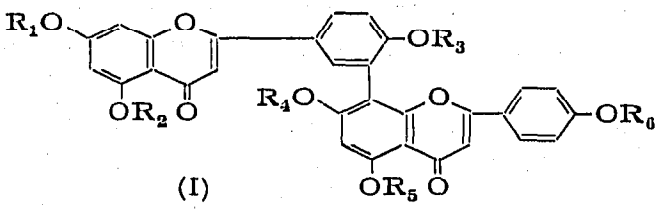
Chromatographic separation of biflavonoid compounds

Some pigments have recently been isolated from plants of the Coniferae family (in particular from *Taxus, Torreja, Sciadopitys* genus). Such compounds can be considered as flavonoids, and structure (I) has now been proposed for them[1-3].



(I)

We have isolated from *Taxus baccata* L. some pigments of this class: one of them has been identified as sciadopitysin (I) where $R_1 = R_2 = R_6 = CH_3$ and $R_3 = R_4 = R_5 = H$, while the formulae of the two remaining pigments are still being discussed[4-6].

In order to obtain a quick separation and a satisfactory identification of the various biflavones, isolated from vegetable extracts, we have developed an analytical method based on the different mobilities of the above compounds on paper.

With the classical solvent systems the separations, using either the ascending or the descending technique on different chromatographic papers, have not been satisfactory, because of the too high migration speeds of the examined biflavones; the R_F values of the test substances are too close together, as shown in Table I.

Among the various solvent systems tested chloroform saturated with water is recommended as the most satisfactory.

Table II shows that the R_F values of biflavones with this solvent system are well spaced, thus allowing an efficient separation not only of the above biflavones, but also of biflavonoid from flavonoid compounds, which do not move under these conditions.

Flavonoid compounds

Sciadopitysin, m.p. 294–296°, from leaves of *Taxus baccata* L.[4].
Flavone 212, m.p. 212–215°, from leaves of *Taxus baccata* L.[5].
Flavone 310, m.p. 310° (dec.), from leaves of *Taxus baccata* L.[5].

TABLE I

R_F VALUES OF SOME FLAVONOID COMPOUNDS

Compound	*Acetic acid–water (2:3)*	*Isopropyl alcohol–water (3:2)*	*Ethyl acetate satd. with H_2O*
Quercetin	0.28	0.74	0.82
Sciadopitysin	0.80	0.95	0.95
Flavone 212	0.81	0.96	0.95
Flavone 310	0.81	0.96	0.95
Demethylsciadopitysin	0.69	0.95	0.93
Ginkgetin	0.94	0.96	0.95
Morin	0.61	0.78	0.74

TABLE II

R_F VALUES OF SOME BIFLAVONES

Solvent: Chloroform saturated with water.
Paper: Whatman No. 1. Detection: Visible and U.V. light.

Compound	R_F	Colour	
		Visible	*Ultraviolet*
Quercetin	—	yellow	bright yellow
Sciadopitysin	0.77	—	brown
Flavone 212	0.21	—	brown
Flavone 310	0.71	—	brown
Demethylsciadopitysin	—	—	brown
Ginkgetin	0.76	—	brown
Morin	—	yellow	bright yellow

Demethylsciadopitysin, m.p. 260–262°, prepared by demethylation of sciadopitysin[4].
Ginkgetin, m.p. 342–344°, from leaves of *Gingko biloba*.
Quercetin Merck for chromatography.
Morin Merck for chromatography.

Solvents

The solvent systems were:
(a) acetic acid–water (2:3, v/v);
(b) acetic acid–water (1:5, v/v);
(c) isopropanol–water (3:2, v/v);
(d) isopropanol–water (1:1, v/v);
(e) ethyl acetate saturated with water;
(f) chloroform saturated with water.

Chromatographic procedure

The flavonoid compounds were separated by one-dimensional chromatography with the descending method, using Whatman No. 1 paper sheets of 24 × 52 cm. The developing time at room temperature varied from 6 to 12 hours, according to the solvent system. About 0.5 mg of material per spot was applied to the sheets of paper as approximately 0.5 % solutions in methanol. For sciadopitysin, insoluble in methanol, a mixture of ethanol–methylene chloride (1:1) was used as solvent.

The chromatograms were developed, dried, then observed in U.V. light, where spots of flavonoid compounds exhibit a bright fluorescence and can be outlined in pencil.

The R_F values of the biflavones were then calculated on the basis of these results.

Istituto Chimico della Università,
Torino (Italy)

G. DI MODICA
A. M. RIVERO

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