402. *Highly Unsaturated Compounds. Part VI. The Triene Acid from the Seeds of Pomegranates.*

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ALTHOUGH at least five forms of elæostearic acid, all apparently *cis-trans*-isomerides, have at various times been reported to have been isolated, yet until recently the authenticity of only the two originally known forms (α and β) of the acid could be asserted with any degree of confidence. Last year, however, the isolation of two new crystalline forms of elæostearic acid from vegetable oils was reported by Toyama and Tsuchiya (*J. Soc. Chem. Ind. Japan*, 1935, **38**, 182B, 185B), and the evidence adduced went far to substantiate the claims of these authors as to the nature of their products. The recognition beyond question of error of new members of the elæostearic and related series presents some difficulties, and particularly in the present case there is the danger of confusing mixtures of the α- and the (secondarily formed) β-isomeride of elæostearic acid, or of α-elæostearic and traces of saturated acids, for genuine new compounds. In view of the economic importance of elæostearic glycerides, and the biological interest attaching to the relative distribution of conjugated and non-conjugated types of polyene acids it seemed desirable to obtain additional evidence to that advanced by the Japanese authors. At present, however, a source of only one of the new acids has become available.

One of Toyama and Tsuchiya's new isomerides was obtained by saponification of the oil extracted from the seeds of pomegranates (*Punica granatum*, L.). This substance, punicic acid, was reported to melt at 43·5—44° and to show a considerable exaltation (6·9 units) of the molecular refraction; it yielded a crystalline tetrabromide, recognised as a tetrabromo-derivative of elæostearic acid; furthermore it yielded *n*-valeric acid, azelaic acid (methyl hydrogen azelate from the methyl ester) and probably oxalic acid on ozonolysis, and gave decided depressions of melting point (8—9° and 3—4·5° respectively) when admixed with α- and with β-elæostearic acid.* All identifications, however, of the derivatives and degradation products of the acid were carried out by the method of mixed melting point and no single analysis appears to have been made : nevertheless the evidence strongly suggested that the reported new substance consisted wholly or mainly of a member of the elæostearic group and the chief points of doubt concerned the homogeneity of the acid and the genuineness of its isomeric relationship to the already-known α- and β-forms.

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* The m. p. of punicic acid as given by Toyama and Tsuchiya (43·5—44°) lies close to that of the eutectic mixture of the pure α- and β-elæostearic acids.

We found no difficulty in isolating an acid similar to that described by the Japanese authors from the seeds of pomegranate, and this, after every endeavour had been made to remove traces of other solid acids which might reasonably have been present in the oil, melted constantly at 44°. This acid, as judged by its analysis, its degree of unsaturation (amounting to three double bonds per molecule as determined by hydrogenation), and the facts (a) that it gave reasonably good yields of well-authenticated valeric and azelaic acids, together with some oxalic acid, on oxidation with permanganate, (b) that it gave a quantitative yield of stearic acid on complete hydrogenation, and (c) that it undoubtedly underwent facile isomerisation into genuine β-elæostearic acid (capable of giving the appropriate maleic anhydride derivative) under the influence of ultra-violet light, could not be other than an elæostearic acid.

Concerning the comparison of the new acid with the known α- and β-forms of elæostearic acid we find that punicic acid of m. p. 44° when mixed with highly purified α- and β-elæostearic acids (m. p. 48·5° and 70° respectively) is capable of depressing the melting point of the former to a point as low as 35° and that of the latter to 38°. Careful determination of the mixed melting point curve of pure α- and β-elæostearic acids shows that the eutectic mixture of these two acids melts at 43·4°. Additional evidence of difference between the three acids is afforded by their *X*-ray patterns, for the following report on which we are indebted to Mr. J. Thewlis, M.Sc.

"*X*-Ray powder photographs have been taken of α- and β-elæostearic and of punicic acids. It has been found that the spacings of punicic acid and β-elæostearic acid are quite distinct and that the two substances are separate and distinct individuals. The patterns for α-elæostearic acid and punicic acid are similar, but appear to differ slightly in spacing; furthermore, there are, in the punicic acid pattern, two well-defined lines in the region of 40 A. which are absent in α-elæostearic acid. The patterns suggest that whilst the sizes of the α-elæostearic and the punicic acid molecules are closely similar, there is a difference of atomic arrangement; and the punicic acid pattern is not that to be expected for a mixture of α- and β-elæostearic acids."

Although punicic acid readily passes into β-elæostearic acid, which, like its isomeric α-form, readily gives a crystalline maleic anhydride addition product, it does not itself give a maleic anhydride derivative—or at least no such derivative has at present been obtainable. Moreover punicic acid, although oxidisable in the air, yet possesses a considerable degree of stability compared with α-elæostearic acid: indeed it resembles the β- rather than the α-elæostearic acid, and on this account can be kept in nitrogen without serious deterioration for many weeks.

