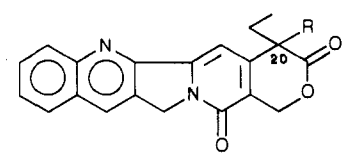
Plant Antitumor Agents. 28.[1] Resolution of a Key Tricyclic Synthon, 5′(*RS*)-1,5-Dioxo-5′-ethyl-5′-hydroxy-2′*H*,5′*H*,6′*H*-6′-oxopyrano[3′,4′-*f*]$\Delta^{6,8}$-tetrahydroindolizine: Total Synthesis and Antitumor Activity of 20(*S*)- and 20(*R*)-Camptothecin

Mansukh C. Wani,* Allan W. Nicholas, and Monroe E. Wall*

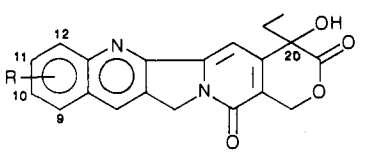
Research Triangle Institute, Research Triangle Park, North Carolina 27709. *Received June 16, 1987*

The resolution of the tricyclic ketone (**3a** + **3b**) by the separation of diastereomeric adducts **4a** and **4c** of the precursor ketal **5** is described. The regenerated enantiomers **3a** and **3b** of 100% optical purity represent the key intermediates from which 20(*R*)-camptothecin (**1a**) and 20(*S*)-camptothecin (**1b**), respectively, have been prepared. The 20*R* analogue **1a** was 10–100 times less active than the natural 20(*S*)-camptothecin (**1b**) in 9KB and 9PS cytotoxicity assays and almost inactive in in vivo L-1210 leukemia tests as compared to the highly potent and active natural compound **1b**.

20(*S*)-Camptothecin (**1b**) is a potent antitumor agent isolated by Wall and co-workers[2] 20 years ago. During subsequent years, a number of synthetic approaches to this novel pentacyclic system have been reported.[3] The majority of these methods invariably lacked the versatility of generating analogues of potentially greater activity and lower toxicity than the parent **1b**. Among these approaches of limited practical utility, only one[4] has afforded the actual 20*S* system rather than the 20*RS* racemate **2a**.



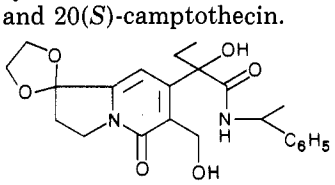
1a: 20(*R*); R = ◀OH
b: 20(*S*); R = ıılıOH



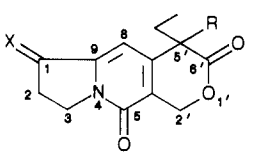
2a: R = H[20(*RS*)]
b: R = 10-NH_2[20(*RS*)]
c: R = 10,11-OCH_2O-[20(*RS*)]

Ongoing research in our laboratory has led to the development of a facile synthetic route to the racemic tricyclic ketone **3**,[5,6] a key intermediate that was employed to give ready access to a host of ring A substituted racemic camptothecin analogues such as **2b** and **2c**.[5-8] Even though some of these racemic analogues (e.g., **2b** and **2c**) showed extremely high activity and potency, 20(*RS*)-camptothecin (**2a**) was only half as potent as natural 20-(*S*)-camptothecin (**1b**).[5] This finding suggested that only the 20*S* isomer exhibited the desired antitumor activity.

As part of a study designed to generate only the presumed "active" enantiomers of analogues **2**, we planned the resolution of the key intermediate **3** into **3a** and **3b**, thereby giving access to each *R* and *S* enantiomer for biological evaluation. In this paper, we present the details of resolution of the tricyclic ketone **3** and determination of the absolute stereochemistry by conversion into 20-(*S*)-camptothecin (**1b**) and 20(*R*)-camptothecin (**1a**). We also present for the first time a comparison of the in vitro cytotoxic and in vivo L-1210 antileukemic activity of 20(*R*)- and 20(*S*)-camptothecin.



4a: *RR*
b: *SR*
c: *SS*
d: *RS*



3a: 5′(*R*); X = O; R = ◀OH
b: 5′(*S*); X = O; R = ıılıOH
5a: 5′(*R*); X = OCH_2CH_2O; R = ◀OH
b: 5′(*S*); X = OCH_2CH_2O; R = ıılıOH

Chemistry. The racemic ketal **5** was prepared as described earlier[5,6] and reacted with (*R*)-(+)-α-methylbenzylamine to give the diastereomeric amide pair (*R*,*R*)-**4a** and (*S*,*R*)-**4b**. The isomers were well-resolved by normal-phase HPLC analysis, and a partial separation of the two components on a preparative scale (100–150 mg) was achieved.

