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Constituents of the Insecticidal Resin of the Yam Bean (*Pachyrrhizus erosus*)[1]

BY L. B. NORTON[2] AND ROY HANSBERRY[3]

The seeds of the yam bean show a toxicity to several species of insects comparable with that of the rotenone-bearing roots of Derris and Lonchocarpus.[4] They are of interest as a potential source of insecticidal material because of their ease of cultivation and harvesting, their annual bearing, and their occurrence in areas in and close to the United States. The present investigation is correlated with a clarification of the taxonomy of the genus *Pachyrrhizus*[5] and a chemical and toxicological survey of a number of seeds from known sources collected during the botanical studies.[6]

Greshoff[7] isolated from the seeds a toxic resinous material which he called "pachyrrhizid," and which resembled a corresponding resin "derrid" from Derris root, now known to consist largely of rotenoids. Van Sillevoldt[8] obtained pachyrrhizid as a more homogeneous non-crystalline resin, and three pure crystalline compounds from the seeds.

Nag, *et al.*,[9] made a detailed study of the composition of the seeds and of their ash and oil. They considered that the toxic principle might be a saponin. Hwang[10] reported the presence of rotenone on the basis of color tests, and isolated two insecticidally inactive crystalline compounds. Norton[11] confirmed the occurrence of rotenone by isolation of the pure compound. Most of this earlier work, as well as that reported here, has been carried out on seeds of *P. erosus* (synonymous with *P. angulatus*[5]), although the seeds of other species appear to be very similar in composition.[6]

In the present investigation, attempts were first made to separate the constituents by crystallization. This method soon proved inadequate, because of the complexity of the mixture and the presence of much non-crystalline material. Chromatographic adsorption on alumina proved more successful, and was adopted as the basis for the fractionation of the yam bean resin into the components reported here.

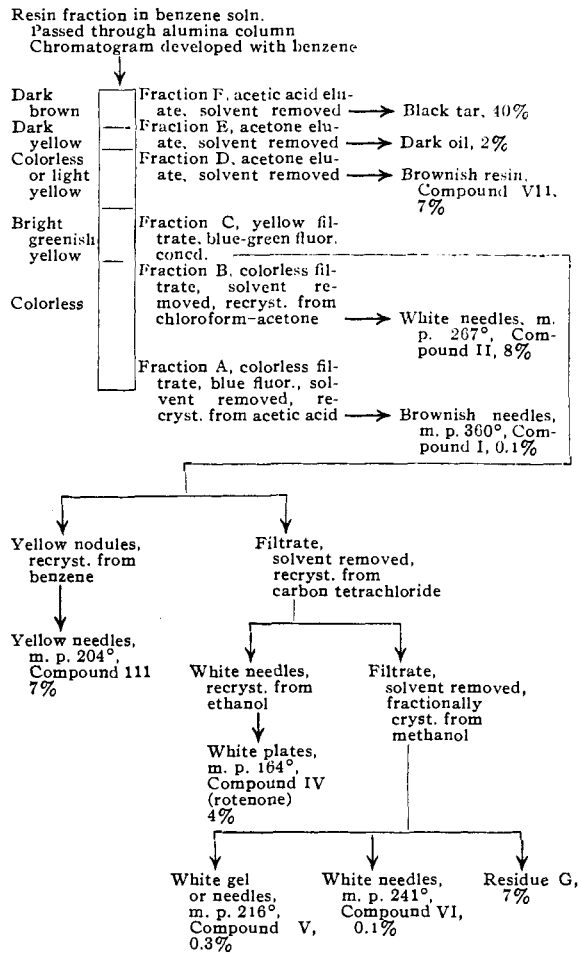

Fig. 1.—Fractionation of yam bean resin.

Experimental

Isolation of Components

Preparation of Resin.—Yam bean seeds (2350 g.) grown in Mexico were coarsely ground in a hammer mill and extracted for forty-eight hours with ethyl ether in a Soxhlet-type extractor.[12] The marc was dried, reground to pass a 60-mesh sieve, and extracted for an additional forty-eight hours with fresh solvent. The extracts were combined and most of the solvent distilled off. On standing overnight, the concentrated extract deposited 3.5 g. of crystalline material which was later found to consist mainly of compounds II and III, and was added to the appropriate fractions during the chromatographic separation (Fig. 1). Most of the remaining ether was removed under reduced pressure, leaving 670 g. of a brown oil.

The oily extract was dissolved in a mixture of 500 cc. of glacial acetic acid and 2000 cc. of petroleum ether (30–60°), 50 cc. of water added, the mixture shaken in a separatory funnel, and the layers separated.[13] Each layer was washed with several fresh portions of the other solvent to

(1) Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 633.

(2) Present address, Department of Entomology, Cornell University, Ithaca, N. Y.

