Experiments on the Synthesis of dl-Camptothecin. 4. Synthesis and Antileukemic Activity of dl-Camptothecin Analogues

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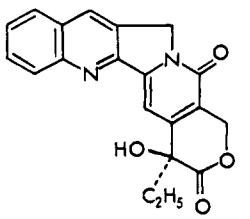
Several *dl*-camptothecin analogues in which the ethyl group in the E ring of the parent compound 1 is replaced by other side chains were synthesized and their life-span activity in L1210 leukemic mice was tested. *dl*-Desethylallylcamptothecin (13) was more active than *dl*-camptothecin and others.

In spite of intensive investigation of the chemotherapeutic properties of camptothecin (1), its high toxicity in



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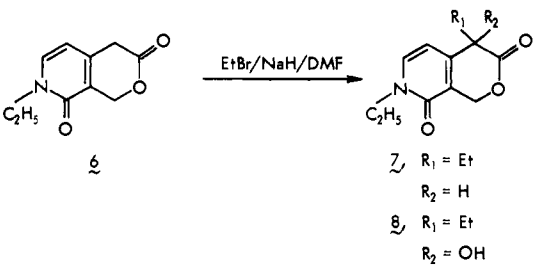
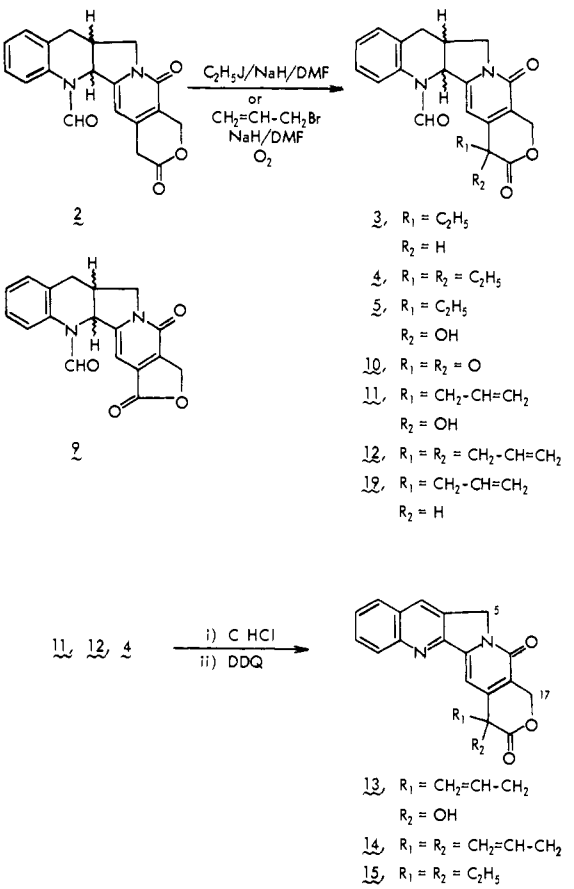
both animals and man seems to have led to its being rejected from consideration as a potentially useful drug.[1] Consequently, structural modification of 1 is necessary if the compound is to have any future promise. In continuation of our total-synthetic study of *dl*-camptothecin[2a,b] we have synthesized some modified *dl*-camptothecin compounds, which may open the way to chemotherapeutic use.

Chemistry. As pointed out in our previous report,[2b] a disadvantage of our synthetic route is the unavoidable coformation of the diethyl lactone 4 in the alkylation step from 2 to 3. A possible way to improve the yield of the desired hydroxyethyl lactone 5 by supression of the coformation of the undesired diethyl lactone 4 has already been suggested in our experiments on the synthesis of a DE ring analogue[2c] of 1. Namely, considerable susceptibility of the monoethyl lactone 7 to oxygen was indicated by the observation that 8 was directly obtained to some extent (10%) in the alkylation of 6, as shown in the Scheme I. Although it was not clear whether the latter was formed during the alkylation step or during the workup, ethylation of 2 was carried out with ethyl iodide and sodium hydride in dimethylformamide with a stream of oxygen bubbled through the reaction mixture, with the expectation that oxygenation of the initially formed 3 might more readily take place than further alkylation to give the undesired 4. The oxygenated product 5[2b] was actually obtained in 42% yield after chromatographic separation (mixture of the two epimers, ca. 2:1), accompanied by 4[2b] and by the γ-lactone 9 in 7 and 4% yield, respectively. The ir spectra of two epimers of 5 and 4 were superimposable with those of authentic samples, respectively. The thin-layer chromatographic properties [chloroform–methanol (10:1)] were also identical. The structure of 9 was verified by elemental analysis and the γ-lactone band at 1780 cm^{-1} in the ir spectrum. The same product was also obtained in the one-step allylation–oxygenation of 2 (7%) (see later). 9 was probably formed via the supposed intermediate, α-keto lactone 10, being generated by direct oxygenation of 2, followed by decarbonylation under ring contraction. Neither 3 nor unreacted 2 was obtained in this experiment. The overall yield of 5 from 2 via isolated 3 followed by oxygenation was ca. 27% as shown previously.[2b] Thus the one-step

