Journal of Natural Products
Vol. 58, No. 7, pp. 1015-1023, July 1995

IMMUNOENZYMATIC METHODS APPLIED TO THE SEARCH FOR BIOACTIVE TAXOIDS FROM *TAXUS BACCATA*

YANWEN GUO, RENÉE VANHAELEN-FASTRÉ, BILO DIALLO, MAURICE VANHAELEN,*

Laboratory of Pharmacognosy and Bromatology, Pharmaceutical Institute, Université Libre de Bruxelles, Campus Plaine CP 205/4 Bld Triomphe, 1050 Brussels, Belgium

MONDHER JAZIRI, JACQUES HOMÈS,

Laboratory of Plant Morphology, Université Libre de Bruxelles, Chaussée de Wavre 1850, B-1160 Brussels, Belgium

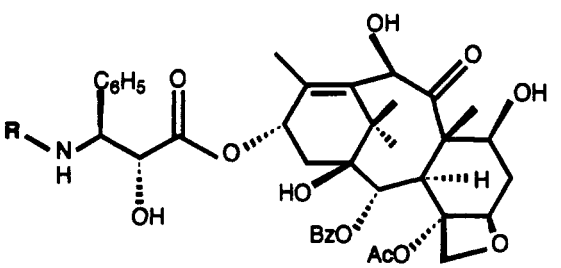
and ROBERT OTTINGER

Department of Organic Chemistry, Ecole Polytechnique, Université Libre de Bruxelles, CP 165, av. F.D. Roosevelt, 50, B-1050 Brussels, Belgium

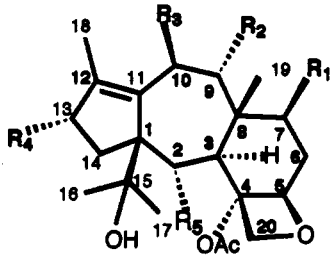
ABSTRACT.—Polyclonal antibodies raised against 2′-succinyltaxol-bovine serum albumin (BSA) conjugate were used for the immunodetection of bioactive taxoids in chromatographic fractions of the stem bark extract of *Taxus baccata*. In addition to taxol, cephalomannine, and baccatin III, two taxoids were isolated and their structures were elucidated as 4α,7β-diacetoxy-2α,9α-dibenzoxy-5β,20-epoxy-10β,13α,15-trihydroxy-11(15→1)-*abeo*-tax-11-ene [**5**] and taxol C [**6**] using spectroscopic methods.

Taxol (now known as paclitaxel) [**1**], a diterpenoid initially isolated from the stem bark of *Taxus brevifolia* Nutt. (Taxaceae) (1), has been found to promote the assembly of tubulin and to inhibit the microtubule disassembly process (2,3). The search for alternative sources of **1** has led to the development of several programs devoted to the identification of *Taxus* species and varieties containing new taxoids useful as key intermediates for the hemi-synthesis of **1** and allowing a better understanding of the complex taxoid biosynthetic pathway. Many new taxoids have been isolated from *Taxus* spp. plant material as a consequence of screening; a recent review (4) listed over 100 taxoids and new ones are being described on a regular basis.

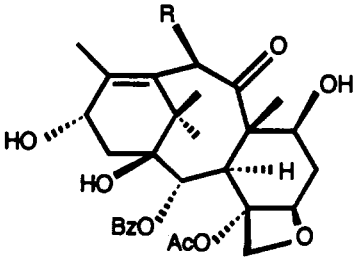
As part of ongoing studies on the constituents of *T. baccata* L. cv. *stricta*, we report here a combination of chromatographic and immunoenzymatic methods applied to the search of bioactive taxoids structurally related to **1**. We previously reported the development of a competitive enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative determination of taxoids in *Taxus* plants and tissue cultures using rabbit antisera raised against 2′-succinyltaxol-bovine serum albumin conjugate (5). The cross-reactivity study showed that, among several naturally occurring taxoids, the antibodies cross-react with cephalomannine [**2**] and baccatin III [**3**], and that no detectable reaction of the antisera was observed with 10-deacetylbaccatin III [**4**] at a



1 R=Bz
2 R=CO-C(CH$_3$)=CH-CH$_3$
6 R=CO-(CH$_2$)$_4$-CH$_3$



	R_1	R_2	R_3	R_4	R_5
5	OAc	OBz	OH	OH	OBz
9	OAc	OBz	OBz	OAc	OBz
10	OAc	OAc	OBz	OAc	OBz
11	OBz	OAc	OAc	OAc	OBz



	R
3	OAc
4	OH

concentration below 1000 ng/50 µl. Furthermore, the hplc fractionation of an extract from *T. baccata* showed an impressive array of constituents with an immunopositive response.

