

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

Part IV. Resinols of *Decalepis Hamiltonii* and *Hemidesmus indicus*

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Received July 17, 1941

IN Part I¹ it was shown that the petroleum ether extract of the *Decalepis* roots contained a ketonic substance melting at 83°, resinol fractions melting between 130° and 185°, a definite compound of the same group melting at 235–36°, sterols and *para*-methoxysalicylaldehyde. The term resinol was used to indicate tentatively substances which were alcoholic in nature and which did not give the correct reactions of sterols and its use was justified by their similarity with substances of the type of amyryl. Their chemical composition approximated closely to the formula $C_{30}H_{50}O$. They gave a pink colour with Liebermann-Burchard reagent and a yellow solution with green fluorescence in Salkowski's reaction. Their exact nature has now been investigated.

As already described¹ the alcohol-soluble portion of the petroleum ether extract was separated into three fractions and from the middle fraction (B) melting at about 205–10° could be obtained after repeated crystallisations using ethyl acetate, besides fractions melting at 210–22°, a definite compound (M) melting at 235–36°. Further crystallisation of this compound from the same solvent raised the melting point to 239–40°. It has the formula $C_{32}H_{52}O_2$ and it is found to be an ester yielding on saponification acetic acid and a tri-terpene alcohol which has been identified as β -amyryl from a detailed study of its properties and the preparation of its derivatives. It therefore follows that the original substance (M) is β -amyryl acetate. Fractions melting between 210–22° could not be further purified but they had the same composition as (M). They were obviously mixtures of β -amyryl acetate with compounds of the same type which are isomeric with it.

The resinols were found to be present to a minor extent in the waxy portion and to a much higher degree in fraction (A) of the alcohol-soluble portion. The preliminary saponification and careful separation into various fractions of the unsaponifiable matter rendered identification of various components easy. By this means the sterols were effectively removed from

the resinols. The separation and identification of the resinol mixtures have been carried out by the acetate as well as by the benzoate methods. Fraction I melting at 175–85° has been found to consist mostly of α -amyrin admixed with small amounts of β -amyrin. The second fraction melting at 160–65° contained lupeol as the main component and it was best isolated as the benzoate. The amyrins were present only in small quantities.

Fractions III, IV, V and VI melting at 155–57° and below were individually examined. Fraction III melting at 155–57° contained β -amyrin and lupeol and very little of α -amyrin. The other lower fractions (IV, V and VI) were very similar to it having the three above compounds in varying proportions. Further in all of them were present small quantities of substances whose acetates were markedly more soluble in alcohol than those of the above resinols and which differed from them in giving a display of colours (pink-blue-green) with the Liebermann-Burchard reagent though they gave the same yellow colour with green fluorescence with the Salkowski's reagent. Thus they exhibited characteristics, partly of sterols and partly of resinols. They are being further investigated.

Hemidesmus indicus.—It was pointed out in Part III² that the composition of the petroleum ether extract of this root agrees closely with that of the *Decalepis* and that what Dutta, Ghosh and Chopra considered to be new sterols were really mixtures of resinols with small quantities of sterols. This is now definitely supported by careful examination of the various resinol fractions. The presence of lupeol and of α - and β -amyrins has been established. The compound melting at 235–36° has been identified as β -amyrin-acetate. During the careful examination of the various fractions, the special type of compounds mentioned in the case of the *Decalepis* as giving display of colours with the Liebermann-Burchard reagent and only yellow colour with Salkowski's reagent was also met with here. Besides these a small quantity of a substance which is characterised by the yellow coloured solution having a brilliant green fluorescence which it gives with the Liebermann-Burchard reagent is obtained. With the Salkowski's reagent it gives only an yellow colour. This probably belongs to the tetracyclic triterpene type and this surmise gains support from the fact that on boiling with formic acid and benzene it undergoes transformation into a product of the pentacyclic type whose formation was indicated by the typical resinol colour reactions. The yield however was very small and a detailed study could not be carried out so far.

The results so far obtained may be summed up as follows : Though the two roots contained the same resinols the *Decalepis* has in it more of

amyryns than lupeol whereas in the case of the *Hemidesmus* roots the position is just the reverse.