Scheme I

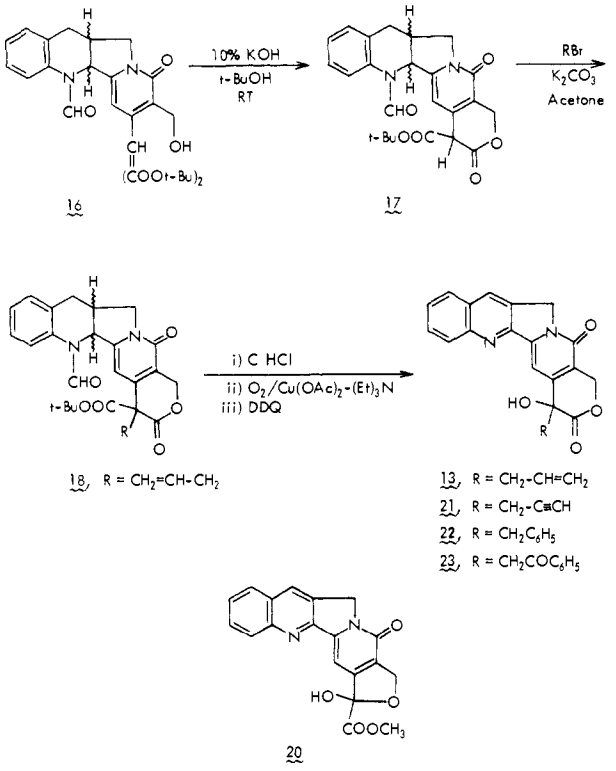


Scheme II



ethylation–oxygenation method has improved the yield of 5 to some extent (ca. 1.6 times). This method was now applied to the synthesis of *dl*-desethylallylcamptothecin (13). Treatment of 2 with allyl bromide by the one-step method followed by thin-layer chromatographic separation gave 11 (two epimers, ca. 7:2, total yield 30%), the diallylated product 12 (19%), the γ-lactone 9 (7%), and the starting material (4%). The epimer of 11 obtained in lesser amount was crystallized to give a pure sample of 11, whose ir spectrum and elemental analysis confirmed the structure (see Experimental Section). Crude 11, 12, and 4 were

Scheme III



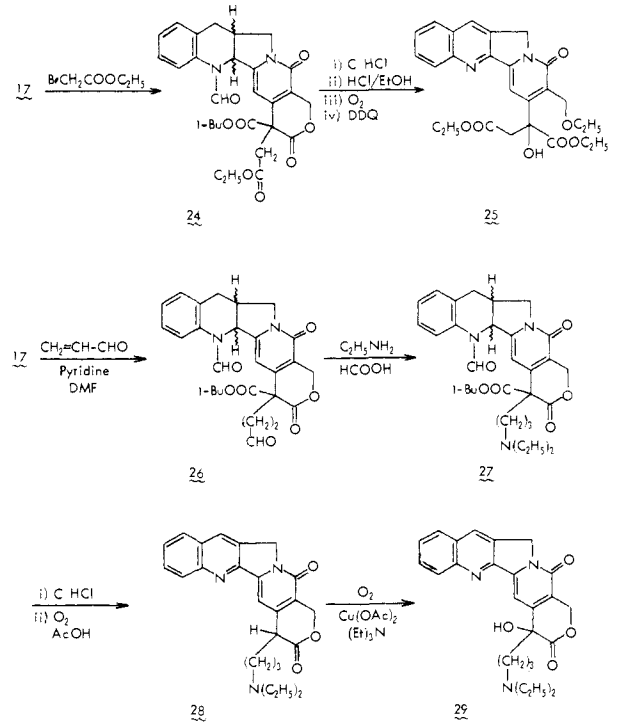
converted to **13**, **14**, and **15** after deformylation with concentrated hydrochloric acid followed by aromatization with dichlorodicyanoquinone in 52, 40, and 70% yield, respectively (Scheme II). Their spectral data and elemental analysis satisfied the structure (see Experimental Section).

