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# THE CONSTITUENTS OF CLUYTIA SIMILIS

BY

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AND

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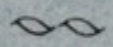

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# CCXXXII.—*The Constituents of* Cluytia similis.

By Frank Tutin and Hubert William Bentley Clewer.

The small shrub, *Cluytia hirsuta*, Muell. Arg., belonging to the *Euphorbiaceae*, is reputed in South Africa to be of value as an antidote for anthrax and for the disinfection of "milt-ziek," or anthrax-infected meat, and is apparently much used by the natives for this purpose (Smith, "A Contribution to South African Materia Medica," Cape Town, 1895, p. 57). This author also mentions, as being more valuable than the above-mentioned species, a smaller form of *Cluytia*, which is either a variety of *C. hirsuta* or else a distinct species. A quantity of this smaller species, which grows in the open veldt, attaining a height of 10 inches or less, was therefore specially collected for us by Mr. G. E. Oliver, of East London. The identity of this material has been determined by Mr. Hutchinson, of Kew, through the kindness of Mr. E. M. Holmes, F.L.S., as *Cluytia similis*, Muell. Arg.

Mr. Oliver also ascertained that the root of *Cluytia similis* was eaten by certain of the natives as an antidote for snake-bite poisoning, and a quantity of this material, as well as the above-ground portions of the plant, has therefore been submitted to examination.

## Experimental.

### I.—*Examination of the Stems and Leaves.*

The material employed in this investigation consisted of the entire above-ground portions of *Cluytia similis*, Muell. Arg., which had been specially collected in South Africa.

*Test for an Alkaloid.*—A quantity (20 grams) of the finely-ground, dried material was digested with Prollius's fluid, and the resulting extract subjected to the usual tests for an alkaloid, but with a negative result. In view of the statement of Smith (*loc. cit.*) that this species of *Cluytia* gave a "green reaction" with

chlorine water and ammonia, thus indicating the presence of quinine or an allied alkaloid, the last-mentioned test was repeated, but with a negative result.

*Preliminary Extraction of the Plant.*—A quantity (25 grams) of the dried and crushed material was extracted successively in a Soxhlet apparatus with various solvents, the following amounts of extract, dried at 100°, being obtained:

| | | | | |
|---|---|---|---|---|
| Petroleum (b. p. 35—50°) extracted | 0·83 gram | = | 3·32 | per cent. |
| Ether | ,, 0·34 ,, | = | 1·36 | ,, |
| Chloroform | ,, 0·22 ,, | = | 0·88 | ,, |
| Ethyl acetate | ,, 0·41 ,, | = | 1·64 | ,, |
| Alcohol | ,, 2·52 ,, | = | 10·08 | ,, |
| Total | = 4·32 grams | = | 17·28 | per cent. |

For the purpose of a complete examination a quantity (7·42 kilograms) of the crushed and dried material was extracted by continuous percolation with hot alcohol, when, after the removal of the greater part of the solvent, 2·17 kilograms of a dark-coloured extract were obtained.

The entire amount of the above-mentioned extract was mixed with water, and steam passed through the mixture for several hours, when a quantity (1·24 grams) of an essential oil was removed. This oil possessed a pale yellow colour and a strong, rank odour.

After the distillation of the extract with steam, as above described, there remained in the distillation flask a dark brown, aqueous liquid (*A*) and a quantity of dark green resin (*B*). The latter was separated, and repeatedly washed with boiling water, the concentrated washings being added to the main portion of the aqueous liquid.

### *Examination of the Aqueous Liquid* (A).

The aqueous liquid (*A*) was concentrated somewhat, and extracted ten times with ether, the ethereal liquids being united, washed, and evaporated to a small bulk. A large volume of petroleum (b. p. 35—50°) was then added to the residue, after which the clear liquid was separated by decantation from the precipitated resinous material. The petroleum solution was then evaporated, the residue dissolved in ether, and the resulting solution shaken successively with aqueous ammonium and sodium carbonates and potassium hydroxide.

