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SHORT COMMUNICATION

WAXES AND STEROLS OF *ERYTHRINA SUBEROSA* BARK

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Abstract—The petroleum ether extract of the bark of *Erythrina suberosa* was fractionated into wax esters, wax alcohols and acids, alkyl ferulates, stigmasterol, sitosterol, campesterol and cholesterol.

IN INDIA, *Erythrina stricta* Roxb., *E. suberosa* Roxb., and *E. variegata* L. var. *orientalis* (L.) Merr. (=*E. indica* Lam.) (Leguminosae) have been used in the treatment of various ailments.[1] During systematic investigation of these species for alkaloidal and nonalkaloidal constituents, the bark of *E. suberosa* has been examined and is reported here. This bark had not been studied previously. However, the constituents of *E. indica* bark have been studied. Alkaloids were reported present, and although none was isolated in pure form,[2] the presence of hypaphorine was later indicated.[3] Docosyl alcohol and sitosterol have also been isolated.[4]

In this work, only traces of alkaloids were detected. The petroleum ether extract of the bark instead deposited a granular solid which on chromatography over alumina yielded aliphatic wax esters, *n*-aliphatic wax alcohols, alkyl ferulates, and *n*-aliphatic wax acids. The residual oily petroleum ether extract was saponified and chromatographed over alumina yielding *n*-aliphatic wax alcohols and sterols.

The wax alcohols isolated from the granular solid and from the oily extract were both shown by GLC to consist solely of an approximately equal mixture of *n*-hexacosyl and *n*-octacosyl alcohols. In sharp contrast, the alcohols obtained on saponification of the wax esters and alkyl ferulates contained no wax alcohols, but rather a mixture of fatty and other unknown saturated alcohols of which *n*-octadecyl alcohol predominated. The free and esterified wax acids were analyzed by GLC of their methyl esters and shown to be an essentially identical mixture of homologs with *n*-hexacosanoic acid predominant plus a large amount of *n*-octacosanoic acid and a small amount of *n*-tetracosanoic acid. Waxes such as

* Maintained at Madison, Wis., U.S.A., in co-operation with the University of Wisconsin.

[1] R. N. CHOPRA, S. L. NAYAR and I. C. CHOPRA, *Glossary of Indian Medicinal Plants*, p. 111, C.S.I.R., New Delhi (1956).

[2] R. N. CHOPRA, S. GHOSH and B. N. SEN, *Ind. J. Med. Res.* **22**, 265 (1934).

[3] P. S. RAO, C. V. RAO and T. R. SESHADRI, *Proc. Ind. Acad. Sci.* **7A**, 179 (1938).

[4] D. S. BHAKUNI and N. M. KHANNA, *J. Sci. Ind. Res.* **18B**, 494 (1959).

[5] J. W. ROWE, C. L. BOWER and E. R. WAGNER, *Phytochem.* **8**, 235 (1969); and references cited therein.

these and ferulic acid esters appear to be common in tree barks,[5] although fatty rather than wax alcohols in the esters is unusual.

The sterols were analyzed by GLC of the TMS ethers which showed stigmasterol (63 per cent), sitosterol (31 per cent), campesterol (5 per cent), and cholesterol (1 per cent). The presence of stigmasterol was confirmed by the i.r. spectrum of the crude sterols, which was identical to sitosterol, but showed the characteristic peak at 971 cm^{-1} for the *trans*-disubstituted double bond of stigmasterol. Small amounts of cholesterol in plants have been reported,[6] and this is probably quite common.

EXPERIMENTAL

Fractionation of the Petroleum Ether Extractives

The bark powder (1·2 kg) was soxhlet extracted with petroleum ether (b.p. 60–80°). During the extraction process, a granular solid (5·5 g) deposited which was removed by filtration. The rest of the extract was concentrated and allowed to cool to room temperature. The granular solid (1·0 g), which again separated, was removed. The supernatant liquid was concentrated to give an oily mass (16 g).

The granular solid (6·5 g) was chromatographed on an alumina column (S. Merck, 195 g). Elution with petroleum ether–benzene (5:8) gave a residue (0·60 g), which was crystallized from $CHCl_3$, m.p. 87–88° (wax esters). Elution with petroleum ether–benzene (1:4) gave a residue (1·10 g), which was crystallized from acetone, m.p. 84–85° (wax alcohols). Elution with $CHCl_3$ and with $CHCl_3$–EtOH (9:1) gave a residue (1·80 g), a portion of which was crystallized from petroleum ether, m.p. 79–80° (alkyl ferulates). Finally, elution with $CHCl_3$–HOAc gave a residue (1·0 g) which was refluxed with petroleum ether. The petroleum ether soluble part was crystallized, m.p. 83–83·5° (wax acids).

