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Extraction and Identification of New Taxoids from the Moroccan Yew

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The needless of the Moroccan yew (*Taxus baccata*) afforded a new taxoids 19-debenzoyl-19-acetyltaxinine **1** and the 5-cinnamoylbrevifoliol (=13-deacetyltaxuspine) **2**. The different fractions of methanolic extract were separed by high performance liquid chromatography. Both compounds **1** and **2** were identified by gas chromatography-mass spectrometry.

Key Words: *Taxus baccata*, 19-Debenzoyl-19-acetyltaxinine, 5-Cinnamoylbrevifoliol (=13-deacetyltaxuspine).

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 is very frequent. Taxol **3** is a mitotic inhibitor used in cancer chemotherapy. It was discovered in 1971 by Wani *et al.*[3]. They isolated it from the bark of the Pacific yew tree. Taxol is now used to treat patients with lung, ovarian, breast, head and neck cancer[4,5]. Since, several researches were carried out on this product[6-10]. But the others taxoides also exist in the leaves and the bark of yew (*Taxus baccata*).

We now report the separation of different fractions of the methanolic extract by column of silica gel and isolation of two taxoids from the needles of the Moroccan yew (*Taxus baccata*) 19-debenzoyl-19-acetyltaxinine **1** and the 5-cinnamoyl brevifoliol (=13-deacetyltaxuspine) **2**. The identification of these alkaloids was established by high performance liquid chromatography HPLC and gas chromatography-mass spectrometry CG/MS methods.

EXPERIMENTAL

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

Extraction of the methanolic extract: The extraction of the alkaloids was carried out according to the method described by Barboni *et al.*[11].

72 g of the crushed needles of Yew (*Taxus baccata*, L.) are extracted with MeOH by agitation at room temperature. The resulting solution is filtered, concentrated then treated by three 100 mL of CH_2Cl_2. Finally, the combined organic phases are evaporated.We obtained a dry extract with an output of 5.04 %. This methanolic extract is separate on column filled out of silica gel in order to obtain its various fractions.

Separation of the various fractions of the methanolic extract on column: 3.63 g of the product obtained is then passed on a column of silica gel by using the CH_2Cl_2 as eluant the fraction A is obtained, with the mixture CH_2Cl_2-acetone (4-1) we obtained the fractions B' and B'' and with eluant acetone, the fraction C is obtained.

The fraction B' then passed in column chromatography on silica gel to separate its various components. By using a $CHCl_3$, we obtained five fractions.

The fraction B'' is subjected to the column chromatography with the solvent $CHCl_3$-MeOH (99-1), we obtained four fractions.

The separation of the various components of the fraction C by column chromatography on Silica gel with eluant $CHCl_3$-MeOH (5-10) gave three fractions.

High performance liquid chromatography: The analysis of the various compounds of the methanolic extract 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 at 280nm. The column is semi preparative reversed phase of type Whatman (PRE HPLC - PACKED column) Partisil 10.ODS-2 (250 × 4.6 mm) protected by a precolonne Varian C18 (30 × 4.6) mm. The eluant is the tertiary phase of ACN- MeOH-H_2O (35-1-15 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 methanolic extract 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 methanolic extract in its silylee form[12-14].

The analysis of the various components of the product 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 mm 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 methanolic extractis carried out by using the MeOH on the dry needles of the Yew (*Taxus baccata*). This extraction gives an average output of 5.04 %.

Separation of the various fractions of the extract methanolic on column: The methanolic extract obtained is then chromatography on a silica gel column. Several mixtures of dichloromethane-acetone were tested, only the following proportions gave a good separation:

With CH_2Cl_2 we obtained the fraction A with an output of 13.16% of the initial product; With the mixture CH_2Cl_2-acetone (4-1) we collected the fractions B' and B" with an output respectively about 25 % and 27.6 %; With acetone as eluant we obtained the fraction C with an output of 22.9 %.

TABLE-1
OUTPUT OF THE VARIOUS FRACTIONS OBTAINED OF SEPARATION THE METHANOLIC EXTRACT ON SILICA GEL COLUMN

Fractions	Rendement (%)
Fraction A	13.1
Fraction B’	25.0
Fraction B’’	27.6
Fraction C	22.9

The fraction B' then passed in chromatographic Silica gel column to separate its various components: With $CHCl_3$, we obtained 5 fractions F1B', F2B', F3B', F4B' and F5B'. According to these results, the major product of the B' fraction is the 2B' fraction with an output of 39.1 %.

The fraction B" separated on chromatographic column with the solvent $CHCl_3$-MeOH (99-1) gave four fractions F1B", F2B", F3B"and F4B", whose majority product is the fraction 3B"with an output of about 35.7 %.

