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# LXXXI.—*A Colouring Matter from* Lomatia ilicifolia *and* Lomatia longifolia.

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## Introductory and Theoretical Part.

Some time ago, by the kindness of Mr. J. H. Maiden, Director of the Technological Museum, Sydney, the author was supplied with a quantity of the seeds of *Lomatia ilicifolia* and *Lomatia longifolia*, two species of the genus Lomatia (order Proteaceæ) growing on the elevated portions of south-eastern New South Wales and north-eastern Victoria. These seeds are more or less surrounded by the yellow substance which forms the subject of this investigation. A preliminary examination soon showed that the colouring matter is the same for both species, and that it is closely allied to the "lapachol" described by Dr. Hooker (Trans., 1892, **61**, 611).

On boiling with water slightly acidified with acetic acid, the yellow substance is easily extracted, and may be purified by crystallisation from hot water (slightly acidified with acetic acid). It forms yellow needles, melting at 127°, soluble in hot water, and separating in the crystalline form on cooling, easily soluble in alcohol, ether, and the ordinary solvents. It dissolves easily in alkalis, forming a deep red solution, from which it is precipitated unchanged on the addition of an acid. Its formula, as ascertained by several combustions and by a molecular weight determination, as also by collateral evidence (detailed below), is $C_{15}H_{14}O_4$. It contains two hydroxyl groups, as evidenced by the formation of a diacetyl derivative on treatment with acetic anhydride. It forms also metallic derivatives, of which the barium compound is the most important yet examined, exhibiting,

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as it does, remarkable similarity to the barium salt of hydroxyhydrolapachol described by Hooker (*loc. cit.*).

So great is this similarity, and so close is the percentage of barium found to that required for the barium salt of hydroxyhydrolapachol, that at first there seemed to be a probability of the identity of the substance under examination with hydroxyhydrolapachol. As, however, the results of ultimate analysis pointed to a distinct difference, the author applied to Dr. Hooker, who very kindly forwarded small specimens of hydroxyhydrolapachol and of some other lapachol derivatives for comparison. On comparing the substances, it was soon seen that they are not identical. Hydroxyhydrolapachol seems to be but very sparingly soluble even in boiling water (nothing is said on this point in Dr. Hooker's paper), whereas, as stated above, the lomatia colouring matter dissolves to a considerable extent and crystallises on cooling. Further, the specimen of hydroxyhydrolapachol forwarded by Dr. Hooker melted sharply at 125°, the same thermometer being used as was employed in determining the other melting points in this paper. In fact, when specimens of the two substances were tried simultaneously with the same thermometer, the one melted at 125° and the other at 127°.

On oxidation with chromic mixture the new substance yields acetic acid, and sometimes, if the oxidation be gently managed, phthalic acid.

If the substance is dissolved in a small quantity of concentrated sulphuric acid, and if the solution, *after standing for about* 20 *minutes*, be poured into a quantity of cold water, a small quantity of tarry matter separates; on filtering this off and allowing the clear solution to remain for several days, beautiful rosettes of *red* crystals gradually form on the bottom and sides of the beaker, and can be easily purified by crystallisation from somewhat diluted alcohol. This new substance melts at 204—205°, and is isomeric with the original yellow substance. If it be boiled for a few minutes with a strong solution of caustic potash, it dissolves forming a deep red solution, and this solution on acidification deposits a yellow crystalline substance, which, after purification, melts at 182°. The two last-mentioned compounds would seem, judging from the results of analysis, and from the melting points, to be identical with Hooker's hydroxy-$\beta$-lapachone and dihydroxyhydrolapachol respectively. It is true that the melting point of the red substance is about 3° higher than that given by Hooker (201·5°) for hydroxy-$\beta$-lapachone, but that might easily arise from some slight difference in purity. The melting point of the yellow substance is, however, identical with that given by Hooker for dihydroxyhydrolapachol. If this supposition be correct (it is to be regretted that the quantity of material at the author's

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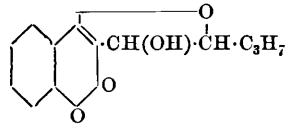


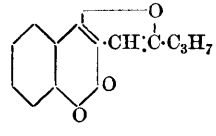
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disposal was too small for an exhaustive examination), it would seem to follow that the lomatia colouring matter is *hydroxylapachol*. Just as lapachol on treatment with concentrated sulphuric acid yields $\beta$-lapachone, so hydroxylapachol should yield hydroxy-$\beta$-lapachone, and the latter on treatment with caustic potash and subsequent acidification should yield, as Hooker has shown, dihydroxyhydrolapachol.

