# WAX AND RESIN COMPONENTS OF *LEPTADENIA RETICULATA*

BY P. BHASKARA RAMA MURTI AND T. R. SESHADRI

*(From the Departments of Chemistry and Chemical Technology, Andhra University)*

Received October 3, 1944

A NUMBER of medicinal plants belonging to the family Asclepiadaceæ have been recently examined and found to contain resinols. *Asclepias syriaca* seems to have been the earliest to be studied. The milky sap of this plant was examined at various times by Marek[1] who noticed the presence of butyric acid esters of various alcohols whose identity was indefinite. The colour reactions of these substances as noted by him were not entirely in agreement with those possessed by sterols. At a later stage he was able to isolate from this mixture of esters a compound melting at 240–41° and having the formula $C_{30}H_{49}OCOCH_3$. Van Romburgh identified this as β-amyrin acetate. It was therefore possible that resinols existed in the sap. Working with the same plant Schmid *et al.*,[2] definitely showed that the main bulk of the material did not possess the prominent characteristics of sterols, *e.g.*, precipitation with digitonin, and that the alcohols consisted of a mixture of α- and β-amyrins.

A similar result was obtained by Matzuret[3] in regard to the composition of *Asclepias carnuti.* It contained esters of aliphatic acids with complex alcohols among which could be identified α- and β-amyrins. Earl and Doherty[4] in a paper on *Sarcostemma australe* which also belongs to this family, recorded the analysis of the wax obtained by extraction with ether. Though direct crystallisation of the unsaponifiable matter was not satisfactory, acetylation and subsequent fractionation enabled the isolation of α- and β-amyrins to be made.

*Hemidesmus indicus,* also known as Indian Sarasaparilla, was investigated by Dutta *et al.*,[5] who stated that it contained besides the 4-methyl ether of β-resorcylic aldehyde, two sterols, hemidesterol and hemidesmol. This root along with that of *Decalepis Hamiltonii* both of which belong to the family Asclepiadaceæ was carefully investigated by Murti and Seshadri.[6] These are closely allied plants and possess similar components. The unsaponifiable part of the waxy matter consisted mainly of resinols and only minor quantities of sterols seemed to be present. The resinol portion

contained α- and β-amyrins primarily with lupeol as a minor component in the case of *Decalepis Hamiltonii* whereas with *Hemidesmus indicus* the reverse was the case. They seem to occur as esters of steam volatile lower fatty acids and also of fatty acids of higher chain-lengths. β-Amyrin acetate could be isolated from the mixture of esters. In this connection it may be mentioned that lupeol was known to occur in the plants belonging to the families Sapotaceæ, Rutaceæ and Leguminoseæ[7]; from the above results it is established that some members of Asclepiadaceæ also contain lupeol.

Another well-known plant of this family is *Calotropis gigantea.* The root bark was first investigated by Hill and Sirkar[8] who employed its alcoholic extract. They reported that besides gutta-percha like material two alcoholic substances, mudarol and akundarol existed as esters with isovaleric acid and they believed that these substances were definitely different from resin alcohols characterised by Cohen, Van Romburgh and others. More recently Basu and Nath[9] carried out a study of the milky juice of *Calotropis gigantea* and reported the isolation of a new sterol which they named calosterol. Its properties were abnormal for a sterol and in general resembled those of resinols. The study of the various parts of *Calotropis gigantea* that has been carried out by us[10] has definitely established the existence of resinols in this plant also.

Another species of Calotropis is *Calotropis procera.* A recent study of the latex of the plant has been made by Hesse *et al.*[11] They have recorded that α-lactucerol is the sole alcoholic component and that it exists as acetate and isovalerate. It is capable of isomeric change into isolactucerol. Though there has been considerable variation in the description of α-lactucerol, it is considered to be a triterpene alcohol.

