B) in the mole ratio of 20 to 1 formed wrinkled films comparable to nonheat-treated linseed oil.

In contrast, the dilinolenate ester (Type B) prepared from polyoxyethylene methyl glucoside containing five ethylene oxide groups per mole produced a clear and coherent film that was essentially tack-free after 12 hr. Type B dilinseedate esters in which methyl glucoside was first etherified with 5- and 10-mole equivalents of ethylene oxide also showed good film-forming properties, but the films remained tacky. Type A dilinolenate ester containing 19 moles of ethylene oxide per mole of product produced a grainy film. Methyl glucoside esters such as these, which have water dispersibility and yet retain some drying oil properties, could conceivably be valuable for making emulsion paints in that the emulsifying agent would become a part of the paint film (9).

Surface Tension. Surface-tension measurements were made with the Du Noüy tensiometer. The results of these measurements show that the surface tension of water is appreciably lowered by the addition of as little as 0.01% of the polyoxyethylene methyl glucoside esters. The values are comparable to those reported for the methyl glucoside diesters (1) and for polyoxyethylene sorbitan monoesters (5).

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REFERENCES

1. Gibbons, J.P., and Swanson, C.J., J. Am. Oil Chemists' Soc., *36*, 553–555 (1959).
2. Gibbons, J.P., Paint Varnish Production, *48*, 57–63 (Oct. 1958).
3. Griffin, W.C. (Atlas Powder Co.), U.S. Patent 2,380,166 (1945).
4. Johnston, N.F. (R.T. Vanderbilt Co. Inc.), U.S. Patent 2,422,486 (1947).
5. Komori, S., Okahara, M., and Okamoto, K., J. Am. Oil Chemists' Soc., *37*, 468–473 (1960).
6. Gibbons, J.P., Private Communication.
7. Ballun, A.T., Schumacher, J.N., Kapella, G.E., and Karabinos, J.V., J. Am. Oil Chemists' Soc., *31*, 20–23 (1954).
8. Wrigley, A.N., Smith, F.D., and Stirton, A.J., J. Am.. Oil Chemists' Soc., *31*, 20–23 (1954).
9. Kubie, W.L., Stolp, J.A., Schwab, A.W., Teeter, H.M., and Cowan, J.C., Abstr. Papers, 139th Meeting Am. Chem. Soc., St. Louis, Mo., March 21–30, 1961.

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Chemical and Physical Characteristics and Possible Configuration of Toxins from Tung Kernels

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Based on limited chemical and physical data, including the infrared and ultraviolet spectra of the toxins and their saponification products, it is suggested that Tung Toxin I and Tung Toxin II are diesters of a 2,3-unsaturated-5-keto acid, a polyhydroxy acid and a 3,4-unsaturated-5-keto tertiary alcohol. The 2,3-unsaturated-5-keto acid is assumed to be in equilibrium with its enol tautomer, the keto form possessing a conjugated diene and the enol form a conjugated triene structure. The polyhydroxy acid is probably very similar to gluconic acid.

THE LITERATURE on the toxicity of tung meal has been reviewed by Mann, Hoffman and Ambrose (6) and by Balthrop, Gallagher, McDonald and Camariotes (1). More recently Holmes and Rayner (5) described a procedure for isolating two nitrogen-free toxins, designated as Toxin I and Toxin II, from oil-free kernels, and reported certain of the characteristics of the toxins. They found Toxins I and II to have the empirical formulas $C_9H_{14}O_2$ and $C_{11}H_{16}O_3$, respectively. Both toxins were optically active. Toxin I contained 5.7% hydroxyl and had a saponification equivalent of 268. Toxin II contained 5.6% hydroxyl and had a saponification equivalent of 401.

This paper reports additional characteristics of the toxins.

Experimental

Small amounts of Toxins I and II were isolated from tung kernels using the procedure developed by Holmes and Rayner (5). These gave single spots in glass paper chromatography with the same Rf values as the toxins reported in the previous paper.

Numerous qualitative tests were applied to the toxins but few were found to be positive. The molecular weights (7), hydrogen iodine values (8), and optical activities of the toxins were also determined by established procedures.

