CHEMICAL EXAMINATION OF *AMARANTHUS SPINOSUS* LINN.

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ABSTRACT

The petroleum-ether extract of *Amaranthus spinosus* L. on application of chromatographic techniques afforded *n*-alkanes C_{23}–C_{33} and iso (-2-methyl)–alkanes C_{29}–C_{33}; esters : transesterified and resolved into acids C_{18}–C_{32} (as methylesters) and free alcohols C_{20}–C_{26}; aliphatic alcohols C_{10}–C_{32}; sterols (*β*-sitosterol, stigmasterol, campesterol and cholesterol) and free acids C_4–C_{32} (C_{18} group contains stearic acid, oleic acid and linoleic acid). The free amino acids identified are leusine, valine, tryptophan, alanine and serine in the aqueous ethanolic extract.

AMARANTHUS SPINOSUS LINN. (Amaranthaceae) is an important medicinal plant growing wild locally and used to cure various diseases. The ash of the leaves is used in dyeing and the plant as a whole is used in snake-bite and as fodder[1–2]. The leaves of the plant contains oxalic acid[3] and sugar[4] while hemaglutins[5] and betacyanin[6] are present in seeds. In view of the important medicinal properties of the herb and the fact that almost no systematic work has been done so far, a chemical examination of this herb has been undertaken, the results are presented in this paper.

EXPERIMENTAL

Air-dried and powdered plant material (2 kg) collected from surroundings of Aligarh District (India) was extracted thrice with 15 lit. distilled light petroleum (60–80°) at its boiling temperature, which after removal of the solvent under reduced pressure gave a semi-solid mass (1·18%). This extract was separated into acidic (25·2%) and neutral parts (65·4%) by washing with KOH solution (10%). The purified neutral fraction (15 g) was taken in benzene and subjected to chromatographic separation over alumina (~ 400 g) and two major fractions were collected by eluting the column with petroleum-ether (60–80°) and benzene.

Petroleum-ether fraction (4·2 g) on crystallisation from acetone-alcohol yielded a colourless waxy solid (400 mg), m.p. 62–63 C°. It showed two spots on T.L.C. (silica gel-2% $AgNO_3$) and therefore was resolved on a column of slicica gel into two parts. Hydrocarbon fraction was eluted with hexane and the ester fraction with chloroform. The separated portions were further analysed by G.L.C. [Gas chromatography was done on PYE series 104 Chromatograph Model 124 with flame inonisation detectors, two column packed with 3% SE 30 on Gas Chrom Z at programmed temperature 150–250 °C (2° C min^{-1}). For identification of homologues the graphical method was employed[7]. The comparison of the retention values with those of standards was used for identification of the gas chromatographic peaks. For a quantitative evaluation areas under the peaks have been calculated.]

Hydrocarbons form two homologous series. One of the series consists of *n*-alkanes (C_{23}–C_{33}) with maximum occurrence of *n*-hentriacontane (C_{31}) (18·1%), *n*-non-acosane (C_{29}) (15·4%), *n*-heptacosane (C_{27}) (9·0%), *n*-octacosane (C_{28}) (8·3%), *n*-triacontane (C_{30}) (8·0%), *n*-dotriacontane (C_{32}) (4·9%), *n*-tritriacontane (C_{33}) (4·5%) and *n*-hexacosane (C_{26}) (3·9%) while *n*-tricosane (C_{23}), *n*-tetracosane (C_{24}) and *n*-pentacosane (C_{25}) were in traces. The other series consists of iso (= 2-methyl)-alkanes (C_{29}–C_{33}) with a maximum of C_{31} (21·1%), C_{29} (3·4%) and C_{33} (2·1%) while C_{30} and C_{32} were in traces; the structure of 2-methyl ocotacosane (C_{29}) and 2-methyl triacontane (C_{31}) in this series was established by G.L.C. combined with mass spectrum. Odd numbered members were prevalent[8–9] in both the series.

Ester fraction showed characteristic i.r. bands at 1176, 1727 cm^{-1} and was transesterified with methanol (+HCl) and the resulting products were gas chromatographed (as methyl esters of the acid and free alcohols). The series of *n*-acids (C_{18}-C_{32} in the form of methyl esters) contained mainly C_{30} (47·7%), C_{28} (28·9%) C_{32} (8·6%); C_{18}, C_{20}, C_{22}, C_{24} and C_{29} were in small amounts (1·0–3·0%) while C_{19}, C_{21}, D_{22}, C_{25}, C_{26}, C_{27} and C_{31} were in traces. The even numbered members were found to be predominating. The series of *n*-alcohols (C_{20}, C_{22}, C_{24}, C_{26}) appeared in traces.

