THE WAXY MATTER OF THE FLOWERS OF *HIBISCUS SABDARIFFA* AND *CARTHAMUS TINCTORIUS*

By P. Bhaskara Rama Murti and T. R. Seshadri

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

Received September 25, 1945

Only a few cases of wax from flowers seem to have received attention. Rose petal wax was studied by Prophete.[1] It contains about 50% of hydrocarbons and the results suggest that paraffins of chain length shorter than C_{25} and possibly olefines are present. Amongst the constituents of the flowers of *Arnica Montana* was found the substance Arnidendiol having the formula $C_{30}H_{50}O_2$.[2] This probably belongs to the triterpene group. Paraffins were isolated from the flowers of *Tagetes grandiflora* by Kuhn *et al.*[3] The mixture melted at 68·5° to 69·5° and was considered to be made up of the C_{31} compound with a small amount of the higher homologue (C_{33}). The wax components of the flowers of *Butea frondosa*[4] and *Pongamia glabra*[5] have recently been examined by Murti *et al.* The former seems to consist entirely of wax esters whereas the latter consists predominantly of these esters along with minor quantities of paraffins.

Small quantities of the waxy matter of the flowers of *Hibiscus sabdariffa* and *Carthamus tinctorius* were obtained as by-products in the course of the study of their pigments. The results of their chemical examination are given below. Both of these waxes consisted predominantly of hydrocarbons.

Hibiscus sabdariffa.—

This plant belongs to the family Malvaceæ. The dry petals were repeatedly extracted with boiling alcohol. On concentrating the alcohol extract and allowing it to stand for several weeks hibiscitrin separated out along with some quantity of wax. Subsequently the clear alcoholic solution was thrown into a large excess of boiling water whereby most of the colouring matter went into solution and the insoluble sticky solid contained the main part of the wax and some resin. About 30 grams of this material was available. As much colouring matter as possible was again removed by repeatedly boiling the solid with water. By subsequently extracting it with ether, it was separated into the ether-insoluble portion (A) and the ether-soluble portion (B).

Fraction (A): Phytosterolin.—The light brown solid (3·0g.) thus obtained melted at about 230°. It was found to be insoluble in water and the

ordinary organic solvents, but it dissolved in hot glacial acetic acid and in pyridine. The clear solution in acetic acid, on gradual dilution with water, deposited a crystalline solid which after one more crystallisation from the above solvent yielded the compound in the form of hexagonal plates melting at 251–52° (decomp.). With the Liebermann-Burchard reagent it produced a play of colours, pink-blue-green and with the Salkowski reagent the chloroform layer assumed a blood red colour while the sulphuric acid layer exhibited a powerful green fluorescence. The high melting point and the colour reactions coupled with the sparing solubility of the compound in the ordinary solvents indicated the possibility of its being a glycoside of a sterol. (Found in a sample dried at 130°: C, 72·4; H, 10·2; $C_{35}H_{60}O_6$ requires C, 72·9 and H, 10·4%.).

The sterolin (0·2g.) was dissolved in amyl alcohol (25 c.c.), hydrochloric acid (2 c.c.) added and the mixture boiled under reflux for 5 hours. After removing the solvent by steam distillation, the products of hydrolysis were taken up with water and ether-extracted. The ether solution was washed with water, dried over anhydrous sodium sulphate and distilled to remove the solvent completely. The residue on crystallisation from alcohol was in the form of colourless needles and it responded to the usual sterol colour reactions. It melted at 136–37°. (Found: C, 83·8; H, 12·2; $C_{29}H_{50}O$ requires C, 84·1; H, 12·1%.); $[\alpha]_D^{30°}$, −35·0° in chloroform solution. The crystalline sterol (0·12 g.) was boiled with acetic anhydride in presence of sodium acetate for three hours. The resulting acetate, on crystallisation from alcohol, was obtained in the form of colourless needles and melted at 126–27°. (Found: C, 81·4; H, 11·4; $C_{31}H_{52}O_2$ requires C, 81·6; H, 11·4%.); $[\alpha]_D^{28°}$, −20·0° in chloroform solution. The above properties are characteristic of sitosterol. The presence of a reducing sugar was detected in the aqueous acid solution by testing it with Fehling's solution and its identity with glucose was established by preparing its osazone.

