

## CONSTITUTION OF POPULNIN

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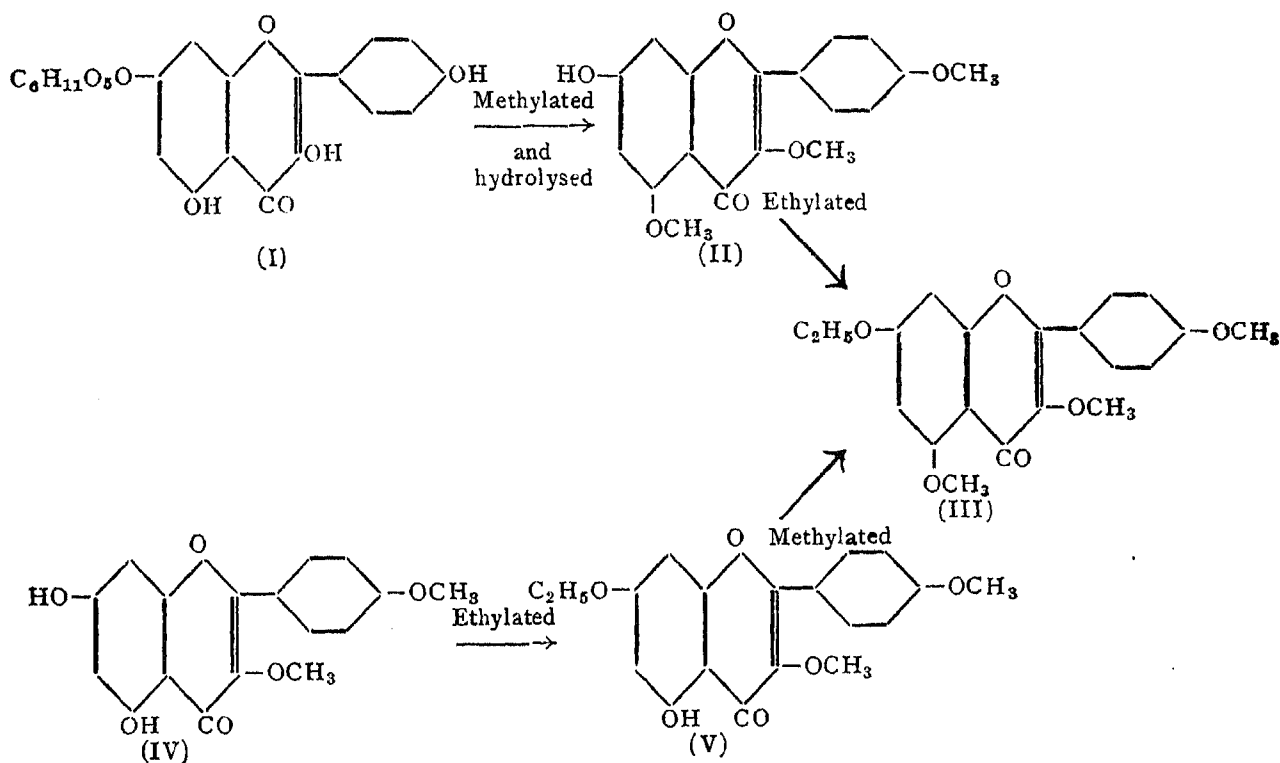
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THE glucoside, Populnin,<sup>1</sup> was obtained as the main crystalline component of the flower petals of *Thespesia populnea* and from its composition it was considered to be a monoglucoside of a tetrahydroxy-flavone which was named populnetin. This has now been confirmed by the yield of glucose produced by acid hydrolysis. Populnetin exhibited considerable stability to cold 50% potash and air. As a matter of fact this property was used for its purification. It was therefore considered to be a flavone and not a flavonol. This is now found to be incorrect. Comparison of populnetin and its derivatives with synthetic samples of scutellarein<sup>2</sup> and 5:7:8:4-tetrahydroxy flavone<sup>3</sup> and their derivatives has shown that it is not one of them. The fission of the tetramethyl ether with alcoholic potash yields anisic acid and methoxy fisetol-dimethyl ether establishing definitely that populnetin is a flavonol and should be identical with kaempferol. The identity has been confirmed by comparison with authentic samples, synthetic as well as natural, of kaempferol and its derivatives. It is therefore clear that the criterion of differentiation between flavones and flavonols based on stability to cold alkali and air is not reliable.