During an attempt to purify the less polar diastereomer by trituration with toluene, the solid obtained was unexpectedly found by HPLC to be enriched in the more polar isomer. This result suggested that toluene may effectively precipitate the more polar isomer. Indeed, the partially purified polar isomer was completely freed of the other isomer by two such treatments. In fact, trituration of an equimolar mixture of the (*R*,*R*)-**4a** and (*S*,*R*)-**4b** diastereomers with toluene gave a solid, which was found to be predominantly (85–90%) the more polar isomer by HPLC.

Mild acid treatment of the remaining crude less polar diastereomer gave partially resolved tricyclic ketal **5**, which was then reacted with (*S*)-(−)-α-methylbenzylamine as before. The more polar *S* amide now in excess was the optical isomer of the polar *R* amide from before.

The configurations of the two pure amides from (*R*)- and (*S*)-α-methylbenzylamines were established as follows. The pure polar isomer from the *R* amine, (*R*,*R*)-**4a** or (*S*,*R*)-**4b** was treated with acetic acid to give the *R* tricyclic ketal **5a** or *S* ketal **5b**. More vigorous acid hydrolysis gave the *R* tricyclic ketone **3a** or *S* ketone **3b**. A one-step acid-catalyzed Friedlander condensation of this tricyclic ketone with *o*-aminobenzaldehyde gave camptothecin with

(1) For preceding paper of this series, see: Wall, M. E.; Wani, M. C.; Taylor, H. *J. Nat. Prod.*, in press.
(2) 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.
(3) For reviews of these methods, see: Cai, J. C.; Hutchinson, C. R. In (a) *The Alkaloids*: Brossi, A., Ed.; Academic: New York, 1983; Vol. XXI, Chapter 4; (b) *Chem. Heterocycl. Compd.* **1983**, *25*, 753.
(4) Corey, E. J.; Crouse, D. E.; Anderson, J. E. *J. Org. Chem.* **1975**, *40*, 2140.
(5) Wani, M. C.; Ronman, P. E.; Lindley, J. T.; Wall, M. E. *J. Med. Chem.* **1980**, *23*, 554.
(6) Wall, M. E.; Wani, M. C.; Natschke, S. M.; Nicholas, A. W. *J. Med. Chem.* **1986**, *29*, 1553.
(7) Wani, M. C.; Nicholas, A. W.; Wall, M. E. *J. Med. Chem.* **1986**, *29*, 2358.
(8) Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Wall, M. E. *J. Med. Chem.*, in press.

0022-2623/87/1830-2317$01.50/0

Table I. Comparison of the L-1210 Mouse Leukemia Life-Prolongation Activity of 20(*S*)-Camptothecin, 20(*S*)-Camptothecin Sodium Salt, and 20(*R*)-Camptothecin

20*R*		20*S*		20*S* sodium salt	
dose,[a] mg/kg	T/C[b]	dose,[a] mg/kg	T/C[b]	dose,[a] mg/kg	T/C[b]
20	132	10	250	80	215
10	118	5	185	40	181
5	114	2.5	147	20	167
2.5	114	1.25	129	10	126

[a] A total of two doses were administered ip on the first and fifth days of the study. [b] T/C is defined as the mean survival time of treated animals divided by the mean survival time of the controls times 100.

$[\alpha]^{21}_D$ −39°. Since natural camptothecin has a known 20*S* configuration and $[\alpha]^{25}_D$ +34°,[2] this sequence leading to unnatural 20(*R*)-camptothecin can now be represented unequivocally as follows: (*R*,*R*)-**4a** → (*R*)-**5a** → (*R*)-**3a** → (*R*)-**1a**.

In like manner, when the pure polar amide isomer from the *S* amine was carried through this sequence, natural 20(*S*)-camptothecin (**1b**), $[\alpha]^{21}_D$ +39°,[9] resulted. Hence, the following sequence was confirmed: (*S*,*S*)-**4c** → (*S*)-**5b** → (*S*)-**3b** → (*S*)-**1b**.

