Journal of Pharmaceutical Sciences

FEBRUARY 1974
VOLUME 63 NUMBER 2

REVIEW ARTICLE

Camptothecin

MAURICE SHAMMA[x] and V. St. GEORGIEV

Keyphrases □ Camptothecin—structure elucidation, synthesis, pharmacology □ Alkaloids, pentacyclic—structure elucidation, synthesis, and pharmacology of camptothecin □ Antitumor activity, pentacyclic alkaloid camptothecin—structure, synthesis, pharmacology

Camptothecin is an alkaloid with a novel ring system. As will be discussed later, it has been reported to possess antileukemic and antitumor activities in animals and humans. In 1966, Wall *et al.* (1) described the isolation and structural determination of this new alkaloid, which was assigned the unusual structure I. The alkaloid was obtained from the stem wood of the tree *Camptotheca acuminata* (Nyssaceae), which grows almost exclusively in mainland China (2, 3). This review will focus on the isolation and structural determination of camptothecin and some related compounds and the biogenesis and synthesis of camptothecin.

ISOLATION AND STRUCTURAL ELUCIDATION

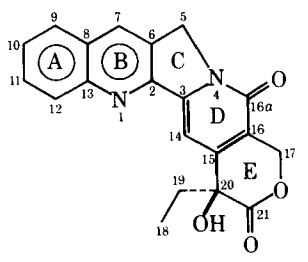
Camptothecin—Camptothecin, mp 264–267° dec., $[\alpha]_D^{25}$ +31.3° [chloroform–methanol (8:2)], showed in its mass spectrum a molecular ion at *m/e* 348.1117 corresponding to the formula $C_{20}H_{16}N_2O_4$ (calc. 348.1111). The UV spectrum exhibited λ_{max} 220, 254, 290, and 370 nm (ϵ 37,320, 29,230, 4980, and 19,900). The IR spectrum had bands corresponding to the presence of hydroxyl (3440 cm^{-1}), δ-lactone (1760–1745 cm^{-1}), lactam (1660 cm^{-1}), and aromatic (1610 and 1588 cm^{-1}) functions. In the NMR spectrum (dimethyl sulfoxide-d_6), signals were present at δ 0.91 (3H, $-CH_2-CH_3$), 1.90 [2H, >C(—OH)—CH_2—CH_3], 5.45 (2H, Ar—CH_2O—), and 5.28 (2H, Ar—CH_2N<).

A mono-*O*-acetyl derivative could be obtained following acetylation, while exhaustive hydrogenation with Adams catalyst in acetic acid gave rise to the dodecahydro compound II (1). In the presence of sodium hydroxide, the sodium salt III was readily formed. Treatment of camptothecin (I) with thionyl chloride yielded chlorocamptothecin (IV), which could be hydrogenolized with palladium-on-carbon to 20-deoxycamptothecin (V). Finally, reduction of camptothecin with sodium borohydride led to the lactol VI (4).

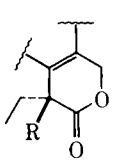
The structure and absolute configuration of the alkaloid were conclusively established through X-ray analysis of the iodoacetate salt (1, 5). The molecular formulas and melting points for camptothecin and a number of its derivatives and analogs are given in Table I.

Very recently, the Indian plant *Mappia foetida* Miers. was found to be an alternative source of camptothecin (6).

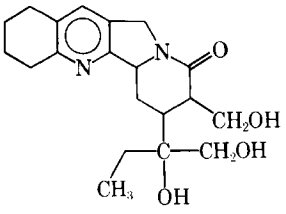
10-Hydroxycamptothecin and 10-Methoxycamptothecin—Further examination of the alkaloidal ex-



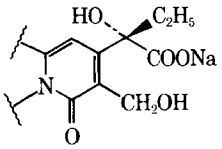
I



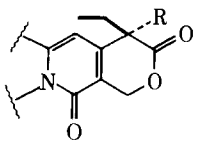
Ia: R = $OCOCH_3$
Ib: R = $OCOCH_2Cl$
Ic: R = $OCOCH_2I$
Id: R = Cl



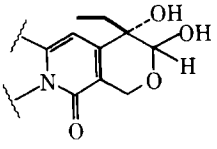
II



III



IV: R = Cl
V: R = H



VI

Table I—Molecular Formulas and Melting Points of Camptothecin and Derivatives

Alkaloid	Formula	Melting Point	Reference
Camptothecin (I)	$C_{20}H_{16}N_2O_4$	264–267°	1
Camptothecin acetate (I*a*)	$C_{22}H_{18}N_2O_5$	271–274°	1
Camptothecin chloroacetate (I*b*)	$C_{22}H_{17}ClN_2O_5$	245–248°	1
Camptothecin iodoacetate (I*c*)	$C_{22}H_{17}IN_2O_5$	238–240°	1
Chlorocamptothecin (I*d*)	$C_{20}H_{15}ClN_2O_3$	248–250°	1
10-Hydroxycamptothecin (VII)	$C_{21}H_{18}N_2O_5$	268–270°	7
9-Methoxycamptothecin (XIII)	$C_{21}H_{18}N_2O_5$	258–260°	6
10-Methoxycamptothecin (VIII)	$C_{21}H_{18}N_2O_5$	254–255°	7

tract of *C. acuminata* resulted in the isolation of two additional alkaloids of the same group: *(a)* 10-hydroxycamptothecin (VII), $C_{20}H_{16}N_2O_5$, mp 268–270°; and *(b)* 10-methoxycamptothecin (VIII), $C_{21}H_{18}N_2O_5$, mp 254–255° (7).

The exact positions of the hydroxyl group in VII and of the methoxyl group in VIII were established from the NMR spectra of VIII and the synthetic analogs IX–XII possessing a methoxyl function at each possible site in ring A (7, 8).

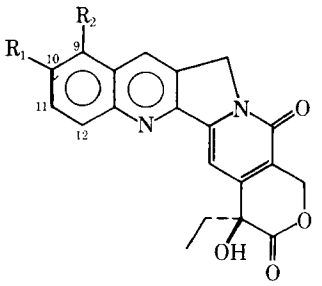
9-Methoxycamptothecin—This alkaloid has been isolated from *M. foetida* Miers. as a minor component of an alkaloidal mixture consisting chiefly of camptothecin (I). Structure XIII was suggested for the new alkaloid (6).

BIOGENESIS

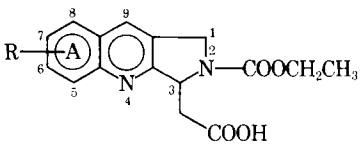
The biogenesis of camptothecin (I) involves the formation of the 1,3-dihydro-2*H*-pyrrolo[3,4*b*]quinoline system (XIV) which was unknown in natural products chemistry prior to the characterization of the alkaloid. The numbering system is based on the Le Men–Taylor model (9).

Careful inspection of the camptothecin molecule reveals structural similarities with such indole alkaloids as rubescine (XV) (10), hunterburnine methiodide (XVI) (5), and dihydrocorynantheol (XVII) (5) as well as with several *Adina* alkaloids (11). Although less obvious, camptothecin (I) and ajmalicine (XVIII) are also related in that both contain the familiar 10-carbon unit XIX characteristic of the Corynanthe family of indole alkaloids (9, 12).

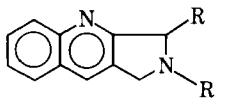
This relationship suggested that camptothecin, like the indole alkaloids, is of tryptamine–terpene origin. Using this premise, Wenkert *et al.* (13) then treated the biogenesis of camptothecin as simply an extension of that for the indole alkaloids (14–22). Two rearrangements of the indole alkaloid isositsirikine (XX) were envisioned to lead to camptothecin (13).



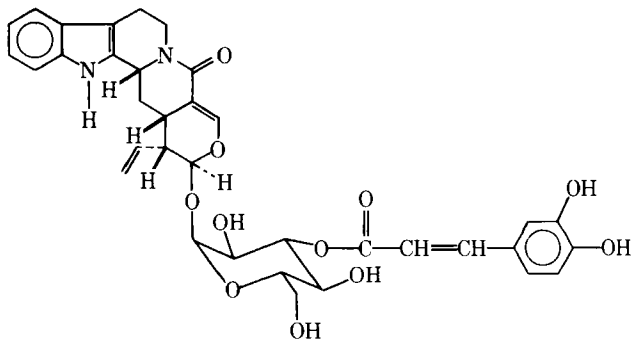
VII: R_1 = OH, R_2 = H
VIII: R_1 = OCH_3, R_2 = H
XIII: R_1 = H, R_2 = OCH_3



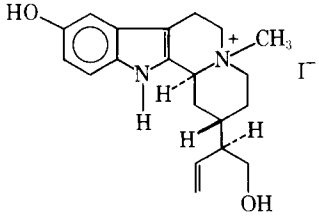
IX: R = 5-OCH_3
X: R = 6-OCH_3
XI: R = 7-OCH_3
XII: R = 8-OCH_3



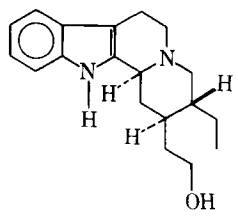
XIV



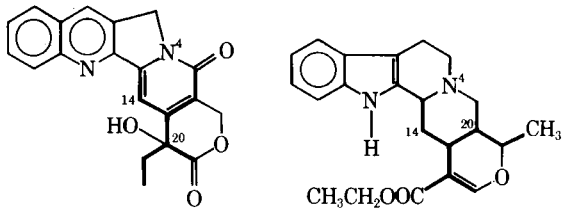
XV



XVI

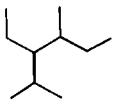


XVII

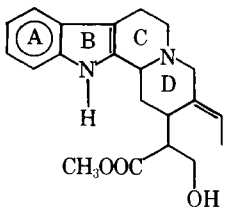


I

XVIII

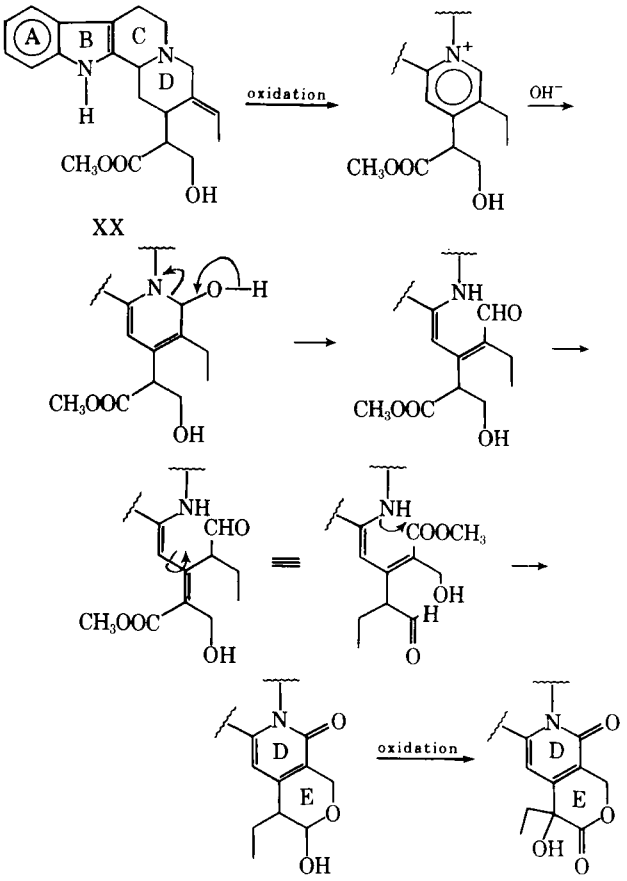


XIX



XX

One sequence involves the conversion of the tetrahydrocarboline system (rings A, B, and C) into the pyrroloquinoline moiety through a series of known reactions (Scheme I) (23–25). The second sequence includes cleavage of ring D of isositsirikine followed by recyclization to give the carbon skeleton appropriate to rings D and E of camptothecin (Scheme II).

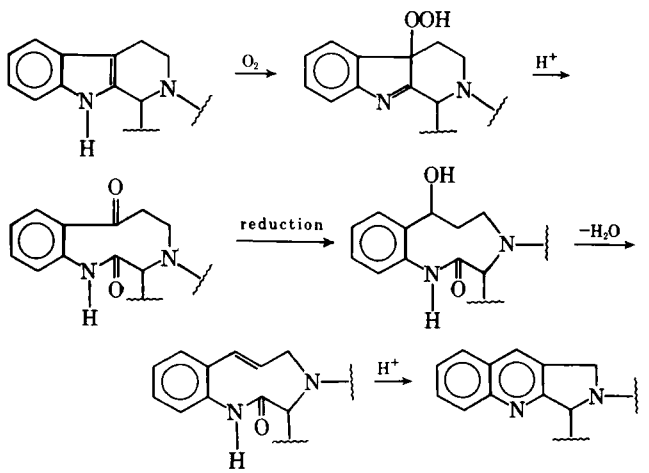


Scheme II

SYNTHESIS OF CAMPTOTHECIN

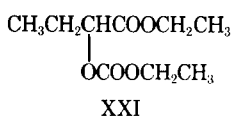
Early reports ascribing promising antitumor activity to camptothecin, coupled with the inaccessibility of *C. acuminata*, generated considerable interest in the total synthesis of the alkaloid. Additionally, a structure involving an α-hydroxy-δ-lactone together with a pyridone system fused to a five-membered ring presents an interesting challenge in organic synthesis. Synthetic efforts culminated recently in several independent preparations of (±)-camptothecin.

The Stork–Schultz Synthesis—Stork and Schultz (26) reported the first total synthesis of (±)-camptothecin in 1971. The starting material was the tricyclic quinoline carboxylic acid XXV, incorporating potential rings A, B, and C of camptothecin, which was obtained by Friedländer condensation of anthranilaldehyde with the known pyrrolidinone XXIV. Following hydrolysis and esterification, the resulting amino ester XXVI was condensed with carbethoxyacetyl chloride to give the diester amide XXVII. Dieckmann cyclization furnished the β-keto ester XXVIII, and hydrolysis and decarboxylation afforded the β-keto lactam XXIX. The β-hydroxy lactam XXX, obtained through reduction of XXIX with sodium borohydride, was then dehydrated to the unsaturated lactam XXXI.

