1. Introduction

Dragendorff’s reagent (DR) was introduced by Johann Georg Noel Dragendorff (1836–1898), who acted as a full-time professor in Pharmacy at the University of Dorpat, Tartu, Estonia (Fig. 1). DR was described for the first time in 1865/1866 (Fig. 2), thus it has more than a 150-year history as a reagent (Dragendorff 1866; Past et al. 2009). Since then, DR has been widely applied, e.g., in pharmacy, medicine, forensic chemistry and related sciences all over the world. DR is a color reagent to detect alkaloids in a test sample. Alkaloids, if present in the solution of the sample, react with DR and yield an orange or orange red precipitate. The reagent is a solution of potassium bismuth iodide prepared from basic bismuth nitrate (Bi(NO₃)₃), tartaric acid, and potassium iodide (KI). In this review article, we make a historical overview on the biography of Professor J. Georg N. Dragendorff and his scientific research work, when he acted as a full-time professor in pharmacy at the University of Dorpat, Tartu, Estonia. The chemistry, method of preparation, mechanism of action, and practical uses of DR in various disciplines and in various European countries including the Baltic countries (Estonia, Latvia, Lithuania), Finland, Ukraine, Moldova, and in Asia (Vietnam), are also discussed. Over several decades, DR and its modifications have found uses in many new applications and disciplines, and a number of commercial DRs are also currently available on the market. Today, DR is used for example in the production of surfactants, where non-ion surfactant is precipitated in water solution with modified DR (KB₄I₄+BaCl₂+glacial acetic acid). Total six different potassium iodobismuthate (DR) solutions are also presented in the European Pharmacopoeia. In conclusion, DR (after more than 150 years of its invention in Estonia) has still an important role in pharmaceutical and related sciences all over the world.
In 1799, Russian Tsar Paul I approved the university plan, which also scheduled the joint professorship in chemistry and pharmacy. In 1800, the chemist Philipp Arzt came from Tallinn to Tartu as the first professor of Chemistry and Pharmacy. Unfortunately, Philipp Arzt drowned in August 1802, and thus the position was void for the next one-two years. After this, a German chemist, Alexander Nicolaus Scherer (1772–1824), was nominated as the first professor of theoretical and applied chemistry to the UD. After Scherer, the following scholars held a professorship in chemistry and pharmacy at the UD: David Hieronymus Grindel from Riga, Latvia (1805–1814), Ferdinand Giese from Harkiv, Ukraine (1814–1821), and Gottfried Osann from Weimar, Germany (1823–1828). G. Osann was a well-known chemist and researcher of the plataunium ore of the Urals, and his scientific work led Carl Claus to discover Ruthenium (Hinrikus et al. 2005, 2007; Raal et al. 2009). Unfortunately, the UD had serious financial difficulties by the time of Dragendorff’s stay in Estonia, limiting the research work of his group. In those times, many Russian universities and universities abroad had much more resources and funding than the UD. On the other hand, Dragendorff noticed that the limited funding resulted in the higher commitment of the students to make a scientific research work. He also showed that all difficulties and challenges related to resources can be overcome with a bright and strong personality (Raal et al. 2006; Kapp et al. 2012).

In Tartu, Dragendorff focused his teaching and research activities solely on pharmacy, pharmacognosy and forensic chemistry for 30 years. He wrote numerous textbooks and monographs, and supervised a total of 90 master’s and 87 doctoral theses in the fields of pharmaceutical chemistry, pharmacology, pharmacognosy, forensic, environmental and food chemistry, physiology and bacteriology. The abovementioned number of master and doctoral dissertations written under his supervision will probably remain as insurmountable forever (Hinrikus et al. 2005; Tankler et al. 2002). In those times, it was quite usual that research work and doctoral dissertations in pharmacy were interdisciplinary combining especially pharmacy with forensic chemistry. The key attribute to the successful research work of Dragendorff and his students was their success in implementing both qualitative and quantitative analytical methods for alkaloids, glycosides and the other biologically active substances (Tankler et al. 2002; Hinrikus et al. 2005, 2007; Raal et al. 2009). Unfortunately, the UD had serious financial difficulties by the time of Dragendorff’s stay in Estonia, limiting the research work of his group. In those times, many Russian universities and universities abroad had much more resources and funding than the UD. On the other hand, Dragendorff noticed that the limited funding resulted in the higher commitment of the students to make a scientific research work. He also showed that all difficulties and challenges related to resources can be overcome with a bright and strong personality (Raal et al. 2006; Kapp et al. 2012).
In the 30-year period under Dragendorff, the UD became one of the most important higher-education and research centers in Europe. Hermann Thoms, a former Professor of pharmaceutical chemistry from Berlin, Germany wrote about J.G.N Dragendorff: “From afar and extensively, students came to the famous man to write researches under his supervision. They spread the fame of Dragendorff to all civilized countries” (Raal et al. 2006). Many teachers and lecturers at the UD were former students of Dragendorf (i.e., Melchior Kubli, Emil Masing, Edwin Johanson, Carl Mandelin). A number of graduated pharmacists worked also as university teachers at higher-education schools abroad (i.e., Oskar Zinoffski in Kiev, Ukraine; Wilhelm Adolphi in St. Petersburg, Russia; Friedrich Beckmann and Bernhard Grewing in Warsaw, Poland) (Raal et al. 2006).

