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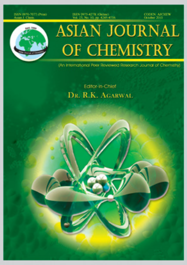

Studies of the Taxine From the Needles of the Moroccan Yew

HIND CHAKCHAK[1,*], HAMID ZINEDDINE[2] and AZIZ CHAOUCH[3]

[1]Centre National Pour la Researche Scientifique et Technique (CNRST), UATRS, Rabat, Morocco
[2]Faculté des Sciences, Universite Moulay Ismail, Meknès, Morocco
[3]Faculté des Sciences, Universite Ibn Tofail, Kénitra, Morocco

*Corresponding author: E-mail: chakchak@cnrst.ma

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The taxine, alkaloids precursor of the taxol, have been isolated from the needles of the Moroccan yew (*Taxus baccata*) and characterized through the use of high performance liquid chromatography. The taxine B, isotaxine B and taxoide basique 2-deacetyltaxine A were identified by gas chromatography and mass spectrometry.

Key Words: *Taxus baccata*, Taxine, Extract, HPLC, GC/MS.

INTRODUCTION

Yews (*Taxus spp.*, Taxaceae) are evergreen, gymnospermous shrubs commonly used for ornamental landscaping. The most common horticultural varieties are English yew (*Taxus baccata*), Pacific or western yew (*Taxus brevifolia*), American yew (*Taxus canadensis*) and Japanese yew (*Taxus cuspidata*). These plants are toxic and have been implicated in human and animal poisonings[1,2].

In Morocco, the yew moves in the forests of the means and high Western Atlas and Rif, in particular in the rich stations of the wet mountains where it grows frequently.

Taxol (paclitaxel) **1** was initially extracted from the bark of the Pacific yew *Taxus brevifolia* by Wani *et al.*[3]. Since that time its use as an anticancer drug has become well established and has been the subject of intense research[4-8].

Taxol, an important and expensive diterpene anticancer drug is extensively used in hospitals and clinics, and is especially used in treatment of ovarian, breast and lung cancers[9,10]. Its primary mechanism of action is related to the ability to stabilize the microtubules and to disrupt their dynamic equilibrium[11]. The presence of taxol was confirmed by spectroscopic and chromatographic methods of analysis[5,12].

However, the extraction of the traxol remains difficult and gives poor yield[13]. The research on finding alternatives to taxol extracted from the bark of the Pacific yew is ongoing. Taxol has been chemically synthesized and semi-synthetic versions have been developed using needles from the yew[14].

Taxine, extracted the needles of the yew (*Taxus baccata* L.), is a precursor of the taxol[15]. This taxine showed a remarkable antitumor activity. They are the principle toxic alkaloids derived from yew[16,17].

The first phytochemistry studies of the taxines of *Taxus baccata* were done by Lucas[18]. It isolated from the needles of the yew the alkaloid which it names taxine. Graf[19] discovered that taxine was a mixture of heterogeneous compounds.

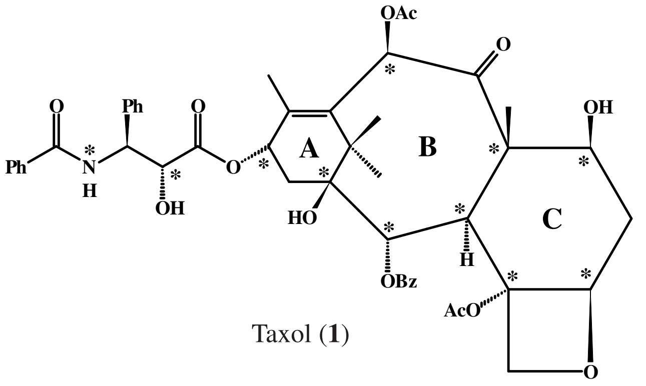
Further investigations relating to the distribution of total taxines determined spectrophotometrically, chromatographically and by infrared analysis made it possible for Graf to recognize two major types of taxine alkaloids: taxine B **2**[20]. Ettouati *et al.*[21] determine the structure of the taxine B by the spectroscopic methods and the experiment of EON. Poupat *et al.*[22] isolated the isotaxine B **3** and an analogue of taxine A **5**, 2-deacetyltaxine A **4** from the leaves of *T. baccata*. The structure of 2-deacetyltaxine A **4** was established using chemical ionization mass spectroscopy (CIMS) and ^{1}H NMR/^{13}C NMR.

