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F.T.R. 3 (Feb. 1963)

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T I T L E P A G E

Subject of Research:

An investigation of certain West Indian Hardwoods.

2. Name of Contractor:

Professor Wesley Cocker, Trinity College,
University of Dublin.

3. Contract Number:

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Final Technical Report

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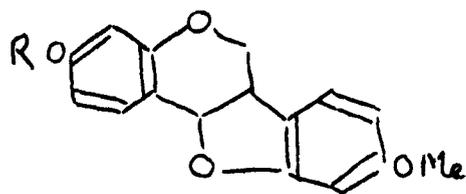
1. SUMMARY

During the year ended 31st January 1963, the following heartwoods have been investigated: - Andira inermis, Araucaria cunninghamii, Copaifera officinalis, Julbernardia globiflora, Lonicera periclymenum, Manilkara bidentata, Shorea meranti, Simaruba amara, Symphoricarpus rivularis, Tabebuia pentaphylla, Ulex europaeus, and Vitex divaricata. Papers have been published or have been accepted for publication on five of these woods namely Andira inermis (J. Chem. Soc., 1961, 4906), Vitex divaricata (Ibid., 1961, 5194), Manilkara bidentata (Ibid., 1963, 667), Symphoricarpus rivularis and Ulex europaeus (Perfumery Essent. Oil Record, early in 1963).

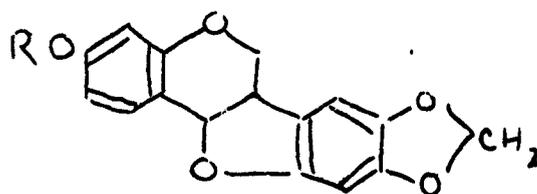
1. Andira inermis (Leguminosae).

In our previous Final Technical Report (Feb., 1962) we described the isolation of inermin and biochanin A from this wood. During the year fatty acids, β -sitosterol, vanillin and glucose have also been isolated.

The synthesis of trifolirhizin (II; R=glucose), the glucoside of inermin, has not been accomplished. We are currently attempting the synthesis of (\pm) demethylhomopteroicarpin (I; R=H) which we believe may be identical with one of the phenolic substances described in F.T.R. (Feb., 1962, p.2 and 8).



(I)



(II)

2. Araucaria cunninghamii (Cupressaceae).

This wood was disappointing. We had hoped to find lignans and the unidentified dundathic acid, $C_{21}H_{32}O_3$ to which Professor Erdtman had drawn our attention. Only small quantities of alkanes, long chain alcohols and β -sitosterol were found.

3. Copaifera officinalis (Leguminosae).

Work on this wood was restarted during the year and some headway has been made. Its extractives afford a steam volatile fraction containing humulene, β -caryophyllene, probably farnesene and probably isocaryophyllene in fair quantities. β -Sitosterol and an unsaturated acid, m.p. $119-121^\circ$ which analysed for $C_{20}H_{30}O_2$ were also obtained. This may be the unidentified copaltic or congocopaltic acid which has previously been isolated from congo copal resin, a fossilised resin from Copaifera spp. This acid is now under study.

4. Julbernardia globiflora (Leguminosae)

This wood afforded long chain normal alkanes, long chain

normal, saturated, fatty acids, β -sitosterol, β -amyrin, a small quantity of a mixture of alcohols, m.p. 234-236°, a red water soluble phenolic substance, m.p. 160-170°, containing sodium and potassium which was hydrolysed enzymatically to sucrose, fructose and glucose. The phenolic compound is probably a lignin.

5. Lonicera periclymenum (Honeysuckle - Caprifoliaceae).

Normal and iso long chain saturated fatty acids, and di- and triunsaturated acids of the normal series were isolated. In the neutral fraction several terpenoids, long chain alkanes and waxes were found. The latter were hydrolysed to β -sitosterol and long chain normal and iso saturated acids and an unsaturated acid fraction. Several sugars were also found.

6. Manilkara bidentata (Mimusops globosa - Sapotaceae).

Normal alkanes from tridecane to tetracosane inclusive, palmitic and stearic acids, β -amyrin, bassic acid, cyclolaudenol and α -spinasterol have been isolated from this wood. The so-called balatol or resiniferol or balataresinol described by earlier workers is a mixture in which cyclolaudenol is the predominant component. We have not identified the other component.

7. Shorea meranti (Dipterocarpaceae).

Work has not long been in progress on this compound.

However it has been shown to contain terpenoids, ketonic components of triterpene type, long chain fatty acids and possibly aromatic unsaturated acids.

8. Simaruba amara (Simarubeae).

This wood gave only normal alkanes, saturated normal long chain acids and β -sitosterol. It is an uninteresting wood.

9. Symphoricarpus rivularis (Caprifoliaceae)

We have identified a glycoside of aesculetin, several terpenoids, β -sitosterol, numerous saturated and unsaturated acids, glucose, fructose, sucrose and sorbose in the extractives of this wood. An unidentified alcohol is also present in small amounts.

10. Tabebuia pentaphylla (Bignoniaceae)

This wood contains pentacosane and heptacosane, veratric acid, long chain acids still to be identified, β -sitosterol, a very small quantity of an acid, m.p. 225-240°, glucose and fructose.

11. Ulex europaeus (Leguminosae)

The wood of this bush contains normal alkanes, long chain saturated and unsaturated acids and waxes which on hydrolysis yield β -amyrin, β -sitosterol, probably lupeol and saturated and unsaturated acids. Several terpenoids, glucose, fructose, maltose

and sucrose have also been found.

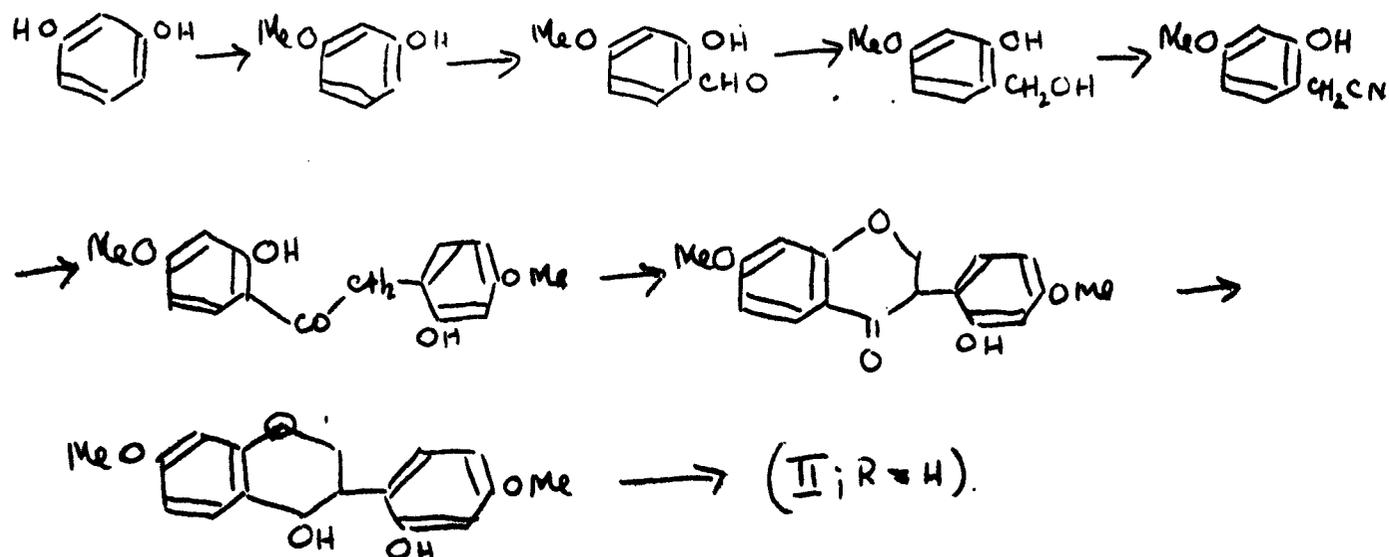
12. Vitex divaricata (Verbenaceae).

The extractive from this wood consists primarily of triacontanyl triacontanoate and the corresponding acid and alcohol, with other waxes, alcohols and acids in smaller amounts. Glucose was also isolated.