### *Isolation of Chrysophanol,* $C_{15}H_{10}O_4$.

The material soluble in ammonium carbonate was small in amount, and consisted chiefly of amorphous products. It also

contained, however, an acid soluble in warm water, which gave a violet coloration with ferric chloride, and appeared to be salicylic acid. The sodium carbonate extract was very small, and was discarded, but on acidifying the extracts obtained by means of potassium hydroxide a quantity (0·15 gram) of a substance was obtained, which separated from ethyl acetate in golden-coloured leaflets. This substance melted at 191°, and proved to be chrysophanol (compare Trans., 1911, **99**, 948). (Found, C = 70·6; H = 4·1. Calc., C = 70·9; H = 3·9 per cent.) It yielded diacetylchrysophanol, melting at 206°. This occurrence of chrysophanol is remarkable, for, so far as known to the present authors, it is the first recorded instance of an anthraquinone derivative occurring in a euphorbiaceous plant, and, moreover, it is very unusual to find this substance unassociated with emodin monomethyl ether.

The portion of the ethereal extract of the aqueous liquid which was undissolved by petroleum consisted only of amorphous products, together with a trace of chrysophanol.

The original aqueous liquid (*A*) which had been extracted with ether was then shaken with twenty successive portions of amyl alcohol. The amyl alcohol solution so obtained, when evaporated to a small bulk under diminished pressure, deposited a quantity (30 grams) of an amorphous, yellow solid. This product could not be crystallised, but after heating with dilute sulphuric acid it yielded a small amount of a sugar which gave *d*-phenylglucosazone, thus indicating that a little glucosidic substance was present.

### *Isolation of a New Acid,* $C_{10}H_{10}O_4$.

The remainder of the amorphous solid which separated from the amyl alcohol extracts on concentration, as above described, was boiled for one minute with 10 per cent. aqueous potassium hydroxide, after which the mixture was acidified and extracted with ether. The ethereal solution was washed, and shaken successively with aqueous ammonium carbonate, sodium carbonate, and potassium hydroxide. The material removed by the last-mentioned two alkalis consisted only of amorphous products, but the product obtained on acidifying the ammonium carbonate extract slowly crystallised on keeping. The solid so obtained was crystallised from benzene containing a small proportion of ethyl acetate, when a quantity (0·08 gram) of a substance separated in pale brown, prismatic needles, which melted without decomposition at 159°:

0·0790 gave 0·1794 $CO_2$ and 0·0377 $H_2O$. C = 61·9; H = 5·3.

$C_{10}H_{10}O_4$ requires C = 61·9; H = 5·2 per cent.

A further and larger amount of this acid was obtained by the alkaline hydrolysis of the product yielded by the lead subacetate precipitate, as subsequently described, when it was again analysed, and the above-given result fully confirmed. The only known compound with which this acid agrees at all in its properties is 2:5-dihydroxy-β-methylcinnamic acid, which is stated to melt at 155—156° (*Ber.*, 1907, **40**, 2731). A quantity of the latter acid was therefore synthesised, but it was found not to be identical with the acid here described. It is evident, therefore, that the above-mentioned *acid* melting at 159° is a new compound; it yields a brown-coloured solution when treated with ferric chloride, which, on keeping, becomes turbid.

The original amyl alcohol filtrate from the amorphous solid, the investigation of which has just been described, contained a quantity of amorphous material which was soluble in water. This product was thoroughly investigated, and submitted to hydrolysis, both with acid and alkali, but nothing crystalline could be obtained from it.

The original aqueous liquid (*A*), which had been extracted with amyl alcohol, as above described, was freed from this solvent and treated with an excess of basic lead acetate solution. A very bulky, yellow precipitate was thus produced, which was collected and washed.