The presence of the hydroxyl group in hydroxylapachol, however, renders possible another reaction under the action of concentrated sulphuric acid, to which there appears to be no parallel in the case of lapachol. If the solution of hydroxylapachol in concentrated sulphuric acid, instead of being allowed to stand for 20 minutes, be *immediately* poured into cold water, a red crystalline substance is precipitated, which after recrystallisation from somewhat diluted alcohol melts at 110—111°; this contains the elements of a molecule of water less than hydroxylapachol, the combustion numbers indicating the formula $C_{15}H_{12}O_3$. This substance must be either identical or isomeric with Paternò's *isolapachone* if Hooker's revised formula for that substance be accepted as correct. That it is not identical is obvious from the melting point (isolapachone, according to Paternò, melts at 140—141°: Hooker does not mention its melting point), and also from the following reaction. If the substance melting at 110—111° is boiled for a few minutes with strong caustic potash it dissolves, forming a deep red liquid from which acids precipitate a yellow crystalline substance, *apparently isomeric with hydroxylapachol*, and melting at 109—110°. Now Hooker has shown (*loc. cit.*) that isolapachone separates unchanged on acidifying its solution in alkalis, the acid substance first formed being apparently converted into its internal anhydride on liberation from its salts.

The formation of the red substance melting at 110—111° appears to constitute, as might be expected from what is stated above, an intermediate stage in the formation of hydroxy-$\beta$-lapachone, for the mother liquor after filtering off the former deposits, after many days' standing, a small quantity of red crystals melting at 204—205°, and easily recognised as identical with hydroxy-$\beta$-lapachone. This being so, it seems to the writer that if Hooker's formula for hydroxy-$\beta$-lapachone (I)

I. 

II. 

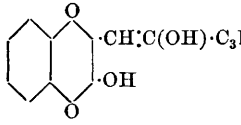
be correct, then the most probable formula for the red substance melting at 110—111° will be II, but this is the formula suggested by Hooker for Paternò's isolapachone, though he says (*loc. cit.*) "the

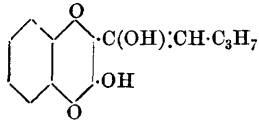
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formula must be accepted with some caution until it is based on further experimental evidence." Obviously there is need for further investigation as to the relationship of these two substances. It may be pointed out, however, that a substance of the above formula might be expected (according to Hooker's experiments in similar cases) to yield a substance of the formula III,

III. 

IV. 

This would be isomeric with the original hydroxylapachol, which as it can be converted into Hooker's hydroxy-$\beta$-lapachol by treatment with sulphuric acid should have the formula IV. The writer puts forward these views with some diffidence as he has had but a small quantity of material to work on, and no more is available at present. Should any more substance be obtained later on, the writer proposes to hand it over to Dr. Hooker, who, he understands, is still investigating lapachol and its derivatives.

## Experimental Part.

*Extraction and Purification of Hydroxylapachol.*—The seeds to which the yellow substance adheres are exhausted with boiling water, slightly acidified with acetic acid, and the liquid rapidly filtered while hot; on cooling, it deposits the hydroxylapachol in a crystalline form. Two or three recrystallisations from slightly acidified boiling water usually suffice for complete purification. The substance is then seen to be perfectly homogeneous when examined by the microscope, and to consist of slender, yellow needles; the melting point after several crystallisations from varying solvents remains constant at 127°, and when submitted to combustion it gave the following results.

