SOME CHEMICAL CONSTITUENTS OF THE LEAVES OF Mirabilis jalapa L.

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Chromatographic and spectroscopic methods were employed for analysis of the leaves of *Mirabilis jalapa* L. Light petroleum extract contains hydrocarbons (17.8%), ketones (18.0%), alcohols (12.1%), sterols (21.2%), acids (7.0%) and an unidentified residue (23.9%). The presence of leucine, valine, tryptophan, alanine, glycine, tartaric and citric acids in the aqueous-ethanolic extract were detected by co-descending paper chromatography.

Mirabilis jalapa L. (Nyctagenaceae) is an important Indian medicinal plant widely used. The juice of the fresh leaves is very soothing and allays the itching when applied against urticaria^{1,2}. Trigonelline³, betaxanthins and betacyanin^{4,5} have been isolated from this plant. The seeds has been mentioned to contain cinnamic acid and mixture of quercetin and campherol glycosides⁶. In the view of the important medicinal properties of the leaves and the fact that almost no systematic work has been done so far a chemical study of the leaves of this plant has been undertaken to obtain the interesting compounds, the results being presented in this communication.

EXPERIMENTAL

Extract. Air-dried leaves (2 kg) of Mirabilis jalapa L. from the surroundings of Aligarh district (India) were extracted thrice with 15 l light petroleum $(60-80^{\circ}\text{C})$ at boiling temperature. A total of 6.0 g (0.3%) purified extract was obtained from which an acidic portion (7.0%) was separated.

Chromatography. Column chromatography and thin layer chromatography were done on activated alumina and silica gel respectively. Elution of the plate with tetrachloromethane with a trace of ethyl acetate gave spots of the following R_F values: hydrocarbons 0.8, ketones 0.7. Elution with tetrachloromethane-ethyl acetate (95:5) gave spots of R_F values: alcohols 0.57 and sterols 0.47. Gas chromatography was done on PYE series 104 Chromatograph Model 124 with flame ionisation detectors, two columns packed with 3% SE-30 on Gas Chrom Z at 230°C or at programmed temperature 150-250°C (2°C min⁻¹). For identification of homologues the usual graphical method was employed⁷. For a quantitative evaluation areas under the peaks have been calculated. The comparison of the retention values with those of standards was used for identification of the gaschromatographic peaks. For descending paper chromatography (Whatman No 1) the system 1-butanol-formic acid-water (4:1:5 v/v, organic layer) was used for deve-

loping. Bromophenol blue⁸ (for carboxylic acids) and ninhydrin^{9,10} (for amino acids) served as spraying agents after drying the paper.

Spectroscopy. 1R spectra were recorded in a Perkin Elmer spectrometer in KBr discs. The 1 H-NMR spectrum (δ -scale) was recorded on Varian spectrometer A-60A in CDCl₃ using tetramethylsilane as the internal standard. The mass spectrum was recorded at 75 eV with direct inlet system at 160° C.

RESULTS

The extract solved in ether was treated with 5% KOH and separated into neutral and acidic fractions. The purified neutral portion was taken in benzene and subjected to chromatographic separation on alumina. Following fractions — homogeneous at thin layer chromatography — were obtained:

Hydrocarbons. Elution with light petroleum (17·8%) and crystallisation from ethanol-acetone gave a waxy solid m.p. $59-60^{\circ}$ C. It represents according to GLC-analysis the homologous series of n-alkanes $C_{23}-C_{35}$ with a maximum occurrence of odd numbered members C_{31} , C_{29} , C_{27} and C_{25} (Table I). An other series of compounds probably branched alkanes¹¹ is also present in small amounts.

Ketones. Futher elution with light petroleum-benzene (80:20) (18·0%) and crystallisation from ethyl acetate-methanol gave a compound m.p. $69-70^{\circ}$ C. It exhibited positive test for carbonyl compounds and has been identified as 12-tricosanone (lit. 12 m.p. 69° C). In its IR spectrum bands at 1725 (CO) and 722 cm⁻¹ (CH₂)_n appeared. The preparation of 2,4-dinitrophenylhydrazone (m.p. $75-76^{\circ}$ C) and oxime 13 (m.p. $35-36^{\circ}$ C) confirmed its identity.

Alcohols. Another fraction (12·1%) was obtained on elution with light petroleum-benzene (60:40). GLC analysis indicated the presence of the homologous series of primary alcohols $C_{20} - C_{32}$ where even numbered members were prevailing. After

TABLE I
Composition of n-Alkanes According to GLC Analysis

| | Number of C-atoms | % | Number of C-atoms | % | |
|---|----------------------|------------|-------------------|-------------|--|
| 1 | 23 | 0.7 | 20 | 17.2 | |
| | 23 24 | 0·7 4·4 | 29 30 | 17·3 8·0 | |
| | 25 | 10.3 | 31 | 17.9 | |
| | 26 | 9.8 | 32 | 2.4 | |
| | 27 | 17.0 | 23 | 2.2 | |
| | 28 | 10.0 | 34-35 | traces | |

some crystallisations from methanol n-hexacosanol m.p. 80°C could be isolated (lit. 14 m.p. 79·5°C) exibiting bands at 1050, 3420 (OH) and 721 cm⁻¹ (CH₂)_n in the IR spectrum. Its acetate 15 had m.p. 65-66°C.

Sterols. On elution with light petroleum-benzene (40:60) the fraction of sterols was obtained. It gave positive Liebermann-Burchard test and yellow colour with tetranitromethane. Repeated crystallisation from methanol afforded a compound, m.p. $134-135^{\circ}$ C, identified as β -sitosterol by the IR. PMR (refs^{16,17}) and mass spectrum^{18,19} by mixed m.p. determination and by comparison of retention values on thin layer chromatography. Its acetate and benzoate were also identical with authentic derivatives²⁰. According to GLC-analysis of the crude fraction β -sitosterol (90%), stigmasterol (7%) and camposterol (2%) were the main components.

Free acids. The purified acidic fraction was taken in benzene and subjected to chromatographic separation on silica gel. Elution with benzene supplied a compound m.p. $84-85^{\circ}$ C (methanol). It has been identified as tetracosanoic acid (lit.²¹ m.p. $84\cdot15^{\circ}$ C), possessing in IR spectrum bands at 725 (CH₂)_n, 1720 (CO), 2855 and 2935 cm⁻¹ (CH). The methyl ester was identical with authentic specimen (m.p. $55-56^{\circ}$ C, lit.²¹ $56\cdot5^{\circ}$ C). GLC analysis of mother liquors after crystallisation (in form of methyl esters) showed that other homologues are also present in minute amounts.

Non-volatile carboxylic acids and amino acids. The defatted leaves of the plant material were extracted with aquous ethanol (1:1) at its boiling temperature and concentrated in vacuo. A syrupy liquid was obtained which showed the presence of non-volatile carboxylic acids and amino acids. The extract was employed along with authentic specimens of the various non-volatile carboxylic acids by means of descending paper chromatography. The presence of citric and tartaric acids was thus established. With chloroform decoloured concentrated extract was used for characterisation of amino acids together with the reference samples^{9,10}. The presence of glycine, alanine, tryptophan, valine and leucine was thus confirmed.

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