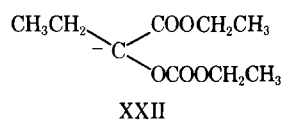


Scheme I

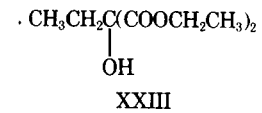
The stage was now set for the critical annelation of ring E of the camptothecin molecule. To begin with, the use of 1 equivalent of lithium diisopropylamide at dry ice–acetone temperature on the carbonate ester XXI generated the desired anion XXII. Low temperature was necessitated by the fact that the anion tended to rearrange to the tartronic ester XXIII at room temperature. Michael addition of the anion XXII to the unsaturated lactam XXXI generated the γ-lactone XXXII in 85% yield. The carbox-



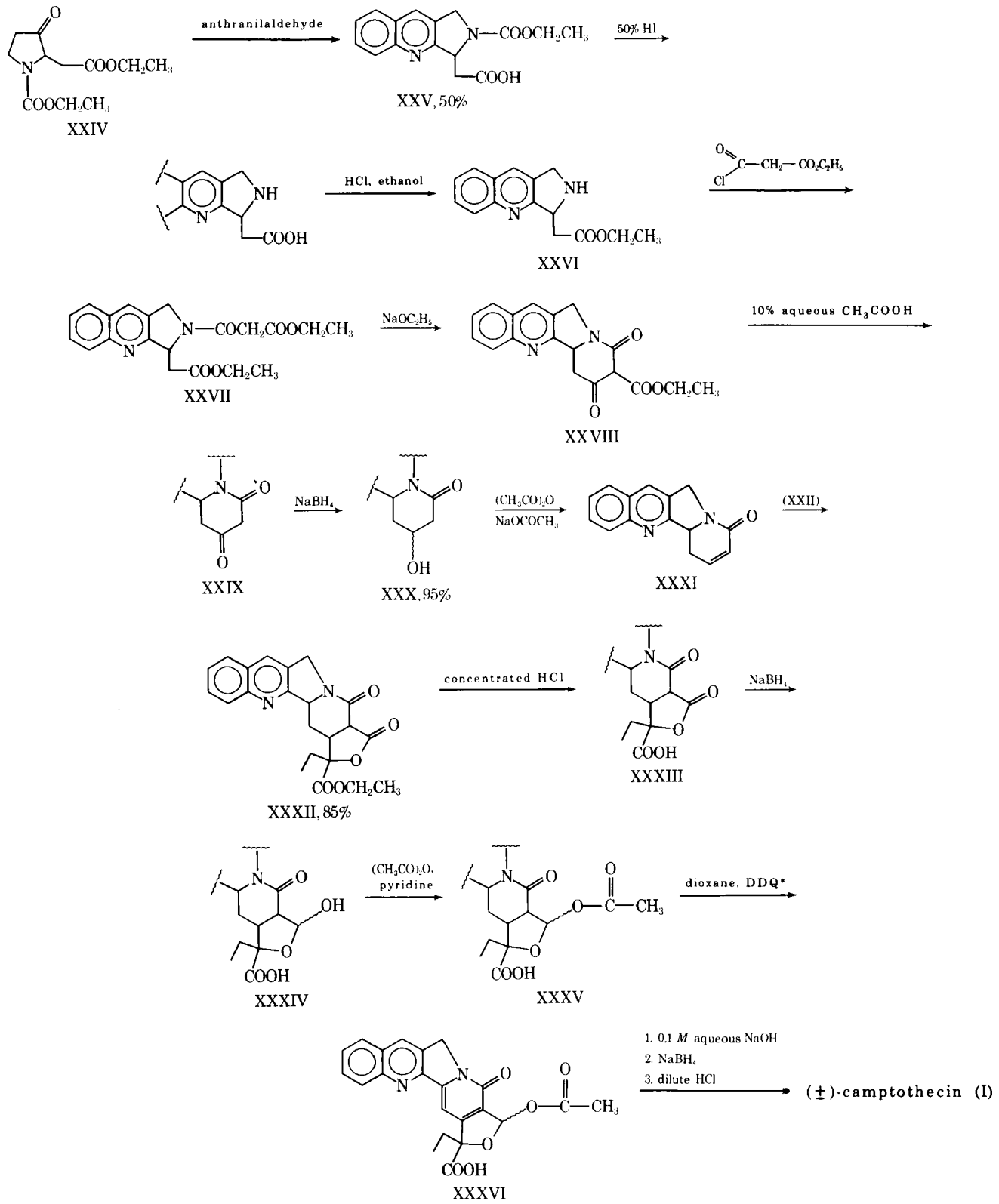
XXI



XXII



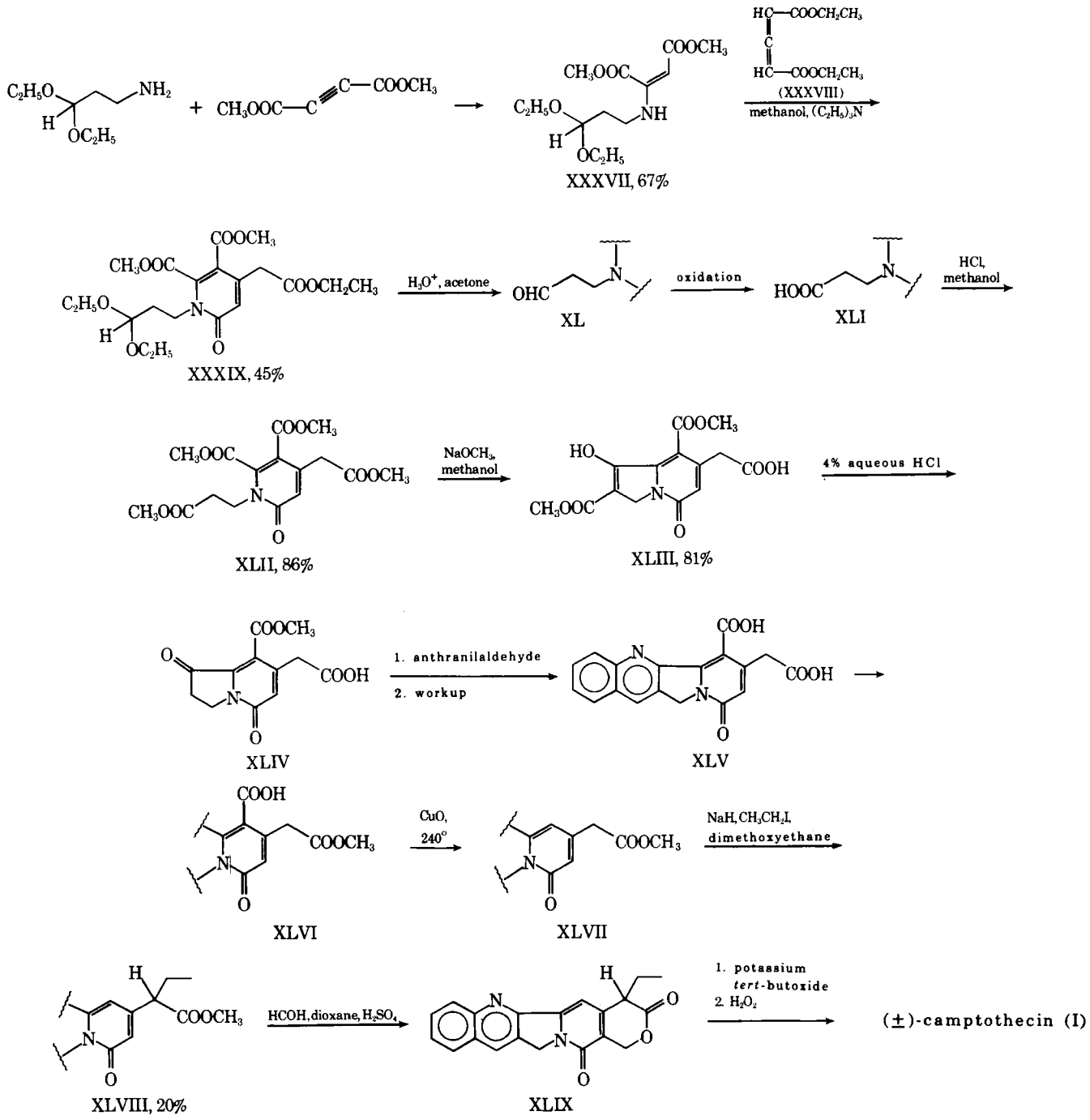
XXIII



*Scheme III—*DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone*

ylic acid XXXIII derived from hydrolysis of the ethyl ester function in XXXII was then reduced to the hemiacetal XXXIV. *O*-Acetylation (XXXV) and dehydrogenation subsequently supplied the pyridone XXXVI. The pyridone XXXVI was converted to (±)-camptothecin through hydrolysis with dilute sodium hydroxide, sodium borohydride reduction, and acidification with dilute hydrochloric acid (Scheme III).

The Volkmann-Danishefsky-Eggler-Solomon Synthesis—The first step in this synthesis (27) was the preparation of the enamino ester XXXVII through the reaction of β-aminopropionaldehyde di-

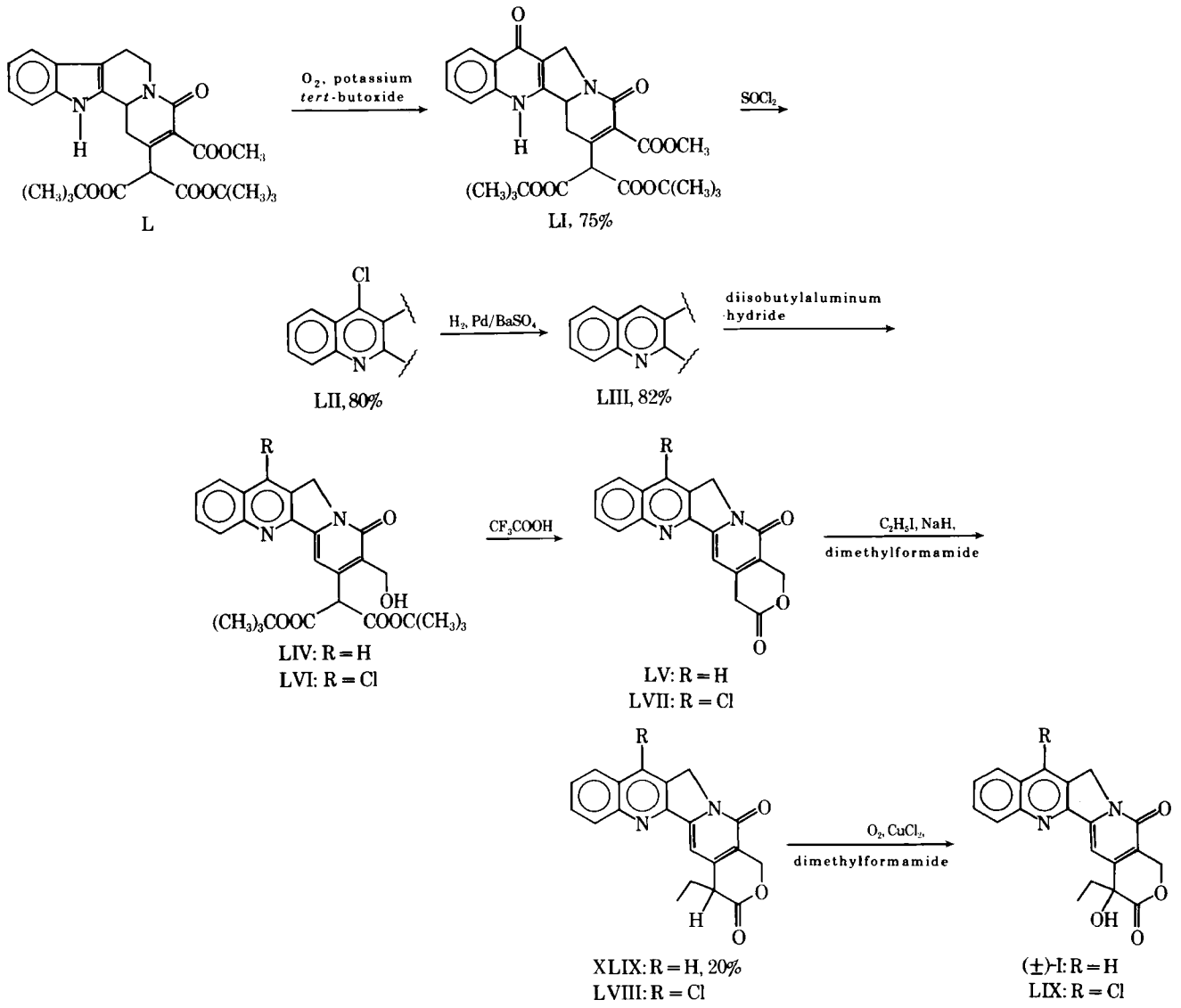


Scheme IV

ethyl acetal with dicarbomethoxyacetylene. Condensation of XXXVII with dicarbethoxyallene (XXXVIII) in methanol containing triethylamine afforded the pyridone triester XXXIX in what amounts to a new pyridone synthesis. Acid hydrolysis achieved the quantitative transformation of this material to the aldehyde XL.

The next two steps, oxidation and esterification–transesterification, were directed toward the formation of the tetraester XLII, which possesses the appropriate substituents for the construction of ring C of camptothecin. Dieckmann ring closure of XLII led reproducibly, in 81% yield, to the enolic acid ester XLIII which, following hydrolysis and selective decarboxylation, gave rise to the keto acid XLIV. Friedländer condensation with anthranilaldehyde was accompanied by hydrolysis of the ester function. The resulting tetracyclic diacid XLV was selectively esterified to the monocarboxylic acid ester XLVI, and subsequent decarboxylation provided the tetracyclic ester XLVII in 29% yield from the enolic acid ester XLIII.

C-Ethylation of XLVII gave the ethylated monoester XLVIII, which was converted to (±)-20-deoxycamptothecin (XLIX) after treatment with paraformaldehyde in dioxane containing a trace of sulfuric



Scheme V

acid. Hydrogen peroxide oxidation of the anion of XLIX then afforded (±)-camptothecin (I) (Scheme IV).

The Winterfeldt Synthesis—It was noted earlier in this review that the biogenetic pathway to camptothecin most probably involves the oxidation of an indole double bond followed by recyclization and aromatization to a quinoline system (9, 13). A biogenetically patterned synthesis of (±)-camptothecin based on such a series of transformations was carried out by Winterfeldt and coworkers (28–30).

The previously known (31) indolic intermediate L readily underwent the indole-quinolone conversion upon oxidation (30) so that Compound LI was obtained in 75% yield. The required quinoline analog LIII was then obtained through the classical sequence involving treatment with thionyl chloride to give LII and hydrogenolysis using palladium–barium sulfate.

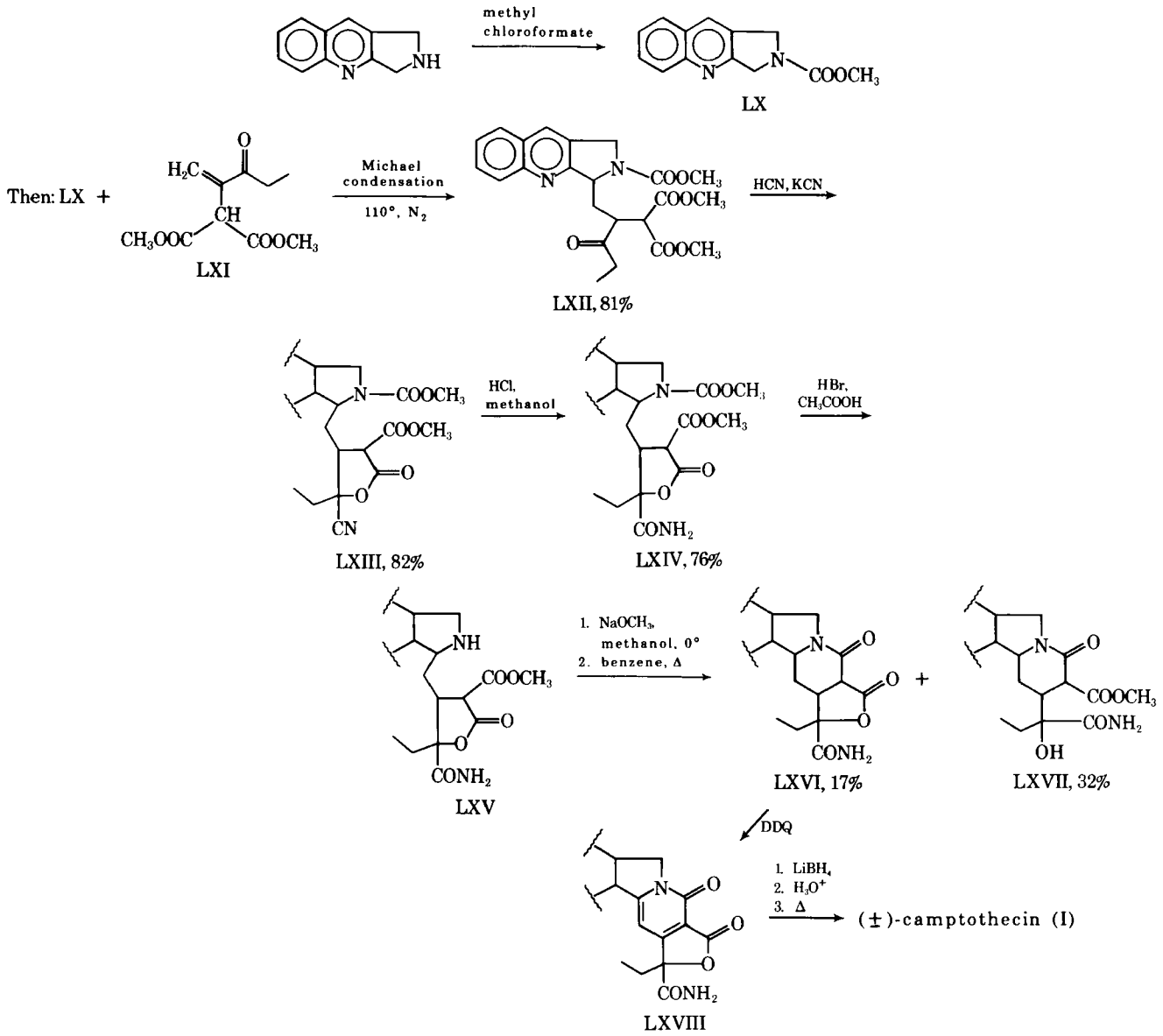
Selective reduction of LIII using diisobutylaluminum hydride led to the alcohol LIV which, in turn, led to the δ-lactone LV. C-Alkylation of LV followed by oxidation of the resulting (±)-20-deoxycamptothecin (XLIX) with oxygen and cupric chloride supplied (±)-camptothecin (Scheme V).