*Isolation of Fumaric Acid.*

The basic lead acetate precipitate, which was found to contain a considerable amount of lead chloride owing to the presence of potassium chloride in the original aqueous liquid, was decomposed by means of hydrogen sulphide, and filtered. The filtrate was then evaporated to a small bulk under diminished pressure, and extracted with ether in order to remove any ether-soluble substances which had been formed by the hydrolysing action of the hydrochloric acid derived from the lead chloride. The ethereal liquid so obtained was then extracted successively with aqueous ammonium carbonate, sodium carbonate, and potassium hydroxide. On acidifying the liquid obtained by means of the first-mentioned alkali, and extracting it with ether, a small amount of a brown-coloured product was obtained. The latter, when crystallised from a mixture of ethyl acetate and alcohol, yielded small, light brown prisms, which sublimed at about 200°, and were identified as fumaric acid. (Found, C = 41·6; H = 3·7. Calc., C = 41·4; H = 3·4 per cent.)

The sodium carbonate extract, obtained as above described, yielded nothing but amorphous material, but on acidifying the potassium hydroxide extract a small amount of chrysophanol was obtained.

To the original acid aqueous liquid which had been extracted

with ether as above described, potassium hydroxide was added until it contained about 8 per cent. of this alkali, when it was boiled for a short time, acidified, and again extracted with ether. The ammonium carbonate extract of the ethereal solution then yielded about 0·5 gram of the previously-described new acid, $C_{10}H_{10}O_4$, which was analysed. (Found, C = 61·8; H = 5·3. Calc., C = 61·9; H = 5·2 per cent.) The remainder of the material dissolved by the ether consisted only of amorphous products.

The filtrate from the lead subacetate precipitate, obtained as above described, was concentrated, when, on keeping, it deposited some potassium chloride and sodium acetate. It furthermore readily yielded *d*-phenylglucosazone (m. p. 216°), but no other definite compound could be obtained from it.

### *Examination of the Resins* (B).

The resin (*B*) was a hard, dark green mass, and amounted to 350 grams, being thus equivalent to about 4·7 per cent. of the weight of the drug employed. It was dissolved in alcohol, mixed with purified sawdust, and the thoroughly dried mixture extracted successively in a large Soxhlet apparatus with light petroleum (b. p. 35—50°), ether, chloroform, ethyl acetate, and alcohol.

### *Petroleum Extract of the Resin.*

### *Isolation of a New Ester, Cluytyl Cluytinate,* $C_{49}H_{98}O_2$.

The petroleum extract of the resin was dark green, and, when deprived of solvent, amounted to 207 grams. It was digested with 2 litres of ether, and the mixture kept for some time, and filtered. A quantity (9 grams) of a dark green solid was thus separated, which was washed with ether and then distilled under diminished pressure, when the greater part of the material passed over at a very high temperature as a light-coloured distillate. The latter, which solidified on cooling, was crystallised from glacial acetic acid with the employment of animal charcoal. Two fractions were thus obtained, one of which was very sparingly soluble, and was separated by rapid filtration from the warm solution. This solid was repeatedly crystallised from various solvents, when the melting point remained unchanged at 76·5°. The *substance* formed nacreous leaflets, and amounted to 0·5 gram:

0·0758 gave 0·2266 $CO_2$ and 0·0932 $H_2O$. C = 81·5; H = 13·7.

$C_{49}H_{98}O_2$ requires C = 81·9; H = 13·6 per cent.

This compound was found to be a new *ester* formed by the combination of cluytyl alcohol and cluytinic acid, two new compounds described below, and is therefore *cluytyl cluytinate*.

*Isolation of Cluytyl Alcohol,* $C_{28}H_{57}\cdot OH$, *and Cluytinic Acid,* $C_{20}H_{41}\cdot CO_2H$.

A quantity of cluytyl cluytinate was hydrolysed by boiling with alcoholic potassium hydroxide for two hours, after which water was added and the alkaline mixture extracted with ether. The ethereal liquid was evaporated, when a quantity of an alcohol was obtained, which crystallised from ethyl acetate in small, glistening leaflets, melting at 82·5°, and this melting point was unchanged by further recrystallisation:

0·0942 gave 0·2826 $CO_2$ and 0·1204 $H_2O$. C=81·8; H=14·2.

$C_{28}H_{58}O$ requires C=82·0; H=14·1 per cent.

