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Structures of the Major Human Metabolites of Docetaxel (RP 56976 - Taxotere®)

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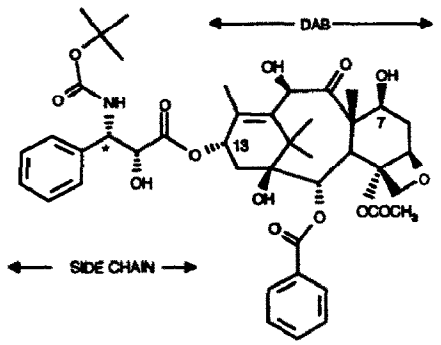
Abstract : The structures of the main human metabolites of docetaxel (RP 56976-Taxotere®) isolated from human feces have been established using HPLC/MS-MS and 600 MHz NMR experiments. These metabolites are C-13 side chain oxidation derivatives of the parent compound.

Docetaxel 1 (RP 56976, Taxotere®) is a semisynthetic compound obtained via direct acylation at the C-13 position of 10-deacetyl baccatin III (DAB) extracted from the leaves of *Taxus baccata L.* [1]. Docetaxel as well as paclitaxel (Taxol®) are currently considered as among the most promising drugs in Cancer Research [2]. Docetaxel interferes with the microtubule - tubulin system in eukaryotic cells. It shows excellent anti-tumor activity and is actually in phase II clinical trials.



1 : ^{14}C labelled docetaxel

Pharmacokinetics and metabolism studies of docetaxel have recently been reported both in human and animals [3]. It has been demonstrated that docetaxel excretion mainly occured in the feces via the biliary route during the first 48 hours after administration [4]. The excretion balance indicates that more than 60 % of the administered ^{14}C labelled drug is recovered in human feces as metabolites and parent compound.

A reversed phase HPLC radiochromatogram of organic extracts indicates the presence of 4 major metabolites (M_{16}, M_{17}, M_{18}, M_{23}) in respectively 7.5 ; 6.5 ; 7.7 and 23.3 % of the initial administered dose. A minor compound (M_{15}) is also detected.

Due to a large amount of impurities, the direct HPLC-MS coupling [5] leads to the restitution of a very broad Total Ionic Current curve (TIC). The overlap of numerous peaks of various masses hampered any reliable interpretation. Because the electrospray mass spectrum of docetaxel showed a fragment ion at m/z = 527 amu - corresponding to the loss of the C-13 side chain - we performed HPLC/MS-MS experiments in order to obtain a better selectivity. In the parent ion mode [6], while the first quadripole (Q_1) is scanning from 500 to 1000 amu, the third quadripole (Q_3) only filters m/z = 527 ions. Therefore only ions leading to m/z = 527 by fragmentation into the collision cell (Q_2) are detected. These MS-MS experiments restitute a TIC curve nearly superimposable with the HPLC radiochromatogram (Figure 1), showing that all the previously detected metabolites correspond to C-13 side chain structural modification of docetaxel.

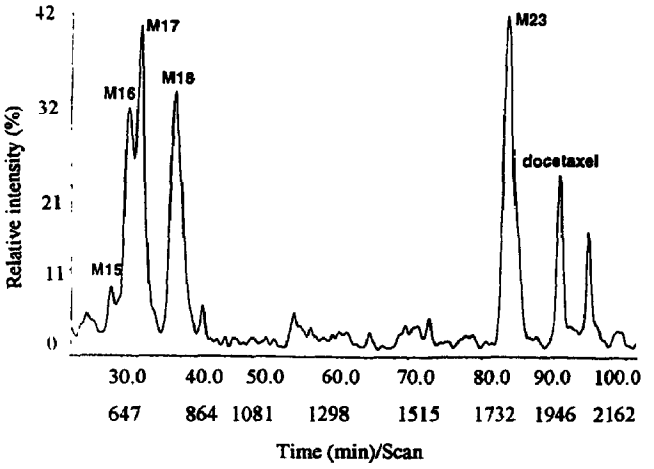


Figure 1 :Total Ionic Current of HPLC/MS-MS experiments

The masses obtained for the five metabolites are the following : M_{15}, m/z = 838 (MH^+) ; M_{16}, m/z = 822 (MH^+) ; M_{17}, m/z = 824 (MH^+) ; M_{18}, m/z = 822 (MH^+) and M_{23}, m/z = 820 (MH^+).