In the present paper, we report on the strategy employed for the immunodetection and isolation of the two taxoids **5** and **6**, as well as their structure elucidation using ^{1}H-nmr, ^{13}C-nmr, and mass spectrometry, and their cytotoxic evaluation against P-388 leukemia cells.

RESULTS AND DISCUSSION

The fractionation scheme for the immunodetection and isolation of compounds **5** and **6** is shown in Figure 1. The dried powder of *T. baccata* stem bark was extracted with MeOH-CH_2Cl_2 (1:1). After solvent partition of the extract, the petroleum ether, aqueous MeOH, and CH_2Cl_2 fractions were assayed by ELISA using polyclonal antibodies raised against 2′-succinyltaxol-BSA conjugate. The results showed that 70% of the total immunoresponse was recovered in the CH_2Cl_2 fraction; 20% and 5% of the total immunoresponse were recorded in the petroleum ether and in the aqueous MeOH fractions, respectively (Table 1).

The CH_2Cl_2 fraction was further subjected to Si gel cc and 13 fractions obtained were assayed by ELISA (Table 2). Fraction 11, which eluted with hexane-Me_2CO (7:3) showed the highest immuno-signal value. Analysis of this fraction by tlc and hplc indicated that taxol [**1**] and cephalomannine [**2**] were the major constituents. Purification of both compounds was achieved using high-speed countercurrent chromatography (hsccc) as described previously (6). A part of fraction 10 from the cc, which showed a relative high immunoresponse, was subjected to hplc for further investigation. The ELISA-inhibition study of the seven fractions isolated by semi-prep. hplc was carried out and the distribution of the immunoreactivity of these fractions was compared to that of **1**. Two groups of compounds could be distinguished among the different tested fractions. The first group included taxol [**1**], cephalomannine [**2**], and compound X_4 (Figure 1) which showed a high affinity to the anti-taxol antibodies; the second group included baccatin III [**3**] and compounds X_1 to X_3 which showed a much lower affinity to the antibodies than the first group. It was, therefore, postulated that compound X_4 should have a structure closely related to **1**, and further purification of this compound was achieved using hsccc of fraction 10. This compound was identified as taxol C [**6**].

The same strategy was followed for the detection and isolation of the new taxoid **5** which was isolated by prep. tlc from fraction 9.

Compound **5** had a molecular formula of $C_{38}H_{44}O_{12}$ as determined by fabms and

