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Technologies of Isolation and Separation of Anthraquinone Derivatives

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***Abstract*—**In review the generalized data about different methods of extraction, separation and purification of natural and modify anthraquinones is presented. The basic regularity of an isolation process is analyzed. Action of temperature, pH, and polarity of extragent, catalysts and other factors on an isolation process is revealed.

***Keywords*—**Anthraquinones, chromatography, extraction, phytopreparation, precipitation.

I. Introduction

CREATION of high-performance low-toxicity medicine preparations is one of the most important challenges for medicine, pharmacology and organic chemistry. Scientists in many countries search for biologically active compounds extracted from natural materials with synthetic methods as well as synthesized as a result of structure change in well-known biologically active compounds by inclusion of new functional groups, replacement of heteroatoms, creation of new types of chemical bonds and other processes.

Chemical modification of any natural anthraquinone as a way of searching for or targeted synthesis of biologically active compounds may be justified only if raw sources for their production are accessible. In many plants of only one family *Polygonaceae* concentration of the sum of anthraquinones varies from 1.5 to 10% (*Rumex gmelinii*); thus, raw sources and undemanding conditions of their growth enable to suppose that source of raw materials for such research is available. The other side of the problem is technology of extraction of natural anthraquinones from raw materials. In this regard, search of effective methods of extraction of bioactive anthraqionone derivatives is actual.

II. Results and Discussion

Most of natural anthraquinones have been found in higher plants of the *Polygonaceae, Rubiaceae, Rhamnaceae, Leguminosae, Bignoniaceae* and other families, and a significantly smaller number have been isolated from algae, lichens, fungi, insects, sea animals, and so on.

Non-substituted anthraquinone has been found in the essential oi1 of tobacco, the wood extracts of *Quebrachia lorentzii* Criseb. and *Acacia decurrens* Willd.; however, in these cases, this compound does not apparently occur in the initial plant material, but is formed during isolation [1].

The content of anthraquinone in plants varies depending on the vegetation phase, the age, areal, soil, and the climatic conditions of growing [2].

In higher plants, anthraquinones are localized predominantly in the heart wood, cortex, roots, and rhizomes, or, more rarely, in stems, fruits, and flowers. Unlike many other natural substances, quinones are produced by the callus and suspension cultures of the higher plant cells.

A characteristic feature of oxidized forms of anthracenes (note that it is these forms that are found in plants most frequently) is their stability to air and to temperature and pH variations. Hydroxyanthraquinones are yellow, orange, and red substances producing brightly coloured solutions in alkalis and in concentrated sulfuric acid; the colour of the solutions becomes deeper with increasing the alkali concentration or on addition of boric acid to solutions of hydroxyanthraquinones in concentrated sulfuric acid. All types of hydroxyl-anthraquinones form salts with alkali metals and stable lacs and complexes with Al, Fe, Cr, Ba, Mg, Cu, etc. [3].

Depending on the number and location of OH groups, different colour develops when hydroxyanthraquinones react with magnesium acetate in an alcoholic solution: 1,2-dihydroxyanthraquinones (the alizarin type) give violet, 1,4-dihydroxyanthraquinones (quinizarin type) produce purple, and 1,3- 1,6-, and 1,8-dihydroxyanthraquinones (chrysazin type) give orange-red colour. For 1,8-dihydroxy-anthraquinones, the method of obtaining coloured compounds by interaction with magnesium acetate is more sensitive and selective than the reaction with an alkali. The two OH groups in the p-positions are responsible for the fluorescence in an acetic acid solution and for the intense blue colour in sulfuric acid [4]. The qualitative reaction for o-hydroxy groups (alizarin) with zirconium nitrate (a red-violet precipitate in an acidic solution) is not out of date yet [5].

In alkaline solutions the dimeric forms of natural anthraquinones give yellow and green colour, which changes to red-brown upon oxidation. Acidifying the alkaline solution and extracting with ether makes it possible to isolate a fraction that turns red on addition of pyridine or piperidine and develops green colour in concentrated sulfuric acid. This colouring is imparted by compounds with the dianthrone structure of a hypericin type, which decompose to give anthraquinones and anthrones. This phenomenon is also characteristic of the dimers with the 1,1'-type of bonding and free 2- and 4-hydroxy groups. A similar colour has also been

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observed for skirin (8,8'-diemodin) [6].

