# STUDIES ON THE STRUCTURE OF ASEBOTIN, A COMPONENT OF ANDROMEDA JAPONICA THUMB.

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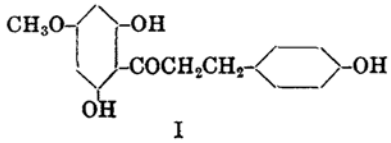
Asebotin is a component of *Andromeda japonica* Thumb, and has been investigated by several workers.[1] According to an investigation by Eykman it is a glucoside, which is decomposed by mineral acid in the following way:

$$\underset{\text{Asebotin}}{C_{24}H_{28}O_{12}} + H_2O = \underset{\text{Asebogenin}}{C_{18}H_{18}O_7} + \underset{\text{Glucose}}{C_6H_{12}O_6}.$$

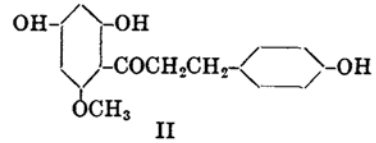
The results obtained by the present author have altered the equation as follows:

$$\underset{\text{Asebotin}}{C_{22}H_{26}O_{10}} + H_2O = \underset{\text{Asebogenin}}{C_{16}H_{16}O_5} + \underset{\text{Glucose}}{C_6H_{12}O_6}.$$

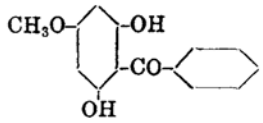
Asebotin (m. p. 147.5°) gave a reddish brown tinge with ferric chloride, but no colouration with metallic magnesium and hydrochloric acid. Asebogenin (m. p. 168°) has one methoxyl group and gave triacetylasebogenin (m. p. 66–67°) by acetylation. Triacetyl-asebogenin neither gave ferric chloride reaction nor decolourised potassium permanganate. Asebogenin gave phloroglucinol-monomethylether (b. p. 175–178°/7 m.m., m. p. 74–77°) as a phenolic substance as well as phloretic acid (m. p. 128°) as an acidic substance, when it was fused at 170–180° with potassium hydroxide in an atmosphere of hydrogen. Thus, it is possible to represent asebogenin by either of the two following formulas I and II:



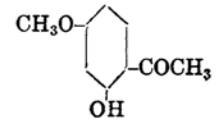

I




II




III

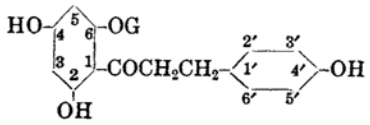



IV

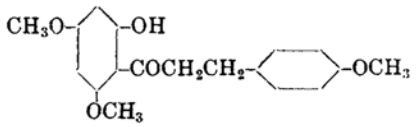
(1) Eykman, *Ber.* **16** (1883), 2769; E. Bourquelot and A. Fichtenholz, *Chem. Zentr.*, **83** (1912), I, 585, 733, 1224; Marc Bridel, *ibid.*, **102** (1931), I, 468; Marc Bridel and A. Kramer, *ibid.*, **103** (1932), I, 396; *ibid.*, **104**, (1933), II, 3289.

Generally speaking, hydroxyl groups occupy ortho-positions in relation to the carbonyl group in natural substances having both hydroxyl groups and methoxyl groups, as in cotoin (III)[2], Päonol (IV)[3], and flavones. Then it is possible to state that formula I represents the structure of asebogenin. This conclusion was confirmed by the following synthesis: iso-asebogenin (II) was synthesised by condensation of phloroglucinol-monomethyl-ether and phloretic nitrile in the presence of freshly fused zinc chloride (Hoesch's reaction)[4], which melted at 201-202°.

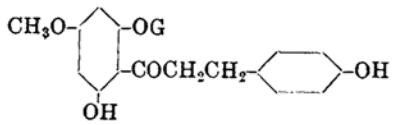
The position of glucose was confirmed by methylation of asebotin. When asebotin was methylated with methyl iodide and potassium carbonate in dry acetone, a colourless transparent syrup was obtained, which gave no ferric chloride reaction. On hydrolysis of this syrup with dilute sulphuric acid, colourless prismatic crystals (from alcohol) were obtained. These crystals melted at 109-110° alone and in admixture with 6-hydroxy-2, 4, 4′-trimethoxy-phenyl-propiophenone (VI) (m. p. 109-110°) which had been prepared by F. R. Johnson and A. Robertson[5] to determine the position of glucose in phloridzin (V) in the same way. Therefore the position of glucose in asebotin proved to be " 6 " as in phloridzin.




