

## CONSTITUTION OF PATULETIN—PART I

BY L. RAMACHANDRA ROW AND T. R. SESHADRI

(From the Department of Chemistry, Andhra University)

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It has been shown in previous papers<sup>1</sup> that patuletin<sup>2</sup> is not identical either with 5:6:3':4'-tetra-hydroxy-flavonol or with the 6:7:3':4'-isomer as was originally considered probable. Consequently the compound has been reinvestigated, particular attention being paid to the identification of the products of fission.

For this purpose patuletin had to be methylated. Since during the period of this work, methylating agents such as methyl iodide and dimethyl sulphate were not available in the market due to the conditions of war and had to be prepared for use, attempts were made to effect strict economy in the reagent. As methyl iodide is volatile and losses are heavy, it was unsuitable. Further it does not invariably bring about complete methylation. The method of methylation adopted by Rao and Seshadri<sup>2</sup> using the acetyl derivative of the pigment had to be given up in view of the large quantities of dimethyl sulphate required. However, methylation with dimethyl sulphate and anhydrous potassium carbonate in anhydrous acetone medium was found to be quite suitable. Dimethyl sulphate being non-volatile, losses of the methylating agent are negligible. Further acetone is an excellent medium for methylating hydroxy-flavones and flavonols. In this process even the resistant 5-hydroxyl group gets methylated readily and yields are uniformly satisfactory.

When patuletin was methylated in this way, a colourless crystalline product melting at 141–42° (I) was obtained. It was insoluble in aqueous alkali, cold or warm, and gave no ferric chloride colour. However, this product was not identical with the ether (II) obtained previously by Rao and Seshadri.<sup>2</sup> The new product depressed the melting point of the older one. On closer examination, it was discovered that the latter was only a partially methylated derivative of patuletin. Though insoluble in the cold, it was soluble in warm aqueous alkali and gave a brown colour with neutral alcoholic ferric chloride. These reactions are characteristic of a free 5-hydroxyl. A repetition of the older method of methylation using patuletin acetate yielded the same product (II) giving all the reactions for

a free 5-hydroxyl. It is extraordinary and difficult to explain the formation of this partially methylated product in view of the fact that the method yielded completely methylated derivatives in the case of quercetagenin, herbacetin, quercetin, cannabiscetin and gossypetin acetates.<sup>3</sup> However, the formation of methyl ether (II) established the presence of a 5-hydroxyl group in patuletin.

