

ISOLATION AND CONSTITUTION OF QUERCETAGITRIN, A GLUCOSIDE OF QUERCETAGETIN

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Received August 5, 1941

THERE has been some confusion in the past regarding the use of the common and the botanical names of the flowers of the Tagetes. The correct designation should be *T. erecta* for the African Marigold and *T. patula* for the French Marigold.¹

The flowers of the African Marigold (wrongly named as *T. patula*) were first examined by Latour and Magnier De La Source.² They were extracted with boiling 85% alcohol, and the flavonol, quercetagetin was isolated in an impure state by concentrating the extract. Later Perkin³ carried out a more detailed investigation. The alcoholic extract of the flowers was concentrated, diluted with water and extracted with ether which dissolved the free quercetagetin. Though Perkin realised that the aqueous solution contained a glucoside of the flavonol, he did not attempt to isolate it but directly hydrolysed it for reasons of economy to produce more quercetagetin. Thinking that Perkin and others examined the flowers of *T. patula*, Mahal⁴ recently studied the flowers of *T. erecta* (wrongly designated as French Marigold) and showed that they contained the same pigment. He too did not attempt to isolate the glucoside. It is obvious that both Perkin and Mahal investigated the same species, *T. erecta*, Perkin having given the wrong botanical name and Mahal the wrong common name. This presumption of ours has been confirmed by the examination of the flowers of *T. patula* also, whose chemical composition is very different. The present investigation has been undertaken with a view to isolate the glucoside and establish its constitution, and these objects have been achieved.

The chemical examination of the petals of *T. erecta* was carried out on the same general lines as had been adopted in the case of the cotton flowers.⁵ When the alcoholic extract was concentrated and allowed to stand, a yellow crystalline solid was obtained, and this was found to consist of the glucoside of quercetagetin, now called quercetagitrin. The mother-liquor was diluted with water and ether extracted, whereby quercetagetin was isolated. The

neutral and the basic lead acetate fractions were insignificant, and hence they were not worked up.

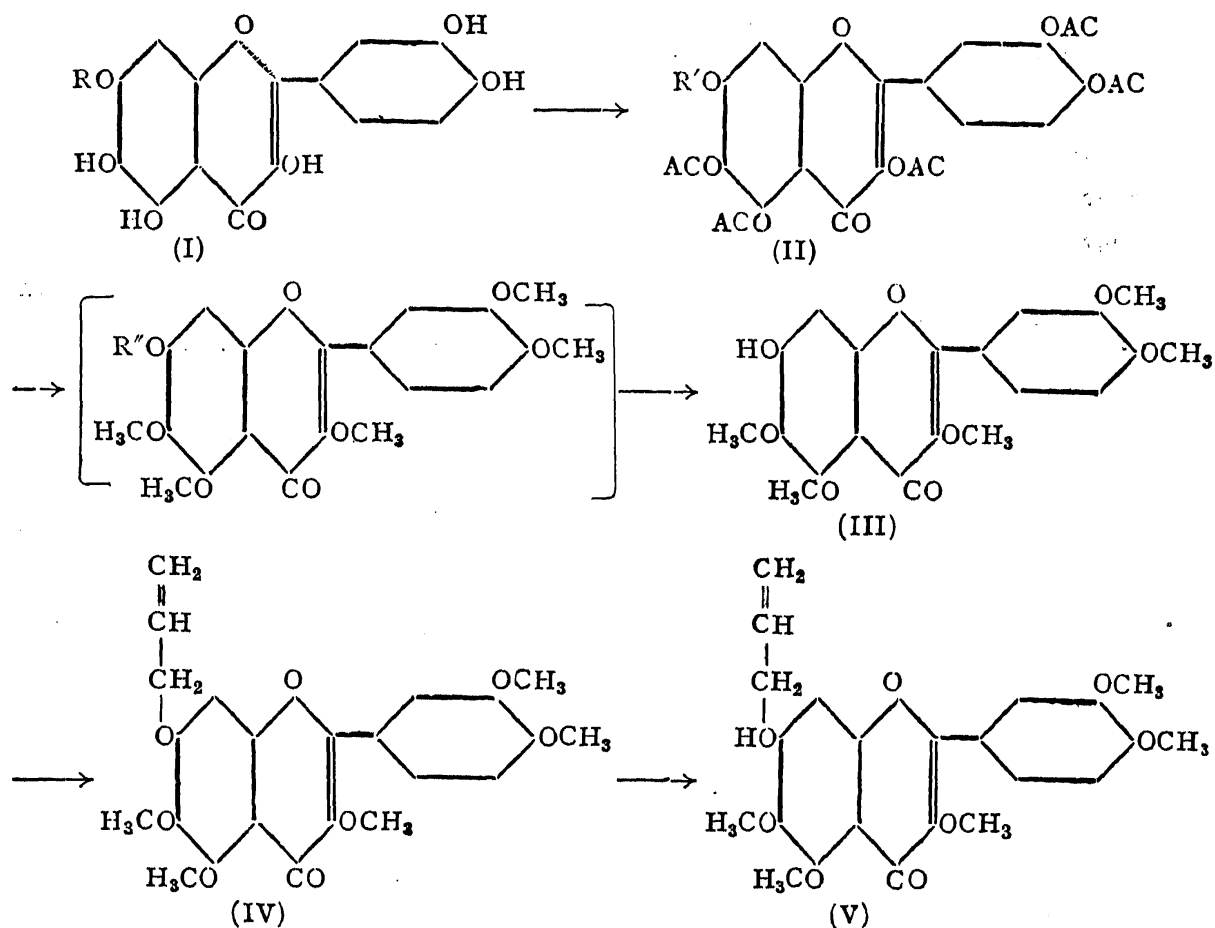
Quercetagitrin is a monoglucoside ($C_{21}H_{20}O_{13}$) yielding on hydrolysis one molecule of glucose and one of quercetagetin. It crystallises in the form of yellow needles and melts with decomposition at $236-38^\circ$. Its non-acetyl derivative melts at $225-27^\circ$. Sodium hydroxide readily dissolves the substance to yield a yellow solution, and ferric chloride imparts a brownish-green colour to an alcoholic solution. With alkaline buffer solutions, the most prominent colour of the glucoside is pink, and this test enables the substance to be easily distinguished from its aglucone, quercetagetin which gives a transient green and a final brown or brown-red as the characteristic colours.

