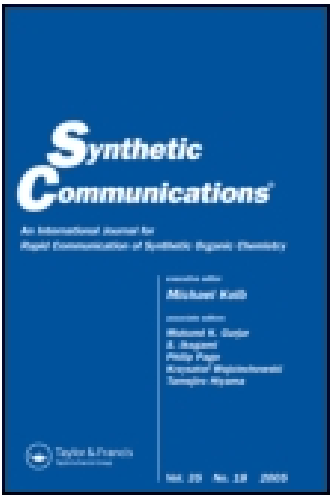

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Urea-Mediated Regioselective Nitration of (20*S*)-Camptothecin[#]

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ABSTRACT

A facile and efficient procedure for the regioselective nitration of (20*S*) camptothecin, using urea mediated reagent system under relatively mild experimental conditions, yielding promising anticancer drug 9-nitro-(20*S*)-camptothecin in 40% yield with purity of 94.5% (on HPLC) is being reported.

Key Words: Anticancer drugs; Camptothecin; Nitration; 9-Nitro-(20*S*)-camptothecin.

[#]Indian Patent 1559/DEL December 21, 1999.

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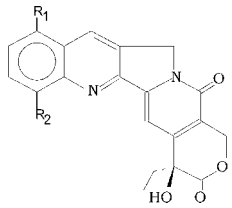
The present work relates to the preparation of a semisynthetic derivative 9-nitro camptothecin from the natural (20*S*)-camptothecin isolated from *Nothapodytes foetida* (wight) Sleumer.[1,2] It is reported to exhibit strong anti-tumor activity and results in complete tumor regression followed by no further growth of tumor.[3]

Metabolic studies on 9-nitrocamptothecin (9-NC) indicated that it gets converted into 9-amino-(20*S*)-camptothecin (9-AC). Preclinical studies have revealed that administration of 9-NC, which being more stable and less toxic than 9-AC, produces the desired effect, which being qualitatively and quantitatively superior to 9-AC.[4]



Camptothecin= R_1=R_2=H

9-Nitrocamptothecin= R_1=NO_2, R_2=H

12-Nitrocamptothecin= R_1=H, R_2= NO_2

9-Aminocamptothecin= R_1=NH_2, R_2=H

Conventional nitration (mixture of conc. HNO_3 and H_2SO_4),[5,6] when employed for nitration of camptothecin yielded 12-nitrocamptothecin (60%) and the desired product 9-nitrocamptothecin was obtained in extremely poor yield along with undesired polynitrated products. In order to select a suitable reaction medium for the nitration of camptothecin to get better yields of 9-nitrocamptothecin, a mild mediated-nitrating method was used.[7] It is well known that a molecule like crystalline tetragonal urea (or thiourea) does not remain rigid and acts as a host molecule and forms hexagonal lattice containing the guest in longitudinal channels (5 A^0 dia.). Since position 12 in the aromatic ring of camptothecin is on relatively hindered side, it was visualized to use urea as the "host" molecule for the "guest" nitronium ion so that the total volume of the reagent becomes larger to prefer position 9 (which lies on least hindered side of the ring) to 12. The controlled nitration of camptothecin has been achieved by sustained release of NO_2^+ trapped in flexible urea channels due to interaction of Vander-Waal forces between the host and guest.[8] It is reported that using organic media reaction kinetics shifts from 2nd order to 1st order with respect to NO_2^+ ion concentration. To the best of our knowledge zero order nitration with respect to camptothecin has not been reported till date.

$$\mathrm{CPT} + \mathrm{excess(CH_3CO)_2O} \xrightarrow{\mathrm{Urea/HNO_3/AcOH}} 9\mathrm{-nitroCPT} + 12 - \mathrm{nitroCPT} + \mathrm{CH_3CO_2NO_2} + \mathrm{CH_3COOH}$$

The reaction may proceed by in-situ generation of acetylnitrate, which may dissociate to yield nitronium ion.

$$(CH_3CO)_2O + \text{urea} + HNO_3 \longleftrightarrow CH_3C(O)ONO_2 + NH_3 + CO_2$$
$$CH_3C(O)ONO_2 \longleftrightarrow CH_3COO^- + NO_2^+$$

Conc. HNO_3 on ionization forms 4% nitronium ions which enter into urea channel as tiny guest molecules thereby protecting camptothecin from vigorous nitration which leads to the formation of undesirable products.

Table 1 gives a comparative account of nitration carried out under different temperature conditions.

A method has been developed for the nitration of natural (20*S*)-camptothecin $C_{20}H_{16}N_2O_4$, mol. wt. 348.35 C 69.96%, H 4.63%, N 8.04%, O 18.37%, mp 255–277°C dec. 264–267°, $[\alpha]\ ^{25}D + 31.3°$ (in MeOH–$CHCl_3$) isolated from woody stem of the Indian tree *Nothapodytes foetida* (wight) Sleumer (Fam. Icacinaceae). TLC, HPLC, MP, IR, UV, ^1H NMR and mass spectrometry established its purity (98.5%). The stereochemistry of the natural compound, which is an essential requirement for biological activity, was ascertained by optical rotation.

