Taxane-Type Diterpenes from a New Caledonia endemic member of Taxaceae, Austrotaxus spicata Compton

Laurent Ettouati, Alain Ahond, Christiane Poupat, Pierre Potier

Taxane-Type Diterpenes from a New Caledonia endemic member of Taxaceae, *Austrotaxus spicata* Compton

Laurent Ettouati, Alain Ahond, Christiane Poupat and Pierre Potier†

ABSTRACT

Austrotaxus spicata Compton is an uncommon austral Taxaceae native to New Caledonia. The study of its leaves and trunk bark has allowed the isolation of 31 taxanes. Most of them are alkaloids owing to the presence of a dimethylaminophenylpropionic acid side-chain. Contrary to the *Taxus* species, *Austrotaxus* has shown the presence of alkaloids in the trunk bark, but no oxetane compounds were found.

THE TAXACEAE FAMILY

Review— In the Old World, the European yew tree (*Taxus baccata* L.) has been known for a long time, due mainly to the toxicity of its foliage especially for cattle and sometimes for human beings. Therefore, it has been the subject of early phytochemical studies (Lythgoe 1968, Miller 1980). From the European yew tree, a member of the Taxaceae family, nitrogenous taxane diterpenes have been isolated, *e.g.* taxines A **1** (Graf *et al.* 1986) and B **2** (Graf *et al.* 1982, Ettouati *et al.* 1991a) and, in particular from the Pacific yew tree, *Taxus brevifolia* Nutt., a well-known antitumoral compound, taxol **3** (Wani *et al.* 1971, Kingston 1991). All these compounds share the same diterpene skeleton called taxane **4**. However, in addition to *Taxus*, several less common genera belong to the Taxaceae family *i.e.* *Amentotaxus* Pilger, *Pseudotaxus* Cheng, *Nothotaxus* Florin, *Torreya* Arnold and finally, *Austrotaxus* Compton (Cope 1998).

Biology of *Austrotaxus spicata*— In contrast to the first four genera mentioned above which are mainly septentrional, the last one *Austrotaxus* thrives only in the South hemisphere on the island of New Caledonia. *Austrotaxus spicata* Compton (De Laubenfelds 1972), the only species of the genus, is an uncommon tree of 3 to 25 meters high with a fibrous red-brown bark and linear leaves.This species grows in the shady forest of the septentrional part of New Caledonia to a height of 500 to 1350 meters.

Laurent Ettouati is Assistant Professor, Université Claude Bernard Lyon I, Institut des Sciences Pharmaceutiques et Biologiques, Laboratoire de Chimie Thérapeutique, 8 avenue Rockefeller, 69873 Lyon cedex 08, France.

Alain Ahond and Christiane Poupat are Research Directors, Institut de Chimie des Substances Naturelles du CNRS, 91198 Gif-sur-Yvette Cedex, France.

†Prof. Pierre Potier, Research Director, has been Director of the "Institut de Chimie des Substances Naturelles du CNRS" until 2000.

Conclusion— So far, only *Taxus*, *Torreya* (Li *et al*. 2003) and *Amentotaxus* (Chen *et al.* 2006) had been the subject of phytochemical works, but taxanes had only been isolated from yew trees. Therefore, the close relationship of *Austrotaxus spicata* with yew tree species prompted us to study this plant.

PHYTOCHEMICAL STUDY OF *AUSTROTAXUS* (Ettouati *et al.*, 1988, 1989, 1991b)

Methods— Preliminary tests had shown the presence of alkaloids in *Austrotaxus* leaves and trunk bark. Accordingly, dried powdered samples of trunk bark and leaves were extracted by dichloromethane after basification (aqueous ammonia). After an acid-base extraction, the crude alkaloidal extract was then separated by a combination of chromatography on silica gel column, preparative TLC, and the structure of the isolated compounds determined by classical analytical methods, mainly from ^{1}H and ^{13}C NMR data.

Anemonin— Besides taxanes, anemonin **5**, a non-alkaloid compound derived from ranunculin, a glycoside mainly known in Renonculaceae (Ruijgrok 1966, Bai *et al*. 1996), has been found in very large amounts (nine percent of the crude basic extract) in the leaves. This presence is an outstanding example of biochemical parallelism.