It follows from the above results that populnin is a monoglucoside of kaempferol. In order to locate the position of the sugar group it was methylated by means of dimethyl sulphate and potassium carbonate in anhydrous acetone medium. The product, when hydrolysed with mineral acid, yielded a compound melting at 283–85°. It was a trimethyl ether and did not give any prominent reaction with alcoholic ferric chloride. These properties indicated that the free hydroxyl was not in the 3 or 5 position. The melting points of the corresponding 4'-hydroxy and 7-hydroxy compounds as recorded in the literature are nearly the same as above. But a mixture of the degradation product with the 4'-hydroxy compound recently synthesised in this laboratory<sup>4</sup> melted lower and indefinitely. By elimination it should therefore be concluded that the partial methyl ether obtained from populnin has the hydroxyl group in the 7-position (II) which also indicates the position of the sugar group (I).

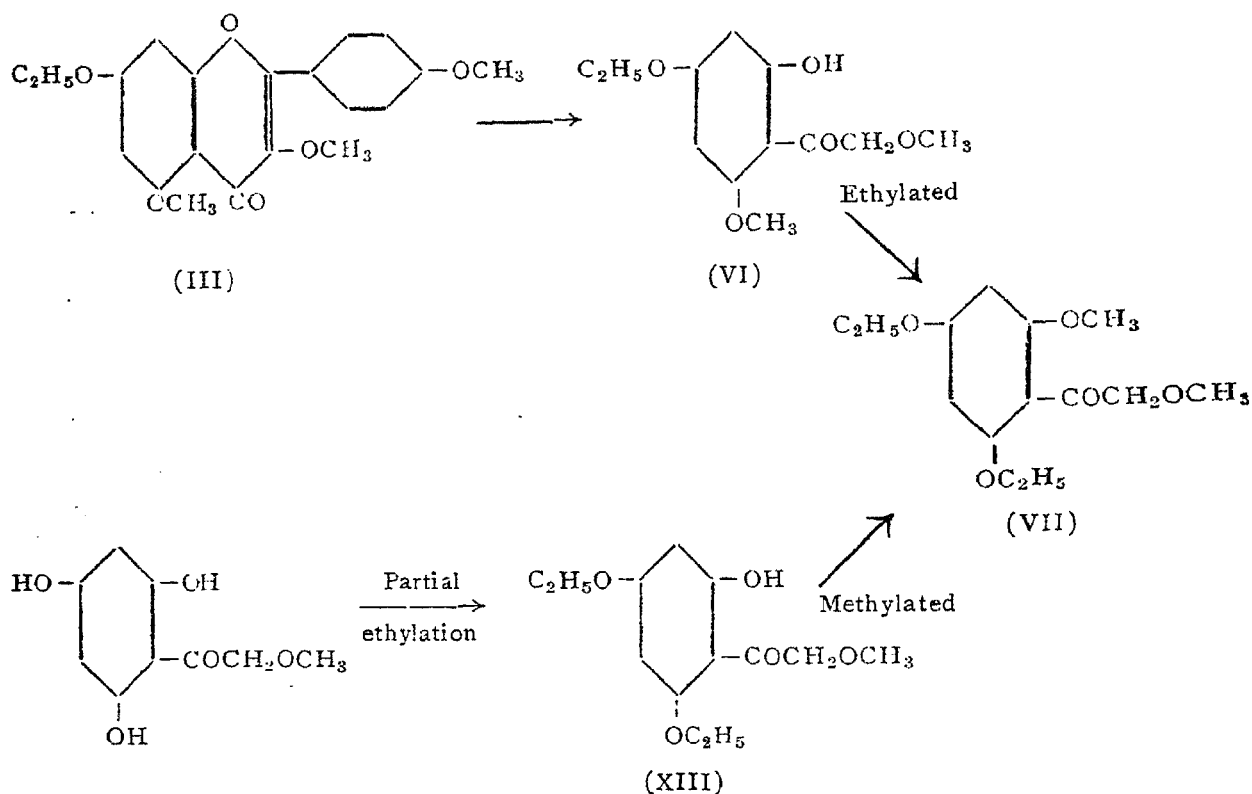
Name of the partial methyl ether	Melting point of hydroxy compound	Ferric chloride reaction	M.P. of acetate
5:7:4'-trimethoxy-3-hydroxy flavone <sup>5</sup>	151-52°	Violet brown	190-91°
3:7:4'-trimethoxy-5-hydroxy flavone <sup>6</sup>	152-53°	Olive green	..
3:5:4'-trimethoxy-7-hydroxy flavone	283-85°	Nil	159-60°
3:5:7-trimethoxy-4'-hydroxy flavone	278-80°	Nil	147-49°

