by the straight line curve drawn from the plot of percentage of cheuri fat vs R. Table III shows the actual and experimental values of cheuri fat.

Although the color present in a vegetable fat or oil is unstable, the presence of this band in a three-year-old sample is quite interesting. As such, it can be suggested that presence of band in visible spectra of ghee may be due to adulteration and if the band is between the 640 to 680 nm region, it may be due to cheuri fat. This information, along with other physical constants and values, would be helpful in confirming the presence of cheuri fat in ghee.

ACKNOWLEDGMENT

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Functionalization at the Double Bond Region of Jojoba Oil: I. Bromine Derivatives

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ABSTRACT

Addition of bromine to jojoba oil and its *trans*-isomer yielded tetrabromojojoba, which upon elimination afforded the acetylenic and allenic components, respectively, when reacted with excess of base. The bromoolefinic products were obtained when limited amount of base was used. Allylic bromination of the liquid wax and its *trans*-isomer, and subsequent HBr elimination, yielded the two conjugated diene systems on both parts of the ester (jojoba tetraene).

INTRODUCTION

Jojoba oil (I) is a promising agricultural product, as are its derivatives (1,2). Its chemical structure dictates the chemical reactions and the possible products and derivatives which can be derived from it. In our previous studies, we have concentrated on the ester function of the wax and prepared the quaternary ammonium salts (3), the amide (4) and muscalure (5). Introduction of new groups at the double bond region enables one to transform the hydrophobic wax into much more polar compounds with different chemical, physical and technological properties. In this paper, we present the results of bromination reactions at the double bond region on the natural oil with Z-double bonds, as well as the isomerized oil, with E-double bonds. Elimination of HBr from these products increases unsaturation of the hydrophobic ester.

$$(\underline{Z},\underline{Z})\text{-}CH_3(CH_2)_7CH{=}CH(CH_2)_mC(=O)O(CH_2)_nCH{=}CH(CH_2)_7CH_3$$

I: m = 7,9,11,13; n = 8,10,12,14.

EXPERIMENTAL PROCEDURES

General

The general procedure of solvent purification, work-up of reactions and nuclear magnetic resonance (NMR) technique for monitoring the reactions have been described earlier (3-5). LiCl and Li_2CO_3 (CP) were dried prior to the reaction, at 120-130 C for 2 hr. Melting points were taken on a Thomas Hoover Capillary melting point apparatus, and are not corrected. The NMR spectra were determined on a Varian XL-100 in CCl_4 or $CDCl_3$ solution, with chemical shifts expressed in δ. The infrared (IR) spectra were determined with a Perkin Elmer 377, the sample usually being neat or in $CHCl_3$ solution. The ultraviolet (UV) spectra were taken on a Bausch and Lomb Spectronic 210 in C_6H_{12} solution. Iodine value (Wijs) of 81.5 indicates ca. 95-96% double bond equivalence. Microanalyses were performed in the Microanalytical Laboratory of the Applied Research Institute, Ben-Gurion University of the Negev, Israel.

Double Bond Isomerization and Separation of *trans*-Jojoba (Ia)

A solution of 500 g of jojoba oil in 500 mL petroleum ether (60-80 C) and 50 mL of 2 M $NaNO_2$ was heated until reflux and then 16 mL of 6 M HNO_3 was added by drops within 5 min, after which heating was continued for 15-20 min. The hot solution was immediately transferred to a separatory funnel and was washed with hot water (50 C) (5 × 50 mL), until pH 7 was reached. The solvent was evaporated and the residue was left in a beaker to solidify

(mp 48-51 C). Chromatography of a sample of the crude product (1.8 g) on 30% $AgNO_3$ on silica gel (140 g) yielded 1.32 g of *trans*-jojoba, which was eluted by 2% ether in petroleum ether. The mp of the purest fraction was 53-55 C. NMR spectrum was identical to that of jojoba oil, except for the olefinic hydrogens, which appeared at 5.25 as multiplet.

Hydrolysis of Jojoba Oil (I) and *trans*-jojoba (Ia)

A mixture of 3 g of jojoba oil (0.005 mol), 3 g KOH, 10 mL H_2O and 30 mL *i*-PrOH was heated at 60 C for 4-5 hr. The crude reaction mixture was extracted with petroleum ether to yield 1.2 g jojobyl alcohol (**III**). The residue was acidified and extracted with $CHCl_3$ to yield 1.0 g jojoboic acid (**II**): IR 3400-2700, 1700 cm^{-1}; NMR 5.20 (2H, -C<u>H</u>=C<u>H</u>-, q, J=5 cps), 2.22 (2H, -C<u>H</u>$_2$COOH, t, J=7 cps), 1.92 (4H, allylic H).

