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A brief history of taxol

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ABSTRACT

Traditional application of plant in folk medicine is a base for discovery of new active chemical substances. In the second half of 20th century, significant antitumor activity of extract from bark of the Yew tree was recognized. The compound that was responsible for this kind of properties has called taxol and had structure of diterpene with acyl groups. Low availability forced researchers to find different ways of taxol gaining. In the further years, many synthetic and semi-synthetic methods were developed. Nowadays, biotechnological methods with use of cell suspension cultures are introduced.

Key words: cancer, taxol, chemotherapy.

For a very long time, raw plant materials were used for medical purposes [1, 2]. The knowledge considering directions and ways of their therapeutic applications was mainly based on traditional folk medicine. With development of different kinds of qualitative and quantitative chemical analysis, the knowledge of chemical composition of natural therapeutic agents was broadened. Nowadays, besides of very good development of chemical synthesis technology, raw plant materials are still use for isolating many active substances, mainly in enantiomerically pure form. Very often this kind of natural products are the starting point for new compounds synthesizing in belief, that structural analogies may be applicated to gain more effective drugs.

The one of the more interesting examples illustrating this type of attitude of searching for new, biologically active substances is a history of taxol – acylated diterpene which was isolated from yew tree bark with affirmed strong antitumor activity.

Therapeutic properties of yew tree were applicated in practice since the ancient times [3]. Then its toxic activity was also found. In XIX century, on the base on many hundred years experience of traditional folk medicine, a lot of compounds responsible for biological activity of yew tree were isolated. In sixties of XX century, cytostatic properties of extracts from yew tree bark (*Taxus brevifolia*) were developed and confirmed. The structure of active substance named taxol was publicated for the first time in 1971 [4] (Figure 1).

Initially, the only one source of this compound was *Taxus brevifolia* bark; nevertheless taxol is a metabolite formed by each species of yew trees in different but small amounts. Unfortunately, the whole procedure of isolation of this substance from raw plant material *T. brevifolia* was hardly arduous work and underwent with low yield. Isolation of 1 kg of raw natural product required the use of 10 tons of yew tree bark. It means that 3000 of trees ought to be cut out. Also, it shoud be point out, that 1 kg of taxol is sufficient for 500 patients; moreover *Taxus brevifolia* is one of the slowest growing trees in the world. The total number of this yew species in the Northwest of United States was then estimated to one million. Further exploitation of *T. brevifolia* for experimental purposes endangered liquidation to natural places of yew tree. Moreover, the obvious ecological aspect of this problem, it was automatically connected with abandon of very promising research on taxol. It was essential to elaborate some different methods – more costless and higher yielding to obtain this valuable substance from acylic diterpene taxanes group. Within the confines of searching of optimal solution, it was found that in a result of extraction of fresh *T. baccata* needles, different taxane diterpene was gained. It was 10-deacetylbaccatin, which was

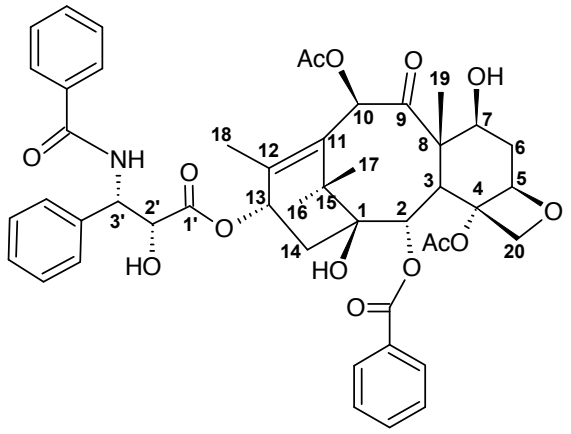


Figure 1. Structural formula of taxol with terpenic skeleton carbon atoms numeration

structurally closed to taxol [5]. The process of isolation underwent from more favourable material and with higher yield – from 3 tonnes of needles, about 1 kg of substance was obtained. Moreover, the procedure did not require destroying of yew trees, because needles are easily renewable. From the moment of elaborating an effective method of transformation of 10-deacetylbaccatin in taxol, it might be obtain in semisynthetic way. This method is based on side chain addition as a result of esterification reaction of C-13 hydroxyl group in 10-deacetylbaccatin with N-benzoylphenylisoserine. This process requires earlier selective esterification of secondary C-7 and C-10 hydroxyl groups. Because of the different reactivity of hydroxyl groups in molecule, first step concerns triethylsilylation of C-7 OH; additionally C-10 hydroxyl group has to be acetylated. This semiproduct may be coupled in reaction with enantiomeric O-protected N-benzoyl-α-hydroxy-β-aminoacid. After deprotection of hydroxyl groups with HCl, taxol is obtained with yield up to 90% (Figure 2).

