THE PREPARATION OF TRITIUM AND DEUTERIUM-LABELLED CAMPTOTHECIN

Peter E. Ronman*, Mansukh C. Wani, and Monroe E. Wall+
Research Triangle Institute, Research Triangle Park,
North Carolina, 27709

SUMMARY

The synthesis of d,ℓ,-12-bromocamptothecin (2d) from camptothecin (1) is described. Reduction of the bromo derivative 2d with tritium gas in the presence of palladium on carbon afforded d,ℓ-camptothecin 12-^{3}H having a specific activity of 29 Ci/mmol. A simpler labelling procedure was subsequently discovered whereby deuterium in the presence of palladium on carbon reduced ring B of camptothecin and also exchanged the C-5 hydrogen with deuterium. The reduced camptothecin aromatized to camptothecin in the presence of air to give deuterium incorporation in the C-5 and C-7 positions.

Key Words: Tritium, camptothecin, cmr.

INTRODUCTION

Camptothecin++ is a novel pentacyclic alkaloid[1] with highly significant activity in animal leukemia systems[2] and which exhibits marked *in vitro* activity in inhibition of RNA syntheses and "depolymerization" of DNA.[3] In studies involving the pharmacological behavior of camptothecin specifically tritium labelled camptothecin was required. In this paper,

---

*Norwich Pharmaceutical Company, Norwich, New York.

+To whom correspondence should be addressed.

++Obtained from National Cancer Institute, NIH through the courtesy of Dr. John Douros.

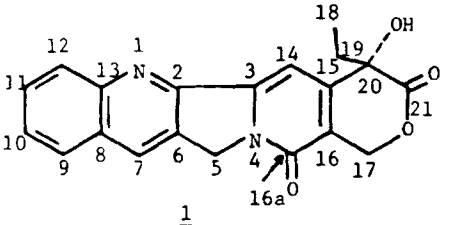
0362-4803/81/030319-11$01.00

Received August 10, 1979
Revised October 5, 1979

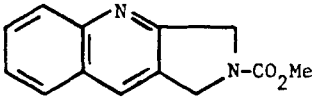
we wish to report two procedures for the preparation of labelled camptothecin (1). The first, involving four steps, introduces a label primarily at the C-12 position. This synthesis proceeds through the known d,ℓ-12-bromocamptothecin (2d)[4], a compound for which experimental details were lacking. Therefore, in this paper full experimental details are reported. The second is a one step procedure and provides optically active camptothecin (1) labelled at the C-5 and C-7 positions.

RESULTS AND DISCUSSION

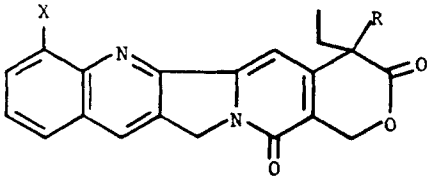
d-Camptothecin (1) was nitrated using concentrated sulfuric acid and fuming nitric acid to give a complex mixture from which d,ℓ-12-nitrocamptothecin (2a) could be obtained by chromatography in low yields (26%). Chinese workers report that 12-nitrocamptothecin (2a) has a blue fluorescence whereas the isomeric 9-nitrocamptothecin gives a yellow fluorescence.[4] We isolated a compound with a blue fluorescence and a

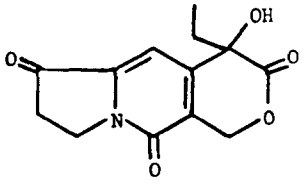


1



3





4

2a X = NO_2, R = OH

2b X = NH_2, R = OH

2c X = $\overset{+}{N}_2Br^-$, R = OH

2d X = Br, R = OH

2e X = ^3H, R = OH

5, X=R=H

melting point of 271-273°C (lit.[4] 268-270°C). Reduction of 2a with hydrogen in the presence of platinum proceeded without difficulty to give amine 2b in good yield. Diazotization of 2b was carried out in 48% hydrobromic acid with sodium nitrite. Amine 2b was only partially soluble in the acid, but the diazonium salt 2c formed a clear orange solution. Oxygen was purged from the system using argon and freshly prepared cuprous bromide was then added. If these precautions were not taken, an unknown tribrominated product and a compound containing one bromine and one extra oxygen were formed as the principal products as determined by mass spectrometry.