In natural objects, anthracene derivatives may occur as anthracene glycosides, incorporating residues of various saccharides, or as aglycones without these residues. The former are readily soluble in water and alcohols and almost insoluble in nonpolar organic solvents, which dissolve aglycones. Hence, to separate them, extraction should be conducted first with nonpolar solvents, in order to isolate anthracene aglycones, and then with polar solvents, to extract all other anthracene derivatives [2].

To ensure the most complete extraction of anthracene derivatives, the glycosided forms should first be hydrolyzed by dilute hydrochloric, sulfuric, or acetic acid and then the overall anthraquinone aglycones should be extracted with ethyl acetate, acetone, or alcohol [7].

The phytochemical analysis of fresh plant slices for the presence of anthraquinone derivatives is conveniently performed using a 5% aqueous solution of alkali or ammonia (red shades). Specific colour reactions for various anthraquinones, anthranols, and anthrones based on the use of more than 40 reagents have been described. The most popular reagents for paper chromatography are ammonia vapour (pink colouring), 3-5% aqueous solutions of sodium carbonate or caustic soda (from pink to cherry-red), a 1% alcoholic solution of magnesium acetate, or the same with addition of ammonia (from pink to violet shades depending on the number and positions of substituents). A 5% borax solution, a 1% iron chloride solution, 25% phosphoric acid, a solution of silver oxide in ammonia, a 5% zinc acetate solution, and some other reagents are used more rarely because they give colouration with any phenolic OH group.

To identify the reduced forms, a 1% pyridine solution o-nitrosodimethylaniline is used (green tints on heating). The colours developed in an alcoholic solution of magnesium acetate, as noted above, might be indicative of the number and position of hydroxy groups.

The anthraquinones with a COOH group are soluble in aqueous solutions of sodium bicarbonate or carbonate and in alkali; the derivatives with α- and β-hydroxy groups differ in solubilities and in the colour produced in these solutions; this can be used for their preliminary identification and separation. However, one should take into account that the colours of solutions may be non-specific when different forms of anthraquinones are present simultaneously.

Hydroxyanthraquinones and anthrones are well soluble in nonpolar organic solvents; therefore, the above-mentioned differences in their solubilities can be used for the complete recovery of all the possible anthraquinone derivatives and for their rough separation.

Anthraquinones have also been extracted from raw materials using liquefied gases and ultrasound, fractional extraction and countercurrent with infusion [8].

From sea lily, urchins, ophiuroideas, sea cucumbers, and starfish, anthraquinones, including their sulfo and halogen derivatives, are extracted with ethanol or acetone after treating the animal objects with hydrochloric acid [9].

A particular solvent and extraction conditions should be chosen taking into account the type of raw material and the nature of the accompanying compounds. Any solvent (polar, nonpolar, or mixed) usually extracts not only anthraquinone derivatives but also substances of non-anthraquinone nature which must be separated.

Since natural anthraquinones by their chemical structure are polyphenols and/or O-glycosides, vegetable material is treated by water, alcohols (ethyl, methyl) or water-alcohol mixtures in order to extract them. Aglycones are better soluble in organic solvents but they have selective solubility. In order to obtain aglycones, glycosides contained in vegetable material are subjected to hydrolysis (10% H_2SO_4) or enzymatic splitting after which free aglycones are extracted by ether, benzene or chlorophorm. Anthraquinones with a carboxyl group as a substitute dissolve in water solutions of carbonates and hydrocarbonates of alkaline metals and their hydroxides with formation of salts. Anthraquinones with a hydroxyl in β-position do not interact with hydrocarbonates but interact with water solutions of carbonates and hydroxyls of alkaline metals with formation of phenolates. Substances containing α-hydroxyl form phenolates in alkaline solutions only [3].

Raw material is grinded to the sizes of particles of 3-5 mm and extracted during heating and mixing in ordinary extractors, as a rule, in 2–3(4) runs of 2-5-hour cycles or by the counterflow.

In order to separate accompanying substances, extracts are combined, concentrated to a small volume and fractioned according to the chemical nature of accompanying substances.