*Leptadenia reticulata* is another plant of this family commonly available in the hilly tracts of the East Coast of India. It is a tender creeper and seems to find an occasional use in nose and ear troubles. With a view to make sure if all the members belonging to Asclepiadaceæ invariably contain resinols, a composite sample of this creeper consisting of stems and roots has now been examined. Since in all the cases that have been studied so far resinols come in the ligroin soluble fraction, only this solvent has now been used for extraction. The ligroin extract yields about 1·5% of waxy matter which has been separated into fractions and studied in detail. As the result it has been found that the major portion of the wax consists of aliphatic components which are esters of long-chain alcohols, $C_{28}$–$C_{34}$ with long-chain acids, $C_{28}$–$C_{32}$. A very small quantity of a substance melting at 115° was also obtained. It could not be completely characterised.

Further, the extract contains about 12·0% of sterols and it is remarkable that these are found to occur entirely free and not in the form of esters. For purposes of separating the individual sterols, bromo-derivatives of their acetates were prepared and fractionated according to the method of Windaus and Hauth.[12] The top fraction, forming about 75% of the mixed bromo-compounds, melted round about the temperature recorded in the literature for the tetra-bromide of stigmasteryl acetate, and the crystal structure also agreed. Further the acetate and the sterol obtained from the bromo-compound on de-bromination and subsequent hydrolysis, possess properties agreeing closely with those of stigmasteryl acetate and stigmasterol; consequently this fraction is considered to consist of stigmasterol. The more soluble bromoacetates, the minor fraction, on de-bromination and repeated purification, yielded an acetate which closely resembled that of $\gamma$-sitosterol. The properties of the free sterol obtained from it are also in close agreement with those of the above-mentioned sterol. Hence, this fraction is considered to contain $\gamma$-sitosterol. It is therefore concluded that the sterol mixture consists of stigmasterol as the major component (75%) and $\gamma$-sitosterol (25%) as the minor component. No resinol could be found. It is not definite if this could be correlated with the fact that the plant does not yield a milky latex. However, the conclusion may be drawn that though most plants of the family Asclepiadaceæ contain resinols there are others devoid of them.

## *Experimental*

The whole plant excepting the leaves was employed for the investigation. A composite sample of the stems and roots was shredded into small bits, dried in the sun and then ground into coarse powder. About 4 kilograms were extracted in kilogram lots with ligroin for about 20 hours. Most of the solvent was removed by distillation and the resulting residue was heated on a water-bath to remove the last traces of the solvent. The dry extract (42·0 g.) thus obtained appeared as a dark green semi-solid. It was extracted 5 times using each time 200 c.c. of boiling alcohol; the clear solutions were decanted hot and mixed together. The undissolved residue was marked (A) (5·0 g.). The combined alcoholic solution when kept for a day deposited a bulky solid melting between 60–85°. This was separated and marked (B). When the mother-liquor was concentrated to about 200 c.c. a soft crystalline looking solid contaminated with resin was obtained. A little more produced by further concentrations to 50 c.c. was also added. This fraction was marked (C) (7·0 g.). When tested by means of the Salkowski and Liebermann-Burchard reagents fractions (A) and (B) produced no colour indicating that they were free from resinols and sterols and fraction (C) gave the tests characteristic of sterols.

Fraction (A) was rather sticky and repeated purification did not yield a definite solid; even after saponification the products could not be satisfactorily purified.

*Fraction B (aliphatic wax esters).*—This fraction (27·0 g.) was obtained as a green solid with very indefinite melting point. In order to separate it into fractions it was digested with boiling acetone (1000 c.c.). A small portion was insoluble; additions of ether (400 c.c.) did not make any difference. The insoluble solid was filtered off and marked $B_1$. Concentration of the filtrate to 300 c.c. resulted in the separation of a pale green solid melting at about 87°. After digestion with boiling ethyl acetate and crystallisation successively with benzene-alcohol mixture and chloroform a colourless solid melting at 90–92° was obtained (6·0 g.). This was marked $B_2$. By working up the acetone and ethyl acetate mother liquors and purifying the product another fraction ($B_3$) melting at 86–89° was collected.