(3) Department of Entomology, Cornell University, Ithaca, N. Y. Present address, Shell Oil Co., Agricultural Laboratory, Modesto, Calif.

(4) Hansberry and Lee, *J. Econ. Entomol.*, **36**, 351 (1943).

(5) Clausen, Cornell Univ. Agr. Expt. Sta. Memoir **264**, Nov., 1944.

(6) Hansberry, Clausen and Norton, in preparation.

(7) Greshoff, *Meded. uit's Lands Plantentuin*, **7**, (1890).

(8) van Sillevoldt, *Arch. Pharm.*, **237**, 595 (1899).

(9) Nag, Banerjee and Pain, *Trans. Bose Research Inst.*, Calcutta, **11**, 83 (1935–1936), *C. A.*, **33**, 3422 (1939).

(10) Hwang, *Kwangsi Agr.*, **2**, 269 (1941), in Chinese. English summary in *Rev. Appl. Entomol.*, **30A**, 418 (1942).

(11) Norton, THIS JOURNAL, **65**, 2259 (1943).

(12) Rapp, Woodmansee and McHargue, *Ind. Eng. Chem., Anal. Ed.*, **15**, 351 (1943).

(13) Goodhue and Haller, THIS JOURNAL, **62**, 2520 (1940).

increase the sharpness of separation and the washings added to the corresponding main solution. A light brown powder remained insoluble in both layers. When shaken with water, this powder formed a colloidal suspension showing marked surface activity. It showed no toxicity to insects, and was not further investigated.

The petroleum ether solution was washed with water, sodium bicarbonate solution, and again with water, dried with anhydrous sodium sulfate, and the solvent evaporated under reduced pressure. The residue was a brown mobile oil (627 g., 26.7% of original beans), showing very faint color tests for the rotenoids and no significant toxicity to insects. It was not further investigated.

The acetic acid solution was diluted with 4 volumes of water and extracted with five 500-cc. portions of ether and two of chloroform. The aqueous layer was discarded. The ether–chloroform solution was washed twice with water containing sodium sulfate to reduce emulsification, dried with anhydrous sodium sulfate, and the solvent evaporated under reduced pressure. The residue was twice taken up in benzene and evaporated to dryness under reduced pressure to remove traces of the other solvents The residue was a brittle brown resin (30 g., 1.3% of original beans; 33.5 g., 1.4% including the crystalline matter previously separated) showing intense color tests for the rotenoids and a marked toxicity to insects. All of the fractions described were isolated from this resin.

Preparation of Adsorbent.—From preliminary tests, aluminum oxide "Ignited Powder, Reagent Grade, General Chemical Co.," proved to have adequate adsorptive properties. To remove alkali, the presence of which in alumina has been shown[14] to cause alteration in the rotenoids, the material was washed by decantation with distilled water until the washings were neutral to litmus (about 12 times), filtered, and dried for several hours at 125°. This heat-activated material, however, caused extensive decomposition of the yam bean resin, while air-drying yielded a product insufficiently adsorptive for good fractionation. Partial activation by controlled heating gave some samples of excellent properties, but was not reproducible on a larger scale. The procedure finally adopted was a full activation by heat, followed by moistening with methanol and exposure to the air in a thin layer overnight. Used adsorbent could be recovered by burning off organic matter at dull red heat in a muffle furnace and repeating the methanol treatment. This partially deactivated product required several repetitions of the chromatographic procedure for satisfactory separations, but was easily reproducible and caused no detectable change in the resin constituents. Evidence for the lack of alteration of the resin constituents was furnished by the isolation of optically active, alkali-sensitive and toxic compounds after adsorption, and by the isolation of several compounds which could be recognized in the original resin by color tests, fluorescence, or direct crystallization.

The adsorbent was packed in the chromatographic columns by inserting a small cotton plug and pouring in portions of a slurry of the prepared alumina in benzene. Each portion was allowed to settle, with tapping, under gravity drainage or slight suction. The bed of adsorbent was kept completely covered with solvent throughout its preparation and use, and the top of the finished bed was protected from mechanical displacement by a closely fitting disk of filter paper.

A 5 × 80 cm. column was used for the preliminary separation, and assorted smaller columns for the sharper division of individual fractions and mother liquors. The quantity of adsorbent needed for the desired separation in the larger columns was determined by small-scale tests in 4-mm. columns.

Color Tests.—Several of the tests which have been proposed for the detection or estimation of rotenone and related compounds were used qualitatively for a sharper division of the final chromatographic fractions than that permitted by the preliminary criteria given in Fig. 1. These tests were also made quantitatively on the individual compounds isolated, both as a means of identification and as a basis for their tentative quantitative estimation in mixtures.[6]

The qualitative Durham test was made by evaporating a test portion of the substance to dryness on a spot-plate, adding a drop of concentrated nitric acid, and neutralizing the acid after thirty seconds with concentrated ammonia.