This alcohol thus appears to be a new compound, and it is proposed to designate it *cluytyl alcohol.* A further and larger amount of it was subsequently obtained from the unsaponifiable matter contained in the petroleum extract of the resin, when its properties and composition were confirmed. *Cluytyl acetate,* $C_{28}H_{57}\cdot O\cdot CO\cdot CH_3$, was prepared by the action of acetic anhydride on the respective alcohol, when it formed small leaflets melting at 64°.

The alkaline aqueous liquid from which the cluytyl alcohol had been removed by means of ether, as above described, contained a quantity of a sparingly soluble potassium salt. This was collected, and heated with dilute sulphuric acid and chloroform, after which the chloroform solution was separated, washed, and evaporated, when a solid of low melting point was obtained. The latter was crystallised several times from ethyl acetate, when it formed colourless, lustrous leaflets, melting sharply at 69°. Further crystallisations from alcohol and from glacial acetic acid failed to effect any change in the melting point:

0·0865 gave 0·2446 $CO_2$ and 0·1013 $H_2O$. C=77·1; H=13·0.

$C_{21}H_{42}O_2$ requires C=77·3; H=12·9 per cent.

This substance possessed all the attributes of homogeneity, and thus appears to be a new fatty acid possessing the formula $C_{20}H_{41}\cdot CO_2H$, which it is proposed to designate *cluytinic acid.* A further and larger quantity of the same acid was subsequently obtained by the fractional distillation of the esters of the fatty acids present in the petroleum extract, as described below, when its composition and properties were confirmed.

Cluytinic acid has approximately the same melting point as stearic acid, and is indistinguishable in appearance from the latter, but when the two substances are mixed fusion occurs at 61—62°.

The material more readily soluble in glacial acetic acid, from which the above-described cluytyl cluytinate had been separated,

was found to consist chiefly of cluytyl alcohol, and was examined in connexion with the unsaponifiable matter described below.

The original ethereal solution from which the crude cluytyl cluytinate had been separated was shaken with aqueous ammonium carbonate, but nothing was removed. It was then shaken with aqueous potassium carbonate, when a quantity of a viscid, green product separated, which was found to consist essentially of cluytyl alcohol. The ethereal liquid was subsequently washed with water, and after separating the dark green aqueous liquid, the former was extracted with aqueous potassium hydroxide, when 0·8 gram of chrysophanol was removed. After this treatment the ethereal liquid was again washed with water, when a further amount of green material was removed, and was found to be similar in character to that removed by water after extraction with potassium carbonate. Both these liquids were acidified, extracted with ether, and the resulting material, which consisted of chlorophyll and free fatty acids, esterified with methyl alcohol. The resulting esters, after being deprived of a large amount of chlorophyll by shaking first with aqueous alkali and subsequently with water, were distilled, and examined in connexion with the esters of the combined fatty acids subsequently described.

*Isolation of Cerotic Acid*, $C_{27}H_{54}O_2$.

The ethereal solution of the neutral portion of the petroleum extract of the resin was evaporated, and the residue heated for two hours with an excess of alcoholic potassium hydroxide. Water was then added, and the mixture repeatedly extracted with ether. During this operation a quantity of a flocculent solid separated at the juncture of the aqueous and ethereal layers. This was collected, when it was found to consist chiefly of the potassium salt of a higher fatty acid. It was freed from some neutral material, and the fatty acid isolated and crystallised from ethyl acetate. Small, glistening leaflets were thus obtained, which melted at 82—83°. Analysis indicated this substance to be cerotic acid, although the melting point observed is somewhat higher than that usually recorded for this acid. (Found, C = 78·7; H = 13·3. Calc., C = 79·0; H = 13·2 per cent.)

The ethereal solution of the unsaponifiable matter, which had been separated from the alkaline, aqueous liquid and the potassium cerotate as above described, was washed, dried, and evaporated, when the residue (25 grams) was dissolved in alcohol. On cooling the solution a quantity (about 5 grams) of an apparently amorphous

product separated. This was distilled under diminished pressure, the distillate dissolved in pyridine, and the mixture heated with an equal weight of phthalic anhydride for a short time. Water was then gradually added to the warm solution, and the resulting liquid extracted with ether. The ethereal solution was deprived of pyridine, and then shaken with aqueous sodium carbonate, when a quantity of the solid sodium salt of an acid phthalic ester of an alcohol separated. The latter was collected and hydrolysed with alcoholic potassium hydroxide, when a quantity (2·5 grams) of an alcoholic substance was obtained. The latter formed small, glistening leaflets, melting at 82·5°, and proved to be cluytyl alcohol.

