

A STUDY OF THE CHEMICAL COMPONENTS OF THE ROOTS OF *DECALEPIS HAMILTONII* (MAKALI VERU)

Part I. Chemical Composition of the Roots

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Decalepis Hamiltonii belongs to the family Asclepiadaceæ of which *Hemidesmus indicus* better known as Indian Sarsaparilla is also a member. It grows largely in South India in the hilly and forest areas of the Western Ghats. The root is cylindrical and measures from 1 to 6 cms. in diameter. It is markedly fleshy and has a strong aroma which is more prominent than that of *Hemidesmus indicus*. It is largely used in making pickles along with limes. The fresh root tastes sweet accompanied by a tingling sensation on the tongue. Barring a note on the isolation of paramethoxysalicylaldehyde by the steam distillation of the roots¹ no systematic chemical examination of this plant product seems to have been carried out. In the fresh root there is about 82% of moisture; the inner woody core constitutes about 8% and the remaining 92% forms the fleshy portion. The air dried and powdered material can be preserved for a considerable length of time in the absence of a preservative without any deterioration. A description of the extracts obtained by means of various solvents used in succession is given in the experimental part. Since the most considerable portions were extracted by means of petroleum ether and alcohol and the rest did not extract significant amounts, several kilograms of the root were extracted with these two solvents alone and the products studied in detail.

The petroleum ether extract constituted about 3% of the air-dry root and contained all the oily and waxy matter besides certain crystalline compounds. It was at first separated into two main fractions by means of alcohol, (1) the alcohol soluble and (2) the alcohol insoluble portion. The alcohol insoluble fraction was waxy in appearance. After saponification of the wax and careful fractionation of the products, the following substances were obtained: (1) a ketonic substance melting at 83°, (2) alcoholic

substances belonging to the resinol* group melting between 151–165°, (3) phytosterol mixture melting at about 110° the acetates of which melt between 130–60° and (4) solid and liquid fatty acids. It is possible that the resinols and the phytosterols exist in the wax as esters. From the alcohol soluble portion three definite fractions were isolated. The least soluble fraction (A) consisted mostly of the alcohols of the resinol type contaminated with a little wax. It melted at about 175–85° and simple crystallisation was not enough to purify it. It was therefore subjected to a preliminary saponification before fractional crystallisation. Resinols more or less similar to those contained in the waxy portion were found to be present here also. From a rough estimate the total quantity of resinols was about 17.0 g., whereas the amount of phytosterols existing mostly as esters was about 3.0 g. in 4 kg. of the roots. From the middle fraction (B) melting at about 205–10° could be obtained after repeated crystallisations from ethyl acetate, besides some fractions melting at 210–22°, a definite compound melting at 235–6° having the formula $C_{32}H_{52}O_2$. The most soluble fraction (C) consisted of an essential oil more than 80% of which was found to be para methoxysalicylaldehyde.

The ordinary reagents that were employed for characterising the sterols such as the Liebermann, the Hesse and the Liebermann-Burchard reagents easily distinguish between the sterols proper and the resinols obtained from the petrol extract. The details are given in the experimental section.

From the alcoholic extract of the root the following substances could be isolated: (1) *Saponins*.—The alcohol extract was concentrated and treated with excess of ether thereby precipitating saponins. (2) *Tannins*.—The alcohol-ether solution was distilled in order to remove the solvents and then diluted with a large volume of water when tannins were precipitated. (3) *Resin acids*.—By extracting the aqueous solution with ether and carefully examining the ether extract a crystalline resin acid melting at 245° and having the composition $C_{22}H_{28}O_{10}$ along with small quantities of an amorphous acid melting at about 180° were obtained. (4) *Inositol*.—The final aqueous solution yielded a precipitate with basic lead acetate from which after removing lead with hydrogen sulphide inactive inositol could be isolated.

* The term resinol has been used here to indicate tentatively those substances which are alcoholic in nature and which do not give the correct reaction of sterols. Their exact constitutions are being investigated. But since they exhibit similarity with substances of the type of amyirin the use of the term resinol seems to be appropriate.