Although this one-step alkylation–oxygenation method does seem to somewhat improve the yield of the monoalkylhydroxy lactone (**5** and **11**) by supressing formation of the undesired dialkyl lactone (**4** and **12**), exclusive formation of the monoalkylated product could not be achieved. For this purpose generation of the ester lactone **17** seemed desirable. This was readily obtained in 97% (possibly a mixture of two epimers) by treatment of **16**[2b] with potassium hydroxide in *tert*-butyl alcohol for 30 min at room temperature, as shown in Scheme III. Elemental analysis and the lactone and ester band at 1750 and 1730 cm^{-1} in the ir spectrum verified the structure. Allylation of **17** with allyl bromide and potassium carbonate in refluxing acetone gave crude **18**, which appeared as one spot on its thin-layer chromatogram but, like **17**, probably contained two epimers. A portion of **18** was treated with trifluoracetic acid to give the monoallyl lactone **19** (Scheme II). The ir spectrum was superimposable with that of an authentic sample, though both were probably epimer mixtures. The authentic material was obtained by treatment of **2** with allyl bromide and sodium hydride in dimethylformamide without an oxygen stream. Thus the exclusive monoalkylation seemed to be realized.

Crude **18** was treated with concentrated hydrochloric acid (deformylation and simultaneous decarboxylation), followed by oxygenation with an oxygen stream in the presence of cupric acetate and triethylamine,[2b] and aromatization with dichlorodicyanoquinone successively. After chromatographic purification of the resulting residue, **13** was obtained as the main product in ca. 30% overall yield from **17**. Further chromatographic purification of minor fractions afforded only a small amount of **14** (Scheme II) and possibly **20** according to its elemental analysis and spectral data (see Experimental Section). **14** was probably formed by decarboxylation of **17** and/or **18** followed by diallylation because of the long reaction time (16 h). The precise pathway of the formation of **20** is unclear; it can be only supposed that the α-keto lactone **10** (NH for NCHO, Scheme II) formed in the course of oxygenation underwent methanolysis followed by lactone cleavage and recyclization under ring contraction. The postulated intermediate **10** (NH for NCHO) might be formed by oxidative cleavage of the allyl side chain of the α-oxyallyl lactone **11** (NH for NCHO) or direct oxygenation of **2** contaminated only slightly with crude **18**. In this way **13** was obtained practically without coformation of the undesired **14** and consequently without any chromatographic separation in the intermediate steps. *dl*-Desethylpropargyl- (**21**), *dl*-desethylbenzyl- (**22**), and desethylphenacylcamptothecin (**23**) were synthesized analogously from **17** via corresponding intermediates. Their physical data confirmed the structure (see Experimental Section). An analogous reaction of **17** with bromoethyl acetate gave **24** almost quantiatively according to its ir and NMR spectrum [ν_{max} 1750–1770 cm^{-1} (very strong), δ ~1.1–1.2 and ~3.9–4.1 ppm for methyl and methylene proton signals in the ethyl ester]. Deformylation and simultaneous decarboxylation with concentrated hydrochloric acid caused, however, hydrolysis of the ethyl ester, and back esterification with saturated hydrogen chloride–ethanol accompanied the cleavage of the lactone ring to give the crude ethyl ether diethyl ester. Oxygenation and aromatization analogous to the production of **13**, **21**, **22**, and **23** gave **25**, the mass spectrum (M^+ 480) and elemental analysis suggesting its structure.

In order to obtain a modified camptothecin having a basic side chain in the E ring, the following attempt was made. Michael addition of acrolein to **17** in pyridine and dimethylformamide afforded an epimeric mixture of **26**, as shown in Scheme IV. The NMR spectrum showed *N*-formyl and *C*-formyl proton signals at δ 8.5 and 9.7, respectively, confirming structure **26**. Reductive carbonyl amination of **26** with diethylamine and formic acid af-

Scheme IV



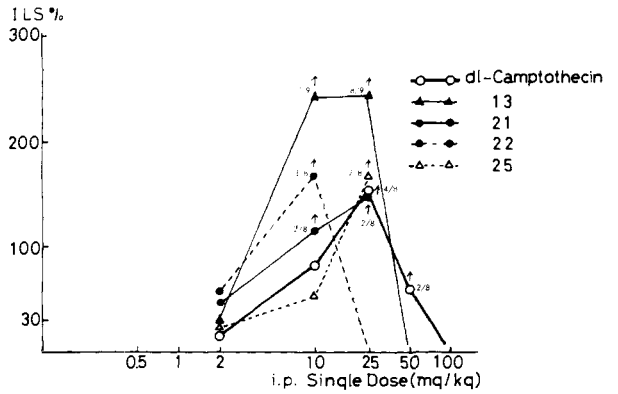


Figure 1. Groups of BDF_1 mice, eight to nine animals to a group, were inoculated intraperitoneally with 5×10^5 cells of L1210 in 0.1 ml of a saline solution. Test compound was injected intraperitoneally once on the day following tumor inoculation. Antitumor activity was evaluated by the increase in life span over controls. Animals surviving for over 30 days were counted as 30 days and shown as the fraction of survivors at the arrow mark.

