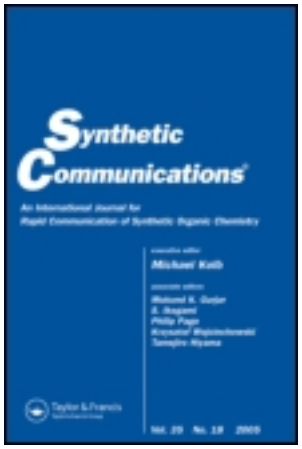

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SYNTHESIS OF 10-HYDROXYCAMPTOTHECIN: EVALUATION OF NEW MODERATORS FOR THE CHEMOSELECTIVE REDUCTION OF CAMPTOTHECIN

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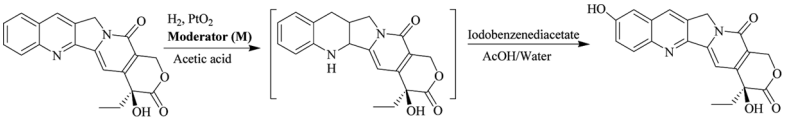
GRAPHICAL ABSTRACT



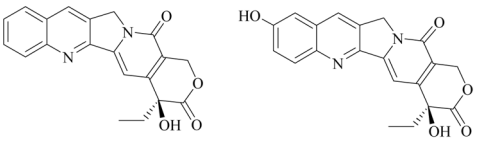
Camptothecin (1) **1,2,6,7- tetrahydrocamptothecin (1A)** **10-hydroxycamptothecin (2)**

Abstract *10-Hydroxycamptothecin is prepared by chemoselective catalytic hydrogenation of the B-ring of camptothecin over PtO_2 with sulfur moderators followed by oxidation using iodobenzenediacetate. New moderators (*viz. *thioanisole, dimethyl sulfide, diphenyl sulfide, 2-mercapto ethanol), which moderate the hydrogenation of the B- ring of camptothecin, are being explored.*

Keywords Camptothecin; DMSO; 10-hydroxycamptothecin; moderator; thioanisole

INTRODUCTION

Camptothecin (**1**), an alkaloid with efficacy in animal tumor models, was isolated from Chinese tree *Camptotheca acuminata* by Wall and coworkers in 1966[1] and from the Indian tree *Nothapodytes foetida* by Govindachari and Viswanthan.[2]



Camptothecin (1) **10-Hydroxycamptothecin (2)**

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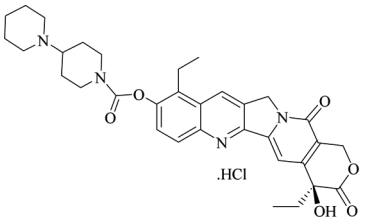
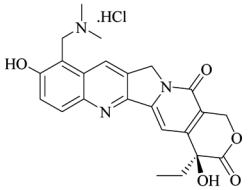
Many camptothecin derivatives were prepared, and their biological activities were successfully studied and reported.[3–11] Among the camptothecin derivatives, 10-hydroxycamptothecin (**2**) is a key intermediate for the preparation of drugs, viz. irinotecan (**3**) and topotecan (**4**), which are used for the treatment of different types of cancer, such as colon cancer, ovarian cancer, cervical cancer, and small cell lung cancer.



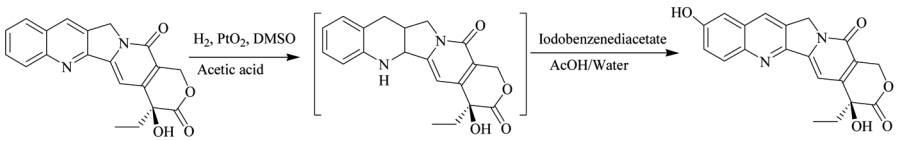
Irinotecan hydrochloride (3)



Topotecan hydrochloride (4)

10-Hydroxycamptothecin (**2**) gained importance in 1985 when it was reported that it acts as an inhibitor of DNA topoisomerase.[11–14] Subsequently, it has been a target for synthesis by numerous research groups because of its impressive biological activity and paucity of naturally derived material. There are several synthetic schemes reported in literature[11,14–16] for the synthesis of 10-hydroxycamptothecin (**2**), and many of them are not suitable for commercial synthesis. Jeffery et al.[14] have reported an efficient conversion of camptothecin (**1**) to 10-hydroxycamptothecin (**2**) through chemoselective catalytic hydrogenation of the B-ring of camptothecin (**1**) over PtO_2 with dimethylsulfoxide (DMSO)[14,15] as moderator followed by oxidation with iodosobenzenediacetate (Scheme 1).