In efforts to provide confirmation of the above constitution the mono-hydroxy compound (II) was subjected to fission with alcoholic potash; it was found to be unaffected by this treatment. It was therefore ethylated and the constitution of the ethyl ether (III) established in two ways. (1) 5:7-Dihydroxy-3:4'-dimethoxy-flavone (IV) obtained by the condensation of  $\omega$ -methoxy phloracetophenone and the sodium salt and anhydride of anisic acid, was subjected to partial ethylation which should be normally expected to take place in the 7-position (V). Subsequently it was methylated and the final product was found to be identical with the abovementioned ethyl ether (III) obtained from the glucoside. The reactions can be explained as given below.



(2) The second method of confirmation involved fission of the ethyl ether (III) whereby anisic acid and a dimethyl-monoethyl phloraceto

phenone (VI) were obtained. Further ethylation of the ketonic product yielded a diethyl ether (VII) whose constitution was again established by synthesis as given below starting from  $\omega$ -methoxy phloracetophenone.



The formation of the 2:4-diethyl ether (VII) as the degradation product is possible only if in (III) the ethoxyl were in the 7-position, thus confirming that populnin is a 7-glucoside of k ampferol (I).

A glycoside of k ampferol with a disaccharose unit in the 7-position has been recently described by Nakamura and Hukuti.<sup>7</sup> The 7-hydroxy compound obtained as the result of methylation and subsequent hydrolysis agrees in its properties with the compound described here in the course of the study of populnin. The Japanese authors report the synthesis of this compound starting from the monomethyl ether of phloroglucinol. But they record the melting point of the acetate as 205° which is too high. We get its melting point as 159–60°. More recently the abstract of the paper of Zemlen and Bognar<sup>8</sup> on the constitution of robinin has become available. As the result of enzymic hydrolysis robinin yielded k ampferol-7-1-rhamnoside from which by methylation and subsequent hydrolysis 3:5:4'-trimethyl ether of k ampferol was obtained. Its melting point is given as 288–89° and that of its acetate as 157–58°. These melting points agree with ours closely.

## EXPERIMENTAL

*Hydrolysis of Populnin.*—Populnin (2 g.) was boiled under reflux with 7% sulphuric acid (200 c.c.) for two hours. In about half an hour the glucoside was completely dissolved and the aglucone began to separate out within an hour. The contents were filtered and the filtrate extracted with ether so as to render it free from aglucone. It was then neutralised with sodium carbonate, clarified with neutral lead acetate and the sugar formed was quantitatively estimated by the method of Munson and Walker (Found: glucose, 40.2;  $C_{21}H_{20}O_{11}$ ,  $H_2O$  requires glucose, 38.6%). The aglucone was crystallised from aqueous alcohol when it was obtained as fine needles, yellow in colour and melting at 275–76° (Found in sample dried at 120°: C, 63.3; H, 3.9;  $C_{15}H_{10}O_6$  requires C, 62.9; H, 3.5%). Mixed melting points of populnetin with both synthetic and natural samples of k ampferol were undepressed. Populnetin Acetate was prepared by boiling populnetin with acetic anhydride and a few drops of pyridine for two hours. The product was finally recrystallised from ethyl acetate when it came out as colourless short rectangular plates. When heated, the substance sintered strongly and became glassy at about 120° and melted down at 180°, a behaviour characteristic of k ampferol acetate.

