WAX AND RESIN COMPONENTS OF THE PERICARP OF JAMBUL FRUITS (*EUGENIA JAMBOLANA*)

BY P. BHASKARA RAMA MURTI AND N. V. SUBBA RAO

(*From the Department of Chemical Technology, Andhra University*)

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IN a publication by Rao and Seshadri[1] a preliminary report on the chemical components of the fleshy pericarp of the fruits of *Eugenia jambolana* was made. The note also contains a brief resume of past work on the different parts of the plant. They found that the sweetness of the pericarp was entirely due to the presence of reducing sugars and that there was a total absence of sucrose. The sourness and astringency were attributed to gallic acid and tannin. As partly responsible for the attractive colour the presence of an anthocyanin, Cyanin, was established. But a considerable portion of the colour seemed to be due to the presence of a second pigment which was phenolic in nature and which crystallised from dilute alcohol as snuff-coloured plates melting above 300°. In addition to the colouring matter, two colourless crystalline substances one melting at 149° and another at 232° were also reported to be present. In the present paper the results of a detailed investigation of the wax and resin components of the pericarp are presented.

The dried pericarp was first extracted with methyl alcoholic hydrogen chloride in the cold and on the addition of excess of ether to the extract the colouring matter was completely precipitated. From the alcohol-ether solution was obtained a semi-solid brown residue after complete evaporation and dilution with water. Purification of this using extraction with ether and recrystallisation of ether-soluble matter with alcohol gave an almost colourless solid. Much more of it was obtained by further extraction of the dried pericarp with acetone-ether mixture. The purified solid melted at about 232° with decomposition. This was identical with the substance reported by Rao and Seshadri as melting at this temperature. On close examination it was found to be a mixture, and repeated trials to separate it by crystallisation were unsuccessful. It was then subjected to saponification in the presence of benzene. The unsaponifiable matter contained a sterol and a very small amount of an essential oil and they were separated

by steam distillation. After purification, the sterol melted at 135° and formed an acetyl derivative melting at 120-21°. It seemed to have the same characteristics as the phytosterol obtained by Power and Callan[2] and by Hart and Heyl[3] from the seeds of Jambolana. No detailed identification was possible since the quantity was very small. It was probably a mixture of sterols.

The saponified portion yielded a crystalline potassium salt which was sparingly soluble. The corresponding acid could be easily purified through the potassium salt. It was sparingly soluble in ligroin and could be crystallised from boiling ethyl alcohol. It then melted at 298-300°. Its solution in concentrated sulphuric acid exhibited a strong greenish fluorescence. Though it did not respond to Salkowski reaction it gave a play of colours, pink to green, with the Liebermann-Burchard reagent. It gave no colour with ferric chloride. These properties and the results of analysis indicated that it may be a triterpene acid of the formula $C_{30}H_{48}O_3$ and this was supported by a molecular weight determination by titration (520). It exhibited optical activity, $(\alpha)_D + 78{\cdot}3$ in chloroform solution. It formed a crystalline acetyl derivative but its melting point was indefinite, 150–70°. Its methyl ester was prepared by two methods: (1) heating with methyl iodide and potassium carbonate in anhydrous acetone solution and (2) with diazomethane in anhydrous alcoholic solution. The same substance was produced. Its melting point also was indefinite 161–99°. This was found to be due to hydration, since after drying at 110° for 2 hours the melting point went up and it melted at 195–99°. The ester could further be acetylated and the acetate of the methyl ester melted at 214-16°. Thus it was clear that the original compound was a hydroxy acid of the triterpene series. Tests with fish indicated that it was toxic.