$B_1$ was sparingly soluble in ordinary solvents; it was purified by boiling with a mixture of benzene and chloroform when it melted at about 110°. It was finally crystallised from a mixture of chloroform and acetic anhydride and was obtained as colourless rhombs which melted at 115°. It gave negative Salkowski and Liebermann-Burchard reactions (Found: C, 80·1; H, 11·7%; $C_{12}H_{20}O$ requires C, 80·0; H, 11·1%.). The results of analysis agree with the above empirical formula. The final yield was very small and no further data could be obtained due to dearth of material.

$B_2$ had an acid value of 6·9 and seemed to consist mostly of wax esters (Found: C, 82·5; H, 13·4; $C_{62}H_{124}O_2$ requires C, 82·7; H, 13·8%.). It (5·0 g.) was subjected to saponification by boiling its benzene solution (300 c.c.) with 8% alcoholic potash (300 c.c.) for 20 hours. Subsequently an alcoholic solution of calcium chloride (30·0 g. in 400 c.c.) was added and the boiling continued for 2 more hours. After concentrating the mixture, the resulting solid was filtered hot and washed with small amounts of alcohol. It was then repeatedly extracted with boiling acetone (200 c.c.) to remove the unsaponifiable matter from the soap. On keeping the acetone solution for a day a colourless solid separated out and it was purified by recrystallisation from chloroform. The first crop (I) (2·1 g.) melted at 92° and the melt set to a solid at 91°. The subsequent crop (II) was negligible in amount and melted at 88–90°. Solid (I) was subjected to acetylation by boiling with acetic anhydride and sodium acetate. The resulting product was found to be an acetate and after crystallisation from ether-alcohol mixture it melted at 76° (Found: C, 80·2; H, 13·3; $C_{36}H_{72}O_2$ requires C, 80·6; H, 13·4%.). The melting point of the acetate corresponds to an

alcohol mixture of average chain length $C_{34}$ and the melting point of the alcohol mixture also supports the conclusion that this fraction consisted of $C_{34}$ alcohol and near homologues.

The calcium soap left after extraction of the unsaponifiable matter was decomposed by boiling with acetic acid (300 c.c.) and the mixture was largely diluted with water. The resulting solid was again digested with boiling acetic acid and the free acids thus obtained were purified by dissolving in acetone-chloroform mixture. On keeping the solution for a day a colourless crystalline solid (2·5 g.) melting at 90-92° was obtained. Its melting point could not be raised by further crystallisation (Found: C, 79·8; H, 13·3; $C_{30}H_{60}O_2$ requires C, 79·7; H, 13·3% and $C_{32}H_{64}O_2$ requires C, 80·0; H, 13·3%.). It had a molecular weight of 467·8. Consequently it had a mean chain length of $C_{31}$ and probably consisted of $C_{30}$ and $C_{32}$ acids.

Fraction ($B_2$) thus seemed to be mostly made up of esters of alcohol of $C_{34}$ dimension and its near homologues with acids having chain lengths of $C_{30}$ and $C_{32}$.

