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Note

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

II. Blankophor® BA 267%, BBU neu and KU, and Tinopal® CBS-X and 5 BMS-X

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In Part I¹ we mentioned that Calcofluor M2R New (American Cyanamid, New York, N.Y., U.S.A.) is a sensitive and specific thin-layer chromatographic (TLC) detection reagent for glycoalkaloids and steroid alkaloids in *Solanum* species. Unfortunately, this optical brightener is no longer produced, although it may still be obtainable. Therefore, we have now investigated the application of some other optical brighteners which are, like Calcofluor M2R New, derivatives of diaminostilbene-disulphonic acid. Spray solutions of the optical brighteners were applied to steroid alkaloids, glycoalkaloids and related substances. We also determined the minimal detectable amounts (MDA) of some steroid (glyco)alkaloids.

EXPERIMENTAL

All TLC was carried out on pre-coated silica gel plates with a layer thickness of 250 μm (Merck, Darmstadt, G.F.R.). Spray solutions of Tinopal 5 BMS-X and Tinopal CBS-X (Ciba-Geigy, Arnhem, The Netherlands) and Blankophor BBU neu and Blankophor BA 267% (Bayer, Arnhem, The Netherlands) were 0.02% in methanol and that of Blankophor KU (Bayer) was 0.02% in chloroform.

The glycoalkaloids were extracted from the same *Solanum* species as was used in Part I^{1,2}. These species contain virtually all known structures of glycoalkaloids and steroid alkaloids present in the genus *Solanum*³.

The steroid alkaloids were obtained by hydrolysis of the glycoside mixtures⁴. We also extracted a few saponin-containing drugs (Table I)⁵. The TLC of the steroid (glyco)alkaloids and of the saponins was carried out as described in Part I¹.

For the determination of the response of the optical brighteners, 5.7 nmol (equivalent to 5 μg of solanine) of each of the substances listed in Table II, dissolved in the appropriate solvent, was spotted on a plate.

Detection

After drying for 30 min at 120°, the plates were sprayed with one of the spray solutions, observed under longwave UV light (365 nm), sprayed with 0.01 *N* sulphuric

TABLE I
SAPONIN-CONTAINING DRUGS AND SAPONINS EXAMINED

<i>Saponin-containing drug</i>	<i>Part used</i>	<i>Saponin type</i>
<i>Phytolacca americana</i>	Berries	Triterpene
<i>Polygala senega</i>	Roots	Triterpene
<i>Smilax</i> species	Roots	Steroid
<i>Trigonella foenumgraecum</i>	Seeds	Steroid
<i>Saponins:</i>		
Aescin		Triterpene
Saponinum purum		Triterpene

TABLE II
RESPONSE OF GLYCIALKALOIDS, STEROID ALKALOIDS AND RELATED SUBSTANCES TO THE OPTICAL BRIGHTENERS

All of the responses are compared with the very weak fluorescence given by of 5- μ l spots of water and of chloroform-methanol (1:1). - = No response; \pm = weak; + = positive; ++ = strong; +++ = very strong.

<i>Compound</i>	<i>Response</i>				
	<i>Tinopal</i>		<i>Blankophor</i>		
	<i>5 BMS-X</i>	<i>CBS-X</i>	<i>BA 267%</i>	<i>BBU neu</i>	<i>KU</i>
<i>Alkaloids:</i>					
Aconitine	-	+	\pm	-	-
Atropine	\pm	\pm	\pm	-	-
Pilocarpine	-	+	-	-	-
Strophantine	-	+	-	-	-
<i>Steroid saponins:</i>					
Diosgenin	\pm	+	\pm	\pm	-
Tigogenin	\pm	+	\pm	\pm	-
<i>Saccharides:</i>					
Glucose	-	-	-	-	-
Galactose	-	-	-	-	-
Rhamnose	-	-	-	-	-
Lactose	-	\pm	-	-	-
Mannose	-	\pm	-	-	-
Saccharose	-	\pm	-	-	-
<i>Steroid alkaloids:</i>					
Demissidine	+++	++	+++	++	+
Solanidine	+++	++	+++	++	+
Solasodine	+++	++	+++	++	+
<i>Glycoalkaloids:</i>					
Solanine	+++	+++	+++	+++	+
Tomatine	+++	+++	+++	+++	+
<i>Glycosides:</i>					
Aesculin	-	-	-	-	-
Aloin*	-	-	-	-	-
Amygdaline	-	+	-	\pm	-
Apigenin	-	-	-	-	-
Monoglucoside*	-	-	-	-	-
Arbutin	-	-	-	-	-
Digitoxin	+	+	+	+	\pm
Frangulin*	-	-	-	-	-
Gitoxin	+	+	+	+	\pm
Rutin	-	-	-	-	-
Salicin	-	+	-	-	-
Sennoside B*	-	-	-	-	-

*The fluorescence of these spots is very weak.

acid and observed again under UV light. One plate with the steroid (glyco)alkaloids was also sprayed with Dragendorff reagent.

Minimum detectable amount

The MDA of solanine, solanidine, solasodine and tomatine was determined by applying decreasing amounts, in steps of 0.01 μg , to plates. After development, the plates were dried, sprayed with a 0.02% methanolic solution of Blankophor BA 267%, Tinopal 5 BMS-X or Calcofluor M2R New and observed under longwave UV light.

RESULTS AND DISCUSSION

The responses of the substances to the optical brighteners are listed in Table II. The glycoalkaloids gave a distinct, light blue fluorescence and the steroid alkaloids had a fluorescence of lower intensity. Chromatograms of the steroid (glyco)alkaloids gave the same pattern of spots with the optical brighteners as with Dragendorff reagent. When observed immediately after spraying, each of the chromatograms of aesciin, saponinum purum and *Trigonella foenumgraecum* showed one band, having the same intensity as the band of solanine. After 5–10 min more bands became visible, but they had lower intensities and different colours.

For Blankophor BA 267% and Tinopal 5 BMS-X the difference between the spots and background was enhanced after spraying with 0.01 *N* sulphuric acid. Spraying with Blankophor KU gave poorly visible spots.

The MDA of the steroid (glyco)alkaloids is about the same with Blankophor BA 267% and Tinopal 5 BMS-X as with Calcofluor M2R New (Table III), which is 10 times lower than with Dragendorff reagent¹.

Blankophor BA 267% and Tinopal 5 BMS-X gave the best results, and are sensitive TLC detection reagents for steroid (glyco)alkaloids.

TABLE III

MDA OF GLYCOALKALOIDS AND STEROID ALKALOIDS WITH THE OPTICAL BRIGHTENERS

<i>Compound</i>	<i>Calcofluor M2R New</i>		<i>Tinopal 5 BMS-X</i>		<i>Blankophor BA 267%</i>	
	$\mu\text{g} \times 10^{-2}$	$\mu\text{mol} \times 10^{-5}$	$\mu\text{g} \times 10^{-2}$	$\mu\text{mol} \times 10^{-5}$	$\mu\text{g} \times 10^{-2}$	$\mu\text{mol} \times 10^{-5}$
Solanine	1	1.1	2	2.2	2	2.2
Solanidine	14	34	9	23	14	34
Solasodine	14	34	14	34	20	46
Tomatine	1	1.1	3	3.3	3	3.3

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