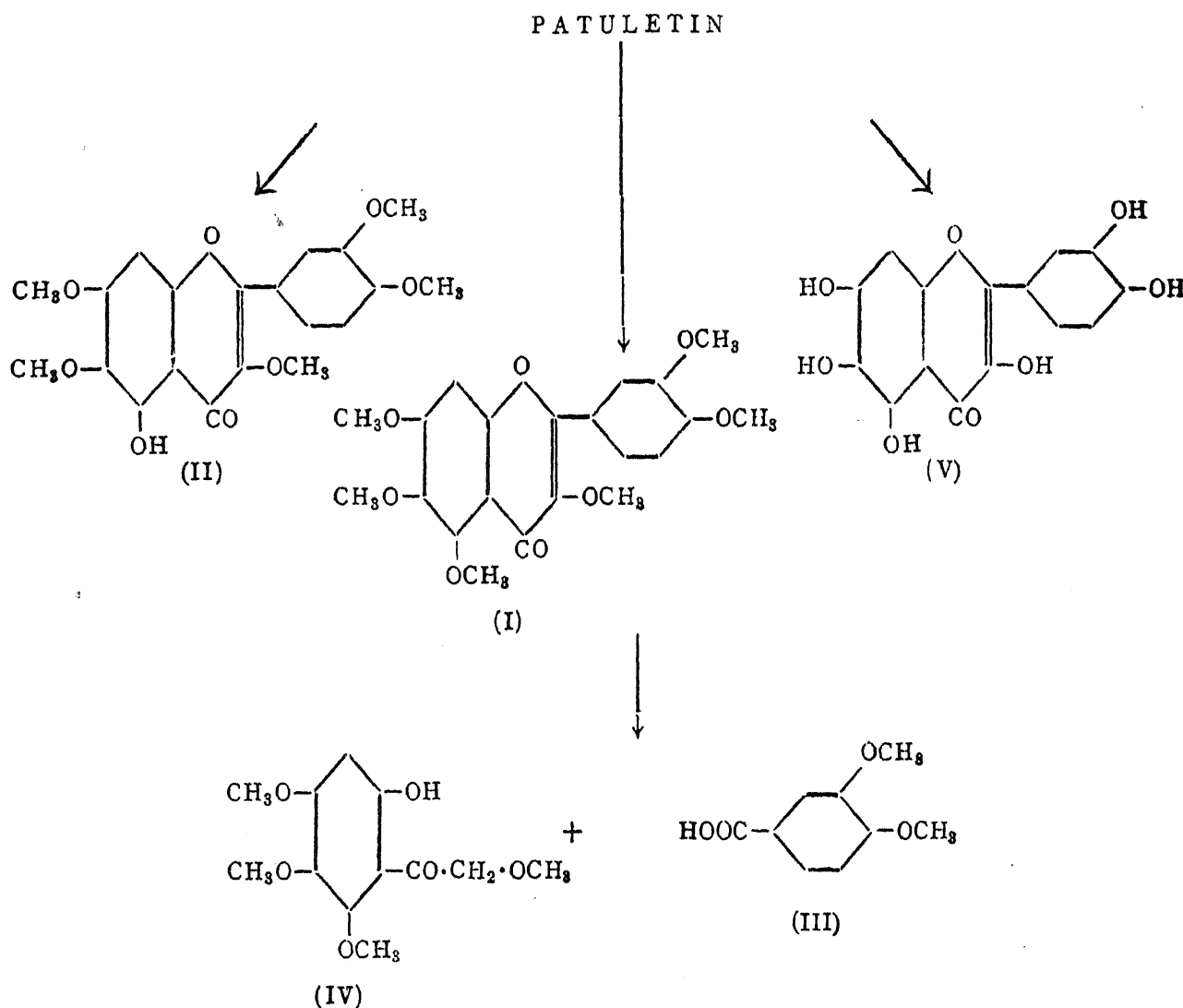
When subjected to fission with 50% aqueous potash, the completely methylated derivative of patuletin (I) yielded an acid which was identified to be veratric acid (III) both by analysis and by direct comparison with an authentic sample. An ortho-hydroxy-ketone (IV) was also isolated as another product of degradation and it had the formula  $C_{12}H_{16}O_6$ . Estimation of methoxyls gave evidence for four methoxyls in the ketone. It was, therefore, considered to be a tetra-methoxy-ortho-hydroxy-acetophenone. From the composition of fission products, it could be inferred that the new methyl ether of patuletin (I) should be a hexamethyl ether. This was supported by analysis both for methoxyls and carbon and hydrogen. Rao and Seshadri's compound (II) has now been shown to be a pentamethyl ether with a free hydroxyl still left. On further methylation using dimethyl sulphate and aqueous alkali in acetone medium at 60° it yielded a product identical with the new methyl ether (I).

The formation of hexa- and penta-methyl ethers necessitated a closer and more detailed analytical study of patuletin. Careful Micro-Zeisel estimation showed the presence of one methoxyl. This important fact was somehow missed by the previous workers and the matter escaped careful check probably because the analytical values for C and H agreed closely with the requirements of a penta-hydroxy-flavonol. Patuletin has now, therefore, been assigned the elementary formula  $C_{16}H_{12}O_8$  instead of the previous formula  $C_{15}H_{10}O_7$ . The new formula is in greater accord with the analytical results for patuletin and its acetate. It is a monomethyl ether of a flavonol having six hydroxyl groups. The isolation of veratric acid and a tetramethoxy-ortho-hydroxy-acetophenone (IV) as the degradation products of the methyl ether (I) proves that two of the six hydroxyls are situated in the side-phenyl nucleus and the remainder in the benzo-pyrone part.

Of the naturally occurring flavonols containing six hydroxyl groups only quercetagenin and gossypetin possess two hydroxyl groups in the side-phenyl nucleus. A close resemblance between the former and patuletin has already been noted by previous workers. The following table gives the comparative data between patuletin and quercetagenin:

	Patuletin	Quercetagetin
1. Molecular formula	$C_{16}H_{12}O_8$	$C_{15}H_{10}O_8$
2. Melting point	261-62°	315-18°
3. Acetate, m.p.	171-172°	209-10°
4. Methyl ethers	(a) Partially methylated compound, m.p. 159-60° (b) Completely methylated compound, m.p. 141-42°	(a) Penta-methyl ether, m.p. 159-60° (b) Hexamethyl ether, m.p. 141-42°