*Populnetin Methyl Ether.*—As the older method of methylation using sodium hydroxide and dimethyl sulphate was found to be not productive of good yields, the following method was adopted. Populnetin (2 g.) was dissolved in anhydrous acetone (50 c.c.); the solution was treated with anhydrous potassium carbonate (25 g.) and dimethyl sulphate (8 c.c.) and the mixture boiled under reflux for 30 hours. A further quantity of dimethyl sulphate (4 c.c.) was added in small quantities during the first 24 hours. The mixture was finally filtered under suction while still hot and the residue was washed with a small quantity of hot anhydrous acetone. The filtrate was concentrated and on the addition of excess of water a bulky precipitate of the methyl ether was produced. It was filtered and recrystallised from alcohol when it came out as long rectangular plates melting at 165–66° (Found: C, 60.5; H, 6.1;  $OCH_3$ , 32.4; and loss on drying at 120° *in vacuo* for 2 hours, 9.2;  $C_{19}H_{18}O_6$ ,  $2H_2O$  requires C, 60.3; H, 5.8;  $OCH_3$ , 32.8 and loss on drying 9.5%). The substance did not dissolve in alkali and gave no colour with ferric chloride. With concentrated sulphuric acid it gave a yellow solution with weak green fluorescence. The mixed melting point of tetramethyl populnetin with a synthetic sample of tetramethyl k ampferol (m.p. 165–66°), obtained by methylating 5:7-dihydroxy-3:4'-dimethoxy flavone, was undepressed. The above partially methylated flavone was

prepared by condensing  $\omega$ -methoxy-phloracetophenone with anisic anhydride and sodium anisate according to the method of Robinson and Shinoda.<sup>9</sup>

*Decomposition of Populnetin Methyl Ether.*—(a) 50% Aqueous potash: O-Tetramethyl populnetin (0.5 g.) was boiled under reflux in a silver flask with 20 c.c. of 50% aqueous potash for 8 hours. The product was then diluted and acidified with concentrated hydrochloric acid when a colourless solid separated out. The contents were ether-extracted and the ether solution was washed repeatedly with 5% sodium bicarbonate solution till no more acid could be extracted. The bicarbonate extract was acidified and the acid that precipitated out was filtered and recrystallised from alcohol. It was found to be identical with anisic acid. The remaining ether solution contained the ketonic part but the yield of the ketone was not sufficient for identification. Hence the following method of decomposition was employed.

(b) Absolute alcoholic potash.—O-Tetramethyl populnetin (1.0 g.) was boiled under reflux with absolute alcoholic potash (30 c.c.; 8%) for a period of six hours. At the end of the experiment, as much of alcohol as possible was removed by distillation and the residue was dissolved in water. The solution was then filtered free from any suspended insoluble impurities and the clear filtrate was acidified with dilute sulphuric acid. The contents were then ether-extracted and the ether solution was repeatedly washed with 5% sodium bicarbonate solution till no more acid could be extracted. The solvent was then distilled off and the solid obtained was recrystallised from alcohol. It appeared as irregular plates melting at 104–6° and was found to be identical with methoxy fisetol dimethyl ether by a mixed melting point determination with a synthetic sample obtained by partial methylation of  $\omega$ -methoxy-phloracetophenone.<sup>10</sup>

The sodium bicarbonate extract was acidified and the acid that precipitated out was filtered and recrystallised from alcohol. It was found to be identical with anisic acid.

*Methylation of Populnin and Hydrolysis.*—Populnin (2 g.) was suspended in anhydrous acetone (50 c.c.) and the contents were refluxed for 30 hours after adding dimethyl sulphate (10 c.c.) and potassium carbonate (25 g.). The mixture was finally filtered under suction while still hot and the residue was washed with a small quantity of hot anhydrous acetone. The filtrate was concentrated and finally as much of acetone as possible was evaporated off. The residue was a viscous semi-solid and it could not be crystallised even after repeated attempts. Therefore it was hydrolysed by boiling with 200 c.c. of 7% sulphuric acid for 2 hours. A clear solution was first obtained

and an almost colourless bulky solid began to separate out at the end of the first half hour. The product (II) was filtered and was recrystallised using a large quantity of alcohol. It came out as colourless short needles melting at 283-85° (Found: C, 66.1; H, 5.2;  $C_{18}H_{16}O_6$  requires C, 65.9; H, 4.9%). The substance was soluble in alkali to form a light yellow solution and gave no colour with ferric chloride in alcoholic solution. It was stable to alcoholic potash and could be recovered almost completely even after refluxing with 10% absolute alcoholic potash for 8 hours. The mixed melting point with 4'-hydroxy-3:5:7-trimethoxyflavone (m.p. 278-80°) was considerably depressed and was not sharp (235-55°). Its acetyl derivative was prepared by boiling with acetic anhydride and a few drops of pyridine. It crystallised from ethyl acetate or acetone in the form of colourless flat needles and narrow rectangular plates melting at 159-60°. Mixed melting point with 4'-acetoxy-3:5:7-trimethoxy flavone was considerably depressed.