Experimental

Decalepis Hamiltonii: Identification of β -amyryn acetate.—The crystalline compound (M) melting at 235–36° (0.5 g.) was heated under reflux with 6% alcoholic potash (25 c.c.) for 5 hours and the contents were largely diluted with water. The crystalline precipitate that was formed was filtered and recrystallised from alcohol when it was obtained as needles melting at 196–97°. [Found: C, 83.8; H, 11.2; $C_{30}H_{50}O$ requires C, 84.4; H, 11.7%.] This was identified as β -amyryn by comparison with a sample described later on; the mixed melting point was undepressed. The filtrate was concentrated to a small bulk, acidified with dilute sulphuric acid and steam-distilled. In the distillate the presence of acetic acid was established by performing the lanthanum nitrate test carried out according to the procedure of Neelakantam and Row.³ Hence the original compound (M) was identified as β -amyryn acetate. The melting point rose to 239–40° after further crystallisation and it was unaffected by admixture with a sample of β -amyryn acetate whose detailed study is given later on in this paper. The results of combustion analysis have been given in Part I and they agree with the formula $C_{32}H_{52}O_2$.

As already described in Part I the resinols from (A) were fractionated into six fractions and the corresponding fractions from (W) were added to them; fraction I had m.p. 175–85°; II, 160–65°; III, 155–57°; IV, 151–53°; V, 135–45° and fraction VI, 130–35°.

Resinol fraction I (m.p. 175–85°): Separation by the acetate method.—The solid (2 g.) was boiled with acetic anhydride (10 c.c.) and sodium acetate (4 g.) for 2½ hours and the mixture poured into water. After allowing it to stand for 12 hours the solid product was separated and crystallised from alcohol whereby a crop of crystals melting at 215° were obtained. Further repeated crystallisation from ethyl acetate raised the melting point to 222–23° and at this stage the solid appeared as colourless shining elongated plates. [Found: C, 81.6; H, 10.9; $C_{32}H_{52}O_2$ requires C, 82.1; H, 11.1%.] $[\alpha]_D^{25}$, +81.5° in chloroform and that already recorded for α -amyryn acetate is +83.0°.

The acetate was hydrolysed by heating with 5% alcoholic potash for 3 hours. After dilution with excess of water the resulting solid was crystallised from alcohol when glistening colourless needles melting at 185–87° were obtained. [Found: C, 84.0; H, 11.6; $C_{30}H_{50}O$ requires C, 84.4; H, 11.7%.] $[\alpha]_D^{25}$ in benzene, +92.1° and the value noted by previous workers for α -amyryn +93.3°. Thus the presence of α -amyryn in this fraction was established. This was further confirmed by the preparation of the benzoate

which melted at 192° (described later). The mother-liquor and washings from the crystallisation of the above-mentioned acetate were mixed and the solvent removed. The solid residue thus obtained melted at $160-70^{\circ}$. It was first boiled with a small amount of methyl alcohol and filtered and subsequently recrystallised from ethyl acetate. It now melted at $215-18^{\circ}$ and it could not be further purified by the ordinary methods. [Found: C, 81.9; H, 11.9; $C_{32}H_{52}O$ requires C, 82.1; H, 11.1%.] On hydrolysis and purification of the resulting alcohol could be obtained a small quantity of β -amyrin melting at $196-97^{\circ}$ and a larger quantity of α -amyrin melting at $185-87^{\circ}$.

Separation by benzoate method.—The crystalline solid (2 g.) was dissolved in dry benzene (20 c.c.) and treated in the cold with benzoyl chloride (10 c.c.) and pyridine (7 c.c.). The mixture was allowed to stand overnight and finally heated under reflux for 2 hours. The contents were then diluted with water and extracted with excess of benzene. The benzene solution was evaporated to dryness, the residue taken up in ether and shaken successively with aqueous sodium hydroxide, aqueous sulphuric acid and water. On concentrating the ether solution and cooling in ice colourless needles of a solid melting at about 190° were obtained. When further recrystallised from ligroin and benzene-alcohol mixture, the substance finally melted at $192-93^{\circ}$. [Found: C, 83.6; H, 10.2; and $C_{37}H_{54}O_2$ requires C, 83.8; H, 10.2%.] $[\alpha]_D^{28}$ in benzene, $+90.0^{\circ}$ and that recorded by previous workers for α -amyrin benzoate in benzene solution is $+87.3^{\circ}$. Further crops of the same substance could be obtained on concentrating the ether solution and recrystallising the solid from benzene-alcohol mixture. The final fractions, however, melted at $165-75^{\circ}$ and no pure compound could be obtained from them. The benzoate was hydrolysed by heating with 5% alcoholic potash for 5 hours. By diluting the contents with a large excess of water and crystallising the resulting solid from alcohol, shining colourless needles melting at $185-87^{\circ}$ were obtained and these were found to be identical with the sample of α -amyrin obtained from the acetate method.