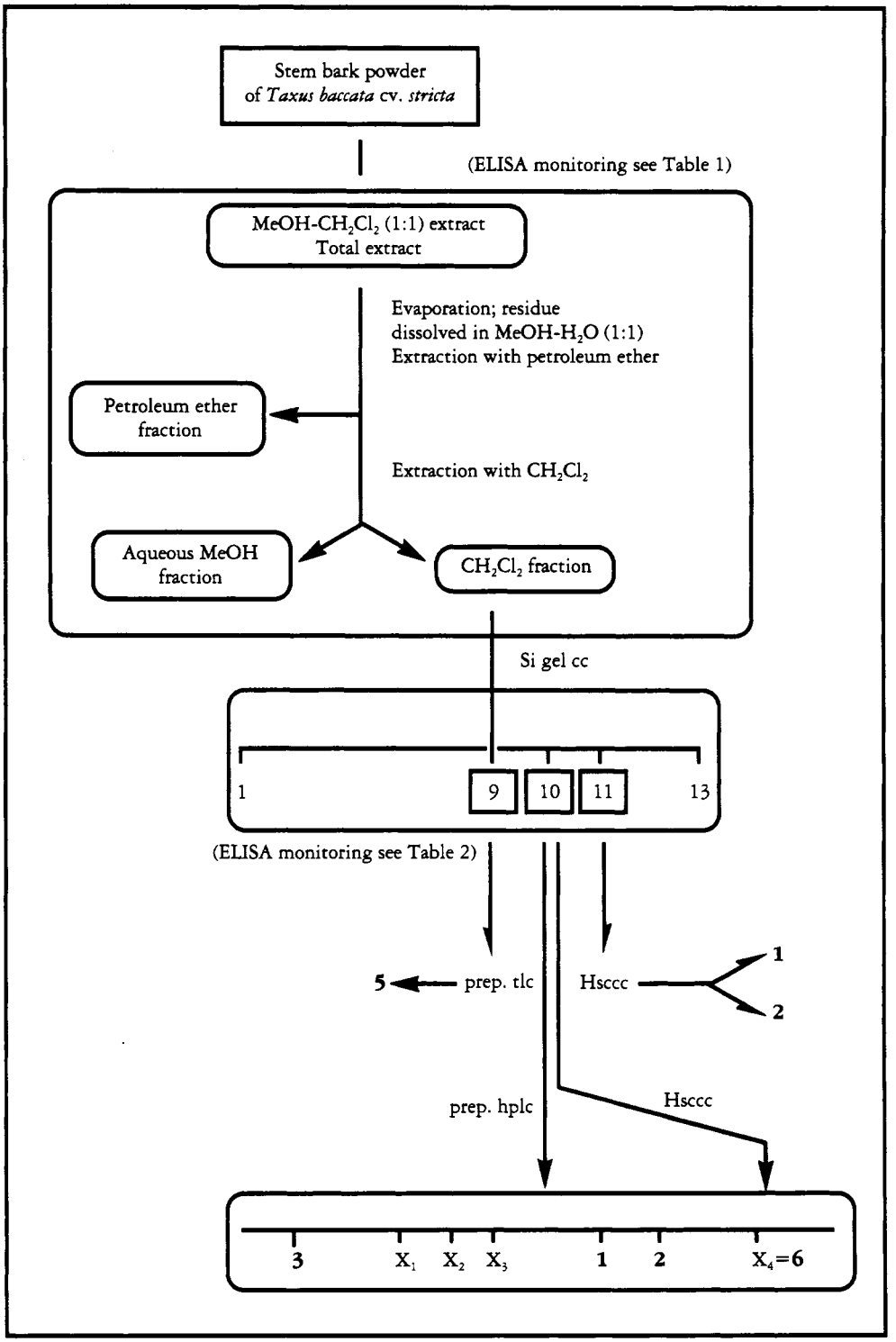


FIGURE 1. Experimental procedure for the isolation of taxoids from stem bark of *Taxus baccata* L. cv. *stricta*.

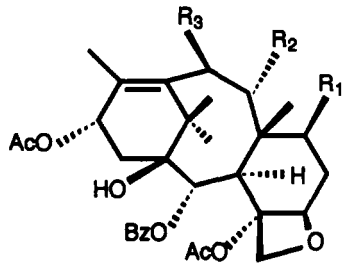
TABLE 1. Taxol Equivalent Content in Fractions Obtained After Solvent Partition of the Total Extract of *T. baccata* cv. *stricta*.

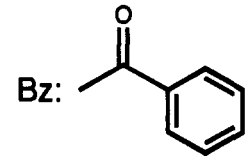
Fraction designation	Relative taxol equivalent content (% of total)
Total extract	100
Petroleum ether fraction	20
Aqueous MeOH fraction	5
CH_2Cl_2 fraction	70

cims. The ^{1}H-nmr spectrum was closely related to those of baccatin VI [7] and 9-dihydro-13-acetylbaccatin III [8] (7,8). However, signals corresponding to an additional benzoxy and only two acetoxy groups were observed. Comparison of the ^{1}H-nmr chemical shifts and coupling constants, as well as the ^{13}C- and the 2D nmr (^{1}H-^{1}H COSY, HMQC, and HMBC) data with those previously reported for closely related taxoids (8–11), allowed the assignment of the ^{1}H- and ^{13}C-nmr signals as well as the location of the acetoxy and benzoxy substituents. The studies by Chu *et al.* (9), who used double-quantum COSY and HECTOR nmr analysis to unambiguously assign the ^{1}H- and ^{13}C-nmr signals in the spectra of compounds **9–11**, were particularly helpful. Compounds **9–11** were originally assigned the normal taxane skeleton, but were found later to possess an A-nortaxane skeleton (12). The distinction between signals corresponding to the methylene protons at C-6 and C-14 was not obvious. However, close comparison of the geminal coupling constant values and analysis of the ^{1}H-^{1}H COSY and HMBC nmr spectra allowed the assignment of these signals. The spin-system derived from H-5β, H-6α, H-6β, and H-7α was, thus, readily established; a 1H multiplet at δ 2.64 was coupled with a 1H multiplet partly overlapping with a methyl signal at δ 2.02; the multiplet at δ 2.64 was also coupled with a 1H broad doublet at δ 4.93 (J=7.6 Hz) and with a 1H triplet at δ 5.70 (J=7.9 Hz). This 4-spin-system is solely attributable to H-6α, H-6β, H-5β, and H-7α, respectively. The 1H doublet of doublets at about δ 1.79 (J=14.9 Hz) was related to a 1H multiplet centered at δ 2.30; these two signals were both correlated to the 1H triplet at δ 4.57. This 3-spin-system was assumed to derive from H-14β, H-14α, and H-13α, respectively. The C-20 methylene protons of the oxetane bridge were observed as a characteristic AX system at δ 4.17 and δ 4.53 (J=7.8 Hz). The