Experimental

Large quantities of the fresh and fully grown roots of *Decalepis Hamiltonii* were obtained from the Coimbatore district in the month of December. They were cut into thin slices, dried in the sun and stored in well stoppered containers. The material kept very well for over two years without the help of any preservative. When required for use the material was ground to coarse powder and employed.

With a view to determine the moisture content of the fresh root, a representative sample of the roots was shredded by means of a cane shredder and using this material the loss in weight when dried at 100–05° for 3 hours was determined. The moisture was found to be 82% of the fresh root. The woody parts of the roots were separated from the fleshy portion by scraping with a knife and separately weighed. They constituted only 8% of the fresh root.

100 g. of the air-dried powdered root were extracted successively with different solvents. The quantity of each extract and the properties are given below:

1. Petroleum ether	3.4%
2. Ether	0.8%
3. Chloroform	0.8%
4. Ethyl alcohol	3.7%
5. Water	2.3%

1. The petroleum ether extract was a brown, sweet smelling waxy mass. It consisted of fatty and waxy matter, some crystalline compounds and the sweet smelling aldehyde, para methoxysalicylaldehyde. It gave a pink colour with alcoholic ferric chloride.

2. The ether extract was an orange-yellow semisolid mass with a weak bitter taste. It contained some resin acids and gave the same colour reaction with ferric chloride solution as the petrol extract.

3. The chloroform extract was brown in colour and tasted bitter. The alcoholic solution of the extract gave a greenish blue colour with ferric chloride.

4. The alcohol extract was a dark brown semisolid mass. It tasted bitter mixed with a little sweetness. It gave a blue black precipitate with alcoholic ferric chloride. It reduced Fehling's solution thereby indicating the presence of reducing substances. On the addition of water a good amount of an amorphous brown solid was thrown down which gave positive colour reactions for tannins.

5. The water extract did not possess any marked taste. It gave a pale green colour with ferric chloride solution. When an equal volume of alcohol was added to it a copious precipitate of gums and pectins was thrown down. The extract was easily attacked by fungus.

The above extracts were individually tested for the presence of alkaloids with the usual reagents but no alkaloid could be detected.

Extraction of the Roots with Light Petroleum.—

For a complete examination of the root, 4 kg. of the powder were extracted in a big Soxhlet extractor in batches of 1 kg. with petroleum ether (B.P. 60–80°) until further extraction gave no solid matter (about 20 hours per batch). Most of the petrol was recovered from the extract by distillation and the last traces of the solvent were removed by bubbling nitrogen gas. The yellowish brown solid (120 g.) was then boiled with alcohol (200 c.c.) and the mixture allowed to cool down to about 60° when most of the waxy matter solidified. The clear solution was then decanted. More alcohol was added to the solid residue and the process repeated till about a litre of alcohol was employed for the extraction. All the alcohol extracts were collected together. The insoluble waxy material was named (W) and its analysis is described separately. When the alcoholic solution was concentrated to nearly half its bulk and allowed to stand for a day at the room temperature a colourless high melting solid separated out. This weighed about 15 g. and was termed (A). The mother liquor was subsequently slowly concentrated in stages and cooled in the ice chest and the colourless solid material separating out was collected till no more was obtained. The aggregate of this product was designated (B). The final mother liquor left behind was called (C).

Examination of the Fractions of the Petrol Extract.—

*Wax (W) (60 g.).—*This was a sticky mass melting between 60–70°. It was dissolved in about 50 c.c. of benzene and heated under reflux for about 12 hours with 200 c.c. of N/2 alcoholic potash. The solvents were distilled off as completely as possible, the residue mixed with pumice stone and dried on a steam-bath. The dry material was extracted completely with petroleum ether and acetone in succession. But it was noticed that the petroleum ether had removed all extractable matter and nothing was left for acetone to extract. The petrol extract was washed with water and dried over anhydrous sodium sulphate. This procedure had to be adopted for separating the unsaponifiable matter since the ordinary method using the aqueous suspension gave rise to very difficult emulsions when extracted with petroleum ether.

