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NEW TROPICAL SEED OILS. PART I. CONJUGATED TRIENOIC AND TETRAENOIC ACIDS AND THEIR OXO DERIVATIVES IN THE SEED OILS OF *CHRYSOBALANUS ICACO* AND *PARINARIUM LAURINUM*

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The seed oils from *Chrysobalanus icaco* and *Parinarium laurinum* contain the following acids (% wt.): palmitic (4,4), stearic (18,7), oleic (11,2), linoleic (6,2), arachidic (1,0), conjugated octadecadienoic (0,1), α-eleostearic (22,22), α-parinaric (10,62), α-licanic (10,0) and a hitherto unknown acid (18,0) which is proved to be 4-oxo-octadeca-*cis*-9, *trans*-11, *trans*-13, *cis*-15-tetraenoic acid. α-Parinaric acid from both these sources is the *cis*-9, *trans*-11, *trans*-13, *cis*-15 isomer.

Introduction

We are presently engaged in screening a large number of tropical seed oils with a view to finding crops of potential commercial value. Early in this work samples of *Chrysobalanus icaco* (coco plum) and *Parinarium laurinum* seeds were obtained from Fiji. The seed oil of this latter has been examined before[1,2] though the detailed configuration of the parinaric acid (18:4; 9, 11, 13, 15) has not been established; *Chrysobalanus icaco* seed oil (abbreviated in this paper to chryso oil) appears not to have been examined before. Preliminary investigation showed it to contain conjugated acids like those in *P. laurinum*, accompanied by two oxo acids one of which resembled licanic acid. We now report our examination of these two seed oils. It is of interest that *C. icaco*, *P. laurinum*, and *Licania rigida* (oiticica: used in this investigation as a convenient source of licanic acid) belong to the same order (Rosales) and the same tribe (Chrysobalaneae).

Discussion

C. icaco kernels when extracted with ether gave about 51% of oil from which the methyl esters were prepared by interesterification. The glycerides and the methyl esters gave informative ultraviolet spectra with six peaks

(table 1) indicative of conjugated triene and tetraene unsaturation; they also showed oxo carbonyl (1720 cm^{-1}) and ester carbonyl (1743 cm^{-1}) absorption in their infrared spectra. On the basis of this evidence we examined, by gas–liquid chromatography, the methyl esters of chryso oil, oiticica oil (known to contain eleostearic and licanic acid), and the seed oil of *P. laurinum* (known to contain eleostearic acid and parinaric acid). On an Apiezon L column chryso esters gave ten peaks with the carbon numbers shown in table 2. Some, but not all, of these peaks were present in the other two ester mixtures and we concluded that chryso esters contain palmitic (carbon number 16.0), unsaturated C_{18} (17.6), stearic (18.0), eleostearic (19.1 and 19.5), parinaric (19.9 and 20.3), licanic (20.3 and 20.6), and an unidentified ester (21.1 and 21.3), or esters of closely related structure. Chromatography on polar columns was less informative but showed the presence of palmitic, stearic, oleic, linoleic, and arachidic esters in addition to the conjugated compounds.

Chromatographed on thin layers of silica, chryso esters separated into two bands: so did oiticica esters, but parinarium esters gave only an upper band. This procedure effectively separates non-oxygenated esters (upper band) from oxygenated esters (lower band). Both fractions still contained esters with conjugated triene and tetraene absorption (table 1) but the esters in the upper band no longer showed carbonyl absorption at 1720 cm^{-1}. There was considerable enrichment of conjugated tetraene in the (lower) oxygenated fraction. G.L.C. (table 2) confirmed that a useful separation had been effected.

The chryso esters were also separated by silver ion thin layer chromatography into seven bands corresponding to two oxygenated esters, linoleate, conjugated tetraenoate, conjugated trienoate, oleate, and saturated esters. Difficulty in making the bands of conjugated esters visible with dichlorofluorescein was overcome by exposure to ammonia vapour as described in the experimental section. A more useful separation of conjugated triene and tetraene esters was obtained by applying silver ion chromatography separately to the non-oxygenated and oxygenated esters.

