Synthetic Studies Leading to DE-Ring Analogs of Camptothecin (1)

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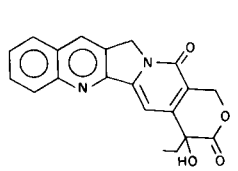
A synthetic scheme leading to an isomeric DE ring (**14**) of the anti-tumor alkaloid, camptothecin, has been achieved. However, the method failed to give the correct pyridone lactone (**2**) found in this alkaloid.

Although there are now a number of totally synthetic approaches (2-8) to the alkaloid camptothecin (**1**) isolated by Wall in 1966 (9), interest in this potential antitumor agent continues in an effort to prepare suitable analogs. In two recent reports, Wall (10) and Sugasawa (7) described synthetic approaches to the DE ring system of camptothecin (**2**). Interestingly, the bicyclic pyridone (**2**, R = H) was found to have weak but definite cytotoxic activity (1/100th that of **1** (10). We wish to describe our own



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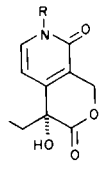
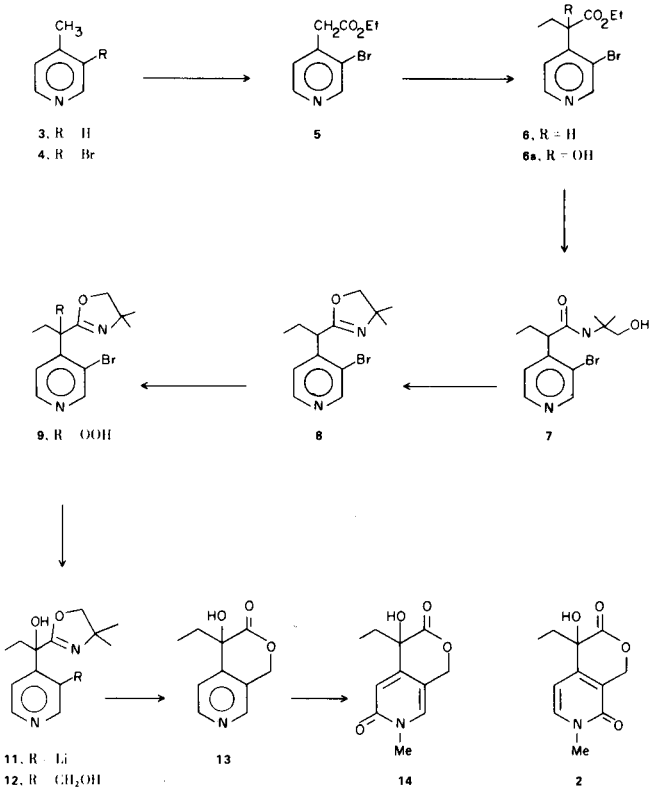


2 (R = H, Me)

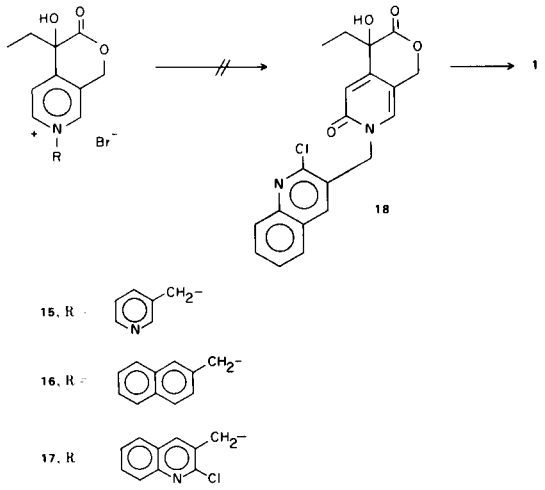
efforts toward the synthesis of DE ring analogs (**13**,**14**) by a different approach which arose from our studies on the total synthesis of camptothecin (8).