2. INTRODUCTION

1. Andira inermis.

In our Final Technical Report of February 1962 (p. 2 and 8) we referred to two unidentified phenolic compounds, m.p. 118° and 124-126°. Since homoptercarpin (I; R=Me) is frequently found with pterocarpin (II; R=Me) it is possible that the compound (I; R=H) will be found with inermin (II; R=H). We are now engaged on the synthesis of (I; R=H) by the following route.



We have reached the stage of the substituted benzyl alcohol.

Many attempts were made to condense tetraacetylbromoglucose with inermin (II; R=H) but in every case starting materials were recovered. The synthesis of trifolirhizin will thus have to await the availability of further supplies of inermin.

The fatty acids isolated from Andira by ligroin extraction were esterified and the mixture was analysed by gas-liquid chromatography on silicon rubber and silicon S.E. 30 columns. In each case the esters of the following acids were identified: - Arachidic (C₂₀; 72%), heneicosanoic (15.7%), behenic (3.7%), tricosanoic (2.3%), lignoceric (C₂₄; 3.6%), pentacosanoic (1.5%), cerotic (C₂₆; 1.0%), nonacosanoic (0.2%), and melissic (C₃₀; 0.1%).

The phenolic acid mentioned in F.T.R., 1962 (p.2,9) has not been further investigated for want of material.

2 2. Araucaria cunninghamii

This wood which belongs to the family cupressaceae was obtained from the State Botanic Gardens in Dublin. The family to which it belongs is rich in terpenoids, lignans and tropolones (compare for example the excellent review by H. Erdtman in Progress in Organic Chemistry, Butterworths Scientific Publications, London, 1952, p.22-63). An unidentified acid known as dundathic acid, C₂₁H₃₂O₃, is present in some cupressales. This was sought but we failed to find it. In fact we found only normal

hexacosanol and octacosanol, β -sitosterol and alkanes which have not been investigated yet.

3. Copaifera officinalis.

This wood of the leguminosae family was examined earlier without much success. A new worker in the team however has found some very interesting substances in it. Separation of the ligroin extract into neutral and acidic fractions gave an acid, m.p. 119-121°, $[\alpha]_D + 36^\circ$. In the infrared it had bands at 1690 (CO_2H , probably α, β -unsaturated) 1640 ($\text{C}=\text{C}$ of $-\text{C}=\text{C}-\text{CO}_2\text{H}$), 925 (acid OH), 720 cm^{-1} (CH_2 groups in chain). In the ultraviolet a maximum at 2190 Å is characteristic of the $\text{C}=\text{C}-\text{CO}_2\text{H}$ group. Reduction of the acid gave a new acid with a maximum at 1710 cm^{-1} characteristic of an isolated carboxyl group. It would seem therefore that the acid is α, β -unsaturated.

In 1908, Engel (Arch. Pharm., 1908, 246, 293) isolated from congo copal (Copaifera spp.) an acid "congocopalic acid", m.p. 115-118° which he assumed to be monocarboxylic of formula $\text{C}_{19}\text{H}_{30}\text{O}_2$. In 1926, Bauer and Gonsor (Chem. Umschau, 1926, 33, 250) suggested that it had the formula $\text{C}_{38}\text{H}_{60}\text{O}_4$ and that it was a dicarboxylic acid, with two double bonds per molecule. Mertens and Hellinckx (Chem. Abs., 1940, 34, 3934) referred to "congocopalic acid" as $\text{C}_{36}\text{H}_{58}(\text{CO}_2\text{H})_2$ and to "congo-copalolic acid" as $\text{C}_{21}\text{H}_{32}(\text{OH})\text{CO}_2\text{H}$. Sutton (J. Chem. Soc., 1949 /isolated a ⁶⁷⁶)

"congoic acid", from the same source, whose methyl ester analysed for either $C_{21}H_{34}O_2$ or $C_{21}H_{36}O_2$ containing two double bonds. He also isolated "congo-copalic acid", m.p. $140-165^\circ$ whose methyl ester, m.p. $65-70^\circ$ had a molecular weight of 1400 and formula $(C_{29}H_{47}OCO_2H)_3$. Sutton isolated "congo-copalolic acid" of formula $(C_{30}H_{48}O_3)_n$. From data given by the isothermal distillation method n appears to be 32.

It may be possible to relate our acid with one of those from congo copal in which case the latter will be used as source of the acid.

Copaifera officinalis also afforded β -sitosterol and a steam volatile oil. The latter distilled between $90-95^\circ/2$ mm., $[\alpha]_D - 4.8^\circ$ which analysed satisfactorily for $C_{15}H_{24}$ and showed maxima at 3100, 1775, 1645 and 885 cm^{-1} (terminal CH_2 group). By gas-liquid chromatography this compound was shown to be almost pure β -caryophyllene. Smaller peaks on the spectrogram corresponded to humulene (α -caryophyllene). The other two peaks are probably farnesene and isocaryophyllene.

4. Julbernardia globiflora

This wood was obtained from Tanganyika where its bark is used for making ropes, beehives, sacks, etc., and a decoction as an eye lotion for conjunctivitis. The bark, root and leaf are said to be highly toxic and in Northern Rhodesia the tree is used

for trial by ordeal among the Lamba (Watt and Breyer-Brandwijk, "Medicinal and Poisonous Plants of Southern and Eastern Africa", Livingstone, Edinburgh and London, 1962). We have not yet found an extractive which would merit the statements made above.

The ligroin extractives consist of β -sitosterol, all the normal alkanes from pentadecane to octacosane, the normal saturated fatty acids from eicosanoic to octacosanoic acid inclusive, and octadecenoic^{to}/heneicosenoic, tetracosenoic, nonacosenoic and triacontenoic acids. A small quantity of a so far unidentified "alcohol", m.p. 234-236° was obtained but thin layer chromatography showed it to be a mixture.

Ether extraction of the wood yielded a dark red, water soluble phenolic substance, m.p. 160-170°, which contained sodium and potassium ions and sugars. It had maxima at 2250 and 2840 Å. Attempted acid hydrolysis of the glycoside gave tars, but the hydrolysis was satisfactorily carried out with the enzyme, mycozyme, giving glucose, fructose and sucrose which were identified chromatographically. The aglycone could not be crystallised, but it gave an acetyl compound (no Fe^{+++} reaction), m.p. 164-167°, λ_{max} 2170 and 2750 Å and ν_{max} 1786 (phenolic acetate), 1266, 1249, and 1205 cm^{-1} (acetate). The acetate analysed for $(\text{C}_{10}\text{H}_{10}\text{O}_4)_x$ and its acetyl content showed that two acetyl groups were present in each $\text{C}_{10}\text{H}_{10}\text{O}_4$. We thus account for all the oxygen. It contained no methoxyl. The author is not aware of any lignin which

lacks methoxyl so that more attention will have to be given to this compound.

5. Lonicera periclymenum.

Several species of Lonicera have been investigated previously Goodwin (Biochem. J., 1952, 51, 458) showed that the berries of L. japonica contain carotenoids. Alkaloids were detected in L. iberica (Zolotnitskaya, Izvest. Akad. Nauk Armian. S.S.R., Biol. i Sel' khoz. Nauki, 1954, 7, (no 5), 27). L. xylostrum (Béguin, Pharm. Acta. Helv., 1931, 6, 195), L. gigantea (Igolen, Parfums France, 1937, 15, 298), L. prunus and L. viburnum (Ionesco, Ann. Sci. univ. Jassy, 1940, Sect. 2, 26, 75) have also been investigated, but little of interest has been found.

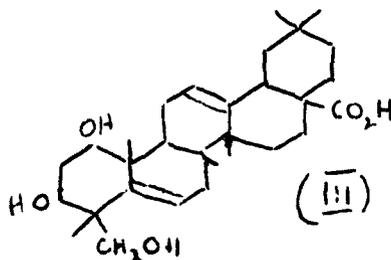
Acidic and neutral fractions, but no phenolic fractions were found in L. periclymenum. The acids were investigated as methyl esters by gas-liquid chromatography. Normal dodecanoic to octadecanoic acid inclusive, arachidic acid, isoundecan-, isododecan-, isotridecan-, isopentadecan- and isoheptadecanoic acids were found. Palmitic acid (hexadecanoic acid) constituted over half of the mixture. The unsaturated acids, hexadecen-, octadecen-, octadecadien-, and octadecatrienoic acids were also present.