In a parallel sequence, 7-chlorocamptothecin (LIX) was prepared through similar reactions (LVI→LVII→LVIII→LIX).

The Wani–Wall–Levine Synthesis—1,3-Dihydro-2-methoxycarbonyl- 2*H*-pyrrolo[3,4*b*]quinoline (LX), which served as the starting material for this synthesis (32), undergoes a crucial Michael condensation with the keto diester LXI to produce the urethan LXII in 81% yield. This urethan possesses nearly all of the functionalities necessary for the eventual formation of rings D and E of camptothecin.

Treatment of LXII with potassium cyanide in liquid hydrogen cyanide generated a cyanohydrin, which immediately lactonized to a mixture of diastereoisomeric cyano lactones LXIII. The diastereoisomeric mixture of amides LXIV was obtained upon treatment of LXIII with methanolic hydrogen chloride.

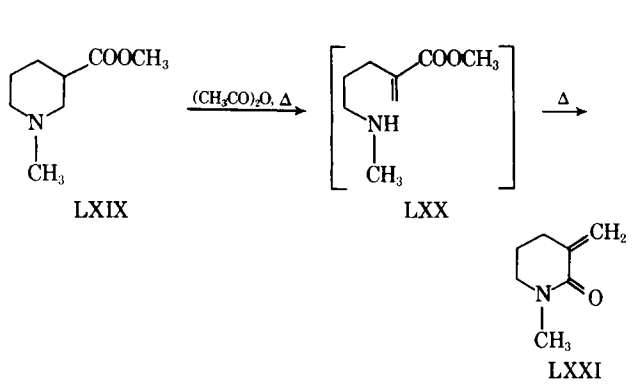
To construct ring D of camptothecin, the *N*-carbo-



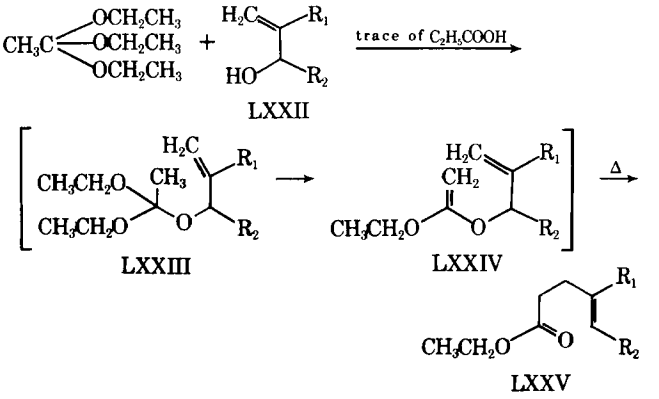
Scheme VI

methoxy group of LXIV was selectively removed using hydrogen bromide in glacial acetic acid, and the resulting amine LXV was refluxed with base. A separable mixture of lactams LXVI and LXVII was thus obtained. The remainder of the synthesis involved the oxidation of LXVI into LXVIII and reduction of the lactone carbonyl in the latter compound with lithium borohydride. Acidification of the reaction mixture followed by heating on a steam bath gave rise to (±)-camptothecin (I) (Scheme VI).

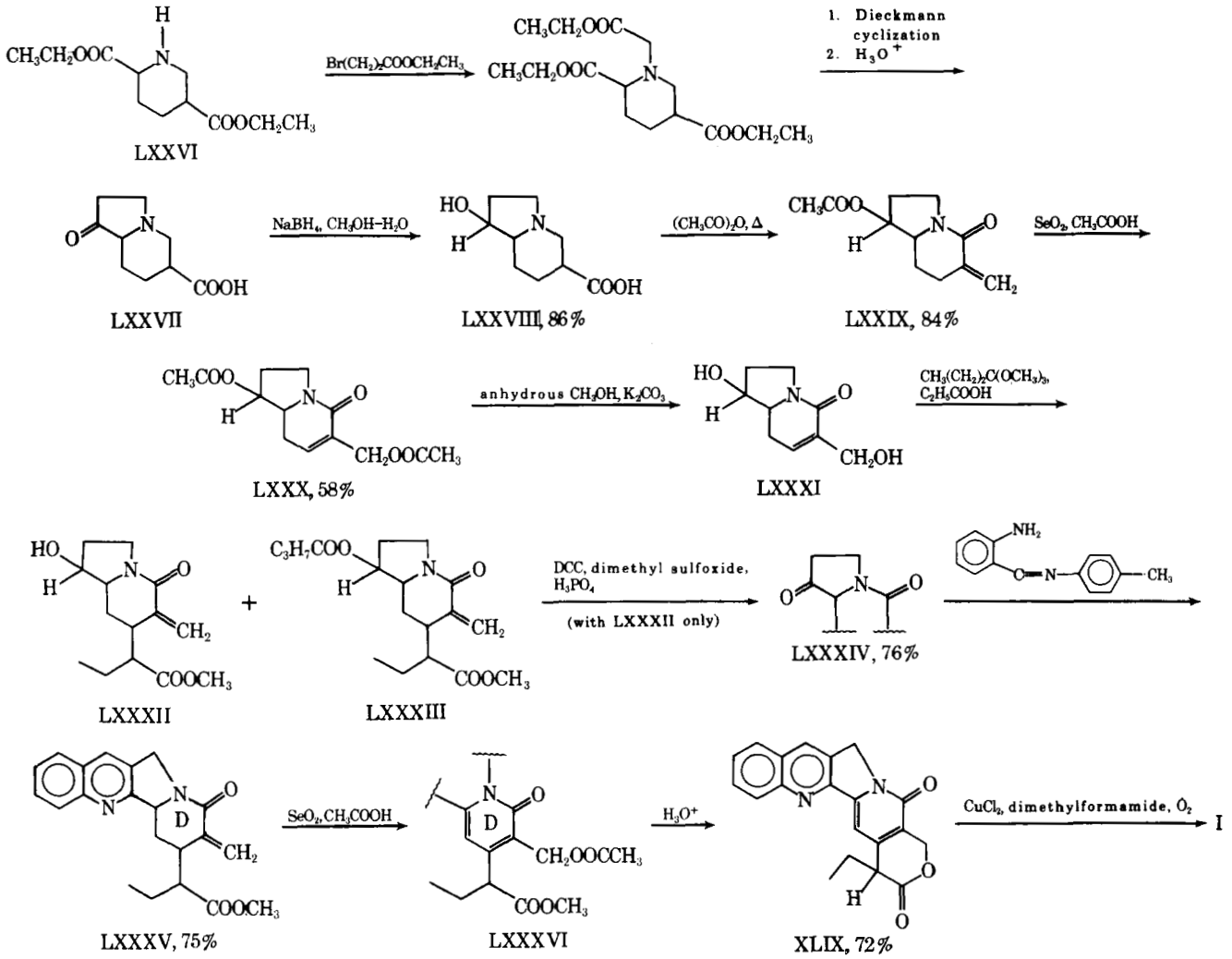
The Rapoport–Tang Synthesis—Two known reactions were of primary importance in this synthesis (33). First, the α-methylene-lactam rearrange-



Scheme VII



Scheme VIII



Scheme IX

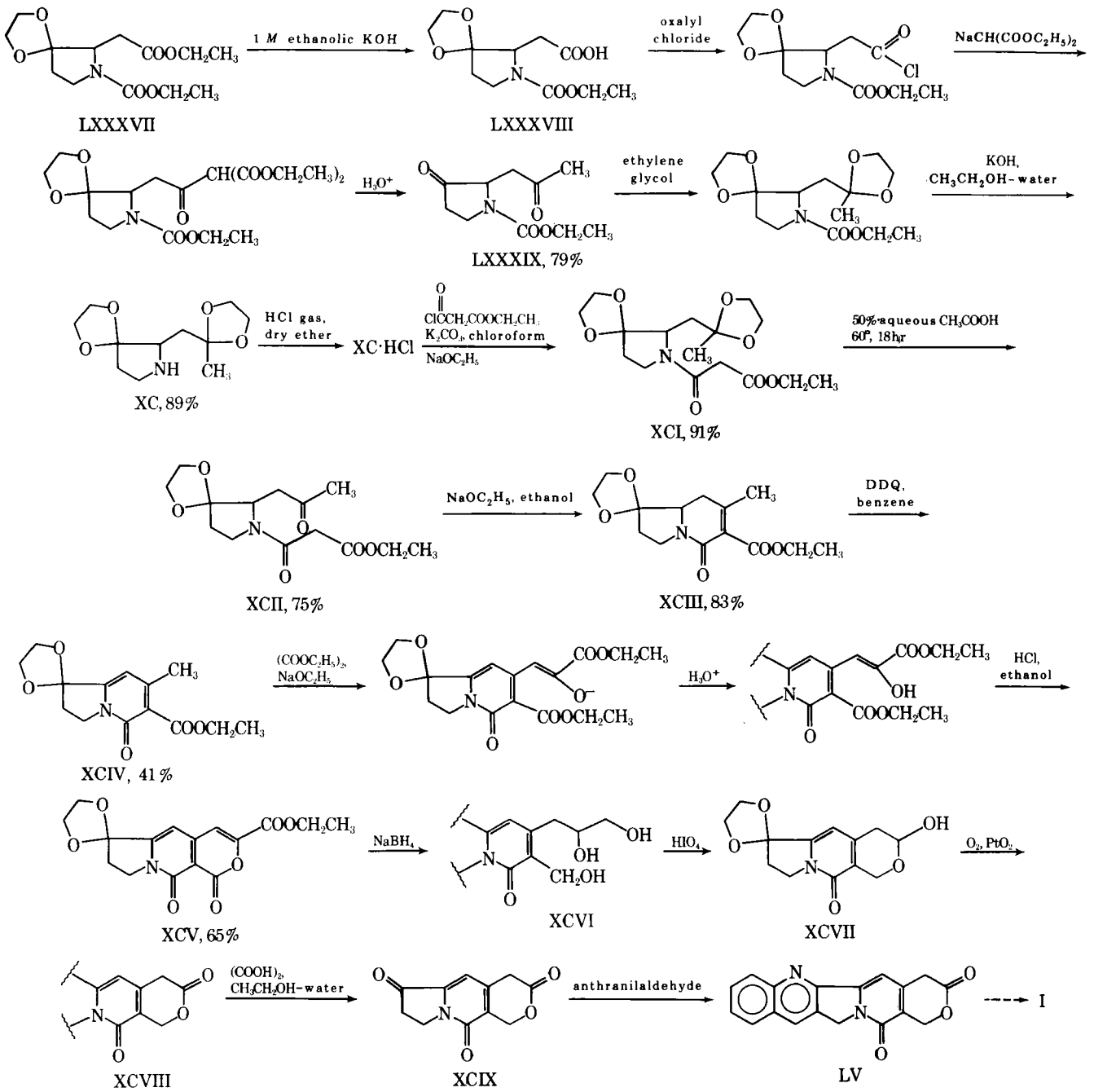
ment (34), when applied to a simple β-amino ester such as 1-methylnipecotinate (LXIX), led to the unsaturated lactam LXXI through the probable intermediacy of the α,β-unsaturated ester LXX (Scheme VII).

Second, the new Johnson *et al.* (35) modification of the Claisen thermal rearrangement had been proven to be an efficient method for the preparation of substituted olefins. The method simply involves heating an allylic alcohol, *e.g.*, LXXII, with an excess of an orthoester such as ethyl orthoacetate in the presence of a trace of a weak acid, usually propionic acid. Evidently, the mixed orthoester LXXIII is formed, which loses ethanol to form the ketene acetal LXXIV, which then rearranges to the olefinic ester LXXV (Scheme VIII).

Immediately after initial trials involving the above two transformations using model compounds (described later), a total synthesis of (±)-camptothecin was initiated (33). The first steps of this synthesis dealt with the formation of rings C and D of the alkaloid. The ketonic β-amino acid LXXVII obtained from the diester LXXVI was reduced with sodium borohydride in methanol–water to the alcoholic β-amino acid LXXVIII. When refluxed with acetic anhydride, LXXVIII underwent the α-methylene lactam rearrangement to afford the piperidone acetate LXXIX as a diastereoisomeric mixture. The diacetate LXXX, obtained *via* selenium dioxide in acetic acid oxidation of LXXIX, was converted to the diol LXXXI in an anhydrous methanol–potassium carbonate medium. Application of the Johnson *et al.* (35) modification of the Claisen rearrangement, using trimethyl orthobutyrate, then gave rise to two products, the free alcohol LXXXII and the butyrate ester LXXXIII, which could readily be converted to the desired alcohol LXXXII.

Following oxidation of LXXXII with dicyclohexylcarbodiimide (DCC) in dimethyl sulfoxide, the AB ring system of camptothecin was constructed *via* Friedländer condensation of the ketone LXXXIV and *N*-(2-aminobenzylidene)-*p*-toluidine.

The remaining transformations in the synthesis, involving aromatization of the ring D as well as formation of the ring E, were accomplished through selenium dioxide oxidation of the α-methylene lactam LXXXV, hydrolysis, lactonization to XLIX, and finally introduction of the C-20 tertiary alcohol function using oxygen–cupric chloride in dimethylformamide (Scheme IX).