The same procedure yielded **IIa** and **IIIa** from **Ia**. Acid **IIa**, mp 47-50 C (from CH_3OH); IR 3350-2700, 1700, 970 cm^{-1}; NMR 5.20 (2H, -C<u>H</u>=C<u>H</u>-, m), 2.30 (2H, -C<u>H</u>$_2$-COOH, t, J=7 cps), 1.96 (4H, allylic H). Alcohol **IIIa**, mp 49-51 C (from CH_3OH); IR 3400-3300, 970 cm^{-1}; NMR 5.20 (2H, -C<u>H</u>=C<u>H</u>, m), 3.46 (2H, -C<u>H</u>$_2$OH, t, J=7 cps), 1.92 (4H, allylic H).

Dibromojojobyl Alcohol IV, and IVa

A solution of 4.8 g Br_2 (0.03 mol) in 15 mL CCl_4 was added to a solution of 8.7 g jojobyl alcohol (**III**) (0.03 mol) in 50 mL CCl_4 at 5 C. Density of the crude product, **IV**, (13.5 g) is 1.04 g/cm^3, n_D^{27} 1.4948; IR 3300 cm^{-1}; NMR 4.14 (2H, -C<u>H</u>(Br)-, m), 3.54 (2H, -C<u>H</u>$_2$OH, t, J=7 cps). Calcd. for Br 34.8%; found 33.4%.

The same procedure on **IIIc** ($LiAlH_4$-reduction of ester functionality of *trans*-jojoba [**Ia**]) yielded **IVa**: IR 3300 cm^{-1}; NMR 4.10 (2H, -C<u>H</u>(Br)-, m), 3.52 (2H, -C<u>H</u>$_2$OH, t, J=7 cps). Calcd. for Br 34.8%; found 34.1%.

Tetrabromojojoba (V) and Tetrabromo-*trans*-jojoba (Va) (Scheme I)

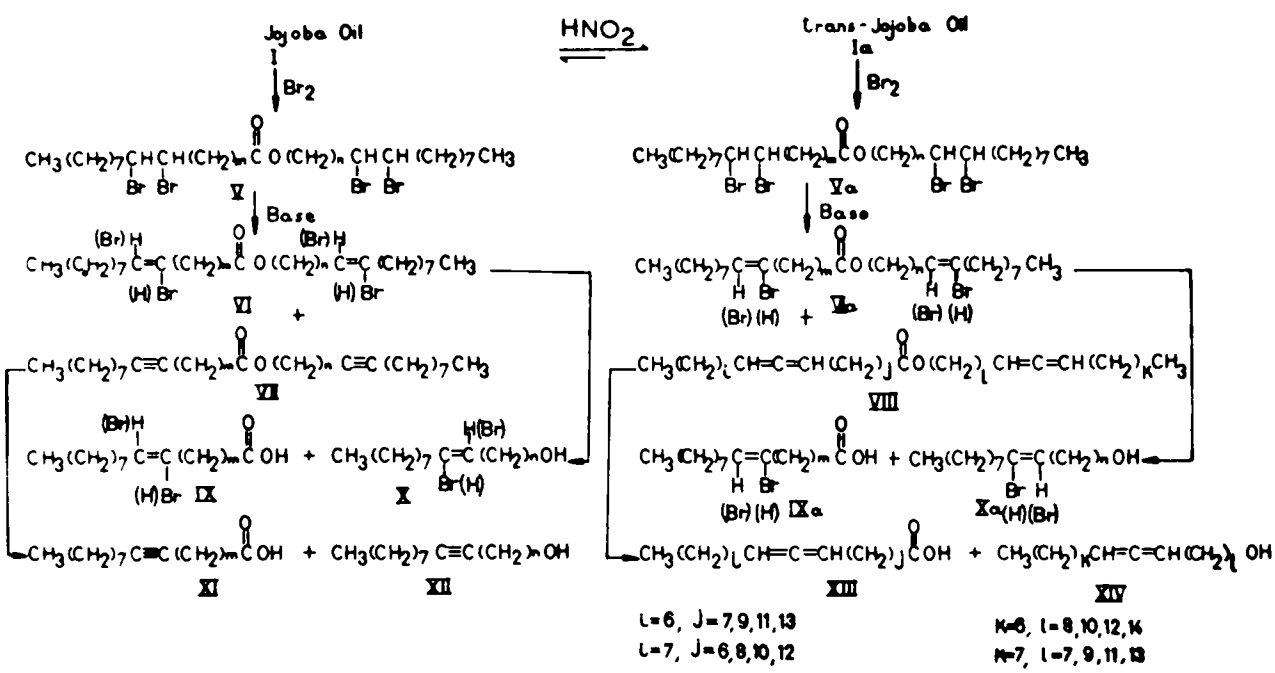
To a solution of 59 g jojoba oil (0.1 mol) in 250 mL CCl_4 a solution of 32 g Br_2 (0.2 mol) in 50 mL CCl_4 was added by drops. The inside temperature was kept at 17-20 C by cold water bath and the addition took 3 hr. The reaction mixture was almost colorless during the addition, but at the end, a small amount of unreacted bromine was left. The colored solution was washed with Na_2SO_3 solution and after work-up yielded 91 g of **V**, density, 1.19 g/cm^3, n_D^{21} 1.4909; NMR 4.15 (4H, -C<u>H</u>(Br)-,d,d, J=10 and 4 cps), 3.98 (2H, -C<u>H</u>$_2$OCO, t, J=7 cps), 2.26 (2H, -C<u>H</u>$_2$COO, t, J=7 cps). Calcd. for Br, 35.2%; found, 35.4%.