A steam volatile fraction yielded cineol, limonene, α - and β -pinene, myrcene and alloocumene, whilst other neutral

components were the normal alkanes from heneicosane to triacontane inclusive and waxes. On hydrolysis, the latter gave β -sitosterol and the following acids: - dodecan-, tetradecan-, octadecan-, isoundecan- and isoheptadecanoic acids and an unsaturated fraction which on hydrogenation afforded isononadecanoic acid. Glucose, fructose and sucrose were also isolated.

6. Manilkara bidentata.

Little can be added to the report made in F.T.R. (Feb., 1962, p.11-17). In short it can now be stated that in the neutral fraction the normal alkanes from tridecane to tetracosane, β -amyrin, α -spinasterol, cyclolaudenol, and an unidentified compound, in very small quantities, are present. Two long chain fatty acids are present and basic acid (III) occurs as a saponin in which the sugars are glucose, rhamnose and xylose.



It seems clear that the substance variously described as balataresinol and formulated as $C_{27}H_{46}O_2$ or $C_{27}H_{44}O_2$ (Tschirch and Schereschewski, Arch. Pharm., 1905, 243, 358) or $C_{30}H_{50}O$ (Cohen, ibid., 1907, 245, 245; 1908, 246, 358), balatol formulated as

$C_{32}H_{52}O_2$ (Tanake, Kuwata and Suzuki, J. Soc. Chem. Ind., Japan, 1935, 38, 504B) and resiniferol formulated as $C_{30}H_{50}O$ (Dupont, Julia and Wragg, Bull. Soc. chim. France, 1953, 852, 504B) which were isolated from balata resin and Euphorbia resinifera are mixtures similar in composition to the "alcohol" which we isolated from M. bidentata. The latter was finally shown by gas-liquid chromatography to be cyclolaudenol and another compound present in relatively small amounts. This other compound is not α - or β -amyrin, cycloartenol, cycloartenone, lupeol, β -sitosterol or α -spinasterol.

We obtained cyclolaudenol in a pure condition by oxidation of the above mixture to ketone from which cyclolaudenone was isolated pure. Reduction with sodium and isopropanol gave cyclolaudenol.

7. Shorea meranti

Shorea meranti was obtained from Malaya Extraction with ligroin gave a fairly high yield of a white solid, m.p. 77-90° which had maxima at 1710-1750 (C=O), 720 and 740 cm^{-1} (chain of CH_2 groups). It therefore appeared to be a mixture, possibly of fatty acids and esters. This mixture, on distillation in steam, gave an oil with a terpenaceous odour which has not yet been investigated. The non-volatile residue has been divided into ether soluble and ether insoluble acids and esters. The ether

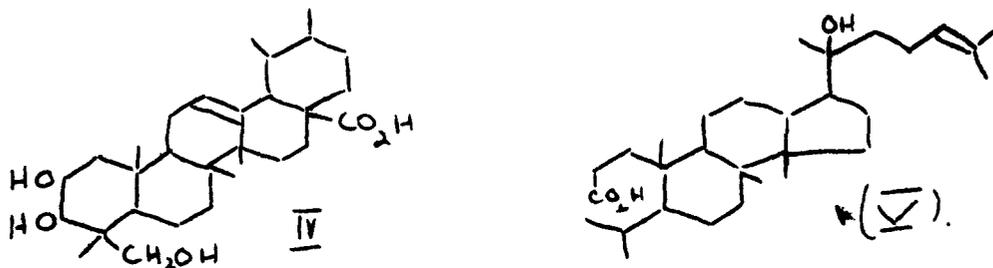
insoluble fraction consists mainly of esters having a maximum at 1740 cm^{-1} in the infrared. The ether soluble mixture has given acids, which are bicarbonate soluble and therefore not very long chain acids, whose spectrum in the infrared indicates some unsaturation. These acids are being purified by thin layer chromatography through their acetoxymethylmercurimethoxy adducts. The ether soluble esters have been saponified and the products are under examination.

The main ligroin extract afforded a steam volatile terpenaceous fraction and a gummy neutral fraction whose infrared spectrum showed unsaturation and the presence of hydroxyl groups.

Although none of the terpenes have been positively identified, there is evidence for the following: - 1,8-cineol, α - and β - pinene, limonene, terpinolene, citral and phellandrene.

The gummy neutral fraction is proving difficult to resolve, but we might expect it to contain similar compounds to those found in dammar resin. The work on this resin has been described by Brewis and Halsall (J. Chem. Soc., 1961, 646), Dunstan, et. al., (Croat. Chim. Acta., 1957, 29, 173), Fazackerley, Halsall and Jones (J. Chem. Soc., 1959, 1877), Baddeley, Halsall and Jones (ibid., 1960, 1715) and by Mills and Werner (ibid., 1955, 3132). The acidic constituents of the resin are asiatic acid (IV), dammarenolic acid (V) investigated by Arigoni et. al. (J. Chem. Soc., 1960, 1900), and two other acids isolated by Brewis

and Halsall (loc. cit.) as their methyl esters. These analysed for $C_{32}H_{48-50}O_6$ or $C_{33}H_{50-52}O_6$ and $C_{32}H_{50-52}O_6$ respectively.



Neutral components of dammar resin include hydroxyhopanone.

8. Simaruba amara

Another batch of this wood was delivered. We therefore took another look at it, but found it to be of little interest. It contains cineol, α -terpineol, β -sitosterol, normal alkanes from tetracosane to dotriacontane, and the normal saturated acids from octadecan- to hexacosanoic acid.

9. Symphoricarpus rivularis

This climbing plant gave a ligroin extractive which contained only insignificant amounts of acids and phenols, but it contained modest amounts of a neutral fraction. Alkaline hydrolysis of this gave β -sitosterol and another alcohol in very small amount. The latter, m.p. 193° (acetate, m.p. 165°) gave a positive Liebermann-Burchard reaction, it showed maxima at

875(RRC = CH₂) and 1640 cm⁻¹(C=C), but chromatography on paper showed it to be a mixture of two substances. When further supplies are available the investigation of these two compounds will be continued.

The mixture of acids released by hydrolysis was complex. It contained saturated, mono-, di-, and triethenoid types. The saturated fraction consisted partly of branched types. After esterification the mixture was treated with mercuric acetate in methanol giving the acetoxymercurimethoxy complexes (cf. Cocker, Dahl and McMurry, J. Chem. Soc., in Press) of the unsaturated esters. These were then separated by thin layer chromatography and the complexes decomposed. In this way the following acids were identified: - normal nonoic to octadecanoic inclusive, isodecan-, isotridecan to isohexadecan- inclusive, isooctadecan- to isopentacosanoic inclusive, tetradecen- to hexadecenoic inclusive, octadecenoic, tetradecadien- to hexadecadienoic inclusive and hexadecatrienoic.

Several terpenoids were present in the ligroin extract. Using a diethyleneglycol succinate column and also a castor wax column cineol and/or limonene, myrcene and/or phellandrene, alloocimene and/or methyl heptenone, α - and β - thujone, nerol, α -terpineol and citral were identified.

Extraction of the wood with methanol gave a lignin-rich mixture containing free glucose, fructose, sorbose and sucrose.

* See Experimental Section

Acid hydrolysis of the extract afforded aesculetin (cf. McCullagh, Walton and White, Trans. Roy. Soc. Canada, 1929, 23(V), 159) identified by paper chromatography.

10. Tabebuia pentaphylla.

The heartwood of this West Indian timber, extracted with ligroin, gave acidic, 'hydroxide soluble' and neutral fractions. Veratric acid was isolated from the acidic fraction and identified by spectroscopy and by mixed m.p. with an authentic sample. β -Sitosterol was the only compound isolated from the hydroxide soluble fraction. This can only be due to incomplete resolution of emulsions which readily form with steroids and triterpenes in alkali.

The neutral fraction gave a small amount of steam volatile terpenaceous oil which has not yet been investigated, but the main neutral fraction was a wax. Saponification of this gave acids still under examination, β -sitosterol and normal penta- and heptacosanes.

