Methyl Punicate, *Alpha* and *Beta* Eleostearate. *Cis, Trans* Isomerism and Structure[1]

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Methyl esters of *beta* eleostearic, *alpha* eleostearic, and punicic acids have been isomerized with iodine and light to the same equilibrium mixture of 64% *beta*, 33% *alpha*, and 2.6% punicate structures. The course of isomerization is in agreement with the following structures: *trans, trans, trans* for *beta*; *cis, trans, trans* for *alpha*; and *cis, trans, cis* for punicate, in agreement with structures proven *via* synthesis by Crombie and Jackson. There is some theoretical and experimental evidence that the center double bond of this type of conjugated triene isomerizes less readily than the outer double bonds.

It was shown by several of the authors (1) that isomeric *trans, trans; cis, trans, and cis, cis* conjugated linoleates were readily isomerized by iodine and light to the same equilibrium mixture of 64% *trans, trans*; 32% *cis, trans,* with little if any *cis, cis* isomer. Recently Chipault and Hawkins (2) have confirmed the equilibrium of the *cis, trans* to the *trans, trans* isomer. They report 71% *trans, trans* as the equilibrium concentration with little or no *cis, cis* isomer.

The present study is an extension of the techniques used on the conjugated dienes to the three isomeric conjugated 9,11,13 trienoic esters, methyl *alpha* eleostearate, *beta* eleostearate, and punicate. Theoretically there are eight isomeric 9,11,13 trienoic C_{18} acids:

1. *ttt*	3. *ttc*	5. *cct*	7. *ctc*
2. *ctt*	4. *tct*	6. *tcc*	8. *ccc*

Of these, 2 and 3 would undoubtedly have essentially identical infrared and ultraviolet absorption, as would the pair 5 and 6.

Beta eleostearate has been assigned structure 1, *ttt,* based on the ease of formation of the maleic anhydride adduct and on the presence of an isolated *trans* double bond in the adduct (3,4).

Alpha eleostearate has been assigned structure 2, *ctt,* based on ready formation of an adduct with maleic anhydride (two adjacent conjugated *trans* double bonds, slower than *beta* eleostearate) combined with the absence of isolated *trans* double bond in the adduct (2,3). Ahlers also proposed these structures for *alpha* and *beta* eleostearate, based on infrared spectra and the fact that *beta* eleostearate is the highest melting isomer, formed when *alpha* eleostearate is isomerized (5).

Punicic acid, a third isomeric conjugated trienoic C_{18} acid which occurs in pomegranate-seed oil, has been examined spectroscopically by Ahlers *et al.* (5,6). They concluded that it had only one *trans* double bond because of the low intensity of the 10.11 μ band. Of the two possible structures, *cct (tcc)* and *ctc,* with one *trans* and two *cis* double bonds, they preferred the *cct (tcc)* structure, based largely on the similarity of the relative intensities of the 10.1, 10.63 μ doublet to the 10.17, 10.53 μ doublet of the *cis, trans* diene, alkali-conjugated linoleate.

The present study reports the equilibration of the double bond systems of methyl *alpha* eleostearate, *beta* eleostearate, and punicate with iodine and light. The equilibrium composition is approximately 64% *beta* eleostearate, 33% *alpha* eleostearate, and 2.6% punicate. A consideration of the course of the isomerization suggested that punicate is the *ctc* structure rather than the *cct* proposed by Ahlers.

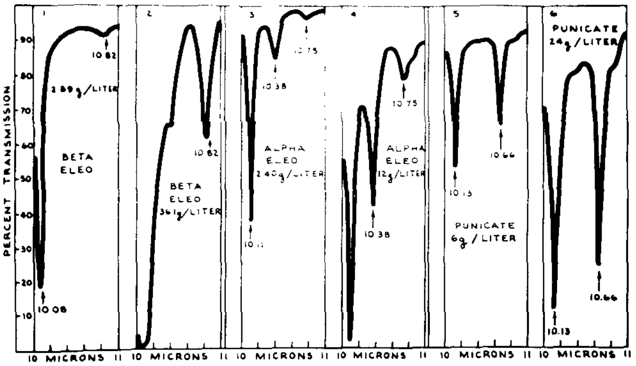

Fig. 1. Infrared curves of eleostearate isomers.

