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OCCURRENCE OF 2,6-DIMETHOXY CINNAMALDEHYDE IN *TAXUS FLORIDANA* AND STRUCTURAL REVISION OF TAXIFLORINE TO TAXCHININ M

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Key Word Index—*Taxus floridana*; Taxaceae; taxiflorine (taxchinin M); 2,6-dimethoxy cinnamaldehyde; taxchinin L; 10-deacetyl paclitaxel-7-xyloside.

Abstract—Taxiflorine, originally isolated from the needles of *Taxus floridana* and described previously, has its structure revised to that of taxchinin M. Four other known taxanes also isolated were: 1-deoxy baccatin IV, 1-hydroxy baccatin 1, 10-deacetyl paclitaxel-7-xyloside and 13-deacetyl taxiflorine (taxchinin L), together with: trans-2,6-dimethoxy cinnamaldehyde, rhododendrol, ponasterone A and α-conidendrin. © 1998 Elsevier Science Ltd. All rights reserved

The isolation of a taxane from *T. floridana*, and assignment of its structure as taxiflorine **1a** was reported in 1996 [1]. In a re-isolation of **1a**, we found that the NMR spectrum of acetyl taxiflorine **2** did not match with that of the 13-acetyl-13-decinnamoyl taxchinin B [2], although the two must be identical. This brought into question whether the structure is **1a** or **1b**. The spectral data of **2** were reanalyzed using the long range HETCOR (HMBC) spectrum (Table 1A) to obtain an unambiguous assignment.

The C═O signal at 166.2, assignable to C_6H_5CO- based on the cross peak with the ^{1}H-signal at δ 7.92 (ArH-2,6), also showed interaction with that at δ 6.32 (H-9, on the basis of COSY and HETCOR), thereby locating the benzoate at C-9, and pointing to **1b**. One of the five acetate signals in **1**, (δ 170.1) showed a cross peak with the ^{1}H-signal at δ 5.61 assigned to H-13. The other four acetates remain at 2α, 4α, 7β, and 10β.

Additionally, the ^{1}H-signal at δ 1.87 interacted with the signals at δ 135.8 (C-11), δ 146.8 (C-12), and δ 78.4 (C-13), whereas the ^{1}H-signal at δ 1.77 interacted with those at δ 70.2 (C-7) and δ 77.2 (C-9). This permitted an unambiguous and revised assignment of the signal at δ 1.87 to the C-18-methyl, and that at δ 1.77 to the C-19-methyl, and leaving the signals at δ 1.65, 1.82, 2.03 and 2.13 × 2 to the five acetyl functions.

To confirm the structure **1b**, the compound was also oxidized with Jones reagent to the ketone **3**, and its spectra compared with those of some known C-9 and C-10 keto taxanes.

The uv spectrum of **3** (λ_{max} at 232 nm with a shoulder at 253 nm) was consistent with that of a C═C—C═O system [3] and differed from that of 9-keto-taxanes containing a benzoate ester such as baccatin III. The ^{13}C keto-carbonyl signal of **3** (δ 192.2 assigned to C-10), is also consistent with a C═C—C═O system, and in contrast to the δ 199–204 signal shown by 9-keto-taxanes. Similarly, the C-12 signal is at 156.8 ppm in **3** instead of 147.0 ppm, again showing a C═C—C═O function, and supporting the structure **1b** [4]. A taxane with this structure has recently been reported from *T. chinensis* and was named taxchinin M [5].

Four other crystalline taxanes, isolated for the first time from the extract of *T. floridana*, were identified as the known 1-deoxy-baccatin IV **4** (structures not shown) [6], 1-hydroxy-baccatin 1 **5** [7], 10-deacetyl paclitaxel-7-xyloside **6** [8] and 13-deacetyl taxiflorine **7**. This last one was found to be the same as taxchinin L [5].

Although **6** is present in the bark of *T. brevifolia* to the extent of >0.1% [8], and in the bark of *T. baccata* to a lesser extent [9], its presence in the needles of *T. floridana* (0.003%) is significant, because this adds further to the value of *T. floridana*, already shown as a good source of both paclitaxel, and 10-deacetyl baccatin III [1].