Ether extraction of the wood gave a fraction which was partly water soluble and partly insoluble. The latter afforded more veratric acid and a small quantity (10 mg.) of a solid, m.p. 200-206°. This showed maxima at 2300 and 3150 A in ethanol but these became a single maximum at 2850 in ethanol/sodium acetate; this is a characteristic of a 4-hydroxyphenyl-

ketone. It coupled with diazotised sulphanilic acid giving a red azo compound and with diazotised p-nitroaniline giving a blue azo compound. The spectrum in the ultraviolet region is not unlike that of a coumarin, but the quantity of the substance available was too little to do much work with it.

11. Ulex europaeus

Work on this prickly shrub was completed during the year. Reference to earlier work on the shrub was made in F.T.R. (Feb. 1962, p.24).

The ligroin extract gave neutral and acidic fractions, but no recognisable phenolic compounds. The acids were esterified with methanol and boron trifluoride and resolved into saturated and unsaturated fractions by the use of mercuric acetate in methanol, and thin layer chromatography (see above). The following acids were thus identified: - normal dodecanoic to normal tetracosanoic acid inclusive, octadecenoic, octadecadienoic and octadecatrienoic acid. We are not sure of the stereochemistry of these unsaturated acids but the retention times of their esters are those of oleic, linoleic and linolenic acids respectively.

A steam-volatile oil obtained from the neutral fraction contained α - pinene, β -pinene or myrcene, limonene, phellandrene, cineol or p-cymene and β - thujone.

The remainder of the neutral fraction was hydrolysed

and the 'alcohols' released were acetylated and chromatographed on alumina. The first fractions eluted contained alkanes which were shown by gas-liquid chromatography to be normal tridecane to normal tetracosane inclusive and isotridecane to isoeicosane inclusive. Later fractions from the alumina column contained β -amyrin and β -sitosterol acetates and an acetate, m.p. 180-200° which behaved as lupeol acetate. Another alcohol isolated as its benzoate, in very small amounts, was shown to be a mixture of several unidentified substances.

Extraction of the timber with methanol afforded maltose, sucrose, glucose and fructose.

12. Vitex divaricata

Previous work on this wood was briefly reviewed in F.T.R. (Feb., 1962, p.18). We also reviewed our own work on the wood in that report.

The supposed hexacosanoic acid, m.p. 85-87°, described in the above mentioned report has now been shown to be a mixture of hexa- and octacosanoic, triacontan- and dotriacontanoic acid, in which the C₃₀ acid predominates.

The fraction which we described previously as being a mixture of C₂₈-C₃₀ alcohols has now been shown to be predominantly Triacontan-1-ol (90%). Octacosan-1-ol and dotriacontan-1-ol are also present with traces of octadecan-1-ol, eicosan-1-ol,

docosan-1-ol and pentacosan-1-ol.

The extractive is thus largely triacontanyl tricontoate and the corresponding acid and alcohol.

EXPERIMENTAL.

Experimental details of our work on A. inermis, M. bidentata, V. divaricata, S. rivularis, and U. europaeus will not be given. Preprints of our papers on the first three woods are available. Typescript copies of the papers on the other two woods can be made available.

Ultraviolet spectra were measured for ethanolic solutions, infrared spectra for Nujol suspensions, or as gums, and $[\alpha]_D$ for chloroform solutions. Gas-liquid chromatography was carried out on an Aerograph Hi Fi 600 instrument (Wilkins Instrument and Research Inc.).

1. Araucaria cunninghamii

This wood (15 lbs.) was extracted continuously for 48 hr. with ligroin (15 l.), and the extract was concentrated to 1 l. It was then extracted successively with 5% sodium hydrogen carbonate and 5% sodium hydroxide. The acidic fraction consisted of a semi solid (3 mg.) which was not further investigated. There was no phenolic fraction.

The neutral fraction (20 g.) was hydrolysed during 5 hr. with 5% methanolic sodium hydroxide (200 c.c.) in benzene (20 c.c.). Water was added and unsaponifiables were extracted with ether.

Unsaponifiables

These (10 g.) were dissolved in ligroin, run on a neutral alumina column and eluted with (a) ligroin (fraction A), (b) ether-benzene (1:10 v/v) (fractions B and C).

Fraction A (2 g.) had ν_{\max} 2941 (CH_3 , CH_2), 1460, 1378 cm^{-1} (CH_3 , CH_2). This mixture of alkanes has not yet been satisfactorily resolved by gas-liquid chromatography.

Fraction B (2 g.), m.p. 75-78° gave an acetate, m.p. 68° which had ν_{\max} 1739 (acetate), 1242 (acetate), 730, 720 cm^{-1} (CH_2 groups). This mixture of acetates was chromatographed on a silicone rubber column; Temp. 202° \pm ; N_2 flow 28 c.c./min.; H_2 flow 26 c.c./min.; chart speed 20 in./hr. The results are given below.

Peak No.	Rel. T_R	No. of C atoms in alcohol	Approx Quantity %
1 (Standard)	1.0000	normal 20	
2 "	1.9600	" 22	
3	7.7820	" 26	39
4	15.0000	" 28	61

Fraction C (0.6 g.), m.p. 135° gave no depression with β -sitosterol.

Saponifiables.

The alkaline solution was acidified, extracted with ether giving an acidic brown gum (ca 1 g.). This was esterified in the usual way with diazomethane and chromatographed.

2. Copaifera officinalis.

Heartwood shavings (46 lb.) were extracted continuously with ligroin (15 l.) for 48 hr., then concentrated to 1.5 l. and resolved into acidic and neutral fractions as above. No phenols were encountered. The acidic fraction (80 g.) afforded a resin which on standing gave a positive Liebermann-Burchard reaction. This fraction (5 g.) was dissolved in ligroin, run on a column of silica gel and eluted successively with ligroin, benzene, ether and methanol. Colourless gums were obtained, the early fractions of which became solid on standing at room temperature.

Crystallisation from ligroin gave fine needles, m.p. 119-121°.

$[\alpha]_D + 36^\circ$ (C 0.92), λ_{\max} 2190 Å, ν_{\max} 1690 and 1640 (C=C-CO₂H), 925 and 720 cm⁻¹ (CH₂ in chain) (Found: C, 78.6; H, 10.5; E, 277. Calc. for C₂₀H₃₀O₂: C, 79.4; H, 10.0; E, 302. C₂₀H₃₂O₂: C, 78.9; H, 10.6%; E, 304. C₁₉H₃₀O₂: C, 78.6; H, 10.4%, E, 290. C₁₈H₂₈O₂: C, 78.2; H, 10.2%; E, 276).

Reduction of the acid in ethyl acetate over palladised charcoal gave an acidic gum, ν_{\max} 1710 (CO₂H). Attempts to esterify the unsaturated acid with boron trifluoride in methanol

failed, possibly due to hindrance, but diazomethane gave a colourless oily ester, which gave one peak only on diethyleneglycol succinate and silicone rubber columns.

Later fractions eluted from the silica gel columns gave β -sitosterol, m.p. and mixed m.p. 135° , $[\alpha]_D - 36^\circ$.

Removal of ligroin from the neutral fraction gave a gum (28 g.) which was distilled in steam giving a pale yellow volatile oil (12.8 g.), b.p. $90-95^\circ/2$ mm. $\sqrt{\text{max}} 3100$ (CH_3 , CH_2), 1675 ($\text{C} = \text{C}$), 1645 ($\text{C} = \text{C}$), 885 ($>\text{C} = \text{CH}_2$) (Found: C, 87.8; H, 12.0; Calc. for $\text{C}_{15}\text{H}_{24}$: C, 88.2; H, 11.8%). Chromatographed on the above columns it showed one peak of the same retention time as β -caryophyllene. However on a sucrosehexaacetateisobutyrate column four peaks were revealed (Table I).

TABLE I

Column: 5 ft. x $1/8$ in. Temp. $164 \pm 1^\circ$. N_2 flow 20 c.c./min. H_2 flow 23 c.c./min. Chart speed 15 in./hr.

Peak	Retention Vol.(c.c)	Approx. quantity %	
1	128	5.5	Farnesene?
2	144	65.0	β -Caryophyllene
3	176	10.6	Humulene
4	199	18.7	isocaryophyllene?

