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Isolation and Chemistry of Some of the Active Principles of Piscidia Erythrina L.

Thesis
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To my dear

Mother

I wish to express my deep sense of gratitude to Prof. Dr. J. Büchi for giving me the opportunity of working under his able guidance. His constructive criticism and kind advice have always been a source of inspiration for me.

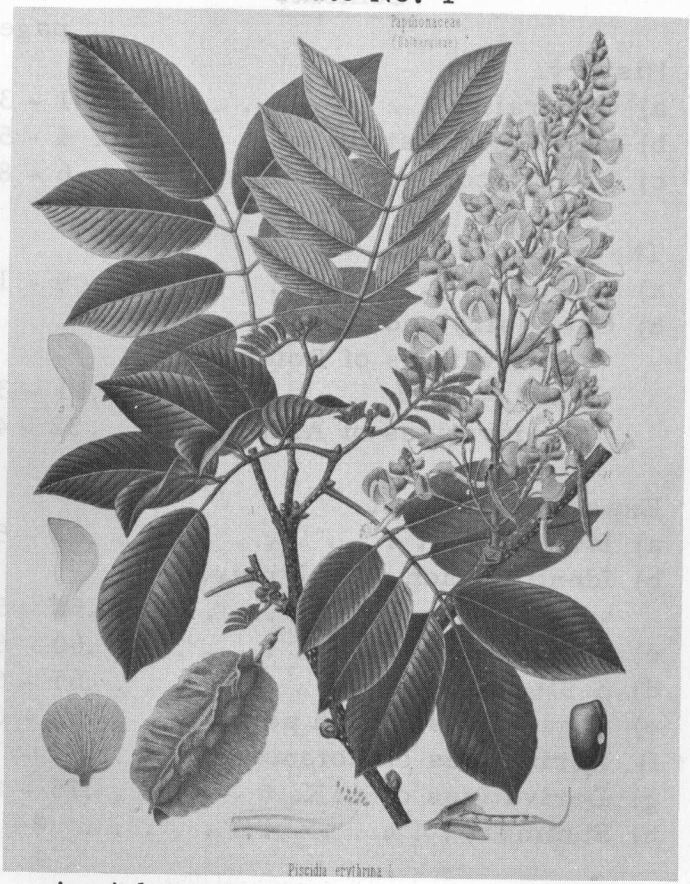
To Dr. A. Aebi I shall always feel obliged for his untiring help and valuable suggestions during my association with him.

I also thank Mr. R. Schwegler of Pharmacy Institute E. T. H. for his helping hand and continuous interest he showed in my work.

Last but not least, thankful acknowledgment is due to the E. T. H., Zürich, and to the House of Dr. A. Wander A.G., Berne, for the financial assistance which they made available for the completion of this work.

Contents

Plate No. I



A. A branch of Piscidia erythrina showing leaves.

B. A branch of Piscidia erythrina showing flowers.

1) Standard. 2) Wing. 3) Keel. 4) Stamens. 5) Pistil. 6) Fruit. 7) Seed.

Reproduced from the Kohler's Medicinal Pflanzen, Band III, page 196.

HISTORY

Piscidia erythrina L. (Plate No. I) more commonly known in the United States as Jamaica Dagwood, is a tree belonging to the family Leguminosae, subfamily Papillionasae, the taxonomic characters of which were first described in 1753 by Linnaeus[1], as Piscidia piscipula. It was more fully described by Sargent under the name Ichthyomethia piscipula. It is also known by the name Piscidia carthaginensis. The common names for this plant are many, among them are 1. "bois ivrant". 2. "Murungu". 3. "Fish poison tree". 4. "bois enivrant". 5. "Jamaica fish-fuddle tree". 6. "Fish catching coral tree". 7. "Guana hedionda". 8. "Flor de papagallo". 9. "Cocinte" and 10. "Barbasco".

It grows in the West Indian Islands, especially in Jamaica and Martinique, and it has been found in Florida, Texas, Southern Mexico and the Northern regions of South America. The root bark has been used by natives for a great many years as an analgesic and as an agent for anesthetizing fish. In 1794, Barham[2] pointed out that he had used the bark of the drug as an astringent tonic in the treatment of ulcers. In 1884, Hamilton[3] tried this drug on himself against tooth

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1. Linnaeus C., Species Plantarum, Vol. 2, 707 (1753).
2. Tschirch A., Handbuch der Pharmakognosie, Vol. 3, 804 (1925).
3. ibid. Vol. 3, 804 (1925).

neuralgia and attributed to it very active narcotic and anesthesic properties.

The first chemical investigation of Piscidia erythrina was reported in 1883 by Hart[4], who obtained a nearly colourless crystalline substance from the fluid extract of its root bark. The substance melted at 192° (uncorr.) and the elementary analysis gave the formula $C_{29}H_{24}O_8$. He called it "piscidia" and considered it to be responsible for fish active poison. In 1898, Berberich[5] confirmed Hart's results. In 1901, Freer and Clover[6] studied the root bark of Piscidia erythrina and showed that Hart's piscidia consisted of two distinct substances, one $C_{23}H_{20}O$· m.p. 201° and the other $C_{22}H_{18}O_6$ m.p. 216°. The former being in the greater proportion. They also isolated a number of other substances, among which was piscidic acid $C_{11}H_{12}O_7$ m.p. 185°. In 1919, Pittenger and Ewe[7] got the same results as Hart. In 1934, Danckworth and Schutte[8] reported the presence of water soluble glycoside of

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4. Hart E., Am. Chem. Journ. 5, 39 (1883).
5. Berberich H., Am. Journ. Pharm. 70, 425 (1898).
6. Freer P. C. and Clover A. M., Am. Chem. Journ. 25, 390 (1901).
7. Pittenger P. S. and Ewe G. E., Am. Journ. Pharm. 91, 575 (1919).
8. Danckworth P. W. and Schutte E., Arch. Pharm. 272, 701 (1934).

saponin character. In 1944, Russell and Kacska[9] reported the possible presence of rotenone and a new compound "ichthynone" $C_{23}H_{20}O_7$ m.p. 203°. In 1948, Costello and Butler[10] found the bark of piscidia erythrina to have authentic properties.

In 1948, Robertson[11] reported the results of their experiments on the structure of piscidic acid (dibasic acid) $C_{11}H_{12}O_7$.

In 1956, while this work was coming to an end, Moore and Eng[12] published an extensive investigation on the principles isolated from Piscidia erythrina. Their results are very similar to ours and are mentioned in detail later on.

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9. Russel A. and Kaczka E. A., Journ. Am. Chem. Soc. 66, 548 (1944).
10. Costello C. H. and Butler W., Journ. Am. Pharm. Assoc., Sci, ed. 37, 89 (1948).
11. Robertson A. and Budge W., Journ. Chem. Soc. 257, (1948).
12. Moore J. A. and St. Eng. Journ. Am. Chem. Soc. 78, 395 (1956).

HISTOLOGICAL STUDIES

The histology of the root bark of Piscidia erythrina has been described by Collin[13], Swaters[14], Moeller[15], Tschirch[2] and Jaeger[16]. An exhaustive report on a pharmacognostic study of Piscidia erythrina was published by Elena Gautier Auxence[17] in 1953.

It was established that the cork is composed of several rows of collapsed tubular cells, regularly superposed, that the cortical paranchyma possesses rectangular or slightly polyhedric cells, without sclerenchyma cells. The phloem is very much developed with slightly tangentially elongated cells, slightly smaller than those of cortical paranchyma, and regularly disposed in radical series. Medullary rays are more or less sinuous, composed of two rows of cells, while in the phloem there are numerous bundles of fibres and soft bast, tangentially elongated and in parallel disposition.

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13. Collin E., Union Pharm. 25, 5 (1884).
14. Swaters A. B., Diss. Utrecht Gottingen (1896)
15. Moeller J., Pharm. Zeitz. p. 567 (1883).
16. Jaeger P., Bull. Soc. Bot. France 87, 130 (1940).
17. Elena Gautier Auxence, Economic Bot. 7, 270 (1953).

At the level of the cortical paranchyma and phloem paranchyma, there are chlorophlic cells, crystal cells and large lucanae containing a resinous substance. Crystal cells surround the fibre-phloem bundles.

The resinous substance is easily soluble in cold absolute alcohol and is saponifiable by the alkalis and does not give a Tannin reaction. The presence of starch in all paranchyma cells is observed. Cells of Calcium oxalate and Tannin are also reported.

PHARMACOLOGICAL STUDIES

The earliest studies were reported by Isaac Ott and Nagle[18] in 1880, who found the drug to have narcotic properties, and that it causes dilation of the pupils, and increases secretion of sweat. Also that it causes a rise in blood pressure. They compared the action a little to that of Morphine. In 1916, Delzell[19] showed that the drug lowers the amplitude of the contractions of the excised intestines of the rabbit. In 1919, Pittenger[7] studied the standardization of the drug, on the basis of the production of incoordination and ataxia because of the hypnotic effect. In 1932, Drake[20] reported that 1 ml. ext. (from 0.2 gm. plant material) caused the death of Goldfish in less than 100 minutes.

In 1936, Hauschild[21] reported the amorphous yellowish substance obtained from the petrolether extract of the drug, to be toxic to fish and for warm blooded animals, strongly toxic for rats and rabbits when administered parenterally, but non-toxic in oral administration in doses up to 5 mg. He also reported the lethal dose of his active substance for fish and found that it killed them in a dilution of 1: 80'000'000.

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18. Ott I. and Nagle K., Detroit lancet 3, 533 (1880).
19. Delzell W. R., Burman G. E. and Pilcher J. D., Arch. Int. med. 18, 752 (1916).
20. Drake M. L. and Spies J. R., Journ. Econ. Ent. 25, 129 (1932).
21. Hauschild F., Arch. Pharm. 274, 388 (1936).

In 1944, Russel and Kacza[9] reported that Ichthynone kills Goldfish at a concentration of one part in a million.

In 1948, Costello[10] pointed out that preparations of piscidia bark are exceedingly potent uterine depressents in vivo and vitro on various laboratory animals. That the depressent effect was observed in alcoholic extracts, petrolether extract, and extract of chloroform, that the petrolether was the single optimum solvent for the depressent principle. They also stated that all parts of the plant contained the depressent principle, the root bark being the most potent.

The drug began to be used more extensively in regular medical practice after publication of the experiments of Ott and Nagle.[18] Thus in 1883, Firth[22], Payne[23], Seifert[24], Wells[25] and Palmer[26] reported that they used it successfully in delirium tremers, for alcoholism, nervous-bilious attacks, in phthisis, hysterical mania and in nervous headaches.

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22. Firth L. B., Therap. Gaz. 4, 101 (1883).
23. Payne A.S., ibid., 4, 59 (1883).
24. Seifert O., Wien med. Blatt (1883).
25. Wells A.K., Therap. Gaz. 4, 501 (1883).
26. Palmer E., ibid., 4, 502 (1883).

In 1935, Reko[27] reported that the drug, together with two other drugs, is an ingredient of the Mexican native tea called "Sinicuichi".

In 1937, Leclerc[28] reported its value as analgesic, especially in the pains originating in the pelvic organs.

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27. Reko V. A., Pharm. Montash 16, 155 (1935).
28. Leclerc H., La Presse med. 45, 1430 (1937).

THEORETICAL PART

ISOLATION

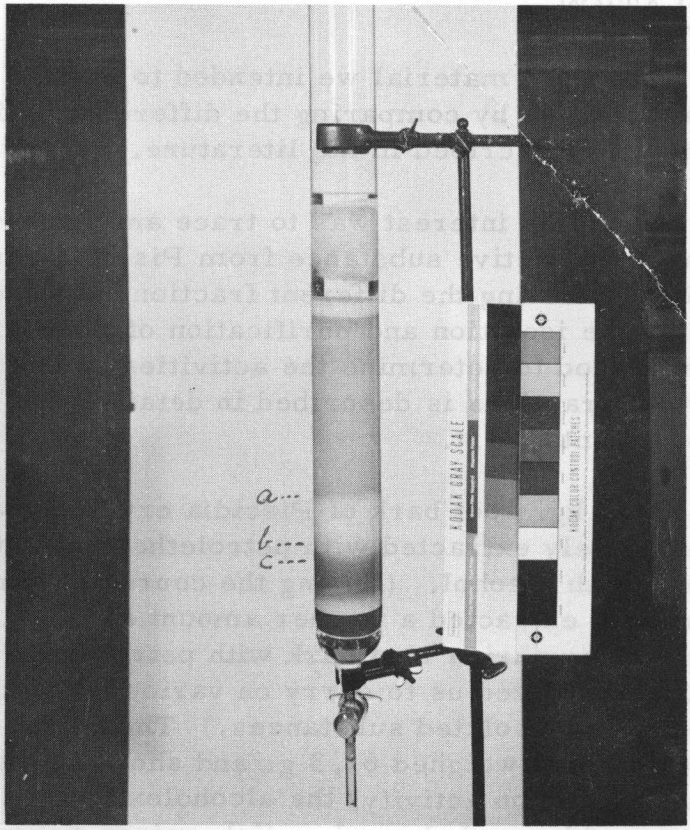
The plant material we intended to extract was identified by comparing the different sections with those described in the literature.

Our main interest was to trace and isolate the fish-poison active substance from Piscidia erythrina by testing the different fractions obtained during the isolation and purification of the extracts. The method to determine the activities of these various fractions is described in detail in the experimental part.

17,7 kg. root bark of Piscidia erythrina were exhaustively extracted with petrolether and afterwards with alcohol. (During the course of our work, we extracted a further amount of 50 kg. of Piscidia erythrina root bark with petrolether which facilitated us to carry on various reactions on different isolated substances.) The petrolether extract weighed 61,3 g. and showed a definite fish poison activity, the alcoholextract gave 490 g residue and showed no fish-poison activity.

The alcoholic extract was chromatographed on alumina and on silicagel in a small scale. There was no fraction isolated which we could crystallize. The main portion stayed on alumina even after eluation with methanol containing 0,1% acetic acid.