V




VI




VII

G = Glucose

Thus, judging from the result obtained, asebotin can be regarded as phlordzin-monomethyl-ether (VII), as evidenced by the fact that asebotin gives the same reaction as phloridzin, turning yellow and precipitating an amorphous substance on treatment with boron acetic acid anhydride.[6] However, the

(2) " Beilsteins Handbuch der Organischen Chemie ", VIII, 419.

(3) *Ibid.*, VIII, 267.

(4) When a side chain is introduced in phloroglucinol-monomethyl-ether by Hoesch's reaction, it enters the ortho-position to methoxyl-groups completely or at least for the most part, *Ber.*, **58** (1925), 1691; **61** (1928), 2300; *Helv. Chim. Acta.*, **2** (1919), 486, 478.

(5) *J. Chem Soc.*, **1930**, 21.

(6) *Ber*, **54** (1921), 3020.

present author's view differs from that held by Marc Bridel and A. Kramer[1] who considered them to be identical, whereas, they differ, according to the present author's investigation, in that asebotin contains a methoxyl group in place of a hydroxyl group in phloridzin.

## Experimental.

**Isolation and Properties of Asebotin.** Fresh leaves of *Andromeda japonica* Thumb. were extracted with warm water for 5 hours. The extraction was repeated again. When the resulting extract was concentrated on the water bath under reduced pressure and left to stand, asebotin separated out gradually in brown crystals. It was filtered, dried, and recrystallised from either water (50 c.c. per 1 g. asebotin) or a mixture of alcohol and water (charcoal). Asebotin was obtained in colourless long plates, m.p. 147.5°. The yield was 30 g. (pure asebotin) from 15 kg. of leaves. Asebotin is soluble in warm water, alcohol, and glacial acetic acid, but sparingly soluble in light petroleum, ether, benzene, and chloroform. It gave a reddish brown tinge with ferric chloride, and neither gave colouration with metallic magnesium and hydrochloric acid, nor reduced Fehling's solution. Yellowish colouration was given with the formation of an amorphous precipitate on treatment with boron acetic acid anhydride in acetic acid anhydride. $[\alpha]_D^{23.5} = -46.7°$ (absolute alcohol, c = 4.904. Substance dried at the boiling point of toluene over phosphorous pentoxide was analysed (Found: C, 58.51, 58.26, 58.37; H, 6.15, 6.36, 6.42. Calc. for $C_{22}H_{26}O_{10}$: C, 58.63; H, 5.82%).

**Hydrolysis of Asebotin.** When asebotin (1 g.) was warmed on the water bath with 5% sulphuric acid (50 c.c.), at first it was dissolved to a great extent, then the solution became turbid with formation of asebogenin crystals. After two hours it was cooled and filtered. Yield of asebogenin was 0.63 g.

**Qualitative Test of Glucose.** Sulphuric acid was removed from the filtrate, from which asebogenin had been separated by filtration, with a solution of barium hydroxide. On drying the filtrate, it gave the sugar with yellowish resinous matter. When a part of the sugar was warmed on water bath with phenylhydrazine hydrochloride (2 parts) and sodium acetate (2 parts) in water (20 parts), yellowish glucosazone was formed, and it was filtered and washed with methyl alcohol and ether, melting point 209° alone and in admixture with the authentic specimen. The sugar gave neither Seliwanoff's reaction with resorcinol nor Ihl-Pechmnn's diphenylamine reaction. On leaving the sugar with phenylhydrazine hydrochloride in water at room temperature, there was found no precipitate of phenylhydrazone.

**Asebogenin.** Asebogenin was crystallised from dilute alcohol (charcoal) in colourless long plates, m. p. 168°. It gave purple-reddish tinge with ferric chloride. It is soluble in acetic ester, acetone, methyl alcohol, ethyl alcohol, ether, and alkali, but sparingly soluble in chloroform, light petroleum, benzene, and toluene. On analysis, the result agreed with $C_{16}H_{16}O_5$, having one methoxyl group (Found: C, 66.19, 66.32; H, 5.55, 5.86; $CH_3O$, 10.6. Calc. for $C_{15}H_{13}O_4 \cdot OCH_3$: C, 66.64; H, 5.60; $CH_3O$, 10.76%).

**Preparation and Properties of Acetylasebogenin.** Acetic acid anhydride (3 c.c.) was added to a solution of asebogenin (0.5 g.) in pyridine (4 c.c.) and the solution was allowed

to stand overnight. When the reaction mixture was treated with water, a white crystalline mass separated. This was collected and dried. It was crystallised from alcohol in colourless prisms, melting at 76–77°. It did not give ferric chloride reaction and did not decolourise potassium permanganate in glacial acetic acid (Found: C, 63.39, 63.58; H, 5.63, 5.87. Calc. for $C_{22}H_{22}O_8$; C, 63.74; H, 5.35%).

