Rearrangement of *O*-Cinnamoyltaxicin I to a Novel C-13 Spiro-Taxane

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During the large-scale synthesis of an *O*-cinnamoyltaxicin I acetonide, an intermediate for the semisynthesis of 7-deoxypaclitaxel derivatives, side-product **3** was formed via a vinylogous retro-aldol reaction and a long-range hydride shift from *O*-cinnamoyltaxicin I (**1**) under alkaline reaction conditions. Compound **3** has two hemi-acetal bridges at C-1,C-9 and C-10,C-13. Compound **4** was formed from side-product **3** under acidic reaction conditions and is the first C-13 spiro-taxane described in the literature. This spiro-taxane has two acetal bridges between C-1,C-13 and C-10,C-13.

Paclitaxel (Taxol, Yewtaxan) is an antitumor drug first isolated from the bark of the Pacific yew (*Taxus brevifolia* Nutt., Taxaceae).[1] Taxine alkaloids are the major alkaloids of the taxine fraction (3−9 g/kg dried needles) from the needles of *Taxus baccata* L., and they are excellent precursors for the semisynthesis of 7-deoxypaclitaxel and 1,7-dideoxypaclitaxel derivatives.[2−5] During the scale-up of this semisynthesis, we detected a C-13 spiro-taxane as a side-product. The formation of this side-product decreased the total yield of 7-deoxypaclitaxel and 1,7-dideoxypaclitaxel derivatives. Furthermore, it was the first C-13 spiro-taxane ever reported. For these reasons, we decided to investigate the formation of this new C-13 spiro-taxane.

Results and Discussion

During large-scale synthesis of new 7-deoxypaclitaxel and 1,7-dideoxypaclitaxel derivatives from taxine alkaloids, formation of a side-product was noticed during the base-catalyzed reaction of taxine methiodides to compounds **1** and **2**.[2,3] This reaction was complete after 2 h with only a very small amount of the side-product **3** formed. However, when the reaction was carried out overnight, compounds **2** and **3** were the only products. Therefore, it was concluded that compound **3** was formed from *O*-cinnamoyltaxicin I (**1**). Under the acidic conditions of the next reaction step (**1,2** to 9,10-isopropylidene derivatives),[3] compound **3** was converted into product **4**. The proposed mechanism for the formation of compounds **3** and **4** from *O*-cinnamoyltaxicin I (**1**) is given in Scheme 1. The first step under basic reaction conditions was a vinylogous retro-aldol reaction that resulted in fragmentation of the C-1,C-15 bond. After the formation of a C-10,C-13 hemi-acetal bridge, a long-range hydride shift took place from C-9 to C-1. Finally, a second hemi-acetal bridge was formed between C-1 and C-9, which resulted in compound **3**. A similar reaction sequence has been published by Appendino et al. for taxicin I.[6] Under acidic reaction conditions, the C-1,C-9 hemi-acetal bridge was opened again, and a C-2,C-13 acetal bridge was formed. This resulted in C-13 spiro-compound **4**. When Appendino et al. brought their rearranged taxicin I into contact with acids, they also observed the opening of the C-1,C-9 hemi-acetal bridge.[6] However, it was claimed that
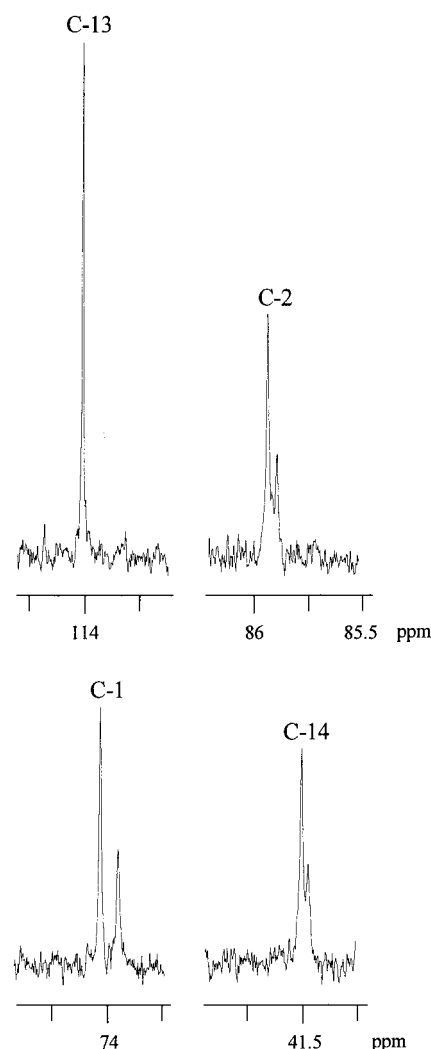

