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Selective Nitration of Paclitaxel and Related Taxanes

Koppaka V. Rao and James H. Johnson Jr.*

Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Box J-100485, Gainesville, FL 32610, U.S.A.

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Abstract: Paclitaxel and some of its natural analogues are readily converted to their respective nitrate esters when reacted with nitric acid in acetic anhydride at 25° C in 30 minutes. Under milder conditions, partially esterified products can be prepared. The regioselectivity of these partial nitrations is studied.

In an attempt to nitrate the phenyl ring of the N-benzoyl phenyl-isoserine side chain in paclitaxel **1**,[1] the compound was subjected to reaction with a 1 : 5 mixture of concentrated nitric acid in acetic anhydride and an equal volume of dichloromethane. Reaction proceeded readily at room temperature in 30 minutes to give a single product which exhibited a much higher R_f on tlc than paclitaxel and even higher than 2', 7-diacetyl paclitaxel **2**,[2] thus eliminating the possibility of acid-catalyzed acetylation. The ^{1}HNMR spectrum showed a considerable downfield shift of the 2' and 7 proton signals. Following characterization by elemental analysis and IR spectroscopy, which exhibited characteristic bands for nitrate esters at 1650, 1270, and 835 cm^{-1}, the structure was determined to be that of paclitaxel-2', 7-dinitrate ester **3**.[10] It was surprising that the product showed no evidence of rearrangement of the A-ring or cleavage of the oxetane ring, both of which have been known to occur in the presence of strong acids.[3]

The reaction was also repeated with three of the natural analogues of paclitaxel, namely 10-deacetyl baccatin III **4**,[4] 10-deacetyl paclitaxel **5**,[5] and 10-deacetyl paclitaxel-7-xyloside **6**.[6] In each case the reaction proceeded to yield the corresponding tri-**7**,[11] tri-**8**,[12] and penta-**9**[13] nitrate esters respectively. All of these compounds crystallized readily from ethyl ether after workup without any need for chromatography to give near quantitative yields (> 95%). In no case was nitration at the sterically hindered 1-hydroxyl observed. Proton and carbon NMR values of the completely nitrated compounds are given in Table 1.

With these results in hand, we decided to run the reaction at 0° C for only 10 - 15 minutes to determine if the reaction was regioselective. Using this protocol paclitaxel yielded the 7-mononitrate **10**[14] of paclitaxel in ~90% yield after crystallization from the crude workup. The position of the nitrate ester was easily determined by ^{1}HNMR and COSY spectroscopy as the nitrate ester causes a downfield shift of 1.3 - 1.7 ppm on the adjacent proton. This result was interesting since the 2'-hydroxyl is much more reactive than the 7-hydroxyl in acetylation reactions.[2] With nitration however the order of reactivity was 7 > 2'.

Similar studies were applied to compounds **4**, **5**, and **6** and they also displayed some regioselectivity, however because these compounds contain more than 2 reactive hydroxyl groups, column chromatography was needed to separate the products. When 10-deacetyl baccatin III was subjected to this protocol three partially nitrated compounds were obtained namely the 10-mononitrate **11**,[15] the 10, 13-dinitrate **12**,[16] and the 7, 10-dinitrate **13**,[17] although **11** and **12** were the major compounds. From this it can be concluded that the order of reactivity is 10 > 13 > 7. This also differs from the acetylation reactivities in which the 7-hydroxyl is the most reactive followed by the 10-hydroxyl and 13-hydroxyl respectively.[7] The reaction of 10-deacetyl paclitaxel likewise gave the 10-mononitrate **14**[18] and the 7, 10-dinitrate **15**,[19] again showing the 10-hydroxyl to be the most reactive and the 2'-hydroxyl the least. With acetylation the 2'-hydroxyl seems to be the most reactive and the 10-hydroxyl the least reactive and the 7-hydroxyl in between.[8] Finally, 10-deacetyl paclitaxel-7-xyloside was used and because of the 5 available hydroxyls many products were observed on tlc and only the major ones were