TABLE 2. Taxol Equivalent Content in Fractions Obtained After Separation of the CH_2Cl_2 Extract by Si gel cc.

Fraction number	Taxol equivalent content (%)
1	4.63
2	4.78
3	6.80
4	7.13
5	2.60
6	1.30
7	3.33
8	7.97
9	11.13
10	12.40
11	22.75
12	9.13
13	8.40





	R_1	R_2	R_3
7	OAc	OAc	OAc
8	OH	OH	OAc

characteristic H-3α doublet at δ 3.28 was correlated with the signal at δ 6.23 assigned to H-2β (J=7.2 Hz). The appearance of H-3α and H-2β as an isolated spin-system confirmed that C-1 and C-4 are fully substituted. In comparison with the ^{1}H-nmr spectrum of **8** (8), the characteristic proton resonances due to the oxetane moiety were not significantly shifted (Table 3); however, slight upfield shifts were observed for the H-3α and H-6α signals. In contrast, the H-2β, H-7α, and H-13β signals were significantly shifted; H-2β resonated at about δ 6.50 whenever a benzoxy substituent was present at the C-2 position. However, in the ^{1}H-nmr spectrum of **5**, the H-2β doublet was observed at δ 6.23. In addition, H-7 resonated at ca. δ 4.40 (H-7α) or at δ 3.70 (H-7β) when OH-7 was not esterified as in **1**–**3** and 7-*epi*-taxol (13,14). The H-7 signal in the spectrum of **5** was observed at δ 5.70 (t, $J_{7\alpha,6\alpha}=J_{7\alpha,6\beta}$=7.9 Hz), indicating acyl substitution at the C-7 position; this acyl substituent should be an acetoxy group as in **9** in which H-7α resonates at δ 5.70 as a triplet (J=8.1 Hz) (9). The H-13β characteristic broad triplet was found at δ 4.57 ($J_{13\beta,14\alpha}=J_{13\beta,14\beta}$=8.6 Hz), in agreement with the absence of an acyl group at the C-13 position as in **3** (8,14). The second acetoxy function was assumed to be located at C-4 as no change in the chemical shift difference between the C-20 proton resonances was observed; indeed, with compounds lacking the C-4 acetoxy function, the AB quartet for these diastereotopic C-20 protons shows a smaller chemical shift difference than that observed in compounds with an acetoxy group at the C-4 position. In addition, the C-7 methine proton signal was not shifted; a C-4 deacylation results in an upfield shift of this signal by ca. 0.4 ppm (15). This conclusion was also supported by the fact that an acetoxy substitution at the C-4 position has always been observed in taxoids bearing the oxetane moiety. The second benzoxy substituent could be therefore assigned to the C-9 or C-10 position.

An HMQC nmr experiment was used to assign carbon signals for all proton-bearing carbons and the quaternary carbons as well as the carbonyl carbons were assigned from the HMBC nmr spectra (J filter set for 5 Hz and 10 Hz, Table 3). It is noteworthy that none of the carbonyl signals showed cross-peaks with any other proton signal, so that these HMBC spectra could not be used to assign the position of the acyl substituents. The H-14β signal at δ 1.17 showed cross-peaks with the C-15, C-13, C-1, and C-2 resonances; this signal was also correlated with the C-11 resonance, whereas the H-14α multiplet at δ 2.30 was coupled to the C-12 resonance. This feature, together with the resonance of the quaternary C-15 carbon at δ 76.2 instead of at ca. δ 44 as in **1** (13) and related derivatives, suggested that **5** has the 11(15→1)-*abeo*-taxane skeleton (10, 11, 16–18). This was further supported by the absence of cross-peaks between the H-16/H-17 Me signals and the olefinic C-11 resonance in the HMBC nmr spectrum, as already shown for the 11(15→1)-*abeo*-taxane structure (with the contracted 5-membered A-