Chryso octadecatrienoic ester

This was isolated by thin layer chromatography, first on silica and then on silica impregnated with silver nitrate. Its ultraviolet spectrum (table 1) showed it to be ~96% pure; on an ApL column it gave a large peak at 19.2, a smaller one at 19.45, and a very small peak at 17.6. Its acid melted at 46.5° (α-eleostearic acid m.p. 48–49°) and when oxidised by the von Rudloff procedure[3]) it gave azelaic acid as the only dibasic acid. The ester readily forms a maleic anhydride adduct which also gave azelaic acid when oxidised. The triene ester had a strong absorption band at 988 cm^{-1} and a weak band at

TABLE 1

Ultraviolet absorption (λ_{max} and $E^{1\%}_{1cm}$) of various fractions.

λ_{max} (mμ)	260	270	281	290	304	319
Chryso oil	459	618	636	548	707	636
Chryso esters	484	645	672	537	690	618
non-oxygenated esters	435	582	509	–	231	203
oxygenated esters	540	805	947	1071	1531	1380
Chryso trienoate	1230	1621	1266	–	–	–
Chryso tetraenoate	–	–	–	1560	2345	2118
Chryso oxo-trienoate	1260	1680	1320	–	–	–
Chryso oxo-tetraenoate	–	–	–	1524	2284	2056
P. laurinum esters	421	610	800	1053	1537	1379
P. laurinum trienoate	1297	1726	1352	–	–	–
P. laurinum tetraenoate	–	–	–	1658	2485	2257
C_{18}-trienoate*	–	1694	–	–	–	–
C_{18}-tetraenoate*	–	(388)	–	–	2503	–
C_{18}-oxo-trienoate*	–	1638	–	–	–	–
C_{18}-oxo-tetraenoate*	–	(371)	–	–	2387	–

* These values, obtained from results quoted in reference [9]), are used to calculate the component esters.

TABLE 2

Carbon numbers (ApL) of methyl esters of seed oils and selected fractions.

Carbon number	16.0	17.6	18.0	19.1	19.5	19.9	20.3	20.6	21.1	21.3
Chryso esters	+	+	+	+	+	+	+	+	+	+
Non-oxygenated esters	+	+	+	+	+	+	–	–	–	–
Oxygenated esters	–	–	–	–	–	–	+	+	+	+
Oiticica esters	+	+	+	+	+	–	+	+	–	–
P. laurinum esters	+	+	+	+	+	+	+	–	–	–

962 cm^{-1} but its maleic anhydride adduct showed little or no absorption in this region. All this indicates that the acid is octadeca-*cis*-9, *trans*-11, *trans*-13-trienoic acid, identical with α-eleostearic acid and this was confirmed by partial reduction with hydrazine. This furnished a mixture of stearate, *cis* and *trans* monoenoates, and conjugated dienoates and trienoate which was separated by silver ion chromatography. The fraction containing only *trans* monoene and *trans-trans* conjugated diene gave C_{11} and C_{13} dibasic acids when oxidised; the fraction containing only *cis* monoene and *cis-trans* conjugated diene gave azelaic acid as the only dibasic acid when oxidised.

TABLE 3

Carbon numbers of isomeric octadecenoates resulting during partial reduction (hydrazine) of α-eleostearic acid.

	Carbon numbers		
Total reduction product[1]	17.65	17.76	17.81
	17.61	17.72	17.77
Cis monoenoates	17.65	–	–
Trans monoenoates[2, 3]	–	17.78	17.83
	–	17.71	17.77
Synthetic Δ^9, Δ^{11}, and Δ^{13} octadecenoates[4])			
cis isomers	17.63	17.68	17.80
trans isomers	17.74	17.78	17.80

[1] With added 9*c* isomer the same three peaks were observed but the first one was enlarged.
[2] With added 9*c* isomer a third peak appeared *before* the two already present.
[3] With added 9*c* and 9*t* isomers two additional peaks appeared *before* the two already present.