Figure 1. Deuterium exchange experiment to determine the number and the locations of hydroxyl groups in compound **4**.

the reaction stopped after this ring opening and that the mono-bridged hemi-acetal **5** was the final product. After performing several NMR and MS experiments on spiro-compound **4**, it could be concluded that the structure published by Appendino et al. was not in accordance with their data.[6]

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Scheme 1. Mechanism for the Formation of Compounds **3** and **4**
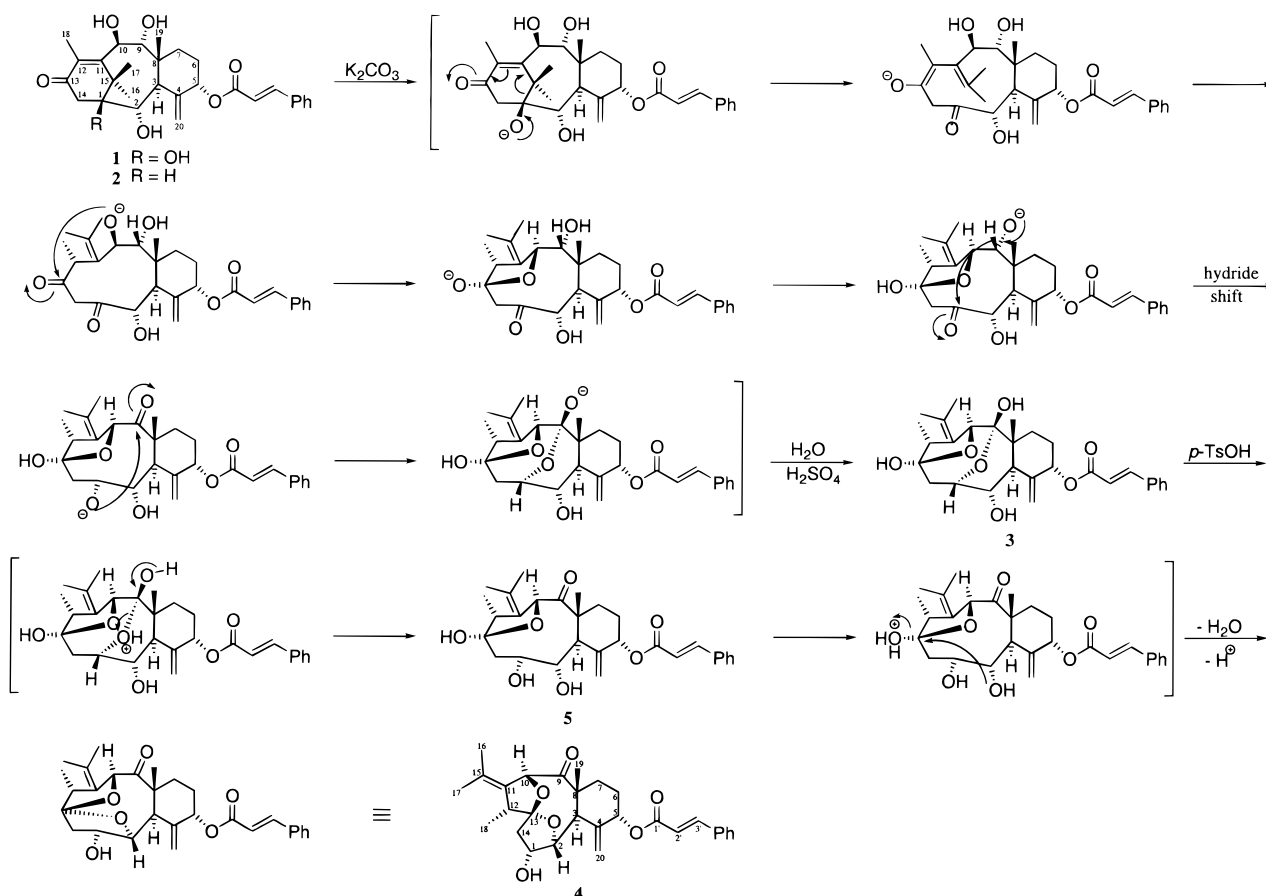
¹H and ¹³C NMR spectra of compound **3** (Table 1) were in good correspondence with the data published by Appendino et al.[6] It was not possible to record EIMS or CIMS of compound **3** because of rapid conversion into **4** during the measurement. However, an FDMS could be measured successfully. The ¹H–¹H COSY and ¹H–¹³C HETCOR spectra of compound **4** supported the structure drawn in Scheme 1. The NOESY spectrum of compound **4** revealed the relative configuration at C-1, C-2, C-10, and C-12. H-1 had a strong NOE interaction with the protons of C-19, which revealed the stereochemistry at C-1. Both H-1 and H-12 showed NOE interactions with H-14β but not with H-14α. Furthermore, H-12 had an NOE interaction with H-10. This proved the stereochemistry at C-10 and C-12. H-2 had an NOE interaction with H-1, H-3, and H-20a. These interactions were only possible with the stereochemistry at C-2 as drawn in Scheme 1. Knowing the configurations at C-2 and C-10, the stereochemistry of C-13 as illustrated in Scheme 1 was the only one consistent with the NMR data of compound **4**. All coupling constants were checked with a 3D model by looking at the corresponding H–H angles, and they completely supported the structure in Scheme 1. The position of the carbonyl group at C-9 was proven with a ¹H–¹³C COLOC spectrum, which showed a strong interaction between C-9 and the protons at C-19. To determine the number and the locations of hydroxyl groups in molecule **4**, a ¹³C NMR spectrum was measured in C_6D_6 with 3 drops of H_2O and 2 drops of D_2O. If a hydroxyl group is partially deuterated, separate ¹³C signals can be observed for C–OH and C–OD. The signal for C-1 was clearly separated (Figure 1). Furthermore, a long-range deuterium isotope effect could be seen on C-2 and C-14. None of the other ¹³C signals was effected in this experiment. This proved that compound **4** contained only

