SOME REACTIONS OF LUFFAMARIN

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The isolation of a crystalline substance from the seeds of *Luffa ægyptiaca* Mill. (Fam.: Cucurbitaceæ) was described in an earlier communication from this laboratory a few years ago.[1] The results of the experiments that we have performed on this substance have not been easy to interpret and hence their publication has been withheld so far. Recently a few papers have appeared in the literature describing the isolation of compounds having more or less the same melting point as ours from a variety of sources.[2–4] These papers contain data of a very superficial nature with regard to one or two properties of the substances discussed. In view of the appearance of these papers from other quarters, we consider it worthwhile to present our data without making any claims to correct interpretation.

Molecular formulæ having 15, 18, 19 and 32 carbon atoms in the molecule have been proposed by the different workers mentioned above for the compounds isolated by them. The data that we have makes it appear that the provisional formula $C_{20}H_{30}O_5$ may be a nearer representation of our substance.

To facilitate easy reference the compound isolated by us is now designated "Luffamarin". It has an acetoxyl group in its molecule. It gives an acetate which analyses for two acetoxyl groups. It also forms a benzoate and a 3:5-dinitrobenzoate. These point to the existence of one acylable hydroxyl group. The substance also forms an oxime and a 2:4-dinitrophenylhydrazone whose nitrogen contents indicate the presence of one ketonic group in the original molecule. Thus of the five oxygen atoms in the molecule four are accounted for, two by the acetoxyl group, one by the hydroxyl group and one by the ketonic group.

The function of the remaining oxygen is not quite obvious. The compound gives a positive Liebermann-Burchard reaction,[5–6] a positive reaction with antimony trichloride[7–8] and Shear's aniline-hydrochloric acid reaction.[9–10] These reactions are known to be answered by terpenoid compounds and by heterocyclic compounds containing oxygen as a hetero-atom. Hence it seems likely that the fifth oxygen exists in a hetero-ring.

Luffamarin reacts with bromine in carbon tetrachloride-chloroform mixture; quantitative titration indicates the addition of approximately two moles of bromine. The substance reacts with perbenzoic acid giving rise to a new compound whose analysis indicates the addition of two oxygen atoms. However, quantitative titration with perbenzoic acid indicated that only about 0·7 atom of oxygen was absorbed and the same value was obtained even on repetition. Luffamarin did not react with maleic anhydride when left with this reagent in benzene solution in the cold for 4 days.

Treatment with chromic acid (1 oxygen equivalent) at room temperature yielded a compound which analysed for $C_{20}H_{30}O_6$ or $C_{20}H_{28}O_6$. This compound yielded only a monodinitrophenylhydrazone. Oxidation with cold neutral potassium permanganate yielded a very small quantity of a neutral substance whose analysis indicated the addition of four hydroxyl groups to the molecule. This probably would indicate the presence of two double bonds.

Treatment with alkaline hydrogen peroxide gave an acid (probable formula $C_{13}H_{18}O_4$) as the main product. As minor products a neutral fraction (probable formula $C_{10}H_{12}O_2$?) and another substance soluble in sodium hydroxide but not in bicarbonate (probable formula $C_{10}H_{16}O_3$?) were obtained. The acid was characterized by the preparation of its methyl ester. When subjected to the action of acetic anhydride in the presence of pyridine, it yielded a new neutral compound which was free from acetoxyl group and whose formula indicated the removal of the elements of a molecule of water. The acid reacted with 2:4-dinitrophenylhydrazine to yield a hydrazone whose analysis indicated the existence of one carbonyl group.

Hydrolysis of luffamarin with weak alcoholic potash also resulted in clean degradation of the molecule. An acidic, a phenolic and a neutral fraction were obtained. Combustion values indicate the probable formulæ $C_7H_{10}O_2$, $C_{10}H_{16}O_3$ and $C_9H_{14}O_3$ respectively for them.

EXPERIMENTAL

Most of the substances described herein proved rather difficult to crystallize and in several cases they could be obtained only as microcrystalline powder.

Luffamarin was crystallised from chloroform-ether as colourless prisms melting at 180–82°. It is not soluble in 20% aqueous sodium hydroxide, cold or hot; but with concentrated alcoholic potash it gives a light reddish-yellow colour which gradually deepens on heating. It does not give any colour with alcoholic ferric chloride. It readily undergoes resinification with

either hot formic acid or hot alcoholic hydrochloric acid. It reduces Tollen's reagent in the cold. No green colour is produced when the substance is treated with concentrated sulphuric acid and gallic acid (absence of methylenedioxy group). [Found: C, 68·1; H, 8·9; $-COCH_3$, 11·4; M.W. (Rast.): 379, 388. $C_{20}H_{30}O_5$ (350·4) requires C, 68·6; H, 8·6; $-COCH_3$ (1), 12·3%.]

