♣HBr-Reactive Acids of *Malva sylvestris* Seed Oil

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ABSTRACT

Malva sylvestris seed oil contained 5.6% sterculic, 11.0% malvalic, 1.6% vernolic, 15.6% lauric, 6.6% myristic, 26.6% palmitic, 5.6% palmitoleic, a trace of stearic, 23.0% oleic and 4.0% linoleic acids. The co-occurrence of malvalic and sterculic acids was established by gas liquid chromatography (GLC) of the silver nitrate-methanol treated esters using *Sterculia foetida* esters as the reference standard. Co-occurrence of epoxy acid (vernolic acid) was confirmed with *Vernonia anthelmintica* as the lipid standard.

INTRODUCTION

Malva sylvestris Roxb. is an erect glabrous annual, 3-5 ft high. The flowers are ca. 1-1½ in. in diameter, pale rose streaked with purple. It is found throughout Punjab and on the W. Himalaya, extending to Europe, North America and Siberia (1).

Many reports have appeared about naturally occurring HBr-reactive (cyclopropene and epoxy) acid. Recently, cyclopropenoid and epoxy fatty acids have been the subject of much investigation because of their profound biological effects on animals and their cocarcinogenic properties. A biological oddity is that malvaceous seed oils containing cyclopropenoid fatty acids usually also contain epoxy fatty acids (2). This paper characterizes and estimates the individual HBr-reactive (cyclopropene and epoxy) acids in the seed oil of *M. sylvestris* and is the first report of its fatty acid composition.

EXPERIMENTAL PROCEDURES

The methyl esters were prepared (3) from *M. sylvestris* seed oil in the same way as for other Malvaceous oils containing cyclopropenoid fatty acids. Gas liquid chromatography (GLC) was done with a Perkin-Elmer Model 154 equipped with a thermal conductivity detector and a diethylene glycol succinate column. Separations were carried out isothermally at 200 C with a hydrogen flow of 70 mL min^{-1}. GLC data are given as area percentages with *Sterculia foetida* esters as a reference standard.

RESULTS AND DISCUSSION

The seed oil, n_D^{30} 1.4880, was obtained from *M. sylvestris* seeds and yielded 16.6%, which contained 1.6% unsaponifiables. It responded to Halphen test (4) and picric acid TLC test (5) indicating the presence of cyclopropenoid and epoxy fatty acids. The oil showed the typical nuclear magnetic resonance (NMR) signal at 9.2τ for the hydrogens of cyclopropene moiety. Both the oil and its methyl esters had the characteristic infrared (IR) absorptions at 1852 cm^{-1} and 1010 cm^{-1} (cyclopropene moiety) and at 848 cm^{-1} and 828 cm^{-1} (epoxy group). Quantification of these acids by HBr-titration at 2 different temperatures (6) demonstrated that 18.2% of the reactive material was present in the glyceride oil. The UV spectrum of the oil indicated that no conjugation existed in the component acids.

The 11.0% malvalate and 5.6% sterculate were calculated by GLC analysis of the silver nitrate-methanol treated *M. sylvestris* esters compared with *S. foetida* esters. The oil showed 0.08% oxirane oxygen equivalent to 1.6% epoxy-oleic acid. Acetylation of the oil, followed by saponification, gave *threo*-12,13-dihydroxyoleic acid (m.p. and mixed m.p. 54-55 C). Quantitative determination of the oxirane content of the oil was in fair agreement with the amount of corresponding isolated dihydroxy acid. The unsaturated dihydroxy acid analyzed for $C_{18}H_{34}O_4$ had an IR absorption at 3400-3500 cm^{-1} (hydroxyl). IR showed no *trans* absorption. The unsaturated diol acid on hydrogenation (Pt catalyst) consumed 0.98 mol equivalent of hydrogen (one double bond) to yield *threo*-12,13-dihydroxystearic acid (m.p. and mixed m.p. 96-97 C). Comparison of the mobility behavior of saturated and unsaturated diols with those of authentic samples on direct and boric acid TLC (7) further demonstrated its identity.

Oxidative cleavage (8) of the saturated and unsaturated dihydroxy acids showed the unsaturated compound was 12,13-dihydroxyoleic acid derived from 12,13-epoxyoleic acid. The dihydroxy acid was the *threo* isomer and, therefore, the epoxide function has the *cis* configuration. The 16.6% total cyclopropenoid acids by GLC is in good agreement with HBr-titration determination.

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