Aglycones – chrysophanol, emodin, physcion, rhein and aloe-emodin – are chromatographed in parallel with eluates (from the extract) on Silufol UV_{254} plates in the system toluene-ethyl ether of formic acid – formic acid (5:4:1). It should be noted that it is quite possible to spray acid hydrolysate on chromatogram without extraction of aglycones by liphophilic solvents. The described method makes it possible to make quick and reliable identification of aglycone part of "bound" anthraquinones (if two or three thin-layer chromatograms are available) [2], [6].

In many plants the sum of the most widely spread aglycones (chrysophanol, physcion and emodin) dominate. It is not difficult to separate them using the scheme:

Crude extract
↓
5-7% $NaHCO_3$ (individual Emodin)
↓
Chrysophanol with Physcion mixture
↓
KI, ethane-1,2-diol
↓
Chrysophanol with Emodin mixture
↓
Al_2O_3, crystallization
↓
Chrysophanol + Emodin

Fig. 1 Scheme of selective extraction of natural anthraquinones

It is also possible to separate anthraquinones using

chromatography on KSL silicagel columns. The columns are washed by the mixtures of solvents which give good separation in thin layers of silicagel, namely, the mixture of toluene, ethyl ether of formic acid and formic acid (5:4:1); the mixture of benzene and carbon tetrachloride (1:1), ethyl alcohol and benzene (92:8) and other mixtures. The ratio of the mixture and adsorbent is 1:150; the volume of fractions is 50 ml. An optimal variant realized in the scheme of dealkylation of the mixture of chrysophanol and physcion was studied using all methods for dealkylation in the aromatic series described in literature [see Table I].

TABLE I
INFLUENCE OF DEALKYLATION AGENTS AND TIME OF REACTIONS FOR CHRYSOPHANOL OUTPUT

Desalkylating agents	Time of reaction, hour	Output of chrysophanol,%	Comments
H_2SO_4	5-18	15-20	resinification
Pyridine hydrochloride	8-9	60-65	no gums
HBr / 100% HAc	5-20	40-45	resinification
KI /ethane-1,2-diol	10-15	55-68	no gums
$AlCl_3$ / 1,4-dioxane	8-15	30-35	no gums
$AlCl_3$/nitrobenzene	6-15	22-25	no gums
KBr / 1,4-dioxane	6-15	15-20	no gums
KBr / H_2SO_4	6-18	30-35	resinification

As it is seen from the Table, the best outputs of chrysophanol were observed in the 9-hour reaction with pyridine hydrochloride and in the 10-15 hour reaction with KI in ethylene glycol. We chose the second method because of inconveniences of work with pyridine.

For the separation and identification of anthraquinones, paper electrophoresis can be applied with the use of 0.05 and 0.1 N sodium borate solutions in 0.1 N sodium hydroxide as the electrolyte. Thus the anthraquinones of Rumex have been successfully separated on Whatman paper N3 at pH 2.2 over a period of 2 hours using an acetate-formate buffer [10].

When working with new plant objects with unknown structures, it is necessary to keep them away from heat and light, because anthrones, anthranols, dianthrones, anthraquinonaldehydes, and some hydroanthracenes are thermo- and light-sensitive, and anthraceneglycosides are decomposed by enzymes, acids, and alkalis.

The methods of countercurrent extraction, liquid-liquid chromatography (LLC), drop countercurrent chromatography, high performance liquid chromatography (HPLC) or a combination of chromatographic and other methods are suitable for the separation of anthracene glycosides [2], [7], [10].

The method of ascending paper chromatography is used with two types of solvents: mixtures of alcohols with acids and water are used to analyze and separate glycosides, whereas low-polarity solvents are used to analyze aglycones. The chromatograms are examined in UV light: aglycones shine yellow and orange, anthrones, anthranols and dianthrones glow yellowish green, glycosides fluoresce pink or orange, and the glow of dimers varies from straw yellow to light brown.

Depending on the oxidation state of the rings, the number, nature, and mutual arrangement of the substituents, anthraquinone derivatives exhibit different mobilities on paper and in a thin layer of a sorbent: the more substituents in a structure, the smaller the R_f value. The hydroxy groups in β-positions decrease R_f in low-polarity solvents. The methylation and acetylation of α-hydroxy groups also result in smaller R_f (compared with those for the corresponding α-hydroxy derivatives); similar derivatives of β-hydroxy-anthraquinones are characterized by larger R_f. The anthrones have lower R_f than the corresponding anthraquinones, apparently, due to the ability to enolyze.

