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PART XXIV

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By

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SUMMARY

The essential oil of *Calythrix tetragona* Lab. from the Glasshouse Mountains, Queensland, has been examined in detail. Two samples distilled in March and July were obtained in 0.3% and 0.39% yield, and were closely similar in the nature and the proportions of the constituents which were found to be α-pinene, β-pinene (the principal constituent), limonene, γ-terpinene, myrtenal, α-terpineol, citronellol aromadendrene, melalinol, a β-diketonic (?) substance $C_{12}H_{16}O_3$, an ester of citronellic acid and other unidentified substances. The composition of these samples of oil differs significantly from that of the oil from *Calythrix tetragona* Lab. var. "A" from Denman, N.S.W.

We have examined two samples of the essential oil of the shrub *Calythrix tetragona* Lab. obtained from the top of Ngungun, which is one of the Glasshouse Mountains which lie about 50 miles north of Brisbane. The two samples, collected in March and July, were closely similar and have been shown to contain α-pinene, β-pinene (the major constituent, about 45 per cent. of both samples), limonene, γ-terpinene, myrtenal, α-terpineol, citronellol, an ester of citronellic acid, aromadendrene, a sesquiterpene alcohol melalinol $C_{15}H_{26}O$ and a substance $C_{12}H_{16}O_3$. It is likely that camphene and pinocarveol and/or myrtenol were also present.

The essential oil of a plant from Denman, N.S.W., tentatively described as *Calythrix tetragona* Lab. var. "A" was examined by Penfold, Ramage and Simonsen (1) and yielded interesting results in that 60 to 70 per cent. of the oil was found to be esters, mainly methyl citronellate, methyl geranate and citronellyl formate. α-Pinene and citronellol were identified also but it appears that no appreciable quantity of sesquiterpenes or sesquiterpene alcohols were present. In containing citronellol and an ester of citronellic acid the Ngungun oil shows a significant likeness to the Denman var. "A" oil, but otherwise there is a closer resemblance to the oil of *C. virgata* which has been found by Penfold and Morrison (2) to contain α-pinene, citronellol and geraniol with their acetates and formates, citronellic and geranic esters, sesquiterpenes and sesquiterpene alcohols.

The samples of oil from Ngungun were examined with the aid of a still of low holdup which contained one metre of the packing described by Bower and Cooke (3) and showed a maximum efficiency equivalent to fifty-five theoretical plates. In accordance with a recent suggestion (4), the distillates were collected in numerous fractions of approximately equal volume and the density, refractive index and rotation of each was measured. From the data so obtained, refractive index/density diagrams were prepared and these are reproduced in Figs. 1A, 1B II and III. In the various diagrams, the fractions are numbered in order, some being omitted to avoid confusion where they fall very close together. Points which according to the data in the literature, represent the properties of the
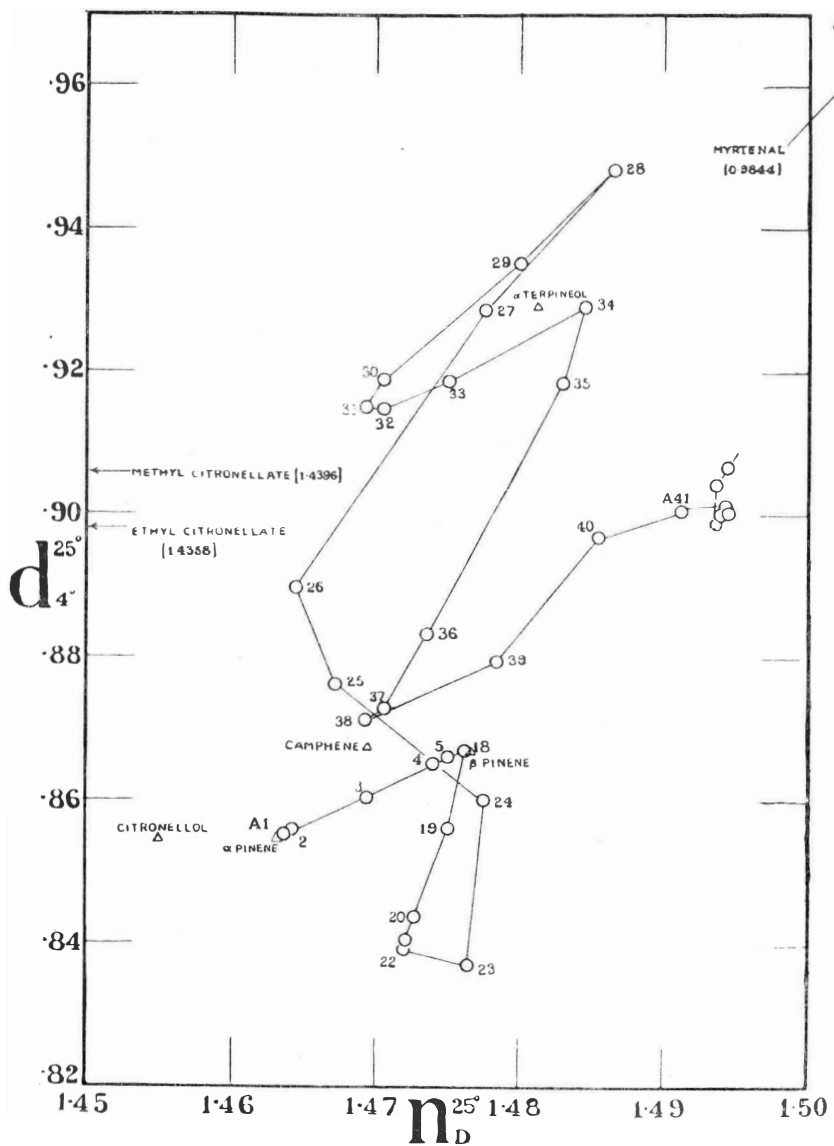
Fig. 1A.