To a glacial acetic acid solution of luffamarin were added acetyl chloride and powdered zinc chloride and heated on a small open flame. Within a few minutes a yellow colour gradually changing to brownish-red was obtained (Tschugaieff reaction). In the Liebermann-Burchard reaction a green colour was obtained. On boiling the substance for half a minute with aniline and concentrated hydrochloric acid, a light red colour was obtained within half an hour, which deepened within 24 hours (Shear's aniline-hydrochloric acid reaction). No colour was obtained when a chloroform solution of the substance was treated with a solution of trichloroacetic acid (Rosenheim test). To a solution of the substance in absolute alcohol were added an absolute alcohol solution of *m*-dinitrobenzene and a solution of potassium hydroxide in absolute alcohol. A deep violet colour was obtained in a few minutes turning to blood-red after three hours (Zimmermann's reaction).[11] When luffamarin was moistened with hydrochloric acid containing ferric chloride and the resulting mixture evaporated to dryness, a violet residue was obtained.

When a small quantity of the substance was dissolved in a few ml. of chloroform and treated with a 25% solution of antimony trichloride in chloroform and left for two days, an orange–red layer separated at the bottom. The test was repeated with the addition of a small quantity of acetic anhydride before adding the reagent. A deep pink colour was obtained when left overnight.

Luffamarin acetate.—This was prepared by treating the substance in pyridine with acetyl chloride. The substance crystallized from dilute methanol as nodules and then from ether-petroleum ether as a microcrystalline powder melting at 129–30°. $[\alpha]_D^{27} = +2° \pm 1°$ ($c = 1·647$ in chloroform). [Found on substance dried in *in vacuo* for two hours at 90°: C, 66·2; H, 9·0; $-COCH_3$, 24·0. $C_{22}H_{32}O_6$ requires: C, 67·3; H, 8·2; $-COCH_3$ (2), 21·9%.]

Luffamarin benzoate.—This was prepared by treating the substance in absolute pyridine with benzoyl chloride. It crystallized from dilute methanol as colourless prisms melting at 140–42°. $(\alpha)_D^{28} = -48·6° \pm 3°$ ($c = 1·023$ in chloroform). (Found: C, 72·1; H, 7·6. $C_{27}H_{34}O_6$ requires; C, 71·3; H, 7·5%.)

Luffamarin 3:5-*dinitrobenzoate*.—This was prepared on the same lines as above using 3:5-dinitrobenzoyl chloride. It crystallized from dilute alcohol as colourless needles melting at 190–93°. $[\alpha]_D^{28} = +23{\cdot}8° \pm 2°$ ($c = 1{\cdot}249$ in chloroform). (Found: N, 5·5. $C_{27}H_{32}O_{10}N_2$ requires: N, 5·2%.)

Luffamarin oxime.—This was prepared by heating the substance in alcohol with a mixture of hydroxylamine hydrochloride and sodium acetate. The substance crystallized from benzene-acetone as colourless prisms melting at 187–89° (slight sintering at 150°). $[\alpha]_D^{27} = +38{\cdot}2° \pm 2°$ ($c = 1{\cdot}415$ in chloroform: acetone 4:1). (Found: N, 4·7. $C_{20}H_{31}O_5N$ requires: N, 3·8%.)

Luffamarin 2:4-*dinitrophenylhydrazone*.—This substance was prepared in the usual manner by treating luffamarin with the phenylhydrazine in alcohol with a trace of hydrochloric acid. It crystallized from alcohol as a reddish-yellow microcrystalline powder melting at 196–99°. (Found: N, 10·6. $C_{26}H_{34}O_8N_4$ requires: N, 10·6%.)

Bromine titration.—This was carried out by treating a known weight of the substance in a mixture of carbon tetrachloride and chloroform with excess of an approximately 2·5% solution of bromine in carbon tetrachloride, keeping the mixture in the dark for 2 hours and determining the excess of bromine in the usual manner by addition of potassium iodide and titrating with standard sodium thiosulphate solution. 200 mg. of the substance consumed bromine equivalent to 21·3 ml. of 0·1130 N thiosulphate (experiment 1) and 21·25 ml. of 0·1130 N thiosulphate (experiment 2) (Found: Number of double bonds: 2·1, in both experiments).