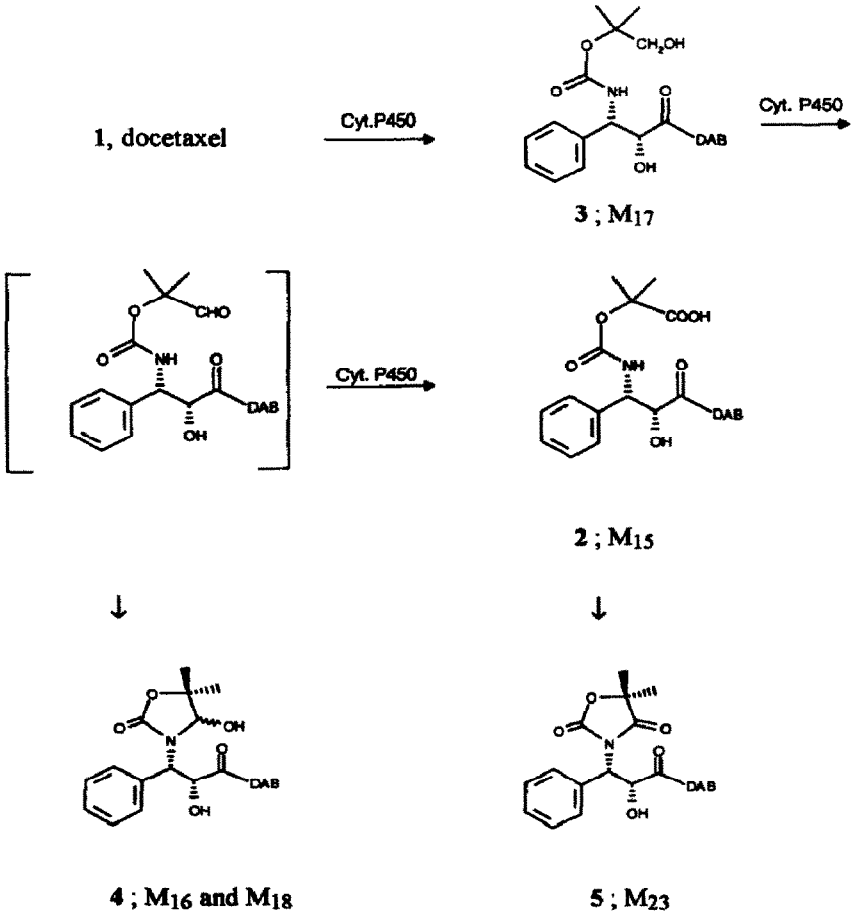
In the case of M_{15} (a very minor metabolite), a difference of + 30 amu - when comparing to the parent compound - could correspond to the usual oxidation of a methyl group into a carboxylic acid function. Therefore the structure of M_{15} is postulated to be **2**.

The difference of + 16 amu means that M_{17} belongs to a monohydroxylated species (either on the phenyl or the t-butyl group). Furthermore the 600 MHz NMR spectra of isolated HPLC fractions presented an additional CH_2O AB pattern ($CDCl_3$, δ = 3.44 and 3.60 ppm, J = 12 Hz), thus confirming that the structure of M_{17} is **3**. This type of hydroxylation as been previously reported when octalin derivatives were submitted to microbial oxidation [7]. An attempt at isolating M_{15} led to its dehydration into M_{23}.

The NMR spectra of M_{16} and M_{18} indicated the presence of an additional singlet proton respectively at δ = 4.74 and δ = 4.4 ppm ($CDCl_3$ solution). Furthermore a methyl group pertaining to the t-butyl group, and the amide proton of the side chain are missing in both cases.

The rather unusual low field resonance of these singlets suggests the presence of an hemi-aminal function. This observation is in good agreement with the possible formation of a cyclic hydroxyoxazolidinone moiety **4**. Due to the presence of an additional asymmetric centre M_{16} and M_{18} are postulated to be diasterisomers.

The NMR spectrum of M_{23} is not very informative except for the losses of a methyl (from the t-butyl group) and of the side chain amide proton. As M_{15} (the carboxylic derivative **2**) is readily transformed into M_{23} by H_2O leakage, structure **5** is proposed for the later.



Scheme 1 - DAB is the abbreviation for 10-deacetyl baccatin III (see structure **1**)

Finally the confirmation of the structures of these metabolites as well as the postulated metabolic pathway for docetaxel were supported by synthetic work [8].

Indeed the synthetic compounds obtained have the same physico-chemical properties (NMR, MS and IR spectra) as the docetaxel metabolites described in this work.

Despite the similarity in their chemical structures, docetaxel and paclitaxel present large differences in their metabolisms since the major human metabolites of paclitaxel are two monohydroxylated derivatives [9]. The first one is hydroxylated at the C-6 position of the diterpene moiety while the second is hydroxylated at the para position of the side chain phenyl.

These modifications did not occur for docetaxel, which presents an original metabolic oxidative pathway (Scheme 1) : first the parent compound is oxidized into the primary alcohol **3**, leading then to the putative aldehyde (unobserved) giving the two cyclic hydroxyoxazolidinones **4**. A further oxidative step leads to the unstable carboxylic acid derivative **2** giving the oxazolidinedione **5** after cyclisation. The three oxidative steps result from the involvement of cytochrome P450 enzymes [3] . The same metabolic pathway has also been reported for rat [10].

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