QUALITATIVE CHARACTERISTICS OF ALKALOIDS OF SPECIES OF THE GENUS Vinca

V. Yu. Vachnadze, V. M. Malikov,
Kh. T. Il'yasova, K. S. Mudzhiri,
and S. Yu. Yunusov

UDC 547.941.1

Methods for the qualitative analysis of alkaloids in a thin layer using various adsorbents – alumina, silica gel, and others – are widely known for their efficiency [1, 2].

For the indole alkaloids a number of systems and characteristic reagents for individual bases and groups of alkaloids have been proposed: concentrated nitric acid, cerium ammonium sulfate, iron ammonium sulfate, and many others [3-5].

Among them the reagent cerium ammonium sulfate may be singled out because of the sensitivity and individual nature of its action; it has proved very valuable in the analysis of the alkaloids of Vinca rosea (Madagascar periwinkle) [5]. In view of this, we have attempted to correlate the nature of the coloration of alkaloids of the genus Vinca produced by the reagent cerium ammonium sulfate with the chemical structures of the substances. This will assist in the preliminary determination of the association of the bases with a given chromophore.

The adsorbents used for thin-layer chromatography were kieselguhr G (Merck) and Silufol, which are mainly used for compounds of a different nature (flavonoids, coumarins, etc.), since the starch present as binding agent in the Silufol plates does not permit the spots of the alkaloids to be detected with the Dragendorff reagent. Consequently, we decided to use the reagent cerium ammonium sulfate for these purposes. Under these conditions, the sensitivity for the individual chromophores did not change.

In view of the fact that the hydroxyindole alkaloids are not revealed by this reagent, for majdine, isomajdine, vinerine, and vineridine we attempted to use a 0.2 M solution of $FeCl_3$ in 35% perchloric acid as reagent [6]. It was found that the reaction was positive when the materials were heated at 70-80°C.

About 40 alkaloids were subjected to qualitative analysis and characterization. The criterion used was the coloration which they gave with the reagent cerium ammonium sulfate, which was observed for 24 h. To evaluate the coloration of the spots of the alkaloids, we used A. S. Bondartsev's scale of colors.

Color on the Scale	Color	Color on the Scale	Color
a-2	Blackish	i-5	Bluish green
a-3	Grayish violet	j-1	Isabella, pale yellow
a-6	Pale grayish	j-3	Pale sandy
b-1	Dark blue	j-5	Walnut
b-6	Cream	k-1	Smoky
c-4	Dark ash	k-2	Straw-yellow
d-4	Orange-pink	k-3	Lemon-yellow
d-6	Ocher-yellow	*l*-5	Carmine red
d-7	Yellowish rust	*l*-7	Golden yellow
e-2	Yellow-orange	m-3	Pale blue

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. I. G. Kutateladze Institute of Pharmaceutical Chemistry, Academy of Sciences of the Georgian SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 72-76, January-February, 1973. Original article submitted June 6, 1972.

Color on the Scale	Color	Color on the Scale	Color
e-4	Leather-brown	m-4	Pinkish violet
e-5	Pale lemon yellow	m-6	Violet carmine
g-1	Rusty	n-1	Plum
g-3	Pinkish lilac	n-5	Flesh pink
g-5	Lead gray	n-6	Dark cream
h-2	Dark violet	o-3	Pale honey
h-3	Ocher	o-4	Orange red
i-1	Gray-yellow	o-5	Yellow
i-2	Yellowish green	o-7	Minium red

In the investigation of the qualitative color reactions of alkaloids with cerium ammonium sulfate, four main groups of them were distinguished according to their chromophoric systems.

The group of α-methyleneindolines that are derivatives of akuammicine can, according to information on the alkaloids analyzed, be subdivided into two classes. The first class of compounds, which is characterized by the presence of an ester grouping in the chromophore, at the moment of contact with the cerium ammonium sulfate reagent gives a bright-blue coloration; after an hour, the edge becomes light blue and the center walnut-colored; and after 24 h, a light-walnut coloration appears. The range of coloration changes in the period from 5 min to 1 h. The alkaloid vinervine is an exception in this class of compounds. Obviously, in this case a decisive influence is also played by the nature of the substituent in the aromatic ring (Table 1).

Compounds of the second class, which are characterized by an aldehyde grouping in the chromophore, form a gray-yellow or ocher color after 1 h. Here, again, the color is affected by substituents in the aromatic ring.