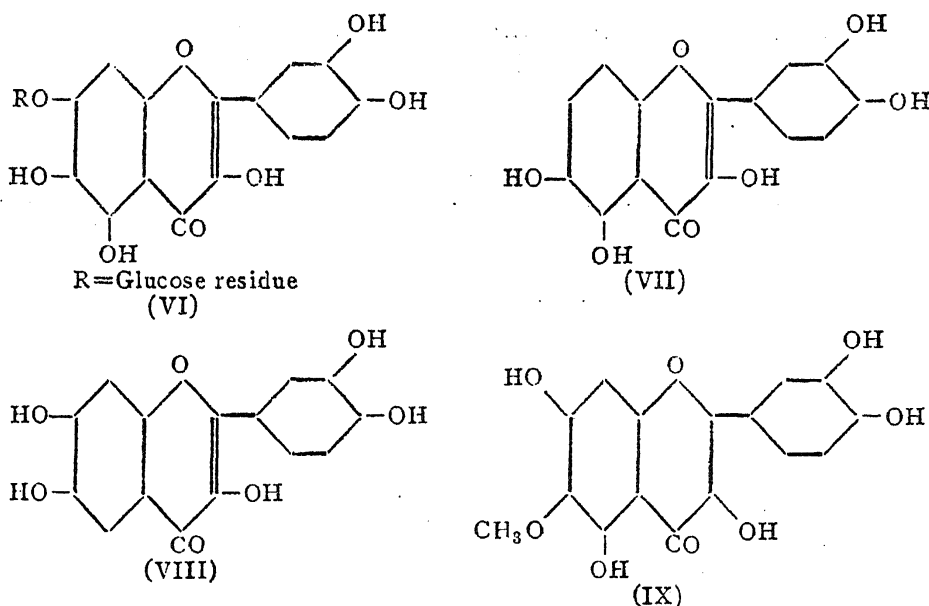
Authentic samples of penta- and hexa-methyl ethers of quercetagetin were obtained from quercetagetin isolated from the flowers of *Tagetes erecta*, and mixed melting points with the partially methylated (II) and completely methylated (I) derivatives of patuletin were determined. In both cases there was no depression. It was thus conclusively proved that patuletin is a monomethyl ether of quercetagetin. The identification was complete when quercetagetin (V) was obtained on demethylating patuletin with hydriodic acid.



It therefore follows that the ketonic fission product (IV) is quercetagetol tetramethyl ether. This has been obtained pure for the first time now; the yield is good if absolute alcoholic potash is employed for the fission and water is avoided. The corresponding tetra-ethyl compound was prepared by Perkin<sup>4</sup> by the alkaline degradation of quercetagetin hexa-ethyl ether. Nelson<sup>7</sup> and Goldsworthy and Robinson<sup>6</sup> in connection with their work on tangeretin characterised the methyl ether as its oxime. The ketonic compound (IV) now obtained, yields an oxime having the properties described by them.

The position of the methoxyl in patuletin was next investigated. A comparative study of the colour reactions of patuletin and quercetagetin in alkaline buffer solutions showed marked differences. Quercetagetin developed a greenish yellow colour initially which changed over to green during the course of an hour. Within 24 hours, the solution became brownish yellow. Except for the increase in intensity and rapidity of the colour change, the sequence of colours was almost the same throughout the range of pH (8.6 to 13.4) employed. Patuletin did not exhibit such a marked change in colour. The initial colour was bright yellow in all cases and showed little or no change during the course of an hour. After 24 hours the colours ranged from orange to orange-brown and pink. Since these colour reactions are primarily controlled by the composition of the benzo-pyrone part, it was considered probable that the marked differences between quercetagetin and patuletin is caused by the presence of the methoxyl in this part of the molecule. The ease with which patuletin could be oxidised in alkaline solution by means of atmospheric oxygen suggests that the 3-hydroxyl is free and not methylated. The formation of the penta-methyl ether further excludes the 5-hydroxyl. Hence the position of the methoxyl should be either 6 or 7.