Dragendorff presented and summarized his achievements in the field of alkaloids in a scientific paper published in 1872 (Dragendorff 1872). However, the history of DR began somewhat earlier: DR was mentioned actually for the first time in a publication in Pharmaceutische Zeitschrift für Russland” (Dragendorff 1866).

2. Chemistry of Dragendorff’s reagent

Interestingly, the European Pharmacopoeia (2019) uses the general name “iodobismuthate reagent” instead of DR. The composition of DR is not fully elucidated and reported in the literature. Commonly, the source of bismuth (III) ions is reported to be bismuth subnitrate (bismuth oxinitrate, 4BiNO3(OH)2,BiO(OH)) or (Nitroxyo)oxobismuthine (Bi(NO3)3). Both of these chemical names are often considered as synonyms. At least six different structures for this substance have been fully characterized with single-crystal studies: BiO3H2O3(NO3)3, 4H2O (Lazarini 1979a; Sundvall 1979), Bi3O4(OH)(NO3)2, 4H2O (Lazarini 1979b), Bi2O2(OH)2NO3, 3H2O (Lazarini 1977), Bi3O2(OH)(NO3)3, NH3 (Christensen and Lebech 2012), Bi2O3(OH)2(NO3)2, 3H2O (Lazarini 1978), Bi2O3(NO3)2(OH) (Henry et al. 2005), and Bi2O3NO3 (Kodama 1994).

DR is prepared by dissolving bismuth subnitrate in diluted acid (acetic or tartaric acid, very rarely hydrochloric or sulfuric acid) and mixing it with a strong solution of potassium iodide. A low pH is mandatory for this reagent.

The formation of reagent is generally described as follows: The bismuth ions from bismuth subnitrate react with potassium iodide and form at first the black precipitate of bismuth iodide

\[ \text{Bi}^{3+} + 3\text{KI} \rightarrow \text{BiI}_3 + 3\text{K}^+ \]

Then, after the complete sedimentation of bismuth(III)iodide, the excess of iodide ions react to latter to form an orange colored, soluble complex of potassiumtetraiodobismuthate.

\[ \text{BiI}_3 + \text{K} \rightarrow [\text{K}[\text{BiI}_4]] \]

So far, very little is known about the mechanism of action of DR. The key search phrases, such as “Dragendorff reagent reaction mechanism” or “iodobismuthate reagent reaction mechanism” give not any result in the scientific literature data bases. It seems that the number of scientific research works published in this topic area is very limited (even negligible). The explanation for the color reaction induced by DR could be following:

Most alkaloids have a tertiary amine group R3N. This chemical group can react similarly to ammonia (NH3) and act as a base, which reacts with an acid to form an ammonium salt.

\[ \text{R}_3\text{N} + \text{HX} \rightarrow [\text{R}_3\text{NH}]^+ \text{X}^- \]

(X = anions of acid = Cl- , NO3-, HSO4-, CH3COO,-...)

Then, an ion-exchange reaction takes place between ammonium salt and potassiumtetraiodobismuthate leading to the formation of an insoluble complex salt.

\[ [\text{R}_3\text{NH}]^+ \text{X}^- + 3\text{K}[\text{BiI}_4] \rightarrow [\text{R}_3\text{NH}]^+[[\text{BiI}_4]]^3- + \text{KK} \]

Depending on the nature of alkaloid (or tertiary amine), this ion pair has a yellow to orange to red to brown color. Secondary amines will create less intensive colors. It is also worth to mention that not all alkaloids are detectable with DR. For example, caffeine and other purine alkaloids do not form a precipitate with DR (Baerheim-Svendsen and Verpoorte 1983; Popt et al. 1990; Pedersen 2006). A number of false-positive alkaloid tests based on DR has been reported in the literature as well. These tests were found to be as a result from the reactions with nitrogenous (e.g. peptides) or non-nitrogenous plant constituents. All non-nitrogenous compounds that were found to give a strong positive reaction with DR, were oxygen containing compounds (Habib 1980).

3. Use of Dragendorff’s reagent in some selected countries

The uses of DR in higher-education teaching and research work in some selected European countries and Vietnam is described below. The major focus is on pharmaceutical applications of DR and the historical time-frame of past 100 years.