forded an epimeric mixture of **27**. Appearance of the *N*-ethyl proton signals at ca. δ 1 instead of that of the *C*-formyl group confirmed the structure. Deformylation and simultaneous decarboxylation of **27** followed by dehydrogenation in an oxygen stream in boiling acetic acid[3] gave crude **28**. Comparison by thin-layer analysis indicated that dehydrogenation with dichlorodicyanoquinone was less preferable than the method using an oxygen stream in boiling acetic acid. Crude **28** was treated with an oxygen stream, as for the production of **12**, to give **29**. After thin-layer chromatographic purification, however, only a small amount of product (mp 235–237° dec) was obtained. The molecular ion peak (M^+ 433) in the mass spectrum coincided with that of the target product **29**, but the elemental analysis showed a product with two more oxygens than **29**. Thus we have abandoned the attempt to obtain modified camptothecin having a basic side chain in the E ring.

Results and Discussion

Five (**13**, **21**, **22**, **23**, and **25**) of the nine camptothecin analogues showed anti-L1210 activity. Four analogues, **13**, **21**, **22**, and **25**, showed higher toxicity than *dl*-camptothecin but were active at lower doses than that of *dl*-camptothecin. **13** was the most active in these compounds; eight out of nine mice dosed with 50 mg/kg and seven out of nine mice dosed with 25 mg/kg survived over 30 days after tumor inoculation (see Figure 1). Contrary to **13**, **21**, **22**, and **23**, **14** and **15** having no hydroxyl group at C-20 showed inactivity, confirming that the α-hydroxy lactone functionally present in the E ring is an absolute requirement for the antitumor activity of **1**.[4] In spite of the lack of the lactone moiety (E ring), however, **25** retained an activity comparable to *dl*-camptothecin, suggesting higher significance of the hydroxy group at C-20 rather than the lactone for the potency (Table I).

Each epimer of **5** (**5a** and **5b**) showed no activity. From this fact it can be deduced that the basicity at N_1 and/or the flat molecular shape play an important role for the activity of **1**.

Next, experiments with single and divided doses on **13** have been done. As shown in Table II, administration by divided dose did not give increased activity; rather, increased toxicity was indicated. Further, we studied the **13** sodium salt, but the results are not up to expectation; activity was not increased. Toxicity was decreased on ip administration, slightly increased on sc, and clearly in-

Table I. Life Span Activity of Camptothecin Analogues. Single Ip Dose against L1210 Leukemia in Mice

Compd	Dose, μmol/kg	ILS, %	Survivors over 30 days	CI value[a]
	5.74	16	0/7	
	28.7	84	0/7	
dl-1	71.8	>155	4/8	25/3
	144	>60	2/8	
	287	−43	0/8	
	26.3	−6	0/8	
5a	65.8	−9	0/8	
	132	−3	0/8	
	26.3	−6	0/8	
5b	65.8	−9	0/8	
	132	−12	0/8	
	5.56	34	0/8	
	27.8	>244	7/9	
13	69.4	>245	8/9	25/2
	139	−21	0/9	
	5.21	−5	0/9	
14	26.0	−8	0/9	
	65.1	−5	0/9	
	130	1	0/9	
	5.56	0	0/8	
15	27.8	−4	0/8	
	69.4	5	0/8	
	139	−11	0/8	
	5.58	50	0/8	
21	27.9	>117	2/8	25/1
	69.8	>149	2/8	
	4.87	58	0/8	
22	24.4	>171	3/8	10/1
	61.0	−34	0/8	
	45.7	12	0/8	
23	22.8	10	0/8	
	57.1	21	0/8	
	4.17	26	0/8	
25	20.8	55	0/8	25/2
	52.1	>170	2/8	

[a] CI value = ILS_{max}/ILS_{30}.

Table II. Life Span Activity of *dl*-Desethylallylcamptothecin and Its Sodium Salt against L1210 Leukemia in Mouse

Compd	Dose, μmol/kg	Route	ILS, %	Survivors over 30 days
13	5.56 × 1	ip	16	0/7
	27.8 × 1		84	0/7
	69.4 × 1		>155	4/8
	139 × 1		>60	2/8
	278 × 1		−43	0/8
	1.11 × 5	ip	14	0/8
	5.56 × 5		42	0/8
	13.8 × 5		>105	0/8
	27.8 × 5		−13	3/8
	1.11 × 5	sc	−1	0/8
	5.56 × 5		4	0/8
	13.8 × 5		26	0/8
	27.8 × 5		51	0/8
	5.56 × 4	po	3	0/8
	13.8 × 4		−1	0/8
	27.8 × 4		−1	0/8
13 sodium salt	1.00 × 5	ip	−2	0/8
	5.00 × 5		13	0/8
	12.5 × 5		58	0/8
	25.0 × 5		>118	3/8
	1.00 × 5	sc	−2	0/8
	5.00 × 5		9	0/8
	12.5 × 5		39	0/8
	25.0 × 5		72	0/8
	5.00 × 5	po	12	0/8
	12.5 × 5		40	0/8
	25.0 × 4		21	0/8

creased on oral administration.

