12,13-Isobaccatin III. Taxane Enol Esters (12,13-Isotaxanes)

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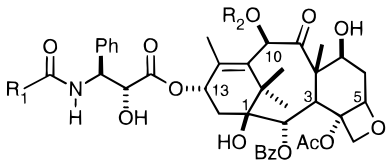
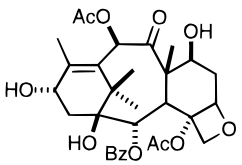
The diterpenoid nucleus of the antitumor agent taxol[1] (**1**) has shown a propensity for unusual and often unexpected chemical transformations.[2] Contraction of rings A and B,[3] oxetane opening,[3,4] epimerization at C-7,[5] and cyclopropane formation[6,7] are among the many fascinating transformations of the taxol molecule reported to date. We report another unexpected result observed during chemical modification of baccatin III (**2**) which has allowed us to develop a new series of taxol analogs having potent antitumor activity.

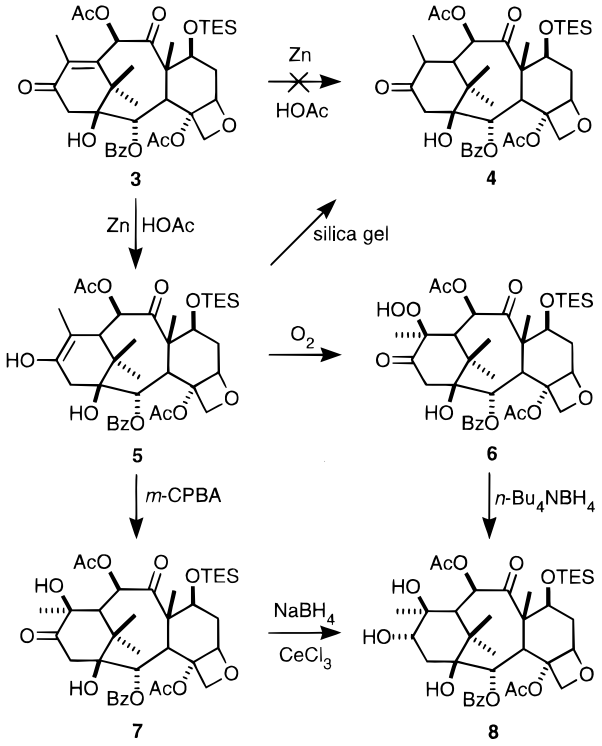


1, R_1 = Ph; R_2 = Ac (Taxol)
11, R_1 = Me_3CO; R_2 = H (Taxotere)
20, R_1 = Me_3CO; R_2 = Ac



2 (Baccatin III)

When attempting to reduce 13-ketobaccatin III-7-O-TES[8] (**3**) with zinc in acetic acid, we did not obtain the anticipated 11,12-dihydro-13-ketobaccatin III-7-O-TES (**4**).[9,10] Instead, following filtration and evaporation of the reaction solvent, we obtained a new compound, **5**, which was found by combustion analysis to be isomeric with **4**. The ^{13}C NMR spectrum of this new compound clearly lacked a signal for the C-13 carbonyl required for structure **4** and had new signals for olefinic carbons at δ 146.0 and 102.4. Further clues to the structure of this unknown compound were provided by its reactivity. Upon direct chromatography over silica gel, the compound was transformed into ketone **4** as deduced from spectral properties.



When shaken with aqueous acid and dichloromethane, the unknown was converted to a second new material, **6**, whose molecular weight was higher by 32 mass units than that calculated for ketone **4**. When allowed to react with *m*-chloroperbenzoic acid under a nitrogen atmosphere, the unknown was slowly converted in modest yield (14%) to a third new compound, **7**. Reduction of **6** with tetra-*n*-butylammonium borohydride gave a crystalline triol whose structure was shown by X-ray crystallography to be that of the 11,12-dihydro-12β-hydroxybaccatin III derivative **8**. The triol **8** also was obtained from **7** by reduction with sodium borohydride/cerium(III) chloride.[11]

We interpret the preceding observations in terms of an enol structure for **5** as follows. The ^{13}C olefinic signals given above for **5** are comparable to those reported for various simple enols, *e.g.*, 2-methylprop-1-en-1-ol[12] (δ 136.2 for C_α and 105.9 for C_β), propen-2-ol[13] (δ 156.8 for C_α and 95.3 for C_β), or 1-cyclohexenol[14] (δ 150.5 for C_α). Conversion of **5** to ketone **4** upon chromatography is consistent with the presence of an enol in **5**. The addition of 32 mass units during workup suggests the addition of molecular oxygen and the formation of a hydroperoxide (as in **6**), a reaction of enols having precedent in the literature.[15] Finally, the X-ray structure of the 1,12,13-triol **8**, obtained by reduction of the hydroperoxide in **6** to a hydroxyl group, clearly defines the configuration of the hydroperoxide as 12β and indicates that the olefinic bond of **5** must be between carbons 12 and 13. Together, these observations are consistent with assignment of an enolic structure to **5**, a compound to which we have given the name[16] 12,13-isobaccatin III-7-O-TES. This new example of a stable unconjugated enol

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(2) Cf.: *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. C., Ojima, I., Vyas, D. M., Eds.; ACS Symposium Series 583; American Chemical Society: Washington, DC, 1995.