The sequence began by brominating 4-picoline (**3**) by the method of Pearson (11) affording 3-bromopicoline (**4**) in quantities sufficient for further use. Treatment of the latter with lithium diisopropylamide in THF followed by addition of diethyl carbonate produced ester **5** in good yield. The α-ethyl derivative **6** was obtained in excellent yield when **5** was converted to its sodium salt (sodium hydride-DMF) and treated with ethyl iodide. The synthetic strategy now called for masking the carboxyl group in **6** and this was done by first forming the hydroxy amide **7** (2-amino-2-methyl-1-propanol, 145°) and then cyclization to the oxazoline **8** using thionyl chloride (0-5°). The overall conversion of **6** to **8** was 77%. In order to introduce the tertiary hydroxyl group to obtain **10**, the oxazoline **8** was oxidized as its potassium salt (*t*-butyl alcohol, THF, oxygen) in 92% yield to the hydroperoxide **9** which was cleanly and quantitatively reduced to the hydroxy derivative **10** merely by stirring with alcoholic sodium borohydride.



3, R = H; **4**, R = Br — **5** — **6**, R = H; **6a**, R = OH — **7** — **8** — **9**, R = OOH — **10** — **11**, R = Li; **12**, R = CH_2OH — **13** — **14** — **2**

Intorduction of the 3-hydroxymethyl substituent in **12** was the next key step in the synthesis. The required transformation was based upon the stability of oxazolines toward organo-metallic reagents (12). First, the hydroxyl proton in **10** was removed by introducing 1.0 equivalent of sodium hydride to a THF solution containing **10**. After hydrogen evolution had ceased, the solution was cooled to -78° and phenyllithium was added to effect a halogen-metal interchange resulting in the lithio derivative **11**. Addition of paraformaldehyde followed affording the hydroxymethyl derivative **12**. Purification of the latter was found to be tedious and inefficient and it was, there-



fore, directly hydrolyzed in ethanol containing 10% sulfuric acid. This treatment produced the pyridine lactone **13** in 60% overall yield from **10**. When the pyridine ester **6** was subjected to the potassium *t*-butoxide-oxide oxidation (as in **8** → **9**), it also proceeded in excellent yield as did the reduction of the intermediate hydroperoxide to give **6a**. However, when **6a** was treated with phenyllithium-paraformaldehyde (as in **10** → **12**), the result was a mixture of many products. Hence, the utility of the oxazoline as a masking group for carboxylic derivatives was clearly demonstrated.

Studies were initiated to transform the now readily available pyridine lactone **13** into the DE-ring system of camptothecin **2**. It was felt that the *N*-methyl salt of **13** should be oxidized to the pyridone **2** or **14**. the latter being an isomer of the camptothecin DE-ring moiety. This indeed proved to be partially correct, since ferricyanide oxidation of the *N*-methyl quaternary salt of **13** gave **14** in 48% yield with only a trace of **2** (7) being detectable. Numerous attempts failed to alter the product distribution of **14** and **2** and this was attributed to steric effects present in **13** which made the "wrong" α-position more susceptible to oxidation. In the event that a larger *N*-alkyl group on **13** might serve to reverse the site of oxidation, the preparation of the *N*-(3-pyridylmethyl) **15**, *N*-(2-naphthylmethyl) **16**, and the *N*-(3-quinolylmethyl) **17** quaternary salts were investigated.

Although **15**, **16** and **18** could only be obtained as hygroscopic salts, they were subjected as such to ferricyanide oxidation under various conditions and failed to produce any significant amount of either pyridone. Unfortunately, the *N*-methyl salt of **13** appeared to have been a unique case. It is not certain, however, whether continued efforts would bear fruit in the sequence leading to **18** since the latter would be, if successfully prepared (13), a reasonable precursor to camptothecin. Additional studies on this subject are not planned.

EXPERIMENTAL (14)

3-Bromo-4-picoline (**4**).

