an isomeric dibromide is present in this oil is shown by its behavior. When treated with a 2% solution of sodium carbonate the oil became cloudy and a white solid separated rapidly. This was washed repeatedly with sodium carbonate solution and with water. The neutral, unsaturated product, insoluble in water but readily soluble in organic solvents, was purified by crystallization from a small volume of boiling glacial acetic acid to which a few drops of water were added. The substance separated in sharp, colorless needles, melting at 81°. It was proved by analysis and by oxidation in cold permanganate solution to *p*-bromobenzoic acid, to be α-bromo-4-bromostyrene.

Anal. Calcd. for $C_8H_6Br_2$: C, 36.64; H, 2.29. Found: C, 36.09; H, 2.33.

The sodium carbonate filtrates deposited the yellow acid on acidification. The fact that no styrene derivative was formed when the pure, crystalline dibromide was similarly treated, shows that the red, oily residues contain a diastereoisomeric dibromide. After standing for several weeks at room temperature the oil had changed to the stable, solid form.

When the pure, crystalline dibromide was shaken with a 10% solution of sodium carbonate a small amount of a solid, similar in appearance to the styrene derivative just described, separated from the solution. It softens, however, at 100° and melts at 120° with vigorous effervescence. Analysis proves it to be a hydrate of the dibromide.

Anal. Calcd. for $C_{10}H_7O_3Br_3H_2O$: C, 27.71; H, 2.09. Found: C, 27.34; H, 2.10.

All of the ketonic acids here described dissolve in concentrated sulfuric acid to give an orange solution slowly changing to a persistent dark brown color. The brilliant color changes characteristic of the methoxy substituted acids do not take place.

Summary

4-Bromobenzalpyruvic acid was prepared for the purpose of studying the influence on the side-chain of the *p*-bromine atom as compared with that of other *para* substituents. The unusual stability of the dibromo addition product has made possible the preparation of isomeric β-bromo-4-bromobenzalpyruvic acids, believed to be chelated and open-chain isomers.

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The Non-crystalline Constituents of *Tephrosia virginiana* Roots

BY LYLE D. GOODHUE AND H. L. HALLER

The increasing demand for insecticides, such as rotenone and related compounds, that are non-toxic to man has intensified the search for new sources of these materials. The isolation of rotenone by Clark[1] from the roots of (*Cracca*) *Tephrosia virginiana* L., the most abundant species of *Tephrosia* indigenous to the United States, has stimulated interest in the development of a domestic source of this group of insecticides. Analytical studies[2] of samples of this plant, commonly known as devil's shoestring, have shown considerable variation in the amount of the insecticidal constituents, but recent selective breeding experiments[3] now make it possible to produce a root of uniformly higher quality. These new varieties are now available in limited amounts and have permitted further studies on the chemical constituents. This paper reports the results of such an investigation.

The extractives from a sample of root that analyzed 2.4% rotenone and 7.4% total extractives was divided into four fractions, namely, rotenone, alkali-soluble portion, neutral resin, and oil. Except for rotenone these fractions are for the most part noncrystalline mixtures.

The alkali-soluble portion, which was removed from an ether solution of the extractives with 5% potassium hydroxide, consists mainly of phenolic substances. In addition to the usual dehydro compounds and tephrosin that separate at this stage, a new substance having a melting point of 76° was isolated. Although it appeared in this fraction, the purified compound is not soluble in aqueous alkali. Attempts to isolate other crystalline substances or to prepare crystalline derivatives from this fraction were unsuccessful.

The neutral-resin fraction contains deguelin, which is easily obtained in the racemic form by treatment of the resin with dilute methanolic alkali. Although deguelin has been found in other species of *Tephrosia*, it has not previously been isolated from *T. virginiana*. Two other crystalline compounds also have been isolated by adsorption on activated carbon. One is an orange-

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(1) Clark, *Science*, **77** (n. s.), 311 (1933).

(2) Jones, Campbell and Sullivan, *Soap*, **11**, [9] 99 (1935).

(3) The material used in these studies was furnished by G. A. Russell, of the Bureau of Plant Industry, U. S. Department of Agriculture, and V. A. Little, of the Texas Agricultural Experiment Station, for whose coöperation we wish to express our appreciation.

yellow compound, m. p. 125°, to which the formula $C_{22}H_{24}O_4$ has been tentatively assigned; and the other, m. p. 131°, is identical with one isolated by Clark to which he has assigned the molecular formula $C_{20}H_{24}O_4$.

