# THE CONSTITUENTS OF TYPHA ANGUSTATA, BORY. ET CHAUB.

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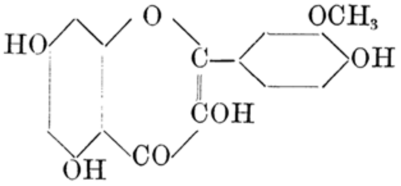
The plant, *Typha angustata, Bory. et Chaub.*, belongs to the *Typhaceae.* Its pollen has been used medicinally from remote antiquity in the East as a diuretic and a styptic. So far as I am aware, no chemical examination of this pollen appears to have been made. For the purpose of investigating its chemical constituents, it was first extracted with hot alcohol. After evaporating most of the alcohol, the residue was separated into two parts by treatment with petroleum ether.

From the soluble part in petroleum ether, a colourless substance, m.p. 62.5–63°, was obtained. The composition of this substance was $C_{16}H_{32}O_2$, and its constitution was found to be palmitic acid by analysis, molecular weight determination, and by the method of mixed melting point.

The insoluble part in petroleum ether gave a characteristic reaction to flavone by treatment with metallic magnesium and hydrochloric acid and, after being hydrolysed with sulphuric acid and then neutralised with sodium hydroxide, it reduced Fehling's solution. From these facts, this part appears to contain a glucoside of flavone. With the view to isolate the glucoside, the part was extracted with hot water, the solution precipitated with lead acetate, and the filtrate treated with basic lead acetate, by which means a second lead precipitate was formed. The latter was decomposed with acetic acid and, after being freed from the soluble lead salt with sulphuric acid, the excess of the sulphuric acid was neutralised with barium carbonate and the barium sulphate thus precipitated was then filtered. Since the glucoside could not be obtained in a crystalline state after these treatments, it was then hydrolysed with dilute sulphuric acid, when a yellow substance (A) separated, which was collected and washed with water. From the filtrate, *d*-phenylglucosazone, m.p. 203–204°, was obtained by treatment with phenylhydrazine and acetic acid, and *p*-bromobenzoylhydrazone was also formed by the action of *p*-bromobenzoylhydrazine on it, which is soluble in a boiling mixture of alcohol, chloroform and water. These facts point to the presence of *d*-glucose in the filtrate.

The yellow substance (A) had a composition, $C_{16}H_{12}O_7$, and melted at 307°. The presence of four hydroxyl groups in the compound was proved by the formation of the tetra-acetyl derivative, $C_{16}H_8O_7(C_2H_3O)_4$, m.p. 205° and the existence of one methoxyl group, by the micro-Zeisel method. The compound, on decomposition with sodium hydroxide, gave phloroglucinol as a phenolic decomposition product and vanillic acid, as an acidic one. From these results, the constitution of substance (A) appears to be *iso*rhamnetin :



Since there was no authentic specimen of *iso*rhamnetin, the author confirmed the identity of substance (A) with it by synthesising the latter by Robinson's method,⁽¹⁾ and comparing their acetyl derivatives.

## Experimental.

About 20 kilos. of the material were extracted by boiling with 70 % alcohol for six hours in the portion of 250 grams, 1250 c.c. of the alcohol being used for each 250 grams of the material. After the removal of most of the alcohol, the remaining alcoholic solution was shaken with petroleum ether, b.p. 50–80°, and the petroleum ether solution (a) and the alcoholic residue (b) were treated as follows:

A portion of the petroleum ether solution (a) was then mixed with acetone, when colourless crystals were deposited, which, after being recrys-

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tallised from methyl alcohol, melted at 60.5–61.5°. The filtrate was evaporated and the residual oil was distilled under a reduced pressure of 2–3 mm., when the distillate melted at 61–61.5° after recrystallisation from methyl alcohol and produced no depression of the melting point by admixture with the above specimen. After this preliminary experiment, the main portion of the petroleum ether solution (a) was evaporated and the residual oil was distilled under a diminished pressure of 2–3 mm. After recrystallising it four times from methyl alcohol, colourless crystals melting at 62.5–63° were obtained (Found: $C = 74.80$; $H = 12.71$; Mol. wt., by Rast's method, 248; 254. $C_{16}H_{32}O_2$ requires $C = 74.92$; $H = 12.59\%$. Mol. wt., 256. 3).