TABLE 3. Nmr Data for Compound **5**.[a]

^{13}C nmr ($CDCl_3$)		HMQC	^{1}H nmr ($CDCl_3$)			HMBC
Carbon	δ		Proton	δ, *J* in Hz	^{1}H-^{1}H COSY	
C-1	66.9					
C-2	68.7	⟷	H-2	6.23, 1H, d, *J*=7.5	⟷	C-3, C-4, C-8, C-15
C-3	44.2	⟷	H-3	3.28, 1H, d, *J*=7.5	⟷	C-1, C-2, C-4, C-8, C-19, C-20
C-4	79.2					
C-5	85.0	⟷	H-5	4.93, 1H, d, *J*=7.6	⟷	C-3, C-4, C-7
C-6	34.8	⟷	H-6α	2.64, 1H, m	⟷	C-7, C-8
			H-6β	2.00, 1H, m	⟷	
C-7	70.4	⟷	H-7	5.70, 1H, t, *J*=7.9	⟷	C-19
C-8	43.1					
C-9	79.4	⟷	H-9	5.80, 1H, d, *J*=8.6	⟷	C-10
C-10	66.7	⟷	H-10	4.73, 1H, t, *J*=8.6	⟷	C-1, C-9, C-12
C-11	138.3					
C-12	145.5					
C-13	77.8	⟷	H-13	4.57, 1H, t, *J*=6.9	⟷	C-12, C-11
C-14	39.7	⟷	H-14α	2.30, 1H, m	⟷	C-1, C-2, C-12, C-13, C-15
			H-14β	1.77, 1H, dd, *J*=14.9, 6.9	⟷	C-1, C-2, C-11, C-13, C-15
C-15	76.2					
C-16	25.9	⟷	H-16	1.18, 3H, s		C-1, C-15, C-17
C-17	27.7	⟷	H-17	1.04, 3H, s		C-1, C-15, C-16
C-18	11.3	⟷	H-18	2.02, 3H, br s		C-11, C-12
C-19	20.5	⟷	H-19	1.45, 3H, s		
C-20	74.8	⟷	H-20α	4.52, 1H, d, *J*=7.8	⟷	C-3, C-4, C-5
			H-20β	4.17, 1H, d, *J*=7.8	⟷	C-3, C-4, C-5
2-CO, 9-CO	165.3					
i-Bz	133.1					
o-Bz	129.7	⟷	o-Bz	8.11, 2H, d, *J*=7.1	⟷	$OCOC_6H_5$
			o-Bz	8.04, 2H, d, *J*=7.1	⟷	
m-Bz	128.6	⟷	m-Bz	7.47, 4H, m	⟷	
p-Bz	133.0	⟷	p-Bz	7.63, 1H, m	⟷	
p-Bz	133.6	⟷	p-Bz	7.56, 1H, m	⟷	
4-COMe	171.7	⟷	4-COMe	2.23, 3H, s		OCOMe
4-COMe	22.4					
7-COMe	171.7	⟷	7-COMe	1.84, 3H, s		OCOMe
7-COMe	13.1					

[a]The ^{1}H-, ^{13}C-(BBD) and inverse-detected spectra were recorded on a Varian-Unit 600 using TMS as internal reference. Observed ^{1}H, ^{1}H-COSY, and HMQC correlations are shown by arrows (⟷).