Humulene was obtained from hop oil for comparison.

Farnesene is being prepared for comparison.

The non-volatile neutral fraction was saponified for 12 hr. with 7% methanolic potash (200 c.c.) in benzene (50 c.c.). The solution was diluted with water to twice its volume and extracted with ether to remove unsaponifiables. The latter were refluxed for 2 hr. with acetic anhydride and pyridine, and the acetate (11 g.) was chromatographed on neutral alumina and eluted successively with ligroin, benzene, ether and methanol. Early fractions (4 g.) consisted of terpenes identical with the steam volatile fraction. Later fractions were β -sitosterol/^{acetate} m.p. and mixed m.p. 125° , $[\alpha]_D - 36^{\circ}$.

The alkaline solution after saponification was acidified giving a resinous material (1.5 g.) similar in infrared spectrum to the acid described above.

3. Julbernardia globiflora

This wood (55 lb.) was extracted in the usual way with ligroin and the extract was concentrated to 2 l. A white solid (A; 11 g.) was deposited and collected. Mother liquors were further concentrated to 500 c.c. (B) and separated into acidic and neutral fractions. There were no phenols.

Fraction A was extracted first with 4% sodium hydrogen carbonate but only a trace of acid was obtained. Extraction with 5% sodium hydroxide yielded a solid (4.5g.), m.p. $72-74^{\circ}$ (needles from ethyl acetate-light petroleum), ν_{\max} 1710 (CO_2H), 1639 and

1600 (C=C), 720 cm^{-1} (CH_2) (Found: C, 78.9; H, 13.0). Esterified with diazomethane it (300 mg.) gave a product, m.p. 65-66°.

Mercuration of the esters.

The reagent was mercuric acetate (14 g.), methanol (250 c.c.), glacial acetic acid (2.5 c.c.) and water (1 c.c.). The mixture of esters (300 mg.) was left in the dark for 24 hr. with the reagent (9 c.c.), methanol was removed under reduced pressure at 20°, and the residue was extracted with ether (4 x 20 c.c.), washed with water and dried.

Fractionation of the complexes.

Silica gel (Merck's Kieselgel G) was deposited as a layer 275 μ thick on plate-glass sheets, 20 x 20 cm., and heated at 110° for 0.5 hr. The ether solution of the complexes was applied by a fine spray as a narrow band near the edge of each plate. The plates were then developed in two systems (a) light petroleum-ether (4:1; v/v), (b) propan-1-ol - glacial acetic acid^(150:1). In system (a) the saturated esters moved rapidly, and the band was scraped from the plates leaving the acetoxymethylmercurimethoxy-complexes near the origin. These were then developed in system (b). Identification of the saturated ester zone was made by exposure of a narrow band to iodine vapour which gives a yellow colour due to absorption of the vapour by the band. The complexes were identified by spraying with diphenylcarbazone in 95% ethanol which gives a purple colour. The esters m.p. 65-66° from fraction A behaved as shown in Table II.

TABLE II

<u>Ester Zones</u>	<u>Solvent System</u>	
	(a) R_F	(b) R_F
Saturated esters.	0.95 - 1.0	
Complexes of:		
mono unsaturated esters		0.95
di-		0.71
tri-		0.45

Recovery of esters.

The zone containing the saturated esters was stirred with ether (4 x 15 c.c.), filtered, dried ($MgSO_4$), evaporated to dryness, and submitted to gas-liquid chromatography in methylene chloride. The zones of the complexes were removed from the plates and stirred with 5% methanolic hydrochloric acid (3 x 15 c.c.). The supernatant solutions were diluted with water (100 c.c.), extracted with ether, dried and concentrated.

TABLE IIIChromatographic separation of the saturated esters obtained from (A)

Column: 5 ft. x 1/8", silicone rubber; Temp, 228°;
 N₂ flow 26 c.c./min., H₂ flow 24 c.c./min.; chart
 speed 20 in./hr.

Peak No.	Rel. T _R	Carbon number of ester [*]	Approx. quantity %
1	0.5370	20	28.1
2	1.0000	22	17.4
3	1.4070	24	30.0
4	1.6020	25	3.3
5	1.7240	26	21.0

TABLE IVChromatographic separation of the monounsaturated esters obtained from A.

Conditions as for Table III except for temp. 186° ± 1°

Peak No.	Rel T _R	Carbon number of ester	Approx. quantity %
1	1.0000	18	11.6
2	1.3349	19	9.0
3	1.6741	20	19.9
4	2.1531	21	16.6
5	3.8516	24	26.5
6	5.0717	27	2.5
7	8.9230	29	3.5
8	16.8181	30	9.9

* Throughout this report the carbon number of the ester does not include the carbon of the OCH₃ group.

The mother liquors (B) were extracted with 5% sodium hydroxide giving an acidic fraction (4.5 g.) which was esterified with diazomethane. The esters were analysed on a S.E. 30 column; temp., 259-260°; N₂ flow, 31 c.c./min.; H₂ flow, 30 c.c./min.; chart speed, 20 in./hr., with the following results.

TABLE V

Peak No.	Rel. T _R	Carbon number	Approx. quantity %
1	0.5990	20	6.8
2	1.0000	21	12.2
3	1.2932	22	6.1
4	1.7430	23	20.6
5	2.2428	24	17.1
6	2.9741	25	30.9
7	3.7540	27	2.5
8	4.8025	28	3.6

The neutral fraction of (B) (12 g.) was hydrolysed for 18 hr. with 8% methanolic potash, benzene being added to give homogeneity. Solvents were distilled off, the residue was diluted and the non-saponifiables were extracted with ether. Ether was removed and methanol was added giving β -sitosterol (3.5 g.), m.p. and mixed m.p. 138°, $[\alpha]_D - 45^\circ$ (C 0.21). The methanol solution was distilled in steam but little volatile material was obtained. The non-saponifiable residue (5.3 g.) (ν_{\max} 3500 [OH], 1733 [CO], 1640 [C=C], 885 [C=CH₂]) was

absorbed on a column of silica gel and eluted successively with ligroin, ligroin-benzene (9:1); (8:2); (7:3). Early fractions eluted consisted of a yellow semi-solid with the spectrum of an alkane. It was analysed by gas-liquid chromatography with the results shown below.

TABLE VI

Column: S.E. 30; Temp. 213°; N₂ flow 23 c.c./min;
H₂ flow 30 c.c./min.

Peak No.	Rel. T _R	Carbon number (normal series)
1	0.0794	15
2	0.1032	16
3	0.1428	17
4	0.1944	18
5	0.2697	19
6	0.3725	20
7	0.5160	21
8	0.7230	22
9	1.0000	23 (standard)
10	2.7350	26 (")
11	5.2300	30 (") Not present in the wood.

Using temp., 251°, the following additional alkanes were identified C₂₄, C₂₅, C₂₇, C₂₈.

When the silica gel column was eluted with ether-benzene (40: 60; 50: 50), more β-sitosterol (0.8 g.) was isolated. Ether alone eluted an alcohol (110 mg.), m.p. 234-236°, [α]_D²⁰ + 98°, (Found: C, 75.5; H, 9.9). Thin layer chromatography on

silica-gel using cyclohexane-ethyl acetate (70:30) as solvent system revealed two spots R_F 0.184 and 0.325, the former being the more intense. These have not been identified.

Ether extraction of the lignin extracted J. globiflora gave^a dark red water soluble phenolic substance (6.7 g.), m.p. 160-170°, (see p.9 for spectral data). Attempted hydrolysis with hot 1% hydrochloric acid gave a tarry product, but the acid solution contained sugars. Reaction of the solid with acetic anhydride and pyridine gave a pale yellow solid, m.p. ca 180° (indefinite),

ν_{\max} 3509, 2740 (CO₂H), 1786 (phenolic acetate), 1626, 1613 (aromatic), 1266, 1249, 1205 (acetate). The product was reacylated by heating for 2 hr. on the water bath with the above reagent. The product was washed with 10% sodium hydroxide, water, dried and crystallised from ethanol. It then gave m.p. 164-7°, λ_{\max} 2750 Å, ν_{\max} 1754 cm⁻¹ (Found: C, 62.18; H, 5.13, OAc, 45.72%).