*Isolation of Triacontane,* $C_{30}H_{62}$.

The ethereal solution from which the sodium salt of the cluytyl hydrogen phthalate had been separated was evaporated, and the residue crystallised, first from ethyl acetate, and subsequently from petroleum (b. p. 90—120°), when a quantity (1 gram) of colourless leaflets was obtained. This substance melted at 65·5°, and was found to be triacontane, $C_{30}H_{62}$. (Found, C = 84·9; H = 14·6. Calc., C = 85·3; H = 14·7 per cent.)

*Isolation of a New Phytosterol, Cluytiasterol,* $C_{27}H_{43}\cdot OH$.

The alcoholic solution of the unsaponifiable matter, from which the crude mixture of triacontane and cluytyl alcohol had been separated, was concentrated, and some ethyl acetate and a little water added. On keeping the mixture for a few days a quantity (0·7 gram) of large, colourless leaflets separated. This product had the properties of a phytosterol, and melted at 136°, but, as it did not appear homogeneous, it was converted into the acetyl derivative. The latter crystallised in leaflets, melting at 131—132°, but, after repeated crystallisation from alcohol and ethyl acetate alternately, the melting point was raised to 139°, at which point it remained constant. The pure acetyl derivative was then hydrolysed, and the resulting alcohol isolated and crystallised from a mixture of ethyl acetate and dilute alcohol, when a quantity (0·35 gram) of colourless leaflets, melting at 159°, was obtained:

0·0942,* on heating at 110°, lost 0·0043 $H_2O$. $H_2O = 4·6$.

0·0899 † gave 0·2769 $CO_2$ and 0·0947 $H_2O$. C = 84·0; H = 11·7.

$C_{27}H_{44}O,H_2O$ requires $H_2O = 4·5$ per cent.

$C_{27}H_{44}O$ requires C = 84·4; H = 11·5 per cent.

This substance was evidently a phytosterol, since it gave the colour reactions exhibited by this class of compounds, and, as it does

* Air-dried substance. † Dried at 110°.

not agree in its properties with any compound previously described, it is proposed to designate it *cluytiasterol*. Its optical activity was determined with the following result:

0·2853,* made up to 20 c.c. with chloroform, gave $\alpha_D$ −1°30′ in a 2-dcm. tube, whence $[\alpha]_D$ −52·6°.

*Examination of the Fatty Acids.*

The alkaline aqueous liquid from which the unsaponifiable matter had been removed, as above described, was acidified, and distilled with steam, but the amount of volatile acid removed was negligible. The mixture was then extracted with ether, when a small amount of an undissolved solid was collected. The latter crystallised from alcohol in needles melting at 248°, and yielded an acetyl derivative melting at 255°, but the amount obtained was too small for further examination. The dark green, ethereal solution containing the fatty acids was then evaporated to a small bulk and treated with a large volume of petroleum (b. p. 35—50°), when the greater part of the chlorophyll was precipitated. The clear petroleum solution was then decanted, evaporated, and the residual fatty acids converted into their methyl esters and distilled, when a quantity (21 grams) of a liquid was obtained. The latter was mixed with the 16 grams of esters previously isolated, and the whole fractionally distilled several times under 60 mm. pressure, when the following fractions were obtained: (i) −240°; (ii) 240—250°; (iii) 250—260°; (iv) 260—270°; (v) 270—280°; (vi) 280°+.

Fraction (i) solidified on cooling, and was found to consist of methyl palmitate, since it yielded palmitic acid (m. p. 63°) on hydrolysis. (Found, C=74·7; H=12·5. Calc., C=75·0; H=12·5 per cent.)

