1. INTRODUCTION

*Origanum* L. is a taxonomically complex genus containing approximately 26-30 species. Its geographical distribution extends from North Africa and Western Europe to Central Asia. One species *Origanum vulgare* has a very wide distribution from the Azores to Taiwan, whereas most species are endemic to a single island or mountain. The centre of diversity is in the Mediterranean basin where over 70% of the species are found. They usually occur in mountainous regions ranging from 500 to 1500 metres in altitude in rocky places with calcareous soils. The species of *Origanum* like so many other Mediterranean labiates, are distinctly shrubby or at least woody at the base, and rich in volatile oils. These oils are found in glands which are distributed mainly in the leaves. The genus has been variously treated in the past, some authors recognizing a number of distinct genera e.g. (2), others recognizing only sections e.g. (1): both treatments are still in current use (3, 11).

2. PROCEDURE

2.1. Material and Methods

In the course of a phytochemical survey of *Origanum* and related genera, dry leaf-material from herbarium specimens and from fresh samples of *Origanum* species grown from seeds in the Plant Science Laboratories glasshouse complex was used.

30 species of *Origanum* were examined for flavones, flavanols, flavone C-glycosides and cinnamic acids in leaf tissue. Most of the flavonoid constituents were completely characterized. Representative species of related genera were also screened for their flavonoid constituents.

2.1.1. Two-dimensional paper chromatography of direct leaf extracts.

One gram of fresh leaves, or 100 mg of dried material was ground and extracted 2 x with MeOH-H₂O (4 : 1). 2D-PCs (Whatman No 1) were spotted with the extract from ca 50 mg of plant material (dry wt) and run in n-BuOH-
-HOAc-H₂O (4:1:5, BAW) and 15% HOAc. UV-absorbing spots were circled then fumed with ammonia vapour, any colour changes recorded and Rf values were calculated (BAW and 15% HOAc). Tables 1, 2, 3 show the distribution of spots 1-19 in the Origanum and related genera species with their Rf values in five different solvents and tentative identification of flavone glycosides.

2.1.2. Identification of glycosides by acid-hydrolysis. For the aglycone moiety of the glycosides and the identity of sugars, a small portion of the purified sample was treated with 2N HCl at 100°C for 1.5 hours (for glucuronides) or the sample was treated with 2N HCl at 100°C for 30 minutes (where no glucuronides were suspected). After acid hydrolysis, resultant mixture was extracted with ethyl acetate (1:1), the upper EtOAc layer containing the (EtOAc) aglycone moiety, and the lower aqueous phase containing sugars, were concentrated separately. The concentrate of the upper layer was diluted with 1-2 drops of 95% EtOH (ethanol), spotted onto Whatman No 1 chromatography paper, run one-dimensionally in the solvents; n-BuOH-HOAc-H₂O (4:1:5, BAW), conc. HCl-HOAc-H₂O (3:3:10, Forstal), phenol-H₂O (4:1), with the aglycone markers.

The lower aqueous layer which contained sugars was dried on a rotatory evaporator to remove HCl, then dissolved in deionized water (1-2 drops) and chromatographed one dimensionally on Whatman No 1 paper in solvents phenol-H₂O (4:1), n-BuOH-benzene-pyridine-H₂O (5:1:3:3, B B P W). Reference sugars were glucose, galactose, xylose, arabinose, rhamnose and glucuronic acid. The developed chromatograms were dipped in aniline hydrogen phthalate reagent (6), dried, and heated (110°C) for 5-10 minutes. Sugar spots appeared brown or orange.

The identity of flavonoid glycosides was established by a combination of per-methylation, mass and UV spectroscopy and co-chromatography with authentic markers.

2.1.3. Identification by UV spectroscopy. Glycoside samples were dissolved in 100% MeOH and their UV absorption spectra measured between 225 and 450 nm (Pye Unicam recording spectrophotometer). Spectra were recorded before and after the addition of small amounts of diagnostic inorganic reagents (aqueous sodium hydroxide (2N), solid anhydrous sodium acetate and boric acid, or 5% aluminum chloride in EtOH). The wavelength maximum shifts, according to the location of various functional groups on the aromatic (A/B) rings (6, 7). (Fig. 1 shows a typical representation of UV