

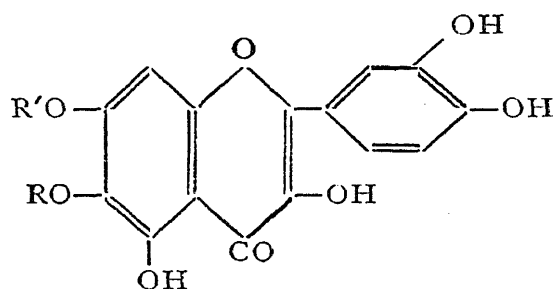
# ISOLATION AND CONSTITUTION OF PATULITRIN

BY N. R. BANNERJEE AND T. R. SESHADRI, F.A.Sc.

(From the Department of Chemistry, University of Delhi, Delhi)

Received September 6, 1956

THE isolation of patuletin<sup>1</sup> from the flowers of *Tagetes patula* (French marigold) and the establishment of its constitution<sup>2</sup> as 6-methoxy-3:5:7:3':4'-pentahydroxy flavone (I a) has already been reported. In the course of this work the presence of a glycoside in small quantities was recorded. Subsequently, from a number of test extractions it was found that the mature flowers, particularly late in the season, contained no glycoside and only early season flowers and especially buds contained appreciable amounts and fresh flowers were the most suitable for extraction. A similar observation has been made with the flowers of *Tagetes erecta* which also yield the glucoside, quercetagitrin (I b) only when collected in the early season. Using fresh flush of flowers and buds of *T. patula*, it has now been possible to isolate the glycoside in a crystalline condition. Its solubility and other properties are similar to those of quercetagitrin. On hydrolysis with mineral acid it yields patuletin and one mole of glucose. The position of the sugar unit has been established by complete methylation of the glycoside and hydrolysis of the product yielding 7-hydroxy-3:5:6:3':4'-pentamethoxy flavone,<sup>3</sup> whose identity has been confirmed by ethylation and mixed melting point determination of the methyl-ethyl ether with a synthetic sample.<sup>4</sup> The new glycoside is, therefore, patuletin 7-mono-glucoside (I c) and has been named patulitrin.



I a R=CH<sub>3</sub>; R'=H

b R=H; R'=glucose residue

c R=CH<sub>3</sub>; R'=glucose residue

The occurrence of quercetageitin (nor-patuletin) also as 7-monoglucoside (I b) in the closely related plant *T. erecta* would appear to be significant and suggest that the partial methylation of quercetagitrin possibly leads to the formation of patulitrin which eventually produces patuletin by hydrolysis. In an earlier publication by Pankajamani and Seshadri<sup>5</sup> various possible methods leading to the formation of partial methyl ethers of flavonoids in nature were discussed. Protection of reactive hydroxyl groups (e.g., 7-hydroxyl) by glycoside formation was recognised as one of the important

methods involved. In the present case of patulitrin the other hydroxyl groups in 3, 3' and 4'-positions should have been protected by other mechanisms.

#### EXPERIMENTAL PROCEDURE

*Isolation.*—Petals of fresh flowers (5 lb.) were extracted thrice with boiling rectified spirits (3 litres) for 12 hours each time. The combined extract (9 litres) was concentrated under reduced pressure to recover most of the solvent whereby reddish-brown petrol-soluble waxy matter separated out of the aqueous alcoholic concentrate. It was filtered off and the filtrate exhaustively extracted with ether. On standing in the refrigerator for a month, it deposited pale-yellow crystals (1 g.). When crystallised from acetone methanol mixture it came out as pale-yellow needles, m.p. 253–54° (d.). It was soluble in ethyl and methyl alcohols, sparingly soluble in acetone and water and insoluble in ether. It gave an olive brown colour with ferric chloride and reddish pink colour with magnesium and hydrochloric acid and readily dissolved in aqueous sodium hydroxide (1%), carbonate (5%) and borax (5%) yielding bright yellow solutions. Neutral and basic lead acetate solutions precipitated red-coloured lead salts from alcoholic solutions of the glycoside.  $R_F$  (circular) values are 0.77 (phenol saturated with water, 34°), 0.64 (water saturated with phenol, 34°), 0.58 (butanol-acetic acid-water, 40:10:50, upper layer, 36°) and 0.59 (butanol-acetic acid-water, lower layer, 36°) (Found in a sample dried at 120°: C, 52.9; H, 4.3;  $C_{22}H_{22}O_{13}$  requires C, 53.4; H, 4.5%).