As a part of research program on camptothecin derivatives, we explored the synthesis of 10-hydroxycamptothecin using DMSO as moderator. However, the hydrogenation reaction was found to be reasonable on a small scale but suffered from inconsistency with regard to both yield and quality on a large scale. This could be due to inconsistent conversion of DMSO to dimethylsulfide (DMS) during the course of hydrogenation.[17–23] Further investigation motivated us to explore a better moderator to bring consistency in the formation of the product. Herein, we report the use of new moderators, viz. thioanisole, dimethylsulfide, diphenylsulfide, and 2-mercaptoethanol in the reduction of the B-ring of camptothecin for consistent reaction profile. The advantage has been demonstrated in the commercial application to irinotecan (**3**)[24] and topotecan (**4**).[25]



Camptothecin (1) **1,2,6,7- Tetrahydrocamptothecin (1A)** **10-Hydroxycamptothecin (2)**

Scheme 1. Synthesis of 10-hydroxycamptiothecin by using DMSO as moderator.

RESULTS AND DISCUSSION

Preparation of 10-hydroxycamptothecin (**2**) was carried out in two steps: catalytic hydrogenation of camptothecin (**1**) to 1,2,6,7-tetrahydrocamptothecin (**1A**) using PtO_2 under hydrogen gas with DMSO as moderator,[14,15] followed by oxidation with iodozobenzenediacetate (Scheme 1).

The inconsistency in the progress of the reaction may be attributed to these factors:

1. Often the reaction was not initiated.
2. Presence of a significant amount of starting material even after prolonged reaction time
3. Over-reduction of ring A along with the presence of a significant amount of starting material.

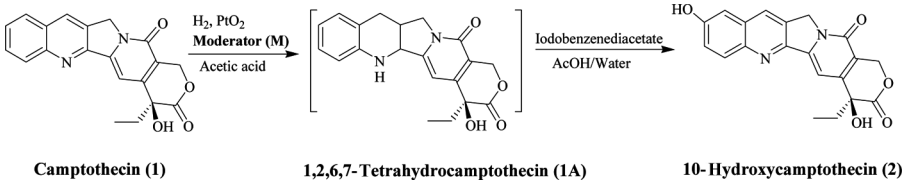
Based on the understanding of the mechanism and effect of sulfur poisoning on the catalyst, we suspected that the moderator could be the reason for the above problems. Also, it is known in the literature that sulfur is the actual moderator that poisons the catalyst and causes selective partial reduction of B-ring. Selective reduction depends on several parameters, such as type of catalyst, stirring, and temperature.

Thus, attempts were taken with representative sulfides to control the poisoning effect and to have a robust and economical process for the preparation of 10-hydroxycamptothecin. When sulfur compounds, viz. thioanisole, dimethylsulfide, diphenylsulfide, and 2-mercaptoethanol were used as moderators, better consistency in yield and quality of the product were observed.

The preparation of 10-hydroxycamptothecin (**2**) from camptothecin (**1**) using new moderators is represented in Scheme **2**. The intermediate 1,2,6,7-tetrahydrocamptothecin (**1A**) was not isolated as it is unstable, air sensitive, and converts back to camptothecin (**1**).[14]

All these sulfide poisoners resulted in good conversion of camptothecin (**1**) to 10-hydroxycamptothecin (**2**) with good purity and yield (Table 1). As depicted in Table 1, the reaction in DMSO was incomplete with 30% of starting material remaining. Other moderators (thioanisole, dimethylsulfide, diphenylsulfide, 2-mercaptoethanol) gave good conversion of the camptothecin (<2%).

Although all sulfide poisoners, mentioned in Table 1, gave encouraging results with regard to conversion of the starting material and purity of the product, one sulfide moderator had been chosen for further optimization. The sulfide 2-meracpto ethanol was not considered further because of its toxic data, while DMS was rejected



Scheme 2. Synthesis of 10-hydroxycamptiothecin using new moderators. Moderator (M) = thioanisole, dimethylsulfide, diphenylsulfide, and 2-mercaptoethanol.

Table 1. Effect of moderators on product quality

Entry	Catalyst	Moderator	Reaction temperature (°C)/pressure (psi)	HPLC purity (%)	Unreacted starting material (%)	Yield (%)
1	PtO_2	DMSO	60–65/60–65	25	30	40
2	PtO_2	DMS	60–65/60–65	98.1	1.6	64
3	PtO_2	Thioanisole	60–65/60–65	98.7	1.1	67
4	PtO_2	Thioanisole	60–65/60–65	98.5	1.3	65
5	PtO_2	2-Mercaptoethanol	60–65/60–65	97.9	1.7	63
6	PtO_2	Diphenylsulfide	60–65/60–65	97.8	1.8	59

for its volatility and flammability. Thioanisole has been chosen for further study and optimization.

Further studies on the hydrogenation with thioanisole resulted in a highly robust transformation with very high reproducibility levels and purity of the product. The reaction has been scaled up to multikilograms with ease and control.

Because of the poor solubility of **2**, purification was quite difficult. Hence, a purification process for crude 10-hydroxycamptothecin (**2**) was established using N,N-dimethylformamide and methanol to achieve the desired purity required for further stages in the synthesis of active pharmaceutical ingredients (APIs). This has been demonstrated in the formal synthesis of irinotecan hydrochloride (**3**) and toptecan hydrochloride (**4**).