The red phenolic substance, m.p. 160-170°, (1 g.) was dissolved in deionised water (30 c.c.), a buffer solution (22.5 c.c.) prepared from 0.2N acetic acid (21 c.c.)- 0.2N sodium acetate (49 c.c.)^{was added}/followed by mycozyme (100 mg.) in 30 c.c. water. The mixture was diluted to 100 c.c. and incubated at 45° for 60 hr. The mixture was then extracted with ether and the ethereal solution was filtered through iron-free kieselguhr. It was then dried over anhydrous magnesium sulphate and the ether removed under reduced

pressure. The residual solid (ca. 50 mg.) was shown to contain fructose, glucose and sucrose by paper chromatography

R_F	0.2	0.17	0.1	} Butanol - EtOH - H ₂ O }
	fructose	glucose	sucrose	
R_F	1.3	0.95	0.5	} Ethyl Ac - Pyridine - H ₂ O }

The solid hydrolysate did not melt below 300°. The infrared spectrum was identical with that of the original phenol.

4. Lonicera periclymenum.

The extraction of this wood (32 lbs.) was described in F.T.R. (Feb., 1962, p.54-55). The free acid fraction (11.7 g.) had ν_{\max} 1725, 1710 ($>C=O$), 1600 ($C=C$), 720-730 cm^{-1} (CH_2 in chain). Acids (2.5 g.) were refluxed for 7 min. with 4M methanolic borontrifluoride (25 c.c.) giving esters (2.55 g.), ν_{\max} 1739 cm^{-1} (CO_2Me). These were then separated into saturated and unsaturated types by the method described under J. globiflora. The R_F values were as follows: - saturated (solvent a), 0.91; monounsaturated (solvent b), 0.80; diunsaturated, 0.60, 0.46 (two bands); triunsaturated, 0.29. Each fraction was separately analysed by gas-liquid chromatography using silicon S.E. 30, quadrol sucroseacetateisobutyrate and diethyleneglycol-succinate columns. The figures for the first column only will

be given.

TABLE VII

Column S.E. 30, 5 ft. x 1/8 in.; Temp. $201^{\circ} \pm 1^{\circ}$;
 N_2 flow 20 c.c./min.; H_2 flow 24 c.c./min.; chart
 speed 20 in./hr. A = authentic standard

Peak No.	Rel. T_R	No. of carbon atoms in ester and type of chain.		Approx. quantity %
		Normal	iso	
1	0.0883		11	3.1
2	0.1765		12	5.1
3	0.1912	12		3.0
4	0.2500		13	3.0 A
5	0.2830	13		2.6
6	0.3560	14		4.1
7	0.4830		15	2.0 A
8	0.6180	15		2.3
9	1.0000	16		53.0 A
10	1.2480		17	4.8
11	1.3970	17		2.9
12	2.0200	18		4.8 A
13	4.2400	20		7.3 A

A small amount of normal undecanoic acid was identified with this column but not with the D.E.G.S. and S.A.I. B. columns. A small amount of iso decanoic acid was identified with the S.A.I.B. column but not with the others.

The monounsaturated esters were analysed on D.E.G.S. and S.E. 30 columns. The results of the latter run are given below.

TABLE VIII

Column S.E. 30, 5 ft. x 1/8"; Temp. $204^{\circ} \pm 1^{\circ}$; N_2 flow: 19 c.c./min.; H_2 , 24 c.c./min.; chart speed 20 in.²/hr.

Peak No.	Rel T_R	No. of C. atoms in ester and type of chain	Approx. quantity %
1	0.7010	normal 16	32
2	1.0000	" 18	68

The remainder of the neutral fraction (18.7 g.) was hydrolysed for 7 hr. with boiling 5% methanolic potassium hydroxide (200 c.c.). On cooling a brown solid (2.5 g.) was deposited. It was crystallised from methanol giving a product (X), m.p. $122-124^{\circ}$, $[\alpha]_D^{14} - 17.7^{\circ}$. Its acetate had m.p. 102° . Both gave a positive Liebermann-Burchard colour. The alcohol (X) was absorbed on a column of neutral alumina and eluted with light petroleum-benzene (9:1; v/v) and (4:1, v/v). A substance (84 mg.), m.p. 58° , $[\alpha]_D = 0^{\circ}$, $\nu_{\text{max}}^{\text{ing}}$ 715 and 727 cm^{-1} (chain of CH_2 groups) was first eluted, having the properties of an alkane. Gas-liquid chromatography on a S.E. 30 column showed the presence of C_{21} to C_{30} normal alkanes in the following relative quantities:- C_{21} (2.5%), C_{22} (1.2%), C_{23} (1.15%), C_{24} (3.1%), C_{25} (8.0%), C_{26} (12.9%), C_{27} (15.0%), C_{28} (14.7%), C_{29} (14.5%), C_{30} (11.2%), C_{31} (8.6%), C_{32} (4.3%), C_{33} (2.9%). Chromatography on a poly-m-phenylether column confirmed these results.

The mother liquors from the crystallisation of (X) were concentrated giving β -sitosterol (130 mg.) as plates, m.p. 135° , $[\alpha]_D^{16} - 41$ (C 0.28) undepressed by an authentic specimen. Its acetate had m.p. and mixed m.p. 126° .

Elution of the alumina column mentioned above successively with light petroleum (40) -benzene (60); (30)-(70); (20)-(80); (10); (90) and pure benzene afforded a series of crystalline fractions, 86 mg., 164 mg., 120 mg., 85 mg., 52 mg. respectively. All were β -sitosterol. Elution with varying concentrations of ether-acetone gave more β -sitosterol (171 mg.).

The diunsaturated esters, analysed on S.E. 30 and S.A.I.B. columns were shown to contain octadecadienoic ester. Conditions were similar to those of Table VIII; authentic linoleic ester was used as standard. Similarly the triunsaturated esters were shown to contain octadecatrienoic ester, linolenic ester being used as standard.

The neutral fraction (20.5 g.) from the ligroin extraction was distilled in steam giving an orange oil (1.8 g.) which was submitted to gas-liquid chromatography, with the results shown below.

I. Column: 5 ft. x 1/8 in., diethyleneglycol succinate; temp. $80^{\circ} \pm 1^{\circ}$; N_2 flow 22 c.c./min.; H_2 flow 23 c.c./min.; charge speed 20 in./hr. A = authentic specimen.

<u>Rel T_R</u>	<u>Component</u>
0.3400	α -pinene
0.5040	β -pinene
1.0000	cineol (A), possibly limonene

Using the same column at 122°; N₂ flow 21 c.c./min.; H₂ flow 24 c.c./min., myrcene and alloocimene were identified (Rel. T_R; myrcene, 0.8000; cineol, 1.0000; alloocimene, 1.8500).

II. Column: castor wax; Temp. programmed from 50° to 200° ± 1° N₂ flow 27 c.c./min., H₂ flow 23 c.c./min.; chart speed as before.

<u>Rel. T_R</u>	<u>Component</u>
0.6620	α-pinene
0.8130	β-pinene and or myrcene
0.8870	limonene
1.0000	cineol (A)
1.2500	alloocimene

In the programmed chromatographic separation many other unidentified peaks were revealed.

Dilution of the methanolic potash solution mentioned above with an equal volume of water afforded a yellow semi-solid (7.6 g.) which on refluxing with methanol and then cooling, afforded β-sitosterol (0.75 g.), m.p. and mixed m.p. 135°, $[\alpha]_D^{18} - 35^\circ$ (acetate, m.p. 123°). The residue from this was a yellow oil (6.5 g.) which has not been resolved.

Acids from the saponification.

The aqueous methanolic potash solution was acidified and extracted with ether giving a semi-solid (4.2 g.), ν_{\max} 1710 (C=O), 724 cm⁻¹ (CH₂). A portion (1.5 g.) was esterified as described above with methanolic boron trifluoride and the

methyl esters were submitted to gas chromatography as in the case of the esters of the free acids. Two columns, namely S.E. 30 and D.E.G.S. were used and esters of authentic normal C₁₂, C₁₄, C₁₆ and iso C₁₄ acids were used as markers. The following acids were identified, iso C₁₁ (2.6%), n-C₁₂ (3.7%), n-C₁₄ (3.3%), n-C₁₅ (1.4%), n-C₁₆ (48.0), iso C₁₇ (1.6%), n-C₁₇ (1.7%) and n-C₁₈ (35.3%).