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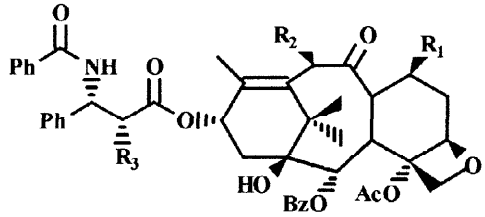
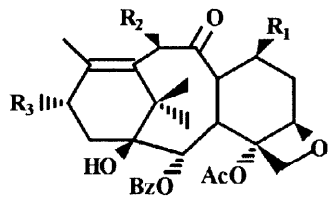
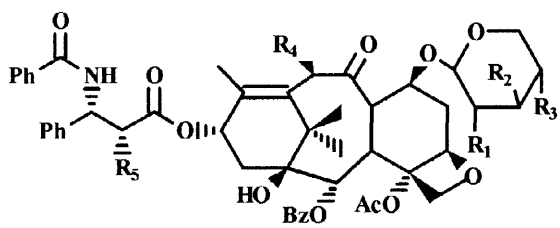
isolated. These included the 2''-mononitrate **16**,[20] the 3''-mononitrate **17**,[21] the 4''-mononitrate **18**,[22] and the 2'', 3'', 4'', 10-tetranitrate **19**.[23] This result indicates that the sugar hydroxyls are about equally reactive and more reactive than the 10-hydroxyl, with the 2'-hydroxyl being again the least reactive.



1. R_1 = OH, R_2 = OAc, R_3 = OH
2. R_1 = OAc, R_2 = OAc, R_3 = OAc
3. R_1 = ONO_2, R_2 = OAc, R_3 = ONO_2
5. R_1 = OH, R_2 = OH, R_3 = OH
8. R_1 = ONO_2, R_2 = ONO_2, R_3 = ONO_2
10. R_1 = ONO_2, R_2 = OAc, R_3 = OH
14. R_1 = OH, R_2 = ONO_2, R_3 = OH
15. R_1 = ONO_2, R_2 = ONO_2, R_3 = OH



4. R_1 = OH, R_2 = OH, R_3 = OH
7. R_1 = ONO_2, R_2 = ONO_2, R_3 = ONO_2
11. R_1 = OH, R_2 = ONO_2, R_3 = OH
12. R_1 = OH, R_2 = ONO_2, R_3 = ONO_2
13. R_1 = ONO_2, R_2 = ONO_2, R_3 = OH



6. R_1 = OH, R_2 = OH, R_3 = OH, R_4 = OH, R_5 = OH
9. R_1 = ONO_2, R_2 = ONO_2, R_3 = ONO_2, R_4 = ONO_2, R_5 = ONO_2
16. R_1 = ONO_2, R_2 = OH, R_3 = OH, R_4 = OH, R_5 = OH
17. R_1 = OH, R_2 = ONO_2, R_3 = OH, R_4 = OH, R_5 = OH
18. R_1 = OH, R_2 = OH, R_3 = ONO_2, R_4 = OH, R_5 = OH
19. R_1 = ONO_2, R_2 = ONO_2, R_3 = ONO_2, R_4 = ONO_2, R_5 = OH

Table 1. ^{1}H and ^{13}CNMR Chemical Shifts for Completely Nitrated Compounds in $CDCl_3$