The Goodhue test was carried out essentially as published.[15] For the quantitative determinations, the color was developed for one hour at 20°.

The Meyer test[16] was modified by adding the sulfuric acid–nitrite reagent directly to an acetone solution of the substance tested without dilution of the latter with water. Quantitatively, the color was developed for one hour at 20°.

The version of the Rogers–Calamari test[17] using acetone solutions and hydrogen peroxide as oxidizing agent was found most convenient. The color was developed for forty-five minutes at 20°.

For the quantitative tests, the light transmission was determined in a Lumetron Model 402E photoelectric colorimeter with filters of the designated transmission maxima. The procedures are given in greater detail elsewhere.[6]

Melting Points.—Like most of the known rotenoids,[18] several of the yam bean constituents showed a marked m. p. depression in soft glass capillaries. All of the values reported here were determined either in Pyrex capillaries in an aluminum block or between quartz plates on a microscope hot stage. Comparison of the latter two methods showed no significant differences, even with the most alkali-sensitive of the compounds. For those compounds showing decomposition or rapid sublimation at the m. p., capillaries containing a few crystals were dropped into the block at successively higher temperatures until complete melting with little preliminary decomposition occurred within thirty seconds.

Fractionation of Resin.—A 6% solution of the resin in benzene was drained through the column either by gravity or by slight suction, and was followed with pure benzene to develop the bands. The general appearance of the chromatogram is indicated in Fig. 1, although the two top bands were sometimes divided into a number of narrow bands of varying dark colors. The three lower fractions were very weakly adsorbed, moving down rapidly on further development, and were most conveniently collected in the filtrate as indicated. The three upper fractions were obtained by mechanical separation of the colored sections of adsorbent and elution of each with the proper solvent.

Each of the preliminary fractions was repeatedly fractionated on fresh columns and similar fractions combined, until no further separation was evident or in some cases until purification so reduced the solubility of the material that further fractionation was impractical.

Fraction A was collected from the first appearance of a blue fluorescence in the filtrate to the appearance of a Meyer color test in a small test portion of the filtrate. The latter test gave a sharper division of fractions than did the gradual diminution of fluorescence. Additional material belonging to this fraction was obtained by elution with acetic acid of otherwise exhausted columns used for the other fractions and in the recrystallization of Fraction B, since the extremely low solubility of this compound apparently led to some crystallization in the columns. These combined materials were evaporated to dryness and recrystallized several times from acetic acid and from large volumes of chloroform and acetone, giving 30 mg. (0.1%) of compound I.

Fraction B was collected from the appearance of a Meyer test in the filtrate to the appearance of a yellow color and a blue-green fluorescence. Chromatographic treatment of

(14) Cahn, Phipers and Boam, *J. Soc. Chem. Ind.*, **57T**, 200 (1938).

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

(16) Meyer, *Rec. trav. chim.*, **55**, 954 (1936).

(17) Rogers and Calamari, *Ind. Eng. Chem., Anal. Ed.*, **8**, 135 (1936).

(18) Jones, *ibid.*, **13**, 819 (1941).

TABLE I

PROPERTIES OF INDIVIDUAL COMPOUNDS FROM THE YAM BEAN

Compound	Form	M. p. (cor.), °C.	$[\alpha]^{25}D$ in benzene[a]	%C[b] obs.	%C calcd.	%H obs.	%H calcd.	%CH_3O[c] obs.	%CH_3O calcd.	Mol. wt.[d] obs.	Mol. wt. calcd.	Empirical formula	CH_3O groups
I	Light brown needles	360 (sbl., dec.)	(insol.)	66.87	67.51	2.52	2.52	0.00	0.00	180 ±	160	$C_9H_4O_3$	0
II	White needles	272 (sbl., dec.)	0	65.32	65.60	3.87	3.85	8.49	8.47	360 ±	366	$C_{20}H_{14}O_7$	1
III	Yellow needles	204	0	67.95	68.96	3.39	3.47	8.92	8.91	350	348	$C_{20}H_{12}O_6$	1
IV	White plates	164	−236°		70.02		5.62		15.74		394	$C_{23}H_{22}O_6$	2
V	White needles or gel	218	+234°	68.31	68.17	4.65	4.57	17.4	17.62	360	352	$C_{20}H_{16}O_6$	2
VI	White needles	242	0(?)	67.75	68.96	3.54	3.47	8.89	8.91	354	348	$C_{20}H_{12}O_6$	1
VII	Light yellow resin	100 ±	+24°	65.59	65.40	4.34	4.29	7.48	7.35	400 ±	422	$C_{23}H_{18}O_8$	1

