TABLE II

Plasticizing Characteristics of the Hydrogenated and Nonhydrogenated Adducts

Plasticizer	Plasticizer (%)	Tensile strength (p.s.i.)	100% Modulus (p.s.i.)	Elongation (%)	Brittle point (°C.)	Compatibility[a] (days)	Volatility loss[b] (%)
Acrylonitrile-eleostearate adduct							
Methyl ester	35	2670	1190	300	−25	150+	0.20
Ethyl ester	30	3140	1540	360	−29	<30	.....
	35	.....[c]	1210	.....[c]	−33	<30	.....
Butyl ester	30	3230	1820	330	−29	< 7	.....
	35	.....[c]	1308	.....[c]	−35	< 7	.....
Hydrogenated acrylonitrile-eleostearate adduct							
Methyl ester	30	3230	1760	330	−15	150+	.....
	35	.....[c]	1190	.....[c]	−25	150+	0.13
Ethyl ester	30	3150	1550	350	−23	150+	.....
	35	.....[c]	1290	.....[c]	−27	150+	0.13
Butyl ester	35	2840	1350	410	−29	150+	0.16
Tung oil-acrylonitrile adduct	35	2950	2520	190	− 3	150+	0.04
Control (DOP)	30	3270	2180	300	−27	.....	.....
	35	3030	1600	370	−33	.....	0.21

[a] + Indicates no bleeding during time specified. [b] Activated carbon method A.S.T.M. D 1203-52T. [c] Beyond limit measurable on IP-4 tester (6 in.) on 1-in. standard test length.

The hydrogenated adducts, on the other hand, appear to be sufficiently compatible to be used as primary plasticizers with vinyl copolymer at the 35% level. Hydrogenation vastly improved the compatibility of these adducts without changing the other plasticizing characteristics appreciably.

The stocks plasticized with the nonhydrogenated and hydrogenated adducts are inferior in tensile strength, superior in modulus, and comparable in ultimate elongation and brittle point. In most instances they exhibit lower volatility than the stock plasticized with DOP (di-2-ethylhexylphthalate).

The outstanding characteristic of all these adducts is their high plasticizing efficiency, as reflected by the low modulus at 100% elongation. In certain instances, *viz.*, the ethyl and butyl adducts and the hydrogenated methyl and ethyl adducts, it was not possible to determine break and elongation at the standard plasticizer concentration of 35% since the extensibillity of the stock exceeded the limits of the IP-4 Scott Tester. These two properties were therefore evaluated at a lower plasticizer level (30%).

Although the tung oil-acrylonitrile adduct was not found to be the equivalent of DOP in certain respects, it does have the advantage of low volatility and is suitable as a primary plasticizer.

Summary

The addition products obtained by the Diels-Alder reaction of acrylonitrile with the methyl, ethyl, and butyl eleostearates have been prepared in good yields and purified by means of high-vacuum distillation. It has been possible to saturate preferentially by hydrogenation both the cyclic and exocyclic double bonds of the acrylonitrile adducts without material reduction of the nitrile group. Also the acrylonitrile adduct of tung oil has been prepared. All these adducts have been intercompared with DOP and evaluated as primary plasticizers for vinyl chloride-vinyl acetate copolymer. These products, with the exception of the nonhydrogenated ethyl and butyl derivatives, were found to be satisfactory as primary plasticizers. The results obtained indicate that hydrogenation greatly improves the compatibility of the adducts without changing their other plasticizing characteristics appreciably. The outstanding characteristic of all the adducts is their high plasticizing efficiency, as reflected by their low modulus at 100% elongation.

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Isolation of Two Nitrogen-Free Toxins from Tung Kernels

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TUNG MEAL AND OIL are produced from the seeds of the trees *Aleurites fordii* and *A. montana.* The meal is highly toxic to animals, including man, and has found little use except as a nitrogenous fertilizer. It contains 22–25% proteins and is a potentially valuable feedstuff. The program under which this research was conducted has as its goal the upgrading of tung meal by detoxifying it to render it acceptable as an animal feed.