After this study had been completed (1956) and reported to the Society (April 1957), the very elegant work of Crombie and Jacklin (7) appeared, confirming by synthesis the *ttt* structure of *beta* eleostearate, the *ctt* structure of *alpha* eleostearate, and the *ctc* structure of punicate. They also showed that the trichosanic acid of snake gourd oil is identical with punicic acid.

Experimental

Methyl *alpha* eleostearate and methyl *beta* eleostearate were those prepared as previously reported (8).

Methyl punicate was prepared from pomegranate seed oil, which was obtained in 3% yield by extracting air-dried ground pomegranate seeds with Skellysolve F. The acids, recovered from alcoholic KOH saponification (room temp., 4 hrs.), were extracted and crystallized once from Skellysolve F at −25°C. and twice from methanol at −25°C. The acid was esterified by dissolving in methanol (25 cc. per gram) that contained 4% of p-toluenesulfonic acid. After 3 hrs. at room temperature the solution was diluted with water and extracted with Skellysolve F. The solution of ester was washed with sodium carbonate solution, dried over sodium sulfate, and evaporated under high vacuum. The resultant methyl ester showed a molecular extinction $\epsilon = 45.8 \times 10^3$ at the maximum of 274 mμ. (Crombie [7] reports $\epsilon = 46.0 \times 10^3$ for the acid.) The methyl punicate showed only two maxima in the 10–11 μ region of *trans* double bond absorption, one at 10.13 μ the other at 10.66 μ (Figure 1 and Table I). The very slight absorption peaks or inflections at 10.38 μ and about 10.8 μ are probably due to *alpha* eleostearate impurity (4% estimated by sloping base line estimation of absorbancy).

The infrared spectra were run on a Beckman IR-3 instrument in a 1-mm. cell. The esters were first scanned in CS_2 in the absence of iodine. Then they were made up in CS_2 and quantitatively diluted with iodine in CS_2 to produce a final concentration of iodine 0.0025 g./100 ml. of solution. The dilution with the iodine solution, the filling of the cell, and insertion in the instrument were done in darkness except for a very faint light from the instrument.

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One eye of the operator was covered for some time, causing it to become sensitive enough to permit accurate manipulation in the dark room. The course of isomerization by the action of the light in the cell compartment of the instrument on the iodine-catalyzed solution in the cell was followed by running successive spectra, leaving the cell in the instrument. When equilibrium was nearly complete, exposure to room light or to a photoflood lamp was sometimes used to ensure complete equilibrium. The results of the study are shown in Table I and Figures 1 and 2. The coefficients were calculated for the nominal 1-mm. cell thickness of the uncalibrated cell, probably accurate to 5% or better.

TABLE I
Specific Extinction Coefficients (g./liter, 1 cm.) of Conjugated Trienoic Esters and Composition of Equilibrium Mixtures

	10.1 μ	10.38 μ	10.66 μ	10.75 μ	10.82 μ
B, *ttt*	2.39	......	......	......	.053
A, *ctt*	1.68	0.23	......	0.044	(0.0225)[a]
P, *ctc*	0.52	......	0.36	......	......
B, equil.	......	0.073 32.0% A	0.0036 1.0% P	......	0.036 (corr.) 68% B
A, equil.	......	0.069 30.0% A	0.0028 0.8% P	......	0.033 (corr.) 63% B
P, equil.	......	0.072 31.0% A	0.004 1.1% P	......	0.0295 56% B
A + B mix. 37.1 g./l.	......	0.059 26.0% A	......	......	0.0394 (corr.) 74.0% B
B, equil. 36 gm./l. vs. A + B mix.	......	0.0233 10.1% A =34.0% A Total	0.0094 2.6% P	......	63.4% B by diff.

[a] Nonmaximal absorption, used for correcting absorption of *beta* isomer at 10.82 μ.

The coefficients of the reference and equilibrated mixtures were calculated from sloping base-lines drawn between adjacent peaks of absorption minima. In the equilibrium data the percentage of *beta* eleostearate was calculated from the absorption at 10.82 μ, correcting for the nonmaximal contribution of *alpha* eleostearate at this wavelength. *Alpha* eleostearate was calculated from the absorption at 10.38 μ, and punicate from absorption at 10.66 μ. Since the calculation of punicate at the low-equilibrium concentration involved measurement of a small absorption from a very highly sloping base-line, a mixture of *alpha* and *beta* eleostearate was made to approximate the ratio of the equilibrium mixture. This was analyzed spectroscopically, then used as a standard against equilibrated *beta* eleostearate. The punicate band was clearly resolved (Figure 2) and permitted a more accurate estimation of punicate in the equilibrium mixture (Table I). The weak *alpha* eleostearate band at 10.36 μ in this spectrum also permitted a calculation of *alpha* eleostearate in the equilibrium. The value thus obtained, 34%, agrees with those obtained directly on the equilibrated mixtures.