3.1. Estonia

Estonia was a part of the Tsarist Russian Empire from the 19th century until 1918, and both the Russian (1880, 1891) and German Pharmacopoeias were used in pharmaceutical practice. In the 20th century, the Russian Pharmacopoeia (the 6th edition, 1910), Russian Military Pharmacopoeia (1913), and German Pharmacopoeia (the 6th edition, 1926) were used in Estonia and the other Baltic countries (Latvia and Lithuania) until the new national pharmacopoeias were published in the 1930’s (Kondratas et al. 2015). Interestingly, DR was included into the Russian Pharmacopoeia, but the reagent was not listed in the Estonian Pharmacopoeia (1937). A Mayer’s reagent has been used instead of DR for the determination of alkaloids in herbal drugs and their preparations (Folium Stramonii, Fructus Papaveris, Rhizoma Hydrastis, Rhizoma Veratii, Tinctura Aconiti, Tinctura et extractum Belladonnae, Tinctura et extractum Cinchonae, Extractione Strychni, Extraitum Opii, etc.), but not for individual alkaloid substances. The Estonian Pharmacopoeia presents in total 54 monographs for plant extracts and 104 monographs for the other dosage forms of plant origin (Kondratas et al. 2015).

In the Soviet-Union (USSR) period (1944–1991), the curriculum of pharmacy at the University of Tartu relied on the Soviet Union Pharmacopoeias (1946, 1952, 1961, 1968, 1990). Perhaps surprisingly, the Soviet Union U.S.S.R. Pharmacopoeia Vol. 8 (1952) does not mention DR, but in the later volumes 9-10 (1961, 1968) DR is described as a mixture of equal parts of solution 1 (0.85 g of basic bismuth nitrate in 40 ml water and 10 ml acetic acid) and solution 2 (5 g of potassium iodide in 20 ml water). Total 100 ml of water and 20 ml of acetic acid were added to 10 ml of solutions 1 and 2 (used at a ratio of 1:1). In the latest version of the U.S.S.R. Pharmacopoeia (1990), however, a modified DR is described: 5 ml of 1% water solution of ascorbic acid and 5 ml of 95% ethanol is added into 5 ml of equal parts of solution 1 and 2.

In the Soviet-Union time textbook of pharmaceutical chemistry (Meleniteva 1976), DR is shown to be applicable for the identification of alkaloids in their aqueous solutions with sulfuric acid or hydrochloric acid. When DR is used as the mixture of bismuth nitrate and potassium iodide, an amorphous, or sometimes also a crystalline orange-red or brick-red precipitate will appear. In another Soviet-Uni Union textbook of pharmaceutical chemistry (Senov 1971), DR is mentioned to indicate the presence of alkaloids in sulfatic acid solutions, if a precipitate of different colors is formed. DR is also mentioned in a more recent Russian university textbook of pharmacognosy (Jakovlev and Blinova 2004), but not in the Soviet-Union time textbook of pharmacognosy published earlier in the 1980’s (Muravjova 1981). In conclusion, DR was
well known and used in the laboratory works in pharmaceutical chemistry, forensic chemistry and pharmacognosy at the University of Tartu within the Soviet-Union period.

Today, the European Pharmacopoeia is being officially used in Estonia. At the University of Tartu, pharmacy students are using an Estonian textbook of pharmacognosy (Raal 2010; 2016), in which DR and the other corresponding reagents, such as Mandelin’s, Marquise’s, Sonnenscen’s and Mayer’s reagents, are described in detail. The Pharmacy students have also one specific laboratory work entitled “Screening of different groups of biologically active compounds in unknown plant material”, where the students first prepare an aqueous or ethanolic extract from an unknown herbal powder and then determine the presence of alkaloids and other substances in the extract. In addition to DR, they use also Fröde’s and Marquise’s reagents for the precipitation of alkaloids to obtain more evidence-based results.

3.2. Latvia

A recent study on higher pharmaceutical education in Latvia suggests that the use of DR was very limited in both teaching and research work within the Soviet-Union (USSR) time period ranging from 1919 to 1953 (Maurina 2008). The only study describing the use of iodine potassium and potassium bismuthiodide (DR) was conducted by Professor E. Svirlovs (Department of Pharmacognosy, Division of Pharmacy, University of Latvia) on the chemical composition of Polygonum hydropiper L. (Svirlovs 1944). He investigated the presence of alkaloids in an ether extract obtained from the plant, and found that the present reagent gave a slight opalescence outcome. In his pharmacognosy textbook he pointed out that alkaloids can be obtained in the form of complexes with potassium bismuthiodide and other compounds (Svirlovs 1940). In 1940, the Latvian Pharmacopoeia (1940) was issued, but DR was not included in the present national pharmacopoeia. This could be explained by the fact that DR was not involved in the study courses provided by the Division of Pharmacy at the University of Latvia. The leading scientific staff (teachers and researchers) in the Division of Pharmacy participated also in the development of the pharmacopoeia. According to Professor J. Maizite, their Pharmacy students verified and compared the medicinal preparations and analytical methods described in the German, Russian, British, USA, Belgian, Swiss, Italian, Romanian, Spanish, and Swedish national pharmacopoeias (Kondratas et al. 2015).