Plate No. 11



Chromatography of the mother liquor portion, after filtering off various crystalline fractions from the petrolether extract of Piscidia erythrina

a) -Sitosterol, b) A new substance A. K. 6, c) Substance showing strong fish poison activity.

Different solubility of the petrolether extract in acetone-petrolether 1: 1 made it possible to filter off some fractions of m.p. 80-85°, 137-140° and 137-185° successively, which showed no biological activity and therefore were not further investigated. The mother liquor still showed definite activity and weighed 50, 1 g. This portion was chromatographed on alumina and gave the following three different compounds (Plate No. II).

I. A substance which showed strong fish poison activity and which we later identified by its analysis, mixed m.p. and its similar IR- and UV-absorption spectra as identical with Rotenone. The formation of the hydrazone and also the isomerization of fish poison gave derivatives which were identical with Rotenone iso-hydrazone[1] and iso-Rotenone[2].

II. β-Sitosterol identified by its acetate.

III. A new substance which we called A.K. 6. This compound showed no fish poison activity and has the formula $C_{23}H_{20}O_6$.

CHEMISTRY AND SOME DERIVATIVES OF ROTENONE AND A.K. 6

Since we only had a comparatively small

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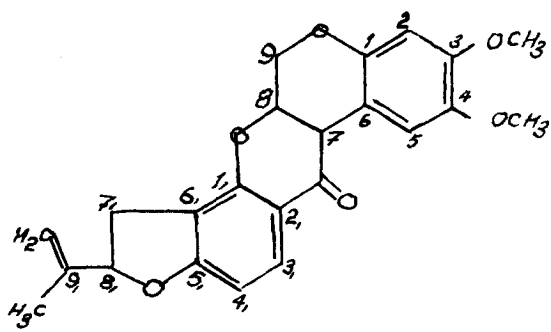
1. Butenandt A., Ann. 464, 265 (1928), and also experimental part page 65.
2. Butenandt A., Ann. 477, 256 (1930).

amount of A. K. 6 we decided to prepare first some suitable derivatives from Rotenone for comparison of their UV- and IR-spectra with the data observed from A. K. 6.

a) Rotenone



Pure Rotenone[1] was made from a British Drug Houses sample (experimental part page 58) and its UV-absorption-spectrum Fig. 1 showed two maxima at 238 mμ (ε = 12800) and 294 mμ (ε = 15600)

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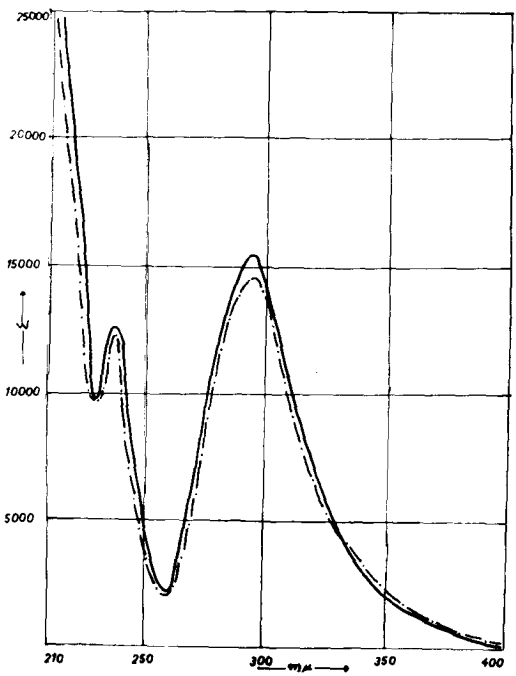
1. For the proof of the structure see: Butenandt A., Inaugural-Dissertation, Gottingen, 1928. Butenandt A. and Hildebrandt, Ann. 477, 245 (1930). Butenandt A. and McCartney, Ann. 494, 17 (1932). La Forge F. B. and Haller H. L., Chem. Reviews 12, 181 (1933).

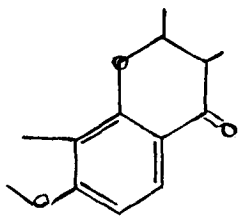
UV-Absorption curve of Rotenone (I) and fish poison

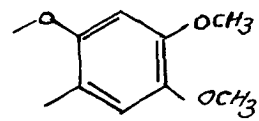


——— Rotenone
-·-·-·- Fish poison

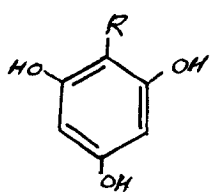
Fig. 1

Structure I would consist of the two independent chromophores II and III, which should add up to the absorption curve in Fig. 1.





But the possibility that enolisation of the keto group of I with the C_7 hydrogen links the two chromophores into conjugation cannot be ruled out. However the IR-absorption curve shows a strong ketone band at 1679 cm^{-1} observed in carbon tetrachloride solution which might point to the preference of the non-enolized form, but the fact that the UV-absorption curve was determined in alcohol excludes a definite comparison of these arguments. We found two compounds which would correspond somehow to the chromophoric systems II and III, Todd[1] mentioned the absorption data of phloroglucine IV and phloroglucine butanone V.



IV R = H max = 270 mμ, ϵ = 623

V R = $CO(CH_2)_2CH_3$ max = 229 mμ, ϵ = 12800, 289 mμ, ϵ = 16000.

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1. Todd A.R. and Birch J., J. Chem. Soc. 3103 (1952).

The addition of the absorption spectra for IV and V should give a curve which shows some similarity to Rotenone absorption. Fig. 2a and 2b show the identical IR-absorption spectra[1] of the isolated fish poison and of Rotenone.

1680 cm^{-1}: ketone band conjugated with aromatic system 1611 cm^{-1} and 1512 cm^{-1}: aromatic bands.

Surprisingly there is no band observed for the $R_2 \diagdown C = CH_2$ group as it occurs in structure I
$R_1 \diagup$
for Rotenone. This band should be at 890 cm^{-1} with moderate intensity. This fact gave us the idea to ozonize Rotenone and prove the presence of the $\diagdown C = CH_2$ group by the formation of formaldehyde. The fragments of the ozonolysis definitely contained formaldehyde and we believe that the $\diagup C = CH_2$ group of Rotenone has a band of very low intensity.

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1. We are very grateful to Siv. Ing. J. Lothe for the careful determination and extensive interpretation of most of the IR-spectra presented in this thesis.

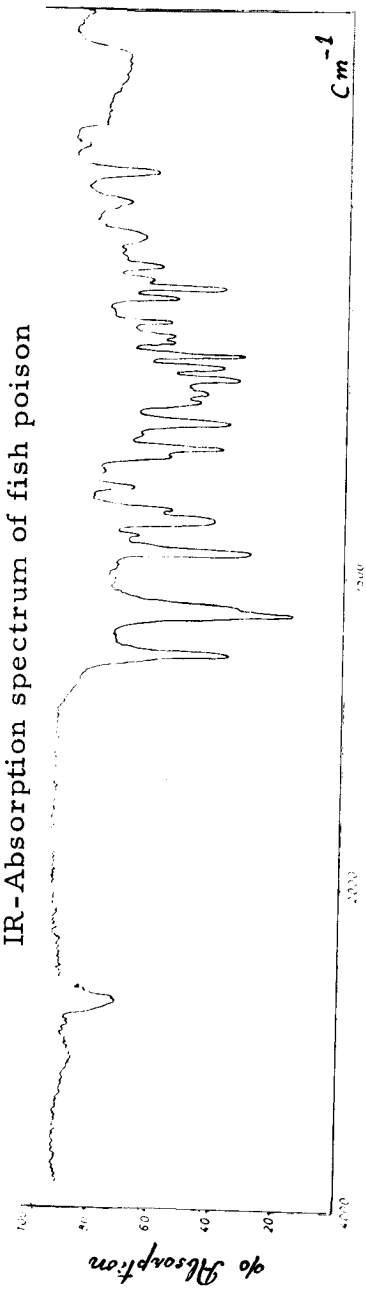

IR-Absorption spectrum of fish poison
% Absorption
100
80
60
40
20
Cm-1

Fig. 2a

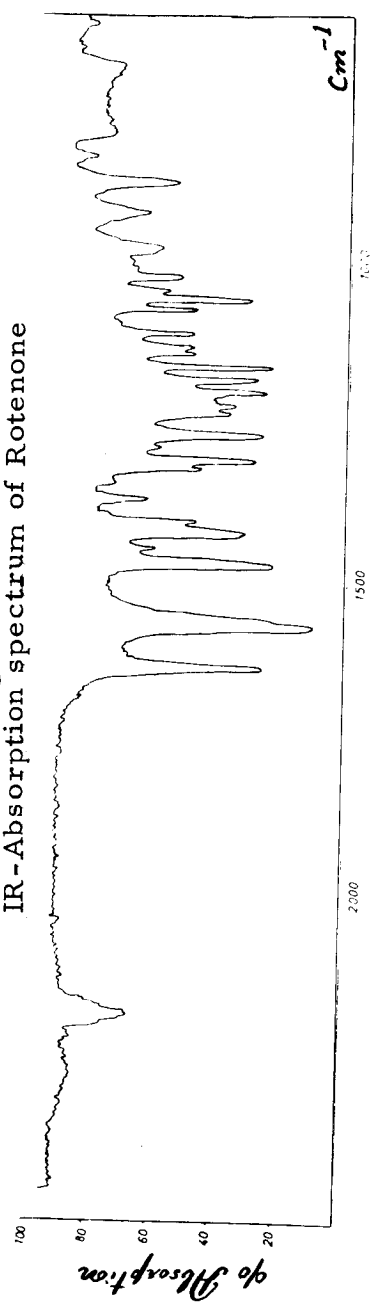
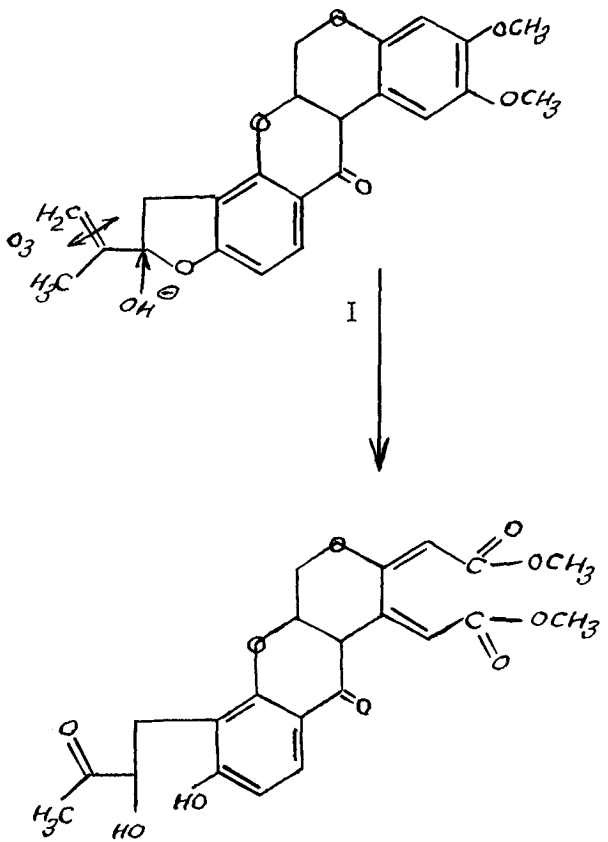

IR-Absorption spectrum of Rotenone
% Absorption
100
80
60
40
20
2000
1500
Cm-1

Fig. 2b

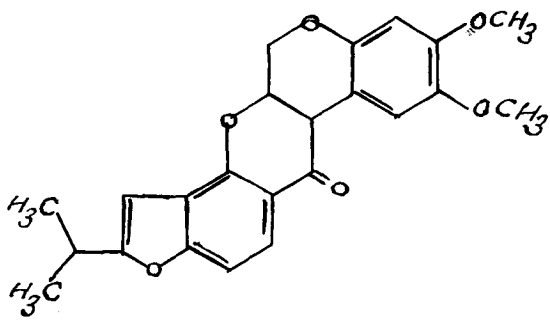
We also isolated the large fragment of the ozonization compounds and found a compound which analysed well on $C_{22}H_{22}O_{10}$. Since this substance also suffered a steam distillation in acid solution the following oxidation and ring opening might have occurred:



VI

VI would correspond to the formula $C_{22}H_{22}O_{10}$ very well. No further investigation on this substance was pursued.

The isomerization of Rotenone I in the presence of concentrated sulfuric acid gave iso-Rotenone VII[1].



This isomerization to the formation of iso-Rotenone gave an entire change in the UV-absorption curve (Fig. 3), 1 mzx. = 242 mμ (ϵ = 34000) as main absorption maxima.

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1. Butenandt A., Ann. 477, 256 (1930).

UV-Absorption curve of iso-Rotenone

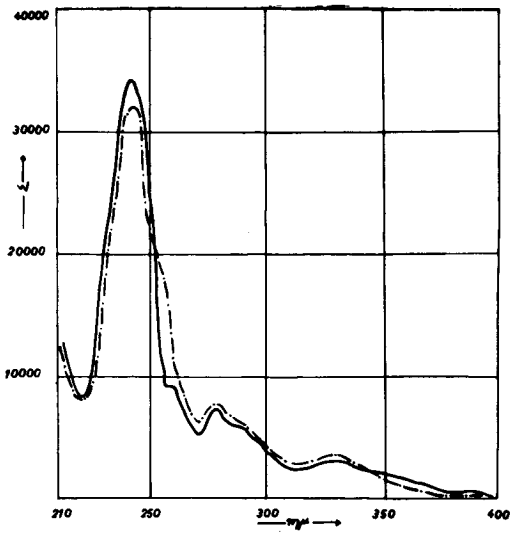


Fig. 3 ——— iso-Rotenone

-·-·-·- iso-Fish poison

The IR-absorption spectra of the isomerized fish poison and of iso-Rotenone were again identical as Fig. 4a and 4b show.