The three monoenoates produced during partial reduction with hydrazine were also identified by a combination of thin layer and gas–liquid chromatography without recourse to oxidative fission. G.L.C. was carried out on a capillary column (50 m) coated with ApL. On this column conjugated dienoates and trienoates have carbon numbers greater than 18.0 but *cis* and *trans* monoenoates are eluted before stearate. The results summarised in table 3 show that the reduction mixture contained the 9*c*, 11*t*, and 13*t* octadecenoic isomers.

Chryso octadecatetraenoic ester

The tetraenoic ester, again isolated by thin layer chromatography on silica and on silica/silver nitrate, showed the expected ultraviolet absorption (table 1) and was about 94% pure. Oxidation gave only azelaic acid so it must be the 9,11,13,15-tetraene. Partial reduction with hydrazine gives a mixture of stearate, octadecenoates, and conjugated dienoates and trienoates. Treatment with maleic anhydride removes part of the dienoate (the *t,t* isomers) and all the trienoates which must therefore be *t,t,t* and/or *c,t,t*, isomers. When oxidised, the *trans* monoene fraction gave only C_{11} and C_{13} dibasic acids whilst the *cis* monoene fraction gave a high proportion of C_9 acid along with some C_{15} and a little C_{13} dibasic acid. This last probably arises from the 13*t*,15*c*-dienoate which, along with the 9*c*,11*t* isomer, contaminates the *cis* monoene fraction. The tetraene must therefore have the 9*c*,11*t*,13*t*,15*c* configuration. Its infrared spectrum showed strong absorption at 993 cm^{-1} and a weaker absorption band at 951 cm^{-1}.

Chryso oxo-octadecatrienoic ester

The two oxygenated esters, separated from non-oxygenated esters by chromatography on thin layers of silica, were further segregated, by silver ion chromatography, into an upper band containing a triene ester and a lower band containing a tetraene ester (see table 1 for ultraviolet absorption).

The trienoic oxo-ester showed ester (1748 cm^{-1}) and carbonyl (1726 cm^{-1}) absorption bands and also bands at 998 cm^{-1} (strong) and 968 cm^{-1} (weak), indicative of conjugated *c,t,t* unsaturation. On an ApL column the ester showed a major peak at 20.3 and a smaller one at 20.6; after hydrogenation there was only a single peak at 19.2. The hydrogenated ester melted at 47.5° and its acid at 95.5–96.2° and these values were unchanged when the samples were mixed with hydrogenated licanic ester and its acid, prepared from oiticica oil. The hydrogenated oxo triene, hydrogenated oxo tetraene, and hydrogenated licanic ester gave mainly C_{14} and C_{15} monobasic acids when separately oxidised with chromic acid. All three are thereby shown to be derivatives of 4-oxostearic acid.

The oxo trienoic ester readily formed a maleic anhydride adduct which, from its infrared spectrum, no longer contains *trans* unsaturation. The triene ester, its maleic anhydride adduct, and licanic ester, when oxidised by the von Rudloff procedure gave the same major product, 4-oxo-azelaic acid. Its ester has a carbon number of 12.9 (ApL). These results prove that the oxo trienoic acid is identical with licanic acid which is again proved to be 4-oxo-octadeca-*cis*-9,*trans*-11,*trans*-13-trienoic acid.

TABLE 4

Component methyl esters (% wt.) derived from *Chrysobalanus icaco* and *Parinarium laurinum** seed oils.