From the results of analysis of the free alcohol, its acetate and benzoate and from their properties particularly specific rotations the presence of α -amyrin in fraction (I) as the predominant component is established. β -Amyrin is present only in minor quantities.

Fraction II (m.p. $160-65^{\circ}$).—This solid (5.0 g.) was dissolved in benzene (25 c.c.) and benzoyl chloride (15 c.c.) and pyridine (10 c.c.) added. The benzylation was effected just as before. The crude benzoate mixture melted at 180° and when this was boiled with a small amount of methyl alcohol and filtered, the melting point rose to $210-20^{\circ}$. Subsequent crystallisation

from ether yielded a compound melting at 249–54°. Final recrystallisation (3 times) from ligroin yielded colourless gritty broad rectangular plates melting at 261–62°. No change was effected by further crystallisation. [Found: C, 83.4; H, 10.5; $C_{37}H_{54}O_2$ requires C, 83.8; H, 10.2%.] $[\alpha]_D^{28}$, + 62.5° in chloroform solution and $[\alpha]_D$ noted in the literature for lupeol benzoate, + 60.9°.

The benzoate was hydrolysed by heating with alcoholic potash for 5 hours according to the procedure already described. The product was subjected to slow crystallisation from a mixture of alcohol and acetone. By this means long needles, melting at 211–12°, were obtained. Further crystallisation did not bring about any change. [Found: C, 84.0; H, 11.1; $C_{30}H_{50}O$ requires C, 84.4; H, 11.7%.] $[\alpha]_D^{28}$ in chloroform, + 41.0° and that recorded for lupeol, + 41.2°. From all the above results it is concluded that lupeol is present in this fraction.

The mother-liquor and washings from lupeol benzoate were mixed and concentrated. First crops of crystals melting at 240–50° were obtained which eventually yielded on purification lupeol benzoate. Subsequent crops yielded on recrystallisation (4 times) from benzene-alcohol mixture a small quantity (0.2 g.) of a solid melting at 231–32°. [Found: C, 84.2; H, 10.4; and $C_{37}H_{54}O_2$ requires C, 83.8; H, 10.2%.] This benzoate on hydrolysis with alcoholic potash yielded a compound melting at 197–98° which was found to be identical with β -amyrin obtained from the acetate melting at 239°. The mixed melting point was not lowered. Hence the presence of a small quantity of β -amyrin in this fraction was established.

The final mother from the above benzoates was evaporated to dryness and the residue dissolved in benzene-alcohol mixture and allowed to crystallise slowly. After removing some indefinitely melting fractions α -amyrin benzoate melting at 192–93°, was obtained in well-defined needles and was found to be identical with the sample of the benzoate obtained from the first fraction. The final residues yielded a solid melting at 140–70°.

Hence fraction (II) contains lupeol as the main component mixed with α - and β -amyrins, the last being in the smallest quantity.

Fractions III, IV, V and VI.—(Resinols melting between 130–57°.) All these fractions gave very similar results. They were first acetylated and the acetates fractionally crystallised from alcohol. A very small quantity of sparingly soluble fraction (1) melting at 235° was obtained. After further crystallisation from ethyl acetate it was found to be β -amyrin acetate. Subsequently several fractions (2) were collected from the alcohol solution. One of these melted fairly sharp at 165–66° and on analysis yielded the following

results. [Found: C, 82.3; H, 10.6; and $C_{32}H_{52}O_2$ requires C, 82.1; H, 11.1%.]

All these gave the pink colour with Liebermann-Burchard reagent and yellow colour with green fluorescence with Salkowski's reagent. The last and the most soluble fraction melted indefinitely at 120° and exhibited certain peculiar colour reactions. With the Liebermann-Burchard reagent it gave a play of colours pink-blue-green and with the Salkowski's reagent the sulphuric acid layer assumed orange yellow with green fluorescence on standing. These characteristics differ from those of well-known sterols and resinols and hence this fraction is being further examined.

