vs. time was determined. A half-life (minutes) was calculated from the slope of the regression line. The half-life values ($t_{1/2}$) for **2b** are 9.9 min (pH 6.0), 3.9 min (pH 6.6), 1.8 min (pH 7.1); for **1b** the values are 5.8 min (pH 5.1) and 1.8 min (pH 6.0). A statistical comparison between the two compounds shows a significant difference ($p < 0.05$) in the $t_{1/2}$ values at pH 6.0 but no significant difference in the dependence of half-life on pH. This indicates that although the two compounds exhibit a difference in their rate of reaction with cysteine, the mode of the reaction is the same.

Acknowledgment. This research was supported by the National Institutes on Drug Abuse. We thank Michael Powers and Mary Schwartz for the capable technical assistance.

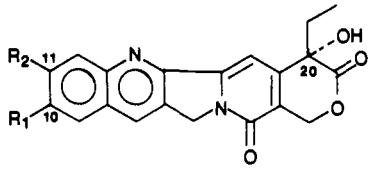
Plant Antitumor Agents. 22.[1] Isolation of 11-Hydroxycamptothecin from *Camptotheca acuminata* Decne: Total Synthesis and Biological Activity

Monroe E. Wall,* Mansukh C. Wani,* Susan M. Natschke,† and Allan W. Nicholas

Research Triangle Institute, Research Triangle Park, North Carolina 27709. *Received January 21, 1986*

The antitumor alkaloid 11-hydroxy-(20*S*)-camptothecin (4) was isolated from the woody tissue of *Camptotheca acuminata*. Structural proof derived from comparison with racemic **4** prepared by total synthesis. Antitumor activity of racemic **4** in L1210 leukemia in mice was considerably greater than that of natural (20*S*)-camptothecin (1) and its sodium salt. There was also no toxic effect observed even at relatively high doses.

Since the initial isolation from *Camptotheca acuminata* of the novel alkaloid camptothecin (**1**) in 1966,[2] there has been a continuing search for related compounds. Moreover, because of the potent activity of **1** in the resistant L1210 mouse leukemia assay, as well as in numerous other rodent leukemia and solid tumor systems,[3] there has also been interest in the total synthesis of **1** and analogues.[4] In addition to **1**, we have previously isolated as minor components 10-hydroxycamptothecin (**2**) and its 10-methoxy analogue **3** from *C. acuminata* bark and wood.[5] Other minor camptothecin analogues with structural differences at C-20 have been isolated by Hutchinson's group.[6] 9-Methoxycamptothecin has been isolated as a minor fraction from *Nothapodytes foetida* (Wight) Sleumer (Icacinaceae) (formerly *Mappia foetida* Miers).[7]

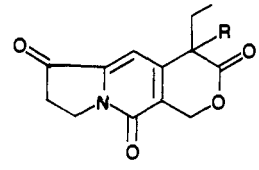


1, $R_1 = R_2 = H$
2, $R_1 = OH$; $R_2 = H$
3, $R_1 = OCH_3$; $R_2 = H$
4, $R_1 = H$; $R_2 = OH$
5, $R_1 = H$; $R_2 = OCH_3$

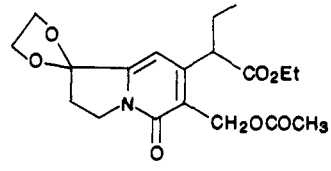
We now report the isolation of a new alkaloid 11-hydroxy-(20*S*)-camptothecin (**4**) in trace quantities from residual fractions of a large-scale extraction of *C. acuminata* bark and wood and the synthesis of the 20*R*,*S*-analogue.

Residual fractions remaining after a large-scale extraction and isolation of **1** from *C. acuminata* were analyzed by HPLC, primarily in search of the 10-hydroxy analogue **2**. A minor impurity was present with R_T values close to those of **2**, which could be concentrated to some extent in mother liquors after recrystallization. After repetitive HPLC, approximately 2 mg of pure compound was obtained. The exact mass determination of 364.1054 ($C_{20}H_{16}N_2O_5$) suggested a positional isomer of hydroxycamptothecin. Direct comparisons of the unknown with authenic 9-hydroxycamptothecin prepared by demethylation of 9-methoxycamptothecin eliminated the 9-position from consideration. The limited ^1H NMR analysis that could be conducted with the small available sample suggested that the compound might have structure **4**.