ring) (10,12,16). This type of A-ring rearrangement of taxol or taxol-like compounds with the C-15 resonance at ca. δ 68, has been shown to arise under acidic conditions (12,19,20). Brevifoliol was initially assigned a normal taxane skeleton (21), but this structure was later corrected to the A-nortaxane skeleton (16,17). Recently, an X-ray crystallographic investigation of 5-brevifoliol acetate confirmed the revision of the structure of brevifoliol as well as those of some taxoids reported earlier, since they all possess a rearranged 11(15→1)-*abeo*-taxane skeleton and not an intact 6-membered A-ring (12). As brevifoliol was shown to be found in nature as the 11(15→1)-*abeo*-taxane (12) and as the isolation procedure of **5** was achieved under neutral conditions, it is unlikely that **5** is an artifact. In the ^{1}H-nmr spectrum of **1**, the isolated spin-system comprising a triplet at δ 4.73 and a broad doublet at δ 5.80 was attributed to H-9β and H-10α. These chemical shift values are similar to those found for H-9β and H-10α in the ^{1}H-nmr spectrum of **8**, which bears an acetoxy group at C-10 and a OH group at the C-9 position. However, the HMBC nmr spectrum of **5** showed cross-peaks between the triplet at δ 4.73 and the signals at δ 66.9 (C-1), δ 145.5 (C-12), and δ 79.4; in the HMQC nmr spectrum, this carbon signal at δ 79.4 was correlated to the proton triplet at δ 5.80, while the proton doublet at δ 4.73 was coupled to the carbon signal at δ 66.7. These observations allowed the assignments of the signals at δ 66.7, δ 79.4, δ 4.73, and δ 5.80 to C-10, C-9, H-10α, and H-9β, respectively. The second benzoxy substituent is therefore assigned to the C-9 position, as H-9β is more deshielded than H-10α. The ^{1}H-^{1}H COSY nmr spectrum showed a correlation between the H-10 signal at δ 4.73 and a broad signal at δ 3.5 attributable solely to the C-10 hydroxyl proton. The multiplicity of the H-10 signal (triplet, J=8.6 Hz) indicated that its coupling with the H-9 and OH-10 protons were similar; this strong coupling with the OH-10 proton suggested that this hydroxyl proton exchange was very slow, maybe because of strong intramolecular hydrogen bonding. The structure 4α,7β-diacetoxy-2α,9α-dibenzoxy-5β,20-epoxy-10β,13α,15-trihydroxy-11(15→1)-*abeo*-tax-11-ene was thus assigned to **5**. As suggested by Chen and Kingston (10), **5** has been named as a derivative of taxchinin C (11), and specifically as 13-deacetyl-10-debenzoyltaxchinin C.

Compound **6** was identified as taxol C according to its ^{1}H-nmr, ^{13}C-nmr, and ms data. The occurrence of **6** in the roots of *T.* x *media* has been reported (23) and it has also been isolated from cell cultures of *T. baccata* (24). Taxol C showed cytotoxic activity two times higher than that of **1** against P-388 leukemia cells in the MTT reduction assay (25) (IC_{50} ca. 20 μg/ml for **6** and ca. 45 μg/ml for **1**). It has been reported that the activity of **6** was similar to that of **1** in a tubulin assembly assay and that it was very active in the NCI human tumor panel in vitro test with an average GI_{50} value of 4.47×10^{-7} M (24). Compound **6** was more selective against cell lines of non-small-cell lung cancer (HOP-62 and NCI H-460), colon cancer (COLO 205), CNS cancer (SF-539 and SNB-75), and ovarian cancer (OVCAR-3) (24). The detection of compound **6**, with significant cytotoxicity, together with **1** and **2**, indicates that immunoenzymatic methods aimed at predicting the presence of active taxoids in chromatographic fractions can be used as a powerful tool in the search for new active constituents in *Taxus* species.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The optical rotations were determined using a Perkin-Elmer 141 polarimeter. The ir spectra were taken on a Bruker IFS 55 instrument. ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian Unit 600; the DEPT sequence was used to distinguish methylene carbon signals from those due to methine, methyl, and quaternary carbons; ^{1}H-^{1}H and one-bond ^{1}H-^{13}C connectivities were determined via COSY and 2D proton-detected HMQC experiments, respectively; two- and three-bond ^{1}H-^{13}C correlations were determined using 2D proton-detected HMBC experiments. Chemical shift data are reported in ppm downfield from TMS. The fabms were obtained using glycerol as matrix; the cims were recorded on a VG 7070 F apparatus. Si gel 60 (Merck, mean particle size, 0.040–0.063 μm) was used for cc.

PLANT MATERIAL.—Stem bark samples of *T. baccata* L. cv. *stricta* (Taxaceae) were collected from trees growing in the Jardin Experimental Jean Massart of the Université Libre de Bruxelles, Brussels, Belgium.

