An Efficient Conversion of Camptothecin to 10-Hydroxycamptothecin

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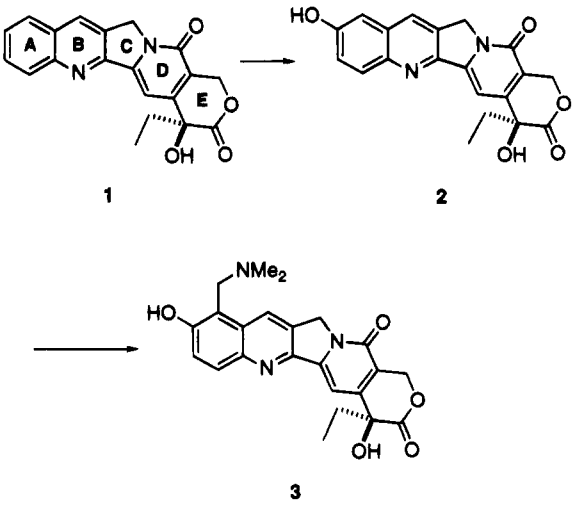
The alkaloid camptothecin (**1**) received intense synthetic and clinical interest following the 1966 report of its isolation, structure determination, and strong antitumor activity.[3] This interest waned when the compound was found to have severe clinical toxicity caused in part by its lack of solubility, but it returned following the 1985 report that **1** acts by inhibiting DNA topoisomerase I, which governs the topology of DNA reassembly following replication.[4] This represented a new and potentially selective mode of action since the enzyme is present in much larger quantities in transformed, neoplastic cells than in normal cells. Consequently, a number of groups began investigating derivatives of **1** in hopes of finding one that retained the ability to inhibit topoisomerase I but avoided toxicity problems.[5]

During the course of our development work on the water-soluble camptothecin derivative topotecan (**3**)[6] we required 100-g quantities of the key intermediate 10-hydroxycamptothecin[7] (**2**) in enantiomerically pure form. There was no commercial source of this compound and no suitable synthetic preparation in the literature,[8] so we set out to develop our own. This note describes the result of our efforts: a high-yielding, two-step, semisynthesis of **2** that we have successfully carried out on a multikilogram scale. In addition to meeting our own needs, this method could be useful for the preparation of other compounds under investigation.[9]

Scheme 1



1 **2** **3**

We were able to obtain reliable quantities of **1** from several sources[10] so we focused on this as our starting material for preparing **2**. Our attempts at direct oxygenation of the A-ring of **1** were unsuccessful. We then tried to activate the A-ring through either *N*-oxide formation[11] or B-ring reduction, and we obtained more encouraging results with the latter strategy.[12] Thus, we set out to develop a selective reduction of **1** followed by a selective oxidation to give **2**.

Catalytic hydrogenation of **1** over platinum in mildly acidic solution was only partially selective for 1,2,6,7-tetrahydrocamptothecin (**4**)[13] as further reduction of the product occurred well before the starting material had been completely consumed. The overreduced material had to be removed by a difficult chromatography, giving **4** in poor yield. We felt that partial poisoning of the catalyst might increase the selectivity of the reduction for the B ring of **1**. Indeed, when DMSO was added to the reaction mixture only **4** was obtained, even on extended reaction at elevated temperatures and pressures. PtO_2 was initially used as the catalyst, but more highly dispersed Pt/C gave a faster and more complete reaction with less overall platinum. The yield of **4** was typically 88–90%.[14]

The second problem was trickier: **2** is more easily oxidized than **4**, so that regardless of the oxidant we used, much of the desired product was destroyed by further oxidation as it was formed in the reaction mixture. In addition, the oxidation was only moderately selective for 10-oxygenation over simple aromatization, leading to significant quantities of **1** which could not be separated from the desired **2**. We investigated a number of oxidants and conditions in attempting to minimize these problems

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(3) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888. Suffness, M.; Cordell, G. A. In *The Alkaloids: Chemistry and Pharmacology*; Brossi, A., Ed.; Academic Press: Orlando, FL, 1985; Vol. XXV, pp 73–88.

(4) Hsiang, Y.-H; Hertzberg, R.; Hecht, S.; Liu, L. F. *J. Biol. Chem.* **1985**, *260*, 14873.

(5) For instance, see Giovanella, B. C.; Stehlin, J. S.; Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Liu, L. F.; Silber, R.; Potmesil, M. *Science* **1989**, *246*, 1046.

(6) Kingsbury, W. D.; Boehm, J. C.; Jakas, D. R.; Holden, K. G.; Hecht, S. M.; Gallagher, G.; Caranfa, M. J.; McCabe, F. L.; Faucette, L. F.; Johnson, R. K.; Hertzberg, R. P. *J. Med. Chem.* **1991**, *34*, 98.

(7) Wani, M. C.; Wall, M. E. *J. Org. Chem.* **1969**, *34*, 1364.