After heating at 90°C for 1 hour, d,ℓ-12-bromocamptothecin (2d) with a melting point of 243-245°C (lit.[4] 239°C) could be isolated in 36% yield. Reduction of 2d in dioxane using 10% palladium on carbon and tritium gas (ca. 600 Torr) for 12 hours gave a product which was purified by column chromatography to give d,ℓ-camptothecin-12-^{3}H (2e) of high specific activity (29 Ci/mmol).

If reduction of 2d was carried out with deuterium (760 Torr) for a longer period (24 hours), a pink fluorescent material which was slightly less polar than camptothecin was formed. After exposure of this compound to air, a product was formed which was not characterized except for the fact that the pink fluorescence was replaced by blue fluorescence and the R_f of this compound was identical to that of camptothecin.

We assumed that in addition to replacement of the 12-bromo substituent by deuterium, partial reduction of ring B by deuterium might have occurred. The latter upon rearomatization yielded camptothecin.

This suggested that camptothecin (1) may be directly deuterated thus leading to a simpler method of labelling 1 than that via the 12-bromo-route.

Accordingly, 1 was subjected to 24-hour deuteration as described above. The pink, fluorescent material was formed as before suggesting that this product arose from the partial reduction of 1 with deuterium. When oxygen was passed through the solution, the pink fluorescence disappeared. The resulting product was purified by chromatography.

Mass spectral peaks in the region of parent ion were observed as follows: 348 (80), 349 (100), 350 (78), 351 (30) indicating that 1 with a mixture of d_0, d_1, d_2, and d_3 species had been formed. It could be calculated that deuterated 1 contained 35% d_0, 35% d_1, 24% d_2, and 6% d_3 species with an average of 1.01 deuterium atoms per molecule.[5] Integration of the C-5 methylene protons at δ 5.29 in the pmr spectrum of deuterated 1 showed only 55% of the expected value. An average of 0.9 (2 x 0.45) deuterium incorporated at this position would account for the reduced peak area. Thus at this position, there were either 0, 1, or 2 deuterium atoms. Since the mass spectrum showed a d_3 species, at least one other position in the molecule must also be deuterated. Because there were almost no d_4 species present, this indicated that ring A was not reduced. Another possible position which was ruled out is C-17 (2 benzylic hydrogens) since deuteration at this position would also give rise to a d_4 species.[6] Reduction of either rings B or D and rearomatization would result in a single deuterium being incorporated at either C-7 or C-14.

A cmr study was undertaken in order to determine the exact position of deuteration. For carbons substituted with deuterium, there is a

decrease in peak area relative to the same carbon in an undeuterated sample. Thus, by comparing the ratio of the peak areas of deuterated camptothecin to camptothecin (1), it should be possible to determine which carbons were substituted with deuterium. However, it was first necessary to assign chemical shift values to the carbons of camptothecin.

Hutchinson and coworkers[7] report chemical shift values for 18 of the 20 carbon atoms of 1 and make partial assignments for these values. We were able to observe signals corresponding to all 20 carbons and, with the aid of spectra of intermediates obtained during the synthesis of d,ℓ-camptothecin[8] and of substituted camptothecin derivatives, make definite assignments (see table 1). Assignments for the aromatic carbons in the quinoline and pyridone nuclei were the most difficult to make.

By comparison with quinoline,[9] 2,6-dimethylquinoline,[9] 2,3-dimethylpyridine,[9] and substituted quinoline 3,[10] assignments could be made for the quinoline portion of the molecule. These assignments were further substantiated by making use of the substituent effects of bromine and applying this information to d,ℓ-12-bromocamptothecin (2d). There was a close agreement with the predicted and observed chemical shift values thus giving further proof that the quinoline carbons of camptothecin (1) were assigned correctly. Low intensity peaks were assigned to quaternary carbons.

Assignment of the pyridone carbons were likewise made by comparison with the cmr spectra of pyridone[9] and intermediates such as 4 obtained during the synthesis of d,ℓ-camptothecin.[8] The spectrum of desoxycamptothecin (5) was useful in assigning the chemical shift value of 150.0 (instead of 148.0) to C-15 in camptothecin (1).

Table 1

Cmr Assignments for Camptothecin (1)[a]

Carbon	Chemical Shift
2	152.6
3	145.4
5	50.3
6	129.8
7	131.6
8	128.0
9	128.5
10	127.7
11	129.0
12	130.4
13	148.0
14	96.7
15	150.0
16	119.1
16a	156.8
17	65.3
18	7.9
19	30.3
20	72.4
21	172.5

[a]The spectrum was recorded at room temperature in DMSO-d_6.