identified constituents, are also included in the diagrams for comparison. Owing to the presence of numerous constituents in quantities comparable with the holdup of the still, the separations obtained were in most cases far from complete. However, in addition to the eleven constituents listed above, the presence of at least fifteen other substances is indicated by the shape of the refractive index/density (n/d) curves. The assumption has been made here and will be tested later that hemiacetal formation (13) does not complicate the interpretation of the n/d curves.

Of the March oil (d_4^{25} 0.8845, n_D^{25} 1.4854 and α_D — 13°), 200 ml. were available for distillation, and were separated into fractions numbered A1, A2, etc., of which A1 and A2 were 2.5 ml. in volume, A3 to A18 averaged 5 ml. each, and A19 and higher averaged 2 ml. Considering the fractions in order, the two lowest boiling A1 and A2, from their relation to the point for pure α-pinene (4, 5) are obviously fairly pure α-pinene. Fractions A7 to A18 are all substantially pure β-pinene (4, 5). The intermediate fractions A3 to A6 all show a slight deviation from the α-pinene/β-pinene line towards the camphene point (4). This is greatest in A4 and suggests that this fraction may contain about 10 per cent. of camphene. The presence of about one millilitre of camphene would be sufficient to cause the total observed deviation. No additional experimental evidence is available to confirm this possibility. From A22 of b.p. 68°/20 mm. γ-terpinene tetrabromide was obtained, but other substances were probably also present. Fraction A23, by the criterion of its boiling point (70°/20 mm.) falls within the terpenes, but it is difficult to suggest a possible constituent from among the known terpenes apart from *allo*-ocimene. Fraction A24 was collected over a considerable rise in boiling point and evidently contains another undetermined substance. The convexity formed by A25, A26 and A27 is also clearly due to an unidentified substance or substances of low refractive index and density 0.88-0.90.

The principal constituent of A28 was identified as myrtenal and in agreement with this the line through fractions A27 and A28 passes close to the point for pure myrtenal. This is perhaps not unexpected in view of the presence in the essential oil of much β-pinene from which substance, myrtenal (together with myrtenol and pinocarveol) is formed by autoxidation (6). Fraction A31 was found to have an ester value of 82 due to the presence of an ester of citronellic acid, and also contained α-terpineol, identified as the phenylurethane. The substance causing the peak at A34 was not identified, but would seem from Fig. II to be an alcohol. The angle at A38 results from the presence of citronellol, confirmed by the preparation of the characteristic allophanate. Above fraction A40 the material consists substantially of sesquiterpenes, but is included in the n/d diagram for comparison with the corresponding portion of the July oil curve.