one hydroxyl group and that it was located on C-1. This was the final proof that compound **4** is the C-13 spiro-taxane as depicted in Scheme 1.

Experimental Section

General Experimental Procedures. Chemicals and solvents were of analytical grade, HPLC grade, or distilled prior to use. NMR spectra were recorded on a Bruker DPX 400 spectrometer. MS were recorded on a Finnigan MAT 95 spectrometer. Melting points were measured with a Olympus BH-2 apparatus.

Plant Material. One- to two-year-old branches of *Taxus baccata* L. (120 kg) were obtained from plants growing on the premises of the Forestry Department (Wageningen Agricultural University, The Netherlands), which are classified as Hi-S/946. After drying for 15 h at 60° in an oven with forced air ventilation, the needles were separated from the branches.

Extraction and Isolation. Dried needles of *T. baccata* (40 kg) were soaked in 0.5% (v/v) H_2SO_4 (215 L) without stirring for 1–2 weeks. The extract was separated from the needles and brought to pH 10–10.5 by addition of 25% aqueous ammonia. Subsequently, the solution was extracted in batches of 15 L, each with two 5-L portions of Et_2O. After evaporation under reduced pressure, the Et_2O was recycled. The total yield of crude taxine alkaloids was 165 g (4.1 g/kg dried needles).

Semisynthesis. The taxine methiodides, compounds **1** and **2**, and the 9,10-isopropylidene derivatives were synthesized as described by Wiegerinck et al.[3] and Jenniskens et al.[2]

Compound 3. A mixture of taxine methiodides (50 g) was taken into 500 mL absolute EtOH, and 62.5 g of K_2CO_3 dissolved in 500 mL of water was added. The mixture was stirred for 20 h at room temperature. During the first 2 h of the reaction only compounds **1** and **2** were formed. After evaporation of EtOH, 500 mL of 0.5% H_2SO_4 and 500 mL of brine were added. The aqueous layer was extracted with four 250-mL portions of $CHCl_3$. The combined organic layers were

