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French pharmaceutical industry and biologically active natural products from Pierre Fabre Laboratories

B. David, R. Bellé, C. Dunouau, P. Fleury, I. Granger-Pouny, P. Négol and **M-J. Serrano**

Centre de Recherche des Substances Naturelles, Institut de Recherche PIERRE FABRE 16, rue Jean Rostand 81603 Gaillac (France)

ABSTRACT The industrial preparation and the bioactivity of the anticancer drug, Navelbine, were discussed. The active components of *Ruscus aculeatus* L., a plant used in the treatment of veinous insufficiency, were also mentioned. Structural elucidation of these compounds was reported.

ABSTRAK Penyediaan industri dan aktiviti ubat antibarah Navelbina dibincangkan. Juga komponen aktif dari pokok *Ruscus aculeatus* L. disebut. Tumbuhan ini digunakan untuk rawatan masalah saluran darah. Elusidasi struktur juga dilaporkan.

(Navelbine. *Ruscus aculeatus* L., NMR, *Catharanthus roseus*, Vinorelbine)

INTRODUCTION

Pierre Fabre Laboratories, an independant French company is particularly active in the natural products research. In thirty years of continuous development, Pierre Fabre has become the 2nd biggest private French pharmaceutical and dermocosmetic company.

For the interest in the natural product field, there are two main reasons. First, in 1961 the founder of our group, a dispensary pharmacist in Castres named Pierre Fabre, discovered the activity of *Ruscus aculeatus* L. against veinous insufficiency and set up Pierre Fabre Medical Laboratory. The second reason is that there is an uninterrupted success in this field from the early sales of *Ruscus* extracts until now. Pierre Fabre activities include ethical drugs, OTC products and dermocos-metics. Major ethical drugs are prepared from plant sources such as *Ruscus aculeatus, Catharanthus roseus,* and *Serenoa repens*. The development of Pierre Fabre Laboratories has increased to a considerable extent during these last years. Today, there are more than six thousand collaborators working internationally.

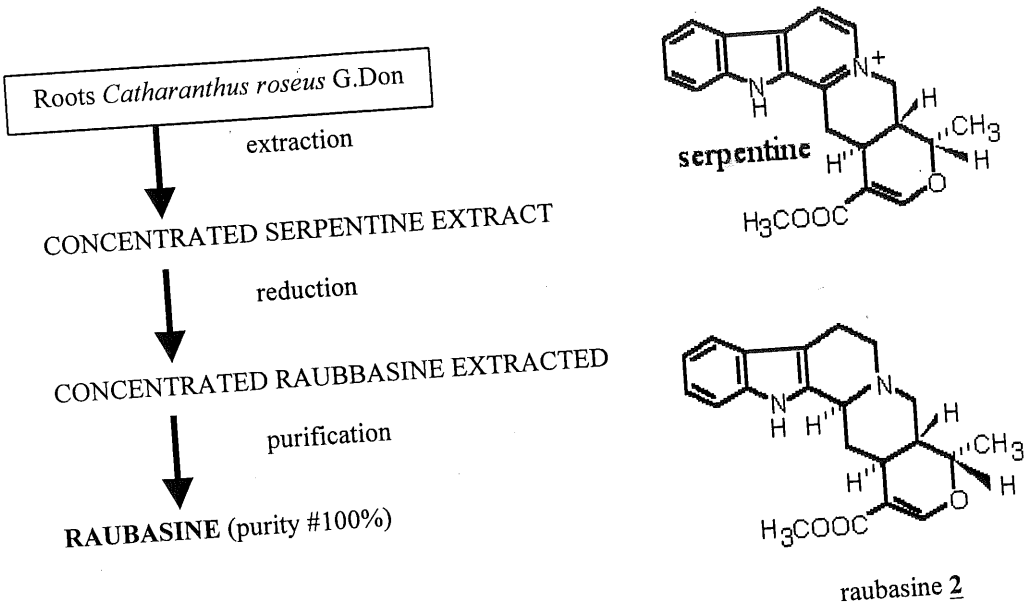
Two major issues will be discussed briefly in this communication which are:

1) *Catharanthus roseus* from which raubasine and vinorelbine are prepared.