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(7) Johnson, R. A.; Nidy, E. G.; Dobrowolski, P. J.; Gebhard, I.; Qualls, S. J.; Wicnienski, N. A.; Kelly, R. C. *Tetrahedron Lett.* **1994**, *35*, 7893.

(8) 13-Ketobaccatin III was first described in ref 1.

(9) Zinc reductions of 13-ketobaccatins, including 10-deacetyl-13-ketobaccatin III, under various conditions have been reported: Marder, R.; Dubois, J.; Guénard, D.; Guéritte-Voegelein, F.; Potier, P. *Tetrahedron* **1995**, *51*, 1985.

(10) 10-Deacetyl-11,12-dihydrobaccatin III-7-O-TES is described in ref 9, as well as in the following: Appendino, G.; Jakupovic, J.; Cravotto, G.; Enriù, R.; Varese, M.; Bombardelli, E. *Tetrahedron Lett.* **1995**, *36*, 3233. For the X-ray crystallographic structure, see: Chiaroni, A.; Riche, C.; Marder, R.; Dubois, J.; Guénard, D.; Guéritte-Voegelein, F. *Acta Crystallogr.* **1995**, *C51*, 2050.

(11) Luche, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 2226.

(12) Capon, B.; Siddhanta, A. K. *J. Org. Chem.* **1984**, *49*, 255.

(13) Sojka, S. A.; Poranski, C. F., Jr.; Moniz, W. B. *J. Magn. Reson.* **1976**, *23*, 417.

(14) Moniz, W. B.; Sojka, S. A.; Poranski, C. F., Jr.; Birkle, D. L. *J. Am. Chem. Soc.* **1978**, *100*, 7940.

(15) (a) Attenburrow, J.; Connett, J. E.; Graham, W.; Oughton, J. F.; Ritchie, A. C.; Wilkinson, P. A. *J. Chem. Soc.* **1961**, 4547. (b) Enslin, P. R. *Tetrahedron* **1971**, *27*, 1909.

(16) While we recognize that a more appropriate trivial nomenclature for this type of structure may be 11,12-dihydro-12,13-dehydrobaccatin III, we prefer and suggest the shortened nomenclature used in this manuscript.

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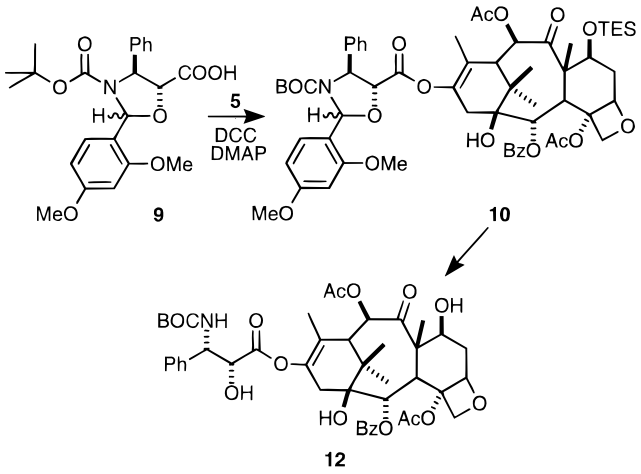
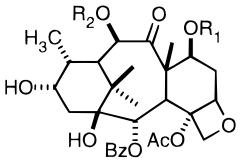
may be added to the small list of such enols previously reported in the literature.[17]

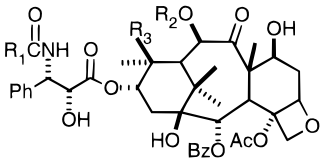
Enol **5** did not react with acetic anhydride but was successfully acylated with the taxotere[18] side chain precursor **9**[7,19] in the presence of dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine in CH_2Cl_2−toluene, giving **10** (89%, two diastereoisomers). We chose to acylate with the *tert*-butyloxycarbonyl (BOC) side chain of taxotere (**11**) rather than the benzamide side chain of taxol (**1**) because, in our experience,[7] and as reported by others,[2] taxotere side chain analogs are consistently more potent in antitumor assays when compared with the taxol analog. Removal of the protecting groups from



10 was achieved by stirring with 4:1 HOAc−H_2O at room temperature for 4 days and gave, after chromatographic purification, the new analog **12** (40%). Evidence that the enol ester has been formed is seen in the spectral data for **12**; for example, the ^{13}C NMR spectrum has signals at δ 143.3 and 121.9 comparable to signals at 144.2 and 124.3 reported[20] for the enol acetate of taxuspine D and are consistent with the enolic ester double bond. A signal at δ 2.76 in the ^1H NMR is characteristic for the C-11 proton in this compound. It is noteworthy that throughout these chemical manipulations, the enol ester functionality remained intact.

