Quantitative Analysis of Cyclopentadiene Compounds

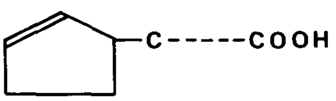
N. TOTANI, Y. TOTANI, and N. MATSUO, Department
of Industrial Chemistry, Faculty of Engineering, Seikei University,
Kichijoji, Musashino-shi, Tokyo, Japan

ABSTRACT AND SUMMARY

A quantitative color reaction of cyclopentadiene was examined when cyclopentadiene was contained in a mixture of benzene, 1,3-cyclohexadiene, cyclohexene, cyclohexane, cyclopentene, or cyclopentane. It was found that cyclopentadiene could be determined in the following manner. A sample was weighed to 1.38 x 10^{-5} mole in a test tube, while a mixture of 10 ml ethyl alcohol plus 4 ml sulfuric acid was cooled in an ice bath. The mixture was poured into the test tube and the tube was heated in a water bath at 95 C for 30 sec followed by immediate cooling. Then the absorbance at 580 nm was measured and the content of cyclopentadiene was calculated with a calibration curve. The color reaction was tested with ethyl linoleate cyclic monomer (ll cm), ethyl linolenate cyclic monomer (lncm), and photo-isomerized tung oil fatty acid ethyl ester cyclic monomer (βcm), which were prepared from each purified fatty acid or from photo-isomerized tung oil by cyclization with sodium hydroxide. It was found that cyclopentadiene compounds were formed from ethyl linolenate, but not from ethyl linoleate or from β-eleostearate, which was a main component of photo-isomerized tung oil.

INTRODUCTION

Many papers have reported that cyclic monomers (mostly 6-membered ring compounds) form from unsaturated fatty acids or their triglycerides during thermal treatment or by catalytic cyclization. But few reports mentioned 5-membered ring compounds in such cyclic monomers, although they seem to form as easily as 6-membered ring compounds. Spener and Mangold (1) reported that *Hydnocarpus antheliminthica* and *Oncoba echinata* contain cyclopentene compounds (I), which were not formed from coexisting unsaturated fatty acids (mostly linoleic acid and α-linolenic acid) by a cyclization reaction.

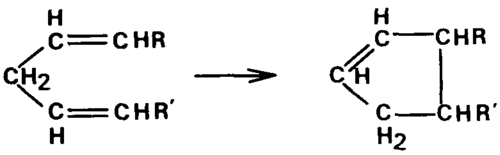


(I)

Meanwhile, Gast et al. (2) suggested the existence of 3,4-disubstituted cyclopentene compounds (II) in fatty acid esters obtained from linseed oil

(II)

heated at 300 C for about 7 hr, formed by the following pathway.



Cyclic monomers prepared from linseed oil and tung oil heated with sodium hydroxide contained 1,3-cyclohexadiene compounds as main components and also ortho-substituted aromatic compounds, and cyclohexene compounds as by-products (3). Furthermore, cyclopentadiene compounds, cyclopentene compounds, and cyclopentane compounds probably may be present with the cyclic monomers. But, it is difficult to isolate 5-membered ring compounds or to determine their structures since their chemical and physical properties are similar to 6-membered ring compounds.

Cyclopentadiene compounds and 1,3-cyclohexadiene compounds are more sensitive to color reactions with their conjugated double bonds than the other cyclic compounds mentioned above. The authors reported previously the spectra of 1,3-cyclohexadiene compounds (4).

In this paper, because of the similarity of cyclopentadiene compounds to 1,3-cyclohexadiene compounds, spectra of cyclopentadiene compounds were studied using cyclopentadiene and 1,3-cyclohexadiene. Colorimetry of cyclopentadiene was also applied to cyclic monomers obtained from ethyl linolenate, ethyl linoleate, and photo-isomerized tung oil by heat cyclization with sodium hydroxide.

EXPERIMENTAL PROCEDURES

Colorimetry of Cyclopentadiene

Seven cyclic compounds, i.e., cyclopentadiene that was newly prepared from dicyclopentadiene by thermal decomposition, 1,3-cyclohexadiene, cyclohexene, cyclohexane, benzene, cyclopentene, and cyclopentane, were accurately weighed (up to 1.0 mg) in separate test tubes, then heated in a water bath at 95 C for 30 sec after addition of a cold mixture of ethyl alcohol (10 ml) plus sulfuric acid (1-20 ml). The heated samples were quickly cooled in an ice bath and absorbances were measured with a Hitachi Spectrophotometer Model 181 using a glass cell (1 cm thickness).