Recently, the structure of the taxine B was confirmed by liquid chromatography high performance HPLC[23-25].

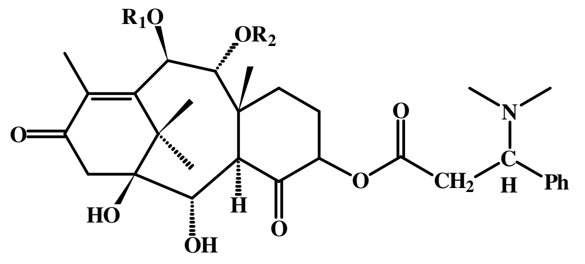
The objective of present study is the extraction of the taxine from the needles of the Moroccan yew and the identification of all the taxines as taxine B **2**, isotaxine B **3** and taxoide basique 2-deacetyltaxine A **4** by high performance liquid chromatography and gas chromatography-mass spectrometry methods.

EXPERIMENTAL

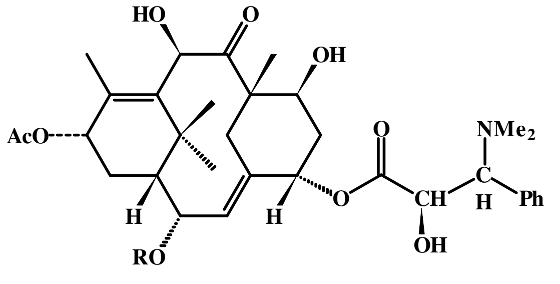
The samples of the yew studied were collected of the Average Atlas. They are then dried during 2 h with 60 ºC. The needles are separated from the branches, are crushed and kept in the air until use.



Taxol (**1**)



Taxine B (**2**): R_1 = OAc, R_2 = H
Isotaxine B (**3**): R_1 = H, R_2 = OAc



2-Deacetyltaxine A (**4**) R = H
Taxine A (**5**) R = OAc

Extraction of the taxine: The extraction of the taxine was carried out according to the method of Jenniskens *et al.*[24]. 50 g of the crushed needles of Yew was soaked in the sulphuric acid with 0.5 %. The solution remains in agitation during 4 days.

The filtrate is extracted with 3 × 300 mL from ethyl ether Et_2O. The organic phase is then adjusted at pH = 10 to 10.5 with an aqueous solution of aq. NH_3 at 25 %. The solution is extracted with 3 × 100 mL from $CHCl_3$. The combined organic layers are dried with Na_2SO_4, are filtered and evaporated. We obtained a yellowish product, with an output of total alkaloid 5.26 g per kilogram of needles of Yew.

Chromatographic analyses

High performance liquid chromatography (HPLC): The analysis of the various compounds of the taxine was carried out using a high performance liquid chromatography HPLC equipped with a pump Jasco 880-PU, a detector U.V. Jasco 870 UV, an integrator varian 4400 and a collector of fraction Gilson FC-203. Detection is carried out with the spectrometer of absorption in the ultra-violet with 280 nm. The column is an analytical column of type 5 μm Microsorb C18 (150 × 4.6 mm) protected by a precolonne Varian C18 3 cm length and 4.6 mm from internal diameter.

The eluant is the tertiary mixture dihydrogen phosphates sodium:acetonitrile:methanol with the proportions (47.5:27.5: 25 v/v/v) (throughout all elution, the composition of the mobile phase remains constant). The flow of the mobile phase is 0.75 mL/min.

The analysis was then supplemented by gas chromatography-mass spectrometry GC-MS.

Gas chromatography-mass spectrometry (GC-MS): The taxine is then subjected to a transformation into product silyle in order to identify the heavy products by gas chromatography-mass spectrometry (GC-MS). We analyzed the extract taxine in its silylee form[26-28].