Mann, Hoffman, and Ambrose (5) and Balthrop, Gallagher, McDonald, and Camariotes (1) recently reviewed the literature on the toxicity of tung kernels and meal. The consensus is that there are at least two toxic substances in the tung kernel. One of these

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substances is insoluble in organic solvents and is easily detoxified by heat. The second toxic material is extractable by ether, ethanol, and many other organic solvents (but not by petroleum ether) and is comparatively heat-stable. In the present investigation it was found that two nitrogen-free toxins could be separated from this soluble material by chromatographic procedures.

Experimental Procedures

Method of Testing for Toxicity. Chicks two to seven days old were used, and regular chick feed and water were kept before the chicks at all times. Known amounts of the material to be tested were weighed into No. 5 gelatin capsules and given to individual chicks at regular intervals. No attempts were made to determine the level of toxicity but simply to determine which fraction was toxic after separation had been made. For this purpose, in early stages of the work, the administration of 40-mg. doses of the material to a chick on two successive days was used as a standard test. If this dosage did not kill the chick, the material had low toxicity as compared to the crude extract. In the later stages of the work the standard treatment was reduced to a single 10-mg. dose.

Extraction of Crude Toxins from Tung Kernels. The crude toxin was extracted from the tung kernels with ethyl ether after the oil had been removed from the meal by exhaustive extraction with commercial hexane (b.p. range 64°–74°C.). The tung fruit *(Aleurites fordii)* used as the starting material was obtained as hulled kernels from commercial mills and in some cases stored for as much as eight months at 4°C. The kernels were completely freed of shells by hand. The hand-shelled kernels were ground first to a fine meal in a Bauer [2] mill set with a clearance of about 0.25 cm. (0.1 in.). This meal was then extracted repeatedly at room temperature with commercial hexane in a Buchner funnel, or in a glass column, 10 cm. x 60 cm., fitted with a porous glass bottom, to an oil content of 2 to 3%. It was then spread out in trays and dried at room temperature, then ground in a ball mill for 2 hrs., after which it was again put into the glass extraction column and extracted further with commercial hexane until the oil content had been reduced to 0.1 to 0.2%. Following this extraction, the meal was again dried at room temperature, put back into the column, and extracted with ethyl ether. About 3,000 ml. of ether were percolated through a column containing 1,000 g. of meal during a period of 24 to 36 hrs. Most of the ether was removed from the extract by distillation, and the remainder was driven off by warming the concentrated solution in a shallow dish over a hot water bath. Heating was continued no longer than necessary to dry the sample. The dry residue, amounting to about 0.5 g. from 1,000 g. of meal, was highly toxic to young chicks.

Extraction of Crude Toxins from Tung Press Cake. Commercial tung press cake, which consists of about equal parts of shell and kernel meal, was batch-extracted on a pilot-plant scale. One hundred and seventy-five pounds of press cake were charged into a cylindrical extractor (2) 13 in. by 60 in. located in the open. The extractor was filled with 35 gal. of benzene (Federal Spec. W-B-231a for Grade II). The cake was allowed to soak for at least two hours at ambient temperature (60° to 75°F.), and the unabsorbed benzene solution was then drained off. An equal amount of fresh benzene was added, and the extraction was repeated four times. The benzene solutions amounting to 60 to 75 gal., were concentrated in a rising film evaporator under reduced pressure at a temperature of 150° to 170°F. to a volume of about 3 gal. containing 50% nonvolatile substances (mostly tung oil).

An equal volume of 80% ethanol was added to the concentrated benzene extract. The resulting mixture was equilibrated in separatory funnels. After the mixture had separated into two phases, the lower phase containing most of the benzene and tung oil was drawn off and discarded as the nonvolatile matter present was not toxic. A dark colored flocculant sometimes formed in the upper phase, which was easily removed by filtration through glass wool. The filtered solution containing the toxins was then mixed with half its volume of commercial pentane (b.p. range 35°–59°C.); and after equilibrium had been reached, the upper phase was discarded. The extraction with commercial pentane was repeated until a nearly colorless upper phase resulted. The 80% ethanol solution (lower phase) was then concentrated by distillation under reduced pressure while nitrogen gas was bubbled through the liquid to prevent bumping and foaming. When the liquid volume had been reduced as much as feasible by this method, the concentrate was then reduced to complete dryness under reduced pressure in a rotary evaporator at temperatures of 50°C. or lower. The dried extract was then taken up in absolute ethanol, and the ethanol was evaporated to give crude toxins. About 70 g. of dry toxic residue were obtained from 175 lbs. of commercial press cake. This material corresponds to the ether extract of oil-free kernels.