H or C#	Compd. **3**	Compd. 7	Compd. **8**	Compd. **9**
1	*****, 78.5	*****, 78.2	*****, 78.5	*****, 78.7
2	5.72 d 6.9 Hz, 74.3	5.64 d 6.6 Hz, 73.5	5.71 d 6.9 Hz, 74.0	5.71 d 6.9 Hz, 74.5
3	4.02 d 6.9 Hz, 47.2	3.92 d 6.9 Hz, 47.7	3.95 d 6.9 Hz, 47.2	3.81 d 6.6 Hz, 45.7
4	*****, 80.7	*****, 80.2	*****, 80.6	*****, 80.6
5	4.99 d 9.0 Hz, 83.6	4.97 d 8.7 Hz, 83.5	4.98 d 9.0 Hz, 83.4	4.88 d 9.0 Hz, 83.5
6α	2.69 m, 32.5	2.72 m, 32.5	2.71 m, 32.5	2.81 m, 35.8
6β	2.04 m, *****	2.04 m, *****	2.07 m, *****	2.04 m, *****
7	5.75 dd 7.2, 10.5 Hz, 79.8	5.78 dd 7.2, 10.5 Hz, 79.9	5.75 dd 7.2, 10.5 Hz, 79.8	4.17 m, 80.1
8	*****, 55.3	*****, 55.8	*****, 55.6	*****, 57.8
9	*****, 200.4	*****, 199.3	*****, 199.5	*****, 199.9
10	6.31 s, 74.5	6.38 s, 81.4	6.36 s, 81.6	6.42 s, 82.1
11	*****, 135.5	*****, 134.2	*****, 135.4	*****, 135.5
12	*****, 140.9	*****, 142.2	*****, 144.2	*****, 143.1
13	6.30 t 9.9 Hz, 72.8	6.23 t 8.1 Hz, 78.1	6.29 t 9.0 Hz, 72.5	6.25 t 8.7 Hz, 72.6
14α	2.38 m, 35.4	2.48 dd 9.9, 15.6 Hz, 34.2	2.39 m, 35.3	2.42 m, 35.3
14β	2.38 m, *****	2.36 dd 7.8, 15.9 Hz, *****	2.39 m, *****	2.30 m, *****
15	*****, 43.3	*****, 42.9	*****, 43.1	*****, 42.9
16	1.23 s, 26.5	1.20 s, 26.6	1.20 s, 26.4	1.19 s, 26.2
17	1.17 s, 21.5	1.15 s, 20.3	1.14 s, 21.5	1.13 s, 21.6
18	1.94 s, 14.3	2.12 s, 15.0	2.00 s, 14.8	1.97 s, 14.8

Table 1. ^{1}H and $^{13}CNMR$ chemical shifts for completely nitrated compounds in $CDCl_3$ continued

H or C#	Compd. **3**	Compd. 7	Compd. **8**	Compd. **9**
19	1.82 s, 11.0	1.82 s, 10.8	1.84 s, 11.0	1.75 s, 10.7
20α	4.35 d 8.7 Hz, 76.2	4.36 d 8.7 Hz, 76.0	4.37 d 8.4 Hz, 76.2	4.35 d 8.7 Hz, 76.5
20β	4.20 d 8.4 Hz, *****	4.11 d 8.4 Hz, *****	4.20 d 8.4 Hz, *****	4.19 d 8.7 Hz, *****
2`	5.69 d 3.0 Hz, 80.3	*****	5.68 d 2.7 Hz, 80.2	5.65 d 3.0 Hz, 80.3
3`	6.14 dd 2.7, 9.6 Hz, 52.1	*****	6.13 dd 2.7, 9.3 Hz, 52.1	6.10 dd 3.0, 9.3 Hz, 52.2
NH	6.97 d 9.6 Hz, *****	*****	6.90 d 9.3 Hz, *****	6.82 d 9.3 Hz, *****
1``	*****	*****	*****	4.76 d 4.5 Hz, 98.9
2``	*****	*****	*****	4.98 dd 4.2, 6.3 Hz, 74.3
3``	*****	*****	*****	5.26 t 6.3 Hz, 72.9
4``	*****	*****	*****	5.08 dd 5.7, 9.6 Hz, 74.1
5`` ax	*****	*****	*****	4.23 m, 59.5
5`` eq	*****	*****	*****	3.68 dd 5.7, 12.9 Hz, *****
4-Ac	2.50 s, 22.6	2.43 s, 22.3	2.51 s, 22.6	2.48 s, 22.7
10- Ac	2.18 s, 20.6	*****	*****	*****
OBz-1	*****, 130.3	*****, 128.6	*****, 130.3	*****, 129.0
OBz-2,6	8.12 d 7.2 Hz, 130.2	8.06 d 6.9 Hz, 130.1	8.13 d 7.5 Hz, 130.2	8.13 d 7.2 Hz, 130.2
OBz-3,5	7.52 t 7.8 Hz, 128.8	7.51 t 7.8 Hz, 128.9	7.52, 128.8	7.40-7.55, 128.8
OBz-4	7.63 t, 133.8	7.65 t 7.5 Hz, 131.9	7.64 t 7.2 Hz, 133.9	7.63 t 6.6 Hz, 133.8
NBz-1	*****, 131.2	*****	*****, 131.3	*****, 131.2
NBz-2,6	7.73 d 7.2 Hz, 127.1	*****	7.73 d 7.5 Hz, 127.1	7.73 d 6.9 Hz, 127.1
NBz-3,5	7.41-7.46, 129.4	*****	7.41-7.47, 129.4	7.40-7.55, 129.4
NBz-4	7.41-7.46, 129.0	*****	7.41-7.47, 129.1	7.40-7.55, 129.1
Ph-1	*****, 133.1	*****	*****, 133.0	*****, 133.1
Ph-2,6	7.41-7.46, 126.5	*****	7.41-7.47, 126.5	7.40-7.55, 126.5
Ph-3,5	7.41-7.46, 128.8	*****	7.41-7.47, 128.8	7.40-7.55, 128.8
Ph-4	7.49-7.54, 132.3	*****	7.52, 132.4	7.40-7.55, 132.3
C=O	170.2, 169.5, 167.7, 166.8, 166.7	171.1, 166.9	170.4, 167.6, 166.7, 166.7	170.3, 167.3, 166.8, 166.7