*Ethylation of (II).*—The partial methyl ether of populnetin (II) (1.0 g.) was dissolved in anhydrous acetone (50 c.c.), ethyl iodide (5 g.) and dry potassium carbonate (15 g.) added and the mixture refluxed for 30 hours. At the end of the experiment, the contents were filtered while hot and the residue was washed with hot anhydrous acetone. The filtrate was concentrated and was kept in an ice-chest after dilution with water. The solid that separated out was filtered and washed with dilute alkali to remove any unethylated compound. The product was first crystallised from alcohol when a brownish crystalline solid melting at 80° was obtained; but a colourless sample could be obtained by crystallising it from ethyl acetate. The compound (III) was finally recrystallised from alcohol from which it slowly separated out as rectangular plates melting at 128-30° (Found: C, 67.5; H, 5.4;  $C_{20}H_{20}O_6$  requires C, 67.4; H, 5.6%).

*Decomposition of the Monoethyl-trimethyl Ether (III).*—The ethylated product obtained above (1.0 g.) was boiled under reflux with absolute alcoholic potash (30 c.c.; 8%) for a period of 6 hours. As much of alcohol as possible was then removed by distillation; the residue was dissolved in water and the clear solution was acidified with excess of dilute sulphuric acid. The contents were then ether-extracted. The ether solution was shaken three times with 5% sodium bicarbonate solution to extract the acid part (A).

*The Ketonic Part (VI).*—The remaining ether solution was then washed with water and on distilling off the solvent the residue was found to be a pale yellow viscous liquid which solidified during the course of a few hours when kept in contact with water in an ice-chest. The ketone was crystallised

from dilute alcohol when it came out as rectangular plates melting at 108–10°. It dissolved in alkali to give a bright yellow solution and gave in alcoholic solution an olive green colour with ferric chloride (Found: C, 59.8; H, 7.0;  $C_{12}H_{16}O_5$  requires C, 60.0; H, 6.7%).

*The Acid Part (A).*—The bicarbonate solution on acidification with concentrated hydrochloric acid gave rise to a precipitate which on recrystallisation from alcohol was found to be identical with anisic acid.

*Ethylation of (VI).*—The ketone (VI) (0.5 g.) was dissolved in anhydrous acetone (25 c.c.) and after the addition of ethyl iodide (2 c.c.) and anhydrous potassium carbonate (5 g.) the mixture was boiled under reflux for 30 hours. The solvent was then distilled off and the residue was treated with water when an insoluble liquid separated out. It was ether-extracted and the ether solution was washed with dilute alkali to remove any unethylated ketone. After washing the ether solution free from alkali and distilling off the ether, a reddish yellow viscous liquid remained behind which could not be solidified and recrystallised even after repeated attempts. It was insoluble in aqueous alkali and an alcoholic solution did not give any colour with ferric chloride. Hence the 2:4-dinitrophenyl-hydrazone derivative was prepared by boiling the ketone with an alcoholic solution of 2:4-dinitrophenylhydrazine. The product was washed with dilute hydrochloric acid and was recrystallised from alcohol when it came out as deep red rhombohedral prisms melting at 198–200° (Found: C, 53.4; H, 5.0;  $C_{20}H_{24}O_8N_4$  requires C, 53.6; H, 5.4%).

*7-Ethoxy-5-hydroxy-3:4'-dimethoxyflavone (V).*—5:7-Dihydroxy-3:4'-dimethoxy flavone (IV) was prepared by condensing  $\omega$ -methoxyphloracetophenone with anisic anhydride and sodium anisate according to the method of Robinson and Shinoda.<sup>9</sup> The flavone (1 g.) was dissolved in dry acetone (50 c.c.), ethyl iodide (0.6 g.) and anhydrous potassium carbonate (10 g.) added and the mixture boiled under reflux for 6 hours. At the end of the experiment, acetone was completely distilled off and the residue was taken up with water and ether extracted. From the ether solution, the partially ethylated flavone was extracted by means of dilute aqueous alkali repeatedly until the extraction was complete. The combined alkali extracts were acidified and the precipitate was filtered and recrystallised from alcohol. The 5-hydroxy compound (V) crystallised out as bright yellow rectangular plates melting at 131–32° (Found: C, 66.8; H, 5.1;  $C_{19}H_{18}O_6$  requires C, 66.7; H, 5.3%). An alcoholic solution of the compound gave an olive green colour with ferric chloride.