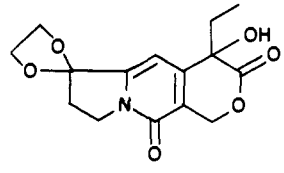
To further confirm structure **4**, racemic 11-methoxycamptothecin (**5**) was prepared by the acid-catalyzed Friedlander condensation of 4-methoxy-2-aminobenzaldehyde[5] with the key tricyclic ketone **7** in a manner previously described for the deoxy tricyclic ketone **6**.[8] The intermediate **7** was prepared from the bicyclic pyridone **8**[8] in two steps. Thus, simultaneous ring closure and oxygenation of **8** gave the tricyclic ketal **9**. Hydrolysis of the ketal moiety in **9** gave compound **7**. The methoxy functionality of **5** was cleaved in refluxing 48% aqueous HBr to afford racemic **4** after chromatographic purification. The 250-MHz ^1H NMR spectra (Me_2SO-d_6) of synthetic and natural **4** were superimposable. The melting points and TLC and HPLC properties were identical, as well as the UV and IR spectra.



6, R = H
7, R = OH



8



9

After our work was completed, a review by Cai and Hutchinson[4] noted that **4** was isolated by Chinese workers

†Present address: School of Pharmacy, The University of North Carolina, Chapel Hill, NC 27514.

(1) For paper 21, see: Meyer, B. N.; Wall, M. E.; Wani, M. C.; Taylor, H. L. *J. Nat. Prod.* **1985**, *48*, 952.
(2) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *94*, 3888.
(3) Private communication, Dr. M. Suffness, NCI.
(4) Cai, J. C.; Hutchinson, C. R. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1983; Vol. XXI, Chapter 4, p 101.
(5) Wani, M. C.; Wall, M. E. *J. Org. Chem.* **1969**, *34*, 1364.
(6) Adamovich, J. A.; Cina, J. A.; Hutchinson, C. R. *Phytochemistry* **1979**, *18*, 1085.
(7) Govindachari, T. R.; Ravindranath, K. R.; Viswanath, N. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1215.
(8) Wani, M. C.; Ronman, P. E.; Lindley, J. T.; Wall, M. E. *J. Med. Chem.* **1980**, *23*, 554.

0022-2623/86/1829-1553$01.50/0

Table I. Comparison of the Activities of (20*S*)-Camptothecin (1), Its Sodium Salt, and 11-Hydroxy-(20*RS*)-camptothecin (4) in L1210 Leukemia (6LE31)[a]

compd (NSC no.)	max % T/C (dose), mg/kg	active dose range, mg/kg	toxic dose, mg/kg
(20*S*)-camptothecin (1) (094600)	164 (8) no cures out of 6	2–8	16
(20*S*)-camptothecin sodium salt (100880)	178 (40) no cures out of 6	10[b]–40	80
11-hydroxy-(20*RS*)-camptothecin (365729)	357 (60) 3 cures out of 6	7.5[b]–60[c]	

[a] These compounds, NSC 094600, NSC 100880, and NSC 365729, were assayed in the 6LE31 tumor system at the Southern Research Institute under the same control. Compounds were administered ip to mice using Klucel emulsifier, treatment schedule Q04D × 02. [b] Lowest dose administered. [c] Highest dose administered.

from the fruit of *C. acuminata* and that a synthesis of **4** had been submitted for publication. No antitumor data for this compound are available from Chinese sources.

Biological Testing. (20*S*)-Camptothecin (1), (20*S*)-camptothecin, sodium salt, and 11-hydroxy-(20*RS*)-camptothecin (4) were simultaneously tested for activity in the L1210 mouse leukemia assay.[9] As shown in Table I, the racemic compound **4** exhibited much greater activity than **1** or the sodium salt, with 3 out of 6 cures and a very high % T/C, 357 (based on survival time). We have shown previously that camptothecin was about 5 times more potent than its sodium salt at equal doses in P388 leukemia.[8] The racemic 11-hydroxy analogue also exhibited a greater therapeutic index than camptothecin or the sodium salt. It should be noted that racemic camptothecin was about half as potent as (20*S*)-camptothecin.[8] Hence, it is likely that compound **4** of 20*S* configuration would be considerably more potent than racemic **4**. Since natural 10-hydroxycamptothecin has been shown to be more active and more potent than camptothecin in P388 mouse leukemia,[8] these results indicate that hydroxylation in ring A, at least at C-10 and C-11, is an important feature to be considered in the design of additional active compounds in the camptothecin series. This also suggests the study of other substituent effects at C-10 and C-11, and research in this area is actively in progress at our laboratory.