The middle fraction of the acetates (2) given above could not be further satisfactorily separated into definite compounds. It was therefore hydrolysed and the free alcohols benzoylated. From this product, benzoates of lupeol, β -amyrin and α -amyrin could be obtained. Lupeol seemed to be the most important component in these fractions also.

Hemidesmus indicus.—Fractions I (m.p. $175-85^\circ$), II (m.p. $165-70^\circ$), III (m.p. $155-65^\circ$) and IV (m.p. $150-54^\circ$) gave very similar results, hence they are reported together. First they were acetylated and the acetates fractionally crystallised from alcohol. The more sparingly soluble portion (A) melting at $190-200^\circ$ was repeatedly recrystallised from ethyl acetate when a very small quantity of β -amyrin acetate melting at 236° separated first. Subsequently the bulk of the acetate portion separated out melting at about 200° . After repeated recrystallisation (6 times) from ethyl acetate pure lupeol acetate (shining flat needles) melting at 214° was obtained from it. [Found: C, 81.8; H, 10.4; $C_{32}H_{52}O_2$ requires C, 82.1; H, 11.1%.] $[\alpha]_D^{25}$, $+45.4^\circ$ in chloroform solution and $[\alpha]_D$ for lupeol acetate is given in the literature as $+47.1^\circ$ in chloroform. The melting point was not depressed when mixed with lupeol acetate from *D. Hamiltonii*. When hydrolysed with alcoholic potash the acetate yielded a product which could be easily crystallised from alcohol. It was obtained as silky needles melting at 212° (Lupeol). $[\alpha]_D$ in chloroform solution is $+41.1^\circ$ and that recorded for lupeol is $+41.2^\circ$. Further no depression in the melting point was observed when mixed with the lupeol obtained from the *Decalepis* roots.

The alcohol mother-liquor from the crystallisation of acetates gave indefinite mixtures melting at $165-95^\circ$ and they were not further examined.

Fractions I and II were also benzoylated and the mixture of benzoates examined as in the case of *Decalepis Hamiltonii*. Pure lupeol benzoate was easily obtained from ligroin in a good yield from the first crop of crystals and the mother-liquor yielded indefinite mixtures melting at $180-220^\circ$. It was

therefore concluded that lupeol was the main component of these fractions and the amyryns probably occurred along with it. The presence of β -amyryn was definitely established.

Fraction V (m.p. 135–40°).—This fraction was acetylated and the acetates were subjected to careful fractionation from an alcohol solution. It was noticed that the first fractions (m.p. 185–200°) gave the usual tests for the pentacyclic resinols, *i.e.*, a pink colour with the Liebermann-Burchard reagent and a yellow solution with the Salkowski's reagent. From the middle portions could be obtained a small quantity of a colourless solid melting at 135–45°. This behaved differently. With the Liebermann-Burchard reagent it gave a yellow solution with brilliant green fluorescence and the solution slowly assumed faint pink colour on standing. No test was given with Salkowski's reagent, only a very pale yellow solution being produced. This has been noted as the characteristic colour reaction of tetracyclic triterpenes and hence a sample of the substance was heated under reflux with a mixture of formic acid and benzene for 2 hours in order to bring about the formation of the pentacyclic triterpenes. After crystallisation from ethyl acetate the product melted at about 195° and it produced the usual pink colour with the Liebermann-Burchard reagent. Hence the existence of the tetracyclic compounds in the fraction V was indicated.

The final fractions of the acetates gave with the Liebermann-Burchard reagent a play of colours pink-blue-green but produced no red colour with Salkowski's reagent. These seem to be the same as the substances obtained in a similar manner from *Decalepis Hamiltonii*. The quantity was however too small to permit detailed study.

Summary

The existence of resinol in the petrol-soluble portions of *Decalepis Hamiltonii* and *Hemidesmus indicus* has been established by the isolation of α - and β -amyryns and lupeol from them. They exist both free and as esters. The various fractions have been analysed using both the acetate and the benzoate methods. The roots of the *Decalepis* contain more of the amyryns and less of lupeol whereas the *Hemidesmus* contains more of lupeol. The more soluble fractions of both roots seem to possess certain compounds having partly the properties of sterols and partly those of resinols. Further in *Hemidesmus* roots small amounts of tetracyclic triterpene alcohols also seem to be present.

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