acids esterified to epidermal cell-wall polysaccharides, lignin in sclerenchyma tissue and cutin in cuticles. Cuticles of many monocot and dicot species fluoresced when excited with UV-A radiation (Harris and Hartley, 1980; Hartley and Harris 1981). It is uncertain, however, if UV-A-excited fluorescence also arises in part from flavonoids which have been reported to occur in cuticular waxes (Wollenweber and Dietz, 1981). Trichomes, both glandular and non-glandular, can also contribute to superficial auto-fluorescence (Karabourniotis and Fasseas, 1996; Meyer *et al.*, 2003; Agati *et al.*, unpublished data).

Tissue localisation of compounds exhibiting weak or undetectable auto-fluorescence, including many polyphenolics, can be obtained by using specific fluorescence intensifiers (Hutzler *et al.*, 1998). A widely used staining procedure for leaf sections, to visualise flavonoids, employs solutions of Naturstoff reagent (NR) which is diphenylboric acid 2-aminoethylester (Dai *et al.*, 1995; Schnitzler *et al.*, 1996; Hutzler *et al.*, 1998). This reagent was previously used to detect flavonoids in thin layer chromatography by inducing secondary fluorescence (Markham, 1989). However, the products formed by reaction of specific flavonoids with NR have not yet been identified, and we must also be aware that NR is not totally specific for flavonoids. Indeed, it reacts also with hydroxycinnamic acids and their derivatives by forming fluorescent products (Agati *et al.*, 2002). Separation of fluorescence from different classes of compounds can be obtained only by using different excitation wavelengths (Tattini *et al.*, 2004).

Alkaline conditions also induce strong fluorescence in both flavonoids (Schnabl *et al.*, 1986; Markham, 1989) and hydroxycinnamic acids (Harris and Hartley, 1976; Meyer *et al.*, 2003). Because of this lack of specificity, this alkaline test cannot be easily applied to localise specific compounds in leaf sections. However, the combination of the alkali treatment with appropriate excitation wavelengths can help to discriminate between different phenolics.

6.3 Electronic absorption of radiation

Electronic absorption of radiation is a key factor determining plant surface properties. Substantial absorption at the short-wavelength edge of natural radiation, that is the UV-B region, depends on the presence of linear or cyclic compounds exhibiting four or more conjugated double bonds (Cockell and Knowland, 1999). The normal components of plant waxes lack such conjugated double bond systems (see Chapter 4). Accordingly, dissolved cuticular wax from needles of two conifer species did not absorb significantly above 300 nm (Bornman and Vogelmann, 1988) and penetration of UV radiation into needles of the conifer *Picea pungens* was not affected by wax removal (Day *et al.*, 1992); similarly, wax extraction had little effect on the UV absorption of isolated cuticles (Baur *et al.*, 1998). Epicuticular matter from *Olea chrysophylla* also absorbed very little natural UV (Karabourniotis *et al.*, 1992) and epicuticular material from *Pinguicula vulgaris* showed only a single peak at 277 nm which is outside the UV-B range (Mendez *et al.*, 1999).