I. 0·2520 gram of substance gave 0·6455 $CO_2$ and 0·1300 $H_2O$.
II. 0·2144 ,, ,, ,, 0·5500 $CO_2$ ,, 0·1115 $H_2O$.
III. 0·2133 ,, ,, ,, 0·5445 $CO_2$ ,, 0·1070 $H_2O$.
IV. 0·2143 ,, ,, ,, 0·5500 $CO_2$ ,, 0·1045 $H_2O$.
V. 0·2137 ,, ,, ,, 0·5449 $CO_2$ ,, —
VI. 0·2204 ,, ,, ,, 0·5634 $CO_2$ ,, 0·1110 $H_2O$.

| | Found. | | | | | | Calculated for |
|---|---|---|---|---|---|---|---|
| | I | II. | III. | IV. | V. | VI. | $C_{15}H_{14}O_4$. |
| G .... | 69·85 | 69·96 | 69·62 | 69·99 | 69·54 | 69·71 | 69·76 |
| H .... | 5·73 | 5·77 | 5·57 | 5·42 | lost | 5·59 | 5·42 |

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A molecular weight determination was made by the freezing point method, using glacial acetic acid as the solvent, with the following result.

| | | |
|---|---|---|
| Weight of substance taken..... | = | 0·382 gram. |
| Weight of solvent ..... | = | 22·872 grams. |
| Depression of freezing point..... | = | 0·244°. |

Using the constant 3860 given by Ostwald for acetic acid, this gives

$$\text{Molecular weight} = \frac{3860 \times 0{\cdot}382}{22{\cdot}872 \times 0{\cdot}244} = 264.$$

The calculated molecular weight for $C_{15}H_{14}O_4$ is 258.

*Metallic Derivatives.*—Hydroxylapachol dissolves easily in solutions of the caustic and carbonated alkalis, but the compounds formed are very soluble, and so far have not been obtained in a pure state.

The barium derivative, as already indicated, exhibits a great resemblance to the barium derivative of hydroxyhydrolapachol described by Hooker (*loc. cit.*), yet it presents some slight differences from that compound. It is readily prepared by boiling hydroxylapachol with water and barium carbonate. A deep crimson solution is formed, which, on cooling, usually deposits orange needles of the barium derivative. Sometimes, however, the liquid instead of depositing crystals sets to a deep claret-coloured jelly. The conditions necessary to produce this result have not, however, from want of material been determined; the usual result is the formation of the crystals referred to. These crystals when dried over sulphuric acid appear at first to be practically anhydrous, as they lose only a trifle in weight even when heated to 110—120°. Above this temperature they are apt to decompose. The following figures obtained on analysis show, however, that just as in the case of the corresponding hydroxyhydrolapachol compound these crystals are not really anhydrous, but contain one molecule of water.

| | | | | | |
|---|---|---|---|---|---|
| I. | 0·2970 | gram of substance | gave | 0·1012 | gram $BaSO_4$. |
| II. | 0·1719 | ,, | ,, | 0·0585 | ,, $BaSO_4$. |
| III. | 0·2148 | ,, | ,, | 0·0740 | ,, $BaSO_4$. |
| IV. | 0·2078 | ,, | ,, | 0·0723 | ,, $BaSO_4$. |
| V. | 0·2075 | ,, | ,, | 0·0718 | ,, $BaSO_4$. |

| | Found. | | | | | Calculated for |
|---|---|---|---|---|---|---|
| | I. | II. | III. | IV. | V. | $(C_{15}H_{13}O_4)_2Ba + H_2O$. |
| Ba.... | 20·04 | 20·01 | 20·25 | 20·46 | 20·34 | 20·47 |

In Experiments I, II, and III the substance was dried at 100°, in Experiments IV and V it was dried at 110—120°. If now the orange