Experimental Section

Melting points were determined by using a Kofler hot-stage melting point apparatus. Thin-layer chromatography was carried out on precoated 0.25-mm layers of silica gel 60F-254 (Merck). Locations of spots on the chromatograms were determined by one or a combination of the following: short-wave UV, long-wave UV, and charring by heating plates sprayed with ceric sulfate and phosphomolybdic acid. ^{1}H NMR spectra were obtained on a Bruker 250 250-MHz nuclear magnetic resonanced spectrometer. Infrared spectra were obtained on a Perkin-Elmer 267 spectrophotometer, and exact mass determinations were made on a Ms-902 mas spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are correct within ±0.4% of theory. HPLC was performed on a Waters system incorporating a Model 6000A pump, a Model 450 absorbance detector at 254 nm, a Model U6K injector, and a Whatman Partisil PXS 10/25 PAC column or Whatman Partisil Magnum 9 PAC column. The PAC column was used in the normal-phase mode, employing mixtures of MeOH and $CHCl_3$ (or CH_2Cl_2).

11-Hydroxy-(20*S*)-camptothecin (4). A portion of Monsanto 1213770-8[10] (2.3 g) was chromatographed through a column of silica gel EM-360 (900 g). Fractions of 50-mL volume were collected by using an elution solvent of 5% methanol in chloroform. Fractions 100–130 gave 10-hydroxycamptothecin (**2**, 135 mg) containing about 10% of 11-hydroxycamptothecin (**4**). Recrystallization of the sample from 13% MeOH in chloroform gave 30 mg of pure **2**. A second crop yielded 53 mg of **2** which was contaminated with **4**. The mother liquor (24 mg) and a portion (18 mg) of crop 2 were processed in a number of portions by a Whatman Magnum 9 PAC column using 3% methanol in methylene chloride. Thus, 3 mg of 11-hydroxycamptothecin (**4**) was obtained as a tan solid. Trituration with chloroform–methanol afforded 2 mg of **4** from which the following data were obtained: mp 320–324 °C dec (lit.[11] mp 327–330 °C dec); $[\alpha]^{21}_D$ −30.7° (c, 0.0326, py); IR (KBr) 3450 (OH), 1742 (lactone), 1654 (pyridone), 1613–1592 (aromatic) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 0.88 (t, 3, *J* = 7 Hz, H-18), 1.85 (m, 2, H-19), 5.20 (s, 2, H-5), 5.41 (s, 2, H-17), 6.51 (br s, 1, 20-OH), 7.26 (dd, 1, *J* = 9, 2.5 Hz, H-10), 7.28 (s, 1, H-14), 7.36 (d, 1, *J* = 2.5 Hz, H-12), 7.95 (d, 1, *J* = 9 Hz, H-9), 8.52 (s, 1, H-7), 10.43 (s, 1, 11-OH). Anal. Calcd for $C_{20}H_{16}N_2O_5$, 364.1059. Found, 364.1054.

1,1-(Ethylenedioxy)-5-oxo-(5′-ethyl-5′-hydroxy-2′*H*,5′*H*,6′*H*-6-oxopyrano)[3′,4′-*f*]Δ^{6,8}-tetrahydroindolizine (9). The diester ketal *8* (405 mg, 1.07 mmol) from previous work[8] as a mixture with anhydrous potassium carbonate (148 mg, 1.07 mmol) and methanol (7.5 mL) was treated by bubbling oxygen for 24 h. The solution was cooled with an ice bath and made acidic (pH 2–4) by the addition of 1 N aqueous H_2SO_4. Most of the methanol was removed by reduced pressure at ambient temperature, and water (20 mL) was added. Extraction with methylene chloride (3 × 10 mL), drying (Na_2SO_4), and evaporation afforded a solid, which was recrystallized from methylene chloride–hexane to give to give **9** (280 mg, 85%) as a white solid: mp 179–181 °C; IR ($CHCl_3$) 1740, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3, *J* = 7 Hz, CH_2CH_3), 1.75 (q, 2, *J* = 7 Hz, CH_2CH_3), 2.35 (t, 2, *J* = 6.5 Hz, CH_2 α to ketal), 4.1 (m, 6, CH_2CH_2O and CH_2N), 5.30 (ABq, 2, *J* = 17 Hz, Δν = 85 Hz, CH_2 α to lactone O), 6.87 (s, 1, vinylic H). Anal. ($C_{15}H_{17}NO_6$) C, H, N.