The July oil (d_4^{25} 0.8714, n_D^{25} 1.4774 and α_D — 14°) was treated in a similar manner to the March oil. In this case, 300 ml. were distilled into numerous fractions of which B1 and B2 were each $2\frac{1}{2}$ ml. B3 to B20 averaged about 8 ml. and B21 were of 2 ml. each.

In comparing the curves of Fig. 1A and Fig. 1B, a general similarity is immediately apparent, but there are important differences in detail. The first two fractions B1 and B2 are notable in indicating a substance boiling only slightly below α-pinene and of lower refractive index and density. The material is clearly

not α-thujene or any other known terpene.   α-Pinene and β-pinene were both present, the latter being the major constituent of the oil.   The absence of a convexity corresponding to that at A23 of the March oil is striking.   Another difference lies in the isolation of both *l*-limonene tetrabromide and γ-terpinene tetrabromide from fraction B24.
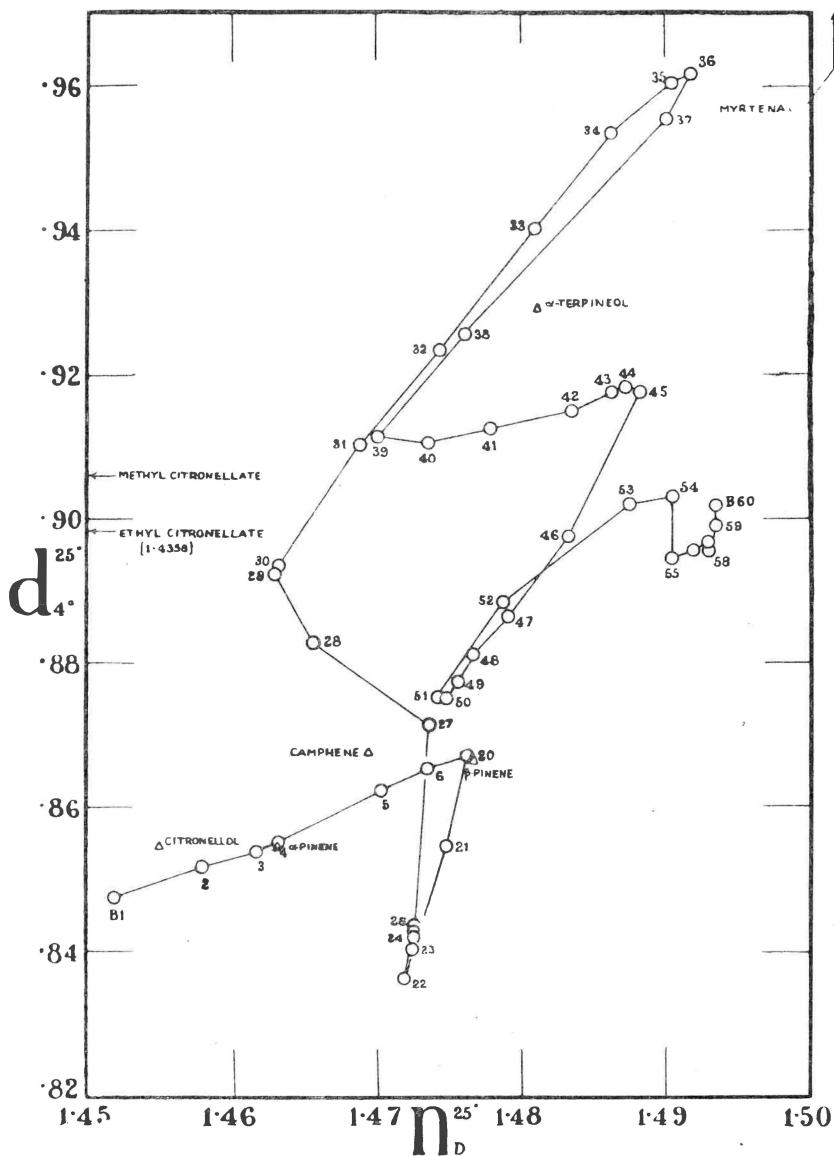

Fig. 1B

Fraction B27 clearly corresponds to A24 of the March oil, and similarly B28, B29 and B30 correspond to A25 and A26. Fraction B36 was shown to contain myrtenal but, as revealed in the experimental section, there are arguments for the presence of pinocarveol and/or myrtenol also. The citronellic ester convexity is similar to that of the March oil, but that about B44 is displaced to a higher refractive index and corresponds to a maximum in the rotation (a_D — 43).