Classification and biogenetic background— Overall, 31 taxanes have been isolated from *Austrotaxus* leaves and trunk bark. The majority are alkaloids, owing to the presence of a 3-dimethylamino 3-phenylpropionic acid ester **6**, also called Winstertein acid, as in the major alkaloid of the European yew tree, taxine B **2**. The instability of the yew leaves alkaloidal mixture has been well known for a long time, due to the desamination of this side-chain **6** in basic medium to give cinnamoylated compound (Lythgoe 1968). However, if the presence of alkaloids in yew tree has been known for a century, it is only in 1982 that Graf *et al*. established the structure of taxine A **2** and shortly thereafter that of taxine B **2** (Graf *et al*. 1982, Graf *et al*. 1986, see also Ettouati *et al*. 1991a). The same instability has been noticed for several *Austrotaxus* alkaloids bearing the same Winstertein acid side-chain **6** (*e.g*. 2'-desacetoxyaustrospicatine **12**). In 1987, Guéritte-Voegelein, Guénard and Potier proposed a biochemical classification for the known taxanes based on the taxane skeleton substitution at the 4 and/or 5 position : group A with an exomethylene in 4 position, group B with an epoxide in 4 position, group C with an oxetane in 4,5 position (Guéritte-Voegelein *et al*. 1987). Thus, only taxanes bearing an epoxide or an exomethylene have been isolated from *Austrotaxus*. A preliminary study looking for the presence of taxol in leaves and trunk bark was not conclusive (Colin 1986).

Taxanes from *Austrotaxus* leaves— 19 taxanes have been isolated from the leaves of *Austrotaxus spicata*; 16 are alkaloids and new (Tables 1, 2 and 3). Their alkaloidal nature is due to the presence of a Winstertein acid ester **6,** hydroxylated or acetoxylated in 2' position. The majority of those taxanes, such as austrospicatine **8**, the major isolated compound (3.5 percent of the crude alkaloids), belong to the group A of the classification of Guéritte-

Voegelein *et al*.. Another example of this group, austrotaxine **20**, as four related compounds, has in addition a ketone in 10 position and one acetoxyle in 14 position. Finally, two taxanes with an epoxide β in 4, spicataxine **28** and nicaustrine **33** bearing a additional nicotinoyl substituent in the 9 position, have also been extracted from the leaves. It can be stressed that it is the first time that nicotinic ester taxanes have been isolated.

Taxanes from *Austrotaxus* trunk bark— Overall, 16 taxanes have been found in the trunk bark of *Austrotaxus*; 12 of them are alkaloidal due to the same Winstertein acid side-chain, as those extracted from the leaves, and belong to groups A and B of the classification of Guéritte-Voegelein *et al*.. Spicaledonine **25** is unique among those taxanes, in that it contains an intramolecular bond between carbons 3 and 11, as austrocaledonine **26** isolated from the leaves.

Comparative distribution of taxanes between yew trees and *Austrotaxus*— 5-decinnamoyl-taxinine J **17** and comptonine **19** are the only taxanes shared by yew tree species (Kingston *et al*. 1982, Shi and Kiyota 2005) and *Austrotaxus*. But one could notice that all taxanes from *Austrotaxus* have the same skeleton substitutions in the 4 position that taxanes isolated from yew tree species. More important is the difference in taxane distribution throughout the plant. Indeed, contrary to *Austrotaxus* no alkaloids have been isolated so far from the trunk bark of yew tree species even if alkaloids have been found in stem barks of several *Taxus* species (Baloglu and Kingston 1999). Conversely, *Austrotaxus* has not been observed to contain oxetane compounds. For the yew tree species, a transformation of the alkaloids into taxol-type compounds in the leaves, following the biogenetic pathway of Guéritte-Voegelein *et al*., may take place with subsequent carrying of these secondary metabolites to the trunk bark. Indeed, the content of taxol **3** and related compounds in the leaves is strikingly low compared to the non-esterified 10-desacetylbaccatine III (Chauvière *et al*. 1981). This might be a detoxification process, oxetane-type taxanes with a phenylisoserine side-chain such as taxol **3** being very powerful antimitotic components.