The same procedure on **Ia** yielded **Va**; NMR, 3.98-4.10 (4H, -C<u>H</u>(Br)-, bd, J=6 cps), partial overlapping on 3.94 (2H, -C<u>H</u>$_2$OCO, t, J=7 cps), 2.20 (2H, -C<u>H</u>$_2$COO, t, J=7 cps). Calcd. for Br, 35.2%; found 34.6%.

Reactions of Tetrabromojojoba (V) and Tetrabromo-*trans*-jojoba (Va) with Different Bases

KOH. A solution of 0.91 g of **V** (0.001 mol), 1.0 g KOH, 15 mL *i*-PrOH, 5 mL H_2O and 2 drops of tetrabutyl phosphonium chloride was stirred for 18 hr at 60 C. Water was added and the crude mixture was extracted with petroleum ether to yield 0.2 g of bromoalcohol **X**, IR 3400 cm^{-1}; NMR 5.56 (1H, -C<u>H</u>=C(Br)-, t, J=7 cps), 3.50 (2H, -C<u>H</u>$_2$OH, t, J=7 cps), 2.40 (2H, -C<u>H</u>$_2$C(Br)=CH-, t, J=7 cps), 2.12 (2H, -C<u>H</u>$_2$CH=C(Br)-, t, J=7 cps), calcd. for Br, 20.5%; found 19.7%. Acidification of the aqueous phase and extraction with $CHCl_3$ yielded 0.220 g of bromoacid **IX**, IR 3400-2700, 1700 cm^{-1}; NMR 5.54 (1H, -C<u>H</u>=C(Br)-, t, J=7 cps), 2.36 (2H, -C<u>H</u>$_2$C(Br)=CH-, t, J=7 cps), 2.30 (2H, -C<u>H</u>$_2$COOH, t, J=7 cps), 2.08 (2H, -C<u>H</u>$_2$CH=C(Br), t, J=7 cps). Calcd. for Br, 20.9%; found 19.9%. The products did not show absorption in the UV above 210 nm.

NH_4OH. A suspension of 10 g of **V** in 50 mL concentrated NH_4OH in a sealed steel reactor lined with teflon (4) was heated in an oil bath at 155-160 C for 50 hr, after which the reaction mixture was extracted with petroleum ether to yield 6 g of crude product **VI**: NMR 5.54 (2H, -C<u>H</u>=C(Br)-, t, J=7 cps) on top of an "envelope" ranging from 6.05 to 5.10 (integration fits to 1H), 4.00 (2H, -C<u>H</u>$_2$OCO, t, J=7 cps), 3.50 (-C<u>H</u>$_2$OH, t, J=7 cps, integration fits to amount of 10-15% of alcohol), 1.90-2.46 (10 H, m). Calcd. for Br,



SCHEME 1.

21.3%; found 13.5%.

$NaNH_2$. A solution of 20 g of **V** (0.022 mol) in 40 mL dry THF was added to $NaNH_2$ (prepared from 2 g of Na [0.087 mol] in 40 mL liquid NH_3 and crystal of $Fe(NO_3)_3$), stirred for 4 hr and left overnight. Addition of 10 mL ethanol, 20 mL of 20% NH_4Cl solution and extraction with petroleum ether yielded 11 g of crude product: NMR 5.54 (2H, -CH=C(Br)-, t, J=7 cps), 3.50 (2H, $-CH_2OH$, t, J = 7 cps), 2.38 (4H, $-CH_2C(Br)=CH-$, t, J=7 cps), 1.90-2.25 (6H, $-CH_2CONH_2$, $-CH_2CH=C(Br)-$, m).

t-BuOK (6:1 ratio). A solution of 1.8 g of **V** (0.002 mol) and 1.33 g of *t*-BuOK (0.012 mol) in 25 mL dry DMSO was heated at 100 C for 5 hr. Usual work-up and separation yielded 0.3 g acid **XI**: IR 3400-2700 cm^{-1}; NMR 2.24-1.95 (6H, $-CH_2C{\equiv}CCH_2-$, $-CH_2COOH$, m); and 0.350 g alcohol **XII**: IR 3300 cm^{-1}; NMR 3.42 (2H, $-CH_2OH$, t, J=7 cps), 2.05-1.95 (4H, $-CH_2C{\equiv}CCH_2-$, m). Both products contained less than 0.5% Br; and contained up to 5-6% of allenic products **XIII** and **XIV**. The same quantities of **Va** and reagents, and same procedure yielded the acid **XIII**; IR 3400-2700, 1925, 890 cm^{-1}; NMR 4.96 (2H, $-CH=C=CH-$, q, 5 cps), 1.90-2.24 (6H, allylic and $-CH_2COOH$, m); and alcohol **XIV**, IR 3300, 1925, 890 cm^{-1}; NMR 4.96 (2H, $-CH=C=CH-$, q, 5 cps), 3.50 (2H, $-CH_2OH$, t, J=7 cps), 1.95-2.05 (4H, $-CH_2CH=C=CHCH_2-$, m).

