It has been further shown that of the two isomeric nitriles produced by this reaction only 3-pentenonitrile is susceptible to acid hydrolysis.

These results and those of a previous study[6] on the carbonation of butenylmagnesium bromide indicate that either an α or γ-substituted butenoic acid may be prepared from either of a given pair of α- and γ-substituted allyl halides by an appropriate choice of method, as shown.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NORTH CAROLINA]

Fish Poisons from *Ichthyomethia Piscipula*. I

BY ALFRED RUSSELL AND E. A. KACZKA[1]

Since the occurrence of toxic principles in various species in many genera of the family *Fabaceae* is well established and covered in several review articles,[2] it is unnecessary to give here a general survey of the field.

In connection with another study[3] there was collected on Key Largo, Florida, several hundred pounds of the roots, and wood, including bark, of the Jamaica dogwood. The powdered bark of the roots has been used extensively throughout tropical and subtropical America to narcotize fish but no exact information is available as to the nature of the toxic materials present.

The Jamaica dogwood has now been identified as *Ichthyomethia piscipula* (L) A. Hitchc. and it appears to be the same species as *Piscidia erythrina* L. Although six species in genus *Ichthyomethia* are stated[4] to occur in tropical and subtropical America, *I. piscipula* is the only member native to the United States.

E. Hart[5] separated, from a commercial liquid extract of *Piscidia erythrina*, a substance of m. p. 192° which he claimed was the toxic principle and named it piscidia. Freer and Clover[6] denied this, stating that Hart's product was not toxic and was not a chemical individual but a mixture of two distinct compounds, one melting at 201° and the other at 216°. They obtained a number of products from *Piscidia erythrina* "bark" by extraction with water and with petroleum ether. None of these was positively identified and apparently no toxicity tests were made since none was claimed to be toxic.

A drug known as "Cortex Piscidiae erythrinae," claimed to be an extract of the bark of *Piscidia erythrina*, was examined by Dankwortt and Schütte.[7] Ten assorted products were isolated by these workers and they came to the conclusion that the toxic principle was an amorphous saponin. Finally, Hauschild[8] made an examination of the bark of *Piscidia erythrina* and concluded that the toxic substance was a crystalline material m. p. 72° and not the saponin of Dankwortt and Schütte.

Obviously there is no way of correlating this earlier work since it appears that four different initial materials were used—root bark, tree bark, a commercial liquid extract and "Cortex Piscidiae erythrinae."

The physiological properties associated with *Piscidia erythrina* may be summarized: Ott[9] states that it is a direct sedative, producing narcotic effects that are refreshing and not followed, like those produced by opium, by hyperaemia of the brain, nausea and general nervous disturbance. It is claimed, also by Ott,[10] to be of value in treatment of bronchitis, asthma, nervous cough, writer's cramp, muscular spasms, chorea, tetanus and toothache. According to Reko[11] some quantity of *Piscidia erythrina* is included in "Sinicuichi"—a Mexican drink which causes rapid stupefaction. Drake and Spies[12] and Hauschild[8] report that an extract of the bark is toxic to goldfish.

In the present work, a few preliminary tests showed that the powdered root bark and root wood were very toxic to goldfish, two grams of either sprinkled into four liters of water producing death in a few minutes. Neither the powdered wood nor the powdered bark of the tree were noticeably toxic. Accordingly the work has been confined to a study of the extractives of the root bark and root wood.

Extraction of the ground bark with cold water followed by evaporation at low temperatures gave a large quantity (30% of the weight of the bark) of a dark colored resinous vitreous material. This extract was toxic to goldfish, the extracted bark was not. No satisfactory analytical separation of the components of aqueous extract could be achieved and the procedure was abandoned. Extractions with methanol, acetone and ethyl

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(1) From a dissertation submitted to the Faculty of the University of North Carolina in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June, 1943.

(2) H. King, *Ann. Rep.*, **29**, 186 (1932); LaForge, Haller and Smith, *Chem. Rev.*, **12**, 182 (1933), etc.

(3) Russell, *et al.*, "Natural Tanning Materials of the Southeastern United States," *J. Am. Leather Chem. Assoc.*, part I, XXXVI, 340 (1942); part II, XXXVIII, 30 (1943); part III, XXXVIII, 144 (1943); part IV, XXXVIII, 235 (1943); part V, XXXVIII, 355 (1943).

(4) Small, "Manual of the Southeastern Flora," New York, N. Y., 1933.

(5) E. Hart, *Am. Chem. J.*, **5**, 39 (1883).

(6) Freer and Clover, *ibid.*, **25**, 405 (1901).

(7) Dankwortt and Schütte, *Arch. Pharm.*, **272**, 701 (1934).