Concentration of Crude Toxins. The initial work was done on the ether extracts from oil-free meal, but for the later work the product from the benzene extraction of commercial press cake was used. With no knowledge of the nature of the toxic material to

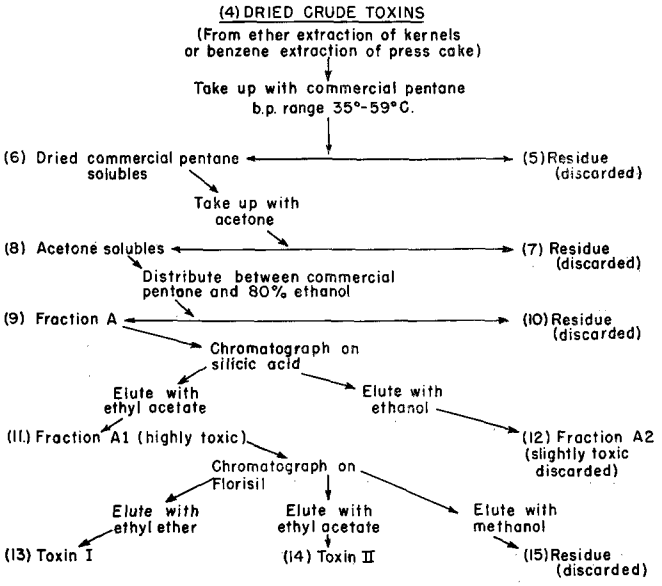

FIG. 1. Isolation of Toxins I and II from crude toxins. *Note:* The numbers assigned to the different materials correspond to those in Table I.

[2] It is not the policy of the Department to recommend the products of one company over those of any others engaged in the same business. This name is furnished merely for convenience and information.

TABLE I

Fractionation of Tung Kernels

Fractionation procedure	Materials	% on kernels	Results of chick tests
Ground kernels	Kernels	100	
Extract kernels with commercial hexane	1. Oil (discarded)	66	Meal is highly toxic to chicks
	2. Meal	34	
Extract meal with ethyl ether	3. Meal (discarded)	33.82	Half of chicks died in 122 hrs. on rations containing 12.5% tung meal
	4. Ether extract (crude toxins)	.18	40-mg. doses daily killed chicks in 24 to 36 hrs. after 1st dose
Take up ether extract in petroleum ether[a]	5. Petroleum ether insolubles (discarded)	.03	Chicks given 40-mg. doses on two successive days thrived
	6. Petroleum ether solubles	.15	40-mg. doses daily killed chicks in 18 to 66 hrs. after 1st dose. No more than two doses given to any chick
Take up petroleum ether solubles in acetone	7. Acetone-insolubles (discarded)	.12	Chicks given 40-mg. doses on two successive days thrived
	8. Acetone-solubles	.03	40-mg. doses daily killed chicks in 16 to 30 hrs. after 1st dose
Dissolve in 80% ethanol, extract 4–6 times with petroleum ether which has been equilibrated with 80% ethanol	9. Fraction A (remaining in 80% ethanol)	.015	Chicks given 3- to 20-mg. doses on two successive days died
	10. Material extracted by petroleum ether (discarded)	.015	Chicks given 40-mg. doses on two successive days thrived
Fraction A chromatographed on silicic acid column			
Fraction A1 is eluted with ethyl acetate	11. Fraction A1	.0087	20-mg. doses on 2 successive days killed chicks in 24 to 70 hrs.
Fraction A2 is eluted wih ethanol	12. Fraction A2	.0063	Chicks usually survived 20-mg. doses on 2 successive days
Fraction A1 chromatographed on Florisil			
Toxin I is eluted with ethyl ether	13. Toxin I	.0009	Single 10-mg. dose kills 4-day old chicks
Toxin II is eluted with ethyl acetate	14. Toxin II	.0017	Single 10-mg. dose kills 4-day old chicks
Residue eluted with methanol	15. Residue	.0057	Chicks survived 40-mg. doses on 2 successive days

[a] b.p. range 35°–59°C.

be isolated, various methods of fractionation were tried; and whenever an apparent fractionation was obtained, the different fractions were tested on chicks for toxicity. If one fraction proved toxic and the other not toxic, the nontoxic fraction was discarded. The scheme of extraction, concentration, and isolation of Toxins I and II from tung kernels together with data on toxicity and the proportions of different fractions obtained is given in Table I. The scheme of concentration and isolation from the crude toxins is also shown in Figure 1.