Of the four non-taxane compounds isolated from the extract of *T. floridana*, three were identified as rhododendrol **8**, (previously from the needles of *T. brevifolia* [10] and *Betula pendula* [11]) ponasterone A

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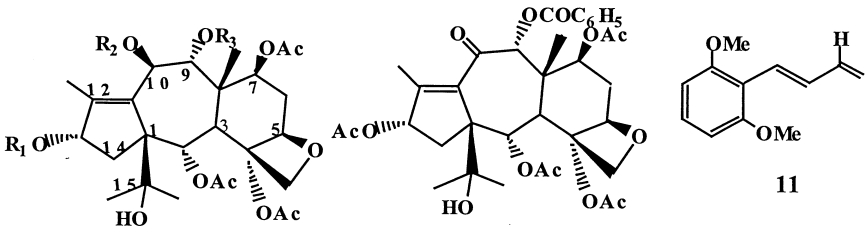

1a: R 1 = Ac, R 2 = C 6 H5 CO, R 3 = H
1b: R 1 = Ac, R 2 = H, R 3 = C 6 H5 CO
2: R 1 = R 2 = Ac, R 3 = C 6 H5 CO
7: R 1 = R 2 =H, R 3 = C 6 H5 CO

Fig. 1. Some taxane and non-taxane components of *Taxus floridana*.

Table 1. A: HMBC Correlations of Compound **2B**: ^{1}H and ^{13}C Spectra of Compound **3**

	^{1}H	^{13}C	HMBC	#	^{1}H-ppm	^{13}C-ppm
1	***	67.3	Me-16, Me-17	1	***	65.5
2	6.16, *d* 7.5 Hz	67.8	H-3	2	6.22, *d* 7.5 Hz	68.7
3	3.00, *d* 7.5 Hz	43.7	H-5	3	3.12, *d* 7.8 Hz	44.1
4	***	78.6	H-5	4	***	79.0
5	4.96, *d* 7.2 Hz	84.6	H-20α	5	5.00, *d* 6.0 Hz	84.9
6α	2.67, *m*	34.5	***	6α	2.74, *m*	34.3
6*β*	1.77, *m*	***	***	6*β*	1.84, *m*	***
7	5.54, *t* 7.8 Hz	70.2	H.5, Me-19	7	5.16, *t* 7.5 Hz	71.0
8	***	43.5	H-2, H-3	8	***	44.5
9	6.32, *d* 10.8 Hz	77.2	H-10, Me-19	9	6.32	83.6
10	6.43, *d* 10.5 Hz	67.6	H-9	10	***	192.2
11	***	135.8	Me-18	11	***	137.5
12	***	146.8	Me-18	12	***	156.8
13	5.61, *t* 6.9 Hz	78.4	H-14α, Me-18	13	5.72, *t*	78.9
14α	1.68, *m*	36.6	H-2	14α	1.76, *dd* 8.1, 14.7 Hz	37.1
14*β*	2.26, *m*	***	***	14*β*	2.40, *dd* 7.2, 14.4 Hz	***
15	***	75.2	Me-16, Me-17	15	***	76.3
16	1.17, *s*	27.3	Me-17	16	1.22, *s*	25.5
17	1.20, *s*	24.9	Me-16	17	1.18, *s*	27.4
18	1.87, *s*	11.5	***	18	2.08, *s*	13.8
19	1.77, *s*	13.0	***	19	1.91, *s*	13.6
20α	4.49, *d* 7.2 Hz	129.5	H-3	20α	4.56, *d* 7.2 Hz	74.7
20*β*	4.41, *d* 7.2 Hz	***	***	20*β*	4.45, *d* 7.2 Hz	***
Bz-1	***	129.3	***	Bz-1	***	129.8[#]
Bn-2,6	7.92, *d* 7.2 Hz	129.5	H-para	Bz-2,6	8.07, *d* 7.2 Hz	129.8
Bz-3,5	7.43, *t* 7.8 Hz	128.2	***	Bz-3,5	7.45, *t* 8.1 Hz	133.2
Bz-4	7.56, *t* 7.5 Hz	133.1	H-ortho	Bz-4	7.58, *t* 7.2 Hz	133.2
C═O	***	166.2	H-9, H-ortho	CO–Ac	***	470.4, 170.3, 169.6
C═O	***	167.8	Ac(Me)-1.65	CO–Bz	***	166.8
C═O	***	168.9	Ac(Me)-2.03			
C═O	***	169.7	Ac(Me)-1.82			
C═O	***	170.1	Ac(Me)-2.03, H-13			
C═O	***	170.3	Ac(Me)-2.13			