[a] The concentrations were as close to 1 g./100 cc. as the solubility or the amount available permitted and were as follows: II, 0.28 g./100 cc.; III, 0.3 g./100 cc.; IV, 1 g./100 cc.; V, 0.35 g./100 cc.; VII, 1 g./100 cc. The rotations were measured in 2-dm. tubes (V in a 1-dm. tube) in a Schmidt and Haensch triple-field polarimeter with a sodium vapor lamp. [b] Carbon and hydrogen were determined by microcombustion by the Carl Tiedcke Laboratory, New York. [c] The methoxyl content was determined by a micro adaptation of the volumetric hydriodic acid method.[19] [d] Determined by a modification of the Rast method, using camphor as solvent.[20] The constant for the solvent was determined against pure rotenone.

the crystalline material separating from the original extract yielded further quantities of this fraction. Concentration of the benzene solution yielded white needles which were best recrystallized from chloroform–acetone mixtures. Separation from traces of compound I was difficult, and the melting point gradually rose during purification from 250° to a constant value of 272° (with decomposition). The material (2.5 g., 8%) was designated compound II. It was extremely sensitive to alkali, and the use of soft glass capillaries reduced the m. p. by about 50°.

Fraction C was collected from the appearance of yellow color and blue-green fluorescence in the filtrate to the disappearance of the Goodhue test. The fluorescence disappeared too gradually for use as a sharp indicator of the fraction limit. Some evidence was noted of a partial segregation of the components of this complex fraction on the column, but no practical separation could be obtained with the adsorbent used. Further quantities of this fraction were obtained in addition to Fraction B by chromatographic treatment of the crystals separating from the original extract.

On concentration, the benzene solution of Fraction C deposited several crops of yellow nodules. They were recrystallized from benzene to yield 2 g. (7%) of light yellow fluorescent needles, designated compound III.

When further concentration of the benzene solution led to the deposition of an amorphous material, all of the benzene was evaporated off under reduced pressure and the residue taken up in hot carbon tetrachloride. On cooling for several hours at 0°, a brown amorphous material separated and was filtered off. On scratching, shaking, or seeding with rotenone–carbon tetrachloride solvate, the solution deposited a mass of white needles. Recrystallization from carbon tetrachloride and finally from alcohol yielded 1.2 g. (4%) of white plates, designated compound IV and later identified as rotenone.[11]

After crystallization of all the rotenone possible, the carbon tetrachloride was evaporated from the filtrate under reduced pressure and the residue subjected to a systematic fractional crystallization from methanol or chloroform–methanol. The first fraction came out as a gel or gelatinous precipitate, which was finally converted to silky white needles as purification progressed. The final product (80 mg., 0.3%), designated compound V, showed a marked tendency even when pure to separate from solution as a gel, which could be converted to crystals by seeding.

The next fraction consisted of white needles (30 mg., 0.1%) designated compound VI. Until fairly pure, it tended to separate very slowly over a period of weeks as white nodules, accompanied by much non-crystalline residue from which it was separated mechanically. On standing in the light, solutions tended to turn pink.

No further new crystalline compounds were obtained by continuation of the fractional crystallization, which yielded only small residual quantities of compounds II and III and a large non-crystalline residue G. The latter contained some VII, as indicated by its behavior on the adsorption column, and from its toxicity (Tables II and III) very probably contained one or more toxic compounds not isolated.

Fraction D was collected mainly by removal of the colorless or light yellow layer of adsorbent remaining in the lower part of the column after the preceding fractions had been carried through completely with benzene, and elution with acetone. A small proportion was carried through into the filtrate following the disappearance of the Goodhue test which characterized Fraction C. The upper limit of Fraction D was taken as the point where a test portion of the adsorbent eluted with a few drops of acetone began to show a brown color or none at all in the Meyer test, instead of the clear purple characterizing the main fraction. Evaporation of the solvent from this fraction left a light brown resin from which no crystals could be obtained from a wide variety of single and mixed solvents. Treatment with alcoholic alkali failed to induce crystallization. Further chromatographic treatments, fractional precipitation, and molecular distillation failed to yield demonstrably different subfractions, although some of the color could be eliminated. The whole fraction was finally adsorbed on an alumina column, developed thoroughly with benzene, an appreciable quantity of the material at each end of the fraction arbitrarily discarded, and the remainder eluted with acetone. The resin thus obtained (2 g., 7%) was tentatively assumed to be homogeneous, and was designated compound VII.

Fraction E was collected as the acetone eluate of the darker yellow area between the upper limit of Fraction D and the dark brown bands of Fraction F. Both fractions E and F were undoubtedly heterogeneous and were not sharply divided. Some additional material having the properties of Fraction E could be obtained by acetone elution of the brown adsorbent containing F. Removal of the solvent from the eluate left 0.5 g. (2%) of a brown oil from which no crystalline material could be obtained. Molecular distillation yielded a small fraction apparently identical with compound VII. Fraction E as a whole had no appreciable toxicity, and was not further investigated.