It will be noted from Table I that the total of equilibrated *beta* plus *alpha* eleostearate plus punicate totals 101% for *beta* elleostearate, 94% for *alpha* eleostearate, and 88% for punicate, suggesting that the *alpha* eleostearate and punicate were less pure than the *beta* eleostearate. It was of interest to determine whether ultraviolet absorption of equilibrated mixtures would confirm this indication. The isomers were made up in isooctane solution at a concentration suitable for measurement (*ca.* 0.01 g./liter) in isooctane containing .0004 g./liter of iodine and equilibrated by exposure to artificial fluorescent room light or to photoflood lamps. The absorption maximum was at 268 mμ, identical to that of the *beta* eleostearate, the major component. The equilibrated *beta* eleostearate showed the highest extinction coefficient. That of the equilibrated *alpha* ester was 97.5% of the *beta* ester; and the equilibrated punicate was 91% of the coefficient of the *beta* eleostearate. These results are in fairly good agreement with the values obtained by infrared measurement.

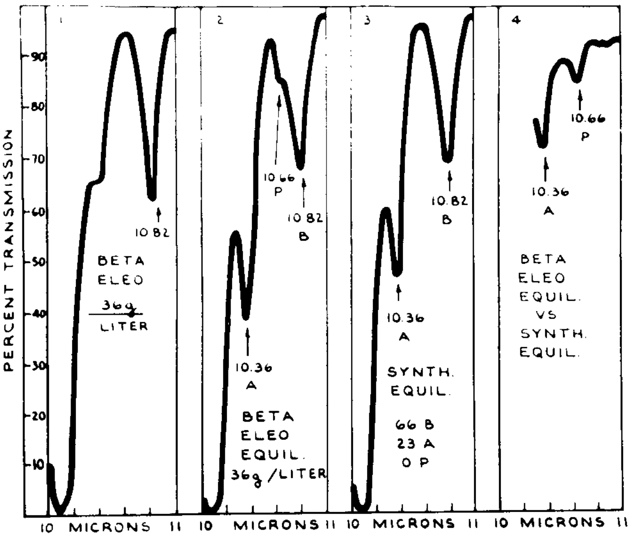

Fig. 2. Infrared curves of equilibrated *beta* eleostearate.

Discussion of Results

The intensity of the *trans* bands near 10.1 μ is in agreement with the structures assigned to the isomers in view of the data on conjugated dienes (1). Punicate, with one conjugated *trans* double bond, $k = 0.52$, is of the same order as *cis, trans* linoleate, $k = 0.495$. *Alpha* eleostearate, with two *trans* double bonds in conjugation, $k = 1.68$, is of the same order as *trans, trans* linoleate, $k = 1.43$, while *beta* eleostearate with 3 conjugated *trans* double bonds, $k = 2.39$, has the highest coefficient. Because these maxima are not at the same wavelength but are close enough not to be resolved in mixtures, it was decided to use the "secondary" bands in the 10–11 μ region for estimating the isomers in the equilibrium mixture. The 10.82 μ band of *beta* eleostearate was suitably resolved at the concentrations used but was corrected for absorption because of the *alpha* eleostearate, which has a band at 10.75 μ that is not compensated by the base-line drawn from the 10.6 μ to 11.0 absorption minima. The *alpha* eleostearate was estimated from its band at 10.38 μ with a base-line drawn between the 10.35 μ and the 10.6 μ absorption minima. Punicate in the original equilibria was calculated from absorption at 10.66 μ, estimated from a base-line drawn between the 10.6 μ and 10.7 μ absorption minima. When the equilibrated *beta* eleostearate was run differentially against the synthetic *alpha* plus *beta* eleostearate, the adjacent *beta* and *alpha* bands were diminished so that a much more satisfactory base-line between 10.6 μ and 10.8 μ was used (Figure 2).

During the course of the isomerization of the three isomers no bands between 10 μ and 11 μ were observed in the successive spectra other than those which could be assigned to the three isomers. This suggested, but did not prove, that other spectroscopic isomers were absent.