t-BuOK (2.5:1 ratio). A solution of 0.9 g of **V** (0.001 mol) and 0.28 g of *t*-BuOK (0.0025 mol) in 15 mL dry DMSO was heated at 100 C for 4-5 hr. Usual work-up yielded 0.6 g of **VI**, IR 1710, 740 cm^{-1}; NMR 5.54 (2H, $-\underset{\underset{Br}{|}}{\overset{\overset{H}{|}}{C}}=C-$, t, J=7 cps), 3.96 (1.6 H, $-CH_2OCO$, t, J=7 cps), 2.30 (3.6H, $-CH_2C(Br)=CH-$, t, J=7 cps), "contaminated" with 10-20% of the acetylenic product **VII**. Calcd. for Br, 21.3%; found 18.5%.

The same reaction on **Va** yielded 0.570 g of **VIa**; IR 1710, 740 cm^{-1}; NMR 5.66 (1.6H, $-\underset{\underset{H\ Br}{|\ |}}{C=C}-$, t, J=7 cps), 5.52 (0.4H, $-\underset{\underset{Br}{|}}{\overset{\overset{H}{|}}{C}}=C-$, t, J=7 cps), 3.90 (2H, $-CH_2OCO$, t, J=7 cps), 2.35 (4H, $-CH_2C(Br)=CH-$, t, J=7 cps). Calcd. for Br, 21.3%; found 18.7%.

NBS Bromination of I and Ia to Yield XV (XV a) (Scheme II)

A solution of 11.8 g of jojoba oil (0.02 mol) and 7.1 g NBS (0.04 mol) in 100 mL CCl_4 was refluxed for 4 hr. Filtration of succinimide and evaporation of the solvent yielded 14.8 g of crude **XV** (allylic bromides): IR, 980 and 740 cm^{-1}; NMR 5.30-5.80 (3.9-4H, $-CH=CH-$, m, with an intense doublet at the center, 5.58, J=4 cps); 4.60-4.80 (10-15% of 2H, $-CH(Br)CH=CHCH(Br)-$, m), 4.42 (1.7-1.8H, $-(Br)CHCH=CH-$, m), 4.00 (2H, $-CH_2OCO$, t, J=7 cps), 2.26 (2H, $-CH_2COO$, t, J=7 cps).

The same procedure for **Ia** yielded the same crude product as above, **XV** (identical IR and NMR spectra).

Jojoba Tetraene (XVI, XVIa)

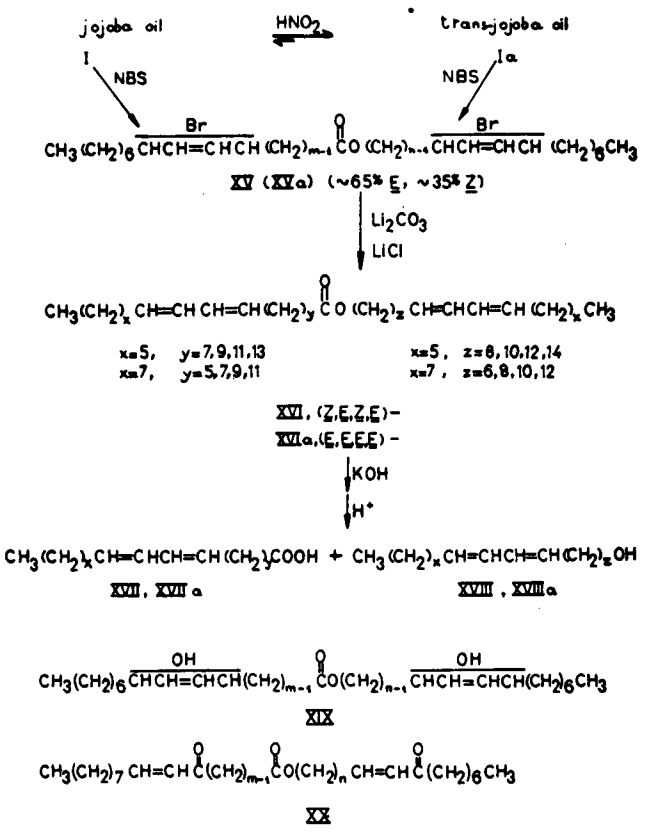
A mixture of 8 g of **XV** (0.011 mol), 8 g of dry LiCl (0.19 mol) and 8 g of dry Li_2CO_3 (0.11 mol) in 100 mL dry dioxane (or chlorobenzene) yielded 6.5 g of crude **XVI-XVIa** (80% elimination as determined by NMR). Purification on TLC plate (elution with 10% ether in petroleum ether solution) yielded **XVI-XVIa** in 80% purity, UV $\lambda_{max}^{C_6H_{12}}$ 230 (ϵ ~20,000). IR 985, 980, 950, 720 cm^{-1}; NMR 6.30-5.02 (6.4-6.5H, $-CH=CH-CH=CH-$, m), 4.40-4.30 (0.4H, $CH(Br)CH=CH$; or $-CH(OH)CH=CH-$), m), 3.95 (2H, $-CH_2OCO-$, t, J=7 cps), 2.20 (2H, $-CH_2COO$, t, J=7 cps); anal. of Br, 4.8-5.3%. Hydrolysis of the crude product (1.1 g) with 0.5 g KOH in 5 mL H_2O and 20 mL *i*-PrOH at room temperature for 20 hr yielded 0.32 g of alcohol **XVIII-XVIIIa** (90-95% purity as determined by NMR); IR 3,330 cm^{-1}; NMR 5.00-6.26 (3.6-3.8H, $-CH=CHCH=CH-$, m) 4.16-4.36 (0.1 H), 3.48 (2H, $-CH_2OH$, t, J=7 cps), 1.90-2.20 (4H, allylic H, m); anal. for Br, 2.9%; and 0.33 g of acid **XVII-XVIIa** (contaminated with hydroxylic products, such as **XIX**); IR 3400-2700, 1700, 985, 965, 720 cm^{-1}; NMR 5.04-6.08 (2-2.2H, $-CH=CHCH=CH-$ and $-CH=CHCH(OH)$, m), 4.00-4.40 (1H, $-CH=CHCH(OH)$, m), 2.26 (2H, $-CH_2COO$, t, J=7 cps), 1.92-2.16 (3H, allylic H, m).