IR-Absorption spectrum of iso-Fish poison

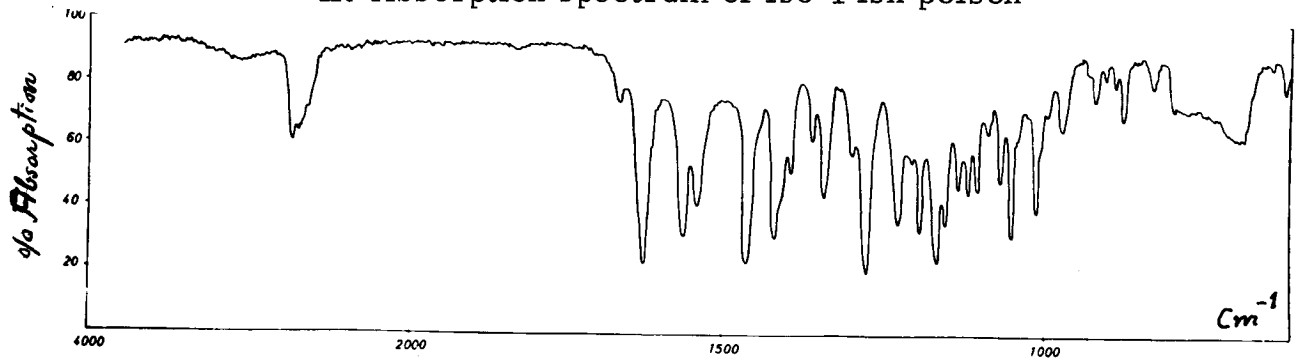


Fig. 4a

IR-Absorption spectrum of iso-Rotenone

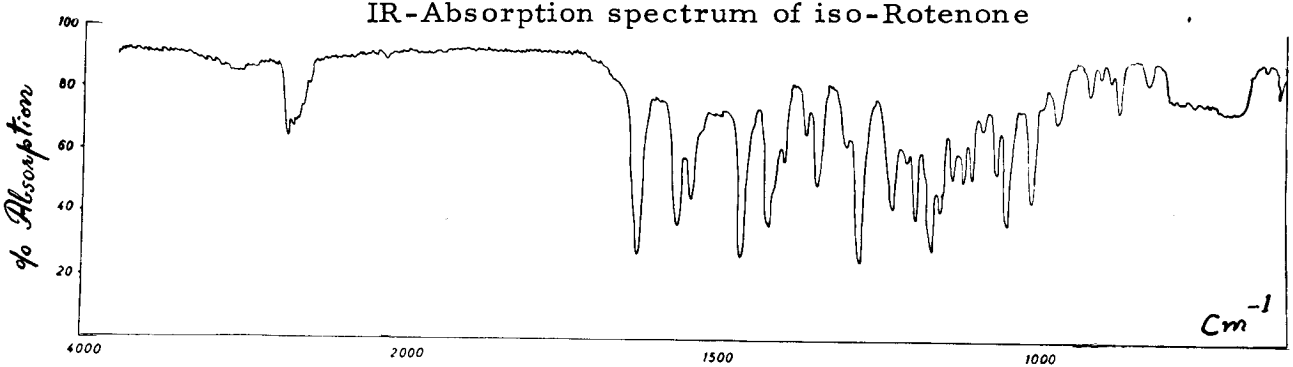
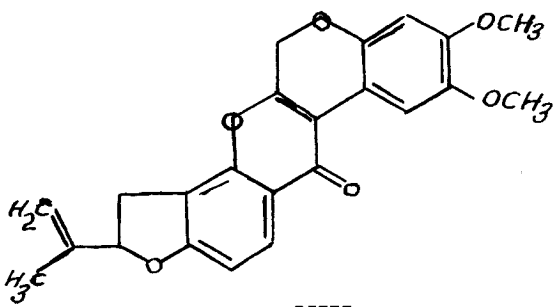


Fig. 4b

The frequencies of the ketone group and of the above mentioned aromatic bands did not change much compared to Rotenone. Oxidation of Rotenone with Iodine gave dehydrorotenone VIII[1].



VIII

This substance shows already a very complicated aromatic UV-spectrum with three typical maxima: 240 mμ (ϵ = 24000), 280 mμ (ϵ = 19000) and 310 mμ (ϵ = 15000). Fig. 5

1. Butenandt A., Inaugural-Dissertation, page 38, Gottingen (1928).

UV-Spectrum of dehydrorotenone VIII

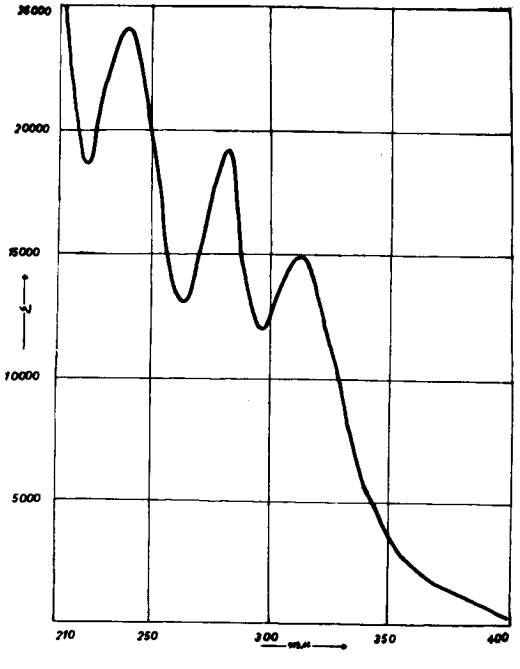
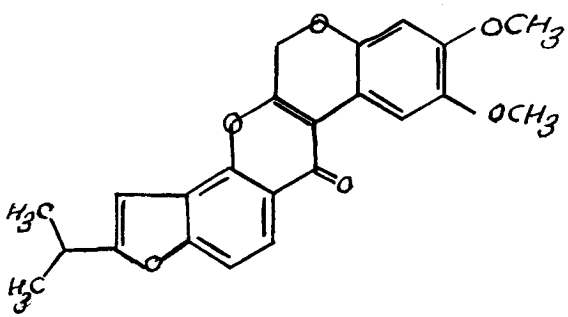


Fig. 5

In this case the carbonyl band in the infrared shows the additional conjugation with the C-C double band, by absorbing at a lower frequency of 1650 cm^{-1}. The band at 1638 cm^{-1} is probably an aromatic band.

By treatment of the above dehydrorotenone VIII with concentrated sulfuric acid we obtained iso-dehydrorotenone IX (see page 66 experimental part)



IX

IR-Spectrum of dehydrorotenone

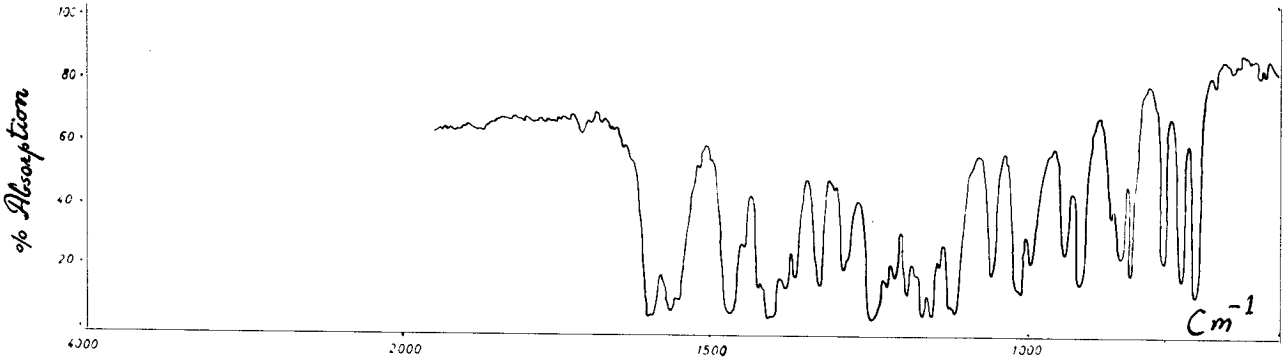


Fig. 6

The UV-absorption spectrum shows two maxima at 237 mμ (ε = 26500) and at 305 mμ (ε = 12000). Fig. 7

UV-Absorption of iso-dehydrorotenone IX

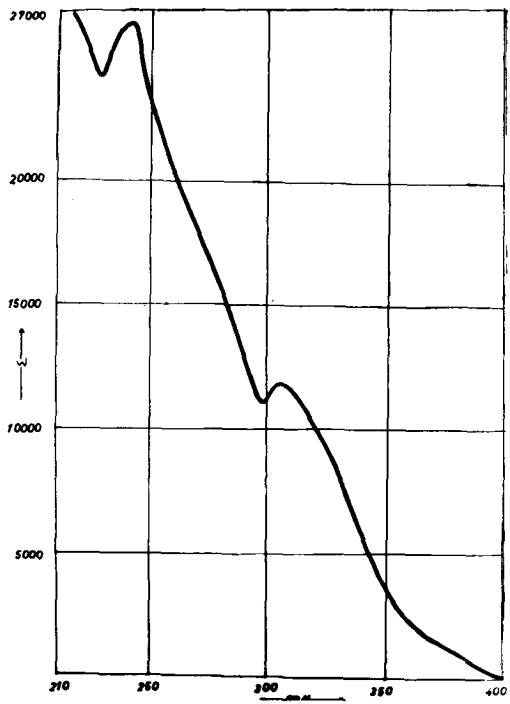
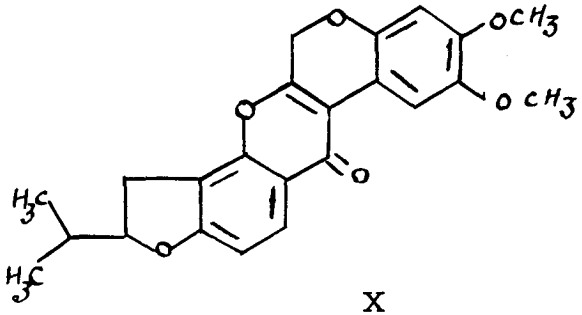


Fig. 7

The difference from the absorption of iso-Rotenone (Fig. 3) is very distinct. The IR-absorption of this substance was not determined.

We also hydrogenated dehydrorotenone VIII with platin oxide in acetic acid and obtained

dihydrodehydrorotenone X[1].



X

As we expected this compound showed an UV-absorption almost identical with dehydrorotenone VIII. It has three maxima at 240 mμ (ε = 27500), 280 mμ (ε = 23000) and 310 mμ (ε = 15000). Fig. 8

1. La Forge F. B. and Smith, J. Am. Chem. Soc. 52, 1091 (1930).

UV-Absorption spectrum of
dihydrodehydrorotenone X

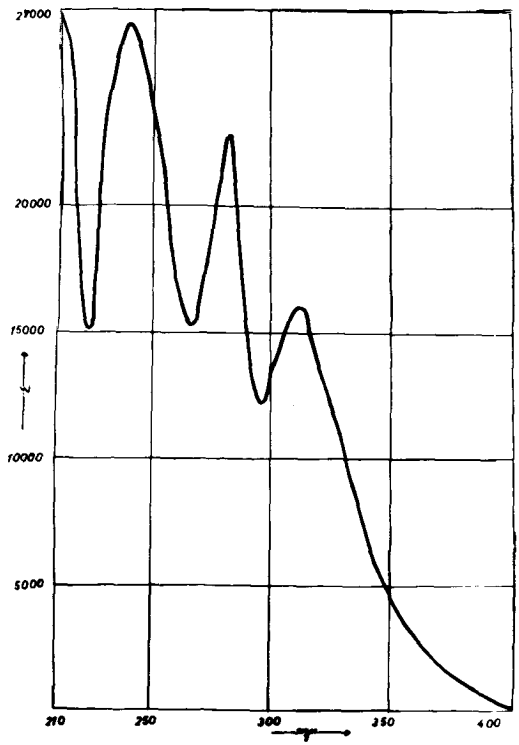
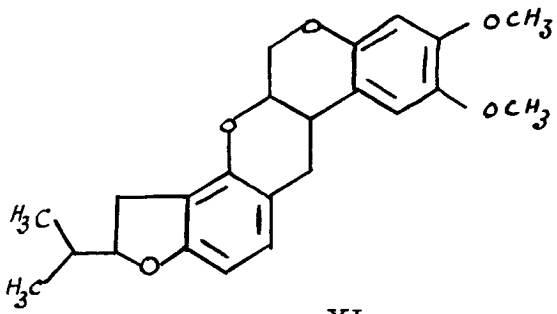


Fig. 8

In this connection we were interested in the absorption spectrum of the compound obtained by similar hydrogenation of Rotenone. Under these conditions dihydrodesoxyrotenone XI[1] is formed, which showed only one maximum at 290 mμ (ε = 8300) and a high end absorption towards 210 mμ. Fig. 9

1. Butenandt A., Inaugural-Dissertation, page 33, Gottingen.



XI

UV-Absorption of dihydrodesoxyrotenone XI

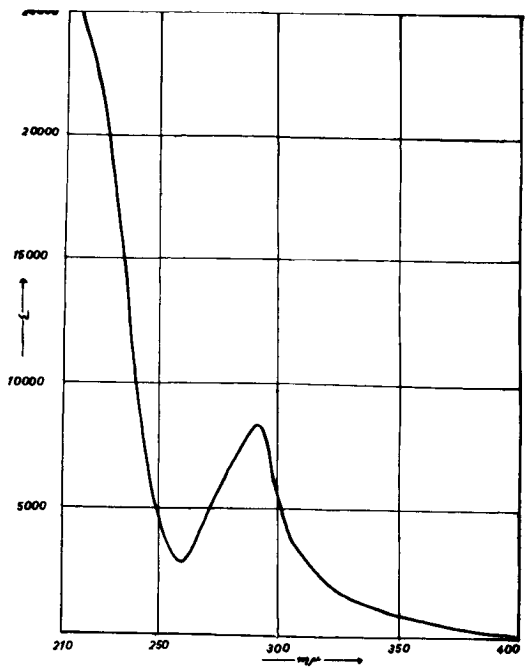
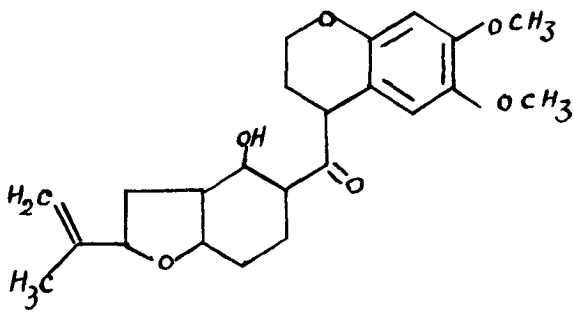


Fig. 9

We would like to mention here that Rotenone I hydrogenated in the presence of palladium/carbon in acetic acid takes up two moles of hydrogen (experimental part page 68). The obtained substance was not analytically determined for the purpose was only to compare the amount of hydrogen-uptake with the unknown A. K. 6 (see page 78).