Oxidation with perbenzoic acid.—The substance (100 mg.) was dissolved in chloroform (5 ml.) and a chloroform solution of perbenzoic acid (10 ml.) was added. The solution was left in the ice-chest for 2 days with intermittent shaking. At the end of the period, a solution of potassium iodide (1 g.) and glacial acetic acid (2 ml.) in water (50 ml.) was added, the mixture thoroughly shaken and titrated against standard sodium thiosulphate solution. A blank experiment was also conducted. 100 mg. of the substance consumed perbenzoic acid equivalent to 3·2 ml. of 0·1130 N sodium thiosulphate solution (experiment 1) and 3·4 ml. of 0·1130 N sodium thiosulphate solution (experiment 2). [Found: No. of double bonds: 0·63 (experiment 1); 0·67 (experiment 2).]

The solution left at the end of the titration was transferred to a separating funnel, the lower chloroform layer was drawn off and the aqueous layer was extracted with chloroform (2 × 20 ml.). The mixed chloroform solution was diluted with three times its volume of ether, washed with 5%

sodium bicarbonate solution and then with water till neutral. It was dried over sodium sulphate, the solvents were removed and the residue was dissolved in benzene, concentrated and set aside, when a colourless sticky mass separated. This was dissolved in hot benzene, cooled and excess of petroleum ether was added when a white precipitate was obtained. It was filtered and washed with excess of petroleum ether, m.p. 108–12°/132–34°. $[\alpha]_D^{28} = +18{\cdot}9° \pm 4°$ ($c = 0{\cdot}9683$ in chloroform: acetone 4: 1). (Found: C, 62·9; H, 8·3. $C_{20}H_{30}O_7$ requires: C, 62·8; H, 7·9%.)

The same compound was obtained even when the solution, at the end of two days, was directly worked up without previous titration (mixed m.p.).

Chromic acid oxidation.—To a solution of the substance (0·2 g.) in glacial acetic acid (2 ml.) was added a 2% solution of chromium trioxide (2·1 ml.) in the same solvent (one atom of oxygen per mole of substance). The mixture was left for 3 hours at room temperature, diluted with dilute sulphuric acid (50 ml.), extracted with ether (4 × 30 ml.) and the combined ethereal extract washed with 2 N sodium carbonate solution and then with water till neutral. It was dried over sodium sulphate, the solvent was removed and the residue was purified by chromatography over alumina. By dissolving in ether and adding excess of petroleum ether, the substance was obtained as a powder melting at 108–11°. $[\alpha]_D^{28} = +4{\cdot}2° \pm 3°$ ($c = 1{\cdot}013$ in chloroform). (Found on sample dried *in vacuo* at 80° for two hours: C, 65·1; H, 8·2. $C_{20}H_{30}O_6$ requires: C, 65·6; H, 8·3%.)

The 2: 4-dinitrophenylhydrazone of the above oxidation product was prepared in the usual way. It crystallized from absolute alcohol as brownish red rods melting at 148–51°. (Found: N, 10·0. $C_{26}H_{34}O_9N_4$ requires: N, 10·3%.)

Permanganate oxidation.—To an acetone solution of the substance (0·25 g.) was added an aqueous solution of potassium permanganate till a permanent pink colour was obtained. Water was added and the solvent was removed under reduced pressure. Sulphur dioxide was passed through the resulting aqueous solution till the precipitated manganese dioxide went into solution. The solution was extracted with ether, the ethereal extract washed neutral and dried over sodium sulphate. Removal of the solvent left only a trace of residue. The aqueous layer left after ether extraction, when kept in the ice-chest for a month, deposited a small quantity of a crystalline material. This was filtered, washed and crystallized from methanol-ether when colourless needles melting at 318° were obtained (5 mg.). The substance was insoluble in 5% sodium hydroxide solution. (Found: C, 57·0; H, 8·5. $C_{20}H_{34}O_9$ requires: C, 57·4; H, 8·2%.)

Alkaline hydrogen peroxide oxidation of luffamarin.—To a solution of the substance (2·0 g.) in alcohol (60 ml.) were added a solution of potassium hydroxide (4 g.) in alcohol (20 ml.) and a solution of hydrogen peroxide, 100 vols. (24 ml.) and the mixture kept for 1 hour with occasional shaking. Next the solution was warmed until there was no more effervescence, neutralized with 1 : 1 hydrochloric acid to congo-red and the alcohol was removed under reduced pressure adding small quantities of water to keep the total volume approximately constant. The solution was extracted with ether (5×50 ml.). The combined ethereal extract was washed with water till neutral and extracted successively with 5% sodium bicarbonate solution (5 × 25 ml.) and 5% sodium hydroxide solution (3 × 20 ml.). The remaining ethereal layer was washed with water till neutral, dried and the solvent removed. The residue on crystallization from chloroform-ether yielded needles melting at 255–58° (0·02 g.). (Found: C, 71·7; H, 8·5%.)