LXXXVII LXXXVIII LXXXIX, 79% XC, 89% XC·HCl XCI, 91% XCII, 75% XCIII, 83% XCIV, 41% XCV, 65% XCVI XCVII XCVIII XCIX LV

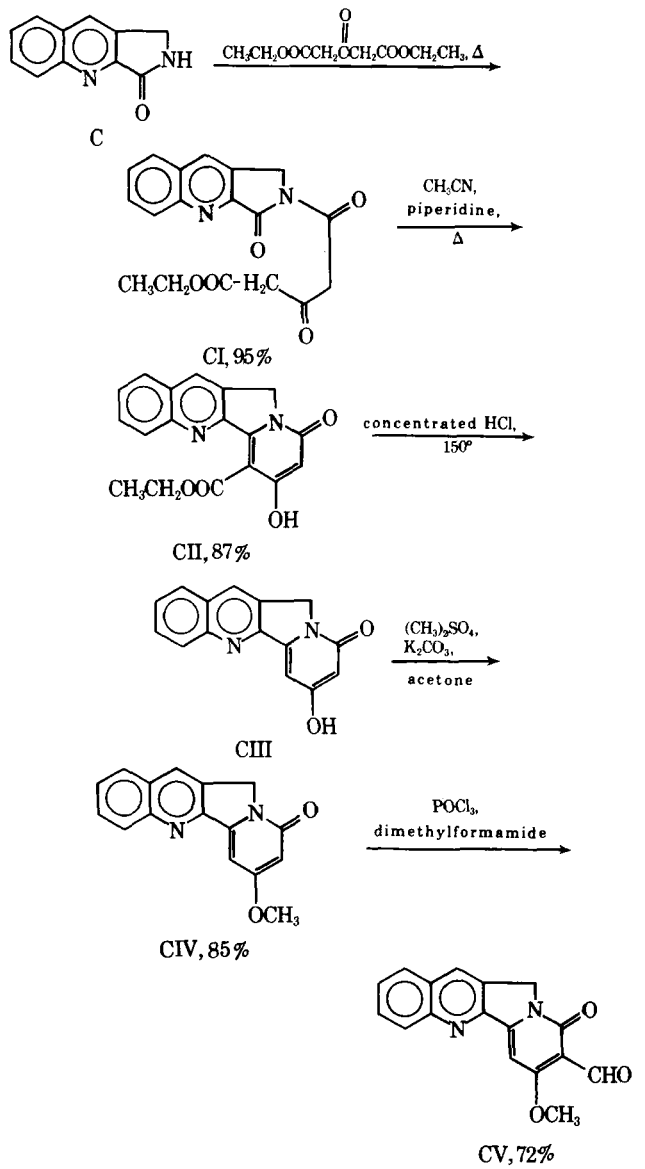
Scheme X

The Shamma–Smithers–Georgiev Synthesis—Since rings A and B in camptothecin primarily impart to the alkaloid its near total insolubility in the usual organic solvents, the construction of these two rings was reserved for the terminal stages of the synthesis (36).

Prolonged treatment of the urethan LXXXVII with 1 *M* ethanolic potassium hydroxide gave the carboxylic acid LXXXVIII in 91% yield. The acid chloride of LXXXVIII was converted to the dione LXXXIX by reaction with the sodium salt of diethyl malonate and acid hydrolysis of the resulting diester. The amine XC, obtained in 89% yield after alkaline hydrolysis of the diethylene ketal of LXXXIX, was acylated to the amide XCI in 91% yield, using carbethoxyacetyl chloride in basic solution.

The critical selective hydrolysis of XCI to the monoketone XCII was achieved in 75% yield using 50% aqueous acetic acid. Treatment of XCII with sodium ethoxide furnished the dihydropyridone XCIII in what was, in effect, a new synthesis of dihydropyridones. Subsequent oxidation of XCIII with excess 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) produced the pyridone XCIV containing rings C and D of camptothecin.

Attention was then turned to the construction of the δ-lactone ring E of camptothecin. Base-catalyzed condensation of the pyridone XCIV with diethyl oxa-

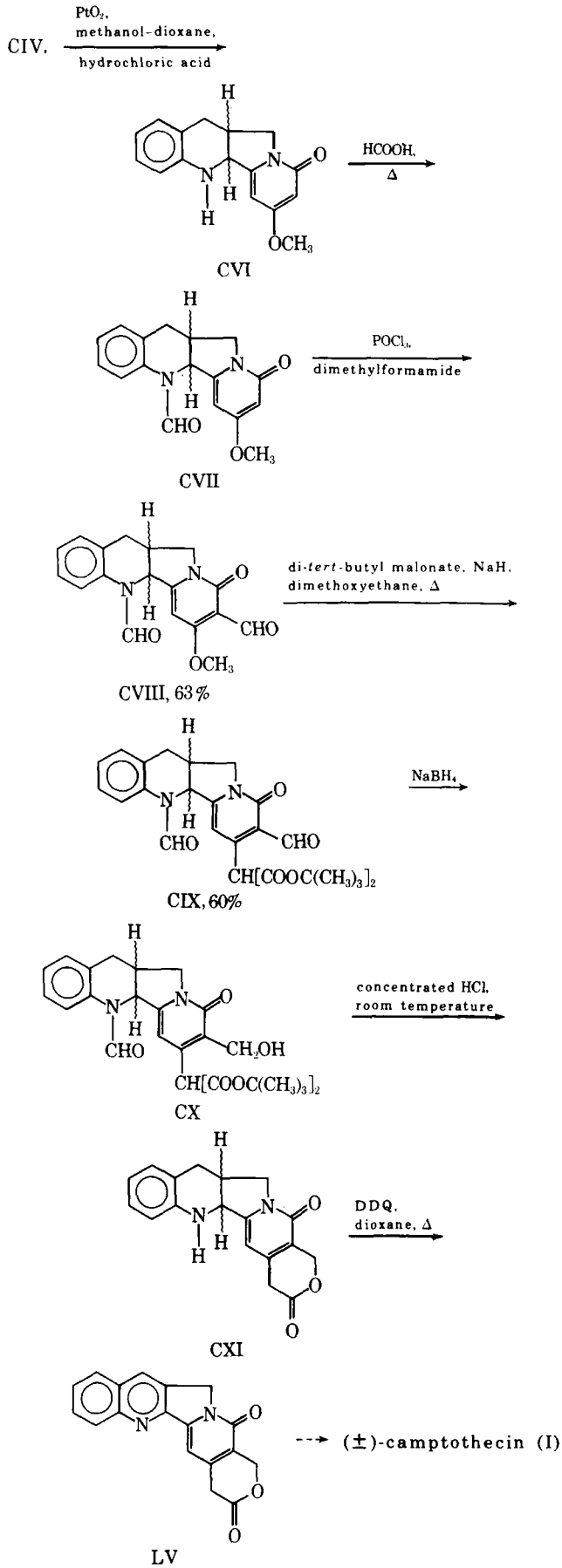


Scheme XI

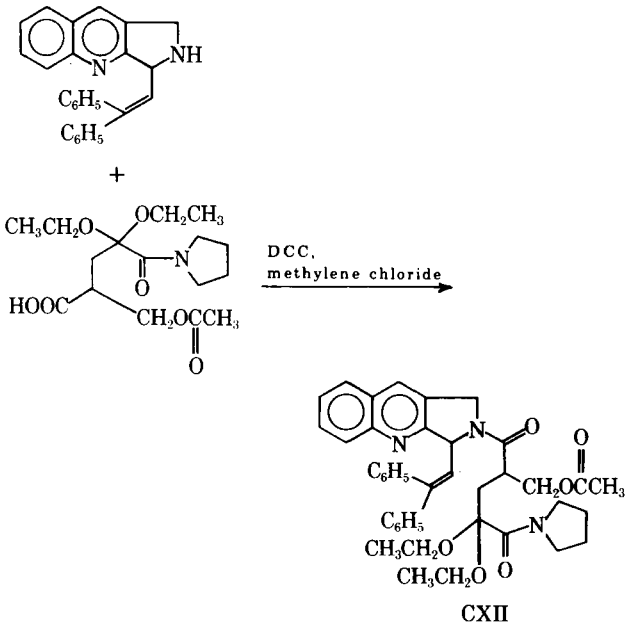
late and acidification of the resulting product afforded the vinyl lactone XCV in 65% yield. The conversion of the latter compound to the δ-lactone XCVIII was carried out utilizing sodium borohydride succeeded by periodic acid. The catalytic oxidation of the resulting hemiacetal XCVII led to the desired δ-lactone XCVIII. The sodium borohydride reduction of the vinyl lactone XCV proceeded through the intermediacy of the triol XCVI, thus indicating a marked susceptibility of the pyrone carbonyl to nucleophilic attack.

Hydrolysis of the ketal function of XCVIII and condensation of the resulting keto lactone XCIX with anthranilaldehyde afforded the known 20-deoxy-2-deethylcamptothecin (LV) (28, 29), which had been previously converted to (±)-camptothecin (I) (28, 29) (Scheme X).

The Sugasawa-Toyoda-Sasakura Synthesis—3-Oxodihydro-2*H*-pyrrolo[3,4*b*]quinoline (C) served as a starting material for this synthesis (37). Heating of C in diethyl acetonedicarboxylate at 160–165° gave rise to the imido ester CI. Intramolecular cycli-



Scheme XII



Scheme XIII

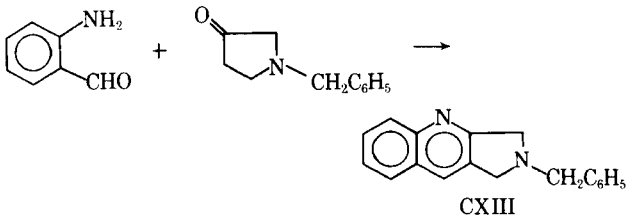
zation of CI to the hydroxypyridone ester CII proceeded smoothly. Hydrolysis and decarboxylation of CII, followed by methylation of the hydroxyl group of the resulting Compound CIII, furnished the 4-methoxy-2-pyridone CIV which possesses the ABCD ring system of camptothecin.

To construct ring E of the alkaloid, the generation of an active substituent next to the methoxyl group was desirable. Thus, treatment of CIV with phosphorus oxychloride in dimethylformamide afforded the methoxyaldehyde CV (Scheme XI).

Initial attempts to build ring E according to an annelation method previously developed (37) (discussed later) were not successful because of the lability of CV under basic conditions. The 4-methoxy-2-pyridone CIV was, therefore, reduced with Adams catalyst in methanol–dioxane containing hydrochloric acid. *N*-Formylation of the resulting Compound CVI, followed by a Vilsmeir C-formylation, gave rise to the corresponding aldehyde CVIII. When the latter compound was treated with di-*tert*-butyl malonate and sodium hydride in refluxing dioxane, the expected malonate ester CIX was obtained. Crude CIX was hydrogenated with sodium borohydride to give CX, which was treated directly with concentrated hydrochloric acid at room temperature to furnish CXI through lactone formation and *N*-deformylation. Dehydrogenation of CXI with 2,3-dichloro-5,6-dicyanobenzoquinone afforded 20-deoxy-2-deethylcamptothecin (LV), which was subsequently ethylated (ethyl iodide–sodium hydride) and oxidized (O_2, copper acetate, triethylamine), thus giving (±)-camptothecin (I) (Scheme XII)

SYNTHESIS OF CAMPTOTHECIN ANALOGS

A variety of simpler analogs of camptothecin have been prepared, and these preparations are discussed in the present section. The work described here is essentially a summary of transformations carried out as models preceding the total synthesis of the alkaloid.



Scheme XIV

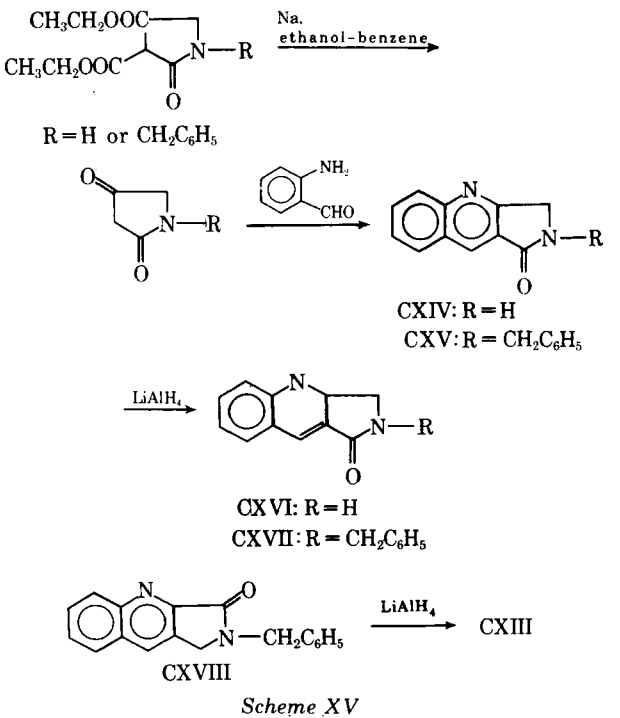
Analogs Containing the ABC Ring System of Camptothecin—In an early attempt at a total synthesis of camptothecin, the preparation of the polyfunctional analog CXII, which incorporates the ABC ring system of the alkaloid as well as some structural features of rings D and E, was described (38) (Scheme XIII).

In a separate study, analog CXIII was prepared *via* Friedländer condensation of anthranilaldehyde and 1-benzyl-3-oxopyrrolidine (39) (Scheme XIV). Lithium aluminum hydride reduction of the tricyclic analogs CXIV and CXV gave lactams CXVI and CXVII, while analog CXVIII was converted mainly to the amine CXIII (39) (Scheme XV).

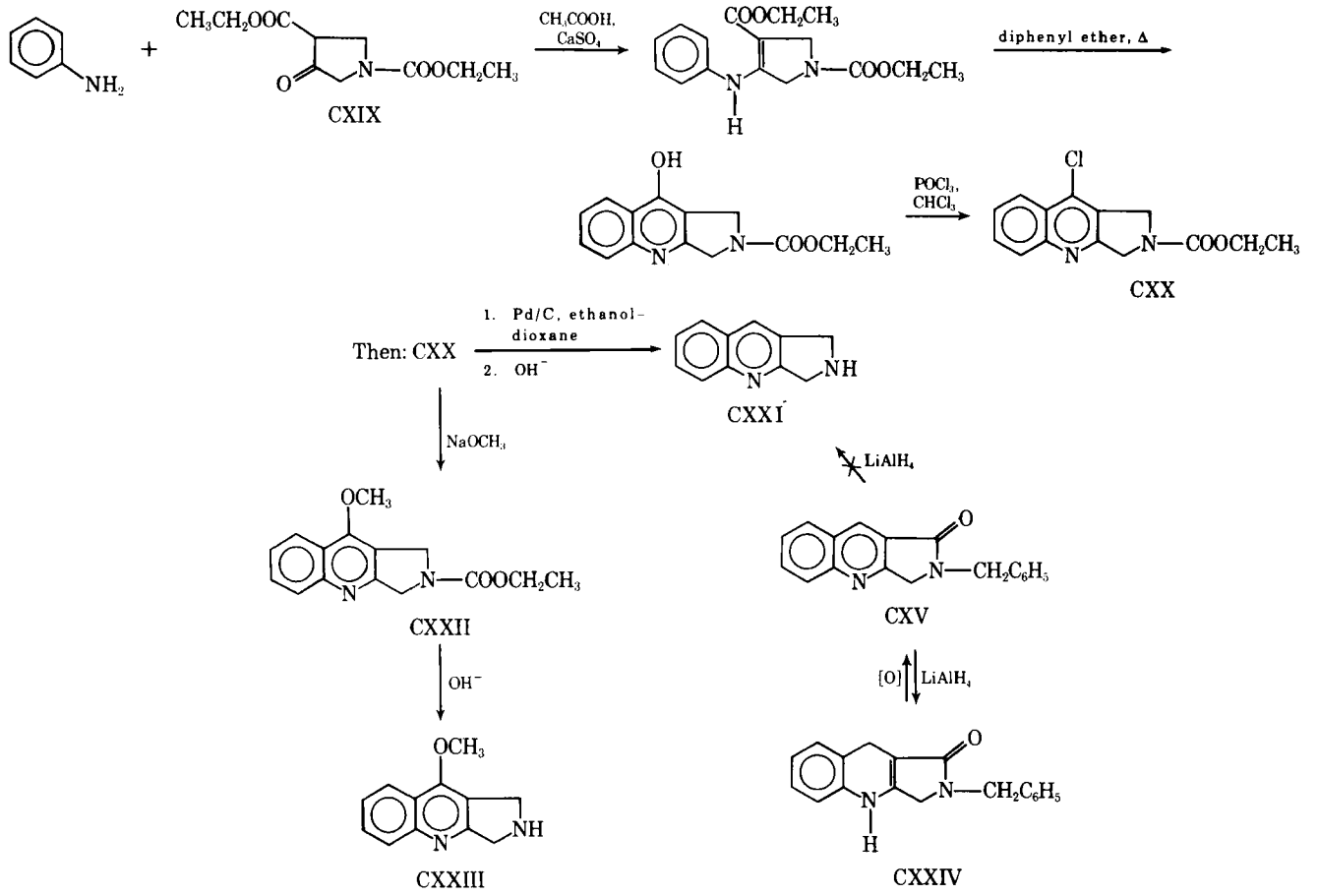
The tricyclic bases CXXI and CXXIII have been prepared using aniline and 1,4-diethoxycarbonyl-3-oxopyrrolidine (CXIX) as starting materials (40). Lithium aluminum hydride reduction of Compound CXV afforded 2-benzyl-1-oxo-2,3,4,9-tetrahydro-1*H*-pyrrolo[3,4*b*]quinoline (CXXIV) instead of the desired CXXI (40) (Scheme XVI). These results are in close analogy with an earlier observation already discussed (39).