Both C-5 and C-7 suffered a reduction in peak areas in going from camptothecin (1) to deuterated camptothecin. From this information, it could be concluded that deuteration was taking place at these two positions. The palladium catalyst was then responsible for a two-fold effect: (1) reduction of ring B and (2) exchange of the benzylic hydrogens at C-5.[11] Previous work from our laboratories has shown that it is possible to exchange the hydrogens at the C-5 position, but only under drastic basic conditions.[12] Thus the C-5 position should not be particularly vulnerable to further exchange under in vivo conditions. This second method of labelling camptothecin avoids the tedious synthesis of d,ℓ-12-bromocamptothecin (2d) and also provides material which is optically active.

EXPERIMENTAL

Radioactive samples were counted in a Packard Tri-Carb 3375 liquid scintillation spectrometer using an Omnifluor-toluene (6 g/liter) cocktail. Developed TLC plates were scanned on a Varian Berthold Radioscanner fitted with a model LB 242 K ratemeter. Pmr spectra were recorded on a Varian HA-100 spectrometer and cmr spectra were recorded on a JEOL JNM-PS-100 spectrometer. Mass spectra were obtained on an AEI MS 902 instrument. Tritium gas was purchased from New England Nuclear, Boston, Mass.

d,ℓ-12-Nitrocamptothecin (2a). Camptothecin (1, 1.276 g, 3.67 mmol) was dissolved in concentrated sulfuric acid (93%, 25 mL). The solution was cooled to -5°C in an ice-salt bath and fuming nitric acid (90%, 1.3 mL) was added over a 10 min period. The yellow solution which had darkened considerably was allowed to warm to room temperature and stirred until TLC [silica gel; $CHCl_3$-acetone-MeOH (70:20:10)] showed the disappearance of starting material (ca. 7 hr.). The reaction mixture

was poured over ice (150 g) and extracted with CH_2Cl_2 (3 X 150 mL). The organic phase was dried (Na_2SO_4) and evaporated to yield 1.47 g of a crude material which was passed through a silica gel column (20 g, 10% MeOH-$CHCl_3$) to obtain 1.22 g of a yellow solid. This material was mixed with silica gel 60 (10 g) using 13% MeOH-$CHCl_3$, dried and then applied to a larger column (200 g) which was eluted with 3% MeOH-$CHCl_3$. The desired material with an R_f = 0.40 (camptothecin's R_f = 0.50) in $CHCl_3$-acetone-MeOH (70:20:10) was isolated as a yellow solid which was crystallized from 13% MeOH-$CHCl_3$ and EtOAc to yield 0.38 g (26%) of 2a. Required for $C_{20}H_{15}N_3O_6$: m/e 393.0961, found: m/e 393.0965.

d,ℓ-12-Aminocamptothecin (2b). A mixture of 2a (127 mg, 0.323 mmol) and platinum oxide (110 mg) in absolute EtOH (75 ml) was hydrogenated in a Parr Shaker during 2.5 hr. at 35°C. The mixture was filtered through Celite and evaporated to yield an orange solid which was crystallized from 13% MeOH-$CHCl_3$ and EtOAc to yield 105 mg (90%) of 2b. Required for $C_{20}H_{17}H_3O_4$: m/e 363.1219. Found: m/e 363.1213.

d,ℓ-12-Bromocamptothecin (2d). Amine 2b (90 mg, 0.248 mmol) was suspended in hydrobromic acid (48%, 3.3 mL) and cooled in an ice bath. Sodium nitrite (20.5 mg, 0.297 mmol) dissolved in 0.20 mL of H_2O was added portionwise over a 10 min period and the mixture was allowed to stir at room temperature for 1 hr. at which time it turned a clear orange. The system was purged with argon and cuprous bromide (46 mg, 0.231 mmol) was added. After heating at 90°C for 1 hr., the reaction mixture was cooled and added to H_2O (30 mL), extracted with CH_2Cl_2 (3 X 30 mL), dried (Na_2SO_4) and evaporated to give 118 mg of crude material. This was eluted from a silica gel 60 column (20 g) using 1% MeOH-$CHCl_3$ to give a yellow solid (a quenching spot with the same R_f as 1) which

was crystallized from $CHCl_3$ to yield 38 mg (36%) of 2d. Required for $C_{20}H_{15}BrN_2O_4$: m/e 426.0212. Found: m/e 426.0213.