Two samples of each toxin were saponified, one under mild conditions by allowing sample to stand overnight at room temperature in absolute ethanol adjusted to about pH 11 with KOH, the other by refluxing the sample with alcoholic KOH (40 g./l. in 95% ethanol) for 1.5 hrs. The saponified samples were separated into three fractions; the ethyl ether extract of an aqueous alkaline solution referred to as the "unsaponifiables," the acids extracted by ethyl ether after acidification of the solution with hydrochloric acid, and the residue that remained in the water solution. In none of the fractions of the saponified samples could the spots characteristic of Toxins I and II in glass paper chromatography be detected. This was taken as evidence that saponification was complete.

Ultraviolet spectra of the toxins and their saponification products were obtained from 99% ethanol solutions over the region 220 to 360 mμ with a Cary Automatic Recording Spectrophotometer Model 14. The instrument was first balanced throughout this range with the solvent in two matched 2-cm. path-length cells.

Infrared absorption curves were obtained both from chloroform solutions and as KBr discs of the toxins and as KBr discs of their saponification products, with a Perkin-Elmer Infrared Spectrophotometer Model 21. Spectra were obtained of chloroform solutions in concentrations from 20 to 40 g. per l. depending upon the amount of sample available and of KBr discs containing approximately 2 mg. per 350 mg. of KBr. The chloroform solutions were measured over the region 2 to 12 μ, the KBr discs from 2 to 15 μ. In each case the instrument was balanced with either the chloroform solvent in two matched 0.499 mm. cells or blank KBr discs in both beams. Settings used were resolution, 927; suppression, 3; gain, 6; response, 1; speed, 0.5 microns per min.

Data and Discussion

The molecular weight of Toxin I was 500–600 and its hydrogen iodine value 147, the corresponding values for Toxin II were 700–800 and 89.

Positive qualitative tests for esters (4a), enols (4b), and unsaturation (2) were found for both toxins. The optical activity of successive preparations of the toxins varied (5). The optical activity of a sample decreased with time and as the sample was handled in the laboratory. The optical activity $[\alpha]_D^{24}$ (C, 0.6, abs. ethanol) of a sample of Toxin I sealed in a polariscope tube and stored at 24°C. exposed to laboratory light gradually dropped from +75° to +22° in 82 days; that of a sample of Toxin II under the same conditions dropped from $[\alpha]_D^{24}$ (C, 0.8 abs. ethanol) +64° to +39°.

Ultraviolet Absorption of Toxin I. Ultraviolet absorption spectra were obtained on five preparations of Toxin I. All spectra showed good agreement exhibiting three maxima in the 260 to 280 mμ region and a single maximum at 231–233 mμ region, as shown in Table I. The three bands at longer wavelengths

TABLE I

Ultraviolet Absorptivities of Tung Toxin I

Band	Wavelength (mμ)	Range in absorptivities (5 samples)
Maximum	279	20.11–23.50
Minimum	274–275	19.39–22.29
Maximum	269	20.78–24.03
Shoulder	260	18.01–19.78
Minimum	252–253	17.14–17.79
Maximum	231–233	22.42–25.18

indicate triene conjugation. The spectra in this region are very similar to that of a material containing about 12% α-eleostearic acid in ethanol solution. The band at 231–233 mμ indicates a conjugated diene moiety, about 20% when calculated as conjugated linoleic acid. The spectra thus resemble that of a tung oil where the eleostearic acid has been reduced (as by polymerization) to 12% and which contains about 20% conjugated octadecadienoic acid.

Ultraviolet Absorption of Toxin II. The ultraviolet spectra of Toxin II were also obtained on five preparations. The maximum and minimum absorptivities are shown in Table II. The spectra differ somewhat from those of Toxin I. In the spectra exhibiting the more intense bands, maxima are found at 279 mμ and 272 mμ, the latter being merely a "shoulder." In spectra with weaker bands, these two bands are unresolved as a broad maximum extending from about 270 to 279 mμ. In all samples the spectra reveal a more intense band which appears from 232 to 234 mμ in the various preparations.