While being a part of the Soviet Union, the U.S.S.R. Pharmacopoeia was official pharmacopoeia also in Latvia. As already mentioned, DR was not included in the Soviet Union U.S.S.R. Pharmacopoeia Vol. 8 (1952), but in the subsequent volume 9 (1961) DR was described among the reagents used for the microscopical analysis of medicinal herbs. It is evident that the inspiration for adopting this new reagent to the Soviet Union U.S.S.R. Pharmacopoeia was originated from the monographs of international scientists, which were translated into Russian language in the 1950’s. For example, in the foreword to the monograph on organic compound analysis (published in Russian in 1953), it was stated that this monograph is the most comprehensive publication in the relevant field of organic chemistry (Bauer 1953). In 1954, a monograph on paper chromatography was published in Russian language (Block et al. 1954). In this monograph, the use of a modified DR in the chromatographic determination of alkaloids was described. Obviously, the DR-induced reactions described in this monograph to indicate the presence of alkaloids and the ease of their application encouraged the universities to adopt this monograph in the practical work of students as well. Since the 1960’s, the practical laboratory works in pharmacognosy were systematically developed by updating materials and methodologies at the Faculty of Pharmacy of Riga Medical Institute (Dolgova et al. 1966; Rubina et al. 1975). In these research laboratory work teaching materials, DR was described as one key reagent for the detection of alkaloids (together with nine other corresponding reagents). The modification of DR was used to identify alkaloids in the paper chromatograms.

Within the post-Soviet-Union period (from 1991 to date), pharmacy students at Riga Stradins University have carried out the common alkaloid detection reactions in pharmacognosy classes. One of the reagents used in detection reactions is DR. Recently, a tropane-group alkaloid paper chromatography was also adopted in practical classes, and the presence of such alkaloids in herbal medicines was detected using a modified DR.

3.3. Lithuania

The isolation and properties of two alkaloids (morphine and quinine) were described in the journal “Punieniekis Farmaceutyczny Wilcik” (Notes of Vilnius Pharmacy) in 1820 and 1821. Some decades later, Dragendorff’s investigations of alkaloids and his discoveries (including DR) became well known also in Lithuania, and in 1869 he was elected as a member of the Vilnius Medical Society (Gudienė 2017). Furthermore, his personality was well known to the pharmacists of interwar Lithuania. Professor Dragendorff is mentioned frequently as one of the most famous pharmacists in the professional journal of Lithuanian pharmacists “Farmacijos žinios” (Pharmacy News) (1923–1940). The Lithuanian Pharmacopoeia (1938) presents four reagents in total: Esbach, Meyer, Milon, Nesler. The Pharmacopoeia describes five alkaloids (morphine, atropine, quinine, caffeine and codeine), but perhaps surprisingly, the reagents for the identification of these alkaloids are described. Only a few papers on alkaloid-accumulating plants and alkaloids has been published in the „Farmacijos žinios” (Pharmacy News) journal. Elena Paškovičaitė Purtokienė, Assistant Professor in the Department of Pharmacy and Pharmacognosy at the University of Lithuania (known as Vytautas Magnus University since 1930) published an article on the methods for the determination of alkaloids (Gudienė 2017). This research work was inspired and partially based on the article published by the German scientists J. Gadamer and E. Neuhoff, who described the determination of alkaloids according to the IV German Pharmacopoeia and by using a C.C. Keller method. In her article, E. Purtokienė described the use of the German Pharmacopoeia (V and VI) and the other related methods for the detection of alkaloids, but DR was not used in these methods (Purtokienė 1929).

In Lithuania, DR has been applied in the laboratory training courses at the universities since the Soviet-Union (USSR) times. The Lithuanian scientists and students read the Soviet-Union scientific literature in Russian language and learned the methods for the identification of alkaloids developed by Dragendorff. As already mentioned, the pharmaceutical chemistry textbook published by P. L. Senov described the most important alkaloid reagents including DR (Senov 1952). In the 1960’s, the Pharmacy students were taught to identify alkaloids by DR in Lithuania. In the 1970’s, however, the new textbooks authored by Professor E. F. Kramarenko (Kiev) and his prominent student Professor Paulius Vainauskas, Dean of the Faculty of Pharmacy (1996–2006), Lithuanian University of Medicine, replaced the textbook of Senov. In 1975, the Lithuanian scientist in pharmacognosy, Eduardas Kanopka, published a book entitled “Alkaloids and their raw materials”. In this book, he described in detail the methods introduced by Professor J. Georg N. Dragendorff (University of Tartu) for the qualitative and quantitative analysis of alkaloids. E. Kanopka also mentions the textbook of A. Orechov “Alkaloid chemistry” (1938) and emphasizes that until 1938 there was no textbook on alkaloids chemistry available in Russian language (Kanopka 1975). Both authors (Kanopka and Orechov), however, do not describe the use of the DR in their books. In the Toxicology Laboratory of Lithuania, DR was a basic reagent until 2000 in the identification of alkaloids and in the detection of drug poisoning (in urine or liver) caused by biological materials.