d,ℓ-camptothecin-12-^{3}H (2e). A mixture of 2d (4 mg, 0.0094 mmol), 10% palladium on carbon (3.2 mg), p-dioxane (0.4 mL) and triethylamine (0.04 mL) was placed in a small flask connected to a modified high vacuum microhydrogenation apparatus.[13] A break-seal ampoule containing carrier free tritium gas (5.0 Ci, 1.90 mL at STP) was attached. The mixture was frozen in liquid nitrogen, the system evacuated and the ampoule seal was broken. The mixture was warmed to room temperature (19°C) and stirred for 12 hr. During the reaction, the pressure of tritium gas was slightly under atmospheric pressure. The uptake of tritium matched the calculated amount. The mixture was refrozen and the excess tritium gas pumped out. After warming to room temperature, the catalyst was removed by filtration through Celite and washed with 13% MeOH-$CHCl_3$. The solvent was removed *in vacuo* and the residue taken up in a minimum amount of 4% MeOH-$CHCl_3$ and eluted through a silica gel 60 column (6 g) monitoring with a UV light. TLC [silica gel HF_{254}, $CHCl_3$-acetone-MeOH (70:20:10)] of fraction 2 (1.7 mg, 52%)[14] showed a single fluorescent, radioactive spot with an R_f identical with that of camptothecin (1). Its UV spectrum was also identical with that of 1. This material had a specific activity of 29 Ci/mmol and a radiochemical purity of greater than 95%.

d-camptothecin-5,7-^{2}H. A mixture of d-camptothecin (1, 20 mg, 0.086 mmol), 10% palladium on carbon (30 mg), anhydrous p-dioxane (16 mL) and triethylamine (1.2 mL) was stirred at room temperature (19°C) in an atmosphere of deuterium (760 Torr) for 24 hr. The catalyst was removed by filtration, washed with 13% MeOH-$CHCl_3$, and oxygen was bubbled

through the solution for 3 hr. The solvent was removed in vacuo and the resulting solid was mixed with a small amount of silica gel using 13% MeOH-$CHCl_3$, dried and then applied to a larger column of silica gel 60 (8 g). The column was eluted with 2% MeOH-$CHCl_3$. The resulting material was crystallized from 13% MeOH-$CHCl_3$ and EtOAc to yield 11 mg (37%) of light yellow solid which was pure by TLC. m/e: 348(80) 349(100) 350(78), 351(30), 352(5.5).

ACKNOWLEDGEMENT

The support of this investigation by the U. S. Public Health Service Research Grant No. CA20050-03 from the National Cancer Institute is gratefully acknowledged. The authors also thank Dr. D. Rosenthal and Mr. F. Williams for mass spectral data obtained from the Research Triangle Institute Center for Mass Spectrometry.

REFERENCES AND NOTES

1. Wall, M. E., Wani, M. C., Cook, C. E. Palmer, K. H., McPhail, A. T., and Sim, G. A., J. Am. Chem. Soc., 88, 3888 (1966).
2. Wall, M. E. in Symposiumsberichtes, 4th Int. Symp. Biochem. Physiol. Alkaloide, ed. Mothes, K., Schreiber, K., Schütte, H. R., Berlin: Akad. Verlag, pp. 77-87, 1969.
3. "Mechanism of Action of Antimicrobial and Antitumor Agents," J. W. Corcoran, and F. E. Hann, Editors, Springer-Verlag. New York, N. Y., 1975, p. 48-57.
4. Anon, Acta Chim. Sinica, 33, 71 (1975).
5. The percent values were calculated using a computer program. Intensities of M^+, $M^+ + 1$, etc. for 1 were entered into a computer program along with the intensities of M^+, $M^+ + 1$ etc. for deuterated 1. The percent of each deuterated species allowing for the

natural abundance of ^{13}C could then be calculated. The computer program was developed by Mr. George Taylor, Research Triangle Institute from a basic procedure described by Klaus Biemann in, "Mass Spectrometry, Organic Chemical Applications", McGraw-Hill Book Co., New York, N.Y., 1962.

6. Integration of the pmr spectrum of deuterated camptothecin showed no detectable amounts of deuterium at this position.
7. Hutchinson, C. R., Heckendorf, A. H. and Daddona, P. E., J. Am. Chem. Soc., 96, 5609 (1974).
8. Wani, M. C., Ronman, P. E., Lindley, J. T. and Wall, M. E., J. Med. Chem., in press.
9. Johnson, L. F. and Jankowski, W. C., "Carbon-13 NMR Spectra," John Wiley and Sons, New York, N. Y., 1972.