1,5-Dioxo-(5′-ethyl-5′-hydroxy-2′*H*,5′*H*,6′*H*-6-oxopyrano)[3′,4′-*f*]Δ^{6,8}-tetrahydroindolizine (7). The intermediate ketal **9** (3.88 g, 12.6 mmol) as a solution in 2 N aqueous H_2SO_4 (50 mL) and dimethoxyethane (150 mL) was heated at 50 °C for 24 h under N_2. Half of the solvent was removed by reduced pressure, and the resulting solution was diluted with water (100 mL). Extraction with methylene chloride (5 × 50 mL), drying of the extract (Na_2SO_4), and evaporation gave a solid, which was recrystallized from methylene chloride–hexane to yield **7** (2.68 g, 80%) as a light-brown solid: mp 185–187 °C; IR ($CHCl_3$) 1750 (shoulder, ketone), 1745 (lactone), 1660 (pyridone) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3, *J* = 7 Hz, CH_2CH_3), 1.80 (q, 2, *J* = 7 HZ, CH_2CH_3), 2.93 (t, 2, *J* = 6.5 Hz, $CH_2C{=}O$), 4.30 (t, 2, *J* = 6.5 Hz, CH_2N). 5.35 (ABq, 2, *J* = 17 Hz, Δν = 85 Hz, CH_2 α to lactone O), 7.17 (s, 1, vinylic H). Anal. ($C_{13}H_{13}NO_5$) C, H, N.

11-Methoxy-(20*RS*)-camptothecin (5). A mixture of 4-methoxy-2-aminobenzaldehyde[5] (180 mg, 1.19 mmol) and the tricyclic ketone **7** (300 mg, 1.14 mmol) in toluene (18 mL) was heated under N_2 in a flask equipped with a Dean-Stark trap. At reflux, *p*-toluenesulfonic acid (5 mg) as added, and the red-brown solution was heated for an additional 2 h. The toluene was removed under reduced pressure to give a brown solid, which was treated with water (10 mL) and chloroform (20 mL). The aqueous phase was extracted with additional chloroform (3 × 20 mL), and the combined extracts were dried (Na_2SO_4). Evaporation gave a brown solid, which was recrystallized from methanol–chloroform to give **5** (216 mg, 50%) as a tan solid: 275–279 °C; IR (KBr) 3480 (OH), 1745 (lactone), 1660 (pyridone), 1622 (aromatic), 1236, 1152 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 0.87 (t, 3, *J* = 7 Hz, H-18), 1.85 (m, 2, H-19), 3.95 (s, 3, 11-OCH_3), 5.24 (s, 2, H-5), 5.42 (s, 2, H-17), 7.32 (s, 1, H-14), 7.37 (dd, 1, *J* = 9, 2.5 Hz, H-10), 7.56 (d, 1, *J* = 2.5 Hz, H-12), 8.02 (d, 1, *J* = 9 Hz, H-9), 8.60 (s, 1, H-7). Anal.

(9) Tests were carried out at the Southern Research Institute under NCI contract.

(10) This chromatographic fraction, enriched in 10-hydroxy-(20*S*)-camptothecin, was obtained by Monsanto during the large-scale fractionation of extracts from the wood and bark of *C. acuminata*.

(11) Lin, L. T.; Sung, C. C.; Hsu, J. S. *K'o Hsueh T'ung Pao (Chin. Ed.)* **1979**, *24*, 478.

Calcd for $C_{21}H_{18}N_2O_5$, 378.1214. Found, 378.1219. ($C_{21}H_{18}N_2O_5$) C, H, N.

11-Hydroxy-(20*RS*)-camptothecin (4). The methoxy compound **3** (75 mg) was combined with 48% aqueous HBr (2.5 mL) and heated at reflux for 6 h. The red-brown mixture was stripped of solvent under high vacuum. Chromatography of the residue through silica gel (15 g) (7% MeOH–$CHCl_3$) gave compound **4** (33 mg, 45%) as a beige solid, which was further purified by recrystallization from 13% MeOH in $CHCl_3$: mp 323–326 °C (lit.[11] mp 327–330 °C dec); IR (KBr) 3450 (OH), 1742 (lactone), 1654 (pyridone), 1613–1592 (aromatic), 1570, 1245 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.85 (m, 2, H-19), 5.20 (s, 2, H-5), 5.41 (s, 2, H-17), 6.51 (br s, 1, 20-OH), 7.26 (dd, 1, J = 9, 2.5 Hz, H-10), 7.28 (s, 1, H-14), 7.36 (d, 1, J = 2.5 Hz, H-12), 7.95 (d, 1, J = 9 Hz, H-9), 8.52 (s, 1, H-7), 10.43 (s, 1, 11-OH). Anal. Calcd for $C_{20}H_{16}N_2O_5$, 364.1059. Found, 364.1054. ($C_{20}H_{16}N_2O_5 \cdot 1.0H_2O$) C, H, N.