2) *Ruscus aculeatus* and the ongoing studies on steroid saponins. Complete NMR study of one of the active saponoside (desgluconero-ruscin) and recent isolation of new minor compounds.

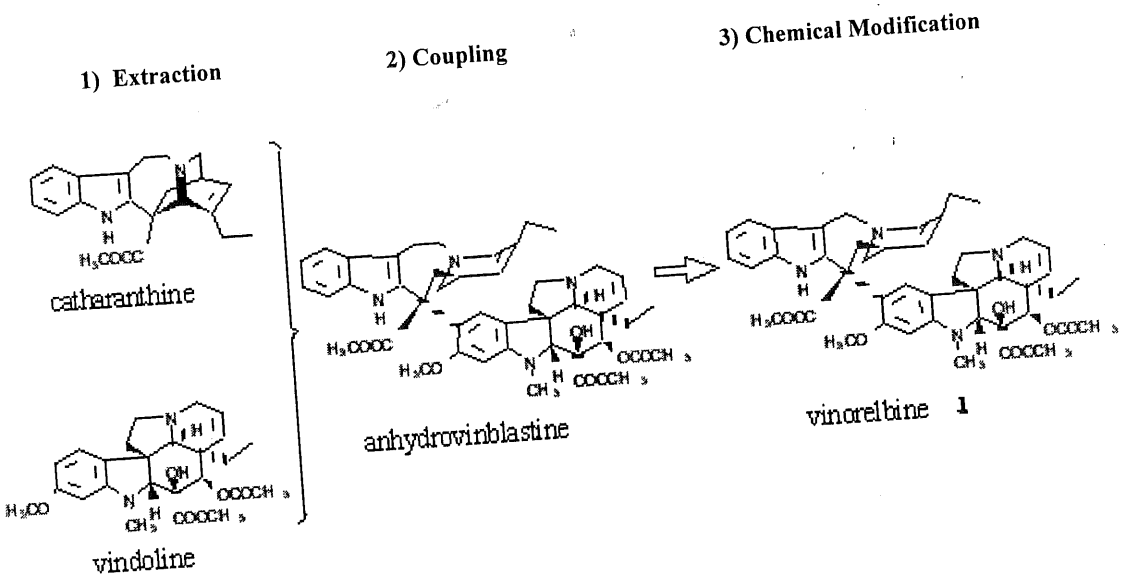
Catharanthus roseus

This plant, which belongs to the family of Apocynaceae, is used to produce from the leaves, vinorelbine **1**, an anticancer compound (Non Small Cell Lung Cancer and Breast Cancer), and from the roots, raubasine **2**, a medicine for cerebral blood circulation.



Scheme 1. Hemisynthesis of raubasine

Scheme **1** shows the process of preparation of raubasine from the roots of the plant. Extraction of crude serpentine leads to raubasine after hydrogenation. The crude raubasine is then purified mainly by crystalisation to attain almost 100% purity.



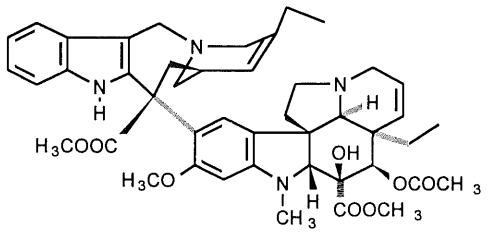
Scheme 2. Preparation of vinorelbine from Catharanthus roseus leaves

Hemisynthesis of vinorelbine is presented in scheme **2**. Two indolic alkaloids are extracted from the leaves and their coupling leads to a hydrovinblastine which is then modified chemically to get active anticancer product. Vinorelbine was first discovered by the group of Professor Pierre POTIER [1] (Institut de Chimie des Substances naturelles CNRS, in Gif sur Yvette) and then developped by Pierre Fabre Laboratories in Castres and Gaillac.