Preparation of Ethyl Linolenate

Linseed oil was esterified by means of sodium ethoxide and was treated by urea adduct separation. Then crude ethyl linolenate was purified by silica-gel column (n-hexane:ether 95:5 as a solvent). The obtained ethyl linolenate was composed of ethyl linolenate (95.3%) and ethyl linoleate (4.7%).

Preparation of Ethyl Linoleate

Ethyl linoleate was prepared from safflower oil through esterification and urea adduct separation, and consisted of ethyl linoleate (96.3%) and ethyl oleate (3.7%).

Preparation of lncm llcm, and βcm

Purified ethyl linolenate was heated to cyclize with sodium hydroxide and ethylene glycol at 295 C for 15 min in a stainless steel autoclave, then acidified and esterified by the usual method. After the polymeric ester and decomposition products were separated from the heated ethyl linolenate by a silica-gel column with n-hexane as the solvent, lncm was purified by urea adduct separation and column chromatographic separation as mentioned above. The yield of lncm was 26.3%.

TABLE I

Relation between Color and Amount of Sulfuric Acid

Sulfuric acid (ml)	0–2	3,4	5	6,7	8–10	11–14	15-20
Cyclopentadiene	Colorless	Purple	Reddish purple	Neutral	Reddish brown	Neutral	Brown
1,3-Cyclohexadiene	Colorless		Blue purple	Reddish purple	Neutral	Reddish brown	Brown

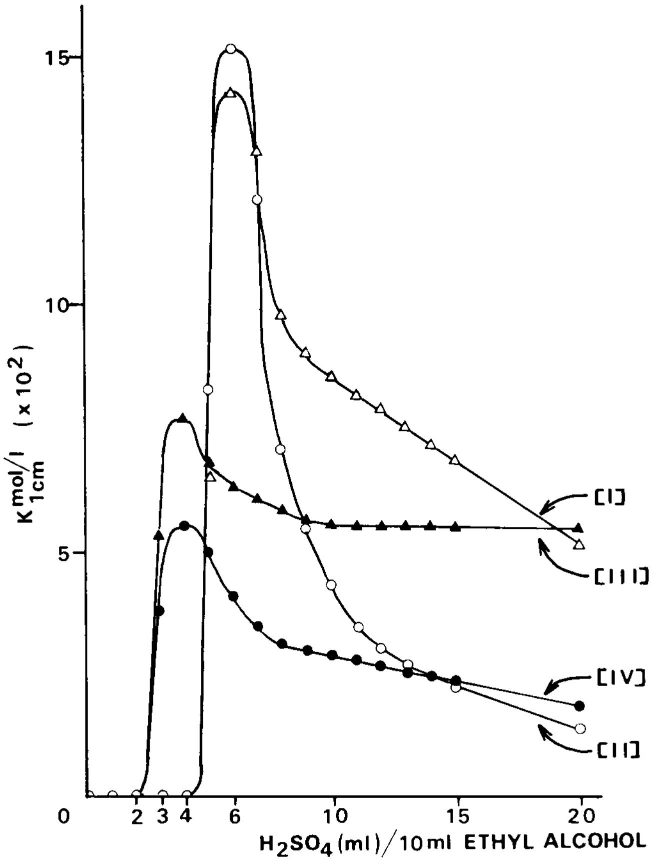


FIG. 1. Relation between absorbance at 515 nm or 580 nm and amount of sulfuric acid. [I] 1,3-cyclohexadiene (515 nm), [II] 1,3-cyclohexadiene (580 nm), [III] cyclopentadiene (515 nm), [IV] cyclopentadiene (580 nm).

Llcm and βcm were prepared as above from purified ethyl linoleate and tung oil photo-isomerized with potassium iodide. The yields of llcm and βcm were 1.2% and 25.0%, respectively.

Gas chromatographic composition of photo-isomerized tung oil before cyclization was as follows: palmitate, 3.1%; stearate, 6.3%; oleate, 11.1%; linoleate, 4.3%; linolenate, 4.0%; α-eleostearate, 9.1%; β-eleostearate, 62.1%.

RESULTS

Colorimetry of Cyclopentadiene

Benzene, cyclohexene, cyclohexane, and cyclopentane were colorless and cyclopentene was slightly yellow in a mixture that contained over 8 ml of sulfuric acid.