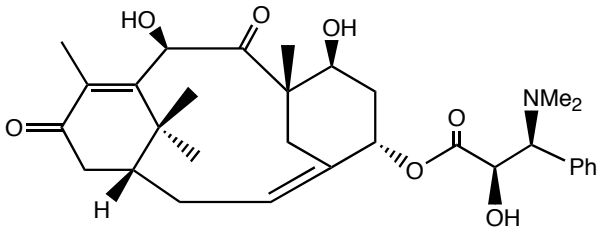
CONCLUSION

The isolation of taxanes from *Austrotaxus* confirms the close botanic relationship between this one and yew tree species in spite of a remote austral habitat. Recent phylogenetic analyses have corroborated these observations (Cheng *et al*. 2000). However, the structures of *Austrotaxus* taxanes are limited to non-oxetanic compounds, mainly alkaloidal ones. *Austrotaxus* does not seem an interesting source of taxanes for possible use in hemisynthesis of taxol, due to the limited habitat of this species, contrary to the European yew tree (Ettouati *et al*. 1991c, Poujol *et al*. 1997).

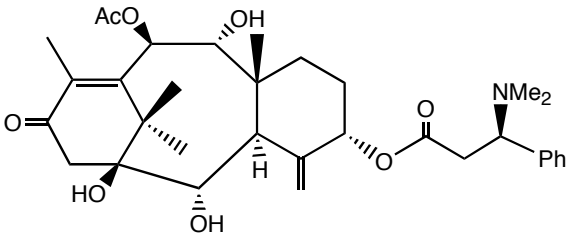
REFERENCES

- Bai, Y.; Benn, M.H.; Majak, W.; McDiarmid, R. 1996. Extraction and HPLC Determination of Ranunculin in Species of the Buttercup Family. Journal of Agricultural and Food Chemistry. **44**(8): 2235-2238.
- Baloglu, E.; Kingston, D.G.I. 1999. The Taxane Diterpenoids. Journal of Natural Products. **62**(10): 1448-1472.
- Blechert, S.; Guénard, D. 1990. *Taxus* Alkaloids. In: Brossi A., ed. The alkaloids. Vol. 39. Chap. 6. San Diego. Academic Press: 195-238.
- Chauvière, G.; Guénard D.; Picot, F.; Sénilh, V.; Potier, P. 1981. Analyse structurale et étude biochimique de produits isolés de l'if: *Taxus baccata* L. (Taxacées). Comptes rendus des Séances de l'Académie des Sciences. **293**, série II, (7): 501-503.
- Chen, H.-L.; Wang, L.-W.; Su, H.J.; Wei, B.-L.; Yang, S.Z.; Lin, C.N. 2006. New Terpenoids of *Amentotaxus formosana*. Organic Letters. **8**(4): 753-756.
- Cheng, Y; Nicholson, R.G.; Tripp, K.; Chaw, S.-M. 2000. Phylogeny of Taxaceae and Cephalotaxaceae Genera Inferred from Chloroplast *mat*K Gene and Nuclear rDNA ITS Region. Molecular Phylogenetics and Evolution. **14**(3): 353-365
- Colin, M. 1986. Personal communication. Institut de Chimie des Substances Naturelles, C.N.R.S., 91198, Gif-sur-Yvette cedex, France.
- Cope, E.A. 1998. Taxaceae : The Genera and Cultivated Species. The Botanical Reviews, **64**(4): 291-322.
- De Laubenfelds D.J. 1972. Flore de la Nouvelle-Calédonie et dépendances. 4-Gymnospermes. Paris: Muséum National d'Histoire Naturelle: 11-14.
- Ettouati, L.; Ahond, A.; Convert, O.; Laurent, D.; Poupat, C.; Potier, P. 1988. Plantes de Nouvelle-Calédonie. 114. Taxanes isolés des feuilles d'*Austrotaxus spicata* Compton (Taxacées). Bulletin de la Société Chimique de France. série II. (4): 749-755.
- Ettouati, L.; Ahond, A.; Convert, O.; Poupat, C.; Potier, P. 1989. Plantes de Nouvelle Calédonie. 124. Taxanes isolés des écorces de tronc d'*Austrotaxus spicata* Compton (Taxacées). Bulletin de la Société Chimique de France. série II. (5): 687-694.
- Ettouati, L.; Ahond, A.; Poupat, C.; Potier, P. 1991a. Révision structurale de la taxine B, alcaloïde majoritaire des feuilles de l'if d'Europe, *Taxus baccata* L. Journal of Natural Products. **54**(5): 1455-1458.
- Ettouati, L.; Ahond, A.; Poupat, C.; Potier, P. 1991b. L'austrocalédonine, nouvel alcaloïde extrait d'un conifère endémique de Nouvelle-Calédonie, *Austrotaxus spicata*. [Abstract] in: Third Pacific-Asia Symposium on biologically active natural products, Nouméa, New Caledonia; 1991 august 26-30: 45.