A further substance we prepared for direct comparison with the chemistry of A. K. 6 was Rotenol XII[1].



XII

We obtained this compound by treatment of Rotenone I with alkali in the presence of zinc. The UV-absorption of the pure Rotenol shows two maxima at 237 mμ (ε = 9500) and 296 mμ (ε = 13200). Fig. 10

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1. Butenandt A., Inaugural Dissertation, page 46, Gottingen (1928).

UV-Absorption of Rotenol XII

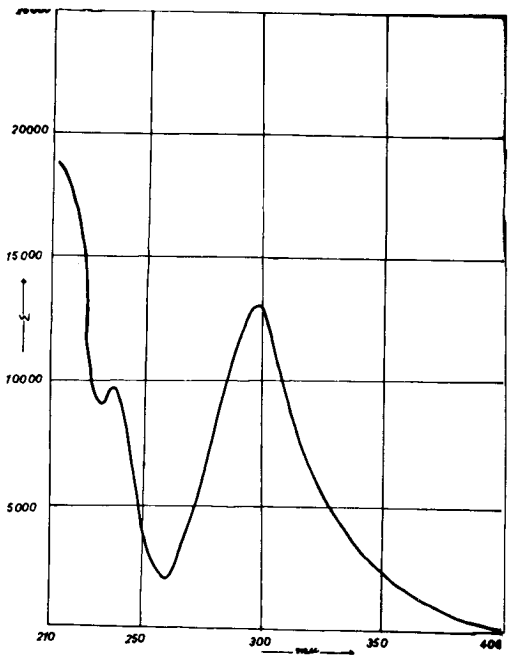
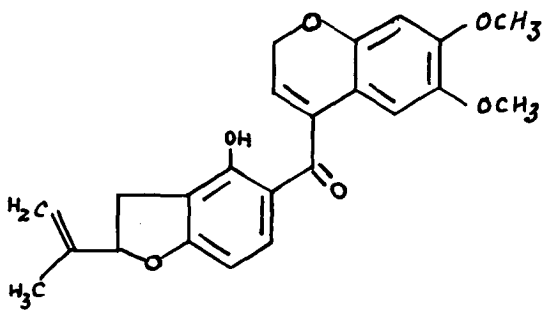


Fig. 10

From Rotenol XII we were able after the treatment with potassium ferricyanide in alkali to isolate dehydrorotenol XIII[1].

1. Haller H. L. and La Forge F. B., J. Am. Chem. Soc. 53, 2271 (1931).



XIII

It shows UV-maxima at 237 mμ (ε = 22800) and 296 mμ (ε = 17800). Fig. 11

UV-Absorption of dehydrorotenol XIII

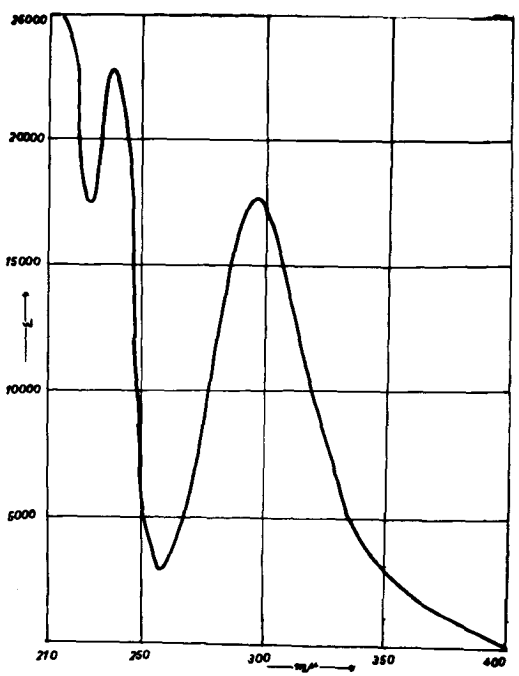


Fig. 11

Rotenone I should give after strong oxidative degradation some lower aromatic acids or phenols. Several attempts were made with chromic acid, sodium dichromate and potassium permanganate in different conditions (for a detailed description see experimental part page 71) but never any clearcut crystalline degradation compound could be isolated.

b) A.K. 6

After several CH-determinations which gave the choice for the formula for A. K. 6 between $C_{22}H_{18}O_6$ and $C_{23}H_{20}O_6$ we decided the latter as being the more likely. (While we were finishing this work, the results of the isolation of several substances from Piscidia erythrina were reported by J. A. Moore and St. Eng in J. Am. Chem. Soc. 78, 395 (1956). The authors also isolated besides Rotenone a substance they called Jamaicine, which we believe is identical with our compound A.K. 6. They prefer the formula $C_{22}H_{20}O_6$.) Some solubilities and other properties are mentioned in the experimental part. It contains only one methoxy-group and the gallic acid test[1] on the methylendioxy-configuration is negative compared to narcotine and hydrastine (no Fehling colour with A.K. 6) but shows a similar colour (bluish green) as Piperonal, Berberin and Apiol. (For a detailed report of this test compare the experimental part page 77). Therefore for the moment we think it possible that A.K. 6 has a methylen-

1. Labert J. A., Bull. Soc. Chim. biol. 15, 1344 (1932).

dioxy grouping. (The methylendioxy grouping in Piperional does not give any OCH_3.) The substance is optically inactive. It does not form an acetate under mild and then stronger conditions and shows no visible ferric chloride-reaction. It does not form an oxime or a hydrazone under different conditions and the active hydrogen determination was negative. After treatment with ozone neither acetone nor formaldehyde could be found in the volatile fractions and no definite substance could be isolated from the non-volatile residue. Dehydration under the condition for the formation of dehydrorotenone with iodine gave only starting material. Oxidation with sodium dichromate gave no crystalline compound. The UV-absorption spectrum shows three maxima at 231 mμ (ϵ = 27000), 266 mμ (ϵ = 26500) and at 307 mμ (ϵ = 14100) (Fig. 12).

UV-Absorption of A. K. 6

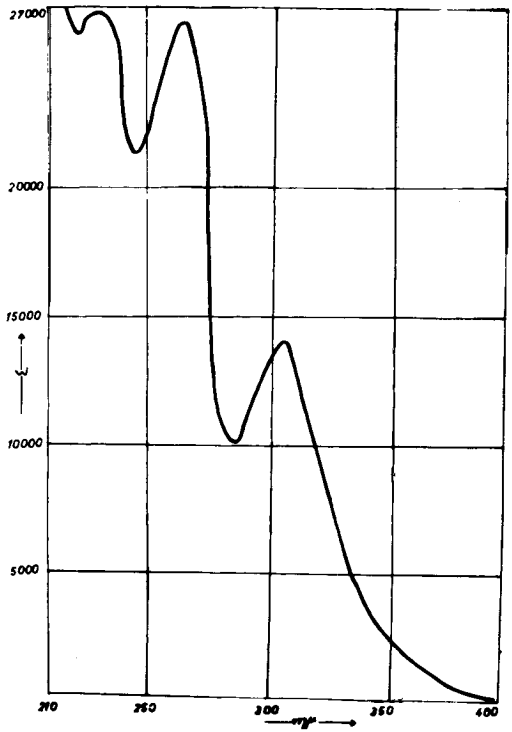


Fig. 12

This spectrum has an extraordinary similarity to the absorption curve of dehydrorotenone VIII (Fig. 5) and naturally also to dihydrodehydeorotenone X (Fig. 8).

The IR-absorption of A.K. 6 (Fig. 13) shows a carbonyl band of low frequency at 1651 cm^{-1}, which could belong to conjugation with aromatic systems, to strong hydrogen bonding or to a carbonyl of a polycyclic quinone system. The strong band at 1636 cm^{-1} can be attributed to an aromatic system, the band being shifted to a higher wave number, which is sometimes observed in polycyclic aromatic systems. There are some further strong aromatic band at 1500 cm^{-1} and some weaker ones near 1575 cm^{-1}. There is no hydroxyl band observed in the region 3600 - 3000 cm^{-1}. The bands at 1042 cm^{-1} and at 1113 cm^{-1} could point out to C-O stretching vibration band, as it also occurs in ethers.

We also carried out some hydrogenation tests on A.K. 6. Microanalytical hydrogenation in acetic acid in the presence of platin oxide showed quick uptake of 4 to 4,5 mole. We isolated a new substance which we called hydro-A.K. 6. It analyzed for the formula $C_{22}H_{22}O_6$. The UV-absorption spectrum shows only two maxima at 234 mμ (ϵ = 19300) and 290 mμ (ϵ = 19000).

This absorption curve shows a surprising similarity to the absorption of Rotenol XII (Fig. 10).

IR-Spectrum of A.K. 6

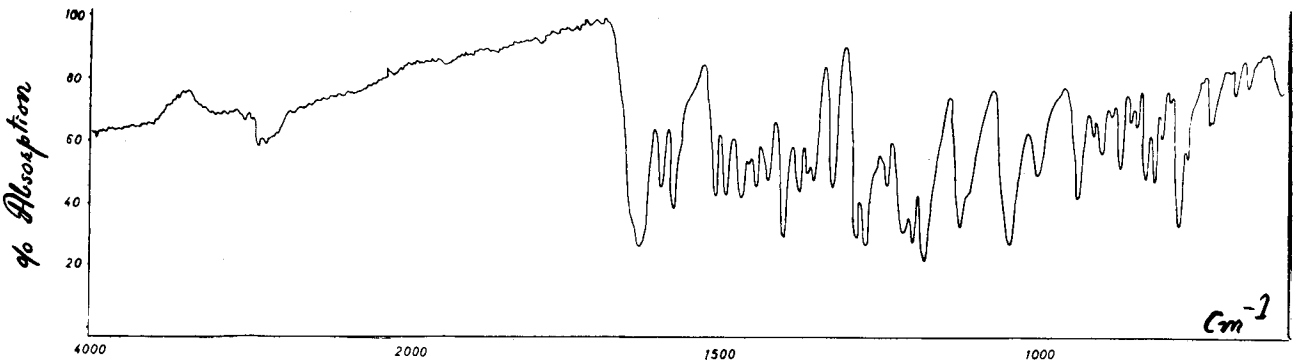


Fig. 13

IR-Spectrum of Hydro-A.K. 6

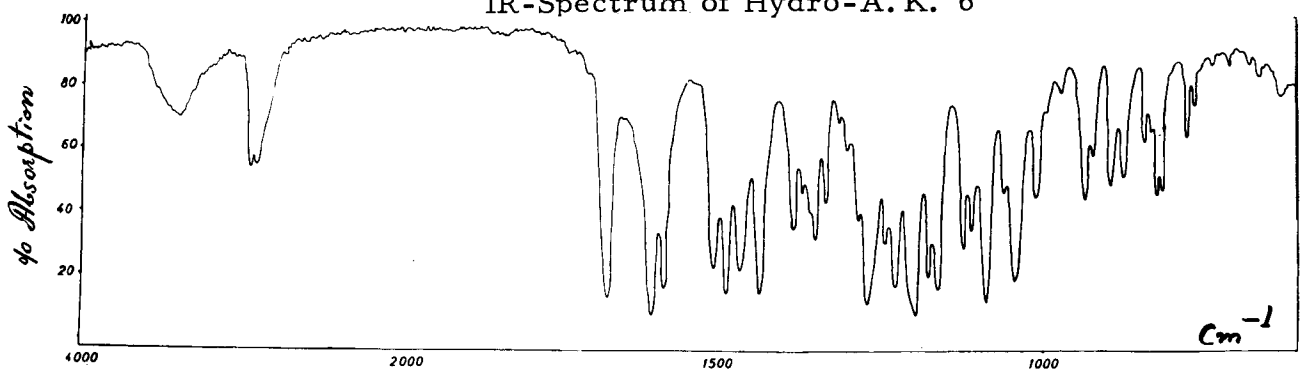


Fig. 15

The IR-spectrum of hydro-A.K. 6 (Fig. 15) shows a hydroxyl band at 3410 cm^{-1}. The carbonyl group has come to the higher frequency of 1678 cm^{-1}. There are strong aromatic bands at about 1600 cm^{-1} and 1500 cm^{-1}. A weak band at 775 cm^{-1} could be due to an aromatic system and two strong bands at 1045 cm^{-1} and 1185 cm^{-1} are probably showing C-O stretching vibration.