The petroleum ether extract of the unsaponifiable matter was distilled in order to remove the solvent and the residue was dissolved in about 500 c.c. of hot alcohol. About 3 g. of an amorphous solid (K) came down on cooling; it was filtered and the filtrate was examined separately. The solid (K) was sparingly soluble in ether and alcohol and readily soluble in ethyl acetate. It could be crystallised from alcohol when a large volume of the solvent was employed for the purpose. But final crystallisations (thrice) from ethyl acetate were necessary to render it quite pure. It was thus obtained in colourless hexagonal plates melting at 83–84°. (Found: C, 80.6; H, 13.6%). When the compound was dissolved in chloroform and concentrated sulphuric acid added, it did not exhibit any colour or fluorescence and gave no colour with the Liebermann-Burchard reagent. When boiled with acetic anhydride and sodium acetate on an oil-bath for 5 hours it remained unchanged. When, however, it was boiled for about 3 hours in alcoholic solution with hydroxylamine hydrochloride and alkali it gave rise to an oxime which could be easily separated from the unchanged ketone by means of the greater solubility of the oxime in alcohol. It was obtained in the form of colourless needles melting at 68°. The nature of this ketonic substance is under investigation.

The filtrate left after the separation of the ketone (K) was concentrated to about half its bulk and treated with 50 c.c. of acetone. This mixture was concentrated and the crystallising solid was obtained in three different fractions: fraction (1) melting between 160–65° (6.5 g.), fraction (2) melting between 155–57° (1.4 g.) and fraction (3) melting between 151–53° (2 g.). All these agreed in properties very closely with similar fractions obtained from component (A). Hence they are described in detail in that connection.

Isolation of Sterols.—The residual mother liquor left after the separation of the above fractions was evaporated to dryness when an orange yellow sticky mass was produced. It was dissolved in a mixture of petroleum ether and alcohol and the solution decolourised using a little animal charcoal. The very pale yellow solution thus obtained was evaporated to dryness and the residue taken up in chloroform. This solution was treated with sufficient amount of alcohol to produce turbidity and allowed to stand in an ice-chest for several days when shining hexagonal plates separated out (1 g.) melting at 105–10°. When a chloroform solution of this substance was boiled with acetic anhydride and a drop of concentrated sulphuric acid added there was a play of colours, pink-blue-green. A chloroform solution of the compound produced blood-red colour with concentrated sulphuric acid with a greenish blue fluorescence. When the mother liquor was evaporated an amorphous

mass giving sterol reactions was obtained. Since ordinary methods of crystallisation failed to yield a crystalline product, the dry mass (2 g.) was acetylated by boiling with acetic anhydride (25 c.c.) and sodium acetate (5 g.) for three hours. The product was treated with 200 c.c. of water and allowed to stand in the cold for a day. The solid that separated out was dissolved in about 100 c.c. of alcohol and cooled in ice. An amorphous semisolid mass soon separated out. The clear alcoholic solution was then decanted and kept in the ice-chest for about 16 hours. A colourless crystalline solid consisting of the acetates was thus obtained melting at 130–60°. It gave the usual sterol reactions. From the melting point of the acetate mixture it may be inferred that the sterol fraction consists of a mixture of stigma and brassica sterols. This point is still under investigation.

Fatty Acids (12 g.).—The soap which had been extracted with petroleum ether and acetone was treated with water and decomposed by means of dilute sulphuric acid. The liberated fatty acids were extracted with ether and the ether distilled off from the extract. The yield of the mixed fatty acids thereby obtained (after drying) was 12 g. When separated by means of Twitchell's lead salt method into alcohol soluble and alcohol insoluble salts and on their subsequent decomposition, it was found to consist of 75% of solid and 25% of liquid fatty acids. The solid acids melted at 80–82° and seemed to consist mainly of higher fatty acids (saponification equivalent 360). The composition of this mixture and that of the liquid fraction is under investigation.