Table 1. ^1H and ^{13}C NMR Data for Compounds **3** and **4**

	3			**4**	
carbon	^1H	^{13}C		^1H	^{13}C
1	3.86 (ddd, $J = 7.2, 1.6, 7.8$ Hz)	77.2 (d)		5.18 (dddd, $J = 5.8, 8.4, 6.7, 7.4$ Hz)	74.0 (d)
2	4.26 (dd, $J = 11.6, 7.2$ Hz)	70.6 (d)		4.11 (dd, $J = 5.8, 5.8$ Hz)	85.9 (d)
3	3.04 (d, $J = 11.6$ Hz)	47.2 (d)		3.39 (dd, 5.8, 1.7 Hz)	54.8 (d)
4		143.4 (s)			141.9 (s)
5	5.50 (dd, $J = 2.6, 2.6$ Hz)	75.5 (d)		5.48 (dd, $J = 2.8, 2.8$ Hz)	76.2 (d)
6α	2.01 (m)	28.6 (t)		1.95 (m)	27.5 (t)
6β	2.01 (m)			1.95 (m)	
7α	2.29 (ddd, $J = 13.9, 14.0, 4.8$ Hz)	30.8 (t)		2.64 (ddd, $J = 14.0, 15.0, 4.8$ Hz)	33.2 (t)
7β	1.59 (m)			1.21 (m)	
8		44.4 (s)			51.6 (s)
9		110.7 (s)			214.4 (s)
10	4.93 (s)	83.5 (d)		4.75 (s)	81.3 (d)
11		131.5 (s)			130.0 (s)
12	2.92 (qq, $J = 7.3, 1.6$ Hz)	49.3 (d)		2.72 (qq, $J = 6.9, 2.0$ Hz)	40.4 (d)
13		101.4 (s)			114.0 (s)
14α	2.18 (dd, $J = 14.7, 1.6$ Hz)	41.9 (t)		2.00 (dd, $J = 11.4, 8.4$ Hz)	41.5 (t)
14β	2.88 (dd, $J = 14.7, 7.8$ Hz)			2.45 (dd, $J = 11.4, 6.7$ Hz)	
15		134.8 (s)			133.3 (s)
16	1.78 (d, $J = 1.6$ Hz)	22.9 (q)		1.77 (d, $J = 2.0$ Hz)	24.1 (q)
17	1.83 (s)	21.4 (q)		1.80 (s)	21.1 (q)
18	1.39 (d, $J = 7.3$ Hz)	17.0 (q)		1.19 (d, $J = 6.9$ Hz)	13.8 (q)
19	1.22 (s)	16.1 (q)		1.52 (s)	15.2 (q)
20a	5.51 (s)	114.7 (t)		5.73 (d, $J = 1.7$ Hz)	118.0 (t)
20b	5.14 (s)			5.44 (s)	
1′		166.7 (s)			166.6 (s)
2′	6.52 (d, $J = 16.0$ Hz)	119.0 (d)		6.44 (d, $J = 16.0$ Hz)	119.0 (d)
3′	7.73 (d, $J = 16.0$ Hz)	145.3 (d)		7.67 (d, $J = 16.0$ Hz)	145.1 (d)
i-Ph		134.9 (s)			134.8 (s)
o-Ph	7.58 (m)	129.3 (2d)		7.54 (m)	129.3 (2d)
m-Ph	7.44 (m)	128.5 (2d)		7.40 (m)	128.5 (2d)
p-Ph	7.44 (m)	130.7 (d)		7.40 (m)	130.6 (d)
O*H*				1.95 (d, $J = 7.4$ Hz)	

dried (Na_2SO_4) and evaporated under reduced pressure to obtain 37 g of a mixture in which **2** and **3** were the major products according to HPLC. Compound **3** was purified as a white powder by column chromatography over silica (petroleum ether 40°/60°−EtOAc = 1:1); mp 120−122 °C; FDMS m/z (rel int) 496 [M]$^+$ of compound **3** and 478 [M]$^+$ due to formation of **4** during the measurement. ^1H and ^{13}C NMR (CDCl$_3$) values are given in Table 1.

Spiro-taxane 4. A crude mixture of **2** and **3** (36 g) was dissolved in 400 mL of anhydrous acetone and stirred with 200 g $CuSO_4$ and 2 g *p*-TsOH. After 24 h the reaction mixture was filtered over Hyflo, evaporated, taken into 500 mL of CH_2Cl_2, and washed with 75 mL of a saturated $NaHCO_3$ solution. Drying, filtration, and evaporation under reduced pressure yielded a mixture of 23 g of spiro-compound **4** and the 9,10-isopropylidene derivative formed from **2**. Compound **4** was purified as a white powder by column chromatography over silica (petroleum ether 40°/60°−EtOAc 5:2); mp 112−114 °C; EIMS m/z (rel int) 478 ([M]$^+$, 62), 330 (53), 148 (30), 131 (100), 105 (29), 103 (40), 91 (52), 77 (33), 69 (96), 51 (51), 45 (86); calcd for $C_{29}H_{34}O_6$ m/z 478.2355, found m/z 478.2348; FDMS m/z (rel int) 478 [M]$^+$. All NMR spectra were measured in CDCl$_3$ except the deuterium exchange experiment (C_6D_6/D_2O/H_2O). Table 1 gives the ^1H and ^{13}C values. COSY, NOESY, HETCOR, and COLOC spectra are available on request from the corresponding author.

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