Experimental Section

Melting points were determined on a Yanagimoto micromelting apparatus and are uncorrected. Ir spectra were recorded by a Koken DS-207B or JASCO IRS supectrophotometer. Wave numbers are expressed in reciprocal centimeters. NMR spectra were taken in $CDCl_3$ solution on a Varian A-60 or T-60 spectrophotometer. Chemical shifts are expressed as δ values (parts per million) from tetramethylsilane. Mass spectra were recorded on a Hitachi RMU-6 mass spectrometer. Analyses are within ±0.3% of the calculated values. Unless otherwise stated, column chromatography was on silica gel (activity II, mesh 0.05–0.2 mm) and thin-layer chromatography on silica gel GF. Unless otherwise noted, the organic layer was washed with saturated NaCl–H_2O or H_2O and dried over Na_2SO_4 or $MgSO_4$. Sodium hydride coated with oil (55%) was used and washed three times with benzene before use.

(1) One-Step Ethylation–Hydroxylation of the *N*-Formyl Lactone 2. To a stirred solution of **2** (50 mg, 0.15 mmol) in DMF (4 ml), NaH (16 mg, 0.38 mmol) was added under ice cooling and N_2 atmosphere. After the mixture had been stirred for 30 min a stream of O_2 was passed through the mixture and then EtI (47 mg, 0.30 mmol) was added. The resulting solution was kept for 30 min under the same conditions and then poured into ice–AcOH. Extraction with $CHCl_3$ and evaporation after drying over Na_2SO_4 gave a yellow foam (56 mg), which was separated by TLC [solvent system $CHCl_3$–MeOH (10:1)] to give **4** (4 mg), **9** (2 mg), one epimer of **5** (8 mg), and the other epimer of **5** (16 mg).

(2) One-Step Allylation–Oxygenation of the *N*-Formyl Lactone 2. A solution of **2** (270 mg, 0.80 mmol) in DMF (16 ml) was treated with NaH (88 mg, 2.0 mmol) and allyl bromide (146 mg, 1.2 mmol) under an O_2 stream as above. Analogous workup gave a residue (291 mg), which was separated by TLC [solvent system $CHCl_3$–MeOH (20:1)] to give **12** (63 mg), a mixture of one epimer of **11** and **9** (60 mg), **2** (11 mg), and the other epimer of **11** (73 mg), the polarity decreasing in this order. The mixed fraction of the epimer of **11** and **9** was again separated by TLC [solvent system cyclohexane–EtOAc–AcOH (5:5:2)] to give the epimer of **11** (21 mg) and **9** (19 mg). The former fraction was recrystallized from MeOH to give a pure sample of the epimer of **11** (14 mg, mp 247–248°): ir ($CHCl_3$) 3540, 1745, 1630 cm^{-1} (br). Anal. ($C_{22}H_{20}N_2O_5$) C, H, N. The latter fraction was recrystallized from $CHCl_3$–MeOH to give a pure sample of **9** (15 mg, mp >290° dec): ir ($CHCl_3$) 1780, 1675 cm^{-1}. Anal. ($C_{18}H_{14}N_2O_4$) C, H, N.

(3) *dl*-Desethylallylcamptothecin (13) from the Epimer of 11. A solution of the more polar epimer of **11** obtained in experiment 2 (73 mg) in concentrated HCl (0.2 ml) was allowed to stand for 16 h at room temperature. The solution was treated with ice–NaOAc and extracted with $CHCl_3$. Concentration of the extract gave crude *dl*-*N*-tetrahydrodesethylallylcamptothecin (67 mg). To a solution of this crude residue in absolute dioxane (8.0 ml) was added DDQ (110 mg) and the mixture was refluxed for 1 h under stirring. The solvent was removed and the resulting residue was suspended in $CHCl_3$ containing 1% MeOH and chromatographed [silica gel, 6 g; each fraction containing 10 ml of $CHCl_3$–MeOH (99:1)]. Fractions 7–13 were collected and concentrated. The residue was recrystallized from $CHCl_3$–MeOH to give **13** (34 mg, mp 276–278° dec): ir (KBr) 3315, 1755, 1650 cm^{-1}; NMR 2.5–2.7 (2 H, allyl H), 4.9–5.9 (7 H, ring aliphatic and vinyl H), 8.0–9.1 ppm (6 H, aromatic H) ($CDCl_3$ + CF_3COOH); MS *m/e* 360 (M^+). Anal. ($C_{21}H_{16}N_2O_4$) C, H, N, O.