Composition of Fraction A (12 g.).—This colourless solid which melted at about 175–85° appeared to be crystalline but it had no definite shape under the microscope. It dissolved readily in ether, acetone, ethyl acetate and chloroform even in the cold but was sparingly soluble in alcohol. A chloroform solution of the substance gave a yellow colour with concentrated sulphuric acid and developed a greenish-blue fluorescence on standing. When a similar chloroform solution was boiled with acetic anhydride and a drop of concentrated sulphuric acid added (Liebermann-Burchard reaction) a deep pink colour was produced and it lasted for several hours. These reactions are somewhat different from those given by sterols. The crude solid was optically active, $[\alpha]_D^{25}$ being +61.9°. Since the solid could not be completely purified by simple recrystallisations from the ordinary solvents it was subjected to treatment with N/2 alcoholic potash just as in the case of the wax (W). On cooling the mixture a crystalline mass separated out even in the presence of the alkali. However the whole mixture was evaporated, mixed with pumice stone, dried

and extracted with petroleum ether and acetone. The petrol extracted all the unsaponifiable matter. The extract was washed with water, dried over sodium sulphate and distilled in order to recover the solvent. The residue was dissolved in about 350 c.c. of hot alcohol and allowed to stand for two days at the end of which a colourless crystalline solid (long narrow rods and needles) melting at 175–85° was found to have separated out. It was filtered off, the mother liquor was concentrated and the solid fractions crystallising out were collected in stages. The following 6 fractions were thus obtained and no further crystalline substance was left in the final mother liquor.

Yields and analysis of the fractions

M.P.	Yield	Analysis
1. 175–185°	3.0 g.	C 84.6; H 11.3
2. 160–165°	1.5 „	C 82.7; H 11.6
3. 155–157°	0.8 „	C 83.8; H 11.5
4. 151–153°	0.5 „	C 81.9; H 12.3
5. 135–145°	0.5 „	
6. 130–138°	0.5 „	

The individual fractions exhibited the same solubility in different solvents and the same colour reactions as shown by the crude mixture. The three fractions obtained previously from the wax (W) were found to be similar in composition with the corresponding fractions (2), (3) and (4) above. They gave the same colour reactions (*i.e.*,) a yellow colour with the Hesse's reagent and a pink colour with the Liebermann-Burchard reagent. It was also noticed that there was no depression in melting points when the fractions 1, 2 and 3 from the wax were mixed with the fractions 2, 3 and 4 respectively described above.

Fraction (1) described above was acetylated, as a typical member of this group, by boiling with acetic anhydride and sodium acetate for 3½ hours on an oil-bath. After treatment with a large excess of water the product was crystallised from alcohol-acetone mixture. Two crops of colourless crystals were obtained one melting at 212–18° and the other at 194–98°. With a view to be sure that they were different from the original substance, and that there had been acetylation the acetate fraction melting at 212–18° was analysed. (Found: C, 79.9; H, 10.5%.) It was therefore concluded that there was acetylation and the various fractions consisted of hydroxy compounds of the resinol type.

After the separation of the unsaponifiable matter the remaining soap was dissolved in water and acidified. Only a very small quantity of fatty acids was obtained and hence the fraction (A) consisted mostly of the unsaponifiable matter.