Fraction $B_3$ (15·0 g.) was subjected to saponification by boiling its benzene solution (500 c.c.) with an equal volume of 8% alcoholic potash for 20 hours. After removing the major bulk of the solvents, the resulting products were dried into a friable mass. The dry material was extracted in a Soxhlet with petroleum ether and acetone in succession to remove the unsaponifiable matter from the soap. To ensure complete saponification the unsaponifiable matter obtained after removal of the above solvents was again subjected to saponification by boiling its benzene solution (150 c.c.) with sodium ethoxide (3·5 g. of sodium in 200 c.c. of alcohol) for 4 hours. Then an alcoholic solution of calcium chloride (20·0 g. in 200 c.c. of alcohol) was added and the boiling continued for two more hours. The contents were filtered hot. The solution was concentrated to half its bulk, largely diluted with water and the resulting precipitate consisting of the calcium soaps (negligible) and the unsaponifiable matter was filtered. The precipitate was dried and was extracted with boiling acetone (500 c.c.) using 100 c.c. at a time. The acetone extracts were all mixed together and on concentrating the combined solution to about 150 c.c. a pale yellow solid (7·0 g.) separated out. It was dissolved in chloroform and filtered. After keeping for a day the chloroform solution deposited a colourless solid (4·0 g.) melting at 89-90° and setting at 88·5°. It appeared as rhombs under the microscope (Found: C, 82·8; H, 13·8; $C_{32}H_{66}O$ requires C, 82·4; H, 14·2%). On acetylation by boiling with acetic anhydride and sodium acetate it yielded

an acetate melting at 73·0°. The melting point of the acetate corresponds to an average chain length of 32 C atoms in the alcohol. The chloroform mother liquor yielded a solid which formed an acetate melting at 70–71° indicating that it consisted of lower homologues. In the first saponification itself, the esters were almost completely decomposed. Consequently, the major bulk of the acids derived from them were obtained as their potassium salts and were liberated by heating the potassium soap with dilute sulphuric acid. The acids (7·5 g.) thus obtained were highly coloured and most of the colour was removed by treating their benzene solution with 'norit'. The solvent was completely removed by distillation and the residue was repeatedly crystallised from acetone and chloroform in succession. The top fraction (3·9 g.) melted at 89–91° and had a molecular weight of 460·4; consequently it may be considered to have a mean chain length of $C_{30\cdot6}$ and probably consisted of $C_{30}$ acid and its higher homologues. The second fraction (2·1 g.) melted at about 88° and had a molecular weight, 422·8. It had a mean chain length of $C_{29\cdot3}$ and the fraction was considered to be a mixture of $C_{30}$ acid and its lower homologues.

Fraction ($B_3$) was therefore considered to be a mixture of esters of long chain alcohols, $C_{28}$–$C_{32}$, with acids of similar chain lengths.

*Fraction (C) (stigmasterol and γ-sitosterol).*—The crude fraction (7·0 g.), on crystallisation from acetone, yielded a colourless solid in the form of tiny plates melting at 132–34°. With the Salkowski reagent it gave a blood red colour in the chloroform layer while exhibiting green fluorescence in the acid layer; with the Liebermann-Burchard reagent a play of colours—pink-blue-green was produced. It formed a precipitate with digitonin in alcoholic solution. In order to test for the presence of esters the crystalline solid was subjected to saponification by boiling with alcoholic potash. The solvent was distilled off as far as possible and the concentrate was diluted with water and ether extracted. After removing the ether, the residue was fractionally crystallised from alcohol and three crystalline fractions were collected. All of them appeared as broad plates and melted at 134-36°. $(\alpha)_D$, −40·2° in chloroform. However, they did not show any depression in their melting points when mixed with the original solid. The aqueous alkali solution obtained after ether extraction, when rendered acidic, did not give either steam-volatile or non-volatile acid.

*Acetylation.*—The above fractions were mixed together and the combined solid (4·0 g.) was subjected to acetylation by boiling with acetic anhydride (25 c.c.) and sodium acetate (5·0 g.) for 3 hours. The resulting product, after crystallisation from ether-alcohol mixture, was obtained as

broad colourless plates melting at 130–32°. No improvement in the melting point could be effected by further crystallisation from various solvents. $(\alpha)_D$, $-48\cdot7°$ in chloroform.