(8) Hauschild, *ibid.*, **274**, 388 (1936).

(9) Ott, *Detroit Lancet*, June, 1880.

(10) Ott, *Archives of Medicine*, February, 1881.

(11) Reko, *Pharm. Monatsh.*, **16**, 155 (1937).

(12) Drake and Spies, *J. Econ. Entomol.*, **25**, 129 (1932).

acetate were similarly unsatisfactory. Finally it was found that prolonged extraction (Soxhlet) of the dried bark with petroleum ether (30–60°) removed all the toxic material with a small quantity of wax. The latter was easily removed from the dried petroleum ether extract by washing with ether. The ether washed residue formed a white crystalline mass. After this preliminary trial, the extraction was carried out on a much larger scale using a continuous type extractor handling about two kilos of bark at one time. The crystalline material was most easily separated by interrupting the extraction when much of the more soluble part had been removed (continued extraction gave the less soluble portion). Final purification was accomplished by repeated crystallizations from ethanol. Two pure compounds A and B were isolated.

The same two compounds occur to the same extent and in about the same proportion in the root wood. Extraction of the root wood by the same process as applied to the bark was, comparatively, very easy and the wood was used as the source of most of the toxic material examined.

Extraction of the root bark or root wood (Soxhlet) with chloroform gave some low melting, wax-like material that had toxic properties. This product has not been further examined.

Both A and B are very toxic to goldfish, causing death in less than two hours at a dilution of about one part in a million. The compound A is identical with rotenone; the compound B is new and is, very likely, closely related to rotenone, its molecular formula is $C_{23}H_{20}O_7$ and it has been named "ichthynone."

Experimental

Qualitative Extractions of Root Bark and Root Wood of *I. piscipula* with Petroleum Ether.—I. Root bark: 50 g. of air dry bark (milled to the consistency of tea) was extracted in a large Soxhlet apparatus. The apparatus functioned about twenty times an hour and the bark was exhausted of toxic materials after twenty-four hours. The solvent was distilled and the residue collected—this was a pale yellow crystalline mass containing a little wax. The wax was removed by washing three times with 10-cc. portions of ether. The wax-free residue formed a white crystalline mass that was very toxic. The yield was 0.04 g.

II. Root wood: 50 g. of air-dry wood (chipped to about the size of rice) was extracted and treated as described for the bark. The yield of mixed crystalline, wax-free, toxic material was 0.039 g.

The large-scale extraction process was run fifteen times and a total of 18 g. of wax-free crystalline toxic material was obtained.

Separation of Rotenone.—The more soluble fraction, obtained when the extraction process was interrupted, was freed from traces of wax by washing with ether—the ether washed residue melted at 150–155°. After eight crystallizations from ethanol, a pure compound of m. p. 164° resulted. Its solubility in various solvents were in agreement with those recorded for rotenone and it gave a positive Durham test identical with that given by rotenone. *Anal.* Calcd. for $C_{23}H_{22}O_6$: C, 70.05; H, 5.58. Found: C, 70.08; H, 5.72. Mixed m. p. (with sample of authentic rotenone) was 163°.

Isolation of Icthynone.—The less soluble fraction obtained during the second extraction period was collected, freed from traces of wax by washing twice with 10-cc. portions of ether and crystallized four times from ethanol. A pure compound crystallizing in stout hexagonal rodlets was obtained m. p. 203–204°. *Anal.* Calcd. for $C_{23}H_{20}O_7$: C, 67.65; H, 4.92; mol. wt., 408. Found: C, 67.50; H, 5.07; C, 67.61; H, 5.14; C, 67.49; H, 5.09; mol. wt. 398, 416, 384, 390, av. 397. (The low solubility of ichthynone in all suitable solvents made it difficult to get consistent values for molecular weight. The above values were obtained by the boiling point method using chloroform as a solvent.)

Ichthynone is insoluble in water, acids or alkalies, very slightly soluble in petroleum ether, diethyl ether and acetone, and rather more soluble in ethanol, chloroform, and carbon tetrachloride. Ichthynone is optically inactive and is an unsaturated ketone containing two methoxyl groups. There is no evidence indicating the presence of either an alcoholic or a phenolic hydroxyl group. This distinguishes ichthynone from toxicarol, sumatrol and tephrosin since toxicarol and sumatrol are phenolic in character while tephrosin contains an alcoholic hydroxyl group that is easily removed by dehydration. However, all attempts to dehydrate ichthynone were unsuccessful. *Estimation of methoxyl:* Calcd. for $C_{21}H_{14}O_5(OCH_3)_2$: OCH_3, 15.2. Found: OCH_3, 15.1, 15.2, 15.8.