The oily fraction has been further separated by distillation into five fractions, four of which are liquid and one is semi-solid. The three lower boiling fractions have been shown to possess the properties of sesquiterpenes. Two of these give the azulene test, but no crystalline derivatives could be prepared. The semi-solid that was obtained by molecular distillation is a complex mixture containing resins, oils and probably some polymerized terpenes. The oil fraction, which was not distillable, was composed largely of glycerides. It had an iodine value of 142, placing it high in the class of semi-drying oils.

Experimental

After being dried and ground, 3 kg. of improved *Tephrosia virginiana* root were exhaustively extracted with chloroform by percolation. The chloroform was removed under reduced pressure, and the extractives were taken up in 1 liter of ether. Most of the rotenone crystallized and was removed by filtration. The ether solution was then extracted with three 100-cc. portions of 5% potassium hydroxide in saturated sodium chloride solution, and washed with water, 2% hydrochloric acid, and again with water. The ether was removed and the residue was taken up in carbon tetrachloride, from which the remainder of the rotenone was allowed to crystallize. A total of 72 g. of rotenone was obtained.

Alkali-soluble Fraction.—The portion soluble in aqueous alkali was recovered by acidifying the 5% potassium hydroxide solution with hydrochloric acid and extracting with ether. Some crystals which separated at this point were found to be a mixture of tephrosin, m. p. 192°, and dehydrorotenone, m. p. 225°, $[\alpha]^{20}D$ −37°. The phenolic material was purified by again dissolving in 5% potassium hydroxide saturated with sodium chloride and extracting with ether. The phenolic substances were again recovered and dried *in vacuo*. Some of this material was distilled in a molecular still, and a small amount of a substance melting at 76° crystallized from the first fraction. It was later found that this substance separated from an alcoholic solution of the phenolic substances. It was purified by adsorption on activated carbon (Darco G 60) from alcohol and elution with ether. When it was mixed with the substance obtained by molecular distillation, no depression of the melting point resulted. This compound was not soluble in alkalies. It did not give the Goodhue red color test[4] for rotenone and related compounds, and contained no methoxyl.

All fractions of these aqueous alkali soluble substances prepared by molecular distillation were highly colored, and no further crystalline material was obtained. Each fraction gave a distinct coloration with ferric chloride. Attempts were made to prepare derivatives with 3,5-dinitrobenzoyl chloride, with bromine, and by nitration, but no workable product was obtained.

Separation of the Oil from the Neutral Resin.—After the removal of the aqueous alkali soluble substances and the last of the rotenone, there remained a mixture of neutral resin and oil in carbon tetrachloride solution. The carbon tetrachloride was removed, and the residue was taken up in 200 cc. of glacial acetic acid; 200 cc. of petroleum ether was added, followed by 20 cc. of water to cause the layers to separate. The petroleum layer contained the oil. Several extractions of each layer with the other solvent were made to obtain a better separation. The acetic acid was removed from the petroleum ether with alkali, and the oil was recovered. The neutral resin was recovered from the 90% acetic acid by precipitation with water and extraction with ether. The ether was extracted with 5% potassium hydroxide and washed with water, 2% hydrochloric acid, and again with water.

Neutral Resin.—The neutral resin, when treated with dilute methanolic potassium hydroxide, deposited about 20% of its weight as racemic deguelin, which, after recrystallizing once from carbon tetrachloride and once from ethanol, melted at 171°. The crystals had the characteristic lathlike shape and other optical characteristics of deguelin. The substances rendered alkali-soluble were removed from the non-crystalline material after racemization. This resin gave a specific rotation of −60° as compared with −90° for the original resin in benzene, and it still gave a red color about half as intense as that given by the original resin in the Goodhue test.

Further proof of the presence of deguelin was given by the isolation of l-dihydrodeguelin from the neutral resin. Upon hydrogenation in alcohol with the Adams catalyst, about 0.75 g. of this material was obtained from 5 g. of neutral resin. It melted at 155°; 0.2045 g. of substance in 5 cc. of benzene rotated −8.59° in a 2-dm. tube, $[\alpha]^{20}D$ −103.2°.

Another portion of neutral resin was treated in ethanol solution with Darco G 60. The carbon was extracted with ether in a Soxhlet for twenty-four hours, and the resulting material in a fairly concentrated ether solution was seeded with tephrosin and inactive deguelin, and placed in the refrigerator to crystallize. A large part of the deguelin was either oxidized to tephrosin or changed to inactive deguelin by this process. These compounds are removed most easily at this point. The non-crystalline portion was recovered and distilled in a molecular still at 180° under 0.0001 mm. pressure. The distillate, when dissolved in alcohol, deposited masses of orange-yellow needles which, after recrystallizing twice from ethanol, melted at 125°.