(8) Four total syntheses of racemic **2** have been reported, as well as three semisyntheses of **2** from **1**. All were unsuitable, for reasons of length, yield, and/or reproducibility. Total syntheses: (a) Shen, W.; Coburn, C. A.; Bornmann, W. G.; Danishefsky, S. J. *J. Org. Chem.* **1993**, *58*, 611. (b) Cai, J.-C.; Yin, M.-G.; Min, A.-Z.; Feng, D.-W.; Zhang, X.-X. *Hua Hsueh Hsueh Pao* **1981**, *39*, 171. *Chem. Abstr.* **1981**, *95*, 133209h. (c) Wani, M. C.; Ronman, P. E.; Lindley, J. T.; Wall, M. E. *J. Med. Chem.* **1980**, *23*, 554. (d) Kametani, T.; Ohsawa, T.; Ihara, M. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1563. Semisyntheses: (e) Miyasaka, T.; Sawada, S.; Nokata, K.; Mutai, M. U. S. Patent 4 545 880, 1985; *Chem. Abstr.* **1983**, *99*, 71068h (covers two methods). (f) Miyasaka, T.; Sawada, S.; Nokata, K.; Mutai, M. Japanese Patent 87 032 749-B, 1987; *Chem. Abstr.* **1984**, *100*, 192141z. (g) The three semisynthetic methods were subsequently described in a single paper: Sawada, S.; Matsuoka, S.; Nokata, K.; Nagata, H.; Furuta, T.; Yokokura, T.; Miyasaka, T. *Chem. Pharm. Bull.* **1991**, *39*, 3183.

(9) 10-Oxygenated camptothecin derivatives are being investigated by at least one other group. See Sawada, S.; Okajima, S.; Aiyama, R.; Nokata, K.; Furuta, T.; Yokokura, T.; Sugino, E.; Yamaguchi, K.; Miyasaka, T. *Chem. Pharm. Bull.* **1991**, *39*, 1446.

(10) **1** can be isolated from *Camptotheca acuminata* (Sino-American Tianjin SK&F Labs Ltd., Tianjin, China) or from *Nothapodytes foetida* (Atul Products Ltd., Gujarat, India).

(11) See reference 8e.

(12) While formation of the *N*-oxide of **1** was straightforward, the subsequent photorearrangement to **2** required high dilution, severely limiting the amount of material that could be prepared. Furthermore, the yield suffered due to poor selectivity and the instability of **2** to the reaction conditions.

(13) **4** is formed as a mixture of two diastereomers in about an 18:1 ratio. Both diastereomers undergo the subsequent oxidation.

(14) This is the yield based only on the major diastereomer of **4**.

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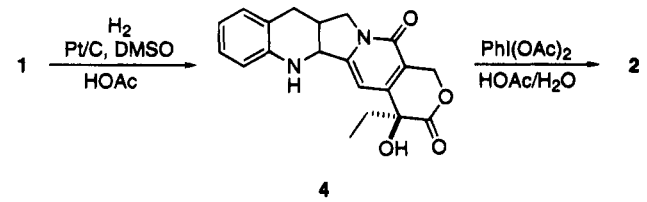
Scheme 2



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with yield and selectivity. The key turned out to be conducting the oxidation in a solvent mixture consisting of water and a miscible organic solvent. Among several systems that gave good results, 1:1 acetic acid/water provided the highest yield and selectivity and was the most convenient as it allowed us to use the filtered solution of the hydrogenation product directly in the oxidation.[15] Addition of water to the reactive intermediate was rapid enough that selectivity for **2** over **1** was about 25:1[16] and **2** precipitated from solution as soon as it was formed, thus being protected from further oxidation. With iodobenzene diacetate as the oxidant, **2** was typically isolated in 88–91% yield.

This procedure provides a significant improvement over existing methods for obtaining 10-hydroxycamptothecin. It is short, simple, reproducible, and high-yielding. As such, it should facilitate the development of promising camptothecin analogs.

Experimental Section

CAUTION: Camptothecin has been established as a significant clastogenic agent, causing chromosomal aberrations. Consequently, it and all structurally related compounds must be considered potential mutagens and potential reproductive hazards for both males and females. Appropriate precautions (use of respirator, gloves, fume hood) must be taken when handling these compounds and any waste streams generated from their use.

General. Unless otherwise mentioned, all solvents and reagents were of reagent grade and were used as received. 5% Platinum on activated carbon powder (dry, uniform, reduced, carbon code CP-37) was obtained from Engelhard Corp. The same catalyst in 50% water-wet form was also used successfully. Iodobenzene diacetate (98%) was purchased from Aldrich Chemical Co. and used as received.

Preparation of 1,2,6,7-Tetrahydrocamptothecin (4). Camptothecin obtained from natural sources may require purification before it can be successfully hydrogenated. We found recrystallization from 15 volumes (mL/g) of DMF at 150 °C and/or recrystallization from 35 volumes of refluxing acetic acid, with an activated charcoal treatment of the hot solution, to be effective.