13 Sodium Salt. A suspension of **13** (23 mg, 0.061 mmol) and 0.05 N NaOH (F 1.235, 0.99 ml, 0.061 mmol) in dioxane (1 ml) containing EtOH (0.1 ml) was warmed for 5 min at 90° (bath temperature). The resulting solution was concentrated, H_2O (ca. 1 ml) was added, and a small amount of undissolved material was filtrated. The filtrate was concentrated, and the residue was dissolved in CH_3OH (0.1 ml). Acetone was added to the solution until it started to become cloudy, and this was again warmed until resolution. After being allowed to stand at room temperature, **13** sodium salt (21 mg, mp >270°) was obtained: ir ~3400 (br), 1640, ~1600 (br).

(4) Diallylated Lactone 14 from 12. Crude **12** obtained in experiment 2 was treated with concentrated HCl (0.2 ml) followed by DDQ (87 mg) in absolute dioxane (6 ml), as in experiment 3. Analogous workup and chromatographic separation [5 g of silica gel, solvent system $CHCl_3$–MeOH (99:1)] gave **14** (23 mg, mp 276–278° dec from CH_3OH): ir ($CHCl_3$) 1735, 1660 cm^{-1}; MS *m/e* 384 (M^+). Anal. ($C_{24}H_{20}N_2O_3$) C, H, N, O.

(5) Diethylated Lactone 15[2b] from 4. Crude **4** (100 mg) obtained in experiment 1 was treated with concentrated HCl (0.2 ml) followed by DDQ (145 mg) in absolute dioxane (8 ml), as in experiment 3. After similar chromatographic separation and recrystallization from MeOH, **15** (59 mg, mp 283–286° dec, 70%) was obtained.

(6) Cyclization of 16 to the Ester Lactone 17. To a stirred suspension of **16** (500 mg) in *t*-BuOH (9.0 ml), 10% KOH (1.0 ml) was added at room temperature. Within 5 min the mixture became a clear solution, which again turned to a thick suspension within 20 min. After being kept for a further 10 min, ice–AcOH was added on the mixture, which was then extracted with $CHCl_3$. The residue of the extract was recrystallized from MeOH to give **17** (417 mg, mp 257–258° dec): ir ($CHCl_3$) 1750, 1730, 1640 cm^{-1}; NMR ~1.4 (two singlets, 9 H, *t*-Bu), 5.3 (2 H, C-17 H), 8.6 ppm (1 H, NCHO). Anal. ($C_{24}H_{24}N_2O_6$) C, H, N, O.

(7) Allylation of 17 to 19 via Crude 18. To a stirred suspension of **17** (25 mg, 0.057 mmol) in acetone (2 ml) were added K_2CO_3 (32 mg, 0.23 mmol) and allyl bromide (14 mg, 0.11 mmol) and the mixture was refluxed for 16 h under N_2 atmosphere. After the mixture had been cooled, it was poured onto ice–AcOH and extracted with $CHCl_3$–MeOH (3:1). The residue of the extract [30 mg, one spot on TLC, solvent system $CHCl_3$–MeOH (10:1)] was refluxed in CF_3COOH for 30 min. The evaporated residue was recrystallized to give **19** (6 mg, mp 208–210° dec).

(8) The Monoallyl Lactone 19 from 2. The experiment was carried out [**2** (50 mg), NaH (8 mg), allyl bromide (27 mg), and DMF (4 ml)] analogously to the production of **3** and **4** from **2**.[2b] After similar workup **12** (22 mg), **19** (13 mg), and **2** (12 mg) were obtained. **19** was recrystallized from MeOH to give an epimeric mixture of **19** (mp 208–210° dec). Anal. ($C_{22}H_{20}N_2O_4$) C, H, N.

(9) *dl*-Desethylallylcamptothecin (13) by Allylation of 17 Followed by Deformylation, Decarboxylation, Oxygenation, and Aromatization. Allylation was performed with **17** (400 mg), K_2CO_3 (506 mg), and allyl bromide (222 mg) in acetone (30 ml), as in experiment 7. Crude **18** (467 mg) was allowed to stand with concentrated HCl (1 ml) for 16 h at room temperature. The solution was treated with ice–AcONa and extracted with $CHCl_3$. Concentration of the extract gave an amorphous residue (319 mg). To a solution of this residue in CH_3OH (25 ml) were added $Cu(OAc)_2 \cdot H_2O$ (37 mg) and $(Et)_3N$ (185 mg), and the resulting slightly cloudy solution was treated with an O_2 stream for 40 min under ice cooling. A small amount of undissolved material was filtered off, and the filtrate was concentrated. The resulting residue was treated with ice–AcOH and extracted with $CHCl_3$. The residue of the extract (359 mg) was dissolved in absolute dioxane (30 ml) containing DDQ (460 mg) and the mixture was refluxed for 30 min under stirring. The evaporated residue was chromatographed as in experiment 3 (silica gel 25 g, each fraction containing 30–40 ml). From fractions 4–14, **13** (100 mg, mp 264–268° dec) was obtained. The residues of fractions **2** and **3** were collected and again chromatographed (silica gel, 8 g) to give **14** (4 mg, mp 273–275° dec). Further chromatographic purification (silica gel, 10 g) of collected fractions 15–32 gave **20** (5 mg, $CHCl_3$–MeOH, mp 260–270° dec): ir (Nujol) 3173, 1748, 1655 cm^{-1}; NMR 3.8 (3 H, s, $COOCH_3$), 5.3 (2 H, s), 5.6 (2 H, s), 7.9–9.0 ppm (6 H, aromatic H) ($CDCl_3$ + CF_3COOH); MS *m/e* 350 (M^+). Anal. ($C_{19}H_{14}N_2O_5$) C, H, N.