The alkali-soluble fraction, recovered from the alkali extract, crystallized from chloroform-ether as feathery needles melting at 274–77° (5 mg.). The substance did not give any colour with alcoholic ferric chloride. (Found: C, 65·6; H, 8·5%.)

The acid recovered from the bicarbonate extract in the usual manner crystallized from ether-petroleum ether in nodules melting at 155–58° (0·7 g.). $[\alpha]_D^{28} = +94{\cdot}2° \pm 3°$ ($c = 1{\cdot}037$ in chloroform). [Found: C, 65·6; H, 7·6. M.W. (titration method): 256·2. $C_{13}H_{18}O_4$ (238·3) requires: C, 65·5; H, 7·6%.]

The methyl ester was prepared by treating an ethereal solution of the acid with excess of ethereal diazomethane. It was purified by dissolving in absolute ether and precipitating with excess of petroleum ether and thus obtained as a powder melting at 97–100° (decomp.). [Found on sample dried *in vacuo* at 60° for 2 hours: $-OCH_3$, 11·0. $C_{14}H_{20}O_4$ requires: $-OCH_3$ (1), 12·3%.)

The anhydro-acid was prepared by treating a solution of the acid (0·12 g.) in absolute pyridine (1·5 ml.) with acetic anhydride (0·75 ml.) and leaving at room temperature for 3 days. It crystallized from chloroform-ether in clusters of needles melting at 235–36°. (Found: C, 70·0; H, 8·0. $C_{13}H_{16}O_3$ requires: C, 70·9; H, 7·3%.)

The 2 : 4-dinitrophenylhydrazone of the acid was obtained from ether-petroleum ether as a micro-crystalline powder, m.p. 170–74°. (Found: N, 12·7. $C_{19}H_{22}O_7N_4$ requires: N, 13·4%.)

Alkali hydrolysis of luffamarin.—The substance (0·5 g.) was refluxed with N/5 alcoholic potash (50 ml.) for 1 hour. The resulting solution was worked up in the usual manner and the product separated into an acid fraction, a sodium hydroxide soluble fraction and a neutral fraction along usual lines.

The acid fraction (0·1 g.) was dissolved in chloroform, excess of benzene was added and concentrated to a small volume when a pasty mass separated. It was first purified through 5% A.R. sodium bicarbonate solution. By dissolving the dry substance in benzene and adding excess of petroleum ether a powder was obtained, m.p. 137–40°. $[\alpha]_D^{27} = +28° \pm 3°$ ($c = 0·995$ in chloroform). (Found: C, 66·7; H, 8·5. $C_7H_{10}O_2$ requires: C, 66·6; H, 8·0%.)

The sodium hydroxide soluble fraction was obtained as a powder by dissolving in benzene and adding excess of petroleum ether, m.p. 146–48°. It gave a violet-red colour with alcoholic ferric chloride and decolourized a solution of bromine in carbon tetrachloride. $[\alpha]_D^{28} = +14·9° \pm 4°$ ($c = 1·076$ in chloroform). (Found: C, 66·0; H, 8·3. $C_{10}H_{16}O_3$ requires: C, 65·2; H, 8·8%.)

The neutral fraction (0·21 g.) was purified through ether. After removal of the small amount (5 mg.) of an ether-insoluble impurity (colourless plates from benzene-methanol, m.p. 282–84°), the main portion (ether soluble) was purified by chromatography over alumina. It was obtained as a powder by dissolving in benzene and adding excess of petroleum ether, m.p. 156–59°. (Found: C, 62·6; H, 8·8. $C_9H_{15}O_3$ requires: C, 63·1, H, 8·8%.)

Summary

The crystalline substance obtained from the seeds of *Luffa ægyptiaca* Mill., now named "Luffamarin", has the probable molecular formula $C_{20}H_{30}O_5$. It contains an alcoholic hydroxyl group, a ketonic group and an acetoxyl group. The function of the remaining oxygen is not clearly known. It is probably present in a heterocyclic ring.

A number of derivatives and degradation products have been prepared and analysed.

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