Hydrolysis of XV to XIX (Allylic Alcohols)

Dibromojojoba **XV** (**XVa**) (500 mg) was eluted on thin layer chromatographic (TLC) plates to yield 3 fractions (elution with 30% ether in petroleum ether). 1st fraction, 42 mg, mainly **XVI-XVIa**; 2nd fraction, 96 mg, mainly **XVI-XVIa** and **XIX**. 3rd fraction, 114 mg, **XIX** (75% pure); IR, 3,400, 1700, 970, 725 cm^{-1}; NMR 5.60-6.10 and 5.20-5.30 (olefinic hydrogens of **XV** and **XVI**, 25% as impurities), 5.34-5.50 (4H, $-CH=CH-$, m), 3.80-4.06 (4H, $-CH(OH)CH=CH-$, and $-CH_2OC(O)$, m), 1.90-2.30 (6H, m); anal. for Br, 4.0%.

Oxidation of XIX to XX

A suspension of 50 mg of **XIX** (75% pure) and 0.2 g freshly



SCHEME 2.

prepared MnO_2 in 5 mL petroleum ether was stirred at room temperature for 50 hr and then refluxed for 4 hr to leave a residue of 40 mg of crude **XX** after filtration and washing with $CHCl_3$: IR 3400-3300, 1720, 1670, 1620, 970, 725 cm^{-1}; NMR 5.20-7.00 (4H, -CH=CH-, m) 3.90 (2H, $-CH_2OC(O)$, t, J=7 cps), 1.95-2.50 (10H, m).

RESULTS AND DISCUSSION

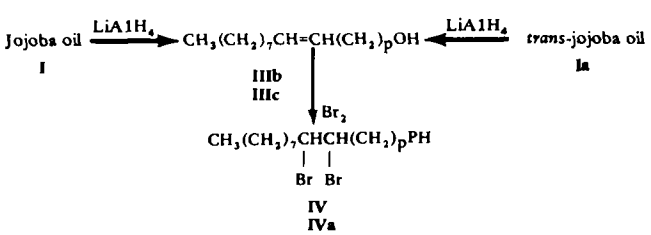
Jojoba oil, as other unsaturated natural fatty acids, contains Z double bonds. The isomerization of the double bonds has been studied earlier (6), and it was found that an equilibrium is attained, composed of 65-70% E and 30-35% of Z double bonds. We tried to enrich the E,E-isomer by column chromatograpy on argentenated silica gel and crystallization of the different fractions. The fractions which contained at least 70% of E-double bonds, as determined by IR (7), melted at 53-55 C. The same results were obtained after repeated purification using a urea inclusion complex (8).

These unsuccessful trials to purify *trans*-jojoba stem from the fact that there are two separate double bonds in one molecule, in which there might be both the original Z, and the E isomer. Thus, any partial separation on the basis of one of the double bonds is balanced by the opposing effect of the other double bond. Only those molecules with two E-double bonds may be separated. The percentage of these molecules is not high, so we do not see Z/E separation. Another reason might be the length of the chain and the distance of the double bonds from the end of the molecule, which reduces the chance of urea separation by inclusion.