Several *N*-substituted analogs of CXXI have also been reported (41, 42).

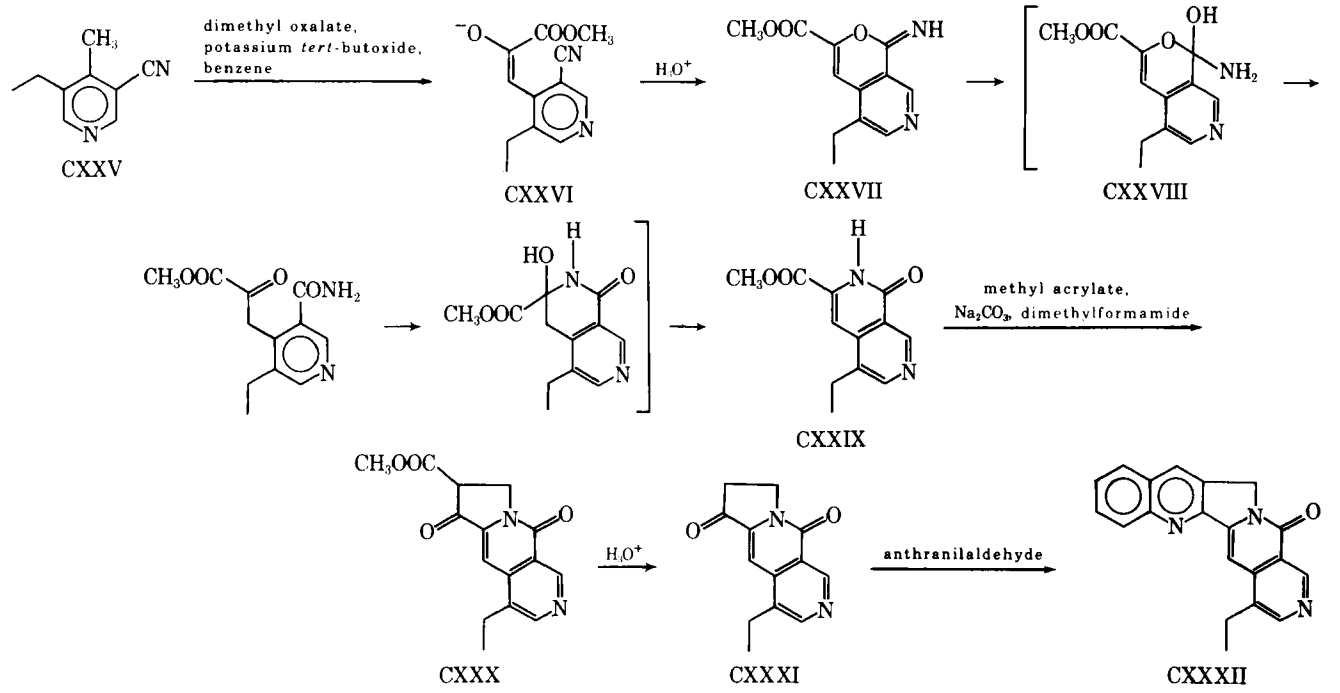
Analogs Containing the ABCD Ring System of Camptothecin—The pentacyclic base CXXXII has been prepared (13) as a potential precursor of the alkaloid. 4-Methyl-5-ethylnicotinonitrile (CXXV) was condensed with dimethyl oxalate, and the resulting



Scheme XV



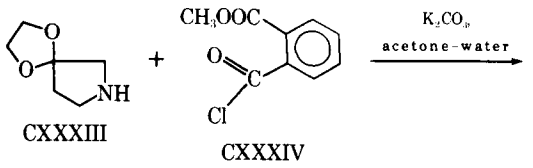
Scheme XVI



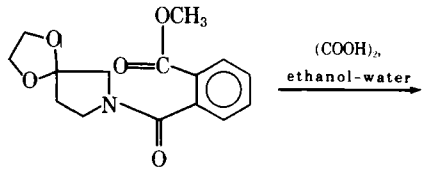
Scheme XVII

enolate salt CXXVI was cyclized in aqueous acid to the azaisocarbostyril CXXIX through the intermediacy of CXXVII and CXXVIII. Condensation of CXXIX with methyl acrylate produced the diester CXXX, which was converted to the ketone CXXXI through hydrolysis and decarboxylation. Finally, Friedländer condensation using anthranilaldehyde led to the desired pentacyclic base CXXXII (Scheme XVII).

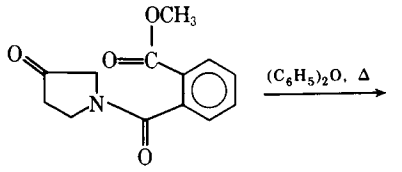
Another approach (43) to a pentacyclic system



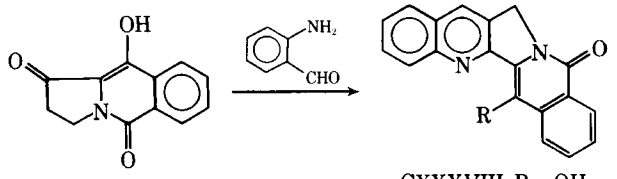
CXXXIII CXXXIV



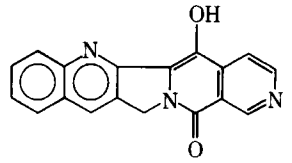
CXXXV



CXXXVI

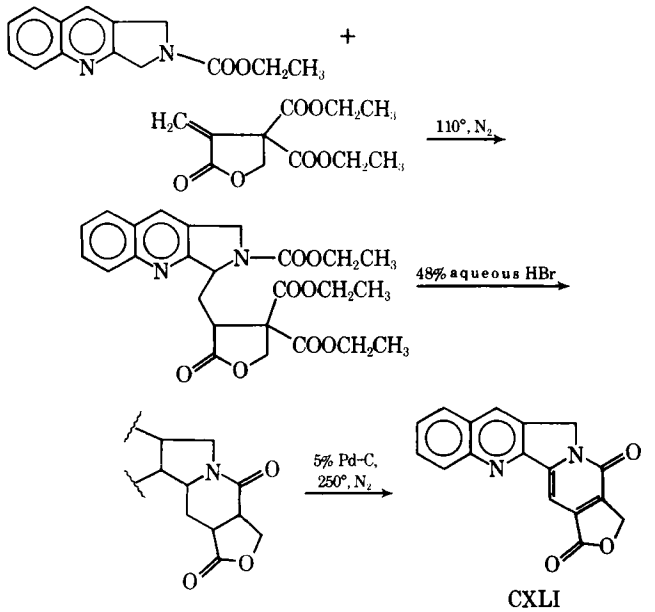


CXXXVII CXXXVIII: R = OH
CXXXIX: R = H



CXL

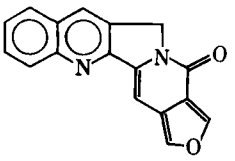
Scheme XVIII



CXLI

Scheme XIX

bearing resemblance to camptothecin involved condensation of the 3-pyrrolidinone ethylene ketal CXXXIII with the acid chloride of phthalic acid monomethyl ester CXXXIV to afford the ester amide



CXLII

CXXXV. Deketalization to CXXXVI followed by cyclization engendered the tricyclic ketone CXXXVII which, upon Friedländer condensation, led to the pentacyclic analog CXXXVIII (Scheme XVIII).

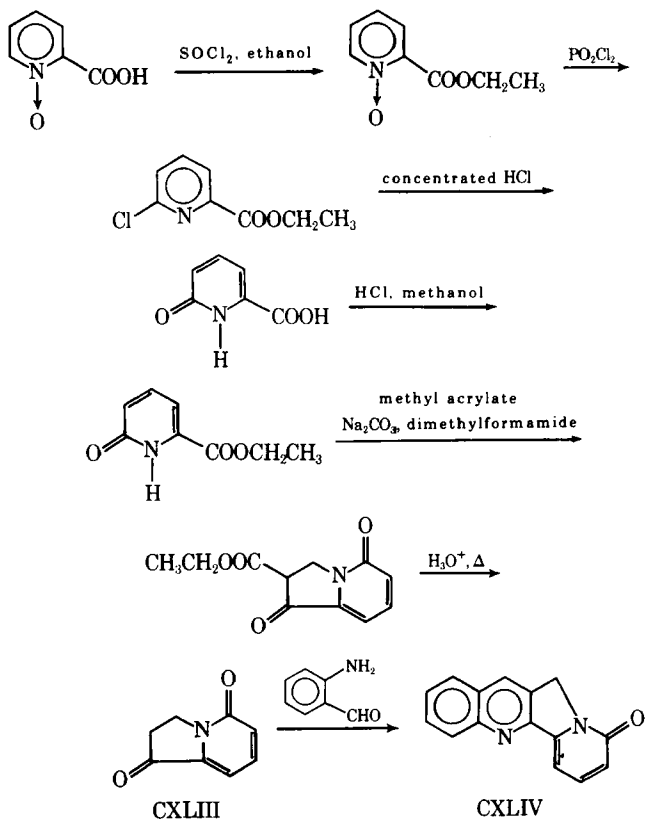
Using parallel routes, the pentacyclic species CXXXIX and CXL have also been prepared (43) (Scheme XVIII). Analog CXLI, which possesses a γ-lactone rather than the required δ-lactone, has also been prepared (44) (Scheme XIX).

Yet another pentacyclic analog of camptothecin is the furanoid derivative CXLII whose synthesis was reported in brief form (45).

Friedländer condensation of anthranilaldehyde with 3-oxopyrrolidino[1,2*f*]-2-pyridone (CXLIII) resulted in formation of the ABCD ring system CXLIV of camptothecin (46) (Scheme XX).

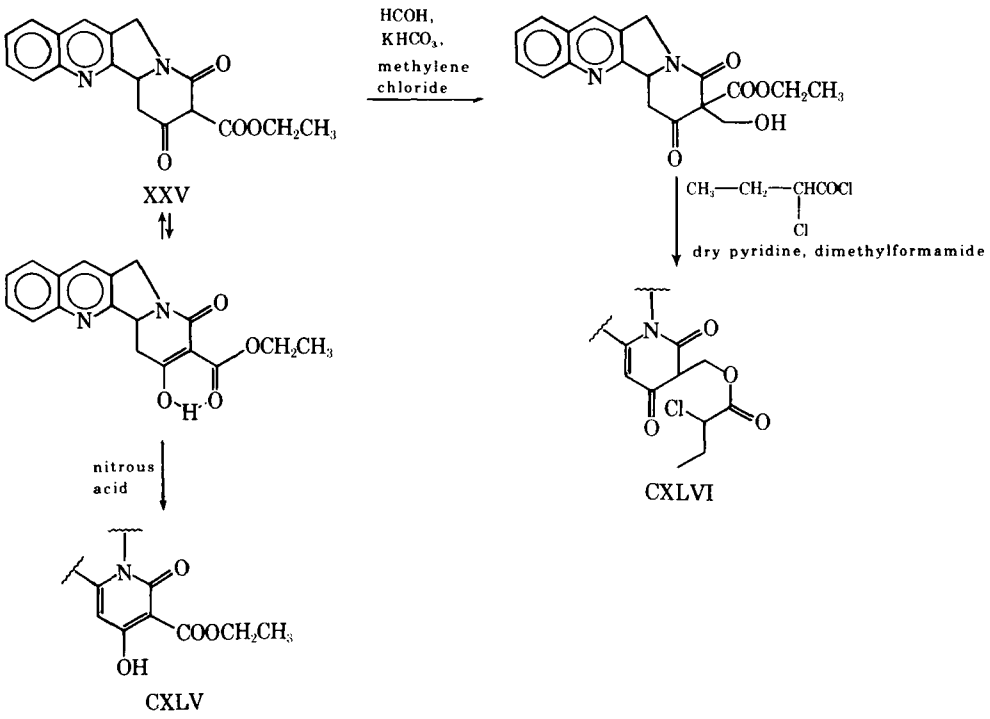
Two camptothecin analogs, CXLV and CXLVI, have been prepared (47) using the key intermediate XXV (Scheme XXI).

Condensation of base CXXII (already discussed) with β-formylpropionic acid diethyl acetal, followed by treatment with dilute hydrochloric acid and then intramolecular cyclization, gave rise to the lactam CXLVII. Following dehydrogenation, the tetracyclic analog CXLVIII was obtained (40) (Scheme XXII).



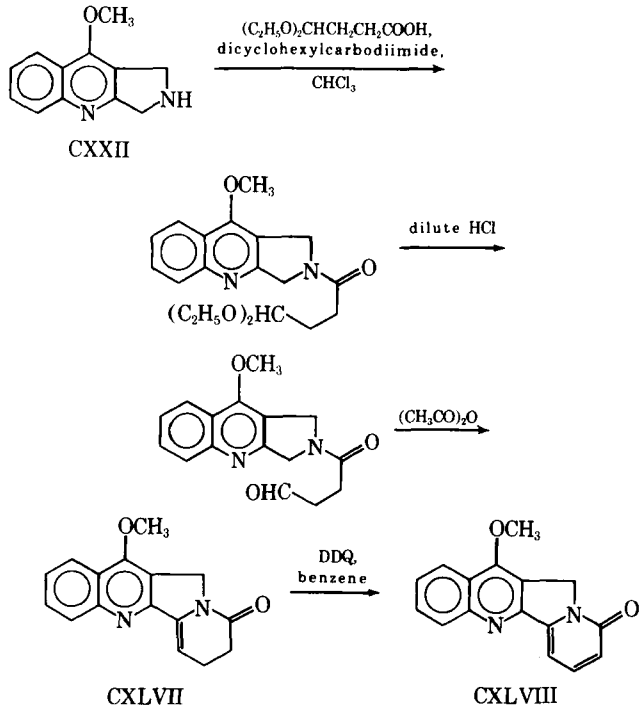
CXLIII CXLIV

Scheme XX



Scheme XXI

An unusual and interesting entry into rings ABCD of camptothecin, provided by Borch *et al.* (48), involved in the key step the condensation of the imminium salt CLI with the anion CLII. *N,N*-Dimethyl-3-cyanoquinaldamide (CL) was first prepared by Friedländer condensation between *o*-aminobenzaldehyde and ethyl 3-cyanopyruvate, followed by treatment of the resulting ester CXLIX with dimethylamine in ethanol. The next step involved the preparation of the enamine CLIII using the imminium salt CLI and the triester ion CLII. Catalytic hydrogenation of CLIII furnished the corresponding C-3 aminomethyl derivative which, without isolation, underwent an intramolecular enamine exchange-cyclization reaction to give the camptothecin analog CLIV (Scheme XXIII).