The dried crude toxins (Material 4, Table I) were taken up in commercial pentane in which the toxic material dissolves (although the toxic material is not extractable from the meal with this solvent). Typically, 5 g. of the crude toxins were triturated with five successive 20-ml. portions of commercial pentane, and the clear and almost colorless supernatant liquid was separated by centrifugation and decantation. Further concentration of the toxins was accomplished by evaporating the commercial pentane solution to dryness and triturating with five successive 20-ml. portions of acetone and drawing off the pale yellow supernatant liquid with a pipette. The toxins dissolved in the acetone, the nontoxic insoluble residue, mostly phosphatide, was discarded.

Tests with various solvent combinations in a Craig countercurrent distributor showed that the toxic material soluble in acetone could easily be separated from much inert material by distribution between commercial pentane and 80% aqueous ethanol. Thereafter the acetone solutions were evaporated to dryness; 200 mg. were dissolved in 100 ml. of 80% aqueous ethanol and extracted four to six times in a separatory funnel with equal volumes of commercial pentane which had been equilibrated with 80% ethanol. The toxins remained in the 80% ethanol, and the petroleum ether extract was discarded.

Additional concentration was achieved by evaporating the ethanolic solution to dryness (Fraction A) and chromatographing the residue on a silicic acid column. The silicic acid was Mallinckrodt's 100 mesh silicic acid specially prepared for chromatography by the method of Ramsey and Patterson, but before use a portion of the fines was removed by stirring the silicic acid into distilled water, allowing the mixture to settle for a few minutes. Then the supernatant liquid containing fines in suspension was poured off. This was repeated until about one-third of the weight of the silicic acid had been discarded as fines. The water was filtered off by suction in a Buchner funnel, and the acid was dried for 24 hrs. at 105°C. and stored in air-tight containers.

Ninety grams of the prepared silicic acid were slurried with 500 ml. ethyl acetate and poured into a column 45 x 200 mm. After the column had settled and the excess ethyl acetate percolated through, 1.5 g. of the residue dissolved in 3 to 5 ml. of ethyl acetate was carefully added to the top of the column, and the column was developed with 500 ml. ethyl acetate. The material eluted with ethyl acetate (Fraction A1) was highly toxic to chicks. The material absorbed by the column could be eluted with ethanol and was only slightly toxic to chicks.

Isolation of Toxins I and II. The ethyl acetate eluate from the silicic acid column (Fraction A1) was evaporated to dryness, dissolved in peroxide-free pure ethyl ether, and chromatographed on a Florisil column. Ethyl ether eluted a highly toxic substance which has been designated as Tung Toxin I. Following the ether elution, another highly toxic substance which has been designated as Tung Toxin II was eluted with ethyl acetate.

In routine isolations of Tung Toxins I and II columns 45 x 200 mm. with fritted glass bottoms were used. A typical procedure was as follows. Forty-two

grams of Florisil activated at 230°F. were weighed and slurried with peroxide-free ethyl ether (Baker, purified anhydrous) poured into the column and stirred to allow all air bubbles to escape; 350-mg. portions of the residue from ethyl acetate eluate were dissolved in 5 ml. of ether. Just as the ether used in slurrying sank into the absorbent, the sample solution was added with a dropping pipette and a thin layer of glass wool was placed in the columns. If part of the sample failed to dissolve in the ether, it was physically transferred to the column. As soon as the sample sank into the absorbent, more ether was poured into the column. Five hundred milliliters of ether were percolated through the column and evaporated to dryness to obtain 35 mg. of Toxin I. In addition, 600 ml. of ether were percolated through the column, and the small amount of material eluted was discarded since paper chromatography showed it to be neither Toxin I nor Toxin II.

As the last of the ether sank into the absorbent, 500 ml. of ethyl acetate were added to the column. This eluate was evaporated to dryness to obtain 75 mg. of Toxin II. Substantially all of the rest of the material added to the column could be eluted with methanol but was found to be nontoxic.