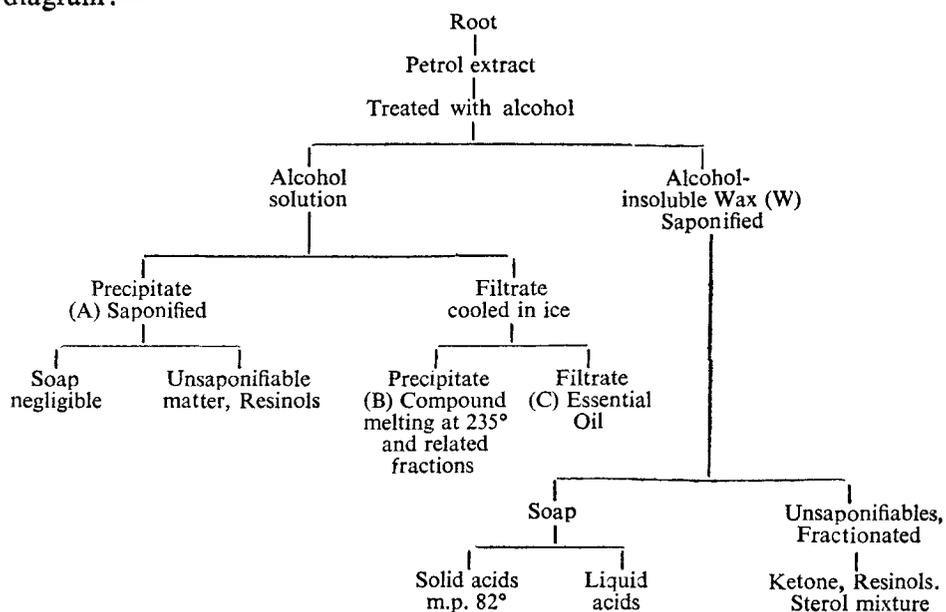
It is interesting to note that the same resinol fractions are obtained from the wax (W) and the fraction (A). It seems probable that they exist both free and in the combined form as esters. The properties of the waxy portion may be due to the existence predominantly of the esters. The high melting point and other characteristics of fraction (A) may be accounted for by their existence in the free form.

Fraction (B) (2 g.).—As already mentioned this fraction was easily soluble in ether and hot ethyl acetate and after repeated recrystallisation (6 times) from the latter solvent a small quantity (0.5 g.) of a colourless crystalline (rectangular rods) compound melting at 235–36° was obtained. There was no rise in melting point after further crystallisation. Besides this, fractions melting between 210–22° were obtained. They seemed to contain the same compound mixed with various quantities of impurities because they had the same properties and reactions. The pure substance and also the lower melting fractions gave a pale yellow colour when treated with chloroform and concentrated sulphuric acid and a greenish blue fluorescence slowly developed on standing. A deep pink colour was produced with the Liebermann-Burchard reagent. (Found: C, 82.0; H, 10.7; $C_{32}H_{52}O_2$ requires C, 82.1; H, 11.1%.) The compound was neutral and did not exhibit the properties of a carboxylic acid or a phenol. It did not undergo acetylation when boiled with acetic anhydride and sodium acetate and consequently it did not contain an alcoholic hydroxyl group. It seems to belong to the group of colourless compounds found in the wax (W) and in the fraction (A) since the same colour reactions are given by both but difference seems to exist with respect to the function of the oxygen atoms.

Fraction (C).—The final alcoholic mother liquor was distilled in order to recover all the alcohol, the brown oily residue was dissolved in ether and the ether solution dried over anhydrous sodium sulphate. After filtering it was distilled to recover the solvent. About 40 g. of a viscous oily substance was left behind. Though this solidified to a crystalline mass on cooling in a refrigerator it rapidly melted at the room temperature. Hence it was extracted with excess of petroleum ether when a small quantity of a dark viscous liquid was left behind. The petrol solution was decanted and on being concentrated and cooled yielded colourless crystals of paramethoxy-salicylaldehyde. It melted at 42° and no depression in melting point was

noticed when mixed with a synthetic specimen of the aldehyde. It had the characteristic odour and gave pink colour with ferric chloride. The yield of the pure aldehyde was 32 g. amounting to 0.8% of the air-dried root. The coloured viscous material left behind still possessed the smell of the aldehyde but no more of it could be isolated from it by extraction. However when it was dissolved in ether and treated with bromine a small quantity of a bromoderivative melting at 120–21° was obtained. This was identified as the 5-bromoderivative of the aldehyde and hence it was concluded that the resin still contained some amount of the above aldehyde.