TABLE II

Ranges in Ultraviolet Absorptivities of Tung Toxin II

	Band	Wavelength (mμ)	Absorptivity
Preparations 1, 2, and 3	Maximum	270–273	10.21–12.43*
	Minimum	264–265	11.67–12.14
	Maximum	233–234	26.33–27.11
Preparations 4 and 5	Maximum	279	15.30–15.89
	Shoulder	272	15.04–15.58
	Minimum	261–262	13.90–14.26
	Maximum	232–233	29.30–29.47

* Spectrum of Preparation No. 2 exhibited only a shoulder at 272 mμ and no minimum at 264–265 mμ.

In the spectra of Toxin II the bands at the longer wavelengths are superimposed on a noncharacteristic absorption which increases rapidly toward the shorter wavelengths. The intensities listed in Table II are maxima (peak heights including background). The actual values of these bands above this sharply changing background are considerably smaller. If we consider these bands as arising from a triene conjugated system (as in Toxin I), we might expect the weakest of the three bands (in a typical eleostearic acid) at the shortest wavelength will be completely covered by the background as the contribution of the background will be greatest at 260 mμ. The band at about 270 mμ should be stronger than that at about 280 mμ but again the background will be stronger at 270 mμ than at 280 mμ and the net result may be that the portion of the band extending above the background might be about equal at the two wavelengths. Under these conditions the spectra would take the appearance revealed for the various preparations of Toxin II. This means that the spectra of Toxin II when corrected for background absorption would resemble those of Toxin I with a triene conjugated group absorbing in the region 260–280 mμ and a diene conjugated group absorbing at 232–234 mμ. Toxin II had a higher percentage of the conjugated linoleic acid-like group, probably about 24%, but con-

TABLE III

Infrared Absorption Bands of Tung Toxins I and II

Wavelength, μ				Probable correlation with functional group
Toxin I		Toxin II		
KBr disc	CH_3Cl	KBr disc	CH_3Cl	
2.96	2.92	2.96	2.93	Bonded –O–H . . . stretching.
.....	3.39	.....	3.39	C-H asymmetric stretching of CH_3(methyl) and CH_2 (methylene) groups.
3.44	3.48	3.45	3.48	
3.51	.....	3.52	.....	
5.78	5.77	5.76	5.76	C=O stretching of ester.
.....	5.83	5.86	5.81	Probably C=O stretching of ketone.
6.14	6.11	6.15	6.10	C=C stretching, probably conjugated (low frequency).
6.86	6.82	6.85	6.81	C–H in-plane-deformation. CH_2 scissoring and/or CH_3 bending.
7.27	7.22	7.29	7.23	CH_3 symmetrical-in-plane deformation and CH_2 bending. Low frequency probably indicating bending about unsaturated groups.
7.57	7.45	7.57	7.48	
7.90	7.85–7	7.95	7.90	C–O stretching, ester probably unsaturated.
8.07	.....	8.09	.....	C–O stretching and/or –OH bending, alcohol.
8.57	8.59	8.70	8.61	C–O stretching, ester.
9.56	9.50	9.35	9.22	C–O stretching and/or –OH bending, alcohol.
.....	10.03	9.87	10.06	C–H bending about cis-trans or trans-trans conjugated C=C groups.
10.15	10.23	10.15	10.21	
.....	10.26	10.62	10.54	