The bromination of 4-picoline was carried out by a slightly modified application of the method of Pearson, *et al.* (11). A 2-liter three-necked flask was equipped with a mechanical stirrer, a thermometer, and a reflux condenser topped with a pressure-equalized addition funnel. To 455 g. (3.4 moles) of aluminum chloride was added, with vigorous stirring under nitrogen, over 60 minutes, 125 g. (1.33 moles) of 4-picoline. The resulting mixture was maintained at 95-105° while 128 g. (0.8 mole) of bromine was introduced through the condenser over about 5 hours. The reaction mixture was stirred at 95-100° for 15 hours, after which an additional 80 g. (0.5 mole) of bromine was added over 3 hours. After stirring for another 20 hours at 95-100°, the mixture was cooled and poured over 3000 g. of ice, slowly, and with vigorous stirring. The aqueous mixture was cooled by occasional addition of ice while saturated sodium hydroxide solution was added until all of the inorganic material dissolved. The dark oil which separated was collected, and the aqueous layer was extracted with 3 x 500 ml. portions of ether. The oil and ether extracts were combined, washed with 250 ml. of 10% aqueous sodium bisulfite and then with water, dried (potassium carbonate) and evaporated. The residual liquid was fractionally distilled under 13 mm pressure to yield 43 g. (34%) of unreacted picoline and 113 g. (80% conversion) of 3-bromo-4-picoline, b.p. 81-83°.

Ethyl 3-Bromo-4-pyridylacetate (**5**).

A solution of 25.2 ml. (0.18 mole, 18 g.) of diisopropylamine in 250 ml. of dry tetrahydrofuran was stirred under nitrogen at room temperature while 2.4 *N* phenyllithium in ether, 75 ml. (0.18 mole), was added over 30 minutes. The resulting solution was stirred at room temperature for 30 minutes and was then treated, over 10 minutes with a solution of 13.76 g. (0.08 mole) of 3-bromo-4-picoline in 10 ml. of THF. Five minutes after addition was complete, the solution was treated with 20 ml. (0.18 mole) of diethyl carbonate and stirred for 60 minutes. The solution was poured into 500 ml. of ether and treated with 50 ml. of water. The organic layer was separated, washed with 4 x 50 ml. portion of water and then with 50 ml. of saturated sodium chloride solution, dried (potassium carbonate) and evaporated. The residual liquid was distilled to yield a low-boiling fraction consisting of 2.28 g. (17%) of recovered 3-bromo-4-picoline, while the major fraction was 13.41 g. (82% conversion) of the desired product, b.p. 91-95° (0.06 mm); ir (neat) 1735 cm^{-1}, nmr (deuteriochloroform): δ, 8.70 (s, 1), 8.47 (d, 1, J = 5 Hz), 7.27 (d, 1, J = 5 Hz), 4.20 (q, 2, J = 7 Hz), 3.80 (s, 2), 1.27 (t, 3, J = 7 Hz).

A picrate was prepared from ethanolic picric acid, m.p. 127-128°.

Anal. Calcd. for $C_{15}H_{13}BrN_4O_9$: C, 38.07; H, 2.77; N, 11.84. Found: C, 38.29; H, 2.87; N, 11.90.

Ethyl 2-(3-Bromo-4-pyridyl)butanoate (**6**).

A solution fo 19.52 g. (0.08 mole) of **5** in 50 ml. of dry *N,N*-dimethylformamide was added dropwise to a stirred mixture of 3.36 g. of sodium hydride (57% dispersion in mineral oil, 0.08 mole) in 200 ml. of DMF. Stirring at room temperature was continued for 60 minutes and the solution was then cooled to 0° and treated over 5 minutes with 13.7 g. (0.088 mole) of iodoethane. The mixture was stirred in ice for 30 minutes and was then allowed to warm to room temperature over 60 minutes. The mixture was poured into 500 ml. of ice water and extracted

with 5 x 100 ml. portion of ether. The combined extracts were washed twice with 50 ml. portions of saturated sodium chloride solution, dried (potassium carbonate) and evaporated. Distillation yielded 20.52 g. (94%) of the desired product, b.p. 87-90° (0.25 mm); ir (neat) 1732 cm^{-1}; nmr (deuteriochloroform): δ, 8.65 (s, 1), 8.43 (d, 1, J = 5 Hz), 7.32 (d, 1, J = 5 Hz), 4.15 (q, 2, J = 7 Hz), 4.00 (t, 1, J = 7 Hz), 1.93 (oct., 2, J = 7 Hz), 1.19 (t, 3, J = 7 Hz), 0.92 (t, 3, J = 7 Hz).

A picrate was prepared, m.p. 79-80° (ethanol).