*7-Ethoxy-3 : 5 : 4'-trimethoxy flavone.*—5-Hydroxy-7-ethoxy-3 : 4'-dimethoxy flavone (V) (1.0 g.) was dissolved in dry acetone (30 c.c.) treated with anhydrous potassium carbonate (15 g.) and excess of dimethyl sulphate (2 c.c.) and the mixture boiled under reflux for 30 hours. The product was worked up in the same way as mentioned for similar cases. When recrystallised from alcohol it came out as thick rectangular plates melting at 128–30° (Found: C, 67.3; H, 6.0;  $C_{20}H_{20}O_6$  requires C, 67.4; H, 5.6%). It was identical with compound III obtained by the degradation of populnin and the mixed melting point was undepressed.

*2-Hydroxy-4-ethoxy- $\omega$  : 6-dimethoxy acetophenone.*—7-Ethoxy-3 : 5 : 4'-trimethoxy flavone (1 g.) was treated with absolute alcoholic potash (2 g. of potash in 30 c.c. of absolute alcohol) and the mixture boiled under reflux on a water-bath for about 6 hours. As much of alcohol as possible was then removed by distillation, the residue dissolved in water and the solution acidified with excess of dilute sulphuric acid. The product was then ether-extracted and the ether solution was washed repeatedly with sodium bicarbonate solution until anisic acid was completely removed. The solvent was then distilled off and the solid obtained was recrystallised from dilute alcohol. The ketone crystallised out as rectangular plates melting at 108–10° (Found: C, 60.1; H, 6.7;  $C_{12}H_{16}O_5$  requires C, 60.1; H, 6.7%). The mixed melting point with the ketone (VI) obtained from monoethyl trimethyl populnetin (III) was undepressed.

*2 : 4-Diethoxy-6-hydroxy- $\omega$ -methoxy acetophenone (VIII).*— $\omega$ -Methoxy-phloracetophenone (1 g.) was dissolved in dry acetone (30 c.c.) and the solution refluxed for about six hours after adding ethyl iodide (1.3 c.c.) and anhydrous potassium carbonate (5 g.). The solvent was then removed by distillation, the residue taken up with water and ether extracted. From the ether extract the partially ethylated  $\omega$ -methoxy-phloracetophenone was separated by extraction with alkali. The alkali solution was then acidified when an almost colourless substance precipitated out. It was filtered and recrystallised from alcohol when it came out as rectangular plates and prisms melting at 110–12° (Found: C, 61.3; H, 7.1;  $C_{13}H_{18}O_5$  requires C, 61.4; H, 7.1%).

*2 : 4-Diethoxy- $\omega$  : 6-dimethoxy acetophenone.*—The above ketone (0.5 g.) was dissolved in acetone (30 c.c.) and methylated by boiling for 30 hours, with dimethyl sulphate (2 c.c.) and anhydrous potassium carbonate (5 g.). The solvent was then removed by distillation and the residue treated with water when an insoluble oily product separated out; it was extracted with ether. The ether solution was washed with dilute alkali to remove any



unmethylated ketone. After washing the ether solution free of alkali the solvent was distilled off; a reddish yellow viscous liquid remained behind and it could not be obtained as a solid even after repeated attempts at crystallisation. Hence the 2:4-dinitrophenylhydrazone was prepared by boiling it with an alcoholic solution of 2:4-dinitrophenylhydrazine. The product was recrystallised from alcohol when it came out as deep red rhombohedral prisms melting at 198-200° (Found: C, 53.4; H, 5.4;  $C_{20}H_{24}O_8N_4$  requires C, 53.6; H, 5.4%). The mixed melting point with the dinitrophenylhydrazone of the ethylated ketone (VII) obtained from populnin was undepressed. Mixed melting point with 2:4-dinitrophenylhydrazine itself was depressed.

#### SUMMARY

Populnin is a monoglucoside of populnetin which is shown to be identical with k mpferol. By the methylation and the subsequent hydrolysis of the glucoside a trimethyl ether of k mpferol is obtained. Its colour reactions and properties indicate definitely that the free hydroxyl group is not in the 3- or 5-position. It has been compared with the isomeric 4'-hydroxy compound and found to be different. Thus the degradation product should have the hydroxyl in the 7-position. This is confirmed by ethylation and comparison of the ethyl ether and of its alkali degradation product (ketone) with synthetic samples.

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