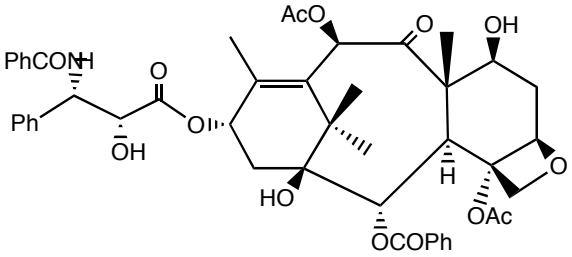
- Ettouati, L.; Ahond, A.; Poupat, C.; Potier, P. 1991c. Première Hémisynthèse d'un composé de Type Taxane porteur d'un Groupement Oxétane en 4(20), 5. Tetrahedron. **47**(47): 9823-9838.
- Graf, E.; Kirfell, A.; Wolff, G.J.; Breitmaier, E. 1982. Die Aufklärung von Taxin A aus *Taxus baccata* L. Liebigs Annalen der Chemie. (2): 376-381.
- Graf, E.; Weinandy, S.; Koch, B.; Breitmaier, E. 1986. ^{13}C-NMR-Untersuchung von Taxin B aus *Taxus baccata* L. Liebigs Annalen der Chemie. (7): 1147-1151.
- Guéritte-Voegelein, F.; Guénard, D.; Potier, P. 1987. Taxol and derivatives: a biogenetic hypothesis. Journal of Natural Products. **50**(1): 9-18.
- Kingston, D.G.I.; Hawkins, D.R.; Ovington L. 1982. New Taxanes From *Taxus brevifolia*. Journal of Natural Products. **45**(4): 466-470.
- Kingston, D.G.I. 1991. The Chemistry of Taxol. Pharmacology and Therapeutics. **52**(1): 1-34.
- Li, S.-H; Zhang, H.-J.; Nui, X.M.; Yao, P.; Sun, H.-D.; Fong, H.H.S. 2003. Chemical Constituents From *Amentotaxus yunnanensis* and *Torreya yunnanensis*. Journal of Natural Products. **66**(1): 1002-1005.
- Lythgoe, B. 1968. The *Taxus* alkaloids. In: Manske R.H.F., ed. The alkaloids. Vol. 10. Chap. 14. New York: Academic Press: 597-626.
- Miller, R.W. 1980. A Brief Survey of *Taxus* Alkaloids and other Taxane Derivatives. Journal of Natural Products. **43**(4): 425-437.
- Poujol, H; Ahond, A.; Al Mourabit, A.; Chiaroni, A.; Poupat, C.; Riche, C.; Potier, P. 1997. Taxoïdes: Nouveaux analogues du 7-déshydroxydocétaxel préparés à partir des alcaloïdes de l'If. Tetrahedron. **53**(14): 5169-5184.
- Ruijgrok, H.W.L. 1966. Distribution of ranunculine and cyanogenetic compounds in the Renunculaceae. In: Swain T. ed. Comparative Phytochemistry. London: Academic Press : 175-186.
- Shi, Q.-W.; Kiyota, H. 2005. New Natural Taxane Diterpenoids from *Taxus* Species since 1999. Chemistry and Biodiversity. **2**: 1597-1623.
- Wani, M.C.; Taylor, H.L.; Wall, M.E.; Coggon, P.; Mc Phail, A.T. 1971. Plant Antitumor Agents. VI. The Isolation and Structure of Taxol, a Novel Antileukemic and Antitumor Agent from *Taxus brevifolia*. Journal of the American Chemical Society. **93**(9): 2325-2327.