Since cyclopentadiene and 1,3-cyclohexadiene were reddish purple in the mixture at certain sulfuric acid volumes (Table I), absorbances were measured at the wavelength of maximum absorbances (515 nm for red, 580 nm for purple), and the molecular extinction coefficients (K) were calculated (Fig. 1). At both 515 nm and 580 nm, K values of cyclopentadiene were maximum in 4 ml of sulfuric acid, whereas those of 1,3-cyclohexadiene started to increase at nearly 4.5 ml and showed maximums at 6 ml. Therefore, spectrophotometric conditions using 3 or 4 ml of sulfuric acid seemed to be optimal for qualitative and quantitative analyses of cyclopentadiene, even if cyclopentadiene was mixed with 1,3-cyclohexadiene.

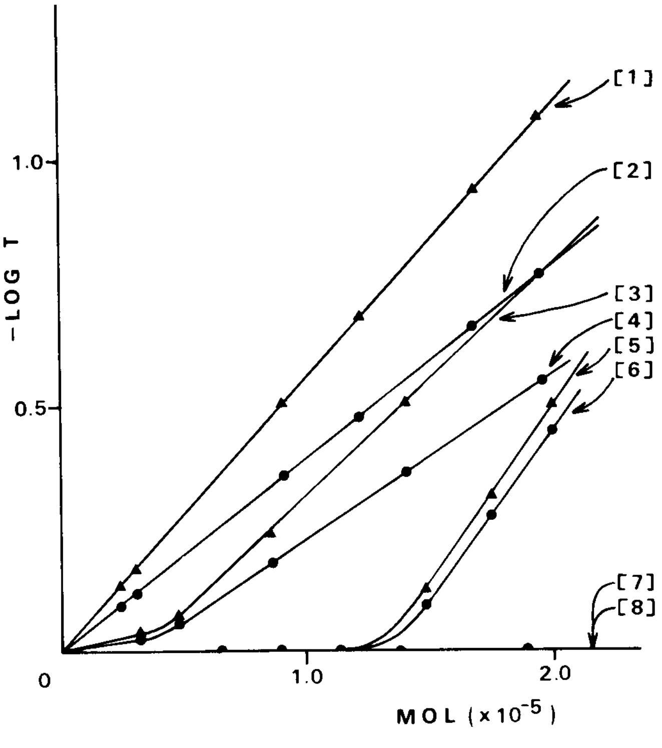


FIG. 2. Relation between absorbance at 515 nm or 580 nm and amount of cyclopentadiene (P) or 1,3-cyclohexadiene (H). [1] P + 4 ml H_2SO_4, 515 nm; [2] P + 4 ml H_2SO_4, 580 nm; [3] P + 3 ml H_2SO_4 515 nm; [4] P + 3 ml H_2SO_4, 580 nm; [5] H + 4 ml H_2SO_4, 515 nm; [6] H + 4 ml H_2SO_4, 580 nm; [7] H + 3 ml H_2SO_4, 515 nm; [8] H + 3 ml H_2SO_4, 580 nm.

Calibration Curve of Cyclopentadiene

Absorbances of various amounts of cyclopentadiene were measured with sulfuric acid volumes of 3 and 4 ml (Fig. 2). When the sulfuric acid volume was 3 ml, relations between absorbance and amount of cyclopentadiene were almost linear (curves 3 and 4), but the absorbance was very low with small amounts of cyclopentadiene. And 1,3-cyclohexadiene showed no absorption till the amount was 5.20 mg (6.50 x 10^{-5} mole) (curves 7 and 8). When the sulfuric acid volume was 4 ml, relations between absorbance and amount of cyclopentadiene were linear (curves 1 and 2), and absorbance of 1,3-cyclohexadiene was 0 till the amount was 1.10 mg (1.38 x 10^{-5} mole) (curves 5 and 6). Thus using 4 ml of sulfuric acid for spectral analysis was optimal to quantitate cyclopentadiene if sample weight was limited, because 1,3-cyclohexadiene had no effect on the analysis, and the absorption of cyclopentadiene was strong.

TABLE II

Mixtures of Cyclopentadiene and 1,3-Cyclohexadiene Used in Model Quantitative Analyses

MN[a]	Cyclopentadiene mol ($x10^{-5}$)[b]	1,3-Cyclohexadiene mol ($x10^{-5}$)[c]	b/b+c
A	0.30	0.63	0.32
B	0.91	0.63	0.59
C	1.06	1.48	0.42
D	1.21	0.63	0.66
E	1.67	0.50	0.77
F	1.82	1.50	0.55

[a] Mixture name.