Owing to the formation of intra- and intermolecular hydrogen bonds and the susceptibility of the reduced forms of anthraquinones to oxidation, there is the possibility of «tail» formation, distortion of spot shapes, and doubling of spots on the chromatograms; in these cases, anthracene derivatives should be better chromatographed in an oxygen-free atmosphere [11]. When dimers or compounds with close structures are separated, the paper is sometimes impregnated preliminarily with alcohols, aqueous alcohols, formamide, dimethylformamide, benzene, soda, oxalic acid, or some other solvents.

The paper quality is of great importance in paper chromatography. On «fast» paper, the spots for the substances are sometimes oblong or bean-like, which is often misinterpreted as a mixture of two substances. With such spot shapes it is useful to compare several chromatograms on different types of paper in the same solvent.

The following systems provide good separation: petroleum ether saturated with a mixture of 97% methanol and benzene (50:1); carbon tetrachloride - benzene (49:1); chloroform - methanol (8:2); buthanol – pyridine - benzene-water (5:3:1:3); petroleum ether – acetone - water (1:1:3); toluene - acetic acid - buthanol (1:1:1); pentane – xylene - methanol (4:2:1); buthanol – methanol - water (5:5:2); amyl alcohol-acetic acid-water (4:1:5) and (2:1:2); petroleum ether (b.p. 40°C) – toluene – xylene - methanol (4:1:1:1); octanol - butyric acid - 2-propanol (3:3:1), pentanol - isobutyric acid - acetic acid - water (15:10:10:10); cyclohexane – benzene - methanol (35:25:10), etc. [1]-[4], [9].

A mixture of the oxidized and reduced forms can be separated on paper impregnated with 11% soybean oil using a methyl cellosolve - water mixture (4:1) as the solvent. When chromatographed in the system buthanol - acetic acid - water (4:1:5), anthranols have lower R_f values than anthrones and the spots of anthraquinones and dianthrones are located lower than those of the corresponding dianthranols [7], [11]. The clearest separation of the indicated derivatives is attained when the ratio of the solvents in this mixture is equal to 40:13:29 and 40:12.5:29.

Chrysophanic acid and physcion can be separated only in specific cases, for example, in 78% ethanol on paper impregnated with sodium bicarbonate. Chrysophanic acid and emodin cannot be separated in this way because of the coincidence of their R_f. If benzene or toluene is used as

solvents, the chrysophanic acid and physcion move along with the solvent front, the emodin has a medium R_f value, and glycosides and dimers remain at the start [8]. Physcion and chrysophanic acid are separable in pentane with the emodin and glycosides remaining at the start. Instead of pentane, petroleum ether or cyclohexane can be used but if even small amounts of benzene, methanol, or chloroform are added to them, the separation of chrysophanol and physcion does not occur any longer. It is acceptable to use benzene (up to half the chromatogram) and then, after drying, to continue chromatography in pentane in the same direction [3]. We propose separating small amounts of physcion in 60% ethanol on paper impregnated with a sodium bicarbonate solution and large amounts, by fractional treatment of a benzene solution with 1%, 3%, and 5% solutions of sodium bicarbonate. Besides physcion, the soda extracts thus obtained also contain emodin; after isolation they are separated in columns with silica gel by elution with petroleum ether and benzene.

A butanol-acetic acid-water (4:1:5) mixture and water-saturated butanol are suitable for separating emodin, frangulin, and glucofrangulin [7]. We propose separating anthraquinones and their respective anthrones on paper in the chloroform - petroleum ether (7:3) system or in a thin silica gel layer in the acetone - carbon tetrachloride (4:1) system.

Separation of anthraquinones, anthrones, and hetero-dianthrones by treatment of an ethereal extract from plants with a bicarbonate solution followed by chromatography on paper impregnated with alcohols or formamide as well as by means of successive treatment of the extracts with solvents of different polarity has been described [12].

Sennosides are separated on soda - treated paper in 70% ethanol or without any treatment in a toluene - methanol (2:1) system; the chrysophanol and physcioanthrones are separated on paper in heptane. Anthrones are conveniently separated by treating an extract or dissolving a sample of substances in a 0.1% pyridine solution of p-nitrosodimethylaniline with the subsequent paper chromatography in the ethyl acetate - methanol - water (100:17:13) system. Paper chromatography is also used for preparative purposes [2], [6].

