A SELECTIVE ONE-STEP INTRODUCTION OF HYDROXYLIC FUNCTIONS AT THE C-5 AND C-7 POSITIONS OF 20(S)-CAMPTOTHECIN, AN ALKALOID HAVING ANTITUMOR ACTIVITY [1]

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Abstract —— 20(S)-Camptothecin was selectively hydroxymethylated at the C-7 position by a simple process using hydrogen peroxide-methanol in the presence of ferrous ion as the radical source. Formation of diastereomers of 5-hydroxycamptothecin was observed by using ammonium persulfate-bromoacetic acid in the presence of ferrous or silver ion. The hydroxylic compounds prepared by the processes were converted further into acyloxyl, alkoxyl, and carboxylic acid derivatives.

20(S)-Camptothecin (1), an alkaloid isolated first from Camptotheca acuminata (NYSSACEAE) by Wall and co-workers in 1966,[2] has attracted much attention because of its significant inhibitory activity toward leukemia L1210 in mice and Walker 256 tumor in rats;[3] however, its high toxicity has restricted its clinical treatments.[4] Although many reports on total syntheses including chemical modifications of the original structure of 1 have appeared to date,[5] no particular progress seems to have been made to improve the therapeutical behavior. Recently Pei-chuang and co-workers successfully introduced hydroxyl, methoxyl, and chloro substituents at C-12 position by nitration and successive reduction and diazotization.[6] We wish to communicate here on a selective introduction of a hydroxymethyl group at the C-7 position and a hydroxyl group at the C-5 position of the naturally occurring 20(S)-camptothecin by a simple homolytic process.

Difficulties experienced in our earier attempts to alkylate 1 were mainly due to its poor reactivity to electrophilic substitution and also due to its insolubility in ordinary organic solvents. As pointed out by Minisci and Porta,[7] homolytic alkylation of heteroaromatic bases like quinoline can be performed in aqueous acidic media, where an alkyl radical adds at the electron deficient α- and γ- positions of the protonated bases. Thus, on treatment with hydrogen peroxide and ferrous sulfate in a methanol-sulfuric acid solution, 1 furnished 7-hydroxymethylcamptothecin (2) {m/e 378.1283 [M^+] for $C_{21}H_{18}N_2O_5$ = 378.1209} in an excellent yield. The formation of 2 was also observed either by heating with hydroxylamine-O-sulfonic acid in methanol-water[8] or by heating with ammonium persulfate in methanol-50% sulfuric acid.[9]

In typical procedure to prepare 2, 30% H_2O_2 (9 ml, 88.3 mmol) was added dropwise during 1 hr at 0°C into a stirred solution of 1 (1.00 g, 8.61 mmol) in methanol-aqueous sulfuric acid (MeOH : 50% H_2SO_4 = 30 ml : 50 ml), After stirring at room temperature for 8 hr, dilution of the reaction mixture (1 l) yielded a precipitate, which was purified by recrystallization from dimethylformamide-dioxane to furnish 890 mg (82% yield) of pale yellow crystals.

Acetylation of 2 with equimolar amounts of acetic anhydride at room temperature afforded 7-acetoxymethylcamptothecin (3) { pale yellow needles from $CHCl_3$-n-C_6H_{14}, m/e 420.1396 [M^+] for $C_{23}H_{20}N_2O_6$ = 420.1314, $[\alpha]_D$ +165.8° (c = 2 x 10^{-3} in EtOH)} . 7-Acetoxy-20-O-acetylcamptothecin (4) and monohemisuccinate (5) were also prepared with acetic and succinic anhydride, respectively. Chromic acid oxidation of 2 furnished camptothecin-7-carboxylic acid (6).