TABLE-2
OUTPUT OF THE VARIOUS FRACTIONS OBTAINED OF SEPARATION THE FRACTION B’ ON SILICA GEL COLUMN WITH $CHCl_3$ AS ELUANT

Fractions	Poids en (mg)	Rendements (%)
Fraction B’	460	
Fraction 1B’	20	4.3
Fraction 2B’	180	39.1
Fraction 3B’	70	15.2
Fraction 4B’	50	10.8
Fraction 5B’	120	26.0

TABLE-3
OUTPUT OF THE VARIOUS FRACTIONS OBTAINED OF SEPARATION THE FRACTION B’’ ON SILICA GEL COLUMN WITH $CHCL_3$ -MEOH (99-1) AS ELUANT

Fractions	Poids	Rendements (%)
Fraction B’’	0.14 g	
Fraction 1B’’	20 mg	14.2
Fraction 2B’’	10 mg	7.1
Fraction 3B’’	50 mg	35.7
Fraction 4B’’	30 mg	21.4

The separation of the various components of the fraction C on chromatographic silica gel column with eluant $CHCl_3$ - MeOH (5-10 %) gave three fractions.

TABLE-4
OUTPUT OF THE VARIOUS FRACTIONS OBTAINED OF SEPARATION THE FRACTION C ON SILICA GEL COLUMN WITH $CHCl_3$-MEOH (5-10) AS ELUANT

Fractions	Poids	Rendements (%)
Fraction C	0.18 g	
Fraction 1C	60 mg	33.3
Fraction 2C	40 mg	22.2
Fraction 3C	60 mg	33.3

The separation of the methanolic extract by the chromatography on column of silica gel gave four fractions A, B', B" and C. the fraction B' provided five fractions, the fraction B" gave 4 fractions and the separation of the fraction C provided 3 fractions. All these results indicated that the methanolic extract contains at least 12 products.

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 HPLC and gas chromatography-mass spectrometry GC-MS.

HPLC analyses: The high performance liquid chromatography HPLC is employed for identification of the various compounds of methanolic extract.

This analysis which was carried out by the tertiary phase of ACN-MeOH-H_2O (35-1-15 v/v/v) gave a good separation (Fig. 1).

The analysis of the chromatogram obtained during these analyses, under fixed experimental conditions provides approximately 13 peaks between 6.6 mn and 78 mn. The fraction B' provides 5 peaks between 10.09 mn and 12.44 mn. The various peaks of the fraction B" appeared between 11.74 mn and 67.24 mn. The three products of the fraction C appeared between 6.60 mn and 8.81 mn. These results confirm those obtained during separation on column filled with silica gel.

TABLE-5
DIFFERENT FRACTIONS DETECTED BY CHROMATOGRAM HPLC OF THE INITIAL PRODUCT

Produit onitial		Fractions B'			Fraction B''			Fraction C	
Pic Nº	TR (mn)	Pic Nº	TR (mn)		Pic Nº	TR (m)		Pic Nº	TR (mn)
1	6.60	1	10.09	$F_{5B'}$	1	6.55		1	6.25
2	8.81	2	10.42		2	10.19		2	7.88
3	10.09	3	11.08	$F_{2B'}$	3	11.87		3	8.8
4	10.68	4	11.72	$F_{3B'}$	4	11.74	$F_{3B''}$		
5	11.49	5	12.44	$F_{4B'}$	5	23.35			
6	12.01	6	13.06		6	24.36			
7	21.70	7	27.97		7	26.81			
8	22.60	8	29.94		8	28.61	$F_{4B''}$		
9	24.42	9	43.86		9	51.78	$F_{2B''}$		
10	26.31	10	54.93		10	64.17	$F_{1B''}$		
11	46.83	11	61.35		11	67.24	$F_{1B''}$		
12	59.97	12	64.92						
13	78.02	13	69.37						

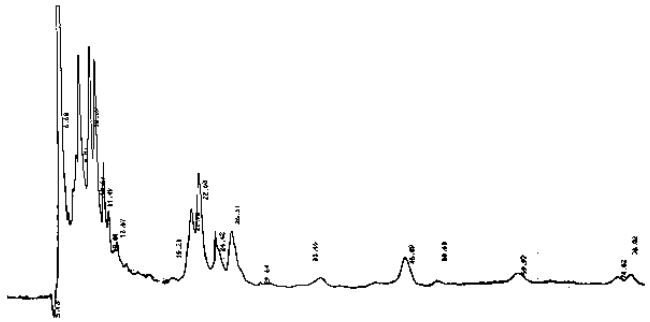


Fig. 1. Chromatogram HPLC of the methanolic extract with a detection UV at 280 nm

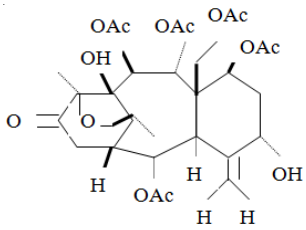
The comparison of the chromatogram of the initial product with chromatograms of the different fractions showed that the products of these fractions constitute the majority of the components of the initial product. These results agree with those of the literature[11].