A comparison has again been made of the colour reactions of patuletin with those of (1) quercetagitrin (VI) having the glucose unit attached to the hydroxyl in the 7-position, (2) 3:5:6:3':4'-penta-hydroxy-flavone (VII) in which there is no hydroxyl in the 7-position and (3) 3:6:7:3':4'-penta-hydroxy-flavone (VIII) having no hydroxyl in the 5-position. The difference is definite. There is marked change of colour from the initial yellow within an hour at the most in these three cases whereas patuletin resembles quercetin (a 5:7-dihydroxy compound) in giving a yellow colour stable for several hours. It may therefore be suggested tentatively that patuletin has the methoxyl in the 6-position (IX).



## EXPERIMENTAL

*Methylation of Patuletin:*

(a) *Hexa-methyl ether (I)*.—A solution of dry patuletin (1.0 g.) in anhydrous acetone (150 c.c.) was treated with dimethyl sulphate (2.0 c.c.) and freshly ignited potassium carbonate (15 g.). The mixture was refluxed on a water-bath for 30 hours. At intervals of eight hours, further quantities (2.0 c.c.) of dimethyl sulphate were added to the refluxing liquid. A total of 8 c.c. of the reagent was thus used. After 30 hours, the acetone solution was filtered and the potassium salts washed with three lots of warm acetone (30 c.c.). The filtrate was concentrated on a water-bath and to the concentrate, water (50 c.c.) was added when fine colourless needles of the methyl ether separated out. The potassium salts were dissolved in water and the solution acidified with hydrochloric acid. There was no appreciable precipitate. The crystalline methyl ether was re-crystallised twice from alcohol when it was obtained as shining colourless prismatic needles melting at 141–42°. Yield: 0.65 g. (Found: C, 62.6; H, 5.4; OCH<sub>3</sub>, 45.9; C<sub>21</sub>H<sub>22</sub>O<sub>8</sub> requires C, 62.7; H, 5.5; and OCH<sub>3</sub>, 46.3%.) The methyl ether was insoluble in cold or warm aqueous sodium hydroxide. It gave no colour with neutral ferric chloride in alcoholic solution. It depressed the melting point of the methyl ether obtained by Rao and Seshadri.<sup>2</sup>

(b) *Penta-methyl ether (II)*.—The methyl ether obtained by Rao and Seshadri<sup>2</sup> was crystallised twice from alcohol. It came out as very pale yellow needles melting at 159–60°. Even when a third crystallisation was carried out using some animal charcoal, the compound retained a yellow tinge. It was insoluble in cold aqueous sodium hydroxide but dissolved

when warmed yielding a bright yellow solution. With ferric chloride, it developed a brown colour in alcoholic solution. These reactions are characteristic of a free 5-hydroxyl in the compound. To verify if the same partially methylated compound would again be obtained, patuletin was acetylated and methylation carried out according to the method of Rao and Seshadri.<sup>2</sup>

Acetyl patuletin (0.5 g.) was methylated in acetone medium by means of dimethyl sulphate (20 c.c.) and aqueous sodium hydroxide (20%, 40 c.c.). The reagents were added alternately in three lots with vigorous stirring and cooling under the tap. The methyl ether was obtained as very pale yellow shining needles melting at 159–60° and was identical with the older sample; the mixed melting point was not depressed. (Found: C, 61.9; H, 4.8; OCH<sub>3</sub>, 40.1; C<sub>20</sub>H<sub>20</sub>O<sub>8</sub> requires C, 61.9; H, 5.2; and OCH<sub>3</sub>, 40.0%. Rao and Seshadri recorded OCH<sub>3</sub>, 39.6%.)

The above experiment was repeated with the following modifications (*cf.* Anderson) to find out if a completely methylated compound could be obtained.

(1) Dimethyl sulphate (20 c.c.) was added to the acetone solution of the patuletin acetate in one lot and then the alkali (20%, 40 c.c.) in small lots. Cooling was employed when the mixture became hot in order to save the solvent and to moderate the speed of the reaction.

(2) Same as (1) above except that the mixture was not cooled under the tap and the loss of acetone due to the high temperature attained during the course of the reaction was made good by adding more of acetone.

The product in both cases was again the partial methyl ether. In these experiments, the yields were frequently low and the product required careful purification.

#### *Methylation of Penta-methyl ether (II).*

The penta-methyl ether (0.1 g.) was dissolved in acetone (20 c.c.) and treated with aqueous sodium hydroxide (10 c.c.) and dimethyl sulphate (2 c.c.) in small quantities alternately, keeping the solution at 60°. Throughout the addition the solution was kept stirred. The pale yellow liquid was left in the water-bath at 60° for an hour more and the acetone was distilled off. The residual solution was then acidified with hydrochloric acid and left in the ice-chest for a number of hours when the completely methylated compound separated. It crystallised from aqueous alcohol (animal charcoal) in the form of white prismatic needles melting at 141–42°. It did not depress the melting point of the hexa-methyl ether (I).

