

NEW TESTS FOR THE IDENTIFICATION OF KHELLIN, VISNAGIN AND KHELLOL-GLUCOSIDE

BY M. Z. BARAKAT AND N. BADRAN

*From the Biochemistry Department, Faculty of Medicine, Abbassia, Ibrahim Pasha
El-Kebir University, Cairo, Egypt*

Received May 21, 1951

AMMI VISNAGA fruit yields three definitely crystalline substances, khellin, visnagin and khellol-glucoside, which have been isolated in a chemically pure form and their molecular and structural formulæ established^{1,2,3,4}. Khellin is the most active pharmacologically, being an antispasmodic^{5,6}. Visnagin is less active and khellol-glucoside is devoid of the antispasmodic action of khellin⁷.

Khellin is generally dispensed in the form of tablets or solutions for injection. The determination of the melting point⁸ of the extracted khellin from injections and tablets is required as a test for purity. The identification tests, so far known, for khellin, are the rose-red colour developed with solid sodium or potassium hydroxide⁹, the deep orange colour obtained with concentrated sulphuric acid¹⁰ and the wine red colour produced with iodine and potassium hydroxide solution¹¹. Up to the present, no identity tests which differentiate between the three well-defined constituents of *Ammi Visnaga* have been given. In this paper, new qualitative tests to identify khellin, visnagin and khellol-glucoside are described. Most of the tests are believed to be due to the formation of oxonium salts of khellin, similar to those obtained by direct treatment of khellin with sulphuric acid and hydrochloric acid¹². The oxonium salts are stable in the dry solid state for a long time, but decompose almost quantitatively in aqueous solution or in dilute ammonia at room temperature to give khellin. The oxonium salts are practically insoluble in organic solvents, e.g., benzene, toluene, acetone and chloroform. Attempts to recrystallise the oxonium salts of khellin from the various solvents were unsuccessful and, therefore, the solutions were simply filtered, washed with dry ethyl acetate, dried and the melting-points determined. The alloxan and ninhydrin tests are believed to be due to a condensation reaction between the active carbonyl group of the reagent and the 2-methyl group of either khellin or visnagin.

These qualitative tests have been applied on pure khellin, visnagin, and khellol-glucoside using 0.1 g. of the substance.

IDENTIFICATION TESTS

(1) *Phosphoric acid*. To 0.1 g. add 2 ml. of phosphoric acid B.P.:

Khellin: orange-red crystals, which dissolve on heating. The oxonium phosphate was also prepared and isolated as follows: 0.5 g. of powdered khellin was dissolved in 1 ml. of syrupy phosphoric acid to give an orange, viscous solution. On the gradual addition of 50 ml. of dry ethyl acetate, with trituration, a yellow, crystalline solid separated, which

KHELLIN, VISNAGIN AND KHELLOL-GLUCOSIDE

was filtered off, washed and dried. Yield 0.7 g. The oxonium salt occurs as yellow crystals which melt with decomposition at 126°C. to form an orange liquid. When treated with distilled water these crystals split into pure khellin (m.pt. 154°C.) and phosphoric acid.

Visnagin : a pale yellow colour.

Khellol-glucoside : an intense yellow colour.

(2) *Ammonium molybdate*. Dissolve 0.1 g., by warming, in 5 ml. of ethanol (50 per cent.), add 1 ml. of an 8 per cent. aqueous ammonium molybdate solution and heat for 2 minutes; add 2 ml. of 20 per cent. sulphuric acid and warm :

Khellin : an orange, crystalline precipitate, m.pt. 195°C. (decomp.).

Visnagin : a yellowish-green colour and a green deposit.

Khellol-glucoside : a blue colour, due to the reduction of molybdic acid to molybdenum blue by the glucose liberated by hydrolysis.

(3) *Sodium tungstate*. Dissolve 0.1 g. in 5 ml. of ethanol (50 per cent.) by warming, add 1 ml. of sodium tungstate solution (10 per cent.) and heat for 2 minutes; add 2 ml. of dilute sulphuric acid (20 per cent.) and warm :

Khellin : an orange, crystalline precipitate, m.pt. 205°C. (decomp.).

Visnagin : a yellow solution, which gives a yellow deposit on cooling.

Khellol-glucoside : a yellow colour, and on cooling colourless crystals of the glucoside deposit.