The HPLC made it possible to have a good separation of the various components of the methanolic extract, 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.

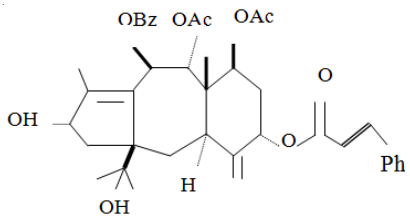
Analysis of the methanolic extract by the GC-MS: The analysis of the methanolic extract silyle by gas chromatography-mass spectrometry (GC-MS) made it possible to identify the various compounds of the product. It shows a different compounds identified by their time of retention. The comparison with the literature[11] concerning the analysis of the components by the mass spectrometry of the extract allowed the identification of two alkaloids *viz.*, the 19-debenzoyl-19-acetyltaxinine bisilyle (**1**) and the 5-cinnamoylbrevifoliol (=13-deacetyl-taxuspine)bisilyle (**2**).

Mass spectrum of 19-debenzoyl-19-acetyltaxinine bisilyle (1): The mass spectrum of 19-debenzoyl-19-acetyl-taxinine bisilyle, which appears at 35.7 mn, present a peak fragment at *m/z* = 588 which indicated the loss of the three molecules HOAc. The others fragments at *m/z* = 530, *m/z* = 471, *m/z* = 397, *m/z* = 261 and *m/z* = 209 (Fig. 2).

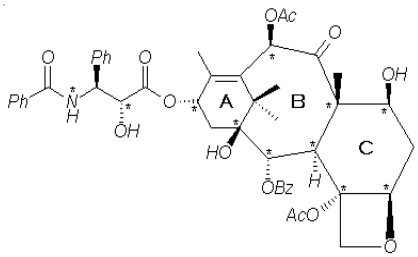
Mass spectrum of 5-cinnamoylbrevifoliol (=13-deacetyl-taxuspine)bisilyle (2): The mass spectrum of 5-cinnamoyl-brevifoliol (=13-deacetyltaxuspine) bisilyle (Fig. 3) at



19-Debenzoyl-19-acetyltaxinine **1**



5-cinnamoylbrevifoliol (=13-deacetyl taxuspine **2**)



Le taxol **3**

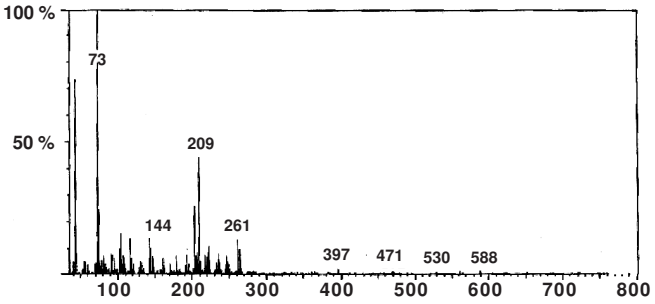


Fig. 2. Mass spectrum of the 19-Debenzoyl-19-acetyltaxinine **1**

retention time to 29.9 mn indicates a peak to *m/z* = 664 which corresponded to the loss of molecules HBz and AcOH. The others fragments at *m/z* = 460, *m/z* =371, *m/z* = 203 and *m/z* = 147.

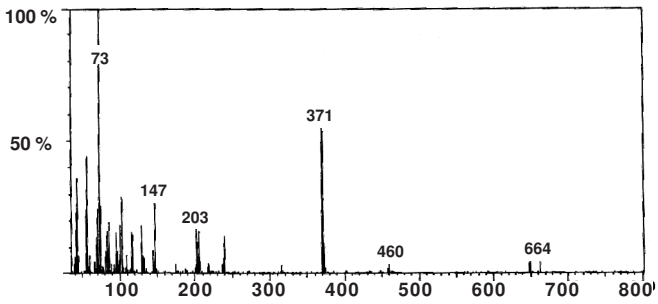


Fig. 3. Mass spectrum of the 5-cinnamoylbrevifoliol (=13-deacetyltaxuspine) **2**

Conclusion

The study of methanolic extract from the yew (*Taxus baccata*) was carried out. From the needles of the yew, we could isolate the methanolic extract by the MeOH with an output of order 5.04 %. The separation of different alkaloids was realized on a column filled out of silica gel. The methanolic extract contains at least 12 products. The analysis of different fractions was carried out mainly by the high performance liquid chromatography HPLC and gas chromatography spectrum of mass GC-MS. We could identify the19-debenzoyl-19-acetyl-taxinine1[15,16] and the 5-cinnamoylbrevifoliol (=13-deacetyl-taxuspine)[17,18].

According to the literature, the compound 5-cinnamoyl-brevifoliol (=13-deacetyltaxuspine) is a constituent minor of the leaves of the yew; but the first taxoide carrying a cinnamoyle grouping which is isolated starting from this source. Generally, this type of compound is obtained starting from a compound carrying an ester in the grouping of acid winterstein[19].

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