Biological Testing. The 9KB and 9PS (in vitro) cell and L-1210 leukemia (in vivo) assays were conducted by contractors for the National Cancer Institute by standard procedures.[10] The ED_{50} values for 20(*S*)-camptothecin (**1b**) and 20(*R*)-camptothecin (**1a**) in the 9KB assay were 4×10^{-2} and 4×10^{-1} μg/mL, respectively, and in 9PS, 3×10^{-2} and 2.4×10^{0} μg/mL, respectively. Thus natural 20(*S*)-camptothecin was approximately 10–100-fold more potent than the 20*R* enantiomer in the two in vitro assays. Table I compares 20(*S*)-camptothecin (**1b**) and its corresponding sodium salt with the 20*R* enantiomer **1a** in the L-1210 mouse leukemia assay. The 20*R* isomer was almost inactive at 20 mg/kg. In contrast, both 20(*S*)-camptothecin (**1b**) and the sodium salt were much more active. It is of interest, as was noted earlier in a P388 leukemia mouse assay,[5] that camptothecin (**1b**) is about 8 times more potent than the corresponding sodium salt in the L-1210 mouse leukemia assay, reemphasizing the importance of the intact α-hydroxy lactone moiety for antitumor activity.[5] In addition, it now is certain that the configuration at C-20 must be *S* for antitumor activity.

Experimental Section

Melting points were taken on a Kofler hot-stage microscope and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 267 spectrophotometer, and ^{1}H NMR spectra were determined on a Bruker 250 spectrometer at 250 MHz. Optical rotations were measured on a Rudolph Autopol III automatic polarimeter with a 1-cm^3 capacity quartz cell (10-cm path length). Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA. Analytical HPLC analyses were performed on a Waters system incorporating a Model 6000A pump, a Model 450 absorbance detector at 254 nm, and a Whatman Partisil 5 silica column (4.6 × 250 mm). The solvent system was 15% ethanol in hexanes at 2.0 mL/min. Radial preparative thick-layer chromatography was performed on a Harrison Chromatotron with a rotor coated to a 2-mm thickness (Merck silica gel 60, PF-254); column chromatography was carried out under medium pressure on a Merck Lobar Lichroprep Si 60 size B column. Preparative separation attempts employed 0.5–1.5% methanol in chloroform solvent mixtures.

(*R*)-(+)- and (*S*)-(−)-α-methylbenzylamine were purchased from Aldrich Chemical Co. Racemic tricyclic ketal **5** was obtained during previous studies in this laboratory.[5,6]

Amide Adduct 4a. A stirred suspension of the racemic ketal **5** (500 mg, 1.629 mmol) in (*R*)-(+)-α-methylbenzylamine (3.0 mL) was heated under nitrogen to 70 °C whereupon a pale yellow solution resulted. Heating at 70 °C was maintained for 20 h, and the excess amine was removed by high vacuum distillation to afford the crude mixture of diastereomers **4a** (*R*,*R*) and **4b** (*S*,*R*) as an orange syrup. Partial separation of **4a** and **4b** (~75% purities) on a 100-mg scale could be effected by radial thick-layer chromatography or medium-pressure silica column with 0.5–1.5% methanol in chloroform. The relative proportions of **4a** and **4b** were readily determined analytically by HPLC on Partisil 5 with 15% ethanol in hexane. The *R*,*R* isomer **4a** was the last to elute (**4b**, t_R 10 min; **4a**, t_R 11 min). A syrupy mixture of **4a** and **4b** obtained from **3** (500 mg) was dissolved in toluene (20 mL), and after 10 min the precipitated *R*,*R* isomer **4a** (215 mg, 62%) of 95% purity was removed by filtration. An optical purity of 100% was achieved by dissolving the sample in a minimum quantity of warm methylene chloride (0.5 mL) followed by dilution with toluene (5 mL). The pure isomer **4a** crystallized as clear, colorless needles (138 mg): mp 121–127 °C; IR ($CHCl_3$) 3410, 3540–3080 (NH, OH), 3000, 2980, 2900 (CH), 1658, 1650 (amide, pyridone), 1588 (aromatic), 1513, 1505, 1318, 1030 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, *J* = 7 Hz, CH_2CH_3), 1.50 (d, 3 H, *J* = 7 Hz, Ar(N)CHCH_3), 2.03 (m, 1 H, HCHCH$_3$), 2.18 (m, 1 H, HCHCH$_3$), 2.33 (t, 2 H, *J* = 7 Hz, CH_2CH_2N), 3.97 (t, 2 H, *J* = 7 Hz, CH_2CH_2N), 4.12 (m, 4 H, OCH_2CH_2O), 4.73 (nd, 1 H, *J* = 13 Hz, ArHCHOH), 5.03 (d, 1 H, *J* = 13 Hz, ArHCHOH), 5.06 (m, 1 H, Ar(N)CHCH$_3$), 5.93 (br s, 1 H, tertiary OH), 6.67 (s, 1 H, pyridone *H*), 7.32 (m, 5 H, aromatic), 7.48 (d, 1 H, *J* = 8 Hz, N*H*); $[\alpha]^{21}_D$ +56° (*c* 0.392, $CHCl_3$/MeOH, 4:1). Anal. ($C_{23}H_{28}N_2O_6 \cdot H_2O$) C, H, N.