For synthesis of benzoyl aminoacid system named side chain, different types of reactions were used. In the first publicated method, the Sharpless epoxidation was used as a key step. The optically pure epoxide opening reaction with azide anion gave product with expected configuration. Further steps: O-benzoylation, reduction connected with transfer of benzoyl group from oxygen atom to nitrogen atom, then protecting hydroxyl group and hydrolysis of ester group gave demanding 3-phenylisoserine derivative.

In 1994 R. Holton with co-workers presented the total synthesis of taxol [6]. (–)-Camphor was the main substrate for whole, forty-steps process. But for the reason of very low yield (0.4%) and lack of profitabil-

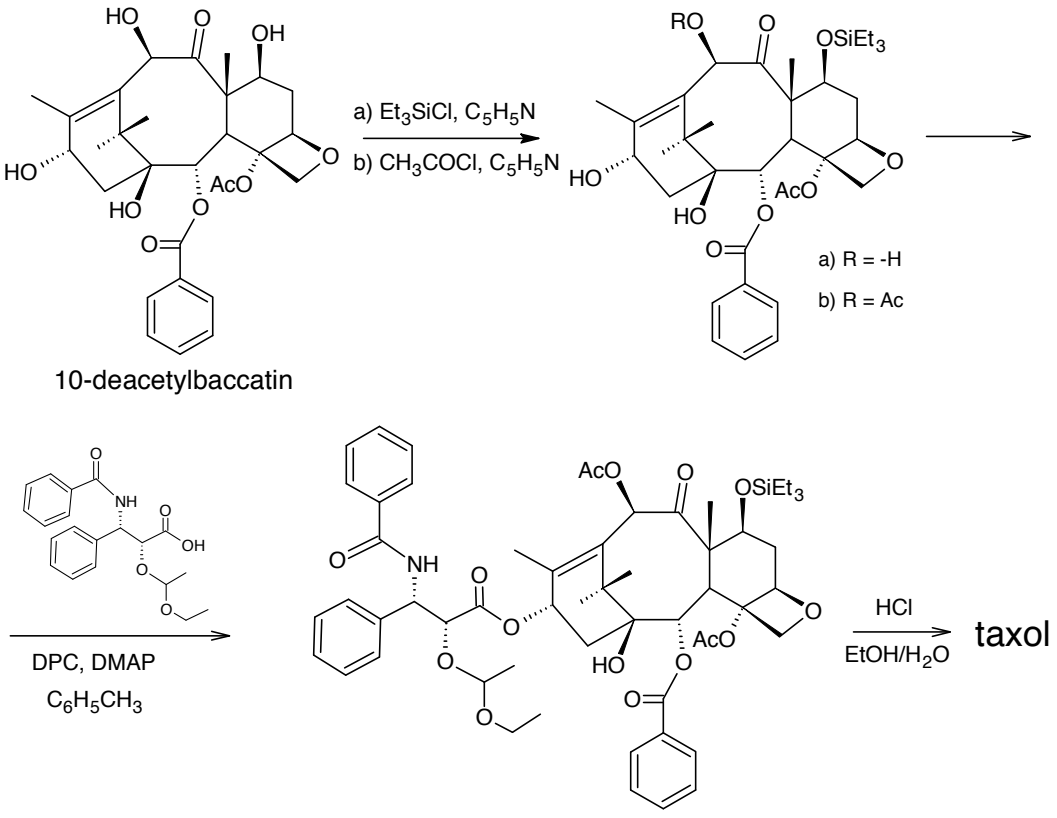


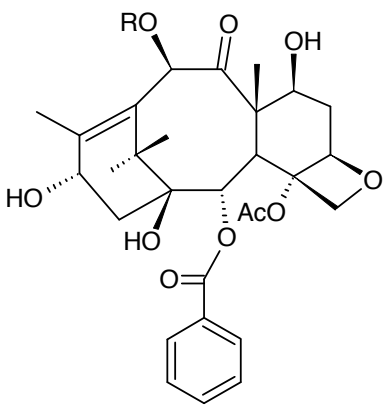
Figure 2. Semi-synthetic method of obtaining taxol from 10-deacetylbaccatine