In conclusion we have developed a mild and effective method for the regioselective nitration of camptothecin in good yields.

Table 1. Comparative nitration of camptothecin

		Yield %					
S. no.	Temp.	9NC	12NC	CPT	Byproduct	Total nitration	Isolated 9NC
1	Room temp.	31	21	23	25	52	25.7
2	0°C	39	14	8	39	53	35.8
3	−20°C	43	35	19	3	78	40.0

Note: Yields were determined by HPLC** before isolation of the products. For each reaction Camptothecin (700 mg, 2 mmole), acetic anhydride (20 mL), nitrating mixture 0.9 mL (0.009 mole) for 3 h.

**HPLC done on Partisil silica column 5 μ, 4 × 260 mm, isocratic mobile phase $CHCl_3$: MeOH (98 : 2) at 366 nm.

EXPERIMENTAL

General

^{1}H NMR, IR spectra and HPLC systematically characterized all products. Melting points determined are uncorrected IR spectra was recorded on a PC-16-FT Perkin Elmer Spectrometer. ^{1}H NMR was recorded on an INOVA-600 "eden." HRMS was recorded on EIL Rpos-359 spectrometer. HPLC was recorded on Gilson. The reagents were purchased from E. Merck and Ranbaxy. The solvents used were of commercial grade and were double distilled. The yields reported are not theoretical yield but are on the basis of HPLC and isolated products.

Preparation of the Nitrating Solution

Stock nitrating solution was prepared by mixing conc. HNO_3 (60% w/w specific gravity 1.41, 0.5 mole, 31.6 mL) and urea (0.5 g) and diluting the solution to 50 mL with glacial acetic acid. The solution is stable and can be kept for 3 months under refrigerated conditions.

General Procedure for Nitration

Natural camptothecin (700 mg, 2 mmole) was suspended in acetic anhydride (20 mL) with stirring at -20° in a round bottom flask (100 mL) equipped with a magnetic stirrer. The mixture was stirred for 30 min, to this suspension was added slowly, above nitrating solution (0.009 mole, 0.09 mL). The resulting orange red mixture was stirred for 3 h. at (18–20°C). Progress of the reaction was monitored on TLC. The reaction mixture was allowed to stand at room temperature for 18 h. and poured into crushed ice (50 mL) when a red oily suspension was obtained and kept for 3 h. with occasional stirring changed into a yellow crude solid, which on desiccation over P_2O_5 for 12 h gave yellow solid (0.25 g). It was crystallized from ethanol, mp 276–279°C, $[\alpha]\ ^{23}D + 26^\circ$ (c 0.2, MeOH–$CHCl_3$), identified by mp, HPLC and ^{1}H NMR data and optical rotation as 12-nitro-(20*S*)-camptothecin. After separating the solid 12-nitro CPT the yellow filtrate was extracted with CH_2Cl_2 (4 × 100 mL). The extract was washed with water (2 × 40 mL) and dried over anhydrous sodium sulphate. Evaporation of the solvent under reduced pressure yielded a yellow solid (0.4 g), which was admixed with celite and loaded on a column of silica gel. The elution was carried out with $CHCl_3$: MeOH (98 : 3–95 : 5) mixture in a gradient fashion. TLC selected eluates on evaporation afforded a yellow solid (280 mg, 40%). It was crystallized from ethyl acetate and hexane.

MP, TLC, HPLC, ^{1}H NMR identified it to be 9-nitro-(20*S*)-camptothecin mp 182–186°C, $[\alpha]\ ^{23}D + 27°$ (c 0.2, MeOH–$CHCl_3$), IR (KBr, cm^{-1}) 3430 (OH), 1750 (lactone), 1660 (pyridone), 1590 (aromatic) 1530 (NO_2).

^{1}H NMR: ($CDCl_3$), $\delta = 1.05$ (3H, t, $J = 7.40$ Hz, C19-methyl protons), 1.92 (2H, m, C18-methylene protons), 3.82 (1H, s, C20–OH), 5.40 (2H, s, C5-methylene protons), 5.55 (2H, dd, $J = 14.21$, 14.21 Hz, C17-methylene protons), 7.70 (1H, s, C14-H), 7.92 (1H, t, $J = 8.40$ Hz, C11-H), 8.48 (1H, d, $J = 8.35$ Hz, C10-H), 8.55 (1H, d, $J = 8.35$ Hz, C12-H), 9.36 (1H, s, C7-H).

MS: m/z (%) = 393 (M^+, 100), 364 (M–C_2H_5, 35), 349 (48), 334 (25), 320 (25), 293 (35), 274 (8), 262 (8), 246 (15), 234 (6), 218 (20), 205 (8), 190 (9), 177 (5), 164 (3), 151 (3), 137 (5), 123 (4), 109 (5), 95 (5), 75 (3), 60 (23).

HRMS: Calcd. M^+ for $C_{20}H_{15}N_30_6$ 393.0960. Found 393.0961.

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