The new glucoside is isomeric with gossypitrin and cannabiscitrin.⁶ It gives a brick-red precipitate with lead acetate and is hydrolysed with difficulty by acids, showing thereby that it is not a 3-glucoside. It undergoes ready oxidation in cold alkali to produce protocatechuic acid, and hence the sugar group should be present in the benzopyrone ring. To definitely establish its position the glucoside is completely methylated through its acetyl derivative⁷ (II) and the product hydrolysed with a view to identify the resulting pentamethyl quercetagetin (III). This pentamethyl ether cannot have the free hydroxyl group in either 3- or 3'- or 4'-position since the glucoside does not have the sugar unit in those positions. Further the compound gives rise to veratric acid on oxidation in boiling 50% potash. As shown in the following table it is entirely different from 3: 6: 7: 3': 4'-pentamethyl quercetagetin originally prepared by Perkin³ by the methylation of the flavonol and later synthesised by Baker, Nodzu and Robinson⁸ during the course of the synthesis of quercetagetin.

	Solubility in cold sodium hydroxide	Colour with ferric chloride	Melting point
3: 6: 7: 3': 4'-pentamethyl quercetagetin	Almost insoluble	Intense olive green colour	159-60°
The new pentamethyl quercetagetin	Easily soluble	No prominent colour	234-35°

Hence the free hydroxyl group is not in position 5; it should be in either 6 or 7 position. To settle this point, the pentamethyl ether is allylated and the allyl ether (IV) subjected to Claisen Rearrangement, when the allyl group undergoes smooth migration (V). This is possible only if the allyl group were originally present in the 7th and not the 6th position as otherwise the

migration cannot occur for want of a free ortho- or para-position. Hence the free hydroxyl group should have been in position 7 in the pentamethyl ether and so the glucoside should be a 7-glucoside (I). The changes involved are diagrammatically represented below:



(R is glucose residue and R' is the fully acetylated sugar group.)

Experimental

Isolation of Quercetagitrin.—6 Kg. of the dry petals of the African Marigold flowers were extracted with methylated spirit in batches of 500 g. Each batch was refluxed with alcohol two times, each treatment lasting for about 12 hours. The combined alcoholic extract was concentrated by distillation to about 1000 c.c. The waxes and the resins that separated out were carefully filtered off through fluted filters, and after the addition of a few drops of toluene, the concentrate was allowed to stand for a long time. After four months some yellow crystalline solid began to appear. The supernatant liquid too became a thick syrup by the separation of further quantities of wax and resin, and consequently the filtration of the pigment became extremely slow and difficult. The residue which was brown in colour and sticky to the touch was thoroughly washed with alcohol, when all the impurities were removed and the solid assumed a golden yellow colour.