ity, this method did not find any practical application in pharmaceutical industry. During last a dozen or so years, many reports has been appeared describing the use of *in vitro* cell suspension cultures for biosynthesis of taxol [7–10]. Cell cultures ensure continuity of supplying the starting substances and are independent on quality of raw plant material. Because of control possibility of culture conditions, it may lead to increase of bioproduction of baccatin and 10-deacetylbaccatin. Both are taxol precursors. The structures of these compounds are shown on Figure 3.

Biotransformation methods are very favourable due to the final product of this kind of synthesis is characterized by high purity. Also, the yield of this process is usually high enough. These reactions are highly specific and they let to obtain large amounts of natural compounds which often appear in small quantities in plants. Lastly, R. Venilla and J. Muthumary [8] presented the method of gaining taxol from endophytic fungi cultures *Pestalotiopsis sp*. isolated from tropical tree bark named *Tabebuia pentaphylla*. To confirm the presence of taxol, the fungal samples were subjected to HPLC method. The quantity of final product was estimated to be 208.6 µg/L. Other authors [9, 11, 12] presented the way of obtaining 10-deacetylbaccatin, taxol and other taxane derivatives with use of plant cell suspension cultures isolated from eg. eucalyptus (*Eucalyptus perriniana*), common liverwort (*Marchantia polymorpha*), Indian snakeroot (*Rauwolfia serpentina*), tobacco (*Nicotiana tabacum*) and soya bean (*Glycine max*). Nowadays, there are still some research to improve the production methods of diterpene compounds eg. with use of jatropha (*Jatropha curcas*) [7].

There are many reports describing relationships between taxol structure and its pharmacological activity (SAR) [13, 14]. The basic diterpene skeleton includes ten chiral centers and additionally C-13 side chain has two asymmetric carbon atoms. Depicted on Figure 1 stereochemical system is essential for cytostatic activity of taxol. Several publications have concerned the influence of presence of particular hydroxyl groups, that are substituents in main diterpene skeleton, on SAR. It was found, that removal of C-7 OH and acetoxyl group at C-10 position did not change significantly the cytotoxicity of molecule. However, 4-deacetyl- and 4-deacetoxytaxol had lowest antitumor activity. Also, 1-deoxytaxol was 10-fold less active in comparison with taxol. Substitution in para- position at 2-benzoyl group also decreased the level of biological activity. Interestingly, analogs with ortho- and particularly meta- substituted aroyl substituents were usually more active than taxol. Some of them were even sixfold more cytotoxic. The summary of taxol SAR is presented in Figure 4.

Calculated partition coefficient value (ClogP) for taxol molecule is 4.73. It indicates on high lipophilicity of this compound. Its aqueous solubility is less than 0.01 mg/L, so this substance is practically insoluble in water. The solubility difficulties made its application to patients very limited. This problem was tried to be dissolved in several ways. At the beggining, taxol was administered as a 6 mg/mL surfactant – Cremophor EL and ethanol mixture diluted with normal saline or 5% dextrose in water to the required final concentration [15]. Because of the relatively high taxol doses, patients also received large doses of surfactant, which was not neutral for people health. Several years ago, nanotechnology gave an effective possibility of supplying highly hydrophobic drug to the organism [16, 17]. With use of nanotechnology, methods were elaborated concering incorporation of active agent, insoluble in water, into biocompatible nanomolecules that