Nowadays, pharmacy students of the Lithuanian University of Health Sciences (Kaunas) still are familiar with the identification and testing methods of alkaloid-like substances developed by Dragendorff. The method based on DR is included in the study programs of pharmacognosy, toxicology and pharmaceutical chemistry. Qualitative testing of alkaloid containing plant material is presented in the lectures of pharmacognosy and in the
More recently, Liukkonen (1960) analyzed atropine, papaverine, thebaine, narceine, papaverine, and narcotine were well separated in preparations (Krogerus and Tuovinen 1957). Morphine, codeine, and the detection of the abovementioned drugs on a two-dimensional paper chromatogram of alkaloids. Qualitative determination of alkaloids. Assay of alkaloids. The set of suitable reagents include Mayer’s reagent, Wagner’s-Bushard’s reagent, and Marne’s reagent, tannin, sili- cotungstate, phosphomolybdiate, and picric acid. The fingerprints of the alkaloids on paper chromatography (Thermopsisid herba, semen) and thin-layer chromatography (TLC) (Thermopsisid herba, semen, Atropae belladonae folium, Daturae semen) plates are visualized by spraying them with a DR solution. The application of DR and its modifications (i.e., Munier-Mache- boeuf) in toxicology and forensic chemistry are described in detail. The lectures are given to the 4th and 5th-year pharmacy students at the Lithuanian University of Health Sciences. In addition, these reagents are also presented in the textbook of toxicology and forensic toxicology (Vainauskas and Kazlauskienė 2008). DR is presented not only as an agent for the visualization of TLC spots of various alkaloids (atropine, codeine, quinine, caffeine, cocaine, pseudoephedrine, methamphetamine, arcoleine, etc.), but also for the analysis of benzodiazepines, amidopyrin and other substances. The application of DR for the identification of some pharmaceuti- cally active substances (under colour reaction) is also discussed in the lectures of pharmaceutical chemistry. DR is also listed as a reagent to be applicable in the research part of some master-level (MSc) courses. The part of MSc students perform their experiments using DR to identify alkaloids in plant materials and for the TLC analysis of alkaloid-like markers and reagent-sensitive drugs, such as benzodiazepines.

3.4. Finland

We did not find very much information about the status of DR in the former Finnish and Nordic Pharmacopoeia and the use of the reagent in the higher education in Pharmacy. In Finland, the bachelor’s, master’s and postgraduate higher-degree education in pharmacy are offered by two universities: University of Helsinki and University of Eastern Finland, Kuopio. In addition, the Åbo Akademi University, Turku offers bachelor of science in pharmacy, BSc (Pharm) programme only in Swedish language. At the University of Helsinki, DR (also called as a “iodobismuthate reagent”) has been applied in the laboratory training courses in pharmacognosy for the identification of alkaloids. Pharmacognosy (today known also as pharmaceutical biology) has a long-term history as a teaching subject at the University of Helsinki, and the subject has established its position as one of the major subjects in pharmacy. To our best knowledge, the University of Eastern Finland and Åbo Akademi University do not provide any special courses in pharma- cognosy, and thus the information on the application of DR in their teaching courses is negligible.

A review of the literature reveals several examples about the use of DR in the scientific research works published by Finnish scien- tists mainly in 1950’s and 1960’s. Krogerus and Tudenman (1954) studied the mixtures of morphine-HCl, codeine phosphate, scopol- amine-HBr, strychnine nitrate, ethylmorphine-HCl, diacetylmor- phine-HCl, atropine sulfate, cocaine-HCl, quinidine sulfate, and papaverine-HCl by means of two-dimensional paper chromatog- raphy. The authors found that the minimum quantities necessary for the detection of the abovementioned drugs on a two-dimensional chromatogram with a diluted DR were in the range of 10-60 g. In another study, papaverine-HCl and narcotine-HCl were separated in an ascending chromatography by using a dioxane-water-formic acid (90:9.5:0.5 ml) mixture, and the chromatogram was developed with DR (Krogerus 1955). Professor V.E. Krogerus, who was later in the 1960’s an acknowledged professor in galenic pharmacy at the University of Helsinki, reported the use of DR in the paper chromatographic analysis of opium alkaloids in some galenic preparations (Krogerus and Tuovinen 1957). Morphine, codeine, thebaine, narceine, papaverine, and narcotine were well separated and subsequently identified by comparison with pure alkaloids.

More recently, Liukkonen (1960) analyzed atropine, papaverine and morphine in tablets and suppositories by paper electrophoresis, and the separated bands in the dried paper strips were detected with DR. Levonen (1960) applied DR for the identification of seminal stains by paper chromatography in the field of forensic sciences. The simultaneous detection of choline and spermine was presented. As the samples of interest were sprayed with DR, spermine gave a pink color and choline a deep purple. The scientists at the Univer- sity of Tampere, Finland applied benzidine-Dragendorff’s reagent in TLC for rapid testing alkaloids (drugs) and their metabolites in human urine samples in emergency situations (Kärkkäinen and Vapaatalo 1978).