Fractions (ii) and (iii), when hydrolysed, yielded a small amount of a mixture of unsaturated acids, but the greater part of the material was a mixture consisting essentially of palmitic and stearic acids. (Found, C=75·2; H=12·6. Calc., for $C_{16}H_{32}O_2$, C=75·0; H=12·5; for $C_{18}H_{36}O_2$, C=76·1; H=12·7 per cent.)

Fraction (iv) became partly solid on keeping. On hydrolysis it yielded a further quantity of the previously-described new fatty acid, cluytinic acid, $C_{21}H_{42}O_2$, which was identified by its melting point (69°) and by analysis. (Found, C=77·3; H=13·1. Calc., C=77·3; H=12·9 per cent.)

Fraction (v) was too small for examination, but fraction (vi), after being hydrolysed and freed from traces of unsaponifiable matter, yielded an acid melting at 74°. The amount of the latter, however, was too small for investigation.

* Dried at 110°.

*Ethereal Extract of the Resin.*

*Isolation of a New Tetrahydric Alcohol, Cluytianol,* $C_{29}H_{46}O(OH)_4$.

The ethereal extract of the resin was dark green, and amounted to 39 grams. A portion of it (about 1 gram) was a black powder, vary sparingly soluble in ether, which was collected and separately examined. This sparingly soluble solid was treated with animal charcoal in pyridine solution, the product which separated on cooling the filtered liquid being then acetylated by boiling with acetic anhydride containing some pyridine. The solution so obtained was concentrated and cooled, when a substance separated in small plates. This solid was recrystallised seven times from alcohol, when small, colourless leaflets, melting constantly at 160°, were obtained:

0·0919 gave 0·2316 $CO_2$ and 0·0753 $H_2O$. C = 68·7; H = 9·1.

$C_{37}H_{58}O_9$ requires C = 68·7; H = 9·0 per cent.

This substance proved to be the *tetra-acetyl* derivative of a tetrahydric alcohol isomeric with the trihydric alcohol, ipuranol, which it greatly resembles in its general properties. This new alcohol has been designated *cluytianol*, and the above-described substance is therefore *tetra-acetylcluytianol*.

A quantity of tetra-acetylcluytianol was hydrolysed, and the resulting alcohol crystallised from pyridine, when very small, colourless crystals were obtained, which, when heated fairly rapidly, decomposed and melted at 300—305°:

0·0985 gave 0·2628 $CO_2$ and 0·0929 $H_2O$. C = 72·8; H = 10·5.

$C_{29}H_{50}O_5$ requires C = 72·8; H = 10·5 per cent.

This is the first time that a substance has been isolated which resembles ipuranol and the other related alcohols in its general characters, but contains four hydroxyl groups.

*Tetrabenzoylcluytianol,* $C_{29}H_{46}O_5(CO{\cdot}C_6H_5)_4$.—A small quantity of cluytianol was benzoylated in pyridine solution, and the resulting compound crystallised from a mixture of chloroform and alcohol, when long, flattened needles, melting at 192°, were obtained:

0·1029 gave 0·2878 $CO_2$ and 0·0713 $H_2O$. C = 76·3; H = 7·7.

$C_{57}H_{66}O_9$ requires C = 76·5; H = 7·4 per cent.

The ethereal solution of the more readily soluble portion of the ethereal extract of the resin deposited on keeping a further quantity (about 1 gram) of crude cluytianol. The clear ethereal solution of the resin was then shaken with aqueous ammonium carbonate, which removed a quantity of dark green, viscid material, from which nothing crystalline could be obtained. On subsequently shaking the ethereal solution with aqueous potassium hydroxide,

however, a red liquid was obtained, which, when acidified, yielded a yellow powder. The latter was collected, when it was found to be almost insoluble in alcohol or ethyl acetate, but crystallised from dilute pyridine in small rosettes of brownish-red needles, which decomposed and melted at 315°. This substance dissolved in concentrated sulphuric acid with a crimson colour, and possessed the properties of a phenolic anthraquinone derivative:

0·0904 gave 0·2134 $CO_2$ and 0·0331 $H_2O$. C = 64·4; H = 4·1.
0·0957 „ 0·2265 $CO_2$ „ 0·0352 $H_2O$. C = 64·5; H = 4·1.
$C_{14}H_{10}O_5$ requires C = 65·1; H = 3·9 per cent.