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crystals be vigorously rubbed up in an agate mortar, they undergo a change similar to that described by Hooker in the case of the hydroxyhydrolapachol compound; this rubbing up is tedious and difficult, but the compound gradually assumes a very deep maroon colour. In this condition it is apparently amorphous, but it does not swell up and become converted into the orange crystals on mere contact with water like the corresponding hydroxyhydrolapachol compound. On boiling with water it dissolves, and on cooling the solution deposits the orange crystals. Several experiments with small quantities of material indicated that in the rubbing up process water is absorbed from the atmosphere, and that the triturated material on being heated loses more water than can be accounted for by this absorption, that in fact, it becomes converted into the anhydrous barium compound. The heating, however, needs to be carefully managed, and the temperature must not rise much above 100°, or decomposition is liable to set in. Moreover, if heat (even a temperature of 100°) be applied too suddenly the substance is partly converted into the original orange crystalline condition, and the escape of water is in some measure interrupted. Whereas if the heating be gradual the substance will retain its deep maroon colour until water ceases to come off. The following are some of the results obtained in these experiments, all of them being carried out with the triturated material, and the heating to 100° being in most cases gradual, though in some instances the material was placed at once in a water oven round which the water was boiling.

| | | | | | |
|---|---|---|---|---|---|
| I. | 0·1888 | lost (at 100°) | 0·0100 $H_2O$ | and yielded | 0·0635 $BaSO_4$. |
| II. | 0·2970 | ,, | 0·0170 $H_2O$ | ,, | 0·0980 $BaSO_4$. |
| III. | 0·5855 | ,, | 0·0315 $H_2O$. | | |
| IV. | 0·3412 | | | ,, | 0·1138 $BaSO_4$. |
| V. | 0·2773 | ,, | 0·0128 $H_2O$ | ,, | 0·0925 $BaSO_4$. |
| VI. | 1·1660 | ,, | 0·0585 $H_2O$. | | |
| VII. | 0·2134 | | | ,, | 0·071 $BaSO_4$. |

| | I. | II. | III. | IV. | V. | VI. | VII. |
|---|---|---|---|---|---|---|---|
| Percentage of water lost at 100° | 5·29 | 5·72 | 5·39 | — | 4·62 | 5·02 | — |
| Percentage of Ba on undried material | 19·77 | 19·40 | — | 19·61 | 19·61 | — | 19·56 |
| Percentage of Ba on material dried at 100° | 20·88 | 20·58 | — | 20·80 | 20·56 | — | 20·68 |

The absorption of water during the process of trituration is, so far as these experiments are concerned, deduced from the diminished percentage of barium; the percentage in the orange crystals being about 20·4, and in that in the triturated material 19·59 (average).

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It was thought desirable, however, to confirm this by direct experiment. A weighed quantity of the orange crystals was therefore taken and very carefully rubbed up in an agate mortar on a sheet of white paper in such a way that no particles were lost. The material was then very carefully scraped out of the mortar and weighed. The mortar, pestle, and spatula used for scraping were then treated with pure hot water till all adhering substance (very small in amount) was dissolved, and the solution was evaporated in a platinum dish and converted into barium sulphate. From the weight of $BaSO_4$ thus found, the equivalent weight of the barium derivative was calculated, and this was added to the weight of the main quantity. In an actual experiment conducted in this way, 1·146 grams of the orange crystals gave 1·166 grams of triturated substance actually weighed *plus* 0·0125 gram, calculated from $BaSO_4$ as just indicated, or a total of 1·1785 grams. This is a gain of 0·0325 gram or 2·83 per cent. This is the material used in Experiment VI above, in which the total loss on heating to 100° was 5·02 per cent. It may be added that while these experiments were being conducted, the atmosphere was almost constantly humid. The figures agree fairly closely and seem to be consistent with the supposition that during trituration the orange crystals of the barium derivative absorb $1H_2O$, but that on heating the triturated material to 100°, it loses not only the water taken up, but another molecule in addition, becoming, in fact, anhydrous, or nearly so. The following figures bear out these statements when compared with the experimental results. The addition of $1H_2O$ to $(C_{15}H_{13}O_4)_2Ba + H_2O$ requires an increase in weight of 2·69 per cent., and the percentage of barium in the substance so formed would be 19·94. If, then, this substance loses $2H_2O$, the decrease in weight would be 5·24 per cent., and the percentage of barium in the anhydrous derivative would be 21·04. That the percentages of barium found in the dried material fall, in some cases, considerably short of the latter figure, is doubtless due to the fact above stated, that sometimes even with careful heating, the dark maroon-coloured compound is partly converted into the original orange crystalline condition, the escape of water being thereby prevented and the percentage of barium consequently lowered.