Other peaks whose retention time/carbon number did not fall on the line for either the normal or iso acids, were found to be unsaturated. We could not identify them however from the G.L.C. trace. The mixture of esters (1.0 g.) was therefore hydrogenated in ethyl acetate (20 c.c.) over 10% palladised charcoal for 14 hr. The product was inert to tetranitromethane and showed no unsaturation in the infrared region. When submitted to gas chromatography on the two columns mentioned above, another peak was revealed namely that corresponding to the ester of the iso C₁₉ acid. An iso C₁₉ unsaturated acid is therefore present in the mixture (ca 17%).

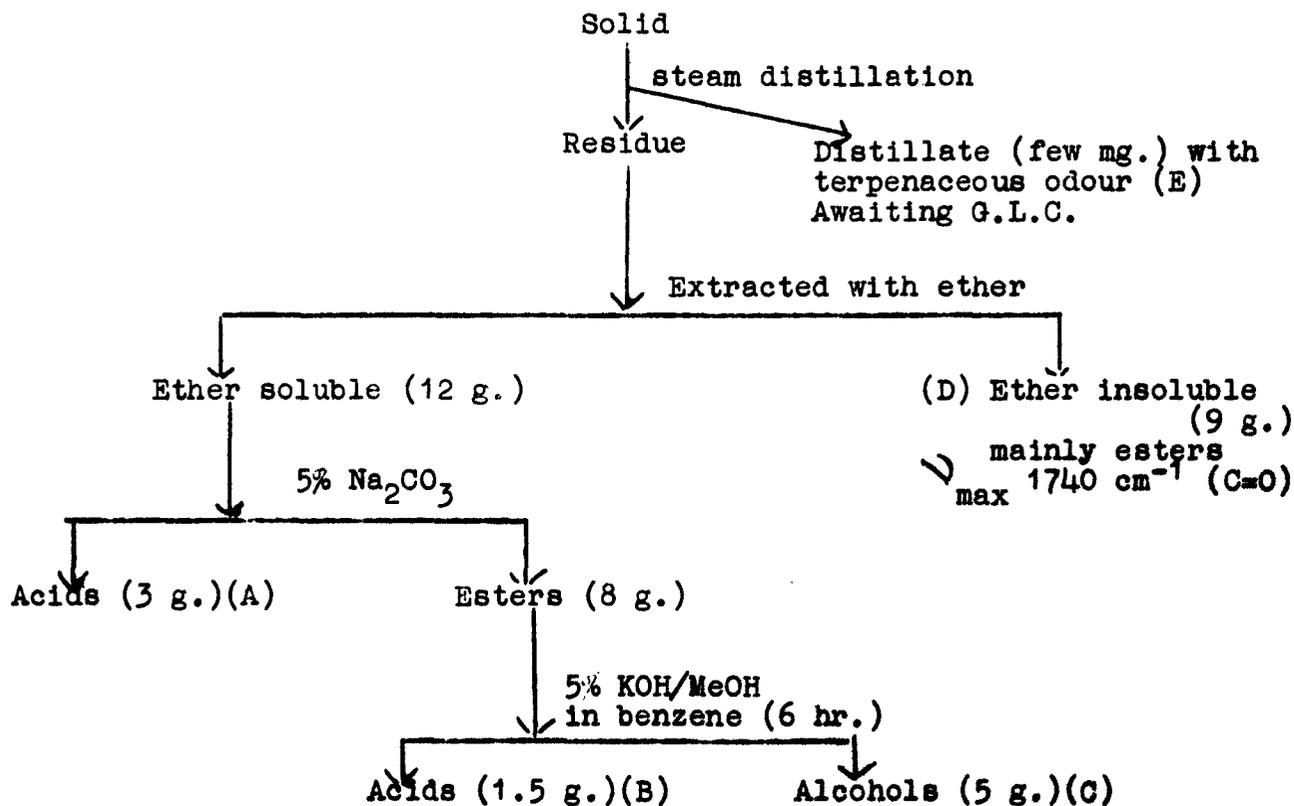
Extraction of L. periclymenum (32 lbs., ligroin extracted) with methanol gave a tar (71.5 g.) consisting largely of lignin. An aqueous extract of this gave a positive Molisch test. Chromotography on paper using two solvent systems (a) butanol-ethanol-water (5:1:4; v/v), (b) ethyl acetate-pyridine-water (8:2:1; v/v) gave the following R_F values: - sucrose, 0.08 and 0.51 respectively; glucose, 0.14 and 1.0; fructose, 0.19 and 1.32. The aqueous extract gave spots with the following R_F values 0.08 and 0.52;

0.14 and 1.0; 0.185 and 1.33 respectively.

Hydrolysis of the tar (50.4 g.) with a 5% solution of hydrochloric acid in 50 % aqueous methanol (250 c.c.) for 18 hr. gave glucose and fructose, identified as above.

5. Shorea meranti

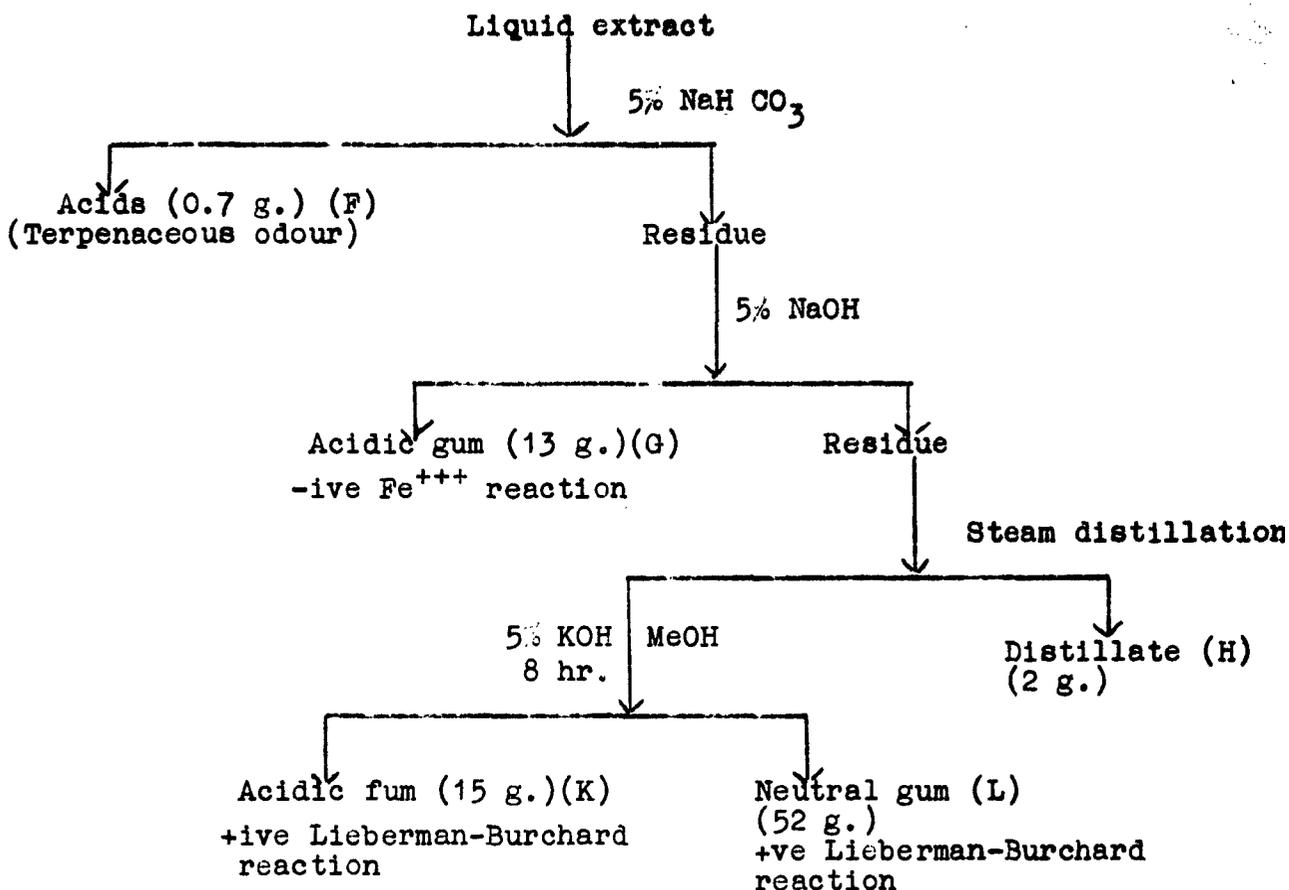
Shavings (19.75 Kg.) were extracted with ligroin in the usual way and the extract was concentrated to 2 l., when a white solid (48 g.), m.p. 77-90° was deposited. It showed ν_{\max} 1710-1750 (C=O), 720-740 cm^{-1} (CH₂ in chain). Some of this solid (21 g.) was investigated according to the following scheme.