*Hydrolysis.*—The glycoside (0.15 g.) was boiled under reflux with aqueous sulphuric acid (50 c.c.; 7%) for 2 hours. The aglucone was taken up in ether and the aqueous solution exhaustively extracted with ether. On removing the solvent, the extract gave a yellow solid residue (0.1 g.) which crystallised from aqueous alcohol as yellow needles, m.p. and mixed m.p. with patuletin, 260–61°.  $R_F$  and mixed  $R_F$  0.77 (phenol saturated with water, 34°) and 0.45 (water saturated with phenol, 34°). Acetate, m.p. and mixed m.p. with patuletin acetate, 176–78°. The aglucone was methylated using dimethyl sulphate and anhydrous potassium carbonate in dry acetone medium. The methyl ether crystallised from aqueous alcohol as colourless needles, m.p. and mixed m.p. with O-pentamethyl patuletin, 141–42°.

The aqueous acid solution was divided into two equal portions. One portion was neutralised with barium carbonate, filtered and the filtrate was concentrated to small bulk (25 c.c.).  $R_F$  and mixed  $R_F$  with glucose 0.56 (phenol-water, 9:1, 30°). With phenyl hydrazine acetate, the solution yielded glucosazone, m.p. and mixed m.p., 205° (d.). The second portion

was neutralised with sodium bicarbonate and titrated against standard Benedict' reagent; the yield of glucose agreed with the requirements of a monoglucoside.

*Methylation of the glucoside and hydrolysis.*—A finely powdered suspension of the glucoside (0.1 g.) in anhydrous acetone (50 c.c.) was boiled with dimethyl sulphate (1 c.c.; excess) and anhydrous potassium carbonate (3 g.) for 50 hours till the product gave no ferric chloride colour. The semi-solid residue, obtained by filtration and distilling off the solvent from the filtrate, was boiled with aqueous sulphuric acid (50 c.c.; 7%) for 2 hours. There was clear solution and a colourless solid separated out gradually from it. It was taken up in ether, the solution shaken up with aqueous sodium carbonate (5%) and the carbonate extract acidified. When the product was extracted with ether and the solution evaporated a colourless solid (50 mg.) was obtained. It crystallised from alcohol as colourless long plates and needles, m.p. 231–33° (Found in a sample dried at 110°: C, 62.1; H, 5.3;  $C_{20}H_{20}O_8$  requires C, 61.9; H, 5.2%). It did not give any ferric reaction. Rao and Seshadri<sup>5</sup> reported 234–35° as the melting point of 7-hydroxy-3:5:6:3':4'-pentamethoxy flavone obtained by a similar treatment of quercetagitrin.

*Ethylation.*—The partial methyl ether (25 mg.) was boiled with diethyl sulphate (0.2 c.c.) in anhydrous acetone (25 c.c.) in the presence of anhydrous potassium carbonate (1 g.) for 4 hours. The ethyl ether crystallised from acetone-benzene as colourless rectangular plates, m.p. 128–30°, and mixed melting point with a synthetic sample<sup>4</sup> of 7-ethoxy-3:5:6:3':4'-pentamethoxy flavone was undepressed.

#### SUMMARY

A new glucoside has been isolated from the flowers of *Tagetes patula*. It yields by direct hydrolysis patuletin and glucose and by hydrolysis after complete methylation 3:5:6:3':4'-O-pentamethyl quercetagetin, characterised also as its ethyl ether. The glycoside is, thus, patuletin 7-mono-glucoside and is, therefore, named patulitrin.

#### REFERENCES

1. Rao and Seshadri .. *Proc. Ind. Acad. Sci.*, 1941, **14 A**, 643.
2. ————— .. *Ibid.*, 1945, **22 A**, 215.
3. ————— .. *Ibid.*, 1941, **14 A**, 289.
4. Rajagopalan and Seshadri .. *Ibid.*, 1948, **28 A**, 36.
5. Pankajamani and Seshadri .. *Jour. Sci. and Industr. Res.*, 1955, **12 B**, 127.