(4) *Potassium ferrocyanide*. To 0.1 g. dissolved in 5 ml. of ethanol (50 per cent.) by warming, add 2 ml. of potassium ferrocyanide solution (5 per cent.) and heat for 2 minutes; add 2 ml. of 20 per cent. sulphuric acid and warm :

Khellin : an orange, crystalline precipitate, the supernatant liquid assumes a greenish-blue colour.

Visnagin : a yellow, crystalline precipitate.

Khellol-glucoside : a blue colour.

(5) *Mayer's reagent*. Dissolve 0.1 g., by warming, in 5 ml. of ethanol (50 per cent.), add 2 ml. of Mayer's reagent B.P. and heat for 2 minutes; add 2 ml. of 20 per cent. sulphuric acid :

Khellin : an orange, crystalline precipitate.

Visnagin : a yellow, crystalline precipitate.

Khellol-glucoside : a yellow colour, but no deposit.

(6) *Potassium pyroantimonate*. Dissolve 0.1 g. of substance, by warming, in 5 ml. of ethanol (50 per cent.), add 2 ml. of potassium pyroantimonate solution (5 per cent.), and warm for 2 minutes; add 2 ml. of 20 per cent. sulphuric acid :

Khellin : an orange-yellow precipitate.

Visnagin : a greenish-yellow precipitate.

Khellol-glucoside : a white precipitate.

(7) *Platinic chloride*. Dissolve 0.1 g., by warming, in 5 ml. of ethanol (50 per cent.), add 1 ml. of platinic chloride solution (10 per cent.) and 2 ml. of concentrated hydrochloric acid and heat for 2 minutes :

Khellin: a red solution, which deposits orange crystals on cooling; m.pt. 202° to 204° C. (decomp.). The crystals decompose in distilled water at room temperature, giving pure khellin, m.pt. 154° C.

Visnagin: an orange solution, which deposits, on cooling, orange crystals m.pt. 183° to 185° C. (decomp.). The crystals decompose in distilled water at room temperature giving pure visnagin m.pt. 142° to 143° C.

Khellol-glycoside: an orange solution; colourless crystals of the glucoside on standing.

(8) *Antimony trichloride*. To 0.1 g. dissolved, by warming, in 5 ml. of ethanol (50 per cent.) add 2 ml. of antimony trichloride reagent (prepared by dissolving 15 g. of antimony trichloride in 40 ml. of distilled water and 20 ml. of concentrated hydrochloric acid):

Khellin: an orange, crystalline precipitate; recrystallised from dry ethyl acetate, m.p.t. 160° C. to 161° C., decomposing to give a red melt. The crystals decompose in distilled water at room temperature giving pure khellin.

Visnagin: a yellow precipitate.

Khellol-glucoside: a white precipitate, which dissolves on shaking and deposits again on standing.

(9) *Alloxan*. To 0.1 g. in a dry test tube add 0.1 g. of alloxan, 2 ml. concentrated sulphuric acid (poured down the side of the test tube), and warm for 1 minute:

Khellin: a dark green colour; on pouring 2 drops into 10 ml. of distilled water, a purple colour is obtained and on making alkaline with sodium hydroxide solution (30 per cent.) the purple colour changes to yellowish-green.

Visnagin: a dark red colour; on pouring 2 drops into 10 ml. of distilled water a buff precipitate is obtained; and on making alkaline with sodium hydroxide solution (30 per cent.) the precipitate dissolves giving a yellow solution with a red tinge.

Khellol-glucoside: a dark red colour; on pouring 2 drops into 10 ml. of distilled water, a pale red solution is obtained, which darkens on adding sodium hydroxide solution (30 per cent.).

(10) *Acetic anhydride*. To 0.1 g. in a dry test tube add 0.5 ml. of acetic anhydride, 2 ml. of concentrated sulphuric acid (poured down the side of the tube) and heat for 2 minutes:

Khellin: a dark red colour; on pouring a few drops into 10 ml. of distilled water, a yellowish-green fluorescence is observed and on making alkaline with sodium hydroxide solution (30 per cent.), the solution assumes a red colour.

Visnagin: a dark green colour; on pouring a few drops into 10 ml. of distilled water, a pale purple colour with a colloidal reddish-brown precipitate appears; on making alkaline with sodium hydroxide solution (30 per cent.) the solution shows a green fluorescence.