Acids (A) showed unsaturation (\checkmark max 2700 (CO₂H), 1710 (CO₂H), ca 1600 (C=C), 1520 (C=C), 720, 730 cm⁻¹ (CH₂), which could be aromatic in character. They were chromatographed, but with little success, on silica gel, twelve fractions being collected. No crystalline material was obtained until ether-benzene (10:90) was used. Thin layer chromatography of each fraction using light petroleum-ether (2:1) as solvent system suggested that fractions 1 to 5, 6 to 8, and 9 - 12 be combined in three fractions. Infrared absorption of these fractions suggested that they were respectively mixtures of long chain saturated acids, saturated and unsaturated acids of acyclic and aromatic types, whilst the third has not yet been investigated. The unsaturated and saturated and aromatic acids? are now being separated through the adducts of the **unsaturated esters with mercuric acetate in methanol.**

Fractions B - E shown on the chart await investigation.

The main ligroin extract was concentrated to 250 c.c., ether (400 c.c.) was added and the extract was investigated as shown below.



The acid fractions (F) and (G) have not yet been examined. The distillate (H) has a highly terpenaceous odour. Chromatography as described on p.22 shows the presence of a number of compounds which have not yet been positively identified but the following are indicated: - 1,8- cineol, α - and β - pinene, limonene, terpinolene, citral and phellandrene.

The neutral gum (L) is unsaturated and it contains

hydroxyl groups (I.R. spectrum). Its Liebermann-Burchard colour reaction - green with a blue fluorescence—is characteristic of many tetracyclic terpenes. Chromatography on neutral alumina failed to effect any purification. Ketonic or aldehydic material could not be extracted with Girard reagents. Work is still in progress.

The acidic gum (K) seems also to be a tetracyclic triterpene. Its spectrum (ν_{\max} 1710, 1620, 1600, 1510 cm^{-1}) indicates a high degree of unsaturation.

6. Tabebuia pentaphylla

The wood (50 lbs.) extracted with ligroin gave a solution which was divided into acidic, hydroxide soluble and neutral fractions. The acidic fraction afforded a solid acid (0.26 g.), m.p. 165-170° which was sublimed under reduced pressure giving a white solid which crystallised from acetone as needles, m.p. and mixed m.p. with veratric acid, 175°, λ_{\max} 2900 (log ϵ 4.16), 2600 Å (log ϵ 4.5), ν_{\max} 1600 and 1520 ($\text{C}=\text{C}$, aromatic), 1050 cm^{-1} (OCH_3) (Found: C, 59.9; H, 5.35. Calc. for $\text{C}_9\text{H}_{10}\text{O}_4$: C, 59.3; H, 5.5%).

The hydroxide soluble fraction was chromatographed on a silica gel column using successively ligroin, benzene, ether, acetone, and methanol separate and in combination as solvents. Only β -sitosterol (ca 10 mg.), m.p. and mixed m.p. 134° was isolated.

β -Sitosterol forms fine emulsions with sodium hydroxide which cannot be easily resolved.

The neutral fraction was distilled in steam giving a terpenaceous oil (0.4 g.) which has still to be analysed. The non-volatile fraction (8.0 g.) was hydrolysed with 5% methanolic potash (150 c.c.) in benzene (20 c.c.) giving acids (2.2 g.) which were esterified with diazomethane. Both saturated and unsaturated esters were present and these were separated by using mercuric acetate in methanol and thin layer chromatography as described previously. These esters still require investigation.

The non-saponifiables (5.6 g.) were absorbed on neutral alumina and eluted with the solvents mentioned above. Using ligroin, alkanes (0.01 g.), m.p. 55° were eluted and gas-liquid chromatography on a S.E. 30 column (see above) showed the presence of normal C_{25} and C_{27} alkanes. β -Sitosterol (0.9 g.), m.p. and mixed m.p. 132° was isolated from later fractions.

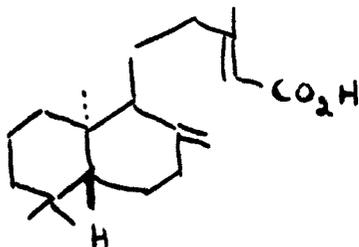
Extraction of the ligroin extracted T. pentaphylla with ether gave a solution which was extracted successively with water, sodium hydrogen carbonate and sodium hydroxide, dried and concentrated. A dark neutral gum (15.0 g.) remained. The aqueous extract when run on paper as previously described revealed the presence of glucose and fructose. The sodium hydrogen carbonate extract gave an oily acid fraction (2.6 g.), which on standing became partly solid. The solid on sublimation in a vacuum gave veratric acid (few mg.),

m.p. 167° and a non sublimable brown solid (10 mg.), m.p. $200-206^{\circ}$.
 λ_{\max} 2300 and 3150 Å (ethanol), 2850 Å (Ethanol + sodium acetate).
 This coupled with diazotised sulphanilic acid and diazotised p-nitroaniline.

The above neutral fraction was hydrolysed with alkali. From the acid fraction thus obtained a white solid (16 mg.), λ_{\max} 1700 (CO_2H) was obtained. Recrystallisation from ethanol gave a solid m.p. $225-240^{\circ}$ (decomp.). It gave a positive Liebermann-Burchard reaction.

Addendum to *Copaifera officinalis* (see p.7,8, and 21)

Since the report was written the author has seen a paper by Nakano and Djerassi (J. Org. Chem., 1961, 26, 167) on^a copalic acid from Brazil Copal. This copalic acid was liquid, but it was laevorotatory in contrast to our dextrorotatory acid. However it had a maximum at 2200 Å in the ultraviolet and at 1698, 1653, 833 and 873 cm^{-1} in the infrared. These are not vastly different spectral characteristics from those we record. Djerassi's copalic acid is considered to be a mixture of several double bond isomers which have the skeleton of agathanedicarboxylic acid but with the less usual A/B ring stereochemistry shown below.



Conclusions.

It is unfortunate that the contract is not to be renewed. Some of the work described in this report would merit further investigation. Even more important is the fact that a body of young post-graduates are now trained in the investigation of wood extractives, and this work should be expanded considerably so as to cover many more woods.

It is not opportune yet to dwell on the phytochemical implications of the results we have described. It is significant however that in the majority of the woods investigated phenolic substances are absent when alkanes and ester waxes are present as the main components of the extractives.

We hope to be able to continue the work as funds permit.

Personnel Professor W. Cocker, Miss Anne Cantan, Dr. Carmel Dempsey, Mr. E. Webster have been engaged on the work throughout the year. Mr. Stanley Shaw and Mr. E. Simmons left the team in October. Dr. McMurry returned to the team in September and Mr. Donald Sainsbury joined it then. Mr. Thor Dahl spent about half a year on the project.

Time expended. Professor Cocker spent about 600 hours, Miss Cantan, Dr. Dempsey and Mr. Webster about 1800 hours each, Mr. Shaw and Mr. Simmons about 1300 hours each, Mr. Sainsbury

about 1000 hours and Dr. McMurry about 300 hours. Mrs. Hannaford (Secretary) has spent about 100 hours on the reports.

Cost. During the year a second Aerograph Hi Fi Gas liquid chromatography apparatus was purchased at the cost of about £500. The cost of consumable materials etc. was of the order of £800.

This is the last of our reports under this contract. I would like to thank the U.S. Army through its European Research Office for making this work possible. It has been most productive work.