Anal. Calcd. for $C_{22}H_{24}O_4$: C, 74.96; H, 6.87; 2MeO, 17.60; mol. wt., 352.2. Found: C, 75.03, 74.88; H, 6.74, 6.65; MeO, 17.66, 17.66; mol. wt. (Signer's method[5]), 350. In benzene solution the compound was optically inactive.

After the mother liquor from the yellow compound had stood overnight, large masses of white needle-shaped crystals were deposited which, after several crystalliza-

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(4) Goodhue, *J. Assoc. Official Agr. Chem.*, **19**, 118 (1936).

(5) Signer, *Ann.*, **478**, 246 (1930).

TABLE I

PHYSICAL AND CHEMICAL DATA ON THE FRACTIONS OF THE OIL FROM *Tephrosia virginiana*

Fraction	B. p., °C.	Density (28° C.), G. per cc.	Refractive index (28°C.)	Sp. rotn. in acetone (20°C.)	Sapn. no.	Unsaponifiable residue	Iodine No. (Wijs)
Terpene I	80– 95 (1 mm.)	0.928	1.4995	−10	...	....	...
Terpene II	95–115 (1 mm.)	.948	1.5005	+ 6.25	...	....	...
Terpene III	115–140 (1 mm.)	.929	1.4925	− 1.24	...	....	...
Semi-solid	150–200 (0.0001 mm.)	1.162	1.5465	....	145	52.2	138
Residual oil	Non-volatile	0.954	1.4810	0 (in Bz)	186	12.4	142

tions from ethanol, melted at 131°. This is apparently the same compound as that isolated by Clark, for a mixed-melting-point determination with a sample furnished by him gave no depression.

Anal. Calcd. for $C_{21}H_{22}O_4$: C, 74.51; H, 6.55; 1MeO, 9.17; mol. wt., 338.2. Found: C, 74.26, 74.46; H, 6.51, 6.61; MeO, 9.21, 9.22; mol. wt. (Signer's method[5]), 339. 0.106 g. in 5 cc. of benzene solution rotated 4.05° in a 2-dm. tube; $[\alpha]^{20}_D$ −95.5.

After the removal of these two crystalline compounds considerable resin remained, which upon treatment with dilute methanolic alkali deposited no crystalline material.

The activated carbon after elution with ether still contained a substantial amount of material which could be eluted with chloroform. No crystalline substance has yet been obtained from this fraction.

Oil.—The oily substances in *Tephrosia virginiana* which were separated from the 90% acetic acid solution of the neutral substances by extraction with petroleum ether were further fractionated. The first fraction, which appeared to be composed mainly of sesquiterpenes, was removed at 150° under 1 mm. pressure, and later redistilled and divided into three fractions. By reducing the pressure to 0.0001 mm. a semi-solid fraction was obtained at this temperature. The liquid left in the still had many characteristics of a vegetable oil. The data on these fractions are assembled in Table I.

The first and second terpene fractions gave the azulene test, which indicated the presence of a sesquiterpene or sesquiterpene alcohol. The first fraction was insoluble in 90% ethanol, whereas the second was completely soluble. The first fraction is probably composed mostly of hydrocarbons; the second is probably hydroxyl derivatives. No solid derivatives could be obtained from any of the terpene fractions.

The semi-solid fraction was a mixture of resin and oil, as indicated by the high unsaponifiable residue.

The oil fraction as obtained by this treatment was probably polymerized to some extent, since it would be expected to distil at 150° under 0.0001 mm. pressure. Glycerol could be detected in the original oil, but the water-soluble substances from the saponification of 1.0 g. gave the test with less difficulty.

Summary

1. The insecticidal constituents of *Tephrosia virginiana* have been studied further. The total chloroform extractives were divided into four fractions: rotenone, alkali-soluble portion, neutral resin and oil.

2. A small amount of a substance melting at 76° was isolated from the alkali-soluble fraction. The non-crystalline material appeared to be composed largely of phenolic substances.

3. The neutral-resin fraction yielded deguelin, which is reported for the first time as one of the constituents of this species. By adsorption on activated carbon and molecular distillation, two other compounds were isolated from this fraction in considerable quantity. One of these, melting at 131°, has been reported by Clark; the other is an orange-yellow compound, m. p. 125°, not previously reported.

4. The oil fraction was composed largely of liquids having the characteristics of sesquiterpenes. The remainder was a drying oil from which a small amount of semi-solid resin was removed by molecular distillation.

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