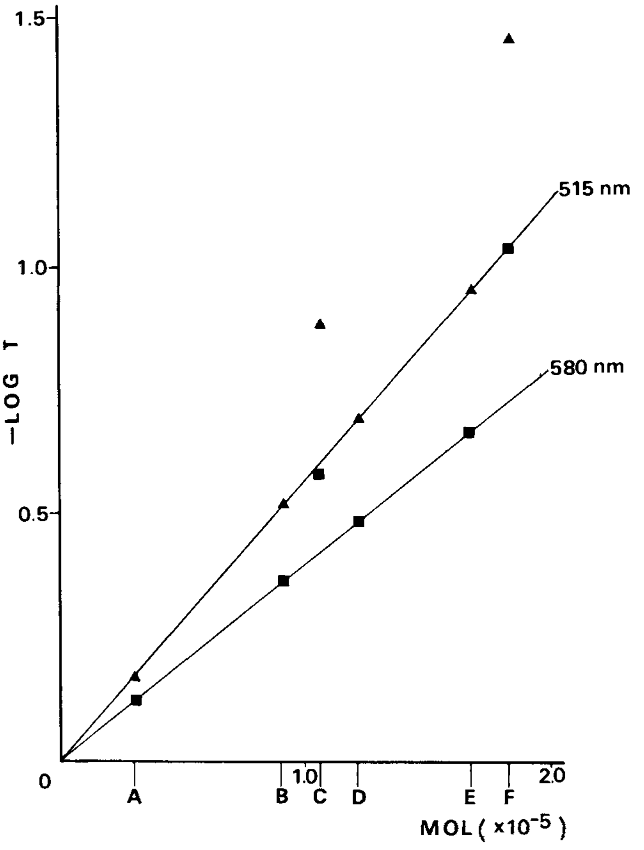


FIG. 3. Quantitative analyses of cyclopentadiene in six mixtures (A–F) shown in Table II.

Application of Spectral Analysis to Standard Mixtures

Mixtures of cyclopentadiene and 1,3-cyclohexadiene in various ratios (Table II) were prepared to determine whether the spectral analysis mentioned above was capable of quantifying cyclopentadiene. These mixtures were subjected to the procedure and the results are shown in Figure 3. When the weight of 1,3-cyclohexadiene in the mixture was under 1.10 mg (1.38 x 10^{-5} mole), quantitative values were just on the calibration curve inserted above. But, the values deviated from the curve when weight of the 1,3-cyclohexadiene was over 1.10 mg (C and F in Table II and Fig. 3).

GLC of Cyclic Monomers

Cyclic monomers were analyzed by gas liquid chromatography (3 mm x 3 m s.s., with 15% DEGS on Celite 545 at 200 C) (Fig. 4). In a previous paper (3), the authors reported ranges on gas chromatograms of cyclohexene compounds, cyclohexadiene compounds, and ortho-substituted aromatic compounds in βcm by means of retention ratios (α) which were based on the retention time of ethyl β-eleostearate (i.e., α-value of ethyl β-eleostearate was 1.00); cyclohexene compounds, 0.24-0.36; cyclohexadiene compounds, 0.30-0.56; ortho-substituted aromatic compounds, 0.56-1.09. Llcm had a peak at α = 0.27, but had no peak in the ranges of cyclohexadiene compounds or ortho-substituted aromatic compounds. Lncm had almost the same pattern as βcm. A main component was located in the range of cyclohexadiene compounds.

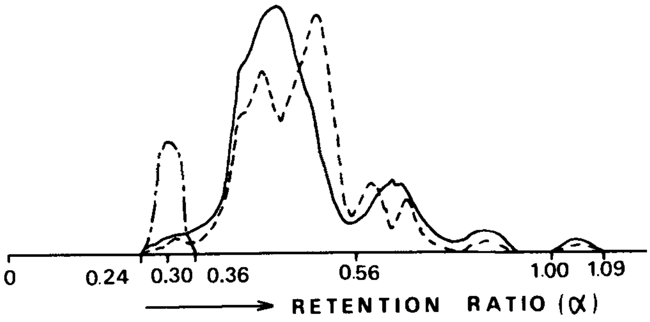


FIG. 4. Gas liquid chromatogram of cyclic monomers from ethyl linoleate (llcm, ---), ethyl linolenate (lncm, — — —), and tung oil (βcm, ——).