Benzene eluate (8·5 g) was further chromatographed over alumina (~ 250 g) and yielded two products.

Alcohols.—Elution with light petroleum-benzene (60:40) gave the alcohol fraction. It exhibited bands at 1050, 3400 (OH) and 715, 725 cm^{-1} $(CH_2)_n$ in i.r. spectrum (KBr phase). When analysed by G.L.C. it gave a series of aliphatic alcohols C_{10}-C_{34}; even numbered members were predominating, with maximum occurrence of C_{28} (45·3%), C_{30} (19·1%), C_{22} (11·6%), C_{32} (9·6%), C_{26} (6·4%) and C_{24} (2·9%) while C_{14} C_{21} were present in small amounts and other members were in traces. However it is interesting to mention that even numbered members were not prevalent between C_{14} C_{21} in the same series. Branched chain alcohols were also present in minute amounts.

From this mixture *n*-octacosanol (C_{28}), m.p. 83–84° C (300 mg) (lit.[10] m.p. 83·4 °C) could only be crystallised from methanol (T.L.C.-homogeneous) and confirmed by preparation of its acetate[11] m.p. 75–76° C.

Sterols.—On further elution with light petroleum benzene (20: 80) the fraction of sterols (3·4 g) was obtained. It gave positive Liebermann-Burchard test and yellow colour with TNM. Repeated crystallisation from ethanol afforded shining crystals (350 mg) m.p. 158–69° C (T.L.C.-homogeneous). The i.r. spectrum of the sterol exhibited peaks at 3410 (OH) and 1610 cm^{-1} (C = C). It showed depression in m.p. when mixed with authentic specimen of β-sitosterol and gave different R_f values (Co-T.L.C.). The crystalline sterol yielded its acetate m.p. 173° C (ν_{max}^{KBr} 1730, 1245 $cm.^{-1}$) and benzoate m.p. 185–86 °C G.L.C. analysis of the product indicated it to be a mixture of β-sitosterol (83·0%), stigmasterol (12·3%) campesterol (2·9%) and cholesterol (1·6%). It is worth mentioning that the presence of cholesterol is a unique finding in a lower plant (herb).

Acids.—The acidic fraction (2·2 g) was subjected to chromatographic purification over silica gel (~ 80 g). Elution with light petroleum-benzene (1 : 3) afforded a colourless waxy substance (330 mg) m.p. 78–81 °C and showed peaks in i.r. spectrum (KBr) at 718, 728 $(CH_2)_n$; 1710 (CO); 2850 and 2920 cm^{-1} (CH). T.L.C. indicated it to be a mixture which could not be resolved further even by repeated column chromatography. This product on G.L.C. analysis (after esterification) represented a series of acids C_4–C_{32} with C_{16} (27·5%), C_{18} (17·2%), C_{28} (15·5%), C_{30} (10·7%), C_{24} (9·8%), C_{22} (6·0%), C_{26} (6·1%) and C_{23} (2·5%) while the other members were found in traces. C_{18} group contained saturated and unsaturated acids and was further analysed by G.L.C. (3% diethyleneglycol succinate, at 140°C on polar phase) which indicated it to be a mixture of stearic acid (32·6%), oleic acid (52·6%) and linoleic acid (14·7%). Branched chain acids were also present in minute amounts.

Aqueous ethanolic extract.—The defatted plant material was also extracted with aqueous ethanol (1 : 1) at its boiling temperature and concentrated *in vacuo*. A dark brown syrupy liquid was obtained which showed the presence of amino acids. The concentrated extract was extracted with chloroform to remove colouring matter. Descending paper chromatography[8,12] (Whatman No. 1) of the aqueous alcoholic extract in *n*-butanol : acetic acid : water (4: 1: 5, v/v organic layer) indicated the presence of leucine, valine, tryptophan, alanine and serine (when sprayed with ninhydrin 0·1% acetone) as free amino-acids and confirmed by direct comparison with their respective reference samples.

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