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Note

Unexpected specificity of iodine–potassium iodide spray reagent for closely related flavones

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Iodine is used as its vapour or in solution for the detection of many organic compounds in thin-layer and/or paper chromatography[1–4]. Most compounds give a yellow to brown coloration, but in some cases an intense blue colour is obtained comparable to that in the reaction of iodine with starch. Such blue complexes are given by structurally totally unrelated compounds, *e.g.*, the alkaloid narceine[3], some steroids[4], the flavones apigenin and isovitexin-7-glucoside[5] and some quinones and phthaleins[5]. On the other hand, a close structural relationship does not necessarily result in similar complex formation. Of a series of 37 closely related Δ^4-3-keto-C_{21} steroids only four compounds gave blue spots with iodine[4].

The long-known reaction with isovitexin-7-glucoside[5] attracted our attention in connection with work on a number of chemical races of *Silene pratensis* (Rafn) Gordon and Gren[6,7]. These plants contain flavone glycosides based on isovitexin and/or vitexin[8]. Differences among populations are found in the flavone glycosylation pattern, and a colour reaction to distinguish these glycosides, and thus the different genotypes, would be very helpful.

EXPERIMENTAL

The main source of our compounds are the petals of genetically well defined races of *Silene pratensis* and *Silene dioica*, although some compounds were isolated from leaves of the same species. The basic flavones in these species are isovitexin and/or vitexin; these compounds can also be hydroxylated and methylated in the B-ring to give (iso)orientin and (iso)scoparin. O-Glycosylation occurs in the 7-position and at the 6- (or 8-)-2″-OH. In addition to the *Silene* compounds a number of

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randomly chosen flavonoids were investigated which were mainly obtained from commercial sources. Kaempferol-3-glucoside was isolated from *Larix* needles.

Methanolic extracts of petals or solutions of flavonoids were spotted on Whatman No. 1 or Schleicher & Schull No. 2043a chromatography paper or on Baker-flex cellulose thin-layer sheets and developed with 1 % hydrochloric acid or butanol–27 % acetic acid (1:1). The flavonoids were detected under UV light both in the presence and absence of ammonia vapour. Afterwards the sheets were sprayed with a solution of 0.01 *M* I_2 in 0.025 *M* KI. Iodine vapour or solutions of iodine in organic solvents could also be used.

When necessary the quantity of the flavone solution was measured spectrophotometrically using the absorption at 335 nm and a test curve of vitexin, assuming a more or less identical molar extinction for the different (iso)vitexin glycosides.

RESULTS AND DISCUSSION

The results have been summarized in Table I for a number of randomly chosen flavonoids, and Table II for the closely related *Silene* flavones. As expected, those compounds giving a blue precipitate with iodine in Barger and Starling's investigations[5] of 1915 (flavone, chrysin, apigenin) also appeared as blue or bluish grey spots on the chromatograms. The flavonols kaempferol and quercetin also gave a bluish grey colour. All corresponding glycosides, however, became yellow to brown-yellow.

The opposite effect is obtained with isovitexin-7-glucoside which gave an intense blue colour, whereas its "aglycone" isovitexin became yellow (Table II). Remarkably, of all 23 flavones investigated, isovitexin-7-glucoside appeared to be the only one with the "starch reaction". Substitution of the 7-O-glucose by xylose or galactose leads to a loss of this capacity. The possibility to distinguish the 7-glucoside and 7-galactoside in this way was an unexpected result since these compounds have otherwise practically identical properties.

TABLE I

COLOUR REACTION OF RANDOMLY CHOSEN FLAVONOIDS WITH IODINE–POTASSIUM IODIDE

Compound	*Colour*
Flavone	Intense blue
Chrysin	Bluish grey
Apigenin	Blue
Apigenin-7-glucoside	Brown yellow
Apiin	Brown-yellow
Acacetin	Bluish grey
Luteolin	Yellow
Luteolin-7-glucoside	Yellow
Naringenin	White
Naringin	Colourless
Kaempferol	Bluish grey
Kaempferol-3-glucoside	Yellow
Quercetin	Bluish grey
Rutin	Yellow
Myricetin	Brown-yellow

TABLE II

COLOUR REACTION OF CLOSELY RELATED C-GLYCOSYLFLAVONES WITH IODINE–POTASSIUM IODIDE

Compound	*Colour*
Isovitexin	Yellow
6-O″-Arabinosylisovitexin	Yellow
6-O″-Rhamnosylisovitexin	Yellow
6-O″-Glucosylisovitexin	Yellow
7-Glucosylisovitexin	Intense blue
7-Galactosylisovitexin	Light yellow
7-Xylosylisovitexin	Yellow
7-Glucosyl-6-O″-arabinosylisovitexin	Light yellow
7-Glucosyl-6-O″-rhamnosylisovitexin	Light yellow
7-Glucosyl-6-O″-glucosylisovitexin	Light yellow
7-Xylosyl-6-O″-arabinosylisovitexin	Yellow
7-Xylosyl-6-O″-rhamnosylisovitexin	Light yellow
7-Xylosyl-6-O″-glucosylisovitexin	Violet
Vitexin	Yellow
8-O″-Rhamnosylvitexin	Yellow
8-O″-Glucosylvitexin	Yellow
8-O″-Xyloxylvitexin	Yellow
Isoorientin	Brown
7-Glucosylisoorientin	Yellow
6-O″-Rhamnosylisoorientin	Yellow
Isoscoparin	Yellow
7-Glucosylisoscoparin	Yellow
6-O″-Rhamnosylisoscoparin	Yellow

The sensitivity of the reaction depends on the local concentration. Less than 1 μg of isovitexin-7-glucoside is sufficient when the compound is just spotted on paper without development, but for a developed paper chromatogram about 20 μg are necessary for an intense colour. For comparison, 80 μg of isovitexin-7-galactoside on the same sheet only gave a light yellow colouration.

The specificity of the reaction with isovitexin-7-glucoside in the group of closely related C-glucosylflavones provides us with a rapid screening method in population-genetic studies on *Silene*[7].

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