

KHELLIN AND ITS ASSAY IN INJECTIONS AND TABLETS

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THE most important constituent of *Ammi Visnaga* fruits, as regards pharmacological activity, is khellin, $C_{14}H_{12}O_5$, m.pt. 153° to $155^\circ C$. Khellin is a furano-chromone derivative with a pronounced antispasmodic action on smooth muscle viz.: ureter, bronchial muscle, intestine and bile duct^{1,2}. It has, moreover, a specific dilator action on the coronary arteries which makes it useful in the treatment of angina pectoris².

Besides khellin, the fruits contain two other constituents which have been isolated in chemically pure form and their molecular and structural formulæ established viz.: a glucoside, khellol-glucoside³, $C_{13}H_9O_5 \cdot C_6H_{11}O_5 \cdot 2H_2O$, m.pt. $175^\circ C$. and visnagin⁴, $C_{13}H_{10}O_4$, m.pt. 142° to $145^\circ C$., both of which are also furano-chromone derivatives. The glucoside is devoid of the antispasmodic action of khellin while visnagin has a much lower activity than khellin³. The fruits contain about 1 per cent. of pure khellin, 0.1 per cent of pure visnagin and 0.3 per cent. of pure khellol-glucoside.

Khellin is usually obtained from the powdered fruits by extracting with ether or light petroleum, concentrating the extract and leaving it to crystallise; or alternatively the powdered fruits are extracted with alcohol, the alcohol is distilled and the residue extracted with chloroform. After distillation of the chloroform, crude khellin is obtained. By both methods, khellin is extracted in association with visnagin; the glucoside being insoluble in ether, in light petroleum and in chloroform. Khellin must be purified by several crystallisations from alcohol to free it from impurities and from visnagin. Nowadays, a number of pharmaceutical laboratories in Egypt prepare khellin on a semi-large scale. It is generally dispensed in the form of tablets or injectable solutions.

Up to the present, no standard description of khellin has been given. In this communication, the description, solubilities, identification, purity test of khellin and the method of its assay in the above preparations are described.

Description.—Khellin (2-methyl-5:8-dimethoxy-6:7-furano-chromone, Mol.Wt. 260), occurs in colourless needle-shaped crystals, odourless, taste bitter. It should contain not less than 99 per cent. of $C_{14}H_{12}O_5$.

Solubilities.—Khellin is very soluble in chloroform, less soluble in cold ether and in light petroleum, more soluble in the hot liquids. Soluble at $25^\circ C$. in 130 parts of alcohol (95 per cent.) in 6750 parts of water⁵, in 500 parts of a saturated aqueous solution of theophylline, and in 33 parts of a saturated solution of sodium benzoate. It is soluble in glacial acetic acid and in dilute mineral acids from which it is regained unchanged.

Identification Tests.—(a) When one drop of 0.01 per cent w/v solution

in alcohol or in water is added to a piece of solid sodium or potassium hydroxide, a rose red colour is developed within 2 minutes^{7,8}.

(b) When a few crystals are treated with 1 drop of concentrated sulphuric acid on a white porcelain plate, a deep orange colour is developed which on dilution with water becomes yellow⁸.

(c) When a solution of 10 mg. in 2 ml. of alcohol (50 per cent.) is poured on a freshly prepared mixture of 0.5 ml. of N/2 iodine and 0.5 ml. of 10N potassium hydroxide solution, a yellow colour is formed followed by a yellow precipitate, which redissolves gradually on shaking, imparting to the solution a wine red colour.

Test for Purity.—m.pt. 153° to 155°C.

INJECTION OF KHELLIN

In consequence of the greater solubility in water in presence of theophylline or sodium benzoate, solutions for injection in an aqueous medium containing one or both of these compounds are already to be found on the Egyptian market.

Identification and Test for Purity.—Place in a separating funnel a volume of the injection equivalent to about 0.2 g. of khellin. Extract the khellin from this solution with three successive quantities, each of 10 ml. of pure benzene. Evaporate the combined benzene extracts to dryness on a water-bath and dry the residue at 100°C. The residue should comply with the tests for khellin.

Assay.—In solutions for injection, khellin may be assayed colorimetrically by the sulphuric acid method⁶. Dilute a volume of the solution equivalent to about 0.1 g. of khellin with distilled water to 100 ml. Dilute 10 ml. of this dilution to 50 ml. Measure 2.5 of this dilution in a dry colorimeter tube, add 10 ml. of 10 N sulphuric acid, leave to stand for about 5 minutes and read the percentage transmission in a photoelectric colorimeter against water as the blank, set at 100 per cent. transmission using blue filter No. 420. Read the amount of khellin corresponding to the percentage transmission from a calibration table, prepared under the same conditions, using standard dilutions of pure khellin. The result obtained, multiplied by 200, gives the amount of khellin present in the original volume taken.

TABLETS OF KHELLIN

Identification and Test for Purity.—Triturate a quantity of the powdered tablets, equivalent to 0.2 g. of khellin, with two successive quantities, each of 10 ml., of chloroform. Filter, evaporate the chloroform to dryness, on a water-bath, and dry the residue at 100°C. The residue should comply with the tests for khellin.

Assay.—Weigh and powder 20 tablets; treat an accurately weighed quantity of the powder, equivalent to about 0.1 g. of khellin, on a dry filter with successive small quantities of hot alcohol (95 per cent.) until the khellin is completely extracted. Concentrate the alcoholic extract to about 15 ml. and transfer it to a volumetric flask of 100 ml. capacity, washing the flask with two successive quantities, each of 5 ml., of alcohol

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(95 per cent.) and make up to volume with distilled water. Dilute 10 ml. of this solution to 50 ml. with distilled water. Carry out the assay, using 2.5 ml. of this dilution, as described for injections. The result obtained, multiplied by 200, gives the amount of khellin in the original weight taken.

DISCUSSION

The determination of the melting point of the extracted khellin from injections and tablets is required as a test for purity. If the khellin used is contaminated with visnagin or with other impurities from the fruits, the product will begin to melt below 140°C. In such cases the result of the assay may be reported as "total chromones of *Ammi Visnaga* fruits calculated as khellin."

In the extraction of khellin from injections, benzene is used instead of chloroform, to avoid the extraction of any theophylline present, theophylline being insoluble in benzene. Theophylline and sodium benzoate do not interfere with the method of assay.

SUMMARY

- (1) The characters and identification tests of khellin are described.
- (2) The standards of purity of khellin used in pharmaceutical preparations is given.
- (3) The method of assay of khellin in injections and tablets is described.

REFERENCES

1. Samaan, *Brit. J. Urol.*, 1933, **5**, 213.
2. Anrep, Barsoum, Kenawy and Misrahy, *Lancet*, 1947, 557; *Brit. Heart J.*, 1946, **8**, 171.
3. Spath and Gruber, *Ber. dtsh. chem. Ges.*, 1941, **74**, 1549.
4. Spath and Gruber, *ibid*, 1941, **74**, 1492.
5. Anrep, Kenawy, Barsoum and Riad Fahmy, *Gaz. Fac. Med., Cairo*, 1947, **14**, No. 1.
6. Fahmy Badran and Messeid, *J. Pharm. Pharmacol.*, 1949, **1**, 529.
7. Fahmy and El-Keiy, *Rep. Pharm. Soc. Egypt*, 1931, Vol. **3**.
8. Abdel-Rahman, *Thesis, Faculty of Medicine, Fouad Ist University*, 1943.