Estimation of Unsaturation: 1. Ichthynone Dibromide.—0.25 g. ichthynone was dissolved in 26 cc. of carbon tetrachloride containing 0.12 g. of bromine and the solution refluxed for three hours. The carbon tetrachloride was evaporated, the white crystalline residue washed with a little ether and dried, m. p. 234–235°. *Anal.* Calcd. for $C_{23}H_{20}O_7Br_2$: Br, 28.2. Found: Br, 27.2. 26.0.

II. Tetrahydroichthynone.—1.00 g. of ichthynone, dissolved in 150 cc. ethyl acetate containing 0.1 g. of platinum black, was reduced (room temperature) in a low pressure (semi-micro) bomb (30 cm. pressure). After three hours no more hydrogen was taken up and the reduced product, which had precipitated, was collected, separated from the catalyst by dissolving in sufficient hot alcohol and filtering. On cooling tetrahydroichthynone separated in slender white needles, m. p. 233–234° after one recrystallization. *Anal.* Calcd. for $C_{23}H_{24}O_7$: C, 66.99; H, 5.82. Found: C, 67.30; H, 5.83.

Carbonyl Derivatives. I. Ichthynone Hydrazone.—0.20 g. of ichthynone and 0.20 g. of hydrazine hydrate were dissolved in 50 cc. of ethanol and 2 cc. of 0.1 *N* KOH added. The solution was refluxed for four hours, cooled and neutralized, whereupon the hydrazone crystallized. It was collected, washed with a little ethanol and crystallized twice from ethanol as pale yellow needles, m. p. 215–217°. *Anal.* Calcd. for $C_{23}H_{22}O_6N_2$: N, 6.63. Found: N, 6.70, 6.77.

II. Ichthynone Phenylhydrazone.—0.50 g. of ichthynone was dissolved in 100 cc. of ethanol and 5 cc. of phenylhydrazine, followed by 2.5 cc. of 0.2 *N* KOH, added. After refluxing for four hours the reaction mixture was cooled and neutralized. After standing for twelve hours the phenylhydrazone separated. Recrystallized twice from ethanol it formed brilliant yellow needles m. p. 195–200° (d.). *Anal.* Calcd. for $C_{29}H_{26}O_6N_2$: N, 5.62. Found: N, 5.76, 5.77.

Toxicity Tests on Goldfish.—0.001 g. of ichthynone dispersed (by dissolving in 1 cc. of acetone and then adding the solution to water) in 1000 cc. of water was fatal to goldfish in less than two hours. Ichthynone is, then, toxic to goldfish at a concentration of approximately one part in a million.

Summary

Extraction of the root bark or root wood of *Ichthyomethia piscipula* with petroleum ether gives a mixture of crystalline materials very toxic to goldfish.

Two pure compounds have been isolated, rotenone and a new compound "ichthynone." Ichthynone crystallizes difficultly from ethanol in sturdy hexagonal rodlets m. p. 203–204°. It

has the molecular formula $C_{23}H_{20}O_7$ and is an unsaturated ketone containing two methoxyl groups.

Ichthynone kills goldfish at a concentration of, approximately, one part in a million.

CHAPEL HILL, N. C. RECEIVED NOVEMBER 29, 1943

[CONTRIBUTION NO. 330 FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF TEXAS]

The Nitrogen Compounds in Petroleum Distillates. XXV. Isolation and Identification of 3- and 4-Cyclopentylpyridines from California Petroleum

BY H. L. LOCHTE, E. D. THOMAS[1,2] AND PRICE TRUITT[3]

In a recent communication from this Laboratory[4] the chloroform extraction method of Bailey and co-workers[5] was extended to the hydrochlorides of petroleum bases boiling below the quinolines in the hope that, just as in the higher boiling range where the quinolines are found in the water layer and those of the substituted pyridines in the chloroform layer, so in the lower range the water layer might selectively dissolve the hydrochlorides of pyridines carrying cyclic substituents while the chloroform layer might hold the alkyl substituted pyridines. The chloroform layer was indeed found to contain alkyl substituted pyridines of which *dl*-2-(*s*-butyl)-4,5-dimethylpyridine was identified and several others isolated and analyzed.[4]

The water layer from the chloroform extraction of hydrochlorides of bases boiling at 210–213° reported in the previous communication[4] has been studied to determine what type of bases predominate.

Using a combination of fractionation by distillation and by fractional neutralization two isomeric cyclopentylpyridines were isolated. Since 2-cyclopentylpyridine is easily prepared by the interesting reaction of Emmert,[6] this compound was synthesized but it was found that neither of the petroleum compounds was identical with it.

