantly lower levels of resins and contain the trans polyisoprene isomer (Tezuka *et al.* 1980).

The quantitative variation in composition of latex between individual pulai trees is not particularly large for the randomly selected, uncultivated trees. A study of the factors affecting latex composition from individual trees is beyond the scope of this paper. However, the reasonably low level of variability in composition means that the results from this study probably give a realistic indication of the general composition of *A. angustiloba* latex, at least in western Peninsular Malaysia.

Table 1. Fractional composition of four samples of latex from *Alstonia angustiloba*

	Percentage of whole latex by weight				
Tree	A	B	C	D	Mean
Water content	69.8	64.9	66.2	66.6	66.9
Acetone solubles (resins)	26.4	29.6	25.6	25.8	26.8
Petroleum ether solubles (rubber)	2.1	3.0	4.9	4.7	3.7
Insoluble residue	1.7	2.5	3.4	2.7	2.6
Total solids content	30.2	35.1	33.8	33.4	33.1

Tree A = Segambut, Kuala Lumpur; Trees B, C and D = Kepong, Kuala Lumpur

Table 2. Comparison of the mean composition of solids obtained from four samples of *A. angustiloba* latex with reported composition of jelutong latex

	Percentage of dried latex by weight		
	A. angustiloba	Jelutong (1)*	Jelutong (2)*
Acetone solubles	81.1	77.5	79.5
Hydrocarbon solubles	11.0	20.1	17.4
Insoluble residue	7.7	2.4	3.1

* Data from Tezuka *et al.* (1980).

Isolation and identification of the resin fraction

As the petroleum ether soluble portion has been identified as 1,4-*cis* polyisoprene rubber in an earlier paper, attention was turned to the predominant acetone soluble portion which was reported as consisting of five components by TLC (Halimahton & Morris 1990). By changing the TLC eluant it was possible to identify seven distinct bands, two of which were much more intense than the rest (Figure 1). The acetone extract was separated by column chromatography on silica gel. The two predominant fractions that were obtained were fraction 2 (eluant hexane:ethyl acetate 17:3) and fraction 4 (eluant hexane:ethyl acetate 7:3). These two fractions constituted between 70 and 80% of the total acetone extract (Table 3). Both of the two major fractions appeared as single spots by TLC after chromatographic separation and both gave crystalline white solids after removal of the solvent.

Analysis of fraction 2 by GCMS indicated two distinct peaks (retention times 13.1 and 16.9 *min*) plus a shoulder on the second peak. Mass analysis of the two peaks showed both to give a strong molecular ion signal at m/e 468 and a prominant signal at 408 (M-60) which was indicative of loss of acetic acid. From these results, it was postulated that fraction 2 consisted of a mixture of isomers of triterpene acetates with the molecular formula $C_{32}H_{52}0_2$. The molecular formula was confirmed by results from elemental analysis which found C: 81.93%, H: 11.54%, and O: 6.66% (calculated C: 82.05%, H: 11.11%, and O: 6.83%), whilst the

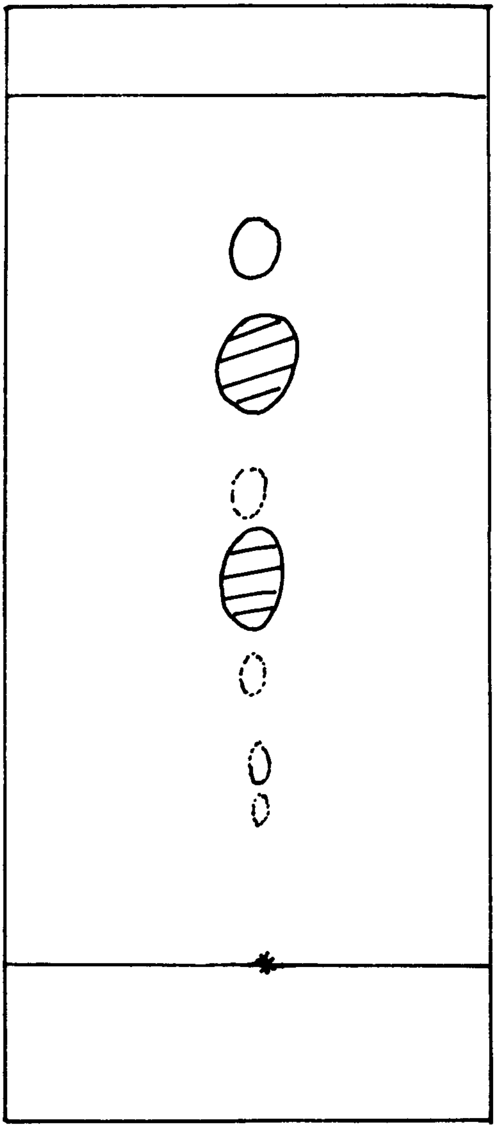


Figure 1. Thin-layer chromatography of the acetone solubles of the resin from the latex of *A. angustiloba* (silica; hexane:ethyl acetate, 7:3; detected with 20% sulphuric acid)

presence of isomers was confirmed by the ^{13}C NMR spectrum (Figure 2). Comparison of the ^{13}C NMR spectrum with literature data (Wenkert *et al.* 1978, Scholichin *et al.* 1980) enabled the complete assignment of the major signals to lupeol acetate (1) (Table 4). The minor peaks corresponded to the isomeric α-amyrin acetate (2) (Seo *et al.* 1975) (Table 4).