3.5. Ukraine

The Department of Pharmacognosy of the National University of Pharmacy originates from the laboratory of the Imperial Kharkiv University, which was founded on the initiative of V. N. Karazin (1805). Its history has something in common with the history of the University of Tartu. According to the Charter (§22, 1804), the Department of Medicinal Theology, Pharmacy and Medicinal Literature was established at the University. Such departments in Europe were called “materia medica”. The Department was headed by the famous chemist Johann Emanuel Ferdinand Giese before his moving to the UD (Estonia) in 1814. By the resolution of the Medical Faculty in 1839, the teaching course of pharma- cognosy was created in the Department of Medicinal Theology, and in 1847-1848 it was separated from pharmacology (Bagaley 1893-1898). The Kharkiv Pharmaceutical Institute was established on the basis of the laboratory in 1921. In the beginning of the 1950’s, the new directions for studying the alkaloid-based raw material began. The method of isolating alkaloids from the plant raw materials and identification with the help of DR were used (Dragendorff 1872). The methods were described in the theses of I.V. Manko “The chemical study of blueweed (Echium vulgare) and hound’s-tongue (Cynoglossum officinale) of the heliotrope family” (1954) and P.M. Lyapunova “The phytochemical study of common periwinkle”, (1964). Today, our department is studying alkaloids in the wild species of plants of the Ukrainian flora, in particular the Borag- inaceae family. The presence of alkaloids with different chemical properties and pharmacological action was found in blueweed (Echium vulgare). An alkaloid that showed a curare-like activity in animal tests was found. A new alkaloid named cynoglossofine was isolated from hound’s-tongue (Cynoglossum officinale). The alkaloid composition of individual organs of Vinca minor L. of the Apocynaceae family was studied. For the first time, the alkaloid reserpine was isolated from periwinkle; vinca and vincamine were extracted from the root of the medicinal raw material. The laboratory regulations for the antihypertensive and antisepsic drug “vimcin” were developed (Koshovyi and Kovalov 2018). The 1st edition of the State Pharmacopoeia of Ukraine (SpU, 2001, 2004, 2008, 2011) (amendments 1-4), contains ten monographs on the determination of alkaloids by TLC using DR. The SpU, 1st edition (2001) recommends to use DR (R1), its modification (R2) and potassium bismuth iodate diluted solution for the identifica- tion of alkaloids. Potassium bismuth iodate solution R1. 1070601: Dissolve 100 g of tartaric acid R in 400 ml of water R, add 8.5 g of basic bismuth nitrate R, shake for 1 hour, add 200 ml of the solution of 400 g/L of potassium iodate R and shake vigorously. Stand for 24 hours and filter. Store in a place protected from light.

Potassium bismuth iodate solution R2. 1070602: Starting solution. Suspend 1.7 g of basic bismuth nitrate R and 20 g of tartaric acid R in 40 ml of water R. To the suspension add 40 ml of the solution of 400 g/L of potassium iodate R, shake for 1 hour and filter. The shelf life of the solution is several days when stored in orange glass vials. The solution is for spraying. Immediately before use, mix 5 ml of the initial solution with 15 ml of water R. Potassium bismuth iodate diluted solution. 1070603: Dissolve 100 g of tartaric acid R in 500 ml of water R and add 50 ml of potassium bismuth iodate solution R1. Store in a place protected from light.

According to some articles, the SpU (2008, 2009, 2011) recom- mends treating the chromatograms with DR followed by treating

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the plate with the solution of sodium nitrite. In this case, the detection sensitivity increases to 0.1 μg, allowing one to differentiate hyoscyamine from atropine, as well as impurities. In the SPbU (1st edition, 2001), DR is recommended for the identification of biologically active substances by means of chromatography in the following ten monographs: “Atropine Sulfate” (p. 323); “Econazole Nitrate” (p. 369); “Ethylmorphine Hydrochloride” (p. 570). In the SPbU, 1st edition (2004), there are monographs “Bupivacaine Hydrochloride” (p. 300) and “Codeine” (p. 380); in the SPbU, 1st edition (2008) – “Greater celandine”; in the SPbU, 1st edition (2010) – “Belladonna Leaf” (p. 156-158); in the SPbU, 1st edition (2011), “Standardized Belladonna Leaf Tincture” (p. 293), “Clowernine Hydrochloride” (p. 446), “Stramonium Leaf” (p. 307). The SPbU! (the 2nd edition in three volumes and three amendments, 2011-2018) contains the same monographs with the identification of alkaloids and nitrogen-containing compounds. New monographs on Boldo leaves (Peumus boldus) appear in the SPbU 2.2 (p. 142, 2014), Boldo leaf dry extract (p. 140); in the SPbU 2.3 the monograph on celandine (Chelidonium majus) has the national part concerning the celandine herb (Chelidionii herba) (p. 490, 2014). In the educational process (Bezugly 2008) and in the research of phytochemistry (Kolysnyk 2015; Avidzba et al. 2018; Golopyorova et al. 2019; Kovalyova et al. 2019) concerning alkaloids of different nature, DR and its modifications are currently used in the Department of Pharmacognosy, National University of Pharmacy in Kharkiv. For example, the DR by R. Munier; if the sensitivity of chromatographic developer is 1-3 μg of substances in the sample, DR in the modification of D. Vagujfalvi is used. The reagent diluted in the ratio of 1:4 with the subsequent treatment of the chromatogram with sulfuric acid increases the sensitivity of the detection of alkaloids. In biological materials, DR is used in the Moldaver modification (the minimum detection of 0.30-1 μg, Silifol) and A. S. Tschchenko (1966) modification (the reagent composition: 1.5 g of NaBiO₃, 7.5 g of KI, 100 ml, 2% H₂SO₄) (Bolotov et al. 2011; Bondar and Bagulya 2013).