A 1-gallon, stainless steel, stirred autoclave was charged with recrystallized **1** (200 g, 98.3% w/w, 564 mmol), 5% Pt/C (equivalent to 100 g dry weight), glacial acetic acid (2.00 L), and DMSO (15.0 mL). Hydrogenation was carried out with vigorous stirring at 65 °C and about 65 psi of hydrogen pressure. After 22 h the reaction was cooled and filtered. The reactor was rinsed with an additional 2.00 L of acetic acid, and the rinse was used to wash the filter cake. HPLC assay of the combined filtrates vs a reference standard (see below) showed the presence of 176 g (497 mmol, 88% solution yield) of **4** (major diastereomer; minor also present but not quantified).

An analytical sample of the major diastereomer of **4** was isolated by vacuum concentration of the filtrate (about 480 mL, containing about 17 g of **4**) from an earlier hydrogenation of **1**, giving an oil which was taken up in methanol (150 mL). The resulting thick, white suspension was treated with 4 N HCl (37 mL, 3 equiv) to give an even thicker (mechanical stirring required) suspension. Cooling to −20 °C gave, after filtration, the HCl salt of **4** as a very fine, white solid which was washed with 40 mL of −20 °C methanol and air-dried. The solid (19 g) was suspended in a mixture of CH_2Cl_2 (1.42 L) and saturated aqueous $NaHCO_3$ (1.00 L) and stirred vigorously until it had completely dissolved. The aqueous layer was extracted once with CH_2Cl_2, and the combined organic layers were dried with Na_2SO_4 and concentrated to about 100 mL. The resulting slurry was cooled to −20 °C and filtered to give **4** (free base) as a white solid, which was dried under vacuum and stored at −20 °C. Final weight: 13.8 g. mp 216–219 °C dec; ^1H NMR agreed with published[6g] data; ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 157.4, 153.5, 150.2, 143.6, 128.5, 127.1, 119.6, 116.4, 116.3, 113.3, 97.8, 72.4, 65.2, 56.8, 48.7, 31.7, 30.3, 28.4, 7.9; IR (KBr) 3538, 3466, 1732, 1648, 1163, 1118 cm^{-1}; mass spectrum (DCI/NH_3) m/z 353 (MH^+, 100), 309; (DCI/ND_3 shows m/z 356 and 311, indicating two exchangeable protons in the parent and only one in the fragment). Anal. Calcd for $C_{20}H_{20}N_2O_4 \cdot H_2O$: C, 64.85; H, 5.99; N, 7.56. Found: C, 64.57; H, 5.84; N, 7.33.

Preparation of 10-Hydroxycamptothecin (2). A 12-L flask was charged with the acetic acid solution of **4** (176 g, 497 mmol, in 4.60 L of solution) from the above hydrogenation. The solution was concentrated under vacuum[17] at 25–35 °C to about 1.95 L, and an equal volume of distilled water was added to give a heavy, white precipitate. The rapidly stirred suspension was treated with iodobenzene diacetate (320 g, 993 mmol) added over about 5 min. An exotherm from 22 °C to 34 °C was observed over 10 min. On addition of the oxidant, the suspension turned dark green and most of the solid went into solution. The green color faded to dark brown and then yellow over about 20 min. Additional iodobenzene diacetate was added in portions of 80.0 g (248 mmol) and 64.0 g (199 mmol) at about 1 h intervals. After stirring overnight, the dark-yellow suspension was concentrated by distillation at atmospheric pressure, while adding 5.25 L of 1:1 acetic acid/water in portions during the distillation, until a total of 7.44 L of distillate had been removed. Iodobenzene was removed during this procedure. The remaining yellow slurry was cooled to rt, filtered, and washed with methanol. Drying at 45 °C/0.5 mmHg gave **2** as a fine, yellow powder (172 g, 96.1% w/w, 454 mmol, 91% yield): mp 267–268 °C dec; ^1H NMR agreed with published[5,6g] data; ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 156.8, 156.6, 150.1, 149.4, 145.9, 143.2, 130.6, 129.8, 129.6, 129.2, 123.0, 118.1, 108.8, 95.8, 72.4, 65.3, 50.1, 30.3, 7.8. Anal. Calcd for $C_{20}H_{16}N_2O_5 \cdot H_2O$: C, 62.82; H, 4.74; N, 7.33. Found: C, 62.97; H, 4.59; N, 7.17.

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(15) In addition to the labor saved by avoiding a product isolation, direct use of the solution of **4** minimized the opportunity for the facile air-oxidation of **4** back to **1**.

(16) No product from addition of acetic acid (that is, 10-acetoxycamptothecin) was observed under these conditions, though it was observed when the reaction was conducted in glacial acetic acid.

(17) Vacuum is used to exclude oxygen. While **4** does not appear to be thermally labile, in solution it is readily oxidized to **1** by dissolved oxygen.