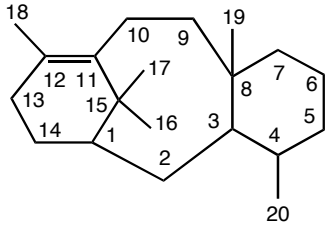
taxine A **1**



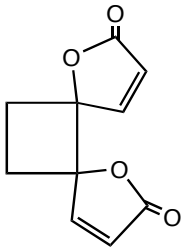
taxine B **2**



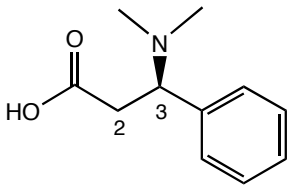
taxol **3**



4

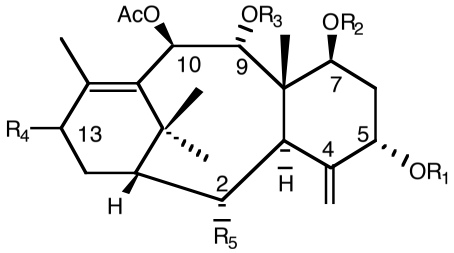


anémonine **5**



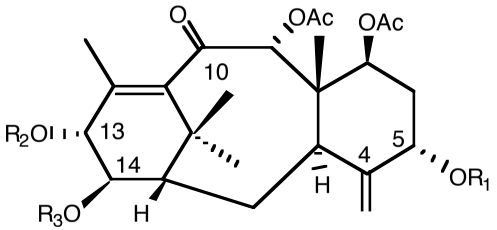
6

Table 1

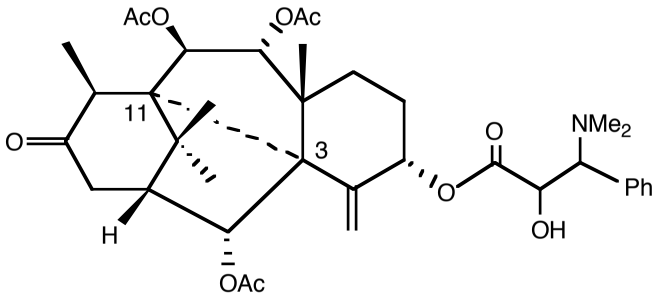


	R1	R2	R3	R4	R5	distribution
2'-desacetylaustrospicatine **7**	-CO-CHOH-CH(NMe_2)Ph	Ac	Ac	OAc(α)	H	leaves, bark
austrospicatine **8**	-CO-CHOAc-CH(NMe_2)Ph	Ac	Ac	OAc(α)	H	leaves
7β-desacetylaustrospicatine **9**	-CO-CHOAc-CH(NMe_2)Ph	H	Ac	OAc(α)	H	leaves
7β,9α-bisdesacetylaustrospicatine **10**	-CO-CHOAc-CH(NMe_2)Ph	H	H	OAc(α)	H	leaves
2',7β,9α-trisdesacetylaustrospicatine **11**	-CO-CHOH-CH(NMe_2)Ph	H	H	OAc(α)	H	leaves
2'-desacetoxyaustrospicatine **12**	-CO-CH_2-CH(NMe_2)Ph	Ac	Ac	OAc(α)	H	leaves, bark
2α-acetoxyaustrospicatine **13**	-CO-CHOAc-CH(NMe_2)Ph	Ac	Ac	OAc(α)	OAc	leaves
2α-acetoxy-2'-desacetylaustrospicatine **14**	-CO-CHOH-CH(NMe_2)Ph	Ac	Ac	OAc(α)	OAc	leaves
2α-hydroxy-2'-desacetoxyaustrospicatine **15**	-CO-CH_2-CH(NMe_2)Ph	Ac	Ac	OAc(α)	OH	leaves
2α-desacetyl-5α-decinnamoyltaxinine J **16**	H	Ac	Ac	OAc(α)	OH	leaves
5α-decinnamoyltaxinine J **17**	H	Ac	Ac	OAc(α)	OAc	leaves
2α,5α,13α-trihydroxy-7β,9α,10β-triacetoxy-taxa-4(20),11-diene **18**	Ac	Ac	Ac	OAc(α)	OH	leaves
comptonine **19**	-CO-CHOH-CH(NMe_2)Ph	Ac	Ac	=O	H	bark