A comparison with known compounds showed close similarity with oleanolic acid. Oleanolic acid was obtained from several sources and has been examined by various workers who gave it a number of names. Power and Tutin[4] first isolated 'oleanol' from the leaves of olive tree and characterised it as a dihydric alcohol having the molecular formula $C_{31}H_{48}O(OH)_2$. From *Eugenia caryphyllata* (cloves), caryophyllin[5] was isolated in a pure form and its properties were compared with urson, a compound which was isolated from the skins of grapes and which was nearly identical with caryophyllin. Van der Haar[6] showed that the two substances oleanol and caryophyllin were identical, in spite of the slight variations in the recorded properties of the various derivatives and that they were not dihydroxy compounds but monohydroxy monocarboxylic acids. In view of this he assigned the name 'oleanolic acid' to the substance. The following table furnishes the

comparison between the properties of the substance isolated from Jambul fruits and those of oleanolic acid :—

	Colourless substance from Jambul fruits	Oleanolic acid
Molecular formula	$C_{30}H_{48}O_3$	$C_{30}H_{48}O_3$
M.P. of the substance	298–300°	306–308°
Specific rotation	$[a]_D$+78·3°	$[a]_D$+79·5°
Methyl ester (m.p.)	195–199°	196–198°
Acetyl derivative of methyl ester (m.p.) ..	214–216°	223°
Toxicity to fish	Toxic	Toxic

Experimental

About 2 Kg. of the dried pericarp of the fruits of *Eugenia jambolana* were extracted with 4 litres of methyl alcoholic hydrogen chloride (2.5%) at room temperature for a period of 2 weeks. The extract was filtered and treated with 10 litres of ether to precipitate the colouring matter, which was separately examined. The ether-alcohol mixture free from the colouring matter was distilled to recover the solvents. The last traces of alcohol were removed in an open basin and then diluted with water (600 c.c.). The entire product, pasty and resinous in nature, was extracted repeatedly with small amounts of ether and the combined ether extracts concentrated. The light greenish brown residue was then taken up in alcohol and recrystallised. The resulting substance was almost colourless and began to melt at 232° (decomp.).

The pericarp residue left behind after methyl alcoholic hydrogen chloride extraction was refluxed twice with 3 litres of acetone-ether mixture (1 : 1) and the combined extracts distilled off to recover the solvents. The substance obtained was taken in hot alcohol and recrystallised. The resulting product was found to be identical with the above and therefore they were combined (yield 5 g.) and treated further.

The substance was saponified for 12 hours with alcoholic potash (8%) in the presence of benzene so as to effect complete saponification. The alcohol was then distilled off and the soap taken in a large excess of water and repeatedly extracted with ether. The filtered ether extracts on evaporation left behind a semi-solid unsaponifiable matter (A). The aqueous caustic potash solution contained a large amount of colourless solid. It was filtered and the residue of potassium salt was collected (B). The alkaline solution on acidification and boiling gave rise to a solid (C) which was filtered.

Unsaponifiable matter (*A*) (*Sterol and essential oil*).—It had a sweet smell which indicated the presence of an essential oil. Further it gave the Salkowski reaction as well as the play of colours with Liebermann-Burchard reagent. This gave a clue that it might contain a sterol. Therefore, with a view to separate the essential oil, it was subjected to steam distillation. The essential oil obtained had a refractive index, 1·464. Nothing more could be done since it was very small in quantity.

The residue in the flask left after steam distillation was repeatedly extracted with ether and the combined extracts concentrated. The product thus obtained was twice recrystallised from alcohol and a shining crystalline substance melting at 135° was obtained (yield 0·5 g.). It gave all the sterol reactions and its acetate melted at 121°.

Potassium salt (*B*) (*isolation of a triterpene acid*).—The crystalline potassium salt was treated with dilute hydrochloric acid, boiled and filtered. The solid acid thus obtained (3 g.) was then heated with 40 c.c. of $\frac{1}{2}$ N alcoholic potash and 15 c.c. of water for an hour and a half, the solution filtered while hot and then allowed to cool slowly, when only a very small amount of the porduct separated. Therefore, most of the alcohol was removed and the salt allowed to crystallise. The crystalline salt (rectangular rods) was dissolved in 3 parts of cold methyl alcohol, filtered and the salt precipitated from the alcoholic solution by the addition of water. The salt thus obtained was taken in 10 parts of methyl alcohol and to it was added a slight excess of strong HCl in order to liberate the free acid.