The new hydroxyl group which is formed after hydrogenation of A.K. 6, as is clearly shown in the infrared analysis, is very inactive. None of

UV-Absorption of Hydro-A.K. 6

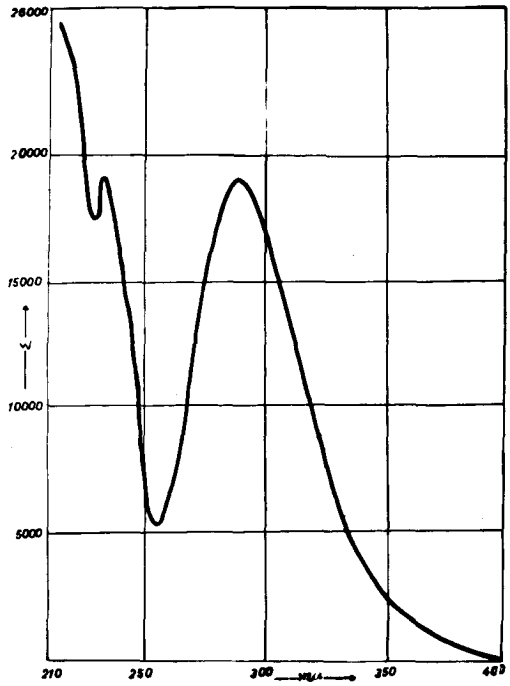


Fig. 14

the usual reactions, which show the presence of a phenolic hydroxyl, were positive. A primary or secondary aliphatic hydroxyl is also excluded (see experimental part page 75). Treatment of A. K. 6 with zinc and alkali gave a neutral and an alkali-soluble substance. The neutral compound has the formula $C_{20}H_{20}O_6$ and shows quite a different aromatic absorption in the UV from A. K. 6. It has a main maximum at 268 mμ (ϵ = 27000) and a lower maximum at 305 mμ (ϵ = 16000) (Fig. 16).

UV-Absorption of neutral substance split from A. K. 6

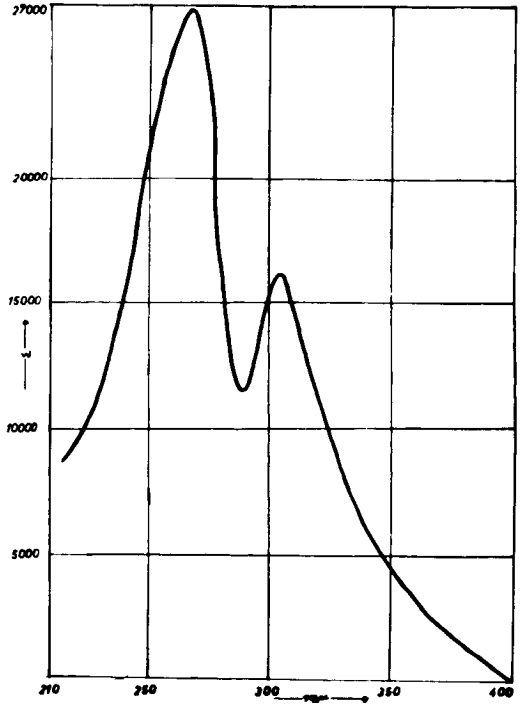


Fig. 16

The IR-spectra (Fig. 17) is more or less like the IR-spectra of A.K. 6. The carbonyl band appears at a relatively low frequency, i.e. 1645 cm^{-1}. The strong band at 1620 cm^{-1} can be attributed to the aromatic system. Some further strong aromatic bands are observed at 1510 cm^{-1} and at 1490 cm^{-1}. There is no hydroxyl band observed between the region 3000-3600 cm^{-1}. The bands at 1250 cm^{-1} and 1120 cm^{-1} could point out to C-O stretching vibrations.

The alkali-soluble portion showed the behaviour of a phenol (see experimental part page 82) and analyzed well for the formula $C_{21}H_{22}O_6$ (this

IR-Spectrum of neutral substance split from A. K. 6

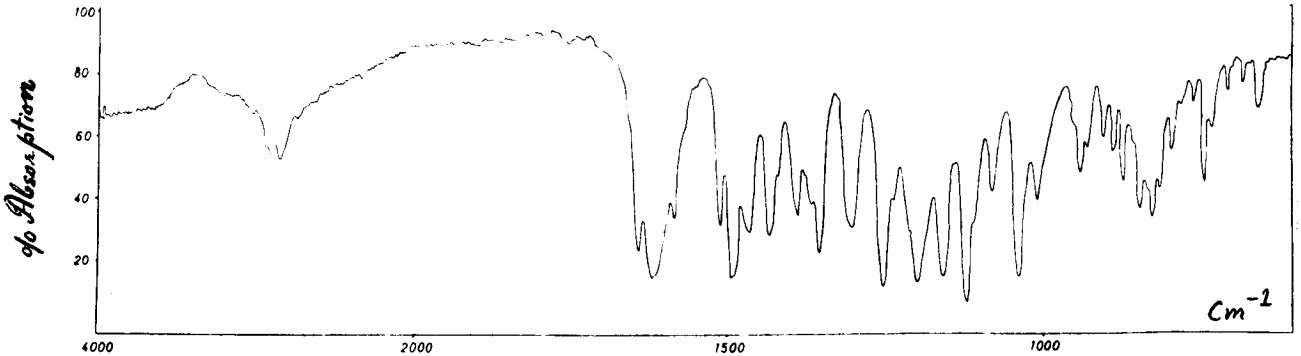


Fig. 17

analysis result does not exclude a $C_{14}H_{14}O_4$ compound). It showed again a different UV-absorption with one maximum at 265 mμ (ϵ = 22500) (Fig. 18). Both, the neutral and the alkali-soluble substances showed the same reaction colour in the gallic acid test as A.K. 6.

UV-Absorption of alkali-soluble substance split from A.K. 6

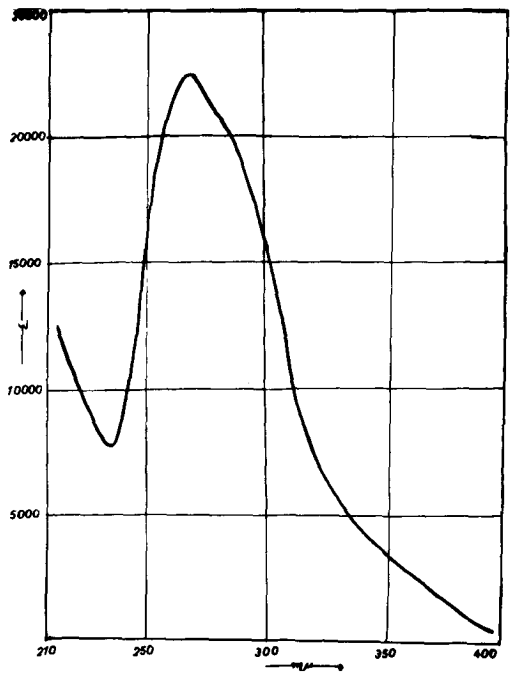
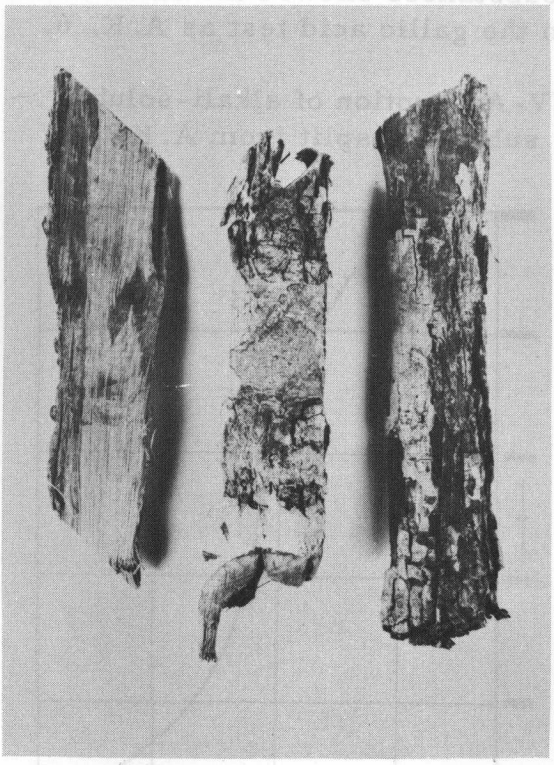


Fig. 18

Plate No. III



Piscidia erythrina,
Root bark.

EXPERIMENTAL PART

ISOLATION

1. Identification of the plant material.

A thorough macroscopical and microscopical study of Piscidia erythrina root bark, bought from Pennic and Co., New York, was made and compared with literature.

Macroscopical

The root bark after complete drying (Plate III) has a greenish outer appearance. The cork is irregularly broken, and small black spots are present in irregular distribution on the outer surface. The inner surface has a dark brown colour and presents more or less a fibrous appearance.

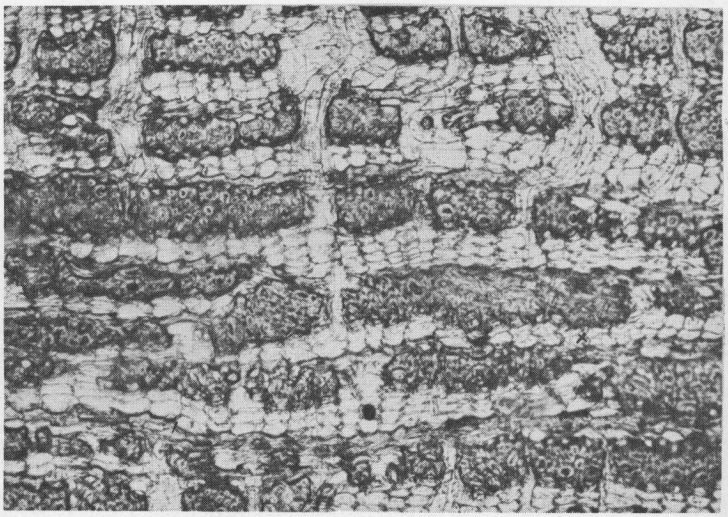
Microscopical

The bark was softened by treating it with a hot mixutre of glycerine: water: 95% alcohol; 1: 2: 2, for 12 hours. Transverse and radial longitudinal sections were cut with sliding hand microtome at 10-15μ, and stained with phloroglucin and hydrochloric acid.

Transverse Section (Plate IVA) shows on the outermost the cork which is composed of several layers of collapsed tubular yellowish brown regu-

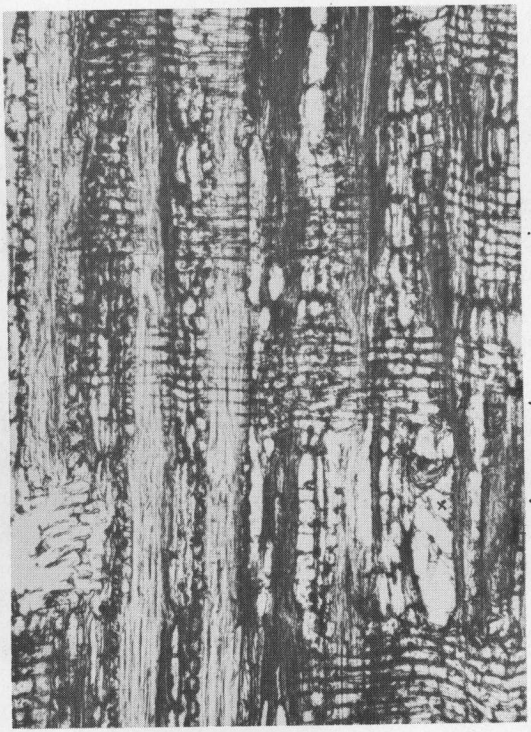
Plate No. IV

A



Transverse section
Piscidia erythrina, Root bark.
a) Medullary rays, b) Phloem fibers,
c) Phloem paranchyma.

B



Radial longitudinal section
Piscidia erythrina, Root bark,
a) Medullary rays, b) Phloem fibers,
c) Phloem paranchyma

larly superposed cells. The outer and inner cell walls show some lignification. Cortex consists of pranchymatous, non-lignified, rectangular or slightly polyhedric cells, which contain starch grains and tannin. The starch grains are single to 2-5 compound. The individual grains are elliptical, rounded-polygonal with a distinct central hilum and measure between 8 μ - 12.6 μ in diameter. Scattered monoclinic crystals of calcium oxalate were also observed. Large secretory cavities, elliptical to lens shaped, with brownish red amorphous substance also occur in this region.

The Phloem is a broad region consisting of Phloem parenchyma tissue, composed of tangenitally elongated and non-lignified cells. It is traversed by sinous interrupted medullary rays, which consist of up to six rows of cells radially elongated, containing large amounts of tannins. Also were observed numerous alternating groups of lignified fibers composed of polygonal cells, with thick lignified walls and comparatively larger and irregular polygonal lumina.

Radial longitudinal Section (Plate IVB) shows many layers of cork cells, of the same form as in the transverse section. The cortex is a broad zone composed of rounded to polygonal parenchymatous cells, some of which have lignified walls and contain a monoclinic prism of calcium oxalate; others contain tannin or starch. Large elongated secretion cavities with brownish-red amorphous substance are observed in some of the sections. The phloem fibers appear in longitudinally elongated discontinuous bundles, accompanied by

calcium oxalate crystal rows. The phloem consist of rounded to polygonal non-lignified cells. The medullary rays are discontinuous and cross at right angles to the other tissues. The cells of medullary rays are rectangular and radially elongated, and many of them contain numerous irregular brown masses of tannin.

The Micro-Chemical tests.

Transverse sections of the root bark which was softened in humid chamber (desiccator with a water saturated atmosphere), were used for all the micro-chemical tests.

1. The abundance of starch grains was observed in the cortical region, when a drop of iodine in potassium iodide solution which stains them black, was put on a section.
2. 1% ferric chloride test solution stains the tannin bluish-black, in the cortical and phloem region.
3. No red colour was observed when a section was treated with Sudan III, showing the absence of fats and volatile oils.
4. Crystals of calcium oxalate were observed in the cortex and to a lesser extent in the phloem region.
5. Concentrated nitric acid dissolved the brownish-red contents of the secretory cavities, found in the cortical region. This substance is not soluble in boiling water and 95% alcohol, also it is not soluble in 50% potassium hydroxide solution, when cold but dissolves on boiling.

6. When a section is treated with dilute sulfuric acid and Denges solution (mercuric oxide in sulfuric acid) no colour was observed on warming and then boiling the preparation. Rotenone gives red colour under these conditions.
7. No bluish green colour was observed when a section was boiled with concentrated sulfuric acid and a drop of 10% gallic acid. Substance A.K. 6 gives an intense bluish-green colour under these conditions.

As the histological results correspond to those found in literature, the plant material was confirmed to be genuine.