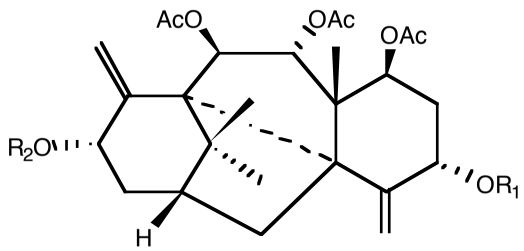
Table 2



	R1	R2	R3	distribution
austrotaxine **20**	-CO-CHOAc-CH(NMe_2)Ph	Ac	Ac	leaves
2'-desacetylaustrotaxine **21**	-CO-CHOH-CH(NMe_2)Ph	Ac	Ac	leaves
2'-desacetoxyaustrotaxine **22**	-CO-CH_2-CH(NMe_2)Ph	Ac	Ac	leaves
2',13α,14β-desacetylaustrotaxine **23**	-CO-CHOH-CH(NMe_2)Ph	H	H	bark
5α,13α,14β-trihydroxy-7β,9α-diacetoxy-10-oxo-taxa-4(20),11-diene **24**	H	H	H	bark

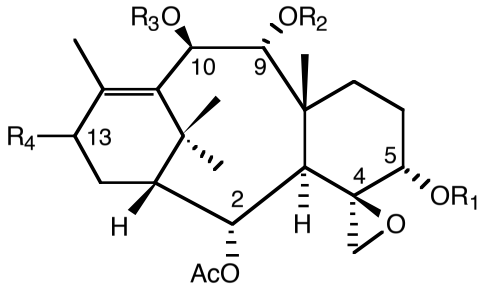


spicaledonine **25**

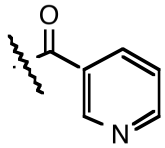


austrocaledonine **26**
R_1 = -CO-CHOH-CH(NMe_2)Ph
R_2 = Ac
compound **27**
$R_1 = R_2$ = H

Table 3



nicotinyl :



	R_1	R_2	R_3	R_4	distribution
spicataxine **28**	-CO-CH_2-CH(NMe_2)Ph	H	Ac	OAc(α)	leaves, bark
9α-acetyl-10β-desacetylspicataxine **29**	-CO-CH_2-CH(NMe_2)Ph	Ac	H	OAc(α)	bark
spicatine **30**	-CO-CH=CH-Ph	H	Ac	OAc(α)	bark
9α-acetyl-10β-desacetylspicatine **31**	-CO-CH=CH-Ph	Ac	H	OAc(α)	bark
10β-desacetylspicatine **32**	-CO-CH=CH-Ph	H	H	OAc(α)	bark
nicaustrine **33**	-CO-CH_2-CH(NMe_2)Ph	Nicotinyl	Ac	OAc(α)	leaves, bark
N-demethylnicaustrine **34**	-CO-CH_2-CH(NHMe)Ph	Nicotinyl	Ac	OAc(α)	bark
nicotaxine **35**	H	Nicotinyl	Ac	=O	bark
5α-*O*-(3'-methylamino-3'-phenylpropionyl)-nicotaxine **36**	-CO-CH_2-CH(NHMe)Ph	Nicotinyl	Ac	=O	bark
5α-*O*-(3'-amino-3'-phenylpropionyl)-nicotaxine **37**	-CO-CH_2-CH(NH_2)Ph	Nicotinyl	Ac	=O	bark