As punicate isomerized, a definite maximum for *alpha* eleostearate was observed, analogous to the maximum for conjugated *cis, trans* linoleate observed during isomerization of *cis, cis* linoleate (1). The concentration of the *alpha* isomer then decreased to the equilibrium value while that of the *beta* isomer rose steadily to the equilibrium value.

As *alpha* eleostearate isomerized, the *beta* isomer increased regularly to its equilibrium content. Punicate concentration apparently went through a slight but definite maximum, greater than the final low equilibrium value.

As *beta* eleostearate isomerized, the content of the *alpha* and punicate isomers increased regularly to equilibrium values.

As previously mentioned, no bands between 10 and 11 μ were observed during the isomerization of any of the three isomers studied other than those which could be attributed to these isomers. If one assumes from this observation that spectroscopic isomers other than the three already characterized by secondary 10–11 μ bands are absent during equilibration, the structure of punicate may be deduced to be *ctc*. This follows from the fact that punicate has only one *trans* double bond (weakest 10.1 μ band) leaving *cct (tcc)* and *ctc* as alternate structures. The *cct (tcc)* structures would be expected to give a *tct* structure as one of the primary products of isomerization, which might be expected to have other secondary bands in the 10–11 μ region. Since no such bands were observed, this structure seems less probable than the alternate *ctc* structure. On the other hand, the *ctc* punicic structure should give the observed maximum of the *alpha* isomer *(ctt)* during its isomerization. The synthetic proof of the structure of punicate by Crombie made this presumptive "proof" of little consequence. However the apparent absence of other isomers in the equilibrium would also mean that the center double bond (at least if *trans*) does not readily isomerize; otherwise the equilibrium should contain some of all six spectroscopic species.

Speculation as to the cause of this presumed inactivity of the center double bond to *cis, trans* isomerization led to the following suggestion by M. M. Kreevoy of the University of Minnesota: when a double bond forms the intermediate complex or activated state with the catalyst, it loses much of its double bond character. If this occurs on an "outer" double bond of a conjugated triene, the two remaining double bonds are still conjugated and the resonance due to their conjugation should give appreciable stability to this intermediate. When the "center" double bond forms the intermediate complex, conjugation is essentially lost so this unstabilized form would be less probable than that from an "outer" double bond. This leads to the interesting prediction that isomerization of an isomer with a *cis* center double bond might lead to a mixture of the *tct, cct, tcc,* and *ccc* isomers with none of the known isomers, each of which has a *trans* center double bond. The all-*cis* isomer would be the most interesting one to isomerize since it should have no bands in the 10–11 μ region by analogy to the *cis, cis* linoleate. The *cct (tcc)* isomer should be the first to appear, followed by the *tct* isomer.

Summary

Methyl *beta* eleostearate, *alpha* eleostearate, and punicate isomerize readily with iodine and light to an equilibrium mixture of 2.6% punicate, 33% *alpha* eleostearate, and 64% *beta* eleostearate. The course of isomerization is in agreement with the following structures: *ttt* for *beta* eleostearate, *ctt* for *alpha* eleostearate, and *ctc* for punicate. These structures have been proven *via* synthesis by Crombie and Jacklin. There is some evidence that the center double bond of a conjugated triene isomerizes less readily than the outer double bonds.

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Bleaching of Off-Colored Cottonseed Oils[1]

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The adsorption of fixed red pigments from refined off-colored cottonseed oil by several adsorbents is described by the empirical Freundlich adsorption isotherm. Numerical values for the coefficient and the exponent in the Freundlich equation were determined for several oils and several adsorbents. Activated alumina was found to be a superior adsorbent for removing the red color bodies. It was observed that the variations from oil to oil in the numerical values of the coefficient and the exponent of the Freundlich equation were smaller for the several aluminas than they were for the other adsorbents studied. The particle size and moisture contents of the alumina, and the temperature of activated adsorption were of importance in determining the effectiveness with which the red color bodies were removed from the oils. The conditions required for optimum bleaching with alumina had no detectably adverse effect on oil quality.

THE VALUE and usefulness of about 25% of the cottonseed oils produced in the United States are impaired because of the presence in the oil of red pigments that are not removed by the conventional refining and bleaching processes. The evidence developed during the past several years suggests very strongly that the alkali-fast, red color bodies in off-colored oils are derivatives of gossypol and that they

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