Khellol-glucoside: a dark greenish brown colour; on pouring a few drops into 10 ml. of distilled water, a wine red colour is obtained, which

KHELLIN, VISNAGIN AND KHELLOL-GLUCOSIDE

remains unchanged when made alkaline with sodium hydroxide solution (30 per cent.).

(11) *Ninhydrin*. To a few mg. of ninhydrin in a dry test-tube, add 2 ml. of concentrated sulphuric acid (poured down the side of the tube); a pale rose-red colour is developed. On adding a few crystals of the substance under test:

Khellin: a brilliant green colour.

Visnagin: a purple colour, which darkens on standing.

Khellol-glucoside: a wine red colour.

SPOT TESTS FOR KHELLIN, VISNAGIN AND KHELLOL-GLUCOSIDE

(1) *Phosphoric acid test*. To a few crystals, on a white porcelain slab, add 2 drops of syrupy phosphoric acid (sp. gr. 1.750).

Khellin: orange red crystals.

Visnagin: a yellow colour.

Khellol-glucoside: an intense yellow colour.

(2) *Alloxan test*. To a few crystals, on a white porcelain slab, add a few crystals of alloxan and 5 drops of concentrated sulphuric acid, and triturate with a thin glass rod until the solids go into solution.

Khellin: initial colour orange; after 20 minutes a violet colour with an orange edge appears.

Visnagin: initial colour yellow; after 20 minutes, a red colour with a green edge is observed.

Khellol-glucoside: initial colour yellow, remaining unchanged after 20 minutes.

(3) *Ninhydrin test*. To a few crystals, on a white porcelain slab, add a few crystals of ninhydrin and 5 drops of concentrated sulphuric acid, and triturate with a thin glass rod.

Khellin: an intense green colour.

Visnagin: a purple colour which darkens on standing.

Khellol-glucoside: a wine-red colour.

Ninhydrin alone, gives with concentrated sulphuric acid, a rose-red colour.

(4) *Antimony chloride test*. To a few crystals, on a white porcelain slab, add 3 drops of the antimony chloride reagent, previously described.

Khellin: deep orange crystals.

Visnagin: a light yellow colour.

Khellol-glucoside: a dull yellow colour.

SUMMARY

(1) New qualitative tests which distinguish between khellin, visnagin and khellol-glucoside in the pure state are described; they are applied on 0.1 g. of the substance.

(2) These tests, at least in the case of khellin, are believed to be due to the formation of oxonium salts. Khellin phosphate occurs as yellow crystals m.pt. 126°C. with decomposition to an orange melt.

(3) Spot tests for khellin, visnagin and khellol-glucoside are described. They can be carried out on a few mg. of the substance.

REFERENCES

1. Fantl and Salem, *Biochem. Ztschr.*, 1930, **226**, 166.
2. Wahba Saleh Abdel-Malek, *Thesis on the constitution of Ammi Visnaga fruit, Faculty of Science, Fouad 1st University*, 1932.
3. M. Khairy Hassan, *Thesis on the structural formula of the glucoside of Ammi Visnaga fruit, Faculty of Science, Fouad 1st University*, 1932.
4. Spath and Grüber, *Ber. dtsh. chem. Ges.*, 1938, **71**, 106; 1941, **74**, 1492; 1941, **74**, 1549.
5. Samaan, *Brit. J. Urol.*, 1933, **5**, 213.
6. Anrep, Barsoum, Kenawy and Misrahy, *Lancet*, 1947, **254**, 557; *Brit. Heart J.*, 1946, **8**, 171.
7. Anrep, Kenawy, Barsoum and Riad Fahmy, *Gaz. Fac. Med. Cairo*, 1947, **14**, No. 1.
8. Fahmy, Badran and Messeid, *J. Pharm. Pharmacol.*, 1949, **1**, 535.
9. Fahmy and El-Keiy, *Rep. Pharm. Soc. Egypt*, 1931, **3**, 36.
10. Abdel Rahman, *Thesis, Faculty of Medicine, Fouad 1st University*, 1943.
11. Fahmy, Badran and Messeid, *J. Pharm. Pharmacol.*, 1949, **1**, 535.
12. Moubasher and Barakat, *J. Amer. chem. Soc.*, 1950, **72**, 2807.