3.6. Moldova

Today, the European Pharmacopoeia (Ph.Eur., the 9th edition) is in use in the Republic of Moldova. The provisions of European Pharmacopoeia are established as mandatory quality standards for both domestic and imported medicinal products for human use (ORDER Nr. 776, 2017). In addition, the references to the provisions of the US Pharmacopoeia (USP), British Pharmacopoeia (BP) and Japan Pharmacopoeia (JP) are in force. These pharmacopoeias are valid in the cases, if there is a need to use the methods of analysis and control of medicinal substances, and if the corresponding methods are not included in the current 9th edition of the European Pharmacopoeia. The relevant monographs of the Romanian Pharmacopoeia, the Pharmacopoeia of Republic of Belarus and the Pharmacopoeia of Russian Federation (current editions) are also valid for assessing the quality of medicinal vegetable products, which are not included in the Ph.Eur., USP, BP or JP (ORDER Nr. 113, 2011).

In Moldova, the students of the Faculty of Pharmacy at the Nicolae Testemitanu State University of Medicine and Pharmacy are using a national textbook of pharmacognosy, which describes the application of DR in a qualitative reaction for the identification of alkaloids (Nistreanu and Calab, 2016). The present reaction is also one of the most common sedimentation reactions used with alkaloids. According to the textbook, DR forms a brown precipitate with most alkaloids in weakly acidic solutions. A number of other reagents intended for the identification of alkaloids, are also listed in this textbook (i.e., Mayer’s, Wagner’s, Bouchardat’s-Marme’s reagents). The preparation of DR is described in the textbook a follows: For preparing Solution 1, 0.85 g of bismuth sub-nitrate is dissolved in 40 ml of water and 10 ml of acetic acid is added. For preparing Solution 2, 20 g of potassium iodide is dissolved in 50 ml of water. Equal volumes of Solutions 1 and 2 are mixed. Finally, 100 ml of water and 20 ml of acetic acid are added in 10 ml of the abovementioned mixture of Solution 1 and 2 (Nistreanu and Calab, 2016). Today, DR has found uses also in other applications. For example, in Romania (a neighboring country of the Republic of Moldova) DR was applied for the detection of beta-lactam antibiotics. Beta-lactam antibiotics, however, proved to be less sensitive to this commonly used chromatographic reagent (penicillin gave a very pale orange spot) (Hancu et al. 2013).

3.7. Asia (Vietnam)

The alkaloids isolated from many Asian herbs are reported to have antibacterial, antiviral, antiproliferation and anticancer (cytotoxicity) effects, and consequently, the use of alkaloids as drugs has gained a wide interest in Asian countries (Amirkia and Heinrich 2014; Cushnie et al. 2014; Gyawali and Ibrahim 2014; Qui et al. 2014). Screening the phytochemical properties of plant extracts (from an unknown plant material) is usually the first step to indicate it, if there are any interesting phytochemicals present in the plant. Mayer reagent, Boucharad reagent and DR are the most common reagents for detecting alkaloids through a precipitation reaction in many Asian countries. DR, however, is especially mentioned in many textbooks as the most important reagent for the identification of alkaloids.