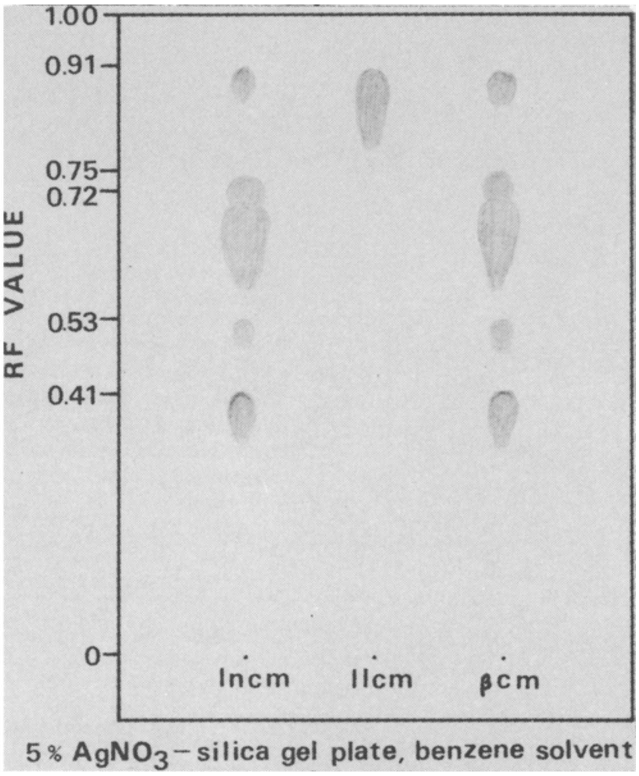


FIG. 5. Thin layer chromatogram of cyclic monomers from ethyl linolenate (lncm), ethyl linoleate (llcm), and tung oil (βcm).

TLC of Cyclic Monomers

The cyclic monomers were analyzed by thin layer chromatography on 5% (wt:wt) silver nitrate-silica gel plates (thickness 0.5 mm, 20 cm x 20 cm, benzene as a solvent) (Fig. 5).

It had been reported (3) that, on the thin layer chromatogram of βcm, there were 5 spots, i.e., Rf = 0.41, 0.53, 0.72, 0.75, and 0.91, where 0.91 was a cyclohexene compound, 0.72, 0.53, and 0.41 were cyclohexadiene compounds, 0.75 was an ortho-substituted aromatic compound. Lncm had almost the same pattern as βcm. Llcm produced a large spot at Rf = 0.91.

TABLE III

Quantitative Analyses of Cyclopentadiene Compounds in lncm, βcm, and llcm

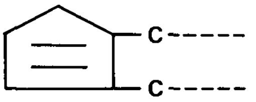
Cyclic monomer	Sample amount ($x10^{-5}$ mole)	Absorbance at 580 nm	%	Average %
Lncm	1.23	0.0178	4.1	4.1
	1.41	0.0172	4.2	
	1.63	0.0223	4.1	
	1.73	0.0223	4.0	
	2.45	0.0353	4.1	
	3.20	0.0540	4.5	4.5
	3.73	0.0645	4.6	
βcm	1.30	0.0035	0.08	0.08
	1.34	0.0038	0.07	
Llcm	1.56	0	0	0
	3.44	0.0004	0	

IR of Cyclic Monomers

All three cyclic monomers showed methylene (2900 cm^{-1}), ester (723 cm^{-1}), and cyclohexene (663 cm^{-1}) by IR. Furthermore lncm and βcm showed ortho-substituted aromatic structure (1603, 750 cm^{-1}) and cyclohexadiene (695 cm^{-1}).

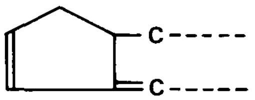
Spectra of Cyclic Monomers

Application of spectrophotometry to analysis of cyclic monomers required examination of two more details. If cyclopentadiene compounds were formed from unsaturated straight chain fatty acids (esters), they must have the structure below (III), which have side chains on adjacent

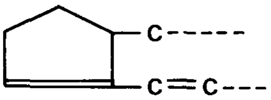


(III)

carbons. The first point was whether two side chains of the cyclopentadiene ring had an effect on the spectra. The second was whether the structures where conjugated double bonds migrated to a side chain, as below, influenced the spectra, although they were not known to exist.



(IV)



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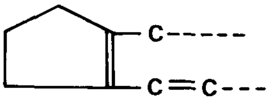

(VI)

For the first point, the side chains were conjectured to have no effect on the spectra since ethyl stearate, ethyl oleate, and ethyl linoleate were negative to the procedure with a sample amount of 1.38 x 10^{-5} mole. For the second point, it was desirable to synthesize all expected compounds to examine probability of coloring, but that is beyond the scope of this report. It was presumed that this color reaction closely related to number, distance, and position of double bonds. In the structures mentioned above [(IV), (V), (VI)], there was one double bond in the ring and another on the side chain. The relation of the two double bonds was considered to be similar to conjugated double bonds on straight chain.

