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Note

Optical brighteners as thin-layer chromatography detection reagents for glycoalkaloids and steroid alkaloids in *Solanum* species

I. Calcofluor® M2R New*

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In the development of a thin-layer chromatographic screening method for the glycoalkaloids and their aglycones, the steroid alkaloids, in *Solanum* species, a sensitive and specific detection reagent is required. As most of the detection reagents for these substances, reported elsewhere[1–4], did not fulfill our requirements regarding sensitivity and specificity, we investigated the use of an optical brightener, Calcofluor® M2R New.

Optical brighteners are used in mycology as microscopic reagents for visualizing cell walls[5]. To test the specificity of Calcofluor, we applied the reagent to substances related to the steroid (glyco)alkaloids, such as alkaloids, glycosides, and saponins. Also, the effect of the optical brightener on glycoalkaloids and steroid alkaloids was compared with the effect of some other reagents. Additionally, we determined the minimal detectable quantity (MDQ) of a few steroid (glyco)alkaloids.

EXPERIMENTAL

All thin-layer chromatography (TLC) was carried out on pre-coated silica gel plates, 250 μm layer thickness (Merck, Darmstadt, G.F.R.). The detection reagents are listed in Table I.

For the determination of the response of Calcofluor M2R New, 5.7 nmol (equivalent to 5 μg solanine) of each of the substances listed in Table II, dissolved in the appropriate solvent, was spotted on a plate. The plate was dipped in reagent 4 and immediately observed under long-wave (365 nm) UV light.

Because only a few steroid (glyco)alkaloids were at out disposal, several *Solanum* species (Table III) were extracted. Part of the glycoside mixtures were hydrolyzed. Of the extracted glycoalkaloids, 5 μl of a 0.1% methanolic solution and 5 μl of a 0.1% chloroform solution of the steroid alkaloids, obtained by hydrolysis, were spotted.

For the glycoalkaloids the plates were developed in *n*-butanol–formic acid–

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TABLE I

DETECTION REAGENTS APPLIED TO STEROID (GLYCO)ALKALOIDS, SAPONINS AND RELATED SUBSTANCES

No.	*Reagent*	*Application*	*Heating*	*Substances*	*Colours*			
					Daylight		*Long-wave UV (365 nm)*	
					Spots	*Background*	*Spots*	*Background*
1	5% Sulphuric acid in diethyl ether[6]	Dip	5 min, 100°	Steroid alkaloids, glycoalkaloids	Rose-grey	White	Light blue	Dark pink
2	Antimony trichloride–glacial acetic acid (1:1)[7]	Spray	5 min, 100°	Steroid alkaloids, glycoalkaloids	Rose	White	Orange-yellow	Pink
3	Dragendorff reagent[8]	Dip	–	Steroid alkaloids, glycoalkaloids	Orange-red	Orange-yellow	–	–
4	Calcofluor® M2R New, 0.02% – methanol	Dip	–	See Table II	–	–	Light blue	Blue
5	Anisaldehyde*	Spray	5 min, 100°	Saponins	Various	White	–	–
6	Blood gelatine[9]	Pour	–	Saponins	White	Red	–	–

* Anisaldehyde reagent: Dissolve 0.5 ml anisaldehyde in 80 ml of methanol and 10 ml of glacial acetic acid, then add 5 ml of conc. sulphuric acid.

TABLE II

RESPONSE OF GLYCOALKALOIDS, STEROID ALKALOIDS AND RELATED SUBSTANCES TO CALCOFLUOR® M2R NEW

All the responses are compared with the very weak fluorescence of 5-μl spots of water and of chloroform–methanol (1:1). — = No response; ± = weak; + = positive; ++ = strong.

Compound	*Response*	*Compound*	*Response*	*Compound*	*Response*
Alkaloids		*Saccharides*		*Glycosides*	
Aconitine	—	Galactose	—	Aesculin	—
Atropine	—	Glucose	—	Aloin**	—
Lobeline	—	Rhamnose	—	Apigenin	
Morphine	—	Lactose	—	Monoglucoside	—
Nicotine	—	Mannose	—	Arbutin	—
Pilocarpine	—	Saccharose	—	Digitoxin	±
Quinine*	—	*Steroid alkaloids*		Frangulin**	—
Reserpine*	—	Solanidine	+	Gitoxin	±
Steroidsapogenins		Solasodine	+	Hesperidin	—
Diosgenin	—	Demissidine	+	Rutin	—
Tigogenin	—	Tomatidine	+	Salicin	—
Saponins		*Glycoalkaloids*		Sennoside B**	—
Aescin	+	Solanine	++		
Saponinum purum	+	Tomatine	++		