baccatin: R= Ac

10-deacetylbaccatin: R= -H

Figure 3. Structure of baccatine and 10-deacetylbaccatine

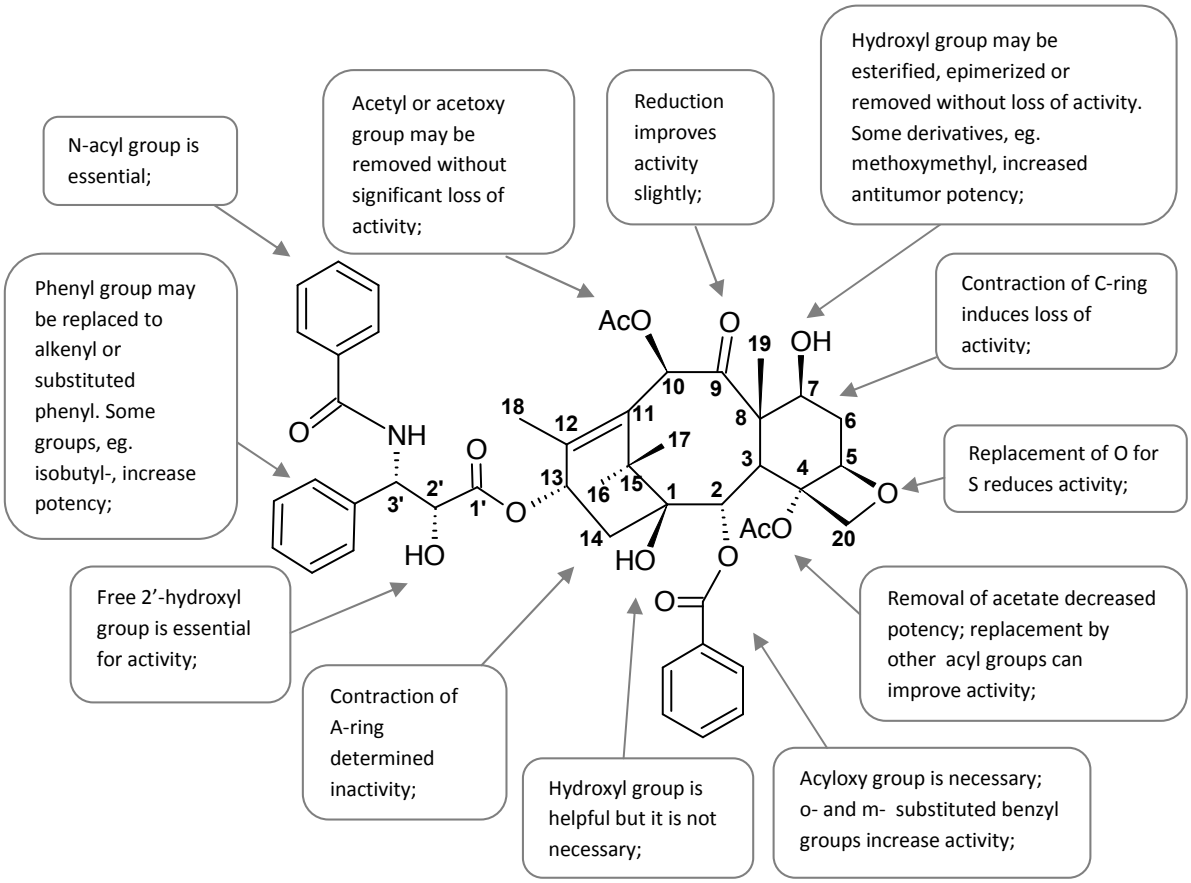


Figure 4. Structure-activity relationships of taxol

were obtained on the base of silica [18] or functionalized carbon [19]. Some others named block copolymer micelles have structures as core – shell type. They have capacity to transport large therapeutic payloads and passively target tumor sites [20]. Their active surface area is about 1000 m^2/g, that is why block copolymer micelles may serve as a carrier for hydrophobic active substances. Moreover, micellar shell is hydrophilic, so these structures are highly soluble in polar medium. It allows to deliver safely and in non-invasive manner the hydrophobic drug to organism cells [21]. In case of taxol, the use of nanotechnology method had additional advantage. Because of specific targeting of nanomolecules, the retention time was prolonged and accumulation of active substance in tumor tissue was increased. What is important, the healthy biological systems were not destroyed [22]. Another way of improving bioavailability of hydrophobic molecule is making some modifications of functional groups and obtaining derivatives with better solubility in aqueous media and with comparable or improved range of therapeutic activity. After making chemical modifications at the end fragment of C-13 side chain and deprotecting C-10 hydroxyl group, a new taxol derivative was obtained. This substance named taxotere [23]. It has t-butoxycarbonyl moiety instead of benzoyl fragment on the amino group of phenylisoserine chain at C-13 and a hydroxyl group instead of acetoxy group at C-10, as a result of selective hydrolysis reaction (Figure 5).