In all reactions of *trans*-jojoba, the equilibrium mixture of 65-70% of E-jojoba and 30-35% of Z-jojoba was used (see later). It should be emphasized that many molecules could contain one Z- and one E-double bond along the chain.

Hydrolysis of the *trans*-jojoba yielded *trans*-jojoboic acid (**IIa**) (mp 47-50 C), and the *trans*-jojobyl alcohol (**IIIa**) (mp 49-51 C), whose mp are in the range of pure unsaturated fatty acids and the alcohols, which comprise the esteric mixture.

Introduction of bromine atoms into the molecule enables one to increase the polarity of the oil, to increase its specific gravity and thus form a very hydrophobic and lypophilic solvent heavier than water, and to increase unsaturation by elimination of HBr. We thus added bromine to jojoba oil (**I**), *trans*-jojoba (**Ia**), jojobyl alcohol (**IIIb**) and *trans*-jojobyl alcohol (**IIIc**) (which is formed by $LiAlH_4$ reduction of the ester group of Ia). Different proportions of C_{18}, C_{20}, C_{22} and C_{24} chains in the acidic and alcoholic components of jojoba oil (1) afford different compositions of the alcohol, which is derived either from reduction of the ester function of the oil or its hydrolysis (3). The addition is instantaneous with immediate decoloration of the solution (it is slowed down only at the end of the addition process) to yield tetrabromojojoba (**V**), tetrabromo-*trans*-jojoba (**Va**) (see Scheme I), dibromojojobyl alcohol (**IV**), and dibromo-*trans*-jojobyl alcohol (**IVa**).



The density of **V** and **IV** are 1.19 g/cm^3 and 1.04 g/cm^3 at 20 C, respectively. Tetrabromojojoba (**V**) is stable toward hydrolysis of the bromine atoms even at high temperature (H_2O+ decalin, 110 C) for 120 hr, although slow coloration of the mixture occurs.

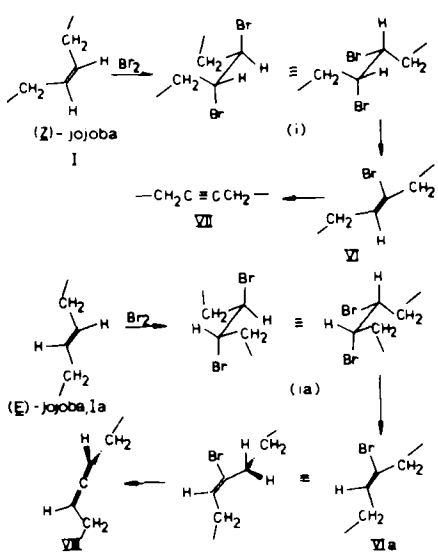
Bromine adds *trans* to double bonds. Thus, jojoba oil (**I**) would yield (i) (partial structure of **V**), whereas **Ia** would form (ia) (partial structure of **Va**) (see Schemes I and III). The more acidic hydrogen is eliminated first, by base, so that **VI** and **VIa** are formed at the initial step of elimination of HBr. In the second step, with more base, anti-elimination in **VI** would lead to acetylene **VII**, or less probably, to the allene **VIII** (9). Anti-elimination in **VIa** would form the allene **VIII**, whereas only syn elimination might lead to acetylene **VII**. We thus expect to get both **VI** and **VII** from **V**, whereas **Va** would form **VIa** and **VIII**, when strong base is applied on each of the isomers of tetrabromojojoba. We were aware of the fact that either hydrolysis, transesterification, or amidation at the ester function might occur under these conditions, when we applied H_2O, ROH or RNH_2.

Different basic reagents, under either mild or more drastic conditions, have been applied (Scheme I). As expected, KOH in H_2O and *i*-PrOH caused both elimination to **VI** and hydrolysis of the ester. Some nucleophilic substitution of Br by OH has taken place. When tetraalkyl ammonium or phosphonium salts were used, the hydrolysis was enhanced at the expense of the elimination reaction. The crude reaction mixture, after complete hydrolysis, was separated into a neutral fraction, which contained bromojojobyl alcohol **X**, and basic aqueous fraction which, upon acidification, yielded bromojojoboic acid **IX**. The NMR spectra clearly showed elimination of only one equivalent of HBr on each side of the ester (see Experimental Procedures).

Reaction of sodamide in liquid ammonia on **V** yielded the same bromolefinic products, together with amidation to yield a mixture of alcohol **X** and the amide of acid **IX**.

Concentrated NH_4OH solution and **V**, under autogenous pressure at 155-165 C (oil bath temperature [4]), led to elimination, together with low conversion (~10-15%) to amide. In this case, too, no acetylenic product was formed. The lower bromine content (13% instead of 21.3%) indicates some bromine exchange by OH.