The calcium derivative prepared by a method similar to that used for the barium compound forms an almost black, granular mass, which under the microscope is seen to consist of very dark red crystalline grains. After having been left in a desiccator over sulphuric acid for several days, it loses water slowly at 100°, more rapidly at 120°. In one experiment 0·2712 gram prepared in this way yielded 0·0645 $CaSO_4$, which represents 6·99 per cent. of calcium;

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the theoretical amount for $(C_{15}H_{13}O_4)_2Ca + H_2O$ being 6·99 per cent. One rough experiment seemed to indicate that this salt also, when rubbed up in a mortar, behaves in somewhat the same manner as the barium compound, but the data are, from want of material, insufficient to establish the point.

The silver derivative can be prepared by precipitating a solution of silver nitrate with a solution of the barium derivative. It forms a maroon-coloured, crystalline powder. On analysis 0·2304 gram gave 0·065 metallic silver = 28·21 per cent.; the theoretical number for $C_{15}H_{13}O_4Ag + H_2O$ being 28·19 per cent.

*Acetyl Derivative.*—To prepare the acetyl derivative, hydroxylapachol is boiled with acetic anhydride and a minute quantity of zinc chloride for two or three minutes; water is then added, the contents of the vessel heated to decompose the excess of acetic anhydride, and the whole is allowed to stand. In this way, a yellow solid is obtained, which on recrystallisation forms yellow needles, appearing quite homogeneous when examined by the microscope. After being several times recrystallised from alcohol and allowed to dry in the air, its melting point remains constant at 82°.

0·2265 gave 0·5503 $CO_2$ and 0·118 $H_2O$. C = 66·26; H = 5·79.
$C_{15}H_{12}O_4(C_2H_3O)_2$ requires C = 66·66; H = 5·26 per cent.

Two saponification experiments were performed as follows.

1. A small portion of the substance = 0·3542 gram was boiled with *pure* caustic soda till complete solution was effected, the liquid acidified with sulphuric acid, and distilled with the usual precautions, the distillate being collected in a flask containing pure barium carbonate. The distillate was then boiled with the barium carbonate, filtered, and the barium in the filtrate estimated. The $BaSO_4$ obtained weighed 0·2189 gram, and this corresponds to 0·1127 acetic acid or 31·8 per cent. The theoretical quantity obtainable from a diacetyl derivative is 35·08 per cent. and from a monacetyl derivative 20 per cent.

2. A weighed quantity of material = 0·2175 gram was boiled for some time with water and a slight excess of barium carbonate in a flask attached to a reflux condenser. As soon as saponification was complete, the excess of barium carbonate was filtered off and the barium estimated in the filtrate. The quantity of $BaSO_4$ found was 0·2094 gram, but of this only two-thirds is due to the presence of the acetyl groups, the remainder being due to the formation of the soluble barium derivative of hydroxylapachol. This is evident from the following equation.

$$2C_{15}H_{12}O_4(C_2H_3O)_2 + 3BaCO_3 + H_2O = (C_{15}H_{13}O_4)_2Ba + 2(C_2H_3O_2)_2Ba + 3CO_2.$$

Two-thirds of 0·2094 = 0·1396 $BaSO_4$, which corresponds to

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0·0719 gram acetic acid or 33·06 per cent. Theory, 35·08 per cent. There can be no doubt, therefore, that a diacetyl derivative is formed.

*Oxidation.*—On boiling with a 5 per cent. solution of potassium dichromate in dilute sulphuric acid for some hours, hydroxylapachol is almost completely oxidised, only a small quantity of tarry matter being formed. Acetic and phthalic acids were easily recognised as the products of oxidation.