Characteristics of Toxins I and II. Toxins I and II were shown to be chromatographically homogeneous but different by chromatography on glass filter paper (No. x-934-AH, H. Reeve Angel and Company, 52 Duane street, New York, N. Y.) with cyclohexane containing 2% or 3% absolute ethanol. The paper was cleaned by igniting in a furnace at 600° (3) and impregnated with monopotassium phosphate (4a). The spots were visualized by spraying with concentrated sulfuric acid as described by Dieckert and Morris (4b).

Chromatography was carried out in 35 x 200 mm. test tubes. Ten milliliters of freshly prepared solution were put into a test tube, and a stopper carrying a bent glass rod through the center was inserted. By tilting the tube and shaking vigorously to saturate the air, the walls of the tube could be wet to within an inch of the stopper without wetting it. Strips of the paper, 30 x 190 mm., were spotted with 5 micrograms of the toxins about 30 mm. from the bottom of strip and about 15 mm. apart at equal distances from the edges when two spots were applied to the same strip. Strips were allowed to dry at room temperature for 15 min., fastened at the top with paper clips, and suspended from the rods in the test tubes for development. The R_f values were so sensitive to the exact conditions under which development was carried out that comparisons between the two substances were always made by chromatographing them on the same strip of paper.

Single spots were obtained for Toxins I and II. The R_f value of Toxin I was about 0.84 and that of Toxin II about 0.71 when developed with cyclohexane containing 3% absolute ethanol. The respective values were about 0.74 and 0.56 when developed with cyclohexane containing 2% absolute ethanol. Toxin I gives a less diffuse spot when developed with 2% ethanol solution and Toxin II when developed with 3% ethanol solution. Both toxins streaked in paper chromatography to some extent, and a trace of material nearly always remained at the origin.

Toxin II was shown to be unstable by paper chromatography. Two spots of the toxin were applied to a strip of glass paper and developed in cyclohexane containing 3% absolute ethanol until the solvent front rose about halfway to the top of the strip. The strip was then removed, dried at atmospheric temperature, and split down the middle into two strips. One strip was sprayed with sulfuric acid and heated to locate the spot. A dot was then put on the second strip to correspond to the position of the toxin on the first strip, and the second strip was cut off about an inch below this dot. If the second strip was allowed to dry at room temperature for about 4 hrs. and re-developed in the same solvent, the toxin no longer migrated to the expected position but remained at the origin, showing that some change had taken place in the material during the several hours of drying.

Toxin I, obtained by evaporation of the ether eluate from the Florisil column, dried to a viscous and tacky material, light yellow to orange in color. Toxin II obtained by evaporation of the ethyl acetate eluate dried to a clear, almost colorless, friable film. Both toxins gradually softened to very viscous liquids as the temperature was raised to 100°. Selected analytical data pertaining to Toxins I and II are given below.

Toxin I contained 70.6% carbon, 9.0% hydrogen, and 20.4% oxygen (by difference) and no nitrogen, phosphorus, sulfur, or halogen. The empirical formula $C_9H_{14}O_2$ corresponds to a composition of 70.1% carbon, 9.2% hydrogen, and 20.8% oxygen. Toxin I contained 5.7% hydroxyl and had a saponification equivalent of 268. It was optically active with a specific rotation of +65° to +78° in ethanol.

Toxin II contained 67.5% carbon, 8.2% hydrogen, and 24.3% oxygen (by difference) and no nitrogen, phosphorus, sulfur, or halogen. The empirical formula $C_{11}H_{16}O_3$ corresponds to a composition of 67.3% carbon, 8.2% hydrogen, and 24.5% oxygen. Toxin II contained 5.6% hydroxyl and had a saponification value of 401. It was optically active with a specific rotation in ethanol of +55° to +68°.

Summary

A procedure is described for isolating two nitrogen-free toxins from tung kernels and from press cake. Chick-feeding tests were used to determine which fraction was toxic at every separation. Both substances were highly toxic as 10-mg. doses killed 4-day old chicks. These substances were shown by chromatography on glass paper to be different and chromatographically homogeneous, but also unstable. The elementary composition, hydroxyl content, saponification value, and specific rotation for the two toxins are given.

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