Excess of *t*-BuOH in DMSO or THF could force complete elimination of 4 mol of HBr from **V** and **VA** to yield



SCHEME 3.

mixtures of the acetylenic acid (**XI**) and alcohol (**XII**), and allenic acid (**XIII**) and alcohol (**XIV**), respectively. When a limited amount of this base was used (2.5 equivalents) **VI** and **VIa** were formed. It is notable that, in the latter case, while **V** yielded **VI** and small amount of **VII**, **Va** yielded a mixture of bromoolefinic products **VIa** (∿75-80%) and **VI** (20-25%), as found in the NMR (see Experimental Procedures). No allenic product **VIII** could be detected in the NMR. The lower content of bromine (18.5-18.7% instead of 21.3%) suggests some acetylenic components production.

The net result is that we now can prepare brominated jojoba oil, which still contains the two double bonds, or their acetylenic and allenic components. The difference in the chemical shifts of the olefinic protons of the bromo-derivatives of Z-jojoba and E-jojoba is notable:

VI, IX, X: -C(H)=C(Br)-, δ 5.54, t, J=7 cps.

VIa, IXa, Xa: -C(H)=C(Br)-, δ 5.66, t, J=7 cps.

This deshielding effect of Br atom on *cis*-olefinic proton has been observed (10).

It seems that the reluctance of the long chain system to react readily with reactive reagents might stem from either folding of the chains, thus forming artificial stereochemical hindrance, or else it is the result of less fruitful collisions, which is expected for long chains and a relatively small reaction center. This retardation effect on the rate, or even some chemical inertness, has already been found by us in the reactions of jojobyl amines and halides (3) or in amidation of jojoba oil (4).

Another approach for introducing bromine, and subsequently unsaturation, is by allylic bromination with NBS. In principle, the bromine atom can be introduced on either side of the double bond. When two equivalents of NBS were applied to one equivalent of **I**, we could detect a small amount of product with two bromine atoms allylic to one

-CH(Br)-CH=CH-CH(Br)-

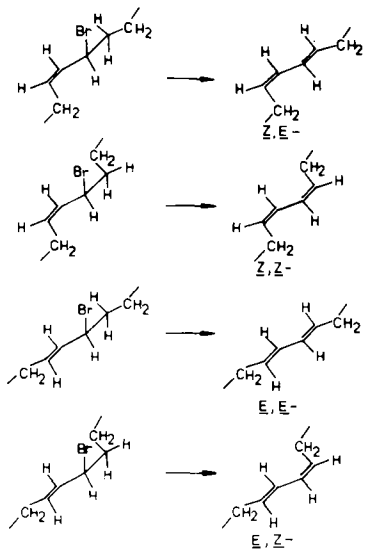
(a)

double bond such as (a), as indicated by the NMR of crude **XV** (δ 4.60-4.80) (see Scheme II and Experimental Procedures). It is notable that isomeration of jojoba oil to *trans*-jojoba (∿65% *trans*), upon reaction with NBS, has taken place. The allylic radical formed is probably sufficiently long-lived to yield the equilibrium mixture.

Elimination of HBr from **XV** might lead, altogether, to a mixture of 4 possible isomers, on each diene unit, of the tetraene chain: Z,E- and E,Z, which are identical for practical purposes, E,E, which will be predominant, and very little of Z,Z-isomer, if at all (see Scheme IV).

Concentrated NH_4OH solution led mostly to nucleophilic substitution of the bromine atoms by OH groups. The percentage of Br dropped to 4-5% (from 21%) and strong absorption of OH in the IR appeared. The NMR spectrum of the olefinic hydrogens changed in shape, but no increase in integration took place. The crude product showed similar polarity on a TLC plate as jojobyl alcohol (R_f 0.38 in 20% ether in petroleum ether; jojobyl alcohol 0.31; jojoba oil 0.9).

In other aqueous solutions (KOH+H_2O) some elimination took place, but the major path was displacement of the



SCHEME 4.

bromine atoms by OH, to yield **XIX**. We have also found supporting evidence for this process on TLC plates or on basic alumina. Oxidation of the allylic alcohol **XIX** with fresh MnO_2 afforded an unsaturated ketone such as **XX** (one of 4 possible isomers).

Heating with *t*-BuOK in DMSO was needed in order to achieve elimination, together with alcoholysis, as occurred with tetrabromojojoba (V) (see above).

The best results were obtained by refluxing **XV** (or **XVa**) with Li_2CO_3 and LiCl in polar and aprotic solvent such as dioxane, DMF, DMSO or chlorobenzene, to yield **XVI-**

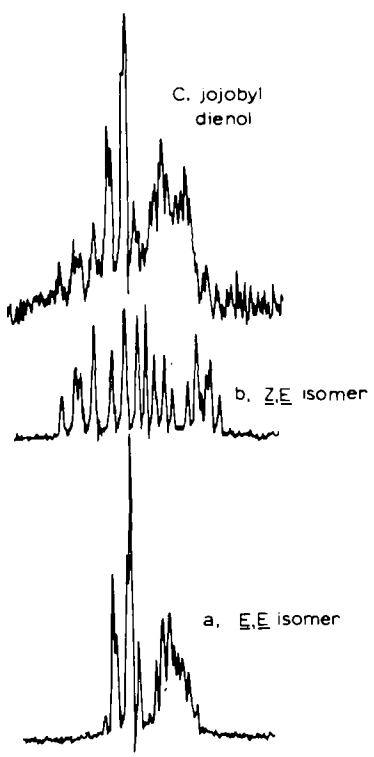


FIG. 1. NMR spectrum of the olefinic hydrogens region of XVI-XVIa, as compared with pure (E,E)- and (Z,E)-isomers (Shani and Klug, unpublished data; see also ref. 11).