5′(*R*)-1,5-Dioxo-5′-ethyl-5′-hydroxy-2′*H*,5′*H*,6′*H*-6′-oxopyrano[3′,4′-*f*]Δ^{6,8}-tetrahydroindolizine (3a). A solution of **4a** (33 mg, 0.0771 mmol) in glacial acetic acid (1 mL) was heated at 70 °C for 2 h, and the solvent was removed in vacuo. The gummy product was dissolved in methylene chloride (5 mL), and the solution was washed with water (1 mL). Drying (Na_2SO_4) and evaporation afforded **5a** as a beige foam (23 mg, 97%), which crystallized as colorless short needles from ethyl acetate. Compound **5a** was chromatographically identical with the authentic racemic **5**:[6] mp 166–167 °C [lit.[6] mp for racemate 179–181 °C]; $[\alpha]^{21}_D$ −70° (*c* 0.333, $CHCl_3$/MeOH, 4:1).

The ketal group was cleaved by treatment of **5a** (23 mg, 0.0749 mmol) with dimethoxyethane (1 mL) and 2 N aqueous sulfuric acid (0.4 mL) at 50 °C under nitrogen for 8 h. The organic solvent was removed in vacuo, and the aqueous phase was extracted with chloroform (2 × 5 mL). The extract was dried (Na_2SO_4) and evaporated to yield the tricyclic ketone **3a** (19 mg, 96%) as a pale foam. Recrystallization of the sample from ethyl acetate gave **3a** as near-colorless prisms having chromatographic and spectral properties identical with authentic racemate **3**: mp 169–170 °C [lit.[6] for racemate 185–187 °C]; $[\alpha]^{21}_D$ −96° (*c* 0.3167, $CHCl_3$/MeOH, 4:1). Compound **3a** can be obtained directly from 4a by heating with sulfuric acid/dimethoxyethane.

The *R* configuration of **3a** was established by its reaction with *o*-aminobenzaldehyde by standard methods[5,6] to give 20(*R*)-camptothecin. Thus, a mixture of **3a** (134 mg, 0.510 mmol) and freshly prepared *o*-aminobenzaldehyde (200 mg, 1.653 mmol) was refluxed in toluene (20 mL) and treated with *p*-toluenesulfonic acid (5 mg). After 1 h, the solvent was removed in vacuo and the dark residue was chromatographed (SiO_2, 1% MeOH in $CHCl_3$) to afford 20(*R*)-camptothecin (**1a**) as a tan solid (128 mg, 72%). Recrystallization from methanol/chloroform gave **1a** as a beige powder: $[\alpha]^{21}_D$ −39° (*c* 0.3, $CHCl_3$/MeOH, 4:1). The other physical data measured (mp, TLC, and ^{1}H NMR) were identical with those of the natural alkaloid **1b**.

Amide Adduct 4b. The toluene mother liquor from isolation of **4a** above was concentrated in vacuo to give crude **4b** (*S*,*R* isomer) containing ~20% of **4a** (*R*,*R*) as a yellow syrup (305 mg). The syrup was dissolved in glacial acetic acid (8 mL), and the stirred solution was heated under nitrogen at 70 °C for 7 h to regenerate **5** enriched in the *S* isomer **5b**. The reaction mixture

(9) The observed rotation in the present study is higher than the one reported previously.[2] This discrepancy may be due to one or a combination of several factors such as solvent composition, concentration, temperature, and the instrument.

(10) Geran, R. I.; Greenberg; N. H.; McDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 1.

was evaporated under high vacuum to give a pale orange syrup, which was partitioned between water (2 mL) and chloroform (10 mL). The organic phase was isolated, dried (Na_2SO_4), and evaporated to afford **5b** contaminated with **5a** as an orange gummy foam (240 mg). The mixture was treated with (*S*)-(−)-α-methylbenzylamine (7 mL), and the stirred solution was heated at 75 °C for 20 h. Excess amine was removed by high vacuum distillation to afford an orange-red syrup consisting of the *S,S* isomer **4c** contaminated with the *R,S* compound **4d**. The sample was dissolved in toluene (5 mL) at room temperature, and within 10 min nearly pure **4c** had precipitated. The beige, powdery solid was dissolved in warm methylene chloride (0.5 mL) and diluted with toluene (5 mL). The clear, colorless needles that separated (110 mg) were composed of **4c** with no evidence of contamination by **4d** as determined by HPLC analysis (Partisil 5, 15% ethanol/hexanes). As expected, the enantiomeric pairs **4a** (*R,R*) and **4c** (*S,S*) were chromatographically and analytically identical except for $[\alpha]_D$ values; $[\alpha]^{21}_D$ for **4c** −56° (*c* 0.392, $CHCl_3$/MeOH, 4:1), vs +56° obtained for **4a**. Anal. (**4c**) ($C_{23}H_{28}N_2O_6 \cdot H_2O$) C, H, N.