The resulting acid was filtered and repeatedly boiled with solvent petrol so as to remove any petrol-soluble portions and then recrystallised from ethyl alcohol thrice. The crystalline substance (rectangular prisms) obtained melted at 298–300°. It was soluble in hot alcohol, acetone, ether, chloroform and benzene. It gave a strong greenish fluorescence with conc. H_2SO_4. It did not give the Salkowski reaction but gave a play of colours, from pink to green with Liebermann-Burchard reagent. It contained no methoxyl group [Found: C, 73·4; H, 11·0; loss on drying, 7·2%. $C_{30}H_{48}O_3$ $2H_2O$ requires C, 73·1; H, 10·6; H_2O (loss) 7·3%.] Its minimum molecular weight (*i.e.*, equivalent weight) as determined by titration with alkali was 520. It had a specific rotation in chloroform solution of $(\alpha)_D$ + 78·3°.

Derivatives.—The acetate of the above acid was obtained by boiling with acetic anhydride in presence of sodium acetate. When recrystallised from aqueous alcohol, it was found to melt indefinitely between 150° and 170°. Even after reacetylation the melting point did not improve. The combus-

tion analysis, however, agreed with the monoacetate of the acid. (Found: C, 76·6; H, 10·5; $C_{32}H_{50}O_4$ requires C, 77·1; H, 10·1%.)

The *methyl ester* of the acid was prepared by heating with excess of methyliodide and anhydrous potassium carbonate in dry acetone medium for 30 hours. After distilling off the solvent the residue was treated with water in order to remove inorganic salts. The product was then purified by first extracting with ether in which it was soluble. It was finally recrystallised from aqueous acetone (1 : 1) or aqueous alcohol. It was obtained in the form of needles with indefinite melting point, 163-99°. The ester nature of this product was established by titration with alkali. (Found: OCH_3, 6·1; $C_{31}H_{50}O_3$ requires OCH_3, 6·6%.)

The methyl ester was also prepared in a different way. The acid was dissolved in anhydrous methyl alcohol and treated with excess of diazomethane in ethereal solution. The product when recrystallised was found to be indentical with the sample described above. Suspecting that the presence of water of crystallisation was the cause of indefinite melting point, the ester was dried at 110–15° for 2 hours. It was then found to melt at 195–99°. The melting point of the methyl ester of oleanolic acid found in the literature is 196–98°.

The acetyl derivative of the above methyl ester was prepared by boiling with acetic anhydride and anhydrous sodium acetate. It was first recrystallised from ethylacetate using a little decolourising carbon and finally from ethylacetate-alcohol mixture. It appeared as colourless needles and had a melting of 214–16°. (Found: C. 76·8; H, 10·4; no loss on drying; OCH_3, 6·5; $C_{33}H_{52}O_4$ requires C, 77·3, H, 10·2; OCH_3, 6·1%.)

Solid (*C*).—This on treatment with solvent petrol could be separated into two fractions: (*i*) easily soluble and (*ii*) sparingly soluble. The former was waxy in nature indicating it to be a fatty acid mixture. The latter on recrystallisation from methyl alcohol gave a substance melting at 240° and exhibiting all the properties of the acid obtained from (B). The yield of the fatty acid mixture was, however, very small for a detailed examination.

Toxicity.—35 mg. of the acid (*B*) was dissolved in 5 c.c. of rectified spirit and the solution added to 1000 c.c. of water contained in a wide trough. Fish were introduced into the water and they showed symptoms of toxicity within 2 hours thus indicating the toxic action of the substance to fish.

Summary

The waxy matter of the pericarp of *Eugenia jambolana* was saponified using alcoholic potash. The unsaponifiable matter contained a little

sterol and some essential oil. A sparingly soluble potassium salt was produced which yielded a triterpene hydroxy acid. A study of its properties and of its derivatives indicates that it is oleanolic acid. It seems to be the major component of the waxy matter.

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