Titration: 0.5000 gr., dissolved in alcohol, neutralised 21.04 c.c. of an 0.0926-*N* solution of alcoholic potash, whereas this amount of a monobasic acid, $C_{16}H_{32}O_2$, should neutralise 21.07 c.c.

The identity of this acid with palmitic acid was proved by the fact that the mixture of the acid with an authentic specimen of palmitic acid caused no depression of the melting point.

After being combined with the alcoholic residue (b) from several separate experiments, it was further concentrated to 4 litres under diminished pressure, diluted with 24 litres of water and heated on a water-bath for 2.5 to 3 hours. The light, brown, aqueous solution thus obtained was again evaporated to one-fourth of its bulk.

(1) **The Determination of Reducing Sugar combined with Flavone.** The glucoside of flavone was precipitated by basic lead acetate, the precipitate was decomposed with dilute acetic acid (50 %) and freed from the soluble lead compound with sulphuric acid. The excess of sulphuric acid was then neutralised with barium carbonate, and the barium sulphate thus precipitated was filtered. As the glucoside of flavone was difficult to obtain in a crystalline state, sulphuric acid was added till the solution contained 4 per cent of this acid and heated on a water-bath, when a yellow substance (A) was obtained, which was collected and washed well with water. The yield was 50 grams. A portion of the filtrate was warmed with phenylhydrazine and glacial acetic acid, when brownish yellow crystals separated. After being recrystallised from alcohol, the crystals melted at 203–204° and caused no depression of the melting point by admixture with *d*-phenylglucosazone. In order to decide whether the reducing sugar is glucose or mannose, another portion of the filtrate was treated with acetic acid and *p*-bromobenzoylhydrazine, when colourless crystals were obtained, which were clearly dissolved in a boiling mixture of alcohol, water and chloroform. It thus follows that the reducing sugar is *d*-glucose.

(2) **The Determination of Yellow Substance (A).** After being recrystallised from methyl alcohol, it separated in lemon-yellow crystals, m.p. 307° (decomp.) (Found: $C = 60.61$; $H = 3.99$; $CH_3O$, by the micro-Zeisel method $= 9.58$; Mol. wt., by the Rast method, 303. $C_{16}H_{12}O_7$ requires $C = 60.74$; $H = 3.80$; $CH_3O = 9.80\%$. Mol. wt., 316).

*The Acetyl Derivative.* This compound was prepared by heating the substance with acetic anhydride and fused sodium acetate, and melted at 204–205° (Found: $C=59.59$; $H=4.28$. $C_{24}H_{20}O_{11}$ requires $C=59.51$; $H=4.13\%$).

**The Decomposition of Yellow Substance (A) with Sodium Hydroxide.** The substance (1 gram) was dissolved in a solution of 2 grams of sodium hydroxide in 12 c.c. of water and a current of air was passed through it for three hours, when the colour of the solution turned a brownish yellow. The solution was neutralised with sulphuric acid, and, after an excess of sodium bicarbonate had been added, it was extracted with ether. The ethereal solution (c) and the aqueous residue (d) were treated in the following way:

(c) After the removal of the ether, the residue was recrystallised from water with the addition of animal charcoal, when colourless crystals were obtained, which, drying at 105–110°, melted at 216–217°. This substance did not depress the melting point after being mixed with anhydrous phloroglucinol, being undoubtedly identical with the latter.

(d) The aqueous residue was acidified with hydrochloric acid and extracted with ether. After evaporating the ether from the ethereal solution, the residue was recrystallised from water with the addition of animal charcoal. The substance thus purified melted at 206–207° and its melting point was not depressed by admixture with an authentic specimen of vanillic acid.

**Synthesis of *iso*Rhamnetin.** In order to prove the identity of yellow substance (A) with *iso*rhamnetin, the latter was synthesised by Robinson's method.

For this purpose, vanillic acid was first synthesised by a modification of the method described in the report on the German patent No. 258887. *iso*Rhamnetin was then synthesised from vanillic acid by following exactly the directions given by Robinson and converted into the tetra-acetyl derivative, m.p. 204–205.5°. The melting point of this substance was not depressed by admixture with the acetyl derivative of yellow substance (A), thus leaving no doubt as to identity of yellow substance (A) with *iso*rhamnetin.

In conclusion, the author wishes to express his sincere thanks to Professor Hideo Maniwa, Chiba Medical College, by whose suggestion this investigation was made, and to Professor Hiroshi Nomura for his kind guidance during the investigation.

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