Anal. Calcd. for $C_{17}H_{17}BrN_4O_9$: C, 40.73; H, 3.43; N, 11.18. Found: C, 40.77; H, 3.49; N, 11.23.

N-(Hydroxy-*t*-butyl)-2-(3-bromo-4-pyridyl)butyramide (**7**).

A solution of 20.52 g. (0.075 mole) of the ester, **6**, and 20.5 g. (0.23 mole) of 2-amino-2-methyl-1-propanol was heated at 145-155° under nitrogen. After 48 hours, the unreacted amino alcohol was removed under reduced pressure. The residual oil was dissolved in 75 ml. of benzene and boiled for several minutes with charcoal. After filtration, the hot benzene solution was treated with hexane until cloudy, and enough benzene was added to give a clear solution. On slow cooling, 17.9 g. of white granules formed. Concentration of the mother liquor yielded another 2.2 g. of the product (85% total yield). After sublimation, the product gave white prisms, m.p. 90-91°; ir (potassium bromide) 1665 cm^{-1}; nmr (deuteriochloroform): δ, 8.8 (s, 1), 8.5 (d, J = 5 Hz, 1), 7.5 (d, J = 5 Hz), 1, 6.2 (bd.s., 1), 4.6 (t, J = 6 Hz, 1), 3.8 (mult., overlapping, 1), 3.6 (d, J = 6 Hz, 2), 1.9 (mult., 2), 1.3 (s, 6), 1.0 (t, J = 8 Hz, 3).

Anal. Calcd. for $C_{13}H_{19}BrN_2O_2$: C, 49.53; H, 6.09; N, 8.89. Found: C, 49.67; H, 5.87; N, 8.82.

2-(1-[3-Bromo-4-pyridyl]propyl)-4,4-dimethyloxazoline (**8**).

A solution of 12.94 g. (0.041 mole) of the hydroxyamide **7** in 75 ml. of dichloromethane was maintained at -5° to 0° in an ice-salt bath while a solution of 10.8 g. (0.09 mole) of thionyl chloride in 25 ml. of dichloromethane was added dropwise, over 45 minutes. The resulting solution was stirred at 0° for 45 minutes and was then treated with 75 ml. of iced 20% potassium carbonate solution and stirred for 15 minutes. The mixture was treated with 300 ml. of dichloromethane and the organic layer was separated. The aqueous layer was extracted with another 100 ml. portion of dichloromethane combined dichloromethane fractions were washed with 50 ml. of saturated brine solution, dried (potassium carbonate) and evaporated. The amber liquid was distilled to yield 11.07 g. (91%) of colorless liquid, b.p. 99-100° (0.15 mm); ir (neat) 1660 cm^{-1}; nmr (carbon tetrachloride): δ, 8.7 (s, 1), 8.4 (d, J = 5 Hz, 1), 7.3 (d, J = 5 Hz, 1), 3.9 (m, overlapping, 1), 3.8 (s, 2), 1.9 (m, 2), 1.4 (s, 6), 0.9 (t, J = 7 Hz, 3).

Anal. Calcd. for $C_{13}H_{17}BrN_2O$: C, 52.53; H, 5.79; N, 9.43. Found: C, 52.42; H, 6.00; N, 9.44.

2-(1-Hydroperoxy-1-[3-bromo-4-pyridyl]propyl)-4,4-dimethyloxazoline (**9**).

A solution of 3.36 g. (0.03 mole) of potassium *t*-butoxide in 50 ml. of dry THF was cooled below -35° and stirred while a solution of 5.94 g. (0.02 mole) of the oxazoline, **8**, in 25 ml. of THF was added over a few minutes. Dry oxygen was bubbled through the resulting orange solution at a moderate rate for 60 minutes at -25° to -35° resulting in a discharge of color. The yellow solution was poured into 100 ml. of 20% aqueous ammonium chloride and extracted with 4 x 100 ml. portions of ether. The combined extracts were washed with 2 x 50 ml. portions of saturated sodium chloride solution, dried (sodium sulfate) and evaporated. The residue was triturated with 200 ml. of pentane, and the white crystalline solid was filtered off and dried under vacuum to give 6.05 g. (92%) of the product. The product was pure as isolated, and it could be recrystallized from dichloromethane-pentane as white prisms, m.p. 141-142° dec. The material was soluble in 10% aqueous sodium hydroxide and could be reprecipitated on neutralization, but was insoluble in 10% aqueous sodium carbonate; ir (potassium bromide): (deuteriochloroform): 3110 cm^{-1} and 2800 cm^{-1} (strong, broad), 1655 cm^{-1} (strong, sharp); nmr (deuteriochloroform): δ, 8.7 (bd.s., 1), 8.5 (bd.d., J = 5 Hz, 1), 7.5 (d, J = 5 Hz), 1, 4.1 (s, 2), 2.5 (m, 2), 1.4 (s, 6), 0.8 (t, J = 8 Hz, 3).