The oily mass (16 g) was saponified by refluxing with 100 ml of 1 N alc. KOH, and 5·50 g of unsaponifiables were obtained in the usual way. The unsaponifiables were chromatographed on alumina (S. Merck, 137·5 g). Elution with petroleum ether gave 2·10 g of a complex mixture. Elution with petroleum ether–benzene (1:4) yielded a residue (0·52 g) which was crystallized from acetone, m.p. 84–84·5° (wax alcohols). Elution with benzene gave a solid (0·24 g) which was crystallized from methanol, m.p. 153° (sterols).

Wax Esters

The i.r. spectrum showed an ester carbonyl at 1738 and $(CH_2)_x$ bands at 730 and 720 cm^{-1}. The fraction was saponified by refluxing in 2 N alc. KOH for 4 hr in N_2, poured into water, acidified, and extracted with ether. The ether layer was methylated with CH_2N_2 and chromatographed on alumina (Woelm, neutral, Act. II). Petroleum ether eluted wax acid methyl esters and benzene eluted the wax alcohols.

The wax acid methyl esters were analyzed by GLC on an SE-30 column (3% on Anakrom ABS, 6 ft × $\frac{1}{8}$ in., 237°). The analysis showed 20:0 (trace), 22:0 (2%), 24:0 (14%), 25:0 (1%), 26:0 (50%), 27:0 (trace), 28:0 (31%), and 30:0 (trace).*

The wax alcohols were analyzed by GLC on the same column at 235° (for C_{20} to C_{30} alcohols) and at 185° (for C_{12} to C_{20} alcohols) analogously to the wax acid methyl esters. The analysis showed 14:0 (trace), 15:0 (trace), 16:0 (13%), 17:0 (6%), 18:0 (39%), 19:0 (3%), and two unknowns (13% and 26%).* The two unknowns did not correspond to *n*-aliphatic unsaturated alcohols, and no vinylic hydrogens were visible in the NMR of the wax alcohols.

Wax Alcohols

The wax alcohols from the granular solid and from the oily extract had identical i.r.'s, ν_{max} 3420 (OH) and 730 and 720 cm^{-1} [$(CH_2)_x$]. They were analyzed as before and showed 26:0 (54%) plus 28:0 (46%) and 26:0 (58%) plus 28:0 (42%), respectively.

Alkyl Ferulates

The NMR, u.v. and i.r. spectra were essentially identical to authentic wax alcohol ferulate.[5] The ester was saponified by refluxing in 2 N alc. KOH for 4 hr in N_2, concentrated, diluted with water, and extracted with ether to yield the alcohols. These alcohols were analyzed as before by GLC and showed 14:0 (trace), 15:0 (6%), 16:0 (10%), 17:0 (3%), 18:0 (33%), 19:0 (10%), 20:0 (7%), and the same two unknowns as in the wax esters (10% and 20%, respectively).

* Percentages on GLC are determined by normalization of peak areas.

[6] H. SINGH, V. K. KAPOOR and A. S. CHAWLA, *J. Sci. Ind. Res.* **28**, 339 (1969); J. W. ROWE, *Phytochem.* **4**, 1 (1965).

The aqueous layer, after extraction of the alcohols, was acidified and extracted with ether. The product was identical in R_f to authentic ferulic acid when chromatographed in acetone–water (1:1) 3% NaCl + 0·1 N HCl, and with water-saturated benzene–acetic acid (125:72) and had the same colour reactions.[7]

Wax Acids

The i.r. spectrum showed carboxyl (1715) and methylene (730 and 720 cm^{-1}). The acids were esterified with CH_2N_2 and analyzed as before by GLC and showed 22:0 (trace), 24:0 (8%), 25:0 (trace), 26:0 (62%), 27:0 (trace), 28:0 (29%), and 30:0 (trace).

Sterols

The sterols formed a digitonide, and were identical to crude sitosterol by TLC. The sterols were analyzed by GLC of their TMS ethers on 1% SE-30 on Anakrom SD, 10 ft × ⅛ in., 260°, and compared with standard sterol ethers.

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[7] M. J. Strohl and M. K. Seikel, *Phytochem.* **4**, 383 (1965).