The working up of the petrol extract is summed up in the following diagram:—



Extraction with Alcohol.—The root powder which had been exhausted with light petroleum was dried in a current of air and subsequently extracted for about 30 hours with methylated spirits (distilled over lime) in the same apparatus. The alcohol soluble matter was separated into a number of fractions and each fraction examined in detail. The brown extract was concentrated to one-fourth its bulk (500 c.c.) by distillation under reduced pressure. A small quantity of a brown solid (D) separated out and it was removed by filtration. The filtrate was then treated with excess of ether when a dark slimy substance (E) was thrown out. The alcohol ether mixture was again distilled to remove the solvents as far as possible and the residue was treated with a large excess of boiling water. Thereby a considerable

amount of a brown solid (F) was obtained. This was mixed with (D) for purposes of further examination. The aqueous solution was extracted repeatedly with ether and amyl alcohol in succession. The ether extract (G) and the amyl alcohol extract (H) were also examined separately. The final aqueous extract was subsequently treated with neutral and basic lead acetate solutions and these precipitates also were examined.

Fractions (D) and (F):—The solid weighed about 0.7% on the weight of the dry root. It had a mixture of bitter and acid taste. It was packed in a Soxhlet extractor and subjected to successive extraction with petroleum ether, ether, chloroform and alcohol. Petroleum ether and chloroform extracts were negligible. From the ether solution a very small quantity of a crystalline acid melting at 245° could be obtained by adopting the process described in connection with the fraction (G). The alcohol extract gave a green solution with ferric chloride indicating the presence of tannins. It was noted that considerable quantities of the solid now remained unextracted even by alcohol.

Fraction (E).—This was dark brown in colour and very bitter to the taste and swelled considerably on drying. When shaken up with a little water it exhibited plenty of frothing. It was purified by dissolving in dry alcohol (50 c.c.) and precipitating by the addition of a large excess of ether. The process was repeated four times and finally the solid was treated with water. A small quantity of water-insoluble residue gave tests for tannins and the aqueous solution when concentrated on a water-bath yielded a semi-solid mass which could not be crystallised. It gave tests for saponins. The ether alcohol solutions obtained in the course of the purification of the saponin left a small quantity of solid matter on evaporation and this yielded a crystalline acid melting at 245° under the conditions described in connection with the fraction (G).

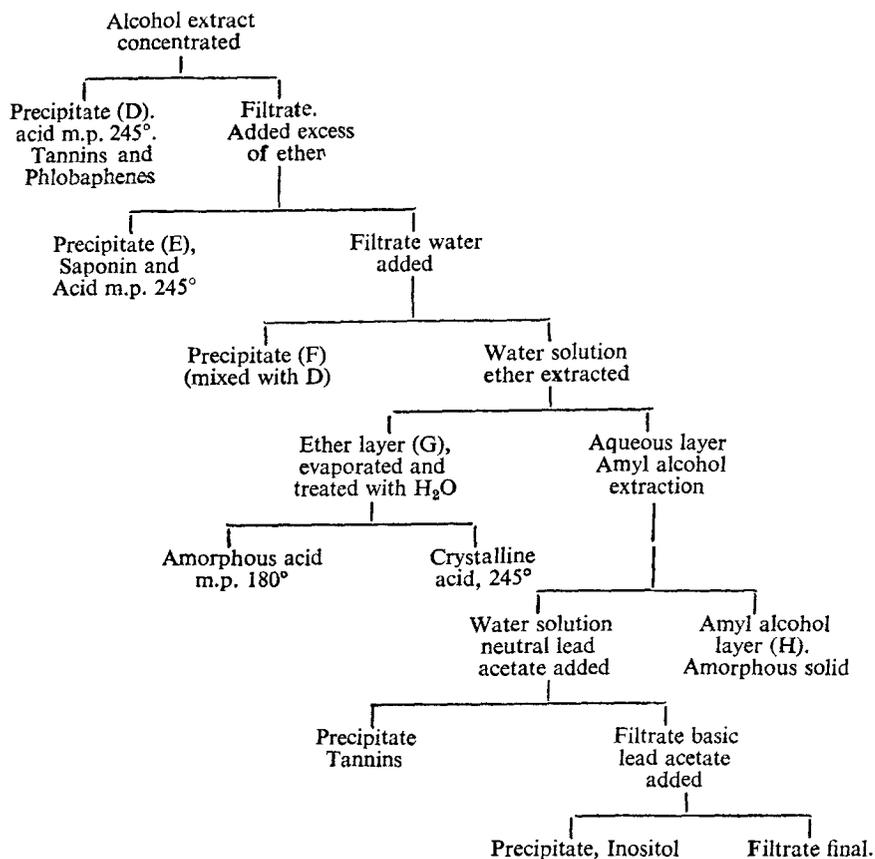
Fraction (G), Ether Extract.—On distilling off the solvent the residue was noticed to have a sweet smell. It gave a greenish blue colour with ferric chloride solution. When treated with ice-cold water it formed a colloidal suspension along with a considerable amount of insoluble brown mass and these were separated. The water suspension was acidified with a few drops of hydrochloric acid and extracted with ether. The solvent was recovered from the ether solution and the residue was treated with a small quantity of water and allowed to stand in the ice-chest for about 12 hours. A yellow crystalline solid (0.5 g.) (plates and rods) melting at 245° was thus obtained. It was filtered and recrystallised from alcohol. The substance was readily soluble in ether and alcohol and aqueous alkali