2. Test method for fish poison activity.

For the location of active fish poison, it was decided to carry out a small scale pharmacological test on fish. For this purpose various five liter flasks were used. Each of the flasks was filled with 5 liters of tap water and kept overnight, to attain a uniform temperature. The arrangement of passing a roughly equal amount of air through each flask was made. To start an experiment an equal amount of each substance to be tested was added to different flasks. The concentration then was 1: 5'000'000, i.e. 1 mg. crystals or extract in 5 liters, in all experiments. For making this dilution, 0,05% alcoholic solution of the substance to be tested was made and 2 cc. of this solution was added to the 5 liters of water. A blind solution was always kept to compare the results. The variety of the fish for the tests was Phoxinus

Vaevis L. or Minnows and care was always taken to select approximately the same size of fish and five fish were used for each flask. The fish collected from the main tank were put into one of such 5 liter water flasks, where temperature and air bubble development were constant. They were kept for about an hour to let them get accustomed to the new surroundings and temperature. After that an equal number of fish were placed into each flask containing the different substances, and the effect was watched closely. When the so-called overturning time came, the effect of the fish poison was regarded as complete and this was the time we used as a measurement for the fish poison strength of each solution. As overturning time we regarded the moment when the fish made a complete turn around its longitudinal axis. From the very beginning the fish swimming in the poison active solution showed themselves to be very restless. There was a tendency for deep breathing and coming closer to the air bubble developer or to the surface of the water. Further indications of the toxicity were difficulty in respiration and laying on one side interrupted by sudden jumps to try to leave the flask. We were

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We are very grateful to Dr. H. Woker, Eidg. Anstalt fur Wasserversorgung, Abwasserreinigung und Gewasserschutz, E.T.H., Zurich (Switzerland) for his help and friendly advice on carrying out the pharmacological tests on fish.

able to make the fish recover completely from the poison effect by taking it out of the flask after its first overturning time and putting it into fresh running tap water.

3. Petrolether Extraction.

17,7 kg. of the powdered drug were extracted by maceration with 36 ltr petrolether at room temperature, overnight. The plant powder was collected by filtration on a Buchner funnel and the filtrate evaporated at 45° at 15 mm. vacuum. The extraction process was repeated five times with 36 ltr petrolether each time and the resulting extracted portions mixed. After complete evaporation of the solvent and residue was a yellowish paste, which weighed 61.3 g. The yield calculated on the amount of plant material was 0.346 %.

4. Alcoholic Extraction.

After exhaustive extraction of the plant material with petrolether it was treated with the same amount of alcohol (denaturated with 1% benzene) in the same way. The removal of the alcohol was also done by evaporation of the combined alcoholic extracts at 45° and in a 15 mm. vacuum. The yield was 490 g. which corresponds to 2,79% calculated on the amount of the plant material.

Both, the petrolether and the alcohol extract were tried for their activity as a fish poison.

Petrolether extract : overturning time 40 min.
Alcohol extract : no overturning observed.

SEPARATION OF THE PETROLETHER EXTRACTION

61,3 g. of petrolether extract was dissolved in a mixture of acetone-petrolether 1: 1 and the insoluble portion filtered off and not further investigated. The filtrate was kept overnight at 0° and a newly crystallized fraction again removed by filtration, m.p. 137° -- with delay -- 185°. This way, two further fractions were obtained from the acetone-petrolether solution with the m.p. 80-85° and 137-140°. None of these fractions showed any fish poison activity and therefore were not further investigated.

The remaining acetone-petrolether solution weighed after complete evaporation 50,1 g. This fraction gave a positive fish poison test.

Some preliminary experiments showed that a chromatography on Alumina (Woelm, neutral, activity I) gave a good separation and did not destroy the fish poison activity.

50,1 g. petrolether extract was chromatographed on 750 g. Alumina, starting with petrolether-benzene 1: 1, each elution being 1000 cm^3.

Table
Chromatography I

No.	Solvent	Weight	Melting-point	Fish poison activity
1	Benzene: petrolether 1: 1	-	-	-
2	Benzene: petrolether 1: 1	5,8776 gm	oil	no activity
3	Benzene: petrolether 1: 1	-	-	-
4	Benzene: petrolether 9: 1	2,4586 gm	oil	no activity
5	Benzene: petrolether 9: 1	7,2278 gm	oil	no activity
6	Benzene: petrolether 9: 1	-	-	-
7	Benzene	-	-	-
8	Benzene	1,2300 gm	oil	no activity
9	Benzene	-		-
10	Benzene: Chloroform 9: 1	-		-
11	Benzene: Chloroform 9: 1	-		-
12	Benzene: Chloroform 9: 1	-		-
13	Benzene: Chloroform 4: 1	3,3476 gm	150-158°	Turning point 7 min.
14.	Benzene: Chloroform 4: 1	3,5386 gm	150-188°	Turning point 10 min.
15	Benzene: Chloroform 4: 1	2,045 gm	188°	no activity
16	Benzene: Chloroform 4: 1	-	-	-

No.	Solvent	Weight	Melting-point	Fish poison activity
17	Benzene: Chloroform 1: 1	2,5705 gm	140°	no activity
18	Benzene: Chloroform 1: 1	10,8430 gm		
19	Benzene: Chloroform 1: 1	-		-
20	Benzene: Chloroform 1: 9	-		-
21	Benzene: Chloroform 1: 9	-		-
22	Benzene: Chloroform 1: 9	-		-
23	Chloroform	-		-
24	Chloroform	1,6048 gm	oil	no activity
25	Chloroform	2,4120 gm	oil	no activity
26	Chloroform	-		-
27	Chloroform: Methanol 9: 1	-		-
28	Chloroform: Methanol 9: 1	-		-
29	Chloroform: Methanol 4: 1	-		-
30	Chloroform: Methanol 4: 1	-		-
31	Chloroform: Methanol 4: 1	-		-
32	Chloroform: Methanol1: 1	0,3130 gm	oil	no activity
33	Chloroform: Methanol 1: 1	0.1230 gm	oil	no activity
34	Chloroform: Methanol 1: 1	1,7230 gm	oil	no activity

No.	Solvent	Weight	Melting-point	Fish poison activity
35	Chloroform: Methanol 1: 1	-	-	-
36	Methanol	0,600 gm	oil	no activity
37	Methanol	-	-	-
38	Methanol	-	-	-

Fraction 13 was soluble in ether, acetone, chloroform, benzene, pyridine, sparingly soluble in petrolether and in alcohol, fairly soluble in hot alcohol. Recrystallization from acetone-ether-petrolether gave white crystals of m.p. 152-162° which again crystallized from the same solvents yielded 687 mg. fish poison of m.p. 163°. This substance is believed to be the pure fish poison, its turning point occurs after 4.5 minutes.

The mother liquor of this fraction gave, after rechromatography further 77 mg. of pure fish poison.

Fraction 14 was crystallized from acetone-petrol-ether and gave 667 mg. crystals of m.p. 150-188°.

Fraction 15 gave after recrystallization from acetone-petrol-ether 1,277 g. of A.K. 6

Fractions 17 and 18 were combined and separated by fractional crystallization. A first fraction from acetone-petrolether gave 327 mg. of a substance of m.p. 70-75°, which was not further investigated. Its mother liquor, again saturated with acetone-petrolether, gave 127 mg. crystals of m.p. 210-212° which was not further investigated either. The remaining mother liquor this time saturated with a small amount acetone and with an excess petrolether yielded 4,56 g. of crude β-Sitosterol of m.p. 133-136°. The filtrate of this fraction was completely evaporated and the residue weighing 6.28 g. was chromatographed on 180 Alumina. The fractions eluated with

Chromatography 2

No.	Solvent	Weight	Melting-point	Fish poison activity
1	Benzene-ether 4: 1	41 mg	158-162°	Turning point 5 min.
2	Benzene-ether 4: 1	50 mg	162-163°	Turning point 5 min.
3	Benzene-ether 4: 1	71 mg	(158)-188°	Turning point 2 hours
4	Benzene-ether 4: 1	103 mg	187-188°	no activity
5	Benzene-ether 4: 1	111 mg	187-190°	no activity
6	Benzene-ether 4: 1	120 mg	190-192°	no activity
7	Benzene-ether 4: 1	150 mg	190-192°	no activity
8	Benzene-ether 4: 1	9 mg	-	-

benzene-chloroform 1: 1 gave a further 3.23 g. of crude β-Sitosterol of m.p. 133-136°.

Further purification of fraction 14 from chromatography I

667 mg. fraction 14 was chromatographed on 30 30 g. Alumina and eluated each time with 70 cc. solvent. (See Table Chromatography 2)

Fractions 1 and 2 were combined and gave after recrystallization 70 mg. fish poison of m.p. 163°.

Fraction 3 was treated with charcoal, filtered through Alumina with benzene as solvent and gave 43 mg. of inactive substance A.K. 6 of m.p. 190-191°.

The remaining fractions 4-7 were combined and gave after recrystallization 393 mg. of A.K. 6 with the m.p. 190-191°.

IDENTIFICATION OF FISH POISON WITH ROTENONE

The total amount of pure fish poison was 834 mg and had m.p. 163-164°. A mixed melting point with a pure authentical sample of Rotenone melted at 163° and showed no depression.

Rotenone obtained from British Drug Houses (England) was purified by several recrystallizations until it melted at 163°.

3,735 mg gave 9.585 mg. CO_2 and 1.947 mg H_2O

$C_{23}H_{22}O_6$ (394.41)
Calculated: C, 70.04; H, 5.62; $2OCH_3$, 15.72%. Found: C, 70.03; H, 5.83; OCH_2, 15.74%.

Rotenone Derris is a sample isolated in these laboratories from Derris elliptica after the method developed in the isolation of the fish poison of Piscidia erythrina (unpublished results).

Rotation

Fish poison in Chloroform $[\alpha]_D^{20} = -122°$ (c=1.36)

in Benzene $[\alpha]_D^{20} = -227°$ (c=1.02)

in Ethanol $[\alpha]_D^{20} = -226°$ (c=1.05)

Rotenone BDH

in Chloroform $[\alpha]_D^{20} = -122^\circ$ (c=1.70)

in Benzene: $[\alpha]_D^{20} = -227^\circ$ (c=1.62)

in Ethanol $[\alpha]_D^{20} = -226^\circ$ (c=0.97)

Rotenone Derris

in Chloroform $[\alpha]_D^{20} = -121^\circ$ (c=1.10)

in Benzene $[\alpha]_D^{20} = -227^\circ$ (c=1,25)

in Ethanol $[\alpha]_D^{20} = -227^\circ$ (c=1.40)

4.205 mg. gave 10.790 mg CO_2 and 2,180 mg H_2O. 4,567 mg used 6.96 cc of 0.02 n-$Na_2S_2O_3$

$C_{23}H_{22}O_6$ (394.41)

Calculated: C, 70.04; H, 5.62; $2OCH_3$, 15.72%. Found: C, 70.02; H, 5.80; OCH_3, 15.76%.

UV-absorption spectra (Fig. No. 1) and IR-absorption spectra (Fig. No. 2a and 2b) are fully mentioned in the theoretical part and are identical with the spectra of an authentical sample of Rotenone.

Potassium permanganate in acetone and bromine in water give a precipitation when added to a solution of fish poison, resp. Rotenone.

SUBSTANCE A. K. 6

The total amount of pure A. K. 6 was 1.713 g. and it melted at 191°. It is soluble in acetone, chloroform, benzene and pyridine, slightly soluble in alcohol, fairly soluble in hot alcohol and ether, insoluble in petrolether. It crystallizes best from acetone-ether-petrolether. It is insoluble in sodium hydroxide, hydrochloric acid and water.

Potassium permanganate in acetone or bromine in water added to a solution of A. K. 6 gives no precipitation.

A. K. 6 has no fish poison activity but is eluated from Alumina together with the fish poison, resp. with Rotenone. $[\alpha]_D=0°$.

4,106 mg gave 10,527 mg CO_2 and 1,787 mg H_2O. 3,860 mg used 3,13 cc 0.02 n-$Na_2S_2O_3$. 4.238 mg used 3,37 cc 0.02 n-$Na_2S_2O_3$

$C_{22}H_{18}O_6$

Calculated: C, 69.83; H, 4.80; OCH_3, 8.20%
Found: C, 69.97; H, 4.87; OCH_3, 8.39% and 8.23%.

3,778 mg gave 9.746 mg CO_2 and 1.800 mg. H_2O. 7.207 mg used 5.43 cc 0.02 n-$Na_2S_2O_3$.

$C_{23}H_{20}O_6$

Calculated: C, 70.40; H, 5.14; OCH_3, 7.90%
Found: C, 70.40; H, 5.33; OCH_3, 7.79%.

5.317 mg gave 0 cc CH_4 at 20°

Found: no active hydrogen

4,171 mg gave at 23° 715 mm Hg 0.013 cc N_2

Found: 0.34% N: no nitrogen

10,510 mg. used 3,579 cc H_2, 0°, 760 mm Hg, Hydrogenation time one hour, catalyst: platin oxide/acetic acid.

Dz: 5.99

It has taken up 6 moles of hydrogen.

UV-spectrum (Fig. No. 14) and IR-spectrum (Fig. No. 15) are discussed in the theoretical part.

β-SITOSTEROL

The total amount of isolated β-Sitosterol was 7.79 g. and melted at 142° after several recrystallizations from ether-petrolether.

$[\alpha]_D^{20} = -37°$ (c = 1.17)

3,970 mg. gave 12.309 mg CO_2 and 4,178 mg H_2O. 3,085 mg. used 0.07 cc 0.02 n-$Na_2S_2O_3$.

Methoxyl determination was negative.

$C_{29}H_{50}O$ (414,69)

Calculated: C, 83.99; H, 12.15%. Found: C, 84.61; H, 11.78%.

Acetylation

50 mg. β-Sitosterol was dissolved in a mixture of 1 cc. pyridine and 1 cc. acetic anhydride and left overnight at ordinary temperature. After evaporation under reduced pressure, the residue was taken up in 50 cc. ether and washed with 5 cc water, 5 cc n-sodium hydroxide and water. The ether was dried with sodium sulfate and evaporated. The residue crystallized from ether-petrolether. White crystals m.p. 127°, yield 33 mg. $[a]_D^{20}$ = -41° (c = 1.04)

$C_{31}H_{52}O_2$ (456.73)

Calculated: C, 81.52; H, 11.48%. Found: C, 81.35; H, 11.44%.