	C.i	*P.l*
Palmitic	4%	4%
Stearic	18	7
Oleic	11	2
Linoleic	6	2
Arachidic	1	–
Octadecadienoic (conjugated)	–	1
Eleostearic (9*c*, 11*t*, 13*t*)	22	22
Parinaric (9*c*, 11*t*, 13*t*, 15*c*)	10	62
Licanic (9*c*, 11*t*, 13*t*)	10	–
4-oxoparinaric (9*c*, 11*t*, 13*t*, 15*c*)	18	–

* For previous analyses of *P. laurinum* seed oil see references [1, 2, 5].

Chryso oxo-octadecatetraenoic ester

The ultraviolet and infrared spectra of this compound show it to contain ester and carbonyl groups and a conjugated tetraene system. Its infrared spectrum in the 950–1000 cm^{-1} region is identical with that for the non-oxygenated tetraene and both tetraenes are therefore considered to have the same *c,t,t,c* configuration. It was shown to be a derivative of 4-oxostearic acid (see above) and since von Rudloff oxidation gave the same product as did licanic ester (4-oxo-azelaic ester of carbon number 12.9 on an ApL column) it is concluded that this acid is 4-oxo-octadeca-*cis*-9,*trans*-11,*trans*-13,*cis*-15-tetraenoic acid. It has the same structural relationship to α-parinaric acid as licanic acid has to α-eleostearic acid.

$$CH_3\cdot[CH_2]_3\cdot\overset{t}{C}H=\overset{t}{C}H\cdot CH=\overset{c}{C}H\cdot[CH_2]_7\cdot CO_2H$$

α-eleostearic acid

$$CH_3\cdot CH_2\cdot\overset{c}{C}H=\overset{t}{C}H\cdot CH=\overset{t}{C}H\cdot CH=\overset{c}{C}H\cdot[CH_2]_7\cdot CO_2H$$

α-parinaric acid

$$CH_3\cdot[CH_2]_3\cdot\overset{t}{C}H=\overset{t}{C}H\cdot CH=\overset{c}{C}H\cdot[CH_2]_4\cdot CO\cdot CH_2\cdot CH_2\cdot CO_2H$$

α-licanic acid

$$CH_3\cdot CH_2\cdot\overset{c}{C}H=\overset{t}{C}H\cdot CH=\overset{t}{C}H\cdot CH=\overset{c}{C}H\cdot[CH_2]_4\cdot CO\cdot CH_2\cdot CH_2\cdot CO_2H$$

new oxo tetraenoic acid from chryso oil

The component acids of chryso oil

Quantitative results are obtained by combining information about the ultraviolet spectra, the preparative separation of oxygenated and non-oxygenated esters on silica, and G.L.C. results. The conclusions given in table 4 show that this oil contains 40% of non-conjugated acids, 32% of non-oxygenated conjugated acids, and 28% of oxygenated conjugated acids.

Parinarium laurinum *seed oil*

We examined this oil at the same time, partly to provide a control for our study of the chryso oil, and partly to settle certain points about parinarium oil which had not been clarified in previous investigations[5]).

The conjugated trienoic and tetraenoic esters were isolated by silver ion chromatography. Their spectra, both ultraviolet (table 1) and infrared, were identical with those of the α-eleostearic acid and α-parinaric acid present in chryso oil.

Both acids were examined by the partial reduction process which has already been elaborated. This confirmed that the trienoic acid is α-eleostearic acid and demonstrated for the first time that parinaric acid from this source has the 9*c*,11*t*,13*t*,15*c* configuration.

Conclusion

It has been demonstrated recently that the parinaric acid in the seed oils of *Impatiens balsamina* (garden balsam)[6] and *I. edgeworthii*[7] is the 9*c*,11*t*, 13*t*,15*c* isomer and our studies show that this same isomer is present in two further seed oils; *P. laurinum* and *C. icaco*. Earlier investigators of the tetraene acid in *P. laurinum* seed oil rejected this structure because parinaric acid does not readily form a maleic anhydride adduct though this has now been achieved[7]). This configuration had been predicted on the assumption that parinaric acid is formed biosynthetically from linolenic acid[8]). It is of interest that only one conjugated octadecatetraenoic acid has been identified whilst five isomeric conjugated octadecatrienoic acids are now known to occur naturally.