XVIa mixture. The product contained ca. 80% of tetraene which was "contaminated" with residual brominated products, or starting material. Analysis showed 4-5% Br, which fitted well with NMR data: 80% tetraene, based on ratio of olefinic hydrogens to others ($\underline{CH_2}OCO$, allylic H). UV absorption showed the typical high intensity of conjugated diene ($\epsilon_{230} \sim 20{,}000$). IR showed absorption at 980 cm^{-1} (conjugated E,E-diene) which replaced the 730 cm^{-1} and 970 cm^{-1} absorption of Z- and E-double bonds, respectively, in **XV-XVa**: NMR spectrum of **XVI-XVIa** showed overlapping of two spectra of E,E-isomer and Z,E-isomer, as compared with known spectra of pheromones (11, and Shani and Klug, unpublished data). By measuring the contribution of each isomer (Fig. 1) one can calculate as much as 30% of Z,E-isomer in the mixture, which fits well with the residual Z-izomer of jojoba oil, after its isomerization with HNO_2, or during the NBS reaction (see above). Qualitative support for this was found in the IR spectrum, where we noticed weak absorptions at 985 and 950 cm^{-1}, typical of Z,E-izomer (Shani and Klug, unpublished data).

Basic hydrolysis of the tetraene **XVI-XVIa** afforded almost pure dien-ol **XVIII-XVIIIa**, which exhibited typical NMR spectrum of E,E-isomer, overlapping Z,E-isomer. A still lower percentage of bromine (2.9%) contaminated the product. The acidic fraction contained, besides the diene-acid **XVII-XVIIa**, hydroxylic products, which always were formed from the dibromide **XV-XVa**.

ACKNOWLEDGMENT

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Calcium-Phytate Complex Formation Studies

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ABSTRACT

A calcium-phytate complex has been prepared. The chemical composition indicates that five calciums combine per phytate molecule. The solubility product of this calcium-phytate is about 10^{-22} in 0.2 M KCl.

INTRODUCTION

Phytic acid, myo-inositol hexaphosphate, is a strong chelating agent that can bind metal ions to form insoluble phytates. Oilseeds have high levels of phytic acid. Much of the extensive literature on the subject stemmed from McCance and Widdowson's (1) demonstration that phytate decreased the absorption and urinary excretion of calcium. Erdman (2) has recently summarized the nutritional implications of phytates. We report here the preparation of a calcium-phytate complex and its solubility properties under certain conditions.

MATERIALS AND METHODS

Phytic acid was prepared from sodium phytate, Lot No. 107C-0066, Sigma Chemical Co., by passage through cation-exchange columns of Rexyn 101 (H), research grade. The sodium content of the acid was ca. 0.2 ppm as judged by atomic absorption spectrophotometry. All other solutions were prepared from reagent-grade chemicals with deionized, distilled water.

An Radiometer PHM64 research pH meter in conjunction with a GK2401C electrode was used for all pH measurements at 25.0 ± 0.05 C. All solubility experiments were carried out at 25 ± 1 C in 250-mL round-bottom flasks rotated at 75 rpm.

For the preparation of the calcium-phytate complexes, 20 to 30 mL of ca. 0.05 M phytic acid was mixed with 100 mL of 0.2 M KCl in a 250-mL beaker. To this solution was added a quantity of ca. 1 M calcium chloride so that the phosphorus/calcium ratio in the reaction mixture varied from 1/1 to 1/4. This solution was then adjusted to between pH 5 and 6 by slowly adding 1 M KOH with stirring. Stirring was continued for an additional 1/2 to 1 hr. The solution was filtered and the precipitate washed with three 20-mL portions of boiling distilled water. The precipitate was allowed to air-dry overnight, and then it was dried at 120 C under vacuum for 1 hr prior to analysis.

The solubility experiments on the calcium-phytate complexes were done in the following manner. From 0.2 to 0.8 g of calcium-phytate was placed into round-bottomed flasks along with 100 mL of a solution 0.2 M in KCl containing from 1.5 to 3.5 mL of 0.1 M HCl. The flask was rotated for

TABLE I

Precipitation of Calcium-Phytate at Varying pH and P/Ca Ratios

pH	P/Ca[a]	%P	%Ca	P/Ca[b]
5.0	1/1	18.85	20.63	1.18
5.3	1/2	18.98	20.64	1.19
5.5	1/3	18.89	20.58	1.19
6.0	1/4	18.56	19.83	1.21

[a]In reaction mixture.
[b]In precipitate.