Acknowledgment. The studies reported in this paper were supported under PHS Grant R01-CA29890, awarded by the National Cancer Institute, DHHS. We wish to thank Harold Taylor for technical assistance, Dr. James Ellard, Monsanto Research Corporation, for the enriched 10-hydroxycamptothecin fractions and data relating to these fractions, and Dr. Matthew Suffness, Chief, Natural Products Research, DTP, DCT, NCI, for valuable discussions and procurement of antitumor assays.

Additions and Corrections

1986, Volume 29

Utpal Sanyal, Saktimoyee Mitra, Prasun Pal, and S. K. Chakraborti*: New α-Methylene γ-Lactone Derivatives of Substituted Nucleic Acid Bases as Potential Agents.

Page 595. This paper was published in error as a Communication. It should have been published as a Note.

Book Reviews

Drug Fate and Metabolism. Methods and Techniques. Volume 5. Edited by E. R. Garrett and J. L. Hirtz. Marcel Dekker, New York. 1985. xv + 345 pp. 16 × 23.5 cm. ISBN 0-8247-7423-x. $79.50.

This book is the latest volume in a continuing series of monographs concerning practical approaches to the study of drug metabolism and pharmacokinetics. As with the previous volumes of this series, the editors have maintained a similar format by reviewing specific areas of interest to those investigators who are interested in recent developments and techniques in this field.

This volume contains six well-written chapters. The first chapter entitled "Pharmacokinetic Procedures and Strategies" written by E. R. Garrett gives very practical suggestions for the design and evaluation of pharmacokinetic studies. Rather than presenting a series of pharmacokinetic equations which can be found elsewhere, Dr. Garrett has developed a general outline for performing pharmacokinetic studies including useful comments on drug assay and protocol design. The section on the evaluation of pharmacokinetic studies is well written and the addition of simulations and literature examples help further to clarify the theoretical equations which are given in the Appendix of this chapter. The second chapter, by D. Riad-Fahmy and G. F. Read reviews the most recent approaches for the performance of radio- and enzyme immunoassay. Although the authors give a table summarizing most of the commercially available kits for performing immunoassay procedures for therapeutic drug monitoring, the emphasis of this chapter is concerned mainly with factors necessary for the development of specific immunoassay procedures within the laboratory. Topics included in this chapter are the production of antisera, the preparation of antigen labels, the general format of immunoassays, and assay validation. An overview on the principles of radioactivity measurement in biological experiments is presented in the third chapter, by L. Botta, H.-U. Gerber, and K. Schmid. This chapter discusses primarily liquid scintillation counting including basic principles, instrumentation, and measurement. A brief note is given by the authors on autoradiography and column chromatography.

In Chapter 4, S. L. Beal and L. B. Scheiner describe in detail data analysis methods applicable to population pharmacokinetic studies, with emphasis on the standard two-stage method and the first-order method. These methods are usable even when the number of responses per patient are small. Each method is given a rigorous mathematical derivation for the general case. In addition, Appendix III of this chapter applies the first-order method to a pharmacokinetic study with specific equations for several pharmacokinetic parameters. This chapter represents a useful reference containing the theoretical framework for a blend of statistics and pharmacokinetics that should have utility for the interpretation of actual patient data. B. F. H. Drenth and R. A. deZeeuw provide an overview of high-pressure liquid chromatography in Chapter 5. A theoretical introduction is followed by sections containing practical comments on stationary and mobile phases, the HPLC apparatus, derivitization, and sample preparation. The longest section emphasizes applications to drug fate and metabolism, including quantitative aspects. Also included in this chapter is a table of stationary phases used for the assay of more commonly used drugs in biological media. The final chapter, by A. Gouyette, gives the current status of mass spectrometry as a universal and sensitive detector for liquid chromatography. The author discusses off-line and on-line techniques and their limitations. Included in this chapter are a summary and three extensive tables of applications of these techniques