Table 3. Proportions of fractions obtained from the chromatographic separation of resins from *A. angustiloba*

	Percentage of acetone soluble resins by weight			
Tree	A	B	C	D
Fraction 2	43.6	37.1	40.3	41.6
Fraction 4	27.5	36.2	30.9	38.0
Fractions 1,3,5,6 and 7	22.9	21.1	22.8	14.2
Uneluted material	6.0	5.6	6.0	6.2

Table 4. Carbon-13 chemical shifts of fraction 2 with reference to published data*

Carbon atom	Observed	Lupeol acetate[1]	Observed acetate[2]	α-Amyrin
1	38.4	38.4	38.5	38.4
2	23.7	23.7	23.6	23.6
3	81.0	81.0	80.6	80.7
4	37.8	37.8	37.7	37.6
5	55.4	55.4	55.3	55.3
6	18.2	18.2	18.2	18.3
7	34.2	34.3	32.9	32.8
8	40.8	40.9	40.8	40.1
9	50.3	50.4	47.6	47.6
10	37.1	37.1	36.8	36.8
11	20.9	21.0	17.5	17.5
12	25.1	25.1	124.3	124.1
13	38.1	38.1	139.6	139.4
14	42.8	42.9	42.1	42.1
15	27.4	27.5	28.7	28.7
16	35.6	35.5	26.6	26.7
17	43.0	42.9	33.7	33.8
18	48.3	48.2	59.1	59.0
19	48.0	47.9	39.6	39.7
20	150.9	150.5	39.6	39.7
21	29.9	29.8	31.3	31.3
22	40.0	39.9	41.5	41.5
23	27.9	28.0	28.1	28.1
24	16.5	16.5	16.9	16.8
25	16.2	16.2	15.7	15.7
26	16.0	16.0	16.7	16.8
27	14.5	14.5	23.4	23.2
28	18.0	18.0	28.1	28.1
29	109.4	109.4	23.3	23.2
30	19.3	19.3	21.4	21.4
CH_3CO	21.3	21.3	21.3	21.2
CH_3CO	171.0	170.8	171.0	170.4

* All figures are in ppm with reference to TMS; [1]Wenkert *et al.* (1978); [2]Seo *et al.* (1975).

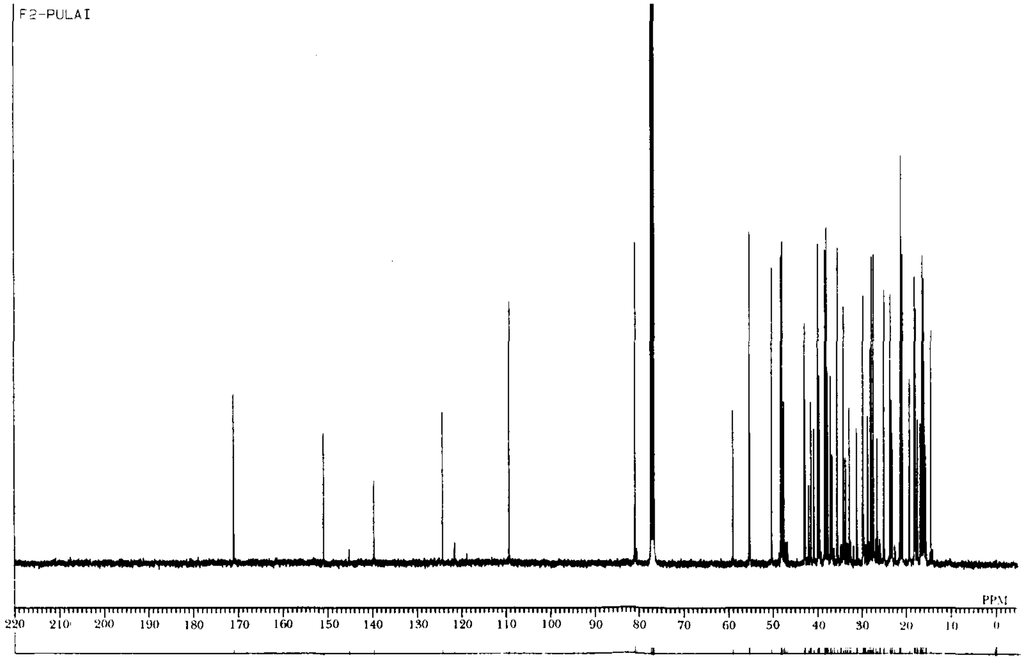
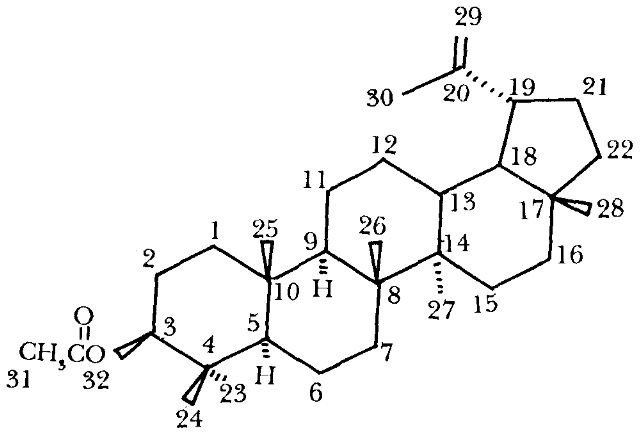