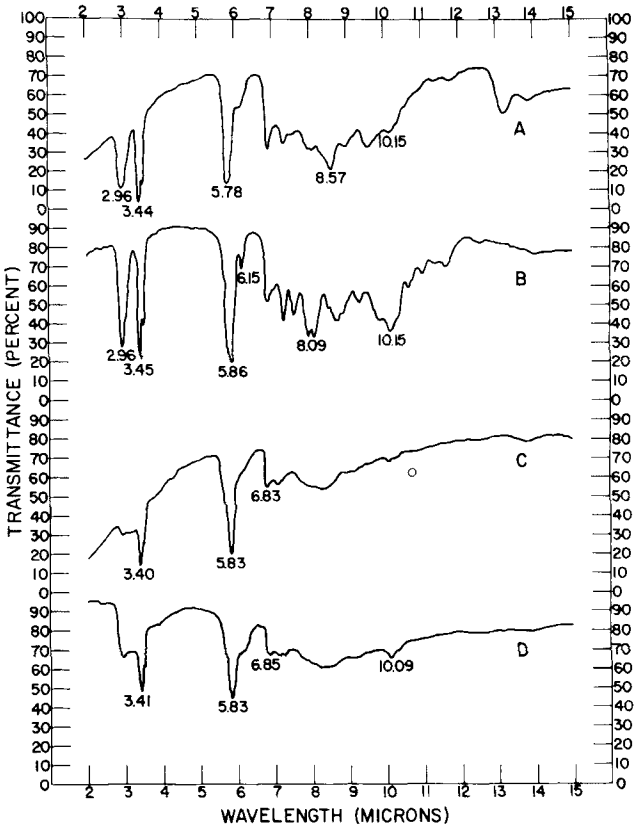


FIG. 1. Infrared spectra as KBr discs of tung toxins and their acids obtained by saponification at reflux temperature of alcoholic KOH; (A) Toxin I, (B) Toxin II, (C) Toxin I acid, (D) Toxin II acid.

siderably less of the eleostearic acid-like group, probably less than the 6–8% calculated as a maximum value without considering background correction.

Infrared Absorption of Toxins I and II. The infrared absorption spectra of the two toxins (not obtained on the same preparations used for the ultraviolet absorption spectra) are very similar qualitatively. A list of the more prominent absorption bands with the functional groups which give rise to them are shown in Table III. The absorption spectra in KBr discs of Toxins I and II are shown in Fig. 1.

The infrared spectra of Toxins I and II along with the conclusions reached from a consideration of their ultraviolet spectra indicate that:

(a) The tung toxins are long-chain unsaturated aliphatic compounds. This is indicated by ultraviolet data and confirmed in the infrared spectra by a lack of bands arising from stretching of aromatic C=C groups at 6.25 μ and 6.70 μ and from bendings about an aromatic ring at 14 μ. The hydrogen iodine values also confirm the presence of unsaturation.

(b) The toxins contain two carbonyl groups, indicated by bands about 5.7 μ and 5.8 μ arising from C=O stretching. The position of the former indicates most likely an ester and that of the latter a ketone.

(c) The toxins contain conjugated unsaturation of both diene and triene types as shown by ultraviolet absorption. This is confirmed by the appearance of a band in the infrared at 6.10 μ for conjugated C=C and weak bands about 10 μ region arising probably from C-H deformation of cis-trans or trans-trans conjugated groups.

(d) The toxins contain −OH groups as indicated by infrared absorption at 2.9 μ and confirmed by chemical analysis.

(e) C-H stretching of CH_2 and CH_3 groups at 3.4 μ and 3.5 μ region and deformations of these groups at 6.8, 7.2, and 7.5 μ further confirm a long chain. Lack of bands arising from C-H stretching below 3.39 μ confirms no aromatic ring and deformations at longer wavelengths, 7.45 and 7.57 μ, may be further indication of unsaturated groups.

Spectra of Saponification Products of Toxins I and II. The ultraviolet absorption of the acids and "unsaponifiable" fractions is summarized in Table IV. The aqueous residue showed no characteristic absorptions in the ultraviolet range. The infrared spectra as KBr discs of the fractions obtained by saponification at reflux temperature of alcoholic KOH are shown in Figures 1 and 2.

The acid fractions from both toxins show ultraviolet absorption for conjugated diene and triene un-