Scheme XXII

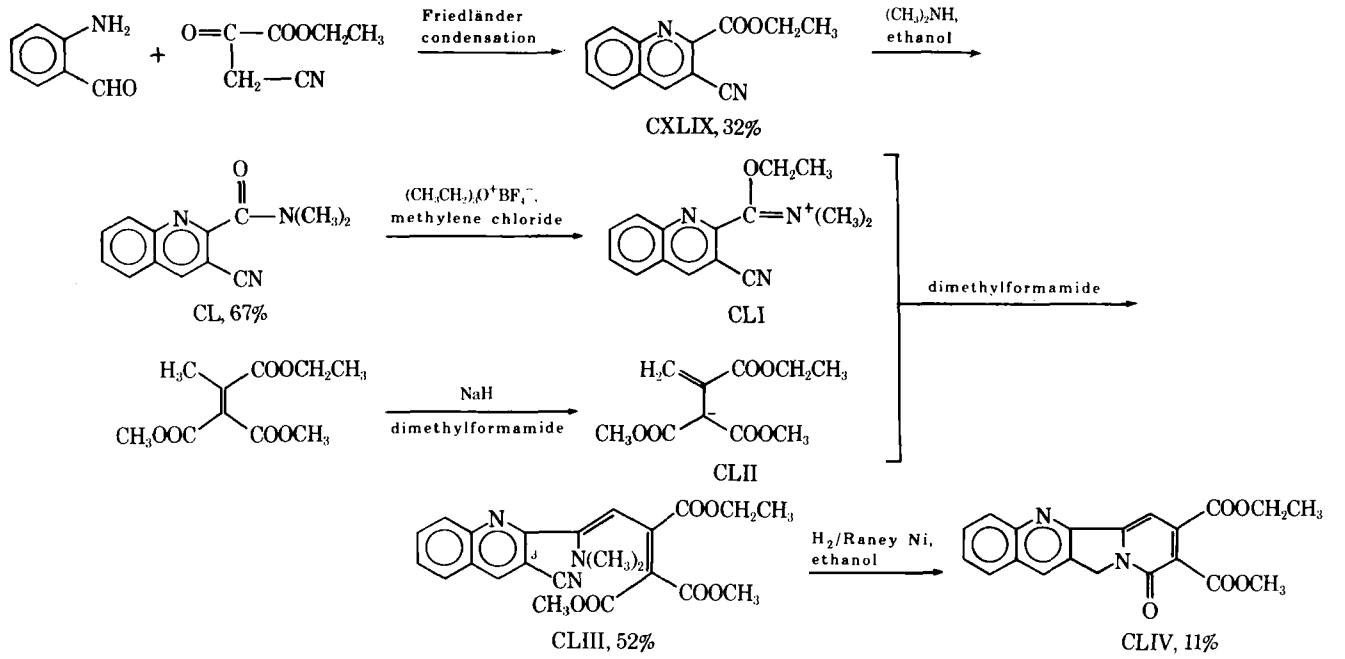
Finally, the tetracyclic pyridone CLVI has been synthesized (49) *via* Friedländer condensation of anthranilaldehyde with the pyridone CLV (Scheme XXIV).

Compounds Possessing Rings D and/or E of Camptothecin—Since the presence of an α-hydroxy-δ-lactone in camptothecin was thought to be responsible for the pharmacological activity of the alkaloid (50), several efforts at the synthesis of this system have been recorded in the literature.

Bromination of the keto diester CLVII (51) under anionic conditions in the presence of sodium hydride followed by dehydrobromination gave a mixture of olefins CLVIII and CLIX. Michael condensation of the olefinic mixture with nitroethane furnished the intermediate CLX. Ethylene ketal formation followed by catalytic reduction of the nitro group then generated lactam CLXI, which was converted to the bicyclic pyridone δ-lactone CLXII through a series of steps paralleling those described under *The Wani-Wall-Levine Synthesis* (Scheme XXV).

The synthesis of the δ-lactone CLXIII has been achieved through the condensation of triethyl 3-ethyl-1(2)-pentene-1,1,3-tricarboxylate with paraformaldehyde (52), and the preparation of the δ-lactone CLXIV has also been reported (53) (Scheme XXVI).

In a study (54) considered as a direct forerunner to a synthesis of camptothecin, the glycol CLXVII was obtained from nipecotic acid (CLXV) *via* methylene



Scheme XXIII

lactam rearrangement to CLXVI, epoxidation, and hydrolysis. The ultimate compound, CLXVIII, which incorporates rings D and E of the alkaloid, was then prepared through a sequence of reactions (Scheme XXVII) bearing close similarity to that applied in the synthesis of camptothecin and described under *The Rapoport–Tang Synthesis.*

Two additional analogs, CLXIX and CLXX, were prepared by a similar route (54). Another variation of this same α-methylene lactam approach, which led to the tricyclic analogs CLXXI and CLXXII, is described in Scheme XXVIII (54).

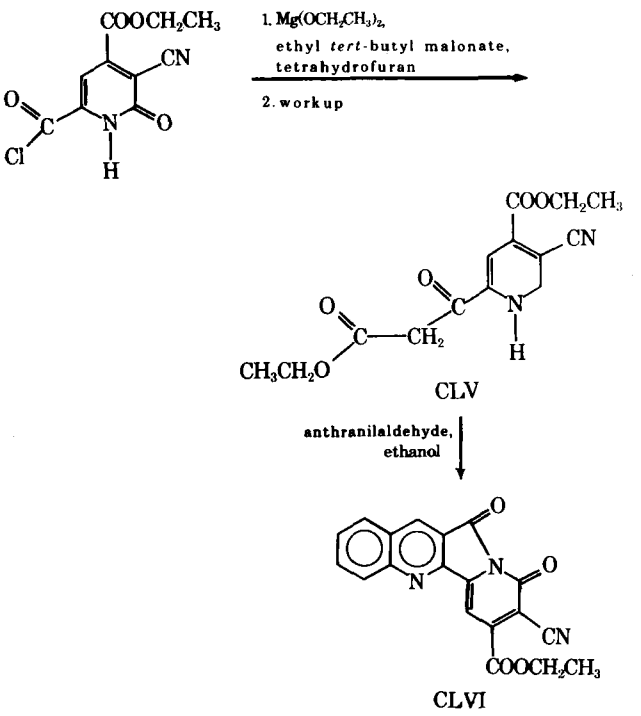
By using 4-methoxy-1-methyl-2-(1*H*)-pyridone (CLXXIII) as the starting material, a method for E ring annelation was developed by Sugasawa *et al.* (37). Vilsmeir formylation (phosphorus oxychloride in dimethylformamide) of CLXXIII supplied the pyridone aldehyde CLXXIV in 73% yield. The terminal compound CLXXV, having the DE ring system of camptothecin, was prepared through a sequence of reactions (Scheme XXIX) similar to that described under *The Sugasawa–Toyoda–Sasakura Synthesis.*

Some efforts aimed at the construction of ring E analogs of camptothecin have also been described (55).

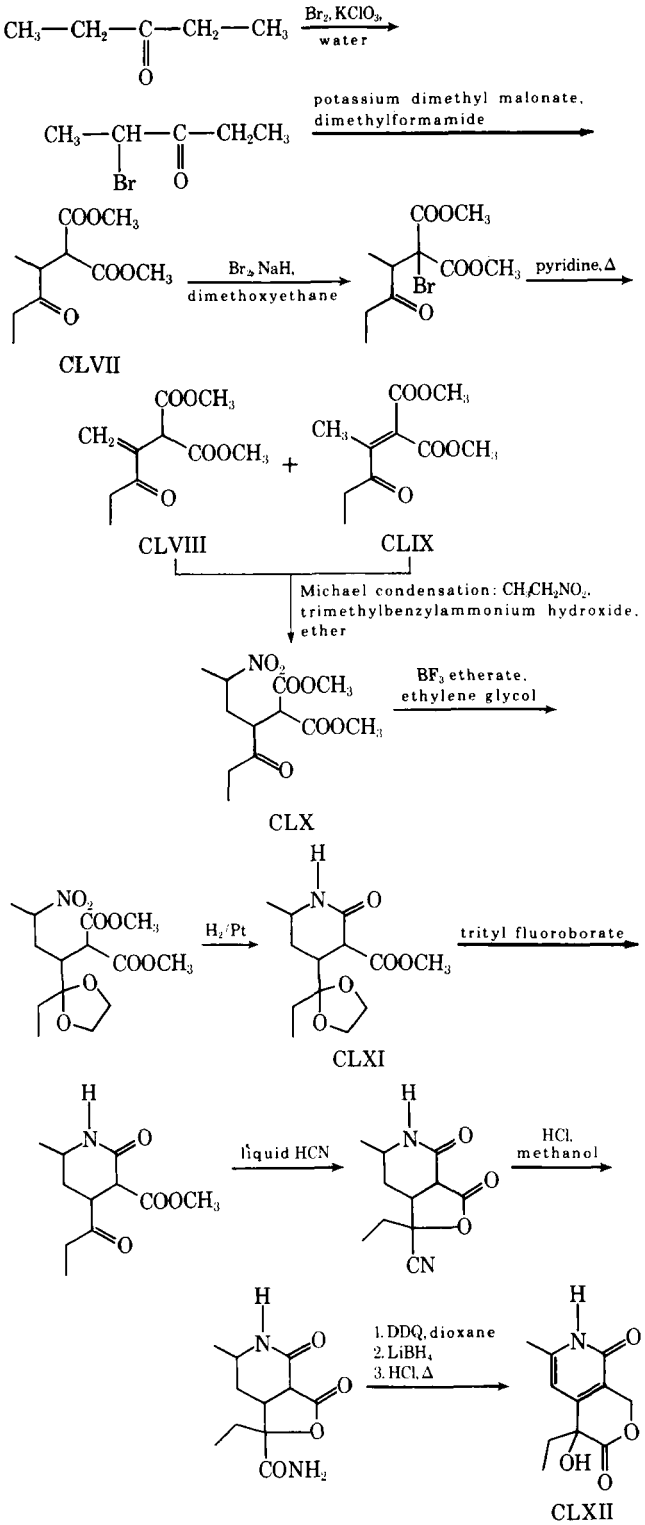
Indole–Quinolone Conversions—Following the biogenetic-type conversion of indole derivatives into quinolones, which culminated in a synthesis of camptothecin (see *The Winterfeldt Synthesis*), it was found that treatment of the tetracyclic indole CLXXVI with potassium *tert*-butoxide in dimethylformamide in the presence of oxygen resulted in formation of the quinolone CLXXVII, which was then transformed to the chloroquinoline CLXXVIII (31) (Scheme XXX). A possible mechanism for the transformation of CLXXVI to CLXXVII is given in Scheme XXXI (30).

In a recent development, the camptothecin analog CLXXX was obtained through the indole–quinolone conversion from the indole compound CLXXIX (56).

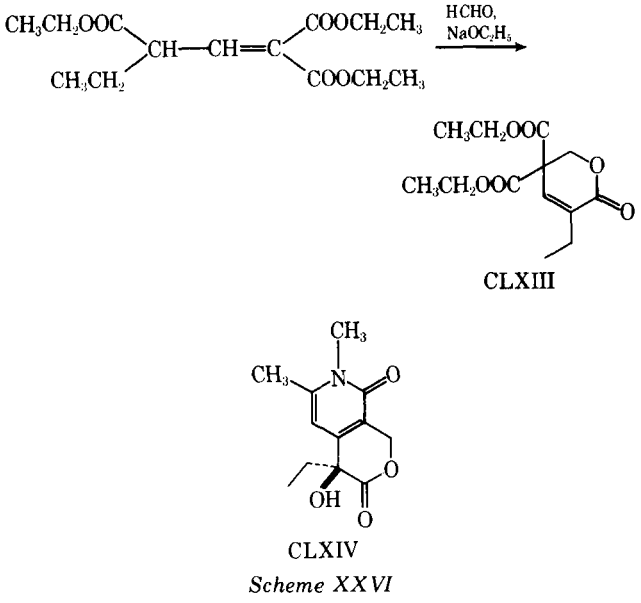
One more indole compound, which was recently synthesized as a potential intermediate to camptothecin, is the hemiketal CLXXXI (57). In a separate study, several model compounds (CLXXXII–CLXXXV) were synthesized through the indole–quinolone conversion of the corresponding indole derivatives (58).



Scheme XXIV



Scheme XXV



Scheme XXVI

PHARMACOLOGY

In the late 1950's, crude extracts of the tree *C. acuminata* (Nyssaceae) were observed to possess antitumor activity during extensive screening (59). At a later time, definite activity was demonstrated for the main alkaloid camptothecin. Tested against leukemia L-1210 in mice, camptothecin gave life prolongation as high as 100% on a daily dose of 0.25–1.0 mg/kg (60). Against rat Walker 256 carcinosarcoma, concentrations as low as 1.25 mg/kg gave significant inhibition of growth (1, 61). Moderate cytotoxic activity, ED_{50} = 0.07 mg/ml, against KB cell culture was also noted.

The effects of camptothecin on DNA have been investigated (62, 63). Some additional biological tests of camptothecin are also available (64, 65).

Following the completion of extensive preliminary testing, camptothecin was administered experimentally to 18 cancer patients at the Baltimore Cancer Research Center (59). The patients were suffering from various advanced solid tumors and were given intravenous injections of camptothecin as the sodium salt. These injections were administered approximately every 2 or 3 weeks in dosages proportional to the body weight of the patient. The most favorable responses were noted in those patients suffering from advanced GI tumors. Improvement was noted in 11 cases. In five of these, at least a 50% mass decrease in the tumor was observed. Side effects noted in these patients were generally tolerable and consisted mainly of alopecia and GI disorders.

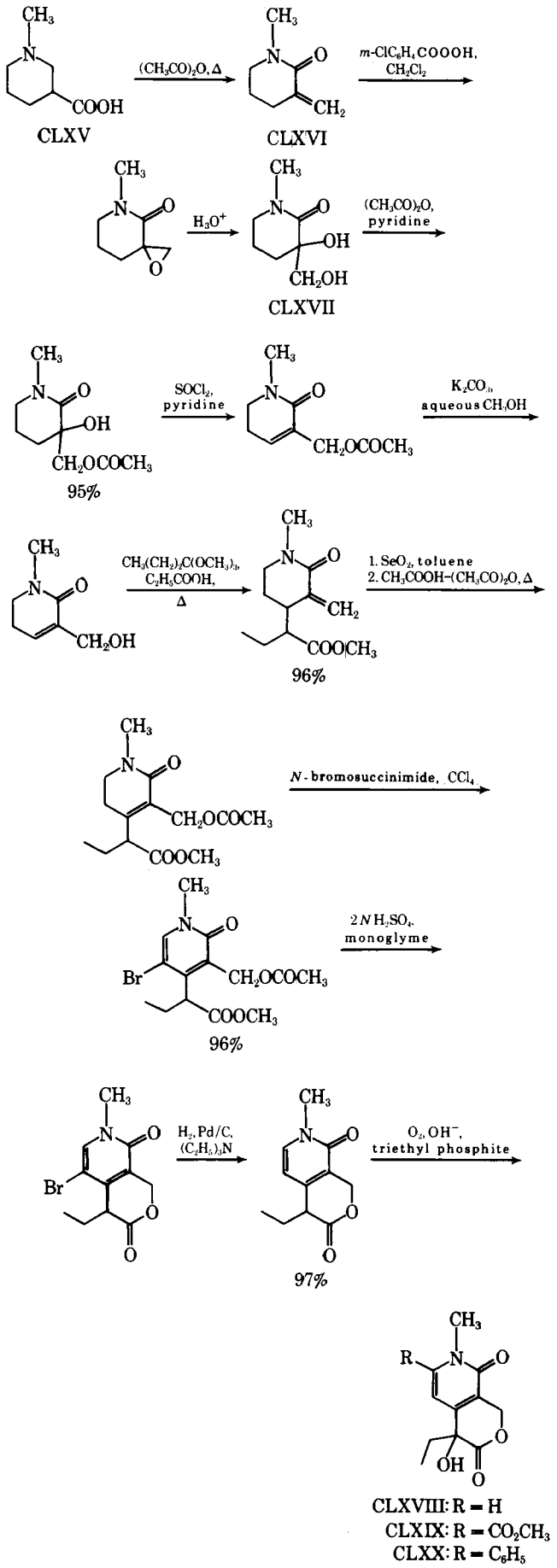
These encouraging results were not corroborated, however, in more recent tests at the Mayo Clinic in Minnesota (66). Camptothecin was used in the treatment of 61 patients with advanced GI adenocarcinoma. Only two of these (3%) showed partial objective responses after 2 months.