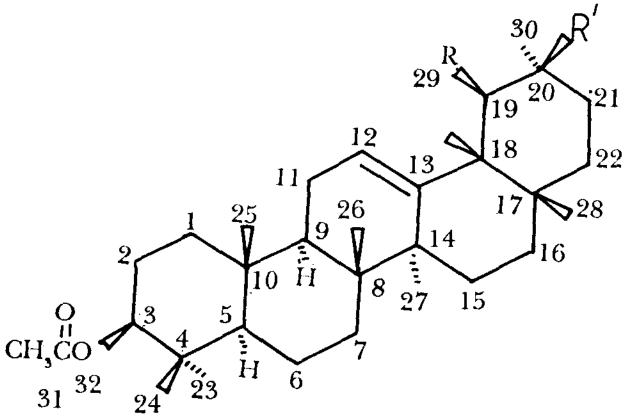
Figure 2. 13C NMR spectrum of the solid from fraction 2 of pulai (*A. angustiloba*) resin

After subtracting the signals for lupeol acetate and α-amyrin acetate from the ^{13}C NMR spectrum of fraction 2, the remaining very small signals corresponded well to those for β-amyrin acetate. For example, the characteristic olefinic resonances were observed at 121.6 and 145.2 ppm for β-amyrin acetate (3) (Seo *et al.* 1975). It was therefore concluded from the ^{13}C NMR data that fraction 2 consisted of lupeol acetate (1), α-amyrin acetate (2) and β-amyrin acetate (3) in the approximate ratio of 10:3:1 (Figure 3). The ^{1}H NMR spectrum, though complex, is consistent with this assignment based on comparison with literature data (Shingu *et al.* 1973).

Following a similar method of elucidation, the solid from fraction 4 of the separation of resins from pulai latex was assigned the molecular formula $C_{30}H_{50}O$. This formula immediately led to the postulation of parent triterpenes lupeol, α-amyrin and β-amyrin as possible components. Comparison of the ^{13}C NMR spectrum of fraction 4 with literature data revealed that both lupeol and α-amyrin were present in a ratio similar to that of their acetates in fraction 2. In addition, a third compound appeared to be present in a concentration intermediate between that of lupeol and α-amyrin. About 30 signals could be picked out for this compound, the most significant being at 185.6, 140.4, 118.9 and 79.0 ppm. This seemed to correspond to the acetate of a different triterpene with an unsaturated bond. The presence of an acetate was confirmed by a singlet resonance at 2.05 ppm in the ^{1}H NMR spectrum of fraction 4 and a peak at 1754 cm^{-1} in its IR spectrum. Identification of this constituent has not yet been achieved.



(1)



(2) R = CH_3', R' = H
(3) R = H, R' = CH_3

Figure 3. Structures of lupeol acetate (1), α-amyrin acetate (2) and β-amyrin acetate (3)

Fraction 2 contained about 40% of the solid material from the resins of pulai latex and, according to NMR signal intensities, lupeol acetate constituted about 62% of this fraction. It is therefore deduced that lupeol acetate accounts for about 25% of the pulai resins. Using a similar method of deduction, lupeol is found to make up about 15% of the total resins, whilst α-amyrin and its acetate together account for a further 15%.

A literature survey on the four compounds revealed that lupeol acetate, the largest single compound of the whole latex, showed antiulcer activity in rats (Gupta *et al.* 1981). It was the most potent of the triterpenoids tested against gastric ulceration induced either by stress or by pyloric ligation. Further studies on Shay rats showed that this antiulcer activity was not due to an effect on the gastric secretion or pH. It seems possible such biological activity could be related to the healing effect on skin sores for which pulai latex is reputed.

Conclusions

The resins or acetone soluble portion of latex from *A. angustiloba* constitutes about 80% by weight of the dry latex. This level of resins is amongst the highest reported for natural latices. The resins consist predominantly of lupeol, lupeol acetate, α-amyrin and its acetate with small amounts of β-amyrin acetate, higher triterpene esters and other terpenoid materials. Lupeol acetate alone accounts for about 25% by weight of the resin fraction or 20% of the total dry weight of the latex. It is hypothesised that lupeol acetate is responsible, at least in part, for the reputed biological activity of *A. angustiloba* latex against skin sores. Biological testing of fractions of the latex would be required to test this hypothesis.

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