The analysis of the various components of the taxine was carried out with a gas chromatography Hewlett Packard HP-5980 coupled to a mass spectrometer HP-5772A. The temperature is programmed with 75 ºC during 5 min of 75 ºC at 275 ºC at a rate of 4 ºC/min and finally 275 ºC during 10 min. The column employed is a capillary tube out of silica molten HP5, a 25 m length and of an internal diameter of 0.3 Misters the thickness of film is of 0.25 μm and the output of the carrier gas used (helium) is of 2 mL/min.

RESULTS AND DISCUSSION

During this study, the determination of the output in taxine is carried out by using sulphuric acid (0.5 %) on the dry needles of the yew. This extraction gives an average output of 0.53 %. This output seems important compared with that obtained from the needles of yew of Europe (*Taxus baccata*, L.) which an output of 0.6 %[21,24].

Chromatographic analyses: chemical composition of the extracts: The chemical composition of the extracts of the needles of the yew was carried out by high performance liquid chromatography and gas chromatography-mass spectrometry.

Analyse high performance liquid chromatograhy: The high performance liquid chromatograhy is employed for identification of the various compounds of taxine, this analysis which was carried out by the tertiary mixture NaH_2PO_4, CH_3CN and CH_3OH in the following proportions (47.5:27.5:25 v/v/v) gave a good separation of alkaloids of the taxine. Fig. 1 represents chromatogram HPLC of the taxine with a detection UV at 280 nm.

The analysis of the chromatogram obtained during these analyses, under fixed experimental conditions, provides six peaks. The majority peaks 1 and 3 correspond, as it was brought back by the literature[24] to alkaloids the taxine B and the isotaxine B. These two products present approximately 20 %.

The HPLC made it possible to have a good separation of the various components of the taxine, but it does not make it possible to identify them. Gas chromatography coupled with the mass spectrometry GC-MS is a technique largely employed to study and identify the chemical components of the extracts of the plants.

Analyse GC-MS

(a) Analysis of the taxines silylees by the GC-MS: The analysis of the taxine silylee by gas chromatography-mass

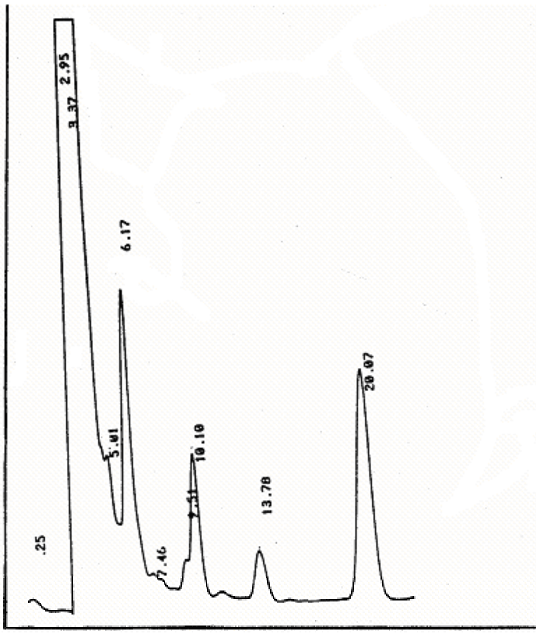


Fig. 1. Chromatogram HPLC with a detection UV at 280 nm

spectrometry GC-MS made it possible to identify the various compounds of the taxine. It shows a different compounds identified by their time of retention. We obtained the spectra of mass of the taxine B **2**, the isotaxine B **3** and the 2-deacetyltaxine A **4**.

(b) Identification of the mass of different taxines: The spectrum of mass of the taxine B bisilylee (Fig. 2) at retention time to 15.5 min indicates a peak to m/z = 727 which corresponds to the molecular ion of the taxine B bisilylee. The fragmentation of this product also indicates an ion fragment to m/z = 384 which corresponds to the loss of molecules $HOCOCH_2CHN (Me)_2Ph$ and $HOCOCH_3$ and $HOSiMe_3$. The ion fragment m/z = 297 implies the loss of a water molecule and $CH_2 = CH\text{-}CO\text{-}CH_2$. This molecule with m/z = 297 is stable by effect of resonance, therefore the rupture becomes difficult.

