

Natural Products from *Ruellia tuberosa* L.

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Natural products have been isolated and identified from petroleum ether extract of *Ruellia tuberosa* L. using chromatographic and spectroscopic methods: (i) *n*-alkanes (C_{11} - C_{27}) with the maximum occurrence of *n*-tritriacontane (C_{32} -18.0%), *n*-pentatriacontane (C_{34} -11.2%), *n*-hentriacontane (C_{31} -9.6%) and *n*-nonacosane (C_{29} -7.7%); (ii) Esters were identified as methyl esters of acid series composed mainly of C_{22} -14.0%, C_{26} -13.7%, C_{28} -16.4% and C_{34} -17.9% and alcohol series mainly of triacontanol (C_{30} -60.0%); (iii) Sterols: stigmasterol 51.0%, β -sitosterol 33.5%, campesterol 11.9% and cholesterol 2.3%.

RUELLIA tuberosa L. (Acanthaceae) is an important widely used Indian medicinal plant¹. Apigenin 7- β -D glucuronoid² and malvidine 3, 5-diglucoside³ have been isolated from this plant. In view of the important medicinal properties of the plant and the fact that almost no systematic work has been done on it, a chemical study of this plant has been undertaken. The present note deals with the isolation and characterisation of chemical constituents from its petroleum ether extract using chromatographic and spectroscopic methods.

Experimental

Air-dried plant material (800 gm) from the surroundings of Aligarh District was extracted thrice with 10 L light petroleum (60-80°) for 36 hrs at its boiling temperature. A total of 3.2 gm (0.4%) extract obtained after removal of volatile impurities was subjected to column chromatography over alumina (30 fold excess) for further purification which yielded two main fractions, one consisting of a mixture of hydrocarbons and esters while the other consisting of sterols.

Fraction 'A':

On elution with light petroleum (60-80°) and crystallisation from acetone-ethanol gave (265 mg) waxy solid, m. p. 65-66° which exhibited two spots (tlc silica gel, R_f values 1.0 and 0.8; developer tetrachloromethane with a trace of ethyl acetate). The mixture was thus rechromatographed over silica gel which yielded two products. The first one, obtained on elution with hexane, was analysed by GLC on PYE series 104 chromatograph model 124 equipped with flame ionization detector and column packed with 3% SE-30 on Gas Chrom Z at 150-300°. A homologous series of *n*-alkanes (C_{11} - C_{27}) accompanied by another series of branched alkanes in small amounts was identified on the basis of comparison of the retention values with

those of the standard. For a quantitative evaluation areas under the peaks have been calculated. The distribution pattern of *n*-alkanes (Table) has been found to be bi-modal⁴ (higher members with odd prevalence, while lower without it).

TABLE—COMPOSITION OF *n*-ALKANES ACCORDING TO GLC ANALYSIS SHOWING bi-MODAL PATTERN

No. of C atoms	% of <i>n</i> -alkanes	No. of C atoms	% of <i>n</i> -alkanes	No. of C atoms	% of <i>n</i> -alkanes
C_{11} - C_{18}	traces	C_{22}	1.0	C_{31}	9.6
C_{19}	0.5	C_{24}	1.8	C_{32}	7.8
C_{17}	0.7	C_{26}	2.6	C_{33}	18.0
C_{16}	1.0	C_{28}	3.5	C_{34}	5.5
C_{15}	0.8	C_{27}	4.4	C_{35}	11.2
C_{20}	1.2	C_{29}	5.4	C_{36}	1.6
C_{21}	1.1	C_{30}	7.7	C_{37}	2.9
C_{23}	1.1	C_{30}	7.1		

The second component obtained on elution with chloroform exhibited characteristic ir peak at 1740 cm^{-1} indicating it to be ester. This ester was transesterified with dry methanol and gaseous hydrogen chloride and the mixture of methyl esters of the acids and free alcohols was gas chromatographed. It was found that the original esters are formed by a mixture of normal acids (C_{18} - C_{38}) with the maximum participation of C_{24} -17.9%, C_{26} -16.4%, C_{30} -13.7% and C_{34} -14.0% homologues, esterified by a series of primary alcohols (C_{18} - C_{38}) with a maximum occurrence of triacontanol (C_{30} -60.0%). Even members of the alcohol series as well as of the acid series were found to be predominant⁴⁻⁵. According to the results obtained earlier⁶⁻⁷ the single chromatographic peak represents mixture of esters.

Fraction 'B':

Light petroleum-benzene (7:3 v/v) eluate yielded a fraction comprising of phytosterol (tlc

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silica gel, Rf value 0.28 ; developer tetrachloromethane with a trace of ethyl acetate). It gave positive Liebermann-Burchard test and yellow colour with tetranitromethane. Its ir spectrum revealed the presence of OH group. Since this sterol even after crystallization was not found to be identical with any of the available authentic samples (co-tlc ; m.m.p.), it was supposed to be mixture of sterols, which could not be resolved further even by repeated column chromatography. This mixture was, therefore, analysed by combined gas chromatography-mass spectrometry (GC-MS) and the sterols were separated as their trimethylsilyl ether (prepared in BSA/pyridine) which exhibited diagnostic ions (M^+)⁸⁻¹⁰.

The trimethylsilyl ethers were shown to contain four sterols which were identified by direct comparison (GC-MS) with those of authentic samples as (i) stigmasterol 51.0%, (ii) β -sitosterol 33.5%, (iii) campesterol 11.9% and (iv) cholesterol 2.3%. However, on repeated crystallization from methanol only β -sitosterol (165 mg) could be isolated (m.p. 137°). This was identified by ir, m.m.p., and by comparison of retention values on thin layer chromatography. Its acetate m.p. 122° and benzoate m.p. 145° were also identical with authentic derivatives¹¹ of β -sitosterol.

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