The mother-liquor continued to deposit more and more of the yellow substance, which was filtered at intervals, and the deposition was complete towards the end of six months. After the preliminary purification with alcohol as stated above, the compound was boiled with chloroform in order to remove carotinoid material. Now it became almost pure, and was found to be practically insoluble in boiling water, alcohol or even acetic acid, though the impure stuff readily went into solution in hot water. It was finally crystallised from aqueous pyridine, when it came out as golden yellow glistening needles and rectangular plates melting at 236–38° (decomp.). The yield was 8 g. [Found in the air-dried sample: C, 48.5; H, 4.8; $C_{21}H_{20}O_{13}$, 2 H_2O requires C, 48.8; H, 4.6%. Found in the sample dried at 120° *in vacuo*: C, 52.3; H, 4.6; $C_{21}H_{20}O_{13}$ requires C, 52.5; H, 4.2%.]

Isolation of Quercetagenin.—The alcoholic mother-liquor remaining after the separation of the glucoside was diluted with a large volume of water and then extracted with ether. On distilling off the solvent, the extract yielded a good amount of a dark brown viscous liquid. When a small quantity of water was added, the liquid changed into a yellow crystalline solid mixed with some resin. The mixture was extracted with cold alcohol which dissolved preferentially the pigment and not the resin. On concentration the alcoholic extract deposited a yellow solid. Further purification was effected by crystallisation from acetic acid, when the substance was obtained as clusters of short needles and plates melting at 314–16°. The yield was 10 g. [Found in the air-dried sample: C, 50.5; H, 4.2; $C_{15}H_{10}O_8$, 2 H_2O requires C, 50.9; H, 4.0%. Found in the specimen dried at 120° *in vacuo*: C, 56.3; H, 3.5; $C_{15}H_{10}O_8$ requires C, 56.6; H, 3.1%.] On acetylation with acetic anhydride and anhydrous sodium acetate the substance yielded an acetyl derivative which was found to melt at 209–10° and agree with acetyl quercetagenin in all its properties.

Properties of the Glucoside.—Quercetagenin was obtained as fine short needles when rapidly crystallised, but on slow crystallisation, it came out as needles and narrow rectangular plates. It produced a brick-red precipitate with lead acetate, and with ferric chloride a brownish green colour. When a small quantity of sodium hydroxide solution was added, the substance immediately dissolved forming a yellow solution. On shaking with air, the yellow colour rapidly changed to brown, and a dark brown precipitate separated out. With alkaline buffer solutions of pH value below 10, the colour changes were slow and not very prominent; but with solutions of higher pH value, the characteristic colour was pink. The formation of this pink colour enables the substance to be distinguished from its aglucone

which gives on the other hand a transient green and a more stable brown or brown-red.

pH	Quercetagetin	Quercetagitrin
9·2	Dissolved to produce a lemon-yellow solution. The colour changed to greenish-yellow within 2 minutes and greenish-brown after 10 minutes. The brown colour slowly intensified and became very prominent the next day.	Immediately dissolved forming a yellow solution. The yellow colour changed slowly to brownish-yellow and the solution assumed a brownish-pink colour after half an hour. After two hours, it was light pink which gradually intensified. After 6 hours the pink colour began to disappear slowly. Even the next day, this tinge was still noticeable.
10·4	Same changes as above but very rapid.	The same changes as above but more rapid. The brownish-yellow colour appeared after 5 minutes and changed to brownish-pink within another minute. The pink colour became prominent after 10 minutes.
11·0	An immediate lemon-yellow solution which became greenish-yellow within half a minute, greenish-brown within a minute and pure brown within 5 minutes. The transition colour between greenish-yellow and greenish-brown was pure green itself. The solution became brownish-red after half an hour and reddish-brown the next day.	The same changes as above but more quickly. The pink was very prominent even after 5 minutes.
12·2	Same changes as in the previous case but very quick. The reddish-brown appeared even within 5 minutes and changed to brownish-red after 10 minutes.	In this case the colour changes were very quick; yellow—brownish-yellow—brownish-pink—pink. The final shade was noticeable even within 3 minutes and was very prominent after 5 minutes. The next day, the solution was still pink.