Anal. Calcd. for $C_{13}H_{17}BrN_2O_3$: C, 47.43; H, 5.22; N, 8.51. Found: C, 47.25; H, 5.28; N, 8.23.

2-(1-Hydroxy-1-[3-bromo-4-pyridyl]propyl)-4,4-dimethyloxazoline (**10**).

A solution of 6.05 g. (18.4 mmoles) of the hydroperoxide, **9**, in 100 ml. of 95% ethanol was cooled in ice-salt to 0° and treated with 1.5 g. (40 mmoles) of sodium borohydride. After stirring at 0-5° for 2 hours the milky mixture was treated with 200 ml. of iced 10% sodium hydroxide solution, stirred for several minutes, and then extracted with 3 x 100 ml. portions of dichloromethane. The combined extracts were washed with 2 x 100 ml. portions of saturated sodium chloride solution, dried (potassium carbonate) and evaporated. The residual white solid was recrystallized from dichloromethane-hexane as white flakes, m.p. 162-165°, 5.60 g. (97%). An analytical sample had m.p. 166-167° after recrystallization from dichloromethane-ether; ir (potassium bromide): 3110 cm^{-1} (strong, broad), 1652 cm^{-1} (strong, sharp); nmr (deuteriochloroform) δ, 8.8 (s, 1); 8.6 (bd.d, J = 5 Hz, 1), 7.7 (d, J = 5 Hz, 1), 4.2 (s, 1), 4.1 (s, 2), 2.4 (m, 2), 1.4 (s, 3), 1.3 (s, 3), 0.8 (t, J = 7 Hz, 3).

Anal. Calcd. for $C_{13}H_{17}BrN_2O_2$: C, 49.85; H, 5.48; N, 8.94. Found: C, 49.59; H, 5.56; N, 8.72.

4-Ethyl-4-hydroxy-3-oxo-3,4-dihydro-1*H*-pyrano[3,4-*c*]pyridine (**13**).

A solution of 9.39 g. (30 mmoles) of the bromopyridine, **10**, in 75 ml. of dry THF was added to a stirred suspension of 1.45 g. of 57% sodium hydride oil dispersion (33 mmoles) in 100 ml. of THF under nitrogen at room temperature at a rate such that moderately rapid hydrogen evolution occurred. When hydrogen evolution was complete the solution was cooled in Dry Ice-acetone (-78°), and 15 ml. of 2.3 *N* phenyllithium in ether (35 mmoles) was introduced in a rapid stream. Addition was accompanied by formation of a thick precipitate so that swirling by hand was required for complete mixing. Shortly after addition was complete, the mixture was treated with 3.0 g. of dry paraformaldehyde (100 m-equivalent of CH_2O, the material was dried under vacuum over phosphorus pentoxide), and the mixture was allowed to warm to room temperature with stirring. The mixture was stirred at room temperature for 45 minutes and was then treated with 5 ml. of absolute ethanol to destroy traces of unreacted sodium hydride or phenyllithium. The solvent was evaporated under reduced pressure, and the residue was extracted with 200 ml. of hot ethanol containing 10% sulfuric acid in two portions. After filtration to remove inorganic salts, the ethanol solution was heated under reflux for 15 hours. The solution was treated with an equal volume of water, and most of the ethanol was distilled off under reduced pressure. The residual aqueous solution was cooled, treated with ice, and carefully neutralized with solid sodium bicarbonate. The mixture was immediately extracted with dichloromethane (6 x 100 ml.