*Alkaline Oxidation of Patuletin*

Degradation of patuletin in 50% aqueous potash was repeated following the general procedure given by Rao and Seshadri.<sup>2</sup> The products of oxidation were methylated with dimethyl sulphate in the usual manner. The liquid was acidified and extracted with ether. The ethereal extract was washed with water and then with aqueous sodium bicarbonate. On acidifying the sodium bicarbonate layer, a colourless crystalline solid was obtained. It crystallised from hot water in the form of colourless rhombic needles melting at 181–82°. It was identified as veratric acid since it did not depress the melting point of an authentic sample.

*Alkaline Hydrolysis of the Hexa-methyl ether (I)*

(a) The methyl ether (1.5 g.) was refluxed in a silver flask with 50% aqueous potash (30 c.c.) for eight hours, the heating being effected in an oil-bath kept at 150–55°. After cooling the flask, the contents were transferred to a conical flask, and acidified with hydrochloric acid. A pale yellow solid separated out. It was thoroughly macerated with sodium bicarbonate solution (10%) and filtered.

*Veratric acid.*—On acidifying the bicarbonate filtrate, a white crystalline solid separated out. It was crystallised twice from hot water using a small quantity of animal charcoal. It was obtained in the form of colourless rhombic needles radiating from a point. It was identical with veratric acid and did not depress the melting point of an authentic sample. Yield: 0.3 g. (Found: C, 59.3; H, 5.2; OCH<sub>3</sub>, 34.6; C<sub>9</sub>H<sub>10</sub>O<sub>4</sub> requires C, 59.3; H, 5.5; and OCH<sub>3</sub>, 34.1%.)

*Ketonic compound (IV): Quercetagetol-tetra-methyl ether.*—The residue which was insoluble in sodium bicarbonate was washed well with water and then crystallised from hot water. It was obtained in the form of colourless narrow rectangular plates melting at 75–76°. Yield: 0.2 g. (Found: C, 56.5; H, 6.2; OCH<sub>3</sub>, 48.0; C<sub>12</sub>H<sub>16</sub>O<sub>6</sub> requires C, 56.3; H, 6.3; and OCH<sub>3</sub>, 48.4%.) It was very easily soluble in cold aqueous sodium hydroxide forming a pale yellow solution. With ferric chloride, it gave a brown colour in alcoholic solution.

(b) The hydrolysis of the hexa-methyl ether (1 g.) was also carried out using absolute alcoholic potash (7%, 30 c.c.). The mixture was refluxed for six hours over a water-bath. After removing the alcohol by evaporation, the residue was dissolved in water (30 c.c.) and acidified with hydrochloric acid. The mixture was extracted five times with ether and the ethereal extract shaken thrice with sodium bicarbonate to remove the acid component.

On acidifying the bicarbonate extract, the acid was obtained in a colourless crystalline condition. It was identical with veratric acid. Yield: 0.3 g. The ether layer was then washed with a little water and evaporated to dryness. On vigorous stirring, the residue solidified into a pale yellow crystalline mass. When crystallised from water, it was obtained as colourless narrow rectangular plates melting at 75–76°. It was identical with the ketone obtained in the earlier experiment; yield: 0.6 g.

*Demethylation of Patuletin: Formation of Quercetagetin.*—A cold solution of patuletin (0.5 g.) in acetic anhydride (3 c.c.) was treated with hydriodic acid (d. 1.7, 10 c.c.) slowly with shaking and cooling under the tap. The mixture was refluxed, in an oil-bath at 150–55° for three hours and then poured into about 50 c.c. of water. The solution was then decolourised by passing sulphur dioxide when a pale yellow compound settled down. It was filtered and crystallised from aqueous alcohol. It was obtained in the form of short needles and plates melting at 315–18° and was found to be identical with quercetagetin. (Found in air-dried sample: C, 50.6; H, 4.0;  $C_{15}H_{10}O_8 \cdot 2H_2O$  requires C, 50.9 and H, 4.0%.)