**Alkali Fusion of Asebogenin.** Asebogenin (2 g.) was added gradually to fused potassium hydroxide which was prepared in a platinum crucible with potassium hydroxide (3 g.) and water (1.5 c.c.), and the mixture was held at 170–180° in an atmosphere of hydrogen. After 30 minutes, the fused mass was cooled. The yellowish product was dissolved in water, the solution was acidified with sulphuric acid, and sodium carbonate was added; the solution was extracted with ether, the ether was evaporated, and the residue was distilled under reduced pressure. A colourless oil distilled at 175–178°/7 mm. After some time it crystallised, m. p. 74–77°. The yield was 0.6 g. On analysis, it corresponded to $C_7H_8O_3$, having one methoxyl group. It is freely soluble in water, alcohol, and ether, and reduces Fehling's solution; it also gave purple-reddish tinge in pine shaving reaction. It tastes sweet. These data show that it is phloroglucinol-monomethyl-ether (Found: C, 59.75; H, 5.98; $CH_3O$, 21.31. Calc. for $C_6H_5O_2 \cdot OCH_3$: C, 59.97; H, 5.76; $CH_3O$, 22.15%).

Sodium carbonate solution, from which phloroglucinol-monomethyl-ether was extracted, was acidified with sulphuric acid, and extracted with ether; the ether was evaporated and the residue was crystallised from benzene in colourless crystals, m. p. 128°. The yield was 1.5 g. On analysis, it corresponded to $C_9H_{10}O_3$ and was found to be phloretic acid (Found: C, 64.46; H, 6.36. Calc. for $C_9H_{10}O_3$: C, 65.03; H, 6.07%).

This acid was methylated with dimethyl sulphate and sodium hydroxide to confirm the above view. The product was crystallised from alcohol in colourless needles, melting at 102–103° (Found: C, 66.53; H, 6.93; $CH_3O$, 17.10; molecular weight obtained by titration with N/10 NaOH, 178. Calc. for $C_9H_9O_2 \cdot OCH_3$: C, 66.63; H, 6.72; $CH_3O$, 17.21; mol. wt. 180).

**Synthesis of Iso-asebogenin.** To a solution of phloroglucinol-monomethyl-ether (1 g.) and phloretic acid nitrile (1 g.) in absolute ether (7 c.c.) a freshly fused anhydrous zinc chloride was added, and into this solution was passed a slow current of dry hydrogen chloride gas for 3 hours, while cooling with ice water. The mixture was allowed to stand overnight in a cool place. The reaction mixture separated in two layers, the lower layer consisting of yellowish semi-solid. After decantation of ether, the semi-solid mass was treated with water (12 c.c.). The oil, which separated on addition of water, crystallised gradually and completely. Colourless prisms thus obtained melted at 198–204° with decomposition. When the crystals were burned on a platinum spatula, it left white zinc oxide. Therefore, this substance is considered to be zinc chloride complex of ketimide chloride. When the zinc chloride complex was dissolved in water and the solution was warmed for one hour on the water bath, it became turbid, an oil separating finally. On cooling, this oil became crystalline and colourless needles crystallized out from the aqueous layer. The substance was recrystallised from methanol in colourless prisms, m. p. 201–202°.(7) It gave a purple-reddish tinge with ferric chloride (Found: $CH_3O$, 10.78. Calc. for $C_{15}H_{13}O_4 \cdot OCH_3$: $CH_3O$, 10.76%).

(7) The mother liquor was not investigated further.

**The Position of Sugar.** Methyl iodide (3 c.c.) was added to a solution of asebotin (3 g.) in dry acetone (20 c.c.) containing powdered potassium carbonate (6 g.) in suspension, and the mixture refluxed for 20 hours. After separation from potassium salts, the acetone and the excess of methyl iodide were removed by distillation. The colourless viscous residue, which could not be recrystallised, did not give a ferric chloride reaction. A solution of the product in a mixture of 50% methyl alcohol (25 c.c.) and 15% sulphuric acid (10 c.c.) was refluxed on the water bath for 30 minutes. On cooling, crystals separated, which crystallised from alcohol in colourless squat prisms, melting at 109–110° alone and in admixture with 6-hydroxy-2, 4, 4′-trimethoxy-phenyl-propiophenone,[5] m. p. 109–110° (Found : C, 68.20; H, 6.42. Calc. for $C_{18}H_{20}O_5$: C, 68.32; H, 6.36%).

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