After each of these combustions a small amount (about 0·0005 gram) of inorganic residue was left in the boat, which would account for the somewhat low percentage of carbon found.

The only known compound with which the above-described anthraquinone derivative could be identical is a substance described by Tschirch (*Arch. Pharm.*, 1900, **238**, 427), but as this author does not record the melting point of the anthraquinone derivative he obtained it is impossible to say whether the latter is identical with the substance here described.

The substance obtained from *Cluytia similis*, which is probably a trihydroxydihydroanthraquinone, yielded an acetyl derivative, which crystallised in small, yellow needles, melting at 167°.

The neutral portion of the ethereal extract of the resin, from which the above-described substance had been separated, consisted only of amorphous products

*Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.*

The chloroform extract of the resin was dark green, and amounted to 10 grams. It was thoroughly examined, and was found to consist of amorphous products, together with a small amount of the above-described substance, $C_{14}H_{10}O_5$.

The ethyl acetate extract of the resin amounted to 7 grams, and consisted only of amorphous products.

The alcohol extract of the resin amounted to 72 grams, and was a nearly black powder. It was submitted to both acid and alkaline hydrolysis, but nothing definite could be isolated from it.

## II.—*Examination of the Root.*

A portion (10 grams) of the ground root of *Cluytia similis* was subjected to a preliminary test for an alkaloid by digestion with Prollius's fluid, but with a negative result.

Another portion (25 grams) of the ground material was succes-

sively extracted with various solvents, when the following amounts of extract, dried at 100°, were obtained:

| | | | | | |
|---|---|---|---|---|---|
| Petroleum (b. p. 25—50°) | extracted | 0·23 | gram | =0·92 | per cent. |
| Ether | ,, | 0·27 | ,, | =1·08 | ,, |
| Chloroform | ,, | 0·18 | ,, | =0·72 | ,, |
| Ethyl acetate | ,, | 0·15 | ,, | =0·60 | ,, |
| Alcohol | ,, | 1·03 | ,, | =4·12 | ,, |
| | Total = | 1·86 | grams | =7·44 | per cent. |

For the purpose of a complete examination, a quantity (7·77 kilograms) of the ground root was completely extracted by continuous percolation with hot alcohol, when, after the removal of the greater part of the solvent, 1·54 kilograms of a dark brown extract were obtained. The latter was mixed with water, and distilled with steam, but practically no volatile material was removed by this treatment.

After the steam distillation the mixture remaining in the distillation flask consisted of a dark-coloured, aqueous liquid (*A*) and a dark brown resin (*B*). The aqueous liquid was decanted, and the resin repeatedly washed with warm water, the washings being concentrated and added to the main bulk of the aqueous liquid.

### *Examination of the Aqueous Liquid* (A).

The aqueous liquid (*A*) was repeatedly extracted with ether, and the resulting extract thoroughly examined, but no indication of the presence of a crystalline substance could be obtained. The aqueous liquid was then extracted many times with amyl alcohol, which removed about 6 grams of a yellow, amorphous product similar to that obtained in like manner from the leaves and stems of the plant.

After the extraction with amyl alcohol the aqueous liquid (*A*) was evaporated to a low bulk under diminished pressure, and treated with alcohol until precipitation commenced. On keeping the mixture a quantity (5 grams) of inorganic matter separated, and was collected. This was found to consist chiefly of potassium sulphate, but copper, iron, manganese, aluminium, calcium, strontium, magnesium, and sodium were also present.

The filtrate from the inorganic matter was then deprived of alcohol, diluted with water, and treated with an excess of basic lead acetate solution. Both the precipitate produced by this treatment and the filtrate from it were deprived of lead, and the resulting liquids examined. The material yielded by the lead acetate precipitate consisted of dark brown, amorphous products, which, on alkaline hydrolysis, gave a small amount of a crystalline acid,

melting at 186°, whilst the final aqueous liquid appeared to contain chiefly sugar, since it yielded an abundance of *d*-phenylglucosazone (m. p. 216°), but it also contained some copper, which imparted to it a blue colour.