Hydrolysis of the Glucoside.—The substance (2 g.) was hydrolysed by boiling with 7% sulphuric acid (100 c.c.). After heating for a few minutes, the solid went into solution, and within half-an-hour another yellow solid separated out, giving rise to bumping. The contents were, therefore, heated for an hour and a half more on a boiling water-bath. After cooling, the precipitate was filtered and the filtrate extracted with ether to completely recover the aglucone. The ether-extract, after the distillation of the solvent, yielded a small amount of the substance. The two samples of the aglucone were mixed together, dried in air and weighed accurately. After recording the yield, the solid was crystallised from dilute alcohol, when it came out as short needles and plates melting at 315–16°. [Found in the air-dried sample: C, 50·5; H, 4·0; $C_{15}H_{10}O_8$, 2 H_2O requires C, 50·9; H, 4·0%.]

The substance was identified as quercetagenin and this was confirmed by preparing its acetyl derivative and comparing it with an authentic sample of acetyl quercetagenin.

The acid solution left after the removal of the aglucone was made up to 120 c.c. and divided into two equal portions. One part was further diluted, neutralised with barium carbonate, and treated after concentration to a small bulk with phenyl hydrazine in dilute acetic acid. When the mixture was heated on a water-bath, the osazone separated out. After crystallisation from alcohol, it was identified as glucosazone from a study of its crystalline structure and the melting point ($204-06^{\circ}$). The other part of the sugar solution was made alkaline by the addition of sodium carbonate and the glucose estimated by titration against standard Fehling solution.

The amounts of the aglucone and the glucose produced as a result of the hydrolysis of the glucoside were calculated. [Found: Aglucone, 68.0; Glucose, 34.3%; $C_{21}H_{20}O_{13}$, 2 H_2O requires: Aglucone ($C_{15}H_{10}O_8$, 2 H_2O) 68.6; Glucose, 34.9%.]

Alkaline Oxidation of the Glucoside: Isolation of Veratric Acid.—Quercetagenin (0.5 g.) was treated with 50% aqueous potash (15 c.c.), when it dissolved immediately to form a blood-red solution. On leaving exposed to air with occasional shaking for 24 hours, the solution became opaque and brown. It was then directly treated, after dilution, with excess of dimethyl sulphate (20 c.c.) in small quantities. After shaking for an hour, the contents were heated on a water-bath for about 30 minutes to complete the methylation and decompose the excess of the methylating agent. The solution was then acidified and ether extracted. A crystalline solid was obtained on evaporating the solvent from the ether extract. It was purified by dissolving in sodium bicarbonate solution and precipitating with hydrochloric acid. When crystallised from aqueous alcohol, it was obtained as colourless needles melting at $183-84^{\circ}$, and was found to be identical with veratric acid.

Acetylation of the Glucoside: Nonaacetyl Quercetagenin.—A small quantity of the glucoside was acetylated by boiling with acetic anhydride and anhydrous sodium acetate. The acetyl derivative was crystallised from glacial acetic acid, when it was obtained as colourless needles melting at $225-27^{\circ}$. [Found: C, 54.8; H, 4.8; $C_{21}H_{11}O_4$ ($OCO CH_3$)₉ requires C, 54.5; H, 4.4%.]

3:5:6:3':4'-Pentamethyl Quercetagenin.—The acetyl quercetagenin (0.5 g.) was dissolved in acetone (25 c.c.) and treated alternately with dimethyl sulphate (10 c.c.) and 20% sodium hydroxide (10 c.c.) in small quantities,

The mixture was shaken vigorously after each addition; considerable amount of heat developed during the course of the reaction. Further quantities of the methylating agent (5 c.c.) and the alkali (5 c.c.) were then added, and finally the medium was made definitely alkaline by the gradual addition of more alkali (15 c.c.). The next day the mixture was refluxed for an hour on the water-bath, and the acetone present was then distilled off. Subsequently the clear alkaline solution was just neutralised and then the required amount of concentrated sulphuric acid was added to it so as to make it 7% in the acid content. After boiling under reflux for 2 hours, the clear solution was cooled in the refrigerator, when a colourless crystalline solid was deposited. It was filtered and the filtrate on ether-extraction yielded a little more. On crystallisation from dilute alcohol, it was obtained as long plates and needles melting at 234–35°. It was easily soluble in sodium hydroxide and it gave no colour with ferric chloride. The yield was 0.3 g. [Found: C, 61.8; H, 5.3; OCH₃, 39.4; C₁₅H₄O₂ (OH) (OCH₃)₅ requires C, 61.9; H, 5.2; OCH₃, 40.0%.] By subjecting it to alkaline oxidation using boiling 50% aqueous potash in a silver flask, the pentamethyl ether gave rise to veratric acid.