(19) Niederl and Niederl, "Organic Quantitative Microanalysis," John Wiley and Sons, New York, N. Y., 1938, p. 190.

(20) Niederl and Niederl, *ibid.*, p. 171.

Fraction F was collected as the acetic acid eluate of the upper dark brown section of the columns, following removal of Fraction E components by elution with acetone. Some additional similar material was obtained from the area from which most of Fraction E had been obtained. Removal of the solvent left a dark brown tar (12 g., 40%) containing a very insoluble solid component. Molecular distillation yielded a trace of compound VII. The fraction showed no appreciable toxicity, and was not further investigated.

The purity and homogeneity of each of the compounds was checked by distillation at 0.001 mm. pressure with a 5-mm. distance to the condensing surface. At temperatures ranging from 150 to 200°, each distilled unchanged except for slight decomposition, and a single recrystallization of distillate and residue yielded products identical with the original compound.

The color tests and melting points have already been discussed in connection with the isolation of the compounds.

Dehydro Compounds.—The dehydro compounds were prepared by the usual method of adding iodine to a boiling alcoholic solution of the compound containing potassium acetate, and treating the product with alcoholic sulfuric acid. The acetyl derivative was not isolated. Identical dehydro derivatives of compounds IV and V were also prepared by the Tattersfield and Martin modification of the Takei procedure.[21]

Alkaline Degradation.—The alkaline degradation results are somewhat tentative and incomplete due to lack of both time and material. Samples of each of the compounds (0.5–5 mg.) were boiled for two hours with 5% alcoholic potassium hydroxide. The solutions were diluted with water, acidified and extracted with ether. The ether solution was shaken with 5% sodium bicarbonate, followed by 5% aqueous potassium hydroxide and water, and the ether evaporated from the remaining neutral fraction after drying with anhydrous sodium sulfate. The two alkaline solutions were acidified and extracted with ether, and the ether solutions washed with water, dried with anhydrous sodium sulfate, and the ether evaporated. The principal fractions were taken up in alcohol and a portion tested for phenolic properties with a drop of dilute ferric chloride solution. Where possible, crystalline derivatives were isolated from the remainder.

Small samples of each of the compounds were also boiled for two hours with 5% alcoholic potassium hydroxide to which 50 mg. of zinc dust was added. The solutions were filtered and the reaction products fractionated as above. Rotenone itself was not included in the tests.

Toxicity.—Each of the compounds, Residue G and the combined residues from all of the mother liquors obtained in the separations and purifications were tested for toxicity against the Mexican bean beetle (*Epilachna varivestis Muls.*) and the silkworm (*Bombyx mori L.*). Each material was dissolved or suspended in 200 parts of acetone, and an equal quantity of water added immediately before use. For tests against the Mexican bean beetle, 1 cc. of the acetone–water suspension was applied through a De Vilbiss nose and throat atomizer directed through the top of a bell jar directly into a 110-mm. specimen dish. The bottom of the dish was covered with two layers of paper toweling on which a large bean leaf was closely pressed. Fifty third instar bean beetle larvae were used in each dish, and mortality counts were made each day for four days. The results are shown in Table II.

For tests against the silkworm, mulberry leaves were dipped into the acetone–water suspension, dried, and 10 fourth instar silkworm larvae were placed on each leaf in 110-mm. specimen dishes. Forty larvae were used with each material. Mortality counts were made every twelve hours. The results are shown in Table III.

TABLE II

TOXICITY OF YAM BEAN CONSTITUENTS TO THIRD INSTAR MEXICAN BEAN BEETLE LARVAE

Constituent	Mortality % 1 day	2 days	3 days	% Foliage eaten
Compound I	0	0	0	100
II	0	0	0	100
III	0	0	0	100
IV (Rotenone)	82	100		0
V	0	2	2	5
VI	0	0	0	85
VII	2	4	6	50
Residue G	96	96	96	0
Combined residues from recrystn.	28	64	90	5

(21) Tattersfield and Martin, *Ann. Appl. Biol.*, **22**, 578 (1935).