EXTRACTION AND ISOLATION.—Powdered stem bark (280 g) was percolated with a MeOH-CH_2Cl_2 (1:1) mixture (3 liters). After solvent evaporation, the residue (55 g) was dissolved in MeOH (350 ml) and the solution was diluted with H_2O (350 ml). The resulting H_2O/MeOH solution was extracted with petroleum ether (3×700 ml), then with CH_2Cl_2 (3×700 ml). The CH_2Cl_2 extract was dried on anhydrous Na_2SO_4, evaporated, and the residue (5 g) was adsorbed on cellulose (10 g), and submitted to cc. The column (45 cm×30 mm i.d.) was filled with Si gel (mean particle size 0.040–0.063 μm) (75 g) suspended in *n*-hexane. The column was eluted with *n*-hexane-Me_2CO (75:25) (1000 ml), then with *n*-hexane-Me_2CO (70:30) (1500 ml), and 13 fractions were collected.

High-performance liquid chromatography (hplc).—Analytical hplc was achieved on a Hibar pre-packed Lichrosorb® RP_{18} column (mean particle size 7 μm, 250 mm×4 mm i.d.) using MeCN-H_2O-MeOH (38:42:20) as the mobile phase at a flow rate of 1 ml/min. Compound identifications were achieved by co-chromatography with authentic samples and by spectrometric analysis with a photodiode-array detector. Semi-prep. separation of fraction 10 from the cc was performed with the same solid phase but on a 250 mm×10 mm i.d. column with MeCN-H_2O-MeOH (45:40:20) as the mobile phase at a flow rate of 6 ml/min.

High-speed counter-current chromatography (hsccc).—Hsccc was performed using an Ito Multi-Layer Coil Separator Extractor (PC, Inc., Potomac, MD) equipped with a 66 mm×2.6 mm i.d. column (column capacity: 350 ml). The procedure for the isolation of **1** was described previously (6). For the isolation of **6**, the sample (fraction 10 from the cc; 350 mg) was dissolved in 10 ml of a 1:1 mixture of the two phases of the solvent system and the solution was filtered before loading into the column. The separation was performed at room temperature and 180 fractions of 12 ml each were collected. Fractions were monitored on line by tlc (26) using precoated plates (10×20 cm) of Si gel 60 F_{254} (Merck) and developed in an unsaturated tank with *n*-hexane-Me_2CO (1:1). Fractions 121 to 160 from the hsccc were pooled and evaporated; the residue was further subjected to prep. tlc and the pertinent band (R_f values corresponding to the compound X_4) was isolated, eluted, and the purity checked by analytical hplc (99% pure).

Compound **5**.—Fraction 9 from the cc (200 mg) was subjected to prep. tlc using CH_2Cl_2-MeOH (95:5) as mobile phase to give compound **5**. The purity of this compound was checked by analytical hplc (95% pure): $[\alpha]^{20}D$ −8° (*c*=0.2, $CHCl_3$); ir ν max (KBr) 3409, 2954, 2886, 1727, 1409, 1318, 1136, 1090, 1022, 1000, 932, 845 cm^{-1}; fabms *m/z* $[M+glycerol]^+$ 785, $[M+Na]^+$ 715, $[M+H]^+$ 693, $[M+H-H_2O]^+$ 675, $[M+H-2\times H_2O]^+$ 657; ^1H- and ^{13}C-nmr data, see Table 3.

ELISA PROCEDURE.—The ELISA method used in this study was previously described (5). The anti-taxol antibodies were obtained by immunizing rabbits against 2′-succinyltaxol-BSA conjugate. Aliquots from each chromatographic fraction assayed by ELISA were first dissolved in MeOH and a suitable volume of this solution was diluted in an appropriate buffer for ELISA.

ACKNOWLEDGMENTS

The authors thank Mr. Cl. Maerschalk for recording the HMQC and HMBC nmr spectra. Y.-W.G. thanks the "Foundation Universitaire David et Alice van Buuren" for financial support.