Some difference between the March and the July samples in proportion of constituents at least is apparent here, but no suggestion can be made as to the actual substances involved. Fraction B51 shows a minimum in rotation (a_D — 15.5) corresponding to the presence of citronellol. The higher boiling material contains sesquiterpenes and is included for comparison with the March oil fractions.

After some of the constituents mentioned above had been identified, the remaining fractions from both the March and the July oils were combined to reduce the difficulties arising from the lack of material and the complexity of the mixtures. The bulked material was then separated by the boric ester method into two fractions, alcoholic and non-alcoholic, which were each further fractionated into small (2 ml.) fractions, the resulting n/d curves being reproduced in Fig. II.

The non-alcoholic material was considerably simplified. Fraction C5 (2 ml.) had a high ester number and on saponification yielded an acid identified as citronellic acid. The alcohol combined in the ester was not identified, but from the physical constants of the somewhat impure ester either the methyl or the ethyl ester is indicated. No evidence of esters of geranic acid was found.

The alcohol material was separated into nine (2 ml.) fractions, D1, D2, etc., which reveal the presence of six alcohols. The peak at fraction D3 is possibly due to pinocarveol or myrtenol. From fraction D8 citronellol was separated as the allophanate. It is clear from Figs. 1A and 1B that all of the substances identified, with the possible exception of a-terpineol, were present in both the March and July samples.

The rather small sesquiterpene fractions of both oils were united and fractionated into $2\frac{1}{2}$ ml. fractions, the n/d curve for which is shown in Fig. III. The complexity of the sesquiterpene mixture is obvious from a study of the diagram, no less than six constituents being involved. The separation was very incomplete, but this was unavoidable with the limited quantity available for distillation. It was pleasing to find that a fraction (E9) of appropriate physical properties yielded aromadendrone on ozonisation, but no other constituents were identified. Cadinene hydrochloride could not be obtained from either E11 or E12 in spite of the coincidence of physical properties with those of cadinene. Points corresponding to the properties of a number of selected sesquiterpenes have been included in Fig. III, most of the data being taken from Simonsen's "The Terpenes." It would seem that until the Australian sesquiterpenes have been systematically examined and methods of identification have been developed, the investigation of complex mixtures such as that obtained from *C. tetragona* will be unprofitable.

Sesquiterpene alcohols comprised about 3-5 per cent. of the two oils. More than one substance was present but only one derivative, a crystalline phenylurethane m.p. 141.5-142.5° was obtained in useful form, and this proved to be identical with the phenylurethane of melalinol, a sesquiterpene alcohol previously obtained only from the essential oil of *Melaleuca linariifolia* (7).

A green crystalline substance was obtained from the copper condenser of the still after the steam distillation of both samples of leaves, by washing with ether and evaporating. This substance $(C_{12}H_{15}O_3)_2$ Cu m.p. 216° contained copper and crystallized in needles from methyl alcohol or petrol ether, but was very soluble in acetone, ether and chloroform. By steam distillation from dilute sulphuric acid,