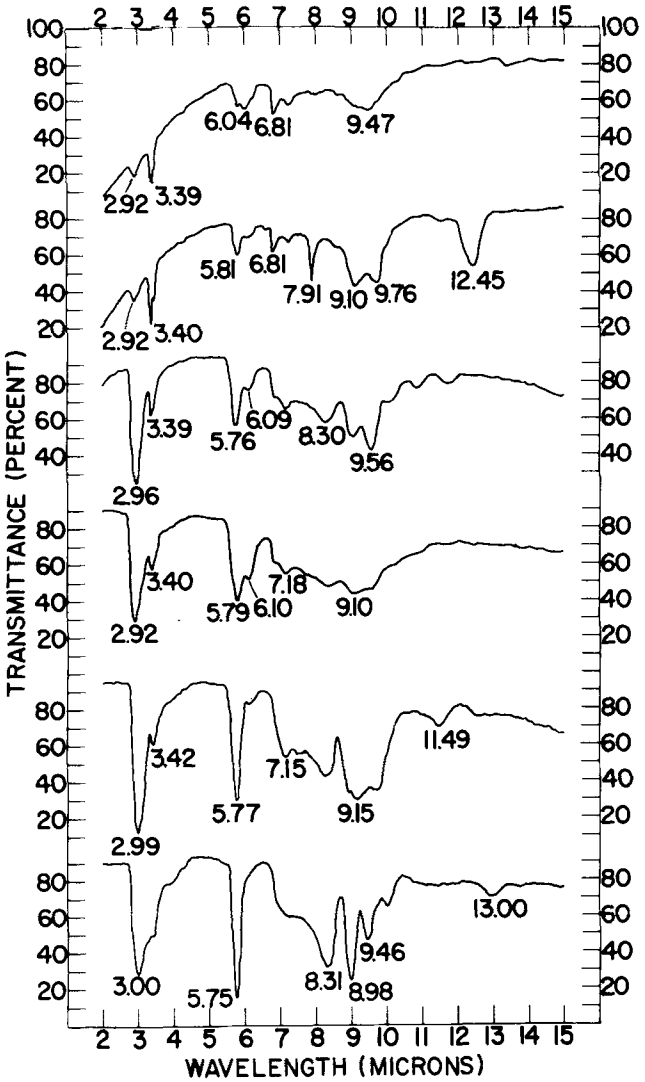


FIG. 2. Infrared spectra as KBr discs of the "unsaponifiable" and aqueous residue fractoins obtained by saponification of Toxins I and II at reflux temperature of alcoholic KOH and of gluconic and glyceric acids; (A) Toxin I "unsaponifiable," (B) Toxin II "unsaponifiable," (C) Toxin I aqueous residue, (D) Toxin II aqueous residue, (E) Gluconic acid, (F) Glyceric acid.

TABLE IV

Ultraviolt Absorptivity Maxima of Saponification Products of Tung Toxins I and II

	Toxin I		Toxin II	
	Wavelength, mμ	Absorptivity	Wavelength, mμ	Absorptivity
A. Acids from saponification of toxins with mild alcoholic KOH at room temperature	275	13.3	.....	.....
	267	14.1	265	11.0
	232	20.8	232	20.6
B. Acids from saponification of toxins with strong alcoholic KOH at reflux temperature	278	8.3	280	23.4
	268	9.9	269	27.2
	.....	.....	260	34.6
	232	20.8	236	34.8
C. "Unsaponifiable" fractions from saponification of toxins with mild alcoholic KOH at room temperature	279	16.1	.....	.....
	268	17.3	265	5.3
	260	15.6	.....	.....
	232	16.9	225	16.3

saturation. The concentration of diene conjugation in the acid from Toxin I is the same whether sample was saponified at room or reflux temperature; the concentration of the triene conjugation is greater in the sample saponified at room temperature than at reflux temperature. The concentrations of both types of conjugation are greater in the acids from Toxin II saponified at reflux temperature than in the sample saponified at room temperature, and also than in both samples of acids from Toxin I. The infrared spectra for both toxins show absorption bands at 2.93 μ for hydroxyl and at 5.82–5.83 μ for carboxyl (and probably ketone carbonyl). A 2,3-unsaturated-5-keto aliphatic acid in equilibrium with its enol form would account for the infrared and ultraviolet absorption characteristics of the acid fractions.