(10) *dl*-Desethylpropargylcamptothecin (21) from 17. **17** was treated analogously as in experiment 9: **17** (50 mg), K_2CO_3 (63 mg), propargyl bromide (27 mg) in acetone (3 ml), concentrated HCl (0.2 ml), $Cu(OAc)_2 \cdot H_2O$ (5 mg), $(Et)_3N$ (2 mg) in MeOH (5 ml) with O_2 stream (20 min), and DDQ (65 mg) in dioxane (7 ml). Chromatographic purification (silica gel, 9 g) gave **21** (9 mg, $CHCl_3$–MeOH, mp 266–267° dec): MS *m/e* 358 (M^+). Anal. ($C_{21}H_{14}N_2O_4$) C, H, N.

(11) *dl*-Desethylbenzylcamptothecin (22) from 17. **17** (50 mg) was treated analogously as in the above experiment with K_2CO_3 (63 mg) and benzyl bromide (39 mg) in acetone (4 cc), followed by concentrated HCl (0.2 ml). To a solution of the resulting residue (45 mg) in DMF (3 ml) was added NaH (7 mg),

and the mixture was stirred for 20 min in N_2 atmosphere. An O_2 stream was then introduced to this mixture for 20 min under ice cooling. The mixture was poured onto ice–AcOH and extracted with $CHCl_3$. The residue (56 mg) of the extract was refluxed with DDQ (40 mg) in dioxane (6 ml) for 30 min. Chromatographic purification (silica gel, 8 g) gave **22** (13 mg, MeOH, mp 275–276° dec): MS m/e 410 (M^+). Anal. ($C_{25}H_{18}N_2O_4$) C, H, N.

(12) *dl*-Desethylphenacylcamptothecin (23) from 17. 17 was treated analogously as in experiments 9 and 10: 17 (50 mg), K_2CO_3 (64 mg), phenacyl bromide (46 mg) in acetone (6 ml), concentrated HCl (0.2 ml), $Cu(OAc)_2 \cdot H_2O$ (4 mg), $(Et)_3N$ (21 mg) in MeOH (6 ml) with an O_2 stream (20 min), and DDQ (64 mg) in dioxane (8 ml). Chromatographic purification (silica gel, 12 g) gave **23** (7 mg, $CHCl_3$–MeOH, mp 267–268° dec): MS m/e 438 (M^+). Anal. ($C_{26}H_{18}N_2O_5$) C, H, N.

(13) 25 from 17 via 24. 17 (50 mg) was treated analogously as in the above experiment with K_2CO_3 (64 mg) and ethyl bromoacetate (38 mg) in acetone (6 ml), followed by concentrated HCl (0.2 ml). The resulting residue (42 mg) was kept in saturated HCl gas–EtOH (4 ml) for 30 min. EtOH was evaporated and the residue was treated with ice–AcONa and extracted with $CHCl_3$. The residue (38 mg) of the extract was treated analogously as in the above experiment: $Cu(OAc)_2 \cdot H_2O$ (4 mg), $(Et)_3N$ (20 mg) in EtOH (5 ml) with O_2 stream (20 min), and DDQ (38 mg) in dioxane (5 ml). Chromatographic purification (silica gel, 6 g) gave **25** (7 mg, MeOH, mp 234–235° dec): MS m/e 480 (M^+). Anal. ($C_{26}H_{28}N_2O_7$) C, H, N.