Fig. II
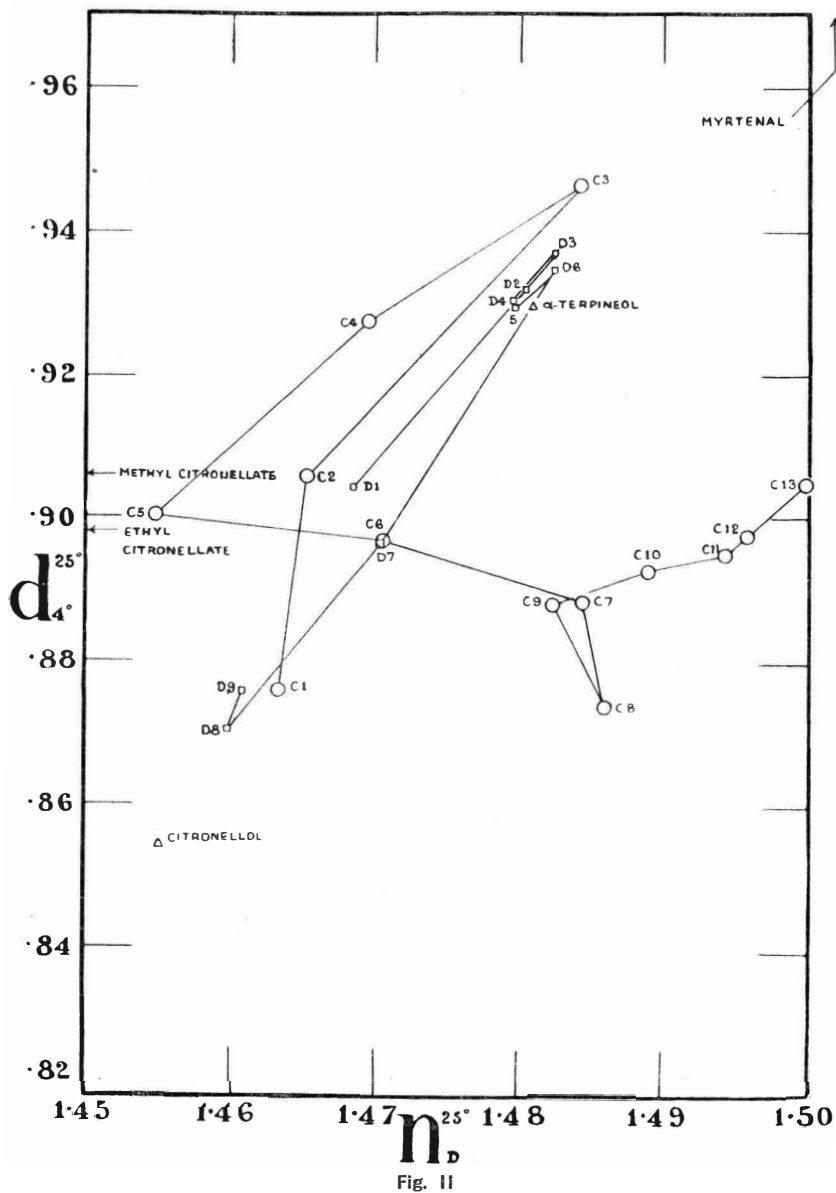
a pale yellow liquid substance $C_{12}H_{16}O_3$ was obtained. This oil was optically inactive, contained no methoxyl groups, gave an intense red ferric reaction and, though insoluble in water, dissolved readily in dilute sodium carbonate solution yielding an orange coloured solution from which yellow needle shaped crystals separated. This substance, which possibly contains a cyclic β-diketonic group and may be related to augustione, leptospermone, etc., is being further investigated.

Consideration of all the data available suggests the following approximate percentages by volume for the principal identified constituents.

				March, 1947	July, 1946
α-Pinene	...	...	...	4	8
β-Pinene	...	...	...	45	45
Other terpenes	...	...	...	5	3
Myrtenal	...	...	...	1	0.5
Methyl (?) citronellate		...		1	1
α-Terpineol	...	...	...	2	1
Citronellol	...	...	...	2	2
Sesquiterpenes	...	...	...	11	6
Sesquiterpene alcohols		...		5	5
Substance $C_{12}H_{16}O_3$	...		...	1	3

EXPERIMENTAL

Leaves and branchlets (172 lb.) of *C. tetragona* were collected in March, 1947, from the top of Ngungun after a period of heavy rain and on steam distillation yielded 230 ml. of a dark brown oil of pleasant odour. The crude oil was redistilled under reduced pressure without rectification in a glass apparatus to remove a small quantity of non-volatile material, and thereby yielded a pale yellow oil of ester value 5, ester value after acetylation 32, d_4^{25} 0.8840, n_D^{25} 1.4854 and α_D —13°. By washing out the copper condenser of the leaf still with ether a green solution was obtained and on removal of the ether, several grams of a copper compound were obtained in an impure state. A further small quantity was obtained by refrigerating in petroleum ether the non-volatile residue from the crude essential oil.

