# SHORT COMMUNICATIONS

## STUDIES ON MELILOTUS INDICA (LINN.) ALL

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### Introduction

*M. indica*, locally known as Sinji, belonging to the family *Leguminosae* grows abundantly in the plains of Pakistan[1] and throughout the tropical areas of Pakistan and India.[2] The seeds are used in bowel complaints and infantile diarrhoea. The whole plant is used as discutient and emollient; externally it is used for fomentation, and as a poultice or plaster for swellings.[2] The other species of the plant namely *M. alba* and *M. officinalis* have been studied by many workers and coumarin reported as the major component.[2] M. indica was taken up for detailed chemical investigation as no work on it has been reported so far. As a result of the present study three crystalline compounds have been isolated melting at 69°, 75° and 139°. The compound melting at 69° was identified as coumarin from its colour reaction[3] as well as by taking its mixed melting point with an authentic sample and comparing the m.p. of their derivatives. The infrared spectrum was also found to be identical with that of the authentic sample. The second compound melting at 75° was shown to be a saturated non-reducing, aliphatic alcohol. It forms acetyl derivative m.p. 60-61° which on hydrolysis yielded the original compound. The identification of the compound is in progress. The third compound melting at 139° gave positive Liebermann Burchard Test and was identified as β-sitosterol by preparing its acetyl and benzoyl derivatives. It was also observed that the coumarin content is higher in the fresh plant and decreases gradually when the plant dries. It may be due to the fact that coumarin sublimes at ordinary temperature. The results are given in the Table 1.

### Experimental

The fresh plant (1.72 kg.) was cut into small pieces and percolated with 95% alcohol. The percolate was concentrated under reduced pressure to a syrupy liquid. This was extracted with ether and the aqueous layer preserved for identification of the sugars.

TABLE 1.—DECREASE IN COUMARIN CONTENTS OF THE PLANT ON DRYING IN THE AIR AT ROOM TEMPERATURE.

| S. No. | Weigh of the plant kg. | Period of drying days | Weight of coumarin g. |
|---|---|---|---|
| 1. | 1.72 | 1 | 1.96 |
| 2. | 1.72 | 15 | 0.991 |
| 3. | 1.72 | 30 | 0.4317 |
| 4. | 1.72 | 60 | 0.1391 |
| 5. | 1.72 | 120 | Practically nil. |

The ether extract of the concentrate was dried over anhydrous sodium sulphate and filtered. The residue on complete removal of the solvent was taken up in petroleum ether. A small quantity of a dark brown insoluble residue was discarded.

The petroleum ether solution was concentrated and adsorbed on a column (24 × 3 cm.) of alumina (May and Baker Ltd. Alumina for Chromatography). It was first eluted with petroleum ether and four fractions of 100 ml. each were collected, and then with acetone collecting two fractions of 100 ml. each.

*Petroleum Ether Fractions.*—Each fraction was charcoaled, dried over anhydrous sodium sulphate and solvent removed. The first fraction was oily in nature (0.081 g.) and was not pursued further. The second and third fraction, on removal of the solvent gave a crystalline deposit, m.p. 65°. On recrystallisation from petroleum ether colourless, shining needles m.p. 69°C. (1.96 g., 0.80%) were obtained. The last fraction gave another crystalline compound m.p. 65° which on recrystallisation from petroleum ether yielded colourless aggregate crystalline compound melting at 74-75°.

*Acetone Fractions.*—Both the fractions were charcoaled and concentrated to yield a solid, melting at 132°, which on recrystallisation from petroleum

ether yielded shining plates, m.p. 139°C. (0.52 g., 0.20%).

*Identification of Isolated Products.*—Compound Melting at 69°C: This was identified as coumarin by taking its mixed m.p. with the authentic sample. Its infra-red spectrum is identical with that of an authentic sample of coumarin. The compound gave a positive colour test for coumarin as follows. It gave green colour when dissolved in hot potassium hydroxide, which when viewed through UV light showed a yellow green fluorescence.[3]

Further it forms an adduct with mercuric chloride like coumarin. Mercuric chloride (2.0 g.) was dissolved in 30 ml. of 25% alcohol and 0.5 g. of the compound was added. This was warmed to dissolve the substance and allowed to stand at room temperature. Long shining, colourless needles appeared after an hour which were filtered, m.p. 158°. On recrystallisation from 25% alcohol finally melted at 163° (m.p. of the adduct reported in literature is 164°.[4])

*Compound Melting at* 75°.—This was soluble in cold chloroform, benzene, and carbon disulphide, in hot alcohol, ether, ethyl acetate, and petroleum ether, and insoluble in water. It does neither decolourise potassium permanganate solution and bromine water nor does it reduce Fehling's solution showing it to be saturated and non-reducing. Its acetyl derivative was prepared by normal method and the product m.p. 54° was recrystallised from alcohol (m.p. 61°). On hydrolysis the original compound was obtained showing that it is an alcohol.

*Compound Melting at* 139°.—This compound was identified as β-sitosterol by taking a mixed m.p. with an authentic sample which showed no depression. The acetyl and benzoyl derivatives were prepared according to normal methods and melted at 127.0° and 144.0°, respectively (m.p. of acetyl derivative and benzoyl derivative recorded in literature are 128° and 145.5°, respectively.)[5]

*Identification of Sugars.*—The original water soluble fraction after extraction with ether was examined by paper chromatography. Ten sugars were indicated by using n-butanol :acetone: water (4:5:1) and Whatman chromatography paper No. 2. Of these only glucose, fructose, sorbose and raffinose could be identified by comparison with the spots of authentic samples. Glucose and fructose were confirmed by preparing their osazones. Two more osazones in crystalline form melting at 198° and 226° were obtained. The first one was identified as cellobiose from its m.p. and also comparing its crystalline shape under microscope with the crystalline shape of an authentic sample given in literature. This could not be confirmed due to the non-availability of an authentic sample of cellobiose. The other osazone melting at 226° could not be identified.

*Preparation of Osazones.*—The original aqueous extract (50 ml.) was placed in a 100 ml. conical flask and 1.0 g. of phenylhydrazine hydrochloride and 1.5 g. of sodium acetate were added. The flask was loosely stoppered and warmed in a boiling water bath with occasional shaking. The precipitate appeared after about 45 minutes. It was cooled and filtered. To the filtrate more of phenylhydrazine hydrochloride (0.5 g.) was added and the same process was repeated when another crop of precipitate appeared after 15 minutes. This was filtered off. To the filtrate again phenylhydrazine hydrochloride was added, and the third and fourth fractions of osazones were obtained after intervals of 15 minutes. All these were crystallised from 60% alcohol. The osazones were those of glucose, fructose, cellobiose and lastly an unidentified osazone, m.p. 226°.

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## References

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