Another tactic of hydroxymethylation by glycolic acid in the presence of ammonium persulfate and silver ion[9] resulted in the formation of 5-hydroxycamptothecin (7) {m/e 364.1041 [M^+] for $C_{20}H_{16}N_2O_5$ = 364.1053} (10% yield) in addition to 2 (19% yield) and camptothecin 1-oxide[10] (trace amount). The nuclear magnetic resonance (NMR) spectra of 7 [δ ppm in DMSO-d_6 : 5.25 (1H. d, J = 17 Hz; C-17 H), 5.52 (1H, d, J = 17 Hz; C-17 H'), 6.66 (0.5H, s; C-5 H), 6.72 (0.5H, s; C-5 H'), 7.37 (1H, br s; C-14 H), 8.52 (1H, br s; C-7 H)] suggested the presence of approximately 1 : 1 mixture of anomers with respect to the hemiacetal configuration at the C-5 in solution. Attempted methylation and bromomethylation at C-7 in the presence of ammonium persulfate and silver or ferrous ion[9] resulted in oxigenation at C-5 to give 7 as the major product, neither 7-methyl- or 7-bromomethylcampto-

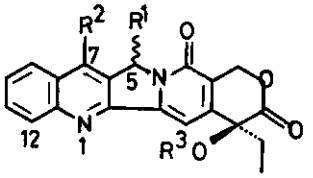
thecin being isolated.

In a typical procedure to prepare 7 consistently in 50-60% yield, a solution of ammonium persulfate (3.80 g, 3.69 mmol) in water was added dropwise during 3-4 hr at 100-110 C into a stirred solution of 1 (1.00 g, 2.87 mmol), $FeSO_4 \cdot 7H_2O$ (0.80 g, 2.87 mmol), and bromoacetic acid (13.9 g, 100 mmol) in 50% H_2SO_4 (40 ml). The aforementioned work-up followed by recrystallization from $CHCl_3$-n-C_6H_{14} afforded 661 mg (58.5% yield) of pale yellow crystals.

In the absence of bromoacetic acid in the above procedure,[11] the yield of 7 was extremely low, indicating that the bromomethyl radical plays an important role in abstraction of the hydrogen at the C-5 position.

By heating in methanol and in n-butanol in the presence of boron trifluoride etherate, 7 was converted quantitatively into 5-methoxy- and 5-n-butoxycampto-

Table. Camptothecin Derivatives.



	R^1	R^2	R^3
1	H	H	H
2	H	CH_2OH	H
3	H	CH_2OCOCH_3	H
4	H	CH_2OCOCH_3	$COCH_3$
5	H	$CH_2OCO(CH_2)_2COOH$	H
6	H	COOH	H
7	OH	H	H
8	OCH_3	H	H
9	OC_4H_9-n	H	H
10	$OCOCH_3$	H	H
11	$OCOCH_3$	H	$COCH_3$
12	$OCOC_6H_5$	H	H

thecins (8 and 9), respectively. Acetylation of 7 with equimolecular amounts of acetic anhydride at room temperature gave 5-acetoxycamptothecin (10). Diacetate (11) and 5-benzoylcamptothecin (12) were prepared in the usual manner. The NMR spectra of these alkoxy- and acyloxycamptothecin (8--12) indicated presence of approximately 1 : 1 mixture of a pair of epimers with respect to the anomeric center at the C-5 position.

The separation of the monoacetate (10) into two anomers was effected by means of high performance liquid chromatography (HPLC):[12] the first isomer to elute 10a {pale yellow needles, m/e 406.1134 $[M^+]$ for $C_{22}H_{18}N_2O_6$ = 406.1158, $[\alpha]_D$ -123° (c = 3.3 x 10^{-3} in EtOH)}, and the second isomer to elute 10b {pale yellow needles, m/e 406.1176 $[M^+]$, $[\alpha]_D$ +117° (c = 5.2 x 10^{-3} in EtOH)}, each showing a singlet methine signal at 7.91 and 7.96 ppm, respectively in the NMR spectra.

The present method provides a simple and facile process of modification of 1 to obtain completely novel derivatives without effecting any change on the functional groups on the D and E rings of the molecule. Detail discussions on the reaction mechanisms and other similar functionalizations together with the special feature of the activity and lower toxicity of these derivatives will be published elswhere.

References and Notes.

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12) Separation through HPLC was conducted under the following conditions: RiChrosorb SI-60, 8 mm x 50 cm, with $CHCl_3$ as an eluent, flow rate 6 ml/min. The retention times for 10a and 10b were 9.3 min and 10.9 min, respectively.

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