TABLE III

TOXICITY OF YAM BEAN CONSTITUENTS TO FOURTH INSTAR SILKWORM LARVAE

Constituent	Mortality % 12 hr.	24 hr.	36 hr.	48 hr.	60 hr.	72 hr.	84 hr.
Compound I	0	0	0	0	3	3	3
II	0	0	0	0	0	3	3
III	0	0	0	0	0	0	3
IV (Rotenone)	33	65	77	90	95	95	98
V	50	65	68	70	78	78	83
VI	0	0	0	0	0	0	0
VII	33	45	63	83	90	95	100
Residue G	70	95	100				
Combined residues from recrystn.	55	80	95	100			

Nature of Compounds

As might be expected from their occurrence with rotenone itself (Compound IV), most of the materials isolated show some evidence of a relationship to the rotenoids. With the exception of I, all have a similar type of empirical formula, contain methoxyl groups, and respond to one or more of the color tests for the rotenoids. Several react with alkali to form phenols and phenolic acids. Two in addition to rotenone are optically active, toxic and form dehydro derivatives. After rotenone itself, compound V appears most closely related to the known rotenoids, followed by VII, II, VI and III which contain but one methoxyl group and show decreasing resemblance in other respects, and no relation to the rotenoids has become apparent for I. No direct relationship between the different compounds isolated has been established as yet either by interconversion or by common derivatives.

None of the compounds is appreciably soluble in dilute aqueous acid or alkali. Attempts to prepare oximes with hydroxylamine hydrochloride and potassium acetate in alcoholic solution have been unsuccessful or inconclusive.

Compound I.—This compound is the least abundant of the materials isolated. Its unusually high m. p. and low solubility are noteworthy. Its intense blue fluorescence, with a maximum at about 410 mμ., furnishes a very sensitive test for its presence, being easily visible in daylight at 0.1 p. p. m. Unlike that of III, its fluorescence is of approximately equal intensity in different solvents. It gives a negative Durham, Goodhue, Meyer and Rogers–Calamari test, although a fugitive grass-green color appears when the Meyer reagent is first added. It gives no color with ferric chloride in alcoholic solution.

Compound I shows no toxicity, contains no methoxyl groups, and shows no apparent relation to the other compounds isolated. The analytical data appear to warrant only a tentative choice of empirical formula. Although insoluble in dilute aqueous alkali, the compound dissolves in boiling alcoholic alkali to form a water-soluble product from which the original compound is precipitated by acids unchanged. This behavior suggests the presence of an

enol or a lactone group, the latter being possibly more likely in view of the low hydrogen and high oxygen content of the compound.

Compound II.—This compound appears to correspond to the white needles having a m. p. over 240° reported by van Sillevoldt.[8] The compound responds to all of the rotenoid color tests. In the Durham test, only an instantaneous green flash appears when the nitric acid is first neutralized with ammonia, and is followed by a reddish brown color. The color in the Goodhue test has 20% of the intensity given by an equal weight of pure rotenone; that in the Meyer test is equal at 520 mμ. and 136% at 430 mμ.; that in the Rogers–Calamari test is 107% in intensity and contains considerably more blue than the color given by rotenone. It gives no color with ferric chloride. When boiled with alcoholic potassium hydroxide, the solution turns dark red. The principal product is extracted from ether by 5% sodium bicarbonate, and forms white prisms from acetone–water having a m. p. 195° with decomposition, and giving a deep blue color with ferric chloride. When *II* is boiled with alcoholic alkali and zinc dust, the solution remains light yellow, and the principal product is insoluble in bicarbonate but is extracted from ether by 5% aqueous potassium hydroxide. It forms yellow needles from alcohol–water, m. p. 175°, giving a blue-green color with ferric chloride. No dehydro compound has been obtained from II.

Although non-toxic, optically inactive, and containing but one methoxyl group, this compound appears to be related to the rotenoids because of its type of empirical formula, its response to all of the rotenoid color tests, and particularly its degradation by alkali to form a phenolic acid and a phenol. It differs from the tentative formula for compounds III and VI by H_2O, and might therefore be a hydroxyl derivative, but is not dehydrated by alcoholic sulfuric acid and gives no color with ferric chloride. The presence of only one methoxyl group leaves one oxygen unaccounted for in a structure of the rotenone type. The low hydrogen content makes it probable that this oxygen is present as a carbonyl group, possibly as a structure of the rotenonone type.

Compound III.—From its method of isolation, appearance, and m. p., this compound appears to be identical with the yellow needles of m. p. 196° reported by van Sillevoldt[8] and those of m. p. 202–204° reported by Hwang.[10] The analyses of III also agree well with those of van Sillevoldt's compound. Hwang's compound was not analyzed. No compound corresponding to the straw-yellow needles of m. p. 189–192° reported by Hwang was isolated in the present work. Since Hwang did not investigate this material thoroughly, because of its non-toxic nature, it is possible that it represents a further fraction of compound III with its m. p. lowered by a small content of compound II, which is likely to separate under similar conditions.

Compound III shows an intense blue-green fluorescence in benzene solution, having a maximum at about 480 mμ. and being visible in daylight below 1 p. p. m. Unlike that of I, its fluorescence is usually weaker or absent in solvents other than benzene. It gives no response to the Durham, Goodhue or Rogers–Calamari tests. In the Meyer test, it gives a gray-brown color instead of a purple, having 37% of the intensity of the color given by rotenone at 520 mμ. and 136% at 430 mμ. It gives no color with ferric chloride. It resembles I in its behavior toward alcoholic alkali, dissolving either with or without the presence of zinc to give a water-soluble compound from which the original compound is precipitated by acid. No dehydro compound has been obtained.