In addition to paper chromatography, column and thin layer chromatography are extensively used for analysis and separation [12]. As an example, about 20 sorbents were tested for the separation of anthraquinones from sorrel; the best results were obtained with magnesium carbonate and alumina using benzene, petrol, and chloroform as eluents. However, it was pointed out that alumina can be used only at a low content of emodin, because the latter is tightly sorbed and cannot be completely washed out by any solvent.

The most widely used sorbents are polyamide, silica gels treated with oxalic acid or without treatment, ion-exchange resins, calcium phosphate, silica deactivated with water (5%), cellulose impregnated with 0.5 M phosphate and without impregnation, hydroxides, carbonates, phosphates, sulfates, mixtures of sorbents, cement, etc. [9].

The dimeric structures are readily separated on silica saturated with 0.01 or 0.5 N oxalic acid in a benzene-acetone (9:1) mixture, on Sephadex LH-20, on cellulose [7], more clearly separated zones being obtained in the case of «wet»-filled columns.

As eluents for the separation of anthracene glycosides, mixtures of lower alcohols or acetone with water in different proportions are used; polar and low-polarity solvents are employed for the separation of aglycones. The clearness of the separation is controlled by one-dimensional chromatography of eluates from the zones in a thin silica gel layer or on paper; the use of Silufol plates eliminates the necessity to visualize chromatograms.

For quantitative analysis and separation of anthracene compounds, the methods of gas, liquid, liquid-liquid, and high performance liquid chromatography are used [3].

Gel filtration is acceptable for separating anthraquinones and glycosides on Sephadexes, molecular sieves, celluloses, and ion-exchangers. The column chromatography on polyamide and silica gel in combination with the preparative high-pressure reversed-phase liquid chromatography is appropriate for separating anthracene glycosides. In this case, the retention time of an anthraquinone compound in a column increases with an increase in the number of hydroxy groups, on passing from α-hydroxy derivatives to β-derivatives or from α,α-disubstituted to α,β- or β,β-disubstituted derivatives as well as from compounds with methyl groups to the corresponding methoxy, hydroxymethyl, and carboxyl derivatives [1], [6].

Thus, practically all types of chromatography are applicable to separation and identification of anthraquinone derivatives. These methods can also be used for preparative purposes, quantitative analysis, and the removal of concomitant impurities.

III. Conclusion

Thus, possibility of selective extraction and isolation of anthraquinone derivatives from plants is shown.

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References

[1] R.A. Muzychkina, D. Yu. Korulkin, *The anthraquinones*, Almaty: MV-Print, 2013.
[2] R.A. Muzychkina, *Natural Anthraquinones. Biological Properties and Physicochemical Characteristics*, Moscow: Phasis, 1998.
[3] D.A. Whiting, *Nat. Prod. Rep*., no. 6, pp. 583-606, 2001.
[4] J. Lokvam, P.D. Coley, T.A. Kursar, *Phytochem*., vol. 65, no. 3, pp. 351-358, 2004.
[5] A.A. Eleganus, C. Bates, A.I. Gray, *Phytochem*., vol. 63, no. 6, pp. 727-730, 2003.
[6] I.-L. Huang, P.-I. Ieh, C.C. Shen, C.C. Chen, *Phytochem*., vol. 64, no. 7, pp. 1277-1279, 2003.
[7] Z. T. Zhang, Y. J. Du, Q. G. Liu, Y. Liu, *Nat. Prod. Res. Dev*., vol. 13, pp. 45-47, 2001.
[8] C. H. Chen, C. Y. Shaw, *J. Nat. Prod.,* vol. 65, pp. 740-741, 2002.
[9] L. Frances, *Phytochem*., vol. 35, no. 3, pp. 685-686, 1994.
[10] B. Botta, *Phytochem*., vol. 22, no. 2, pp. 539-542, 1983.
[11] Wu. Tuang-Shung, T.-T. Jond, H.J. Then, *Phytochem*., vol. 26, no. 6, pp. 1623-1625, 1987.

[12] J. Messawa, F. Ferrari, *Phytochem*., vol. 30, no. 2, pp. 708-710, 1991.