From 190 lb. of plant material collected in July, 1946, after a prolonged dry season, 330 ml. of oil and about 10 g. of copper compound were obtained. For this oil the constants were d_4^{25} 0.8714, n_D^{25} 1.4774, α_D —14°, ester value 9 and ester value after acetylation 45.

All fractionations were carried out at reduced pressure with the still mentioned earlier and at a high reflux ratio. In the following account all densities and refractive indices are for the conditions d_4^{25} and n_D^{25} respectively. The optical rotations of the fractions of the July oil were not measured as no polarimeter was then available. *α-Pinene.*

This hydrocarbon (0.8543, 1.4628, α_D +18°) was identified as pinonic acid and its semicarbazone in the usual way.

β-Pinene.

For middle fractions of the β-pinene plateau, the constants 0.8668, 1.4765 and a_D —17.4° were found and nopinic acid m.p. 126-127° was prepared in the usual way.

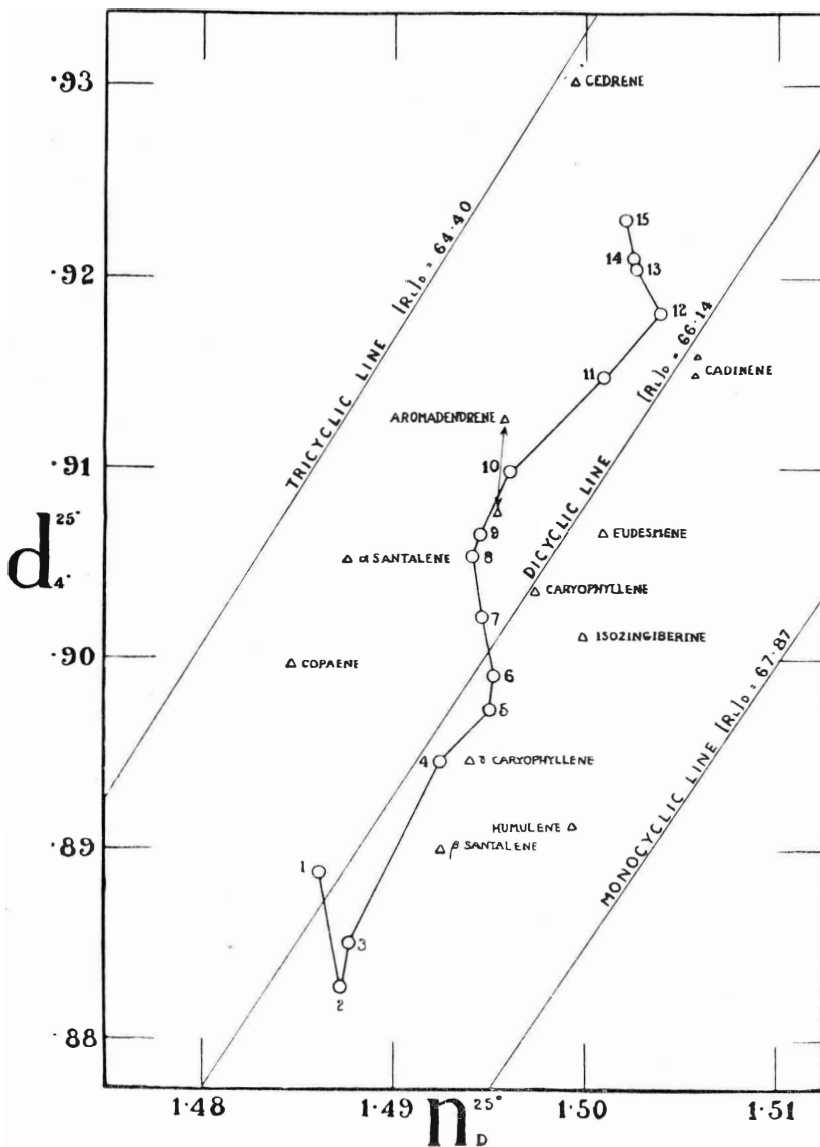Fig. III

l-Limonene and · γ-Terpinene.