There is little direct evidence of a relationship with the rotenoids except for an inconclusive off color with the Meyer reagent, a similarity of empirical formula type, the presence of methoxyl, and occurrence in association. As suggested for I, the behavior with alcoholic alkali might indicate the presence of an enol or a lactone group. Although further evidence is lacking, the rotenonone type of structure suggested for II, with its lactone group, might account both for this reaction and for the high oxygen–hydrogen ratio, if the γ-pyrone group of the rotenoids were stabilized by some means to alkali.

Compound IV.—Identification of this compound as rotenone has already been noted[11] on the basis of identical m. p., optical rotation, toxicity, color tests and dehydro compound.

Compound V.—This compound is unquestionably a member of the rotenoid group. It gives a green Durham test; a Goodhue test of 20% the intensity of that of rotenone; a Meyer test of equal intensity to that of rotenone at 520 mμ. and 126% at 430 mμ.; and a Rogers–Calamari test 136% that of rotenone, but more blue. It contains two methoxyl groups. It has a high optical activity, and is racemized by boiling with alcoholic potassium acetate to give white needles of m. p. 232–234°. It forms a dehydro compound crystallizing in yellow needles, m. p. 267° with decomposition. Treatment of the dehydro compound with zinc and alcoholic alkali yields a bicarbonate-soluble material m. p. 206°, giving a blue-green color with ferric chloride. Treatment of the original compound with alcoholic alkali forms a dark red solution and yields a bicarbonate-soluble fraction giving a deep blue color with ferric chloride. In the presence of zinc, the solution is light yellow, and the main product is a bicarbonate-insoluble but potassium hydroxide-soluble fraction giving a green color with ferric chloride. No crystalline materials were isolated from the alkaline treatments.

All of the properties of V, including its toxicity (Table III) resemble closely those of the known rotenoids. Its empirical formula is identical with that of elliptone,[22] but its m. p. and optical rotation are widely different. It resembles elliptone closely in the colors given by the various alkaline degradation products with ferric chloride and in the m. p. of the dehydro derivative, but differs in the m. p. of the racemic compound (234° *vs.* 177°) and of the phenolic acid from the alkaline treatment of the dehydro compound (204° *vs.* 190°).[23] From the present evidence, V is apparently an isomer of elliptone, but differs from it in more than the configuration of the asymmetric centers. The name Erosone is suggested for V, since it is derived from *Pachyrrhizus erosus* and the generic name has been preempted by pachyrrhizid (VII).

Compound VI.—Like III, this compound gives no Durham, Goodhue or Rogers–Calamari tests, and gives an off color in the Meyer test. The Meyer color is bright yellow, having an intensity 18% that of the rotenone color at 520 mμ. and 86% at 430 mμ. It gives no color with ferric chloride. Although the amount available was not sufficient for direct measurement, it is probably optically inactive since it was unchanged by boiling with alcoholic potassium acetate. No dehydro compound has been obtained. Treatment with alcoholic alkali either with or without zinc forms a neutral product crystallizing in yellowish needles having a double m. p. at 148 and 164°.

Like III, compound VI shows little evidence of close relationship to the rotenoids. It appears to have the same empirical formula as III, although the formula of both is somewhat uncertain from the present analytical data.

Compound VII.—This material appears to be the same as the "pachyrrhizid" of van Sillevoldt,[8] although complete identity is difficult to establish because of the non-crystalline nature. The analyses for carbon and hydrogen agree well, but the methoxyl analyses differ. Van Sillevoldt's assumption of an empirical formula in the C_{30} range with two methoxyl groups appears in error both because of the present results indicating C_{23} with one methoxyl group and because of his similar proved error in the molecular complexity of the compounds from Derris and Lonchocarpus.

Compound VII gives no Durham or Goodhue test, but responds to the Meyer test with an intensity 83% that of rotenone at 520 mμ. and 122% at 430 mμ., and to the Rogers–Calamari test with 24% the intensity of rotenone. No color is given by ferric chloride. Racemization with alcoholic potassium acetate or with dilute alkali has failed

(22) Harper, *J. Chem. Soc.*, 1099 (1939).