and was not affected by boiling with 7% hydrochloric acid. (Found: C, 58.8; H, 5.8; and $C_{22}H_{28}O_{10}$ requires C, 58.3; H, 6.2%.)

The insoluble brown solid was dried on a porous plate, dissolved in ether and the ether solution was extracted with aqueous sodium bicarbonate. The alkaline solution was acidified and ether extracted. By repeated transference between bicarbonate and ether and finally evaporating the ether solution an amorphous solid acid melting at about 180° was obtained. It did not give any colour with alcoholic ferric chloride and could not be obtained crystalline. Further extraction of the original ether solution with aqueous sodium carbonate yielded highly coloured amorphous substances from which nothing definite could be obtained.

Fraction (H).—The amyl alcohol extract was mainly examined for the existence of glycosides. It was concentrated under reduced pressure and the brown residue examined with various solvents but no crystalline solid could be obtained.

Lead Salts.—The precipitated neutral lead salt was decomposed by means of hydrogen sulphide in aqueous suspension. The aqueous solution on concentration under reduced pressure gave a dark brown mass which gave tests for tannins. The basic lead precipitate however under similar treatment yielded an amorphous solid which dissolved partly in hot absolute alcohol. The insoluble residue was dissolved in a little quantity of water and treated with sufficient alcohol to produce turbidity and allowed to stand, occasionally adding a little alcohol in order to promote crystallisation. After two months a colourless crystalline solid was obtained. It was filtered and washed with alcohol. The impure product was finally recrystallised by dissolving it in a small quantity of water and adding excess of alcohol when clusters of glistening crystals were obtained melting at 221° . It was sweet to the taste and was optically inactive. Its identity with inactive inositol was established with the mixed melting point determination with an authentic sample. The alcohol soluble part of the basic lead salt further contained a little amorphous matter which exhibited the properties of tannins.



Summary

The general chemical composition of the roots of *Decalepis Hamiltonii* has been studied. The petroleum ether extract of the *Decalepis* roots was found to contain (1) a ketonic substance melting at 83–84°, resinol fractions melting between 130 and 185°, a definite compound of the same group melting at 235–36°, sterols and para methoxysalicylaldehyde. The subsequent alcohol extract contained besides saponins and tannins a crystalline resin acid melting at 245°, an amorphous resin acid melting at 180° and inactive inositol.

REFERENCE

Rao and Iyengar . . . *Perf. Essent. Oil Rec.*, 1923, 14, 300–01.