portion), and the combined extracts were washed with saturated salt solution, dried (sodium sulfate), and evaporated. The residue was washed with hexane and then recrystallized from ethanol to yield 3.46 g. (60%) of tan crystals of the pyridine lactone, **13**. Recrystallization from etherdichloromethane gave colorless prisms, m.p. 175-176°; ir (potassium bromide): 3100 cm^{-1}, 2830 (strong, broad) 1736 cm^{-1} (strong); nmr (deuteriochloroform): δ, 8.7 (d, J = 5 Hz, 1), 8.6 (s, 1), 7.7 (d, J = 5 Hz, 1), 5.6 (d, J = 16 Hz, 1), 5.5 (d, J = 16 Hz, 1), 4.0 (br.s., 2), 1.8 (q, J = 7 Hz, 2), 1.0 (t, J = 7 Hz, 3).

Anal. Calcd. for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.20; H, 5.63; N, 7.06.

Formation of Methiodide of **13**.

A mixture of 1.00 g. (5.2 mmoles) of **13** in 20 ml. of methyl iodide was heated to reflux for 1.3 hours. After cooling to room temperature, the excess methyl iodide was removed *in vacuo* and the residue was triturated with dichloromethane ether (1:1). The solid was collected by filtration, washed with ether and dried to give 1.62 g. (93%) of the methiodide salt. Recrystallization from 2-propanol-ethanol (2:1) gave colorless crystals, m.p. 177° dec.; ir (nujol): 3415, 3290, 1760 cm^{-1}; uv (ethanol): 264 nm; nmr (DMSO-d_6): δ, 9.2 (s, 1), 9.0 (d, J = 6 Hz, 1), 8.2 (d, J = 6 Hz, 1), 5.9 (d, J = 14 Hz, 1), 5.7 (d, J = 14 Hz, 1), 3.3 (s, 3), 1.9 (q, J = 7 Hz, 2), 0.8 (t, J = 7 Hz, 3).

The compound darkened on standing and, thus, a satisfactory elemental analysis could not be obtained.

4-Ethyl-4-hydroxy-3,4,6,7-tetrahydro-7-methyl-1*H*-pyrano[3,4-*c*]-pyridine-3,5-dione (**14**).

The methiodide of **13**, 1.10 g. (3.28 mmoles), dissolved in 8 ml. of water, was cooled in an ice bath. Solutions of potassium hydroxide, 1.48 g. (26.4 mmoles), in 5 ml. of water, and potassium ferricyanide, 3.26 g. (9.9 mmoles) in 9 ml. of water, were prepared. At intervals of 15 minutes, 1.0 ml. of the potassium hydroxide solution and 1.5 ml. of the potassium ferricyanide solution were added to the methiodide solution with stirring. After the final addition of the potassium ferricyanide solution, the reaction mixture was stirred in an ice bath for 1 hour, then allowed to warm to room temperature, and stirring was continued overnight. The mixture was cooled and acidified with 6*N* hydrochloric acid. To the resulting mixture was added 300 ml. of ethanol. The yellow-green precipitate was removed by filtration and washed with ethanol. The filtrate and the washings were combined and evaporated under reduced pressure. The residue was again treated with isopropanol to give a white precipitate which was removed by filtration and washed with 2-propanol. The combined filtrate and washings were evaporated under reduced pressure. Chromatography of the residue on silica gel yielded 357 mg. (48.7%) of the pyridone, **14**, upon elution with benzene-acetone (3:2). Recrystallization of the eluate from acetone gave colorless prisms, m.p. 213-214°; ir (potassium bromide): 1655 cm^{-1}; nmr (DMSO-d_6): δ, 7.80 (s, 1), 6.47 (s, 1), 6.23 (s, 1), 5.41 (d, J = 14 Hz, 1), 5.09 (d, J = 14 Hz, 1), 3.45 (s, 3), 1.81 (q, J = 7 Hz, 2), 0.85 (t, J = 7 Hz, 3); uv (ethanol) 309 nm; m/e 223 (M^+).

Anal. Calcd. for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87. Found: C, 59.10; H, 5.86.

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