DERIVATIVES OF FISH POISON

Identification of iso-fish poison with iso-Rotenone

100 mg. fish poison was treated with 5 cc. conc. sulfuric acid under cooling in an ice bath.

The solution was immediately transferred into 70 cc. cold water and kept at 0° for 4 hours. The precipitation was filtered and crystallized from acetone-petrolether. We obtained 30 mg. white needles of m.p. 176°.

$[\alpha]_D^{20} = + 7.8°$ (c = 1.02)

3.660 mg. gave 9.357 mg CO_2 and 1.875 mg H_2O

$C_{23}H_{22}O_6$ (394.41)

Calculated : C, 70.04; H, 5.62; 2 OCH_3, 15.72%. Found: C, 69.77; H, 5.73; OCH_3, 15.48%.

The UV- (Fig. No. 3) and IR-spectra (Fig. No. 4a and 4b) are discussed in the theoretical part and are identical with the spectra of iso-Rotenone. Mixed melting-point with iso-Rotenone gave no depression.

Identification of fish-poison-iso-hydrazone with Rotenone-iso-hydrazone

100 mg. fish poison and 100 mg. hydrazine-hydrate were dissolved in 20 cc. alcohol and the mixture refluxed at boiling water bath temperature for one hour. On cooling down white needles crystallized out, which, after recrystallization in hot alcohol, melted at 228°. The yield was 67 mg. A mixed melting point with authentical Rotenone-iso-hydrazone showed no depression.

3.379 mg. gave 8.366 mg. CO_2 and 1.761 mg H_2O. 5.115 mg. gave at 23° 707 mm Hg, 0,325 cc N_2

$C_{23}H_{24}O_5N_2$ (408.44)

Calculated: C, 67.52; H, 5.92; N, 6.86%. Found: C, 67.57; H, 5.83; N, 6.83%.

Ferric chloride solution gave a blue-green colour.

DERIVATIVES OF ROTENONE

Iso-Rotenone

200 mg. Rotenone was treated with 5 cc conc. sulfuric acid at 0°. The reaction solution was immediately added to 100 cc cold water and the mixture left at 0° for 4 hours. The crystalline precipitation was filtered off, washed with water and recrystallized from acetone-petrolether. It gave 54 mg. with m.p. 176°.

$[\alpha]_D^{20} = +7.8°$ (c = 1.15)

3,500 mg gave 9,008 mg CO_2 and 1.745 mg H_2O

$C_{23}H_{22}O_6$ (394.41)

Calculated: C, 70.04; H, 5.62%. Found: C, 70.24; H, 5.58%.

The UV- (Fig. No. 3) and IR-spectra (Fig. No. 4) are mentioned in the theoretical part.

Rotenone-iso-hydrazone

200 mg Rotenone and 200 mg. hydrazine-hydrate were dissolved in 25 cc. alcohol and the mixture refluxed for one hour. On cooling white needles precipitated, which were filtered, washed with cold alcohol and gave after recrystallization from hot alcohol 150 mg. Rotenone-iso-hydrazone of m.p. 229°.

3,970 mg gave 9,790 mg CO_2 and 2,128 mg H_2O. 2,664 mg gave at 23° 713 mm Hg, 0.171 cc N_2. 3,183 mg used 4,68 cc 0.02 n-$Na_2S_2O_3$

$C_{23}H_{24}O_5N_2$ (408.44)

Calculated: C, 67.63; H, 5.92; N, 6.86; OCH_3, 15.20%. Found: C, 67.30; H, 6.00; N, 6.94; OCH_3, 15.21%.

Ferric chloride solution gave a blue-green colour.

Dehydrorotenone

1 g Rotenone and 2.5 g Potassium Acetate were dissolved in 50 cc alcohol. The mixture was heated up to reflux and there was slowly added a solution of 1 g iodine in 15 cc alcohol. After the addition was completed, the mixture was heated up for a further half hour until the solution was only slightly yellow. After cooling down yellow

needles crystallized out, which gave after recrystallization from chloroform-petrolether 453 mg Dehydrorotenone of m.p. 218-219°.

3.725 mg gave 9.396 mg.CO_2 and 1.740 mg H_2O

$C_{23}H_{20}O_6$ (392.39)

Calculated : C, 70.40; H, 5.14%. Found: C, 68.78; H, 5.22%.

UV- (Fig. No. 5) and IR-spectra (Fig. No. 6) are discussed in the theoretical part.

Iso-dehydrorotenone

80 mg dehydrorotenone was treated with 5 cc conc. sulfuric acid by slow addition under efficient cooling in ice water. The mixture was diluted at once with 100 cc ice water. The precipitation was filtered, washed and crystallized from acetone-petrolether. We obtained 25 mg. yellow needles of m.p. 190-191°.

3,337 mg. gave 8.434 mg CO_2 and 1.568 mg H_2O

$C_{23}H_{20}O_6$ (392.39)

Calculated: C, 70.40; H, 5.14%. Found: C, 68.97; H, 5.26%.

The UV-spectrum (Fig. No. 7) is shown in the theoretical part.

Dihydrodehydrorotenone

100 mg Dehydrorotenone was dissolved in 15 cc acetic acid (stable to chromic acid). After the addition of a catalytic amount of platin oxide the solution was shaken in a hydrogen atmosphere. Only one mole of hydrogen was taken up. The solution was filtered and evaporated totally under reduced pressure and at 40°. During the evaporation some alcohol was added to facilitate the removal of acetic acid. The residue weighed 88 mg. and was chromatographed on 3 g. alumina. The fractions eluated with benzene-petrolether 9: 1 gave after recrystallization from acetone-petrolether 40 mg light-yellow needles of m.p. 224°.

4, 142 mg gave 10, 561 mg CO_2 and 2, 110 mg H_2O

$C_{23}H_{22}O_6$ (394, 41)

Calculated: C, 70.04; H, 5.62%. Found: C, 69.58; H, 5.70%.

The UV-absorption curve (Fig. No. 8) is shown in the theoretical part.

Hydrogenation of Rotenone

a) Reduction with platin oxide for formation of Dihydro-desoxyrotenone

1 g Rotenone was dissolved in 30 cc stabilized acetic acid and hydrogenated in the presence

of platin oxide and excess hydrogen. In one hour 3,5 moles hydrogen were taken up. The reaction mixture was filtrated, evaporated and the 0.983 g. residue chromatographed on 30 g alumina. The fractions eluated with benzene-chloroform 1: 1 gave 680 mg dihydrodesoxyrotenone of m.p. 167-168°.
4,330 mg. gave 11,451 mg CO_2 and 2,670 mg H_2O

$C_{23}H_{26}O_5$ (382,44)

Calculated: C, 72.23; H, 6.85%. Found: C, 72.17; H, 6.90%.

The UV-absorption curve (Fig. No. 9) is shown in the theoretical part.

b) Reduction with palladium/carbon

100 mg. Rotenone were dissolved in 15 cc. acetic acid and the solution hydrogenated in the presence of palladium on charcoal as a catalyst. An uptake of 2 moles of hydrogen was observed. The reaction mixture was worked up as indicated above. After filtration through alumina it crystallized in white rhombic crystals of m.p. 190°.

Rotenol

To a boiling alcoholic solution of 1 g Rotenone there was added 2 g zinc dist. After further refluxing for 5 minutes a solution of 10% potassiun

hydroxide in alcohol was added dropwise and the reaction mixture kept boiling. The solution turned yellow immediately. After 4 hours refluxing, the reaction mixture was cooled down, filtered from the zinc dust and the major portion of the alcohol was evaporated under reduced pressure. The concentrated solution was acidified with dil. hydrochloric acid and extracted with ether. The ethereal solution was extracted with potassium hydroxide and washed with water. The remaining ether solution was dried and evaporated and gave 312 mg residue which was chromatographed on 10 g alumina. The fractions eluated with benzene-chloroform 9: 1 and benzene-chloroform 4: 1 gave after recrystallization from acetone-ether-petrolether 247 mg of m.p. 117-118°.

2,670 mg gave 6,821 mg CO_2 and 1,503 mg H_2O

$C_{23}H_{24}O_6$ (396.42)

Calculated: C, 69.68; H, 6.10%. Found: C, 69.72; H, 6.30%.

The UV-absorption curve (Fig. No. 10) is shown in the theoretical part.

Dehydrorotenol

100 mg Rotenol was dissolved in 10 cc methylalcohol and to this hot solution we added 1 cc 10% alcoholic potassium hydroxide. This mixture was

immediately added to a solution of 200 mg potassium ferricyamide in 5 cc water. The reaction mixture was then allowed to stand overnight and then diluted with 70 cc water. The precipitation was filtered off, washed with water and dried. Recrystallization from hot alcohol gave 30 mg white needles, m.p. 78-80°.

3,762 mg gave 9,680 mg CO_2 and 2,020 mg H_2O. 3,647 mg gave 9.399 mg CO_2 and 1,950 mg H_2O

$C_{23}H_{22}O_6$ (394.41)

Calculated: C, 70.04; H, 5.62%. Found: C, 70.22, 70.33; H, 6.01, 5.98%.

The UV-absorption curve (Fig. No. 11) is shown in the theoretical part.

Ozonalysis of Rotenone

In an ozonator producing 436.8 mg. ozone per hour we ozonized 550 mg Rotenone dissolved in 25 cc acetic acid by passing a stream of oxygen containing the mentioned amount of ozone through this solution. The theoretical time for the absorption of one mole ozone was 9 minutes. We ozonized the solution for 15 minutes at ordinary temperature. The theoretical amount we needed for the formation of the ozonide was 66 mg. After titrating of the potassium iodide solution where the unused ozone was reduced, we found that 75.7 mg had been absorbed by Rotenone.

The ozonized solution was steam distilled and the first 50 cc of the distillate was mixed with 10 cc of a 1% dimedon-solution. This solution was left overnight at 0°. The precipitate was filtered, washed and was identical with a sample prepared from formaldehyde and dimedon. They both consisted of white needles with m.p. 180° and gave no depression on mixed m.p.

The residual part of the steam distillation was completely evaporated and the oily residue chromatographed over Alumina. The fractions eluated with chloroform-methanol4: 1 gave 160 mg of a compound with the m.p. 100-102°. Filtered in acetone it was sent for analysis as an amorph substance.

2.927 mg gave 6.351 mg CO_2 and 1,235 mg H_2O

$C_{22}H_{22}O_{10}$ (446.40)

Calculated: C, 59.19; H, 4.97%. Found: C, 59.21; H, 4.72%.

Oxidation of Rotenone

I. Oxidation with sodium dichromate

a) 1 g Rotenone was added, with constant shaking, to a solution of 4 g sodium dichromate in 10 cc water. To this mixture we added carefully 6 cc con. sulfuric acid and left the solution overnight at room temperature. After the addition of 30 cc water the

reaction mixture was extracted with ether. The ether residue weighed 543 mg and could not be crystallized. We tried to obtain a further purification by distillation in a 0.001 mm Hg vacuum. Until we reached 220° bath temperature nothing remarkable distilled.

b) The above experiment was repeated but the reaction mixture was heated to reflux at 130° bath temperature for one hour. The usual isolation gave 576 mg foam which we could not crystallize. High vacuum distillation gave no fraction up to 220° bath temperature.

c) In both the above cases Rotenone was not completely dissolved, so we repeated the two experiments a and b and added to each reaction mixture 5 cc acetic acid. Nothing could be isolated which was crystallized or distillable.

d) The conditions of a and b were used but we replaced the solvent by acetic acid. An additional small amount of water was added to bring the sodium dichromate in solution. After refluxing for two hours and main portion of the acetic acid was evaporated and the solution diluted with water and extracted with ether. The ether residue gave 450 mg which we distilled under reduced pressure. At 200-10° a yellowish substance distilled and had m.p. 87°. Yield 40 mg. The substance is soluble in alkali and gives a brown

colour with ferric chloride. The UV-absorption spectrum showed two maxima: λ_{max} = 234 mμ (ε = 12800) and 293 mμ (ε = 14200). This substance was not further investigated because this method gave no results on the oxidation of A.K. 6.

II. Oxidation with chromic acid.

1 g Rotenone was dissolved in 30 cc. hot acetic acid. To this solution we added 7 g chromic acid dissolved in a small amount of water and 20 cc acetic acid. The mixture was heated for 6 hours on the steam bath. After cooling down the solution was evaporated, diluted with water and extracted with ether. The etheral residue weighed 420 mg. 30 mg of a substance with the m.p. 87° distilled in the vacuum. This compound was identical in m.p. and mixed m.p. isolated after the oxidation of Rotenone with sodium dichromate in acetic acid.

III. Oxidation with potassium permanganate.

a) In acetone:
1 g Rotenone dissolved in 10 cc acetone was added to a solution of potassium permanganate dissolved in acetone-water 4: 1. A further amount of the potassium permanganate-acetone solution was added until the colour stayed violet and no more brown precipitation occurred. The reaction mixture was left overnight, filtered and evaporated. The re-

sidue was extracted with ether and evaporated. The resulting 590 mg. was distilled in vacuo but no fraction could be collected till 220° bath temperature.

b) In alkali solution:
A solution of 1 g Rotenone in 15 cc acetone was mixed with a solution of 0,25 g. potassium hydroxide in 2 cc water and 1 g potassium permanganate and the mixture refluxed for two hours. The acetone was removed, the solution treated with sodium bisulfite acidified with dil. sulfuric acid and extracted with ether. Usual isolation process furnished 38 mg of the above isolated substance of m.p. 85-90°.

The same experiment was carried out with a large excess of potassium permanganate and potassium hydroxide without any positive result.