Attempted Synthesis of 29 from 17. A solution of 17 (100 mg, 0.23 mmol) in pyridine (2 ml) and DMF (1 ml) containing acrolein (38 mg, 0.69 mmol) was kept for 20 h in the dark. After concentration of the solvent followed by codistillation with toluene, the residue was chromatographed [silica gel, 2 g; solvent system $CHCl_3$–CH_3OH (99:1); effluent 150 ml] to give a mixture of epimers of crude **26** (113 mg): NMR ($CDCl_3$) 1.2 (9 H, s, *t*-Bu), 8.5 (1 H, s, NCHO), 9.7 ppm (1 H, s, CHO); ir ($CHCl_3$) 1760, 1740, 1630 cm^{-1}. A mixture of crude **26** (113 mg, 0.23 mmol) in absolute benzene (5 ml) containing formic acid (25 mg, 0.55 mmol) and $(Et)_2NH$ (20 mg, 0.28 mmol) was refluxed for 2 h under stirring. After being cooled, the mixture was extracted with $CHCl_3$–MeOH (3:1). The residue of the extract was chromatographed (silica gel, 1 g, solvent system $CHCl_3$ containing 1–5% MeOH) to give a mixture of epimers of crude **27** (94 mg): ir ($CHCl_3$) 1760, 1740, 1640 cm^{-1}; NMR 0.9–1.2 (15 H, *t*-Bu + $2CH_3$), 8.6 ppm (1 H, s, NCHO). A solution of crude **27** (94 mg) in concentrated HCl (0.2 ml) was allowed to stand for 16 h at room temperature. The solution was then treated with AcONa and extracted with $CHCl_3$–MeOH (3:1). The residue (78 mg) of the extract was dissolved in AcOH (2 ml) and the solution was treated with an O_2 stream for 20 min under refluxing. The solution was then reduced and the residue was treated with ice–Na_2CO_3 and then extracted with $CHCl_3$–MeOH (3:1). The residue (72 mg) of the extract was chromatographed (silica gel, 1 g; solvent system $CHCl_3$ containing 3–10% MeOH) to give a residue (27 mg, tentatively **28**). A suspension of this residue (27 mg), $Cu(OAc)_2 \cdot H_2O$ (2 mg), and $(Et)_3N$ (5 mg) in MeOH (2 ml) was treated with an O_2 stream for 40 min. CH_3OH was removed at room temperature and the residue was treated with ice–AcOH and then extracted with $CHCl_3$. The residue of the extract was purified by TLC [solvent system $CHCl_3$–MeOH (3:1)]. The main fraction (5 mg) was recrystallized to give the product (2 mg, mp 235–237° dec): MS m/e 433 (M^+); ir ($CHCl_3$) 1750, 1640 cm^{-1}. Anal. ($C_{25}H_{27}N_3O_6$) C, H, N.

Pharmacology. Material and Method. Animals and Tumor. BDF_1 mice of 5 weeks of age were used, four males and four females for test groups, five males and five females for control groups. Mice were inoculated intraperitoneally with 5×10^5 cells of L1210. Test compound was injected intraperitoneally once on the day or five times for 5 days following tumor inoculation. Antitumor activity was evaluated by the increase in life span over controls.

Test Compounds. Chemical structures are listed in Table I. Since the compounds except 13 sodium salt are insoluble, they were suspended in the suspending vehicle[5] before using.

References and Notes

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(5) NaCl, 0.9%; Polysorbate 80, 0.4%; CMC, 0.5%; benzyl alcohol, 0.9% in 100 ml of water.

Solid-Phase Synthesis of Drug Glucuronides by Immobilized Glucuronosyltransferase

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Rabbit liver glucuronosyltransferase immobilized on beaded agarose has been used to synthesize glucuronic acid conjugates of meprobamate, diethylstilbestrol, bilirubin, borneol, benzoic acid, and *p*-nitrothiophenol. The immobilized enzyme exhibited a high degree of specificity for UDPGA as cofactor when *p*-nitrophenol is used as substrate. Other cofactors tested were less effective, all producing less than 10% conjugation relative to UDPGA. The effects on agarose-bound enzyme activity of a variety of cosolvents and emulsifiers have been studied. Ethanol, dimethyl sulfoxide, propylene glycol, and bovine serum albumin are among the cosolvents and emulsifiers which can be used within limited concentration ranges to solubilize lipophilic substrates for conjugation. Concentrations of calcium and magnesium cations between 1.5 and 10.0 mM were found to enhance glucuronosyltransferase activity of the immobilized enzyme.

A nearly universal theme in the metabolism of low-molecular-weight drugs and pesticides is their conjugation to glucuronic and sulfuric acids. The resulting conjugates are water soluble, ionized, and usually more readily cleared from the body than the aglycon. The critical role that glucuronide and sulfate conjugates play in controlling the availability of steroid hormones, catecholamines, heme catabolites, and other endogenous compounds is also currently being explored.

Identification of conjugates in most contemporary studies is usually inductive, not direct, based on confirmation of the presence of aglycon after treatment with β-glucuronidase, sulfatases, or acid hydrolysis.[1] In the last several years a number of reports demonstrate that glu-

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