CONCLUSION

We developed an improved, cost-effective, and robust scalable process for 10-hydroxycamptothecin starting from naturally occurring camptothecin and commercially available new sulfide moderators (dimethyl sulfide, diphenyl sulfide, 2-mercapto ethanol), and preferably with thioanisole and commercial solvents. The established procedure was carried out on a pilot scale successfully, which increased the yield and purity of 10-hydroxycamptothecin. Hence, further studies on the application of prepoisoned catalyst with moderators will be reported in due course. The utility of 10-hydroxycamptothecin has been demonstrated in the synthesis of irinotecan and topotecan in achieving the quality required for preparation of dosage forms.

EXPERIMENTAL

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 hazardous for both males and females. Appropriate precautions (use of respirator, gloves, fume hood) must be taken while handling these compounds.

General

All solvents and reagents were obtained from commercial sources and used without further treatment or purification. The NMR experiments were recorded

on a 400-MHz Varian Mercury Plus Fourier transform (FT)-NMR spectrometer using dimethylsulfoxide (DMSO-d_6) solvent. ^{1}H chemical shifts are reported on the δ scale in parts per million (ppm) relative to tetramethylsilane (TMS) (δ 0.00) as internal standard. The electrospray ionization (ESI) and MS-MS studies were performed on a triple quadrupole mass spectometer PE Sciex model API 3000 instrument.

Preparation of 1,2,6,7-Tetrahydrocamptothecin Using Thioanisole as a Moderator

Camptothecin (2 kg, 5.74 mol) was mixed with glacial acetic acid (20 L) containing 60 mL (0.3% v/v) of moderator (thioanisole), and the resulting suspension was charged into a 50-L stainless steel, high-pressure, stirred autoclave. This was followed by addition of 670 g of PtO_2 (33% w/w), and the reactor was sealed. The stirred reactor (300 rpm) was pressurized with hydrogen gas to a pressure of 60–65 Psi. This approximate pressure of hydrogen was maintained throughout the reaction. The reactor was gradually heated to a temperature of about 60–65 °C. After 4.5 h, the reactor was gradually cooled to about 30 °C, and hydrogenation was continued at that temperature for 16 h. The reactor was then vented to the atmosphere, and the reaction mass was removed from the reactor. The reactor was washed with glacial acetic acid (about 6 L); the total volume of acetic acid was filtered to remove suspended catalyst to obtain 1,2,6,7-tetrahydrocamptothecin as its solution in acetic acid, which was oxidized directly.

Preparation of 10-Hydroxycamptothecin by Oxidation of 1,2,6,7-Tetrahydrocamptothecin

Water (26 L) was added to the solution of 1,2,6,7-tetrahydrocamptothecin in 26 L of acetic acid, as prepared in example 1. This was followed by addition of iodosobenzene diacetate (4 kg, 12.41 mol) with rapid stirring in one portion. The slurry turned dark green as nearly all of precipitate dissolved. Over a few minutes, the color of the slurry faded to yellow as more precipitate formed. The slurry was stirred at room temperature for 18 h. The mixture was then heated to distill the solvent at atmospheric pressure. A total of 52 L of a 1:1 solution of acetic acid–water was periodically added to maintain an approximately constant volume through most of the distillation; the slurry was eventually concentrated to a final volume of approximately 300 mL. The initial distillate was cloudy and separated into two phases upon collection. The heavier phase of the distillate consisted of mostly iodobenzene. Distillation was stopped, and the yellow slurry was cooled to 25–30 °C. The solid was collected by filtration and rinsed with methanol (1.5 L × 2) and dried at 50 °C under high vaccum to obtain 1.36 kg (yield: 65%) of 10-hydroxycamptothecin as a fine yellow powder. The purity of the obtained product is >95% as determined by high-performance liquid chromatography (HPLC) versus a standard sample. The isolated product contained less than approximately 2% camptothecin.

The obtained product was optionally purified if necessary by dissolving the wet cake in N,N-dimethylformamide (54 L) at 70–75 °C, transferred to another vessel containing methanol (25 L), stirred for 6–12 h at 25–30 °C, filtered, slurred with methanol (40 L) under reflux for 2 h, filtered, and dried at 55–60 °C to

obtain 1.26 kg (yield: 60%) of yellowish powder with >98% purity by HPLC with about 1% camptothecin.

Spectral Data

ES-MS: m/z 365.2 $(M+H)^+$, 387.2 $(M+Na)^+$, 402.3 $(M+K)^+$, 729.4 $(2M+H)^+$, 751.2 $(2M+Na)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.88 (t, $J=7.2$, 3H), 1.87 (m, 2H), 5.23 (s, 2H), 5.41 (s, 2H), 6.46 (s, OH), 7.26 (s, 1H), 7.28 (d, $J=2.4$, 1H) 7.42 (dd, $J=9.2$, 2.4, 1H), 8.02 (d, $J=9.2$, 1H), 8.45 (s, 1H), 10.3 (s, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 7.83, 29.89, 49.77, 64.87, 72.21, 95.53, 108.53, 117.87, 122.82, 129.05, 129.50, 129.85, 130.53, 142.99, 145.72, 149.33, 149.81, 156.52, 156.71, 172.28.

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