The above experiment was repeated but under very mild conditions, i.e. at room temperatur The distillation gave again 35 mg. of the substance melting at 85-87°.

c) In pyridine:
1 g Rotenone was dissolved in 10 cc pyridine and treated with potassium permanganate dissolved in pyridine and water. The reaction mixture was left overnight, treated with bisulfite and acidified with dil. sulfuric acid. Extraction with ether gave the above mention-

ed substance of m.p. 87°. The experiment was also carried out with an excess potassium permanganate.

Determination of active hydrogen

Freshly prepared methyl magnesium iodide was used to determine the amount of methane development. The reaction was carried out for a semi-quantitative determination in the well known Zerewitinoff-Apparatus under a nitrogen-atmosphere. The substances to test were Rotenone, Rotenol and A.K. 6, each one weighed 100 mg.

Rotenone:	no development of methane; no active hydrogen.
Rotenol:	development of 9 cc methane; 1,5 mole active hydrogen.
A.K. 6:	no development of methane; no active hydrogen.

DERIVATIVES OF A. K. 6

Acetylation

a) 50 mg. A. K. 6 dissolved in a mixture of 1 cc acetic anhydride and 1 cc pyridine. After leaving it for two days only starting material was isolated.

b) 50 mg. A. K. 6 was refluxed in 15 cc acetic anhydride. After evaporation the residue was worked up in the usual way. Only starting material of m.p. 191° was isolated.

Formation of hydrazone

50 mg. A. K. 6 was dissolved in 6 cc alcohol containing 50 mg. hydrazinhydrate. The mixture was refluxed for one hour. After cooling and evaporating of the solvent we obtained 45 mg. substance of m.p. 191° which proved to be starting material. This reaction also proved to be unsuccessful in basic or acid solution.

Formation of Oxime

50 mg. A.K. 6 dissolved in 10 cc alcohol was added to 100 mg hydroxalamine .hydrochloride and 115 mg sodium acetate. The solution was refluxed for 8 hours. Usual working up process gave 45 mg starting material with m.p. 190-191°. Attempts to make the oxime in basic or acid solution were unsuccessful.

Isomerization

50 mg. A.K. 6 was dissolved in 2 cc conc. sulfuric acid and added immediately into 50 cc ice-cold water. The precipitation was filtered off after 4 hours standing in the refrigerator. This fraction was very insoluble in organic solvents and did not melt until 320°.

This insoluble substance was sent for analysis without recrystallization.

4,290 mg. gave 10,062 mg CO_2 and 1,885 mg H_2O

$C_{11}H_{10}O_4$ (206.19)

Calculated: C, 64.07; H, 4.89%. Found: C, 64.01; H, 4.92%.

Gallic acid test

Test method:

2 cc conc. sulfuric acid was added to 0.1 cc. alcoholic solution of A.K. 6 and 0.1 cc alcoholic solution of gallic acid (1: 20). The mixture was heated on a boiling water bath. The solution first became light greenand formed, after a while, a distinct bluish-green persistent colour.

The following samples were tested:

Substance	Colour
Narcotine	Fehling blue
A.K. 6	Bluish-green
Piperanol	Bluish-green
Berberine	Bluish-green
Hydrastine	Fehling blue
Apiol	Fehling blue
Rotenone	No colour

Hydrogenation

(a) 80 mg A.K. 6 dissolved in 20 cc acetic acid was hydrogenated with pladdium/carbon as catalyst. After 6 hours no hydrogen had been taken up. The solution was filtered, evaporated and

69 mg of the starting material isolated.

(b) 130 mg A.K. 6 was dissolved in 20 cc acetic acid and hydrogenated in the presence of platin oxide (Adams) as catalyst. 4 to 4.5 mole were rapidly taken up (7 minutes) when the uptake slowed down and finally stopped. After filtration and evaporation we obtained 121 mg residue which was chromatographed on alumina. The fractions eluated with benzene gave 87 mg of a substance melting at 178-180°. After recrystallization from chloroform it melted at 178-180°.

$[\alpha]_D^{20} = 0$

3.709 mg gave 0.115 ,g CO_2 and 1,920 mg H_2O

3,152 mg gave 7,953 mg CO_2 and 1,695 mg H_2O

4,670 mg gave 0.11 cc CH_4 19°/754 mm

Additional treatment for 2 1/2 hours at 100° gave no further development of methane.

$C_{22}H_{22}O_6$ (382.40)

Calculated: C, 69.10; H, 5.80; H^+, 0.282%. Found: C, 68.92; 68.86. H, 5.95; 6.02. H^+, 0.096%.

$C_{18}H_{22}O_6$ (382.40)

Calculated: C, 68.78; H, 5.77%. Found: C, 68.92; 68.86. H, 5.95; 6.02%.

The substance is soluble in benzene, acetone, chloroform, hot alcohol, slightly soluble in ether, insoluble in petrolether. Insoluble in n-sodium hydroxide and n-hydrochloric acid. No colour formation with ferric chloride, no precipitation with bromine water.

Acetylation (for conditions see A.K. 6 p. 51) was unsuccessful.

The starting material was isolated, when an ethereal sol, of this substance was treated with diazo-methane in ether, and kept for 24 hours at room temperature.

The UV- and IR-absorption spectra (Fig. No. 14 and 15 respectively) are discussed in the theoretical part.

Alkalidegradation

a) A.K. 6 + potassium hydroxide:
100 mg A.K. 6 dissolved in 10 cc alcohol was mixed with 5 cc 10% alcoholic potassium hydroxide and refluxed for 4 hours. After evaporation of the solvent the residue was separated into a neutral (57 mg) and an alkalisoluble fraction (25 mg) in the usual way. None of these fractions crystallized. The neutral fraction was chromatographed on alumina, but could not be eluated anymore even with a mixture of methanol: acetic acid = 99.5: 0.5.

b) Hydrogenated A.K. 6 + potassium hydroxide: 80 mg of hydrogenated A.K. 6 was refluxed with 5 cc 10% alcoholic potassium hydroxide for 4 hours. The separation as in a) gave 37 mg neutral oil and 28 mg alkali soluble oil which could not be crystallized, Chromatography on alumina gave no eluate till methanol.

c) A.K. 6 + potassium hydroxide + zinc: To the boiling solution of 100 mg A.K. 6 in 10 cc alcohol we added 200 mg zinc dust and the mixture again heated for 10 minutes. To this boiling solution we gave 1 cc 10% methanolic potassium nydroxide. This reaction mixture was then refluxed for a further 4.5 hours. A greenish-yellow colour appeared. The reaction mixture was filtered and the main portion of the alcohol was evaporated. It was then acidified with dil. hydrochloric acid and extracted with 200 cc ether. The ether solution was washed with n-sodium hydroxide and with water, dried and evaporate The remaining 62 mg residue were chromatographer on alumina. The fractions eluated with benzene-petrolether 9: 1 and recrystallized from hot methanol gave 35 mg rhombic crystals of m.p. 126-127°, called neutral compound.

The UV- and IR-absorption spectra (Fig. No. 16 and 17 respectively) are discussed in the theoretical part.

The alkali soluble portion was acidified with n-hydrochloric acid and extracted with ether. The ether solution gave, after evaporation, 25 mg of an alkali soluble compound. The whole amount was filtered through 1 g alumina with chloroform as a solvent. 17 mg were eluated, recrystallized from acetone-petrolether and gave m.p. 147-148°. This substance is called alkali soluble compound.

The UV-absorption spectrum (Fig. No. 18) is discussed in the theoretical part.

Neutral compound:

4,015 mg gave 9.962 mg CO_2 and 1.970 mg H_2O

3.687 mg gave 9.122 mg CO_2 and 1.810 mg H_2O

$C_{20}H_{20}O_6$ (356.36)

Calculated: C, 67.40; H, 5.66%. Found: C, 67.71; 67.52. H, 5.49; 5.49%.

The compound is soluble in acetone, benzene, chloroform and other organic solvents. It is insolbule in petrolether, sparingly soluble in hot methanol and fairly soluble in ether. It is insoluble in sodium hydroxide and n-hydrochloric acid. Ferric chloride-test is negative and there is no precipitation with bromine in water.

The test for methylenedioxy grouping with gallic acid is similar to A.K. 6.

Alkali soluble compound:

3,145 mg gave 7.845 mg CO_2 and 1.602 mg H_2O

$C_{14}H_{14}O_4$ (246.25) Calculated C, 68.28; H, 5.73%
$C_{21}H_{22}O_6$ (370.39) Calculated C, 68.09; H, 5.99%
Found C, 68.07; H, 5.70%

Insoluble in water and n-hydrochloric acid. Completely soluble in alkali. Ferric chloride-test gives a bluish colouration. The test with gallic acid gives a colour similar to A.K. 6.

d) Hydrogenated A.K. 6 + potassium hydroxide + zinc:
80 mg. hydrogenated A.K. 6, 200 mg zinc dust and 1 cc 10% methanolic potassium hydroxide were refluxed for 4 hours. The reaction mixture was worked up as indicated in c). We obtained 42 mg. alkali insoluble and 8 mg. alkali soluble residues. Neither of them crystallized after careful chromatography.

Oxidation

a) Iodine + potassium acetate:

150 mg potassium acetate and 50 mg A.K. 6 were dissolved in 10 cc alcohol and heated to reflux. Slowly we added a solution of 75 mg iodine in 5 cc alcohol to the boiling solution. The reaction mixture refluxed for one.hour.

No change in iodine colour was observed. The alcohol was evaporated and the residue taken up in ether and washed with water. The ether left 45 mg which proved to be starting material of m.p. 191°.

b) Sodium dichromate were dissolved in 15 cc acetic acid under the addition of a small amount of water. The mixture was heated on a steambath for one hour. The main portion of acetic acid was evaporated and the residue taken up in ether and a small amount of water. The residue weighed 71 mg. Distillation in vacuum gave oily fractions again.

Ozonolysis

100 mg A.K. 6 was dissolved in 15 cc glacial acetic acid and for 5 minutes at ordinary temperature treated with a stream of ozone. The amount of ozone taken up was 31 mg. The reaction mixture was steam distilled into a solution of 1% dimedon. After leaving the solution overnight no precipitation occurred. This fact should show that no formaldehyde and no acetone was formed. The residue of the steam distillation was chromatographed over alumina. No visible quantity of substance could be eluated, even with 0.5% acetic acid in methanol.

Summary

Pure fish poison was isolated from the petrol-ether extract of Piscidia erythrina L. and was identified as Rotenone.

β-Sitosterol was also isolated from the same extract and was identified by the formation of its acetate.

We were able to isolate a further substance, we called A.K. 6 and which eluates quite close to Rotenone from the alumina column. Certain similarities of UV- and IR-absorption spectra of this substance with those of dehydro-rotenone made us suggest a possible similarity between the structural formulae of A.K. 6 and Rotenone. For the purpose of conforming this idea, we made a series of Rotenone derivatives and compared their UV- and IR-absorption spectra with those of A.K. 6, hydrogenated A.K. 6 and two compounds we isolated from zinc and alkali treatment of A.K. 6.

Though definite similarities were observed in the case of Rotenol and hydrogenated A.K. 6, we could not get any concrete results to formulate the structure of A.K. 6.

Zusammenfassung

Aus dem Petrolaetherextrakt von Piscidia erythrina L. wurde der schon seit einiger Zeit als Fischgift bekannte Inhaltsstoff rein isoliert und als Rotenon identifiziert.

Aus dem gleichen Extrakt isolierten wir auch β-Sitosterin und identifizierten es als Acetat.

Schliesslich gelang es uns, noch eine dritte Substanz, genannt A.K. 6 zu isolieren, die bei der chromatographischen Trennung sehr nahe mit Rotenon eluiert wurde. Gewisse Aehnlichkeiten der UV- und IR-Absorptionsspektren dieser Substanz mit Dehydrorotenon liessen uns eine mogliche strukturelle Verwandtschaft der Strukturen von A.K. 6 und Rotenon vermuten. Um diese Idee zu bestatigen, stellten wir eine Reihe von Rotenon-Derivaten her und verglichen deren UV- und IR-Absorptionsspektren mit denjenigen von A.K. 6, hydriertem A.K. 6 und zweier Verbindungen, die aus der Zink- und Alkali-Behandlung von A.K. 6 isoliert wurden.

Obwohl zwischen Rotenol und A.K. 6 gewisse Aehnlichkeiten gefunden wurden, gelang es uns nicht, eine Struktur fur A.K. 6 vorzuschlagen.

Résumé

Dans un extrait d'ether de petrole de Piscidia erythrina nous avons isolé une substance connue depuis longtemps comme a l'etat pur toxique pour poisson. Nous l'avons identifiè comme roténone.

Du même extrait nous avons isolè le β-Sitosterole et l'identifiè sous forme d'acètate.

Une troisieme substance, très proche du roténone sur la colonne chromatographique a pu être isolee et a été nommée A.K. 6. Differentes analogies des spectres ultraviolets et infrarouges de A.K. 6 et de dihydroroténone laissent supposer une parente structurelle entre ces corps. Afin de prouver notre hypothese nous avons préparé quelques dérivés de la rotenone et compare leurs spectres ultraviolets et infrarouges. avec ceux de A.K. 6, du dérivé hydrogene de A.K. 6 et de deux corps, isolés apres traitement de A.K. 6 avec du zinc et de l'alcali.

Bien que nous ayons pu constater certaines analogies entre le roténol et le dérivé hydrogéné de A.K. 6, nous n'avons pas réussi a formuler la structure de A.K. 6

CURRICULUM VITAE

I, Amrit Lal Kapoor (born October 15, 1931, at Amritsar, India) received my elementary and secondary schooling in that city between 1937 and 1947, In 1949 I took the pre-medical course Diploma in Science, qualifying for the degree of Bachelor in Pharmacy of the University of Punjab in 1952. From October 1952 to April 1954 I worked for my Master's degree in Pharmacognosy also at the University of Punjab, under Prof. Dr. P. N. Mehra, F.N.I., while between October 1954 and July 1956 I carried out a further research programme which is the subject of the accompanying thesis, at the E.T.H., Zurich, under the able guidance of Prof. Dr. J. Büchi.

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