* These substances already fluoresce before applying the optical brightener.
** The fluorescence of these substances disappears with the optical brightener.

water (4:1:5, upper layer) in a saturated tank over 15 cm. In the case of steroid alkaloids, the plates were also developed with *n*-hexane–acetone (1:1) in an unsaturated tank over 15 cm. After drying the plates, we applied reagents 1, 2, 3, or 4. We also extracted a few saponin-containing drugs (Table IV)[10]. Of these extracts 20 μl was spotted on a plate as a band, together with 10 μl bands of 0.1% solution of aescin and of saponinum purum in 70% alcohol. The plate was developed in an unsaturated chamber with *n*-butanol–acetic acid–water (5:1:4, upper layer) over 15 cm. After drying the plates, the spots were visualized with reagents 4, 5, or 6.

For the determination of the MDQ, we applied decreasing quantities, in steps of 0.01 μg, of solanine, tomatine, solanidine, and solasodine, on a plate. After development, the plate was dried and dipped in reagents 3 or 4.

TABLE III

SURVEYED *SOLANUM* SPECIES AND THEIR CONSTITUENTS AS CITED BY SCHREIBER[11]

Solanum species	*Glycoalkaloids*	*Steroid alkaloids*
S. acaule *S. demissum* *S. polyadenium*	Tomatine, demissine	Tomatidine, demissidine
S. chacoense *S. tuberosum* *S. vernei* *S. verrucosum*	Solanine, chaconine	Solanidine
S. dulcamara *S. triflorum*	Solasonine, solamargine	Solasodine

TABLE IV

SURVEYED SAPONIN-CONTAINING DRUGS

Saponin-containing drugs	*Parts used*	*Saponin type*
Phytolacca americana	Berries	Triterpene
Polygala senega	Roots	Triterpene
Smilax species	Roots	Steroid
Trigonella foenumgraecum	Seeds	Steroid

RESULTS AND DISCUSSION

The responses of the substances to Calcofluor are listed in Table II. The two glycoalkaloids gave a distinct fluorescence, the steroid alkaloids and a few saponins had a fluorescence of lower intensity. The cardiac glycosides, digitoxin and gitoxin, gave a weak fluorescence. All the other compounds listed in Table II gave spots having weak or no fluorescence. The fluorescence of the background changed in time from blue to yellow-green and back to blue.

The chromatograms of the saponin-containing drugs treated with reagents 4, 5, or 6 showed some similarity. When observed immediately after dipping in Calcofluor reagent, the chromatograms of aescin, saponinum purum and *Trigonella foenumgraecum* each showed one band. After 5–10 min more bands became visible, but had a much lower intensity and were of different colours. The bands in the chromatogram of *Smilax* root that fluoresced with Calcofluor gave hemolysis with blood gelatine, as did the bands of aescin, saponinum purum, and *Trigonella foenumgraecum*.

Chromatograms of the glycoalkaloids and the steroid alkaloids from the *Solanum* species gave the same spots with Calcofluor reagent as with Dragendorff reagent. Sulphuric acid and antimony trichloride gave more spots, but these reagents are less specific for steroid (glyco)alkaloids and do not have the same sensitivity for all steroid (glyco)alkaloids.

The MDQ of the steroid (glyco)alkaloids (see Table V) with Calcofluor is at least 10 times less than with Dragendorff reagent.

The results show that Calcofluor is more specific for steroid (glyco)alkaloids than Dragendorff reagent, sulphuric acid, or antimony trichloride. Calcofluor is also more sensitive than Dragendorff reagent.

TABLE V

MDQ OF GLYCOALKALOIDS AND STEROID ALKALOIDS WITH CALCOFLUOR® M2R NEW AND DRAGENDORFF REAGENT

	Calcofluor M2R New		*Dragendorff*	
	$\mu g \cdot 10^{-2}$	$\mu mol \cdot 10^{-5}$	$\mu g \cdot 10^{-2}$	$\mu mol \cdot 10^{-5}$
Solanine	2	2.3	20	23
Tomatine	4	4.0	50	50
Solanidine	5	12.8	230	580
Solasodine	7	16.9	>230	>600

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