### *Examination of the Resin* (B).

The resin (*B*) was a dark brown mass, and amounted to 311 grams. It was mixed with alcohol, brought on to purified sawdust, and the dried mixture extracted successively in a large Soxhlet apparatus with petroleum (b. p. 35—50°), ether, chloroform, ethyl acetate, and alcohol.

The petroleum extract of the resin was a green, viscid mass, amounting to 48·5 grams. It was fully examined in a manner analogous to that employed for the examination of the petroleum extract of the resin from the leaves and stems, when it was found to consist of amorphous products, together with palmitic acid (m. p. 62·5°), apparently some stearic acid, a higher fatty acid (m. p. 73°), and a small amount of unsaturated acids. A quantity of cluytiasterol was also present, which was isolated in the form of its *acetate*, melting at 139°:

0·0955 gave 0·2851 $CO_2$ and 0·0966 $H_2O$. C = 81·4; H = 11·2.

$C_{29}H_{46}O_2$ requires C = 81·7; H = 10·8 per cent.

The ethereal extract of the resin amounted to 40 grams, and was a dark green, wax-like mass. It was exhaustively examined, but nothing crystalline could be isolated from it.

The chloroform, ethyl acetate, and alcohol extracts of the resin were nearly black, amorphous solids, and amounted to 66, 17, and 70 grams respectively. They were submitted to both alkaline and acid hydrolysis, and the products carefully examined, but nothing definite could be isolated.

### *Summary.*

The results of this investigation may be summarised as follows: The plant regarded by Smith (*loc. cit.*) as probably a small variety of *Cluytia hirsuta*, Muell. Arg., has been identified as *C. similis*, Muell. Arg. All parts of the plant are devoid of alkaloid.

An alcoholic extract of the above-ground portions of the plant, when distilled in a current of steam, yielded a small amount of an essential oil, possessing a strong, rank odour.

From the portion of the extract which was soluble in water there were isolated: (i) Chrysophanol; (ii) a new *acid*, $C_{10}H_{10}O_4$, which forms light brown, prismatic needles, melting at 159°; (iii) fumaric acid; (iv) apparently a trace of salicylic acid. The aqueous liquid

furthermore contained a large amount of a sugar, which yielded *d*-phenylglucosazone and a quantity of potassium chloride.

The portion of the alcoholic extract which was insoluble in water was a dark green resin. From this material the following compounds were isolated: (i) A new ester, *cluytyl cluytinate*, $C_{49}H_{98}O_2$ (m. p. 76·5°); (ii) triacontane, $C_{30}H_{62}$; (iii) a new phytosterol, *cluytiasterol*, $C_{27}H_{44}O$, melting at 159°, and having $[\alpha]_D$ −52·6°. *Cluytiasteryl acetate* melts at 139°. (iv) *Cluytyl alcohol*, $C_{28}H_{58}O$, m. p. 82·5°; (v) palmitic, stearic, and cerotic acids, together with a small amount of a mixture of unsaturated acids; (vi) a new fatty acid, *cluytinic acid*, $C_{21}H_{42}O_2$ (m. p. 69°); (vii) a new tetrahydric alcohol, *cluytianol*, $C_{29}H_{46}{\cdot}O(OH)_4$ (m. p. 300—305°). Cluytianol is isomeric with the trihydric alcohol, ipuranol, which it resembles in its general properties. *Tetra-acetylcluytianol* melts at 160°, and *tetrabenzoylcluytianol* at 192°; (viii) a compound, $C_{14}H_{10}O_5$, which is probably a trihydroxydihydroanthraquinone.

The alcoholic extract of the root of *Cluytia similis* consisted chiefly of amorphous material, but it yielded palmitic and stearic acids; a higher fatty acid (m. p. 73°); a small amount of a mixture of unsaturated acids; and a quantity of cluytiasterol. It also contained some inorganic matter, in which the presence of copper, iron, manganese, aluminium, calcium, strontium, magnesium, sodium, and potassium was established.

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