3': 4': 3: 5: 6-Pentamethyl-7-allyl Quercetagetin.—The pentamethyl quercetagetin (0.5 g.) was dissolved in dry acetone (50 c.c.), anhydrous potassium carbonate (2 g.) and excess of allyl bromide (2 c.c.) were added to the solution and the mixture boiled under reflux on a water-bath for about 6 hours. The potassium salts were then filtered off and all the solvent was completely removed by distilling the clear solution, when a brown-coloured oil was left behind. It was dissolved in ether, and the solution extracted with 5% sodium hydroxide to remove any unattacked pentamethyl quercetagetin. On the removal of the solvent, the ethereal solution yielded again only an oil, which, however, was much less coloured and turned solid after the addition of a little water and leaving overnight in the ice-chest. It was finally purified by crystallisation from dilute alcohol using a little animal charcoal, and was obtained as almost colourless needles. It sintered at about 118° and melted at 122–24°. [Found in the sample dried at 120° *in vacuo*: C, 64.2; H, 5.7; C₂₃H₂₄O₈ requires C, 64.5; H, 5.5%.] The compound did not dissolve in dilute sodium hydroxide and gave no colour with ferric chloride. The yield of the pure product was 0.3 g.

3': 4': 3: 5: 6-Pentamethyl-8-allyl Quercetagetin.—The above pentamethyl-7-allyl quercetagetin (0.3 g.) was taken into a pyrex filter-tube provided with a tight-fitting rubber stopper. The tube was then exhausted as much as possible using a "Cenco" pump for 15 minutes and then heated on

an oil-bath at 195–200° for 1½ hours. By this treatment a brown semi-solid was obtained, which completely solidified on the addition of a small quantity of water. It crystallised from dilute alcohol (animal charcoal) as colourless narrow rectangular plates melting at 188–90°. [Found: C, 64.2; H, 5.8; C₂₃H₂₄O₈ requires C, 64.5; H, 5.5%.] It was freely soluble in dilute sodium hydroxide. The yield of the pure substance was 0.2 g.

Summary

Quercetagitrin, a monoglucoside of quercetagetin, has for the first time been isolated from the flowers of the African Marigold (*Tagetes erecta*). It is not a 3-glucoside since it gives a red precipitate with lead acetate and does not undergo easy hydrolysis with acids. Further it is easily oxidised in cold alkali, and after methylation the products of decomposition yield veratric acid. The glucose group, therefore, is not present in the side phenyl nucleus. By complete methylation and subsequent hydrolysis of the glucoside, a pentamethyl quercetagetin is obtained. This ether is a new compound, and differs from 3:6:7:3':4'-pentamethyl quercetagetin in all its properties, and hence the free hydroxyl group in it is in either the 6th or the 7th position. The allyl ether of this new pentamethyl quercetagetin smoothly undergoes the Claisen Rearrangement, indicating thereby that the ortho-position with respect to the allyloxy group is unsubstituted. Hence the allyl ether should be 3:5:6:3':4'-pentamethyl-7-allyl quercetagetin, and the new pentamethyl ether should have the constitution of 3:5:6:3':4'-pentamethyl quercetagetin. Consequently quercetagitrin is the 7-glucoside of quercetagetin.

REFERENCES

1. (a) .. *Firminger's Manual of Gardening for India*, 7th Edition, Revised and Edited by W. Burns, 477.
- (b) .. *The Treasury of Botany*, 1120.
2. Latour and Magnier De la .. *Bull. Soc. Chim.*, 1877, (ii) 28, 337.
 Source
3. Perkin .. *Proc. Chem. Soc.*, 1902, 18, 75; *J. C. S.*, 1913, 103, 209.
4. Mahal .. *J. I. C. S.*, 1938, 87.
5. Neelakantam, Ramachandra .. *Proc. Ind. Acad. Sci.*, 1935, 2, 490.
 Rao and Seshadri
6. ——— Suryaprakasa Rao .. *Ibid.* 1941, 14, 105.
 and Seshadri
7. Suryaprakasa Rao and .. *Ibid.*, 1939, 9, 177.
 Seshadri
8. Baker, Nodzu and Robinson .. *J. C. S.*, 1929, 74.