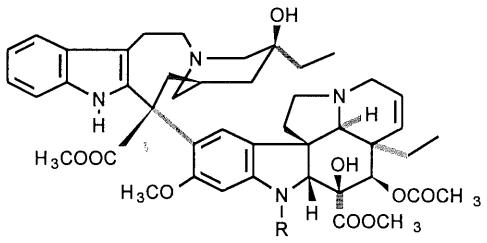
The scaling up of production from a few grams to many kilograms, represented a huge amount of work. Environmental impact, security, optimization of the reactions, compliance with the strictest laws and regulations such as FDA were the mains issues. Therefore, when developing vinorelbine from the historical synthesis in CNRS, Pierre Fabre researchers had to change many parameters. Our production factory got the US-FDA Agreement and at present Navelbine (vinorelbine) is distributed worldwide (in the United States since 1994).

Structural differences between Navelbine, vinblastine and vincristine are minor. However, despite those minute differences, Navelbine produces better results in clinical oncologic assays in terms of toxicology and spectra of action than other "*Vinca*" alkaloids. For example, in breast cancer a far better response average percentage is obtained with Navelbine than with the products in the same family.

The superiority of Navelbine as a product against Non Small Cell Lung Cancer is evidenced by the 29% overall response rate compared to the 17% of vindesine (a classical *Vinca* alkaloid).



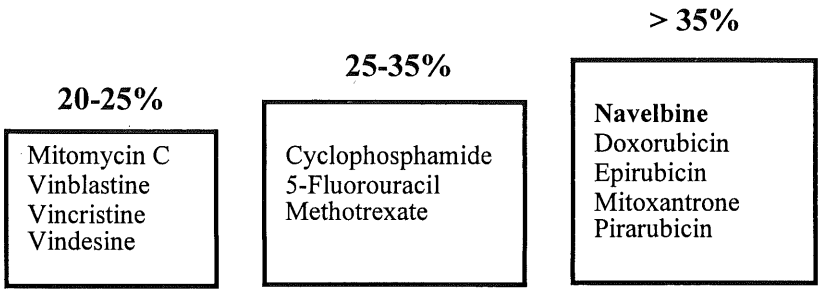
vinorelbine



vinblastine (R = CH_3)
vincristine (R = CHO)

Navelbine as single agent in Breast Cancer

Main cytostatics in Phase II studies (1st line) Overall Response Rates



One of the reasons why Navelbine is better in this family of componds could be explained by a lower neurotoxicity due to a weaker specific affinity for axonal microtubules.

Ruscus aculeatus. L.

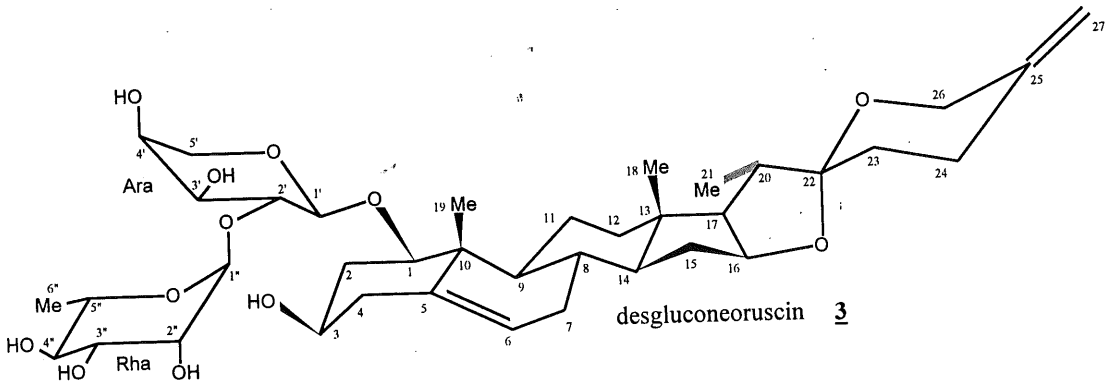
The rhizome of Butcher's broom, a common Mediterranean Liliaceae, is used to produce an ethical medicine that treats veinous insufficiency. Active compounds are a group of steroidal saponosides which we are studying extensively [2-6]. For example, complete and unambiguous ^{1}H and ^{13}C-NMR studies of one major saponoside desgluconeoruscin has been performed [2].