Today, chemistry laboratories in many Asian countries (including Vietnam) are using routinely DR for the detection of alkaloids in TLC. The non-selective methods intended for the detection of a wide range of different organic compounds in TLC, are usually very fairly sensitive (such as iodine vapor or iodine spray reagents and concentrated sulfuric acid). DR, however, is selective and specific only for alkaloids (Toshiyuki and Fujimura 1975). Indian scientists used a simple spectrophotometric method to estimate the total amount of alkaloids precipitated by DR in plant materials (Sreevithya and Mehrotra 2007). In both the Vietnamese Pharmacopoeia (2018) and the Chinese Pharmacopoeia (2015), DR is described as a primary reagent for identifying alkaloids. In the Indian Pharmacopoeia (2018), however, potassium mercuric iodide is presented as a precipitating reagent for alkaloids. The first Vietnamese Pharmacopoeia (the 1st edition, Vol. 1) was issued in 1971 with various difficulties due to a war period. Subsequent editions were published in 1978, 1994, 2002, 2010 and 2017. In the Vietnam Pharmacopoeia (the editions ranging from the 2nd to 5th one), a DR solution is described as follows: Solution 1 (0.85g of bismuth nitrate base in 40 ml of water and 10 ml of acetic acid), and Solution 2 (8 g of potassium iodide in 20 ml of water). Then, mix the volume of Solution 1 and Solution 2. Add 100 ml of water and 20 ml of acetic acid to every 10 ml of the obtained mixture. According to Vietnamese Pharmacopoeia, the 5th edition (2017), about 20 medicinal herbs out of total 372 medicinal herbs and herbal medicines can be determined by DR. In these analyses, a few drops of a DR solution are added into the obtained mixture of medicinal herbs to yield a precipitate with a specific color ranging from red brown (orange red) to orange yellow. The examples of medicinal herbs yielding such a colored precipitate by DR include: Radix Stemonae tuberosae, Tuber Stephaniae, Rhizoma Typhonii trilobati, Herba Hyoscyami capitellatae, Radix Cantonarthise rosei, Radix Streptocauli, Rhiizoma Cyperi, Herba Leonuri japonici, Herba Passiflorae foetidae, Herba Clerodendri philippini, Cortex Holarrhenae, Folium Crini asiatici, Radix Acini, Radix Acini lateralis, Semen Quisqualis, Radix Gentianae, Folium Crini latifoli, Dipsaci, Folium Erythrinae auriegatea, and Rhizoma Liguistici wallichii.

In some cases, DR has been reported to produce false-positive results. The positive test may not always indicate the presence of alkaloids, since the presence of other plant constituents, such as purines, proteins, betaines and ammonium salts, could also yield positive test results with DR. Moreover, DR does not give precipitates with all alkaloids, such as caffeine, strychnine, and brucine. In our experience, it is necessary to carry out the reaction at least with 4-5 reagents to confirm the presence of alkaloids. In addition to the detection of alkaloids, DR is also used in TLC for the determination of other active ingredients in herbal medicines (by spraying color reagents on a chromatographed thin plate). In this procedure, 5-20 μl of standard solutions and the herbal extracts
are separately applied onto TLC plates. After developing a solvent system, the thin plates are allowed to dry in air. After this, the thin plates are sprayed with DR, and inspected under a normal light. On the chromatogram of a test solution, there should be the traces of the same color, and the Rf value with the main active trace on the chromatogram of a reference solution. In Vietnam Pharmacopoeia, the 5th edition (2017), the present spraying method (i.e., combining DR with TLC) is applied for the analysis of numerous active ingredients, such as tetrahydrodalmatin in Tuber Corydalis, ajmalicine in Radix Cantharanthi rosei, palmatin in Caudis et radix Fibraureae, strychnin and brucin in Cortex Strychnyi wallachianica, stachydrin hydrochloro in Herba Leonuri japonicae, strychnin and brucin in Semen Strychi, conessin in Cortex Holarrhenae, lycorine in Folium Crini asiatici, acosinin in Radix Aconiti lateralisi, and cinaminin in Folinium Crini latifoli. In Vietnam, the Pharmacy students at the universities learn to know DR as one of the three specific reagents for the determination of alkaloids. They also get to know the test intended for analyzing alkaloid extracts, such as the extract of Datura (Vietnamese Pharmaco- poecia, 2017). The present test was described in more detail below (two alternative test protocols):

(1) Alkaloids are extracted with water or with the mixture of ethanol and water. After extraction at least 3 times, take 0.1 ml to 0.2 ml of the next extract, acidify with 2 M hydrochloric acid solution, add 0.05 ml of potassium tetraiodocondomeric solution (Mayer’s reagent), or add 0.05 ml of potassium iodobismuthate solution (DR) to the alkaloids belonging to a tomato family. There should be no precipitate or cloudy solution. In the case of an extract with an alcohol content greater than 25% or more, after acidification, the solvent should be evaporated before adding a precipitate reagent.

(2) Alkaloids are extracted by organic solvent. After extraction at least 5 times, take 1 ml to 2 ml of the next extract, add 1 ml to 2 ml of 0.1 M hydrochloric acid solution, evaporate run out of the organic solvent, transfer the remaining liquid into a test tube, and add 0.05 ml of potassium tetraiodocondomeric solution (Mayer’s reagent), or add 0.05 ml of potassium iodobismuthate solution (DR) to the alkaloids belonging to a coffee family, or add 0.05 ml of sodium-potassium iodide solution (Bouchardet’s reagent) to emetin. No obvious turbidity may form in solution.

4. Commercial Dragendorff’s reagents and modifications

To date, DR has been widely used in various pharmaceutical and forensic chemistry applications and the use of this versatile reagent is steadily increasing. For example, a Google search by the key phrase “Dragendorff reagent” gives total 47,700 results (hits) (17/02/2020). One example of the alternative applications of DR is that it is widely used today for the production of surfactants. In this process, a non-ionic surfactant is precipitated in water solution R and its variations R1 to R5.

References


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