[#] Did not appear in the APT spectrum.

9 [(12], also from the bark of *T. brevifolia* [13]), and α-conidendrin, (earlier from *Taxus wallichiana* [14] and others (15)).

The spectral properties of the fourth compound showed that it was trans 2,6-dimethoxy cinnamaldehyde **11**, and this was confirmed by synthesis.

Although **11** is listed in the Chemical Abstracts with an accession number, and cited in two references [16, 17], a close examination of these two references failed to show this compound. Since neither its isolation nor its synthesis has been described so far, **11** is a novel natural product. Furthermore, no other 2,6-oxygenated (hydroxy, methoxy, or hydroxy-methoxy) cinnamyl or cinnamoyl entities have been isolated, either as the alcohol, the acid or the ester. Only 2,6-dimethoxy cinnamic acid [18–21] and its methyl and ethyl esters [22] are known as synthetic products. Thus **11** represents the first such example of a naturally occurring 2,6-oxygenated cinnamoyl moiety. In general, the most common naturally occurring examples of 1:3-dioxy benzenoid compounds contain a carbon substituent at the 4-position (e.g. resacetophenone, umbelliferone) or at the 5-position (e.g. orcinol, resveratrol). The biogenetically most favored cinnamic acids, contain the 3,4-dioxy substitution (e.g. caffeic acid).

EXPERIMENTAL

^1H and ^{13}C NMR, COSY and the HETCOR spectra: Varian VXR-300 and Varian Gemini-300 spectrometers. FAB mass spectra: Finnigan Mat 950 Q spectrometer. IR spectra: Perkin-Elmer 1420 ratio recording spectrophotometer. UV spectra: Perkin-Elmer Lambda 3B spectrophotometer. Mps (uncorr): Fisher apparatus. TLC: silica gel, 60 HF_{254} (E. Merck and Aldrich) with MeOH–Me_2CO–CH_2Cl_2 (1:4:15) or EtOAc/Ligroin (2:5) as solvents, visualization by UV (254 nm) and charring with dilute H_2SO_4 spray. Column chromatography: Silica gel, 100–200 mesh (Aldrich).

Fractionation of the extract of T. floridana

Fresh needles and small twigs of *T. floridana*, collected from the campus of the University of Florida, (2 kg) were extracted and processed by reversed phase column chromatography using C-18 bonded silica and acetonitrile/water as described earlier [1]. Those fractions from the 25–40% acetonitrile/water were freed from 10-deacetyl baccatin III and concentrated to a syrupy solid (10 g).

On standing, this became a crystalline semi-solid (mostly rhododendrol **8**), and 3 g of this was chromatographed on a normal phase silica column (40 g) in dichloromethane. The column was eluted successively with dichloromethane containing 2, 5 and 10% acetone, and then with the addition of 2, 5 and 10% methanol.

The following compounds were obtained in succession, with the yields being calculated from the fresh needles: (2% acetone), 1-β-hydroxy baccatin 1 **5** (0.01%) [7], and taxchinin M (previously taxiflorine) **1b** (0.006%) [1]; (5% acetone), rhododendrol **8**, (0.05%) [10] and taxchinin L **7** (0.01%) [5], (10% acetone) 10-deacetyl baccatin III, (2% methanol), (ponasterone A **9** (0.006%) [12] and (5–10% methanol), 10-deacetyl paclitaxel-7-xyloside **6** (0.003%) [8].