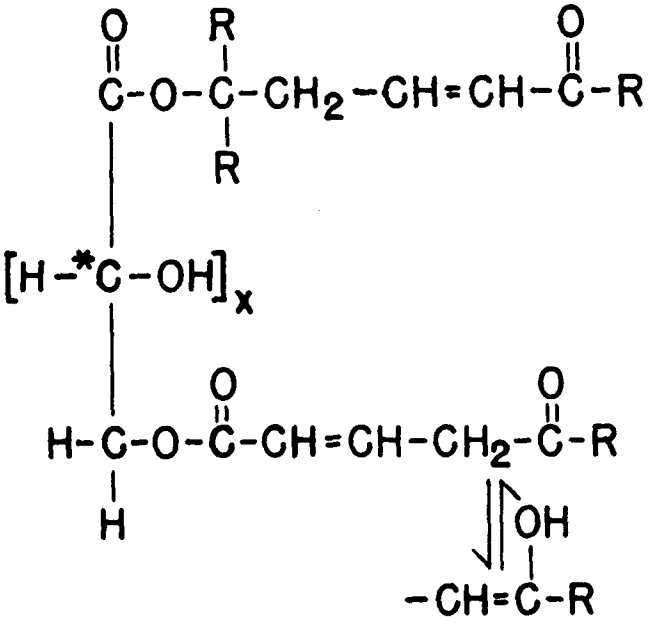
The aqueous residues of the saponified samples showed no characteristic ultraviolet absorption. Hence they contain no conjugated unsaturation.

The infrared spectra for both aqueous residues show absorption bands at 2.92–2.97 μ for hydroxyl and at 5.77–5.78 μ probably for carboxyl, thus indicating a hydroxy acid. Comparison of the spectra of the residues with that of glyceric acid showed a similarity but the ratio of absorption for hydroxyl to carboxyl was less for glyceric acid than for the residues. The spectra of the residues were then compared with that for gluconic acid which they resembled very closely. Hence the residues appear to be a polyhydroxy acid similar to gluconic. Because the infrared spectra for glyceric and gluconic acids could not be found in the literature they are given in Figure 2. Both spectra were determined as KBr discs, gluconic acid 0.22 mg. to 350 mg. KBr and glyceric acid 0.50 mg. to 350 mg. KBr.

The ultraviolet spectra of the "unsaponifiable" fraction of both toxins show the presence of both diene and triene conjugation. The infrared spectra show a band at 2.92–2.93 μ for hydroxyl and at 5.81–5.82 μ for carbonyl. If a 3,4-unsaturated-5-keto tertiary alcohol is split off by saponification it could be partially dehydrated by refluxing with alcoholic KOH to give a mixture of ketones having diene and triene conjugation and a tertiary alcohol having diene conjugation (3) and thus account for the carbonyl, hydroxyl, and diene and triene conjugation in the fraction.

Conclusions

Based on chemical and physical data presented the following is suggested as the possible configuration of the toxins, where R is an alkyl or an alkenyl radical and it is assumed that the keto-acid is in equilibrium with its enol tautomer:



Such a configuration would account for the presence of keto-enol tautomerism, diene and triene conjugation, ester and ketone carbonyls, hydroxyl, and the properties of the various fractions obtained by saponification. Such a structure could also undergo polymerization thus accounting for discrepancies in hydrogen iodine value, molecular weights, changes in optical rotation and decrease in toxicity.

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REFERENCES

1. Balthrop, E., Gallagher, W.B., McDonald, T.F., and Camariotes,S., J. Florida Med. Assoc., *40*, 813–820 (1954).
2. Cheronis, N.D., "Micro and Semimicro Methods" (Technique of Organic Chemistry, vol. 6), pp. 456 and 472, Interscience, New York, 1954.
3. Dupont, G., and Darmon, M., Bull. soc. chim. France *1954*, 240–244.
4. Feigl, F., "Spot Tests in Organic Analysis," 5th English ed., Elsevier, New York, 1956, (a) p. 237. (b) p. 196.
5. Holmes, R.L., and Rayner, E.T., J. Am. Oil Chemists' Soc., *35*, 586–589 (1958).
6. Mann, G.E., Hoffman, W.H., Jr., and Ambrose, A.M., J. Agr. Food Chem., *2*, 258–263 (1954).
7. Menzies, A.W.C., and Wright, S.L., Jr., J. Am. Chem. Soc., *43*, 2314–2323 (1921).
8. Pack, F. C., and Planck, R. W., J. Am. Oil Chemists' Soc., *30*, 461-463 (1593).

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