*Bromo-acetates.*—The crystalline acetate (3·0 g.) was dissolved in ether (150 c.c.) and excess of bromine dissolved in glacial acetic acid was added in drops, all the while keeping the ether solution at 0° C. After allowing it to stand for 12 hours in ice, a colourless crystalline bromo-derivative (2·2 g.) had separated out and it melted at 199° (decomp.). On crystallisation from glacial acetic acid and then from chloroform-alcohol mixture, it was obtained in the form of well-defined rhombic plates melting at 205° (decomp.). It was considered to be the tetra-bromide of stigmasteryl acetate. The ether solution, obtained from the crude bromo-compound, on concentration to about 15 c.c. deposited a smaller amount of a crystalline product melting at about 155°.

*Stigmasteryl acetate.*—The pure bromo-acetate (1·5 g.) was debrominated by carefully boiling with glacial acetic acid and zinc dust. The resulting product, after crystallisation from alcohol, was obtained in the form of broad rectangular plates melting at 143°. $(\alpha)_D$, $-51\cdot7°$ in chloroform.

*Stigmasterol.*—The pure acetate (1·0 g.) was hydrolysed by boiling with alcoholic potash and the resulting sterol was recovered by ether extraction. It crystallised from alcohol in the form of broad rectangular plates and melted at 168° (Found: C, 80·6; H, 12·0; $C_{29}H_{48}O$, $H_2O$ requires C, 80·9; H, 11·6%.). $(\alpha)_D$, $-49\cdot2°$ in chloroform.

*γ-Sitosterol.*—The more soluble bromo-acetate fraction (0·5 g.), m.p. about 155°, was crystallised from acetic acid and the top fractions melting at about 179° were rejected. From the mother liquor, a small amount of a crystalline solid melting at about 135° was collected and purified by crystallisation from ether-alcohol mixture. It was debrominated and the resulting acetate was repeatedly crystallised from alcohol. Finally it was obtained in the form of broad plates melting at 140°. After saponification, it yielded a sterol which crystallised from alcohol as plates and melted at 142°. $(\alpha)_D$, $-41\cdot2°$ in chloroform. From the above data, this sterol was considered to be γ-sitosterol. It is possible that the more soluble bromo-compounds were a mixture of the derivatives of sitosterols.

From the results recorded above, fraction (C) should be considered to consist almost completely of free sterols. Stigmasterol was found to be the major component of the sterol mixture, and γ-sitosterol probably along with small quantities of other sitosterols formed the rest.

*Summary*

Past work on the chemical components of a number of plants of the family Asclepiadaceæ is reviewed. They are found to contain resinols though many of these were originally mistaken for sterols. With a view to test if this is an invariable rule another member, *Leptadenia reticulata,* has now been studied. The major portion of the wax consists of aliphatic wax esters derived from long-chain alcohols ($C_{28}$-$C_{34}$) and long-chain acids ($C_{28}$-$C_{32}$). Considerable amount of sterols occur free and the mixture seems to consist mostly of stigmasterol along with smaller amounts of sitosterols of which $\gamma$-sitosteral could be characterised. No resinols could be detected.

REFERENCES

1. Marek .. *J. Pr. Chem.*, 1903 (ii), **68,** 385 and 449.
2. Schmid and Stohr .. *B. C. A.*, 1927, 248.
3. Matzuret .. *Ibid.*, 1935, 1180.
4. Earl and Doherty .. *Ibid.*, 1937, 3963.
5. Dutta, Ghosh and Chopra *Arch. Pharm.*, 1938, **276,** 333.
6. Murti and Seshadri .. *Proc. Ind. Acad. Sci.*, A, 1941, **13,** 221, 263 and 399; **14,** 93.
7. Heilbron *et al.* .. *J. C. S.*, 1938, 332.
8. Hill and Sirkar .. *Ibid.*, 1915, 1437.
9. Basu and Nath .. *Biochem. J.*, 1934, **28,** 1561.
10. Murti and Seshadri .. *Proc. Ind. Acad. Sci.* A, 1943, **18,** 145.
11. Hesse *et al.* .. *B. C. A.*, 1942, **A 2,** 43.
12. Windaus and Hauth .. *Ber.*, 1906, **39,** 4378–84.