LITERATURE CITED

1. M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, and A.T. McPhail, *J. Am. Chem. Soc.*, **93**, 2325 (1971).
2. P.B. Schiff, J. Fant, L.A. Auster, and S.B. Horwitz, *J. Supramolec. Struc.*, **8**, Suppl. 2, 328 (1978).
3. S.B. Horwitz, L. Lothsteia, J.J. Manfredi, W. Mellado, J. Parness, S.N. Roy, P.B. Schiff, L. Sorbara, and R. Zeheb, *Ann. New York Acad. Sci.*, **466**, 733 (1986).
4. D.G.I. Kingston, A.A. Molinera, and J.M. Rimoldi, in: "Progress in the Chemistry of Organic Natural Products." Ed. by W. Herz, G.W. Kirby, R.E. Moore, W. Steglich, and Ch. Tamm, Springer-Verlag, New York, 1993, Vol. 61, pp. 1–206.
5. M. Jaziri, B. Diallo, M. Vanhaelen, R. Vanhaelen-Fastré, A. Zhiri, A. Becu, and J. Homès, *J. Pharm. Belg.*, **46**, 93 (1991).
6. R. Vanhaelen-Fastré, B. Diallo, M. Jaziri, M.L. Faes, J. Homès, and M. Vanhaelen, *J. Liq. Chromatogr.*, **15**, 697 (1992).
7. D.P. Della Casa de Marcano and T.G. Malsall, *J. Chem. Soc., Chem. Commun.*, 365 (1975).
8. G.P. Gunawardana, U. Premachandran, N.S. Burres, D.N. Whittern, R. Henry, S. Spanton, and J.B. McAlpine, *J. Nat. Prod.*, **55**, 1686 (1992).
9. A. Chu, J. Zajicek, L.B. Davin, N.G. Lewis, and R.B. Croteau, *Phytochemistry*, **31**, 4249 (1992).

10. R. Chen and D.G.I. Kingston, *J. Nat. Prod.*, **57**, 1017 (1994).
11. K. Fuji, K. Tanaka, B. Li, T. Shingu, H. Sun, and T. Taga, *J. Nat. Prod.*, **56**, 1520 (1993).
12. A. Chu, M. Furian, L.B. Davin, J. Zajicek, G.H.N. Towers, C.M. Soucy-Breau, S.J. Rettig, R. Croteau, and N. Lewis, *Phytochemistry*, **36**, 975 (1994).
13. G.N. Chmurny, B.D. Hilton, S. Brobst, S.A. Look, K.M. Witherup, and J.A. Beutler, *J. Nat. Prod.*, **55**, 414 (1992).
14. R.W. Miller, R.G. Powell, and C.R. Smith, Jr., *J. Org. Chem.*, **46**, 1469 (1981).
15. G. Samaranayake, K.A. Neidigh, and D.G.I. Kingston, *J. Nat. Prod.*, **56**, 884 (1993).
16. G.I. Georg, S.R. Gollapudi, G.L. Grunewald, C.W. Gunn, R.H. Himes, B.K. Rao, X.-Z. Liang, Y.W. Mirhom, L.A. Mitscher, D.G. Vander Velde, and Q.-M. Ye, *Bioorg. Med. Chem. Lett.*, **3**, 1345 (1993).
17. G. Appendino, L. Barboni, P. Gariboldi, E. Bombardelli, B. Gabetta, and D. Viterbo, *J. Chem. Soc., Chem. Commun.*, 1587 (1993).
18. K. Fuji, K. Tanaka, B. Li, T. Shingu, H. Sun, and T. Taga, *Tetrahedron Lett.*, **33**, 7915 (1992).
19. A. Wahl, F. Gueritte-Voegelen, D. Guenard, M.T. Le Goff, and P. Potier, *Tetrahedron*, **48**, 6965 (1992).
20. G. Samaranayake, N.F. Magri, C. Jitrangari, and D.G.I. Kingston, *J. Org. Chem.*, **56**, 5114 (1991).
21. F. Balza, S. Tachibana, H. Barrios, and G.H.N. Towers, *Phytochemistry*, **30**, 1613 (1991).
22. V. Sénilh, S. Blechert, M. Colin, D. Guénard, F. Picot, P. Potier, and P. Varenne, *J. Nat. Prod.*, **47**, 131 (1984).
23. L. Barboni, P. Gariboldi, E. Torregiani, G. Appendino, B. Gabetta, and E. Bombardelli, *Phytochemistry*, **36**, 987 (1994).
24. W.W. Ma, G.L. Park, G.A. Gomez, M.H. Nieder, T.L. Adams, J.S. Aynsley, O.P. Sahai, R.J. Smith, R.W. Stahlhut, and P.J. Hylands, *J. Nat. Prod.*, **57**, 116 (1994).
25. R.I. Geran, N.H. Greenberg, M.M. McDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep.*, **3**, 11 (1972).
26. B. Diallo, R. Vanhaelen-Fastré, and M. Vanhaelen, *J. Chromatogr.*, **558**, 446 (1991).

Received 2 November 1994