Finally it was shown that these nitrate esters can be easily removed by stirring with Zn/acetic acid at room temperature for 30 minutes to give the parent compound quantitatively.[9]

References and Notes

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10. **3**: colorless needles, mp 166-168° C, IR (KBr) 3450,1725,1650,1365,1270,1230,1065,835,700 cm^{-1}, Anal. Calc. for $C_{47}H_{51}N_3O_{18}$: C 58.69; H 5.31; N 4.37. Fd. C 58.83; H 5.15; N 4.11.
11. 7: colorless needles, mp 159-161° C, Anal. Calc. for $C_{29}H_{33}N_3O_{16}$: C 51.25; H 4.90; N 6.18. Fd. C 51.63; H 5.25; N 5.83.

12. **8**: colorless needles, mp 159-162° C, Anal. Calc. for $C_{45}H_{46}N_4O_{19} + H_2O$: C 56.02; H 5.01; N 5.81. Fd. C 56.07; H 4.91; N 5.64.
13. **9**: colorless needles, mp 187-188° C, Anal. Calc. for $C_{50}H_{52}N_6O_{27}$: C 51.38; H 4.48; N 7.19. Fd. C 51.53; H 4.59; N 6.82.
14. **10**: colorless needles, mp 163-165° C, Anal. Calc. for $C_{47}H_{50}N_2O_{16} + H_2O$: C 61.57; H 5.72; N 3.06. Fd C 61.95; H 6.10; N 2.91.
15. **11**: white crystalline powder, mp 169-171° C, Anal. Calc. for $C_{29}H_{35}NO_{12}$: C 59.08; H 5.98; N 2.38. Fd. C 58.90; H 6.28; N 2.19.
16. **12**: colorless needles, mp 202-204° C, Anal. Calc. for $C_{29}H_{34}N_2O_{14}$: C 54.89; H 5.40; N 4.41. Fd. C 55.17; H 5.77; N 4.07.
17. **13**: white amorphous powder, Anal Calc. for $C_{29}H_{34}N_2O_{14}$: C 54.88; H 5.40; N 4.14. Fd. C 55.01; H 5.63; N 4.29.
18. **14**: white amorphous powder, Anal Calc. for $C_{45}H_{48}N_2O_{15}$: C 63.08; H 5.65; N 3.27. Fd. C 62.75; H 5.97; N 3.90.
19. **15**: colorless needles, mp 170-172° C, Anal. Calc. for $C_{45}H_{47}N_3O_{18}$: C 58.76; H 5.37; N 4.57. Fd. C 59.11; H 5.27; N 4.49.
20. **16**: white amorphous powder, Anal. Calc. for $C_{50}H_{56}N_2O_{19} + H_2O$: C 59.64; H 5.81; N 2.78. Fd. C 60.02; H 6.18; N 2.40.
21. **17**: colorless needles, mp 209-211° C, Anal. Calc. for $C_{50}H_{56}N_2O_{19} + H_2O$: C 59.64; H 5.81; N 2.78. Fd. C 59.27; H 5.87; N 2.89.
22. **18**: colorless needles, mp 193-195° C, Anal. Calc. for $C_{50}H_{56}N_2O_{19} + H_2O$: C 59.64; H 5.81; N 2.78. Fd. C 59.96; H 6.18; N 2.56.
23. **19**: white crystalline powder, mp 182-184° C, Anal. Calc. for $C_{50}H_{53}N_5O_{25}$: C 53.43; H 4.75; N 6.23. Fd. C 53.67; H 4.82; N 5.87.