There is growing evidence that many highly unsaturated straight-chain compounds arise from unsaturated C_{18} acids such as oleic, linoleic, and crepenynic by processes of desaturation and chain-shortening[11]). The predominance of C_{14} members (and C_{13} compounds arising from them by decarboxylation) in plants and of C_{10} and C_9 members in micro-organisms[12]) suggests that loss of four carbon atoms is a common occurrence. This could result from two β-oxidation sequences or, alternatively, through a single cycle of reactions proceeding through 4-oxo acids of the type present in the oils we have been examining.

Experimental

Wherever possible reactions were carried out under nitrogen and samples were stored at 0° in a nitrogen atmosphere.

Oil was extracted from crushed seeds with ether in a Soxhlet extractor until no more oil was removed. The kernels of *C. icaco* and *P. laurinum* gave 51% and 17% of oil respectively.

Infrared spectra were recorded on liquids or carbon tetrachloride solutions using Perkin Elmer spectrophotometers (137 or 621); ultraviolet spectra were measured using methanolic solutions and a Unicam SP. 800.

Gas liquid chromatography was carried out with a Perkin Elmer Fractometer using an Apiezon L (5%, 1 m) or diethyleneglycol succinate column (20%, 2 m). The capillary column was 50 m long, coated with Apiezon L, and fitted into a Perkin Elmer F11 chromatograph.

Preparative thin layer chromatography was effected on 20 cm × 20 cm

plates with 1 mm layers of silica or of silica and silver nitrate (20%). Plates were activated by heating to 105° for 2 hr. Thinner layers were used for analytical purposes. When the plates were sprayed with dichlorofluorescein the separated bands were clearly visible on the silica layer but the esters with conjugated unsaturation did not show clearly on the silver ion plates. This difficulty was overcome by placing the sprayed plates in a tank containing 2 ml of concentrated ammonia. Esters were extracted from the silica by extraction with ether in a Soxhlet and from silica/silver nitrate with benzene or mixtures of ether and benzene.

Glycerides were converted into methyl esters by interaction at room temperature overnight with an excess of 0.17 molar methanolic sodium methoxide[10]). Acids were converted to methyl esters by reaction with methanolic boron trifluoride.

Von Rudloff oxidations were carried out with mixtures of potassium permanganate and sodium periodate in aqueous *tert*-butanol solution[3]). The oxidation products were recovered, methylated, and identified by gas liquid chromatography.

Isolation of the four conjugated unsaturated acids from chryso oil

Chryso oil (1.86 g) was converted to its methyl esters (1.65 g) which showed oxo (1720 cm^{-1}) and ester (1743 cm^{-1}) carbonyl bands in the infrared and a characteristic ultraviolet spectrum (table 1). These esters were examined by G.L.C. on ApL (table 2) and DEGS columns (see discussion).

Using ten silica plates the esters (1.36 g) were separated into non-oxygenated (0.87 g) and oxygenated esters (0.34 g) by development with a 1:1 mixture of ether and petrol (b.p. 40–60°). Both showed ester carbonyl bands (1743 cm^{-1}) but only the oxygenated ester fraction showed an oxo carbonyl band (1720 cm^{-1}). These two fractions were subsequently re-chromatographed on silver ion plates using ether-petrol mixtures as developing solvents (1:3 for the non-oxygenated ester, 1:1 for the oxygenated esters). Information about the ultraviolet spectra of these separated esters is recorded in table 1.

Chryso octadecatrienoic acid

The ester isolated from the silver ion plates was hydrolysed with methanolic potassium hydroxide at room temperature (15 hr). After crystallization from petrol the acid melted at 46.5°. It gave azelaic acid as the only dibasic acid when oxidised.