Favourable synthesis of taxotere, its high antitumor activity and relatively good way of application contributed to further development of experiments, directed to obtain even more effective taxol modification products. A series of taxol esters were tested. They were gain as a result of hydrogen atom substitution in OH group at C-2' in side chain and in OH group at C-7 of main diterpene skeleton with aminoacid aryl rests [24]. After biological examination of obtained derivatives, it was found that substitution of C-2' hydroxyl group lead to compounds with comparable pharmacological properties with taxol. It is connected with higher susceptibility of this group on the hydrolysis reaction under *in vivo* conditions and transformation of these esters into starting diterpene or its active metabolites.

Both taxol and taxotere are substances registered as therapeutic agents, named respectively as Paclitaxel

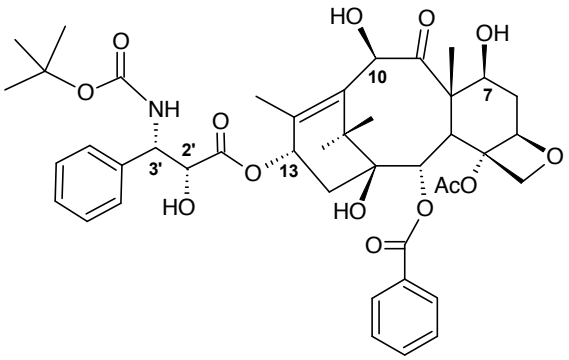
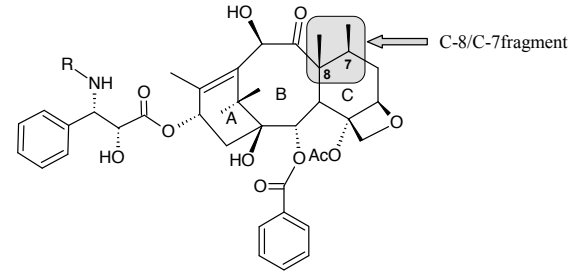


Figure 5. The structure of taxotere



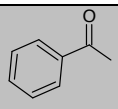
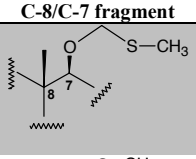
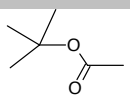
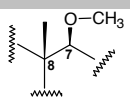
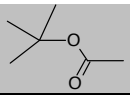
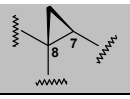
	R	C-8/C-7 fragment
BMS 184476		
TXD 258 (Cabazitaxel)		
XRP 9881 (Larotaxel)		

Figure 6. Structure of active, second generation diterpenes

and Docetaxel. Mechanism of action for both drugs consist in stabilization of microtubules in cell, which counteract their depolimerization [25]. This process inhibits correct separation of two identical sets of chromosomes and their transfer during cell division. Docetaxel and Paclitaxel cause blockage of cell mitosis and consequently induce cell death.

Nowadays, several different compounds of second generation from taxol diterpene group undergoing the sequence of clinical trials. Among them are BMS-184476 and XRP-9881 (Larotaxel) [26]. Moreover TXD-258 has finished successfully clinical trials and has been registered as Cabazitaxel [27] (Figure 6). It can be used in therapy of some tumors that are resistant to Docetaxel.

Conclusion

Studies on taxol are conducted broadly since 1967, so practically since the moment of it development. The methods of taxol obtaining are still improved. Also, there are some effects in fighting against aqueous solubility limits. Each year, there is more knowledge about it mechanism of action and structure – activity relationships that have influence on pharmacological activity of its derivatives. It is necessary to be hopeful that these factors will contribute to further development of antitumor chemiotherapy and will be fruitful in the future in discovering a series of new, even more potent substances in this group.

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