Literature survey revealed limited NMR information on **3**. Partial ^{13}C NMR information [7] has been published but assignments were based on comparison with model compounds. The complete ^{1}H NMR assignment of **3** was unavailable. With the advancement of the two-dimensional NMR experiments (HMBC, HMQC and homonuclear COSY H-H) and the availability of high magnetic fields (>7 T), every carbon and proton signals of this compound can be assigned for the first time [2]. For example, carbons involved in the linkage between the aglycone and the first sugar are unambigously identified using HMBC correlation between C-1 and proton H-1' and between C-1' and proton H-1. The interglycosidic junction is also established by a HMBC correlation between C-1" and proton H-3".

CONCLUSION

The presented examples demonstrate the importance of natural products in the pharmaceutical industry. Natural substances will continue to be an important reservoir of molecular diversity and new pharmaceutical leader compounds in the next millenium.

It should also be emphazised that academic institutions and pharmaceutical companies have complementary expertise, which upon fruitful cooperation can lead to a successful discovery of biologically active natural products as in the vinorelbine case.



desgluconeoruscin **3**

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR, (75MHz) of desgluconeoruscin **3**

^{1}H NMR	δ(ppm)
1a	3.84, dd (11.8, 3.7)
2	2.71, m
2b	2.33, q (11.8)
3	3.85, m
4a	2.67, dd (12.6, ~11)
4b	2.57, dd (12.6, 4.8)
6	5.58, d (5.6)
7a	1.54, m
7b	1.87, dd (11.1, 3.2)
8	1.58, dd (6.6, 3.2)
9	1.55, m
11a	2.97, dd (13.7, 3.0)
11b	1.61, ddd (13.7, 13.5, 3.1)
12a	1.54, m
12b	1.30, t (7.0)
14	1.12, ddd (14.4, 8.8, 6.0)
15a	2.00, ddd (12.7, 6.2, 6.0)
15b	1.42, td (12.7, 6.2)
16	4.51, q (7.3)
17	1.72, m
18	0.85, s
19	1.43, s
20	1.91, t (6.9)
21	1.04, d (6.9)
23a	1.78, td (8.3, 4.9)
23b	1.71, m
24a	2.23, d (11.9)
24b	2.70, m
26a	4.00, d (12.1)
26b	4.44, d (12.1)
27a	4.75, s
27b	4.80, s
1'	4.72, d (7.9)
2'	4.58, t (8.0)
3'	4.14, m
4'	4.14, m
5'a	4.26, d (12.2)
5'b	3.65, d (12.2)
1''	6.32, s
2''	4.71, s
3''	4.62, dd (9.4, 2.5)
4''	4.30, t (9.4)
5''	4.81, m
6''	1.74, d (6.3)

^{13}C NMR	δ(ppm)/ ^{1}H correlated in HMBC
1	83.53/2a, 2b, 6, 19, 1'
2	37.42/4b
3	68.27/1, 2b, 4a, 4b, 6
4	43.91/2a, 6
5	139.67/4a, 7a, 7b, 4b, 19
6	124.71/4a, 4b, 7a
7	32.05/6, 8
8	33.20/6, 7a, 7b, 14
9	50.47/7b, 8, 11a, 11b, 12a, 12b, 14
10	42.97/1, 2b, 4b, 6, 19
11	24.10/12a, 12b, 14
12	40.28/11a, 12a, 12b, 14, 15a, 17, 20
13	40.35/11a, 12a, 12b, 14, 15a, 17, 20
14	56.90/8, 12a, 15a, 18
15	32.43/14
16	81.51/15a, 15b
17	63.09/12b, 14, 15a, 20, 21
18	16.72/12a, 12b
19	15.09/1, 6
20	41.91/18, 21, 23
21	14.99/20
22	109.48/21, 23a, 26b
23	33.29/24a, 24b
24	29.01/23a, 26a, 27a, 27b
25	144.58/23a, 24a, 27b
26	65.03/27a, 27b
27	108.61/24a, 24b
1'	100.36/1, 2', 5'a, 5'b
2'	75.26/1', 1''
3'	75.86/2', 5'a, 5'b
4'	70.08/5'a, 5'b
5'	67.32/1'
1''	101.70/2'
2''	72.55
3''	72.69/2'', 4'', 5''
4''	74.28/3'', 5''
5''	69.45/1'', 4''
6''	19.02/4'', 5''

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