α-Methyleneindoles that are derivatives of aspidospermine, may, like the compounds described above, be subdivided into two classes. Of these, compounds without a substituent in the aromatic ring acquire in the first moment of contact with the agent a dark-blue coloration of the spot in which either an ocherous or a dark orange-yellow shade is interspersed. The first color is characteristic for compounds having no double bond in the 6, 7 position and the light color for those having a double bond in the 6, 7 position. Towards the end of the reaction, the former acquire a pale-lemon and the latter a pale-sandy shade. Compounds with methoxy groups in the aromatic ring and no double bond in the 6, 7 position, such as methoxyvincadifformine become colored deep blue in the first moment of contact with the reagent, but less intensively with a transition to pale blue, and for compounds with a double bond in the 6, 7 position, such as methoxytabersonine, the initial coloration is the same, but after 5 min it is interspersed with lemon. After 24 h, both colorations change to the pale lemon-yellow shade or disappear completely.

For compounds of this group including a carbonyl function, when there is no substituent in the aromatic ring, a dark coloration is observed, and when there is a substituent, a less intense one.

In the group of indole alkaloids, although not so many compounds have been analyzed, the same dependence of the transition of the intensity of the coloration from light lemon-yellow to gray-yellow according to the chromophore is observed. Vincamine gives a bright lemon-yellow coloration and methoxyvincamine (vinicine) a less intense one which appears during the first 5 min. Apovincamine, which has a double bond in the 14,15 position, gives a yellow-green color changing to gray-yellow. Derivatives of δ-yohimbine also differ in the nature of the reaction for the chromophore. Reserpinine, with a substituent ($-OCH_3$) in the aromatic ring, gives a cream-yellow color and ervine, without a substituent in the aromatic ring, a gray-yellow color.

In the group of indole alkaloids, various shades of orange-pink and carmine-red colors predominate. Ajmaline derivatives can be divided, according to the coloration with the cerium ammonium sulfate reagent, into compounds giving an orange-red color at the moment of contact with the reagent and those giving a carmine-red color. Thus, for example, characteristic for herbamine is a carmine-red coloration and for herbadine, an orange-red one. Herbadine is a methyl derivative of herbamine. A similar change in color is observed in vincamajnine and majoridine. The picrinine series is characterized by the same range of colors.

Thus, the results of the investigations performed permit the conclusion that by using the reagents and adsorbents given above, it is possible to make a preliminary determination of the chromophoric group of alkaloids and to perform their corresponding identification.

TABLE 1

Alkaloids	Color with a 1% solution of cerium ammonium sulfate in 85% orthophosphoric acid				
	after spraying	after 5 min	after 15 min	after 1 h	after 24 h
			α-Methyleneindoles		
Vincanine (norfluorocurarine)	g-5	Edge g-5 Center i-1	i-1	i-1	i-1
Vincanidine	h-3	h-3	h-3	o-5	o-5
Akuammicine	b-1	b-1 interspersed with j-5	Edge b-1 Center j-5	Edge b-1 Center j-5	h-3
Vinervinine	b-1	b-1	Dark m-3	Edge m-3 Center j-1	Light j-1
Vinervine sulfate	a-3	a-3	a-3	n-6	–
Ervamine (*l*-vincadifformine)	b-1	b-1	b-1 interspersed with j-5	Edge g-5 Center j-5	e-5
Ervinceine (*l*-methoxyvincadifformine)	b-1	Light b-1	m-3	k-1	–
Tabersonine	b-1	h-2 edge n-1	h-2, interspersed with e-2	j-3	Light j-3
16-Methoxytabersonine	m-3	m-3	m-3, interspersed with k-2	k-2	Light k-2
Ervinidinine	b-1	b-1	n-1, interspersed with c-5	k-2	Light k-2
Ervincinine	b-1	m-3, interspersed with k-3	Edge m-3 Center k-3	i-5	i-5
Ervinidine	b-1	b-1, interspersed with e-2	Edge m-3 Center e-2	Edge m-3 Center e-2	Pale e-2
			Indoles		
Copsinine	d-4	d-4	d-4	d-4	d-4
Copsinilam	d-4	d-4	Dark d-4	Dark d-4	Dark d-4
Copsanone	Pale a-3	Pale a-3	Pale a-3	Pale a-3	Pale a-3
Pseudocopsinine	*l*-5	Dark d-4	*l*-5	*l*-5	*l*-5
Herbamine	*l*-5	*l*-5	*l*-5	*l*-5	b-6
Majdinine	*l*-5	*l*-5	*l*-5	n-5	n-5
Ajmaline	o-7	*l*-5	*l*-5	n-5	–
Vincamajine	*l*-5	*l*-5	*l*-5	Edge n-5 Center k-3	k-3
Akuammine	d-6	d-7	d-7	d-7	d-7
Vincarine	o-4	o-4	o-4	Pale o-4	–
Herbadine	o-4	n-5	n-5	n-5	d-6
Picrinine	g-1	g-1	Edge g-1 Center e-2	Edge e-4 Center k-3	m-4
Vincaricine	*l*-7	Edge d-6 Center g-6	Edge d-6 Center g-6	d-6	d-6
Vincarinine	*l*-7	n-7	d-6	Pale d-6	g-3