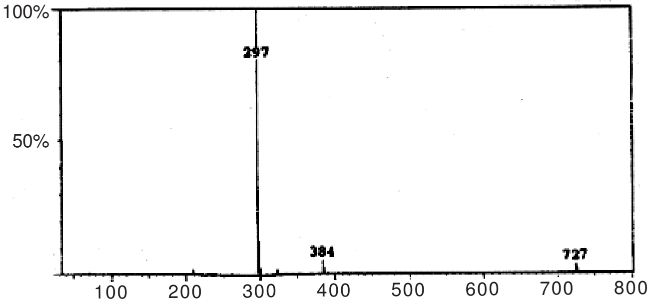


Fig. 2. Spectrum of mass of the taxine B bisilylee **2**

The spectrum of mass of the isotaxine B bisilylee (Fig. 3) which appears at 15.8 min, present a molecular ion at m/z = 727 which corresponds to the isotaxine B bisilylee.

On this spectrum, a peak fragment with m/z = 384 which indicates the loss of the molecules: $HOCOCH_2CHN(Me)_2Ph$ and $HOCOCH_3$ and $HOSiMe_3$. The peak fragment m/z = 297 indicates the loss of a water molecule and $CH_2=CH\text{-}CO\text{-}CH_2$. The peak fragment m/z = 223 corresponds to the loss of a molecule of $HSiMe_3$.

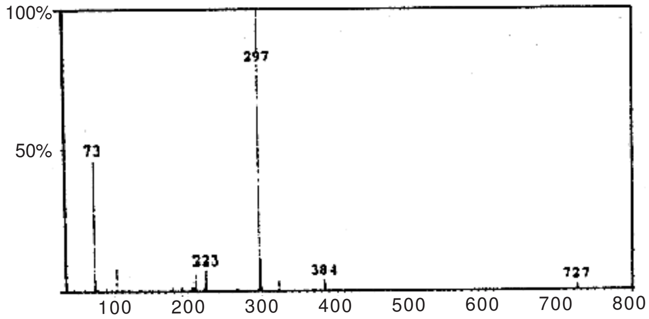


Fig. 3. Spectrum of mass of the isotaxine B bisilylee **3**

The spectrum of mass of the 2-deacetyltaxine A **4** (Fig. 4), which appears at 16.8 min, present a peak fragment m/z = 680 which indicates the loss of a molecule of AcOH and of a molecule of $HOSiMe_3$.

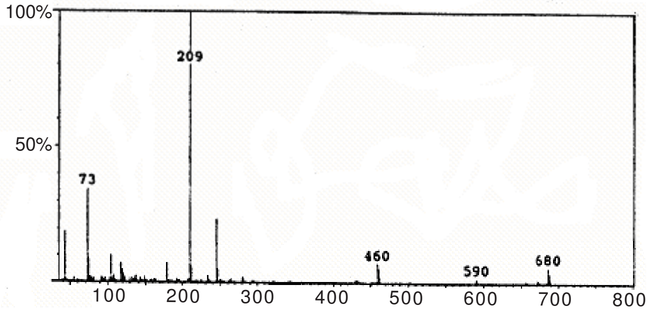


Fig. 4. Spectrum of mass of the 2-deacetyltaxine A trisilylee **4**

On this spectrum, there is a peak fragment m/z = 590 which indicates the loss of a molecule of $HOSiMe_3$, the peak fragment m/z = 460 indicates the loss of the molecule $OSiMe_3C = COH$ and the peak fragment m/z = 209 corresponds to molecule $HOCOCHOHCHN(Me_3)_2Ph$.

The analyses of the taxine by gas chromatography-mass spectrometry GC/MS can identify the majority components of the taxine: the taxine B **2**, the isotaxine B **3** and the 2-deacetyltaxine A **4**. According to the literature, the taxine B **2** and the isotaxine B **3** are majority of the alkaloids[22,24].

Conclusion

During this work, the study of differents taxines from the yew (*Taxus baccata*) was carried out. From the needles of the yew, we could isolate the taxine, precursor of the taxol, by the sulphuric acid with an output of order 0.53 %. The analysis of different compounds was carried out mainly by the high performance liquid chromatography HPLC and gas chromatography spectrum of mass GC-MS. We could identify the taxine B, the isotaxine B and 2-deacetyltaxine A. This work is justified by the interest of the taxol in the treatment of certain cancerous and by extracting a precursor of the taxol, which is the taxine, from needles to renew easily.

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