The initial dichloromethane eluate (0.25 g) was applied to another silica column (3 g) using 25% ethyl acetate/ligroin. This gave the trans 2,6-dimethoxy cinnamaldehyde **11** (0.002%). Elution continued with 50% ethyl acetate/ligroin, which gave α-conidendrin **10** (0.005%) [14], followed by 1-deoxy baccatin IV **4** (0.003%) [6].

Taxchinin M (*Taxiflorine*) *M* **1b**. This was obtained as a crystalline solid (acetone/ligroin), m.p. 254–255°C, $[\alpha]_D$, −26.1°.

Acetyl taxiflorine **2**. Acetylation of **1b** with acetic anhydride/pyridine gave **2** as a glassy solid [1]. The HMBC spectrum is shown in Table 1.

Oxidation of **1b** *to* **3**. To **1b** (0.1 g) in acetone (2 ml) was added Jones reagent. After 30 min, water was added and the product recovered by extraction with dichloromethane. It was crystallized from ether/ligroin, m.p. 201–204°C, yield, 0.055 g; uv, λ_{max}, 232 and 253; ir (KBr pellet), 3520, 2980-2920, 1750-1720, 1430, 1365, 1235, 1020 and 705 cm^{-1}. Anal. Calc. for $C_{35}H_{42}O_{13}$: C, 62.68; H, 6.31. Fd. C, 62.37; H, 6.58.

13-Deacetyl taxiflorine (taxchinin L) **7**. This was obtained as a crystalline solid from ether/ligroin, yield, 0.18 g, 0.01%, m.p. 248–250°C. The spectral data agreed with those given for taxchinin L [5].

Trans 2,6-dimethoxy cinnamaldehyde (**11**). Concentration of the appropriate fractions gave **11** as a glass, yield, 0.02 g; λ_{max} 313 nm; IR (KBr): 3100, 2940-3000, 2810, 2740-2700, 1660, 1605-1585, 1475, 1260, 1140, 1100-1080, 970, 840, 725 cm^{-1}., ^1H NMR, δ 3.90, *s*, 6H, (OMe); 6.58, *d*, $J = 8.4$ Hz, 2H, (Ar-3, 5H), 7.17, *dd*, $J = 7.8$, 16.0 Hz, 1H, (H-1); 7.33, *t*, $J = 8.4$ Hz, 2H, (Ar-4H); 7.93, *d*, $J = 16.0$ Hz, 1H, (H-3); 9.64, *d*, $J = 8.1$ Hz, 2H, (H-1). ^{13}C NMR, δ 55.8×2, (OMe); 103.6, (Ar-3, 5); 112.1 (Ar-1); 131.6, (C-2); 132.6, (Ar-4); 144.5, (C-3); 160.5, (Ar-2,6); 196.4, (C-1); MS, 192 (30%), 161 (100%), 149 (17%), 91 (15%). The IR and NMR spectral data agreed with those for the synthetic aldehyde prepared as shown below.

Trans 2,6-Dimethoxy cinnamic acid **12**. This was prepared by heating 2,6-dimethoxy benzaldehyde (1.2 g, Aldrich), malonic acid (1.4 g, 2 eq.) in pyridine (14 ml) and piperidine (0.2 ml) under reflux for 20 h. The product was crystallized from ether/ligroin, yield 1.84 g (84%), m.p. 144–146°C, (lit. (18) 141–143°C).

Methyl trans 2,6-dimethoxy cinnamate **13**. Compound **12** (1.25 g) was methylated in acetone with dimethyl sulfate (0.7 ml) and potassium carbonate (2 g). The solid was crystallized from ether/ligroin, to give the ester, yield, 1.25 g (92%), m.p. 86–88°C.