Since one of the petroleum pyridines must then be the 3- and the other the 4-cyclopentylpyridine and since there was not enough pure base available to warrant a study of conditions required to obtain nicotinic or isonicotinic acids by oxidation, the synthesis of 3- and 4-substituted pyridines was studied. The new method of Crouch and Lochte[7] was finally used to prepare both 3- and 4-cyclopentylpyridines and thus show that the base yielding a picrate melting at 117.5° is 3-cyclopentylpyridine and the other one is the 4-cyclopentyl isomer.

Experimental

Fractional Distillation of Bases from Water Layer of Chloroform Extraction of 210–213° California Petroleum Bases.—Approximately three liters of bases were obtained from the water layer of the extraction reported in the previous paper.[4] These bases were distilled through a 12-foot Berl saddle-packed steel column at 20 mm. pressure using a 10:1 reflux ratio to yield 13 fractions of 240 cc. boiling at 207–219° with 9 fractions boiling between 212.5 and 214.0°. The index of refraction ranged from 1.502 to 1.516, that of the 9 fractions lay between 1.510 and 1.511, thus indicating that unsaturated or cyclic substituents might be expected.

Fractional Acid Extraction.—Fraction 6 (b. p. 213°; $n^{20}D$ 1.5102) was dissolved in 500 cc. of petroleum ether and extracted in the counter-current apparatus described in the previous paper,[4] Fig. 1, to yield 21 fractions with an average volume of 8 cc. and index of refraction, $n^{20}D$, ranging from 1.505 to 1.515.

Isolation of 3-Cyclopentylpyridine.—Since the index of refraction of the acid extraction fractions indicated that the alkyl substituted pyridines preceded those with cyclic side chain, fraction 19, with a volume of 25 cc. and $n^{20}D$ 1.5152, was selected to determine what type of substituents are present in the fractions with the high index of refraction. The base was dissolved in sulfurous acid and treated with picric acid. A crystalline picrate formed on standing in the ice-box. It melted at 117.5° after repeated recrystallizations from dilute ethanol and from 50% acetic acid. Additional amounts of the same picrate were obtained from fractions 17 and 18 of the same series.

Anal. Calcd. for $C_{16}H_{16}O_7N_4$: C, 51.06; H, 4.26; N, 14.89. Found: C, 50.97; H, 4.16; N, 15.03.

One gram of the picrate was heated with concentrated ammonium hydroxide to liberate the base which was extracted in benzene, dried and distilled in a semi-micro distillation apparatus. A middle fraction gave the following constants: b. p. 215.5° (747 mm.); $n^{20}D$ 1.5173.

Anal. Calcd. for $C_{10}H_{13}N$: N, 9.52. Found: N, 9.55.

Isolation of 4-Cyclopentylpyridine.—Fraction 21, volume 30 cc., $n^{20}D$ 1.5116, yielded a new crystalline picrate which after repeated recrystallizations from dilute ethanol and from 50% acetic acid melted at 145–146° but only a few grams of the pure crystalline picrate were obtained. Since this new base was isolated from the higher boiling fraction of fraction 6 of the distillation series it seemed probable that greater amounts could be isolated from a higher boiling fraction and fraction 11 was fractionally neutralized in the counter current column to yield 21 neutralization fractions. Fractions 10 through 12 yielded only oily picrates but 13 through 21 yielded larger amounts of a picrate melting at 145–146° and showing no depression when mixed with the previous picrate.

Anal. Calcd. for $C_{16}H_{16}O_7N_4$: C, 51.06; H, 4.26; N, 14.89. Found: C, 51.20; H, 4.45; N, 14.96.

One gram of the picrate was decomposed as before and fractionated, b. p. 218° (744 mm.); $n^{20}D$ 1.5167. Both of the cyclopentylpyridines were difficult to analyze, probably because of their tendency to take up moisture.

Anal. Calcd. for $C_{10}H_{13}N$: N, 9.52. Found: N, 9.30.

The chloroplatinate of this base crystallizes readily, forming orange needles decomposing at 225–227°.

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[1] A part of this material was presented in partial fulfillment of requirements for the Master's Degree at the University of Texas by E. D. Thomas, 1942.

[2] Present address: Harvard Medical School.

[3] General Aniline Fellow, 1942–1944.

[4] Lochte, Crouch and Thomas, THIS JOURNAL, **64**, 2753 (1942).

[5] Perrin and Bailey, *ibid.*, **55**, 4136 (1933).

[6] Emmert and Pirot, *Ber.*, **74**, 714 (1941).

[7] Crouch and Lochte, THIS JOURNAL, **65**, 270 (1943).