(23) Harper, *ibid.*, 1424 (1939).

to produce a crystalline product. VII forms a dehydro compound, as noted by van Sillevoldt, but contrary to his results the compound having the higher m. p. of 262° with decomposition was obtained as the principal product. The same compound in approximately the same yield (35%) was obtained on treatment of VII with alcoholic sulfuric acid either with or without previous treatment with iodine and sodium acetate. On boiling the dehydro compound with alcoholic alkali, a bicarbonate-soluble material is formed, giving a blue-green color with ferric chloride. Treatment of VII with alcoholic alkali gives little reaction, most of the original compound being precipitated apparently unchanged from the reaction mixture on the addition of water. A small alkali-soluble fraction was obtained. In the presence of zinc a reaction occurs, yielding a bicarbonate-insoluble but alkali-soluble oil giving a reddish-brown color with ferric chloride.

The compound resembles the rotenoids in empirical formula, toxicity (Table III), optical activity and response to the Meyer and Rogers–Calamari color tests, but differs in response to the Goodhue and Durham tests and reaction with alkali. The formation of the dehydro compound by dehydration alone suggests that VII may be a hydroxy derivative of the tephrosin type. This interpretation is supported by the high oxygen content, the irregular responses to the color tests, and the normal reaction of the dehydro compound with alkali to be expected in the rotenoid series.

No compound corresponding to van Sillevoldt's red-yellow crystals of m. p. 207° was isolated.

Toxicity (Tables II and III).—A number of points of interest are apparent from the toxicity data. Two of the compounds, V and VII, show a toxicity to the silkworm equal or nearly equal to that of rotenone itself. Great specificity is shown by these two compounds, however, since they showed a negligible mortality on the bean beetle under comparable conditions. The reduction in the feeding of the bean beetle caused by these two materials indicates that they have some effect on this insect also, even though less intense. The other compounds are either conclusively non-toxic or of such small effect that the presence of toxicity is highly questionable. The very high toxicity shown by the residues, particularly residue G, is very probably not accounted for by their content of rotenone or the other isolated compounds, because their toxic action on the silkworm is even more rapid than that of any of the pure compounds. Accordingly, unless the compounds show synergism, which was found by Martin[24] to be negligible in several mixtures of rotenoids, one or more highly toxic materials in addition to those isolated are probably present.

Summary

The ether extract of yam beans was divided into a non-toxic oil and a resin toxic to insects. The resin was fractionated by chromatographic methods, yielding one non-crystalline and six crystalline compounds, and three heterogeneous fractions.

One of the crystalline compounds was identified as rotenone, and a second, designated "erosone," was shown to be closely related to elliptone.

Four of the compounds showed evidence of a relation to the rotenoid structure, but differed in containing a single methoxyl group.

Three of the compounds and one of the heterogeneous fractions were toxic to the silkworm, but two of these compounds were of low toxicity to the Mexican bean beetle.

The toxic heterogeneous fraction probably contained at least one toxic compound not isolated.

(24) Martin, *Ann. Appl. Biol.*, **29**, 69 (1942).

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NOTES

Minimum Explosive Concentration of Chlorine Monoxide Diluted with Oxygen

BY GEORGE H. CADY AND ROBERT E. BROWN[1]

Although it has long been known that chlorine monoxide is an explosive compound, no determinations have been made of the explosive limits of mixtures of this substance with other gases. Since the compound is used in high dilution for the industrial production of hypochlorous acid and calcium hypochlorite,[2] it is desirable to know the minimum explosive concentration of the gas. This limit has been measured for mixtures of chlorine monoxide and oxygen at one atmosphere pressure and at 23°. Under these conditions an explosion can be obtained only when the mixture contains over 23.5% chlorine monoxide by volume. Since this amount is much greater than the concentration used commercially, there appears to be no chance that the gas will explode in the plant.

Experimental

Each mixture containing chlorine monoxide was obtained by passing a dry mixture of chlorine in oxygen over a large excess of yellow mercuric oxide which had been dried in an oven at about 110°. The reaction proceeded so far toward completion that no free chlorine was found in any of the mixtures when analyzed by the iodometric technique described by Spinks.[3] The mixture passed through a sampling bulb, an explosion chamber, and finally through a second sampling bulb. Mixtures ranging in concentration from 22 to 40% by volume were tested, the compositions being known to within 0.3%. The explosion chamber was a Pyrex glass tube having a length of 90 cm. and an internal diameter of 34 mm. It was mounted in a vertical position and was equipped with two tungsten wire electrodes located 3 cm. from the bottom of the tube with a spark gap of 2 mm. A hot spark produced by an induction coil was used to initiate an explosion. Tests were made in faint day-light at a temperature within 1° of 23°.

(1) Present address: Lt.(j.g.) Robert E. Brown, U.S.N.R., c/o C.O.P., Navy 163, F.P.O., San Francisco, California.

(2) Geo. H. Cady, U. S. Patent 2,157,524, May 9, 1939; I. E. Muskat and Geo. H. Cady, U. S. Patent 2,240,344, April 29, 1941; U. S. Patent 2,240,342, April 29, 1941.

(3) J. W. T. Spinks, THIS JOURNAL, **53**, 3015–3016 (1931).