TABLE 1 (continued)

Alkaloids	Color with a 1% solution of cerium ammonium sulfate in 85% orthophosphoric acid				
	after spraying	after 5 min	after 15 min	after 1 h	after 24 h
			Indoles		
11-Hydroxypleiocarpamine	–	–	–	Pale b-6	Pale b-6
Akuammidine	a-3	a-2	c-4	a-6	a-6
Tombosine	a-3	c-4	c-4	a-6	a-6
Quebrachamine	m-4	m-4, interspersed with i-1	Edge m-4 Center i-1	Edge g-3 Center i-2	Pale n-1
Vincamine	k-3	k-3	k-3	k-3	k-3
Vincine	–	Pale k-3	Pale k-3	k-3	Pale k-3
Apovincamine	i-1	i-1	i-1	i-1	i-1
Ervine	i-1	i-1	i-1	i-1	i-1
			Hydroxyindoles		
Vinerine	–	g-5	Edge g-5 Center b-1	n-1	–
Vineridine	–	g-5	" "	n-1	–
Majdine	–	m-6	n-1	h-2	–
Isomajdine	–	m-6	n-1	h-2	–

So far as concerns the hydroxyindole alkaloids, the use of Silufol has created the possibility of their qualitative identification in a thin layer together with other alkaloids by means of the reagent 0.2 M $FeCl_3$.

EXPERIMENTAL

To prepare the plates with the adsorbent kieselguhr G (Merck), an instrument of the Desaga type was used. The emulsion for the plates (150 × 150 mm) was prepared in the proportions of 4 g of adsorbent and 9 ml of distilled water for each plate. The prepared plates were first dried in the air for 6 h and then in a drying oven at 105°C for 30 min.

The following systems of solvents were used for chromatography: 1) benzene–ethyl acetate–methanol (2 : 3 : 1); 2) benzene–ethyl acetate (2 : 3); and 3) benzene–methanol (3 : 2).

The changes in the colors of the spots of the alkaloids on the chromatograms with the cerium ammonium sulfate in 85% orthophosphoric acid reagent were recorded immediately after spraying, in the first 5 min, after 15 min, after 1 h, and after 24 h.

The second adsorbent used consisted of Silufol-prepared plates with dimensions of 150 × 150 mm. To reveal the hydroxyindole alkaloids on the Silufol a 0.2 M solution of $FeCl_3$ in 35% perchloric acid (freshly prepared reagent) was used. After spraying, the plates were heated at 70-80°C until the colored spots of the alkaloids appeared. The materials were deposited on the chromatograms with a micropipette in amounts of 0.005-0.06 ml.

The alkaloids subjected to analysis were in the form of the free bases, with the exception of vinervine sulfate. Solvents for the alkaloids were chloroform, chloroform–methanol, and methanol. The concentration of the alkaloids was 0.0001 g in 10 ml of solvent.

SUMMARY

The qualitative characterization of 38 indole alkaloids has been performed by chromatography on kieselguhr G and Silufol with the reagents cerium ammonium sulfate and ferric chloride.

The possibility of a preliminary determination of the chromophoric group (α-methyleneindole, indoline, indole, and hydroxyindole) of alkaloids by means of the reagents cerium ammonium sulfate and ferric chloride has been shown.

LITERATURE CITED

1. A. A. Akhrem and A. I. Kuznetsova, Thin-Layer Chromatography [in Russian], Moscow (1964).
2. Sh. Z. Kasymov, Kh. N. Aripov, T. T. Shakirov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 352 (1967).
3. I. M. Jakovljevic, L. D. Seay, and R. W. Shaffer, J. Pharm. Sci., 53, 553 (1964).
4. N. J. Cone, R. Miller, and N. Neuss, J. Pharm. Sci., 52, 688 (1963).
5. N. R. Farnsworth, R. N. Blomster, and D. Damrateski, Lloydia, 27, 4, 302 (1964).
6. E. J. Shellard and M. Z. Alam, J. Chromatography, 33, No. 2, 347 (1968).
7. A. S. Bondartsev, Scale of Colors (Manual for Biologists in Scientific and Applied Scientific Investigations) [in Russian], Moscow (1954).