Refluxed with maleic anhydride (40 mg) in benzene solution the trienoic acid (20 mg) furnished an adduct which showed no absorption in the 950–975 cm^{-1} region and gave azelaic acid as the sole dibasic acid when oxidised.

Partial reduction: A mixture of the trienoic ester (11 mg), a 15% methanolic solution of hydrazine hydrate (6 ml), and acetic acid (2 drops) was stirred in an open flask for 2 hr at 45–50°. The mixture was then acidified with dilute hydrochloric acid and extracted with ether. G.L.C. examination showed the presence of stearate, monoenoates, and conjugated dienoates. This mixture was separated by silver ion chromatography using a 1:7 ether-petrol mixture, and bands isolated corresponding to *cis* monoene (along with conjugated *cis-trans* diene) and *trans* monoene (along with conjugated *trans-trans* diene). Oxidation of these fractions gave azelaic acid from the *cis* monoene and undecanedioic and tridecanedioic acid from the *trans* monoenes. The reduction products were also examined by capillary G.L.C. (table 3).

Chryso octadecatetraenoic acid

Oxidation of this acid gave azelaic acid as the only dibasic acid.

Partial reduction of the tetraene ester (30 mg) was effected as already described except that oxygen was bubbled through the reaction mixture during reduction. To remove conjugated *trans trans* diene which contaminates the *trans* monoenes even after silver ion chromatography, the reaction product was refluxed with maleic anhydride (40 mg) in benzene (5 ml) for 2 hr. Thereafter the recovered product was separated by silver ion chromatography and appropriate fractions oxidised. The *trans* monoene band gave only C_{11} and C_{13} dibasic acids; the *cis* monoene band gave much azelaic acid, a little C_{15} dibasic acid, and a small amount of C_{13} dibasic acid which is considered to result from the *cis-trans* dienes (9,11 and 13,15) which contaminate this fraction.

Chryso oxo acids

The oxygenated esters isolated by thin layer chromatography on silica were separated by silver ion chromatography into two fractions consisting of conjugated triene and conjugated tetraene (table 1).

The triene ester (9 mg), hydrogenated in acetic acid solution using palladium charcoal (5 mg) as catalyst, furnished an oxo stearate which gave a single peak on an ApL column (carbon number 19.2) and whose infrared spectrum showed strong absorption for oxo and ester groups. This saturated ester melted at 47.5° after crystallisation from petrol and the acid derived from it had m.p. 95.5–96.2° after crystallization from ether-petrol mixture.

Samples of 4-oxostearic ester and acid obtained from authentic licanic acid isolated from oiticica oil had melting points of 48° and 96–96.5°. Mixed melting points of 48° and 95.8° were observed. Identical products were obtained from the chryso oxotetraenoic ester.

The triene ester readily formed an adduct with maleic anhydride when these two reagents were refluxed in benzene for 2 hr.

The triene ester, licanic ester, the maleic anhydride adducts of both these, and the tetraenoic ester were oxidised by the von Rudloff procedure. All five gave the same product of carbon number 12.9 (ApL) and this is believed to be 4-oxo-azelaic acid.

Chromium trioxide (124 mg), dissolved in acetic acid (1.0 ml) and water (0.1 ml), was gradually added to a stirred solution of oxostearate (7 mg) in acetic acid (1 ml). After four hours the mixture was diluted with water, extracted with ether, and the organic product methylated. G.L.C. showed it to consist mainly of tetradecanoic and pentadecanoic esters along with unreacted oxostearate. Identical results were obtained with the oxostearate from licanic acid and from chryso oxo-trienoic and tetraenoic acids.

Conjugated trienoic and tetraenoic esters in Parinarium laurinum *seed oil*

The oil was converted to its methyl esters and the trienoic and tetraenoic compounds isolated by silver ion chromatography. These were shown to be identical with the corresponding acids from chryso oil on the basis of their ultraviolet (table 1) and infrared spectra, their oxidation to azelaic acid, and the products resulting from partial reduction with hydrazine.

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