In our view, therefore, the claim of the Japanese authors to have isolated a third (doubtless geometrically isomeric) form of elæostearic acid is a sound one.

EXPERIMENTAL.

Punicic Acid.—The seeds of pomegranate were disintegrated in a mincing machine and extracted with ether at room temperature. The yellow oil obtained by evaporating the ether was saponified with alcoholic potash and the free fatty acids derived from the product were converted into their magnesium salts as described by Toyama and Tsuchiya (*loc. cit.*, p. 183). The portion of the magnesium salt most soluble in alcohol yielded a solid acid which after two recrystallisations from 80% alcohol and one from petroleum (b. p. 60—80°), followed by thorough drying in a vacuum, melted sharply at 44° (Found: C, 77·25; H, 10·5. Calc. for $C_{18}H_{30}O_2$: C, 77·6; H, 10·85%). The acid absorbed oxygen from the atmosphere, but suffered only slight deterioration during several months when kept in the dark in an atmosphere of nitrogen. It had a neutralisation value of 201·0 (calc. for elæostearic acid, 201·7) and an iodine value, by the Wijs method, of 217·5 (calc., 273·7).

A number of attempts were made to form the maleic anhydride derivative of punicic acid. Under all the conditions used, a sticky product, similar to that obtained by heating punicic acid alone, was formed. In no case did this appear to consist of, or contain, any substantial amount of the desired addition compound.

Comparison with α- and β-Elæostearic Acids.—Comparison of the mixed melting point curves of (a) punicic and α-elæostearic acids and (b) punicic and β-elæostearic acids with that of α-and β-elæostearic acids * left no doubt as to the essential difference of punicic acid from both the α- and the β-form of elæostearic acid. The eutectic points observed were 35° for punicic acid mixed with α-elæostearic acid, and 38° for punicic acid mixed with β-elæostearic acid.

Conversion into β-Elæostearic Acid.—When a 10% solution of punicic acid in xylene was exposed in contact with a little sulphur (nitrogen atmosphere) for 4 hours to the light from a mercury vapour lamp, complete conversion into β-elæostearic acid took place. The product was taken up in 20% aqueous caustic potash, and the filtered solution acidified. The acid thus precipitated was dissolved in ether, dried and recrystallised once from light petroleum. The crystals melted at 70°, alone or mixed with authentic β-elæostearic acid, m. p. 70°.

The maleic anhydride derivative of the irradiated product was formed in the usual way: this melted at 77° and did not depress the m. p. of the maleic anhydride derivative of authentic β-elæostearic acid (Found: C, 70·1; H, 8·4. Calc. for $C_{22}H_{32}O_5$: C, 70·25; H, 8·55%).

Hydrogenation.—On the assumption that punicic acid is isomeric with elæostearic acid (*M*, 278·24), sufficient hydrogen was absorbed in presence of a platinum catalyst to saturate 3·0 double bonds. The product was a homogeneous saturated acid, identified as stearic acid, which melted at once at 70° (mixed m. p. 70°) and gave a methyl ester, m. p. 38° (mixed m. p. 38°).

Oxidation.—The acid, dissolved in 10% aqueous acetone, was oxidised at 0° with a 3% solution of permanganate containing 2% of sodium carbonate. Twelve atoms of oxygen per molecule of acid were supplied in this way. The liquid was filtered, the manganese mud twice extracted with boiling water (50 c.c. portions), and the combined filtrate and aqueous extracts acidified with hydrochloric acid and distilled until only 50 c.c. of liquid remained; the distillate dropped into excess of dilute caustic potash solution. From the residue in the flask, azelaic acid mixed with gummy material separated and was filtered off. The azelaic acid was extracted from the crude mass with boiling water and crystallised successively from benzene and water (m. p. 105°; mixed m. p. 105°. Found: C, 57·6; H, 8·55. Calc. for $C_9H_{16}O_4$: C, 57·5; H, 8·55%); the gummy residue yielded a further quantity of azelaic acid on renewed treatment with permanganate (total yield, 55%). From the aqueous filtrate, calcium oxalate was precipitated in moderate yield. The alkaline liquor containing the distillate was concentrated on a water-bath to 50 c.c., acidified with concentrated hydrochloric acid, and extracted five times with ether. The ethereal extract yielded, after evaporation of the solvent and fractionation of the residual liquor, about 50% of pure valeric acid, b. p. 185—186° (*p*-bromophenacyl ester, m. p. and mixed m. p. 72·5°. Found: C, 52·1; H, 5·3. Calc. for $C_{13}H_{15}O_3Br$: C, 52·2; H, 5·05%).

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