Trans 2,6-Dimethoxy cinnamyl alcohol **14**. Compound **13** (1.25 g) in THF(20 ml) was reduced with lithium aluminum hydride (0.5 g). The product **14**, a colorless liquid (0.7 g, 64%), was directly used for the next step.

Trans 2,6-Dimethoxy cinnamaldehyde **11**. Oxidation of **14** (0.7 g) with chromic anhydride (4 g)

and pyridine (6.5 ml) in dichloromethane (10 ml) was carried out at R. T. for 20 h. The product **11** was recovered by acidification and extraction with dichloromethane and crystallized, yield, 0.22 g, (40%), m.p. 77–78°C. It was identical with the natural product described above. Anal. Calc. for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Fd. 68.52; H, 6.52.

REFERENCES

1. Rao, K. V., Bhakuni, R. S., Juchum, J. and Davies, R. M., *J. Liq. Chromat*., 1996, **19**, 427.
2. Das, B., Rao, S. P., Srinivas, K. V. N. S., Yadav, J. S. and Das. R., *Phytochem*., 1995, **38**, 671.
3. Silverstein, R. M., Bassler, G. C. and Morrill, T. C., *Spectrometric Identification of Organic Compounds*, 4th edn, John Wiley, 1981, p. 100.
4. Fuji, K., Tanaka, K. and Li, B., *J. Nat. Prod*., 1993, **56**, 1520.
5. Tanaka, K., Yokoi, T., Shingu, T., Li, B. and Sun, H., *Chem. Pharm. Bull*., 1996, **44**, 1770.
6. Miller, R. W., *J. Nat. Prod*., 1980, **43**, 425.
7. Della Casa de Marcano, D. P. and Halsall, T. G., *Chem. Commun*., 1970, **21**, 1381.
8. Rao, K. V., Hanuman, J. B., Alvarez, C., Stoy, M., Juchum, J., Davies, R. M. and Baxley, R., *Pharm. Res*., 1996, **12**, 1003.
9. Senilh, V., Blechert, F., Picot, F., Potier, P. and Varenne, P., *J. Nat. Prod*., 1984, **47**, 131.
10. Chu, A., Furlan, M., Davin, L. B., Zajicek, J., Towers, G. H. N., Soucy-Breau, C. M., Rettig, S. J., Croteau, R. and Lewis, G. N., *Phytochem*., 1994, **36**, 975.
11. Smite, E., Lundgren, L. N. and Andersson, R., *Phytochem*., 1993, **32**, 365.
12. Yakemoto, T., Ogawa, S., Nishimoto, N. and Hoffmeister, H., *Z. Naturforsch*., 1967, **22b**, 681.
13. Rao, K. V., Bhakuni, R. S., Hanuman, J. B., Davies, R. M. and Johnson, J., *Phytochemistry*, 1996., **41**, 863.
14. Miller, R. W., McLaughlin, J. L., Powell, R. G., Plattner, R. D., Weisleder, D. and Smith, C. R. Jr., *J. Nat. Prod*., 1982, **47**, 131.
15. Cambie, R. C., Pang, T. M., Parnell, J. C., Rodrigo, R. and Weston, J. R., *Aust. J. Chem*., 1979, **32**, 2741.
16. Boon, J. J., Stout, S. A., Geruit, W. and Spackman, W., *Acta Bot. Neerf*., 1989, **38**, 391.
17. Stout, S. A., Boon, J. J. and Spackman, W., *Geochemica et Cosmochimica Acta*, 1988, **52**, 405.
18. Terrain, D. L., Mohammad, T. and Morrison, H., *J. Org. Chem*., 1995, **60**, 1981.
19. Snook, M. E., Mason, P. F., Arrendale, R. F. and Chortyk, O. T., *J. Chromatography*, 1985, **324**, 141.
20. Shah, H. A. and Shah, R. C., *J. Chem. Soc*., 1938, 1832.
21. Limaye, D. B., *Proc. Ind. Acad. Sci*., 1934, **1**, 163.
22. Heindl, A., Rou, O. and Soiteller, G., *Biomed. Mass Spectrometry*, 1985, **12**, 59.