*Action of Sulphuric acid on Hydroxylapachol and Formation of Hydroxy-β-lapachol.*—Hydroxylapachol is dissolved in a small quantity of concentrated sulphuric acid in the cold, the solution is allowed to stand for from 15 to 20 minutes, and is then thrown into a large quantity of cold water. A small quantity of tarry matter separates which can be filtered off after the liquid has been allowed to stand for a short time; a clear solution is thus obtained from which beautiful rosettes of red crystals begin to separate after an interval of from 7 to 10 days. These crystals are readily purified by two or three crystallisations from slightly diluted alcohol, and they melt at 204—205°.

0·1192 gave 0·3065 $CO_2$ and 0·0615 $H_2O$. C = 70·13*; H = 5·73.
$C_{15}H_{14}O_4$ requires C = 69·76; H = 5·42 per cent.

The melting point of this substance is slightly higher than that given by Hooker (201·5°) for hydroxy-β-lapachone. Two or three different small specimens, however, always melted at the same temperature. From the combustion numbers and the reaction with caustic potash, to be immediately described, the writer believes this substance to be identical with hydroxy-β-lapachone. When boiled for a few minutes with a strong solution of caustic potash, the red crystals dissolve, forming a deep red solution from which on acidification a yellow substance is precipitated; after being recrystallised two or three times from alcohol it melts at 182°. Hooker gives 181—182° as the melting point of dihydroxyhydrolapachol. The following are the results obtained by combustion.

I. 0·2486 gave 0·5938 $CO_2$ and 0·1320 $H_2O$. C = 65·14; H = 5·89.
II. 0·2280 „ 0·5445 „ 0·1198 „ C = 65·13; H = 5·83.
$C_{15}H_{16}O_5$ requires C = 65·21; H = 5·79 per cent.

From want of material, this substance could not be further studied; it was observed, however, that on boiling with concentrated hydrochloric acid it was dissolved, and a red substance was formed, which melted at 202°, and was doubtless hydroxy-β-lapachone.

*Action of Sulphuric acid on Hydroxylapachol and Formation of a Red Substance differing from Hydroxy-β-lapachone.*—Hydroxylapachol is dissolved in the smallest possible quantity of concentrated sul-

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phuric acid and the solution *immediately* poured into cold water; the red crystalline substance which is precipitated is readily purified by one or two recrystallisations from slightly diluted alcohol. As thus obtained, it forms beautiful, silky needles of a somewhat deeper red than hydroxy-$\beta$-lapachone; it melts at 110—111°, is practically insoluble in water, but soluble in alcohol and the ordinary solvents.

0·1544 gave 0·4275 $CO_2$ and 0·0748 $H_2O$. C = 75·52*; H = 5·38.
0·1469 ,, 0·4035 ,, 0·0685 ,, C = 74·91; H = 5·11.
$C_{15}H_{12}O_3$ requires C = 75·00; H = 5·00 per cent.

After filtering off this substance and allowing the mother liquor to stand for a long time, they deposited a small quantity of red crystals melting at 204°, doubtless hydroxy-$\beta$-lapachone. Hence the above-described substance would appear to be an intermediate stage in the formation of hydroxy-$\beta$-lapachone. If the compound melting at 110—111° be boiled for a few minutes with a strong solution of caustic potash, it dissolves, and the solution on acidification deposits a yellow substance which must be recrystallised four or five times from water slightly acidified with acetic acid; it then forms yellow needles melting at 109—110°. It yielded the following results on combustion.

0·128 gave 0·3287 $CO_2$ and 0·064 $H_2O$. C = 70·04*; H = 5·55.
$C_{15}H_{14}O_4$ requires C = 69·76; H = 5·42 per cent.

This substance is obviously formed by the addition of the elements of water to the red compound melting at 110—111°, and is therefore isomeric with hydroxylapachol, but as in the case of some of the other substances described, there was not sufficient material for further examination; its relations to hydroxylapachol and to Paternò's isolapachone have been already discussed in the introductory part.

The author is deeply indebted to Mr. J. H. Maiden, of the Technological Museum, Sydney, for supplies of material which are difficult to obtain, and also to Dr. Hooker for specimens of some of his lapachol derivatives.

* In this combustion and the subsequent ones marked with an asterisk in this paper, the somewhat high percentage of carbon is believed to be due to an imperfection in the combustion apparatus subsequently discovered.—E. H. R.