ADDENDUM

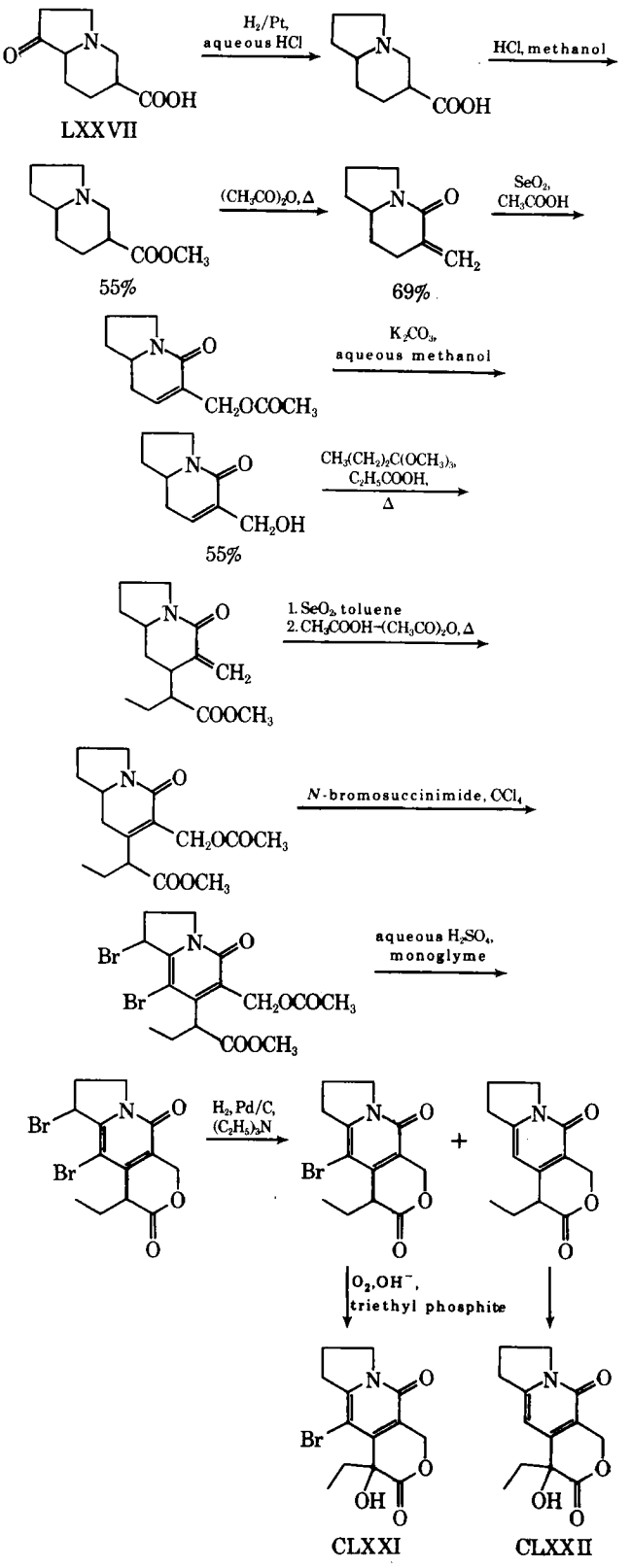
Two additional syntheses of camptothecin have appeared and are discussed below.

The Kende Synthesis—The first goal of this synthesis was the preparation of the furoic acid CLXXXVII starting from furfuraldehyde dimethyl acetal (CLXXXVI) (Scheme XXXII) (67).

The next two steps were aimed toward the construction of the pentacyclic amide CLXXXVIII, which underwent a facile and unusual alkaline hy-

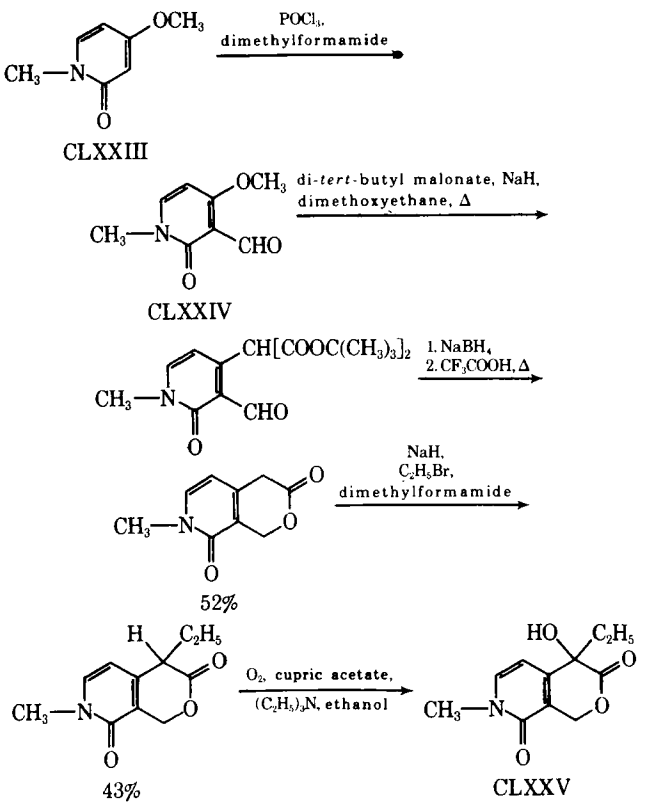


Scheme XXVII

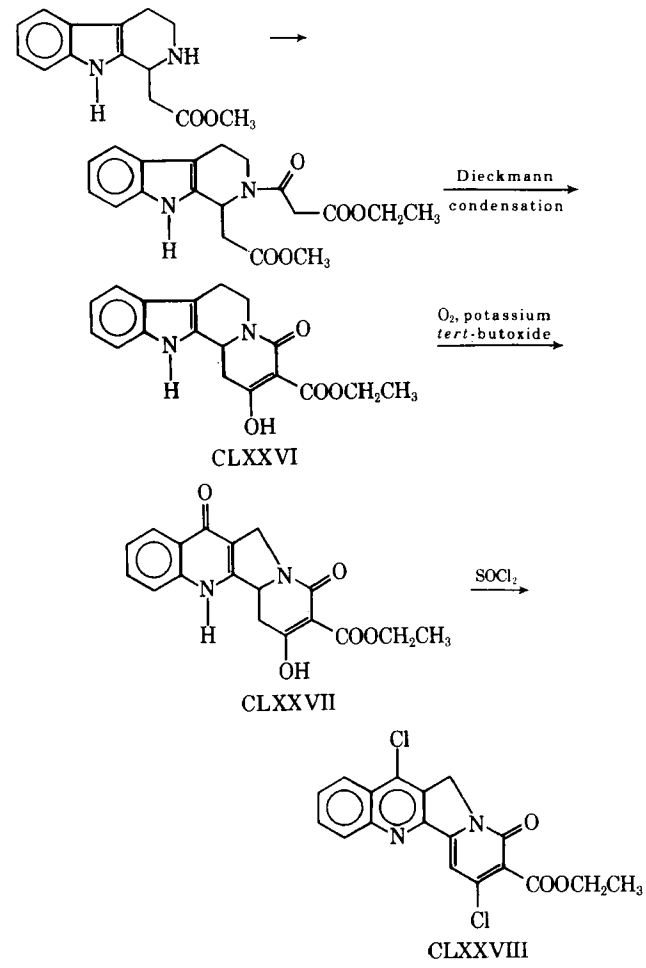


Scheme XXVIII

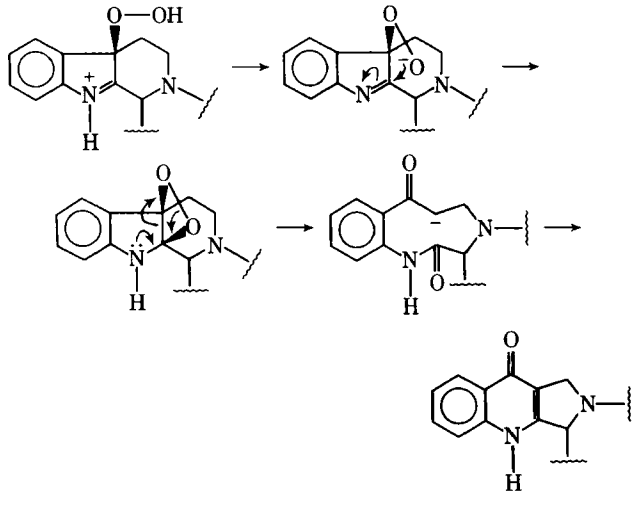
drolysis with loss of one carbon atom to yield the hydroxyamide CLXXXIX, probably by the mechanism indicated. The final steps involved construction of the known tetracyclic ester XLVIII (27), which had been previously converted to (±)-camptothecin (Scheme XXXIII).