Conjugated ethyl linoleate was prepared according to the method of Terry and Wheeler (5), which was analyzed by UV and GLC; conjugated ethyl linoleate, 86.2%; ethyl linoleate, 10.1%; ethyl oleate, 3.7%. This conjugated ethyl linoleate was subjected to our procedure with sample amounts of 2.08 x 10^{-5} mole and 3.44 x 10^{-5} mole, but no absorption resulted. It was concluded that cyclopentadiene compounds only showed absorption by the above color reaction conditions. Therefore the colorimetry was applied to lncm, llcm, and βcm. It was found that coloring of cyclic monomers was a little unstable and that it was better to measure at 580 nm than at 515 nm. Contents of cyclopentadiene compounds, which were calculated with calibration curve (Fig. 2), were 4.1% for lncm, 0.08% for βcm, and 0% for ll cm (Table III).

In conclusion, our procedure of measuring cyclopentadiene compounds contained in a mixture of cyclic monomers was as follows. Sample was weighed (0–1.38 x 10^{-5} mole) in a test tube, while a mixture of 10 ml ethyl alcohol plus 4 ml sulfuric acid was cooled with an ice bath. The mixture was poured into the test tube and the tube was heated in a water bath at 95 C for 30 sec followed by immediate cooling. Then the absorbance at 580 nm was measured and content of cyclopentadiene compounds was calculated with the calibration curve (Fig. 2).

DISCUSSION

The Tortelli-Jaffe reaction (6-10) is positive for cyclic monomers formed from polyunsaturated fatty acids. The procedure of the Tortelli-Jaffe reaction is as follows. A polyunsaturated fatty acid containing cyclic monomers is diluted in chloroform:acetic anhydride solution (6:1) and poured into a bromine-chloroform solution (10%). Then the reactant gradually turns green. This coloring is attributed to a tertiary carbon with a double bond added by a bromic ion. But, this reaction cannot ditinguish cyclopentadiene compounds from other unsaturated cyclic compounds. In the color reaction of this paper, cyclopentadiene and 1,3-cyclohexadiene showed different absorption changes against an increase of sulfuric acid concentration in sulfuric acid-ethyl alcohol mixture. Cyclopentadiene compounds in a mixture of cyclic monomer were quantified relatively easily, making use of the fact that 1,3-cyclohexadiene showed no absorption at the sulfuric acid concentration at which cyclopentadiene showed maximum absorption. Coloring by this color reaction needs conjugated double bonds to exist in a 5-membered ring. If the molecular extinction coefficient changed by the position of two side chains in cyclopentadiene compounds, the effect was neglected. The contents of cyclopentadiene compounds were 4.1% for lncm and 0.08% for βcm, but there was nothing in llcm.

Therefore, it is evident that cyclopentadiene compounds are formed from ethyl linolenate or alkali-conjugated ethyl linolenate as by-products of cyclization (main products are 6-membered ring compounds), not from ethyl linoleate, alkali-conjugated ethyl linoleate, and β-eleostearate. Existence of 0.08% cyclopentadiene compounds in βcm is explained by 4.0% ethyl linolenate contained in the original tung oil. Cyclopentadiene compounds are not considered to be formed from β-eleostearate.

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REFERENCES

1. Spener, F., and H.K. Mangold, Biochemistry 13:2241 (1974).
2. Gast, L.E., W.J. Schneider, C.A. Forest, and J.C. Cowan, JAOCS 40:287 (1963).
3. Totani, N., and N. Matsuo, Yukagaku 24:371 (1975).
4. Totani, N., Y. Totani, and N. Matsuo, Ibid. 23:532 (1974).
5. Terry, D.E., and D.H. Wheeler, Oil & Soap 23:88 (1946).

6. Tortelli, M., and E. Jaffe, Chem. Ztg. 39:14 (1915).
7. Fieser, L.F., and M. Fieser, "Steroids," Reinhold, p. 114.
8. Murase, Y., and Y. Toyama, Research Reports of Nagoya Industrial Science Laboratory, No. 12, 42 (1960).
9. Murase, Y., and Y. Toyama, Ibid. No. 12, 45 (1960).
10. Ono, F., Ibid. No. 12, 47 (1960).

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