Fraction (B).—The ether solution was still contaminated with some flavonols and they were removed by repeated extraction with aqueous sodium hydroxide. The wax recovered from the ether solution had a low and indefinite melting point. It could not be purified by simple crystallisation from any solvent; hence it was subjected to saponification using boiling alcoholic potash. The acid portion was negligible indicating that the wax consisted almost completely of unsaponifiable matter. When the unsaponifiable matter (12·5 g.) was crystallised from benzene-alcohol mixture a colourless product (10·0 g.) was easily obtained. Analysis for carbon and hydrogen indicated that it consisted of hydrocarbons. It was

easily soluble in cold petroleum and was purified by extraction with this solvent. To effect further purification, it was dissolved in hot amyl alcohol followed by the addition of concentrated hydrochloric acid and the contents were boiled for a few minutes. On gradual cooling a cake of hydrocarbons was formed on the top of the liquid and was carefully removed. Subsequently it was boiled with acetic anhydride in the presence of sodium acetate and again it was purified by treatment with sulpuric acid at 130°; during the last process no blackening was noticed. Finally on crystallisation from alcohol and then from petroleum ether, it was obtained as glistening crystals (rhombs). It then melted sharp at 59° and the melt solidified at 58·5°. (Found: C, 84·9; H, 14·8%.). Its melting point corresponds to an average chain length of $C_{27\cdot2}$ on the curve establishing the relationship between the melting points and the chain lengths of hydrocarbons.[6] Hence it was considered to be composed predominantly of C_{27} — hydrocarbons.

When the benzene-alcohol mother liquor was evaporated, the residue gave tests for sterols. But it could not be purified by simple crystallisation. Hence it was acetylated by boiling with acetic anhydride and a few drops of pyridine. After diluting the mixture with water and allowing the product to stand, it was ether extracted. The solvent was evaporated and the residue crystallised from methyl and ethyl alcohols in succession. Finally the substance was obtained as colourless needles melting at 126–27°. It agreed in all properties with the sitosterol acetate obtained earlier from the phytosterolin and their mixed melting point was undepressed. On saponification by boiling with alcoholic potash the free sterol was obtained and it was found to be identical with the sitosterol sample obtained by the hydrolysis of the phytosterolin, the mixed melting point being again undepressed.

Carthamus tinctorius (Safflower):—

The sample of the flower petals (4·0 kg.) was obtained from Bellary in the Deccan Plateau. The wax was isolated from the flower petals by means of carbon tetrachloride. It was contaminated with some essential oil and also some colouring matter. The latter was removed by extracting the carbon tetrachloride solution with aqueous alkali. After removing the solvent the residue (15·0 g.) was first subjected to steam distillation in order to remove the essential oil and then to saponification by boiling its benzene solution with alcoholic potash.

Unsaponifiable matter.—This (13·0 g.) was obtained as an yellow solid and it melted indefinitely at about 50°. It readily dissolved in chloroform, benzene and petroleum ether and sparingly in methyl and ethyl alcohols.

To resolve it into different fractions tests were made using various mixtures of solvents. The following method was found satisfactory. The material was dissolved in chloroform (150 c.c.), an equal amount of alcohol added and the contents were stirred. On allowing to stand for some time a colourless shining solid began to separate out. It was filtered and washed with alcohol in order to remove coloured impurities. It then appeared as rhombs under the microscope and melted at 62–64°. With the Liebermann-Burchard reagent no colour was produced indicating the complete absence of sterols and related compounds. It was purified by repeated crystallisation from ether-alcohol mixture. Finally a solid melting at 64° was obtained. Analysis for carbon and hydrogen showed that it was made up almost completely of hydrocarbons. Further purification was effected adopting the method described under *Hibiscus sabdariffa* followed by treatment with sulphuric acid at 130°. Finally it was crystallised from alcohol and was then obtained as a shining crystalline product which melted at 65° and set at 64·5°. (Found: C, 84·8; H, 15·4%.) Using the curve establishing the relationship between the melting points and chain lengths its average chain length was found to be $C_{29\cdot5}$. Hence the mixture may be considered to consist mostly of C_{29} and C_{31} hydrocarbons.

The soap left after the removal of the unsaponifiable matter was decomposed by boiling with dilute hydrochloric acid. A mixture of fatty acids melting round about 53° was thus obtained in a small amount. Its neutralisation equivalent was 289·6. It was, therefore, considered to be a mixture of stearic acid and its near homologues probably arising out of the fatty oil contained in the wax.

Summary

The waxy matter isolated from the flowers of *Hibiscus sabdariffa* by alcohol extraction yielded phytosterolin as the ether-insoluble component. The ether-soluble portion consisted mainly of hydrocarbons having chain lengths round about C_{27} along with very small amounts of sitosterol. By the extraction of safflower with carbon tetrachloride was obtained a wax made up mostly of paraffin of chain length C_{29} and near homologues.

References

1. Prophete .. *Bull. Soc. Chim.*, 1926, **39**, 600.
2. Dieterle and Engelhard .. *Arch. Pharm.*, 1940, **278**, 225–35.
3. Kuhn *et al.* .. *Zeit. Physiol. Chem.*, 1931, **197**, 152.
4. Murti *et al.* .. *Proc. Ind. Acad. Sci.*, A, 1940, **12**, 472.
5. ——— and Seshadri .. *Ibid.*, 1944, **20**, 279.
6. Piper *et al.* .. *Biochem. J.*, 1931, **25**, 2082.