From 1.0 ml. of fraction B24 by treatment with bromine in ether and amyl alcohol, an immediate precipitate of γ-terpinene tetrabromide (0.10 g.) was obtained. Further crystals (0.30 g.) separated overnight and by crystallization from methyl-alcohol yielded more γ-terpinene tetrabromide and from the mother liquors, rather impure limonene tetrabromide m.p. 95-98°. The tetrabromide of d-limonene only was available for a mixed melting point test and a mixture of the two samples melted at 119-122° which proves that Fraction B24 contained some *l*-limonene in agreement with the high negative rotation (α_D -–75) which was at a maximum in this fraction.

From fraction A22, γ-terpinene tetrabromide only was isolated by similar treatment.

Myrtenal.

Fractions A28, B36 and adjacent fractions readily yielded derivatives of myrtenal. The semicarbazone was formed in pyridine and separated on dilution with water as colourless needles which were crystallized from aqueous alcohol to m.p. 213-215°. The dinitrophenylhydrazone, obtained by the use of Brady's reagent, formed orange needles m.p. 222-223° from alcohol.

β-Pinene (α_D —17.3) was oxidised by using selenium dioxide in alcohol as described by Stallcup and Hawkins (8) and the sodium bisulphite extract of the oxidation products was steam distilled with sodium carbonate. The liberated carbonyl compound which is not pinocarvone as stated by Stallcup and Hawkins (8) but is myrtenal (9), yielded derivatives which appeared identical with those obtained from fractions A28 and B36 and caused no depression of melting points.

Approximate estimations of the myrtenal content of various fractions were attempted by treating 0.1 g. samples with Brady's reagent (2 ml.) and weighing the precipitate after washing with alcohol saturated with myrtenal dinitrophenyl-hydrazone and drying in vacuo. By this method of analysis, A28 was found to contain 50 per cent. of myrtenal from which it may be calculated (assuming ideal mixing) that this fraction, if freed from myrtenal, would show the properties 0.913 and 1.471. Thus the peak in the March oil about fraction A28 is due entirely to myrtenal. In the case of fraction B35, only 16 per cent. of myrtenal was found and the calculated properties 0.956 and 1.488 indicate the presence of additional constitutents of high density, possibly myrtenol or pinocarveol in the July oil. Fraction B34 also exhibited a maximum (α_D +34°) in optical rotation. The positions of fractions D2 and D3 in Fig. II show the influence of these constituents.

α-Terpineol.

α-Terpineol was isolated as the phenylurethane m.p. 109-110° from fraction D5 of the alcohol concentrate obtained by the boric ester method. No depression of melting point resulted on mixing with authentic α-terpineol phenylurethane

Citronellol.

Citronellol was identified in fraction D8 as the allophanate m.p. 105° formed by the action of cyanic acid, and crystallized as colourless needles from aqueous alcohol. No depression of melting point resulted on mixing with authentic citronellol allophanate (10) of the same melting point.

Methyl or Ethyl Citronellate.

The non-alcohol fraction C5 was treated with semi-carbazide acetate in aqueous alcohol and the unreacted oil later distilled off. This removal of carbonyl compounds changed the constants to 0.8950 and 1.4498. Of this material 1.04 g was saponified with alcoholic potash giving a good yield of a liquid acid. This substance (0.9206 and 1.4501) gave a molecular weight value of 169 by the silver salt method and formed a S-benzylthiuronium salt of m.p. 145-146° after crystallization to constant melting point from acetone.

l-Citronellic acid (kindly supplied by Dr. F. H. Reuter of the Sydney Technical College) as the sodium salt in aqueous solution, yielded on addition of an alcoholic solution of S-benzylthiuronium chloride, a precipitate of *S-benzylthiuronium citronellate* m.p. 145-147° from acetone.

Analysis—Found : C, 64.2 ; H, 8.25.

$C_{18}H_{28}O_2N_2S$ requires C, 64.24 : H, 8.38.

No depression of melting point resulted on mixing the derivative from authentic citronellic acid with the material from *C. tetragona*. The calculated molecular weight for citronellic acid is 170.2 and the physical properties have been reported as d_4^{17} 0.9332, n_D^{20} 1.4534 (11) and d_4^{20} 0.9280, n_D^{20} 1.4537 (12).

An unsuccessful attempt was made with a further small quantity of ester to determine the alcoholic radical of the ester. The physical constants of the impure ester suggest the ethyl derivative but the methyl ester is more probable, especially in view of its occurrence in the oil of *C. tetragona* var. "A" (1).