A part of this substance was acetylated by refluxing with acetic anhydride and anhydrous sodium acetate. When the product was crystallised from acetic acid using a little animal charcoal, the acetyl derivative was obtained as long rectangular plates melting at 209–10°. It did not depress the melting point of an authentic sample of acetyl quercetagetin.

*Patuletin and Patuletin-penta-acetate.*—For purposes of analysis, patuletin was crystallised twice from alcohol when it was obtained as yellow needles melting at 261–62° as already recorded. A third crystallisation was also made but it did not improve the melting point. (Found in air-dried sample: C, 52.6; H, 4.4;  $OCH_3$ , 8.4;  $C_{16}H_{12}O_8 \cdot 2H_2O$  requires C, 52.2; H, 4.4; and  $OCH_3$ , 8.4%. Found in the sample dried at 110–20° *in vacuo*: C, 57.7; H, 3.6;  $C_{16}H_{12}O_8$  requires C, 57.8 and H, 3.6%). The penta-acetate crystallised from dilute acetic acid in the form of colourless needles melting at 171–72°. (Found: C, 57.9; H, 4.4;  $C_{26}H_{22}O_{13}$  requires C, 57.6; H, 4.1%. Rao and Seshadri found C, 58.1; H, 3.9%.)

#### *Colour Reactions in Buffer Solutions*

The following buffer solutions prepared from the "Universal Buffer Mixture" (B.D.H.) were employed for studying the colour reactions of the flavonols: (1) pH 8.6; (2) pH 9.8; (3) pH 11.0; (4) pH 12.2; and (5) pH 13.4.



*Patuletin*.—(1-3) Yellow solution undergoing no change in an hour. After 24 hours the colours ranged from orange to brighter orange with a tinge of pink. (4) Stronger yellow with no change in an hour; after 24 hours dull orange with a tinge of pink. (5) The initial yellow colour changed to brown in the course of 30 minutes; after 24 hours brownish yellow.

3:5:6:3':4'-*Penta-hydroxy-flavone*.—(1) and (2) Deep yellow rapidly turning brown; after an hour deep brownish yellow; after 24 hours to pale brown. (3) Brighter yellow; within two minutes reddish brown, stable even after an hour; after 24 hours almost colourless. (4) Bright yellow changing to reddish brown and finally pink within two minutes; after an hour fading; after 24 hours colourless solution. (5) Immediately reddish brown rapidly turning pink; after an hour fading; after 24 hours colourless solution.

3:6:7:3':4'-*Penta-hydroxy-flavone*.—(1) Feeble yellowish green fairly stable; after 24 hours pinkish yellow. (2) and (3) Yellowish green becoming more intense; after 24 hours pinkish orange. (4) Yellow slowly turning brown; after half-an-hour brownish yellow; after 24 hours bright orange. (5) Yellow turning brown rapidly; after an hour brownish yellow; after 24 hours pale brown.

The colour reactions of quercetagetin and quercetagitrin were as already reported.<sup>8</sup>

#### *Bargellini's Test*

The test was carried out by treating a solution of the flavonol (3-5 mg.) in absolute alcohol (10 c.c.) with 3% sodium amalgam and noting the changes. The following hydroxy-flavonols were tested:

(1) *Quercetagetin*.—There was immediate separation of green flocks. When left overnight, they turned brown.

(2) *Quercetagitrin*.—Rapid separation of brown flocks. On the following day, they appeared greenish brown.

(3) *Patuletin*.—Just as in quercetagetin, there was rapid formation of green flocks. After 24 hours, they became yellowish brown.

(4) 3:5:6:3':4'-*Penta-hydroxy-flavone*.—Brown flocks as in quercetagitrin. They turned yellowish brown on keeping overnight.

(5) 3:6:7:3':4'-*Penta-hydroxy-flavone*.—The solution turned bright green only. There was no separation of flocks. Next morning there was only a trace of brown precipitate but no flocks.

(6) *Quercetin*.—Immediately the solution turned yellow with no flocks. The colour slowly changed to brown.

#### SUMMARY

The methylation of patuletin has been reinvestigated. It forms two methyl ethers, one with five methoxyl groups and the other with six. Decomposition of the latter with alcoholic alkali yields quercetagetol-tetra-methyl ether and veratric acid. Demethylation of patuletin produces quercetagetin. Patuletin is shown to be a mono-methyl ether of quercetagetin; from a study of its properties and reactions and from a comparison with related compounds, the methoxyl is tentatively considered to be in the 6-position.

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