5′(*S*)-1,5-Dioxo-5′-ethyl-5′-hydroxy-2′*H*,5′*H*,6′*H*-6′-oxopyrano[3′,4′-*f*]$\Delta^{6,8}$-tetrahydroindolizine (3b). A solution of **4c** (36 mg, 0.0841 mmol) was treated with glacial acetic acid (1 mL) at 70 °C as described for **4a**. The ketal **5b** resulted as a beige foam (26 mg), which crystallized as rosettes of short colorless needles from ethyl acetate. The *S* isomer **5b** was analytically identical with **5a** except for $[\alpha]^{21}_D$, which was determined to be +70° (*c* 0.250, $CHCl_3$/MeOH, 4:1).

As described for **5a**, the ketal functionality in **5b** was hydrolyzed by heating in dimethoxyethane/2 N sulfuric acid at 50 °C for 8 h. Tricyclic ketone **3b** was isolated as pale tan foam (20 mg), which was recrystallized from ethyl acetate to give the pure product as colorless prisms. Except for $[\alpha]^{21}_D$ +96° (*c* 0.4, $CHCl_3$/MeOH, 4:1), isomer **3b** was identical with **3a** and differed from authentic racemic **3** with respect to mp 169–170 °C vs 185–187 °C. As described for ketone **3a**, compound **3b** can be obtained directly from the amide **4c** by using strongly acidic conditions.

The *S* configuration of **3b** was confirmed by the generation of 20(*S*)-camptothecin (**1b**) of $[\alpha]^{21}_D$ +39° from the condensation of **3b** with *o*-aminobenzaldehyde. Thus, a mixture of **3b** (96 mg, 0.365 mmol) and freshly prepared *o*-aminobenzaldehyde (105 mg, 0.868 mmol) was refluxed in toluene (20 mL), and *p*-toluenesulfonic acid (5 mg) was added. After 2 h, the reaction was worked up and the product **1b** was isolated as for the *R* enantiomer **1a**. Synthetic **1b** was obtained as a tan powder (62 mg, 57%): $[\alpha]^{21}_D$ +39° (*c* 0.2917, $CHCl_3$/MeOH, 4:1), with all other properties being identical with natural **1b**.[9]

Acknowledgment. Support of this research by U.S. Public Health Service Research Grant R01-CA38996-02 from the National Cancer Institute is gratefully acknowledged. We thank Drs. Matthew Suffness and Gordon Cragg, NCI, DCT, for their general interest and assistance in procuring in vitro and in vivo bioassays.

Registry No. 1, 31456-25-4; **1a**, 110351-92-3; **1b**, 7689-03-4; **3a**, 110351-91-2; **3b**, 110351-94-5; **4a**, 110314-08-4; **4b**, 110314-09-5; **4c**, 110314-10-8; **5a**, 110351-90-1; **5b**, 110351-93-4; (*R*)-(+)-α-methylbenzylamine, 3886-69-9; *o*-aminobenzaldehyde, 529-23-7; (*S*)-(−)-α-methylbenzylamine, 2627-86-3.

Additions and Corrections

1987, Volume 30

Tai-Shun Lin,* Ming S. Chen, Colin McLaren, You-Song Gao, Ismail Ghazzouli, and William H. Prusoff: Synthesis and Antiviral Activity of Various 3′-Azido, 3′-Amino, 2′,3′-Unsaturated, and 2′,3′-Dideoxy Analogues of Pyrimidine Deoxyribonucleosides against Retroviruses.

Page 441. In Scheme II, structure **19**, the N-3 position of the pyrimidine base is bonded with H; therefore, it should be HN, not ON.

Page 442. Compound **16** listed under the structure (Figure 1) should be compound **15**.

Page 441. Reference 16 "Dube, S. L." should be Dube, S. K.

Page 442. Column 2, line 8 from bottom of the text, "Whereas Wager et al.", should be "Waqer".