With the 11,12-dihydro intermediates **4** and **8** in hand, we were able to prepare 11,12-dihydrotaxol analogs. Reduction of 11,12-dihydro-13-ketobaccatin III-7-O-TES (**4**) with $NaBH_4$/$CeCl_3$ gave 11,12-dihydrobaccatin III-7-O-TES (**13**). The TES protecting group of **13** was removed with $Et_3N{\cdot}(HF)_3$, giving **14**. The 10-acetyl group of **14** was removed with hydrazine,[7,21] giving **15**, whose ^1H NMR spectrum matches the chemical shifts reported by Marder and co-workers[9] for this compound. The





13, R_1 = TES; R_2 = Ac
14, R_1 = H; R_2 = Ac
15, $R_1 = R_2$ = H

16, R_1 = Ph; R_2 = Ac; R_3 = H
18, $R_1 = OC(CH_3)_3$; R_2 = Ac; R_3 = H
19, $R_1 = OC(CH_3)_3$; R_2 = Ac; R_3 = OH

assignments of stereochemistry at C-12 and C-13 in **13**−**15** are thereby confirmed. Coupling of **13** with the taxol side chain precursor, (4*S*,5*R*)-*N*-(benzoyl)-2-(2,4-dimethoxyphenyl)-4-phenyl-5-oxazolidinecarboxylic acid, followed by removal of the protecting groups, gave 11,12-dihydrotaxol (**16**). The deprotection was attempted first in MeOH−HCl, which generated an ortho ester (**17**, structure not shown) with the C-1 and C-2 oxygen atoms. Hydrolysis of **17** with aqueous acid gave the desired **16**. Likewise, coupling of 11,12-dihydrobaccatin III-7-O-TES with BOC side chain precursor **9** and deprotection of the coupled product gave **18**. Finally, coupling of the 11,-12-dihydro-12β-hydroxybaccatin III derivative **8** with **9** and deprotection of the coupled product gave the 11,12-dihydro-12β-hydroxytaxotere analog **19**.

Pharmacological comparison of **12** and **19** with taxotere (**11**) and 10-acetyltaxotere (**20**) gave IC_{50} values of 4.6, 38, 4, and 3.5 nM, respectively, for inhibition of the mouse L1210 leukemic cell assay.[22] The IC_{50} for taxol analog **16** was >0.1 mM in this assay, while for taxol the IC_{50} is 17 nM. These *in vitro* assay results show that the potency of the isotaxol analog **12** is approximately the same as that of "normal" analogs **11** and **20**, whereas the potency of the 11,12-dihydro analogs **16** and **19** is reduced in comparison to the analogous 11,12-olefinic compounds.[23] The approximate equivalent potency of **12** versus that of taxotere (**11**) was confirmed by results from the A2780 human ovarian carcinoma cell growth inhibition assay[24] (**12** inhibits with an IC_{50} of 0.93 nM vs 1.8 ± 0.4 nM for **11**).

Supporting Information Available: Experimental procedures and analytical and spectroscopic data for **4**−**8**, **10**, **12**, **14**−**19**; X-ray crystallographic data with tables of thermal and positional parameters, bond lengths, and bond angles as well as ORTEP drawings (single molecule and stereo representations) for **8** (21 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(18) Mangatal, L.; Adeline, M.-T.; Guénard, D.; Guéritte-Voegelein, F.; Potier, P. *Tetrahedron* **1989**, *45*, 4177. Taxotere is a registered trademark of Rhône-Poulenc Rorer; the generic name for taxotere is docetaxel.

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(22) Cf.: Li, L. H.; *et al. Cancer Res.* **1992**, *52*, 4904. We thank Joan W. Culp for the data included here.

(23) The results described here were presented at the 210th American Chemical Society National Meeting, Chicago IL, August 20−24, 1995, Abstract Medi 175.

(24) The A2780 human ovarian carcinoma cell growth inhibition assay (Lopes, N. M., McGovren, J. P., unpublished results) is based on the cell line described in the following: Perez, R. P.; O'Dwyer, P. J.; Handel, L. M.; Ozols, R. F.; Hamilton, T. C. *Int. J. Cancer* **1991**, *48*, 265. We thank Narima Lopes and Patrick McGovren for permission to publish these results.