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Biosynthetic building blocks of Taxus canadensis taxanes

Lolita O. Zamir*[a], Maria E. Nedea[a] and François X. Garneau[b]

[a]Centre de recherche en Microbiologie Appliquée, Université du Québec, Institut Armand Frappier, 531, boul. des Prairies, Laval, Quebec, Canada, H7N 4Z3

[b]Université du Québec à Chicoutimi, 555 boul de l'Université, Chicoutimi, Quebec, Canada G7H 2B1

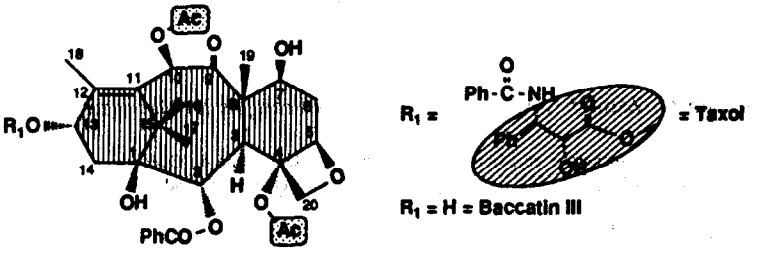
Abstract : Radiolabelling feeding experiments have confirmed that acetate, mevalonate and phenylalanine represent the biosynthetic building blocks of *Taxus canadensis* taxanes.

Studies on the biosynthesis of natural products in intact plants are often hampered by very low incorporations. Radiolabelled precursors are therefore essential. An interesting biogenetic hypothesis was proposed by Potier's research group[1] to rationalize the structural groups of taxanes isolated in yews. The only biosynthetic experiments reported on yews were the elegant studies of Leete[2] and Haslam[3] and their research groups. Both laboratories focused on the origin of the Winterstein acid[4] part of the side chain of *Taxus baccata* taxanes. [3-^{14}C]phenylalanine[2] was incorporated into the mixture of taxanes called taxine and chemical degradations localized the radiolabel into C-3 of 3-dimethylamino-3-phenylpropionic acid[2] (Winterstein's acid). The feeding of specifically labelled (2S)-Phenyl[2-^{14}C,3R-^{3}H] alanine and (2S)-Phenyl[2-^{14}C,3S-^{3}H] alanine respectively to *T. baccata* showed that the 3-pro R proton is lost stereospecifically in the conversion to Winterstein acid[3].

In this publication, we show that the biosynthetic building blocks for taxanes in *T. canadensis* are acetate, mevalonate and phenylalanine. The feedings of radiolabelled precursors were done on newly grown leaves, ground needles and stems as well as cell-free homogenate. The incubations ranged from 2 days to 3 weeks.

Newly grown leaves (light green needles, early June) were found to incorporate the radiolabel better than the older plants (dark green needles, October)[5,6]. Three feeding techniques were compared: i) dipping the newly grown plantlets into a nutrient solution[7] containing the radiolabelled precursor, ii) grinding the newly grown needles and incubating in the same solution[7] and iii) preparing a cell free homogenate[8] and adding the radiolabelled precursor. We found that methods ii) and iii) were comparable and preferable to i). In addition, the best incubation conditions were at room temperature in the dark and for three weeks. The radiolabelled precursors fed respectively to *T. canadensis* new shoots (needles and stems) were [^{3}H]sodium acetate, (3RS)(5RS)[5-^{3}H] mevalonate, (3R)[2-^{14}C] mevalonate. After 1 - 3 weeks incubation in the dark, the taxol was extracted and purified to constant specific activity (dpm/mmol)[9]. The total incorporations[10] of radiolabelled precursors into taxol ranged from 0.02% to 0.12%. The acceptable incorporation of 0.12%[10] was obtained with (3RS)(5RS)(5-^{3}H) mevalonate (0.5mCi fed) when only new growth of *T. canadensis* leaves were used. The feeding method was to add the labelled precursor to the ground leaves kept in nutritive salt solution (method ii) under mechanical agitation. The leaves were harvested after three weeks. In addition, a mixture of [ring-2,6-^{3}H] L-phenylalanine and (3R)[2-^{14}C] mevalonate (^{3}H/^{14}C=3.6) was administered to new growth of *T. canadensis* leaves according to method ii). The derived taxol was extracted and purified according to the published procedure[11]. The ^{3}H/^{14}C ratio obtained in pure taxol was 4.4. In order to confirm that the side chain was phenylalanine derived, we needed to hydrolyse the taxol obtained from that feeding. To facilitate isolation and purification of the products, non-radioactive taxol (1mg) was added prior to the hydrolysis. A major product was baccatin III which after purification showed a constant ^{3}H/^{14}C ratio of 1.75. This experimental result obtained with *T. canadensis* is consistent with previous work[2,3] in *T. baccata*.

This work confirms for the first time the general accepted theory that taxol derives biosynthetically from the building blocks acetate, mevalonate and phenylalanine. The microtechniques utilized for the feeding experiments will enable to test larger intermediates.



Scheme 1: Biosynthesis of taxol: the different shadings emphasize the building blocks: acetate (▒▒▒▒▒▒), mevalonate (||||||||||||) and phenylalanine (//////).

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5. This result is consistent with the findings of Leete' that the incorporations of radiolabelled precursors into *Erythroxylon coca* plants is season dependent (from 0.11% total incorporation in October to 3.9% incorporation in July!). Similar results were recently found in *Taxus brevifolia* (Wheeler, N.C.; Jech, K.; Masters, S.; Brobst, S.W.; Alvarado, B.; Hoover, A.J.; Snader, K.M. *J. Nat. Prod.* **1992**, *55*, 432-440.
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8. Homogenisation of the ground needles and stems (new growth) was done in a blender run at low speed for 3 x 20 s with the following buffer: 0.5 M sucrose, 0.05M Tris-Cl, 0.2mM dithiothreitol, 0.5mM ATP, 0.5mM $MgCl_2$. The radiolabelled precursor was added to the supernatant obtained after filtration through 4 layers of cheese cloth. The incubation was for 2 days.
9. The feeding of [^{3}H] sodium acetate (1.2mCi)(90mCi/mmol) according to method ii) led to [^{3}H] taxol : 3,88 x 10^6 dpm/mmol (0.02% Total incorporation[10]). The low content of taxol in *T. canadensis*[11] (0.005%) explains the levels of total incorporations.
10. Total incorporation : $\frac{\text{Total radioactivity (dpm) in pure taxol x 100}}{\text{Total radioactivity (dpm) administered to leaves}}$. In plants a good incorporation could be in the 0.1% - 0.2% range (Leete E. *Phytochemistry*. **1983**, *22*, 933-935).
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