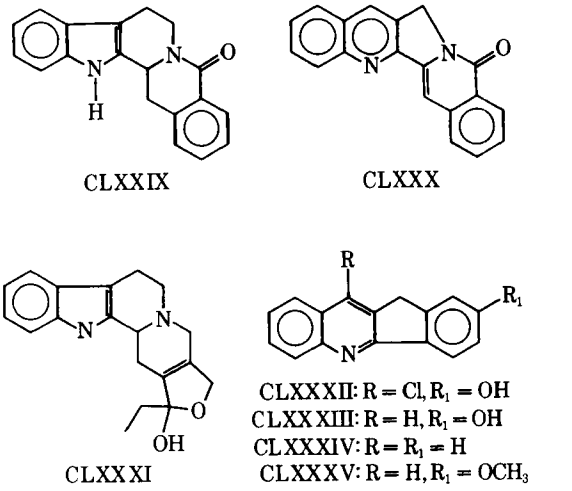
Scheme XXIX

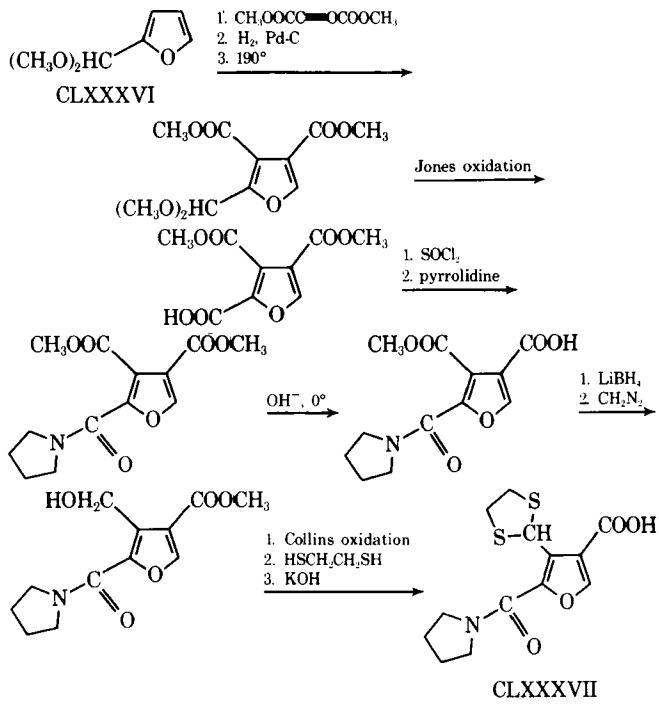


Scheme XXX

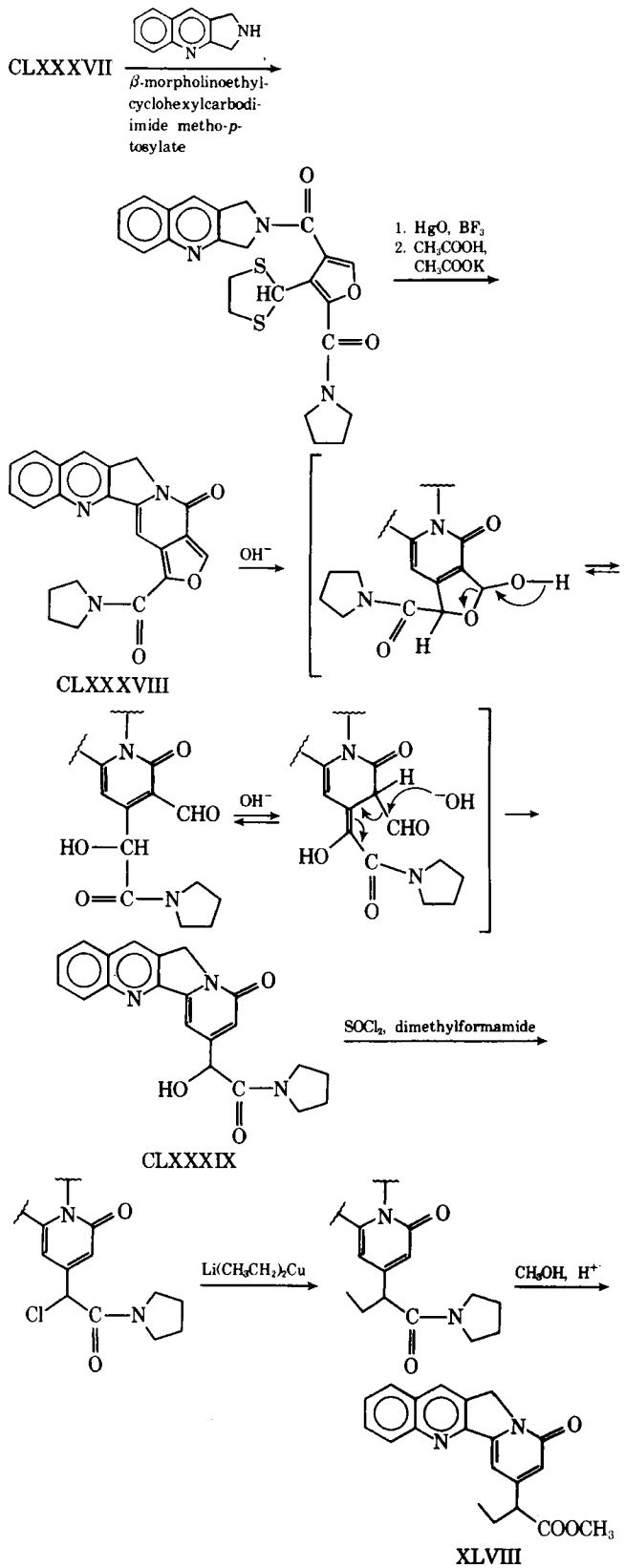


Scheme XXXI

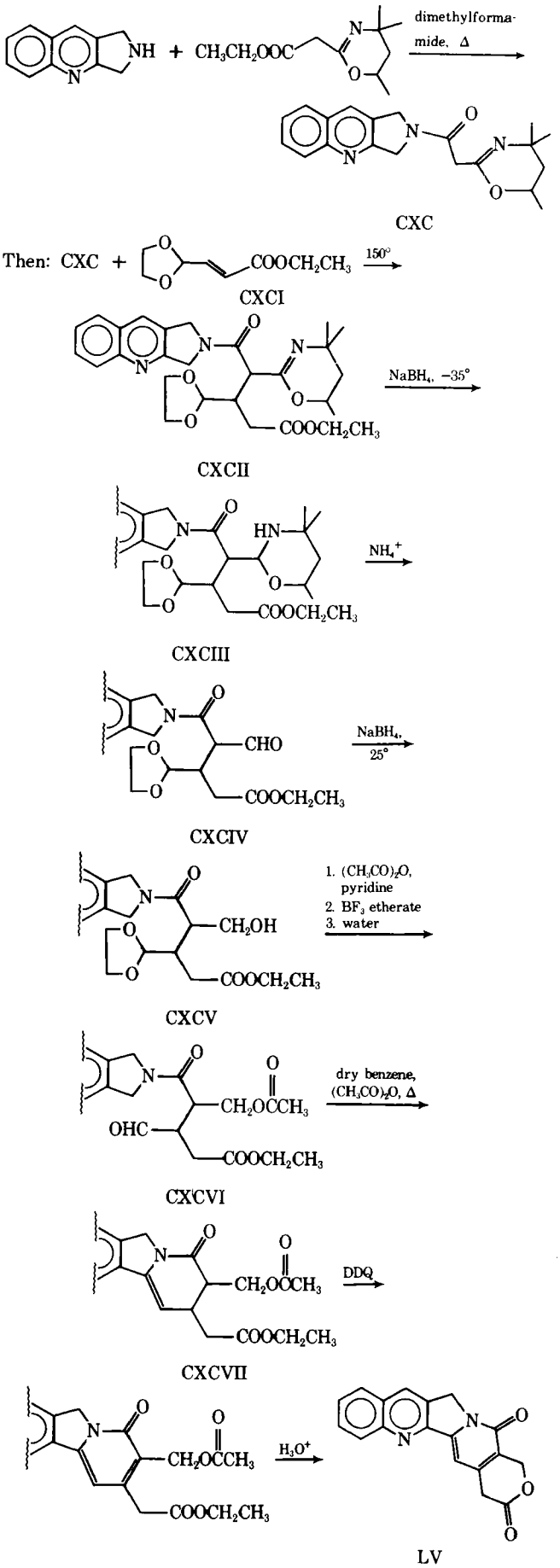




Scheme XXXII

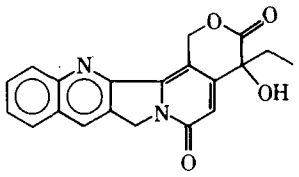


Scheme XXXIII



Scheme XXXIV

The Meyers Synthesis—The synthesis achieved by Meyers and coworkers required initially the preparation of the oxazine amide CXC, which underwent Michael addition with the unsaturated ester CXCI, thus furnishing the oxazine ester CXCII. Sodium bo-



CXCVIII

rohydride reduction to the tetrahydro derivative CXCIII, followed by cleavage to the aldehyde CXCIV, produced, following borohydride reduction, the hydroxy ester CXCV. Cyclodehydration of the aldehyde CXCVI led to the dihydropyridone CXCVII which, after oxidation and hydrolysis, was converted to deoxydeethylcamptothecin (LV). The latter compound had been previously converted to racemic camptothecin (27) (Scheme XXXIV).

Miscellaneous—The synthesis of isocamptothecin (CXCVIII) (69), which is not a natural product, as well as of other camptothecin analogs (70) has been reported. A review of the chemistry, pharmacology, and syntheses of camptothecin has appeared recently (71).

REFERENCES

(1) M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail, and G. A. Sim, *J. Amer. Chem. Soc.*, **88**, 3888(1966).

(2) R. E. Perdue, Jr., R. L. Smith, M. E. Wall, J. L. Hartwell, and B. T. Abbott, Tech. Bull. 1415, Agricultural Research Service, U.S. Department of Agriculture, 1970.

(3) R. E. Perdue, Jr., M. E. Wall, J. L. Hartwell, and B. T. Abbott, *Lloydia*, **31**, 229(1968).

(4) M. E. Wall and M. C. Wani, *Abstracts*, 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., Apr. 1967, p. 6M.

(5) A. T. McPhail and G. A. Sim, *J. Org. Chem. (B)*, **1968**, 923.

(6) T. R. Govindachari and N. Viswanathan, *Indian J. Chem.*, **10**, 453(1972).

(7) M. C. Wani and M. E. Wall, *J. Org. Chem.*, **34**, 1364(1969).

(8) G. Kemper and S. Hirshberg, *Chem. Ber.*, **98**, 419(1965).

(9) M. Shamma, *Experientia*, **14**, 107(1968).

(10) W. T. Blackstock and R. T. Brown, *Tetrahedron Lett.*, **1971**, 3727.

(11) R. T. Brown and W. P. Blackstock, *ibid.*, **1972**, 3063.

(12) V. Snieckus, in "The Alkaloids," vol. XI, R. H. F. Manske, Ed., Academic, New York, N.Y., 1968, p. 1.

(13) E. Wenkert, K. G. Dave, R. G. Lewis, and P. W. Sprague, *J. Amer. Chem. Soc.*, **89**, 6741(1967).

(14) A. R. Battersby, A. R. Burnett, and P. G. Parsons, *J. Chem. Soc. C*, **1969**, 1187, 1193; *Chem. Commun.*, **1970**, 826.

(15) S. Escher, P. Loew, and D. Arigoni, *Chem. Commun.*, **1970**, 823.

(16) A. R. Battersby, S. H. Brown, and T. G. Payne, *ibid.*, **1970**, 827.

(17) A. R. Battersby, R. S. Kapil, J. A. Martin, and L. Mo, *ibid.*, **1968**, 133.

(18) A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Martin, and A. O. Plunkett, *ibid.*, **1966**, 890.

(19) P. Loew and D. Arigoni, *ibid.*, **1968**, 137.

(20) A. I. Scott, *Accounts Chem. Res.*, **3**, 151(1970).

(21) E. Leete, *ibid.*, **2**, 59(1969).

(22) I. D. Spenser, *Comp. Biochem.*, **20**, 231(1968).

(23) M. Janot, R. Goutarel, A. Lettir, G. Tsatsos, and V. Prelog, *Helv. Chim. Acta*, **38**, 1073(1955).

(24) B. Witkop and J. B. Patrick, *J. Amer. Chem. Soc.*, **73**, 2196(1951).

(25) B. Witkop and S. Goodwin, *ibid.*, **75**, 3371(1953).

(26) G. Stork and A. G. Schultz, *ibid.*, **93**, 4074(1971).

(27) R. Volkmann, S. Danishefsky, J. Eggler, and D. M. Solomon, *ibid.*, **93**, 5576(1971).

(28) E. Winterfeldt, T. Korth, D. Pike, and M. Boch, *Angew. Chem., Int. Ed.*, **11**, 289(1972).

(29) M. Boch, T. Korth, J. M. Nelke, D. Pike, H. Radunz, and E. Winterfeldt, *Chem. Ber.*, **105**, 2126(1972).

(30) E. Winterfeldt, *Ann.*, **745**, 23(1971).

(31) E. Winterfeldt and H. Radunz, *Chem. Commun.*, **1971**, 374.

(32) M. C. Wani, H. F. Campbell, G. A. Brine, J. A. Kepler, M. E. Wall, and S. G. Levine, *J. Amer. Chem. Soc.*, **94**, 3631(1972).

(33) C. Tang and H. Rapoport, *ibid.*, **94**, 8615(1972).

(34) M. Ferles, *Collect. Czech. Chem. Commun.*, **29**, 2323(1964).

(35) W. S. Johnson, L. Werthemann, W. R. Bartlett, T. J. Brocksom, T.-t. Li, D. J. Faulkner, and M. R. Petersen, *J. Amer. Chem. Soc.*, **92**, 741(1970).

(36) M. Shamma, D. A. Smithers, and V. St. Georgiev, *Tetrahedron*, **29**, 1949(1973).

(37) T. Sugasawa, T. Toyoda, and K. Sasakura, *Tetrahedron Lett.*, **1972**, 5109.

(38) J. A. Kepler, M. C. Wani, J. N. McNaull, M. E. Wall, and S. G. Levine, *J. Org. Chem.*, **34**, 3853(1969).

(39) M. Shamma and L. Novak, *Collect. Czech. Chem. Commun.*, **35**, 3280(1970).

(40) T. Tanaka, K. Mashimo, and M. Wagatsuma, *Tetrahedron Lett.*, **1971**, 2803.

(41) G. Kempter and S. Hirschberg, *Chem. Ber.*, **98**, 419(1965).

(42) L. H. Zalkow, J. B. Nabors, K. French, and S. C. Bisarya, *J. Chem. Soc. C*, **1971**, 3551.

(43) M. Shamma and L. Novak, *Tetrahedron*, **25**, 2275(1969).

(44) M. C. Wani, J. A. Kepler, J. B. Thompson, M. E. Wall, and S. G. Levine, *Chem. Commun.*, **1970**, 404.

(45) A. S. Kende, R. W. Draper, I. Kubo, and M. Joyeux, *Abstracts*, 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1970, ORGN 10.

(46) T. Kametani, H. Nemoto, H. Takeda, and S. Takano, *Chem. Ind. (London)*, **1970**, 1323; *Tetrahedron*, **26**, 5753(1970).

(47) T. K. Liao, W. H. Nyberg, and C. C. Cheng, *J. Heterocycl. Chem.*, **8**, 373(1971); Compound XXV has been independently synthesized by Stork and Schultz (Ref. 26).

(48) R. F. Borch, C. V. Grudzinskas, D. A. Peterson, and L. D. Weber, *J. Org. Chem.*, **37**, 1141(1972).

(49) T. Kametani, S. Takano, H. Terasawa, and H. Takeda, *J. Pharm. Soc. Jap.*, **92**, 868(1972).

(50) M. E. Wall, *Abstracts*, 4th International Symposium on the Biochemistry and Physiology of Alkaloids, Halle, DDR, Academic Press, Berlin, Germany, 1969, p. 77.

(51) M. E. Wall, H. F. Campbell, M. C. Wani, and S. G. Levine, *J. Amer. Chem. Soc.*, **94**, 3632(1972).

(52) G. R. Pettit, R. J. Quinn, T. H. Smith, and P. Brown, *J. Org. Chem.*, **37**, 2789(1972).

(53) R. H. Wood, P. G. Hoffman, V. S. Waravdekar, and H. B. Wood, *Abstracts*, 162nd National Meeting of the American Chemical Society, Washington D.C., Sept. 1971, ORGN 88.

(54) J. J. Plattner, R. D. Gless, and H. Rapoport, *J. Amer. Chem. Soc.*, **94**, 8613(1972).

(55) T. Kametani, S. Takano, H. Nemoto, and H. Takeda, *J. Pharm. Soc. Jap.*, **91**, 966(1971).

(56) J. Warneke and E. Winterfeldt, *Chem. Ber.*, **105**, 2120(1972).

(57) T. Kametani, S. Takano, and H. Takeda, *J. Pharm. Soc. Jap.*, **92**, 743(1972).

(58) J. A. Beisler, *J. Med. Chem.*, **14**, 1116(1971).

(59) J. A. Gottlieb, A. M. Guarino, J. B. Call, V. T. Oliverio, and J. B. Block, *Cancer Chemother. Rep.*, **54**, 461(1970).

(60) J. M. Venditti and B. J. Abbott, *Lloydia*, **30**, 332(1967).

(61) W. D. DeWys, S. R. Humphreys, and A. Goldin, *Cancer Chemother. Rep.*, **52**, 229(1968).

(62) A. Spataro and D. Kessel, *Biochem. Biophys. Res. Commun.*, **48**, 643(1972).

(63) D. Kessel, H. B. Bosmann, and K. Lohr, *Biochim. Biophys. Acta*, **269**, 210(1972).

(64) D. E. Hunt and R. F. Pittillo, *Appl. Microbiol.*, **16**, 867(1968).

(65) A. M. Guarino and J. B. Call, *Fed. Proc.*, **29**, 543(1970).

(66) C. G. Moertel, A. J. Schutt, R. J. Reitemeier, and R. G. Hahn, *Cancer Chemother. Rep.*, **56**, 95(1972).
(67) A. S. Kende, T. J. Bentley, R. W. Draper, J. K. Jenkins, M. Joyeux, and I. Kubo, *Tetrahedron Lett.*, **1973**, 1307.
(68) A. I. Meyers, R. L. Nolen, E. W. Collington, T. A. Narwid, and R. C. Strickland, *J. Org. Chem.*, **38**, 1974(1973).
(69) S. Danishefsky and R. Volkmann, *Tetrahedron Lett.*, **1973**, 2521.
(70) S. Danishefsky, J. Quick, and S. B. Horwitz, *ibid.*, **1973**, 2525.
(71) A. G. Schultz, *Chem. Rev.*, **73**, 385(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received from the *Department of Chemistry, Pennsylvania State University, University Park, PA 16802*

The authors are grateful to the National Institutes of Health for Grant CA-11450.

*To whom inquiries should be directed.

RESEARCH ARTICLES

Comparison of Criteria for Content Uniformity

BRUCE FLANN

Abstract □ The criterion of lot quality for pharmaceutical unit doses based on the percentage of defectives has been generally used in the evaluation of tests determining content uniformity. This criterion is rejected in favor of joint criteria based on the deviation of the lot mean content from label claim and on the standard deviation of the unit content within the lot. By utilizing methods of simulation by computer, several tests by attributes, including those tests currently in the USP and the NF, are compared with selected tests by variables for reliability, flexibility, and simplicity. (Robustness with respect to type of distribution is rejected as a criterion because it is dependent on the definition of lot quality.) Several tests by variables are quite superior to tests by attributes with respect to reliability and flexibility. Tests for mean content and for weight variation are also examined.

Keyphrases □ Content uniformity—comparison of criteria, percentage of defectives compared to deviation of lot mean content from label claim and standard deviation of within lot unit content, computer simulations □ Statistical evaluation, content uniformity criteria—percentage of defectives method compared to joint use of deviation of lot mean content from label claim and standard deviation of within lot unit content, computer simulations □ Tablets and capsules, content uniformity—comparison of criteria for determination, computer simulations, statistical evaluations

The Pharmaceutical Manufacturers Association of the United States, the United States Pharmacopeial Convention, and the National Formulary Board have pioneered in the field of quality control of the finished pharmaceutical with official recognition of the need for a criterion of content uniformity among single-dosage units. Since 1965, the USP (1) and NF (2) have described, for some tableted pharmaceuticals, a test of this uniformity. Subsequently, they have included a special variation for application to capsules.

A review by Olson and Lee (3) mentioned that the statistics of acceptance sampling was developed during World War II for the armament industry, and shortly after the technique was applied by the pharmaceutical industry to the control of tablet weight (4). In 1960, F. Wiley of the Food and Drug Administration presented data (5) showing that the test controlling tablet weight variation was not controlling content variation. He suggested the replacement of this test with a test for content uniformity. Both old and new tests required counting the number of units beyond an acceptance range (for weight or assay, respectively) and the number beyond double-the-range where the midpoint of the acceptance range was a function either of average tablet weight or average assay.

The tests subsequently accepted by the NF and the USP are of the sequential type requiring 10 or 30 assays. Decisions are based on the number of assays beyond the range 85–115% of label claim. Also, in some cases a sample is automatically rejected if any assays are beyond the range 75–125% of label claim.

Those tests, which are based on the number of measurements (made on single units from a sample) outside an acceptance range, are called tests by attributes. Examples are described in military specifications (6). Other tests, which are based on the magnitude of one or more variables describing the sample, are called tests by variables. A well-known variation of this class of test is based on the magnitude of "the absolute deviation of the mean from the target value plus a multiple of the standard deviation," as described in another set of military specifications (7) and by Lieberman and Resnikoff (8).

Generally, it is accepted that the tests by variables are the more reliable tests of dispersion; *i.e.*, conclusions are less subject to the vagaries of random sampling, at least where the variable is normally distributed. However, there is ample evidence that the content of tablets is not always normally distributed (4, 9–11). Papers comparing the two types of tests can be divided into two groups: those that suggest that most pharmaceutical lots have an approximately