Aromadendrene.

Of fraction E9, 2.2 g. were ozonized in glacial acetic acid and 0.20 g. of crystalline aromadendrene m.p. 81° were obtained, the melting point of which was not depressed by an authentic sample.

All sesquiterpene fractions gave violet to violet-red colours on treatment with bromine in acetic acid. Dehydrogenation of some fractions gave what were apparently mixtures of azulenes but no cadalene or eudalene. Fractions E3, E11 and E15 were treated with hydrogen chloride in ice-cold ether and acetic acid, but no crystals could be obtained from the dark coloured mother liquors.

Melalinol.

After various trial experiments the remaining sesquiterpene alcohols were bulked and treated with an equal volume of phenylisocyanate in the presence of pyridine. After standing two weeks at 5°C. the excess reagent was destroyed by water and the urethane separated from the reaction mixture by extraction with boiling petroleum ether. The residue was then crystallized to constant melting point from methyl alcohol as colourless needles (1.62 g.) m.p. 141.5—142.5°, $[\alpha]_D$ —73.3° (C=2.93 in acetone). Admixture of melalinol phenylurethane (7) of m.p. 141° caused no depression of melting point.

The Copper-containing Compound.

By extraction of the sesquiterpene alcohol fraction of appropriate boiling point with sodium hydroxide solution, a material was obtained which yielded with

copper oxide, a few milligrams of a copper compound identical with that washed from the condenser of the leaf still.

The green copper compound crystallized to a constant melting point of 216° from either methyl alcohol or from petroleum ether but was very soluble in acetone, benzene ether, and ethyl acetate. The mother liquors contained some rather greasy solid of deep green colour.

Ignition in a platinum crucible yielded an ash which gave tests for copper. By ignition, treatment with nitric acid, further ignition and weighing as copper oxide, the following results were obtained :—

Analysis—Found : Cu, 12.2, 12.6 ; C, 60.7, 60.2, 60.5 ; H, 6.25, 6.18, 6.17.

$(C_{12}H_{15}O_3)_2Cu$ requires : Cu, 13.29 ; C, 60.30 ; H, 6.34.

$(C_{13}H_{17}O_3)_2Cu$ requires : Cu, 12.56 ; C, 61.70 ; H, 6.77.

The analytical figures above are inconsistent with any possible formula, and in consequence a loss of copper during the ignitions was suspected, especially since green fumes were evolved soon after heating was commenced. To avoid this difficulty, 2.914 g. of copper compound were boiled with dilute sulphuric acid until decomposition was apparently complete, after which the copper in this solution was estimated by precipitation as cuprous thiocyanate. By this method the proportion of copper was found to be 13.0 per cent. in satisfactory agreement with the formula $(C_{12}H_{15}O_3)_2Cu$ which is supported by the combustion analyses also.

Liberation of the organic moiety from the copper was achieved by boiling with dilute sulphuric acid, the evolved vapours being condensed, extracted with ether and returned to the reaction flask by a suitable apparatus. The decomposition took several hours to complete, the insolubility of the co-ordination compound appearing to slow the process. The ether extract after drying and evaporating yielded an oily residue which was distilled at reduced pressure, the first and last fractions being rejected.

The pale yellow oily distillate had a weak and faintly unpleasant odour, the physical constants being b.p. $_{10.5}$ 138°, d_4^{25} 1.053, n_D^{25} 1.5217 and α_D +0.09°.

Analysis—Found : C, 69.4, 69.5, 69.8 ; H, 7.85, 7.75, 7.73 ; M.W., 188.

$C_{12}H_{16}O_3$ requires C, 69.21 ; H, 7.75 ; M.W., 208.2

The molecular weight was determined ebullioscopically in acetone, using Reiche's apparatus.

The oil was insoluble in water but readily soluble in dilute sodium hydroxide solution to form a strongly yellow solution. Weighed portions of the oil were titrated with 0.0919NNaOH, using phenolphthalein as indicator with the following results :—

Equivalent Weight—Found : 209, 207, 208.

$(C_{12}H_{15}O_3)H$ requires 208.2.

The ferric reaction was intense red, while titration with barium hydroxide solution yielded bright orange crystals which could be recrystallized from hot water but which discoloured and decomposed without melting.

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